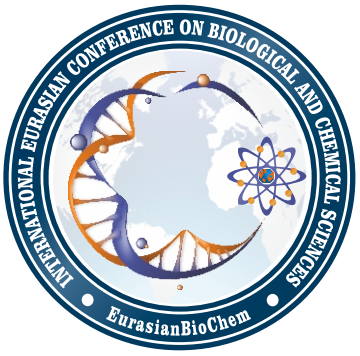


5th
INTERNATIONAL EURASIAN CONFERENCE ON

# BIOLOGICAL AND CHEMICAL SCIENCES

23 - 25 November 2022
Ankara / Turkey

(EurasianBioChem2022)
www.eurasianbiochem.org

# PROCEEDING BOOK

## (Abstracts and Full-Text Papers)

## EurasianBioChem

ISBN : 978-605-72134-0-2

# 5th International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2022)

November 23-25, 2022
Ankara, Turkey
www.EurasianBioChem.org

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## (Abstracts and Full-Text Papers)

**Editor**

Prof. Dr. Muhittin DOĞAN

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**ISBN:** 978-605-72134-0-2

**Publication Date:** December 2022

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* This list is arranged in alphabetical order.

# FOREWORD

*5th International Eurasian Conference on Biological and Chemical Sciences* (*EurasianBioChem 2022*) was successfully held on November 23-25, 2022 with a great participation from many countries.

The objective of *EurasianBioChem 2022* was to bring leading academicians, researchers, scholars as well as industrial professionals together from all over the world to exchange and share their experiences and research results about all aspects of biological and chemical sciences and to discuss the practical challenges encountered and the solutions adopted. In this context, we welcomed precious scientists and the greatest appreciation goes to all participants whose contributions have made this conference a success. I would like to thank the organizing committee and the scientific committee for providing this intense and high quality scientific environment.

I believe that the *EurasianBioChem 2022* has made important contributions to you at large and hope to see you at the *6th International Eurasian Conference on Biological and Chemical Sciences* (*EurasianBioChem 2023*).

**Prof. Dr. Muhittin DOĞAN**

Chairman of *EurasianBioChem 2022*

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# 1. ORAL PRESENTATIONS

# 1.1. ABSTRACTS

## *ORAL PRESENTATION*

# Molecular docking studies of 16-(substituted) dehydroepiandrosterone derivatives as CDK-6 inhibitors for CNS anticancer activity

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**Abstract**

Glucocorticoids have been used in the treatment of brain tumor patients for decades, and they are among the most effective medications for lowering tumor-associated edema, minimizing side effects, and reducing the risk of encephalopathy in patients receiving radiation therapy[9-10] Corticosteroids are commonly used in cancer treatment, and they are especially effective in individuals with substantial peritumoral edema and neurological impairments. The present study was aimed to correlate the 57 novel steroidal derivatives with their ability to inhibit CDK6 protein which is an important target for brain cancer. Molecular docking studies were performed on these 16-substituted epiandrosterone derivatives using Autodock. The best docked compound (S228) is showing Hydrogen bondings with LYSA:43, LYSA29 and ASPA:163, one Pi-Sigma bond with ALAA:162 and alkyl binding with LYSA:29, VALA:27, ILEA:19, ILUA:152. the derivatives possessing 16-(4-nitrobenzylidene) substitution have exhibited higher binding energies in comparison to other substituents. At position 3, the substitution of acetate group, amines or pyrrole ring is preferred.

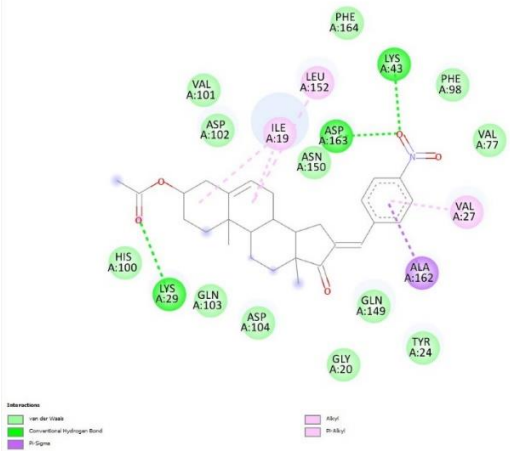


Figure 1: Binding interactions of (S228) structure

**Keywords**: 16-substituted epiandrosterone derivatives, CDK6 inhibitors, brain cancer, Molecular modelling, AutoDock.

## *ORAL PRESENTATION*

# Whole exome sequencing identifies five pathogenic mutations in *ERCC2, MCPH1, CENPJ, ERCC6,* and *CASK* causing primary microcephaly (MCPH) in inbred families of Pakistan

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**Abstract**

Autosomal recessive primary microcephaly (MCPH) is a clinically and genetically diverse neurodevelopmental disorder defined by a diminution in brain volume which results in small head size. There is currently no cure for the disorder, and its exact genetic basis are still unknown. Recent literature reveals 29 genes associated with MCPH. The current study was aimed to better understand the pathophysiology and genetics of the disease. Consanguineous marriages had a higher incidence of MCPH patients, primarily in Pakistan. The study was duly approved from the ethical review committee of Government College University Faisalabad, Pakistan, and the Institute of Biomedicine and Biotechnology SIAT- Chinese Academy of Sciences. Families were collected with the help of special schools, health workers and social media platform. Blood samples of affected and normal subjects were collected after written informed consent of affected family's parents/guardians. DNA was extracted, and whole exome sequencing (WES) was performed on it. Variants were screened and prioritized after data analysis. Sanger sequencing was used to validate the prioritized variants. The results revealed five mutations: ERCC2 (c.2255G>A) in family 1, MCPH1 (c.1254delT) in family 2, CENPJ (c.18delC) in family 3, ERCC6 (c.1178C>T) in family 4, and CASK (c.1289G>A) in family 5. These findings will aid in understanding the genetics and pathophysiology of MCPH, improve molecular diagnostics, pave the way for genetic counselling and the development of novel therapeutics for treating primary microcephaly.

**Keywords***:* Consanguinity; Primary microcephaly; clinical and genetic heterogeneity; DNA extraction; Whole-exome sequencing (WES); Sanger sequencing.

## *ORAL PRESENTATION*

# Cardiomyogenic differentiation of human umbilical cord derived mesenchymal stem cells by overexpression of cardiac transcription factors

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**Abstract**

Stem cell-based therapy is considered one of the most promising disciplines in regenerative medicine. Several clinical trials and meta-analyses of cardiovascular diseases appear to support the **c**ardiac regenerative potential of stem cells. The adult, multipotent mesenchymal stem cells (MSCs) are one of the promising stem cell types. MSCs have multi-differentiation and immuno-modulatory properties. Transcription factors (TFs) play an important role in directing cell fate. In the present study, we aimed to explore the potential of cardiac transcription factors in the cardiac differentiation of human umbilical cord mesenchymal stem cells (hUC-MSCs). hUC-MSCs were isolated and characterized based on their surface marker via immunocytochemistry, flow cytometry, adipogenesis, and osteogenesis assays. The analyses of cardiac potential of transfected hUC-MSCs were analyzed using qPCR and immunocytochemistry. Overexpression of transcription factors in hUC-MSCs greatly enhanced the cardiac genes and protein expression. The results of this study indicate the successful differentiation of hU-MSCs into cardiac-like cells. This research highlights the therapeutic approach for repairing ischemic heart tissue.

**Keywords:** Human umbilical cord, Mesenchymal stem cells, Transcription factors, Heart regeneration, Differentiation.

## *ORAL PRESENTATION*

# A DFT investigation study of the regioselectivity of [3+2] cycloaddition reaction

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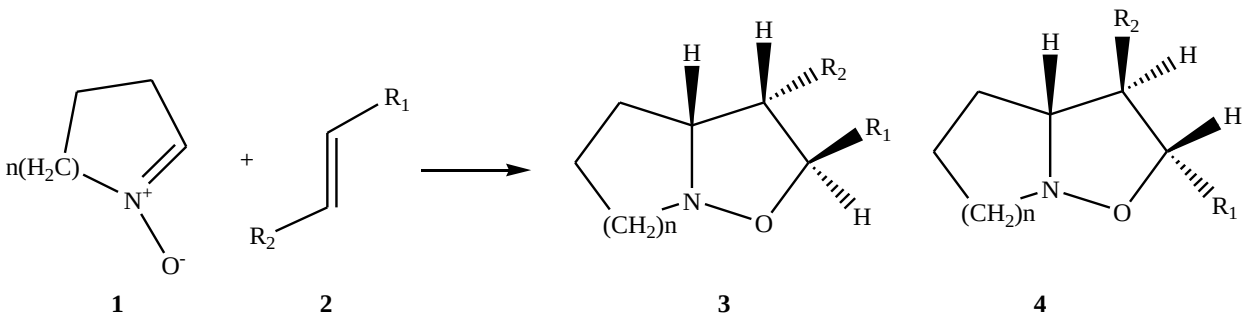
**Abstract**

Cycloaddition reactions of alkenes with cyclic compounds are of such importance in organic chemistry. They are the key role to form five and six membered cyclic compounds. These latter are used in several domains especially in pharmaceutical industry. Particularly, the 1,3dipolar cycloaddition of cyclic nitrones with substituted alkenes shows different forms of regio- and stereoselectivity depending on the electronic and steric effects of the substituents. Normal disubstituted alkenes cycloadditions result in the regiospecific formation cycloadducts having the substituted end of the alkenes attached to the oxygen terminal of the nitrone functionality.
Reactions between nitrones and alkenes leading to the formation of isoxazolidines are well-known due to their great importance in construction processes. Substituted isoxazolidines are interesting biological active compounds that could be used as enzyme inhibitors.




**1** **2** **3** **4**

**Figure 1:** 1,3-Dipolar cycloaddition of nitrones with substituted alkenes

In this study, a presentation of the observed regioselectivity of such reactions is probed theoretically using DFT method and reactivity indices such as Fukui and Parr functions.

**Keywords**: Cycloaddition reactions, Regioselectivity, Parr functions, Fukui functions, DFT calculations.

**References :**

[1] A. Padwa, In *Comprehensive Organic Chemistry*; Pergamon Press: Oxford, 1991; pp 1069-1109.
[2] T. K. Das, S. Salampuria, M. Banerjee, *J. Mol. Struct. Theochem.*, 959 (2010) 22-29.
[3] K. B. G. Torssell, In *Nitrile oxides, nitrones and nitronates in organic synthesis*; New York: VCH, 1998.
[4] S. Majumder, P. Bhuyan, J. *Tetrahedron Lett*., 53 (2012) 762-764.
[5] A. R. Minter, B. B. Brennan, A. K. Mapp, *J. Am. Chem. Soc*., 126 (2004) 10504-10505.
[6]. P. Ding, M. Miller, Y. Chen, P. Helquist, A. J. Oliver, O. Wiest, *Org. Lett*., 6 (2004) 1805-1808.
[7]. K. Marakchi, R. Ghailane, O. Kabbaj, N. Komiha, J. *Chem. Sci*., 126 (2014) 283-292.

## *ORAL PRESENTATION*

# Development and characterization of enriched bioplastics made from whole seaweed and carrageenan for food packaging

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**Abstract**

Recently, seaweed and its hydrocolloids were being promoted as ingredient for biodegradable plastics. In this study, bioplastics were developed from whole seaweed (*Kappaphycus alvarezii*) biomass (WS), semi-refined carrageenan (SRC) and refined carrageenan (RC). To enhance their properties, they were dyed using butterfly pea flower (*Clitoria ternatea L.*), bougainvillea flower; and dragon fruit (*Hylocereus polyrhizus*) peel extracts. The bioplastics development employed material-to-water ratio of 1:50; 2% (v/v) glycerol as plasticizer and 5% (v/v) natural dye. As results, the bioplastics were 0.03 – 0.100 mm in thickness which is in the range of commercial food packaging. Without dye, the WS, SRC and RC bioplastics has opacity ($mm^{-1}$) of 4.14 ± 0.45, 3.50 ± 0.30 and 1.63 ± 0.31, respectively; meanwhile colour difference (ΔE) were 29.6 ± 0.1, 14.2 ± 0.1 and 6.4 ± 0.1, respectively. RC bioplastic has the highest tensile strength (12.80 ± 0.90 MPa) and elongation-at-break (25.7 ± 0.5 %), followed by SRC (8.0 ± 0.4 MPa, 21.3 ± 0.6%) and WS (5.4 ± 0.7 MPa, 17.9 ± 1.0 %). No significant difference in water vapour permeability (WVP); WS degraded the fastest with all bioplastics fully degraded after 18 days; and shelf life (at 25ºC, 56% RH) up to 17 weeks. For the dyed bioplastics, the natural dye blended well into the natural colour of base material resulting in attractive shade. Mechanical properties, WVP and biodegradability were not significantly affected but shelf-life was 20 - 30% longer. Seaweed bioplastics have a potential as green alternative for single-use non-degradable food packaging.

**Keywords:** Bioplastic, seaweed, Kappaphycus alvarezii, antioxidant.

## *ORAL PRESENTATION*

# Study of the mechanical properties of a composite reinforced by waste: polyester and glass

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**Abstract**

The use of composite materials in various applications is experiencing considerable progress because of their excellent mechanical properties, but they have a detrimental impact on the environment during manufacture and after their final use. The objective of this work is the recovery of glass waste from the company "MAGHREB PIPE" M'sila. We prepared composite materials based on two thermosetting matrices (Isophthaleic and Orthophthaleic Unsaturated Polyester) reinforced with glass waste and glass-sand waste with different proportions (10,20, 30, 40, 50% m/m ). The mechanical results show that the waste loading rate has a positive effect on the three-point bending strength and the Young's modulus of this material. The highest values for the resistance and the Young's modulus of bending were respectively 66 MPa, 119 MPa for the composite based on the unsaturated polyester orthopthaleic matrix reinforced with 10% glass waste. Finally, we can say that the waste from the company "MAGHREB PIPE" M'sila has a very promising future for composite materials.

**Keywords:** Composite, unsaturated polyester, waste glass, strength flexural, adhesion.

## *ORAL PRESENTATION*

# The effect of alkali and permanganate treatment on the flexural properties of Clay/ Sawdust composite

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**Abstract**

This study investigates the effect of the chemical treatment of sawdust on the mechanical and thermal properties of a clay composite reinforced with sawdust in variable mass proportions: 0.5, 1, 1.5, 2 and 2.5%, intended for use as brick in rural houses in desert areas. The sawdust was chemically treated with 5 wt% NaOH and 0.01 wt% $KMnO_4$ solutions. The mechanical test results of the reinforced composite with alkali treated sawdust show an increase in flexural and compression strength, reaching a maximum value of 0.89 and 4.85 MPa, respectively.

**Keywords:** Sawdust, clay, thermal conductivity, mechanical properties, chemical treatment.

## *ORAL PRESENTATION*

# Pharmacological drug use aspects related with e Ecotoxicology and Environmental Protection.

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**Abstract**

The use of Pharmaceuticals drugs in humans in animals and in the aquatic environment can impact the environmental health due to their pharmacokinetics and pharmacodynamics process in humans and animals body. The drug excretions can be transform in contaminants of growing importance in environmental health. Pharmaceuticals drugs could affect non-target species and might eventually damage sustainability of susceptible populations in the ecosystem. In these circumstances the health consequences among susceptible human population cannot be ruled out since long-term exposure to cocktails of pharmaceuticals, which might be present in soil, in food and even in drinking water. It is also very important to mention the fact of the antimicrobic gene resistance which circulates in environment through a closed cycle. Taking in consideration these facts it is very important to make a risk analyse for the environmental health issues of pharmaceutical residues. Chronic toxicities, and toxicities caused because of pharmaceutical mixtures and can be a persistent threat for the environment and public health Wastewater and drinking water treatment is essential in prevention of toxicological damage of the environment. One of the aspects of restriction measurements against environment pollution, except the treatment of drinking water and wastewater are also some other restriction measures that can be undertaken in building a guideline of antimicrobic drugs use in humans and animals. Some other measurements include all the instruments that can be used for the prevention of the toxilogical effects caused by drugs residues in environment by reconstruiring a program for unused or expired drugs through the reconsideration of the weakest points of drug monitoring system.

**Key words**: ecotoxicology, pharmaceutical drugs, ecosystem, environment

## *ORAL PRESENTATION*

# The influence of impressed current cathodic protection on the steel pipe coating process

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**Abstract**

Cathodic protection by impressed current coupled with passive protection; require the control and mastery of a whole series of parameters during its operation. The poor cathodic protection which is not well controlled causes various defects on the coatings. This work shows that the potential distribution depends on the precise control of a certain number of characteristic parameters such as the medium, the environment and the protection tools used... For the application of the cathodic protection technique, we must also take into consideration: the value of the imposed currents, the nature of the ground, the grade of the material of the pipelines, the type of coating, the dimensions and locations of the weirs which are all sizes of its implementation. The acceptable distribution of the protection potential along the structure essentially depends on the optimization of the locations and the values of the earth resistances (anodic weirs), and above all on the value of the attenuation coefficient, which is a function of the resistance of insulation and therefore the condition of the coating. The increase in the insulation resistance causes the weakening of the attenuation factor, and therefore a greater withdrawal range. On the contrary, a greater attenuation factor, the range of the withdrawal is reduced and will result in an increase in the withdrawal stations distributed along the pipes when dealing with a structure with a low value of the insulation resistance, hence the need for optimal control of the state of the coating and control of the protection potential.

**Keywords:** Cathodic protection, impressed current, anodic weirs, attenuation factor.

## *ORAL PRESENTATION*

# Modification of a Maghnite by an alkylammonium with different cation exchange capacities

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**Abstract**

Maghnite (Algerian Montmorillonite) was grafted with (3-Mercaptopropyl)triethoxysilane in a water/ethanol mixture. The phenomenon of intercalation of the grafted product was tested following a modification by trimethyltetradecylammonium at different cation exchange capacities. The XRD results revealed a fixation of the d001 value after the grafting reaction, caused by the condensation of the silane molecules with the two adjacent sheets of clay. The blocking effect causes the grafted Maghnite to lose its swelling property despite its modification by trimethyltetradecylammonium which has been successfully carried out.

**Keywords**: Clay, grafted, maghnite, swelling property, alkylammonium.

## *ORAL PRESENTATION*

# Risk factors and oxidative stress among patients with chronic kidney disease from west Algeria

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**Abstract**

Introduction:Chronic kidney disease (CKD) is a major public health problem. Its management is difficult and restrictive on both economic and social level. In Algeria, it has been declared as a major public health priority. The two aims of this research were to first identify the risk factors for CKD in west Algerian patients, and then to measure the oxidative stress level in relation with CKD.
Patients and methods:We have conducted a study of one month including 50 patients, men and women (F/M: 27/23) aged between 20 and 80 years and suffering from CKD. Patients were received at the public hospital of Mostaganem, west Algeria. The socio-demographic characteristics and clinical parameters were determined by a questionnaire containing 26 items. Renal function was evaluated through measurement of plasma creatinine and urea levels, whereas oxidative stress was assessed by measuring thiobarbituric acid reactive substances (TBARS).
Results:Our results showed that women are more likely to have chronic kidney disease than men with high prevalence in patients aged between 35 and 50 years. 38% of patients are overweight, and only 14% have a family history. More than 70% of CKD patients are married compared to only 24% who are unmarried. In our CKD population, arterial hypertension was the main comorbidity (68%) followed by diabetes (20%) and cardiovascular disease (12%). The biochemical profile showed a significant increase in plasma concentrations of urea and creatinine ($p<0.001$). However, no significant increase in the concentration of TBARS was observed.
Conclusion**:**In our population, CKD does not seem to be related to oxidative stress.

**Keywords:** Chronic kidney disease, Hemodialysis, Oxidative stress, TBARS.

## *ORAL PRESENTATION*

# Relationship between pollen rain and vegetation on Bouhachem-Alem mountains (Rifain Mountains, Northern Morocco)

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**Abstract**

A total of 34 modern surface samples were collected from moss polsters on BouhachemAlem mountains (Rifain Mountains, Northern Morocco). The samples were taken from different types of vegetation in Thermo-, Meso-, and supra-Mediterranean belts, within an elevational interval of 1340-205 m a.s.l. the aim of this study is to provide new data on the modern pollen rain and evaluate the pollen and vegetation relationships within the landscapes of Bouhachem-Alem mountains. The statistical ordination of pollen rain shows a good discrimination between different vegetation communities which is represented in eight vegetation types, also indicating in better way, the area which had an anthropogenic influence within the landscape. Our results were discussed to support future interpretations of paleoecological investigations of Rifian landscape.

**Keywords:** Pollen rain, surface samples, relationships, Rifian landscape, vegetation communities, paleoecological investigations.

## *ORAL PRESENTATION*

# Synthesis and spectral analysis of rhodanine-based azo dyes and their use as colorimetric chemosensors for some metal ions

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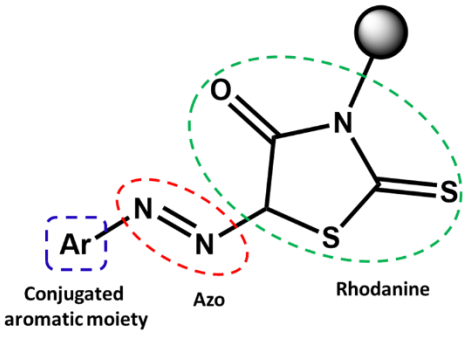
**Abstract**

In this work, novel rhodanine azo-based derivatives containing naphthalene and anthracene ring were synthesized and characterized using FTIR, $^{1}$H-NMR, $^{13}$C-NMR, and elemental analysis spectroscopic techniques. Selectivity and sensitivity of these compounds for some transition metals $Co^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Ni^{2+}$, and $Fe^{3+}$ were studied using UV-vis and fluorescence spectroscopy techniques. **4d** and **4c** showed the highest affinity towards $Fe^{3+}$ ions with association constants of $5.35 \times 10^{8}$ $M^{-1}$ and $4.63 \times 10^{8}$ $M^{-1}$ respectively. In addition, **4d** and **4c** showed high sensitivity toward $Fe^{3+}$ ions with detection limit of 7.05 and 5.14 µM respectively. The formation of complexes between azo **4c,d** and $Fe^{3+}$ was confirmed using molar ratio, and Benesi–Hildebrand methods with 1:2 binding stoichiometry. NMR and IR analysis confirmed the coordination between $Fe^{3+}$ and the nitrogen atom of the NH (hydrazone) group and the oxygen atom of the carbonyl (C=O) group. Owing to their good affinity and high sensitivity for $Fe^{3+}$, **4c** and **4d** show good potential for developing efficient $Fe^{3+}$ chemosensors. Furthermore, the biological activity of the prepared azo derivatives was evaluated against some pathogenic bacteria using three different bacterial species including (*E. coli*., *P. aeruginosa*, *S. aureus*) and compared with amoxicillin as a reference drug. The results showed that our compounds have moderate to good vital activity against the mentioned pathogenic bacteria.

**Keywords:** azo; rhodanine; iron(III); chemosensor; UV/vis spectrometry.




| Product | Ar | ● |
|---|---|---|
| 4a | Naphthalene | $NH_2$ |
| 4b | Naphthalene | $CH_2COOH$ |
| 4c | Naphthalene | H |
| 4d | Anthracene | H |

Scheme 1. Rhodanine-based azo dyes 4c-d.

## *ORAL PRESENTATION*

# Environmentally secure acaricidal composition

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**Abstract**

An environmentally friendly acaricidal composition based on pine resin has been developed. To increase the effectiveness of the resulting botanical insecticide, the composition contains a small amount (no more than 2%) of a synthetic pyrethroid (cypermethrin). The presence of terpentin oil in the composition determines the prolonged action of the pyrethroid, therefore its small amount will be sufficient to cover the duration of the pest development cycle. The effectiveness of the developed composition has been tested against pests of agricultural crops and parasitic mites of animals.
The effectiveness of the acaricidal action of the developed composition was tested on green peach aphids (*Mysodes persicae*, *Hyalopterus pruni*), widespread in Georgia. The American pyrethroid drug Arrivo (Corporation Agriculture Products Group) was taken as a reference standard. The death of aphids was recorded 2, 5, 8 and 14 days after spraying. According to the test results, the acaricidal effectiveness against the pest of the developed composition and the imported preparation is practically on the same level, despite the lower (10 times less) content of cypermethrin in the developed composition.
The effectiveness of the developed composition against parasitic mites of animals was studied both in laboratory conditions and in real conditions. According to the results of laboratory studies, 100% death was observed 96 hours after catamination of hungry individuals with a 0.015-0.02% solution of the developed composition, and after treatment of individuals with a 0.0001-0.0075% solution, mortality (after 96 hours) ranged from 78, 0 - 95.0%. It has been established that when cattle skin is treated with a 0.015-0.02% working solution of a composite preparation, hungry adult ticks completely die within 3-4 days, and well-fed ticks - after 4-5 days. The clinical condition of the animals in the experiment was within the normal range, milk yield did not decrease.

**Keywords:** acaricidal, composition, pests.

## *ORAL PRESENTATION*

# Effect of different pesticides on growth and morphology of *Tetrahymena pyriformis* as a ciliate protozoa model.

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**Abstract**

The use of pesticides plays an important role in agricultural development by reducing agricultural product losses, improving productivity, controlling vector diseases and contributing to diversification of food (Tudi et al. 2021), but their excessive use has been recognized as being possibly hazardous to animal and human health and can generate ecosystem damages essentially when residues persist in the environment (Sharma et al. 2019).The aim of this study is to evaluate the effect of some pesticides on *Tetrahymena pyriformis*. This ciliate protozoon offers several advantages as a test microorganism; it can be easily cultivated in complex or in defined axenic media with the possibility to induce synchronic cultures, it has a high rate of cell division and a great deal is known about its biochemistry. For that, it's an appropriate system for a number of studies including cell morphogenesis, conjugation, cell division, cytotoxicity and proteins regulation (Ziyadi et al., 2022). Several pesticides, used in Moroccan agriculture, have been tested at different concentrations on the growth and morphology of *T. pyriformis*, and compared to a standard culture in normal conditions. The growth (the generation number and the generation time) of the protozoa was perturbed. The minimum inhibitory concentration (MIC) and the inhibitory concentration of 10% ($IC_{10}$) and 50% ($IC_{50}$) were calculated. The effect on the morphological aspect and the mobility of cells at $IC_{10}$ and $IC_{50}$ were also evaluated.

**Keywords:** Pesticides, *Tetrahymena pyriformis*, cytotoxicity, MIC, IC50.

**References:**

M. Tudi, H.D. Ruan, L. Wang, J. Lyu, R. Sadler, D. Connell, C. Chu and D.T. Phung. Agriculture Development, Pesticide Application and its Impact on the Environment. Int. J. Environ. Res. Public Health. 18, 1112 (2021). doi:10.3390/Ijerph18031112.

Sharma, Anket, Vinod Kumar, Babar Shahzad, Mohsin Tanveer, Gagan Preet Singh Sidhu, Neha Handa, Sukhmeen Kaur Kohli, Poonam Yadav, Aditi Shreeya Bali, Ripu Daman Parihar, Owias Iqbal Dar, Kirpal Singh, Shivam Jasrotia, Palak Bakshi, M. Ramakrishnan, Sandeep Kumar, Renu Bhardwaj, and Ashwani Kumar Thukral. 2019. "Worldwide Pesticide Usage and its Impacts on Ecosystem." SN Applied Sciences 1:1446. doi:10.1007/S42452-019-1485-1.

Ziyadi S, Iddar A, Kabine M, El Mzibri M, Moutaouakkil A. (2022) Changes in growth, morphology and physiology of Tetrahymena pyriformis exposed to continuous Cesium-137 and Cobalt-60 gamma-radiation. Current Microbiol. 4;79(2):61.. DOI: 10.1007/s00284-021-02684-6.

## *ORAL PRESENTATION*

# Consumption Of Food Supplements And Their Effect On The Evolution Of The Medical Condition Of Patients During The COVID-19 Pandemic

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**Abstract**

Food supplements (FS) are foodstuffs whose purpose is to supplement the normal diet with a concentrated source of nutrients: vitamins, minerals, Omega3.
The aim of our study is to establish the profile and the behavior of the Oran consumer (Algeria) of food supplements with the most used products and to take stock of the effect of the consumption of food supplements on the evolution of the medical condition of patients during the COVID-19 pandemic.
A prospective survey conducted over a period of 2 months among consumers in an Algerian population and more particularly in Oran city. The collection of information was carried out with social networks with an electronic version due to the conditions of coronavirus. The questionnaire was given to 107 respondents.
The results show a high consumption of multivitamins (zinc and vitamin C) and the survey shows that tiredness and strengthening the immune system are the main reasons for this consumption.
In our study, we have developed the essential aspects of consumption and health issues related to the use of these supplements, the effectiveness of the food supplement, its role in addressing COVID-19 and strengthening the immune system.
In Conclusion food supplements occupy an increasingly important place in the diet and we are therefore seeing a notable change among Algerians in their choices when it comes to strengthening their immune defenses and healing. However, they have significantly increased their consumption of food supplements.

**Keywords:** Food supplements, Survey, Nutrients, Consumption, Covid-19, Immune system.

## *ORAL PRESENTATION*

### *Tritrichomonas foetus* from cattle, cats and pigs show massive genetic differences in their genomes

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**Abstract**

*Tritrichomonas foetus* is a protozoan parasite that colonizes the reproductive tract of cattle as well as the gastrointestinal tract of cats. Bovine tritrichomonosis is a sexually transmitted disease whereas feline tritrichomonosis is thought to be transmitted by the fecal-oral route. Furthermore, *T. foetus* is known as an essentially apathogenic commensal located in the nasal cavity of pigs. Transmission of *T. foetus* between the different hosts has to be considered a realistic scenario that may have important implications for the epidemiology of infections and disease. In our study, we generated whole genome sequencing (WGS) data from bovine, feline and porcine *T. foetus* strains to investigate the genetic (dis)similarities among these diverse strains. As a reference, we used a previously released draft assembly from a bovine *T. foetus* strain K isolated from an infected bull in Brazil. In particular, we identified single nucleotide polymorphisms (SNPs) and the insertion-deletion (indel) variations within the genomes of the different strains. Interestingly, only a low degree of polymorphism (68 SNPs and indels) was found between the bovine and the porcine strains in terms of variants with a predicted impact of moderate or high and where one species is homozygous for one allele and the other homozygous for the other allele. Conversely, however, a 964 times higher number of such differences was detected by comparing the feline with either the bovine (65,569) or the porcine (65,615) strain. These data clearly indicated a close phylogenetic relationship between bovine and porcine *T. foetus* but a remarkable genetic distinctness of these two strains from the feline strain. The latter observation was confirmed by PCR-based sequencing of 20 in silico-selected indel markers and five in silico-selected SNP markers that uniformly demonstrated a relatively distant phylogenetic relationship of three independent feline *T. foetus* isolates in comparison to the bovine and porcine strains investigated. In summary, our comparative genome sequencing approach provided further insights into the genetic diversity of *T. foetus* in relation to the different host origins of the parasite. Furthermore, our study identified a large number of SNP- and indel-containing sequences that may be useful molecular markers for future epidemiological studies aimed at the elucidation of the transmission patterns of *T. foetus* within different host species.

**Keywords:** *Tritrichomonas foetus*, protozoan parasite, WGS, genetic differences.

## *ORAL PRESENTATION*

# Antiepileptic effect of *Cuminum cyminum* and *Rhus pentaphylla* extracts after intra hippocampal injection of kainic acid in rats

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**Abstract**

Epilepsy is one of the most common neurological diseases, characterized by abnormal electrical activity leading to seizures. Currently, antiepileptic drugs are often the cause of undesirable side effects as well it has no effect on drug-resistant epilepsy. In order to study this disease, the kainic acid model is used to identify potential mechanisms of seizure genesis, comorbidities and treatment efficacy.
In the present study, we evaluated the preventive anticonvulsant effect of the ethanolic extract of *Rhus pentaphylla* and the aqueous extract of *Cuminum cyminum*, by oral administration before the intra-hippocampal injection of Kainic acid. The scoring of seizure severity, latency of seizures and duration of total seizures were recorded. The extracts of *Rhus pentaphylla* and *Cuminum cyminum* showed an attenuation of severe convulsive seizures and a significant decrease in the frequency and duration of seizures and prolonged the time of the seizure onset compared to the control.

**Keywords:** Epilepsy, Phytotherapy, Seizures, Anticonvulsant effect.

## *ORAL PRESENTATION*

# Biological activity of essential oil and hydrosol extract of *Pistacias lentiscus*

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**Abstract**

The *Pistacias lentiscus* is a medicinal plant belonging to the anacardiaceae family,this species know under the name of darw, is very widespread in mediterranean countries. The aim of this study is to evaluate the antioxidant activity of essential oil in he aerial parts of this plant from two differents regions of the wilaya of Tlemcen used in medicine traditional. Antioxidant activity is determines by the scavenging of the free radical by different mechanism DPPH and FRAP. Essential oil and plant extracts have a significant free radical inhibitory effect. The stady of the antioxidant activity of essenial oil from *P. lentiscus*, showed that the essenial oil has an antioxidant power expressed by the trapping of the DDPH radical, FRAP.This activity is powerful in the region of OUM EL-LAALOU by compared o HONAIN and this important in aqueous extract and methanolic extract.The separation of the essential oil gave us a major compound that has more antioxydant activity powerful.

**Key words***: Pistacias lentiscus,* essential oil, extracts ,antioxidant activity.

## *ORAL PRESENTATION*

# Effect of earthworm's associated bacteria on plant metabolism

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**Abstract**

Soil-level changes have been shown in various investigations to entail synergistic interactions between earthworms and microorganisms. Knowing the influence of earthworm coelomic fluid (CF) on plant development and defense, we set out to isolate bacteria from this "immune compartment." Bacteria isolated from *Aporrectodea molleri* were investigated for their effects on maize metabolism under abiotic stress conditions such as alkaline soil and nitrogen, phosphate, and potassium (NPK) deficit. A study of metabolomic profiles by gas chromatography coupled to a mass spectrometer (GC-MS) on leaves of plants treated and untreated with bacteria isolated from CF was carried out to reveal potential metabolites that could explain the effect of different treatments on maize under stress conditions (CFB). After 60 days of growth, plants infected with bacteria isolated from the coelomic fluid of the earthworm *Aporrectodea molleri* (CFB1,..., CFB7), as well as those not inoculated (control), were used to extract the lipid metabolites at the leaf level. A total of 114 distinct metabolites have been discovered. Fatty acids were the most prevalent class (45 metabolites, 43 %). Different metabolomic fingerprints indicate that various biochemical mechanisms were engaged in plant response to biostimulants. Metabolic reprogramming induced by bacterial treatments was more evident than in the control group. Abiotic stress did not result in a drop in lipid composition and concentration in plants treated with CFB, in contrast to the control, demonstrating the rigidity of defensive mechanisms. Compounds associated with tolerance-building pathways (glyoxylate and dicarboxylate metabolism, as well as the tricarboxylic acid cycle) accumulated greater in bacterial treatments.

**Keywords:** Earthworm, coelomic fluid, bacteria, plant, metabolomic.

## *ORAL PRESENTATION*

# Evaluation of the impact of loading density associated with thermal stress on the physico-chemical characteristics of meat in camels

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**Abstract**

Meat is one of the foods necessary for the proper functioning of our body; because of its richness in essential components, namely proteins, lipids, vitamins, mineral salts, etc. The dromedary plays a fundamental role in the production of milk and meat in arid and semi-arid regions, and might challenge that the climate change, global warming, drought and desertification. However, camels intended for slaughter are frequently exposed to pre-slaughter stress, namely handling, handling, loading, transport, unloading, travel distance, loading density, thermal stress, the waiting time at slaughterhouses, etc., which can alter its homeostasis, its immunity, its antioxidant status and therefore affect the quality of its meat. The reactivity to this stress is marked by physiological, hormonal, hematological and biochemical responses, and tissue oxidations. Indeed in our work we will focus more particularly on the impact of the loading density associated with thermal stress and their negative influence observed in the group of stressed camels compared to the unstressed concerning the parameters evaluated in particular: the decrease in pH ultimate, the activity of Catalase, superoxide dismutase on the one hand, and an increase in the content of Malondialdehyde, loss in weight, exudation, and water retention capacity on the other hand; hence the lipid peroxidation and the alteration of the enzymatic antioxidant induced by the generation of reactive oxygen species, which originated by oxidative stress; and therefore: affect the quality of the meat, and the health of the consumer. According to this review, to respect the well-being of the dromedary, it seems necessary to ensure conditions conducive to handling, road transport, handling in slaughterhouses... On the other hand, all these recommendations cannot take place without the establishment of urgent legislation on the welfare and protection of camels during all stages of pre-slaughter according to international standards.

**Keywords**: thermal stress, loading density, physicochemical characteristics, dromedary, meat quality, well-being.

## *ORAL PRESENTATION*

# Comparative study of the in-vitro dissolution profiles of atenolol tablets

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**Abstract**

Atenolol is a widely used drug for treating heart conditions available under various trade names by several generic pharmaceutical manufacturers. The aim of the present work was to assess the pharmaceutical equivalence of the generic atenolol tablet (50 mg), available in Algeria, and to compare it to the originator brand using in vitro dissolution study. The dissolution study was carried-out using the paddle apparatus according to the guidelines of US Pharmacopoeia (USP). Results revealed no significant differences in the percent released of the pharmaceutic active ingredient. All tablets released more than 85% of the drug within 15 minutes, which means that the dissolution profiles may be accepted as similar without further mathematical evaluation. Other features such as weight uniformity, content uniformity, tablet hardness, friability and tablet disintegration were also evaluated. All tablets complied with the official specifications. Based on the obtained results, the tested brand is assumed chemically and pharmaceutically equivalent. This study indicated that the generic tablet could be prescribed interchangeably.

**Keywords:** Atenolol, In-vitro dissolution, Paddle method, Generic, quality control.

## *ORAL PRESENTATION*

# Employing solid state fermentation to produce fish protein hydrolysate from *Oncorhynchus mykiss* waste

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**Abstract**

Aquaculture is the fastest-growing animal food production industry in the world, and it is quickly becoming the primary source of aquatic animal food for human consumption. However, enormous amounts of fish waste are generated from beneficiation plants, which might pollute the environment if incorrectly disposed off. To make better use of existing marine resources and to lessen the environmental impact of industrial fish waste, fish protein hydrolysates (FPH) were recovered from fish waste using solid-state fermentation (SSF).
SSF was used to valorize dead and sterile fish *oncorhynchus mykiss* to obtain FPH. The technical technique involves producing hydrolytic enzymes (proteases) and using them in the same reactor unit to hydrolyze fish waste. Both the enzyme synthesis and hydrolysis stages should be optimized. The first tests include assessing the ability of Bacillus cereus inoculum to develop protease activity. Furthermore, *O.mykiss* waste was digested in the absence of any exogenous inoculum, and microbial activity was linked to autochthonous microbiota found in trout. Temperatures of 30 and 37 degrees celsius were tested.
According to the data, fermentation at 30°C without an external inoculum is the more effective for producing 728U/gDM of enzyme during the 120h required for solid-state hydrolysis. The present research is concentrated on scaling up the experiment to manufacture hydrolytic enzymes and FPH in the same reactor based on dynamic temperature and without any external inoculum.

**Keywords:** Solid-state fermentation, *Oncorhynchus mykiss*, Fish protein hydrolysates.

## *ORAL PRESENTATION*

# Copper phosphate nanocatalyst synthesis and their application in organic chemistry

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**Abstract**

Since the intense use of lithium iron battery $LiFePO_4$, the metal phosphate materials have been focused on the development of another cathode[1]. In the last years several studies presented the preparation, characterization and activity of metal phosphate material.The preparation method directly influences on the shape of particles as nanoflower, nanotube, Nanosheets, nanocube[2]. Furthermore, metal phosphate material was used as catalyst. The most known is the $FePO_4$ which demonstrated a high activity for the synthesis of 5-hydroxymethylfurfural[3], and oxidation[4].
In the present study, we prepared iron and copper nanostructure by hydrothermal rout. The catalysts were characterized by XRD, FTIR, MEB-EDS, UV-Vis, Raman spectroscopy, and MET. The nanocatalysts showed a high activity in the propargylamines synthesis via the A3 coupling of aldehyde, amine and alkyne with yield of 100%.

**Keywords:** $FePO_4$, nanostructure, Heteregeneous catalyst, propargylamine.

**References:**
[1] Q. Fan, L. Lei, X. Xu, G. Yin and Y. Sun, *Journal of Power Sources* 2014, *257*, 65-69.
[2] C. Crouzet, N. Recham, F. Brunet, N. Findling, R. David and M.-T. Sougrati, *Solid State Sciences* 2016, *62*, 29-33.
[3] H. Xia, S. Xu, X. Yan and S. Zuo, *Fuel Processing Technology* 2016, *152*, 140-146.
[4] R. Polnišer, M. Štolcová, M. Hronec and M. Mikula, *Applied Catalysis A: General* 2011, *400*, 122-130.

## *ORAL PRESENTATION*

# Development and comparative evaluation of different LAMP and PCR assays for coprological diagnosis of feline tritrichomonosis

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**Abstract**

The protozoan parasite Tritrichomonas foetus may cause severe diarrhea in cats all over the world. In order to evaluate the methodology in coprological molecular diagnosis of feline tritrichomonosis, we compared previously published ("old") and newly developed ("novel") loop-mediated isothermal amplification (LAMP) as well as an old conventional and an old and novel real-time PCR assays regarding their diagnostic sensitivities and specificities. Here, the novel real-time PCR yielded the best methodical performance in that a sensitivity with a detection limit of <0.1 trophozoites (corresponding to ca. <0.13 trophozoites per mg feces) and a maximal specificity for diagnosis of Tritrichomonas spp. was achieved. The other test systems exhibited either an approximately 10-times lower sensitivity (<1 trophozoite corresponding to ca. <1.3 trophozoites per mg feces) (conventional PCR and both LAMP assays) or a lower specificity (old real-time PCR). Conversely, the diagnostic performance assessed with clinical fecal samples from cats demonstrated identical sensitivities (8 of 20 samples tested were positive) for the novel PCR and both LAMP assays. Diagnostic sensitivities were significantly higher than those found for the old real-time (5 positive samples) and conventional PCR (6 positive samples), respectively. Accordingly, our data suggested the novel PCR and both LAMP assays to be well suited molecular tools for direct (i.e. without including an in vitro cultivation step) coprological diagnosis of tritrichomonosis in cats. Interestingly, relative high (novel LAMP, 7 positive samples) to at least moderate (old LAMP, 6 positive samples and 1 sample with equivocal score) diagnostic sensitivities were also achieved by testing clinical samples upon simple visual inspection of colorimetric changes during the LAMP amplification reactions. Accordingly, both LAMP assays may serve as practical molecular tools to perform epidemiological studies on feline (and bovine as well as porcine) tritrichomonosis under simple laboratory conditions.

**Keywords:** *Tritrichomonas foetus*, Tritrichomonosis, Coprology, LAMP, Conventional PCR, Real-time PCR.

## *ORAL PRESENTATION*

# Temporal evolution of the physico-chemical quality of Cheliff dam waters, wilaya of Mostaganem (Case of parameters indicating global warming)

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**Abstract**

Global warming caused by the emission of greenhouse gases, poses several environmental problems including drought which promotes the evaporation of water from dams dedicated to domestic, agricultural and industrial use (thermal fluid). The use of water in the industrial sector requires a very appropriate quality of water in order to avoid scaling and corrosion of equipment caused by the hardness and salinity of the water produced.
Our work is based on the study of the evolution of the concentration of dissolved salts in Cheliff dam waters; located east of the wilaya of Mostaganem over a period of four years (2018-2021), depending on the average temperature of this region and the duration of the dry period. We used Gaussen's Ombro-thermal diagram to fix the beginning and end of the dry period, Langelier's and Larson's index to identify the aggressive and corrosive nature of the water and Piper's diagram to know the hydro-chemical facies.

The results obtained revealed that following the water stress, the average salinity and the total hardness continue to increase, the level of dissolved salts increased from (702, 653, 951, 835 mg/l) to (1248, 1242, 1540, 1395 mg/l) during the last four years (2018-2021). Piper's Diagram shows that all of Cheliff's crudes have a chlorinated, sulphated, calcic and magnesium chemical facies. The significant presence of these ions gives a scaling and corrosive character to the water (average values of total hardness: 570, 620, 700, 670 mg/l in CaCO3 for the years 2018-2019-2020-2021 respectively). The results obtained by the two indices of Langelier and Larson show that the waters of the Cheliff dam have a scaling nature and a clear tendency to corrosion.

**Keywords:** Dam, global warming, water stress, rainfall, salinity, evaporation.

## *ORAL PRESENTATION*

# Square planar Cu(II) Complexes containing Dithiocarbazate Schiff Bases: Synthesis, Structural Evaluation, Cytotoxic Activity, DNA Binding, and Molecular Docking.

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**Abstract**

To solve the issue with the use of platinum-based compounds as therapeutic agents, further research on non-platinum medicines with higher activity and improved selectivity is urgently needed. In light of this, tridentate dithiocarbazate ligands were used to synthesise Cu(II) complexes. The general formula of the produced Cu(II) complexes was discovered to be [Cu(ONS).$H_2O$]. Physical, chemical, and spectroscopic techniques were used to comprehensively characterise the Schiff bases and Cu(II) complexes. The coordination geometry in which the Schiff bases functioned as tridentate ONS donor ligands coordinating via azomethine nitrogen, thiolo sulphur, and phenoxide oxygen atoms was corroborated by the spectroscopic results. The Schiff bases and Cu(II) complexes were optimised using density functional theory (DFT) with the B3LYP hybrid exchange correlation functional with LanL2DZ pseudopotential on Cu and a 6-311G(d, p) Pople basis set for the other atoms to confirm the experimental data. Cu(II) complexes showed the most promising cytotoxicity against two breast cancer cell lines, with $IC_{50}$ values ranging from 0.28 to 4.43 M (MDA-MD-231 and MCF-7). The interactions between DNA and Cu(II) complexes were studied using a UV-vis absorption technique, which was supported by molecular docking analysis, which revealed hydrogen, electrostatic, and hydrophobic binding interactions. Cu(II) complexes may offer hope for the future of developing drugs to treat many illnesses, including cancer.

**Keywords:** ortho-vanillin, Cu(II) complex, DFT, cytotoxicity.

## *ORAL PRESENTATION*

# Investigation of antimicrobial and antioxidant activity of *Tephromela atra* lichen and its chemical isolates, α-alectoronic acid and α-collatolic acid

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**Abstract**

The present study was carried out to investigate in vitro antioxidant and antimicrobial activities of acetone, dichloromethane, petrol ether, and methanol extracts of the lichens *Tephromela atra* and its major metabolites. α-alectoronic acid and α-collatolic acid were identified as bioactive substances in the lichens *Tephromela atra*. The antibacterial activity was investigated using disk diffusion method and the minimal inhibitory concentration values were evaluated against eleven species of bacteria, nine species of fungi, and four species of yeasts using broth microdilution method. α-Alectoronic acid and α-collatolic acid extracts showed significant inhibitory activity against most of the tested bacteria, especially the lowest MIC values of both acids for *Bacillus subtilis*. In addition, the antioxidant activity was performed in vitro by measuring the free radical scavenging capacity of extracts on DPPH. The methanol extracts showed significant antioxidant activity and high DPPH scavenging activity with all concentrations. The results offer a perspective that *Tephromela atra* extracts and the natural substances obtained can be effectively used as a source of antimicrobial and antioxidant agents in the field of pharmaceutical industries.

**Keywords:** *Tephromela atra*, Lichens, Antimicrobial, Antioxidant, α-Alectoronic acid, α Collatolic acid.

## *ORAL PRESENTATION*

# Bibliometric Analysis of ATP Citrate Lyase (ACLY) Studies in Cancer

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**Abstract**

The aim of this study is to examine scientific articles related to ATP citrate lyase (ACLY) enzyme in cancer studies in terms of bibliometric indicators. Connecting two important pathways, glycolysis and lipid metabolism, ACLY synthesizes cytosolic acetyl coenzyme A (acetyl-CoA), an essential cellular building block. Inhibition of ACLY involved in this transition has been proven to induce proliferation suppression and apoptosis in a subset of human cancer cells. This highlights the therapeutic potential of ACLY inhibitors in cancer therapy.

In this study, which was carried out according to the case study method and using the bibliometric analysis technique, bibliometric data from the WoS database produced by Clarivate Analytics were used. The scanning range of the data covers the period between 1994 and 2021. In the analyzes made with the keywords used, it was seen that 159 of 1925 studies registered in the WoS database were studies related to ACLY and cancer. It was determined that 132 of these studies were research articles and 22 of them were review articles. Looking at the research areas, 47 studies were carried out in the field of oncology, 36 studies in the field of biochemistry, molecular biology and 30 studies in the field of cell biology. It has been observed that the majority of the studies are aimed at investigating the use of ACLY, a metabolic central enzyme, as a biomarker in cancer prevention, diagnosis and treatment of cancer. Among the countries where the articles are published, China, which has 62 publications, is followed by the USA with 60 publications. The fact that Turkey ranks 26th among 27 different countries with 1 article shows that the publications on the relationship between cancer and ACLY are not sufficient.

**Anahtar Kelimeler:** ATP citrate lyase, ACLY, cancer, bibliometric.

## *ORAL PRESENTATION*

# Antifungal effect of eucalyptus (*Eucalyptus globulus)* and lavender (*Lavandula officinalis*) essential oils on *Candida albicans* and *Candida glabrata*, the most common fungal factors

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**Abstract**

It is reported that the use of essential oils in the pharmaceutical, cosmetic, food and medical industries in Chinese sources dates back to 3700 BC. According to the data published by the World Health Organization (WHO), more than 20,000 plants species are used for medicinal purposes in the world. It is known that many plants and plant oils, which are known as medicinal plants among the people, which have been found by trial and error method in our country, as in the world, are used in the treatment of diseases. Phenolic compounds found in essential oils are known as the main antimicrobial and antifungal bioactive components. Eucalyptus and lavender oils, which we used in our study, have been found to have antifungal activity on yeasts in previous studies. In recent years, an increase in the incidence of fungal infections has been observed in parallel with the developments in the field of diagnosis and treatment. Candida species are responsible for most of the fungal infections (80 %) in hospitals. The increase in the frequency of fungal infection and the associated mortality and morbidity rates cause the widespread use of empirical antifungals, the emergence of resistant fungal strains and the increase in resistance rates. Therefore, the need for in vitro antifungal susceptibility tests is increasing in the selection of appropriate and effective antifungal therapy. In this context, the way has been paved for the use of supportive phytotherapy and aromatic oil, at least in the treatment of non-systemic superficial infections. Antimicrobial and healing effects of aromatic oils are now used in creams, pomades, band-aids and similar products. For this purpose, we investigated the antifungal activity of Eucalyptus (*Eucalyptus globulus*) and Lavender (*Lavandula officinalis*) oils against C. albicans (ATCC 14053) and C. glabrata (ATCC 15126) strains using REMA (Resasurin Microtiter Assay) method. Accordingly, the MIC (Minimum inhibitory concentration value) of both lavender and eucalyptus oils for C. albicans was determined as 31.25 µg/ml, and for C. glabrata, this value was determined as 62.5 µg/ml for both oils. As a result, it has been determined that the oils have inhibitory activities, especially when used in the required titers against these common fungal agents.

**Keywords:** Essential Oils, MIC, Candida, Eucalyptus, Lavender.

## *ORAL PRESENTATION*

# Bioreactors Characterization

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**Abstract**

The characterization of bioreactors is made to examine the performance of a newly invented bioreactor or to compare different bioreactors in a specific bioprocess. By this way, aeration and mass transfer can be optimized, data is obtained for modeling and scale-up studies. For the characterization of photobioreactors, characterization of liquid and gas phases, characterization of mass and heat transfer, measurement of microorganism growth and product production are made and also light intensity distribution is examined. Studies in the literature that document the comparative performance of different bioreactors and their effect on bioprocesses are scarce, and the available ones often compare two bioreactors (one newly recommended, the other control). In this project, all major bioreactor types (four types) will be examined comparatively: stirred tank bioreactor, internal loop air lift bioreactor, bubble column bioreactor, panel bioreactor. The determination of the hydrodynamic, light distribution and mass transfer properties of these photobioreactors as well as the production of microalgae species in them will allow this study to be the first to compare the performance of four bioreactors in microalgae growth in the literature. The project consists of five workpackages, all of the studies will be carried out by the graduate student under the supervision of the thesis supervisor and will be completed in one years time.

**Keywords:** Microalgae, Photobioreactor, Bioprocess characterization.

## *ORAL PRESENTATION*

# An Enzyme-Free Technique Enables the Isolation of a Large Number of Adipose-Derived Stem Cells at the Bedside

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**Abstract**

Since they can be readily isolated in large quantities, ADSCs are essential for research and applications of regenerative medicine. Nevertheless, depending on how they were removed from the body, their purity, pluripotency, ability for differentiation, and expression of stem cell marker genes may change significantly. The literature lists two procedures for separating regenerative cells from adipose tissue. Enzymatic digestion is the first method, which uses a variety of enzymes to separate stem cells from the tissue they are found in. The second technique entails employing non-enzymatic, mechanical separation techniques to separate the concentrated adipose tissue. Processed lipoaspirate's stromal-vascular fraction, or its aqueous part, is where ADSCs are extracted (SVF). Human cell-based treatments benefit from mechanical process methods to SVF production since there are less regulatory restrictions on its clinical application. This work's goal was to describe a special commercial microlyzer device for producing SVF from adipose tissue utilizing a mechanical method that needed little assistance. Ten distinct patients' tissue samples were used to study the Microlyzer. The recovered cells were assessed for their capacity for colony formation, stem cell potential, phenotypic characterisation, and cell survival. As a result, the quantity of progenitor cells obtained alone from the microlyzed tissue is nearly equal to the quantity obtained using the most effective enzymatic method (according to the CD 90). The cells that were isolated from each group have very similar levels of vitality and capacity for colony formation. These cells entered the differentiation pathways more quickly and demonstrated a higher degree of marker gene expression than the other cells, according to research done on the differentiation potentials of cells produced from the microlyzed tissue. These results imply that mizrolyzer will enable rapid and high rate cell separation at the bedside, notably in regeneration research.

**Keywords:** Adipose-derived stem cell, stromal vascular fraction (svf) isolation, microlyzed adipose tissue, non-enzymatic extraction, regenerative medicine.

## *ORAL PRESENTATION*

# Çoban çantası (*Capsella bursa-pastoris* L.) bitkisinin etil alkol ile oluşturulan karaciğer hasarları üzerine etkisinin histopatolojik ve biyokimyasal olarak araştırılması

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**Özet**

Sunulan bu çalışmanın amacı etil alkol ile oluşturulan karaciğer toksikasyonuna karşı çoban çantası (*Capsella bursa-pastoris* L.) bitkisi çayının koruyucu etkinliğini araştırmaktır. Bu amaçla 32 adet erkek wistar albino rat rastgele 4 gruba ayrıldı. Kontrol grubu; Standart pelet yem ile beslendi. Alkol grubu; Günde bir defa 4 ml/kg % 30'luk etil alkol orogastrik gavaj ile verildi. Alkol + Çoban çantası grubu: Günde bir defa 4 ml/kg % 30'luk etil alkol ve 1 ml/kg bitki çayı günlük orogastrik gavaj ile verildi. Çoban çantası grubu; Günde bir defa 1 ml/kg bitki çayı orogastrik gavaj ile verildi. 60 günlük deneme süresi sonunda sıçanların nekropsileri yapılarak doku ve kan örnekleri alındı. Alınan doku örnekleri Hematoksilen & Eozinle (H&E) ile boyanarak histopatolojik inceleme yapıldı. Alınan kan örnekleri santrifüj edilerek serumları çıkarıldı. Serumlardan karaciğer harabiyeti serum enzimlerinden Alanin transaminaz (ALT), Aspartik transaminaz (AST), Alkalen fosfataz (ALP) ve Laktat dehidrogenaz (LDH) seviyeleri ve Kolesterol, Trigliserit, HDL kolesterol, LDL kolesterol ve Açlık kan şekeri (AKŞ) düzeyleri çalışıldı. Histopatolojik olarak alkol grubu ratların karaciğerlerinde meydana gelen dejeneratif-nekrotik değişikliklerin alkol + Çoban çantası grubunda kısmen azaldığı belirlendi. Biyokimyasal olarak, alkol grubunda kontrole göre artan AST, ALT ve ALP düzeylerinin alkol + Çoban çantası tedavi grubunda azaldığı görüldü. Çoban çantası çayı ile tedavinin trigliserid ve HDL düzeylerine olumlu etki ettiği görülürken, LDL ve kolesterol değerlerini olumsuz etkilediği görüldü. Sonuç olarak, çoban çantası çayı uygulamasının karaciğerde meydana gelen tahribatı azalttığı, ancak LDL ve kolesterol düzeylerinde yükselmeye sebep olduğu belirlendiğinden, çoban çantası çayının etil alkol ile oluşturulan karaciğer toksikasyonuna karşı tam bir koruma sağladığı sonucuna kesin olarak varılamadı.

**Anahtar Kelimeler:** Çoban çantası, etil alkol, histopatoloji, karaciğer, rat.

Bu araştırma Yüzüncü Yıl Üniversitesi Bilimsel Araştırma Projeleri Başkanlığı tarafından TYL-2018-6759 nolu proje olarak desteklenmiştir.

## *ORAL PRESENTATION*

# Evaluation of The Results of Therapeutic Drug Monitoring and In Silico Pharmacokinetic Analysis of Fluvoxamine

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**Abstract**

Objectives: Fluvoxamine (FLUV) is used in the treatment of patients with major depression and obsessive-compulsive disorder. The therapeutic range of FLUV is 60–230 ng/mL. The pharmacokinetic properties of drugs can affect the drug plasma level. In this study, it is aimed to determine the pharmacokinetic properties of FLUV using the *in silico* analysis methods and to evaluate the plasma level of FLUV in patients taking FLUV
Materials and Methods: Pharmacokinetic analyzes were performed by using the SwissADME, and pkCSM. The Therapeutic Drug Monitoring (TDM) analysis reports of the 70 patients (taking FLUV) admitted to NPIstanbul Brain Hospital were evaluated.
Results: The mean TDM values of the female, male and total patients were 71.48±15.32 ng/mL and 79.43±20.45 ng/mL, 78.96±17.58 ng/mL, respectively. There was no significant difference in drug plasma levels as a function of gender. The mean TDM values of the 100 mg/day and 200 mg/day were 40.01±17.63 ng/mL, and 107.29±13.48 ng/mL, respectively. There was a significant difference between the two dose groups ($p<0.05$). 70 patients were classified as three age groups (≤ 25, 26-45, and 46-65). 26-45 age group had higher drug plasma level than ≤ 25 age group ($p<0.05$). There was a linear relationship between FLUV dose and plasma level. According to the results of the *in silico* analysis, FLUV has rapid absorption, easily passes Blood Brain Barrier, and is a Cytochrome P450 enzyme inhibitor.
Conclusion: Obtained data can assist in the interpretation of TDM in various daily drug doses for each patient and it can be a valuable reference.

**Key words:** Fluvoxamine, Therapeutic Drug Monitoring, In Silico.

## *ORAL PRESENTATION*

# Investigation of antimicrobial activity of microbial secondary metabolite loaded cryogel membranes

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**Abstract**

Recently, the increase in the development of multidrug resistant microorganisms led the researchers to investigate novel antimicrobials for treating both antibiotic resistant and susceptible infections. In this concern, natural bioactive compounds derived from microorganisms have a broad range of applications in the field of medicine and tissue engineering. The bacterial secondary metabolites that show numerous features such as antibacterial, antifungal and anti-inflammatory effect, have capability to enhance wound healing process. For this purpose, phenazine derivative secondary metabolite obtained from bacteria was loaded to biocompatible cryogel membrane for developing support material as a wound dressing. The unique properties such as impressive water holding capacities and interconnected macro porous structure make the cryogel materials good candidates for enhanced wound healing process. On the one hand the macro porous structure is offering improved cell adhesion and increased $O_2$ permeability, on the other hand due to the massive water absorption capability, wound exudate containing nutrients that allow microorganism growth can be absorbed quickly. In this study, poly hydroxyethyl methacrylate P(HEMA) cryogel membranes were synthesized and loaded with bacterial seconder metabolite. Characterization studies of cryogel membrane and natural bioactive compound were realized by Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance spectroscopy (H-NMR). Additionally, antimicrobial properties of developed material were examined using cell counting method and disc diffusion method. As a result, support material which does not exist antimicrobial properties alone, gained ability to inhibit bacterial growth when combined with the phenazine derivative secondary metabolite.

**Keywords:** Bacterial secondary metabolite, cryogel, antimicrobial activity, wound healing.

## *ORAL PRESENTATION*

# Novel Clinically Useful Weight Optimized Dynamic Conformal Arcs (WO-DCA) in Stereotactic Body Radiotherapy for Localized Prostate Cancer

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**Abstract**

Interplay effects may influence dose distributions to a moving target such as localized prostate when using volumetric modulated arc therapy (VMAT). To evaluate the feasibility of shortening the duration of prostate stereotactic radiotherapy (SBRT) with increase dosimetry by utilizing weight-optimized dynamic conformal arcs (WO-DCA).

Our study include to selected thirty patients who had been treated to the total dose of 37.5 Gy in 5 fractions of 7.5 Gy on every other day delivered with VMAT technique using double full arcs in Linac. Treatment plans of all patients were re-planned using novel technique of weight optimized based on DCA (dynamic conformal Arcs) with double full arc having multi-leaf collimator (MLC) aperture around the target volume. Weight of the two arcs using optimization method was adjusted to maximize tumor coverage and protect organs at risk (OAR). The clinical VMAT and WO-DCA treatment plans were compared via ASTRO Guideline on hypofractionation in localized prostate cancer for maximize tumor coverage and OAR. Additionally, monitor unit (MU) of radiation values, and beam-on time (BOT) were recorded.

The WO-DCA plans successfully matched VMAT, our institutional standard treatment planning technique, for OAR (rectum, bladder and femurs head) and PTV ($D_{mean}$, $D_{98\%}$, $D_{2\%}$). The values of beam-on time (BOT) [2.23 (1.97-3.02) vs 3.83 (3.05-4.79), $p<0.001$] and monitor units (MU) [1260 (1089-1894) vs. 2405 (2055-3760), $p<0.001$] were clinically significant decreased with WO-DCA in comparison to VMAT.

Due to omission of MLC beam modulation through the target, WO-DCA plans could potentially minimize small-field dosimetry error without interplay effects of interval organ motion with lower radiation exposure in critical structures away from prostate and a faster treatment delivery. This technique may facilitate same day, faster SBRT with potentially eliminating patient-specific VMAT quality assurance.

**Keywords:** Prostate Cancer, Stereotactic Body Radiotherapy, Weight Optimized Dynamic Conformal Arcs, VMAT.

## *ORAL PRESENTATION*

# Influence of past climate changes on the current distribution of a rare and endemic species: Anatolian spiny mouse

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**Abstract**

This research focuses on species distribution modelling (SDM) to have an idea of distribution of Anatolian spiny mouse, *Acomys cilicicus* in the Last Glacial Maximum (LGM), the mid-Holocene and present by using coordinates along Silifke which is the only location the species found. Three ensembled species distribution models (generalized additive models, maximum entropy and boosted regression trees) were used to project Anatolian spiny mouse environmental suitability. Results indicate that current distribution of *A. cilicicus* populations shifted east to west since during the Last Glacial Maximum and apparently eastern part of the Mediterranean region of Turkey is potential refugia for this species.

**Keywords:** Species distribution modelling, *Acomys cilicicus*, endemic species, LGM, Holocene.

## *ORAL PRESENTATION*

# Predicting past population dynamics of broad-toothed field mouse using species distribution modelling

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**Abstract**

Species distribution modelling is a quite useful method to predict the potential past, current, and future distribution of organisms in response to climate change. From this point of view, I tested the influence of Last interglacial, Last glacial maximum, and Mid-Holocene climatic fluctuations and the potential effect on current distribution using 4 ensembled models (GLM, BRT, RF, MAXENT). Performance of the four models generated for broad-toothed field mouse assessed area under the curve (AUC) validation methods and obtained significant results (0.7 and up). The findings indicated that the species distribution was mainly determined by precipitation of wettest month. Depend on past modelling, apparently, this species maintains approximate current distribution since Mid-Holocene.

**Keywords:** Species distribution modelling, *Apodemus mystacinus*, LGM, Mid-Holocene.

## *ORAL PRESENTATION*

# Alzheimer Hastalığının Tedavisinde Kritik Bir Rol Oynayan Kolinesteraz İnhibitörlerindeki Son Gelişmeler (2020-2022)

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**Özet**

Alzheimer hastalığı (AD) oldukça sık rastlanılan ve majör yıkıcı bir etkiye sahip olan nörodejeneratif bir hastalıktır. Özellikle 65 yaş üstü nüfusu büyük oranda tutan, hasta kişinin nöron ölümü, hafıza kaybı, bilişsel ve kolinerjik işlev bozuklukları gibi sıkıntılarla günlük yaşamını önemli derecede etkileyen bir durumdur. Fakat son dönemde yapılan ilaç geliştirme çalışmalarının oldukça yoğun yürütülmesi rağmen halen kişileri tamamen iyileştirebilecek veya hastalığın evresini tamamen durdurabilecek etkili bir ilaç molekülü maalesef bulunamamıştır. Bunun sebeplerinden biri de hastalığın patolojisinin nasıl ortaya çıktığının anlaşılamaması ve karmaşıklığı ile ilgilidir. Alzheimer hastalığı için kabul gören en önemli teori kolinerjik hipotezdir. Önemli bir nörotransmitter olan asetilkolin (ACh), asetilkolinesteraz (AChE) ve butirilkolinesteraz (BuChE) isimli iki kolinesteraz enzimi (ChE) tarafından metabolize edilir. AD ile birlikte ise ChE aktivitesi artmış ve dolayısıyla asetilkolinin yıkımı tetiklenmiştir. Ayrıca AChE'in periferal anyonik bölgesi (PAS)'nin Aβ-peptid fibrillerinin oluşmasına da neden olduğu anlaşılmıştır. Bu sebeple bu hipoteze dayanarak tedavideki en etkili yaklaşım, kolinerjik sinapslarda ACh seviyesini arttırarak eski haline döndürmeye çalışan kolinesteraz inhibitörlerinin (ChEI) kullanılmasıdır. Üstelik multifonksiyonel moleküllerin tasarımları üzerine de odaklanılmıştır. Sonuç olarak hastalığın karmaşık yapısı ve olumsuz etkileri göz önüne alındığında bu nörodejeneratif hastalık ile ilgili daha fazla sayıda çalışma yapılmasının gerekliliği açıktır. Söz konusu çalışmamızda bu alanda son yıllarda yapılan önemli ve dikkat çekici güncel araştırmalara yer verilmiş olup gelecekteki ilaç araştırma ve geliştirme çalışmalarına katkı sağlaması amaçlanmaktadır.

**Anahtar Kelimeler:** Alzheimer, Asetilkolinesteraz, Butirilkolinesteraz, Multifonksiyonel ilaçlar, Çoklu hedefe yönlendirilmiş ligandlar.

## *ORAL PRESENTATION*

# Different Applications of Combination of Ellagic Acid and Carnosic Acid in Hyperglycemic Rats: A Perspective of Protein Dysfunction in Wound Healing Process

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**Abstract**

Oxidative stress is widely recognized in the development and progression of diabetes and its complications, and increased oxidative stress is known to be associated with diabetic wounds. High protein Carbonyl levels (PC), which occur due to the increase of reactive carbonyl groups in protein oxidation, indicate increased oxidative stress as well as protein dysfunction. In addition, Advanced Oxidation Protein Products (AOPP) determination is used to determine the degree of protein oxidation. In this study, we was aimed to reveal the effects of natural phenolic compounds Elagic acid (EA) and diterpene Carnosic acid (CA) on oxidative events occurring in the wound healing process in excisional wounds created in hyperglycemic experimental animals, with both topical and oral gavage applications. 54 female Wistar-albino rats with streptozotocin-induced diabetes were divided into five groups: control group, untreated, treated with carbopol 974P, topical treatment group, and oral treatment group. In the treated groups, wounds were treated after injury by topical application with 2% carbopol 974P gel and 2% carbopol 974P gel containing EA and CA (10 mg/kg) and by oral gavage application with EA and CA (10 mg/kg). Then, on the 3rd and 7th days of wound healing, the animals were sacrificed, and Protein Carbonyl (PC) levels in the wound tissues were determined spectrophotometrically; serum AOPP levels were determined by ELISA method. Comparing the untreated groups (3 and 7 days) with the treated groups (3 and 7 days), a significant decrease in PC levels was observed in the treated groups ($p<0.05$). An increase in AOPP values was observed in the untreated groups compared to the control group ($p<0.05$). When the untreated groups (3 and 7 days) were compared with the topical and oral treatment (3 and 7 days) groups, AOPP levels were statistically decreased in the treatment groups ($p<0.05$). Combination of EA and CA has successfully contributed to wound healing by reducing protein carbonyls during the healing process of diabetic wounds. The strong synergistic effect of these two antioxidants may be beneficial for reducing or eliminating oxidative stress in all phases of diabetic wound healing.

**Acknowledgement:** This research was supported by Gazi University Scientific Research Projects (BAP), Ankara, Turkey (Project Number: 05/2020-07).

**Keywords:** Oxidative stress, Diabetic wounds, Protein Carbonyl, Advanced Oxidation Protein Products.

## *ORAL PRESENTATION*

# New Threat for Fisheries: Microplastic Contamination

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**Abstract**

Within last decade, thousands of studies have been conducted to assess the microplastic contamination levels in marine and ecosystems together with its impact on marine biota. To date, more than 8000 species have been identified as contaminated with microplastic. Major sources of microplastic particles in aquatic environments are identified as river discharges, textile, cosmetic and electronic products, city dust, wastewater treatment plant effluents, agricultural activities such as the application of sewage sludge, vinyl coverings and atmospheric deposition. However, when aquaculture facilities are concern, aquaculture equipment like fattening farms, nets, ropes, pipes and fish feeds that are made from the gastrointestinal tracts of other fish are also reported to be a source of microplastic particles. Studies reveal that there is an intense microplastic pollution starting from sea grass to fish in places where aquaculture is intensive. Also, many studies reported the existence of microplastic particles inside the gastrointestinal tracts of commercially important farmed fishes. All of these concerns has led to the discussions about the potential hazards of seafood consumption on human health. Yet, there is still no regulation which aims to prevent or regulate seafood products for microplastic contamination. Therefore, this study was designed to determine microplastic pollution level and major sources in the aquaculture industries by examining the current knowledge in order to show the necessity of protection measures in aquaculture industry.

**Keywords:** seafood, pollution, fish farm, risk assessment.

## *ORAL PRESENTATION*

# Effects of selected plant secondary metabolites on the pupal parameters of *Hyphantria cunea* Drury, 1773 (Lepidoptera: Arctiidae) larvae infected by *Hyphantria cunea* granulovirus

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**Abstract**

In this study, the effects of various secondary metabolites in the economically important plants (mulberry, apple, walnut, and plum) on the pupal weight, the pupal total protein and the pupal total lipid contents of *Hyphantria cunea* larvae infected by *Hyphantria cunea* granulovirus were investigated. The larvae used in the study were collected from the Kızılırmak Delta in Samsun, Turkey, in 2020. In this study, the larvae of the 2nd generation obtained as the offspring of the 1st generation's adults were used. The larvae were fed daily with fresh plant leaves until pupae. Except for the control group, the plants were infected with the virus. The phenolic compounds and gallotannin amounts of the plants given to the larvae were measured. As a result of the study, it was determined that the highest pupal weight, pupal protein and pupal lipid amounts among the control groups were in the mulberry group with the minimum amount of gallotannin, as well as having catechin and rutin. Again among the control groups, the lowest pupal weight, pupal protein and pupal lipid amounts were in the apple group with the highest rosmarinic acid and protocatechuic acid. Among the infected groups, the highest pupal weight, pupal protein and pupal lipid amounts were in the mulberry group, while the lowest amounts were in the apple group. With viral infection, pupal weights, pupal protein and pupal lipid amounts of all groups decreased compared to the control. As a result of this study, the effects of both viral infection and plant secondary metabolites on pupal parameters were determined.

**Keywords:** Granulovirus, *Hyphantria cunea*, Secondary metabolites, Pupal parameters.

## *ORAL PRESENTATION*

# Green extraction of spent tea waste

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**Abstract**

Tea is cheap and non-alcoholic beverage that is widely consumed throughout the world [1,2]. The consumption of tea has reached almost 6 million tones around the world. Along with the high consumption, considerable amount of tea waste is accumulated. Black tea contains various types of healthful molecules such as polyphenols, flavonoids, phenolic acids and alkaloids [3].

For the last few decades researchers are mainly interested in developing environmentally friendly processes since the community has become more conscious about pollution. In this aspect, we aimed to evaluate the waste of tea using an environmentally green process. Two types of waste are accumulated by the consumption of tea; one is the waste of tea plant during the production of tea and the other one is the dreg of tea after brewing. In this study we used the dreg after brewing which is called spent tea.

Spent tea was collected, dried, and kept for further use. The extraction of bioactive compounds was performed using microwave technology. For this purpose, different types of deep eutectic solvents (DES) that are green solvents, were utilized. It was found that maximum exposure time differed significantly depending on the type of DES. The effect of DES type, exposure time, DES percentage, and solid-liquid ratio were investigated on total phenolic yield, total flavonoid yield and antioxidant activities. The results indicated that spent tea still contained considerable amount of phenolics that could be extracted with DESs -rather than water- and these extracts have the potential to be used as antioxidant additives serving various fields of research.

Acknowledgement: This study was funded by the Scientific and Technological Research Council of Turkey (TUBITAK) 2209-A Project no: 1919B012102396.

**Keywords:** Spent tea, waste valorization, green technology, deep eutectic solvents, microwave.

**References**:

1. Guo, S., Kumar, M., Wang, Y., Xu, P.: Bioresource Technology Current understanding in conversion and application of tea waste biomass: A review. Bioresour. Technol. 338, 125530 (2021).
2. Sarıca, Ş., Tea (Camellia sinensis); Composition , The Effects on Metabolism and Health , Antioxidant Activity and The Use . Heal. (San Fr. 25, 79–85 (2008)
3. Dreosti, I.E.: Bioactive ingredients: Antioxidants and polyphenols in tea. Nutr. Rev. 54, (1996).

## *ORAL PRESENTATION*

# Black tea kombucha extends the lifespan of *Drosophila melanogaster*

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**Abstract**

The search for anti-aging substances is a crucial challenge. Recent studies showed that kombucha has several health-promoting effects. Thus, we evaluated whether the black tea kombucha can prolong the lifespan of *Drosophila* fed with accelerating-aging substances including hydrogen peroxide or potassium dichromate. The longevity assay showed that flies fed with black tea kombucha with hydrogen peroxide compared to flies fed only with hydrogen peroxide displayed longer mean and maximum lifespan. Similar results were recorded with flies fed with black tea kombucha with potassium dichromate compared to flies fed only with potassium dichromate. These results may have occurred since black tea kombucha have phenolic compounds and antioxidant activities. Taken together, we report the pro-longevity effect of black tea kombucha on *Drosophila melanogaster*.

**Keywords:** aging, *Drosophila*, hydrogen peroxide, kombucha.

## *ORAL PRESENTATION*

# Value-added products from biowaste

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**Abstract**

Rapid urbanization simultaneously increasing the use of fossil fuels and organic waste formation has raised the need for food, animal feed, and chemicals. Because of their considerable quantity, renewability, and accessibility, organic wastes might be benefitted as a valuable solution to waste management issues. Many industrial wastes are used to manufacture value-added products such as biofuels, biofertilizers, biohydrogen, enzymes, and bioplastics using microbial fermentation processes because of their organic and nutrient-rich constitution. Pre-treating the waste biomass, creating a product with added value in reactors, and processing the product later to recover it are steps in the process. However, the majority of biowaste processes have only been investigated in the laboratory; commercial applications have not yet been completely employed. The transition to a circular economy will be accelerated by the adoption of new encompassing technologies for the exploitation of organic waste. Thus, the potential and sustainability of creating various value-added products from biowaste and by-product streams are discussed in this presentation.

**Keywords:** Organic waste, value-added products, biomass, biofuels, bio-conversion, biorefinery.

## *ORAL PRESENTATION*

# Orthogonal BODIPY Dimer-Substituted Cyclotriphosphazene Derivatives As Novel Photocatalysts

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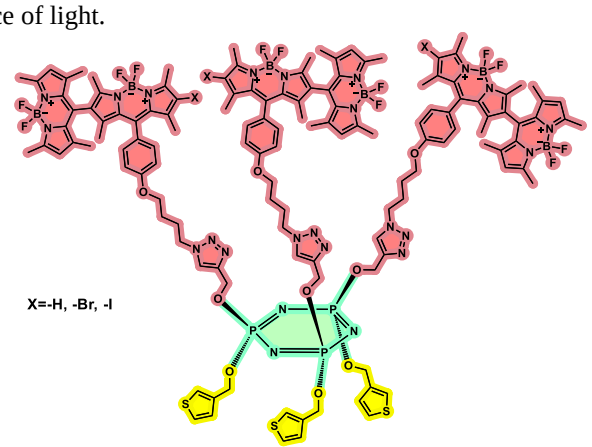
**Abstract**

Materials that can produce reactive oxygen species (ROS) by using molecular oxygen in the medium when excited with light at the appropriate wavelength are called photosensitizer (PS). PS is an important component of photoredox catalytic reactions [1], photovoltaic application [2] and photodynamic therapy [3]. BODIPY derivatives are frequently used as photosensitizer due to their advantages such as allowing functionalization on the molecule, increasing the singlet oxygen production capacity depending on the functionalization, exhibiting good thermal and photo stability, having a long absorption wavelength range and high solubility [3].

In this work, novel triad systems, including cyclotriphosphazene, thiophene and orthogonal BODIPY units, were prepared and their molecular structures were characterised using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, $^{1}H$, $^{13}C$ and $^{31}P$ NMR techniques. The photophysical properties like absorption, emission wavelengths, molar extinction coefficients and fluorescence quantum yields of novel six compounds were investigated by using UV-visible absorbance and fluorescence spectrophotometry. The singlet oxygen generation capacities of BODIPY monomers and orthogonal BODIPY dimer-substituted cyclotriphosphazene derivatives were examined via chemical methods. Additionally, the photocatalytic abilities of the target molecules were determined by oxidizing 1,5-dihydroxynaphthalene (DHN) to juglone in the presence of light.




The triad systems showed stronger ability to generate singlet oxygen and photooxidize DHN to juglone than methylene blue as conventional photosensitizer. Consequently, the orthogonal BODIPY dimer-substituted cyclotriphosphazenes are proposed as photocatalysts that are able to use in organic reactions.

**Keywords:** Photocatalyst, BODIPY, cyclotriphosphazene, photosensitizer, singlet oxygen.

**Acknowledgement:** This work was supported by Ataturk University The Coordination Unit of Scientific Research Projects, project number FDK-2021-9632.

**References:**

**[1]** Wang L., Cao J., Wang J., Chen Q., Cui A., He M., *RSC Adv.*, 2014, 4, 16786-14790.
**[2]** Aguiar A., Farinhas J., Silva W., Santos I.C., Alcácer L., Brett C.M.A., Morgado J., Sobral A.J.F.N., *Dyes and Pigments*, 2021, 193, 109517.
**[3]** Kamkaew A, Lim SH, Lee HB, Kiew LV, Chungc LY, Burgess K., *Chem. Soc. Rev*., 2013, 42, 77-88.

## *ORAL PRESENTATION*

# Influence of *E. coli* cell density on antibiotic resistance

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**Abstract**

*E. coli* is one of the best-known members of the lower gastrointestinal tract in humans and is also known as a pathogen leading to several human diseases. In this study, it is aimed to investigate the relationship between the bacterial cell density of a clinical *E. coli* strain and its resistance to some selected antibiotics. Tested bacterial *E. coli* cells were adjusted to 0.5, 1.0, and 2.0 McFarland (MF) optical density standards. Antibiotic susceptibilities of *E. coli* strain with different cell density levels, against Amikacin (AK), Amoxicillin/Clavulanic acid (AMC), Cefepime (PM), Cefoperazone (CFP), Cefoperazone/Sulbactam (SCF), Cefotaxime (CTX), Ceftriaxone (CRO), Chloramphenicol (C), Ciprofloxacin (CIP), Clindamycin (DA), Imipenem (IPM), Nitrofurantoin (F), Rifampicin (RA), Tobramycin (NN) and Trimethoprim/Sulfamethoxazole (SXT) were determined by Kirby Bauer Disc Diffusion Method. Breakpoint tables for diameter of Zone of Inhibitions (ZOIs), determined by the European Committee on Antimicrobial Susceptibility Testing (Version 12.0, valid from 2022-01-01) were used for the interpretation of antibiotic susceptibilities. According to our results; *E. coli* strain with all 3 different cell densities, was determined as multi-drug resistant and was only susceptible to Nitrofurantoin (F), Imipenem (IPM) and Chloramphenicol (C) antibiotics with diameter of ZOIs ≥ 20 mm. Apart from this, in parallel with the increase in *E. coli* cell density, a slight decrease in ZOIs of Nitrofurantoin (F), Imipenem (IPM), Amikacin (AK) and Chloramphenicol (C) antibiotics was observed. In conclusion, although the increase in *E. coli* cell density influenced ZOIs, it did not influence the antibiotic resistance of the *E. coli* strain.

**Keywords:** Antibiotic resistance, *E. coli*, Multi-drug resistance, Quorum sensing.

## *ORAL PRESENTATION*

# Commercial *Spirulina platensis* tablets and enzyme inhibition

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**Abstract**

The thioredoxin system consists of thioredoxin reductase (E.C 1.6.4.5; TrxR), thioredoxin (Trx) and reduced nicotinamide adenine dinucleotide phosphate (NADPH). TrxR is a flavin adenine dinucleotide (FAD)-containing NADPH-dependent disulfide reductase. *Spirulina platensis* (SP) is a phototrophic, filamentous, and multicellular blue-green microalga, belonging to the class of Cyanobacteria. Spirulina with its high nutritional value has been consumed as food. The importance of the system, which has a wide variety of functions for the cell, is due to the role of TrxR in protecting against oxidant damage, cell growth and transformation, p53 activity, and recycling of ascorbate from its oxidized form. In this study, the *in vitro* effects of some vitamins and drugs on TrxR purified from commercial SP tablets were investigated. For this purpose, the TrxR was purified from commercial SP tablets. Purification processes were performed in two steps: preparation of the homogenate and 2',5'-ADP Sepharose 4B affinity chromatography. The effects of vitamins and various drugs on the purified TrxR activity were investigated. The $IC_{50}$ value, the amount of substance required for the enzyme to have a 50% inhibition effect, was calculated. The inhibition types of compounds were determined with the help of Lineweaver–Burk graphs. As a result of these experiments, it was found that the vitamins and drugs had inhibitory effects on the TrxR enzyme.

**Keywords:** Thioredoxin reductase, *Spirulina platensis*, enzyme purification, inhibition.

## *ORAL PRESENTATION*

# Nucleic acid detection-based biosensor technologies for smart agriculture

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**Abstract**

Biosensor technologies enabled conventional, laboratory-based testing to be rapidly and reliably performed in the field for agriculture, farming, and food processing. Smartphone detection have accelerated biosensor applications in conjunction with Internet-of-Things technology (IoTs). In recent years, biosensors have been evolved to be user and environment safe by containing non-hazardous chemicals, using automation they have been more robust to work under varying environmental conditions. Thanks to smartphones and machine learning applications they become more user friendly for untrained people. Still, affordability, portability, high sensitivity, flexibility, and specificity demands remains among the indispensables of diagnostic technologies.
Here, we have developed simple, portable, low-cost, colorimetric, and remotely controllable pathogen detection platforms based on nucleic acid detection. These tools contain low weight thermally isolated a fast prototyped case, low-cost electronic components, a sample plate, or standard tubes that can be easily scaled for high throughput readouts. Controlling the steady-state temperature error of the system less than <1%, LAMP, Colony-LAMP, Real-time-LAMP reactions can be performed for crude samples of bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), virus (Potato Virus Y), or genetically modified organisms (gts40-3-2/RSS soybean). In our system the end-point colorimetric readouts can be detected either under day light by a naked eye or by a smartphone camera. The obtained row images of the samples and processed data with digitized readouts can be shared among the users and experts without requiring any data interpretation while being stored in a server for forthcoming analysis.
We strongly believe that our platforms can be implement for food processing and they can contribute for rapid and reliable diagnosis in versatile operational environments.

**Keywords:** Internet of things (IOTS), agriculture. biosensors.

## *ORAL PRESENTATION*

# Analysis of Salicaceae Collections Data in Some Herbaria of Türkiye

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**Abstract**

Building on centuries of research based on herbarium specimens gathered through time and around the world, many research on analysis of collections data in herbarium are being done. In this study, we have analysed the herbarium sheets from the family Salicaceae. According to herbaria in Türkiye, within herbaria sheets (family Salicaceae) there are 2 genera. Collection which made up of 917 herbarium records for *Salix* genus and 242 for *Populus* genus was taken into consideration from 6 biggest herbaria of Türkiye as ANK, ISTO, HUB, AEF, ISTE and GAZI. The topics charted are number of taxa in herbaria collections, distribution and number of taxa according to family, distribution of taxa by collection date and locations, and comparison of Turkish data with well-known herbaria from the world. Python programming language was applied for the data cleaning and data visualization with the use of pie charts, donut plots, bar graphs and map plots. ISTO herbarium holds the majority of the *Salix* genus with 287 records, followed by AEF with 221 records and finally HUB with 148 samples. The oldest herbarium sheet date goes back to mid-1800s whereas the vast majority of herbarium sheets were collected during the period 2000-2001 as far as Salicaceae is concerned. Total *Salix* genus collections include 53 taxa. 26% of the collection is represented by species, *Salix alba.* The majority of samples for both genus *Salix/Populus* collected from Ankara province. It is followed by Bolu and Kars provinces respectively. Furthermore, the locations from which the Salicaceae members have been collected are presented with the help of Turkish province map in the current study.

**Keywords:** Salicaceae collections, *Salix* L., *Populus* L., Herbaria, Türkiye, Python.

## *ORAL PRESENTATION*

# Total phenol and flavonoid contents of two different *Salix* species interpreted by *Python*

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**Abstract**

The aim of this study is to determine the strains of *Salix caprea* (goat willow) and *Salix purpurea* subsp. *leucodermis* (endemic to Türkiye) in flower, leaf and bark extracts and to interpret the total phenolic contents and flavonoids. When the ethnobotanical studies on *Salix* species are examined, it is seen that they are used for medical purposes on a large scale such as rheumatoid arthritis, malaria, hemorrhages, gout, neuralgia, internal medicine, antipruritic, analgesic, anti-inflammatory, antibacterial, hemostatic, sedative and anthelmintic. Since both *Salix* species have been seen to have medicinal potential, the study investigated the potential of them. *Salix caprea* and *S. purpurea* subsp. *leucodermis*' flowers, leaves and bark were collected in the spring and early summer from willow garden of National Botanical Garden of Türkiye, Muğla/Köyceğiz and Ankara/ Kızılcahamam districts. Fresh leaves/flowers/bark were dried at room temperature for 2 weeks at least 10-30grams. The samples were pulverized and kept in the desiccator until the time of analysis. The analysis was done in Batı Akdeniz Agricultural Research Institute Labs/Antalya. Total phenolic and flavonoid contents were determined with some modifications of the Folin-Ciocalteu and Zhishen methods, respectively. Total phenolic and flavonoid contents for methanolic extracts of samples ranged from 0.95 to 5.94 g of gallic acid equivalent/100 g dry weight (DW) and from 0, 39 to 2.67 g of catechin equivalent/100 g DW, respectively. According to interpreted preliminary results in Phython with the samples (3 different organs of 2 species from 2 habitats), it is found that the amount of flavonoids and phenolics are varied in regards to organ or habitat type rather than species selected. Especially, the bark included more amounts of flavonoids and phenolics than other organs. The species (*Salix purpurea* subsp. *leucodermis* and *S.caprea*) distributed naturally in Türkiye might have high antioxidant potential.

**Keywords:** Phenolic contents, Flavanoids, *Salix caprea, Salix purpurea* subsp. *leucodermis*, Phython graphics.

## *ORAL PRESENTATION*

# Role of plant growth-promoting rhizobacteria in soilless agriculture and biotechnological advances

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**Abstract**

Microbial fertilizers including plant growth-promoting rhizobacteria (PGPRs) are one of the most important of the environmentally friendly approaches. The mode of action of the effects of PGPRs on plants can be direct or indirect. Direct mechanisms are biofertilization (nitrogen fixation), the production of phytohormone, siderophore and phosphate solubilizing enzyme, indirect mechanism; it stimulates plant development by reducing the harmful impacts of plant pathogens through induced systemic resistance (ISR) and the production of antimicrobial compounds. While rhizobacteria are often used in traditional practices to increase productivity in agricultural production, their use in soilless agriculture is limited. A recent trend is the inclusion of PGPRs into soilless agriculture practices. However, despite several studies to date, there is a huge knowledge gap in terms of the commercialization of PGPRs for soilless agriculture. Therefore, the development of proper strategies, and additional research and trials are considered necessary. This study focuses on a critical discussion from different perspectives of the use of PGPRs in soilless agriculture and biotechnological advances related to these beneficial bacteria.

**Keywords:** Soilless agriculture, Microbial biotechnology, Plant growth-promoting rhizobacteria.

## *ORAL PRESENTATION*

# Detection of The Effect of CYP2C19*4 Mutation on Clopidogrel Response by *In Silico* Methods

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**Abstract**

Single nucleotide polymorphisms cause amino acid change, and protein structure and function are changed. Thus, the patient improves drug resistance and does not respond to therapy. Clopidogrel is an antiplatelet drug and is used for cardiovascular disease therapy such as heart failure, atherosclerosis, and myocardial infarction.CYP2C19 gene is a CYP450 enzyme and metabolizes clopidogrel. Polymorphism of the CYP2C19 gene cause clopidogrel response. A homology modeling study was carried out using the Swiss-Model database and the Chimera program. The selection of models was made with the evaluation of the QMEAN values of the three-dimensional structures. The physicochemical properties of the wild type and CYP2C19*4 mutant type of the CYPC19 gene were analyzed by the ExPASy-ProtParam Portal. The effects of the mutation on the protein and calculation probably damage of mutation on the protein structure were performed by the HOPE database. The AutoDock program was used to demonstrate interactions between clopidogrel and wild-type protein and, mutant type protein. CYP2C19*4 mutant residue is smaller than the wild-type residue. The mutated residue is located in a domain that is important for the binding of other molecules. Mutation of the residue might disturb this function. This mutation causes the loss of interactions and affects the drug response. In this study, it was shown that Clopidogrel drug interactions between mutant type protein by docking study. Possible drug conformation is designed for the effective treatment of patients carrying the common mutation.

**Keywords:** Heart failure, drug resistance, clopidogrel, homology modeling, single nucleotide polymorphism (SNP).

## *ORAL PRESENTATION*

# Kidney Damage Related with Pulmonary Toxicity in Mice

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**Abstract**

Bleomycin (BLM), an antitumor effective drug used in cancer treatment, often causes pulmonary toxicity due to use, and therefore, studies are continuing to reduce this effect in research subjects due to its strong side effects. In addition, patients treated with BLM may develop pulmonary fibrosis. More recently, an increased prevalence of chronic kidney disease has been reported in elderly people with pulmonary fibrosis (IPF). Administration of atorvastatin (ATR) to mice and rats with pulmonary fibrosis or lung fibroblasts reduces fibrosis formation. The current study was designed to investigate the protective effects of ATR on renal oxidative damage in kidney tissue of mice with pulmonary fibrosis. Adult male C57BL/6J mice were used in the present study and divided into four groups: (1) control group, (2) ATR group, (3) BLM group, and (4) BLM + ATR group. At the end of the experiment, kidney tissues were taken and homogenized in cold 0.9% NaCl with glass equipment to obtain 10% (w/v) homogenate and centrifuged at 10.000g at +4°C for 10 min. The supernatants were used for biochemical analysis. Reduced glutathione (GSH), lipid peroxidation (LPO), total antioxidant status (TAS), total oxidant status (TOS), reactive oxygen species (ROS), oxidative stress index (OSI) levels and lactate dehydrogenase (LDH), glutamate dehydrogenase (GDH) and arginase activities in addition to the presence of cell death were determined in kidneys tissues. In BLM groups, LPO, TOS, ROS, OSI levels, and LDH activity were found to be increased, while GSH, TAS, Bax and active-caspase-3 levels, and GDH and arginase activities were decreased. The administration of ATR to mice treated with BLM reversed these changes but, apoptosis was induced in kidney of these mice. These results show to be the ameliorative effect of ATR only on oxidative damage in kidney tissue of mice with pulmonary fibrosis.

**Keywords:** Bleomycin, Atorvastatin, Mice, Enzymes, Apoptosis, Kidney.

## *ORAL PRESENTATION*

# Plum pox virus (PPV)'nin tek adımlı kolorimetrik izotermal amplifikasyon ile tanısı için uygun RNA izolasyon yöntemi üzerine araştırmalar

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**Özet**

Plum pox virus (PPV) sert çekirdekli meyvelerin en önemli viral hastalıklarından biri olarak bilinmektedir. Hastalıkla mücadelede çeşitli bitki koruma faaliyetleri uygulanmakla birlikte, eradikasyon hala en etkili yöntem olarak uygulanmaktadır. Günümüzde hastalık etmenin kesin ve hızlı tanısı, eradikasyon çalışmaları için kilit rol üstlenmiştir. Bu çalışmada PPV'nin tek adımlı kolorimetrik RT-LAMP (Reverse Transcription Loop-mediated Isothermal Amplification) tanısı için en uygun RNA izolasyon yönteminin belirlenmesi amaçlanmıştır. Çalışmada izotermal reaksiyonun yürütülmesinde WarmStart® Colorimetric LAMP 2X Master Mix tanı kiti kullanılmıştır. Yürütülen çeşitli RNA izolasyon kombinasyonları ile tüpler içerisinde oluşan renk değişimleri doğrudan gözlem yöntemi ile değerlendirilmiştir. PPV'nin tek adımlı RT-LAMP tanısı için doğrudan bitki öz suyunun kullanımının reaksiyonu inhibe ettiği ve false-pozitif sonuçlara yol açtığı görülmüştür. Bu sonucun PPV'nin LAMP yöntemi ile arazi koşullarında tanısını zorlaştıracağı değerlendirilmiştir. Çalışma sonucunda renk değişikliğini görülebilir kılan en uygun RNA izolasyon yöntemi belirlenerek, pozitif amplifikasyon için optimal koşullar yeniden revize edilmiştir. Bu araştırma 60 °C sıcaklık 45 dk'lık inkübasyon süresi ile yeni izolasyon yönteminin PPV tanısı için güvenilir olduğunu göstermektedir.

**Anahtar Kelimeler:** Plum pox virus, izotermal amplifikasyon, RNA izolasyonu.

## *ORAL PRESENTATION*

# Evaluation of theToxicity Test of Southern Grape Hyacinth *(Muscari neglectum* Guss. ex Ten.) Plant Extracts in *Wistar albino* Rats

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**Abstract**

Southern grape hyacinth (*Muscari neglectum* Guss, ex Ten.) exhibits anti-inflammatory, anti-allergic, antibacterial, antirheumatic, and antioxidant properties because of its phtochemical compounds (homoisoflavan, flavonoid, alkaloid, terpenoid, steroid, quinic acid, fumaric acid, gentisic acid, caffeic acid, campherol, apigenin and other compounds). People use it as a diuretic, expectorant, to treat stomach problems, and as an aphrodisiac. However, no *in vivo* or toxicity studies on this plant have been conducted thus far. The aim of this study is to add to the literature by conducting extensive toxicity test assessments. *Muscari neglectum* aerial (MNA) and *M. neglectum* bulb (MNB) part extracts were given to the rats at doses of 100, 500 and 1000 mg/kg/day for 7 days by oral way. 42 *Wistar albino* female rats were divided into 7 groups (6 rats per group): Control; MNA-100 mg/kg/day; MNA-500 mg/kg/day; MNA-1000 mg/kg/day; MNB-100 mg/kg/day; MNB-500 mg/kg/day and MNB-1000 mg/kg/day groups. Rats were observed in different time periods ($6^{th}$, $12^{th}$, $18^{th}$ and $24^{th}$ hours after first application and every 24 hour) for signs of toxicity and mortality. Biochemical parameters in serum and hematological parameters in whole blood were examined following a 7-day toxicity test. According to the biochemical data, the increase in alanine aminotransferase (ALT) MNB-1000 mg/kg group, lactate dehydrogenase (LDH) MNA-1000 mg/kg group, and glucose MNB-100 and MNB-1000 mg/kg groups was statistically significant when compared to the control group. Only the statistically significant decrease in mean corpuscular hemoglobin concentration (MCHC) of MNB-1000 mg/kg dosage was determined when compared to the control group. When the toxicity test research is taken as a whole, it can be concluded that *M. neglectum* extracts do not typically produce harmful effect, but because high dosages induce a rise in AST and LDH, it is advisable to intake the plant at lower levels.

**Keywords:** *Muscari neglectum*, toxicity test, biochemical, hematological, rats.

## *ORAL PRESENTATION*

# A systematic study on eco-friendly photodegradation of lipophilic dye Oil Red O using urea-based graphitic carbon nitride-coated cotton fabrics

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**Abstract**

Dye-containing environmental pollutants have irrecoverable hazardous impacts on the ecosystem cycle. Among these dyes, Oil Red O (ORO) is widely used as a fat-soluble diazo dye to stain oils and waxes. In this study, urea-based graphitic carbon nitride (g-$C_3N_4$), coated on cotton fabrics, was used as a nanophotocatalyst for efficient photodegradation of ethanolic ORO. g-$C_3N_4$ is known as a cost-effective, non-toxic, metal-free, and sustainable photocatalyst due to its structural stability and environmentally friendly composition. g-$C_3N_4$ nanosheets were prepared by thermal treatment of urea (calcination at 600 °C at a heating rate of 5 °C $min^{-1}$ for 4 h). The structural analysis of as-prepared g-$C_3N_4$ was performed by FT-IR, XRD, Raman, and SEM, while TGA was used to evaluate thermal stability. A coating time of 1h, 2h, and 4h was applied using a solution of 1 w% of g-$C_3N_4$ to activate the pre-treated fabrics to evaluate the effect of coating time on the complete degradation of the ORO. Dye-degradation studies were performed using a UV-Vis solar simulator with an intensity of 100 mW/$cm^2$ as the irradiation source. UV-Vis absorption spectroscopy was employed to evaluate the photocatalytic efficiency of g-$C_3N_4$ and monitor the degradation of ORO. The optimized photodegradation time to fully degrade the ORO on 2h-coated cotton fabrics was 8h, which was more efficient than previous reports using $TiO_2$ (24h). For 1h- and 4h-coated fabrics, the photodegradation time was 12h and 8h, respectively. A similar photodegradation time of 8h for both 2h- and 4h-coated fabrics might be due to the accessible active surface area of the fabrics.

**Keywords:** Photodegradation, Graphitic carbon nitride, Nanophotocatalyst, Oil Red O, Cotton fabrics.

## *ORAL PRESENTATION*

# Investigation of the effect of alternative filler on tire compound with natural rubber

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**Abstract**

The basic materials of the tire compound are rubber, curing agent and fillers. Fillers are used to provide physical properties as well as to reduce direct material costs. While choosing alternative fillers in this study, attention was paid to the selection of waste materials to contribute to sustainability. For this reason, the effects of charcoal powder and fly ash are being investigated on tire compound with natural rubber to reduce direct material cost of tire compound. The charcoal to be used as filler for the determined tread recipe was obtained from oak. Also, fly ash was obtained from Çatalağzı Thermal Power Plant. To express the ratio of the materials in the tire compound, the phr (parts per hundred rubber) proportioning method was used. In the study, the effects of charcoal, fly ash and the combined of charcoal and fly ash were investigated. According to the experimental results, charcoal and fly ash did not affect the viscosity and scorch time. As a result of the tensile and elongation test, charcoal caused to increase the modulus under room conditions, while fly ash did not cause a significant change. Both charcoal and fly ash greatly decreased the tensile and elongation of the tire compound. The high decrease in tensile and elongation showed that charcoal and fly ash cannot be used in this form and at this rate in tire compounds, since it will cause performance loss.

**Keywords:** Tire, Rubber, Filler materials, Charcoal, Fly ash.

## *ORAL PRESENTATION*

# Santrali çevreleyen arazilerde bazı ağır metaller (Pb, Cd, Ni, Co, As) ile toprak kirliliği

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**Özet**

Çalışma, Irak'ın Samarra kentindeki elektrik santrali yakınlarındaki tarım arazilerinde ağır metallerden kaynaklanan toprak kirliliğini değerlendirmek amacıyla yapılmıştır. Bu çalışma için kirlilik faktörü (CF) ve zenginleştirme faktörü (EF) gibi modern toprak kirliliği standartları kullanılmıştır. Bu çalışma için santrale uzaklıklarına göre 500, 1000, 1500 ve 2000m olmak üzere dört lokasyon belirlenmiştir. Bu sahalardan iki derinlikte (0-30 ve 30-60 cm) toprak örnekleri alınmış ve Pb, Cd, Ni, Co ve As gibi bazı ağır metaller tahmin edilmiştir. Sonuçlar, S1D1, S1D2, S2D1 ve S2D2 bölgeleri için toprakta yüksek Pb konsantrasyonu ve sırasıyla 167.8, 135.1, 129.3, 101.2 mg kg-1'e ulaşmıştır. Kadmiyum en yüksek değerleri S1D1, S1D2, S2D1, S2D2, S3D1, S3D2 bölgelerinde kaydetti ve bu değerler sırasıyla 9.52, 6.05, 6.82, 5.01, 4.11, 3.10 mg kg-1'e ulaşmıştır. S1D1, S1D2, S2D1 ve S2D2 bölgelerindeki nikel konsantrasyonu sırasıyla 104.71, 85.11, 81.32, 77,3 mg kg-1'e bulunmuştur. S1D1 bölgesindeki arsenik konsantrasyonu 21.2 mg kg-1'e ulaştı. Bu sonuçlar, WHO/FAO'ya (2011) göre izin verilen sınırları aşarken, Co konsantrasyonunun değerleri değişti ancak izin verilen sınırları aşmadığı tespit edilmiştir. Sonuçlar, 0-30 ve 30-60 cm derinliklerde S1, S2 siteleri için (Cd, Pb, As) için yüksek EF değerinde bulunmuştur. Değerler orta ($3 < EF \leq 5$) ile yüksek ( $5 < EF < 20$) EF değerleri arasında, S3 bölgesindeki değerler düşük düzeyden ($EF < 2$) orta derecede EF değerleri ($3 < EF \leq 5$) arasında bulunmuştur. Tüm sahalarda ve derinliklerde Ni ve Co değerleri az düzeyden orta dereceye kadar EF değerlerinde bulunmuştur ($EF < 2$, $3 < EF \leq 5$). Sonuçlar, tüm sahalar ve derinlikler için (Cd, Pb, As) için yüksek CF değerleri göstermiştir ve değerler önemli ($3 < CF < 6$) ila çok yüksek kirlenme ($CF > 6$) değerleri arasında, Ni ve Co değerleri, 30-60 cm derinliğinde S1, S2 sahasında önemli ($3 < CF < 6$) ve S3 sahasında orta derecede kontaminasyon ($1 < CF < 3$) arasında olduğu saptanmıştır.

**Anahtar Kelimeler:** Kontaminasyon faktörü, zenginleştirme faktörü, ağır metaller, enerji santrali, toprak kirliliği.

## *ORAL PRESENTATION*

# A microwave-assisted fluidic system for continuous synthesis of polymeric nanocomposite

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**Abstract**

For further usages, the synthesis of narrow-size nanoparticles inside the matrix of polymer is significant. A microwave-powered fluidic system (MWFS) paves the way for continuous production of nanocomposite (NC). In this study, the effect of microwave power (MWP), flow rate, and concentration of the reagents are scientifically investigated. With formic acid, continuous and green synthesis of nylon-6 NC (silver nanoparticles (AgNPs) with a mean size of ~2.59 ± 0.639 nm distributed evenly inside nylon-6) at low temperatures (50–55 °C) was obtained. The gradual increase of the polymer concentration along with the reduction in microwave power (MWP) resulted in a reduction of AgNP size. Also, the size of nanoparticles (NPs) affects the crystallinity of nylon 6 inside synthesized NCs. Regarding the antibacterial efficiency, AgNPs synthesized by MWFS show superior activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* compared to large AgNP (~50 nm) inside NCs synthesized by a conventional heating fluidic system.

**Keywords:** Nanocomposite, Silver, Polymer, Microwave, Fluidic System.

## *ORAL PRESENTATION*

# Preparation of open-cellular carbons from polyester-based foams

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**Abstract**

High Internal Phase Emulsion (HIPE) is a type of concentrated emulsion with an internal phase volume higher than 74% of the total emulsion volume. This volume ratio is the critical ratio required for the most effective packaging of the internal phase droplets [1]. HIPEs are emulsions that are widely used in food, pharmaceutical and cosmetic applications. In recent years, they have attracted great interest especially in the synthesis of hierarchical macroporous polymers. Porosity is an important property sought for materials that serve applications such as separation, purification, chromatography, tissue engineering and catalysis, as well as liquid, gas and even energy storage. HIPEs allow the synthesis of thermoset polymers with controllable open-cellular pore morphology. If a HIPE contains monomer(s), it can be polymerized under suitable conditions. The drying process followed by removal of the internal phase after polymerization leads to the formation of a specific pore morphology dominated by macro-sized cavities interconnected by small pore throats. Polymers obtained by polymerizing precursor HIPEs are named as polyHIPEs [1]. The morphological properties of polyHIPEs reflect the precursor HIPEs used as templates. Thereby, morphological and physical properties can be easily controlled by controlling the experimental parameters during emulsion preparation [2, 3]. In this study, it is aimed to prepare macroporous carbons, which have the potential to be used as a support material in thermal energy storage applications. In this context, polyester-based polyHIPEs were obtained by polymerization of water-in-oil type HIPEs, which were prepared by using a commercial polyester resin and crosslinking comonomers. Afterwards, resulting foams were post-crosslinked by Friedel-Crafts alkylation and then carbonized under inert atmosphere. Finally, chemical structure, pore morphology and BET specific surface area of the obtained polyHIPEs and polyHIPE-derived-carbons were investigated.
Acknowledgement: This study is supported by Yalova University Scientific Research Projects Coordination Unit (Project No: 2021/YL/0016).

**Keywords:** Porous carbons, emulsion templating, thermal energy storage.

**References:**
[1] Mert H.H., Mert E.H., Emulsion Templated Hierarchical Macroporous Polymers *In: Advanced Functional Porous Materials from Macro to Nano Scale Lengths* (2022), Page: 44. Springer Nature, Eds.: Uthaman, A., Thomas, S., Li, T., Maria, H.,
[2] Mert H.H., Mert M.S., Mert E.H. (2019). A statistical approach for tailoring the morphological and mechanical properties of polystyrene PolyHIPEs: looking through experimental design. Materials Research Express, 6, 115306.
[3] Mert E.H, Mert H.H. (2021). Preparation of polyHIPE nanocomposites: Revealing the influence of experimental parameters with the help of experimental design approach. Polymer Composites, 42(2), 724-738.

## *ORAL PRESENTATION*

# Design, Synthesis and *in vitro* Biological Activities of Heterocyclic Amine Derivatives as FABP4 Inhibitors

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**Abstract**

The fatty acid binding proteins (FABPs) belong to intracellular lipid chaperones family and bind lipophilic substances especially long chain fatty acids. FABPs are thought to facilitate the transfer of fatty acids between extra- and intracellular membranes. FABP4 (A-FABP or aP2) is one of the subtypes of this proteins and predominantly expressed in adipocytes and macrophages. It plays a central role in integrating metabolic and inflammatory responses, therefore, the inhibition of FABP4 might have potential therapeutic effect for the treatment of metabolic syndrome, inflammation, atherosclerosis and cancer. Although studies on FABP4 inhibitors (FABP4i) have been carried out for more than 20 years, there is not any compounds in phase trials yet.
Literature survey indicates that many of the compounds reported as FABP4 inhibitors have pyrazole, imidazole, triazole, benzimidazole, pyrimidine, bicyclic pyridine or quinoxaline rings. Evaluating these results, we designed novel heterocyclic compounds bearing amine function and have better solubility properties. The FABP4 inhibitory activity of synthesized compounds was measured with human FABP4 ligand binding assay kit. The obtained results showed that the compounds have promising activities comparing with positive control, arachidonic acid, and the results evaluated with virtual tools.

**Keywords:** FABP4, heterocyclic, metabolic syndrome, adipocyte, macrophage.

## *ORAL PRESENTATION*

# PEGylation of g-$C_3N_4$ and Its Effect on Photocatalytic Activity

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**Abstract**

Graphitic carbon nitride (g-$C_3N_4$, referred to CN) is a well-known semiconductor used as a photocatalyst in the visible region. In this study, CN was synthesized by a one-step heat treatment method using a dicyandiamide precursor, and its surface was modified (called as PEGylated) with a short polymer chain, poly(ethylene glycol) methyl ether (PEGME550, referred to as PEG). The synthesized and PEGylated CN samples were characterized by X-ray diffraction (XRD), thermogravimetric analysis (TGA), Fourier transform infrared spectroscopy (FTIR), UV-vis diffuse reflectance spectroscopy (DRS), photoluminescence spectroscopy (PL), Brunauer–Emmett–Teller (BET) specific surface area analysis, and scanning electron microscopy (SEM). The XRD patterns well-demonstrated the formation of graphitic carbon nitride layers. The grafting density of the PEG chain to the CN surfaces was determined as 4.48 x $10^{-2}$ mmol/g by TGA. The optical bandgap energies of synthesized and PEGylated CN were estimated as 2.75 and 2.80 eV, respectively, using the Tauc plot from the DRS spectra. Photodegradation of organic dye RhB evaluated the effect of PEGylation on the photocatalytic activity of CN under visible light illumination. Based on the reduction in RhB absorption intensity centered at 553 nm, the synthesized CN degraded RhB by 22% after 6 hours of light irradiation, while the PEGylated CN decomposed RhB by 48%. A pseudo-first-order reaction kinetic model was applied to investigate the photocatalytic activity of the samples quantitatively. Accordingly, the degradation rate constants of RhB over the PEGylated and synthesized CN were 0.1064 and 0.0415 $h^{-1}$, respectively, meaning that PEGylated CN showed 2.56 times higher photocatalytic performance compared to that of the pure CN. As a result, this study shows that the photocatalytic activity of CN is significantly increased by grafting oxygen-containing functional groups (herein PEG) to the CN unit without disturbing the crystal structure.

**Keywords:** Graphitic Carbon Nitride (g-$C_3N_4$), PEGylation of g-$C_3N_4$, photodegradation of RhB, photocatalyst, semiconductor.

## *ORAL PRESENTATION*

# Effect of Humic Acid Applications on Physiological and Biochemical Properties of Soybean (*Glycine max* L.) Grown in Salt Stress Conditions

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**Abstract**

In the past decade, salinity became a serious issue causing crop loss especially in arid and semi-arid regions. The impact of this abiotic stress varies depending on the species, severity, and the stress period. Recently, humic acid has been documented as a significant method to increase plant's systematic tolerance under abiotic stress conditions. Therefore, a study was conducted to determine the tolerance level of soybean (*Glycine max* L. cv İlksoy) to salt stress through applying humic acid.

The study was carried out in the growth room chamber of Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Field Crop. 3 seeds were planted in a 500 cc plastic pots contain mix of 1/3 perlite and 2/3 soil under growth condition of 16/8-hour light/dark photoperiod at 25ºC and 65% humidity. The experiment design was factorial complete random design. The treatments were 4 humic acid doses (0, 500, 1000, 2000 ppm), and 3 NaCl salt doses (0, 125, 250 mM). When the plants were grown, 1 healthy seedling was remained for measuring. Stress were applied after one month. Plants were harvested for data measuring at the point plants showed physiological problems (after 7-8 weeks). Root and stem length (cm), fresh weight and dry weight (g), leaf area (cm), chlorophyll content, ion leakage in leaf tissues (%), lipid peroxidation level, relative water content (%), and membrane resistance index in leaf tissues (%) were examined.

As a result of the study, the longest root was 38 cm and plant height was 30.5 cm for the control pots. The highest root fresh weight was 2.082 g and the stem fresh weight was 1.87 g from the pots where 500 ppm humic acid dose applied. In addition, the plants with the highest chlorophyll ratio was 51.05 SPAD UNITS under 250 mM salt without humic acid application.

**Keywords:** *Glycine max* L., Humic Acid, Salt stress.

## *ORAL PRESENTATION*

# PEG-keratin hidrojellerin hazırlanması ve karakterizasyonu

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**Özet**

Doğada en çok bulunan biyopolimerden birisi olan yün keratin, mükemmel biyouyumluluk özellikleri ve yapısında yer alan hücre yapışma motifleri dolayısıyla biyomalzeme olarak büyük potansiyele sahiptir. Çözünür keratin ekstraktları veya bu biyopolimerin diğer polimerlerle karışımları kullanılarak lifler, filmler, süngerler ve hidrojeller hazırlanabilmekte ve bu işlenmiş formlar ilaç salımı ve doku mühendisliği gibi birçok uygulamalarda incelenmektedir. Keratin hidrojellerin oluşturulmasında fiziksel, kimyasal ve ışıkla çapraz bağlama yöntemleri uygulanmaktadır [1]. Daha önceki çalışmalarımızda kendiliğinden düzenlenen ve düşük molekül ağırlığına sahip çapraz bağlayıcı kullanılarak elde edilen çapraz bağlanmış oksitlenmiş keratin (keratoz) hidrojeller test edilmiştir [1,2]. Bu çalışmada ise Michael katılma reaksiyonu kullanılarak PEG-keratin hibrit hidrojeller hazırlanmıştır. Keratin proteinleri, Karacabey Merinos koyun yününden sülfitoliz yöntemi ile ekstrakt edilmiştir. Keratindeki serbest tiol miktarı DTT ile indirgenerek artırılmıştır. İndirgenmiş keratin, 2000 Da PEG-$(C_2H_4\text{-mal})_2$ veya 6000 Da PEG-$(C_2H_4\text{-mal})_2$ ile çapraz bağlanarak ağırlıkça %10 (keratin + PEG) içeren hidrojeller hazırlanmıştır. Keratin ve çapraz bağlayıcı ajanların oranları 1:0.5 olarak kullanılmıştır. Hidrojellerin viskoelastik özellikleri reolojik testler ile analiz edilmiş ve depolama modülleri 2000 Da PEG-$(C_2H_4\text{-mal})_2$ ve 6000 Da PEG-$(C_2H_4\text{-mal})_2$ keratin hidrojeller için sırası ile 2613 ± 254 Pa ve 1313 ± 345 Pa olarak ölçülmüştür. KRT-6000 PEG-$(C2H4\text{-mal})_2$ hidrojel, döngüsel gerinim zamanı süpürme analizinde kendi kendini iyileştirme davranışı sergilemiştir. Dondurularak kurutulmuş hidrojellerin PBS içinde şişme deneyleri sonucunda 2000 Da PEG-$(C_2H_4\text{-mal})_2$ ve 6000 Da PEG-$(C_2H_4\text{-mal})_2$ keratin hidrojeller için şişme oranları sırasıyla %13.7 ± 1.0 ve %17.6 ± 0.5 olarak belirlenmiştir. Son olarak, L929 fare fibroblast ile yapılan CCK-8 testi, 4 güne kadar her iki hidrojelin de hücre proliferasyonunu boş TCPS kuyularından daha yüksek bir oranda desteklediğini göstermiş ve bu hidrojellerin sito-uyumluluğu teyit edilmiştir.

**Anahtar Kelimeler:** Keratin, Hidrojel, Çapraz bağlama, PEG

**Kaynakça:**

1. Yalçın, D., & Top, A. (2022). Novel biopolymer-based hydrogels obtained through crosslinking of keratose proteins using tetrakis (hydroxymethyl) phosphonium chloride. *Iranian Polymer Journal*, 1-11.
2. Pakkaner, E., Yalçın, D., Uysal, B., & Top, A. (2019). Self-assembly behavior of the keratose proteins extracted from oxidized Ovis aries wool fibers. *International journal of biological macromolecules*, *125*, 1008-1015.

## *ORAL PRESENTATION*

# İzmir kıyılarında yayılış gösteren *Gobius* (Gobiidae) cinsine ait türlerin genetik ilişkileri

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**Özet**

*Gobius* genusunun dahil olduğu Gobiidae familyasına ait türler bentik yaşam şekilleri ve besin ağındaki işlevleri nedeniyle kıyısal bölge besin zincirinde büyük önem taşımaktadır. Morfolojik olarak birbirlerine çok fazla benzerlik göstermekte, dolayısıyla tür teşhislerinde güçlükler yaşanmaktadır. Bu çalışmada, İzmir ili kıyılarında konumlu Dikili, Foça, Mordoğan ve Karaburun ilçelerinde yayılış gösteren *Gobius* (Gobiidae) cinsine ait türlerin tespiti edilmesi ve bu türler arasındaki genetik ilişkinin ortaya konması amaçlanmıştır.
Sualtı çalışmalarında hedef türlerin yayılış derinliğine göre scuba (aletli dalış) ya da serbest dalış tekniği kullanılmıştır. Balıklar öncelikle morfolojik özelliklerine, ekolojik ve davranışsal özelliklerine dikkat edilerek tür tanımlamaları yapılmaya çalışılmıştır. DNA izolasyonu için sağ pektoral yüzgeçleri kesilerek %96 lık etil alkolde muhafaza edilmiş, örneğin geri kalanı ise morfolojik çalışmalar için %4 formaldehit solüsyonuna fikse edilmiştir.
Çalışmada *Gobius* cinse ait *G. auratus*, *G. bucchichi*, *G. cobitis*, *G. fallax*, *G. geniporus*, *G. incognitus*, *G. niger*, *G. paganellus*, *G. roulei* ve *G. vittatus* türleri tespit edilmiştir. Genetik analizler neticesinde, sahada ön değerlendirme aşamasında *G. fallax* ve *G. bucchichi* olarak tayin edilen bireylerin *G. incognitus* oldukları ortaya çıkarmıştır. Ayrıca Genbanktan referans olarak alınan *G. fallax* bireylerinin *G. auratus* kompleksi içinde kümelendiği ve bu bireyler arasında genetik farklılığın ortalama %0,07±0,002 olduğu tespit edilmiştir. Buna ek olarak, *G. auratus* kompleksin içinde yer alan iki farklı renklenmenin sadece morfolojik varyete olduğu ortaya konmuştur.

**Anahtar Kelimeler:** *Gobius*, DNA, sualtı, kaya balığı.

## *ORAL PRESENTATION*

# The Design of GH11 Xylanases for Industrial Applications by Enzyme Engineering

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**Abstract**

Xylanases (3.2.1.8) are the main biocatalysts used for the reduction of xylan, which is the major component of the hemicellulose from lignocellulosic material, randomly breaking down the β-1,4-glycosidic bonds. They are classified in 17 glycoside hydrolase (GH) families, especially GH10 and GH11, based on the similarities of their amino acid sequences and catalytic cleft of their three-dimensional structures. Xylanases are industrially important enzymes and their market is enlarged for three decades. These enzymes are favored in a wide range of industrial fields such as animal feeding, food processing, biofuel production, and pulp and paper industry. Among many GH family xylanases, GH11 members are considered advantageous in industrial applications and understanding of the molecular mechanism, because they have greater activity, substrate specificity, and diffusion into the fiber because of their low size. More stable xylanases against extreme conditions are preferred in the industrial fields, but almost all the xylanases are not resistant to severe conditions, and are biotechnologically not suitable. Enzyme engineering is a powerful technology for improving more proper features of xylanases towards the meet of the requirement of industrial processes. The present work considers the biotechnological features of GH11 xylanases upgraded by enzyme engineering approaches (rational design, directed evolution, and semi-rational design) for meeting the need for second-generation biofuel production, prebiotics generation, and pulp biobleaching.

**Keywords:** GH11 xylanase, enzyme engineering, directed evolution, rational design, semi-rational design, industrial application.

## *ORAL PRESENTATION*

# Effects of The Active Ingredient of Green Tea Epigallocatechin gallate (EGCG) Polyphenol on Cell Metabolism and Aging

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**Abstract**

Aging is a normal process in all living organisms due to the error-prone molecular processes that occur as a result of changing environmental conditions over time. As a result of studies at the molecular level, it has been determined that the cellular aging mechanisms between organisms are extremely common and conserved. Green tea has a very high antioxidant activity compared to other teas obtained from the same plant. Considering the ratios of polyphenols, which make up approximately 37% of its composition, it is striking that Epigallocatechin Gallate (EGCG) polyphenol is present in the maximum amount; For this reason, the focus of studies on green tea is EGCG, which is considered the active ingredient of green tea. In this study, the effect of EGCG, the active ingredient of green tea, on cellular aging was investigated in *Schizosaccharomyces pombe*, which is accepted as a model organism, by evaluating parameters such as lifespan, carbohydrate consumption, intracellular oxidation, and it was observed that EGCG delays aging and improves some cellular parameters.

**Keywords:** Epigallocatechin gallate, Green tea, aging, *Schizosaccharomyces pombe.*

## *ORAL PRESENTATION*

# Biyoaktif cam içeren sentetik polimerik nanofiber membranların üretimi, karakterizasyonu ve in vitro hücre canlılığı

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**Özet**

Periodontal dokuları etkileyen periodontitis tedavisinde, enflamasyonun ve enfeksiyonun cerrahi işlemlerle uzaklaştırılması sonrasında dişi saran yumuşak dokuların azalması ile dişin kemik dokusunda meydana gelen boşluğun yerini epitel dokunun hızlıca doldurmaması ve bu süre zarfında kemik dokunun optimal sürede rejenerasyonu için klinik uygulamada bariyer membranlar kullanılmaktadır. Yönlendirilmiş doku rejenerasyonunu sağlayan bariyer membranlar epitel doku ve kemik doku arasına yerleştirilerek, kemiğin rejenerasyonu için gerekli olan boşluğa epitel dokunun göç etmesi engellenmiş olur. Bariyer membranların hem sert hem de yumuşak doku hücrelerinin tutunabilmesi için uygun bir yüzeye, gelişmelerini destekleyebilecek ancak mikroorganizmaların geçişine izin vermeyecek uygun gözenekliliğe ve işlevini tamamladıktan sonra ikinci bir cerrahi operasyonla bölgeden alınmasına gerek kalmaması adına kendiliğinden degrede olabilecek bir yapıya sahip olması gerekmektedir. Fiziksel, kimyasal ve mekanik olarak bu özellikleri taşıyabilecek bariyer membranların üretiminde sentetik ve doğal olmak üzere pek çok farklı polimer çeşidi, biyoaktif bileşenler ve antibiyotiklerin farklı kombinasyonları kullanılarak, elektrospinning yöntemiyle nano boyutlu fiberlerden oluşan membran yapıları üretilebilmektedir. Polimerik tabanlı membranların içeriğine eklenen farklı formlarda biyoaktif camlar, trikalsiyum fosfat, hidroksiapatit gibi inorganik seramikler kemik hücrelerinin büyüme ve gelişimlerini uyarmakta ve hızlandırmaktadır. Ayrıca klorheksidin glukonat gibi antimikrobiyal bileşenlerin membranlara eklenmesi veya sonradan yüklenmesi de doku gelişimi sırasında bölgede enfeksiyon oluşumunun önüne geçebilmektedir. Bu çalışmada; sol-jel yöntemiyle 45S5 nano boyutlu biyoaktif camlar üretilmiş, karakterizasyon, biyoaktivite ve hücre canlılığı testleri gerçekleştirilmiştir. Üretilen 45S5 nano boyutlu biyoaktif camlar, poli(vinil alkol) ile klorheksidin glukonat içeren çözeltilere farklı oranlarda eklenerek elektrospining cihazında nanofiber membranların üretim optimizasyon çalışmaları yapılmış, membranların karakterizasyonları, *in vitro* hücre canlılıkları ve *in vitro* antibakteriyal özellikleri test edilmiştir. Nano boyutlu 45S5 biyoaktif cam ve klorheksidin glukonat içeren nanofiber dental membranların klinik uygulamalarda kullanılabilmesi için fiziksel yapısının uygun olduğu, *in vitro* hücre canlılığı testleri sonucunda L929 fibroblast hücreleri üzerinde toksik etkisinin olmadığı ve ayrıca *in vitro* agar disk difüzyon testlerinde *Escherichia coli* ve *Staphylococcus aureus* üzerinde antibakteriyel etkinliğinin olduğu gösterilmiştir.

**Anahtar Kelimeler:** 45S5 Biyoaktif Cam, Dental Membran, Elektrospinning, Klorheksidin, Nanofiber.

## *ORAL PRESENTATION*

# Ehrlich ascites tumor oluşturulan farelerde kan parametreleri üzerine gilaburu'nun etkisi

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**Özet**

Giriş ve Amaç: Dünya Sağlık Teşkilatına göre kanser küresel olarak kardiyovasküler hastalıklardan sonra ikinci en büyük ölüm sebebidir. Kanser organizmada hücrelerin kontrolsüz bir biçimde büyüyüp çoğalması olarak tanımlanan hastalık grubunun genel ismidir. Kanser araştırmalarının sayısı gün geçtikçe artmakta olup bu araştırmalar için deneysel hayvan modelleri özellikle metabolizma cevabi acısından gereklidir. Ehrlich Ascites Tumor (EAT) fare meme adenokarsinomundan spontan olarak izole edildikten sonra transplante edilebilen forma dönüştürülmüş, sıklıkla kullanılan bir deneysel kanser metodudur. Türkiye`de endemik bir takson olarak varlığını sürdüren, antioksidan etkisi bilinen ve çeşitli hastalıkların tedavisinde kullanılan gilaburu bitkisinin (*Viburnum opulus*) tümör oluşumunu yavaşlatma etkisi daha önceki çalışmalarda bildirilmiştir. Mevcut çalışmada gilaburu meyve suyunun kanser modeli oluşturulmuş farelerde bazı kan parametreleri üzerine etkisinin araştırılması amaçlanmıştır.
Gereç ve Yöntem: Çalışmada 21 adet Balb-C ırkı erkek fare kullanıldı. Fareler rastgele üç gruba ayrılarak standart koşullarda ad libitum beslendi. Tüm hayvanlara $1x10^5$ EAT hücresi intraperitoneal (i.p) olarak verildikten sonra birinci gruptaki (konrol grubu) hayvanlara sadece günlük 0,2 ml i.p. PBS (Phosphate Buffered Solutuon) ikinci gruptaki hayvanlara günlük 15mg/kg 5-Fluoro Uracil, üçüncü gruptaki hayvanlara ise günlük 300 ml/kg i.p. gilaburu 9 gün boyunca verildi. 10. Gün tüm hayvanlar kalplerinden kan alındıktan sonra sakrifiye edildi. Kanlarda bazı biyokimyasal parametreler ölçüldü.
Bulgular: Hayvanlardan alınan kan örnekleri incelendiğinde ak kan hücresi, kırmızı kan hücresi, monosit, granülosit sayıları, hematokrit değer ve hemoglobin miktarı bakımından gruplar arasında istatistiksel olarak anlamlı bir farklılığın bulunmadığı, ancak lenfosit sayısı bakımından gilaburu grubunda kanser kontrol grubuna göre anlamlı bir azalmanın olduğu görüldü ($p<0,005$). Sonuç: Gilaburu grubundaki hayvanların lenfosit değerlerinin sağlıklı hayvan referans aralığında oluşu gilaburunun tedavi edici etkisi olabileceğini düşündürmektedir. Çalışmamızın bu alanda yapılacak araştırmalar için bir basamak niteliği taşıyacağını düşünmekteyiz.

**Anahtar Kelimeler:** kanser, Ehrlich ascites tumor, viburnum opulus, lenfosit.

## *ORAL PRESENTATION*

# Ak zambak (*Lilium candidum*) polenlerinin farklı çözgenlerle ekstraksiyonunun antimikrobiyal aktivite üzerine etkisi*

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**Abstract**

Bitkiler; gıda sanayii başta olmak üzere, ilaç, parfüm, kozmetik gibi çeşitli sanayi dallarında hammadde olarak kullanılmaktadır. Bitkilerin üretmiş oldukları sekonder metabolitler, bitkiyi çevresel stres faktörlerine karşı koruma görevi üstlenmektedir. Bu bileşenlerin etkisi ile, antimikrobiyal etki gösterebilmektedir. Literatür incelendiğinde bitki ekstraktlarının ve uçucu yağlarının antimikrobiyal aktivitesini araştıran çalışmaların sayısının her yıl artmakta olduğu görülmektedir.

*Lilium candidum, Liliaceae* familyasına ait geofit, çok yıllık bir bitkidir. Türkiye'nin biyolojik zenginliklerinden birisi olan *Lilium candidum*, "Ak Zambak, Beyaz Zambak, Mis Zambak, Desti Zambağı, Mezarlık Zambağı ve Bey Zambak'' olarak da isimlendirilmektedir. Türkiye coğrafyasında Güneybatı Anadolu Bölgesi, Akdeniz Bölgesi, Aydın, Muğla, Antalya ve çevresinde dağılım göstermektedir. *Lillium candidum*'un halk arasında tedavi amaçlı kremlerin yapılmasında, kökleri ve çiçeklerinin ülser tedavisinde, cilt yanıkları ve kızarıklıklarında ise antienflamatuar olarak kullanıldığı bilinmektedir. Yapılan çalışmalarda, *Lilium candidum'*dan elde edilen ekstraktın antimikrobiyal aktivite gösterdiği belirlenmiştir ve bu özelliği nedeni ile gıda ve/veya gıda katkı maddesi olarak kullanılabileceği ifade edilmektedir.

Bu çalışma kapsamında *Lillium candidum*'un polenlerinin metanol:su (10:0 v/v, 7:3 v/v, 5:5 v/v, 3:7 v/v), aseton:su (10:0 v/v, 7:3 v/v, 5:5 v/v, 3:7 v/v) ve su ekstraksiyonu yapılmıştır. Test mikroorganizması olarak Gram negatif *Escherichia coli* ATCC25922 kullanılmıştır. Antimikrobiyal aktivite, Kirby Bauer (1966)'ın disk difüzyon metodu temel alınarak incelenmiştir. Ekstrakt örnekleri 10 µL ve 20 µL hacimlerde disklere damlatılmış ve inkübasyon sonunda zon çapları ölçülerek antimikrobiyal etki karşılaştırılmıştır. Aseton ekstraktlarının antimikrobiyal etkisi incelendiğinde en olumlu sonuç sırası ile: aseton:su 3:7 (v/v); aseton:su 5:5 (v/v); aseton:su 10:0 (v/v) olarak gözlemlenmiştir. Metanol ekstraktları incelendiğinde en iyi antimikrobiyal etki metanol:su 3:7 (v/v) olarak bulunmuştur. Disklere uygulanan miktarın artması ile antimikrobiyal etkinin arttığı görülmüştür.

**Keywords:** *Lillium candidum,* antimikrobiyal özellik, fonksiyonel gıda, ekstraksiyon.

** Bu çalışma B.U.Ü. Bilimsel Araştırmalar Birimi tarafından FHIZ-2022-869 numaralı proje ile maddi olarak desteklenmiştir.*

## *ORAL PRESENTATION*

# Comparison of antimicrobial activities of endemic *Hypericum malatyanum* and *Hypericum perforatum* species grown in Malatya

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**Abstract**

Plants, which have been used for various purposes since ancient times, have an important place in improving human health. It has been shown in studies that herbal materials contain many phytochemical compounds with strong antioxidant and antimicrobial activities. Natural plants and their essential oils have been studied in many research areas, especially since 1940, in terms of their antimicrobial effects and important results have been achieved. The disadvantages of the use of antibiotics due to the increase in bacterial resistance have further increased the studies on the use of such plants. *Hypericum perforatum*, also known as Kantoron, is one of these medicinal plants, traditionally used and considered a valuable herbal medicine for more than 2000 years. It is known that *H. perforatum* has been used since ancient times, especially for its wound healing and antidepressant properties. With the recent emergence of antimicrobial properties and high antioxidant capacities, it has a very important place in terms of being an alternative in treatment. In this study, the antimicrobial property of *H. perforatum* (yellow kantoron) was determined by comparing it with *Hypericum malatyanum* (Malatya kantoron), an endemic plant native to Malatya. The antimicrobial activity of these plant extracts on bacterial strains such as *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* was investigated by disc diffusion method using Cefadroxil antibiotic discs as a control. As a result, the zone diameters were measured and it was determined that *H. perforatum* showed antimicrobial activity on four bacteria and *H. malatyanum* showed antimicrobial activity on *P. aeruginosa* and *B. subtilis.*

**Keywords:** *Hypericum perforatum, H. malatyanum*, antimicrobial activity.

## *ORAL PRESENTATION*

# Sodium fluoride-induced kidney cell line magnesium administration effect on IL-1β gene expression

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**Abstract**

Since the kidneys are the primary excretion site of orally ingested fluoride, the kidneys are exposed to more flora than other stays organs. In this study, it was aimed to investigate the effect of magnesium application on IL-1β gene expression as a result of the induction of the kidney cell with sodium fluoride (NaF). Kidney cell line was grown in RPMI 1640 medium containing 10% FBS, 1% L-Glutamine (2mM) and 1% penicillin/streptomycin at 37°C, 5% $CO_2$ and 95% humidity. The $IC_{50}$ dose of NaF to be applied to kidney cells for 24 hours was 4250 µM and the proliferative dose of Magnesium was determined as 5 µM by MTT test. The control was divided into 4 groups as NaF ($IC_{50}$), Mg and NaF+Mg. Substances were applied to cells and total mRNA isolated. The resulting mRNA were translated into cDNA. Expression levels of target gene (IL-1β) were determined by RT-qPCR determined method. It was determined that the $IC_{50}$ toxic dose of NaF applied to kidney cells increased approximately 40 times compared to the control gene. As a result, in this study, it was determined that IL-1β gene became more active and increased in NaF-induced cell death, and decreased this effect with Mg administration, bringing it closer to the control level.

**Keywords:** IL-1B, kidney cell, NaF, Mg

## *ORAL PRESENTATION*

# The Effect of Salinity-Resistant Biofilm-Forming *Azotobacter* spp. on Salt Tolerance in Maize

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**Abstract**

Increasing salinity is among the most detrimental threats restricting crop growth and productivity. In recent years, root inoculation with biofilm-forming plant growth-promoting rhizobacteria (PGPR) is of great interest to improve abiotic stress tolerance. This study examined the plant growth-promoting and biofilm-forming potential of *Azotobacter* spp. obtained from maize rhizosphere, tested the salt effect on biofilm and exopolysaccharide production, and evaluated their effects on maize growth at varying levels of NaCl (0, 50, and 150 mM) under greenhouse conditions. The isolates produced varying levels of IAA (from 0.96 to 7.51 µg $mL^{-1}$) and ACC deaminase (from 2.10 to 19.50 µmol α-ketobutyrate $mg^{-1}$ $h^{-1}$). The isolates conferred varying degrees of resistance to different salt concentrations and produced biofilms at different levels. On average, the highest biofilm formation was observed in *A. chroococcum*. Both biofilm and exopolysaccharide production significantly increased at either 150 and 300 mM NaCl in *A. chroococcum* SC8, *A. beijerinckii* SC10, and *A. tropicalis* SC4 ($p<0.05$). These strains also significantly enhanced root depth by 44.1-55.9%, shoot height by 52.3-58.8%, fresh root weight by 42.8-52.4%, and fresh shoot weight by 44.6-53.6% at 150 mM NaCl in comparison with the uninoculated salt-stressed control ($p<0.05$). Among plant growth regulators, higher biofilm formation along with exopolysaccharide production under increasing salinity exhibited better performance on maize growth under salt stress. These respective strains might be promising bioinoculants for the alleviation of salinity stress in field studies.

**Keywords:** Biofilm, Salinity, Stress, Inoculation, Biofertilizer.

## *ORAL PRESENTATION*

# Thermoregulation of Biofilm and Exopolysaccharide Production by *Ensifer* spp.

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**Abstract**

Biofilm formation can be briefly defined as the community of microbial cells enclosed by a self-produced exopolymeric matrix and adhered to a surface. Biofilm mode of growth provides many advantages to biofilm-producing cells such as increased protection against environmental stresses. Biofilm lifestyle also increases the survivability and persistence of cells under adverse conditions, therefore has been considered a selective survival strategy of microbial cells. In the present study, fifteen *Ensifer* isolates were obtained from rhizosphere soils and tested their ability to form biofilms and produce exopolysaccharide (EPS) under different temperatures (16, 20, 24, 30, and 37°C). As a result of maximum likelihood analysis in MEGA11, of fifteen isolates, six were identified as *Ensifer adhaerens*, five as *Ensifer meliloti*, and four as *Ensifer medicae*. Biofilm formation in *Ensifer* spp. was thermoregulated. Thermoregulation of biofilm formation by the tested strains was similar to that of EPS production. The growth rates of the isolates were lower at lower temperatures, but the highest biofilm and EPS production were observed at 20°C in all the strains tested. Although biofilm formation was the greatest at 20°C, EPS production was at a comparable level between 20 and 24°C, which might be related to the sticky properties of EPS rather than its amount. Overall, EPS was the major contributing factor in biofilm formation. This study concluded that biofilm formation and EPS production in the tested strains of *Ensifer* spp. are regulated by temperature and decreased with increasing temperatures.

**Keywords:** Biofilm, Thermoregulation, Temperature, Exopolysaccharides.

## *ORAL PRESENTATION*

# Cytotoxic, Genotoxic and Antigenotoxic Activity of Royal Jelly and Chestnut Honey Mixture

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**Abstract**

Recently, studies on the use of bee products in the treatment of diseases have been increased. Honey, known as the most common bee product and also royal jelly (RJ), honey bee secretion used in the nutrition of larvae and adult queens, separately have many biological activities such as cancer prevention and wound healing. Today it is tried to maximize their activities by using these products in a mixture. The aim of this study is to examine the cytotoxic, genotoxic and antigenotoxic activity of RJ and chestnut honey (CH) together in a mixture.
Neutral red uptake (NRU) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were used to evaluate cytotoxic effects of RJ-CH mixture (1:5 ratio and 0.5, 1, 5, 10, 25, 50% mg/ml concentrations). Also alkaline comet assay was used for assessing geno/antigenotoxic effects. All testes were applied on both healty lung epithelial cells (BEAS-2B) and lung cancer (A549) cells.
According to the NRU and MTT assays, it was concluded that the mixture had no cytotoxic effect on either cell line. As a result of comet test, it was determined that the mixture had a genotoxic effect only at 10% concentration on both cell lines compared to the untreated control group alone. In order to evaluate antigenotoxic activity, cells pretreated with RJ-CH mixture further treated with $H_2O_2$ to cause DNA damage and comet assay was applied. Results showed that RJ-CH mixture repaired DNA damage only at 1% concentration in A549 cells but at 1%, 5% and 25% in BEAS-2B cells.
Based on findings, it was determined that RJ-CH mixture has a strong biological activity. Planning more in vitro and in vivo studies on the biological activity of the mixture and investigating its chemical content will more clearly reveal the effect of the mixture of these two products, both of which have strong activities.

**Keywords:** royal jelly, chestnut honey, cytotoxicity, antigenotoxicity, mixture toxicity.

## *ORAL PRESENTATION*

# Determination of Sun Protection Potential of *Hibiscus rosa-sinensis* as Natural Additive for Cosmetic Industry

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**Abstract**

Harmful rays coming from the sun cause negative effects on the human body. Sunscreens and UV protective products are used to reduce and prevent these negative effects. Plants have been used in medicine and cosmetics for centuries, and have the potential to treat skin diseases. Consumers tend to use sunscreens that contain natural ingredients instead of synthetic sunscreens. In this study, the in vitro SPF values of water extracts from flowers and leaves of *Hibiscus rosa-sinensis* were investigated. In addition, the SPF values of the water extracts and commercially available cream mixtures were also investigated. The SPF values of the extracts were measured at the wavelengths of 290-320 nm of UV-B ultraviolet rays reaching the earth from the sun. SPF values of *H. rosa-sinensis* flower and leaf extracts were determined as 11.80 and 22.13. The leaf extract has the highest SPF value (21.70) in 10 mL concentration among the prepared extract and cream mixtures. Therefore, *H. rosa-sinensis* extracts may have the potential to be used as natural additives in the cosmetic industries.

**Keywords:** Camellia, sun protection factor (SPF), extracts, cream

## *ORAL PRESENTATION*

# Sülfonamid Esaslı İmin Ligantları ve Onların Nitroaromatik Patlayıcılardaki Sensör Özellikleri

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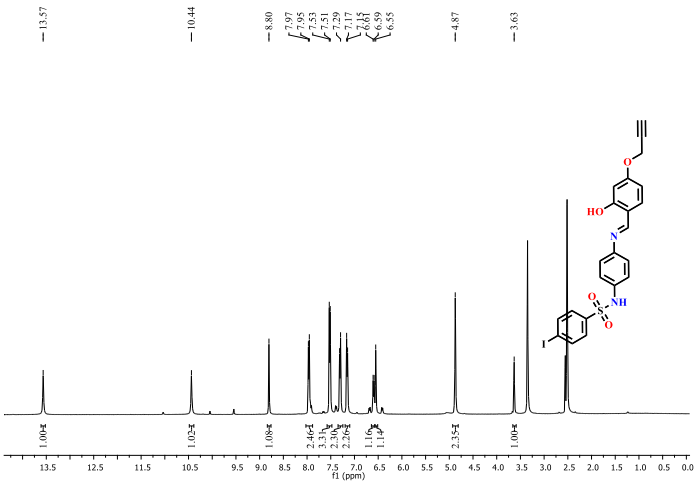
**Özet**

Trinitrotoluen (TNT), dinitrotoluen (DNT), dinitrobenzen (DNB) ve 2,4,6-trinitrofenol (TNP) gibi nitroaromatik bileşikler patlayıcılık özelliğine sahip bileşikler olup patlayıcıların ana bileşenleridir. Nitroaromatik bileşiklerin buhar basınçları, oda sıcaklığında genellikle çok düşüktür. Düşük buhar basınçlı patlayıcıların, uygun kimyasal sensörlerin kullanıldığı füoresans söndürme yöntemleriyle tespit edilmesinin çok etkili olduğu kanıtlanmıştır. Nitroaromatik patlayıcılar, elektronca fakir olmalarından dolayı genellikle iyi bir floresan söndürücü olarak bilinmektedirler. Fotolüminesansa dayalı sensörler, nitroaromatik patlayıcıların tespiti için en etkili araç olarak kabul edilmiştir. Konjuge polimerlerin (CP'ler), elektron donörleri olarak nitroaromatik bileşiklere yüksek bağlanma güçleri nedeniyle nitroaromatik patlayıcılara karşı yüksek hassasiyete ve seçiciliğe sahip oldukları gösterilmiştir[1]. CP'ler, delokalize π* uyarılmış durumları nedeniyle donör yeteneklerini önemli ölçüde artırabilir. Diğer taraftan porfirinler[2] ve oligopirinler[3] gibi konjuge aromatik bileşikler de π-π etkileşimleri yoluyla nitroaromatik bileşiklerle güçlü bir şekilde etkileşime girebildikleri için iyi adaylardır. Ayrıca piren esaslı piridin amidler ve kaliks[4]aren bazlı malzemelerin nitroaromatik patlayıcıların tespiti için sensörler olarak kullanıldığı belirlenmiştir[4]. Mikro gözenekli metal-organik malzemeler (MOF'lar) bu amaç için çekicidir, çünkü prensipte, sadece spesifik analitleri algılar, böylece iyi bir seçicilik sunarlar ve yüksek yoğunlukta analit adsorpsiyon/bağlanma bölgeleri sergileyebilirler, böylece daha düşük tespit limitleri sağlarlar. Yapmış olduğumuz çalışmada sülfonamid esaslı bir seri imin ligandı sentezlenerek yapısal karakterizasyonları yapıldıktan sonra nitroaromatik patlayıcılara karşı kemosensör özellikleri incelenmiştir. Testlerde kullanılan ligantlardan birine ait [1]HNMR spektrumu aşağıdaki şekilde görülmektedir. Yapısal karakterizasyonları yapılmış olan bileşiklerin patlayıcılara karşı sensör özellikleri floresans ve UV-vis spektroskopi metotları ile incelenmiştir.




**Şekil.** N-{4-[(E)-({2-hidroks-4-[(prop-2-yn-1-yl)oksi]fenil}metiliden)amino]fenil}-4-iodobenzen-1-sulfonamit bileşiğine ait 1HNMR spektrumu.

**Anahtar Kelimeler:** Sülfonamid, Nitroaromatik, Sensör

**Kaynakça:**

1. S. W. Thomas, J. P. T. M. Amara, R. E. Bjork and T. M. Swager, Chem. Commun., 2005, 4572-4574.
2. S. Y. Tao, G. T. Li and H. S. Zhu, J. Mater. Chem., 2006, 16, 4521-4528.
3. H. Bai, C. Li and G. Q. Shi, Sens. Actuators, B, 2008, 130, 777-782.
4. S. K. Kim, J. M. Lim, T. Pradhan, H. S. Jung, V. M. Lynch, J. S. Kim, D. Kim and J. L. Sessler, J. Am. Chem. Soc., 2014, 136, 495-505.

## *ORAL PRESENTATION*

# Köpek oral florasındaki *Candida* türlerinin identifikasyonu ve antifungal duyarlılıkları

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**Özet**

Araştırmamızda 60 adet köpek oral svap numunesinde *Candida* türlerinin varlığı araştırılmıştır. Svap numunelerinin Candida kromojenik agara ekimleri yapılarak 30°C'de 48 saat inkübasyona bırakılmıştır. İnkübasyon sonunda 30 (%50) petride üreme tespit edilmiş ve 57 *Candida* kolonisi elde edilmiştir. Kolonilerin Candida kromojenik agarda tek veya çoklu etkenler şeklinde üreme gösterdiği görülmüştür. *Candida* kolonileri, kromojenik agarda oluşturdukları renklere göre tanımlanmış ve 6 farklı *Candida* türü bulunduğu tespit edilmiştir. Elde edilen kolonilerden 16 (%28,1)'sı *Candida krusei*, 10 (%17,5)'u *Candida glabrata*, 10 (%17,5)'u *Candida parapsilosis*, 9 (%15,8)'u *Candida utilis*, 7 (%12,3)'si *Candida tropicalis*, 5 (%8,8)'i *Candida albicans* olarak identifiye edilmiştir. Elde edilen izolatların PCR ile ITS3- ITS4 primerleri kullanılarak moleküler olarak doğrulanmaları yapılmış ve 57 izolatın da *Candida* cinsine ait olduğu belirlenmiştir. *Candida* izolatların antifungal duyarlılıkları disk diffuzyon tekniği ile araştırılmıştır. İzolatların (n=57) Flycytosine %100, Fluconasole %93,4, Miconasole %43,3, Ketoconasole %26,6 dirençli olduğu belirlenirken Nystatine %93,4 oranında duyarlı oldukları tespit edilmiştir. Araştırmamız sonucunda köpek oral florasında patojen Candida türleri tespit edilmiş ve antifungal direnç oluşumları ortaya konmuştur. Bağışıklığın baskılandığı durumlarda köpeklerin oral florasında bulunan Candida türlerinin hem hayvan sağlığı hem insan sağlığı açısından tehdit oluşturabileceği düşünülmektedir.

**Anahtar Kelimeler:** Köpek, Candida, Kromojenik agar, İdentifikasyon, Antifungal.

## *ORAL PRESENTATION*

# Photophysicochemical properties of pyrazoline-fused axial silicon phthalocyanine, both peripheral and non-peripheral zinc phthalocyanines

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**Abstract**

Cancer is still one of the most serious health problems threatening public health today. Although various medical methods such as surgery, chemotherapy and radiation therapy have traditionally been used in the initial diagnosis of cancer, new treatment methods are needed due to the difficulties in treating the more advanced stages of this disease. Photodynamic therapy (PDT) is one of the methods used in the treatment of cancer and is a two-stage treatment that combines light with a photosensitizer (PS) designed to destroy cancer cells after light activation. This treatment method has the advantages of minimal side effects and relatively high therapeutic selectivity compared to chemotherapy and radiotherapy. Phthalocyanine (Pcs) compounds have been preferred as second generation photosensitizers (PSs) for PDT due to their many advantages such as strong efficiency in singlet oxygen production, no toxicity in the dark, and chemical stability. In this study, pyrazoline-fused axial silicon and peripheral and non-peripheral zinc phthalocyanine compounds were synthesized and their photophysicochemical properties were investigated [1,2].

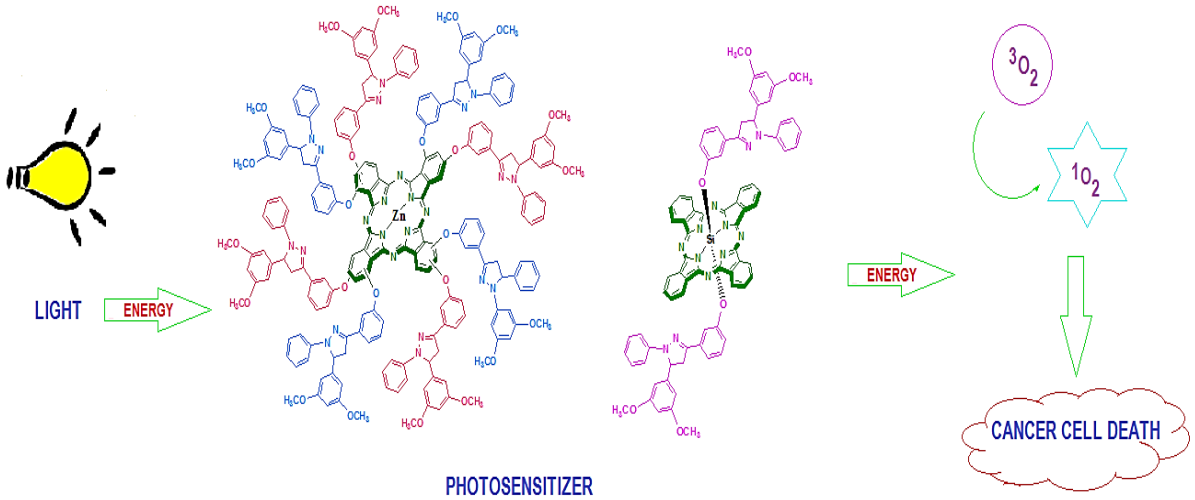


**Figure:** Pyrazoline-fused axial silicon phthalocyanine, both peripheral and non-peripheral zinc phthalocyanines

**Keywords**: Cancer, Phthalocyanine, PDT.

**Acknowledgements:** *This work was supported by Office of Scientific Research Projects of Karadeniz Technical University. Project number:* FDK-2021-9351.

**References:**

[1] H. Yalazan, M. Köç, S. Fandaklı, A. Nas, M. Durmuş, H. Kantekin, Polyhedron 170 (2019) 576–583.
[2] E. Ahmetali, B. Yıldız, E.E. Ahi, M. Durmuş, M. Kasım Şener, Polyhedron 226 (2022) 116111.

## *ORAL PRESENTATION*

# Phosphate recovery from water by $CeO_2$ nanofibers derived from Ce-BTC metal-organic frameworks

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**Abstract**

Phosphate concentrations in water have increased drastically after industrialization. High phosphate concentration causes eutrophication in waters. Eutrophication is known as nutrient pollution in waters. This pollution causes increasing of algal bloom, and algal bloom causes reducing the oxygen concentration in waters, and oxygen concentration decrease causes killing different species living in waters. Agricultural and industrial actions cause high concentration of phosphate discharging to surface and ground waters. The decreasing of the phosphate concentration is important and adsorption is one of the most chosen process for this purpose. The main advantage is that can be applied to low concentration phosphate polluted wastewaters. Different types of adsorbents have been used for phosphate adsorption. The center metal ions in MOF structures are important for phosphate removal. In this study, $CeO_2$ nanofibers derived from Ce-BTC metal-organic frameworks were used as adsorbent. Initial concentration of phosphate, adsorbent dose, temperature, contact time, co-existing ion (NaCl) concentration and pH value of adsorbate solution were investigated to optimize the adsorption process. Langmuir and Freundlich isotherms and pseudo 1$^{st}$ order, pseudo 2$^{nd}$ order and Elovich kinetic models were investigated. The $q_e$ value of $CeO_2$ at 1 mg was determined as 31.52 mg/g. Optimum contact time and pH are determined as 180 min and 9, respectively. Co-existing chloride ions do not affect phosphate adsorption dramatically. As a result of the equilibrium studies, it was determined that the process was compatible with the Freundlich isotherm model ($R^2 \geq 0.99$). The theoretical maximum adsorption capacity ($q_m$) for phosphate removal was calculated as 144.93 mg/g at 308 K. Applied kinetic models were found to be compatible with the adsorption process ($R^2 \geq 0.98$).

**Keywords:** Adsorption, $CeO_2$ nanofibers, Metal-organic frameworks, Phosphate.

## *ORAL PRESENTATION*

# Pros and cons of using micro RNAs as biomarkers

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**Abstract**

In recent years, the number of studies on microRNAs (miRNAs) has increased considerably. miRNAs are short RNA molecules 19 to 25 nucleotides in size that regulate posttranscriptional silencing of target genes (1). It has been revealed that these small structures containing a small number of nucleotides can effect as critical points in organism. Conventional cancer biomarkers are mainly produced by tumor tissues or normal embryo tissues, whereas they are absent or present in small amounts in tissue organ and blood of healthy adults. However, more effective, accurate, specific and sensitive screening biomarkers to perform detective and predictive functions in the treatment of cancer patients are still lacking. Circulating miRNAs in this situation have a particular advantage as a potential clinical application. Almost most of the potential miRNA biomarkers are found in both healthy individuals and cancer patients. It is stated that the difference in expression levels between healthy people and patients is generally quite small. Therefore, it has been reported that studies on comprehensive samples are required to accurately distinguish individuals with cancer from healthy conditions or other benign injuries (2). Given the ability of miRNAs to target multiple genes and key biological mechanisms, these molecules are under intense investigation as both biomarkers and therapeutic agents. Targeting multiple genes simultaneously and miRNA has both advantages and disadvantages for its therapeutic effect. Achieving target organ specificity while limiting off-target effects remains a major challenge in translating new discoveries in research into the successful therapeutic effect of miRNA in the clinic (3). Even though high cost, demand on qualified person to practice wet lab and stability problems count on limitation list of microRNAs, their usage as biomarker opened a new page in epigenetic research with it's superiorities like specificity and ability to design personal and targeted treatment.

**Keywords:** biomarkers, micro RNA, cancer, epigenetic

**References:**

1. Lu TX, Rothenberg ME. MicroRNA. Journal of allergy and clinical immunology. 2018;141(4):1202-7.
2. Wang H, Peng R, Wang J, Qin Z, Xue L. Circulating microRNAs as potential cancer biomarkers: the advantage and disadvantage. Clinical epigenetics. 2018;10(1):1-10.
3. Nana-Sinkam SP, Croce CM. MicroRNA dysregulation in cancer: opportunities for the development of microRNA-based drugs. IDrugs. 2010;13(12):843-6.

## *ORAL PRESENTATION*

# Modified Levan Hydrogels for Bone Tissue Regeneration

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**Abstract**

Defects in bone tissue may occur due to several factors and have ability to regenerate itself after injury however, large defects may not heal itself totally without any additional Support. Thus, new scaffolds and supportive materials are in focus. Despite the effort, researchers could not find any "perfect" bone reconstruction material yet due to lack of bioactivity of synthetic based ones and stability and ethical problems of animal origin ones together with limited biomaterial production and high costs of those who dominate market limits their usage. Biopolymers of microbe origin have superiorities among other biological sources with their easiness in production and downstream at high and controlled yield and low cost. Polymeric hydrogel networks that consist of crosslinked structures with huge water holding capacity, biocompatibility, flexibility, pH and temperature sensitivity and can be proposed as strong candidates for medical applications. Biopolymers are highly preferred in market with their good cell surface interaction, rheological and biological properties. Extreme halophilic environment survivor microorganism, *Halomonas smyrnensis* AAD6T, originated levan, is a β (2 → 6) – linked fructan homopolysaccharide and its potential to be used in different biomaterial studies are investigated previously. In this study, chemically modified levan hydrogels are tested from the perspective of bone regeneration process *in-vitro* with human osteoblast cell line (HOB) as a preliminary scan. Results showed that hydrogels can be proposed as a candidate to be used in bone regeneration with high cell proliferating abilities.

**Keywords:** Bone tissue engineering, levan, hydrogel.

**Acknowledgement**: Special thanks to 119N756 project for financial support.

## *ORAL PRESENTATION*

# Akciğer kanseri tanısında kullanılabilecek aday miRNA'lar

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**Özet**

Kanser küresel boyutta önemli bir halk sağlığı sorunu olup çözümü ve tedavisi için üzerinde araştırmalar hızla devam etmektedir. GLOBOCAN 2020 verilerine göre Asya kıtası her iki cinsiyet ve tüm yaş gruplarında kanser görülme ve buna bağlı ölüm sıralamasında diğer kıtalara göre ilk sırada yer almaktadır. 2020 yılında, Türkiye her yaş ve her cinsiyetten tahmini vaka sayısı 41.264, ölüm sayısı 37.070 kişi ile ilk sırada yer almaktadır. 1993-2020 yılları arasında yayımlanan akciğer kanseri ile ilgili 120 çalışmayı kapsayan bir araştırmada aday genleri belirlemek için yapılan analiz sonucunda TYMS, BRCA1, TP53, CASP3, PLK1, VEGFA, MDM2 ve CCNB1 genlerin biyo belirteç olarak kullanılabileceğini belirtilmiştir. İlaç-gen etkileşimini içeren araştırmalar sonucunda ise özellikle PLK1'in akciğer kanseri hastalarının yaşam süresi bakımından öneme sahip olduğu ve bu hastalık için biyo belirteç olabileceği belirtilmiştir. Bu araştırmanın amacı akciğer kanserinde PLK1'ile ilişkili ve aday biyo belirteç olabilecek miRNA'ları belirleyebilmektir. PLK1'in hedef yolakları arasında RNA ile gen susturulması, hücre döngüsü ve mitoz bölünme yer almaktadır. Kanserin başlamasında ve ilerlemesinde özellikle genlerin ifadesinin düzenlenmesinde rol oynayan miRNA'lar tümör supressör ve onkogen olarak görev alabilmektedir. PLK1 ile ilişkili miRNA'lar; miRSystem, miRDB ve Firefly Discovery Engine veri tabanlarından taranmış olup hsa-miR-296-5p ve hsa-miR-509-3-5p'nin bu veri tabanlarında ortak olarak yer aldığı bulundu. Akciğer kanseri tanısında biyo belirteç olarak önerilen PLK1 geninin yanı sıra belirlenen hsa-miR-296-5p ve hsa-miR-509-3-5p'nin de tanıyı belirlemeye yardımcı biyo belirteçler olarak kullanılmaya aday olabileceği görüldü. Hastalığın seyri ve tedavi cevabının olumlu seyretmesi bakımından belirlenecek olan yeni belirteçler için bu alanda daha fazla çalışma yapılmasının gerekli olduğu görülmüştür.

**Anahtar Kelimeler:** Akciğer kanseri, PLK1 geni, miRNA.

## *ORAL PRESENTATION*

# Comparison of phenolic compositions and beneficial bioactivities of pinecone syrups

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**Abstract**

In recent years, a great deal of research has been conducted on various fruits, vegetables, and spices to identify new ingredients with functional properties. In addition to these edible resources, the evaluation of waste products such as pine tree cones as a source of bioactive molecules is important for the effective use of natural resources. In different regions of our country, traditional pinecone syrup is made from pinecones and used by the people in local treatment.
The aim of this study is to reveal the potential for use for pharmaceutical purposes by determining the bioactive properties of syrups obtained by traditional method from cones belonging to different pine species.
For this purpose, pinecones from different pine species (scotch pine (*Pinus sylvestris*), black pine (*Pinus nigra*), and Turkish red pine (*Pinus brutia*)) grown in Türkiye were collected and pinecone syrups were prepared. Phenolic compounds of these syrups were determined by LC-MS/MS. In addition, antioxidant activity with ABTS method, antidiabetic activity with alpha-glucosidase enzyme inhibition and antimicrobial activity with disc diffusion method were investigated. Although the amounts vary, the same phenolic compounds were found in all three pinecone syrups. Especially gallic acid, cinnamic acid and gentisic acid have higher amounts than other compounds. ABTS•$^{+}$ radical scavenging antioxidant activities of pinecone syrups: scotspine>blackpine>Turkish redpine. $SC_{50}$ values were 0.1373, 0.1899, 0.1949 mg/mL, respectively. Antidiabetic activities by inhibition of alpha-glucosidase enzyme: blackpine>Turkish redpine>scotspine. $IC_{50}$ values were 0.885, 1.173, 1.561, mg/mL, respectively.

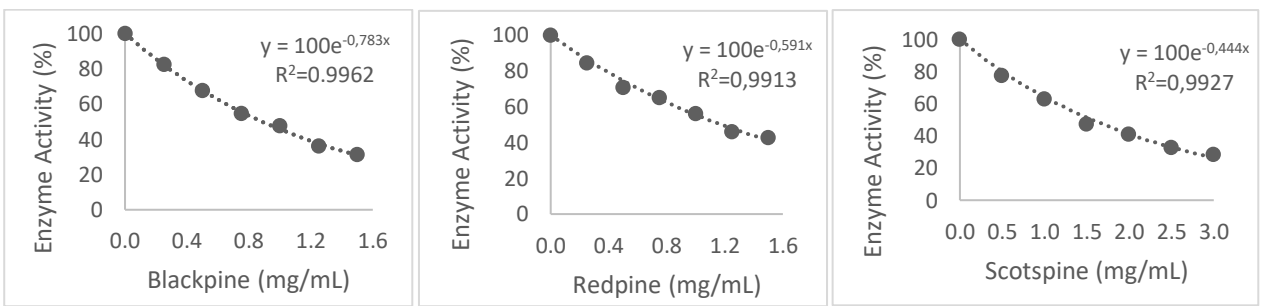


Figure 1.Enzyme-concentration graph of the pinecone syrups studied by the α-glycosidase inhibition method.

Redpine sample showed against *Escherichia coli*, and blackpine sample showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*. Scotspine sample did not show any antimicrobial activity against the microorganisms used in the study.

*This study was supported by Gümüşhane University Scientific Research Projects Coordination Unit (Project no. 21.A0114.01.01).

**Keywords:** Pinecone syrup, bioactivity, phenolics, LC-MS/MS.

## *ORAL PRESENTATION*

# Triazol-oksim bileşiklerinin sentezi ve biyolojik özelliklerinin incelenmesi

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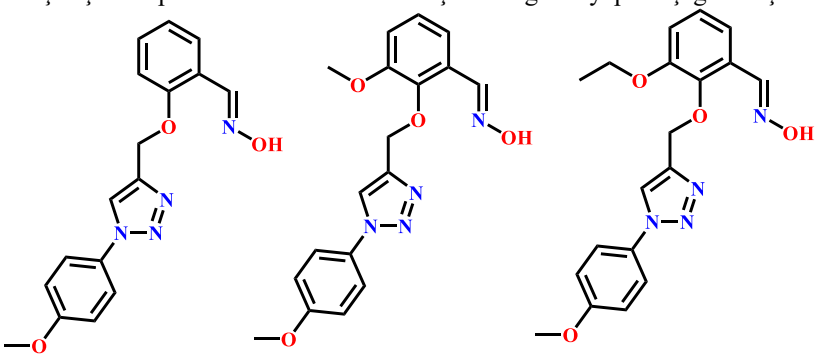
**Özet**

Triazoller, çok sayıda biyolojik aktivitesi nedeniyle heterosiklik kimyada benzersiz bir konuma sahip önemli bir organik bileşik sınıfını oluşturular. 1,2,4-triazol ve 1,2,3-triazol olmak üzere iki izomerik formda bulunur ve birçok tıbbi bileşiğin tasarımı ve sentezi için çekirdek molekül olarak kullanılır. 1,2,4-Triazol, analjezik, antiseptik, antimikrobiyal, antioksidan , anti- üreaz , anti-inflamatuar, diüretikler, antikanser, antikonvülsan, antidiyabetik ve antimigren ajanları gibi terapötik açıdan ilginç ilaç adaylarının geniş spektrumuna sahiptir. Oksim bileşikleri yapısında bulunan -C=N-OH grubu sayesinde çeşitli biyolojik aktivitelere sahip oldukları bilinmektedir. Sunmuş olduğumuz çalışma kapsamında biyoaktif özelliklere sahip oksim ve triazol gruplarınını tek bir iskelette birleştirildiği içeren yeni organik bileşiklerin sentezlenmesi planlanmıştır.

Çalışma kapsamında sentezlenecek bileşiklerin molekül yapıları $^{1}H/^{13}C$ NMR, FT-IR ve diğer enstrümental yöntemler ile karakterize edilmiştir. Ayrıca sentezlenen bazı bileşikleri kristal yapıları tek kristal X-ışını kırınım çalışmaları ile belirlenmiştir. Son olarak yapısal karakterizasyonları tamamlanan bileşiklerin DNA bağlanma, kolin esteraz inhibitör özellikleri araştırılmıştır. Çalışma kapsamında sentezlenen bileşiklerin genel yapısı aşağıdaki şekilde verilmiştir.



**Şekil**: Sentezlenen triazol-oksim bileşiklerinin yapısı

**Anahtar Kelimeler:** Triazol-oksim, biyolojik aktivite, DNA bağlanma, kolin esteraz inhibitör.

## *ORAL PRESENTATION*

# Phenolic compositions, mineral contents, and antidiabetic activities of black locust (*Robinia pseudoacacia* L.) and bristly locust (*Robinia hispida* L.) leaves.

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**Abstract**

Today, the incidence of lifestyle diseases is increasing due to improper nutrition. There are bioactive compounds named polyphenols in plants that effectively prevent these diseases without harmful side effects. *Robinia pseudoacacia* (white-flowered) and *Robinia hispida* (pink-flowered) are known as the thorn tree. Their flowers are excellent sources of polyphenols. In the current literature, there is no study showing the comparison of the phenolic compounds found in the leaves of these plants.

In this study, phenolic compounds were determined by LC-MS/MS in the leaf extracts of these two plant species, and the mineral content was examined by ICP-MS. In addition, antidiabetic activity with alpha-glucosidase enzyme inhibition in leaf extracts has been investigated. The phenolic component types and amounts of extracts differ in different types of leaves. Pelargonidin-HCl, rutin, quercetin-3-beta D-glycoside, and quercetin dihydrate, were high in both plant leaves. Apigenin was found in high amounts (3895.69 ppm) only in *R. hispida* leaf. In the mineral analysis, potassium element content was high in both *R. pseudoacacia* (15867.93mg/kg) and *R. hispida* (12886.78mg/kg) leaves. It is followed by calcium and magnesium elements respectively. % activity-[extract] graphs of *R. pseudoacacia* and *R. hispida* leaf extracts showing similar α-glucosidase enzyme inhibitions are given in Figure 1. $IC_{50}$ values were 1.409 and 1.351 µg/mL, respectively.

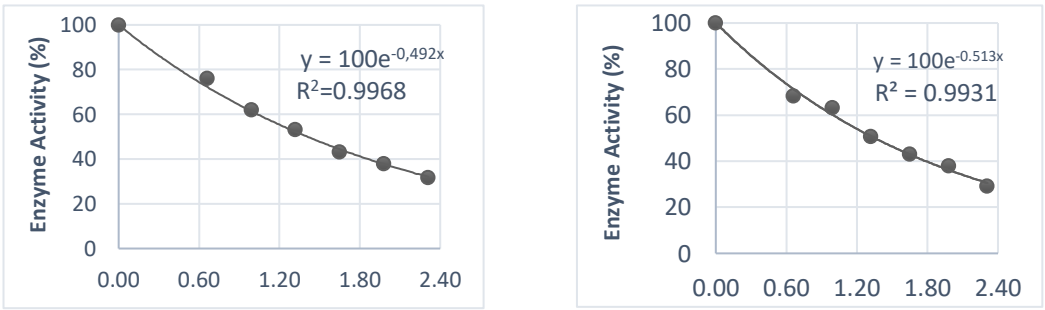


Figure 1. Graphs of *R. pseudoacacia* and *R. hispida* leaf extracts, examined by the enzyme activity method α-glucosidase.

As a result, leaf extracts belonging to these species, which grow naturally in our country, have the potential to be used for pharmaceutical purposes as herbal drugs. Study data have scientifically demonstrated the effectiveness of these plant leaves in the treatment of diabetes, as well as being rich in bioactive components and mineral content.

*This study was supported by Gümüşhane University Scientific Research Projects Coordination Unit (Project no. 21.E0102.07.01).

**Keywords:** Phenolics, flavonoids, LC-MS/MS, mineral content, alpha-glucosidase.

## *ORAL PRESENTATION*

# Preparation of a Molecular Imprinting Technology Based Chronoimpedimetric Urea Sensor

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**Abstract**

Urea[$CO(NH_2)_2$] is a nitrogen-containing organic compound found in body fluids. $NH_3$ formed as a result of metabolic reactions is converted to urea in the liver, less frequently in the kidneys, by the urea cycle. For this reason, the amount of urea in body fluids such as blood and urine can be used as an indicator of kidney and liver dysfunction. Increased urea concentration (normal level in serum is 8-20 mg/dL) can cause kidney failure, urinary tract obstruction and conditions such as gastrointestinal bleeding, while decreased urea concentration can cause liver failure and nephritic syndrome. For the determination of urea, methods such as spectrophotometry, gas chromatography and calorimetry are used. However, these traditional methods have disadvantages such as requiring sample preparation, not being convenient for online monitoring, and taking time. For this reason, it is necessary to develop new methods for faster and more accurate estimation of urea in desired samples. Biosensors can be an alternative to these methods due to their simplicity, low cost and high sensitivity. In this study, a molecularly imprinted polymer-based sensor was prepared for the determination of urea by screen-printed electrodes(SPE). For this aminophenyl boronic acid was chosen as monomer and a specific sensor for urea was designed by using fullerene as nanomaterial by electropolymerization method. In addition, the operability of the system was demonstrated with a non-imprinted(NIP) sensor prepared without target molecule urea. SEM and XPS devices were used for the characterization. The response of the MIP sensor to Tau protein was monitored by the chronoimpedance and the response time was found to be 200 seconds. When the MIP and NIP sensor chronoimpedance results were compared, it was seen that the NIP sensor did not respond to urea. As a result, a selective, sensitive and short response time sensor system was prepared for urea determination.

**Keywords:** Chronoimpedance, Molecular Imprinting Technology, Urea, Sensor,

## *ORAL PRESENTATION*

# Effect of nickel oxide and nickel oxide nanoparticles on lung tissue in rats

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**Abstract**

Nanoparticles (NPs) have important usage international attention due to their widespread advantages ranging from aerospace engineering and nanoelectronics to environmental remediation and medical healthcare. Nickel (II) oxide (NiO) is notable as being the only well-characterized oxide compound of nickel. NiO NPs are used in different applications such as catalysts, light-emitting diodes, lithium-ion batteries, solar cells, electrochemical sensors and biosensors. Such widespread use of NiO and its nanoparticles (NP) increases the risk of oxidative damage. Superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and, Glutathione-S-transferase (GST) are intracellular antioxidant compounds that prevent the formation and damage of reactive oxygen species in the body. This experimental study was carried out with the approval of the Animal Experiments Local Ethics Committee, by applying appropriate protocols within ethical standards for the use of laboratory animals (G.Ü.ET-19.025). In this study, 42 male Wistar rats were used. Experimental animals were divided into 7 groups including control, NiO-orally, NiO-intraperitoneal, NiO-intravenous, NiO-NP-orally, NiO-NP-intraperitoneal and, NiO-NP-intravenous. The study results showed that NiO-orally, NiO-intraperitoneal, NiO-intravenous, NiO-NP-orally, NiO-NP-intraperitoneal and, NiO-NP-intravenous groups caused increased the levels of MDA while a decrease in SOD, CAT, GPx and GST activities were observed in comparison with the control group rats. When the MDA level was compared with the control group and other groups, it was observed that there was a significant increase in NpIV treated group. Similarly, we showed the highest decrease in NpIV treated group.

**Keywords:** Nickel oxide, nanoparticle, lung, rat, oxidative stress.

## *ORAL PRESENTATION*

# Evaluation of Apoptosis After Treatment of Vincristine in AGS Cancer Cells Under Hypoxic Conditions

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**Abstract**

Apoptosis plays an essential role as death-inducing mechanisms. Vincristine (VCR) is a chemotherapeutic drug that binds tubulin dimers and induces apoptosis in cancer cells. However, Vincristine can also protect cancer cells from apoptosis. Here, we aimed to show the effect of VCR on apoptosis in gastric cancer AGS cells under hypoxic conditions.
In this study, the effect of VCR on mRNA levels of apoptosis-related Bax and Bcl-2 has been observed in AGS cells by using reverse transcription real-time polymerase chain reaction (qRT-PCR). Optimum VCR concentration was selected by cell viability analysis.
The optimum VCR concentration was selected as 300 nM for 48 hours period. Bax and Bcl-2 mRNA expressions increased significantly after VCR application to AGS cells under hypoxic conditions compared to the control cells. Also, when it comes to hypoxic conditions, Bax and Bcl-2 gene expressions significantly increased compared to the VCR treatment under hypoxic conditions.
In light of these results, in the presence of VCR, apoptosis seems to increased in AGS cells under hypoxic conditions.

**Keywords:** Gastric Cancer, Vincristine, Apoptosis, Angiogenesis, Hypoxia.

## *ORAL PRESENTATION*

# Antifungal effect of salicylic acid on *Fusarium oxysporum* and *Alternaria alternata in vitro*

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**Abstract**

Salicylic acid, a crucial plant hormone, is well recognized for facilitating host reactions in response to pathogen invasion. Its function in the initiation of plant defense is well known. When bacteria, fungi, insects, or viruses invade a pathosystem, the levels of salicylic acid are known to rise, and exogenous salicylic acid treatment strengthens the host's defense mechanism. *In vitro* research was done on salicylic acid's antifungal effects on two phytopathogenic fungi: *Fusarium oxysporum* and *Alternaria alternata*. The fungal growth inhibitory effect of salicylic acid was assessed using the agar dilution method. *F. oxysporum* and *A. alternata* were inoculated in Sabouraud Dextrose Agar. The salicylic acid was added to the agar plates at the concentrations of 0, 31.25, 62.50, 125, 250, 500, and 1000 ppm and then kept at 27 °C for eight days. The results showed that salicylic acid had no antifungal effect against both investigated fungi below the concentration of 125 ppm. Above the concentration of 125 ppm, the antifungal effect on both fungi increased gradually, but the results showed that salicylic acid was more effective on the growth of *A. alternata.*

**Keywords:** Salicylic acid, Antifungal effect, *Alternaria alternata, Fusarium oxysporum.*

## *ORAL PRESENTATION*

# Synthesis, Characterization and Evaluation of Adsorption Property of Zn-Al Layered Double Hydroxide

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**Abstract**

Industrial activities, which increased rapidly after the industrial revolution, make life easier in terms of technology and science. However, the increase in industrial wastes poses a great threat to living things, water resources and the environment. In the textile industry, which has an important place in the industry, water consumption is very much and materials with many complex structures such as dyestuffs and bleaches are used. For this reason, studies on the treatment of wastewater in the textile industry are important. It is very important for the environment and human health to remove synthetic dyes, especially "azo dyes", in textile wastewater by different methods, as they have potential carcinogenic effects and adversely affect photosynthetic activity in aquatic life as they prevent light penetration. Although many methods such as adsorption, coagulation, chemical, physical, photolysis and advanced oxidation techniques are used for the removal of dyestuffs from wastewater, adsorption is one of the most widely used methods.

In this study, the synthesis and characterization of Zn-Al Layered Double Hydroxide (Zn-Al LDH) as an adsorbent for the adsorption of Reactive Sumifix Supra Yellow dyestuff from wastewater was carried out. For this purpose; first of all, the optimum adsorption conditions (adsorption time, ambient pH, adsorbent amount, pollution concentration and adsoption volume) were determined. It was determined that Zn-Al LDH adsorbed 95% of sumifix supra yellow dyestuff at the end of 60 min adsorption time under optimum experimental conditions.

**Keywords:** Layered double hydroxide, waste water treatment, adsorption, sumifix supra yellow.

## *ORAL PRESENTATION*

# Camera trapping survey of mammals in Çığlıkara Nature Reserve, Turkey

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**Abstract**

This study is based on the mammal records of camera trapping study in Antalya-Çığlıkara Nature Reserve (15654 ha.), conducted between 2019-2021. It was realized by placing camera traps belonging to the General Directorate of Nature Conservation and National Parks with the opportunist method. In the obtained camera trap images, 9 mammal species wolf (*Canis lupus* L.), golden jackal (*Canis aureus* L.), fox (*Vulpes vulpes* L.), lynx (*Lynx lynx* L.), badger (*Meles meles* L.), stone marten (*Martes foina* Erxleben), wild boar (*Sus scrofa* L.), european hare (*Lepus europaeus* Pallas) and squirrel (*Sciurus anomalus* Gmelin) have been identified. *Lynx lynx* has the highest detection rate among the species in Antalya-Çığlıkara Nature Reserve.

**Keywords:** Çığlıkara nature reserve, mammal species richness, camera trapping.

## *ORAL PRESENTATION*

# Antihipertansif ilaç etken maddesi olan amlodipine'nin genotoksik etkilerinin kromozomal anormallik testi ile incelenmesi

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**Özet**

Küresel halk sağlığı sorunları arasında ilk sıralarda yer alan hipertansiyon, kan basıncının uzun süre yüksek seyretmesi ile kendini gösteren kardiyovasküler sistem hastalığıdır. Günümüz yaşam koşullarına bağlı olarak prevalansı (hasta sayısı) gittikçe artmakta, progresif (ilerleyen), komplikasyonları olan ve kontrolü için uzun süre ilaç kullanılması gereken kronik bir hastalıktır. Amlodipine, kalsiyum kanal brokerları grubundan olup, hipertansiyonun kontrolünde sıklıkla kullanılan ilaç etken maddelerinden bir tanesidir. Genotoksisite, bir ajanın genetik materyalde meydana getirdiği değişiklikleri inceler. Bu çalışmanın amacı, ülkemizde de kullanılan Amilodipine'nin olası genotoksik etkilerinin, geçerliliği uluslararası boyutta kabul gören kromozom anormallikleri testi ile insan periferal lenfositlerinde *in vitro* olarak incelenmesi, ayrıca mitotik indeks belirlenerek söz konusu ilaç etken maddesinin sitotoksik aktivitesinin belirlenmesidir. Bu amaçla 23-27 yaş arası sağlıklı, sigara, alkol, ilaç kullanmayan iki bireyden alınan kan 72 saat süreyle kültüre edilmiştir. Kültüre edilmiş lenfositlere 24 ve 48 saat süreyle, farklı konsantrasyonlarda Amilodipine (6,25; 3,13; 1,56; 0,78 ve 0,39 µg/mL) ile muamele edilmiştir. Ayrıca bir negatif, bir çözücü kontrol (DMSO 4,81 µl/ml) ve bir de pozitif kontrol (Mitomisin-C=MMC, 0,20 µg/mL) kullanılmıştır. Yapılan incelemeler sonucunda Amlodipine'nin 24 ve 48 saatlik muamele sürelerinin her ikisinde de kontrol ve çözücü kontrole kıyasla kromozomal anormallik frekansında anlamlı bir değişikliğe sebep olmadığı görülmüştür. Mitotik indeks değerlendirmesinde ise her iki muamele süresinde mitotik indeksin kontrole göre en yüksek iki konsantrasyonda anlamlı düşüşe neden olduğu tespit edilmiştir. Söz konusu düşüş çözücü kontrole kıyasla ise yine her iki uygulama süresinde sadece en yüksek konsantrasyonda önemlidir. Elde edilen veriler, Amlodipine'nin insan periferal lenfositlerinde önemli bir genotoksik etki sergilemediğini göstermektedir. Ancak bu sonuçlar farklı hücre tipleri ve farklı genotoksisite testleri ile desteklenmelidir.

**Anahtar Kelimeler:** Hipertansiyon, Antihipertansif, Genotoksisite, Amlodipine, Kromozomal anormallik.

**Teşekkür**: Bu çalışma Amasya Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimince Desteklenmiştir. Proje Numarası: FMB-BAP 21-0439.

## *ORAL PRESENTATION*

# Antifungal activities of biocompatible cryogels as potential wound dressing materials

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## Abstract

The fungus can act as a pathogen and disruptive effect the immune system. Therefore, materials with antifungal properties can provide an essential deterrent against fungal infections. Saccharomyces cerevisiae, known as sourdough and widely used in the bakery industry, is also used as a probiotic for treating gastroenteritis and regulating the endogenous flora and immune system. However, in recent years, there has been an increase in cases of fungemia caused by *S.cerevisiae* and its subspecies *S.boulardii*, and the use of probiotics in immunocompromised individuals is emphasized that it should be applied with caution in patients with the disease and low weight infants. Cryo-hydrogels, called cryogels, are mainly used in biotechnological fields due to their fast mass flow properties supported by the elastic morphological structures combined with mechanical and chemical stability. Also, these polymers are a valuable option for wound-healing materials. In this study, HEMA-based cryogels (PHEMA, PHEMA-NVP, and PHEMA-NVF) were prepared to investigate their anti-fungal activities on the eukaryotic yeast cell model *Saccharomyces cerevisiae*. Among these, PHEMA-NVF was found to be much more effective. So, PHEMA-NVF can be used as wound dressing material in future studies.

**Keywords:** Cryogels, fungemia, anti-fungal activity, *Saccharomyces cerevisiae*

## *ORAL PRESENTATION*

# Adsorption of fluoroquinolone antibiotic molecule on the surface of X-doped $C_{60}$ fullerenes (X=Mg, Ca, Fe, and Zn)

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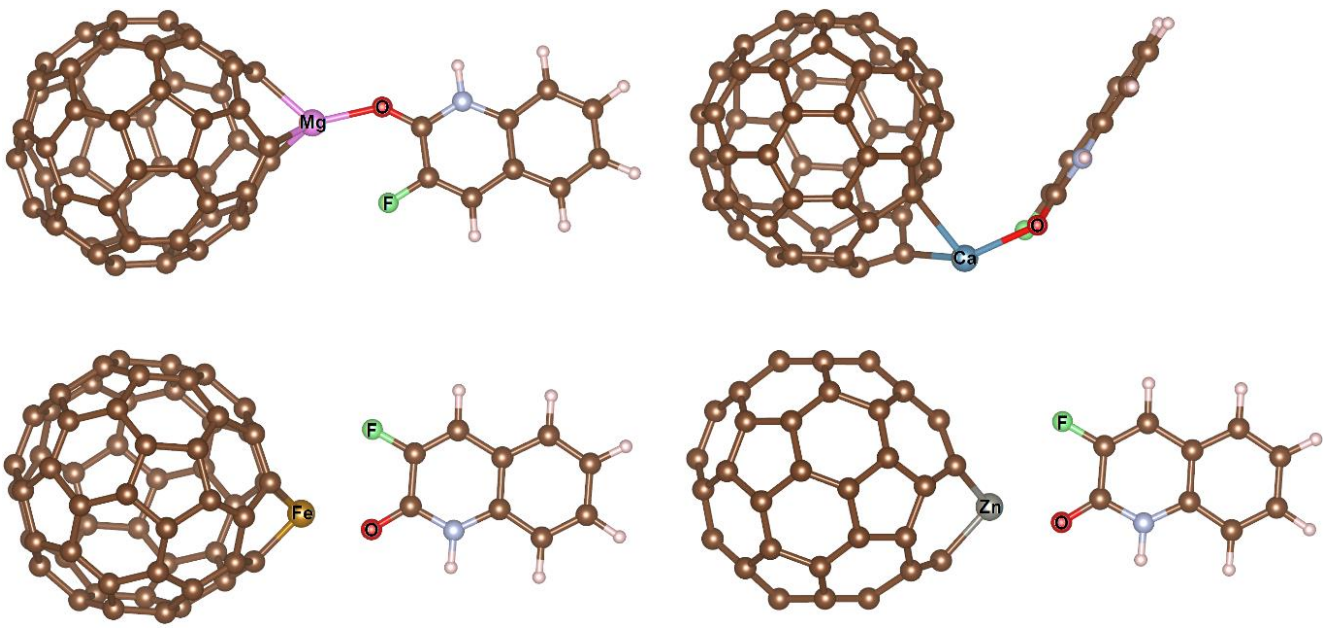
**Abstract**

The electronic sensitivity of magnesium (Mg-), calcium (Ca-), iron (Fe-) and zinc (Zn-) doped $C_{60}$ fullerene with fluoroquinolone (FQ) antibiotic molecule were investigated using density functional theory. Theoretical calculations show that the doping of Mg, Ca, Fe and Zn atoms increases chemical sensitivity. FQ antibiotic molecule was found to be absorbed physically on doped fullerenes. Besides, significant HOMO destabilization in Ca-doped fullerene through the FQ adsorption increases the electrical conductivity of Ca-doped fullerene while generating electrical signals and reduces its $E_g$ from 2.06 to 1.43 eV. The DOS, NCI-RDG, WBI, FBO analyses were conducted to investigate the nature of interactions between antibiotic molecule and doped fullerenes. Results from the present study indicate that Ca-doped fullerene may be proposed as a promising biosensor.

**Keywords:** $C_{60}$, Fluoroquinolone, Doping, Biosensor, DFT.



## *ORAL PRESENTATION*

# Effect of Sulfur Doping on Electronic Structure of ZnO Nanoparticles

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**Abstract**

ZnO nanoparticles (NPs) have attracted tremendous interest in much scientific research due to their excellent properties to use in various biomedical in biomedical, optical, and electronic fields. The doping process are crucial, and the interactions were found to be responsible for the significant changes in the chemical and physical characteristics with doping. In this context, the changes in electronic structure of ZnO NPs have been investigated with Sulfur doping. The structural properties, density of states, HOMO, LUMO and energy gap are examined, and the obtained results are compared with undoped ZnO NPs and then are discussed in detail.

**Keywords:** Doping, Electronic Structure, ZnO Nanoparticles, DFTB.

## *ORAL PRESENTATION*

# Kağıt Kökenli Gıda Ambalajlarında Element İçeriği

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**Özet**

Bu çalışmada gıda maddelerinde ambalaj ürünü olarak kullanılan karton kökenli ambalaj malzemelerinde bulunması şüphe duyulan ağır metallerin, İndüktif Eşleşmiş Plazma Kütle Spektrometre (ICP MS) cihazı kullanılarak metot validasyon çalışmaları ile güvenilir analiz sonuçları ele alındı. Gıda ambalajlarında element tayini için matrikse spike yöntemi ile kontrol çalışmaları ve sertifikalı referans madde kullanılarak geri kazanım çalışmaları yapıldı. Amacımız metot validasyon güvenirliliği ile çalışma sırasında karşılaşılan sorunlar ve çözümleri ele alınarak literatüre katkı sunmaktı. Gıda ambalajı olarak kullanılan karton kökenli ambalaj ürünlerine migrasyon işlemi uygulanmadan bünyelerinde barındırdıklarından şüphe duyulan As, Pb, Cd, Cr ve Hg gibi ağır metallerin konsantrasyonlarının metot validasyon çalışmaları ile belirlenmesi için matrikse (çalışmada kullanılan karton ambalaj) tespit edilmesi hedeflenen sertifikalı standart maddeden örneklerde bulunması tahmin edilen düşük, orta ve yüksek konsantrasyonları temsil edecek şekilde spike yapıldı. Daha sonra ön hazırlık ve uygulanan metotların güvenirliğinin test edilmesi için matriks üzerine sertifikalı referans madde spike yapıldı. Elde edilen veriler doğrultusunda kullanılan metotların ve ön hazırlık işlemlerinin doğruluk çalışmaları gerçekleştirildi. Gıda ambalajı olarak kullanılan ürünlerin ICP MS cihazı kullanılarak ağır metal tayini sırasında numune bazlı kontaminasyon, torch, nebulizer tıkanması, cihaz kalibrasyon parametrelerinde belirsizlik gibi numune sonucumuza etki eden faktörler belirlendi. Mikrodalga cihazı kullanılarak numunelere uygulanan parçalama işleminin güç ve büyük moleküllü olan veya ani reaksiyon verme ihtimali bulunan organik bileşikler içeren örneklere uygulanarak prosedürün doğruluk çalışmaları yapıldı.

**Anahtar Kelimeler:** Element analizi, ICP MS, Metot validasyonu, Gıda ambalajları.

## *ORAL PRESENTATION*

# The New Method Development For The Determination of Fluoroquinolones by HPLC

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**Abstract**

Fluoroquinolones are the main antibacterial drugs that are used against Gram-positive, Gram-negative, and atypical bacteria. They are divided into four generations. Monitoring concentrations of antibacterial agents provides effective therapy and prevents the increase of bacterial resistance to antibiotics. Also, it will give preservative treatment approach against the probably toxicological effects. The aim of the study is finding a new, easy method that allow the separation and simultaneous determination of four fluoroquinolones; ciprofloxacin, ofloxacin, enrofloxacin and moxifloxacin. The separation of fluoroquinolones studies in aqueous media are tried in many methods in which samples placed as single or their binary mixtures. In our study we aimed to find the most effective separation technique for mentioned above four fluoroquinolones. Different acidic and basic mobile phases, buffer solutions and elution types were tried. The most effective and selective method was performed on XTerra, $C_{18}$ (100 x 4.6 mm, particle size 3.5 µm) analytical column and methanol : borate buffer (pH=9.1, 100 mM) (50:50, v/v) containing mobile phase at 0.6 mL/min of flow rate. Generally, fluoroquinolones were determined by using HPLC-Fluorescence detector. In our study detection was realized by DAD detector at 280 nm. Any interference peaks were not observed and obtained good resolution. In the study, separation, and simultaneous determination of ciprofloxacin, ofloxacin, enrofloxacin and moxifloxacin separation were realized. The calibration curves were linear over the concentration ranges of 0.25-10 µg/ml for studied quinolones. Validation studies of the developed method were also studied for these quinolones. This developed method describes a simple, sensitive, and practical HPLC-DAD method which permits to simultaneous determination of ciprofloxacin, ofloxacin, enrofloxacin and moxifloxacin in a mixture. We think that the method can also be applied in biological fluids.

**Keywords:** Fluoroquinolones, HPLC-DAD, ciprofloxacin, ofloxacin, enrofloxacin, moxifloxacin.

## *ORAL PRESENTATION*

# İçme ve Kullanma Sularında Uçucu Organik Bileşiklerin (UOB) Analiz Yöntemi ve Metot Validasyonu

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**Özet**

Uçucu organik bileşikler (UOB) önemli çevre sorunlarından biridir. UOB'ler, mevcut çevresel koşullar altında oldukça uçucu yapıya sahiptirler. Kimyasal özellikleri ile (suda düşük çözünürlük, yüksek lipid çözünürlüğü, yarı uçuculuk ve biyolojik zarlardan geçme ve yağ dokularında birikme yeteneğine sahiptir.) Bu bileşikler, çevrede büyük bir endişe kaynağı haline gelmektedir. Sularda UOB'lerin mevcudiyeti, yüksek miktarda organik madde içeren doğal suların klorlanması sırasında bu bileşiklerin, özellikle THM'lerin küçük miktarlarda üretildiğini keşfetmesinden bu yana, kamuoyunun ilgisini çekmeye ve aktif araştırmaların konusu olmaya devam etmektedir. UOB'lerin ana alt grupları halojenli organikler, monosiklik aromatik hidrokarbonlar, organik sülfitler ve sülfoksitler, BTEX'ler, THM'ler, Aseton ve Esterleri içerir. UOB'ler biyojenik veya antropojenik kökenli olabilir. Antropojenik kaynaklar, doğal kaynaklardan daha fazla endişe yaratır. Su ortamına yönelik başlıca antropojenik VOC kaynakları, boyalar ve kaplamalar, benzin, solventler, endüstriyel ve kentsel atık sular, kentsel ve kırsal akışlar ve atmosferik birikimlerdir. Hava kütlesi taşınımları ile gerçekleşen olaylar ile taşınabilirler. Çevrede birikip kalıcı olmasının yanı sıra, UOB'ler artan sera etkilerine ve buna bağlı ozon incelmesine katkıda bulunur. Toplam çözünmüş organik karbonun ~%10'u nispeten kirlenmemiş sulardaki UOB'lerdir ve konsantrasyonları farklı antropojenik kaynaklardan gelen ham sularda çok daha yüksektir. UOB'ler suya alındıktan sonra fizikokimyasal özelliklerinden etkilenen şekillerde davranacaktır (örneğin, seyreltme, toksikolojik değişiklikler ve biyolojik bozunma, sudaki stabilite ve konsantrasyonlarda önemli roller oynayacaktır). Ayrıca halojenli UOB'ler için halojenlerin ikamesinin sudaki kimyasal ve toksikolojik özellikleri etkilediği bilinmektedir. Bu nedenle, UOB'lerin oluşumu ve davranışı hakkında derinlemesine bilgi edinmek için çevresel arka plan seviyelerinde (µg/L ve ng/L) analitik teknikler çok önemli hale gelmektedir. Bu bağlamda insani tüketim amaçlı kullanılan içme ve kullanma sularında uçucu organik bileşiklerin analizlerinde kullanılmak üzere ön hazırlık işlemleri ve analiz yöntemlerinin metot validasyon çalışmaları yapıldı.

**Anahtar Kelimeler:** Uçucu organik bileşikler, GC MS, Metot validasyonu, İçme suları.

## *ORAL PRESENTATION*

# Synthesis of some novel Schiff base derivative 5-substituted-4-amino 1,2,4-triazole 3-one compounds with potential biological activity

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**Abstract**

In this study, 5 new Schiff base compounds containing triazole-imidazole rings were synthesized. The synthesis of compounds containing triazole and imidazole rings was attempted. Compounds containing this binary system have been realized for heterocyclic imine derivative compounds. According to literature information, these obtained compounds are expected to have potential biological activities such as anticonvulsant and antimicrobial activities. In the first step, iminoster derivative compounds **(1a-e)** were obtained from aryl/alkyl nitrile compounds by the *Pinner* method. In the second step, ester ethoxycarbonyl derivative compounds **(2a-e)** were obtained by the reaction of aryl/alkyl iminoester **(1a-e)** compounds with ethoxycarbonyl hydrazine compound. The resulting ester ethoxycarbonyl hydrazones were reacted with hydrazine hydrate and the corresponding triazole-amine **(3a-e)** compounds were obtained using the method given in the literature. In the original step of the study, the 5-substituted-4-amino-1,2,4-triazol-3-one compounds **(3a-e)** were reacted with 4-imidazole carboxyaldehyde and five new Schiff bases 4-{[((1*H*-imidazol-4-yl)methylene]amino}-5-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-one compounds **(4a-e)** were obtained. A series of 5 new 5-substituted-4-{[(1*H*-Imidazol-4-yl)methylene]amino}-2,4-dihydro-3*H*-1,2,4-triazole-3-one **(4a-e)** were synthesized and their physical properties and IR, $^{1}$H-NMR and $^{13}$C-NMR spectral analyses were performed to elucidate the structures of the compounds. The potential biological activities of the obtained new Schiff base compounds are still being studied.

**Keywords:** Iminoester, Schiff base, triazole-imidazole, ester ethoxycarbonyl hydrazones, synthesis.

## *ORAL PRESENTATION*

# The effect of direct current applications on the cultivation period of microalgae *Chlorella vulgaris*

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**Abstract**

In this study, the effect of direct current applications on the cultivation period of microalgae *Chlorella vulgaris* was investigated. For this research, microalgae *Chlorella vulgaris* was cultured in TAP medium and direct current was applied to the culture medium at 3 different voltages (10V, 20V and 30V) and 3 different durations (5s, 30s and 60s). The process of giving direct current was continued every other day during the cultivation period, starting from the 3rd day of cultivation. In addition, cultures were not mixed during the cultivation period in order to determine the effect of direct current applications. As a result, while the cultivation period of *Chlorella vulgaris* is known as 2 to 3 weeks in the literature, this process extended up to 33 days with the effect of non mixing. It was determined that direct current applications shortened the cultivation period and the cultures entered the death phase earlier. As the duration and current intensity increased, the cultures entered the earlier death phase and direct current applications had a mixing effect on the cultures. In particular, the culture applied 30V 60s direct current entered the death phase on the 17th day. It was concluded that direct current applications shorten the cultivation period.

**Keywords:** *Chlorella vulgaris*, direct current applications, cultivation period.

## *ORAL PRESENTATION*

# The design of modern tacrine-based drug candidates in the treatment of Alzheimer's disease

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**Abstract**

Alzheimer's disease is a common neurological disease among the elderly that leads of morbidity and mortality globally (1). The development of Alzheimer's disease is being relevant with several hypotheses suggested including low acetylcholine levels, formation of β-amyloid and tau protein, oxidative stress, metal ions, neuroinflammation, glutamate over expression, etc. (2). Extensive research has been conducted on cholinergic mechanisms since the observation of loss of cholinergic activity in the brains of Alzheimer's patients. N-methyl-D-aspartate receptor antagonists and cholinesterase inhibitors are used in the clinic to treat Alzheimer's disease. While tacrine, donepezil, galantamine, and rivastigmine are cholinesterase inhibitors, memantine acts as N-methyl-D-aspartate receptor antagonist (3). Recently, aducanumab was approved as a novel treatment for Alzheimer's disease (4). Unfortunately, available drugs in the clinic show limited efficacy and symptomatic effects. Tacrine was approved by the FDA as a cholinesterase inhibitor for the treatment of AD. However, tacrine was withdrawn due to hepatotoxicity from usage. Moreover, it shows limited therapeutic window and short half-life (5). In the scope of this study, multi-targeted tacrine-based ligands were reviewed. *In vitro* and *in vivo* studies indicated that tacrine-based drug candidates showed less hepatotoxicity and high selectivity compared to tacrine as potent drug candidates.

**Keywords:** Alzheimer's disease, tacrine, acetylcholinesterase.

**References:**

1. Cummings, J.; Lee, G.; Nahed, P.; Kambar, M.; Zhong, K.; Fonseca, J.; Taghva, K., Alzheimer's disease drug development pipeline: 2022. Alzheimers Dement., 2022, 8 (1), e12295.
2. Tiwari, S.; Atluri, V.; Kaushik, A.; Yndart, A.; Nair, M., Alzheimer's disease: pathogenesis, diagnostics, and therapeutics. Int. J. Nanomedicine, 2019, 14, 5541-5554.
3. Ashford, J. W., Treatment of Alzheimer's Disease: The legacy of the cholinergic hypothesis, neuroplasticity, and future directions. J. Alzheimers Dis., 2015, 47 (1), 149-156.
4. Beshir, S. A.; Aadithsoorya, A. M.; Parveen, A.; Goh, S. S. L.; Hussain, N.; Menon, V. B., Aducanumab therapy to treat Alzheimer's disease: A narrative review. Int. J. Alzheimers Dis., 2022, 2022, 9343514.
5. Madden, S.; Spaldin, V.; Park, B. K., Clinical pharmacokinetics of tacrine. Clin. Pharmacokinet. 1995, 28 (6), 449-557.

## *ORAL PRESENTATION*

# Chemical and biological stabilities of manually produced-novel and traditional tobacco product extracts up to 8 weeks *in vitro*

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**Abstract**

Smoking is one of the leading causes of preventable deaths and lung cancer worldwide. Hence, tobacco companies produce novel tobacco products with new marketing strategies aiming at “less toxic” smoking. For this purpose, recently developed heated-tobacco products (HTPs) are remarkably popular due to their marketed “less harmful” profile among users. To assess their safety profile, *in vitro* toxicity studies are quite beneficial pre-screening tools for future clinical studies. In the present study, it was aimed to determine the chemical and biological stability of manually produced HTP and research cigarette extracts (3R4F) through their nicotine content by LC-MS analysis, as well as their cytotoxicity via MTT assay and intracellular GSH level in BEAS-2B human bronchial epithelial cells up to 8 weeks. Briefly, CSE and HTP were prepared by pumping smoke. One 3R4F cigarette and one no-flavored HTP stick connected to a heating device were puffed into the culture medium. Followingly, the prepared extracts were considered as 100% (v/v) CSE and HTP, and aliquots were kept at -80°C for 8 weeks. Each aliquot was thawed just before the experiment to assess chemical and biological stability. According to the results, it was shown that nicotine contents of manually produced -CSE and HTP extracts were not changed significantly at the end of week-8. However, cytotoxic and GSH depletive potential of both extracts in BEAS-2B cells are significantly decreased from week-6, particularly with 25-50% (v/v) of extracts, probably due to the loss of volatile organic content of extracts. Therefore, it might be suggested that GSH depletion plays a critical role in the assessment of tobacco product cytotoxicity, and extracts might be considered biologically stable for up to 6 weeks when they were stored at -80ºC. To conclude, these findings might represent a valuable background for future *in vitro* inhalation toxicity studies on tobacco products.

**Keywords:** Cigarette smoke, heated tobacco product, nicotine, cytotoxicity, glutathione, bronchial epithelia.

## *ORAL PRESENTATION*

# Anti-diyabetik ilaç olan linagliptinin karaciger kanser hücresine sitotoksik etkileri

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**Özet**

Dünya Sağlık Örgütü, tümör oranlarının son yıllarda hızla artmakta olduğunu bildirmiştir. Bu sebeple, anti-tümör ilaç pazarı da buna paralel olarak artmaktadır. Elimizdeki imkanları kullanarak yeni ilaçlar keşfetmek gittikçe zorlaşmaktadır. Güncel teknolojiyi kullanılarak yapılan yeni ilaç araştırma ve geliştirme maliyetlerinin artması sebebiyle başarı oranı yıldan yıla düşmektedir. Halihazırda pazarlanan ilaçlar için yeni kullanım alanlarının geliştirilmesi, yeni anti-tümör ilaçları geliştirmenin etkili bir yolu olduğu kanıtlanmıştır. Anti-diyabetik ilaç olan linagliptin (LG) diğer birçok hastalıkta etkinliği makalelerce kanıtlanmıştır. Özellikle LG; otoimmün miyokardit, hepatik steatoz, hepatik fibroz ve diyabetik hayvan modellerinde olumlu anti-inflamatuar ve antioksidan etkiler gösterdiği bildirilmiştir. Çalışmamızın amacı; anti-diyabetik bir ilaç olan LG'nin anti-kanser ilaç olarak yeniden konumlandırılması ve yeni biyolojik özelliklerinin literatüre kazandırılmasıdır. Çalışmamızda LG'nin karaciğer kanser hücre hattında (huh-7) sitotoksik etkisi ve hücre ölüm yolakları incelenmiştir. LG'nin kanser hücre (huh-7) hattında uygulanan hücre canlılığına olan etkileri farklı derişimlerinde MTT testi ile % canlılık kontrolü yapılmıştır. Hücre ölüm yolakları ile apoptoz ve nekroz oranları belirlenmiştir. Tek Yönlü Varyans Analizi (ANOVA) ile, Tukey-Kramer Çoklu Karşılaştırma Testi ise post hoc testi ile istatistiksel analizi yapılmıştır. Bu araştırmadan elde edilen verilerin, ilaç sektöründe yapılacak yeni anti-kanser ilaç çalışmalarında araştırmacılara ışık tutacağına inanılmaktadır. Sonuç olarak, yapılan ön çalışmalara göre LG'nin bu karaciğer kanser (huh-7) türünde sitotoksik etkisi olduğu sonucuna varılmıştır.

**Anahtar Kelimeler:** İlaç yeniden konumlandırma, linagliptin, karaciğer kanseri, Huh-7.

## *ORAL PRESENTATION*

# Investigation of *in vitro* antidiabetic and antioxidant activity of hawthorn vinegar obtained from Endemic *Crataegus tanacetifolia*

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**Abstract**

In this study, the *in vitro* antidiabetic, antioxidant activity and total flavonoid content (TFC) and total phenolic content (TPC) of vinegar obtained from Endemic *Crataegus tanacetifolia* (Lam.) Pers. (Rosaceae), (hawthorn) were examined. Two different commercial products of vinegar were used as study material: one is Kemal Ünlü brand hawthorn vinegar supplied from Malatya province (MS), another one is Temmuz Organic Farm vinegar from Konya (TS). Their antidiabetic activity was determined by α-amylase and α-glucosidase inhibitory methods. Antioxidant activities were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferrous iron chelating (FCA) assays. The results were read in the Elisa reader and evaluated with Excel and GraphPad programs. The MS has been found to have higher α- amylase (95.12± 3.71%) and α-glucosidase inhibitory (81.62 ± 0.33%) effects. The TS demonstrated 94.13 ± 3.85% α-amylase and 75.35 ± 2.19% α-glucosidase inhibitory activity, respectively. The highest TPC was found to be 467.59 ± 6.73 mg GAE/mL in TS vinegar, it was followed by MS vinegar with TPC content of 328.46 ± 5.50 mg GAE/mL. The TFC was found as 1.94 ± 10.36 mg CE/mL and 1.32 ± 10.96 mg CE/mL in TS and MS vinegar, respectively. The FCA was found to be higher in TS (33.37 ± 0.53%) than in MS (31.08 ± 10.87%). The DPPH radical scavenging activity was found as 73.82 ± 2.12% in TS and 80.12 ± 4.45% in MS. ABTS radical scavenging activity was found to be the highest in TS with 82.51± 0.78% and in MS found as 78.65 ± 0.55%. The antidiabetic, antioxidant activity, TPC and TFC determinations of these vinegars were performed for the first time with these methods.

**Keywords:** *Crataegus tanacetifolia*, vinegar, bioactivity, phytochemical

## *ORAL PRESENTATION*

# Toprak Kökenli Patojenlere Karşı Bazı Bitkisel Ekstraktların Etkilerinin Belirlenmesi

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**Abstract**

Havada, toprakta, bitki üstünde çok sayıda mikroorganizmalar bulunmakta ve bunların bir kısmı bitkilerde hastalık oluşumuna neden olmaktadır. Funguslar bitkilerin çeşitli aksamlarında hastalık oluşturabilmektedir. Özellikle toprak kökenli funguslar bitkilerde kök çürüklüğü ve solgunluk gibi hastalıklarla ciddi ürün ve kalite kayıplarına ya da bitkinin ölümüne neden olabileceğinden bu hastalıklarla mücadele önemlidir. Ancak, zirai ilaçlar insan sağlığı ve hayvan sağlığını olumsuz etkilemekte, havada ve suda kalıntı oluşturmaktadır. Bu ve benzeri olumsuzluklar araştırmacıları yeni mücadele yöntemleri geliştirmeye teşvik etmiştir. Böylece insana ve çevreye zarar vermeyecek, kalıntı süresi kısa, kolay elde edilebilen, tıbbi bitkilerin ekstraktlarının kullanımı için çalışmalar başlatılmıştır. Bitki ekstraktlarının patojen gelişimi üzerine antifungal etkilerinin olduğu görülmüştür. Bu çalışmanın amacı Toprak kökenli bazı fungal patojenlere karşı (*Rhizoctoni solani, Sclerotinia sclerotiorum, Sclerotium rolfsii, Fusarium oxysporum* f.sp. *lycopersici* FOL ve *Fusarium oxysporum* f.sp. *radicis- lycopersici* FORL Anason, rezene ve sarımsak ekstraktlarının etkisini belirlemektir. Bu patojenlere karşı Anason, Rezene, Sarımsak ekstraktları kullanılmıştır. Petrilerin kapaklarına bitkisel ekstraktların 1000, 500 ve 250 ppm lik dozları mikropipetle damlatılmış ve petri kapları hemen parafilmle kapatılmıştır. Denemeler 3 tekerrürlü olarak kurulmuş ve petriler 22-25°C'deki inkübatörde gelişmeye bırakılmıştır. Bitkisel ekstrakt içermeyen petrilerdeki herhangi bir fungusun misel gelişimi petri çapına ulaştığında deneme değerlendirilmiştir. Petrilerdeki kolonilerin çapları ölçülerek aşağıdaki formül yardımıyla bitkisel yağların engelleyici etkileri hesaplanmıştır. % Engelleme = (Kont. gelişme – Uyg.gelişme)/Kont. Gelişme X 100 Araştırma sonucunda denemede ele alınan fungusların misel gelişimleri üzerine sarımsak ekstraktının engelleyici etkisinin daha yüksek olduğu belirlenmiştir. Tüm funguslara bitki ekstraktaların 1000 ppm lik dozları etkili bulunmuştur. *R. solani* ve *S. rolfsii* ye karşı 500 ppm lik dozlar etkili bulunmuştur.

**Anahtar Kelimeler:** Ekstrakt, Fungus, antifungal.

## *ORAL PRESENTATION*

# Paraoxonase Enzyme Inhibition Effects Of Sulfonamide Bearing Compounds

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**Abstract**

The human body has various enzymatic mechanisms for removing free radicals (1). Paraoxonase (EC.3.1.8.1), aryl dialkyl phosphatase, is one of the important enzymes in the antioxidant defense system (1,2). PON family is consist of three enzyme types, PON1, PON2, and PON3. PON1 is synthesized in the liver. Since PON1 is associated with high-density lipoproteins (HDL), the primary physiological role of PON1 enzyme is to protect low-density lipoproteins against oxidation. As a result, all three PONs act as antioxidants and have anti-inflammatory effects in various disease states such as atherosclerosis and cardiovascular diseases (1). On the other hand, sulfonamides continue to take attention because of their newly discovered pharmacological properties, as well as their use for antibacterial properties for years. Studies have shown that sulfonamides exhibit selective inhibition properties not only on carbonic anhydrase (CA) isozymes but also on cancer-associated CA isoenzymes, glutamate carboxypeptidase II, lactoperoxidase, and acetylcholine esterase) enzymes. The aim of this study is to investigate the effect of some primary sulfonamide derivatives on human serum PON1 enzyme activity. According to *in vitro* enzyme assays, $IC_{50}$ values of sulfonamide derivatives (1, 2, and 3) were found in the range of 0,62-1,1 μM. According to the results, compound 4 affected enzyme activity in a positive manner, whereas 1-3 compounds inhibited the activity of the enzyme. Since the PON1 enzyme exhibits antimicrobial, antioxidative, antidiabetic and anti-inflammatory as well as lowering the risk of developing coronary artery disease, PON1 could be considered as a promising target to develop new and potent candidates (4).

**Keywords:** Paraoxonase, enzyme inhibition, sulfonamide.

**References:**

(1) Mohammed, C.J.; Lamichhane, S.; Connolly, J.A.; Soehnlen, S.M.; Khalaf, F.K.; Malhotra, D.; Haller, S.T.; Isailovic, D.; Kennedy, D.J. A PON for All Seasons: Comparing Paraoxonase Enzyme Substrates, Activity and Action including the Role of PON3 in Health and Disease. Antioxidants 2022, 11, 590. https://doi.org/10.3390/antiox11030590
(2) Tomatir AG, Pehlivan S, Sahin HH, Balci SO, Budeyri S, Pehlivan M. Q192R and L55M Polymorphisms of Paraoxonase 1 Gene in Chronic Myelogenous Leukemia and Chronic Lymphocytic Leukemia. Anticancer Res. 2015 Sep;35(9):4807-12. PMID: 26254371.
(3) Köksal Z, Kalin R, Camadan Y, Usanmaz H, Almaz Z, Gülçin İ, Gokcen T, Gören AC, Ozdemir H. Secondary Sulfonamides as Effective Lactoperoxidase Inhibitors. Molecules. 2017 May 24;22(6):793. doi: 10.3390/molecules22060793. PMID: 28538675; PMCID: PMC6152724.
(4) Aggarwal G, Prajapati R, Tripathy RK, Bajaj P, Iyengar AR, Sangamwar AT, Pande AH. Toward Understanding the Catalytic Mechanism of Human Paraoxonase 1: Site-Specific Mutagenesis at Position 192. PLoS One. 2016;11(2):e0147999..

## *ORAL PRESENTATION*

# The Antimicrobial Potential of Biosurfactant Produced with *Staphylococcus capitis* and *Achromobacter marplatensis* Strains

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**Abstract**

Biosurfactants are known as biologically based surface-active compounds produced by various bacteria, yeasts, and fungi. It is an undeniable fact that biosurfactants, especially produced as secondary metabolites, have a unique surfactant power with their antimicrobial activities. The anti-bacterial, anti-fungal, anti-viral and antiadhesive/antibiofilm properties of biosurfactants make them useful against various microbial infections. Therefore, the use of biosurfactants as a new treatment method attracts a great attention. For this reason, this study aimed to investigate the potential of biosurfactant production with two bacterial strains as *Staphylococcus capitis, Achromobacter marplatensis*. While it was detected that the biosurfactant was effective on Gram-negative and Gram-positive test bacterial strains according to the results of agar disc diffusion and broth micro dilution assays, a difference was also obtained on its antimicrobial activity against tested bacterial strains. The antimicrobial activities of biosurfactants vary between Gram-negative and Gram-positive bacterial strains. Additionally, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were detected for all tested microorganisms by evaluating the results of broth micro dilution assay. As a result, the biosurfactant can be used as an alternative to commercial antibiotics with its powerful antimicrobial properties.

**Keywords:** Biosurfactant, Bacterial strains, Gram-negative, Gram-positive, Antimicrobial activity,

## *ORAL PRESENTATION*

# Asma Hidrolik İletkenliği: Aquaporinler

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**Özet**

Asma, Transkafkasya Bölgesinden ilk kez kültüre alınan ve günümüzde Çinden Amerikaya, Avusturalya dan Güney Afrikaya ve tüm Akdeniz ülkelerine kadar Dünyanın pek çok yerinde yetiştiriciliği yapılan önemli bir tarım ürünüdür. Asma bitkisi çevresel faktörlere karşı toleranslı olması dünya üzerinde yayılmasına imkân sağlamıştır. Özellikle kuraklık bölgeler de ekonomik anlamda üzüm yetiştiriciliği yapılabilmektedir. Asma bitkisinde kuraklığa karşı toleransının yüksek olmasının en önemli nedenlerinden bir tanesi bitki hidrolik iletkenlik ile ilgili mekanizmaları iyi kullanabilmesidir. Bitkideki bu hidrolik iletkenliği temel düzenleyicisi ise aquaporinlerdir. Stomaların kapanmasından, emboli ve kavistasyonyan kaçınmaya, pasif su akışının düzenlenerek köklerin topraktan daha kolay su almasını sağlamaya, yapraklardan transpirasyonla suyun uzaklaşmasını önlemeye ve hücrelerdeki ozmotik derişimi düzenlemeye kadar pek çok faaliyette aquaporinler görev almaktadırlar. Bu derlemede asma bitkisinin kuraklık gibi abiyotik stres faktörlerine karşı hidrolik iletkenliğinin düzenlemesinde önemli rol oynayan aquaporinlere yer verilmiştir.

**Anahtar Kelimeler:** Pasif su taşınımı, Vitis vinifera, abiotik stres.

## *ORAL PRESENTATION*

# Kalsiyum-Aljinatta tutuklanmış *Paracoccus denitrificans* ile bir sabit yatak biyoreaktörle içme sularından nitrat giderimi üzerine karbon/azot oranı ve farklı karbon kaynaklarının etkisi

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**Özet**

Bu çalışmada, kalsiyum-aljinatta tutuklanmış *Paracoccus denitrificans* hücreleri ile bir sabit yataklı biyofilm reaktörde nitrat giderimi üzerine karbon/azot oranı (C/N) ve farklı karbon kaynaklarının etkisi araştırılmıştır. Çalışmada başlangıç nitrat içeriği 200 mg/L, akış hızı ise 119,52 mL/h olarak sabit tutulmuştur. Farklı karbon kaynaklarının nitrat giderimine etkisi araştırılırken; besleme çözeltisine karbon kaynağı olarak etanol, metanol, gliserol, glukoz ve asetik asit ilave edilmiş ve en yüksek nitrat giderim verimi %97,75 ile etanolün ilave edildiği denemelerden elde edilmiştir. C/N oranının nitrat giderimine etkisinin incelendiği denemelerde karbon kaynağı olarak etanol kullanılmış, C/N oranı 0,5; 1,0; 1,5; 2,0; 2,5; 3,0 olarak değiştirilmiş ve C/N oranı artıkça reaktör verimliliğinde de artış gözlenmiştir.

**Anahtar Kelimeler:** Biyolojik nitrat giderimi, dolgu yataklı biyoreaktör, immobilizasyon, kalsiyum aljinat.

## *ORAL PRESENTATION*

# Exosome isolation and characterization from PID-PC, INS-1, and MiaPaca-2 cells and investigation of the effect of isolated exosomes from PID-PC on pancreatic cancer

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**Abstract**

Pancreatic cancer is one of the deadliest malignant neoplasms and the leading cause of death because of cancer. Exosomes may play a role in the diagnosis and treatment of many different diseases, especially cancer. The purpose of our work is to characterize exosomes isolated from the PID-PC, MiaPaca-2, and INS-1 cell lines and make an analysis comparatively of the size of this exosome for the first time. The second aim is to research the effects of first-time isolated exosomes from PID-PC on the INS-1 and MiaPaca-2 cells. Exosomes were isolated with exosome isolation kits. The cell viability was determined with an MTT assay. Alix, TSG101, CD9, and CD81 proteins were analyzed with Simple WES. The size and concentration of exosomes were detected with NTA. Alix was detected in isolated exosomes from PID-PC, MiaPaca-2, INS-1, and cell lysates obtained from MiaPaca-2, INS-1. TSG101 was detected in obtained cell lysates from PID-PC, MiaPaca-2, and INS-1. CD9 was shown in PID-PC, MiaPaca-2, INS-1 cell lysates, and isolated exosomes from INS-1. CD81 was detected only in obtained lysate from the MiaPaca-2 cell line. The dimensions of isolated exosomes from PID-PC, MiaPaca-2, and INS-1 were analyzed as 103.6±28.6; 100.7±10; 147.2±12.3 nm. Exosome-treated MiaPaca-2 cells proliferated at low concentrations at 24 hours, while decreased cell viability of cells at 72 hours. In exosome-treated INS-1 cells, there was no difference in cell viability until 5 µg/ml for 24 hours, and 2.5 µg/ml for 72 hours, while cell viability began to decrease at higher doses. Alix for isolated exosomes from the three cells and CD9 for isolated exosomes from INS-1 may be a specific marker. Exosomes isolated from the PID-PC cells caused cell death in the MiaPaca-2 cells in a time- and dose-dependent manner.

**Keywords:** Pancreatic island-derived progenitor cell, Pancreatic cancer, Exosomes, Biomarker, Cell death.

## *ORAL PRESENTATION*

# Determination of encapsulation and release profile of thyme extract in alginate particles

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**Abstract**

Today, the use of active ingredients obtained from natural sources is increased. Carrier systems and encapsulation technology are used to increase the bioavailability of active ingredients, to ensure that they are not affected by environmental conditions and released in a controlled manner. In this study, it was aimed to use alginate, which is a natural sourced polymer, as a carrier system and to investigate the encapsulation efficiency and release profile of alginate particles prepared with different concentrations of crosslinker. Thyme plant (Thymus vulgaris L.) extract was used as an active ingredient due to its antioxidant and antimicrobial properties. The total phenolic content of the obtained thyme extract was determined as 14.6 mg GAE/g by Folin-Ciocalteu method. The sizes and morphological properties of alginate particles synthesized with different concentrations (1-3%) of crosslinker ($CaCl_2$) were determined by caliper measurements and Scanning Electron Microscopy analysis. It was determined that the dimensions of the alginate particles were generally 2.13 mm and had an rough surface. Both the encapsulation efficiency and the release profile of the particles loaded with the extract by the Folin-Ciocalteu method were investigated. According to the encapsulation efficiency analysis, increasing the crosslinker concentration increased the efficiency. The use of low concentrations of crosslinker resulted in higher total phenolic content released in the first hour. A total phenolic content of 38±3% was released when 1% crosslinker was used in the preparation of extract-loaded alginate particles; When 2 and 3% crosslinker was used, it was released at the rates of 11±4 and 18±3%, respectively. According to the obtained data; the use of 3% crosslinker in the preparation of alginate particles both increased the encapsulation efficiency and provided a lower initial release amount.

**Keywords**: Alginate particles, Encapsulation,Thyme extract.

## *ORAL PRESENTATION*

# Development of conjugated polymer based amperometric AChE biosensor for pesticide detection

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**Abstract**

Excessive use of pesticides and their persistence in the agricultural fields have raised greater consideration and regarded as harmful for humans and animals. Conjugated polymers are suitable materials for biosensor designs due to their superior properties. Combinations of conjugated polymers with biosensor technology enable the enzymes to be immobilized to the electrode surface in a practical, while shortening the electrochemical response time with fast electron conduction and increasing the stability of the biosensor. Herein, synthesis of the polymer called poly-2,2′-(9,9-dioctyl-9h-fluorene-2,7-diyl)bistiophene, Poly(BT) on the graphite electrode surface was carried out by electrochemical method. After the polymer was deposited on the graphite electrode surface, it was used as an immobilization matrix for the AChE enzyme. To examine the analytical performance of the Poly(BT)/AChE biosensor, amperometric measurements were taken at a constant potential using a three electrode system. The surface and electrochemical properties of the biosensor system were characterized using SEM, EDX, and CV techniques. After working conditions were optimized, analytical performance was identified for AThCl detection. In addition, the developed biosensor was used for pesticide detection in milk and tap water samples, effectively.

**Keywords:** AChE, Conducting Polymer, Electrochemical Biosensor, Enzyme Immobilization, Organophosphorus Pesticides.

## *ORAL PRESENTATION*

# Synthesis and characterization of diblock copolymers of poly(N-2-hydroxypropylmethacrylamide) and oligoethyleneglycol methacrylate

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**Abstract**

In recent years, amphihilic block copolymers drew attention with their ability to form micelles and nanoaggregates, high structural stability, loading capacity and water solubility in drug delivery applications. Polymers that water-soluble, easily functionalized and rapidly synthesized such as poly(N-2-hydroxypropylmethacrylamide) (pHPMA), oligoethylene glycol methyl ether methacrylate (OEGMA) form conjugates with various drugs, they become prominent by increasing the solubility, stability and half-life of drugs in the blood circulation and EPR effect for drug delivery systems. Also pHPMA stand out in biomedical applications with its high biocompatibility and non-immunogenic properties, in addition to pHPMA's easy functionalization and rapid synthesis thanks to its secondary alcohol group.
This study presents, for the first time in literature, synthesis and characterization of diblock copolymers of poly(N-2-hydroxypropylmethacrylamide) and oligoethyleneglycol methacrylate. pOEGMA macro-RAFT agent synthesis were performed successfully. By using pOEGMA as RAFT agent, pHPMA-*b*-pOEGMA copolymer were synthesized by RAFT polymerization method. Diblock copolymers with narrow polydispersity index (PDI=1.2) value and desired molecular weight ($M_n$=41.2 kDa) were determined by gel filtration chromatography (GPC). The chemical structure of the polymer were characterized by FTIR and $^1$H-NMR spectroscopies. The results showed that the newly synthesized diblock copolymers were accomplished to conjugate with various drug to several drug delivery applications.

**Keywords:** polymer, RAFT polymerization, pHPMA, OEGMA.

## *ORAL PRESENTATION*

# Production and Characterization of Three-layered Semi-resorbable Composite Hernia Mesh

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**Abstract**

Hernia repair is the most common surgical operations applied all over the world. The first option in hernia therapy is the surgical operation. If there exist insufficient amount of healthy tissue or the size of the defect is large, mesh prostheses are used to support weakened or the damaged tissue. Using mesh substantially decrease the risk of hernia recurrence. Hence, it is an effective method for therapy of hernia and abdominal wall defect. However, patches currently used in the clinic applications have significant short-term and long-term risks. For this reason, the search for the ideal mesh still continues today. And therefore, studies on composite hernia meshes, which combine materials with different advantages, have been increasing recently. Within the scope of the presented study, it was aimed to produce an ideal hernia mesh. For this purpose, the design, production, and characterization of a three-layered semi-resorbable composite hernia mesh was described. Chlorinated Polypropylene (PP-Cl), Polycaprolactone (PCL) and Polyethylene glycol (PEG) polymers and *H. perforatum* oil were used in the mesh structure. In the three-layered semi-resorbable composite hernia mesh, all layers were produced by electrospinning technique, and the upper layer (parietal side) was non-resorbable, the middle layer was partially resorbable, and the lower layer (visceral side) was fully resorbable. The upper layer composed of electrospun PP-Cl fibers, the middle layer composed of counter-electrospun (double electrospinning) interwoven PP-Cl and PCL fibers, and the lower layer composed of counter-electrospun PCL and oil-containing PEG fibers. Some characteristics of the meshes produced within the scope of the study such as, chemical structure (FTIR), surface morphology (SEM), density, porosity, wettability (% Swelling ratios), hydrophilic/hydrophobic properties (contact angle measurements), time-dependent mass loss, release profile of herbal oil from the mesh, and mechanical strength ( elasticity) were determined by performing various in-vitro characterization studies and patch performance was evaluated.

**Keywords:** Hernia mesh, *H. perforatum* oil, Electrospining, Chlorinated Polypropylene (PP-Cl), Polycaprolactone (PCL), Polyethylene glycol (PEG).

## *ORAL PRESENTATION*

# Production and characterization of PCL/$Ni_2O_3$ NP/PLA nanocomposite cardiac patches

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**Abstract**

One of the leading causes of death worldwide, heart attack, in other words acute myocardial infarction (MI), is the main cause of coronary heart failure. It usually occurs when the cardiac muscles cannot receive enough oxygen and nutrients due to clogged coronary arteries. After MI, massive cell death occurs in the cardiac muscles and scar tissue is formed. Scar tissue cannot transmit the electrical signals produced during pumping of blood, contrary to what should be in a healthy cardiac muscle. Recently, studies on cardiac patches that are designed to at least provide functional support to the dead cardiac tissue, thus increasing the quality of life of patients after MI and reducing their vital risks, have started to gain importance. Cardiac patches are usually designed as three-dimensional porous membranes using biocompatible biomaterials. Biomaterials made from synthetic or biological polymers help the cells to organize with the surrounding functional tissues. However, due to the poor electrical conductivity of biopolymers, the ability of the patch to perform its electrical conductivity function is limited. In the presented study, conductive and elastic PCL/$Ni_2O_3$NP/PLA nanocomposite cardiac patches that can overcome these limitations were produced by simultaneous and counter elecrospinning of poly(lactic acid) (PLA) and poly(ε-caprolactone) polymers which are widely used in tissue engineering applications and approved by the FDA (Food and Drug Administration). Ni2O3 nanoparticles were included to provide conductivity to the patch structure during electrospinning. The chemical structure analysis of the produced patches was made by taking the spectra from the patch samples (FTIR). Surface morphology and fiber structure were evaluated by taking Scanning Electron Microscopy (SEM) images. Density and porosity values of the patches were calculated. Wettability (% Swelling ratios) and biodegradation of patches were determined under in-vitro conditions (pH 7.4, 25°C) in Phosphate Buffer Solution (PBS). The hydrophilic/hydrophobic character of the patch surfaces was determined by taking contact angle measurements. Patch elasticity was characterized using standard tensile tests and patch conductivity was characterized using the 4-point probe technique.

**Keywords:** Cardiac patch, Nanocomposite, Poly(lactic acid), Poly(ε-caprolactone), $Ni_2O_3$ Nanoparticule.

## *ORAL PRESENTATION*

# Doğal bileşenlerin vücut içi uygulamalarda kullanımının incelenmesi

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**Özet**

Vücut içi tedavi amaçlı hazırlanan biyomalzemelerin büyük bir çoğunluğu ilaç salım sistemleri olarak tasarlanmaktadır. Bu sistemler, inorganik nanomalzemelerden, polimerik nanokompozitlere; uyaranlara duyarlı taşıyıcılardan, ilaç etken maddelerine kadar pek çok farklı malzemeden oluşabilmektedir. Biyomalzemelerde doğal bileşenlerin birçoğunun özellikle insan sağlığına zararsız olması, kullanım ömrü sonunda bozunabilmesi sebebiyle sentetik malzemelere alternatif olarak sektörel ve akademik araştırmalarda öne çıkmaktadır. Bu malzemeler arasında doğal polimerler; yüksek oranda biyouyumluluk, biyolojik olarak parçalanabilirlik, yaygın olarak bulunma, dayanıklılık, toksik olmama ve uygun maliyetli olma gibi özelliklerinden dolayı ilaç salım sistemlerinde tercih edilmektedir. Vücut içi ilaç salım sistemleri sürekli olarak vücut akışkanları ile temas halinde olduğu için biyomalzemenin akışkanlarla etkileşimi, sıvı sorpsiyon karakteristikleri, şişme ve boyutsal kararlılık özelliklerinin ortama uygun olması gerekmektedir. Doğal bileşenlerle hazırlanan biyomalzemelerin formülasyonu sırasında; bileşenlerin seçimi, süreç içinde dikkat edilecek parametreler ve parametrelerin birbiri ile etkileşimi için optimizasyon basamakları ve karşılaşılan problemlerin çözüm önerileri, örnekleri ile bu çalışmada paylaşılacaktır.

**Anahtar Kelimeler:** Biyomalzeme, Doğal Polimer, İlaç Salım, Kompozit.

## *ORAL PRESENTATION*

# Demir Oksit ($Fe_2O_3$) Nanopartikül ve Mikropartikül Formunun *Allium cepa* Üzerindeki Genotoksik Etkisinin Comet Testi ile İncelenmesi

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**Özet**

Nanopartiküller (NP) eşsiz elektronik, optik, termal ve fotokatalitik özelliklere sahip oldukları için çok farklı alanlardaki uygulamalarda çok sayıda ürün için ideal adaylardır. Demir oksit ($Fe_2O_3$) nanopartikülleri, son yıllarda özellikle klinik uygulamalarda kullanılan, fiziksel ve kimyasal açıdan kararlı ve ayrıca çevre için güvenli kabul edilen partiküllerdir. Sahip oldukları özellikler nedeniyle manyetik rezonans görüntülemede (MRG) kontrast ajan olarak, hücre ayrıştırılmasında ve algılanmasında, hipertermi tedavisinde kullanılmaktadır. Ancak, yapılan bazı araştırmalarda, bu partiküllerin toksik, özellikle genotoksik etkilere sahip olabileceği tespit edilmiştir. Bu çalışmada demir oksitin nanopartikül (50nm) ve mikropartikül (5µm) formunun *Allium cepa* üzerindeki genotoksik etkisi, komet (kuyruklu yıldız) testi kullanılarak incelenmiştir. Kök uçları, her iki partikülün 125, 250, 500 ve 750 g/mL'lik konsantrasyonları ile 48 saat muamele edilmiştir. DNA hasarı, komet testi kullanılarak üç farklı parametre (komet kuyruk uzunluğu, kuyruk yoğunluğu ve kuyruk momenti) ile değerlendirilmiştir. Negatif kontrole kıyasla, $Fe_2O_3$ NP'leri, 125 ve 250 µg/mL'lik konsantrasyonlarda kuyruk uzunluğunda, 125 µg/mL'lik konsantrasyonda ise kuyruk yoğunluğu ve kuyruk momentinde anlamlı artışa sebep olmuştur. Demir oksit mikropartikülleri ise, bütün konsantrasyonlarda kuyruk uzunluğunda anlamlı artışlar oluştururken, kuyruk yoğunluğu ve kuyruk momentinde sadece en yüksek iki konsantrasyonda (500 ve 750 µg/mL) anlamlı artışlar oluşturmuştur. Bu test sonuçlarına göre her iki partikül de bazı konsantrasyonlarda DNA' da hasarlara sebep olmakla birlikte, nanopartiküllere kıyasla mikropartiküllerin daha genotoksik olduğu gözlenmiştir. Bu etkinin komet testinde elde edilen bu sonuç, aynı partiküllerin incelenen test sistemine göre toksik etkilerinde de bazı farklılıklar gösterebileceğine işaret etmektedir.

**Anahtar Kelimeler**: Demir oksit nanopartikül mikropartikül Allium testi komet testi genotoksik etki.

## *ORAL PRESENTATION*

# Immobilization of Pancreatic Lipase onto Bio-Waste Eggshell Membrane to Improve Enzymatic Stability Properties

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**Abstract**

Lipases (EC 3.1.1.3) are serine hydrolases that catalyze synthesis reactions such as interesterification, esterification, transesterification, acidolysis, aminolysis and alcohollysis in anhydrous medium. In addition, lipases break down long-chain triglycerides into glycerol, free fatty acids, monoglyceride and diacylglycerides in aqueous media. Due to their ability to catalyze both hydrolysis and synthesis reactions, lipases are one of the important biocatalysts used in many biotechnological fields, especially in the detergent, cosmetics, textile and pharmaceutical industries.
Immobilization is the binding of water-soluble enzyme molecules on a water-insoluble solid support by physical and chemical interactions. The carriers used in immobilization are extremely important and the stability properties of the enzyme may improve after the immobilization process, depending on the properties of the groups on the surface of the carriers. For this reason, many natural and synthetic polymer support materials have been used as carriers in the immobilization process. Eggshell membrane (ESM) is a natural biopolymer used in immobilization studies due to its porous structure, biodegradability, large surface area and high structural strength.
In this study, lipase (from porcine pancreas) was immobilized on ESM using adsorption and cross-linking methods. Enzyme amount (0.75 mg/mL), ESM amount (12.5 mg), adsorption time (20 min) and crosslinker concentration (2% GA) were determined as optimization parameters of immobilization process. In addition, optimum temperature, optimum pH, thermal stability and pH stability properties were determined for both free lipase and lipase immobilized ESM. As a result, it can be said that a new, alternative and reusable method is presented for waste oil removal and biodiesel production with lipase immobilization on ESM, which is a natural biopolymer.

**Keywords:** Eggshell membrane, pancreatic lipase, immobilization.

## *ORAL PRESENTATION*

# Electrochemical determination of imipramine with silver nanoparticle-modified electrodes

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**Abstract**

Imipramine-based drugs are tricyclic antidepressants with low level of anticholinergic and alpha-adrenergic effects, mostly used in the treatment of depression. Rapid and accurate determination of the concentration of imipramine in body fluids is important for the treatment of disease. No studies were found in the literature that a pencil graphite electrode was used as a working electrode for the investigation of the electrochemical behavior of imipramine and its voltammetric determination. The developed electrodes were designed for the first time and the determination of imipramine was made with these electrodes for the first time.
Analyses were performed with Autolab Potentiostat/Galvanostat PGSTAT-302 N device. During the preparation of the electrodes, the cyclic voltammetry (CV) method was applied. Optimization of the electrodes and drug analyses were performed with differential pulse voltammetry (DPV). Aqueous Ag/AgCl, Pt wire, and silver nanoparticle-modified electrodes were used as reference electrode, a counter electrode, and working electrode, respectively. Two methods were applied for the modification. Firstly, the bare PGE was activated in 0.05 M buffer solution and at a potential of +1.4 V. Then, the electrodes were kept in solutions of nAg and made ready for measurement (Activated-nAg-PGE). In the second method, the electrochemical coating on PGE was performed by polymerization of pyrrole (Py) and made ready for measurement (nAg-PPy-PGE). Britton-Robinson buffer solutions with different pH values were used in the investigation of the electrochemical behavior. The optimum conditions for Activated-nAg-PGE were determined as 60 sec. for activation, 15 min. for modification, and 10 ppm for nAg. The optimum cycle number for nAg-PPy-PGE were determined 7, scanning speed was 60 mV/s and pH value was 7. Surface characterization of the electrodes was performed by SEM analysis and elemental analysis was performed by SEM-EDS analysis. In addition, electrochemical determinations of imipramine-containing pharmaceutical samples were also carried out under optimum conditions.

**Keywords:** Imipramine, pencil graphite electrode, polypyrrole, silver nanoparticle, voltammetry.

## *ORAL PRESENTATION*

# The synthesis of coke-resistant alumina based catalysts for the production of biofuels from bio-oil

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**Abstract**

The demand for energy has been increasing due to the rapidly changing technologies and the ever-increasing population. Biofuels are alternative clean and renewable energy sources obtained by pyrolysis of biomass. It is an alternative energy source to fossil fuel-derived fuels used in today's motor vehicles. However, the biggest problem in biofuel production reaction is the coke formation. Within the scope of this study, catalysts have been developed that are resistant to coke formation during the reaction. In his study, the mesoporous gamma alumina support was synthesized using the hydrothermal (EISA) synthesis method. In order to increase the Bronsted acidity of the catalysts, materials with a mass content of 10% zirconium were added to the catalyst structure by wet impregnation. Metals (5% tungsten and 5% cobalt) were loaded to catalyst in order to prevent coke formation. From the XRD patterns, the characteristic peaks of γ- $Al_2O_3$, indicating the mesoporous structure, could be determined. In addition, the $N_2$ physisorption of the catalysts were found to be compatible with the Type IV isotherm, which is evidence of the mesoporous structure according to the IUPAC classification. The activity tests of the synthesized mesoporous γ- $Al_2O_3$ and metal-containing catalysts in biofuel production reactions from bio-oil were carried out using a 0.5 g catalyst at a temperature of 400 $^oC$ in an ethanol/reactant volumetric ratio of 70/30 in a continuous flow-filled bed reactor system. As a result of the activity tests, high activity was achieved in the presence of 5Co-5W@101Zr@$Al_2O_3$. Consequently, it has been concluded that the use of mesoporous alumina material in biofuel production reactions from bio-oil is promising.
TUBİTAK 219Z232 grant is acknowledged.

**Keywords:** Alumina, Zirconium, Cobalt. Tungsten, Bronsted acid, Bio-fuel.

## *ORAL PRESENTATION*

# Bio-fuel Production over nickel and cobalt Incorporated HZSM-5 Catalyst

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**Abstract**

Bio-oil produced from biomass is regarded as a promising energy source for traditional transportation fuel in the future. However, the high oxygen content and acidic nature of bio-oil restricted its use in motor vehicles as fuel. Therefore, it is very necessary to transform bio-oil to a liquid fuel that is similar to gasoline or diesel by upgrading. Zeolite cracking and hydrodeoxygenation process are efficient way for upgrading bio-oil to bio-fuel. However, zeolite cracking process is operated under atmospheric pressure. Moreover, zeolite cracking is also able to convert bio-oil to bio-fuel with no hydrogen consumption, which is considered more economical. HZSM-5 have recently become prominent in the production studies of alternative fuels. The aim of the study is the synthesis of cobalt and nickel-incorporated HZSM-5 zeolites for biofuel production. Cobalt (%5 by mass) and nickel (%5-10 by mass) incorporated catalysts are prepared using the wet-impregnation method. The synthesized catalysts are characterized by XRD, $N_2$ adsorption-desorption, SEM/EDS, and TGA-DTA. The zeolite cracking reaction for bio-fuel production is carried out at 400 °C for 3 hours. Low bio-fuel selectivity is obtained with the HZSM-5 and nickel-incorporated HZSM-5 catalysts as 7% and % 9, respectively. This result shows that the HZSM-5 catalyst gave poor performance in the zeolite cracking reaction. The incorporation of cobalt in the structure of HZSM-5 has remarkably improved the catalytic activity performance. As a result of the study, high quality oil phase is obtained with 1Co-25Ni@HZSM-5 catalyst.
TUBİTAK-219Z232 and Gazi University Research Fund (FKB-2021-7446) grants are acknowledged.

**Keywords:** HZSM-5, Bio-oil, Biofuel, Zeolite.

## *ORAL PRESENTATION*

# More Safety Improved Method for Synthesis of Graphene Oxide with Deep Cold

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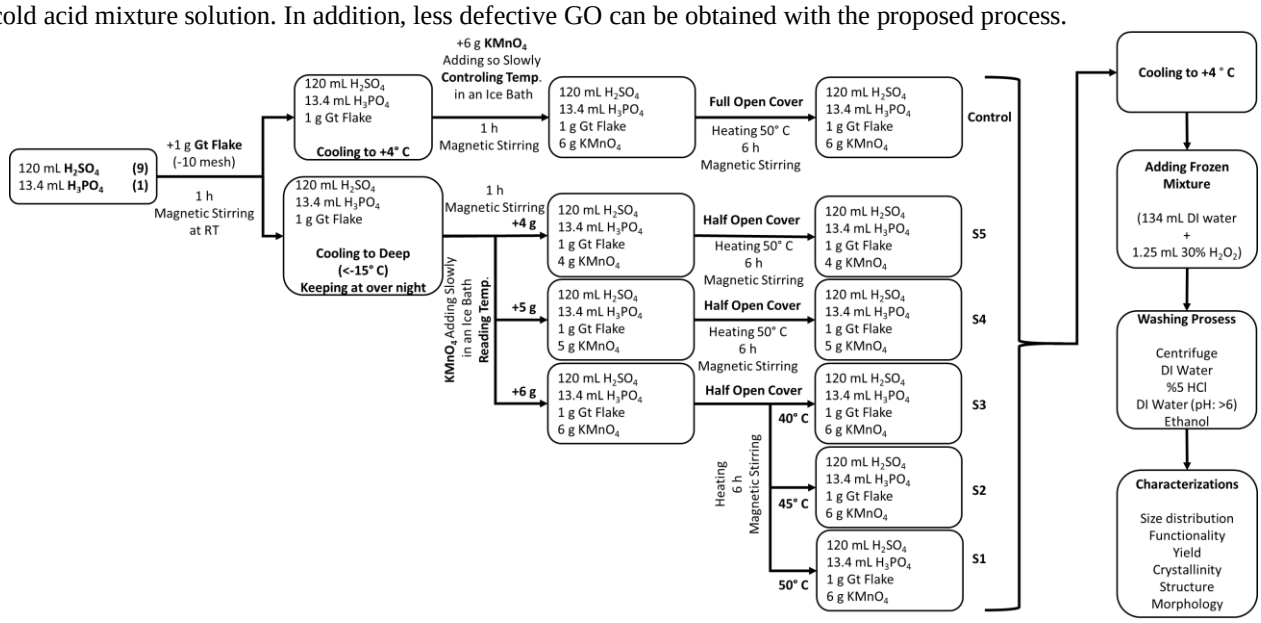
**Abstract**

Various synthetic methods have been developed for the oxidation of graphite (Gt) for the last 160 years [1]. Among them, the Hummers` method (HM) is the most common method in which $KMnO_4$, $NaNO_3$ and $H_2SO_4$ reagents are used [2]. Although many modifications are made to the HM, the formation of toxic gases such as $NO_2$ and $N_2O_4$ is unavoidable, especially at high temperatures. By using $H_3PO_4$ instead of $NaNO_3$, the source of these gases, the reaction can be carried out at low temperatures with better oxidation and less toxic gas elimination. $KMnO_4$, as in many other methods, is used as a powerful oxidizing agent in this "*improved method*" (IM) [3]. While $KMnO_4$ gives a highly exothermic reaction and produces highly reactive intermediates ($Mn_2O_7$) in concentrated $H_2SO_4$ solutions, it dissolves faster with increasing temperature. Since these two events trigger each other, temperature control is required while adding $KMnO_4$. This is essential for GO synthesis because the $KMnO_4$ in high concentrations in the acid mixture can be explosive. Therefore, researchers add $KMnO_4$ to the solution in portions in an ice bath so that the temperature does not exceed 10 degrees. This practice is highlighted in each study, even after 8 years [4].
In this study, we noticed that $KMnO_4$ could be added safely by taking advantage of the low freezing point of the acid mixture ($H_2SO_4$-$H_3PO_4$, 9:1 *v/v*). Therefore, the danger of explosion can be eliminated when $KMnO_4$ is added to the deep cold acid mixture solution. In addition, less defective GO can be obtained with the proposed process.




**Keywords:** graphene oxide (GO), more safety improved method, temperature effect.

**References:**

1. Brisebois, P.P. and M. Siaj, *Harvesting graphene oxide – years 1859 to 2019: a review of its structure, synthesis, properties and exfoliation.* Journal of Materials Chemistry C, 2020. **8**(5): p. 1517-1547.
2. Hummers, W.S. and R.E. Offeman, *Preparation of Graphitic Oxide.* Journal of the American Chemical Society, 1958. **80**(6): p. 1339-1339.
3. Marcano, D.C., et al., *Improved synthesis of graphene oxide.* ACS Nano, 2010. **4**(8): p. 4806-14.
4. Marcano, D.C., et al., *Correction to Improved Synthesis of Graphene Oxide.* ACS Nano, 2018. **12**(2): p. 2078.

## *ORAL PRESENTATION*

# The Future Perspective of Nanovesicular Transport Systems in Drug Delivery

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**Abstract**

In recent years, with the development of nanotechnology, drug research has evolved into a modified multi-featured and tasked drug design rather than the use of a single molecule as a drug. The number of publications published on this subject and the budgets allocated by governments to this subject are increasing every year. Drug molecules are used by means of a carrier molecule by encapsulating into polymers, hydrogel structures, liposomes and niosomes or directly conjugated to a nanoparticle. Thus, some critical features not found in the drug molecules are gained by nanoparticles and nanocarrier systems. The highest potential among these nanocarrier systems is a lipid or surfactant-based nanovesicular transport systems (NTS). NTS have many utility advantages, Lipophilic molecules are trapped within the lipid bilayers, while hydrophilic molecules are retained in the aqueous portions, such as increasing the circulation time and biocompatibility of the drug molecule in the blood, and enhancing their delivery efficiency, biodegradable and do not exert immune-stimulating effects. In addition, they have easily modified surface structures that allow targeted therapy, theranostic therapy and personalized treatment. As a disadvantage, drug aggregation, fusion, and leakage may occur in both liposomes and niosomes . As a conclusion, NTS, which still has some shortcomings, can overcome these handicaps with the further development of engineering and biological sciences and molecules that cannot be used as drugs due to some of their handicaps and side effects can be used by being modified nanotechnologically. With these developments, through to NTS that will be synthesized more personalized and target-sensitive, carrier selection will be among our priorities in the not-too-distant future, as well as drug selection in medical treatments.

**Keywords:** Liposome, niosome, drug delivery.

## *ORAL PRESENTATION*

# Synthesis and molecular docking studies of new schiff base ligand and complexes derived from salicylaldehyde derivate

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**Abstract**

Schiff base ligands are the best condensation products known in the class of organic and inorganic compounds. It is known as a useful ligand to its ease of synthesis, structural diversity, electronic control mechanisms, and coordinating capabilities with metals. In recent years, syntheses and biological activity studies have increased due to their use in cancer treatment and preparation of drugs with chemotherapic properties [1] and it is known that schiff bases containing aromatic amines are used as oxygen transmitters in chemotherapy [2]. These compounds, which are important in terms of biological activities, also have many bioactive features such as antibacterial, antifungal, antimalarial, antimicrobial, antibacterial, antioxidant, antitumor, antiinflammatory, antipyretic, antiulcer, antituberculosis, antiviral, and antipyretics [3]. In this study, Schiff base ligand; synthesized from the reaction of 3,5-dibromosalicylaldehyde, which is a salicylaldehyde derivative and 2-amino-4-chlorophenol in absolute ethyl alcohol, with p-toluenesulfonic acid catalysis. And then M:L ratio 1:2 complexes were prepared with metal acetates such as $Co^{+2}$, $Ni^{+2}$, $Cu^{+2}$, $Zn^{+2}$. The structures of synthesized ligand and complexes were characterized by elemental analysis, IR, $^{1}$H-NMR, $^{13}$C-NMR, UV-Vis, XRD, SEM, magnetic susceptibility, and thermogravimetric analysis methods [4] and in order to elucidate the mechanism of action of the synthesized compounds, some descriptive molecular properties were calculated using the Schrödinger Suite computer-aided molecular modeling program [5].

**Keywords:** Ligand, Metal Complex, Molecular Docking, Salicylaldehyde, Schiff Base.

**References:**

[1] Cai, L., Zhu, P., Huan, F., Wang, J., Zhou, L., Jiang, H., ... & Chen, J. (2021), Toxicity-attenuated mesoporous silica Schiff-base bonded anticancer drug complexes for chemotherapy of drug resistant cancer. Colloids and Surfaces B: Biointerfaces, 205, 111839.
[2] Sharaby CM, Amine MF, Hamed AA (2017), Synthesis, Structure Characterization and Biological Activity of Selected Metal Complexes of Sulfonamide Schiff Base as A Primary Ligand and Some Mixed Ligand Complexes with Glycine as A Secondary Ligand. Journal of Molecular Structure, 1134:208-216.
[3] Alafeefy, A. M., Bakht, M. A., Ganaie, M. A., Ansarie, M. N., El-Sayed, N. N., & Awaad, A. S. (2015), Synthesis, analgesic, anti-inflammatory and anti-ulcerogenic activities of certain novel Schiff's bases as fenamate isosteres. Bioorganic & medicinal chemistry letters, 25(2), 179-183.
[4] Tuna, S., Canpolat, E., and Kaya, M. (2006), Synthesis and Characterization of a New 4-Methoxysalicyliden-p-aminoacetophenoneoxime and Its Complexes with Co(II), Ni(II), Cu(II) and Zn(II), Polish J. of Chem., 80 (10), 1651-1656.
[5] Abdel-Rahman, L. H., Adam, M. S. S., Al-Zaqri, N., Shehata, M. R., Ahmed, H. E. S., & Mohamed, S. K. (2022), Synthesis, characterization, biological and docking studies of ZrO (II), VO (II) and Zn (II) complexes of a halogenated tetra-dentate Schiff base. Arabian Journal of Chemistry, 15(5), 103737.

## *ORAL PRESENTATION*

# Synthesis and molecular docking studies of new schiff base ligand and metal complexes

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**Abstract**

Examination of the chemical basis of the pharmacological properties and mechanism of action of inorganic and organometallic compounds is great importance for drug research [1]. It is also known that Schiff bases are widely used in the synthesis of many drugs [2]. These compounds are in the class of ligands that are used frequently because of their chelates with transition metals. Nitrogen and sulfur atoms are involved in the coordination of metals in the active sites of numerous metallo-biomolecules. In the literature, it has been reported that the binding of the drug to a metallo element increases its activity and in some cases, the complex has more curative properties than the parent drug [3]. In this study a ligand, 3,5-dinitrosalicylidene-2-amino-4-chlorophenol, was synthesized in absolute ethyl alcohol at 60 $^{o}$C under the catalysis of p-toluene sulfonic acid, and ten complexes of the ligand were prepared with M(II) acetates. Their structures were analyzed by IR, $^{1}$H-NMR, $^{13}$C-NMR, UV-Vis, XRD, SEM, magnetic susceptibility and thermogravimetric analysis methods [4]. The mechanism of action of the synthesized compounds and some descriptive molecular properties were calculated by using the Schrödinger Suite computer-aided molecular modeling program. The active region where the coupling would take place with the Receptor Grid Generation module was determined, and the interactions of the possible drug molecule candidates synthesized with the Structure-Based Drug Design (SBDD) method with the target receptor region were calculated [5].

**Keywords:** Ligand, Metal Complex, Molecular Docking.

**References:**

[1] Bakhtiar, R., & Ochiai, E. I. (1999), Pharmacological applications of inorganic complexes. General Pharmacology: The Vascular System, 32(5), 525-540.
[2] Abu-Dief, A. M., El-Khatib, R. M., Aljohani, F. S., Alzahrani, S. O., Mahran, A., Khalifa, M. E., & El-Metwaly, N. M. (2021), Synthesis and intensive characterization for novel Zn (II), Pd (II), Cr (III) and VO (II)-Schiff base complexes; DNA-interaction, DFT, drug-likeness and molecular docking studies. Journal of Molecular Structure, 1242, 130693.
[3] Tyagi, P., Chandra, S., Saraswat, B. S., & Yadav, D. (2015), Design, spectral characterization, thermal, DFT studies and anticancer cell line activities of Co (II), Ni (II) and Cu (II) complexes of Schiff bases derived from 4-amino-5-(pyridin-4-yl)-4H-1, 2, 4-triazole-3-thiol. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 145, 155-164.
[4] Tuna, S., Canpolat, E., and Kaya, M. (2006), Synthesis and Characterization of a New 4-Methoxysalicyliden-p-aminoacetophenoneoxime and Its Complexes with Co(II), Ni(II), Cu(II) and Zn(II), Polish J. of Chem., 80 (10), 1651-1656.
[5] Abdel-Rahman, L. H., Adam, M. S. S., Al-Zaqri, N., Shehata, M. R., Ahmed, H. E. S., & Mohamed, S. K. (2022), Synthesis, characterization, biological and docking studies of ZrO (II), VO (II) and Zn (II) complexes of a halogenated tetra-dentate Schiff base. Arabian Journal of Chemistry, 15(5), 103737.

## *ORAL PRESENTATION*

# Apoptotic Effect of Adipose Tissue-Derived Extracellular Vesicles on Breast Cancer Cells

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**Abstract**

Breast cancer is the most common global malignancy and the second leading cause of cancer death. Although different treatment methods are used, no treatment can eradicate cancer yet and also, it is known that healthy cells can be damaged in cancer treatment with chemotherapy or radiotherapy, which are current treatment methods. Therefore, it is necessary to find that personalized treatment models do not have side effects. Exosomes are natural nanoparticles and are also known as small extracellular vesicles which play important roles in intercellular communication, containing a wide variety of lipids, proteins, metabolites, RNAs and DNAs. This study aimed to investigate the apoptotic effects of exosomes obtained from commercial adipose tissue-derived exosomes (AT-Exo) by precipitation method on human breast cancer MCF-7 and breast epithelial MCF10A at different concentrations by using Annexin V & Dead Cell method. Early apoptotic, late apoptotic, live and dead cells have been evaluated by Annexin V-FITC/PI staining in both cell lines and have found that the percentage of total apoptotic 58.817±4.2%, 56.37±9%, 70.9±4.1% in the $40x10^{10}$, $20x10^{10}$ ve $10x10^{10}$ AT-Exo/mL groups for 24h, 48h and 72h respectively. When MCF-7 cell groups given AT-Exo have been compared both among themselves and with MCF-7 and MCF10A cell groups not given AT-Exo, the difference has been statically significant depending on time and dose ($p<0,05$). MCF10A and MCF-7 control groups have been evaluated, and no significant difference has been observed ($p>0,05$). In conclusion, exosomes may have the potential capacity in cancer therapy by inducing apoptosis in cancer cells.

**Keywords:** Breast Cancer, Adipose Tissue-Derived Exosomes, Precipitation Method, Apoptosis.

## *ORAL PRESENTATION*

# Yüzeyde güçlendirilmiş Raman spektroskopisi (SERS) tekniğinin çeşitli ilaçlardaki dopamin düzeyinin tayininde kullanımı

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**Özet**

Nanoteknoloji; kimyasal, fiziksel, biyolojik, elektronik, elektriksel, mekanik, manyetik, termal, dielektrik ve optik gibi alanlarda çeşitli karakteristik özelliklerinden dolayı son yıllarda yoğun bir şekilde ilgi odağıdır. Dopamin, psikolojik, kardiyolojik ve yaşlılık dönemi rahatsızlıklarının (Parkinson, Alzheimer vb.) gecikmesi ya da oluşumunun önüne geçilmesi için önem arz eden bir nörotransmiter olup kanda ve vücutta tespiti amacı ile kullanılan uzun, zaman alıcı ve eziyetli olan kan testleri, çeşitli laboratuvar testleri, MR, EMG, uyku EEG'si gibi literatürde kullanılan yöntemleri bilinmektedir. Bu yöntemlere alternatif bir yöntemle dopaminin basit tayin edilmesi önemli bir ihtiyaç olarak ortaya çıkmaktadır. Hastalık teşhisinde nanomalzeme kullanımı hızlı ve kolay tespit imkanı sunmaktadır. Bu nedenle bu çalışmada, hidrotermal yöntem kullanılarak altın ve gümüş kaplı yüzeyler üzerinde Ce(IV) katkılanmış $TiO_2$ nanopartikülleri sentezlenerek, FT-IR, SEM ve EDX ile karakterize edilmiştir.
Yüzeyde güçlendirilmiş Raman spektroskopisi (SERS), gerçek örneklerdeki analitlerin düşük konsantrasyonlarının belirlenebilmesini sağlayan güçlü bir tekniktir. SERS tekniğinin uygulanmasında yaygın olarak kolloidal nanopartiküllerden ve katı yüzeylerde oluşturulan plazmonik SERS temelli platformlardan faydalanılmaktadır.
Çalışmada elde edilen sonuçlar üzerinden dopaminin çeşitli ilaçlardaki miktarının belirlenmesine yönelik sentezlenen nanopartikülleri kullanılarak, SERS temelli bir yöntem geliştirilmesinde model çalışmalar tamamlanmıştır. Çalışmanın şuan gerçek ilaç örnekleri üzerindeki uygulamaları devam etmektedir.

**Anahtar Kelimeler:** Yüzeyde zenginleştirilmiş Raman spektroskopisi (SERS), dopamin, hidrotermal sentez.

## *ORAL PRESENTATION*

# Evaluation of insecticidal and enzyme activity potentials of essential oils and extracts of *Chenopodium botrys* against storage products pests

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## Abstract

The essential oil obtained from the aerial part of *Chenopodium botrys* plant by steam distillation was obtained. The remaining aqueous part was extracted with the solvent ethylacetate and n-butanol, respectively. In the end, five extracts were obtained: essential oil (CB-EO), untreated aqueous extract (CB-F), ethylacetate (CB-EA), n-butanol (CB-nBu) and processed aqueous extract (CB-L). The effects of five extracts on both insecticidal (against *Sitophilus granarius* and *Tribolium castaneum*) and enzyme activities (acetylcholinesterase, xanthine oxidase (XO) and tyrosinase) were studied. It has been determined that CB-F extract has an activation effect against tyrosinase enzyme. At the end of 48 hours, CB-EA extract was determined to cause 20% death against the adult insects of *Sitophilus granarius* as a result of contact toxicity test. As a result of GC-MS analysis of essential oil, α-Eudesmol compound was analyzed as the main component.

**Keywords:** *Chenopodium botrys*, Insecticidal activity, Enzyme activity, Essential oils, Phenolic compounds

## *ORAL PRESENTATION*

# Conjugated polymer based amperometric biosensor for glucose detection in real samples

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**Abstract**

There has been growing interest in conducting polymers (CPs) due to a wide range of potential applications in recent years. Special attention has been given to practical applications of CPs as a new class of electroactive materials for technological applications. Conjugated polymer-based biosensors bring simple, accurate, reliable and low-cost determination of various analytes and act as a very effective analytical tool in the food quality control. Since sensor performance largely depends on surface properties, herein, conjugated polymer was used to enhance biosensor performance. In this work a novel approach was reported for glucose detection using conducting polymer and glucose oxidase (GOx). This scaffold was used as the immobilization matrix for the glucose biosensor. The biosensor response at a potential of -0.7 V versus Ag wire was followed by the decrease in oxygen level as a result of enzymatic reaction. Analytical performances of the biosensor were identified by evaluating the parameters of KMapp, Imax, limit of detection, sensitivity and linear range values. Surface characteristics of the biosensor were also evaluated using SEM and CV techniques. The constructed biosensor was applied to determine the glucose content in several beverages.

**Keywords:** GOx, Conjugated Polymer, Electrochemical Biosensor, Glucose, Enzyme Immobilization.

## *ORAL PRESENTATION*

# Synthesis and Characterization of Hydrolyzed Polyoxazolines and Investigation of Their Curing Capabilities

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**Abstract**

Polyoxazoline (POZ) polymers have been gaining ground vastly recently with expanding application range from drug delivery systems to biosensors. This is due to the remarkable tunability and flexibility that these polymers offer in terms of ease of synthesis, thermal properties, hydrophilicity, hydrophobicity and functional groups. Polyoxazoline-Polyethyleneimine (POZ-PEI) copolymers, resulting from hydrolysis of POZ polymers, show curing capabilities with adjustable curing temperature. These make this family of polymers a suitable candidate for use in one-component epoxy resin systems as thermal latent curing agents where both shelf-life and controlled curing temperature are a defining character. In this article, POZ-PEI based curing agents were synthesized as thermal latent curing agents for one-component epoxy resin systems. In the first step, different POZ polymers with alkyl/aryl pendant groups were synthesized and then hydrolyzed to result in POZ-PEI copolymers with preferred compositions. $^{1}$H-NMR, FTIR, GPC, TGA and DSC analyses were used to determine the structural and thermal characteristics of the prepared polymers and following copolymers. Also, for curing studies, DGEBA was chosen as the epoxy resin and DSC analysis was used to determine the curing behavior of curing agent-epoxy resin systems. We investigated the kinetics of POZ hydrolysis to fine-tune the composition of the POZ-PEI copolymers. In addition, the effect of the molecular weight, composition and side chain groups on the curing behavior of epoxy resin systems was studied.

**Keywords:** Polyoxazoline, Polyethyleneimine, Hydrolysis, Curing.

## *ORAL PRESENTATION*

# Contributions to the predaceous diving beetles (Coleoptera: Dytiscidae) fauna of Batman Province, Türkiye

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**Abstract**

This study was carried out in Batman province between September 2018 and June 2019. By reweaving the previous studies it is understood that only one study has been performed with regard of the determination of the predaceous diving beetles (Dytiscidae) of Batman Province, which is located in the Southeastern Anatolian Region of Türkiye. A total of 23 species were collected and identified with this study. 20 of identified species were new records for Batman province. The aim of the study was to determine the diving beetles fauna of our country (Türkiye) in general and Batman province particularly. Specimens were collected from freshwater habitats in Batman province using nets with meshes diameters of 0.5-1 mm. Specimens were fixed and preserved in 70% ethyl alcohol solution at spot and bring to laboratory. The clay and muddy substances on their surfaces were brushed off with a small paintbrush in the laboratory and puts in new bottles with 70% alcohol. The collecting information's of locality were labeled on the bottles. The identification of the specimens was made according to the previous studies using the stereo microscope (Leica S8 APO). The examined materials have been deposited in the private collection of the corresponding author at the Dicle University, Ziya Gökalp Education Faculty, Diyarbakır. The aquatic Coleoptera fauna of our country has not been fully determined. According to the previous studies, up to now about 40 provinces of Türkiye have been studied with this regard. With the further studies in all provinces of Türkiye the diversity of aquatic beetles will be defined in more detailed and with the new species that can be identified trough these studies the diversity of the aquatic beetles of the world will be increase.

**Keywords:** Diving beetles, Aquatic Coleoptera, Fauna, Batman, Türkiye.

## *ORAL PRESENTATION*

# The Nutrition Value of Aquatic Products

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**Abstract**

Total fisheries and aquaculture production has been developed. It has been approximently 214 million in 2020. Personal consumption has been about 22 kg/year. Fish is a very rich food with multiple of high quality amino acids, omega-3-6 fatty acids (DHA and EPA), minerals and vitamins such as vitamins A, D, E, and B (thiamin, niacin, $B_2$, B6 and B12) and, calcium, phosphorus iron, zinc, iodine, magnesium, selenium and potassium minerals. Besindes, AMPK, NMN, Taurine, Ferritin, etc. They supply human body to support the their body functions. Additionally, Fish meat offers a healthy meal to human with high quality protein and various minerals and vitamins and low calories (<20 %) as energy source. Even their protecting or healing effects of fish oil against diseases. Omega -3 fatty acids are good for heart health, reduce of blood thickness by high triglycerides and smoothing the electrical activity of heart. In this study, it is going to elucidate on the benefits of vitamins, minerals, derivatives from eating fish and other aquatic products and indicate the previous studies have been done about the importances of fish and others.

**Keywords:** Aquatic products, Fish, Vitamins, Minerals.

## *ORAL PRESENTATION*

# Association of a Two Marker Haplotype in Neuregulin 1 Gene with Schizophrenia in a Turkish Sample

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**Abstract**

NRG1 is one of the earliest genes that reported to have single nucleotide polymorphisms (SNPs) and haplotypes associated with schizophrenia. In this study, we investigated the associations of haplotypes constructed by the NRG1 SNPs rs4560751 and rs3802160 with Schizophrenia.
We collected DNA samples of 96 patients diagnosed with schizophrenia and 100 healthy controls living in Malatya-Turkey. We analyzed the SNP genotyping results of patients and controls. We estimated the haplotypes by Haploview software and determined the diplotypes of appropriate samples using a spread sheet program. We compared two groups for distributions of haplotypes and diplotypes and tested the statistical significances of genetic associations with Pearson's Chi-Squared method.
The Haploview software estimated 4 different haplotypes and we determined 7 diplotypes which were diploid combinations of those haplotypes. While the distributions of most haplotypes did not show a significant difference between the case and control groups, in the rs4560751-rs3802160 haplotype frame, the T-A haplotype was more frequent in the controls (P=0.02) and G-A/T-A diplotype was seen only in 16 control samples indicating negative associations. Whereas G-A/G-A diplotype was more frequent in the patients (0.03), hence seen positively associated with schizophrenia.
We found one haplotype and one diplotype negatively associated, but one diplotype positively associated with schizophrenia. Since our sample was collected from a limited geographic area, our results may not reflect the whole population of Turkey. Also, the associations we reported here need to be confirmed in different populations to increase the accuracy of our observations because of the complex-multifactorial nature of schizophrenia.
This study has been supported by The Unit of Scientific Research Projects at İnonu University. Project Number: FYL-2021-2450 to Mustafa Mert SÖZEN.

**Keywords:** Schizophrenia, Genetics, Association, NRG1, Neuregulin-1, Haplotype, Turkey.

## *ORAL PRESENTATION*

# Analytical Comparison of CEX Fractions Collected from a Novel Biosimilar Candidate and Its Originator

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**Abstract**

Bevacizumab is a humanized recombinant monoclonal antibody used to treat cancer by targeting VEGF-A to inhibit angiogenesis. A novel bevacizumab biosimilar candidate is fractionated into its acidic and basic charge variants by the CEX method for further structural and functional analysis. The originator product, Avastin, is used as a reference for the comparative study. UPLC-ESI-QToF was used to analyze the modifications leading to charge heterogeneities at intact protein and peptide levels. The C-terminal lysine clipping, and glycosylation profiles of the samples were monitored by intact mAb analysis. The posttranslational modifications, including oxidation, deamidation, and N-terminal pyroglutamic acid formation, were determined by peptide mapping analysis in the selected signal peptides. The relative binding affinities of the fractionated charge isoforms against the antigen, VEGF-A, and the neonatal receptor, FcRn, were revealed by SPR studies. The results show that all CEX fractions from the reference product and the innovator share common glycoforms and posttranslational modifications but at different ratios for some samples. Additionally, the charge variants' binding activity to the antigen and the neonatal receptor molecules varies amongst the samples.

**Keywords**: Biosimilars, biotechnology, CEX, bevacizumab.

## *ORAL PRESENTATION*

# The Impact of Cellulose Pretreated by Tempo on the Properties of CMC/Cellulose Hydrogels Crosslinked by Epichlorohydrin

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**Abstract**

The purpose of the research was to measure the water-absorbing capacity of CMC/Cellulose based hydrogels crosslinked by epichlorohydrin after Tempo oxidation of cellulose. Cellulose and carboxymethylcellulose were mixed in various proportions (10/90, 30/70 and 50/50%, respectively) to produce hydrogels. The hydrogel, produced from cellulose (10%) and carboxymethylcelulose (90%), was demonstrated to have the maximum water absorption capacity in water (2479%) at a pH:7. The absorption behavior of the hydrogel in salt solutions was also examined and the highest value was found as 1746% in $NH_4Cl_2$ solution. The structural and surface properties of the hydrogel were examined by FTIR-ATR and SEM, respectively. It was revealed that the reduction in the hydrogel properties, such as swelling and water absorption, resulting from an increase in cellulose content could be minimized by pretreated cellulose reinforcement.

**Keywords:** Cellulose, Hydrogel, Carboxymethylcellulose, Tempo oxidation.

## *ORAL PRESENTATION*

# Synthesis and cytotoxicity evaluation of novel triazolyl-isatin derivatives

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**Abstract**

Isatin derivatives containing 1,2,4-triazole moiety have been reported to show wide range of biological activities including anticancer. In the present work, novel triazolyl-isatin derivatives have been synthesized and screened for their cytotoxic activity toward T98, human glioblastoma cell line. The structures of the synthesized compounds were characterized by by FT-IR, $^{1}$H NMR, $^{13}$C NMR, elemental analysis and mass spectrometry. The cell viabilities of the tested compounds were measured by MTT assay method. Different concentrations of the synthesized compounds (5, 10, 25, 50 and 100 μM) were incubated for 24h and 48h, respectively. Our results revealed that the presence of electron-withdrawing nitro group at position-5 of the isatin core structure played a significant role in the cytotoxic activity of the synthesized compounds. Among all concentrations of triazolyl-isatin derivatives, 1-[3-(1H-1,2,4-Triazol-1-yl)propyl]-5-nitro-3-[2-(perfluoro-phenyl)hydrazono]indolin-2-one was found to be the most active compound and an $IC_{50}$ value of 189 μM concentration for 24 h incubation period. It could be concluded 1-[3-(1H-1,2,4-Triazol-1-yl)propyl]-5-nitro-3-[2-(perfluoro-phenyl)hydrazono]indolin-2-one increase potential anti-cancer effects and they could be promising novel therapeutic agents for glioblastoma.

**Keywords:** 1,2,4-Triazole, cytotoxicity, isatin, triazolylisatin, brain glioblastoma.

## *ORAL PRESENTATION*

# Valorisation of chicken feet by- products: fabrication of gelatine

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**Abstract**

The valorisation of by-products has become a current requirement for economic and environmental reasons. Poultry slaughterhouses generate as many by-products as red meat industries, especially with the increase of chicken consumption. Our objective is to optimize a protocol for the extraction of gelatine from chicken feet. Fourteen samples of chicken feet were used for gelatine extraction under different conditions (concentrations, time, temperature). The yields of each condition were calculated, and some techno-functional analyses were performed: foaming power, emulsifying power, and viscosity. The Box benken design was used to optimize the extraction parameters including acid acetic concentration (2, 3.5 and 5%), extraction temperature (55, 65 and 75 °C) and extraction time (2, 4 and 6 h). The optimization by response surface method (RSM), Predicted responses for these extraction conditions included: yield of 11.95%, EAI of 32.34$m^2$ $g^{-1}$, viscosity of 4.074 mP. s, and foaming capacity of 173.76%. The optimum extraction parameters were 3.5 %of acetic acid ,74.99°C and for 4.4h. The results showed that chicken feet can be an alternative source of gelatine mammalian.

**Keywords:** feet, Chicken by-product, Gelatine, Optimization, Rsm.

## *ORAL PRESENTATION*

# 2-Metil Naftalinden 2,6 Dimetil Naftalin Üretimine H-Mordenit Katalizörünün Etkisi

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**Özet**

PEN, yüksek oksijen direnci nedeniyle ambalaj sanayinde ve yüksek sıcaklık direnci nedeniyle organik hafıza kartı gibi elektrik ve elektronik teknolojisinde geniş uygulama alanına sahip olmasına rağmen yüksek maliyet sebebiyle istenildiği gibi yaygın kullanıma sahip değildir. PEN'in üretim maliyetinde 2,6-DMN (2,6 Dimetilnaftalin)'nin üretim maliyetinin çok önemli bir yeri vardır. Bunun nedeni ise çıkış maddesi olan 2,6-DMN'in üretim proseslerinin karmaşık, üretim atıklarının fazla ve üretim maliyetinin yüksek olmasıdır. Bu yüzden, 2,6-DMN'in daha az reaksiyon içeren, daha az atıklı, basit ve temiz sentezi ve yüksek seçimlikte üretimi konusunda yoğun araştırmalar yapılmaktadır.
Bu çalışmada 2,6-DMN seçimliliğini yapısal özellikleri sebebiyle destekleyen zeolit tipi H-Mordenit katalizörü üzerinde yapılmıştır. PEN üretiminde önemli bir bileşik olan 2,6-DMN'nin seçimli üretiminin 2-MN'in metilasyonu ile gerçekleşmesi için katalizör geliştirilmesi hedeflenmiştir. Mordenit katalizörünün literatürde 2-MN'den metilasyonla 2,6 DMN üretimi reaksiyonu üzerinde kullanıldığı bir çalışma olmadığından çalışma özgündür.
Bu çalışma ile atık durumundaki (daha değersiz-çok bulunan) ürünlerden daha değerli ürünlerin elde edilmesi, yeni ürünün seçimli olarak daha kısa bir yöntemle (tek reaksiyonla) sağlanabilmesi, çevre konusunda artan duyarlılıklar sebebiyle kaynakların verimli kullanılması konusunda çaba gösterilmesi, ticari ve stratejik bir öneme sahip ürünlerin üretiminin sürekli sağlanması, daha uygun fiyata üretilen ürün sebebiyle piyasa rekabet gücünün arttırılması sağlanacaktır.
Bu çalışmada, H-Mordenit zeolit katalizörü üzerinde 2-MN'nin metanol ile metilasyonu ile 2,6 DMN üretimi amaçlanmıştır. Bu amaç doğrultusunda Alfa Aesar firmasından temin edilen Mordenit (500m2, Si/Al=20) katalizörü pelletlenerek hazırlanıp, H-Mordenit formuna getirilmiş ve reaksiyon sabit yataklı sürekli bir sistemde gerçekleştirilmiştir. Farklı sıcaklık ve boşluk hızlarında (WHSV) çalışmalar yapılmış ve elde edilen sıvı ürünler GC-MS ile analiz edilmiştir.

**Anahtar Kelimeler:** 2,6- Dimetil Naftalin, Metilasyon, 2-Metil Naftalin, Mordenit.

## *ORAL PRESENTATION*

# Investigation of Biodiesel production from Sunflower oil via SrO catalyst

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**Abstract**

As a consequence of increasing energy prices, climatic variations, and environmental degradation, it is anticipated a gradual conversion from the existing fossil fuels to bio-based energy consumption system occurs, and bringing a post-petroleum era. Biodiesel derived from living resources, such as vegetable oils is a non-polluting fuel and considered the most environmentally friendly fuels. Transesterification reaction of triacylglycerol (TG) in animal fats and vegetable oil with short alcoholic chain catalyzed by heterogeneous or homogeneous catalysts used commonly to produce biodiesel. The multiple drawbacks of homogenous catalyst, including high energy consumption, soap formation in high free fatty acid (FFA) content oil, catalyst isolation, high amount of wastewater generation during washing in the transesterification process, and corrosion issues can overcome by heterogeneous catalyst. In this study, the heterogeneous transesterification of sunflower oil to biodiesel over SrO catalyst and the process optimization were investigated. The catalyst was prepared from Sr $(NO_3)_2$ by precipitation method and the highest active catalyst was derived from the nitrate precursor after calcination at 800 $^oC$. Characterization of catalyst was carried out by using Fourier transform-infrared spectroscopy (FT-IR) and Thermogravimetric analyzer (TGA) method. The experimental evaluation of the prepared catalyst was conducted by tranestrification reaction at various conditions like reaction time, temperature, catalyst wt%, methanol/oil molar ratio. According to the results, a 94.4% conversion of fatty acid ethyl ester could be produced in 30 minutes under the optimal reaction conditions of 1 wt.% catalyst loading, 6:1 molar ratio of methanol to oil, and 65 °C. These results indicated that the produced Biodiesel confirmed by (FT-IR) test with SrO heterogeneous catalysts can be successfully applied commercially.

**Keywords:** Biodiesel, Stronium Oxide, Transesterification, $Sr(NO_3)_2$, Catalyst.

## *ORAL PRESENTATION*

# Effect of high dose dimethyl sulfoxide on the brain

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**Abstract**

Dimethyl sulfoxide (DMSO) is currently used as an alternative treatment for a variety of inflammatory conditions. Despite its widespread use, data on its safety and efficacy and its mechanism of action in human cells are insufficient. In this study, inflammatory cytokines from rat brain tissues after subcutaneous injection of 17% DMSO; IL-1, IL-6 and IL-33 were analyzed. In addition, MDA levels were analyzed to demonstrate lipid peroxidation, monoamino oxidase and superoxide dismutase enzyme activities were also measured. Interestingly, IL-1 level was statistically decreased in the 17% DMSO group compared to the control group (***p=0.0007). MDA level increased 17% in the DMSO group compared to the control group (**p=0.0049). Considering that the molecular mechanism caused by DMSO has not yet been fully elucidated, it was concluded that MDA may contribute to the damage caused by DMSO at the cellular dimension. Further studies are needed for changes in interleukins.

**Keywords:** Dimethyl sulfoxide, brain, inflammatory cytokines, MDA.

## *ORAL PRESENTATION*

# Synthesis, Photophysical and Photochemical Properties of Piperazine Substituted Indium(III) Phthalocyanines

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**Abstract**

Photodynamic therapy (PDT) is a combined cancer treatment using capable of generating singlet oxygen following excitation of a photosensitizer with light of a specific wavelength (Motloung et al., 2019). Phthalocyanine compounds are used as photosensitizers in PDT due to their high singlet oxygen quantum yield, high stability, and high triplet quantum state efficiency (Özdemir et al., 2019). The photophysical and photochemical properties of phthalocyanine compounds are affected by the nature of the central metal ion. Since indium(III) metal is diamagnetic and able to host axial substituent, it is one of the central atoms used to improve the photophysical and photochemical properties of phthalocyanines (Çapkın et al., 2020). In addition, piperazine is one of the heterocyclic derivatives used in the development of new drug candidates due to its wide pharmacological activity (Khezami et al., 2021).

In this work, the novel tetra- and octa-piperazine substituted indium(III) phthalocyanine compounds were synthesized and characterized by using standard spectroscopic techniques such as FT-IR, MALDI-TOF, $^{1}$H-NMR and UV-Vis. To investigate the usability in PDT, the photophysical and photochemical properties novel indium(III) phthalocyanine compounds containing different numbers of piperazine groups on the macrocycle and coordinated with iodine atom as axially ligand on the central indium(III) atom were studied in this work.

**Keywords:** Photodynamic therapy, indium(III) phthalocyanine, piperazine group.

**References:**

Çapkın A, Pişkin M, Durmuş M, Bulut M 2020. Spectroscopic, photophysical and photochemical properties of newly metallo-phthalocyanines containing coumarin derivative. Journal of Molecular Structure, 1213, 128145.

Khezami K, Harmandar K, Bağda E, Bağda E, Şahin G, Karakodak N, Durmuş M 2021. BSA/DNA binding behavior and the photophysicochemical properties of novel water soluble zinc (II) phthalocyanines directly substituted with piperazine groups. JBIC Journal of Biological Inorganic Chemistry, 26 (4), 455-465.

Motloung BM, Sekhosana KE, Managa M, Prinsloo E, Nyokong T 2019. The photophysicochemical properties and photodynamic therapy activity of phenyldiazenyl phenoxy substituted phthalocyanines when incorporated into Pluronic® F127 micelles. Polyhedron, 174, 114157.

Özdemir M, Karapınar B, Yalçın B, Salan Ü, Durmuş M, Bulut M 2019. Synthesis and characterization of novel 7-oxy-3-ethyl-6-hexyl-4-methylcoumarin substituted metallo phthalocyanines and investigation of their photophysical and photochemical properties. Dalton Transactions, 48 (34), 13046-13056.

## *ORAL PRESENTATION*

# Recent advances in the use of CRISPR in cancer treatment

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**Abstract**

Mutations in genes are the main cause of cancer. Scientists use different methods to correct these changes in genes. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) can manipulate the DNA of human cells like gene editing tool. CRISPR, which is an immune response created by bacteria against viruses, has many uses today. One of them is for the treatment of cancer, and studies have focused on editing mutated genes or eliminating carcinogenic viruses. This system combines the CRISPR with DNA sequences and a set of Caspase proteins to identify and destroy invading viral DNA and mutated genes. There are two basic parts of CRISPR, the guide RNA that will lead to the bind desired DNA, and the highly preferred DNA cutting enzyme called Cas9. Guide RNA was designed to reflect the DNA of the gene (called the target) to be edited and leads Cas9 to the target. When the CRISPR-bound guide RNA matches the DNA of the target gene, the Cas9 enzyme cuts the desired part of DNA. The common-investigated CRISPR/Cas systems for cancer therapy are almost based on the nucleases including Cas9, Cas12a, Cas13a and their orthologues (1). As a conclusion CRISPR/Cas9 method in cancer research, has new perspective in treatment. Cancer immunotherapy has many advantages over traditional therapy as let the targeted, low cytotoxicity, fast and individual cure. CRISPR-Cas9 has shown great promise for cancer treatment in cancer immunotherapy, manipulation of cancer genome and epigenome and elimination or inactivation of carcinogenic viral infections (2). It might be possible to directly target cancer cells, offering the possibility to eliminate the genetic alterations leading to tumor proliferation and/or metastatic capacity (3). The newly discovered Caspase proteins and enzymes will allow not only the treatment of cancer, but also the elimination of many genetic diseases in the future.

**Keywords:** CRISPR, cancer, immunotherapy, Cas9.

**References:**

1. Song, X., Liu, C., Wang, N., Huang, H., He, S., Gong, C., & Wei, Y. (2021). Delivery of CRISPR/Cas systems for cancer gene therapy and immunotherapy. Advanced drug delivery reviews, 168, 158-180.
2. Cheng, X., Fan, S., Wen, C., & Du, X. (2020). CRISPR/Cas9 for cancer treatment: technology, clinical applications and challenges. Briefings in Functional Genomics, 19(3), 209-214.
3. Martinez-Lage, M., Puig-Serra, P., Menendez, P., Torres-Ruiz, R., & Rodriguez-Perales, S. (2018). CRISPR/Cas9 for cancer therapy: hopes and challenges. Biomedicines, 6(4), 105.

## *ORAL PRESENTATION*

# Responses of pollen performance in olive cultivars to low and high temperature stress

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**Abstract**

In this study, responses of pollen performance in four olive cultivars (Gemlik, Deli, Domat, Çelebi) to low (4 °C) and high (30 °C) temperature stress were determined. The pollen grains were incubated in glass Petri dishes at low (4 °C) and high (30 °C) temperature conditions with 50% relative humidity for 3 h. Control pollen grains were kept at 20 °C with 50% relative humidity for 3h. The results showed no significant difference in pollen germination rate in Gemlik, Domat, and Çelebi after low and high-temperature stress, when compared with the control. However, the pollen germination rate significantly decreased by 24 % after low-temperature stress while the pollen germination rate increased by 38 % after high temperature stress when compared to control. Also, the results showed no significant difference in pollen tube length in Deli and Çelebi after low and high-temperature stress, when compared with the control. However, pollen tube length significantly decreased by 14 % after low-temperature stress while decreased by 17 % after high temperature stress, when compared to control. Also, tube length significantly decreased by 36 % after low-temperature stress and decreased by 12 % after high temperature stress, when compared to control. To determine the relative sensitivity of the different cultivars to low and high temperature stresses, the cumulative stress response index of each group was calculated. According to the cumulative stress response index, Gemlik and Domat were determined as sensitive while Deli and Çelebi were determined as intermediate at low temperature stress. Also, Domat and Çelebi were determined as sensitive while Gemlik were determined as intermediate at high temperature stress. Moreover, Deli was determined as tolerant at high temperature stress.

**Keywords:** cultivars, olive, pollen germination, pollen tube growth, temperature stress.

## *ORAL PRESENTATION*

# *In vitro* Antibacterial Activity of Various Types of Extracts of Streptomyces Isolated from Rhizosphere of the Local Endemic Plants Against Several Plant Pathogenic Bacteria

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**Abstract**

With the increase of antimicrobial resistance in both human and plant pathogenic bacteria, the search for new antimicrobial compounds is gaining speed. Terrestrial actinomycetes are one of the important sources for the discovery of new bioactive compounds. In this study, 6 local Streptomyces isolates (AA50, AS41, BA2, BS40, CA17 and CAH29) isolated and diagnosed previously were grown on 3 different modified media (GYM, TAS, TKN) and metabolite extracts obtained using different solvents were used to investigate *in vitro* antimicrobial effect on different plant pathogenic bacteria. *Pseudomonas syringae pv. lachrymans* (Psl), *Xanthomonas axonopodis pv. phaseoli* (Xap) and *Clavibacter michiganensis ssp. michiganensis* (Cmm) plant pathogens were used as test organisms. Although the antimicrobial activities of microbial organic extracts varied according to the tested organisms and the method used to obtain the extracts, the most effective results were obtained with the ethylacetate method by growing the BA2 isolate in TAS medium. This extract was the most successful with inhibition zones of 20-25 mm on Xap pathogen, 38-42 mm on Cmm pathogen and 23-25 mm on Psl pathogen.

**Keywords:** Streptomyces, Antimicrobial Effect, Plant Pathogen.

**Acknowledgments:** This study is partially supported by Dicle University Research Foundation Project No. FEN.17.016.

## *ORAL PRESENTATION*

# Süt sığırı rasyonuna yağ asit ilavesinin reprodüktif sistem üzerine etkisi

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**Özet**

Günümüzde sütçü sığır işletmelerinde artan süt verimiyle birlikte infertilite problemlerinin de arttığı bilinmektedir. Fertilite sürünün devamını sağlarken, infertilite sürüdeki hayvanların ayıklanma sebeplerinden birini oluşturmaktadır. Fertilitenin arttırılması için yapılması gereken hususlar içinde, geç laktasyon döneminin sonlandırılması ve kuru döneme ayrılması ile başlayan dönemin en iyi şekilde yönetilmesi yer almaktadır. Ayrıca, doğum sonrası süreçte artan süt verimiyle birlikte hayvanlarda meydana gelen negatif enerji dengesi (NED) infertiliteye yol açabilmektedir. Bu dönemin en iyi şekilde yönetilmesi, işletmeye yönelik dengeli rasyonların ve iyi yönetim uygulamalarının sağlanmasıyla mümkün olabilmektedir. Süt sığırı işletmelerinde enerji durumunu ve dolayısıyla üreme performansını iyileştirmeye yardımcı olmak için rasyona yağ ve yağ asitleri ilave edilmektedir. Ruminantlarda rumende olası değişiklikleri önlemek için rasyonda genellikle doymuş yağlar veya by-pass yağlar kullanılmaktadır. Bununla birlikte, uzun zincirli yağ asitlerinin (LCFA), özellikle çoklu doymamış yağ asitlerinin (PUFA) üreme performansını iyileştirdiği öne sürülmüştür. Bu nedenle, süt ineklerinde infertilitede meydana gelen artışa bakıldığında, üreme performansını iyileştirmenin bir yolu olarak rasyona PUFA katılarak desteklenmesi olasılığı büyük ilgi uyandırmıştır. İn vivo çalışmalar, rasyonda kullanılan yağ asitleri profilini değiştirmenin laktasyondaki süt ineklerinde döllenmeyi ve embriyo kalitesini etkileyebileceğini göstermektedir. Ayrıca, sütçü sığırlarda rasyona katılan yağ asitleri immun sistemde, kan pıhtılaşması, enzim aktiviteleri, hücre ve hücre membranlarının oluşumunda da etkilidir. Linoleik asit (C18:2 ω-6), sığırlar tarafından sentezlenemeyen ancak üreme performansını olumlu etkileyen ve bölünmeye yardımcı olabilen esansiyel bir yağ asididir. Diğer bir yağ asiti olan linolenik asitin (C18:3 ω-3) ise, yumurta kalitesi ve embriyo yaşam gücü üzerinde olumlu etkisi olduğu bilinmektedir. Sığırlarda üreme döneminde rasyona lüteolitik etkisi olmayan progesteron sentezini arttırabilen, Ω3 yağ asit düzeyini arttırabilecek katkı maddeleri eklenebilir. Prostaglandin-F2α' da azalma ya da bozulma, gebeliğin tanınması için embriyodan interferon-tau (gebeliğin tanınmasında) üretimi için zaman kazandırır. Sığır rasyonlarına Ω3 yağ asidi ilavesi endometrial prostaglandin-F2α salınımını azaltabilir. Ayrıca, rasyona balık yağı ilaveleri prostaglandin-F2α sentezini azaltıp ve üremeye katkı sağlayabilir. Bu çalışma, süt sığırları işletmelerinde rasyona yağ asitleri ilavesinin fertilite üzerine etkilerini belirlemek amacıyla yapılmıştır.

**Anahtar Kelimeler:** Süt sığırı, yağ asidi, fertilite.

## *ORAL PRESENTATION*

# Effects of nanoparticles ($Al_2O_3$, CuO, $TiO_2$) on the activities of ATPases in the gill and muscle of freshwater mussel (*Unio tigridis*)

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**Abstract**

Mussels are effective bioindicator animals for aquatic environments. For this reason, they were often used to determine the effects of various xenobiotics. This study showed the effects of $Al_2O_3$, CuO and $TiO_2$ NPs on Na-ATPase, Ca-ATPase and Mg-ATPase activities in gill and muscle tissues of mussels after exposure to different concentrations (0, 1, 3.9 mg/L) of nanoparticles for 2 weeks. During exposures, mussels were fed with laboratory culture algae (*Chlorella vulgaris*). Nanoparticles accumulated in the tissues of mussels and the activities of ATPases change significantly. The activities of Na-ATPase and Ca-ATPase decreased significantly ($P<0.05$) in the gill, while Mg-ATPase activity increased. In muscle, the activities of all ATPases were significantly reduced by nanoparticle exposures. The gill seemed more sensitive than the muscle towards nanoparticle exposures and the order of nanoparticle effects was $TiO_2 > CuO > Al_2O_3$. There was no significant change in algae consumption between control and nanoparticle-exposed mussels. This study demonstrated the sensitivity of mussel physiology to nanoparticle exposures and significant accumulation of nanoparticles in mussel tissues.

**Keywords**: Metal, Nanoparticle, ATPase, Mussel, Toxicity.

## *ORAL PRESENTATION*

# Optimum working conditions of ATPases in the gill of freshwater mussel (*Unio tigridis*) and in vitro effects of metals and nanoparticles

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**Abstract**

Since mussels are one of the best bioindicator animals, they have been often used to monitor the water systems for different ecological purposes. However, the characterization of biomarkers should be known before they can be used in environmental monitoring and toxicology studies. This study was carried out to characterize the optimum working conditions of Na-ATPase and Ca-ATPase in mussel gills and to determine. It was also aimed to investigate the in vitro effects of ionic and nanoparticle ($Al_2O_3$, CuO) forms of aluminium and copper (0, 30, 90, 270 µg/L) on Na-ATPase and Ca-ATPase activities. The data showed that there was no ouabain-sensitive ATPase activity in the gill up to 10 mM ouabain concentrations. Na-ATPase and Ca-ATPase activities in the gills of control mussels were 5.124±0.373 and 3.750±0.211 µmol Pi/mg pro./h, respectively. In Vitro exposure to nanoparticles did not significantly ($P>0.05$) change the Na-ATPase and Ca-ATPase activities, while the same concentrations of the ionic aluminium and copper significantly decreased ($P<0.05$) enzyme activities. The data highlighted that aluminium and copper have different modes of action between ionic and nanoparticle forms. The data also suggested that in vivo studies are also needed to better evaluate the effects of nanoparticle and ionic metal forms on the ATPases of mussels.

**Keywords**: Mussel, ATPase, Characterization, Metal, Toxicity.

## *ORAL PRESENTATION*

# A Scale-up Reactor Design for Thermoplastic Polyurethane–Silver Nanocomposite Synthesis via Microwave-promoted Continuous Flow System

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**Abstract**

Composites formed with various nanofillers added to the matrix of thermoplastic polymer materials are used for multiple applications in many fields, from medicine to energy, from health to aviation, with improved properties. Among these composites, thermoplastic polyurethane(TPU)-silver(Ag) nanocomposites with enhanced physical properties attract attention for many medical applications due to their antimicrobial properties [1]. Recently, researchers have focused on microwave-assisted continuous flow (MWFS) systems in producing such composites because it offers a fast, sustainable and efficient synthesis in one step [2].
In this study, as shown in **Figure 1**, a large-scale MWFS was designed, considering a previously published small-scale process for producing TPU-AgNPs, and its commercialization potential was evaluated.

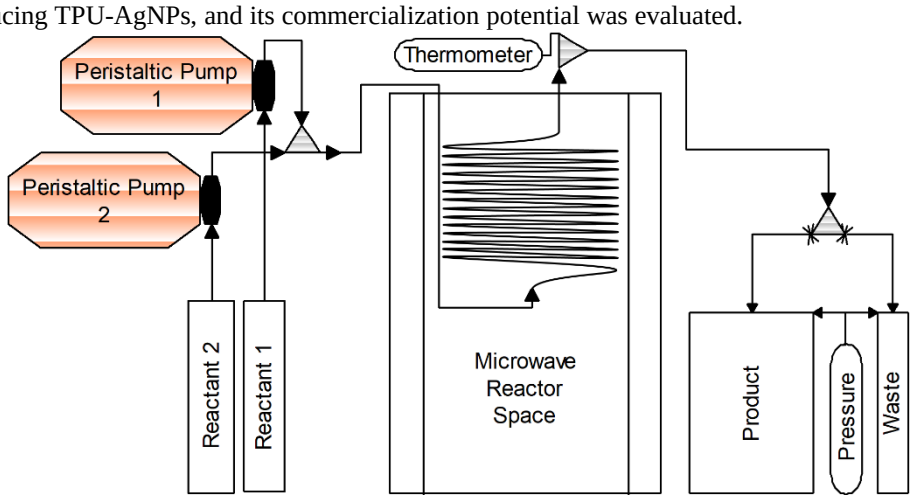


**Figure 1.** A schematic illustration of the scale-up reactor system for synthesis of thermoplastic polyurethane–silver nanocomposites.

**Keywords:** Polymer-based metal nanocomposites, scale-up, microwave-promoted, continuous flow system.

**References:**

1. Saleem, Q., et al., *Microwave-promoted continuous flow synthesis of thermoplastic polyurethane–silver nanocomposites and their antimicrobial performance.* Reaction Chemistry & Engineering, 2022. **7**(7): p. 1510-1524.
2. Saleem, Q., et al., *Microwave-Promoted Continuous Flow Systems in Nanoparticle Synthesis—A Perspective.* ACS Sustainable Chemistry & Engineering, 2021. **9**(30): p. 9988-10015.

## *ORAL PRESENTATION*

# Catalytic effects of metal-loaded SBA-15 on naphthalene methylation with methanol to produce methyl naphthalenes and dimethyl naphthalenes.

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**Abstract**

Selective catalysis to produce 2,6-DMN is an important topic in catalysis due to its importance in the production of high-performance polyethylene naphthalate (PEN) which demonstrates superior properties compared to polyethylene terephthalate (PET). Alternative routes to 2,6-DMN synthesis are of interest due to current complex synthesis procedures which decrease the commercialization of PEN. A suitable procedure lies in the methylation of low-value naphthalene to 2,6-DMN, and an additional isomerization of 2,6-triad isomers for higher yields. In this study, the selective synthesis of 2,6-triad DMNs (1,5-DMN, 1,6-DMN, and 2,6-DMN) was investigated by the methylation of naphthalene on Pt-loaded SBA-15 catalysts. The activity of parent and metal-loaded SBA-15 were investigated by the methylation of naphthalene in a fixed-bed reactor at 400-500 °C and weight hourly space velocity of 2–3 $h^{-1}$. Results show that the suitable pore structure present in SBA-15 catalysts ensured efficient diffusion which resulted in higher naphthalene conversions. In addition, the production of Naphthalene derivatives (MNs and DMNs), selectivity of 2,6-DMN, and 2,6-triad DMNs were enhanced on Pt-impregnated SBA-15. A maximum 2,6-triad DMN selectivity and 2,6/ 2,7-DMN ratio of 25.8 mol % and 1.48 respectively were achieved on the Pt-SBA-15 catalyst.

**Keywords:** Methylation, Naphthalene, 2,6-Triad DMN, SBA-15, PEN.

**I**

## *ORAL PRESENTATION*

# Investigation of PRL (prolactin) and LGB (beta lactoglobulin) gene polymorphism on milk yields traits in Holstein dairy cattle by PCR-RFLP method[#]

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**Abstract**

The aim of this study is to determine the effects of Prolactin (PRL) and Beta lactoglobulin (LGB) genes on lactation milk yield and milk composition in Holstein dairy cows by PCR-RFLP method. A total of 150 Holstein cows from three different dairy cattle farms in the Konya, Manisa and Diyarbakır provinces of Turkey were included in this study. Blood and milk samples were taken from Holstein dairy cows. Dry matter, fat, protein and lactose ratios of milk were determined from milk samples. DNA isolation was made from blood samples by means of ready-made commercial kits. Polymorphic regions of PRL and LGB genes were amplified by PCR. The obtained PCR products were cut with the RsaI enzyme to determine the genotypes of the PRL gene, and with the HaeIII enzyme to determine the genotypes of the LGB gene. The $\chi 2$ test was used to detect the genotypic distribution of polymorphic markers, and the General Linear Model (GLM) was used to determine the effects of PRL and LGB gene regions on lactation milk yield and chemical components of milk. The AA and AB genotypes of the PRL gene were determined in Holstein cows bred in three different farms. The AA genotype of the PRL gene was determined as the most common genotype. AA, AB and BB genotypes of LGB gene were determined. AB genotype of LGB gene was determined as the most common genotype in three different enterprises. In conclusion, while the effect of the PRL gene on the lactation period, lactation milk yield, and 305-day lactation milk yield was significantly in the whole population, the LGB gene was not significantly on these traits. On the other hands, cows with PRL AA genotype showed a shorter lactation period, but cows with AB genotype had higher lactation milk yield and 305-day lactation milk yield. There were no statistically differences among PRL and LGB polymorphic genotypes in terms of the chemical components of milk. These results suggested that the PRL gene may be evaluated in selection programs regarding milk yield.

**Keywords:** Beta lactoglobulin, Holstein, Milk composition, Milk yield, Prolactin, RFLP.

[#]This study has been supported by Van Yuzuncu Yil University Scientific Research Projects Coordination Unit under project number: TYD-2020-8906.

## *ORAL PRESENTATION*

# Model of the Future for Angiogenesis Assessment; Zebrafish

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**Abstract**

Angiogenesis; it is necessary in tumor development and progression, as well one of the most important events is happened in the neoplastic process. Angiogenesis involves the formation of new microvessels in the body. It occurs physiologically or pathologically, depending on the endothelial cell proliferation in capillaries (1). While during the wound healing, physiologically angiogenesis takes a week or more, pathological angiogenesis may continue for months or years. Having a tumor with angiogenic features; it gaves an agresive behaviours and causes faster growth, higher invasion, and faster formation of metastases (2). Cancer is a fatal disease that still has no effective treatment and a high mortality rate. Recent studies are being done for developing a new cure of cancer and a wide variety of *in vivo* models are used. However, due to the fact that *in vivo* models give outcomes late and have more difficult working process, there has been a high interest in zebrafish models in the world. Zebrafish and human genomes share around 70% homology. Moreover, 82% of morbidity-associated genes in human have been linked to at least one ortholog in the zebrafish (3). These similarities support the relevance of the model, moreover the advantages of zebrafish are practical. The zebrafish is a small organism and requires less infrastructure and nursing than mammalian models such as rodents (4). These features make the zebrafish model attractive for researchers and further studies.
In addition to its resemblance to the human organism, the zebrafish model gaves opportunity to easily observe embryogenesis processes via ex utero embryo development and transparent structure during the first period of their life. As a conclusion; Zebrafish is a promising new model for today's research, as it has both economical and easier production conditions and gives fastly outcomes in angiogenesis models.

**Keywords**: Angiogenesis, Zebrafish, Cancer, *In vivo*.

**References:**

1-Welti J, Loges S, Dimmeler S, Carmeliet P, Recent molecular discoveries in angiogenesis and antiangiogenic therapies in cancer, J Clin Invest, 123 (8) (2013), pp. 3190-3200 https://doi.org/10.1172/JCI70212.
2-Danny R. Welch, Douglas R. Hurst; Defining the Hallmarks of Metastasis. Cancer Res 15 June 2019; 79 (12): 3011–3027. https://doi.org/10.1158/0008-5472.CAN-19-0458
3-Howe K, Clar, M.D, Torroja C.F, Torrance J, Berthelot C, Muffato M, Collins J.E, Humphray S, McLaren K, Matthews L, et al. The Zebrafish Reference Genome Sequence and Its Relationship to the Human Genome. Nature 2013, 496, 498–503. (2013). https://doi.org/10.1038/nature12111.
4-Hoareau M, El Kholti N, Debret R, Lambert E. Zebrafish as a Model to Study Vascular Elastic Fibers and Associated Pathologies. International Journal of Molecular Sciences. 2022; 23(4):2102. https://doi.org/10.3390/ijms23042102.

## *ORAL PRESENTATION*

# Anti-bacterial Potential of Genistein According to ESAT6 and OMPA Receptor Interaction via Molecular Docking

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**Abstract**

Daidzein, genistein, and glycitein are the three primary isoflavones found in soybeans in aglycon form. According to many studies have previously described the doenjang isoflavones' antibacterial activity against a variety of microorganisms and bacterial biofilms. Isoflavones are found in many plants including, soybeans and fava beans which are known as medicinal plants, are the main foods of human being. Genistein exhibits anti-bacterial properties and is used for inflectional disseas In this study, we aimed for the first time to determine the possible interactions with genistein to two important tubercosis and outer membrane receptors; 6 kDa early secretory antigenic target (ESAT6) and Outer membrane protein A (OMPA) via the molecular docking method. The molecular structure of Genistein was obtained from PubChem (ID: 5280961). The three-dimensional structure of ESAT6 and OMPA proteins were obtained from the Protein Data Bank (3FAV and 1QJP respectively). BIOVIA Discovery Studio software was used for the minimization and optimization. Docking was done with Autodock Vina in PyRx software. BIOVIA Discovery Studio software was used for analysis and visualization. According to our results, the Genistein molecule exhibited better binding energy to ESAT6 protein than OMPA protein in terms of binding energy. The molecular binding of genistein to one of the important virulence factors of tuberculosis, ESAT6, suggests that it may have anti-tuberculosis activity.

**Keywords:** *Genistein, ESAT6, OMPA, Molecular Docking, Anti-tuberculosis.*

## *ORAL PRESENTATION*

### Development of polymeric nanocarrier systems and *in vitro* studies

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**Abstract**

Lung cancer remains the leading cause of cancer-related deaths in men and women (1). The distribution by level of economic development shows that there is no difference in cancer deaths among men, but lung cancer deaths are higher in industrialized countries than in developing countries. In addition, lung cancer deaths among women in developing countries lag behind breast cancer-related deaths (2). This study aims to encapsulate Doxorubicin into PCL nanoparticles and to investigate the efficacy of these Nano formulations on A549 lung cell line in vitro. PCL (FDA-approved), has been used as a drug delivery system in the development of targeted drugs against cancer in recent years and shows biocompatible, biodegradable properties. The carrier systems were synthesized with 14.000 kDa Polycaprolactone (PCL) according to the Nanoprecipitation method and 1 mg/mL doxorubicin (Dox) was encapsulated by the Double emulsion method. Encapsulation efficiency was evaluated by Ultraviolet-Visible spectroscopy (UV-VIS), Dimensional Analysis Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM), and dialysis membranes at two different pH (6 and 7.4) were used for drug release studies. The particle size of the prepared DOX-PCL is in the range of 200-220 nm. The results of the multidispersion index study showed that the nanoformulations had a monodisperse structure and the PDI value was less than 0.3. Cytotoxicity analyses (MTT) and fluorescence imaging studies were performed to observe the effects on living systems. According to cell culture studies, it was determined that POL-DOX decreased the viability on the A549 cell line more than free DOX and accumulated in the cell nucleus. As a result, it has been observed that POL-DOX has a high potential to be an effective formulation in the chemical treatment of cancer, and also has a theranostic structure that allows systems that can allow secondary treatments such as photodynamic therapy and radiotherapy.

**Keywords:** PCL nanoparticles, drug delivery, theranostic therapy.

**References:**

1- Fitzmaurice, C., Dicker, D., Pain, A., Hamavid, H., Moradi-Lakeh, M., MacIntyre, M. F., ... & Global Burden of Disease Cancer Collaboration. (2015). The global burden of cancer 2013. JAMA Oncology, 1(4), 505-527.
2- Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin; 2015. DOI: 10.3322/caac.21262.

## *ORAL PRESENTATION*

# Curcumin-loaded exosomes inhibit DNMT1 enzyme activity in cervical cancer cells

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**Abstract**

Cervical cancer, the second most common cancer worldwide in women, is responsible for approximately 320,000 deaths per year. The most common treatment method in the treatment of cervical cancer, which is a preventable and treatable disease, is chemotherapy. Drug accumulation in undesirable tissues is the biggest disadvantage in chemotherapy. Therefore, there is an urgent need for the development of new anticancer agents with better efficacy and fewer side effects, and for the controlled release of existing drugs in target tissue with more effective formulations. Exosomes are nano-sized vesicles with a diameter of 30-120 nm, covered with a double phospholipid layer, secreted by the extracellular vesicles of the cell. Due to their biocompatibility and high stability, exosomes are agents with great potential for the transport of anti-cancer drugs.

In mammals, DNA methyl transferases are enzymes that add methyl groups to the 5th carbon atom of cytosine in CpG dinucleotides. Abnormal DNA methylation is observed in cancer. Inhibition of DNMTs is an important approach in cancer treatment because hypermethylation of tumor suppressor genes constitutes an aggressive process for cancer formation. Curcumin has been reported to inhibit DNA methylation in cancer cells.

In this study, exosomes released from HeLa cervical cancer cells were isolated by ultracentrifuge method. These isolated exosomes were loaded with curcumin electroporation technique. The characterization studies of this prepared Exo-Cur formulation were carried out with SEM and zeta sizer. The cytotoxic effects of Curcumin and Exo-Cur formulation on HeLa cervical cancer cells were investigated by MTT method. In addition, the effects of Curcumin and Exo-Cur formulation on HeLa cells methylation were investigated with a colorimetric DNMT activity assay kit. Exosome-specific spherical structures were observed in SEM micrograph analysis. In addition, in zeta analysis, the size of empty exosomes isolated from HeLa cells was found to be 74 nm, while the size of curcumin-loaded exosomes was 119 nm. The half-maximum inhibition concentration (IC50) of Curcumin and Exo-Cur formulation on HeLa cells was calculated as 74.88±2.14 and 33.77±1.87 µg/mL, respectively. It was determined that the total DNMT enzyme activity levels of HeLa cells decreased after Exo-Cur application.

As a result, the potential of the prepared Exo-Cur formulation to be used as a chemotherapy supportive agent in the treatment of cervical cancer through DNMT enzyme inhibition was demonstrated in this study.

**Keywords:** Curcumin, DNMT, Exosome, Cervical Cancer.

## *ORAL PRESENTATION*

# Endoplazmik retikulum stresi inhibisyonunun sıçan beyninde inflamasyon ve otofaji üzerindeki etkilerinin yaşlanmaya ve cinsiyete bağlı incelenmesi

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**Özet**

Yaşlanma, birçok doku ve organda olduğu gibi beyinde de yapısal ve işlevsel birtakım gerilemelere neden olur. Beyin yaşlanması; Alzheimer ve Parkinson gibi birçok nörodejeneratif hastalığın ortaya çıkmasına zemin hazırlar ve altında yatan mekanizmalardan bazıları endoplazmik retikulum (ER) stresi, otofaji ve inflamasyondur. Bu çalışmada ER stres inhibitörü tauroursodeoksikolik asit (TUDCA)'in yaşlanan sıçan beyni üzerindeki etkisinin cinsiyete bağlı olarak araştırılması amaçlanmıştır. Çalışmada genç (4 aylık) ve yaşlı (24 aylık) Wistar albino erkek ve dişi sıçanlar kullanılmıştır. ER stres inhibitörü olarak TUDCA uygulaması 4 hafta süresince yapılmıştır (150 mg/kg/gün, ip, 4 hafta). Süre sonunda hayvanlardan alınan beyin dokuları homojenize edilmiş ve elde edilen homojenatlarda ELİSA yöntemi ile inflamasyon belirteçlerinin (TNF-α, IL-1β) ve otofaji belirteçlerinin (Beclin1, LC3-B) konsantrasyonları ölçülmüştür. Analiz sonuçlarına göre; Beclin1 ve LC3-B seviyeleri yaşlanmayla birlikte hem dişilerde hem de erkeklerde artmıştır. ER stres inhibisyonu genç erkeklerde Beclin1 ve LC3-B düzeylerinde artışa; yaşlılarda ise LC3-B'de azalmaya neden olmuştur. Cinsiyet farkına bakıldığında; Beclin1 konsantrasyonunun yaşlı dişilerde erkeklere kıyasla daha yüksek olduğu bulunmuştur. Gençlerde ise LC3-B seviyelerinin dişilerde erkeklere oranla daha fazla olduğu görülmüştür. TNF-α seviyeleri erkek sıçanlarda yaşlanmayla birlikte artmıştır. ER stres inhibisyonu genç erkeklerde TNF-α ve IL-1β düzeylerinde artışa; yaşlı erkeklerde ise IL-1β seviyelerinde azalmaya neden olmuştur. IL-1β seviyelerinin hem genç hem de yaşlı dişilerde erkeklerden daha yüksek olduğu; TNF-α'nın genç dişilerde erkeklerden daha yüksek olduğu gözlenmiştir. Özetle; yaşlanmayla birlikte beyin dokusunda otofajinin arttığı, TUDCA uygulamasının yaşlılardaki otofaji seviyesinde azalma, gençlerde ise artma eğilimine neden olduğu bulunmuştur. Yaşlanmayla birlikte beyin dokusundaki inflamasyonun arttığı; genç ve yaşlı dişilerde erkeklere kıyasla daha yüksek düzeyde olduğu görülmüştür. TUDCA uygulamasının inflamasyon belirteçleri üzerindeki etkileri hem yaşa hem de cinsiyete bağlı farklar göstermiştir. Sonuç olarak; ER stres inhibisyonunun yaşlı beyinde değişen otofaji ve inflamasyon üzerinde pozitif etkileri olabileceği ve bu etkinin cinsiyete bağlı olarak değişebileceği düşünülmektedir.

*Bu çalışma, Gazi Üniversitesi BAP Birimi tarafından (Proje no: TYL-2022-7522) desteklenmiştir.

**Anahtar Kelimeler:** yaşlanma, endoplazmik retikulum stresi, otofaji, inflamasyon.

## *ORAL PRESENTATION*

# Recent progress in scaffold production with Additive Manufacturing

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**Abstract**

Tissue engineering aims to restore and replace lost tissue functions. For this purpose, three-dimensional (3D) scaffolds with a porous structure that serve as a template for tissue formation are used (1). One of the tissue-engineered products, tissue scaffolds are designed to act as a temporary substitute for tissue defects that induce cell growth and new tissue formation as tissue matures. Although native tissues represent ideal scaffolds in their in vivo form and function, the use of native ECM-based scaffolds can have significant disadvantages. However, thanks to new production techniques, artificial biomimetic structures like natural extracellular matrix (ECM) structure, and morphology can be developed. Choosing an appropriate production method is a critical issue in tissue engineering. Conventional techniques such as solvent casting, gas foaming, electrospinning, freeze drying, phase separation, and freeze casting can produce single homogeneous tissue scaffolds without any control over pore size and shape, porosity, and interconnectivity. Therefore, various problems are encountered in regenerating defective areas with different physicochemical and biological requirements such as subchondral bone and cartilage. To overcome the mentioned limitations, additive manufacturing technology (3D printing) has been developed (2). The structures produced by this method have advantages such as being able to be designed specifically for the physiology of the patient, printing with the desired size, structure, and pore size, being quickly obtained and low in cost, being biocompatible, and not creating a specific immune response. In addition, these scaffolds provide a good microenvironment for cell growth, transport of nutrients, waste byproducts and growth factors, and internal vascularization (3). In conclusion, breakthroughs are expected in fields such as orthopedics, reconstructive surgery, and dentistry because of the joint work of materials science and engineering, the production of new materials, and the development of printing capabilities of 3D printers.

**Keywords:** scaffold, 3D printing, tissue engineering.

**References:**

1. Top, N., & Gökçe, H. Doku Mühendisliğinde Bilgisayar Destekli Yapay Kemik İskelesi Tasarımı, 1.Baskı, İksad, Ankara, 2020
2. Banks, J. (2013). Adding value in additive manufacturing: researchers in the United Kingdom and Europe look to 3D printing for customization. IEEE pulse, 4(6), 22-26.
3. Lavik, E., & Langer, R. (2004). Tissue engineering: current state and perspectives.Applied microbiology and biotechnology, 65(1), 1-8.

## *ORAL PRESENTATION*

# Extraction of Chitosan from Shrimp Shell Waste from Morrocco Local Market

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**Abstract**

Chitin is the second most abundant biopolymer on earth. Its biological and physicochemical properties had a wide range of applications in different fields due to its biological and physicochemical properties. Therefore, researchers have begun investigating new extraction processes of high-purity chitosan from renewable resources. The aim of this work is the extraction of chitosan from shrimp shell waste at room temperature.
The shells, taken from a local fishery market, were deproteinized and demineralized using NaOH and HCl solutions respectively at room temperature. Crude chitin extracted was deacetylated with a concentrated NaOH solution to obtain chitosan. The final product was characterized using IR spectroscopy. The degree of deacetylation was determined by titration and IR spectroscopy procedures and solubility in 1 % acetic acid was also determined.
We concluded that Shrimp shell waste conversion to chitosan is a commercially valuable process and serves as an effective mode of shell remediation.

**Keywords:** Chitosan, shrimp shell, degree of deacetylation, biopolymer.

## *ORAL PRESENTATION*

# Methylation Status of The SYN2 Gene In Schizophrenia

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**Abstract**

Schizophrenia is a neuropsychiatric disease and genetic and environmental factors play a critical role in its etiology and affects early brain development. Although many years of genetic and psychiatric studies, the etiology and molecular mechanisms of schizophrenia have not been fully illuminated. Recent studies have shown that epigenetic mechanisms have important effects on the rise of the abnormal phenotype and showed the importance of these mechanisms on the etiology of schizophrenia. The most common type of DNA methylation, one of the main epigenetic modifications, is addition of the methyl group to the 5th position of cytosine nucleotide of CG dinucleotides found in CpG islands. Considering this information, DNA methylation patterns of 3 different CpG regions located on the promoter of the *SYN2* gene, which is one of the candidate genes for schizophrenia that play an active role in neuronal plasticity, axon elongation and synaptic vesicle release, were compared in the groups of healthy controls and schizophrenia patients. 33 healthy controls and 39 patients with schizophrenia were included in the study. DNA samples obtained from peripheral blood were used for DNA methylation analysis by bisulfite conversion and pyrosequencing methods. The methylation rate of the 3 CpG positions in the *SYN2* gene was compared in the patient and control groups using statistical analysis. Significant DNA methylation difference was detected in the average methylation ratio in 2nd and 3rd CpG regions. The results are important and original in terms of confirming the importance of the role of synapse-related genes in the etiology of schizophrenia, as well as revealing the relationship between DNA methylation in these genes and the disease. This study was performed within the scope of the project #FYL-2021-2719 supported by the Scientific Research Projects Unit of Inonu University.

**Keywords:** DNA methylation, epigenetics, SYN2, schizophrenia.

## *ORAL PRESENTATION*

# Yüzey Geliştirilmiş Raman Saçılması Yöntemiyle Bisfenol–A Tayini

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## Özet

Nanomalzeme, atomların ya da moleküllerin manipülasyonu ile elde edilen teknolojik yapılardır. Malzemeler nano boyuta getirildiğinde, yığın (makro) halinden farklı özelliklere sahip olabilmektedir. Bu durum nano boyutta, kuantum boyut etkilerinin malzeme üzerindeki etkinliği ile açıklanmaktadır. Sağlık, çevre, doğal kaynaklar, inşaat malzemeleri, gıda ve endüstri nanomalzemelerin başlıca uygulama alanı buldukları sektörlerdir [1-5].
Raman spektrumları hedef analite gelen ve saçılan foton arasında ki enerji farkının spektrumlara dönüştürülmüş halidir. Her molekülün kendine özgü Raman spektrumları bulunmaktadır. Hedef analitden alınan sinyallerin düşük olması çeşitli zorluklar oluşturmaktadır. 1977'de Van Duyne ve arkadaşları, nanomalzemelerle oluşturdukları substratları kullanarak Raman sinyallerini arttırmayı başarmışlardır. Bu yönteme ise Yüzey Geliştirilmiş Raman Spektroskopisi (SERS) denilmektedir. SERS, tahribatsız, ultra hassas, hızlı, titreşimsel, spektroskopik bir tekniktir ve analitlerin tayin edilmesinde etkili bir yöntem olarak güncelliğini korumaktadır [6-7].
Bisfenol-A (BPA) günlük hayatımızda kullandığımız plastik ürünlerde sıkça karşımıza çıkmaktadır. Bisfenol A (BPA) polikarbonat plastiklerin ve epoksi reçinelerin sentezinde, ana monomerik malzeme olarak kullanılır, aseton ve fenolden sentezlenmektedir [8]. Epoksi reçineler; termal kağıtlarda [9], şişelerde [10], su borularında, oyuncaklarda, tıbbi ekipmanlarda, elektronik ürünlerde [11], diş dolgu macunlarından konserve ambalajlar gibi metal yüzey ile gıda arasında teması kesmek için kaplama yüzeylerinde kullanılmaktadır [12]. Plastik üretilen sanayi faaliyetleri ve nihai ürünlerin (plastik şişe, biberon vb.) de etkisiyle BPA doğaya, insanlara, gıdalara ve hayvanlara taşınmaktadır [13-15]. BPA; östrojen, progesteron ve androjen reseptörleri dahil olmak üzere steroid hormon reseptörlerini bağlayarak endokrin sistemini bozabilmektedir [14]. Endokrin sistemi ve üremenin bozulması, bağışıklığın zayıflamasına ve çeşitli kanser türlerine yol açmaktadır [13, 16]. Riskler göz önüne alındığında, çevre kirliliği (toprak, su vb.), biyomedikal teşhis ve gıda güvenliği için BPA'nın hızlı, basit, hassas ve seçici bir yöntem ile tespit edilmesi önem arzetmektedir.
Bu çalışmada hızlı, ucuz, güvenilir, yüksek duyarlılıkta BPA tespitinin yapılması için SERS yöntemi kullanılmıştır. Sentezlenen farklı özellikteki nanomalzemeler ile düşük konsantrasyonlardaki BPA tespiti karşılaştırılmalı olarak çalışılmaktadır. Nanomalzemelerin karekterizasyonunda SEM, EDX, UV-Vis ve Raman kullanılmıştır. Gerçek örneklerdeki BPA tayini ise halen devam etmektedir.

**Anahtar Kelimeler:** Nanoteknoloji, Raman, SERS, BPA.

**Kaynakça:**

1. Chen, Hsinchun, et al. "Global nanotechnology development from 1991 to 2012: patents, scientific publications, and effect of NSF funding." Journal of nanoparticle research 15.9 (2013): 1-21.
2. NEDİR, NANOTEKNOLOJİ. "DERLEME REVIEW." Türk Tıp Dergisi (2010).
3. Sanchez, Florence, and Konstantin Sobolev. "Nanotechnology in concrete–a review." Construction and building materials 24.11 (2010): 2060-2071.
4. Teo, Boon K., and X. H. Sun. "From top-down to bottom-up to hybrid nanotechnologies: road to nanodevices." Journal of cluster science 17.4 (2006): 529-540.
5. Adams, Freddy C., and Carlo Barbante. "Nanoscience, nanotechnology and spectrometry." Spectrochimica Acta Part B: Atomic Spectroscopy 86 (2013): 3-13.
6. Büyükgöz, Gülüzar Görkem. "Yüzeyde Güçlendirilmiş Raman Spektroskopisi ile DNA Hassas Tayini ve Nanopartiküller Arası Mesafelerin Optimizasyonu." (2014).
7. Bonyar, Attila, et al. "Investigation of the performance of thermally generated gold nanoislands for LSPR and SERS applications." Sensors and Actuators B: Chemical 255 (2018): 433-439.
8. Matsumoto, Akiko, et al. "Bisphenol A levels in human urine." Environmental health perspectives 111.1 (2003): 101-104.
9. Zimmers, Stephanie M., et al. "Determination of free Bisphenol A (BPA) concentrations in breast milk of US women using a sensitive LC/MS/MS method." Chemosphere 104 (2014): 237-243.
10. Wilczewska, Kamila, Jacek Namieśnik, and Andrzej Wasik. "Troubleshooting of the determination of bisphenol A at ultra-trace levels by liquid chromatography and tandem mass spectrometry." Analytical and bioanalytical chemistry 408.3 (2016): 1009-1013.
11. Chen, Da, et al. "Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity a review." Environmental science & technology 50.11 (2016): 5438-5453.
12. Bingöl, M. *Polikarbonat (PC) su damacanaları ve bebek biberonlarından bisfenol A (BPA) migrasyonuna ve PC degradasyonu sonucu BPA oluşumuna çeşitli faktörlerin etkilerinin belirlenmesi*. Diss. Doktora Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü, Ankara, 2013.
13. Feng, Jingjing, et al. "Building SERS-active heteroassemblies for ultrasensitive Bisphenol A detection." *Biosensors and Bioelectronics* 81 (2016): 138-142.
14. Kim, Andre, et al. "A sensitive and reliable quantification method for bisphenol A based on modified competitive ELISA method." *Chemosphere* 68.7 (2007): 1204-1209.
15. Lin, Pei-Ying, Chiung-Wen Hsieh, and Shuchen Hsieh. "Rapid and sensitive SERS detection of bisphenol A using self-assembled graphitic substrates." *Scientific reports* 7.1 (2017): 1-6.
16. Lin, Li-Kai, and Lia A. Stanciu. "Bisphenol A detection using gold nanostars in a SERS improved lateral flow immunochromatographic assay." Sensors and Actuators B: Chemical 276 (2018): 222-229.

## *ORAL PRESENTATION*

# Potentiometric determination of carbamazepine in pharmaceutical samples

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**Abstract**

Carbamazepine is one of the safest and most effective anti–epileptic drugs used in the treatment of partial and generalized tonic–clonic seizures. The several analytical methods are available in the literature for the determination of carbamazepine in biological and pharmaceutical samples. Compared to the analytical methods used in the determination of carbamazepine, potentiometric methods offer significant advantages such as ease of preparation and use, low cost, high selectivity, wide linear concentration range, low detection limit, short response time and long lifetime [1, 2]. In this study, we developed a potentiometric sensor to perform the determination of carbamazepine in pharmaceutical samples. For this purpose, we synthesized the carbamazepine–tetraphenylborate (CBZ–TPB) ion–pair and used it as an ionophore in sensor production. The proposed sensor has a low cost and is easy to prepare and use. In addition, the sensor is fast, stable, highly selective and can work in a wide pH range without being affected by pH changes. The developed sensor has been successfully applied for the determination of carbamazepine in some pharmaceutical formulations and the obtained results were compared with UV–Vis spectroscopy. The results obtained in both methods are in good agreement. As a result, the sensor can be considered as an alternative to other analytical devices for the determination of carbamazepine.

**Keywords:** Carbamazepine, potentiometry, ion–pair, sensor, anti–epileptic.

**Acknowledgements:**The authors are indebted to the Zonguldak Bülent Ecevit University, Scientific Research Projects Commission (Project Number: 2021-50737594-05).

**References:**

[1] O. Özbek, C. Berkel, Sensors International 3 (2022) 100189.
[2] O. Özbek, C. Berkel, Ö. Isildak, Critical Reviews in Analytical Chemistry 52 (2022) 768–779.

## *ORAL PRESENTATION*

# Mitochondrial phylogeny of *Microtus elbeyli* (Mammalia: Rodentia) recorded from Southeastern Anatolia

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**Abstract**

Based on morphological and karyological differences, *Microtus elbeyli* (Yiğit et al. 2016) was identified as a new species with the specimens from Kilis Province (Southeastern Anatolia) with 2n=46 diploid chromosome number, which had previously been accepted as *Microtus* cf. i*rani*. Any molecular studies on *Microtus elbeyli* has not been conducted so far. In this paper, mitochondrial Cytochrome-b (CYTB) and Cytochrome oxidase-I (COXI) gene analyses were performed using the samples of social voles thought to be closely related to *M. elbeyli* (*Microtus irani*, *Microtus schidlovski*, *Microtus dogramacii*) in order to clarify the taxonomy of *M. elbeyli*. Evidence from the mean genetic distance values, Bayesian Inference trees and Median-joining networks pointed that *M. elbeyli* differed from other *Microtus* species as closer with the *M. dogramacii* samples. Although *M. elbeyli* and *M. dogramacii* are similar in terms of karyological features, they differ regarding external morphology and some skull characteristics. In the light of mitochondrial gene analyzes, karyological and morphological findings, it was supported that *M. elbeyli* is a valid species closely related to *M. dogramacii* which distributed in the northern parts of Central Anatolia.

**Keywords:** *Microtus elbeyli*, vole, Anatolia, mitochondrial DNA.

## ORAL PRESENTATION

# Gıdalarda antioksidan kapasite tayini için uygulanan QUENCHER yaklaşımları

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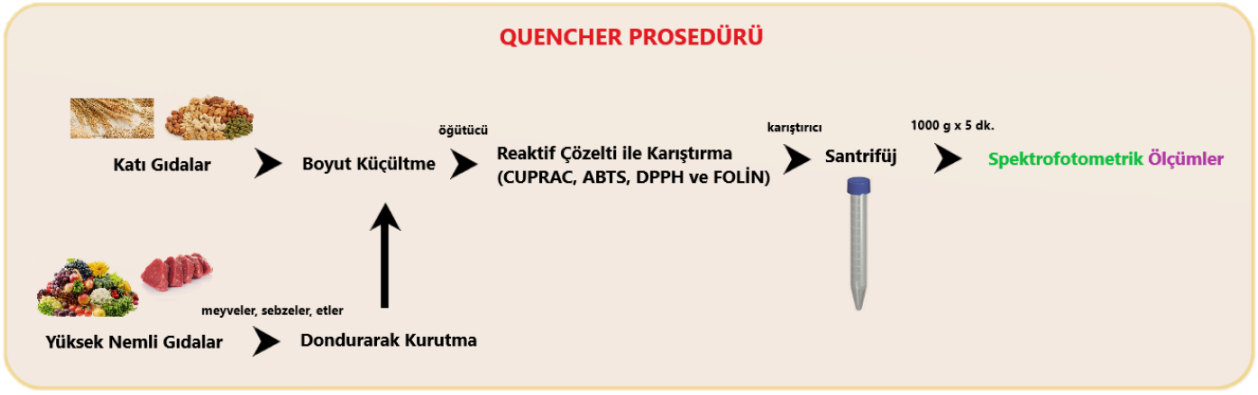
**Özet**

Antioksidanlar serbest radikallerin olumsuz etkilerini önemli ölçüde azaltabilen bileşiklerdir. İnsan sağlığı açısından önemli olan bu antioksidanların biyolojik sıvı, gıda ve saf bileşiklerde antioksidan kapasitelerini ölçmek amacıyla birçok yöntem geliştirilmiştir. Fakat bazı gıda örneklerinde fenolik bileşiklerin hem serbest hem bağlı halde bulunması sebebiyle ayrı ayrı ekstraksiyon ve hidroliz işlemlerinin uygulanması, analiz süresi ve zorluğu, ekstraksiyon koşullarının optimizasyonu vb. durumlardan dolayı analiz için dezavantaj oluşturmaktadır. Toplam antioksidan kapasite (TAC) üzerine yapılan çoğu araştırma, kullanılan organik çözücüye bağlı olarak antioksidanların eksik ekstraksiyonu nedeniyle sınırlı sonuçlar vermektedir. Antioksidanların çeşitli polariteleri ve çoğunun çözünmeyen gıda matriksine kovalent olarak bağlı olması nedeniyle, gerçek "toplam antioksidan kapasitenin" ölçümü zor olmaktadır. Bu eksiklikten yola çıkılarak gıda örneklerinin toplam antioksidan kapasitesini doğrudan tayin etmek için literatürdeki antioksidan kapasite tayin yöntemlerinin öğütülmüş katı örneklere uygulanabilmesini öngören bir işlem olan "QUENCHER" (QUick, Easy, New, CHEap and Reproducible) yaklaşımı literatürde bildirilmiştir. QUENCHER yaklaşımı, TAC reaktifleriyle iç içe dengeler oluşturan redoks reaksiyonları aracılığıyla bağlı fenollerin çözünürlüğünü kolaylaştırır. Metodolojinin çalışma hipotezi, tüm ekstraksiyon adımlarını atlayarak katı gıda ve radikal reaktif çözeltisinin doğrudan temas halinde olmasıdır. Bu şekilde, numunenin çözünür kısmı, normal sıvı-sıvı tipi reaksiyona göre çözücüde bulunan radikalleri süpürerek antioksidan kapasitesini uygular. Bu derlemede "QUENCHER" yaklaşımı ile CUPRAC, ABTS, DPPH ve FOLİN gibi antioksidan kapasite tayin yöntemlerinin literatürde mevcut uygulamalarına yer verilmiştir. Bilinen radikal problar kullanılarak QUENCHER yöntemlerinin optimize edilmesi ve standartlaştırılması, ekstraksiyona bağımlı varyasyonları ortadan kaldırmak ve çeşitli gıda matrikslerinin antioksidan kapasitesi için benzersiz bir veri tabanı oluşturması açışından önemlidir.




**Şema:** QUENCHER prosedürünün şematik gösterimi.

**Anahtar Kelimeler:** Antioksidan kapasite, QUENCHER, fenolikler.

## *ORAL PRESENTATION*

# Bazı Amino Asit Etil Esterlerinin Antimikrobiyal Aktivitelerinin Araştırılması

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**Özet**

Dünya çapında artan mikroorganizma kaynaklı hastalıklar ve bakterilerde gözlemlenen antibiyotik direnci sebebiyle tedavide yeni ve etkin madde arayışları devam etmektedir. Bunlar arasında amino asit türevleri ve peptidler önemli yer tutmaktadır. Bu nedenle bu çalışmanın amacı lösin, metiyonin ve fenilalanin amino asitlerinin etil esterlerinin antibakteriyel ve antifungal aktivitelerinin belirlenmesidir. Bu amaç doğrultusunda antibakteriyel aktiviteyi ölçmek için iki gram pozitif (*Bacillus cereus* ve *Staphylococcus aureus*), iki gram negatif bakteri (*Escherichia coli* ve *Pseudomanas aeruginosa*) ve antifungal aktivitenin belirlenmesi için *Candida albicans* kullanılmıştır. Antimkrobiyal aktivitelerinin belirlenebilmsi için disk difüzyon yöntemine ek olarak Minimum İnhibasyon Konsantrasyon (MİK) ve Minimum Bakterisidal–Fungisidal Konsantrasyon (MBC–MFK) ölçüm metodları kullanılmıştır. Disk difüzyon testinde tüm mikroorganizmalar ve tüm esterler için sadece disk etrafında temiz alan gözlemlenmiştir. MİK deneyinde antibakteriyel aktivite sadece fenilalanin etil esterde her iki gram negative bakteriye (*E. coli* ve *P. aeruginosa*) karşı en yüksek konsantrasyonda (25mg/mL) tespit edilmiş ve diğer esterler için herhangi bir MİK değeri belirlenememiştir. Etil esterleri *C. albicans*'a karşı da antifungal aktivite göstermiştir. Tespit edilen antifungal MİK değerleri fenilalanin etil ester için 13mg/mL iken diğerleri için 25mg/mL'dir. Genel olarak çalışılan amino asit etil esterlerinin antifungal aktivitelerinin olduğunu söyleyebiliriz. Bunun yanında, fenilalanin etil esterin diğerlerinden daha etkin olduğunu ve ilaç geliştirilmesine yönelik yapılacak olan çalışmalara katkı sağlayacağı düşünülmektedir.

**Anahtar Kelimeler:** Amino asit, etil ester, Antimikrobiyal aktivite.

## *ORAL PRESENTATION*

# Sıçanlarda monosodyum iyodoasetat ile oluşturulan deneysel osteoartrit modelinde gelişebilecek inflamatuvar yanıt, oksidatif stres ve dejeneratif süreçte mTOR'un katkısının araştırılması

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**Özet**

Çalışmamızda, sıçanlarda monosodyum iyodo asetat (MİA) ile oluşturulan osteoartrit ve ilişkili patofizyolojik değişikliklerde mTOR'un rolünün olup olmadığı, mTOR etkinliğinde oluşabilecek değişikliklerle ortaya çıkabilecek inflamatuvar yanıt, oksidatif stres ve eklem dejenerasyonu ile bu süreçte rolü olabilecek diz ekleminde ödem oluşumu, eklemde makroskobik ve histopatolojik değişiklikler, IL-1β, TNF-α, NOX4, p22phox ve MMP-13 ekspresyon/etkinlikleri ile ilişkilendirilerek araştırıldı. Sıçanlar 4 gruba ayrıldı: Kontrol (SF; 25 µl, i.a.), OA grubu (MİA; 2 mg/25 µl, i.a.), Rapamisin (1 mg/kg/gün, i.p.), OA + Rapamisin. Kontrol grubundaki sıçanların diz ekleminlerine SF, OA ve OA+Rapamisin grubundaki sıçanların ise 25 µl SF içerisinde çözülmüş 2 mg MİA enjeksiyonu yapıldı. Rapamisin grubundaki sıçanlara sadece rapamisin enjeksiyonu yapıldı. MİA+rapamisin grubunda bulunan sıçanlara yapılan MİA enjeksiyonu ile birlikte 14 gün boyunca iki günde bir seçici mTOR inhibitörü rapamisin uygulandı. MİA enjeksiyonundan önce ve enjeksiyondan sonra 14. Günde, tüm hayvanların diz eklem çapları eklem bölgesindeki ödemin göstergesi olarak ölçülüp, 14. günün sonunda tüm sıçanlara ötenazi uygulanarak diz eklemleri, sinoviyal dokuları ve kan örnekleri alındı. Diz eklemlerinde, eklem dejenerasyonunun göstergeleri olarak, kıkırdak dokusunda yumuşama ve doku zedelenmesi makroskopik bulgular ile kondrosit hücre yapısındaki anormallikler, tidemark devamlılığı, kıkırdağın proteoglikan ve glikozaminoglikan içeriğini gösteren histopatolojik değişiklikler incelendi ve sinoviyal dokuda kıkırdak zedelenmesinin önemli bir göstergesi olan MMP-13 ekspresyonu ölçüldü. Ayrıca sinoviyal dokuda, inflamasyon ve beraberinde gelişen oksidatif stresin göstergeleri olarak IL-1β, p22phox, NOX4 ekspresyonu ve rpS6 fosforilasyonu ile serumda TNF-α düzeyleri ölçüldü. Bulgularımız, mTOR etkinliğindeki artışın, sıçanlarda MİA ile oluşturulan OA ve ilişkili patofizyolojik değişikliklere aracılık eden inflamatuvar yanıt, oksidatif stres ve ilişkili eklem dejenerasyonu ile bu süreçlerde rol alan IL-1β, TNF-α, p22phox, NOX4 ve MMP-13 etkinliğindeki artışa katkıda bulunduğunu göstermektedir.

**Anahtar Kelimeler:** Osteoartrit, MİA, mTOR, İnflamasyon, Oksidatif stres, Eklem dejenerasyonu.

## *ORAL PRESENTATION*

# Traditional use of medicinal plants in İdil (Şırnak)

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**Abstract**

This study was carried out to determine the plants used in folk medicine in Şırnak (İdil) region. During the 2019 vegetation period, the study was conducted in one town and 34 villages in the İdil district. In the survey study, 112 source people were interviewed. Plants used in folk medicine were determined and samples of these plants were collected. As a result of the research, it was determined that 79 plant species belonging to 35 families in the region are used in folk medicine. The first 7 families with the most taxa among the plants found to be used in the research area; Rosaceae 10 (9.6 %), Asteraceae 9 (8.6 %), Lamiaceae 6 (5.7 %), Cucurbitaceae 6 (5.7 %), Solanaceae 4 (3.8 %), Malvaceae 4 (3.8 %), Fabaceae 4 (3.8 %). After the identified plants were scientifically defined, details were provided regarding their local names, usage guidelines, dose, and application times.

**Keywords:** Ethnobotanical, medicinal plants, İdil, Şırnak.

This study was supported by the Van YYU Scientific Research Projects Coordination Unit with the project numbered FYL-2019-7961.

## *ORAL PRESENTATION*

# $Fe_3O_4$ ile CuO Nanopartiküllerinin *Saccharomyces cerevisiae*'da Biyokimyasal Aktivitelerinin Belirlenmesi

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**Özet**

Nanoteknoloji sayesinde, nano boyutundaki malzemelerin üretilmesi ve kullanılması son senelerde yapılan çalışmalar ile hızlı bir artış göstermektedir. Demir-oksit nanopartikülü ($Fe_3O_4$) bir çok alanda yaygın olarak kullanılan metal oksit malzemelerden biridir. Bu nanopartiküllerin biyolojik sistemlerde toksik etkileri üzerine çalışmalar yapılmış olmasına rağmen biyokimyasal parametreler üzerine olan etkilerini araştırmak için az sayıda çalışma yapılmıştır. Bu çalışmada; $Fe_3O_4$ ve CuO nanopartiküllerinin maya (*Saccharomyces cerevisiae)* kültür ortamında bazı biyokimyasal parametreler üzerine etkilerinin belirlenmesi amaçlanmaktadır. Öncelikle deneyde kullanılacak olan *S. cerevisiae* maya hücresinin gelişimi ve çoğalması için YEDP besiyeri ortamı hazırlandı. Besiyeri ortamı içerisine $Fe_3O_4$ ile CuO'den 20 mg/L ve 50 mg/L konsantrasyonları içerecek şekilde deney grubu oluşturuldu. Gerekli sterilizasyon işlemleri yapıldıktan sonra laboratuar şartlarına kadar soğutulan her grup kültür örneğine *S. cerevisiae* hücrelerinin stok kültüründen aşılama yapıldı. Aşılama işleminin ardından inkübasyona bırakılan kültürler, laboratuar şartlarında hücre yoğunlukları ölçülüp santrifüj edilerek hücreler toplandı. Toplanan hücre pelletleri, Tris HCl-baz ve ETDA karışımı ile homojenize edilip santrifüj edildikten sonra süpernatant kısmı ile GSH (Elman reaktifi), total protein (Lowry) ve TBARS ölçümleri (HPLC cihazı ile), geriye kalan pellet kısmıyla da n-hekzan/izopropanol karışımı ile homojenize edilerek yağ asidi ve sterollerin (ergosterol, kampestrol, stigmasteol, betasitosterol) analizi (gaz kromatografi) yapıldı. Sonuçlar SPSS istatistik programı kullanılarak varyans analizi ve gruplar arasındaki farklılık ise LSD testi ile belirlenip istatistik açıdan değerlendirilmiştir. Sonuç olarak; $Fe_3O_4$ ile CuO nanopartiküllerinin *S. cerevisiae*'nın biyokimyasal sistemi üzerinde farklı etkilere sahip olduğu gözlemlendi. Açığa çıkan sonuçların diğer canlı modelleri üzerindeki benzer çalışmalara kaynak olacağı ve *in vivo* sistemler kullanılarak yapılacak ileriki çalışmalara destek olarak, literatür bilgisine katkıda bulunulacağı düşünülmektedir.

**Anahtar kelimeler:** Maya, Nano-Demir Oksit, Yağ asidi, Vitamin, Fitosterol, Malondialdehit.

Bu çalışma, Bitlis Eren Üniversitesi Rektörlüğü Bilimsel Araştırma Projeleri (BAP) birimi tarafından BEBAP **2021.11** nolu proje numarası ile desteklenmiştir.

## *ORAL PRESENTATION*

# The Effect of Trimebutine Malete on the Contractility of Mouse Isolated Prostatic Vas Deferens

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**Abstract**

Trimebutine maleate (TMB) is a widely used drug for the treatment of functional gastrointestinal disorders. TMB has been shown to exhibit spasmolytic activity by interfering with calcium and potassium channels that regulate the smooth muscle contractility of gastrointestinal tract [1]. We recently reported that TMB has vasorelaxant effect in isolated rat thoracic aorta, which is partially nitric oxide dependent and related to the inhibition of L-type calcium channels [2]. In the present study, we performed *in vitro* contractility studies to investigate the effect of TMB on isolated prostatic vas deferens of mice. The contractile responses to KCl (80 mM), phenylephrine (Phe, 0.3 mM), adenosine triphosphate (ATP, 1 mM) and electrical field stimulation (EFS; 50 V, 5 ms, 8-16-32 Hz) were evaluated in the absence or presence of TMB (1, 10 or 100 µM). TMB significantly inhibited the contractions of prostatic vas deferens induced by KCl, Phe, EFS and ATP in a concentration-dependent manner. The lowest concentration of TMB tested did not alter contractile responses ($p>0.05$), however, 10 or 100 µM of TMB caused a marked decrease in KCl, Phe-, EFS- and ATP-induced contractions ($p<0.05$). Our results demonstrated that TMB has an inhibitory effect on the contractility of prostatic vas deferens and its exact mehanism(s) of this action will be investigated in further studies.

**Keywords:** isolated tissue bath, trimebutine, vas deferens.

**References:**

[1]. Lee HT, Kim BJ. Trimebutine as a modulator of gastrointestinal motility. Arch Pharm Res. 34(6):861-864, 2011.
[2]. Kadioglu M, Kaya Yasar Y, Barut EN, Engin S. Trimebutine maleate relaxes the isolated rat thoracic aorta: The role of nitric oxide and L-type calcium channels. Clin Exp Pharmacol Physiol. 46(4):322-328, 2019.

## *ORAL PRESENTATION*

# Optimization of gelatin extraction from chicken feet using Box–Behnken design

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**Abstract**

The valorisation of by-products has become a current requirement for economic and environmental reasons. Poultry slaughterhouses generate as many by-products as red meat industries, especially with the increase of chicken consumption. Our objective is to optimize a protocol for the extraction of gelatin from chicken feet.
Fifteen run of chicken feet were used for gelatin extraction under different conditions. The Box behnken design was used to optimize the extraction parameters including acid acetic concentration (2, 3.5 and 5%), extraction temperature (55, 65 and 75 °C) and extraction time (2, 4 and 6 h). The yields of each condition were calculated and some techno-functional analyses were performed: foaming power, emulsifying power and viscosity.
The optimization by response surface method (RSM), the predicted responses for these extraction conditions included: yield of 11.95%, emulsifier activity index (EAI) of 32.34$m^2$ $g^{-1}$, viscosity of 4.07 mP. s, and foaming capacity of 173.76%. The optimum extraction parameters were 3.5 % of acetic acid at 74.99°C for 4.4h.
The estimation models' ability to forecast the dependent variables reasonably and accurately was confirmed by the discovery that the actual and predicted values were nearly identical.

**Keywords:** Feet -Chicken by-product-Gelatin-Optimization-Rsm.

## *ORAL PRESENTATION*

# The Detection Of Dopamine With High Sensitivity And Selectivity Using Amine- Coated Carbon Dots

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**Abstract**

In this analysis, we explain the straightforward, step-by-step manufacture of N/Fe-CDs using microwave techniques that make use of $FeCl_3$.6H2O, Citric Acid (CA), and Ethylene diamine (EDA) as precursors. The process only requires one step. Analysis using XRD, XPS, and TEM all converged on the same conclusion: the formation was successful. After that, the N/Fe-CDs that had been made were used in an experiment to test the detection of Dopmaine (DA). It has a low detection limit and demonstrates an excellent sensing method toward the detection of DA via the production of catechol–$Fe^{3+}$ complexes. In addition, N/Fe-CDs reacted to selective and sensitive detection of dopamine through the inner filter effect. This detection was brought about by the inner filter effect. In addition, the produced N/Fe-CDs were put to use in actual sample applications, with artificial urine, lake water, and tap water samples being employed in the appropriate applications correspondingly. The existing synthesis method and detection methodologies could be straightforward and economical methods for the improvement of low-cost and sensitive sensors for use in biological and environmental applications.

**Keywords:** Carbon Dot, Dopamine, tap water.

## *ORAL PRESENTATION*

# Au/$Fe_2O_3$ catalyst for propargylamines synthesis under Irradiation

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**Abstract**

In organic synthesis, the irradiation by UV light has attracted many researchers, so several reactions were performed using the photocatalytic route as the conversion of alcohol to aldehyde [1]. The UV visible light is considered as an energy source that can reduce the reaction temperature and modify the metal capacity by the excitation of its electrons leading to strong interaction between molecules and metal [2]. Propargylamines are interesting intermediates to synthesize built and heterocycled molecules such as pyrrole, oxazoles, pyrazoles [3]. Furthermore, a number of propargylamines derivatives were used to treat Parkinson's and Alzheimer's disease such as rasagyline [4]. Their preparation method has drawn the attention of a significant number of researchers. In view of the synthetic procedure, the three coupling component of alkyne, aldehyde and amine (A3) was used in the presence of catalysts[5].
In the present study, two types of mesoporous materials were synthesized: the mesoporous $Fe_2O_3$ was prepared with the hard template using SBA-15, while the mesoporous Au/$Fe_2O_3$ was prepared by DP-NaOH, and DPU. With the last one, different content of gold were used. Then, the effect of preparation method and nanoparticles size on the catalyst activity was demonstrated. The catalyst which gives the best yield was used also to synthesize propargylamines via AHA coupling under ordinary condition and UV light irradiation.

**Keywords:** Au/$Fe_2O_3$, propargylamines, mesoporous materials, UV Irradiation.

**Reference:**
(1) R. Zhang, Y. Liu, Z. Wang, P. Wang, Z. Zheng, X. Qin, X. Zhang, Y. Dai, M.-H. Whangbo and B. Huang, *Appl. Catal. B* (**2019)**, 463-470 *254*.
(2) Z. Jiao, Z. Zhai, X. Guo and X.-Y. Guo, *J. Phys. Chem. C* (**2015)**, 3238-3243 *119*.
(3) K. Lauder, A. Toscani, N. Scalacci and D. Castagnolo, *Chem. Rev.* (**2017)**, 14091-14200 *117*.
(4) M. Sharma, J. Mangas-Sanchez, N. J. Turner and G. Grogan, *Adv. Synth. Catal.* (**2017)**, 2011-2025 *359*.
(5) S. Sadjadi, M. M. Heravi and M. Ebrahimizadeh, *J. Porous Mater.* (**2018)**, 779-788 *25*.

## *ORAL PRESENTATION*

# Phytochemical analysis of essential oil and by-products from *Lavandula angustifolia* Miller

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**Abstract**

The genus Lavandula occurs naturally in the Mediterranean basin and its species are a rich source of phytochemicals that are cultivated for their essential oils, widely used in cosmetics. The aim of this study is to determine the antioxidant activity of waste products after essential oil obtained from *Lavandula angustifolia* Miller flowers by microwave-assisted distillation method, which is one of the environmentally friendly extraction methods, and to determine the essential oil characterization by gas chromatography-mass spectrometry. Total phenolic, total flavonoid, total proanthocyanidin and antioxidant activity tests were performed with the solid waste and liquid waste obtained after hydrolysis of lavandula. Total phenolic, total flavonoid and total proanthocyanidin contents in waste water were determined as 4.936 (mg/g), 4.291 (mg/g) and 0.669 (mg/g) respectively. In solid waste extraction, the total phenolic and total flavonoid contents were identified as 0.649 (mg/g) and 0.467 (mg/g), respectively. ABTS, DPPH and CUPRAC methods were used to determine antioxidant activity. The antioxidant activity of waste water was 10,415 (mg/g) (DPPH), 6,517 (mg/g) (ABTS) and 10,396 (mg/g) (CUPRAC); antioxidant activity of solid waste was 0.804 (mg/g) (DPPH), 0.656 (mg/g) (ABTS) and 1.04 (mg/g) (CUPRAC); the antioxidant activity of the essential oil obtained after distillation was 0.568 (mg/g) (DPPH), 2.93 (mg/g) (ABTS) and 2.918 (mg/g) (CUPRAC). The essential oil components were determined predominantly eucalyptol (10.4%), linalool (30.22%) and borneol (16.22%). On the other hand, total carbohydrate (21.34%), total protein (2.59%) and total lipid (0.74%) contents of solid waste were identified. In line with the results, it is evaluated that besides the rich bioactivity content of the essential oil obtained after the distillation of Lavandula flowers, the wastes obtained after distillation can be used as a source of bioactive compounds.

**Keywords:** Essential oil, microwave-assisted hydrodistillation, antioxidant activity, by-productS.

## *ORAL PRESENTATION*

# Pretreatment techniques for converting black carrot pomace into fermentable sugar

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**Abstract**

Use of agro-food waste products could be a viable alternative for maintaining sustainable development and a zero-waste strategy. Industrial production of bioethanol or enzyme from biomass requires effective pretreatment and hydrolysis step. Moreover, usage of emerging technologies in biomass valorization tends to increase sugar production. Although extraction of polysaccharides to convert sugars from biomass waste by acid or alkali pretreatments, and enzymatic hydrolysis are extensively studied, a few studies have existed about the use of microwave for the extraction of reducing sugars from biomass. The effects of thermal, thermo-chemical and microwave pre-treatment techniques were evaluated and compared relative to the hydrolysis of black carrot pomace in the presence of sulphuric acid. This study proposes three different pretreatment strategies to increase reducing sugar recovery after subjecting black carrot pomace to enzymatic hydrolysis. Initially, three different pretreatment methods such as thermal, thermo-chemical and microwave were performed for black carrot pomace and ~~the~~ changes in its reducing sugar content and structural conformation were analyzed and compared. All the pretreated black carrot pomace samples were then subjected to enzymatic hydrolysis. The reducing sugar content was found as 10.24±0.04 g/L and 10.84±0.50 g/L for thermal and thermo-chemical pretreatment methods, respectively. The most effective pretreatment method was found as microwave assisted with dilute acid (MW-DA) process. Taguchi method was applied to study the effect of several parameters such as microwave power ($X_1$; 300, 600 and 850 W) and pre-treatment time ($X_2$; 30, 60 and 90 s). The optimized reducing sugar content was estimated at 24.62 g/L under 2% $H_2SO_4$ for 30 s at 300 W followed by enzymatic hydrolysis. MW-DA pre-treatment process was observed to lower the cost significantly owing to decrease in power, and pre-treatment time.

**Keywords:** Microwave pre-treatment, fermentable sugar, black carrot pomace, valorization.

## *ORAL PRESENTATION*

# Effect of parsley on oxidative stress-induced brain and intestinal damage and nitric oxide activity

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**Abstract**

Methotrexate (MTX), a folate antagonist, is used in the treatment of diseases such as tumors, psoriasis, and rheumatoid arthritis by inhibiting folate-dependent enzymes. Despite having a broad range of applications, MTX also damages tissues. Studies have demonstrated that oxidative stress is the underlying cause of brain and small intestine damages, and it has been stated that the administration of antioxidants during MTX therapy minimizes these damages. Parsley (*Petroselinum crispum*) contains flavonoids such as apigenin and essential oils such as myristicin and apiol, which should be evaluated because of their strong antioxidant properties. Due to the presence of different flavonoids and essential oils in its composition, parsley has been shown to have hypoglycemic, hypolipidemic, antibacterial, anticoagulant, antioxidant, diuretic effects.

In this study, the effects of parsley extract on the MTX induced-brain and small intestine tissue damages were investigated. For this purpose, 32 Sprague Dawley rats were divided into four groups (control, control + parsley, MTX, MTX + parsley). While the control group (n=8) was given physiological saline, the control + parsley group (n=8) was given parsley (2 g/kg) by oral gavage. MTX was administered intraperitoneally as a single dose (20 mg/kg) to the MTX and MTX + parsley groups. Parsley extract (2 g/kg, 0.5mL) was administered to the MTX + parsley group by oral gavage. At the end of the fifth day, brain and small intestine tissue samples were taken after decapitation. Lipid peroxidation and glutathione level, and nitric oxide activities were determined in the brain and intestinal tissue samples. MTX administration significantly increased LPO level and significantly decreased GSH in brain and small intestine tissues. NO activity did not significantly change in brain tissue and significantly increase small intestine NO activity. In line with the obtained results, parsley extract ameliorated the methotrexate induced-oxidative stress and changed the intestinal NO activity.

**Keywords:** *Petroselinum crispum*, parsley, methotrexate, brain, small intestine, oxidative stress.

## *ORAL PRESENTATION*

# Comparative study of the in-situ physiological stress response induced by Sampling Operation in the European sea bass Dicentrarchus labrax under farming conditions in the Bay of M'diq - the Moroccan Mediterranean

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**Abstract**

Daily or periodic aquaculture operations are essential and necessary during aquaculture production in order to maintain fish welfare as well as to evaluate the stock status, including the monthly sampling operation for the measurement of fish weight and size. The European Sea bass *Dicentrarchus labrax* represents the main species of industrial intensive marine fish farming in Morocco. The present study aims to investigate and compare in-situ the physiological stress response induced by this aquaculture operation in adult and subadult sea bass under rearing conditions in M'diq Bay - Moroccan Mediterranean. Plasma cortisol (Fish cortisol ELISA Kit) and blood glucose and lactate levels (portable readers and specific strips) were measured before and after the sampling operation in both age groups. Our results thus reveal the presence of a primary and secondary stress response to the sampling operation in adult and subadult sea bass *D. labrax*.

**Keywords:** farmed sea bass, cortisolemia, glycemia, lactatemia, stress, aquaculture practices.

## *ORAL PRESENTATION*

# Double Molecular Imprinted Nanoparticles for Controlled Release of Respiratory Drugs

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**Abstract**

Pulmonary drug delivery is an alternative drug delivery system with many advantages over oral or injection delivery methods such as provided easy and fast absorption of soluble substances.[1] Nanocarriers for pulmonary applications have been popular for the past two decades. The use of different nanocarriers such as liposomes, polymeric micelles, dendrimers, mesoporous silica nanoparticles, nanostructured lipid carriers are being invested for the pulmonary drug release.[1-4] Molecular imprinted method is the alternative method for synthesis of nanocarriers and in the drug release systems have increased interest in recent years. Molecular imprinted technology is the process of synthesizing polymers containing binding sites that recognize target molecules specifically.[5] Nowadays, different imprinting strategies such as surface imprinting, segment imprinting, double imprinting are improved for MIP in the different applications.[6]
In this study, with new approach, double molecular imprinted nanoparticles (DMIP) were synthesized by surfactant free emulsion polymerization technics for the investigated to release of the salmeterol xinafoate (SAM) and fluticasone propionate (FLU) drugs. These DMIP nanoparticles were characterized by FTIR, SEM and Zeta sizer. Also, the specific surface areas of DMIP nanoparticles were calculated. Drug loading capacity of DMIP nanoparticles were optimized. The maximum adsorption values of SAM and FLU on the DMIP nanoparticles were found as 537.64 mg/g and 393.32 mg/g respectively. The release of SAM and FLU from DMIP nanoparticles were also investigated with different release conditions such as pH, initial drug concentrations, temperature. In the end of the release studies, SAM and FLU release studies were performed in the simulated lung fluid. At the end of seven days, the amount of released SAM and FLU 4.96 mg /g and 6.15 mg/g in the SLF respectively. Finally, the release behavior of drugs were analyzed by using five different kinetic models and the release of SAM and FLU was determined to be compatible with the Higuchi release model. According to these results, these DMIP nanoparticles were promising nanoparticles for controlled drug release studies.

**Keywords:** Double molecular imprinted nanoparticles, salmeterol xinafoate, fluticasone propionate, controlled drug release, drug release kinetics

**Acknowledgement:** This study was financially supported by Aliye Uster Foundation of Ege University. On the other hand, Dr. Esra FEYZİOĞLU DEMİR was supported by The Scientific and Technological Research Council of Turkey (TUBITAK), 2211/C National PhD Scholarship Program in the Priority Fields in Science and Technology during her doctoral studies.

**References:**

1) Garbuzenko OB, Mainelis G, Taratula O, et al. Inhalation treatment of lung cancer: the influence of composition, size and shape of nanocarriers on their lung accumulation and retention. Cancer Biol Med.2014; 11:44–55.
2) Beck-Broichsitter M, Merkel OM, Kissel T. Controlled pulmonary drug and gene delivery using polymeric nano-carriers. J Control Release. 2012; 161:214–224.
3) Ungaro F, d'Angelo I, Miro A, et al. Engineered PLGA nano- and micro-carriers for pulmonary delivery: challenges and promises. J Pharm Pharmacol. 2012; 64:1217–1235.
4) Zhang J, Wu L, Chan HK, et al. Formation, characterization, and fate of inhaled drug nanoparticles. Adv Drug Deliv Rev. 2011; 63:441–455
5) Büyüktiryaki S, Say R, Denizli A, et al. Mimicking receptor for methylmercury preconcentration based on ion-imprinting. Talanta. 2007; 71:699–705.
6) Chen L, Wang X, Lu W, et al. Molecular imprinting: Perspectives and applications. Chem. Soc. Rev. 2016.

## *ORAL PRESENTATION*

# Retrospective evaluation of parasitic species diagnosed between 2009 and 2022 in the Laboratory of the Department of Parasitology, Faculty of Veterinary Medicine, Kafkas University

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**Abstract**

This study was carried out to evaluate the parasitic species in the samples taken from domestic and wild animals brought to Kafkas University Veterinary Faculty Parasitology Department Laboratory between 2009 and 2022, regardless of gender.

Between 2009 and 2022, a total of 800 materials were sent to the parasitology laboratory. These materials are; 359 (44.9%) intestines, 232 (29%) fecal samples, 68 (8.5%) organs (lung, liver, heart, brain, head, kidney, spleen), 54 (6.8%) skin scrapings, 35 (4.3%) bloods, 23 (2.8%) mix (blood+tick, intestine+larvae, tick+lice, blood+lice, tick+fecal sample+blood), skin scraping+tick, tick+intestine, tick+fecal sample, tick+blood+flea, blood+fecal sample, blood+ fecal sample+lice, fecal sample+blood+nymph), 24 (3%) tick infestation, 2 (0.3%) were infested with larvae, 2 (0.3%) were infested with lice, and 1 (0.1%) were insect infestations. The materials are diagnosed with the routine examination method in accordance with the materials. 800 samples that make up the research material; domestic mammals 640 (80%), 86 (10.8%) domestic birds, 31 (3.8%) wild mammals and, 43 (5.4%) wild birds. As a result of the examination of the incoming samples, it was determined that 313 (39.12%) samples were positive, and 487 samples (60.88%) were negative. Of the 313 positive samples, 159 (50.8%) were protozoan, 92 (29.4%) helminths, 45 (14.4%) ectoparasites and 17 (5.4%) mixed parasites (protozoan+ helminth, ectoparasitic + helminth, ectoparasitic + protozoan).
The fact that many parasite species were found in different animal groups in the Kars region, even during a routine examination, shows us how rich the parasitic fauna is in the region.

With the results of this study, common and economically important parasitic species identified from the samples coming to our routine laboratory have been given priority both in our graduate studies and in the studies carried out by our Department, and data that can always be beneficial in terms of guiding the researches planned to be conducted in the future have also been determined.

**Keywords:** ectoparasite, helminth, Kars, protozoan, retrospective evaluation, routine parasitological examination

We would like to thank them for their contributions. Among the faculty members who worked in our department between the specified years and still do, Prof. Dr. Mukremin Ozkan ARSLAN, Prof. Dr. Zati VATANSEVER, Prof. Dr. Murat KARA, Prof. Dr. Atila AKCA, and Assoc. Dr. Gencay Taskın TASCI.

## *ORAL PRESENTATION*

# Preparation of microgels using microstencil plate for albumin adsorption

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## Abstract

Microfabrication technologies have been utilized in biochemistry, electronic engineering, biotechnology, and biomedical engineering with a variety of applications including solid-phase microextraction (SPME) support materials, tissue scaffolds, MEMS-based sensors, drug delivery systems (DDSs), and so on. Besides their biomedical and tissue engineering applications, microengineered hydrogels could be great materials in SPME of biomolecules such as proteins, peptides, DNA, enzymes, antibodies, because of high-water content, elasticity, biocompatibility, etc. The goal of this study is to design amino acid-based microgels using a microstencil plate for the adsorption of bovine serum albumin (BSA). N-methacryloyl-(L)-histidine methyl ester (MAH), a metal-chelating monomer, was selected as a functional monomer for the target protein, BSA. 2-hydroxyethyl methacrylate (HEMA), and N′-methylenebisacrylamide (MBA), were employed as a main monomer and as a crosslinker, respectively. Polymerization was conducted on a stainless steel microstencil plate having microholes with diameter of 600 μm and with thickness of 500 μm. Optimal conditions for BSA adsorption efficiency of the imprinted microgels were determined by analyzing the effects of medium pH, temperature, initial BSA concentration, incubation time and salt concentration. The maximum BSA adsorption capacity of the microgels was found as 14.6 mg/g microgel. Selectivity studies of the imprinted microgels were applied using non-imprinted microgels as a control group. The relative selectivity coefficients of imprinted microgels for BSA/transferrin and BSA/invertase were 1.8 and 4.2 times higher than the ones of non-imprinted microgels, respectively.

**Keywords:** Albumin, Microgels, Microstencil plate, Molecular imprinting

## *ORAL PRESENTATION*

# Physical, Chemical and Microbiological Examination of Yoghurts Prepared Using Olive Leaf Extract with Antimicrobial and Antioxidant Properties During the Storage Period

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**Abstract**

The most important factor affecting the consumability and reducing the shelf life during storage in industrial yogurts is the development of mold and yeast. In this study, olive leaf (*Olea europaea* L.) extract added to yogurt formulations or sprayed on the surface of yogurts to longer the shelf life of yogurt by inhihibiting the growth of mold and yeast. For this purpose, commercial powdered olive leaf, which is known to contain 20% oleuropein, was added in the yogurt formulation at a rate of 0.4% (D) and 0.6% (E). In surface applied samples, aqueous extracts at concentrations of 0.4% (A) and 0.6% (B) were sprayed onto the yoghurt surface at a rate of approximately 333 µL. The control sample does not contain olive leaf extract. Microbiological and physico-chemical analysis performed during storage period (28 days). According to microbiological analyzes, it was determined that addition of extract did not have a statistically significant effect on the number of lactic acid bacteria in the yogurt samples ($p<0.05$) and was at the level of 8 log cfu/g. During storage, mold-yeast growth was not seen in all yogurt samples, and on day 42, only control mold growth was detected. No statistical difference have seen in A, B, K yogurts in terms of sensory analysis. The addition of extracts directly to the formulation has reduced consumer acceptability. According to Folin-Ciocalteu and ABTS radical scavenging methods, the phenolic substance content of D and E samples was approximately 5 times higher than the A, B, K samples. In the light of these results, it was concluded that the yoghurts produced by the surface spraying method were accepted by the consumer compared to the control yogurt and could prevent the development of mold-yeast on the surface without distinguishing them from the control yogurt.

**Keywords:** Mold, Olive Leaf Extract, Yogurt, Yeast.

## *ORAL PRESENTATION*

# The effect of cerium on the structural and biological properties of composite scaffolds

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**Abstract**

This study reports the effect of varying cerium (Ce) ratios on the structural and biological properties of Ce-doped wollastonite/chitosan (WS/CS) composite scaffolds. For this purpose, undoped and Ce-doped WS nanopowders were synthesized by sol-gel followed by heat treatment dual process. The XRD pattern showed that β-wollastonite phases were formed for the nanopowders synthesized at 1100 ℃. Composites prepared by incorporating the synthesized nanopowders into the chitosan were lyophilized after freezing to fabricate the composite scaffolds. According to the results obtained, the increasing Ce addition seriously affected the mechanical strength and porosity ratios of the scaffolds produced in an interconnected and open porous structure. The porous composite scaffolds were kept in simulated body fluid (SBF) for a while to evaluate their bioactivity. SEM/EDS studies revealed that composite scaffolds formed a uniform layer of apatite similar to bone minerals on their surfaces. When the apatite-forming ability of composite scaffolds was measured inside SBF solutions, they showed excellent biocompatibility and bioactivity due to the release of calcium and magnesium ions. Our research shows that the produced Ce-doped WS/CS scaffolds are promising materials for use in bone tissue engineering applications.

**Keywords:** Cerium, wollastonite, scaffolds, structural properties, biological properties.

## *ORAL PRESENTATION*

# Distribution of entomopathogenic fungi causing natural infection in *Orasanga japonica* (Hemiptera: Ricaniida) in East Black Sea Region of Turkey

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**Abstract**

*Orasanga japonica* (syn. *Ricania japonica*, Hemiptera: Ricaniidae) is one of the most important pests in the Black Sea Region of Turkey. Although various methods and microbial factors from different source have been tried to control on the pest until recently, any fungal pathogen has been detected on it. Entomopathogenic fungi are one of the most important microbial agents in pest control. In this study, we isolated 18 fungal strains from *O. japonica* nymph and adults with fungal infection collected from Rize (Ardeşen, Pazar and İyidere), Trabzon (Of) and Artvin (Hopa) in Turkey. According to morphological and molecular characterization studies based on the internal transcribed spacer (ITS) sequences, all of strains were identified as *Beauveria bassiana* (OJ1-OJ18). The screening test at $1 \times 10^8$ conidia/ml showed that all strains caused 75-100% mortality against nymph and adults after 10 days. Further experiments were performed with the three most effective strains, all belonging to *B. bassiana* (OJ3, OJ7 and OJ15). Dose response of the three fungal strains were carried out on the *O. japonica* nymph and adults at concentrations of $1 \times 10^{5\text{-}9}$ conidia/ml. As a result of the dose response, *B. bassiana* OJ3, OJ7 and OJ15 strains showed that 100, 98.84 and 100% mortality was observed at the highest dose ($10^9$ conidia/ml) on the $10^{th}$ day, and calculated $LC_{50}$ value of OJ3, OJ7 and OJ15 were determined as $0.3 \times 10^5$, $0.2 \times 10^5$ and $0.2 \times 10^4$ conidia/ml, respectively. The presence of *B. bassiana* in detected natural infections and the efficacy trials conducted in the laboratory showed that it has potential against *O. japonica*. These results may have contributed to the decrease in the population of this pest in the eastern black sea region in recent years.

**Keywords:** *Orosanga japonica*, Entomopathogenic fungi, *Beauveria bassiana.*

## *ORAL PRESENTATION*

# Different behaviors of soluble and insoluble biopolymers in electrochemical urea biosensor

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**Abstract**

Polymeric structures play an important role worldwide, from biological studies to industry at large. Rapid advances in chemistry and materials have led to the introduction of a variety of new synthetic polymers. Since most of the polymers are obtained from non-biodegradable and non-renewable sources, many environmental and health problems have occurred with the increasing use. For this reason, interest in protecting the environment has increased in recent years through the use of sustainable resources [1]. Biopolymers have important properties such as good mechanical properties, biocompatibility, non-toxicity and low cost. Thanks to these good properties, biopolymers are exploited for the development of high-performance devices and flexible electronic equipment [2]. In this study, two different biopolymers, gelatin and nanocrystalline cellulose, were used in the electrochemical enzymatic urea biosensor design. It is aimed to determine the morphological and electrochemical effects of these polymers, one of which is soluble and the other is insoluble in common organic solutions, on the electrode surface. The surface morphologies of the electrodes were evaluated by scanning electron microscopy, and their electrochemical performances were evaluated by differential pulse voltammetry. It has been determined that these two biopolymers show quite different effects in the electrochemical sensor study and provide positive contributions to the biosensor in some aspects.

**Keywords:** Urea detection, electrochemical biosensor, enzymatic biosensor, biopolymers, gelatin, nanocrystalline cellulose.

**References:**

1.Abhilash, M. and D. Thomas, Biopolymers for biocomposites and chemical sensor applications, in Biopolymer composites in electronics. 2017, Elsevier. p. 405-435.
2.Wang, Z., et al., Recent advances in natural functional biopolymers and their applications of electronic skins and flexible strain sensors. Polymers, 2021. 13(5): p. 813.

**Acknowledgments:** This work was supported by the TÜBİTAK 1002-B project (Project number: 122Z696). Vildan Sanko is sponsored by the YOK 100/2000 doctorate program and TÜBİTAK 2211-C National PhD Scholarship Programs in the Priority Fields in Science and Technology.

## *ORAL PRESENTATION*

# QSAR study of new HIV-1 protease inhibitors, Molecular Docking, ADMET properties and Molecular Dynamics Simulation

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**Abstract**

The current work is based on a series of thirty-three compounds; their enzymatic inhibitory activities against HIV-1 protease taken from the literature are used in this research to build a mathematical model relating the structure to the biological activity. These compounds were designed by several software, their descriptors were computed by the same various tools, such as Gaussian, Chem3D, ChemSketch and MarvinSketch. Computational methods were used in order to generate the best model using XLSTAT tool, the model obtained has a correlation coefficient of 0.66 for enzymatic inhibitory activities against HIV-1 protease. Internal and external validation confirmed the quality of the proposed model, $Q^2$ = 0.53 and $R^2$ = 0.64 respectively and the model's applicability domain (AD) was determined. Furthermore, one compound has been proposed as efficient against HIV-1 protease with higher biological activity as compared to the existing ones, this drug candidate was evaluated using ADMET properties and Lipinski's rule. Molecular Docking allowed us to determine the best conformation of the complex compound protease – ligand with an affinity value equal to -7.5kcal/mol. Also, molecular dynamics simulation study was carried out in order to investigate the compound's stability. In addition, a comparative study was conducted, using an FDA approved drug.

**Keywords:** HIV-1 protease, QSAR, ADMET properties, Molecular Docking, Molecular Dynamics Simulation.

## *ORAL PRESENTATION*

# Effects of acetyl-L-carnitine administration on sensory gating in rats exposed to chronic ethanol

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**Abstract**

Alcohol is one of the leading causes of preventable morbidity and mortality worldwide. One of the organs negatively affected by chronic alcohol consumption is the brain. It is known that chronic alcohol consumption in humans causes sensory gating deficits. However, no study has been found in the literature on this subject in rats. Acetyl-L-carnitine (ALCAR) is a carnitine metabolite found in the plasma and tissues of mammals and is thought to have a neuroprotective effect. Therefore, in this study it was aimed to determine the effect of chronic alcoholism on sensory gating in rats and whether ALCAR has a protective effect in a possible negative effect.

3 months old 48 male Wistar rats were used in 4 groups: Sham(Sh), chronic ethanol(CE), ALCAR administered(ALCAR), and chronic ethanol+ALCAR(CE+ALCAR). Distilled water to the Sh group, 5g/kg ethanol (25% v/v) to the CE group, 50 mg/kg ALCAR to the ALCAR group, and the same doses of ethanol+ALCAR to the CE+ALCAR group were given by gavage for 4 weeks. Event-related potentials were recorded by using the sensory gating paradigm. Conditioning stimuli(CS) / test stimuli(TS) ratios were used as a measure of sensory gating.

There were no statistically significant differences in CS/TS ratios between Sh vs CE, CE vs CE+ALCAR, and Sh vs ALCAR groups.

Although impaired sensory gating in chronic alcoholism has been shown in humans, it was not observed in rats in our study. This may be due to the low dose of alcohol we give to the rats or another physiological condition. Since sensory gating was not impaired in the chronic alcohol group, it could not be determined whether acetyl-L-carnitine had a protective effect. Repeating this study with higher doses of alcohol in rats would reveal the possible benefit of acetyl L-carnitine against deterioration in sensory gating.

**Keywords:** Chronic alcoholism, rat, acetyl-L-carnitine, sensory gating.

## *ORAL PRESENTATION*

# Kafa travmalarında CGRP düzeyi ile moleküler yaklaşım

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## ÖZET

Kafa travması, kafatası veya beyinde travma sonrası gerçekleşen herhangi bir yaralanmadır. Vücuttaki travmanın gözlenebildiği durumların aksine, kafa travmaları fark edilerek veya fark edilmeden olabilir. Yaygın bir sağlık sorunu olmakla beraber travmatik beyin hasarına tanı konması ve prognozunun tahmini hala sorunlu bir süreçtir.
S100B, Nöron spesifik enolaz (NSE), Glial fibriler asidik protein (GFAP), Myelin-basic protein (MBP) ve tau'yu içeren çeşitli belirteçler orta-ağır TBH sonrası akıbetinde bakılmıştır. Beyin hasarını gösterecek bir belirteç gelistirmek, beynin daha kompleks ve daha az homojen bir organ olmasından daha zor olmustur. Dolay›s›yla tek bir hasar şekli ve ölen tek tip hücre yoktur. Bu nedenle TBH Prognozunda biyobelirteç yanında radyolojik ve klinik bulguların birlikte kullanılması önerilmektedir.
CGRP, kalsitonin geni ilişkili peptit ailesinin bir üyesidir. CGRP hem periferik hem de merkezi nöronlarda üretilir. Güçlü bir peptit vazodilatördür ve nosisepsiyon iletiminde işlev görebilir. Omurilikte, CGRP'nin işlevi ve ifadesi, sentez yerine bağlı olarak farklılık gösterebilir esas motor nöronların hücre gövdelerinden türetilir ve yaralanma sonrası sinir dokusunun yenilenmesine katkıda bulunabilir. Çalışmadaki amacımız, migren hastalarında sıklıkla çalışılan CGRP düzeyinin kafa travmalarında tanı belirteci yönünden değerlendirmektir.
Araştırma DÜ Hastanesine başvuran 19 hafif-orta kafa travmalı hasta, 23 sağlıklı kontrol üzerinde gerçekleştirildi. Travma sonrası istenen rutin radyoloji ve kan parametreleri dışında CGRP düzeyleri serumda spektrofotometrik yöntem kit ile ölçüldü. 4-83 yaş aralığında gelen hastaların ort.yaşı 47 olup, 9 kadın 10 erkek dahil edildi. Biyokimyasal değerlerde anlamlı bir farklılık görülmezken, travma grubu moleküler CGRP(ng/ml) düzeyinde %36,07 anlamlı artış görüldü. Radyoloji verileri ile karşılaştırılarak yorumlanan CGRP seviyesi travma şiddetine göre değişkenlik gösterdi.
Vazodilatasyon, nörojenik enflamasyon, periferik ve santral sensitizasyon durumlarında dikkat çeken CGRP rolünün travmaya bağlı hasar patofizyolojisinde daha detaylı çalışılarak açıklanması beklenmektedir. Buna bağlı olarak, CGRP ligand veya reseptörünü hedef alan ilaçlar kafa travma geçiren bireylerde klinik açıdan yeni bir boyut kazandırabilir.

**Anahtar kelimeler:** Travmatik beyin hasarı, CGRP, moleküler tanı.

## *ORAL PRESENTATION*

# The *Fomes fomentarius* methanol extract and antibiotic interactions in some clinically isolated multi-drug resistant *E. coli* strains with active efflux pumps by using FTIR analysis

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**Abstract**

Studies to prevent antibiotic resistance are continuing rapidly. As it is known, bacteria have various resistance mechanisms. The over-expression of efflux pumps is just one of these resistance mechanisms. In antibiotic intake, the drug taken through this resistance mechanism is expelled out of the cell and the effect of the drug is lost. Various studies are carried out to prevent this. Inhibition of the efflux pumps is one of these methods. With the inhibitory effect of compounds, it is aimed to recover the effect of drugs in the *in vitro* studies. In order to avoid the toxicity problem, the researches for inhibitors from natural sources are under investigation. In this study, methanol extract of *Fomes fomentarius* macrofungus was used to observe its efflux pump inhibition potential in some clinically isolated multidrug-resistant *Escherichia coli* strains. Antibiotics were selected according to the antibiotic resistance profile of the strains used and the study was carried out using a combination with the extract. FTIR analysis was performed to evaluate the mechanism of action for strains with an inhibitory effect as a result of combined use. According to the FTIR analysis; there are significant changes in the band scale. The observed changes were found in proteins, DNA/RNA, phospholipids, polysaccharides, fatty acids, and fingerprint regions. In light of the observed data, it is appropriate to elaborate further studies.

**Keywords:** Bacterial Resistance, *E. coli*, Efflux Pumps, Methanol, FTIR, Multidrug Resistance.

**Acknowledgment:** The authors would like to thank the Kastamonu University Scientific Research Projects Coordination Department under the Grant No. KÜ-BAP01/2021-47 for the support.

## *ORAL PRESENTATION*

# The FTIR analysis of *Fomes fomentarius* ethyl acetate extract-antibiotic interactions in some multi-drug resistant (MDR) *E. coli* strains having active efflux pumps

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**Abstract**

Studies on eliminating multi-antibiotic resistance are continuing and in these studies, the search for inhibitors takes a major role. Due to the toxic nature of synthetic efflux pump inhibitors, new inhibitors having low toxicity are under investigation. Although studies at the *in vivo* level are long-term, studies at the *in vitro* level are provided more easily. In this study, inhibitor trials of some multidrug-resistant *Escherichia coli* strains with active efflux pumps were carried out using ethyl acetate extract of *Fomes fomentarius*, a macrofungi. In addition, antibiotics were selected according to the antibiotic resistance profile of the strains used, and combined use with the extract was tried. According to the results of the combined use of antibiotics and extracts, FTIR analysis was performed for microorganisms presenting an inhibitory effect. Thus, preliminary studies will be carried out by evaluating the mechanism of action. According to the FTIR results; significant changes were observed between the band <900 $cm^{-1}$ and 3000 $cm^{-1}$. While some of these changes occurred in proteins, some changes occurred in DNA/RNA and phospholipids. The obtained data will be detailed and used for further studies.

**Keywords:** Efflux Pumps, Multidrug Resistance, Bacterial Resistance, Ethyl Acetate, *E. coli*, FTIR.

**Acknowledgment:** The authors would like to thank the Kastamonu University Scientific Research Projects Coordination Department under the Grant No. KÜ-BAP01/2021-47 for the support.

## *ORAL PRESENTATION*

# Semi-Automatic Behavior Analysis System For Zebrafish

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**Abstract**

Today, there are many animal behavior analysis programs that are widely used for experimental studies. But these programs are often costly, and these high costs are unattainable for many researchers. For this reason in our laboratory we have developed a semi-automatic behavioral analysis system for zebrafish. With this system, behavioral analyzes of adult zebrafish and zebrafish embryos can be easily performed. We included 3 programs in this system and used these programs for the camera records we captured for our experimental models. The first of these programs is the Wondershare program which is used to edit the camera image. Secondly, the Kinovea program is used to track zebrafish semi-automatically. By following the Kinovea program, the zebrafish is marked and thanks to this label, the tracking of zebrafish can be completed smoothly and efficiently in the Toxtrac program. Finally, numerical data is obtained from the camera image using the Toxtrac program. Using these systems, we are able to perform our behavioral analyzes at low cost.
Due to the environment in which the fish live, reflections from the water may occur while recording which makes it impossible to follow the fish when using a single program. We eliminated these negative situations through the semi-automatic system we developed. As a conclusion, this semi-automatic system allows the conversion of the camera images into numerical data for the performance of behavior analysis in zebrafish.

**Keywords**: Behavior analysis program, semi-automatical, camera, zebrafish.

## *ORAL PRESENTATION*

# Nanoparticle ($Al_2O_3$, CuO and $TiO_2$) accumulation and alterations in the energy reserves in wistar rats

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**Abstract**

The liver is the energy store of mammals and its energy budget can change due to xenobiotic stress. There is no adequate information on the effects of nanoparticles (NPs) on the energy policy of mammals. Thus, this study was undertaken to investigate the accumulation of NPs in the liver of female Wistar rats (Rattus norvegicus var. albinos) following oral administrations (0, 0.5, 5, 50 mg/kg b.w./day) for 14 days. Levels of total protein, lipid and glucose were measured to determine the energy reserves of the liver. ICP-MS (Inductively coupled plasma mass spectrometry) measurement showed that NPs accumulated in the liver, as the concentrations of Al, Cu and Ti increased sharply ($P<0.05$). Data showed that glucose levels decreased significantly ($P<0.05$) after NP exposures at all doses. Similarly, lipid levels also decreased ($P>0.05$) at the highest doses of Al2O3 and CuO exposures. However, total protein levels did not change ($P>0.05$) after any NP exposure. Likewise, the total energy reserves of the liver decreased ($P<0.05$) after the highest NP exposures. Interestingly, data indicated that the first energy sources (glucose and lipid) of the metabolism were decreased by all NPs, indicating possible metabolic stress.

**Keywords:** Metal, Nanoparticle, Rat, Liver, Accumulation, Energy.

## *ORAL PRESENTATION*

# Nanoparticle exposures alter the levels of lipids, carbohydrates and the response of biomarkers in tissues of freshwater mussels

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**Abstract**

This study was carried out to determine the effects of nanoparticles (NPs) on the organic molecules and some biomarkers in the hepatopancreas and muscle of freshwater mussels (Unio tigridis) after 14 days of exposure. Unicellular algae (Chlorella vulgaris) cultured in the laboratory were served to mussels during the experiments. Results demonstrated that the glutathione metabolisms in the hepatopancreas were altered after NP (Al2O3, CuO, TiO2) exposures ($p<0.05$). Likewise, data also indicated that there was lipid peroxidation, as showed that the levels of malondialdehyde increased significantly. Oppositely, acetylcholinesterase activity in the foot muscles significantly. decreased after NP exposures. The levels of protein, glucose and lipid in the foot muscle and hepatopancreas significantly decreased and there were significant decreases in the total energy stores in the tissues. This study demonstrated that NPs might cause toxic effects and consequently reduce the energy stores of mussels and also suggest their availability to be used as a potential biomarker in environmental search.

**Keywords:** Metal-Oxide Nanoparticle, Biomarker, Lipid, Glucose, Mussel.

## ORAL PRESENTATION

# Salamura işleme tekniğinin organik ve konvansiyonel olarak yetiştirilen asma yapraklarının bazı fiziksel ve kimyasal özellikleri üzerine etkilerinin incelenmesi

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**Özet**

Asma yaprağı, kendine özgü aroması ve hafif ekşi tadı nedeniyle yıllardır taze ve salamura edilerek Türk, Balkan ve Orta Doğu mutfaklarında dolmalık olarak kullanılmakta ve tüketilmektedir. Sultani Çekirdeksiz, Narince ve Yapıncak asma yaprakları Türkiye'de en çok salamura yapılan ve tüketilen yapraklardır. Bu çalışmada, organik ve konvansiyonel uygulamalar ile yetiştirilen Sultani Çekirdeksiz asma yaprağının taze ve salamuraya işlenmiş örneklerinde; fiziko-kimyasal özellikler, biyoaktif bileşikler, antioksidan aktivite, mineral ve fenolik madde kompozisyonları incelenmiş ve salamura işleme tekniğinin etkisi ortaya koyulmuştur. Örneklerde pH, titre edilebilir asidite ve kuru madde gravimetrik olarak, toplam fenolik madde, flavonoid ve antioksidan aktivite (ABTS, DPPH ve CUPRAC) spektrofotometrik olarak belirlenmiştir. Mineral kompozisyon ICP OES, fenolik madde kompozisyonu HPLC ile tespit edilmiştir. Taze ve salamura edilen yaprak örneklerinde pH 3.41-3.62, asidite % 0.58-1.58 ve kuru madde % 24.03-26.55 arasında değişmiştir. Salamura fermantasyonu toplam fenolik madde (10.57-13.96 mgCE/g) ve flavonoid (5.14-6.44 mgCE/g) miktarında azalmaya sebep olmuştur. Antioksidan aktivite ABTS için 15.77-19.27, DPPH için 44.92-46.45 ve CUPRAC için 118.37-179.41 $\mu$molTE/g arasında değişmiştir. İncelenen örneklerde mineral madde kompozisyonu üzerine salamura işlemenin önemli derecede etki ettiği tespit edilmiştir ($p \leq 0.05$). İşlenmiş üründeki mineral maddelerin miktarı (P, K, Mg, Ca, Fe, Zn, Mn, Cu) işleme sürecinde salamura suyuna geçiş nedeniyle taze ürüne göre % 16.78 ile 70.11 arasında azalmıştır. İncelenen fenolik bileşiklerden kateşin miktarı üzerine işleme tekniğinin, epikateşin üzerine yetiştiricilik uygulamasının, epikateşin gallat üzerine ise salamura işleme ve yetiştiricilik uygulamalarının sinerjik etkisinin istatiksel olarak önemli olduğu bulunmuştur ($p \leq 0.05$). Epigallokateşin ve epigallokateşin gallat miktarları arasında istatiksel farklılıklar tespit edilememiştir. Yaprak örneklerinde *trans*-resveratrol miktarları 0.84 ile 2.37 $\mu$g/g arasında değişmiştir. Salamura işleme ve yetiştiricilik uygulamaları etkisi örneklerdeki *trans*-resveratrol miktarı açısından istatiksel olarak anlamlı bulunmuştur ($p \leq 0.05$). Sonuç olarak bu çalışma ile asma yaprağı üretimi ve işlenmesindeki uygulanan yetiştiricilik ve salamura fermantasyonunun yaprak bileşimi üzerine etkileri ortaya koyulmuştur.

**Anahtar Kelimeler:** Asma yaprağı, salamura, fermantasyon, mineraller, fenolik madde.

## *ORAL PRESENTATION*

# Termik santral bölgesinde yaşayan kişilerde mikronükleus testi ile genotoksik potansiyelin belirlenmesi

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**Özet**

Termik santraller, dünyada ve ülkemizde elektrik enerjisinin önemli kaynağıdır. Önemli kaynak görevinin yanı sıra muhtemel toksikolojik etkilere neden olabilecekleri de bilinen bir gerçektir. Bu çalışmada, termik santral bölgesinde yaşayan 20 ve bu bölgede yaşamayan 20 kişinin periferal kan lenfositleri mikronükleus testi ile genotoksisite açısından izlendi. Bireylerden alınan kan örnekleri kültür ortamında 37°C'de 72 saat inkübe edildi. Termik santral bölgesinde yaşayan kişilerin mikronükleus oranı (3.24 ± 1.19), bu bölgede yaşamayan kontrol grubu bireylerine göre (1.00 ± 0.38) yüksek bulunmuştur ($P < 0.01$). Mikronükleus oranlarındaki önemli artış, muhtemel genotoksik ve karsinojenik etkileri göstermektedir. Bu etkilerin, termik santral bacalarından çıkan birçok farklı gazdan kaynaklandığını düşünmekteyiz.

**Anahtar Kelimeler:** Termik santral, Mikronükleus, Genotoksik, Karsinojenik.

FLY-2021-902 kodlu proje ile tez çalışmamı destekleyen Yozgat Bozok Üniversitesi, Proje Koordinasyon Uygulama ve Araştırma Merkezi'ne teşekkür ederim.

## *ORAL PRESENTATION*

# Pterjiyum'da hücre iskeleti proteinlerinden ARPC5'in gen ekspresyon düzeyinin analizi

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**Özet**

Pterjiyum, konjunktivanın kornea üzerine doğru limbus boyunca kanat şeklinde büyüyen invaziv fibrovasküler dokusu olarak tanımlanır ve sık görülen bening bir bozukluktur. Yaşla birlikte insidansı artan pterjiyumun etiyolojisi tartışmalı bir konudur. Pterjiyum oluşumunda; hücresel proliferasyon, antiapoptozis, inflamasyon, immün aktivite, hücreler arası matriks (ECM) değişimi, metabolik hastalıklar, anormal hücre kinetiği ve kalıtım gibi olayların rol oynayabileceği ön görülmektedir. Pterjiyum patogenezinin hücresel proliferasyon antiapoptozis, invazyon, migrasyon ve nüks gibi kanserle birçok benzer özelliği barındırması, pterjiyumun bir tümör anoloğu olduğunu düşündürmektedir. Hücresel hareket, birçok biyolojik süreç için esastır. Buna karşın anormal şekilde aktive olmuş hücre göçü, kanser gibi birçok hastalıkta yer almaktadır. Aktin bakımından zengin özel çıkıntılar kanser hücreleri tarafından da oluşturulabilir. WASP/WAVE ailesi proteinlerinin, aktin ile ilgili protein (Arp) 2/3 kompleksinin aktive edilmesi yoluyla aktin polimerizasyonunun kontrolündeki rolü hücre göçünde ve invazyonda görülen aktin bazlı membran çıkıntılarının oluşmasında kritik öneme sahiptir. Farklı kanser tiplerinde metastaz, invazyon, migrasyon, proliferasyon ve hastalığın prognozu ile WASP/WAVE-Arp2/3 gen ailesi ilişkilendirilmiştir. Bu veriler, premalign özelliklere sahip olan pterjiyum oluşumunda ve nüksünde, WASP/WAVE-Arp2/3 gen ailesi üyelerinin rol alabileceğini ve farmakolojik hedef olarak kullanılabileceğini düşündürmektedir. Çalışmamızda, konjonktivada da eksprese olduğu bilinen, WASP/WAVE-Arp2/3 ailesi poteinlerinden olan ARPC5'in pterjiyumlu olgularda ekspresyon düzeyleri incelenerek, pterjiyum oluşumunda olası rollerinin belirlenmesi amaçlanmıştır. Çalışmaya Tokat Gaziosmanpaşa Üniversitesi Tıp Fakültesi Göz Hastalıkları Polikliniğine başvurarak pterjiyum tanısı alan ve opere edilen 27 hasta dahil edildi. Kontrol grupları ise aynı hastalara ait normal konjonktiva dokularından oluşturuldu. ARPC5 gen ekspresyon düzeyleri ise Real-Time PCR metodu ile belirlendi. mRNA düzeylerinin hesaplanmasında $2^{-\Delta\Delta CT}$ metodu kullanıldı. Kontrol dokularına oranla pterjiyum dokularında ARPC5 geni için kat artışı 1.67 olarak belirlendi. Ancak pterjiyum ve kontrol doku örneklerinin ekspresyon düzeyleri arasındaki bu fark istatistiksel olarak anlamlı değildir ($p$=0,45). Bununla birlikte görece örneklem grubumuzun az olması daha fazla örneklem grubu ile çalışarak sonuçların doğrulanmasını gerektirmektedir.

**Anahtar Kelimeler:** ARPC5, Ekspresyon, Hücre iskeleti, Pterjiyum.

## *ORAL PRESENTATION*

# Monitoring culture growth parameters of *Monoraphidium minutum* (Nägeli) Komárková-Legnerová in different nitrogen concentrations

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**Özet**

Microalgae have the most widely used in biotechnological application for obtaining bio valuable substances because of their fast growth rate and quality biochemical compounds. And they can change their growth rates and biochemical structure according to culture conditions. So nowadays their reactions to culture stress parameters are being studied in order to obtain more microalgae biomass or to provide modifications in their biochemical compositions.
In this study, the culture growth parameters of *Monorophidium minitum* in response to different nitrogen concentrations were investigated. For culture monitoring Cell Number (CN), Optical Density (OD) and Dry Weight(DW) values were recorded during the culture period, every other day. For this aim four different nitrogen conditions were used 3 mmol/L (more limitted treatment); 9 mmol/L (limitted treatment); 18 mmol/L (Normal medium); 27 mmol/L (excess treatment).
N limitation causes a decreasing on CN and OD values. Because the lowest CN (492,03x$10^6$ cell/mL) and OD (0,77) values were recorded in 3mmol/L medium at 30$^{th}$ day. In parallel with this the lowest Spesific Growth Rate (0,30841) and highest Doubling Time (2,24726) values were recorded in same medium. On the other hand, while 27 mmol/L medium has the highest CN and OD values, the lowest Biomass Productivity (BP) values were calculated for the same medium. This means that increasing the amount of nitrogen in culture medium causes a rising in CN but a decreasing in biomass productivity.
So for obtaining more microalgae biomass nitrogen excessing in culture medium is not a solution in every time. For obtaining more accurate results and decisions, all culture growing parameters (CN, OD, DW) should have to monitor in the same time.

**Keywords:** Cell number, dry weight, *Monorophidium minitum*, nitrogen, optical density.

## *ORAL PRESENTATION*

# Common errors in the analysis of adsorption data during the transition from a non-linear to a linear model: Isotherm's and kinetic's study as an example

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**Abstract**

Adsorption is considered one of the most commonly used separation and purification processes, in which adsorption occurs by the formation of physical or chemical bonds between a porous solid medium and a mixture of liquid or gas multi-component fluid. Adsorption kinetics and isotherm are the main factors that must be understood before the applicability of any adsorbent.
Therefore, understanding the modelling of the equilibrium data is a very essential way of predicting the adsorption mechanisms of various adsorption systems. The aim of this paper is to establish criteria for choosing the optimum isotherm and kinetic models of different adsorption models and the use of various mathematical error functions during the fitting of a given model to empirical data assessed based on linear or nonlinear regression analysis.
The study intends to describe and demonstrate the degree of distortion of fits during the regression and transformations of nonlinear equations into linear forms using many isotherms and kinetics models. Taking into account the level of error, the values were fitted to error models such as Reduced Chi-Square and Adjusted R-Square, Residual Sum of Squares, and Pearson's r and the correlation coefficient R-Square.

**Keywords:** Adsorption, Distortion, Isotherm, Kinetic.

## *ORAL PRESENTATION*

# Assessment of cytotoxicity of pure glyphosate in two different human cell lines

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**Abstract**

Glyphosate is used as an herbicide on a wide scale throughout the world due to its effectiveness. Although considered non-toxic for humans and animals initially, safety concerns about the usage of glyphosate started to arise in recent years. We assessed toxic effects of active ingredient glyphosate on human bronchial epithelial cells (BEAS-2B) and liver cells (HepG2). To evaluate cell viability in both cell types, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and neutral red (NR) uptake assays were conducted at 24 and 48 h. At 24 h, cytotoxic effects of pure glyphosate to liver cells started from 10 mM concentration for NR assay, whereas from 20 mM to lung cells for both assays. On the other hand, at 48 h, results from two assays showed that glyphosate was cytotoxic to HepG2 and BEAS-2B cells at 2 and 10 mM, respectively. The half maximal inhibitory concentration ($IC_{50}$) for liver cells were found in the range of 25-28 and 15-20 mM at 24 and 48 h, respectively, with similar values calculated from both assays. The corresponding values for BEAS-2B cells were in the range of 34-42 and 21-26 mM at 24 and 48 h, respectively. These findings suggest that liver cells are more sensitive to cytotoxic effects of pure glyphosate than lung cells.

**Keywords:** Glyphosate, lung cells, liver cells, toxicity

## *ORAL PRESENTATION*

# Hybrid nanocomposites elaboration based on graphene and metallic nanoparticles and their environment applications

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**Abstract**

A nanocomposite of copper nanoparticles (Cu NPs) supported graphene (G) was synthesized by the in situ chemical reduction method of a blend composed of graphene and copper (II) sulfate powder using hydrazine hydrochloride as a reductant in an aqueous alkaline ethylene glycol organic solvent. We researched whether the benefit of graphene after chemical modification with Hydrochloric acid (FGA) and with hexamethylenediamine (FGN) to improve the reactivity of graphene surface could achieve a good immobilization and entrapment of copper nanoparticles (Cu NPs). The structure, morphology and thermal stability of the G@Cu, FGA@Cu and FGN@Cu nanocomposites hybrid were investigated by X-ray diffraction, Fourier transform-infrared spectroscopy, Atomic adsorption, Scanning electron microscopy, Transmission electron microscopy and Thermogravimetric analysis. The results demonstrated the successful functionalization of the graphene surface and the effective reduction of Cu (II) ions to nano-zerovalent copper ($Cu^0$). The results indicated that metallic Cu NPs were successfully deposited and dispersed on graphene and functionalized graphene to form an interconnected hybrid network. It was found according to comparative studies with nanocomposites of Cu NPs supported on a graphene, FGA and FGN that the surface morphology, size distribution, and loading of Cu NPs can be modified by controlling the kind of covalent functionalization grafted on graphene nanosheets. Moreover, the hybrids are evaluated for decolorization of methyl blue (MB) solution, a model cationic dye in wastewater generated by the textile industry.

**Keywords:** Functionalized graphene, Nano-zerovalent copper, Nanocomposites, Wastewater.

## *ORAL PRESENTATION*

# General Analysis of Floristic Structure of Iğdır Province

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**Abstract**

In this study, besides the Flora of Turkey records, the floristic, phytosociological, ethnobotanical researches and dissertations were taken into consideration to determine the general floristic structure of the province of Iğdır. The taxa were evaluated in terms of endemism, life form and phytogeographical aspects. According to this; 1020 taxa belonging to 386 genera and 74 families were identified. Two of these taxa belong to the Pteridophyta division; the others belong to the Spermatophyta division, which 3 of them belong to Gymnospermae and the remaining 1015 belong to the Angiospermae subdivision. In addition, 59 taxa were determined as endemic in this study. The distributions of taxa according to phytogeographical regions are as; Irano-Turanian 353 (% 35); Euro-Siberian 88 (% 9); Mediterranean 15 (% 1); Eastern Mediterranean 14 (% 1) and Unknowns 550 (% 54). In terms of number of taxa, respectively the richest genera are; *Astragalus* with 42 taxa, *Verbascum* with 16 taxa, *Trifolium*, *Silene* and *Potentilla* with 12 taxa each; *Allium*, *Bromus*, *Centaurea*, *Nepeta*, *Papaver* and *Ranunculus* with 11 taxa each. The 5 richest families are respectively Asteraceae (128), Fabaceae (100), Poaceae (70), Lamiaceae (69) and Brassicaceae (62).

**Keywords:** analysis, flora, vegetation, Iğdır, Türkiye

## *ORAL PRESENTATION*

# Kemik doku mühendisliğine yönelik biyopolimerik-biyoseramik kompozit iskelelerin mekanik stres şartlarında üretimi ve karakterizasyonu

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**Özet**

Doku mühendisliği yaşam bilimleri, mühendislik ve malzeme bilimi prensiplerini kullanarak, geleneksel tedavi seçeneklerinde ortaya çıkan sınırlayıcı etmenlerden (donör azlığı, immün cevap oluşumu vb.) kaynaklanan sorunlara bir çözüm sunmak amacıyla geliştirilen disiplinler arası bir araştırma alanıdır. Doku mühendisliği çalışmalarının temel bileşenleri biyomalzemeler, hücreler ve biyoaktif moleküllerdir. Hedefe başarılı bir şekilde ulaşmayı sağlayan en önemli unsurlardan biri, hücreler için 'yapay doku ortamını' taklit edebilecek üç boyutlu, biyouyumlu ve biyobozunur biyomalzemelerin geliştirilmesidir. Yürütülen bu çalışmada, biyomimetik kollajen-hiyaluronik asit-kondroitin sülfat-hidroksiapatit (KOL/HYA/KS-HA) kompozit iskeleler geliştirilerek, kemik doku mühendisliğine yönelik kullanılabilirliğini değerlendirildi. Bu amaçla öncelikle uygun konsantrasyonlarda ve iki farklı karıştırma yöntemi kullanılarak hazırlanan polimerik karışımlar mekanik olarak 3 farklı karıştırma süresine (10, 20 ve 30 dk) maruz bırakıldı. Ardından, 6-kuyucuklu petrilere konuldu ve 4 saat -20°C'de, 12 saat -80°C'de bekletildi. Süre sonunda, liyofilizatörde 24 saat süreyle kurutularak iskeleler hazırlandı. Bu işlemi takiben mineralize Kol/HYA/KS iskeleleri elde etmek amacıyla örnekler 28 gün SBF çözeltisi ile muamele edildi. Elde edilen kompozit iskelelerin fiziksel ve kimyasal özellikleri farklı analiz yöntemleri kullanılarak incelendi. Karakterizasyon analizleri sonucunda, artan karıştırma süresiyle ve polimer bileşenlerinin tamamının çözelti formunda kullanılmasıyla üretilen iskelelerin fiziksel dayanımının uygulama için daha yeterli olduğu bulundu. Ayrıca, kimyasal çapraz bağlayıcı kullanılmadan hazırlanan polimer-seramik kompozit iskelelerinin kemik doku mühendisliği uygulamaları için uygun bir biyomalzeme olma potansiyeline sahip olduğu belirlendi.

**Anahtar kelimeler**: Kemik doku mühendisliği, kollajen, hiyaluronik asit, kondroitin sülfat, biyomalzeme.

Bu çalışma TÜBİTAK ARDEB 119S123 nolu proje kapsamında desteklenmiştir.

## *ORAL PRESENTATION*

### *Paronychia pontica* (Borhidi) Chaudhri türünün kromozom sayısının belirlenmesi

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**Özet**

*Paronychia* Mill. cinsi Caryophyllaceae familyasının Paronychioideae Meisn. altfamilyasında yer alan bir cinstir. Cins, Güney Asya ve Güney Afrika hariç tüm dünyada yayılış gösteren, tek veya çok yıllık yaklaşık 110 tür içerir. *Paronychia* türlerinin yayılış merkezleri Türkiye, Peru, Bolivya ve Amerika'dır. Bu çalışmada, *Paronychia pontica* türünün diploid kromozom sayısının belirlenmesi amaçlanmıştır. Bu kapsamda, doğal habitatlarından toplanan örnekler çimlendirildikten sonra, sitogenetik prosedür uygulanmıştır. *Paronychia pontica* türünün diploid kromozom sayısı $2n = 18$ olarak tespit edilmiş ve detaylı kromozomal ölçümler yapılmıştır. Türün kromozom sayısı ile ilgili bu ilk rapor, *Paronychia* sitotaksonomisine önemli katkı sağlayacaktır.

**Anahtar Kelimeler:** *Paronychia*, Kromozom, Sitotaksonomi.

## *ORAL PRESENTATION*

# Determination of radiation shielding properties of novel resin-based composites using Monte Carlo Method

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**Abstract**

Hybrid fillings are used for the mechanical design of hydroxyapatite (HA)-based materials used in dental applications. In most cases, inert silica varieties are employed. The purpose of this research is to calculate the linear attenuation coefficients (μ) of silica-containing fillers in dental composites (SiO2-HA and SYL-HA) using the well-established Monte Carlo method. The simulations utilize a point photon source which emits mono-energetic photons directed as a parallel beam toward the cylindrical absorber, behind which was placed a small disc-shaped vacuum detector. In this manner, the simulation setup ensures that no scattered photons contribute to the flux in the detector. The simulations were carried out using the GAMOS 6.2 program at thirty-six different photon energies ranging from 1 keV to 20 MeV. In addition to the (μ) value, half value layer (HVL), tenth value layer (TVL) and mean free path (MFP) quantities were obtained, which are important in terms of radiation absorption properties. The results of this study indicate very good agreement with theoretical results using the XCOM code. The results show that indicate that the Monte Carlo technique may be used as an alternative for calculations of mass attenuation coefficients, especially for samples that are physically difficult to produce.

**Anahtar Kelimeler:** Monte Carlo, GAMOS, linear attenuation coefficients, dental applications, XCOM,

## *ORAL PRESENTATION*

# Apoptotic Effect of Epigallocatechin Gallate with Epirubicin-HCl in Lung Cancer Cells

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**Abstract**

Lung cancer is the most common type of cancer all over the world. Chemotherapy is one of the most effective therapeutic modalities to treat a variety of tumors, including lung cancer. Epirubicin-HCl is one of the important chemotherapy drugs used in the treatment of lung cancer. The most important problem encountered in the use of this drug is cumulative dose-limiting cardiotoxicity. Drug resistance seen in lung cancer prevents treatment. A combination therapy based on the additive or synergistic effect between chemotherapeutic agents and natural compounds derived from plants may be one of the strategies that can be used to overcome drug resistance. Thus, the therapeutic effect can be increased. Epigallocatechin-3-gallate (EGCG)is a compound, one of the most prominent catechins in green tea leaves, that attracts a lot of attention in medical research.

In our study, the oxidative stress-inducing effects of epirubicin-HCl alone and in combination with EGCG were revealed according to the changes in glutathione peroxidase activity after 48 h of application to A-549 cells. Apoptosis inducing potential was determined by measuring the activity of caspase-3/7, which is one of the key enzymes of the apoptotic pathway, after 48 h of application to cells with epirubicin-HCl alone and also together with EGCG.

Co-administration of epirubicin-HCl with EGCG was found to be more effective in increasing glutathione peroxidase activity and caspase-3/7 activity than epirubicin-HCl alone.

**Keywords:** Epirubicin-HCl, combined therapy, epigallocatechin-3-gallate

## *ORAL PRESENTATION*

# Microwave-promoted continuous fluidic synthesis of thermoplastic polyurethane–metal nanocomposites

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**Abstract**

Thermoplastic polyurethane-metal nanocomposites (NCs) are attractive materials with extensive applications in numerous fields, especially biomedicine to their excellent biocompatibility, versatility, thermal and mechanical properties as well as extraordinary antibacterial performance. Herein, a microwave-promoted fluidic reactor was used for continuous, in-situ synthesis of polyurethane-silver (PU–Ag NCs) and polyurethane-copper (PU-Cu NCs) NCs containing uniformly dispersed Ag nanoparticles (NPs) (~5 nm) and Cu/CuONPs (~9 nm), respectively. Homogeneous solutions of the reactants were fed continuously into a milli-fluidic reactor exposed to microwave (MW) irradiation. The synthetic route was optimized for each NC by exploring the effect of MW power, flow rates, and concentration of PU and metal salt. The presence of NPs in the as-synthesized NCs was confirmed via UV-visible spectroscopy (UV-vis), which showed the characteristic peaks for AgNPs, CuNPs, and CuONPs at 428 nm, ~530 nm, and ~380nm, respectively. Dynamic Light Scattering (DLS) showed that the optimized NCs contained NPs which were small in size (<10 nm) with a narrow size distribution. The size distributions and morphologies of the AgNPs were also characterized using a high-resolution transmission electron microscope with an energy-dispersive X-ray spectrometer (HRTEM-EDS). The increase in thermal stability and crystallinity of the NCs was confirmed using Thermogravimetric analysis coupled with differential thermal analysis (TGA/DTA) and X-ray diffraction spectroscopy (XRD), respectively. The films of PU–Ag NCs exhibited significant antibacterial performance against both Gram-negative and Gram-positive bacteria. Notably, NCs with lower Ag content and a smaller particle size demonstrated antibacterial rates as high as 98.9%, 97.1%, and 99.7% against *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively. A comparison with the MW-heated batch (MWB) and conventionally-heated flow (CHFS) systems established the superiority of the reported MW-promoted continuous flow system as a faster, inexpensive, energy- and material-efficient, up-scalable, and greener synthetic route for polymeric NCs.

**Keywords:** Microwave irradiation, Continuous flow synthesis, Polyurethane-Silver nanocomposite, Polyurethane-Copper nanocomposite, silver nanoparticles, antibacterial activity.

## *ORAL PRESENTATION*

# Synthesis and Characterization of Amine Functionalized Graphitic Carbon Nitrides

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**Abstract**

Surface-modified support materials with light absorption properties, such as graphitic carbon nitrides (g-$C_3N_4$) provide more effective anchoring sites to stabilize nanocatalysts (*e.g.* transition metal dichalcogenides) than their unmodified counterparts. Herein, an elegant surface modification of the urea- (U-g-$C_3N_4$) and dicyandiamide-derived g-$C_3N_4$ (D-g-$C_3N_4$) by primary amine (-$NH_2$) functional groups is described using a sodium naphthalide-based reduction chemistry. The effect of g-$C_3N_4$ type and inert gas purity on surface modification was evaluated. The degree of functionalization and the covalent addition were probed using thermogravimetric analysis (TGA) and X-Ray photoelectron spectroscopy (XPS). The degree of functionalization was 1 -$NH_2$ group per 28 U-$C_3N_4$ carbon atoms by XPS (25 by TGA), and it was obtained using the 7:1 (Na:C) ratio under an argon atmosphere. The $NH_2$-modified U-g-$C_3N_4$ displayed decreased valance (2.25 eV) and conduction (-0.44 eV) bands while almost no change for the band gap (2.97).

**Keywords:** Surface functionalization, reduction reaction, thermal gravimetric analysis, X-ray photoelectron spectroscopy.

## *ORAL PRESENTATION*

# Antimicrobial effect of natural traditional vinegars against *Listeria monocytogenes* strains

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**Abstract**

The interest in natural antimicrobial agents is increasing day by day due to the increasing concern of consumers against chemical agents. Vinegar stands out as a natural antimicrobial agent with its various bioactive components beneficial to human health. In parallel with the increasing in the tendency to healthy foods, the variety of vinegar produced from different components has also increased. In this study, the antimicrobial activities of vinegar samples produced from different raw materials using traditional methods in the home environment were determined by using disc diffusion method against 29 strains of *Listeria monocytogenes* isolated from ready-to-eat foods. Concentrations of 8 vinegars prepared at different grades (50% and 100%) were tested against different cell densities ($10^5$ CFU/mL, $10^6$ CFU/mL, and $10^7$ CFU/mL) of *L. monocytogenes* strains. Antimicrobial effects were evaluated by disc diffusion method by measuring the zone diameters formed around the discs at the end of the incubation period. It was measured that *L. monocytogenes* strains formed zone diameters ranging from 6.0 to 8.8 mm against traditional vinegar samples. Antimicrobial activities of different concentrations of 8 different traditional vinegar samples obtained from different fruits as a natural antimicrobial agent were not found statistically significant, against different concentrations of *L. monocytogenes* strains ($p>0.05$). However, in general, it was observed that the level of efficiency against *L. monocytogenes* strains of traditional vinegar samples at 100% concentration was higher. Antimicrobial activity values of natural antimicrobial agents against different cell densities of *L. monocytogenes* strains were found to be statistically significant.

**Keywords:** *Listeria monocytogenes*, traditional vinegar, antimicrobial activity, disc diffusion method.

## *ORAL PRESENTATION*

# Antimicrobial activity of different selected commercial vinegars against different *Listeria monocytogenes* strains isolated from foods

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**Abstract**

In this study, antimicrobial activity of 29 strains of *Listeria monocytogenes* strains isolated from ready-to-eat foods and 6 different commercially produced vinegar samples which belongs to different companies were used. This study was aimed the antimicrobial effects of vinegar samples produced by commercially against *L. monocytogenes* strains using standard agar disc diffusion technique. Vinegar samples and concentrations of pathogen strains were prepared at different concentrations and cell densities. The zone diameters of *L. monocytogenes* strains were measured between 6.0-11.3 mm against commercial vinegar samples. The antimicrobial activities of different concentrations of commercially produced vinegars against *L. monocytogenes* strains were found to be statistically significant. Moreover, the antimicrobial activity of different cell densities of *L. monocytogenes* strains against commercial vinegars were found to be statistically significant.

**Keywords:** *Listeria monocytogenes*, commercial vinegars, antimicrobial activity, disc diffusion method

## *ORAL PRESENTATION*

# Formulation and Characterization of Ketoprofen Loaded Polycaprolactone, Eudragit S100 and EudragitS100/Polycaprolactone Nanoparticles

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**Abstract**

The aim of the current study was to formulate ketoprofen-loaded EudragitS100 (ES100), polycaprolactone (PCL) and composite ES100/PCL nanoparticles using emulsion solvent evaporation method, to provide prolonged controlled release and to reduce its common side effects in the gastrointestinal tract, which significantly limit its use. Characterization studies (*in vitro* release, encapsulation efficiency (EE), particle size, zeta potential) were also performed. Nanoparticles were produced using biodegredable ES100 and PCL and the effect of polymer type was investigated. In addition, the effect of polymer ratio (100:0,75:25,25:75,0:100) and polyvinyl alcohol (PVA) concentration (0.5-1%) on the characterization of nanoparticles were investigated. The drug release profile from nanoparticles was significantly altered by changing the polymer and producing composite nanoparticles. The ES100 nanoparticles resulted in the highest burst effect and accelerated drug release, while the release from PCL nanoparticles was slowest. The drug release was extended to 24 hours and a more controlled release profile was obtained in composite nanoparticles compared to both PCL and ES100 nanoparticles. The EE of nanoparticles was quite high (55-83%). The increase in PVA concentration also caused an increase in EE. The zeta potential ranged from (-10) to (-19) mV, while the particle size ranged from 188.0 to 686.2 nm. In general, while the zeta potential was acceptable, it enhanced by the addition of PCL. ES100 nanoparticles were the largest nanoparticles (686.2 nm), while PCL nanoparticles were smaller (472.1 nm). However, composite nanoparticles (188.0 and 309.2 nm) had smaller particle size than both PCL and ES100 nanoparticles. As a result, ketoprofen loaded ES100/PCL nanoparticles, which have advantages of both polymers, have been successfully produced with smaller size, improved stability, prolonged controlled release, high EE, and they can be used effectively to provide prolonged controlled release and to reduce the side effects of ketoprofen.

*This study is supported by TÜBİTAK 2209-A [grant-number: 1919B012110764].

**Keywords:** nanoparticle, ketoprofen, polycaprolactone (PCL), Eudragit S100 (ES100), composite nanoparticle, controlled release.

## *ORAL PRESENTATION*

# Creating Snow Washing and Stone Effect on Fashion Denim Fabrics with Alternative Methods

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**Abstract**

Denim washing process is a finishing technique given to denim fabric in order to increase the visuality and to provide strength in the fabric. In the denim washing process, different washing effects such as color fading, shrinkage in the seam, hair removal, wrinkling, and hairiness are made. Traditional blue denim is dyed with a low permanence indigo dye. In order to create a unique denim look, the paint on the surface can be washed with denim washing methods by using very different materials such as stone, sand, different enzymes, sodium hypochlorite or potassium permanganate, hydrogen peroxide, laser, ozone. However, the surface treatment of jeans is not always environmentally friendly, as it creates significant environmental pollution. Today, different ideas are produced as an alternative to denim washing processes with stone effect and snow washing processes. For this purpose, we prepared a homogeneous mixture. This mixture is completely obtained from our own resources and made without the need for any chemicals from abroad. There are products used for this process in the market, but they are not at the desired level or fashion washing, snow washing cannot be done with these products. When the homogeneous mixture we have prepared is interacted with inorganic materials with different properties, it continues to behave like it and carry its properties by carrying the characteristics of that chemical. The mixture we prepared is a homogeneous mixture and was obtained by physical methods. It is expected that this mixture will add a great advantage to the denim industry in terms of cost. Positive results were obtained in snow washings made without stones and without using stones from the denim washing machine. In this study; 123 g of mixture is required for each 0.8 g of denim fabric. With the mixture, both indigo and sulfur dyed fabrics were worn with stone wear effects. The snow washing effect on denim fabrics at the same mixing ratio has been discussed. With this mixture obtained in the studies, the indigo dye was both abraded and the desired effect was achieved, and the desired snow washing effect was achieved by mixing it with different chemicals.



Figure. Denim effect process

**Keywords:** Denim, Effect, Snow washing

## *ORAL PRESENTATION*

# Creating Washing Effect on Denim Fabrics by Electrochemical Method

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**Abstract**

Electrochemical procedures are methods in which electrons are transferred between chemicals through electrodes by means of electric currents. Electrochemical processes in the production of reducing and oxidizing agents are well known and are frequently used in the production of various raw materials. However, today it is not yet used in jeans bleaching. The electrochemical bleaching system (ECB) of indigo dyed denim garments was developed by the institute of textile chemistry and textile physics, based on previous studies of wastewater discoloration by an electrochemical process. Based on comparison with other bleaching systems, the overall echo balance of blue jeans production based on pre-reduced indigo and using the electrochemical bleaching process has been shown to be optimum. ECB has economic and ecological advantages over other bleaching processes. The process is easy to carry out, well reproducible, cost-saving and possibly made possible by low effluent. It can also be used for oxidative or reductive processes. Both processes require a compatibilizer; just like in the laccase process. In the oxidative ECB process, the compatibilizing agent is oxidized at the anode. The activation material oxidizes the water-insoluble indigo on the fabric surface to a water-soluble degradation product. Physical action in the washing machine removes the degraded product from the surface and a dispersing agent suspends it in the bleach bath. Then, the electrodes are pulled towards it, allowing the paint to accumulate. In the literature studies, it has been seen that this process has never been used to create a washing effect. Effecting processes were carried out by applying 2 mV potentials intermittently (once in 2 seconds) to the system prepared by applying 0.1 M EDTA solution (Water:EDTA, 1:10000), electrical potential in a vibrating environment to the washing bath. The effects on 1 cm denim fabric are shown in the figure. Considering the effects on denim, the application of a potential of 2 mV every 2 seconds separates the indigo dye in different regions from the fiber with the effect of vibration and the effecting process takes place.

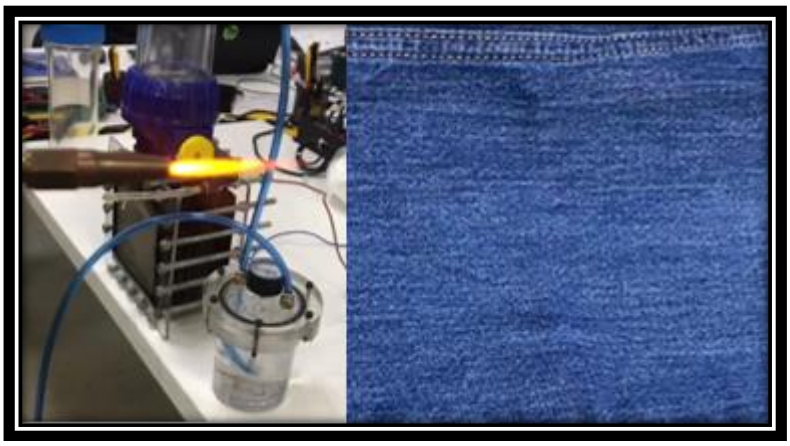

Figure. Electrolysis unit and effecting process

**Keywords:** ECB, Denim, Efeklendirme

## *ORAL PRESENTATION*

# Comparative study of chemical content and antimicrobial activities of propolis extracts obtained from different floral origin: chestnut and forest

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**Abstract**

It is known that propolis have different bioactive compounds including flavonoids and phenolic acids, and its composition varies depending on its geographical and botanical origins. For this reason, in this study, it was aimed to examine the antimicrobial activity, total phenolic-flavonoid content and bioactive compounds analysis of propolis samples collected from different floral sources of the Western Black Sea region of Turkiye. The Liquid Chromatography-Mass Spectrometry (LC-MS/MS) method was used to determine the bioactive components of the samples. Total Phenolic (TP) – Flavonoid (TF) contents were calculated with using Folin-Ciocalteu method and colorimetric aluminum chloride assay. To test antimicrobial efficacy, 7 Gram (-), 9 Gram (+) and 3 yeast-like fungi were selected. Agar well diffusion and minimum inhibitory concentration (MIC) methods were used to detect the inhibitory dose. The bioactive compounds varied between samples and was observed to be higher in the chestnut propolis than forest sample. TP and TF values of chestnut propolis (71.06 mg GAE/mL-11.75 mg QE/mL) were significantly higher than forest sample (36.84 mg GAE/mL-7.04 mg QE/mL). MIC values were in the range of concentrations from >39.06 to 2500 µg/mL for chestnut propolis and >39.06 to 5000 µg/mL for forest sample. The most susceptible strain was *Mycobacterium smegmatis* for both samples. Notably, it was observed that the botanical origins affect the chemical composition of propolis, and this situation can also be effect bactericidal activity in respective propolis due to the synergistic effects of the different bioactive compounds. Morever, suitable levels of effective propolis extract may be protective antimicrobial additives in the food industry to inhibit the growth of selected pathogenic bacteria.

**Keywords:** propolis, total phenol, flavonoid, phenolic compounds, LC-MS/MS, antimicrobial activity.

## *ORAL PRESENTATION*

# Na-Alginat/Nar kabuğu ince filmlerinin sentezi ve karakterizasyonu

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**Özet**

Nar meyvesinin işlenmesi sonucu atık olarak ortaya çıkan nar kabuklarının karatenoid ve polifenol gibi birçok biyoaktif bileşen madde içerdiği bilinmektedir. Nar işleme endüstrisinin % 40'ını oluşturan nar kabuğunun yeniden değerlendirilmesi oldukça önem taşımaktadır. Bu çalışmada nar kabuk atıklarının, biyobozunur film çözeltisi üretiminde kullanılması hedeflenmiştir. Bu amaçla kabukların öğütülmesi ile elde edilen tozun biyobozunur polimer olan Na-Alginat polimeri de kullanılarak ince filminin sentezi gerçekleştirilecektir. Sentezlenen ince film Alginat polimerinin klasik çapraz bağlayıcı molekülü $CaCl_2$ çözeltisine daldırılarak çapraz bağlanacaktır. Ayrıca biyobozunur film çözeltisi sitrik asit çözeltisine de daldırılacak ve klasik yöntemden farklı olarak çapraz bağlanacaktır. Elde edilen filmler FTIR, TGA, DSC, XRD yöntemleri ile karakterize edilecek ve karşılaştırılacaktır. Doğal kaynaklar kullanılarak sentezlenen ve karakterize edilen ince filmlerin gıda ambalaj filmlerine alternatif olacağı düşünülmektedir.

**Anahtar Kelimeler:** atık değerlendirme, alginat, nar kabuğu, ince film.

## *ORAL PRESENTATION*

# Biochemical investigation of methotreaxate-induced oxidative stress on liver tissue

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**Abstract**

Methotrexate is an antifolate and antimetabolite drug used in the treatment of cancer and autoimmune diseases. Methotrexate does not differentiate between normal and malignant cells and thus causes apoptosis and lipid peroxidation even in normal cells. Antioxidants are one of the treatments of reducing the tissue damage which caused by antineoplastic drugs usage. Alpha-lipoic acid is a synthetic version of lipoic acid. It has free radical scavenging properties and has direct antioxidant effects on the recycling of other cellular antioxidants. Quercetin is a flavonol commonly found in edible plants. It has various biological functions such as anticancer, antiproliferative, antioxidant, anti-inflammatory and antimutagenic effects. The aim of this study is to investigate the effect of Alpha lipoic Acid and Quercetin on the damage induced by methotrexate in rat liver

In our study, 24 male Wistar Albino rats were randomly divided in each group equally. The control group given physiological saline, the damage group given Methotrexate (20 mg/kg), the 1st treatment group given Methotrexate (20 mg/kg) + Alpha lipoic Acid (50 mg/kg), and the Methotrexate (20 mg/kg) + Quercetin (50 mg/kg) were 2nd treatment group. 6 days after methotrexate injection, liver samples were taken from rats and LPO levels were analyzed.

While LPO level increased significantly in the methotreaxate group compared to the control group, LPO level was significantly decreased in both treatment groups.

It was determined that the administration of methotreaxate caused an increase in oxidant production by inducing oxidative stress. Alpha Lipoic Acid and Quercetin were effective in repairing the damage. The use of antioxidants may be beneficial in reducing the oxidative stress caused by the use of methotreaxate.

This study was supported by Marmara University BAPKO with the project numbered TYL-2022-10429.

**Keywords:** Methotrexate, Alpha Lipoic Acid, Quercetin, Liver Damage

## *ORAL PRESENTATION*

# Biological Activity Of *Deverra scoparia* Coss & Dur (Apiaceae) and *Commiphora myrrha* Nees. (Burseracae) Extracts

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**Abstract**

*Deverra scoparia* Coss& Dur (Apiaceae) and Commiphora myrrha Nees. (Burseraceae) are two plants that are widely used in Algerian pharmacopoeia for the treatment of inflammatory diseases. Since the main component of immunosurveillance tumoral is the alternative complement pathway (ACV), in this context we were interested in studying the effects of total alkaloid extract of D. scoparia and the crude extract of C. myrrha resinon the activation of VAC. A functional test (AP100) was used to evaluat etheir effect on VAC in vitro on normal human plasma (NHP). The evaluation of AP00 shows that the extract of the total alkaloids of D. scoparia has a significant inhibitory potency and in a dose-dependent manner (94.51 ± 6.82% at 1 mg/ml), whereas the crude extract of the resin C. myrrha is showing an inhibitory effect at a single concentration (86.73 ± 8.54% at 0.25 mg / ml) and their increased toxicity that is dominant. The results of the bioactivity-guided fractionation of the alkaloide xtract of D. scoparia show that fraction C has the highest inhibitory effect (50.93 ± 9.73%) relative to the other fractions.

**Keywords:** Deverra scoparia Coss& Dur, Commiphora myrrha Nees, Alternative pathway of complement, AP100.

## *ORAL PRESENTATION*

# Air quality monitoring system in textile production areas

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**Abstract**

Employee health might be harmed by poor air quality in textile factories. A mask is employed as a preventative measure if the instantaneous values of the ambient air cannot be ascertained. Some particles permeate the respiratory tract due to particle size variations in the working environment and manufacturing. Workers exposed to textile fluff (fine hairs on the surface of fabrics such as wool, velvet, and broadcloth) experience textile-specific lung diseases such as asthma, shortness of breath, and acute pulmonary edema over time in cases where measures against poor air quality are not taken or are insufficient. As a result, dusts with particle sizes ranging from 0.5 to 100 micrometers, which are extremely harmful to human health, create two types of occupational illnesses known as "Bissinosis" and "Silicosis" Byssinosis is a frequent lung illness in the textile sector caused by piles such as cotton, linen, jute, and hemp exposure. Silicosis is described as the buildup of mineral dust in the lungs and the tissue reactivity to this buildup (also known as "Dusty Lung"). As a result, the suggested system for measuring the instantaneous condition of the air comprises of an air sampling pipe, a fan, and a detector for its detection, and can measure micron-sized particles floating in the air in real time. The system to be constructed can continuously aspirate ambient air, take a sample through the particle detector, and measure the sample in the detection chamber. Its goal is to provide an alert as a result of critical level monitoring. The air volume flowing into the system will be estimated, and the data collected from the detector unit will be calculated using the established method, and the speed will be determined instantaneously by utilizing the Direct Current (DC) Fan Motor and the revolution counter in the detecting unit. Image processing, ultrasonic detection, and light absorption techniques will be tested in the detector unit with an air sample (containing a known number of particles), and particle detection sensitivities will be determined individually. Validation phases will be carried out in compliance with ISO 13485 and ISO 14968 standards by guaranteeing system optimization. The prototype's verification controls will be carried out utilizing the provided performance data.

**Keywords:** Textile, Pile, Air Sampling, Rapid Detection, Particle

## *ORAL PRESENTATION*

# Türkiye akar faunası için yeni bir tür kaydı; *Allothrombium adustum* Oudemans, 1905 (Acari: Trombidiidae)

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**Özet**

Antarktika kıtası hariç, dünyanın diğer kara kıtalarına yayılmış ve oldukça zengin tür çeşitliliğine sahip Trombidiidae Leach, 1815 ailesi, şu dört alt familyadan oluşmaktadır; Allothrombiinae Thor, 1935, Dolichothrombiinae Robaux, 1969, Paratrombiinae Feider, 1959 ve Trombidiinae Leach, 1815. Allothrombiinae alt familyasına dahil *Allothrombium* Berlese, 1903 cinsi, dünya geneline yayılmış olup 70'in üzerinde türe sahiptir. Bu türlerden bazıları yalnız larva evresinden, bir kısmı ise sadece larva sonrası aktif evrelerinden (yetişkin ve/veya nimf) bilinmesine karşın, yalnızca 7 tür hem larval hem de larva sonrası evreleriyle birlikte tanımlanmıştır. Bu cinse ait, şu ana kadar ülkemizden sadece 12 tür kaydedilmiştir. 2021 yılı Ekim ayında, Erzincan ili, Refahiye ilçesinden (39° 46' K 39° 01' D, 1300 m) alınan kavak altı döküntü örnekleri, araştırma laboratuvarına getirilmiş ve örnekler Berlese sistemine yerleştirilerek, akar örnekleri ayıklanmıştır. Elde edilen örnekler % 70'lik etilalkol ile muhafaza edilmiş ve Hoyer ortamında preparatları hazırlanmıştır. Preparatlar Olympus BX63 mikroskobunda incelenerek, tür teşhisleri yapılmıştır. Yapılan inceleme sonucunda; *Allothrombium adustum* Oudemans, 1905 türünün nimf evresi teşhis edilmiş ve Türkiye akar faunası için yeni bir kayıt olarak listelenmiştir. Böylece ülkemizden bu cinse ait kaydedilen tür sayısı 13'e ulaşmıştır.

**Anahtar Kelimeler:** Karasal Parasitengona, Trombidioidae, Allothrombiinae, Erzincan

## *ORAL PRESENTATION*

# Biochar ve Toprak Organik Madde Döngüsüne Etkisi

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**Özet**

Biochar (biyokömür), organik atıkların (bitki veya hayvan atıkları, kentsel atıklar, kullanılmış kağıt ve benzeri organik maddeler) oksijensiz veya düşük oksijen içerikli yüksek sıcaklıkta gazlaştırma işlemi sonrasında elde edilen yüksek karbon içeriğine sahip katı formdaki ürünlerdir. Hem çevreyi iyileştirici etkileri hem de bitkisel üretimde sağlayabilecekleri toprak iyileştirici özellikleri ile biochar, organik teknoloji ürünü olan materyaller olarak son yıllarda oldukça önemli bir yere sahip olmuştur. Karbonun toprakta uzun süre tutulmasını sağlayarak organik madde döngüsüne olumlu katkılar sağlaması, tarımsal arazilerde gübre gereksinimi azaltması, toprağın geçirgenliğini arttırması, mikoriza ve mikorizal olarak adlandırılan yararlı mantar hiflerini arttırarak toprak yapısını düzenlemesi gibi toprakta çok olumlu etkilere sahiptir. Karbon içeriği oldukça yüksek, yapısı çok gözenekli ve çoğunlukla alkali karakterde olan biyokömürün toprağa uygulanması ile toprağın organik madde içeriğinin ve pH'ının arttığı ayrıca toprak mikroorganizmalarının çeşidi ve populasyonunun değişmesine neden olduğu bilinmektedir. Böylelikle biochar kaynaklı toprak organik madde içeriği ve mikroorganizma populasyonundaki artış, topraktaki organik madde mineralizasyonuna olumlu katkılar sağlamaktadır. Özellikle organik madde bakımından yetersiz, şiddetli su ve rüzgar erozyonuna maruz kalan topraklara biochar uygulaması, toprakların organik maddece zengin olmasını sağlayacak ve diğer yandan organik atıkların biochara dönüştürülmesi ile bu atıkların değerlendirilmesine katkı sağlayacaktır.

**Anahtar Kelimeler:** Biochar, organik madde, toprak verimliliği, organik atık, mineralizasyon

## *ORAL PRESENTATION*

# Fungisidlerin Topraktaki Mikroorganizma Faaliyetine Etkileri

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**Özet**

Mikroorganizma faaliyetleri topraklarda ayrışma süreçleri açısından hayati öneme sahiptir. Ancak birçok toprakta farklı amaçlarla kullanılan kimyasallar mikroorganizma faaliyetlerini olumsuz yönde etkilemektedir. Toprak ekosisteminin verimliliği ve kararlılığı toprak mikroorganizmaları ve onların aktivitelerine bağlıdır. Bu amaçla, toprak mikroorganizma kommuniteleri üzerinde pestisidlerin yan etkilerinin değerlendirilmesi büyük önem taşımaktadır. Hem mantarlar hem de bakteriler toprak solunumundan sorumlu canlılar olsalar da, karbon ve diğer besin elementlerini kullanım talepleri açısından farklılık gösterirler. Mantarlar, daha yüksek karbon asimilasyonuna sahip olma eğiliminde olduklarından bakterilerden daha fazla karbon depolayabilmektedir. Fungisitler, fungal hastalıkların yok edilmesinde kullanılan bir pestisid olarak bilinmektedir. Toprak karbon, azot mineralizasyonu ve toprak solunumu fungisitlerin ekolojik risk değerlendirmesi açısından indikatör niteliğinde olan parametrelerdir. Pek çok çalışmada toprak karbon ve azot mineralizasyonu üzerine çoğu fungisitin kısa süreli etkileri incelenmiş ve uygulamadan sonra mineralizasyonun uyarıldığı tespit edilmiştir. Ayrıca fungisit uygulamalarının mikroorganizma populasyonunu, katalaz ve üreaz aktivitelerini de olumsuz etkilediği sonucuna varılmıştır.

**Anahtar Kelimeler:** Fungisid, mikroorganizma faaliyeti, toprak, mantar

## *ORAL PRESENTATION*

# Manipulating light through organic materials in solid state

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**Abstract**

Light-driven applications such as photocatalysis and photovoltaics are intriguing since it is possible to operate them by using solar energy, which is the most abundant and green energy source. However, most of these systems can only work efficiently under high energy light (i.e. UV irradiation), therefore, the ultimate goal of utilizing them under solar light is problematic. That being said, a technique named as photon upconversion allows to transform the low energy light to higher energies.[1]

There are several mechanisms for upconversion, amongst which triplet-triplet annihilation photon upconversion (TTA-UC) is effective for the processes involving organic compounds. TTA-UC is driven by two components named sensitizer (e.g. Platinum octaethylporphyrin (PtOEP)) and annihilator (e.g. diphenyl anthracene (DPA)), which are triplet donors and acceptors, respectively.[2] Briefly, the sensitizer compound absorbs light in longer wavelengths (lower energy) and reaches the excited state (one electron travels to the singlet energy level from the ground level). The electron of the sensitizer then goes to its triplet state via intersystem crossing, and make the sensitizer available for triplet energy transfer (TET) with the annihilator molecule. When annihilator receives two TET, its ground state electron will have enough energy to reach the singlet level, of which return to the ground level emits the higher energy (shorter wavelength) light than the sensitizer absorbed.

In this contribution, functionalization of DPA to improve its processability for solid state TTA-UC will be presented. There will be examples of molecular or polymeric structures which were pre- or post-synthetically functionalized for the better processability.

**Keywords:** triplet-triplet annihilation photon upconversion, diphenyl anthracene, porous polymers

[1] P. Bharmoria, H. Bildirir, K. Moth-Poulsen, *Chem. Soc. Rev.* **2020**, *49*, 6529–6554.
[2] F. Edhborg, H. Bildirir, P. Bharmoria, K. Moth-Poulsen, B. Albinsson, *J. Phys. Chem. B* **2021**, *125*, 6255–6263.

## *ORAL PRESENTATION*

# Investigation of the histological structure of the digestive system of *Eurydema spectabilis* Horvath, 1882 (Heteroptera: Pentatomidae)

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**Abstract**

In this study, the anatomy and histology of the digestive system of *Eurydema spectabilis* Horvath, 1882 (Heteroptera: Pentatomidae) were described by light microscopy. In this study, 20 adult male and female E. spectabilis were collected from Kazan, Ankara (June, 2018). The digestive tract of *E. spectabilis* is divided into 3 different regions as foregut, midgut and hindgut. In addition, in light microscopy examinations, it was observed that the salivary gland of *E. spectabilis* consists of the main and accessory salivary gland ducts. The foregut consists of the pharynx, esophagus, and proventriculus. The esophagus is connected to the proventriculus and is seen as a narrow tube. The wall of the proventriculus has an indented structure and is surrounded by a monolayer of epithelium and muscle. The midgut consists of stomach, midgut and bulb. In the midgut, the duct has a monolayer cylindrical epithelium, while the stomach and bulb have a cuboidal epithelium. The hindgut consists of ileum and rectum. The ileum and rectum are composed of a single layer of cuboidal epithelium. Malpighi tubules and gastric cecum are surrounded by a monolayer cubic epithelium. The Malpygian tubules are in two pairs and their distal part lies freely in the hemocoel. As a result, it is aimed to define the digestive system histology of *E. spectabilis*, which is an agricultural pest and has not been studied before, and to form a basis for the control studies to be carried out against this species.

**Keywords:** Foregut, midgut, hindgut, Malpighian tubes, light microscopy

## *ORAL PRESENTATION*

# Multi-omics Data Analysis Oriented Drug Repositioning for Growth Hormone Secreting Pituitary Neuroendocrine Tumors

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**Abstract**

Growth hormone (GH)-secreting tumors are one of the functional tumor subtypes of pituitary neuroendocrine tumors (PitNETs). These tumors can cause the overgrowth of almost any organ due to hypersecretion of GH and insulin-like growth factor-1 (IGF1) which may cause acromegaly. Tumor resection followed by dopamine agonists (DAs), somatostatin analogs (SSAs), and GH-receptor antagonists are prescribed in order to alleviate the effects and reduce hormone secretion. However, some patients may experience aggressive tumor behavior, DA resistance, or relapse after discontinuation of the drug. In addition to all these, the molecular mechanism of GH-PitNETs and the biological processes related to resistance and recurrence have not been fully elucidated yet. There is an unmet need for less side-effective treatment methods for GH-PitNETs using innovative therapeutics that will not reduce the patient's quality of life. In this study, the analysis of omics data obtained by high-throughput techniques at the transcriptome level including mRNA (encoded) and microRNA (miRNA), (non-coding) stages carried out specifically for GH-PitNET. The differential expression analyses revealed 1457 downregulated and 2789 upregulated genes, 49 downregulated and 24 upregulated miRNAs. These differentially expressed genes and miRNAs were used as a query for drug repositioning analysis via the L1000CDS[2] search engine. We presented the transcriptomic landscape of GH-PitNETs through miRNA and mRNA-level data integration via differential expression analysis and proposed repurposed drug candidates based on this integration. In light of these analyses, we elucidated biological processes like TGF-beta and Hedgehog signaling that might have roles in GH-PitNET tumorigenesis. We rationally prioritized and repositioned drug candidates to target these pathways by combining clinical and laboratory data, as well as determining tumor histopathogenesis and transcriptional regulatory properties of these tumors. As a result, we identified sirolimus, an mTOR inhibitor, as a repositioned drug candidate for the management of GH-PitNETs.

**Keywords:** transcriptome, microRNA, drug repositioning, acromegaly, growth hormone, pituitary neuroendocrine tumors

## *ORAL PRESENTATION*

# *MAPK6* genini hedefleyen siRNA yüklü PLGA nanopartiküllerinin meme kanseri hücre dizilerinde antikanser etkilerinin değerlendirilmesi

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**Özet**

Meme kanseri, kadınlarda kansere dayalı ölüme sebep olma açısından ikinci sıradadır. Kanserleşmedeki rolü hala belirsizliğini koruyan Mitojenle Aktive Edilmiş Protein Kinaz (MAPK) ailesinin atipik üyesi MAPK6 (ERK3), meme kanseri hücresi dizisi MCF-7'de aşırı eksprese edilen genlerdendir. Sunulan çalışmada, *MAPK6* genini hedefleyen siRNA yüklü PLGA nanopartikülleri oluşturulması ve bu nanopartiküller aracılı *MAPK6* ekspresyonunun baskılanmasının olası antikanser etkilerinin *in vitro* koşullarda belirlenmesi amaçlanmıştır. Bu kapsamda, siRNA yüklü PLGA nanopartikülleri ikili emülsiyon çözücü buharlaştırma yöntemi ile sentezlenmiştir. Farklı konsantrasyonda dozlar uygulanmış ve hücre canlılığı MTT analizi ile değerlendirilmiştir. Uygun doz belirlendikten sonra hücrelere, MAPK6 siRNA yüklü nanopartikül, boş nanopartikül, *scrambled* siRNA yüklü nanopartikül, çıplak MAPK6 siRNA ve yalnızca medyum uygulanmıştır. Uygulamadan 24, 48 ve 72 saat sonra total RNA izolasyonu ve cDNA sentezi gerçekleştirilmiş, ardından *MAPK6* ekspresyon seviyesi belirlenmiştir. Uygulamanın hücre migrasyonuna etkisi yara iyileşme deneyi ile, hücrelerin koloni oluşturma yeteneğine etkisi ise klonojenik analiz ile incelenmiştir. IC40 değeri baz alınarak, 20 µg/mL MAPK6 siRNA yüklü nanopartikül konsantrasyonu uygun doz olarak belirlenmiştir. MAPK6 siRNA yüklü nanopartikül uygulanan grupta 24, 48 ve 72 saat inkübasyon sonrası *MAPK6* ekspresyonunun, kontrol grubuna kıyasla anlamlı düzeyde ($p<0,001$) azaldığı belirlenmiştir. Ayrıca, MAPK6 siRNA yüklü nanopartikül uygulanan grupta yara kapanma oranının ve oluşan koloni sayısının, kontrol grubuna kıyasla ileri derecede anlamlı düzeyde ($p<0,0001$) düşük olduğu belirlenmiştir. MAPK6 geninin nanopartiküller aracılığıyla baskılanmasının başarılı bir şekilde gerçekleştirildiğinin gösterilmesi ve bu baskılamanın neden olduğu antikanser etkilerin *in vitro* olarak ortaya konulması *MAPK6* genini aday terapötik hedef haline getirmektedir. Çalışmanın sonraki adımında, bu baskılamanın MAPK6 protein seviyesine etkisi ELISA yöntemi ile, hücre proliferasyonuna etkisi immünohistokimyasal inceleme ile, apoptoza etkisi ise akış sitometrisi ile incelenecektir. Sonuç olarak, meme kanseri hücre dizisi MCF-7'de *MAPK6* geninin siRNA yüklü nanopartikül ile baskılanması sağlanmıştır ve bu baskılamanın kanserin ayırıcı özellikleri üzerinde iyileştirici etki gösterdiği *in vitro* olarak ortaya konulmuştur.

**Anahtar Kelimeler:** PLGA Nanopartikül, siRNA, ERK3, MAPK6, Meme kanseri, MCF-7

**Teşekkür**

Bu çalışma, Yıldız Teknik Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmiştir (Proje No: FYL-2022-4887).

## *ORAL PRESENTATION*

# Antimicrobial activity of *Erica manipuliflora* L. methanol, acetone and hexane extracts

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**Abstract**

The aim of this study was to analyze the antibacterial activity of methanol, acetone and hexane extracts obtained using the aerial (flower and leaf) parts of *Erica manipuliflora* L.. The antibacterial activities of these extracts (50 mg/mL) against Gram-positive (*Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis*) and Gram-negative (*Escherichia coli*) bacteria were tested by disk diffusion method. Zones of inhibition (ZOI) were calculated in millimeters (mm), indicating that the microorganisms could not proliferate around the sample-loaded disc. The antimicrobial activity of *E. manipuliflora* extracts was compared with ampicillin (10µL) (positive control) and 10% DMSO (negative control). Findings after incubation revealed that all plant extracts have the potential to inhibit microbial growth of all test microorganisms at varying rates at a concentration of 50mg/mL. Especially, hexane extract showed the highest inhibition against *E. coli* (15.53 mm) among all test microorganisms, while among Gram positive bacteria it showed the highest inhibition against S. aureus (13.8 mm). In addition, the acetone extract was the most effective extract against *E. faecalis* and *S. aureus*, which retarded their microbial growth. Methanol extract showed the greatest ZOI against *B. subtilis* (9.06 mm) compared to other extracts, thus being the most effective inhibitory extract in this bacterium. According to the results obtained, it was concluded that the polar and non-polar extracts formed from the arial parts of *Erica manipuliflora* plant, which has limited studies in the literature, have significant antimicrobial activity.

**Keywords:** Erica manipuliflora, extracts, antimicrobial activity

## *ORAL PRESENTATION*

# Enumeration of *Lactobacillus* and *Bifidobacterium* in Probiotic Dairy Products Sold in Ankara and Çanakkale

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**Abstract**

The objective of this study was to evaluate *Lactobacillus* spp. and *Bifidobacterium* spp. contents of probiotic dairy products sold in in markets in Ankara and Çanakkale. In this study, tested 37 types of probiotic dairy products (plain or fruit-added yoghurt, kefir, ayran, cheese), whose lot numbers and expiration dates differed from each other. A total of 70 samples were evaluated. It was detected *Lactobacillus* spp. in 57 samples and *Bifidobacterium* spp. in 25 of them. Viable bacteria were not detected in 13 of 70 probiotic dairy products. According to the label information, 11 of these samples contained monoculture *Bifidobacterium*, and 2 of them included *Lactobacillus* and *Bifidobacterium* species. Fifty-six products with specific claims of viable bacteria met or exceeded their label claim. Only one of the tested products had a lower number of viable bacteria than declared by the manufacturer. The results showed that 12 products were incompatible with the bacterial species claimed on the label.

**Keywords:** Probiotic dairy foods, *Lactobacillus*, *Bifidobacterium*

## *ORAL PRESENTATION*

# Pathogenic mutation in the *CHAT* gene of a Turkish child with congenital myasthenic syndrome

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**Abstract**

Congenital Myasthenic Syndromes (CMS) are a rare group of diseases that occur as a result of defects in the structure and function of neuromuscular junctions and are very heterogeneous in genetically and phenotypically terms. CMS is mostly early-onset and manifests clinically by abnormal fatigability, or transient or permanent weakness of extra-ocular, facial, bulbar, truncal, respiratory, or limb muscles. Rarely, additional features such as cognitive impairment, dysmorphism, neuropathy, or epilepsy may be present. The genes *CHAT*, *COLQ*, *RAPSN*, *CHRNE*, *DOK7*, *GFPT1*, *CHRNB1*, *CHRND*, and *MUSK* are among the most frequently mutated in CMS. All CMS subtypes share the clinical traits of fatigability and muscle weakness, but the age at which these symptoms first appear, how they manifest, and how they respond to treatment varies depending on the molecular mechanism caused by the underlying genetic defect. The Turkish patient, who is the subject of our presentation, is a 10-year-old male child, who was brought to our center by family members with a complaint of CMS. Whole exome sequencing (WES) analysis was planned for the patient and genetic variations obtained as a result of the analysis were evaluated with the help of *in silico* tools. The patient was found to be homozygous for the *CHAT* gene [NM_020549.5] c.1007T>C (p.Ile336Thr) variation, which is thought to be pathogenic. The existence of variation related to Sanger sequencing was then confirmed. The amino acid alanine at position 336, described in the variation, is conserved across most species and is located in the functional region between amino acids 134 and 713 (CLAT_HUMAN, https://www.uniprot.org/uniprotkb/P28329). As a result of the variation, the amino acid isoleucine at position 336 converts to threonine, and 18 of 25 different *in silico* analysis tools predicted this variation as the cause of disease and 7 of them as uncertain. The variation we detected was never homozygous in gnomAD, ClinVar, and PubMed searches. Variations that disrupt the cellular metabolism by affecting the functional domain of the *CHAT* gene may reveal different phenotypic involvements in patients. There are different treatments depending on the variation in the *CHAT* gene. It has been reported that treatment with acetylcholinesterase inhibitors may be beneficial for CMS6, one of the disease subtypes. The genes mutated in CMS and the age of onset of the disease are variable. At this point, we think that the presented case will contribute to the literature.

**Keywords:** congenital myasthenic syndrome, CMS, endplate myopathies, *CHAT*, whole exome sequencing, WES

## *ORAL PRESENTATION*

# Synthesis of PEG-containing NiCr alloy coatings on stainless steel surface: their corrosion performances in artificial saliva

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**Abstract**

In dentistry, stainless steel is used as implants in reconstructive oral surgery to replace a single tooth or an array of teeth, or in the fabrication of dental prostheses such as metal plates for complete and partial dentures, crowns, and bridges, essential in patients requiring hypoallergenic materials. In clinical orthodontic treatment, different alloys have a wide range of applications [1,2]. Since PEGs are hydrophilic, non-toxic, do not show antigenicity and immunogenicity, hydrophilicity (water-loving), biodegradability and mechanical properties can be improved, they have a wide range of use in the dental field. This study aims to electrochemically synthesize NiCr alloy coatings with and without PEG incorporated alloys on stainless steel electrode, which would increase the corrosion resistance of base stainless steel surface. The presence of PEG in NiCr alloy coating was investigated using SEM and EDX analysis. The corrosion performance of NiCr plated stainless steel substrates without and with PEG was investigated and compared by exposure to corrosive solution of artificial saliva by AC impedance diagrams, $E_{ocp}$-time diagrams and anodic polarization curves. It was found that $NiCr_{PEG}$ alloy plating in artificial saliva exhibited the highest physical barrier behavior on stainless steel among the alloy coatings, in longer exposure time.

**Keywords:** NiCr, PEG, corrosion, alloy, implant

**Acknowledgment**
The research project was funded by the University of Hatay Mustafa Kemal in Turkey, Project No: 20.YL. 040.

**Reference**
[1] Sharifnabi A, Fathi MH, EftekhariYekta B, Hossainalipour M. (2014). The structural and bio-corrosion barrier performance of Mg-substituted fluorapatite coating on 316L stainless steel human body implant. Appl Surf Sci. 2014; 288: 331-340.
[2] Li DG, Wang JD, Chen DR, Liang P. (2015). Influence of Molybdenum on Tribo-corrosion Behavior of 316L Stainless Steel in Artificial Saliva. J Bio TriboCorros. 2015; 1-14.

## *ORAL PRESENTATION*

# An *in vitro* study to investigate the cytotoxic/antiproliferative effects of novel allosteric peptide inhibitors of SHP-2 against MCF-7 breast cancer cells

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**Abstract**

Breast cancer (BC) in women was the most commonly diagnosed cancer worldwide in 2020 and the fifth leading cause of cancer-related death [1]. One of the key enzymes involved in the development and progression of BC is the tyrosine phosphatase SHP-2 [2, 3]. SHP-2, known to be overexpressed in most BC cell lines, is a promising target for the treatment of BC [4]. The highly conserved and positively charged nature of the SHP-2 catalytic domain has led to a shift of interest toward allosteric site inhibitors that offer relatively more selectivity and drug-like properties [5, 6]. In this regard, in our previous study, we identified three novel and potent allosteric peptide inhibitors of SHP-2 by combining structure- and ligand-based virtual screening techniques with *in vitro* biological validation [7]. Here, we examined the cytotoxic/antiproliferative effects of these inhibitors *in vitro* on MCF-7 BC cells using the MTT assay after 24, 48 and 72 hours of incubation. Their selectivity index (SI) was then calculated based on the $IC_{50}$ values on normal MCF10A cells compared to MCF-7. Our results showed that NSC523374 ($IC_{50}$: 1,83 µM and SI≥ 134,3) and NSC374121 ($IC_{50}$: 6,13 µM and SI: 3,7) (48 h) exhibited highly selective cytotoxicity towards MCF-7 cells and had a very potent antiproliferative effect on these cells. Therefore, these imidazole-containing peptide inhibitors could serve as novel lead anticancer compounds for future cancer research and have therapeutic value in the treatment of SHP-2 overexpressing BC.

**Keywords:** Breast Cancer, Cytotoxicity, Antiproliferative, Allosteric SHP-2 Inhibitors, Peptides, Selectivity Index

**Acknowledgement:** We thank the Scientific and Technological Research Council of Turkey (TÜBİTAK) for financial support (Project No: 120Z828). Mine İsaoğlu was supported by the Council of Higher Education (YÖK) 100/2000 and TÜBİTAK-BİDEB 2211/A National PhD Scholarship Programs.

**References:**

1. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 71(3), 209-249.
2. Ma, Y-C., Yang, W-Y., Zhou, L., Wu, J-W., Li, W-Y., Du, S., Ma, Y., Wang, R-L. (2020). Exploring the cause of the dual allosteric targeted inhibition attaching to allosteric sites enhancing SHP2 inhibition. Molecular Diversity, online ahead of print.
3. Shen, D., Chen, W., Zhu, J., Wu, G., Shen, R., Xi, M., Sun, H. (2020). Therapeutic potential of targeting SHP2 in human developmental disorders and cancers. European Journal of Medicinal Chemistry, 190, 112117.
4. Hu, Z., Fang, H., Wang, X., Chen, D., Chen, Z., Wang, S. (2014). Overexpression of SHP2 tyrosine phosphatase promotes the tumorigenesis of breast carcinoma. Oncology Reports, 32(1), 205-212.
5. Xie, J., Si, X., Gu, S., Wang, M., Shen, J., Li, H., Shen, J., Li, D., Fang, Y., Liu, C., Zhu, J. (2017). Allosteric Inhibitors of SHP2 with Therapeutic Potential for Cancer Treatment. Journal of Medicinal Chemistry, 60(24), 10205-10219.
6. Bruder, M., Polo, G., Trivella D. B. B. (2020). Natural allosteric modulators and their biological targets: molecular signatures and mechanisms. Natural Product Reports, 37(4), 488-514.
7. İsaoğlu, M. (2022). Hedefe Dayalı Sanal Tarama Yöntemleri İle Yeni Allosterik SHP-2 İnhibitör Adaylarının Keşfi ve Biyolojik Etkinliklerinin Araştırılması. Doktora Tezi, Fen Bilimleri Enstitüsü, Erzurum.

## *ORAL PRESENTATION*

# Chemical Composition And Antioxidant Activity Of *Asphodelus aestivus* L.

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**Abstract**

*Asphodelus aestivus* L. with the common name Ciris is a plant from Xanthorrhoeaceae family. The plant is popular folk medicines used for treatment of hemorrhoids, rheumatism, menstrual, diuretic, ringworm, milk enhancer, eczema, acne and boilsin [1-2]. *Asphodelus aestivus* L. was extracted with methanol (40 C) in a ultrasonic bath (Heidolph, America) for 1 hours. Filtered extract was evaporated and dissolved with methanol for antioxidant activity. The extract was analyzed by high-performance liquid chromatography with diode array detector (HPLC-DAD) to identify and quantify their phenolic compounds. Antioxidant potential of the methanol extract was characterized with total phenolic content (TPC), total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ABTS [2,20 -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)]. Gallic acid, chlorogenic acid, 2,5-dihydroxybenzoic acid, caffeic acid, vanilic acid, rutin, *p*-coumaric acid, ferulic acid, quercetin, kaempferol and isorhamnetin were detected in the extract. As a result, *Asphodelus aestivus* L., a rich source of phenolic compounds,

**Keywords:** *Asphodelus aestivus* L., antioxidant activity, HPLC-DAD

**References:**

[1] Baytop T., 1999. Türkiye'de Bitkiler ile Tedavi, Nobel Tıp Kitapevleri, İstanbul.

[2] Ugulu I., Baslar S., Yorek N., Dogan Y., 2009. The investigation and quantitative ethnobotanical evaluation of medicinal plants used around Izmir province, Turkey, Journal of Medicinal Plants Research, 3(5): 345-367.

## *ORAL PRESENTATION*

# A Novel Python Script for Genetic Association Analysis at Diplotype Level

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**Abstract**

The genetic association studies are performed to determine if a genetic factor is associated with a disease. Genetic factors associated with diseases can be Single Nucleotide Polymorphisms (SNPs), haplotypes or diplotypes. The haplotypes can be determined or estimated, and their disease associations can be tested by a software like Haploview or PLINK. Diplotype associations of diseases can be tested by previously developed scripts like "Diplotyper", but it also requires use of Haploview and PLINK. Manual analysis' by using a spreadsheet program is also possible but it is time consuming and requires computational power for large files.
In this study, we have developed a new script to determine diplotypes of individuals and test their disease associations by comparing the case and control groups. With a simple and user-friendly interface, this novel script reads pedigree (.ped) files and compatible formats. It automatically creates haplotype and diplotype blocks. It generates all possible diplotype groups and performs statistical analysis on them. Finally, it allows the researcher to easily analyze the results by printing them in a formatted file. This script does not require any additional software, is fully compatible with Python3 and later versions, and offers an uncomplicated and fast alternative for diplotype analysis. The code has been applied for association analysis of existing experimental data and can be easily modified by researchers for different purposes.

**Keywords:** Genetic Association, Diplotype, Disease association.

This study has been supported by The Unit of Scientific Research Projects at İnonu University. Project Number: FCD-2021-2552 to Mustafa Mert SÖZEN.

## *ORAL PRESENTATION*

# Yüksek Molekül Ağırlıklı Polietilen Glikol Diakrilat (PEGDA) Hidrojel Sentezi

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**Özet**

PEGDA non-toksik olması, organik solventlerde ve suda çözünebilirliği, hidrofilikliği, düşük antijenite ve düşük immünojiteye sahip olması gibi özellikleri sebebiyle medikal amaçlarda kullanım için iyi bir adaydır. Düşük toksisitesi ve yüksek biyouyumluluğu sayesinde de FDA tarafından ilaç salım sistemleri ve yara örtüsü uygulamaları gibi biyomedikal alanlarda kullanımı onaylanmış bir malzemedir. Hidrojeller ilaç salım sistemleri, hijyenik ürünler, kontakt lensler, yara örtü malzemeleri, doku mühendisliği gibi pek çok biyomedikal alanda kullanılan su içerisinden çözünmeden şişebilen, çapraz bağlı, üç boyutlu ağ yapılı polimerlerdir. PEGDA hidrojellerin yüksek su tutma kapasitesi, biyouyumluluk ve biyolojik malzemelerle kolay entegre olmasından dolayı biyomedikal uygulamalarda çok kullanılan biyomalzemelerdir (Hemmatgir vd.,2022, Salehi-Abari vd.2019, Ban,2011).
Bu çalışmada, farklı molekül ağırlıklarında polietilen glikolden (PEG) (Mn:2000,4000 ve 6000) yola çıkılarak, PEGDA sentezi yapılmıştır. Sentezlenen PEGDA'ların Fourier Dönüşümlü Kızılötesi Spektroskopisi (FTIR), Nükleer Manyetik Rezonans (NMR) ve Diferansiyel Taramalı Kalorimetri (DSC) ile karakterizasyonu yapılmıştır. Karakterizasyonu tamamlanan PEGDA'lar kullanılarak PEGDA hidrojeller sentezlenmiştir ve FTIR ve DSC ile karakterizasyonu yapılmıştır.
Sentezlenen farklı molekül ağırlıklarındaki PEGDA hidrojellerin şişme ve degredasyon analizleri yapılmıştır. PEGDA'nın molekül ağırlığı değiştikçe hidrojellerin şişme ve degredasyon sürelerinin değiştiği gözlenmiştir. Elde edilen sonuçlara göre PEGDA hidrojellerin kullanım alanları tespit edilmiştir.

**Anahtar Kelimeler:** Polietilen Glikol (PEG), Polietilen glikol diakrilat (PEGDA), PEGDA hidrojel, karakterizasyon

Teşekkür: YÖK 100/2000 programına destekleri için teşekkür eder.

**Kaynaklar:**

Ban, T.J. (2011) Synthesis and Characterisation of PEGDA-based Hydrogels crosslinked with Pentaerythritol Tetrakis (3-Mercaptopropionate). Nanyang Technological University.
Hemmatgir, F., Koupaei, N., Poorazizi, E. (2022). Characterization of a novel semi-interpenetrating hydrogel network fabricated by polyethylene glycol diacrylate/polyvinyl alcohol/tragacanth gum as a wound dressing. Burns, 48,1,146-155. https://doi.org/10.1016/j.burns.2021.04.025.
Salehi-Abari, M., Koupaei N., Hassanzadeh- Tabrizi, S.A. (2020) Synthesis and Characterisation of Semi-Interpenetrating Network of Polycaprolactone/ Polyethylene Glycol Diacrylate/ Zeolite- CuO As Wound Dressing. Materials Technology, 35,5, 290-299. https://doi.org/10.1080/10667857.2019.1678088.

## *ORAL PRESENTATION*

# Comparison of heavy metal concentrations in marine macroalgae of the Northern Aegean Sea, Türkiye

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**Abstract**

This study was conducted to determine heavy metal levels in eight marine macroalgae (*Ulva lactuca, Ulva intestinalis, Ulva linza, Codium fragile, Cystoseira barbata, Padina pavonica, Colpomenia sinuosa, Corallina elongata*) at 15 station in the Turkish coast of the Aegean Sea in 2009. Macroalgae samples were collected in each area to determine total metal concentrations and to gain additional information on both the environmental conditions of the site and possible bioaccumulation patterns. The concentrations (mg/kg dw) of these metals ranged from <0.01 to 15.96 for Co, to <0.01 for Cd, 1.57 to 60.7 for Cu, 77.5 to 5667.3 for Fe, <0.01 to 17.97 for Ni, <0.01 to 14.1 for Pb, and 0.12 to 93.52 for Zn. Fe showed the highest levels in examined tissues of all macroalgae species. Following Fe, Cu generally showed the second highest levels. The data obtained from this study were also compared with those obtained from previous studies.

**Keywords;** Macroalgae, bioindicator, heavy metal, Aegean Sea, Türkiye

## *ORAL PRESENTATION*

# Yenilebilir bir mantar türü olan *Infundibulicybe geotropa*'nın timidin fosforilaz inhibisyonu aktivitesi

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## Özet

Tıbbi mantarlar, kanseri önleyici ve immunomodülatör özellikteki polisakkarit ve triterpenoid kaynaklarıdır. Mantarlardan antikanser, antioksidan, antiviral, antibakteriyal, antiinflamatuar ve immunomodulatör aktiviteleri gösteren lektinler, polisakkaritler, polisakkarit-peptitler, polisakkarit-protein kompleksleri, lanostan triterpenoidler ve fenolik maddeler izole edilmiştir [1]. Japonya'da mantardan üretilen Krestin, toplam kanser ilaç pazarının %25'ini oluşturduğu ve Japonya'ya milyarlarca dolar katkı sağladığı bilinmektedir [2]. Literatürlerde mantarlardan elde edilen maddelerin çeşitli biyo-aktivite gösterdikleri belirtilmektedir. Mantarlardan elde edilen bazı saf maddelerin özellikle sitotoksik ve immunomodülatör oldukları son yıllarda ortaya konmuştur. Metastaz kanser hastalığında ortaya çıkabilen ve tedaviyi zorlaştıran bir durumdur. Kanserle mücadele yapılırken metastaz önlenebilirse tedavi başarıya ulaşabileceği öngörülmektedir. Timidin seviyesini arttırılması metastazın oluşmasının önlenmesinde önemli bir yoldur. Timidin düzeylerini arttırmak için uygulanacak bir diğer yöntem ise timidin fosforilaz enziminin baskılanmasıdır.
Ülkemizde doğal olarak yayılış gösteren, yenilebilir *Infundibulicybe geotropa* mantarının organik solvent ekstreleri ve sıcak su infüzyonun timidin fosforilaz inhibisyonu aktivitesi araştırıldı. Mantar ekstrelerin timidin fosforilaz inhibisyon aktivitesi (TP) ODS-C18 (25 mmx4,6 mm, 5 µm) kolon bağlı HPLC-DAD cihazı kullanılarak timidin ve timin miktarlarının tayini yapılarak ölçüldü. [3-6]. *Infundibulicybe geotropa* mantarının petrol eteri, aseton, metanol ve sıcak suyla ekstrelerinin 2 farklı konsantrasyonlardaki timidin fosforilaz inhibisyonu aktivitesi % inhibisyon olarak belirlendi. Yapılan aktivite test sonuçlarına göre, *Infundibulicybe geotropa* hekzan ekstresinin metastazı engelleme de etkili potansiyel kanser terapötikleri olabileceği düşünülmektedir. Aktivite gösteren kimyasal maddelerin ileri çalışmalarla izolasyonu ve yapılarının tayinlerinin yapılması hedeflenmektedir. Elde edilecek saf maddelerin potansiyel kanser terapötiklerinin geliştirilmesini rol oynayabilecekleri öngörülmektedir.
**Anahtar Kelimeler:** *Infundibulicybe geotropa*, Kanser, Metastaz, Timidin fosforilaz inhibisyonu aktivitesi

## Teşekkür

Bu çalışma TÜBİTAK 1001 Bilimsel ve Teknolojik Araştırma Projelerini Destekleme Programı 121Z551 nolu proje ile desteklenmiştir.
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### Kaynaklar

[1] Öztürk M., Tel-Çayan G., Muhammad A., Terzioğlu P., Duru M.E. (2015). Mushrooms: a source of exciting bioactive compounds. Ed.: Atta-ur-Rahman, Chapter In Studies in Natural Product Chemistry. Amsterdam: Elsevier. 45, 363-456.
[2] Moradali, M.F., Mostafavi, H., Ghods, S., Hedjaroude, G.A. (2007). Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). International Immunopharmacology. 7, 701–724.
[3] Andre B.P. van Kuilenburg, Zoetekouw L. (2005). Determination of thymidine phosphorylase activity by a non-radiochemical assay using reversed-phase high-performance liquid chromatography. Journal of Chromatography B. 820, 271-275.
[4] M. Hill and B. Arrio (1997). Determination of inorganic phosphate by coupling thymidine phosphorylase and reversed-phase high-performance liquid chromatography: Application to tonoplast pyrophosphatase activity. Analytical Biochemıstry. 254, 249–253.
[5] S. Mohamed, L. Caporali, R. De Giorgioc, V. Carelli, M. Contin (2014). HPLC-UV analysis of thymidine and deoxyuridine in plasma of patients with thymidine phosphorylase deficiency. Journal of Chromatography B. 949–950, 58–62.
[6] V. Nuessler, A. Pogrebniak, R. Pelka-Fleischer, K. Boos, W. Wilmanns (1999). Determination of thymidine phosphorylase activity in leukemic blast cells by a high-performance liquid-chromatographic assay. Chromatographia. 49, 173-178.

## *ORAL PRESENTATION*

# *Tricholoma scalpturatum*'un meme kanseri (MCF-7) hücre hattına karşı sitotoksik aktivitesi

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**Özet**

Mantarların halk tıbbında kullanımları eski çağlara dayanmasına rağmen, modern tıpta kullanımları ve ilaç potansiyellerinin ortaya çıkarılmasına dair bilimsel çalışmaların geçmişi fazla değildir. Dünyada kadınlarda en çok görülen kanser insidansı meme kanseridir. Globocan (2012) verilerine göre de Türkiye'de kadınlarda en sık görülen ve en çok ölüme neden olan kanserin meme kanseri olduğu bildirilmiştir (Hatipoğlu, 2007). Mantarlar üzerine yapılan bilimsel çalışmalarda, özellikle onların besinsel değerini ortaya koymak amacıyla; metal-mineral içerikleri, yağ asiti bileşenleri ve aroma bileşenleri üzerine yapılırken son yıllarda mantarların içerdikleri kanseri önleyici ve bağışıklık sistemini destekleyici (immunomodüler) özellikteki bileşenlerin tıbbi aktivitelerinin yüksek olması sebebiyle biyoaktivite çalışmalarına olan ilgi her geçen gün artmıştır. (Moradali vd., 2007; Zhang vd., 2007; Han vd., 2013).
*Tricholoma* türleri tıbbi özellikleri olan *Matsutake* mantarının ailesinden gelmektedir. *Tricholoma matsutake* dünyada hem gıda hem de tıbbi olarak kullanılmaktadır. Tıbbi olarak özellikle kanser hastalıklarının tedavisinde ve bağışıklık sisteminin güçlendirilmesinde tercih edilen türlerden bir tanesidir. Yapılan çalışmalarda *Tricholoma* türlerinin içerdikleri polisakkarit, seskiterpen, lektinler, fenolik bileşikler, $\alpha$-D-glukanlar ve diğer terpenoid sınıfı bileşikler nedeniyle çeşitli insan kanser hücreleri üzerinde antikanserojenik aktivite gösterdiği açıklanmıştır. Bu çalışmada, *Tricholoma scalpturatum* mantarının petrol eteri, aseton ve metanol ekstreleri ve sıcak su infüzyonlarının farklı konsantrasyonlarının (15,625; 31,25; 62,50, 125; 250 mg/mL), MCF-7 insan meme kanser hücresine karşı sitotoksik etkileri araştırıldı. Ekstrelerin ve sıcak su infüzyonlarının sitotoksik etkileri MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) yöntemi ile değerlendirildi. Elde edilen değerler kullanılarak hücrelerin yarısını öldüren konsantrasyon olan $EC_{50}$ değerleri hesaplandı.
Yapılan aktivite test sonuçlarına göre, *Tricholoma scalpturatum* mantarının, aseton ekstresinin konsantrasyona bağımlı olarak MCF-7 hücreleri üzerine etkili potansiyel kanser terapötikleri olabileceği öngörülmüştür.

**Anahtar Kelimeler:** *Tricholoma scalpturatum*, Sitotoksik aktivite, MCF-7 kanser hücre hattı

**Teşekkür**

Bu çalışma TÜBİTAK 1001 Bilimsel ve Teknolojik Araştırma Projelerini Destekleme Programı 121Z551nolu proje ile desteklenmiştir.
*Cansel ÇAKIR destekleri için YÖK 100/2000 Doktora Programı için Türkiye Yüksek Öğretim Komisyonu'na (YÖK) teşekkür eder.

**Kaynaklar**

Han, J.J., Chen, Y.H., Bao, L., Yang, X.L., Liu, D.L., Li, S.J., Zhao F. ve Liu H.W. (2013) Anti-inflammatory and cytotoxic cyathane diterpenoids from the medicinal fungus Cyathus africanus, Fitoterapia 84: 22-31.
Hatipoğlu, A.A. (2007) Kanser erken tanı ve tarama problemleri. Tuncer AM. Türkiye'de Kanser Kontrolü.1. Baskı. Ankara: Onur Matbaacılık, 381-388, 2007.
Moradali, M.F., Mostafavi, H., Ghods, S., Hedjaroude, G.A. (2007). Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). International Immunopharmacology. 7, 701–724.
Öztürk M., Tel-Çayan G., Muhammad A., Terzioğlu P., Duru M.E. (2015). Mushrooms: a source of exciting bioactive compounds. Ed.: Atta-ur-Rahman, Chapter In Studies in Natural Product Chemistry. Amsterdam: Elsevier. 45, 363-456.
Zhang, M., Cui, S.W., Chueng, P.C. ve Wang, K.Q. (2007) Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity, Trends in Food Sci Tech, 18: 4–19.

## *ORAL PRESENTATION*

# Designing organotransition metal complexes with N,S-donor ligands

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**Abstract**

Thiosemicarbazone (TSC) analogs are the most important in the group of ligands containing sulfur donor atoms of Schiff base ligands, which are known to have versatile coordination modes. Thiosemicarbazone derivatives, which contain a ring structure in their skeleton, are studied intensively due to their many biological activities such as antibacterial, antitumor, antifungal and antiviral. The conjugated N–N–S system in thiosemicarbazone scaffolds is shown as the part responsible for the biological activity [1-3]. It is known that thiosemicarbazones containing these systems form stable complexes with transition metals such as Co(II), Ni(II), Zn(II) and Pd(II), and it has been observed in studies that these complexes show biological activities. [4]. In particular, cobalt (II) and palladium (II) complexes obtained with nitrogen and sulfur donor ligands, which are known to contain active ends, are very interesting both with their activity and with their bonding patterns [5]. It is known that thiosemicarbazones form divalent compounds such as monomer, dimer and trimer as a result of the reactions of nickel metal salts. In addition, ligands in these compounds show bi- or monodentate bonding in neutral or anionic form [6]. In this context, nitrogen and sulfur donor ligands containing active sides and Palladium (II) and Cobalt (II) complexes were investigated. Within the scope of the study, a new thiosemicarbazone ligand with S and N donors was synthesized, and Palladium (II) and Cobalt (II) complexes were obtained with the synthesized ligand. The structures of these synthesized compounds were elucidated by elemental analysis, $^{1}$H-NMR, FT-IR spectroscopy and single crystal X-ray analysis.

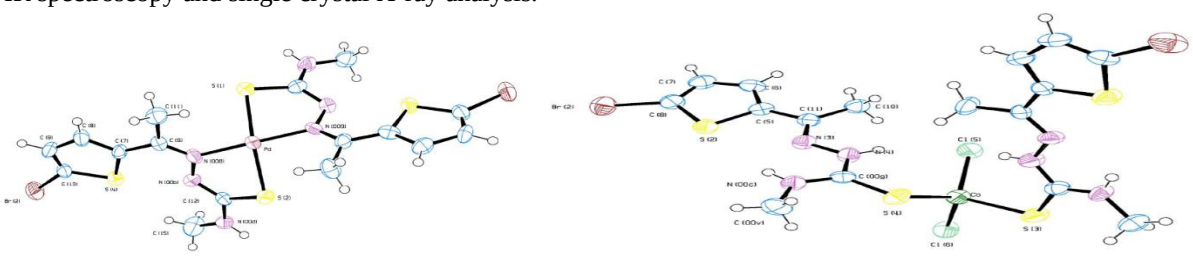

Figure: Single Crystal X-ray Structures of Palladium and Cobalt Complexes

**Keywords:** Thiosemicarbazones, Palladium complexes, Cobalt complexes

**Acknowledgements:** This work is supported by Dokuz Eylül University BAP Coordination Unit (Project No: 2019.KB.FEN.033).

**References:**

1. D. María, Damit, N. S. H. H., Hamid, M. H. S. A., Rahman, N. S. R. H. A., Ilias, S. N. H. H., Inorganica Chimica Acta, 527 (2021), 120557.
2. Bajaj, K., Buchanan, R. M., & Grapperhaus, C. A., Journal of Inorganic Biochemistry (2021), 111620.
3. Guler, S., Kayali, H. A., Sadan, E. O., Sen, B., & Subasi, E., Frontiers in pharmacology (2022), 13, 882756-882756.
4. Lavanya, M., Haribabu, J., Ramaiah, K., Yadav, C. S., Chitumalla, R. K., Jang, J., Jagadeesh, M., Inorganica Chimica Acta, 524 (2021), 120440.
5. Şen, B., Kalhan, H. K., Demir, V., Güler, E. E., Kayalı, H. A., Subaşı, E., Materials Science and Engineering: C, 98 (2019), 550-559.
6. Balakrishnan, N., Haribabu, J., Malekshah, R. E., Swaminathan, S., Balachandran, C., Bhuvanesh, N., Karvembu, R., Inorganica Chimica Acta, (2022), 534, 120805.

## *ORAL PRESENTATION*

# Determination of Cytogenotoxicity of *Sambucus ebulus* L. Aqueous Extract by *Allium* Assay

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**Abstract**

*Sambucus ebulus*, known as dwarf elder or elder berry, is a perennial herb from the Adoxaceae family. Especially fruits and flowers are used in traditionally for the treatment of injuries, bites, sore throat and inflammatory diseases such as hemorrhoids. In this study, the effects of aqueous extract of *S. ebulus* fruits on onion root meristem cells were investigated in terms of cytotoxicity and genotoxicity. Onion bulbs were exposed to 1, 5, 10, 20 and 50% (v/v) concentrations of the extract. Tap water and zinc oxide solution (680 ng/L) were used as negative and positive controls, respectively. Mitotic index was calculated in all groups. It was observed that the mitotic index decreased depending on the increase in concentration, especially in the 20% concentration group and afterwards. The mitotic abnormalities were recorded as disturbed prophase, C-mitosis, choromatid bridge, vagrant and laggard chromosomes. In this study, chromosomal abnormalities, cell membrane disorders and cell death rates in onion meristematic cells and cytotoxic-genotoxic effects of the extract were determined for the first time with *S. ebulus* fruit extract application. With the *Allium* assay, the dose of the extract, is not toxic, was determined and the results were evaluated in terms of the amount of antioxidant substance that the extract has.

**Keywords:** *Sambucus ebulus*, *Allium* test, mitotic index

## *ORAL PRESENTATION*

# Assessing effects of glabridin on cell viability in PC3 androgen-independent prostate cancer cells

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**Abstract**

Natural compounds derived from plants, fungi, and lichens attract the attention of researchers due to their strong biological and pharmacological activities. Glabridin, a polyphenolic flavonoid found as a major component in Licorice root (*Glycyrrhiza glabra*) has been used in traditional medicine in different countries. In previous studies, the anti-cancer, anti-oxidant, anti-inflammatory effects of glabridin were reported. The aim of this study is investigating anti-proliferative effect of glabridin in PC3 androgen-independent prostate cancer cells. Effects of glabridin on cell viability in PC3 cells were detected by colorimetric based MTS assay according to time and dose dependent manner. Total RNA was isolated by Trizol reagent and subsequently cDNA was synthesized. *Akt1* and *NFkB* mRNA gene expression changes were evaluated by Real-Time PCR method. According to our results, $IC_{50}$ value of glabridin in PC3 cells was determined as 14.27 µM at 24th hour. Glabridin downregulates *Akt1* and *NFkB* mRNA gene expressions in dose group PC3 cells compared to control. Glabridin inhibits proliferation of PC3 prostate cancer cells and its effect mechanisms may be associated by regulating the expression of mRNAs that have a role in cell viability. In conclusion, this study may contribute comprehensive pharmaceutical studies about glabridin and its therapeutic potential for prostate cancer treatment.

**Keywords:** Glabridin, PC3 cells, prostate cancer, cell viability

## *ORAL PRESENTATION*

# Koroner Arter Bypass Cerrahisi Sonrasında Arteriyel Filtre Hasarının İncelenmesi

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**Özet**

Koroner arter cerrahisi süresinin uzaması ile birlikte yeterli filtrasyonun sağlanamaması sonucunda hastada çeşitli geçici ve/veya kalıcı hasarlar meydana gelmesi durumunu oluşturabilmektedir. Bu sebeple arteryel filtredeki hasar tespiti ve risk değerlendirmesi hayati önem taşımaktadır. Arteryel filtre vücut dışı dolaşım mekanizmasında kan dolaşımının geçtiği son noktadır. Yapılan çalışmalarda arteryel filtrenin gaz emboli riskini ve buna bağlı komplikasyonları en aza indirdiği ispatlanmıştır. Ancak arteryel filtrenin uzun süre kullanımı filtre yapısında hasara neden olabilir. Oluşabilecek bu hasar ise; emboli riski, inflamasyon, kan hasarı, serebral korumada azalma vb. komplikasyonlara sebep olabilmektedir. Çalışmada kardiyopulmoner bypass devresinde arteryel filtrenin önemi; debi, sıcaklık ve kardiyopulmoner bypass süreleri eşit tutularak ele alınan arteryel filtrenin kardiyopulmoner bypass sürenin uzamasına bağlı oluşan hasarını elektron mikroskubu ile görüntüleyerek hasar tespiti ve risklerin öngörülmesindeki önemi vurgulanacaktır. Bu çalışmada arteryel filtrelerin elektron mikroskobundaki görüntülerini incelenerek meydana gelen hasar tespit edilecek ve tespit edilen hasar sonucunda oluşabilecek riskleri araştırılmıştır.

**Anahtar kelimeler:** Kalp-Akciğer Makinesi, Kardiyopulmoner Bypass, Arteriyel Filtre.

## *ORAL PRESENTATION*

# Investıgation of Polycyclic Aromatic Hydrocarbons (PAHs) in Surface Sediments of the Golden Horn Estuary (Halic), Istanbul, Türkiye: Pollution Characterization, Source Appointment, Environmental and Human Risk Assessments

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## Abstract

The Golden Horn (Turkish: Altın Boynuz or Halic) is a critical urban waterway located at the main entrance of the Bosphorus, which has played an important role in the protection of the maritime merchant ships of the Byzantine and Ottoman empires for thousands of years throughout history. The aim of this study was to find out the pollution profile of surface sediments of the golden horn estuary (Halic) in terms of priority sixteen polycyclic aromatic hydrocarbons (PAHs), to determine their possible sources and to exhibit the potential environmental and human health risks. The determination of PAHs was done with gas chromatography Triple Quadrupole Mass Spectrometry (GC-MS/MS) in selected reaction monitoring (SRM) mode. 1 mL of special internal standard (including Acenaphthene-$d_{10}$, Chrysene-$d_{12}$, Naphthalene-$d_8$, Perylene-$d_{12}$, Phenanthrene-$d_{10}$) (200 µg/L) was spiked in the pre-treatment of samples for the instrumental analysis of PAHs. PAH pollution status in sediment was determined as moderately polluted [1] because total PAH concentrations of surface sediments varied from 206.5 ng/g to 254.5 ng/g. Naphthalene (25.59-34.93 ng/g), Anthracene (20.72-33.62 ng/g), Pyrene (19.88-28.12 ng/g), Fluoranthene (15.87–26.43 ng/g) and Fluorene (12.83-20.69 ng/g) are the dominant PAHs. In pursuant to the results of different type of PAH molecular ratios, they were polluted substantially from petrogenic sources such as Istanbul city traffic [2]. According to the concentrations of PAHs and the related sediment quality guideline values (ERL, ERM, TEL and PEL), the results do not pose any toxic risk to aquatic organisms. In non-carcinogenic risks, HI values changed from 5.70E-05 to 6.56E-01 for child, from 7.23E-06 to 8.32E-02 for adult. In carcinogenic risks for adult, TLCR values ranged from 5.30E-11 to 5.54E-11. In human health risk assessment, there is no risk for non-carcinogenic and carcinogenic hazards for both children and adults considering HQ, HI and TLCR values of PAHs.




**Figure 1.** Satellite images of sampling points in the Golden Horn (Halic)

**Keywords:** Gas chromatography (GC), Pollution, Polycyclic Aromatic Hydrocarbons (PAHs), Risk Assessment, Surface Sediment, Triple Quadrupole Mass Spectrometry (MS/MS)

## References

1. Bemanikharanagh A, Bakhtiari AR, Mohammadi J, Taghizadeh-Mehrjardi R (2017) Characterization and ecological risk of polycyclic aromatic hydrocarbons (PAHs) and n-alkanes in sediments of Shadegan international wetland, the Persian gulf. Mar Pollut Bull 124:155–170.
2. Güzel B, Canlı O, Aslan E (2022) Spatial distribution, source identification and ecological risk assessment of POPs and heavy metals in lake sediments of Istanbul, Turkey. Mar Pollut Bull 175:1-14.

## *ORAL PRESENTATION*

# Optimization studies and application of salicylate-selective carbon paste electrode

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**Abstract**

Many studies have shown that among several instrumental methods potentimetry with ion-selective electrode is one of the most important and widely used electrochemical determination method. Compared to other devices, potentiometric electrodes have several distinctive features such as; short response time, low price, accessibility, sustainability, repeatability and high precision. Therefore, developing a new method for the selective determination of many ionic species is still a point of interest.

Salicylate is one of the common metabolites resulting from hydrolysis of acetylsalicylate and its derivatives, which are widely used as an analgesic, inflammatory, antimicrobial agents, and also as a preventive of heart attack in several pharmaceutical preparations. The levels of salicylic acid higher than 2.2 mmolL $L^{-1}$ are regarded as toxic. Therefore, its determination in dermatological preparations, pharmaceuticals, and in biological fluids of patients who are treated with medicine containing salicylate derivative is very important. For this purpose, a new salicylate-selective carbon paste electrode was fabricated by using a nano composite material including graphite powder, plasticizers, nanoparticles and lipophilic additives.The effects of carbon paste composition and pH, on the performance of the electrode were investigated. Furthermore, the selectivity coefficients were calculated by separate solution method.

The optimization studies of carbon paste revealed that the proposed electrode had super-Nernstian slope of 67.69±0.77mV/pSal in the concentration range of $1.0\times10^{-1}$-$1.0\times10^{-5}$ M at pH 4.0 and 23±1°C. The reponse time and lifetime of the electrode was found as 10s and at least 1 month, respectively. It was successfully applied as an indicator electrode in the potentiometric determination of salicylate.

**Keywords:** Determination of salicylate, ion-selective electrode, nanocomposite, potentiometry.

## *ORAL PRESENTATION*

# Construction of an electrode for the determination of thiocyanate in veterinary medicine

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**Abstract**

Chronically elevated levels of thiocyanate in body fluids are known to be toxic and its relation to local goiter, vertigo, or unconsciousness has been pointed out. At low pH values, thiocyanate present in waste water is converted into cyanide ion in the presence of oxidants and thereby causes profound damage to aquatic life. On the other hand, some drugs having antibacterial effect are administered by thiocyanate and are used for animals. Therefore, the determination of thiocyanate is of practical significance.
Various methods such as spectrophotometry chromatography, polarography, capillary zone electrophoresis, amperometry have been reported previously. However, the use of potentiometric methods based on ion-selective electrodes is increasing in medicinal, environmental, agricultural, and industrial samples due to their advantages such as wide operating range, reasonable selectivity, low detection limit and rapid analysis at low cost.
Nanomaterials promote a greater interaction with targets when they are part of the recognition layer. Their exceptional electrical properties generated at the interfaces are of paramount importance when they are used as the transducing components in potentiometric electrodes. Therefore, we aimed to construct a new thiocyanate-selective carbon paste electrode by using nanoparticles which are very suitable additives to improve the performance of the electrodes. Its response characteristics such as slope, lifetime, response time, optimum working range were determined and the selectivity coefficients were calculated by using separate solution method. The effects of carbon paste composition, pH, conditioning solution on the potentiometric response of the electrode were investigated.
It was found that the electrode showed Nernstian slope of 59.11mV/pSCN within the range of $1.0\times10^{-1}$ – $1.0\times10^{-6}$ M at pH 4.0 for at least 2 weeks with a response time of 10-15s. Moreover, the proposed electrode was satisfactorily used for the determination of thiocyanate in veterinary medicine sample.

**Keywords:** nanoparticles, thiocyanate, ion-selective electrodes

Bu çalışma "İnönü Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi FDK-2022-2949" nolu proje kapsamında gerçekleştirilmiştir.

## *ORAL PRESENTATION*

# In vitro genotoxicity assessment of gadobutrol by comet assay

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**Abstract**

Magnetic resonance imaging (MRI) is a high resolution imaging tool that enables the examination of anatomic, physiologic, biochemical, and molecular details of lesions in the body. In 40% of all imaging, contrast agents are administered to patients to better distinguish the tissues. Gadolinium-based contrast agents (GBCAs) have been widely used in clinical MR imaging studies for over three decades. Since gadolinium is toxic alone, it is bonded a chelating ligand to form GBCAs. Gadobutrol is one of the most commonly used GBCA during MRI, that has non-ionic and macrocyclic chemical structure. According to the recent studies, there are findings showing that GBCAs lead to gadolinium retention in the body resulting in some toxic effects. Evaluations revealed both positive and negative results in terms of genotoxicity as well. Therefore, the aim of this study was to assess the genotoxic potential of gadobutrol on human peripheral lymphocytes by comet assay. For this purpose, the isolated lymphocytes were treated with 7,000, 14,000, 28,000, 56,000, and 112,000 µg/mL concentrations of gadobutrol and incubated for 1 h at 37°C. There were also negative (distilled water) and positive (Hydrogen peroxide) controls in the experiments. It was observed that all the concentrations of gadobutrol (except 14,000 µg/mL) increased tail intensity and tail moment but only the highest concentration (112,000 µg/mL) was significant in tail intensity. Increased in tail intensity and tail moment might be occurred because of the damage like oxidation resulted from free radical formation. Thus, these results can be interpreted like gadobutrol had a weak DNA damaging effect on isolated lymphocytes. Nonetheless, further investigations should be performed for better identification of genotoxic end points of gadobutrol under other *in vitro* and *in vivo* conditions.

**Keywords:** Magnetic resonance imaging, Gadobutrol, Genotoxicity, Comet assay, Human lymphocytes

## *ORAL PRESENTATION*

# Ethical view of anatomical studies in wild animals

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**Abstract**

Anatomy is a fundamental science in the field of animal and human health, and it must be learned completely for clinical works. Without a good knowledge of anatomy, it is very difficult to diagnose diseases, choose a good surgical method and perform the right procedures in the clinic. Recently, there has been an increase in the number of wild animals coming to veterinary clinics. This situation is due to the increasing the number of accidents involving wild animals as a result of the shift of modern life towards to the wild habitats, the increasing the number of wild animals kept at home, and the fact that many wild animal species are kept in captivity in zoos and laboratories. In wild animal studies, ensuring animal welfare is the most important element. Researches should be based on current ethical considerations surrounding the use of wildlife. Consideration should be given to welfare issues in animal capture, handling, anesthesia and anatomical studies. It is impossible to find all wild animals in all anatomy laboratories. It is very difficult to reach especially endangered or endangered animals. Therefore, ethical discussions can be avoided with computer technology including virtual dissection simulations, 3D models, videos, slides and online presentations. It is also ethically appropriate to use plastin specimens and plastic models in learning the anatomy of wild animals.

**Key words:** Anatomy, ethic, research, wild animal

## *ORAL PRESENTATION*

# Green nanotechnologic method using olive leaf extract for determination of phenolic compounds

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**Abstract**

Reactive oxygen species are generated in the human body during metabolic processes and cause oxidative damage to living cells and tissues as a result of attack on biological macromolecules. Antioxidants are defensive substances that can retard or inhibit undesired oxidation reactions by stabilizing or deactivating free radicals. Therefore, it is very important to determine the antioxidant power of the actual specimens in which these compounds are present.

Nanotechnology has become to the fore in important scientific fields such as material science, aerospace engineering, medicinal drug delivery, and biotechnology in recent years. Nanoparticle-based analytical methods have undergone a rapid development at the interface of analytical chemistry, food chemistry, biochemistry, and pharmacy. Due to their small size (1–100 nm) and unique surface characteristics, noble metal nanoparticles (NPs) are used for ultrasensitive sensing and imaging in different research fields. Noble metal NPs dispersed in liquid media exhibit unique surface plasmon resonance (SPR) bands providing selectivity and simplicity for determination of different analytes. It makes them ideal analytical probes for important substances in various fields.

Gold nanoparticles (AuPs) can be synthesized using three different approaches, including physical, chemical, and biological methods. Biological methods have become very popular due to the disadvantages of chemical and physical methods such as high cost, high energy consumption, use of toxic chemicals and harm to the environment. The biological synthesis called “green synthesis” of NPs has several advantages such as being reliable, sustainable, eco-friendly, cost-effective and rapid.

In this study, olive leaf extract was chosen as bioreducing agent due to its polyphenolic composition. In the proposed method, we produced the gold nanoparticles by bioreduction of gold ions to gold atoms in the presence of olive leaf extract. Thus a novel green nanotechnology based method was developed for measurement of total antioxidant capacity of polyphenols.

**Keywords:** Gold nanoparticles, green nanotechnology, olive leaf extract, antioxidant capacity.

## *ORAL PRESENTATION*

# Current EUNIS Habitat preferences of Darevskia derjuguni and its possible fates under different future climate scenarios

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**Abstract**

The Caucasian Biodiversity Hotspot is one of the 36 majorly valuable region in the terrestrial world. Due to its climatological and geological uniqueness, species have adapted well to local dynamics and as a result of it, species richness level is remarkably high. Genus *Darevskia*, which is one of the largest vertebrate group in Western Palearctic, has been represented with a significant number of species in this hotspot. However, *Darevskia derjugini* (Derjugin's lizard) has been relatively less reported than other rock lizards in Anatolian Peninsula. Therefore, in order to better understand the ecological requirements and spatio-temporal dynamics of this species, the EUNIS Habitat preferences and the possible fates under multi-climatological scenarios have been extensively discussed in this study. The results indicated that, only 8 EUNIS Habitat types are suitable for population maintanence of the species and mixed forests are the most preffered habitats with 36.6% ratio. On the other hand, species distribution modeling analysis demonstrated that the distribution range of Derjugin's lizard covers the Eastern Black Sea with the strong interactions of 6 bioclimatic variables. The envelope of these variables was strongly composed of two important parameters (Precipitation of driest month (Bio 14) and Precipation seasonality (Bio 15)) with a rate of 92.3%. In order to speculate its distribution under different climatic scenarios for future, two diverse approaches were taken into account with sub-branches of greenhouse gases representative concentration pathways. The results showed that with the increase of greenhouse gases in atmosphere, the species would limit its distribution to a narrow space along Eastern Black Sea and it would loose more than 50% of its habitats. Additionally, alterations in land cover related to anthropogenic activities would also be a potential serious threat for its survival. In conclusion, this lizard could be an appropraite model species to monitor the health of this biodiversity hotspot and sustainable conservation actions could be taken.

**Keywords:** Derjugin's lizard, The Caucasian Biodiversity Hotspot, Bioclimatology, Land cover, Greenhouse gases

## *ORAL PRESENTATION*

# Synthesis and Characterization of Benzimidazolium Salts Bearing Triflouromethylthioether Group

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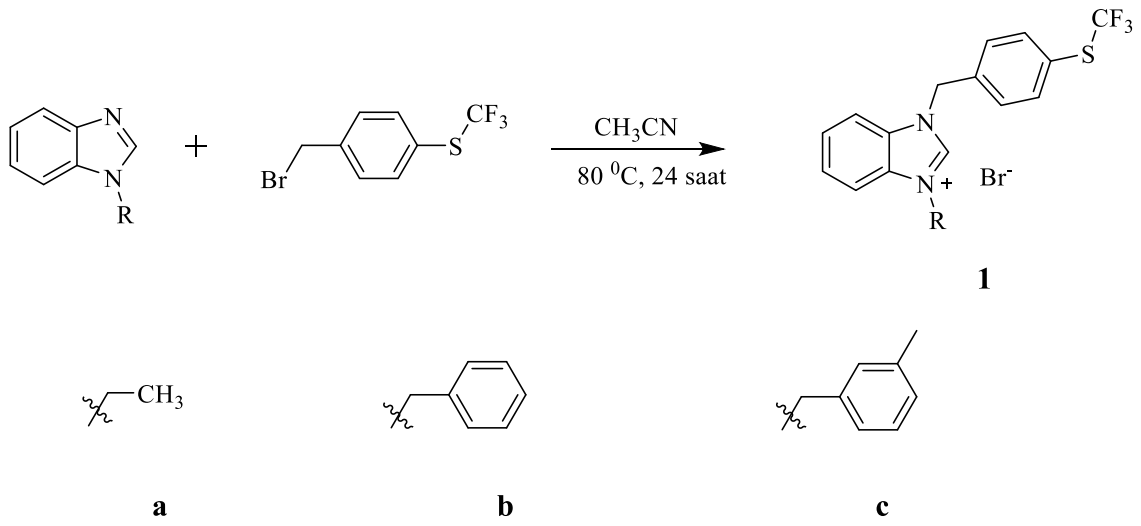
**Abstract**

Stable metal complexes of N-heterocyclic carbenes (NHCs) were independently reported by Wanzlick [1] and Öfele [2] in 1968. NHC ligands, however, were believed to be unstable intermediates at the time [3]. This state persisted until Arduengo et al synthesized stable and isolable NHC in 1991 [4]. Numerous investigations of NHC ligands and their metal complexes have been reported in the realms of organic and organometallic chemistry since this important discovery [5]. In medical chemistry, material sciences, and agrochemistry, fluorinated chemicals play a significant role [6]. For instance, fluorinated chemicals make up around 25% of the molecules in the pharmaceutical industry [7]. Fluorine or fluorinated groups are found in the majority of heteroaromatic substances. Modern Drug discovery techniques for the selective production of fluorinated heteroaromatic compounds are crucial [8].




**1**

**a** **b** **c**

Here, we synthesized the benzimidazolium salts bearing the trifluoromethylbenzyl group as organofluoride compounds, and we used elemental analysis, NMR, and FTIR spectroscopy to characterize them.

**Keywords:** Benzimidazolium salt, FTIR, NMR, Spectroscopy, Synthesis

[1] N. Marion, S. Díez-González, S.P. Nolan, Angew. Chem. Int. Ed. 2007, 46, 2988.
[2] D. Enders, O. Niemeier, A. Henseler, Chem. Rev. 2007, 107, 5606.
[3] W.A. Herrmann, T. Weskamp, V.P.W. Bohm, Adv Organomet. Chem. 2001, 48, 3.
[4] V. Nair, S. Bindu, V. Sreekumar, Angew. Chem. Int. Ed. 2004, 43, 5130.
[5] R.H. Crabtree, J Organomet Chem. 2006, 691, 3146.
[6] P. Kirsch, Modern Fluoroorganic Chemistry: Synthesis Reactivity, Applications, 2nd, Completely Revised and Enlarged Edition; Wiley-VCH, Weinheim, 2013.
[7] Y. Zhou, J. Wang, Z. Gu, S. Wang, W. Zhu, J.L. Acena, V. A. Soloshonok, K. Izawa, H. Liu, Chem. Rev. 2016, 116, 422.
[8] I.S. Kondratov, N.A. Tolmachova, G. Haufe, Eur. J. Org. Chem. 2018, 2018 3618.

## *ORAL PRESENTATION*

# Unraveling the Electron-Donating Ability in Metal-Free Triarylamine-based D-π-A Architecture for Dye-Sensitized Solar Cells (DSSCs): A Detailed DFT/TD-DFT Investigation

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**Abstract**

In this study, computationally designed D-π-A type photosensitizers bearing divergent substituents on their donor units were investigated to enlighten their potential applicability in dye-sensitized solar cell (DSSC) applications. For this aim, a comprehensive library of forty dye molecules was generated by referring to a well-known photosensitizer possessing a triphenylamine ($R^0$) functional group as its donor unit (**Figure 1**).

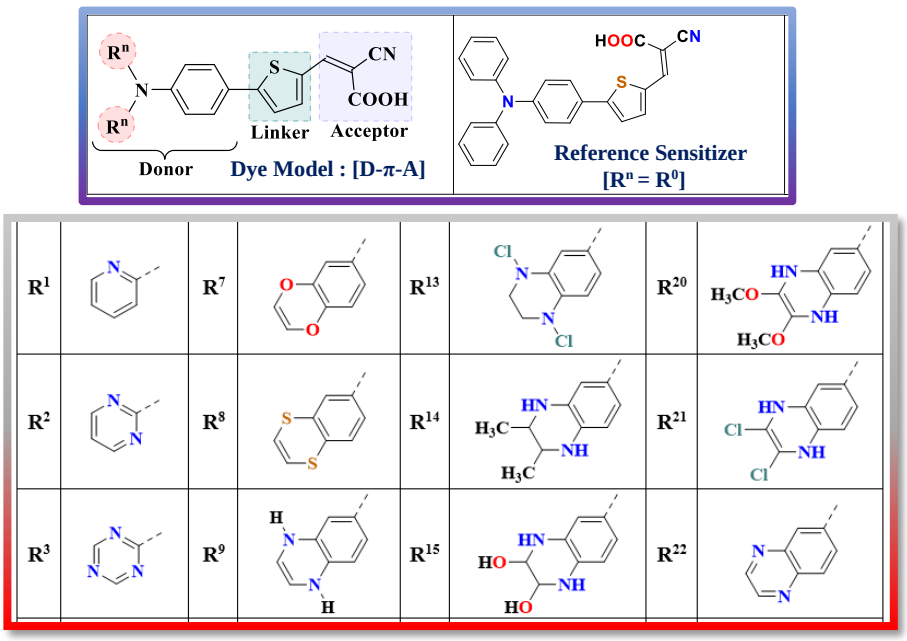
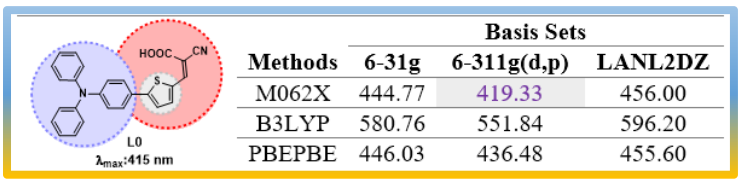



**Figure 1.** Representative scheme of investigated D-π-A type photosensitizers and a series of donor scaffolds.

Target dye molecules were rendered using GaussView 5.0.8, and the requisite computational studies were conducted using Gaussian 09 using a highly parametrized, empirical exchange-correlation density functional Minnesota (M06-2x) with 6-311G(d,p) basis set (**Figure 2**).




| Methods | Basis Sets | | |
|---|---|---|---|
| | 6-31g | 6-311g(d,p) | LANL2DZ |
| M062X | 444.77 | 419.33 | 456.00 |
| B3LYP | 580.76 | 551.84 | 596.20 |
| PBEPBE | 446.03 | 436.48 | 455.60 |

**Figure 2.** Density Functional Theory (DFT) method and basis set dependency of the $\lambda_{max}$ value of the reference dye.

In the concomitant step; global descriptors, $TiO_2$ adsorption, light-harvesting efficiency (LHE), energy conversion efficiency (η), short circuit current density (JSC), open circuit photovoltage ($V_{OC}$), fill factor (FF), and reorganization energy ($\lambda_e$, $\lambda_h$, $\lambda_T$) values were also subjected in detail. Our consecutive efforts have also contributed to DFT and subsequent TD-DFT computations for the D-π-A@$Ti_5O_{10}$ clusters to elucidate the interaction of the investigated photosensitizers with the semiconductor layer ($TiO_2$).

**Keywords:** Triphenylamine, Donor-pi-acceptor, DSSC, DFT, TD-DFT.

## *ORAL PRESENTATION*

# Molecular Identification and Phylogenetic Analysis of the Genus Scaligeria L. from Turkey Based on nrDNA-ITS, trnl-F and trnLintron

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**Abstract**

Globally, the genus *Scaligeria* DC. sensu stricto is represented by about 5 species. The infrageneric classification of *Scaligeria* has been problematic since the early 18th century, and many scientists have conducted taxonomic studies on the genus. *Scaligeria lazica* Boiss., *S. tripartita* (Kalenicz) Tamamsch., *S. meifolia* Boiss., *S. glaucescens* Boiss, *S. hermonis* Post. are nowadays treated synonymous with *Pimpinella L.* (*P. lazica* (Boiss.) M.Hiroe, *P. tripartita* Kalenicz), *Elaeosticta* Fenzl (*E. meifolia* Fenzl.) and *Bunium* L. (*B. hermonis* (Post) Kljuykov.). The genus *Scaligeria* is now represented by single species, *Scaligeria napiformis* Grande., in Turkey.

Total DNA was extracted from leaf tissue by modified CTAB method. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA has been amplified using ITS 4/ITS 5a universal primers. trnL(UAA)-trnF(GAA) and trnL intron regions have been amplified using trnc/f and trnc/trnd universal primers, respectively. Phylogenetic analyses were performed using PAUP 4.0 and MrBayes.

In order to evaluate the monophyly of *Scaligeria* DC., and clarify the species relationships within the genus, sequences of ITS and trnLintron of *Scaligeria* and related genera were studied. ITS region of *Scaligeria* is ranged from 670-680 bp, trnL-F region 900-920 bp and trnL intron is ranged from 430-440 bp. Our phylogenetic analyses revealed that the genus *Scaligeria* sensu lato is not monophyletic. The two species of *Scaligeria* (*S. lazica* Boiss.and *S. tripartita* are nested inside '*Pimpinella* clade' and ***S. meifolia*** and *S. glaucescens* are also nested inside '*Elaeosticta* clade. *S. hermonis* is placed relative to the *Bunium* group. All representative species of *Scaligeria* (*S. napiformis*, *S. halophila* Rech.f, *S. alziarii* Hand, Hadjik. & Zetzsche, *S. moreana* Engstrand) supported monophyletic group (99% bootstrap). In conclusion, our results indicate that molecular study of *Scaligeria* sensu lato could be very useful approach in solving the classification long-standing problems

**Keywords:** Apiceae, DNA, *Scaligeria*, Turkey, phylogeny

## *ORAL PRESENTATION*

# Synthesis and Characterization and Thermal Properties of N-(2,4-dimethyl-pyridine-1-carbonothioyl)-3-nitrobenzamide

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**Abstract**

Thioureas are important compounds in coordination chemistry due to their versatile ability to act as ligands. It has the ability to coordinate various metal center atoms [1]. In addition, thioureas containing sulfur (S) and amine (NH and/or $NH_2$) functional groups in their structure can also bind to the central atom and anions in coordination compounds [2]. Thiourea group compounds have biological and medical uses. The fact that these compounds are biologically active and show stable behavior in physiological conditions attracts the attention of many researchers [3-4]. It has been stated in the literature that the antibacterial properties of these compounds, which have been shown to be active against bacteria such as S. aureus, E. coli, B. subtilis and P. aeruginosa, are due to the sulfur (S) atom in their structure [5-6]. In addition, thioureas show antifungal, antiviral, anti-tumor, antimalarial, anti-tubercular, insecticidal, herbicidal, rodenticidal, anti-helminthic and anti-inflammatory effects [7-8]. Benzoylthioureas are well known among the thiourea groups. It is available in the literature that benzoyl thiourea derivatives and their metal complexes have antibacterial, antifungal, antitumor, antiviral, antithyroid, and antioxidant properties [9]. In this study, N-(2,4-dimethyl-pyridine-1-carbonothioyl)-3-nitrobenzamide was synthesized. Characterization studies were performed with UV-vis and FT-IR spectroscopy measurements. Thermal analysis was performed with the TG/DTG/DTA combined system.

**Keywords:** Benzoylthiourea, Aroylthiouurea, 3-nitro benzoyl chloride, 2,4-dimethyl pyridine.

**References:**
1. Obrzud, M.; Rospenk, M.; Koll, A. Self-Association of N,N0 - Dialkylthiourea Derivatives in Non-Polar Solvents. J. Mol. Struct. 2012, 1018, 54–63. DOI: 10.1016/j.molstruc.2012.01.042.
2. Bregovic, V. B.; Basaric, N.; Mlinaric-Majerski, K. Anion Binding with Urea and Thiourea Derivatives. Coord. Chem. Rev. 2015, 295, 80–124. DOI: 10.1016/j.ccr.2015.03.011.
3. Mukiza, J., Gerber, T. I., Hosten, E. C., & Betz, R. (2015). Crystal structure of bis (μ2-S-(Z)-3-(hydroxido (phenyl) methylene)-1, 1-diphenyl thioureaa-κO, S, S) hexacarbonyl dirhenium (I), C60H52N4O8Re2S2. Zeitschrift für Kristallographie-New Crystal Structures, 230(1), 23-26.
4. Steppeler, F.; Iwan, D.; Wojaczynska, E.; Wojaczy nski, J. Chiral Thioureas—Preparation and Significance in Asymmetric Synthesis and Medicinal Chemistry. Molecules 2020, 25, 401. DOI: 10.3390/molecules25020401.
5. Kumaran, K.; Kamil, S. R. M.; Jaisankar, K. Synthesis, Spectral Characterization and Antimicrobial Studies of New 3-and 4- substituted in 7-Aza Indole Derivatives. Int. J. PharmTech Res. 2012, 4, 169–175.
6. Devineni, S. R., Golla, M., Chamarthi, N. R., Meriga, B., Saddala, M. S., & Asupathri, U. R. (2016). 2-Amino-2, 3-dihydro-1 H-2λ 5-[1, 3, 2] diazaphospholo [4, 5-b] pyridin-2-one-based urea and thiourea derivatives: synthesis, molecular docking study and evaluation of anti-inflammatory and antimicrobial activities. *Medicinal Chemistry Research*, *25*(4), 751-768.
7. Claude, A.; Devi, K. N.; Karthick, S.; Sevvanthi, P.; Sudha, K.; Saraswathi, M.; Balakrishnan, K. Growth and characterization of novel thiourea based NTS, MTS And NMTS crystals. Int. J. ChemTech Res. 2013, 5, 512–521.
8. Emen, F. M., Kutlu, E., Karacolak, A. I., Ali, M. A., Demirdogen, R. E., Yesilkaynak, T., Ayaz, F. (2022). Novel benzoylthiourea derivatives had differential anti-inflammatory photodynamic therapy potentials on in vitro stimulated mammalian macrophages. Photodiagnosis and Photodynamic Therapy, 37, 102685.
9. Yeşilkaynak, T., Özkömeç, F. N., Çeşme, M., Demirdöğen, R. E., Kutlu, E., Kutlu, H. M., & Emen, F. M. (2022). Synthesis of new thiourea derivatives and metal complexes: Thermal behavior, biological evaluation, in silico ADMET profiling and molecular docking studies. Journal of Molecular Structure, 1269, 133758.

## *ORAL PRESENTATION*

# Makine öğrenmesi ile balık tazelik seviyesinin tahmini

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**Özet**

Makine öğrenimi, yapılan bilgi girişlerini işleyerek onları kullanıcıya yararlı bilgilere dönüştürebilen ve bunları tahmin yapmak için kullanabilen çeşitli algoritmalardan oluşan yapay zekâlardır. Gelişen teknoloji ile makine öğrenimi birçok endüstride anlamlı değişiklikler başlatmış ve gösterdiği yüksek performans ile önemli sorunların ortadan kaldırılmasını sağlamıştır. Makine öğreniminin en büyük avantajı büyük miktarda veriyi hızla işlemek ve zamandan tasarruf sağlayan bir kontrol noktasına sahip olmaktır. Gıda kalitesinin belirlenmesinde ise makine öğrenmesi gıdaya temas etmeden ve onu tahrip etmeden uygulanabilecek önemli bir yöntem olabilir. Bu çalışmada çipura, levrek ve alabalık 7 gün buzdolabında (3±1C°) buz içerisinde depolanmış ve her depolama gününde resimleri kaydedilmiştir. Çalışmada, görüntülerden elde edilen CNN (Convolutional neural networks) özelliklerinin kullanıldığı görüntüleri sınıflandırmak için bir destek vektör makinesi olan ve SVM (support vector machine) olarak bilinen algoritma kullanılmıştır. CNN'nin ilk katmanı, görüntülerin giriş boyutlarını belirtmiştir. Doğru sınıflandırma oranları %90,6 ile %100 arasında değişmiş ve veri artışının artması ile doğruluk oranında beklenen artışın oluştuğu gözlenmiştir. Alabalık, çipura ve levrek için ortalama doğru sınıflandırma oranları %80 üzerinde elde edilmiştir. Çalışma sonuçları makine öğrenmesi ile balığa ait taze-bayat ayrımının yüksek doğruluk oranında belirlenebileceğini ve makine öğrenmesinin hızlı kalite belirleme metodu olarak kullanılabileceğini göstermiştir.

**Anahtar Kelimeler:** balık kalite seviyesi, derin öğrenme, makine öğrenmesi

## *ORAL PRESENTATION*

# Synthesis and Characterization of Ag(I)NHC Complexes Bearing Triflouromethylthioether Group

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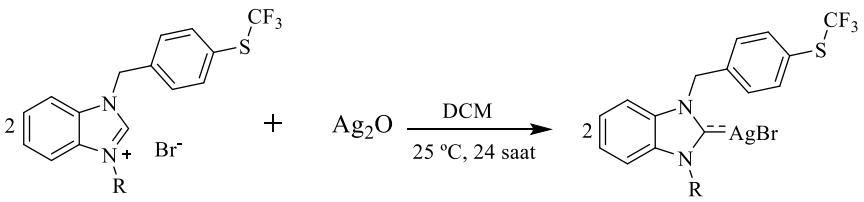
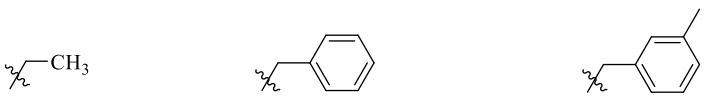
**Abstract**

The organic ligands play a significant role in biological activity by coordinating with the silver metal core. N-heterocyclic carbenes (NHCs) rank among the most significant organic ligands among them. The applications of transition metal complexes containing NHC ligands (M-NHC) are numerous. Applications of Ag-NHC complexes made from these substances for biological activity have attracted interest. Additionally, investigations on the use of Ag-NHC complexes for biological activity have only lately begun [1], and their number is growing daily. Long ago, silver's potential uses in medicine were identified [2]. Silver nitrate and silver sulfonamides are the most widely used silver compounds [1]. Nowadays, it is recognized that the majority of organisms become resistant to antibiotics. Therefore, scientists have worked to create new, more powerful, and multimodal substitutes that have the least negative effects of antibiotics on the human body [3]. Silver has been demonstrated to have widespread activity against antibiotics, even those resistant to lethal bacteria, at the clinical stage [4]. Recent research has shown that silver complexes are less hazardous to humans than other metal complexes, making them suitable antibacterial agents [5].




**1**




**a** **b** **c**

Herein, we synthesized the Ag(I)NHC complexes bearing the trifluoromethylbenzyl group, and we used elemental analysis, NMR, and FTIR spectroscopy to characterize them.

**Keywords:** FTIR, N-heterocyclic carbene, NMR, Silver, Spectroscopy, Synthesis

**Acknowledgment**
This study was financially supported by Inonu University Research Fund. Project Code: FBG-2022-2919.

[1] Melaiye, A.; Simons, R.S.; Milsted, A.; Pingitore, F.; Wesdemiotis, C.; Tessier C.A.; Youngs. W.J. *J. Coord. Chem.* **2014**, *67*, 3649-3663
[2] Habib, A.; Iqbal, M.A.; Bhatti, H.N.; Kamal, A.; Kamal, S. *Inorg. Chem.* **2020**, *111*, 107670.
[3] Haque, R.A.; Iqbal, M.A.; Mohamad, F.; Razali, M.R. *J. Mol. Struct.* **2018**, *1155*, 362-370.
[4] Alisir, S.H.; Sariboga, B.; Caglar, S.; Buyukgungor, O. *J. Mol. Struct.* **2017**, *1130*, 156-164.
[5] Alisir, S.H.; Sariboga, B.; Caglar, S.; Buyukgungor, O. *J. Mol. Struct.* **2017**, *1130*, 156-164.

## *ORAL PRESENTATION*

# The effects of different salt concentrations on the germination rate of *Origanum onites* L. (İzmir thyme) plant.

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**Abstract**

Introduction*: Origanum* is an important genus belonging to the Lamiaceae family and its species are known as thyme. The taxa belonging to the *Origanum* genus, medically and economically important, have an extremely high essential oil content. *Origanum onites* L. is the most developed and exported thyme species in Türkiye. Salinity is one of the important factors affecting plants negatively. Plants exposed to salinity become unable to fully perform their functions. At the same time, salinity is a limiting factor for the yield and development of plants. In this study, it was aimed to determine the effects of different salt concentrations on properties such as germination and emergence rate of *O. onites* (İzmir thyme) seeds, which are economically important.
Materıals and methods: Seeds of *O onites* were obtained from Buldan district of Denizli province. Different salt concentrations of 0, 25, 50, 75, 100, 125, 150, 175 and 200 mM NaCl were applied. The study was carried out in Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Field Crops laboratory in 2022. Germination and emergence rate studies were planned according to the randomized plot design with 4 replications.
Results: Germination rates of İzmir thyme vary between 21% and 77% at different salt concentrations. Germination rate decreased as a result of increasing salt doses. The highest germination rate in the species was determined at 25 mM (67%) except for the control dose. The lowest germination rate was determined at 75 mM salt dose (21%). There was no germination at salt doses of 100 mM and above.
Conclusıon: According to the results of the research, the germination and emergence rate examined at different salt concentrations were found to be significant at the level of 1%. It has been determined that *O onites* are low tolerant to salinity.

**Keywords:** *Origanum onites*, salt, germination rate, medicinal plant

## *ORAL PRESENTATION*

# Antioxidant Properties of *Aronia sp* : A Review

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**Abstract**

*Aronia* genus (Rosaceae family) are a perennial herb that grows in North America. In the form of a bush, it can grow up to 80-140 cm and has purple-black fruits about 6 mm in diameter. In recent years, great attention has been paid to medicinal plants rich in polyphenols with antioxidant and antimicrobial properties. Aronia is one of the most preferred fruits today due to its high antioxidant activity and rich polyphenol content. Aronia berries take part in the removal of free radicals entering the body due to unhealthy diet and environmental conditions, preventing damage to cells and helping cells regenerate. In addition to its high antioxidant content, aronia also contains vitamin C, PP, B, vitamin E and high amounts of anthocyanins. At the same time, it is known that aronia fruit, which contains potassium, magnesium, calcium, manganese and high fiber, stands out in the treatment of certain diseases. Aronia normalizes cholesterol and blood sugar, reduces the risk of atherosclerosis, diabetes, heart attack and stroke, lowers blood pressure, strengthens the immune system and relaxes the nervous system. It is one of the most effective fruits in the treatment of diabetes, cardiovascular diseases, oxidative stress and cancer-related chronic diseases, as it contains very high amounts of various antioxidants. t is emphasized that aronia is a pharmacologically rich source. Polyphenols, especially anthocyanins and procyanidins, form the main group of bioactive components in aronia berries and provide most of the antioxidant properties of the plant. Anthocyanins, which are powerful antioxidants in the content of aronia fruit, are an excellent source for slowing down age-related brain functions and memory deterioration. Aronia berries are used in the production of functional (especially antioxidant-rich) fruit juices and teas with their high anthocyanin content. In this review, the antioxidant properties of aronia are presented.

**Keywords:** antioxidant activity, anthocyanin, health, medicinal plant, nutrition, polyphenol

## *ORAL PRESENTATION*

# Muğla İlinde Yayılış Gösteren *Apis mellifera anatoliaca* Maa, 1953 (Anadolu Bal Arısı)'nın Muğla Ekotipinin Genetik Çeşitliliğinin ISSR Belirteçleri Kullanılarak Belirlenmesi

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**Özet**

Bal arıları Apidae familyası, *Apis* cinsi içerisinde yer alan tarımsal, ekonomik ve ekolojik olarak en önemli böcek türlerinden biridir. Türkiye'nin farklı coğrafik alanlarına adapte olmuş birçok ekotipe sahiptir. Bu ekotiplerden biri Muğla ekotipi *Apis mellifera anatoliaca*'dır. Bu ekotip; üretim dönemini çam ağaçlarının çiçek açma zamanına göre ayarlaması ve Muğla bölgesinin iklim şartlarına daha iyi uyum göstermesinden dolayı diğerlerinden ayrılmıştır.
Fakat *A. m. anatoliaca*'nın Muğla ekotipinin genetik çeşitliliğini ortaya koymaya yönelik çok az sayıda çalışma bulunmaktadır. Biz bu amaçla *Apis mellifera anatoliaca* alttürünün, Muğla ekotipinin ISSR moleküler markör tekniği kullanılarak populasyon içi ve populasyonlar arası genetik benzerlik ve farklılıklarını inceledik.
Bu doğrultuda, Muğla'nın 13 ilçesinde toplamda 10 farklı popülasyonundan toplanan 130 bireyin, popülasyon içi ve popülasyonlar arası genetik çeşitliliği, 10 adet polimorfik ISSR belirteci kullanılarak incelenmiştir. Yapılan analizler sonucunda toplam 425 lokus saptanmış ve primer başına ortalama 42 polimorfik lokus tespit edilmiştir. Popülasyonların polimorfik lokus oranı (P%) %25,18 ile %50,12 arasında değişmektedir.
Bu çalışma sonucunda, tüm popülasyonlar için gözlenen allel sayısı (Na) 1,99±0,04, etkili allel sayısı ortalama (Ne) 1,41±0,35 olarak bulunmuştur. Nei'nin genetik çeşitliliği (H) 0,25±0,18 ve shannon sabiti (I) 0,38±0,24 olarak bulunmuştur. Ayrıca toplam genetik çeşitlilik (Ht) 0,25±0,03, popülasyon içi genetik çeşitlilik (Hs) 0,12±0,01, genetik farklılaşma katsayısı 0,504, gen akış değeri (Nm) 0,49 olarak bulunmuştur.
Analizler sonucunda popülasyonlarda genetik olarak birbirine en yakın olan lokasyonlar Marmaris ve Datça, birbirine en uzak lokasyonlar ise Yatağan ve Marmaris olarak tespit edilmiştir. Ayrıca Moleküler Varyans Analizi (AMOVA) sonucu varyasyonun %51 oranında popülasyon içi ve %49 oranında popülasyonlar arasından kaynaklandığı gözlenmiştir.

**Anahtar kelimeler:** ISSR, genetik çeşitlilik, bal arısı, popülasyon, ekotip

## *ORAL PRESENTATION*

# Model Organizma Olarak Zebra Balıklarında Lipopolisakkarit Uygulanmasının Beyin Dokusunda *lrrk2, dj-1 ve tnf-α* Gen İfadeleri Üzerine Etkilerinin İncelenmesi

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**Özet**

Parkinson hastalığı (PH) dünya üzerinde en çok görülen nörodejeneratif hastalıklardan biridir. Substansiya nigranın kompact parçasındaki dopaminerjik nöronların azalması ile ortaya çıkar. PH gelişiminde etkili başlıca faktörler genetik, yaşlılık ve çevresel tetikleyiciler olarak düşünülmektedir. Araştırmalarda mitokondriyal hasar, oksidatif stres, nöroinflamasyon, protein birikmesi ve otofajik yolaklardaki bozukluklar bildirilen nedenlerdendir. Lrrk2 ve dj-1 dahil olmak üzere bazı genlerin PH gelişimi ile ilişkili olduğu gösterilmiştir. Zebra balıkları son yıllarda kullanımı yaygınlaşan model organizmalardır. Genom yapısının insanlarınkine benzerliği, bakımının kolay ve düşük maliyetli olması ve hızlı çoğalabilmesi gibi nedenlerden dolayı çalışmalarda son yıllarda tercih edilmektedir. Lipopolisakkarit (LPS), gram negatif bakterilerin hücre zarlarında bulunur ve endotoksin bir moleküldür. LPS, insan bağırsaklarında yaşayan bazı bakteriler tarafından da oluşturulmaktadır. Bağırsak epitel duvarının geçirgenliğinin artması ile LPS bu duvarı aşarak kan yoluyla ya da vagus siniri üzerinden beyin dokusunda nöroinflamasyon oluşmasına neden olabilmektedir. Çalışmamızın amacı zebra balıklarına LPS uygulanmasının beyin dokusunda *lrrk2, dj-1 ve tnf-α* genlerinin ifadeleri üzerindeki etkilerinin incelenmesidir. LPS, dört hafta boyunca haftada bir kez intraperitonel olarak 1 µg/gr ve 5 µg/gr dozlarında verilmiştir. İlk ve son hafta olacak şekilde LPS enjeksiyonundan iki gün sonra beyin dokuları alınmış ve RT-PCR için hazırlanmıştır. Yapılan RT-PCR çalışması sonucunda dört hafta boyunca 5 µg/gr LPS verilen grupta kontrol grubuna göre *lrrk2 ve dj-1*, tnf-α genlerinin ifadelerinde anlamlı artışlar gözlemlenmiştir.

**Anahtar Kelimeler:** Parkinson Hastalığı, Lipopolisakkarit, Nöroinflamasyon, Zebra balığı

## *ORAL PRESENTATION*

# Volatile compositions of *Tilia platyphyllos* Scop. infusions by headspace-solid-phase microextraction (HS-SPME), antioxidant activity

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**Abstract**

The genus *Tilia* (Tiliaceae) represent 45 species, of which six species are European. *Tilia* sp. (Tiliaceae) are simple, cordate or deciduous trees with long, silicate shaped, 5-valve fruits and fragrant flowers. It is used as a medicinal tea in traditional medicine for colds, coughs, and hypertension and as an antioxidant (Mabberley, 1997).
Within the scope of this project, *Tilia platyphyllos* Scop. in the culture form from Eskişehir was obtained, and infusions were prepared at different times (including 5 min., 10 min., 15 min. and 30 min.). Volatile compounds of the infusion extracts were analyzed by headspace-solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). *(E)-β*-Ocimene and limonene were determined as major components for infusion extracts (15.7-45.9%) and (11.7-33.4%), respectively. After then, the infusion extracts were lyophilized, and the antioxidant activity of the infusion extracts were performed by DPPH· radical scavenging effect. It was determined that inhibition percentages were relatively high in the concentration range of 10-0.02 mg/mL (5.60-72.45%).
To our knowledge, the infusions' volatile compounds and biological activity were evaluated for the first time using the solid phase microextraction (SPME) method.

**Acknowledgment**
This study was financially supported as a Scientific Research Project (BAP 1905S077) by Anadolu University.

**Keywords:** *Tilia platyphyllos* Scop., infusion, antioxidant activity.

**Reference:**
Mabberley, D.J. 1997. The Plant–Book. Cambridge University Press, Cambridge.

## *ORAL PRESENTATION*

# A Non-Atypical Case of Ductal Hyperplasia and Mastitis in the Dog Mammary Gland

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**Abstract**

A mass of 10x17 cm in diameter was surgically removed from the abdominal second mammary lobe of a 3-year-old, Kangal crossbreed, female dog. The mass was brought to Kastamonu University, Faculty of Veterinary Medicine, Department of Pathology. Macroscopically, numerous nodular formations in yellowish-white color were observed in the mass. The tissue was fixed in 10% formaldehyde solution for histopathological examinations and then paraffin was blocked after the routine tissue follow-up procedure. Sections from paraffin tissues were taken on a 5 µm thick slide in a microtome and stained with Hematoxylin-eosin. Histopathologically, hyperplasia in ductus cuboidal epithelial cells, vacuole formations in the cytoplasm of these cells, and an increase in fibrous tissue were detected. Dilatation and intraluminal secretion were determined in the ducts. In addition, neutrophil infiltration and plasma cells with eosinophilic cytoplasm were seen. As a result, a diagnosis of non-atypical ductal hyperplasia and mastitis was done.

**Keywords:** Dog, Histopathology, Hyperplasia, Mastitis, Mammary Gland

## *ORAL PRESENTATION*

# Dendrimerik Fosfazen Bileşikleri

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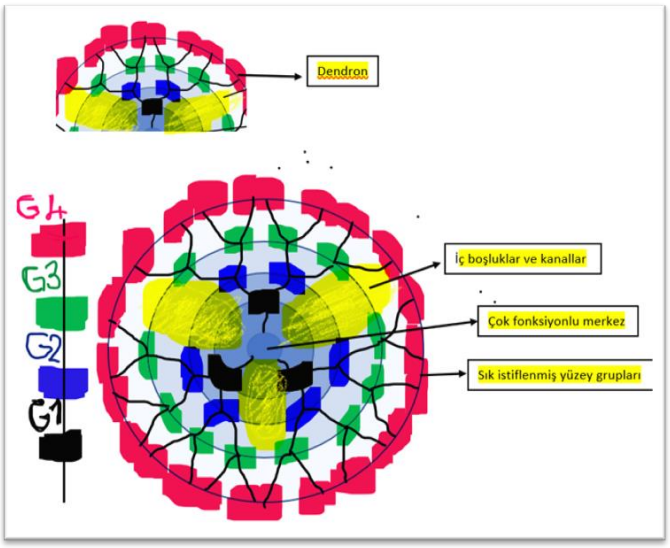
**Özet**

Dendrimerler, düz zincirli polimerlere göre daha iyi tanımlanmış bir boyuta, şekle ve moleküler ağırlığa sahip çok dallı moleküllerdir. Kimyasal olarak aktif uçları olan bir çekirdeğin (bir atom, molekül ya da makromolekül), çekirdeği olmayan dallanmış fonksiyonel gruplarla (dendron) art arda reaksiyonu sonucu elde edilen simetrik ve küresel yapıya sahip polimerlerdir (Şekil 1).
Çizgisel polimerler çözelti içinde silindirik bir yapıya sahipken, dendrimerler içi dolu küreler halindedir ve bu nedenle farklı davranışlar gösterirler. Dendrimerlerin küresel yüzeylerinde çok sayıda aktif grubun bulunması çözünürlüğü, moleküller arası etkileşimi ve kimyasal reaksiyonlara ilgiyi artırır (Hawker, 1990).
Halkalı fosfazenler, kimyasal olarak aktif fosfor-halojen bağı içeren, çok fonksiyonlu anorganik bileşiklerdir. Bu özellikleri nedeniyle dendrimerlerin sentezi için uygun bir çekirdek yapıya sahiptirler. Halkalı fosfazenlerin, dendrimerlerin uçlarında fonksiyonlu grup sayısını arttırmak için kullanıldığı ilk çalışmalar 1990'lı yıllarında gerçekleştirilmiştir (Sournies, 1995; Labarre, 1996). Günümüzde fosfazen dendrimerlerinin, anti-kanser ajan olarak davranışları, antiproliferatif aktiviteleri hakkında yapılan araştırmalar oldukça dikkat çekici sonuçlar sunmaktadır.




**Şekil 1.** Dendron ve dendrimerin şematik gösterimi

Kaynaklar

1. Hawker CJ, Frechet JMJ, Preparation of polymers with controlled molecular architecture. A new convergent approach to dendritic macromolecules. J. Am. Chem. Soc. 1990, 112, 21, 7638–7647
2. Labarre JF, Sournies F, Crasnier F, Labarre MC, Vidal C, Faucher JP and Graffeuil M, Phosphorus, Sulfur Silicon Relat. Elem., 1996, 110, 525–528.
3. Sournies F, Crasnier F, Graffeuil M, Faucher JP, Lahana R, Labarre MC and Labarre JF, Angew. Chem., Int. Ed. Engl., 1995, 34, 578–581.

**Anahtar Kelimeler:** Dendrimer, Halkalı fosfazen, polimer

## *ORAL PRESENTATION*

# Process simulation and design of membrane-based gas separation system

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**Abstract**

Membranes processes are one of the important separation processes used for the separation of liquid and gas phase mixtures. Membrane processes offer significant advantages such as environmental friendliness, lower cost and lower energy consumption compared to other separation applications. Advancement in membrane technology, and membrane process applications in different industrial sectors are increasing rapidly. Today, the solvent recovery from waste gas mixtures containing organic solvents in industrial plants is of great importance due to both the economic and environmental impacts. Polymer membranes have been widely used for the separation of gas mixtures. Although the type of membrane to be used for this purpose varies depending on the process requirements and separation method, hollow fiber type membranes are preferred to others among the various membrane module configurations. At the same time, the membrane material used has an important role on separation performance. In this study, polydimethylsiloxane (PDMS) membrane was preferred for the separation of toluene/air mixture due to its selectivity to volatile organic compounds and its advantageous properties for vapor/gas separation process. Process simulation of the separation of toluene/air gas mixture with membrane unit was carried out using the CHEMCAD software package program in order to separate the toluene from the waste gas mixture with lower energy consumption, higher efficiency and higher purity. The flow regime in membrane module was countercurrent flow, and membrane units were run based on the pressure difference. The separation performances of the existing conventional system and the membrane-based system were compared.

**Keywords:** Membrane, vapor/gas separation, toluene recovery, process design, simulation.

## *ORAL PRESENTATION*

# The effect of additives on mechanical behavior of a composite material

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**Abstract**

The composite was prepared using Alfa fibers a s a reinforcement of the unsaturated polyester matrix and marble as an additive, the aim of this study is to improve the mechanical properties of this composite. Vegetable fibers are characterized by high physical and chemical properties that are abundant in nature light weight biodegradability and low cost these properties made vegetable fibers competitive with synthetic fibers in the preparation of composites, to improve the surface of the allied fibers we treated them with benzoyl, permanganate 0.2 %, alkali treated 9 % for 24 h and placed in the microwave for 10 min. The results obtained from the tests conducted on the composite and fiber showed that the treatment with NaOH at a concentration of 9 % for 24 h had an effect in improving themechanical properties of the composite and the fibers compared to the untreated fibers Other treatments also proved that the added marble had a significant effect in improving themechanical behavior. Among the diagnostic tests used in this study are infrared spectroscopy,tensile test.

**Keywords:** unsaturated polyester, composite, Stipa Tenacissima , Tensile strength, adhesion

## *ORAL PRESENTATION*

# Antifungal activity of essential oils and aqueous extracts of *Rosmarinus officinalis, Origanum compactum and Origanum majorana* on mycelial growth of 5 economically important *phytopathogenic fungi*

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**Abstract**

Various attempts have been made to find an alternative to synthetic fungicides currently used against *Botrytis cinerea, Fusarium oxysporum f. sp. lycopersici, Fusarium solani, Rhizoctonia solani and Rhizopus*, which are important plant diseases that causes significantly yield losses worldwide. The present study aimed to investigate the *in vitro* antifungal effects of aqueous extracts and essential oils of *Rosmarinus officinalis, Origanum compactum, and Origanum majorana* collected from Morocco on the mycelial growth of the five phytopathogenic fungi.

The essential oils were extracted by hydrodistillation using Clevenger apparatus and the aqueous extracts were made by infusion. The antifungal activity against five economically important plant pathogenic fungi was studied *in vitro* using the poisoned food method.

The chemical analysis of the EOs was analyzed using GC-MS. Biochemicals studies were performed for the detection of phytochemicals compounds responsible for the activity of aqueous extracts.

The antifungal activity of essential oil extracts from *the 3 MAP differs* depending on the type of phytopathogenic fungus and the amount of essential oil used against each fungus. The essential oil of *O. compactum* and *O. majorana* reveals a strong effect on *all the fungi* with lowest values of $IC_{50}$ while a moderate effect was obtained with essential oils of *rosemary* with highest values of $IC_{50}$. However, the aqueous extracts show weak activities against all the tested strains. A total of 48 compounds were identified in the 3 EOs. Phytochemical screening revealed the presence of polyphenols and flavonoids, as well as the saponins and catechic tannins.

These preliminary results revealed that the plant extracts are potential and promising antifungal agents which could be used as biofungicide against *B. cinerea, FOL, F.S, R.S,* and *Rhizopus*. Further studies undertaking *in vivo* condition will contribute to confirm the antifungal activity of the three aromatic and medicinal plants.

**Key words:** *R. officinalis; O. compactum; O. majorana*; essential oil; antifungal activity.

## *ORAL PRESENTATION*

# *Echinops borae* ve *Echinops pungens* Türlerinin Antienzim ve Antioksidan Aktivitelerinin Belirlenmesi

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**Özet**

Bu çalışmada, *Echinops borae ve Echinops pungens* türlerinin antienzim ve antioksidan aktivitelerinin araştırmak amaçlanmıştır, *Echinops borae ve Echinops pungens* türlerinin ekstraksiyonları hekzan, kloroform methanol ve su olmak üzere dört farklı çözücü kullanılarak elde edilmiştir. Antioksidan kapasitesi DPPH radikali süpürücü aktivite tayini ve toplam fenolik içeriğin belirlenmesi testleri yapılarak belirlenmiştir. Antienzim aktiviteleri elastaz testi yöntemi kullanılarak araştırılmıştır. Çözücüler ekstraksiyon verimi bakımından değerlendirildiğinde suyun diğer çözücülerden daha etkili olduğu tespit edilmiştir. Bu sırayı methanol takip etmektedir. *Echinops borae ve Echinops pungens* türleri ekstraktlarının antienzim aktivitesi bakımından değerlendirildiğinde bitki ekstrelerinin elastaz üzerinde farklı inhibitör etkilerine sahip oldukları belirlenmiştir. Antioksidan aktivite tayini testleri neticesinde, fenolik madde miktarı en yüksek *Echinops borae'nin* su ile elde edilen ekstraktda gözlenirken, DPPH radikali süpürücü aktivite tayini sonuçlarında ise yine özellikle *Echinops borae'nin* su ile elde edilen ekstraktlarından ve *Echinops pungens* türünün methanollü ekstraktlarından yüksek verim elde edilmiştir. Elde edilen sonuçlar neticesinde de *Echinops borae ve Echinops pungens* türlerinin önemli derecede antioksidan ve antienzim aktiviteye sahip olduğu belirlenmiştir. bu çalışma Erciyes Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından FYL-2021-11067 kodlu proje ile desteklenmiştir.

**Anahtar Kelimeler:** Asteraceae**,** Echinops, Elastaz, Antioksidan, DPPH, fenolik içerik

## *ORAL PRESENTATION*

# Investigation of MOTS-c peptide levels in type 2 diabetes mellitus patients

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**Abstract**

Background: Diabetes is a chronic state of hyperglycemia that can result from inadequate insulin secretion from pancreatic beta cells, insensitivity to insulin in target tissues, or both. It is a metabolic disease whose prevalence is increasing in the world due to sedentary lifestyle and weight gain due to unhealthy diet. Energy homeostasis is the balance between energy intake and energy expenditure in living organisms. MOTS-c is mitochondrial-derived peptide encoded from 12S rRNA in mitochondrial DNA and named as mitochondrial open reading frame of twelve S rRNA- type c. MOTS-c is an important regulator of insulin sensitivity and energy balance. Also, this peptide may protective role of diabetes progression.
Objective: In this context, the aim of our study is to investigate the changes in the levels of MOTS-c peptide in diabetes progression.
Methods: For this purpose, individuals without a diagnosis of Type 2 diabetes and fasting blood glucose values <100 mg/dL were included in the control group, also individuals with fasting blood glucose values of 100-125 mg/dL were added in the prediabetes group, and individuals with fasting blood glucose values of ≥126 mg/dL were included in the type 2 diabetes group. In blood samples taken from patients; fasting blood glucose, HbA1c, total cholesterol, HDL, LDL and triglyceride levels were determined by autoanalyzer. Besides, MOTS-c peptide levels were measured by ELISA.
Results: We observed that glucose, HbA1c, LDL and triglyceride levels in both prediabetic group and diabetic group were significantly higher than compared to control group, however, there was no significant change in MOTS-c levels between the control group and the prediabet group. Also, MOTS-c levels in diabetic patients were significantly decreased compared to control group.
Conclusion: Our results indicate that MOTS-c were regulated by blood glucose. These peptides may have a protective effect on diabetes progression.

**Keywords:** Diabetes Mellitus, HbA1c, Energy homeostasis, MOTS-c

## *ORAL PRESENTATION*

# Synthesis of water-soluble tetra, octa substitute zinc(II) phthalocyanines and their ınvestigation of DNA ınteraction, cytotoxic and phototoxic properties

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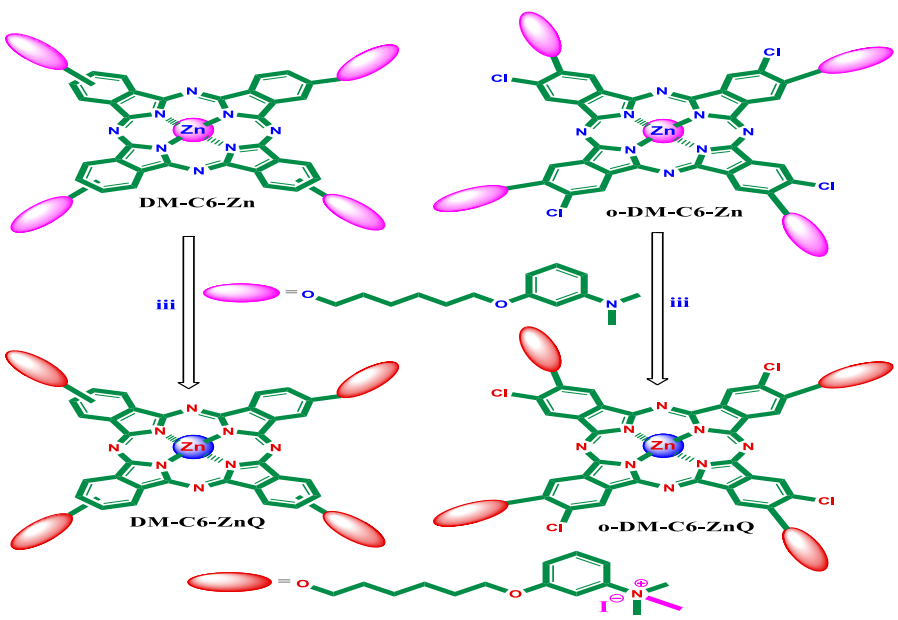
**Abstract**

Phthalocyanines are 16-membered planar molecules consisting of four isoindoline units with an 18-π electron system. Metallophthalocyanines (MPcs) have been extensively studied in many areas including in technology and medical application[1]. Today, cancer is one of the most serious health problems and the second most common cause of death. The number of people living beyond a cancer diagnosis reached nearly 14.5 million in 2014 and is expected to rise to almost 19 million by 2024. Phthalocyanines (Pcs) are the most of second-generation photosensitizers used in PDT[2]. Essential elements of PDT are a photosensitizer agent, light, and oxygen. It has become a very promising treatment method for cancer treatment because it causes little damage to healthy cells. The water soluble photosensitizers are important compounds in photodynamic efficacy in PDT[3]. In this study, new water soluble peripherally tetra and octa-({6-[3-(dimethylamino)phenoxy]hexyl}oxy) groups substituted zinc(II) phthalocyanines (DM-C6-ZnQ, o-DM-C6-ZnQ)was synthesized for the first time. Than we have investigated ct-DNA binding, supercoiled plasmid DNA cleavage properties of DM-C6-ZnQ, o-DM-C6-ZnQ by using spectrophotometric and electrophoretic methods. On the other hand, cytotoxic and phototoxic effects of DM-C6-ZnQ, o-DM-C6-ZnQ were examined using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on human lung adenocarcinoma (A549) cell lines.




**Keywords:** Water soluble, Synthesis, Phthalocyanine, Cytotoxicity, DNA interaction, Phototoxicity.

[1]. Azole Sindelo, Olawale L. Osifeko, Tebello Nyokong, Inorganica Chimica Acta, 476 (2018) 68-76
[2]. T. Keleş, B. Barut, A. Özel, Z. Bıyıklıoğlu, Bioorganic Chemistry 107 (2021) 104637
[3]. Z. Bıyıklıoğlu, T. Keleş, H. Şahin, Journal of Organometallic Chemistry 977 (2022) 122468

## *ORAL PRESENTATION*

# Synthesis and Characterization of Coordination Polymers with 5,5'-(butane-1,4-diylbis(oxy))diisophthalate and 1,4-Bis((2-ethyl-imidazol-1-yl)methyl)benzene Ligands

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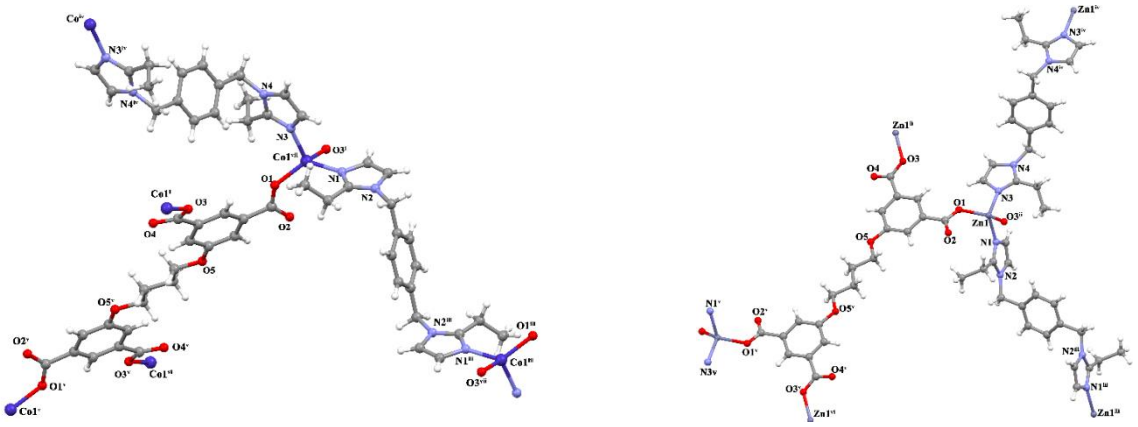
**Abstract**

Recently, multidimensional coordination polymers (CP) and metal-organic framework (MOF) have been used in applications such as magnetism, gas storage and separation, luminescence and catalysis because of their high surface area. In the synthesis of coordination polymers, polycarboxylic acids are generally preferred as anionic ligands and N-donor ligands are preferred as neutral ligands. It has already been established that factors, such as the type organic ligands, metal centres, pH values, reaction time, reaction temperatures and solvents have a profound influence on the final structure of these polymeric compounds. In this study, $[Co_2(\mu_4\text{-L1})(\mu\text{-pbetix})_2]_n$ (**1**) and $[Zn_2(\mu_4\text{-L1})(\mu\text{-pbetix})_2]_n$ (**2**) coordination polymers are synthesized with solvothermal method by using 5,5'-(butane-1,4-diylbis(oxy))diisophthalic acid ($H_4L1$), 1,4-Bis((2-ethyl-imidazol-1-yl)methyl)benzene (pbetix) and $M(NO_3)_2{\cdot}6H_2O$ (M = Co or Zn). The structures of the coordination polymers have been characterized by elemental analysis, IR spectroscopy, single-crystal X-ray diffraction (SCXRD), powder X-ray diffraction (PXRD) techniques. According to X-ray diffraction analyses results, the crystal structures of compounds **1** and **2** are isomorphous. They crystallise in the monoclinic space group $P2_1/c$, and the asymmetric unit contains one M(II) ion (M= Co(II) or Zn(II)), half L1 ligand and one pbetix ligand (Fig. 1a and 1b). The M(II) ion is four coordinated with distorted tetrahedral geometry by two N atoms (N1 and N3), two O atoms (O1 and $O3^i$) from two different L1 ligands. L1 ligands coordinate to M(II) ions to form a wavy two-dimensional layers (2D) and these 2D layers are connected by nitrogen atoms of pbetix as a bridging ligand to give three-dimensional (3D) coordination polymers. Furthermore, the thermal (TG/DTA) and luminescence properties of the complexes were determined. Optical band gaps were calculated with the Kubelka-Munk equation by measuring the UV spectra and reflectance of solid materials with diffuse reflectance.



**(a)** **(b)**

**Fig.1. (a)** Molecular structure of **1 (b)** Molecular structure of **2**

**Keywords :** Coordination Polymer, Substituted bis(imidazole) ligand, oxyisophthalic acid

## *ORAL PRESENTATION*

# Photocatalytic hydrogen evolution by Subphthalocyanine sensitized $TiO_2$

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**Abstract**

Phthalocyanines (Pcs) and their lower homologs subphthalocyanines (SubPcs) have been addressed as optimal candidates for photoinduced electron transfer processes after excitation with visible light (Bottari ve ark., 2021). Subphthalocyanines (SubPc) are aromatic macrocyclic structures containing a boron atom in the center. These molecules; It is used in photodynamic therapy, photovoltaic cells, antibacterial activity and photocatalytic $H_2$ production because it has 14 π-electron systems, molecules absorb light strongly in the visible region (500-700 nm), is chemically and thermally stable, and does not agglomerate unlike many aromatics. SubPc derivatives were used in dye-sensitive photocatalytic hydrogen production (DSSC). Here, in dye-sensitive photocatalytic hydrogen production, the dye molecule binds to the semiconductor ($TiO_2$) and absorbs light of the appropriate wavelength, excites the electron and initiates the hydrogen production mechanism. SubPcs have been investigated for increased activity and stability during the photocatalytic reaction. The photocatalytic activities of two different SubPc sensitized TiO2 photocatalysts were studied in the triethanolamine electron donor at mild condition (pH 7) in the deoxygenetad solution under solar light irradiation. These dye sensitized photocatalysts preserved photocatalytic activity during 8 hour of irradiation due to their 3D structures, which supply nonaggregated properties.

**Keywords:** Subphthalocyanine, photocatalytic, hydrogen evolution

**References**

Bottari, G., de la Torre, G., Guldi, D. M. ve Torres, T., 2021, An exciting twenty-year journey exploring porphyrinoid-based photo-and electro-active systems, *Coordination Chemistry Reviews*, 428, 213605.

## *ORAL PRESENTATION*

# Development of a new spectrophotometric method for the determination of L-ascorbic acid

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**Abstract**

Ascorbic acid (AA), with its chemical formula ($C_6H_8O_6$) or vitamin C, is a six-carbon compound structurally related to glucose. It consists of two convertible compounds. That is, L-ascorbic acid contains a reducing agent and its oxidized derivative, L-dehydroascorbic acid. Its chemical name is known as 2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol [1]. This vitamin is also known as an indirect antioxidant for the regeneration of active states of biologically important antioxidants such as vitamin E and glutathione [2]. While plants and some animals produce their own vitamin C, humans can not produce and must obtain vitamin C from external sources such as foods, particularly fruits and vegetables [3]. AA supports the body in forming connective tissue bone, blood vessel walls, teeth, and also the body in assimilating iron and amino acids [4]. Because of these properties, the determination of the amount of AA has gained increasing importance in various fields of analytical chemistry. In this study, a new spectrophotometric method was developed related to decreasing the color intensity of Fe(III) complex with salicylate derivative with the increasing concentration of the AA. The working principle of the developed method is based on the reduction of Fe(III) (complexed with salicylate derivative) to Fe(II). The optimum parameters of the method were investigated such as time, pH, and concentration of salicylate derivative compound. A wide linear concentration range of AA was obtained to form the calibration equation. In addition, the interference effect of common metal ions and some species were investigated for the developed method.
**Keywords:** Spectrophotometry, Vitamin C, Ascorbic acid, Fe(III)- salicylate derivative complex

**References**

[1]. Moser, U. K. (1990, October).Physiology and metabolism of ascorbic acid. In *Ascorbic Acid in Domestic Animals: Proceedings of the Second Symposium. Basel< 7LOC* (pp. 3-16).
[2]. Jacob, R. A. (1995). The integrated antioxidant system. *Nutrition research*, *15*(5), 755-766.
[3]. Güçlü, K., Sözgen, K., Tütem, E., Özyürek, M., & Apak, R. (2005). Spectrophotometric determination of ascorbic acid using copper (II)–neocuproine reagent in beverages and pharmaceuticals. *Talanta*, *65*(5), 1226-1232.
[4]. Townshend, A. (1995). *Encyclopedia of analytical science* (Vol. 8). Academic Press.

## *ORAL PRESENTATION*

# Tuz stresi ile indüklenmiş *Hypericum perforatum* L. tıbbi bitkisinde MYB-48 ve MYB-101 genlerinin transkripsiyonel düzeyde regülasyonunun incelenmesi

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**Özet**

*Hypericum perforatum* L. halk tarafından nesiller boyunca anksiyete, depresyon, uykusuzluk, gastrit, hemoroid, iltihap, yara, kesik ve yanık tedavisi gibi pek çok amaç için sıklıkla kullanılan tıbbi bitkidir. Literatürde onkolojik çalışmalarda tedaviye yönelik bu bitkinin ekstraktlarının önemini gösteren çalışmalar yer almaktadır. Bitki, Ege bölgesinde hem ticari hem de tıbbi açıdan önemli bir kaynak teşkil etmektedir. Çevresel stres faktörlerinden birisi olan tuzluluk, dünyada olduğu gibi ülkemizde de tarımı son derece ilgilendiren güncel problemlerden biri olarak karşımıza çıkmaktadır. Topraklarımızın gitgide kurak alanlar haline dönüşmesi nedeni ile başarılı bir tarım yapabilmek ve yüksek kalitede ve verimde ürün alabilmek için bitki büyüme ve gelişmesi ile tuzluluk stresi arasındaki ilişkiyi kapsayan çalışmaların artması da önemlidir. Gen anlatımının transkripsiyonel olarak düzenlenmesi çevresel uyaranlara karşı oluşan bitki cevaplarında ve bitkinin fizyolojik ve biyokimyasal gelişiminde önem arz etmektedir. Transkripsiyon faktörleri bitkilerde gen ve gen kümelerinin kontrolünde önemli rol oynamaktadır. Bunların içinde en büyük transkripsiyon faktör ailesini MYB (Miyelobla stomatoz) transkripsiyon faktörleri oluşturmaktadır. MYB proteinler bitkilerde biyokimyasal süreçlerde (bitki gelişimi ve metabolizması) ve bitkilerde savunma mekanizmasında (stres cevaplarında) rol alan bir süperaile transkripsiyon faktörleridir. Günümüzde abiyotik stres altında canlılığını sürdürebilen bitki çeşitlerinin geliştirilmesi ve onların yetiştirilebilmesi için, gen kümelerinin kontrolünde önemli rol alan transkripsiyon faktörlerinin karekterize edilmesi gereklidir.
Bu çalışmada, *H. perforatum* L. tıbbi bitkisi üzerinde tuz stresinin etkisini gözlemlemek üzere, bitki doku kültüründe büyüme ve gelişmesi sağlanan bitkilere 200 mg/L NaCl içeren ve kontrol bitkileri için NaCl içermeyen Hoagland solüsyonu 3 ve 24 saatlik periyotlarda uygulanmıştır. Kontrol grubu olarak direkt *in vitro* bitkiden elde edilen bitki grupları kullanılmıştır. Daha sonra bitkilerden RNA izolasyonu gerçekleştirilip, MYB tipi transkripsiyon faktörlerine ait MYB-48 ve MYB-101 genlerin tuz stresi altında gen ifade seviyelerindeki değişiklikler gözlemlenmiştir. Normalizasyon için Actin geni kullanılmıştır. Çalışma sonunda MYB-101 geninde tuz stresi durumunda gen ifadesinde artış gözlemlenirken MYB-48 geninde anlamlı bir değişiklik bulunmamıştır.

Bu proje 2209/A ‘‘Üniversite Öğrencileri Araştırma Projeleri Destek Programı’’ kapsamında TÜBİTAK tarafından desteklenmiştir.

**Anahtar Kelimeler:** *Hypericum perforatum*, MYB transkripsiyon faktörleri, tuz stresi

## *ORAL PRESENTATION*

# Thermoelectric Properties of Polyselenophene/Zinc oxide Composites

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**Abstract**

Nowadays, researches examining the thermoelectric properties of conductive polymers have become very important. Thermoelectric properties of conductive polymers used for this purpose alone cannot give the desired efficiency. Therefore, thermoelectric properties can be enhanced by adding nanoparticles of inorganic, organic or polymeric structures to conductive polymers. In this study, firstly, polyselenophene was synthesized by chemical oxidative polymerization. Then, polyselenophene was synthesized in the presence of zinc oxide nanoparticles. In the next step, thin films were prepared from polyselenophenes and polyselenophene/zinc oxide composites. The Seebeck coefficient and electrical conductivity of these thin films were measured and the power factor was calculated with these data. It was observed that the Seebeck coefficient of the polyselenophene/zinc oxide composites was higher than that of the polyselenophenes, and the electrical conductivity did not change much. Characterization of synthesized polymers was done by FTIR and UV-Vis. spectroscopy. This study was partially or fully supported by Scientific Research Project Coordination of Yildiz Technical University (FKD-2022-4932).

**Keywords:** Clean energy, Seebeck coefficient, thermoelectric.

## *ORAL PRESENTATION*

# Possible protective effect of alcoholic extract of *Moringa oleifera* on the skin in valproic acid-administered rats

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**Abstract**

Among the main functions of the skin are to protect the body from external factors, to provide water balance, to synthesize vitamin D and to remove various waste materials from the body. Drugs for the treatment of various diseases can adversely affect the structure and functions of the skin. Valproic acid/valproate (VPA) or 2-n-propylpentanoic acid is one of these drugs. Besides being used in the treatment of epilepsy and seizures, VPA is also effective in migraine, bipolar disorder, anxiety and other psychiatric disorders. Among the dermatological side effects of VPA, there are different effects such as hirsutism, hair loss, change in hair color, change in hair growth, and rarely skin rashes. Extracts from *Moringa oleifera* (*M. oleifera)* exhibit multiple nutraceutical or pharmacological functions, including anti-inflammatory, anti-oxidant, anti-cancer, hepatoprotective and neuroprotective functions. The oil obtained from the seed of this plant has cosmetic value and is known to be used as a moisturizer and skin care product in body and hair care. In this study, the possible protective effect of *M.oleifera* alcoholic extract on the skin in VPA-treated rats was investigated. Skin lipid peroxidation values increased significantly in the VPA group compared to the control group. Lipid peroxidation values were significantly reduced with the application of *M. oleifera* extract. *M. oleifera* extract may contribute to the prevention of dermatological side effects of antiepileptic drugs and the development of new therapeutics for skin health. This study was supported by Marmara University Scientific Research Projects Commission (Project No: TYL-2022-10423).

**Keywords:** Valproic acid, *Moringa oleifera* extract, skin, lipid peroxidation.

## *ORAL PRESENTATION*

# Covalent Triazine Frameworks as Oxygen Reduction Reaction Catalysts – Mechanistic and Kinetic Studies in Alkaline Media

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**Abstract**

Cathodic oxygen reduction reaction (ORR) process has paramount importance for determining the performance of the renewable energy technologies (such as fuel cells, batteries).[1, 2] Pt-based alloys are known to be the most active ORR materials in both acidic and alkaline environments, however the sluggish electron transfer kinetics, scarcity, poor methanol tolerance, and high-cost of Pt hinder its widely usage in aforementioned systems, thus limiting the commercialization.[1] Covalent triazine frameworks (CTFs)[3, 4] as N-containing porous carbonaceous polymers have great potential for use as an ORR catalyst or a support material as this class of materials is more advantageous compared to other carbon-based materials. Their structure (such as surface area, porosity, conductivity, degree of graphitization and defects) and N content -most importantly active N-sites towards ORR- can be precisely arranged by the selection of applied monomers and synthesis temperatures.[2] Herein this study, a series of CTF materials were synthesised via ionothermal-route[2] ($ZnCl_2$, 400/600 °C) and electrochemical activities towards ORR were tested via rotating disk electrode (RDE) and compared to Pt black and Carbon Super P. Three CTF materials have shown even higher current densities than Pt black and the ORR process follows the direct 4e− reduction while 2e− reduction is observed at CTF-DCP. Detailed electrochemical analysis of the materials have shown that metal-free CTFs can potentially be used as highly active ORR catalysts.

**Keywords:** CTF, Polymers, ORR, Batteries

**References**

1. Chen, J.; Wang, X.; Cui, X.; Yang, G.; Zheng, W., *ChemComm* **2014,** *50* (5), 557-559.
2. Sönmez, Turgut (2017) Studies of oxygen electrochemistry on spinel oxides. University of Southampton, Doctoral Thesis, 188pp.
3. Sönmez, T.; Belthle, K. S.; Iemhoff, A.; Uecker, J.; Artz, J.; Bisswanger, T.; Stampfer, C.; Hamzah, H. H.; Nicolae, S. A.; Titirici, M.-M. ; Palkovits, R., *Catal Sci & Technol* **2021,** *11* (18), 6191-6204.
4. Artz, J., *ChemCatChem* **2018,** *10* (8), 1753-1771.

## *ORAL PRESENTATION*

# The Investigation of Organometallic Compounds Biological Material Interaction

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**Abstract**

The therapeutic use of organometallic compounds is increasing interest and many compounds are synthesized for this purpose. The interactions of the developed complexes with biological materials are important in elucidating the biological activities of synthesized complexes. In the present study, analytical techniques for elucidating the interaction mechanisms of Gold, Ruthenium, and Copper complexes, which are designed especially for cancer treatment, were reviewed with biological materials. In recent years, the biological activities of the complexes have gained importance, and it is stated that this elucidation plays a key role in the synthesis of new compounds with high selectivity and therapeutic effects. The interaction mechanisms of these organometallic complexes with human serum albumin, bovine serum albumin, and DNA were examined by spectroscopic techniques such as; UV-Vis, Fluorescence, and Circular Dichroism spectroscopy. It is seen that the binding models are interpreted with spectral changes and thermodynamic parameters. For this purpose, the binding constants of the complex-biological material system were determined at a constant temperature, ionic strength, and pH. In addition, for complexes where the binding patterns could not be fully interpreted, compounds with high binding affinity were compared competitively with the intercalative binding model such as ethidium bromide. Moreover, additionally, viscosity and melting experiments have been performed in order to evaluate a binding model.

**Keywords:** Organometalic complexes, DNA, BSA, Spectroscopy, Intercalation

## *ORAL PRESENTATION*

# Ordu ve çevresinde yayılış gösteren *Satureja spicigera* (Lamiaceae) türünün bazı anatomik özellikleri

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**Özet**

*Satureja* L. cinsi Lamiaceae familyasına ait bir cinstir. Cins ülkemizde 5'i endemik olmak üzere toplam 15 takson ile temsil edilmektedir. *Satureja* cinsine ait bazı türler kas ağrıları, hazımsızlık ve ishal gibi çeşitli rahatsızlıkları tedavi etmek için kullanılmaktadır. *Satureja* cinsi yöresel olarak 'kaya kekiği', *S. spicigera* türü ise 'çorba kekiği' ve 'Trabzon kekiği' olarak bilinmektedir. *Satureja spicigera* (K. Koch.) Boiss. türü taşlı veya kayalık ortam koşullarında yetişen, çok yıllık, koyu yeşil yapraklı, beyaz renkli çiçekli bitkilerdir. Çalışmamızın amacı Ordu ve çevresinde yayılış gösteren *Satureja spicigera türünün* anatomik özelliklerini belirlemektir. Bitki örnekleri 2022 yılında Ordu-Perşembe'den toplanmıştır. Anatomik incelemeler için örnekler % 70'lik alkole konularak tespit edilmiştir. Taksonun kök ve gövdesinden enine, yapraklarından hem enine hem de yüzeysel kesitler elle alınmıştır. Ölçümler NIS Elements Imaging Software 3.00 SP5 programı kullanılarak yapılmıştır. Türlerin anatomik kesitlerinin fotoğrafları Nikon FDX-35 marka mikroskop ile çekilmiştir. Bitkideki stoma indeksi hesaplanmıştır. Bitkinin gövdesi dört köşelidir. Gövdenin köşelerinde çok sıralı (5-6), diğer kısımlarında 1-2 sıralı kollenkima hücreleri bulunur. Yaprak tipi ekvifasiyal ve amfistomatik tiptedir Yaprakların üst ve alt yüzeylerinde diasitik ve anizositik tip stomalar bulunmaktadır. Gövde üzerinde örtü ve salgı tüyleri bulunmaktadır. Yaprağın üst ve alt yüzeylerinde bol miktarda salgı tüyleri ve nadiren örtü tüyleri yer almaktadır. Salgı tüyleri kapitat ve peltat tiptedir.

**Anahtar Kelimeler:** *Satureja, Satureja spicigera,* kekik, anatomi, Lamiaceae.

## *ORAL PRESENTATION*

# CuO supported-Polystrene-2-ethylhexyl methacrylate-divinyl benzene copolymer foams for encapsulation of capric-stearic acid eutectic mixture for thermal energy storage application

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**Abstract**

Phase change materials (PCMs) are functional materials for many different thermal energy storage applications that can absorb, release and store large amount of thermal energy. This study based on the design, synthesis, structural and thermal characterization of porous polymer containing copper oxide (CuO) nanoparticles for impregnation of capric-stearic acid eutectic mixture (CA-SA) as PCM. Polystyrene-2-ethylhexyl methacrylate-divinyl benzene copolymer with/without CuO, was produced by high internal phase emulsion polymerization method in a three-dimensional (3D) interconnected porous structure. A series of composite materials were prepared by adding the CA-SA eutectic mixture to the PHP structure by vacuum impregnation method. The porous skeleton structure was able to trap up to 65% by weight PCM without leakage. The structural, morphological, and thermal behavior of composite PCMs was characterized by Fourier transform infrared spectrophotometry (FT-IR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC) analysis, and thermogravimetric analysis (TG). In addition, the thermal conductivity of the produced composite PCMs were determined. The thermal and chemical stability of the synthesized composite PCMs were determined by repeating DSC and FT-IR analyzes after 500 repetitive thermal cycles. The results showed that shape-stabilized CA-SA eutectic mixture-based composite FDMs, which have high thermal energy storage capacity, high reusability, high thermal stability, and can be produced with a one-step fabrication approach, are promising for latent heat thermal energy storage applications.

*The author would like to thank to Tokat Gaziosmanpaşa University Scientific Research Funds (The Project Code: BAP2020/119) for financial support.

**Keywords:** Phase change materials, thermal energy storage, polyhipe, polystrene-2-ethylhexyl methacrylate-divinyl benzene copolymer.

## *ORAL PRESENTATION*

# Chemical Conversion of Waste Biomass to High Value Added Chemicals by Ionic Liquid Catalysis

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**Abstract**

One of the key ways to reduce our global reliance on fossil resources is to conversion biomass resources into valuable platform chemicals or liquid fuels. Biomass-derived 5-hydroxymethylfurfural (5-HMF), levulinic acid and furfural are important raw materials for the production of valuable chemicals and liquid fuels. The direct conversion of lignocellulosic biomass into high value-added chemicals such as HMF, levulinic acid is crucial for process economy and sustainability. For this purpose, several successful studies have been carried out using new type catalysts for more efficient conversion of lignocellulosic sources to HMF, levulinic acid and furfural (Dashtban et al., 2014; Gupta et al., 2018). 1-butyl-3-methylimidazolium hydrogen sulfate ionic liquid was used as catalyst. The effect of reaction conditions such as catalyst amount, temperature and reaction time was investigated. The best reaction conditions was found as 4:1 ionic liquid/biomass ratio, 160 °C reaction temperature and 150 min the reaction time. Furfural was the least formed among these three products, while levulinic acid was formed more. In addition, experiments were also carried out with the microwave method, and a comparison of the microwave method and the thermal method was made. The results showed that the microwave method is more effective than the thermal method.

**Keywords:** Lignocellulosic biomass, Ionic liquid, 5-Hydroxymethylfurfural, Hydrolysis, Chemical conversion.

**Acknowledgments**: The authors thank the Research Fund of Mersin University in Turkey with Project Number: 2021-2-TP2-4453

**References:**

Dashtban, M., Gilbert, A., Fatehi, P. (2014). Recent advancements in the production of hydroxymethylfurfural. Rsc Advances, 4(4), 2037-2050.

Gupta, K., Rai, R. K., & Singh, S. K. (2018). Metal catalysts for the efficient transformation of biomass-derived HMF and furfural to value added chemicals. ChemCatChem, 10(11), 2326-2349.

## *ORAL PRESENTATION*

# Use of Apitherapy Products in Clinical Areas

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**Abstract**

Honey bees (*Apis mellifera*) are social insects that live in colonies, in a hierarchical order, and have economic and ecological importance in nature. In addition, many of the products they produce provide benefits to the health sector. The concept of apitherapy has gained popularity with the protective effect of products such as honey, royal jelly, bee venom, pollen and propolis produced by honey bees against diseases and for treatment purposes. The World Health Organization (WHO) stated that the integration of bee products into the health sector and their therapeutic use should be done by certified physicians. Each bee product contains important ingredients. Studies on this subject clearly show that these products give positive results in strengthening the immune system, certain dermatological and rheumatic diseases. There are also studies reporting positive results for the treatment of some types of cancer. Therefore, the importance of honey bees in the field of health, which has many biological functions in nature, is undeniable. In this study, it is aimed to examine the effects of bee products and their ingredients on diseases and their treatments.

**Keywords:** Honey bee, Apitherapy, Diseases, Treatments

## *ORAL PRESENTATION*

# Determination of the concentration and spatial distribution of heavy metals in the soil surface of Skikda, Algeria

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**Abstract**

The city of Skikda has experienced in recent years rapid urbanization and increased industrialization and density of urban transport. The objective of this study is the determination of the content of metallic elements Cu, Zn, Pb, Cr, Ni in the soil of the city of Skikda. The use of X-ray fluorescence spectroscopy allowed us to identify the following constituents in the collected samples: Mg, Al, Si, P, S, Cl, K, Ca, Ti, V, Cr, Mn Fe, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Sr, Y, Zr, Ba, Pb. The quantitative and qualitative analysis of samples taken from the soil of the city of Skikda by an X-ray fluorescence spectrometer showed that they are characterized by contamination in zinc, lead, nickel and chromium, copper. These heavy metals can result from contamination of human origin linked mainly to agricultural and industrial activities and heavy automobile traffic. On the other hand, natural contamination. Metals from anthropogenic input are present in fairly reactive chemical forms and therefore entail much higher risks than metals of natural origin which are often immobilized in relatively inert forms.

**Keywords:** heavy metals, soil, contamination

## *ORAL PRESENTATION*

# Effects of IL-38 on Adipose Tissue Inflammation and Fibrosis In Obesity

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**Abstract**

The increasing prevalence of obesity is a significant worldwide health concern. The main reason for arising concern is the fact that obesity is a risk factor for a number of chronic diseases (diabetes, cardiovascular disease, cancer). Obesity is a low-grade chronic inflammation. Understanding the inflammatory response will help to develop new therapeutic approaches for metabolic disorders. In obesity, pro-inflammatory cytokines are expressed from adipocytes and adipose tissue macrophages. Chronic inflammation of adipose tissue leads to fibrosis via collagen accumulation. IL-38 is an anti-inflammatory cytokine in the IL-1 family. IL-38 inhibits inflammatory cytokine production by adipocyte cells. There are several studies about the antifibrotic effects of IL-38 on pulmonary fibrosis, myocardial infarction and ophthalmopathy. However, further studies are required for detecting the antifibrotic effects of IL-38 on adipose tissue remodeling. IL-38 gene delivery in mice model reduces weight gain, adipose tissue weight, obesity-induced insulin resistance and inflammatory mediators. Furthermore, IL-38 is also associated with type 2 diabetes mellitus and hyperlipidemia. High IL-38 levels prevent type 2 diabetes mellitus development in mice model. The cellular activity, biological function and mechanisms of IL-38 have not been fully elucidated. Obesity, adipose tissue inflammation and chronic disease association make inflammatory pathways an attractive treatment target for metabolic diseases. Hence, it is possible for IL-38 to be an alternative treatment approach for inflammatory and immunological diseases including obesity. Within the scope of the review, current studies on IL-38 regarding obesity available in the literature are discussed.

**Keywords:** Inflammation, Interleukin-38, Obesity

## *ORAL PRESENTATION*

# Synthesis and Spectral Characterization of New Substitued 3-(4-Nitro-Phenyl)-5-Phenyl-4,5-Dihidro-Pyrazole-1-Carbaldehyde Derivatives

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**Abstract**

Pyrazole is considered a unique heterocyclic compound that possesses a nitrogen atom in the five-membered ring. The pyrazole structure has attracted attention due to its utility in drug design, which has proven to have potential as chemotherapeutic and pharmacotherapeutic agents. The aims of the study were the synthesis and structural characterization of some new substituted pyrazole-carbaldehyde derivatives starting from nitrobenzaldehyde. The most convenient and efficient way to achieve the goal was by synthesizing chalcones as a first step and then from chalcones with hydrazine hydrate in the presence of formic acid the synthesis of substituted pyrazole-carbaldehydes. The main syntheses are using 4-nitroacetophenone as a base together with different benzaldehyde compounds, then to make comparisons, 3-nitroacetophenone and acetophenone were used as a base. The prepared syntheses 3-(4-nitro-phenyl)-5-phenyl-4,5-dihydro-pyrazole-1-carbaldehyde, 5-(3-bromo-phenyl)-3-(4-nitro-phenyl)-4,5-dihydro-pyrazole-1-carbaldehyde, 5-(4-fluoro-phenyl)-3-(4-nitro-phenyl)-4,5-dihydro-pyrazole-1-carbaldehyde, 5-(4-Fluoro-phenyl)-3-(3-nitro-phenyl)-4,5-dihydro-pyrazole-1-carbaldehyde, 5-(3-Nitro-phenyl)-3-phenyl-4,5-dihydro-pyrazole-1-carbaldehyde were analyzed by measuring melting points, recrystallizing them, performing chromatography, recording IK spectra and recording 1H-RBM spectra.

**Keywords:** Chalcones, acetophenones, synthesis, benzaldehyde, pyrazole-carbaldehyde

## *ORAL PRESENTATION*

# Gökkuşağı alabalığı (*Oncorhynchus mykiss*) midesinin morfolojik incelenmesi

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**Özet**

Dünya üzerinde bulunan suyun büyük çoğunluğu okyanuslarda, denizlerde, buzullarda bulunmaktadır. Dünyadaki toplam su kütlesinin yaklaşık olarak yalnızca %0,01'i iç sular olarak nitelendirilen tatlı su gölleri ve akarsularda bulunmaktadır. Hem tatlı sularda yaşayabilen hem de tuzlu suya adapte olabilen Salmonidae familyasının tanınmış üyelerinden biri Gökkuşağı alabalığı (*Oncorhynchus mykiss*)'dir. Gerek geleneksel damak tadına yakın oluşu gerekse kaliteli protein bakımından zengin oluşu Türkiye'de bu balığın tüketiminin fazla olmasının asıl nedenleridir. Dünya üzerinde hızla artan nüfus, kirlilik, kontrolsüz kentleşme vb. sebeplerle gıda arzında birtakım aksaklıklara sebep olmuştur. Bu nedenle gerek denizlerde gerekse iç sularda balık yetiştiriciliği yaygın hale gelmiştir. Balık yetiştiriciliğinde idame hammadde arayışları uzun yıllardan beri yapılmaktadır. Canlılarda yaşamsal faaliyetlerin devamı için gerekli besinler sindirim yolu ile vücuda alınır ve sindirim sisteminin çeşitli kısımlarında çeşitli işlemlerden geçer. Balık rasyonlarında idame edilen yem ve yem katkı maddeleri balıkların büyüme perfomansı üzerinde etkilidir. Sindirim sisteminde yer alan değişiklikler balıklarda büyümeye direkt etkilidir. Bu çalışmada Türkiye'de yetiştiriciliği yapılan Gökkuşağı alabalığı'nın midesinin anatomik açıdan incelenmesi amaçlanmıştır. Bu amaçla 10 adet Gökkuşağı alabalığı (*Oncorhynchus mykiss*)'nın midesi kullanılmıştır. Bu balıklardan 8 tanesi morfolojik inceleme için; 2 tanesi taramalı elektron mikroskobu (SEM) ile incelenmiştir. Mide; cardiac ve pyloric kısım olarak iki kısımda incelendi. Ortalama uzunluklar cardiac kısımda 48,16±3,51 mm; pyloric kısımda ise 22,51±1,12 mm olduğu belirlendi. SEM incelemede ise mide yüzeyinin kıvrımlı bir yapıda olduğu belirlendi. Mide yüzeyi columnar epitel ile kaplı idi. Columnar epitel yüzeyi arasında içerisi microvillus ile kaplı düzensiz poligonal şekilli yapılar gözlendi. Sonuç olarak Türkiye iç sularında yetiştirilen Gökkuşağı alabalığı (*Oncorhynchus mykiss*)'nın midesi anatomik olarak incelenmiştir.

**Anahtar Kelimeler**: Gökkuşağı Alabalığı (*Oncorhynchus mykiss*), mide, morfoloji.

## *ORAL PRESENTATION*

# An *in silico* evaluation of ligands targeting the NLRP3/caspase-1 inflammasome pathway in the management of endodontic diseases

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**Abstract**

The NLRP3/caspase-1 inflammasome pathway is crucial for cellular immunological defense against bacterial infections causing damage to the pulp or periapical tissues in endodontic diseases. In our study, the interactions and binding affinities of several inhibitor ligands with target proteins for the NLRP3 inflammasome and caspase-1 were examined, both of which are crucial components of the inflammatory process. Using computer-aided tools, the loop modeling approach was used to create the human NLRP3 model. Utilizing molecular docking method, the binding affinities, inhibition constants ($K_i$), and ligand-protein interactions of the selected ligands were determined and examined against the NLRP3 inflammasome (PDB ID: 3QF2) and caspase-1 (PDB ID: 1RWK). Autodock 4.0 software was used to do the docking calculations and binding modes. A conformal cluster analysis was performed allowing for a root mean square deviation (RMSD) of 2.0 Å on the docked results. For each ligand used in this study, the predicted binding affinities were within the desirable range (between -5.1 and -11.8 kcal/mol) for inhibitory action. The best binding affinities, $K_i$ values, and strong binding contacts were found for CY-09 and VX-765 in the binding pockets of NLRP3 and human caspase-1, respectively. Therefore, in the treatment of endodontic disorders, the combination of CY-09 and VX-765 ligands can be employed to reduce inflammation. These inhibitors may be utilized to treat endodontic infections in the future as well as to make root canal medications and pulp capping materials more feasible.

**Keywords:** NLRP3 inflammasome inhibition, caspase-1 inhibition, endodontic diseases, molecular docking.

## *ORAL PRESENTATION*

# Gölcük Köyü ve Çevresinin (Kestel, Bursa) Florasına Katkılar

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**Özet**

Yeşilin bin bir tonunu taşıyan Bursa şehri doğası, tarihi, kültürel değerleri ve sahip olduğu biyolojik çeşitliliği ile Türkiye'nin ve Dünyanın önemli şehirleri arasında yer almaktadır. Gölcük, Bursa ilinin Kestel ilçesine bağlı bir köydür. Bursa iline 37 km, Kestel ilçesine 25km uzaklıktadır. Köyün iklimi Marmara iklimi etki alanı içerisindedir. Devam etmekte olan bu çalışmada Gölcük Köyü (Kestel, Bursa) ve çevresinin florasının ortaya çıkarılması amaçlanmaktadır. Araştırma alanında Mayıs 2022 tarihinden itibaren Haziran, Temmuz, Eylül ve Ekim 2022 aylarında arazi çalışmaları yapılmıştır. Bu çalışmalar sonucunda toplanan bitki materyalleri Gölcük Köyü'nde bulunan bitki taksonlarından oluşmaktadır. Bu kapsamda belirli periyotlarla bitki örnekleri optimum vejetasyon dönemlerinde toplanarak herbaryum materyali haline getirilmiştir. Alınan bitki örneklerinin lokalite ve gerekli arazi bilgileri kaydedilerek her birine toplayıcı numarası verilmiştir. Toplanan her bir örneğin teşhislerini kolaylaştırmak adına çiçekli veya meyveli olmak üzere fotoğrafları çekilerek arşivlenmiştir. Bitki örneklerinin teşhisi için "Türkiye ve Doğu Ege Adaları Florası (Flora of Turkey and the East Aegan Islands)'' adlı eserden yararlanılmıştır. Teşhis edilen bitkiler arazi defterinde listelenerek bölgenin florası çıkarılmaya başlanmıştır. Saha çalışmaları sürecinde şu ana dek toplanan bitki örneklerinin değerlendirilmesi sonucunda 39 familyaya ait 131 tür ve türaltı seviyede taksonun özellikleri tespit edilmiştir. Şimdiye kadar süren çalışmalarda Gölcük Köyü (Kestel, Bursa) ve çevresinin florası için toplanan bitki örneklerinden teşhis anahtarları kullanılarak 131 tür tespit edilmiştir.

**Anahtar Kelimeler:** Flora, Gölcük Köyü, Kestel, Bursa

## *ORAL PRESENTATION*

# Phytochemical and biological evaluation of roots extracts from medicinal plant belonging to the Asteraceae family

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**Abstract**

This study aimed to quantify phenolic and flavonoid compounds in ethyl acetate and n-butanol extracts, compare the antioxidant activity of their extracts, and the evaluation of the ant-diabetic activity using α-amylase test.
Firstly, roots of this species were submitted to a phytochemical study, which revealed the presence of several secondary metabolites, such as alkaloids, flavonoids, tannins and others.
Then Soxhlet extraction in three solvents of increasing polarity made it possible to obtain three extracts: $CHCl_3$, EtOAc, and *n*-BuOH .The results of the quantitative analysis reveal that the *n*-BuOH extract is the richest in total polyphenols and flavonoids, with values of 116.298±0.280mgGAE/g of extract and 65.301±0.906mgQE/g of extract, respectively.
The DPPH and ABTS methods used to assess the antioxidant activity reveal that the *n*-BuOH extract has the highest antioxidant activity.
In addition, the α-amylase test shows that all extracts possess dose-dependent antidiabetic activity, Based on the IC50 values, the EtOAc extract has the best inhibitory activity with a value of 200.58±9.95 µg/ml.
**As results** The studied plant which belongs to Asteraceae family is characterized by a fairly large reservoir of secondary metabolites such as alkaloids and flavonoids which are known by their specific therapeutic and pharmacological properties, the Roots of this plant possess large antioxidant ant antidiabetic activity.

**Keywords:** phytochemical screening, polyphenols, flavonoids, antioxidant activity, α-amylase.

## *ORAL PRESENTATION*

# Synthesis of a BODIPY-N-fusedpentaphyrin conjugate and its photophysical evaluation

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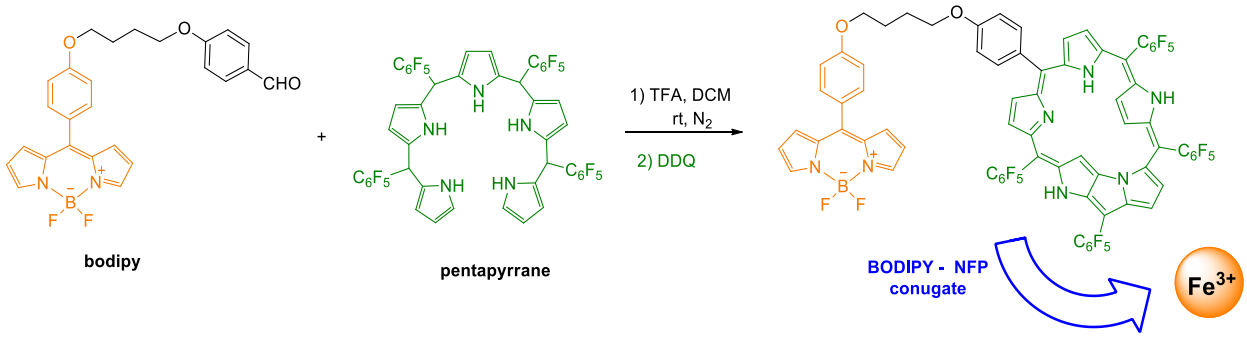
**Abstract**

Development of novel fluorescent organic conjugates to sense specific species is one of the major research area in synthetic organic chemistry. In literature, there are numerous synthetic organic structures having the ability to sense a specific ion, especially the transition metal ions [1-3] in addition the related literature needs to be developed continually. In this work, synthesis of a BODIPY – N-fusedpentaphyrin (BODIPY-NFP) conjugate has been realized and the photophysical properties were screened against several cations and anions. BODIPY-NFP was purified by column chromatography and the structure was characterized with NMR and HRMS techniques. Absorption and emission profile of BODIPY-NFP have revealed the selective sensing of $Fe^{3+}$ ions.




**Keywords:** Organic synthesis, BODIPY conjugates, spectroscopic analysis

**References**

[1] L.-Y. Niu, H. Li, L. Feng, Y.-S Guan, Y.-Z. Chen, C.-F. Duan, L.-Z. Wu, Y.-F. Guan, C.-Ho. Tung, Q.-Z. Yang, BODIPY-based fluorometric sensor array for the highly sensitive identification of heavy-metal ions, Analytica Chimica Acta 775 (2013) 93– 99.
[2] J.-T. Yeh, W.-C. Chen, S.-R. Liu, S.-P. Wu, A coumarin-based sensitive and selective fluorescent sensor for copper(II) ions, New J.Chem., 38, **(**2014) 4434-4439.
[3] J. Nootem, C. Sattayanon, R. Daengngern, A. Kamkaew, W. Wattanathana, S. Wannapaiboon, P. Rashatasakhon, K. Chansaenpak, BODIPY-Pyridylhydrazone Probe for Fluorescence Turn-On Detection of $Fe^{3+}$ and Its Bioimaging Application, Chemosensors, 9, (2021) 165-182.

## *ORAL PRESENTATION*

# Genomic Analysis of *Microbacterium* sp. H37-C3 Synthesizing Silver Nanoparticles

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**Abstract**

Silver nanoparticles are used extensively in biomedicine for various purposes due to their intrinsic therapeutic properties. Plants, algae, fungi, and bacteria have been found to produce silver nanoparticles (AgNPs). In this study, an actinobacterium, *Microbacterium* sp. H37-C3, isolated from a rock sample collected on Horseshoe Island (Antarctica), was used to produce silver nanoparticles extracellularly. The synthesis of silver nanoparticles was characterized by UV–visible spectroscopy (UV–Vis) and scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDX). The silver nanoparticles showed the maximum absorbance peak at 417 nm. The average particle size of the silver nanoparticles ranges from 15 to 30 nm. Moreover, genome characteristics of strain *Microbacterium* sp. H37-C3 were also analyzed to reveal protein-coding genes and secondary metabolite biosynthetic gene clusters as well as the phylogenetic relationship of the strain. The genome annotation performed on the RAST server (https://rast.nmpdr.org/) showed that the number of protein-coding and RNA-coding sequences was 3313 and 53, respectively. The strain has genes related to antibiotic resistance and toxic compounds such as copper homeostasis, bile hydrolysis, cobalt-zinc-cadmium resistance and fluoroquinolone resistance. The genome of strain *Microbacterium* sp. H37-C3 have six biosynthetic gene clusters encoding for two type-III polyketides, a terpene, a betalactone, a lanthipeptide and a thiopeptide as determined by the antiSMASH server (https://antismash.secondarymetabolites.org/#!/start). The phylogenetic analysis based on whole-genome comparison on the TYGS server (https://tygs.dsmz.de/) revealed that strain H37-C3 is representative of a novel species within the genus *Microbacterium*. Given that novel actinobacteria may synthesize unique metabolites, the silver nanoparticles synthesized by novel strains may also have considerable bioactivity and biotechnological potential for the pharmaceutical industry. In future studies, differential gene expression analysis for *Candida albicans* exposed to the silver nanoparticles synthesized by *Microbacterium* sp. H37-C3 will be conducted to reveal its antimicrobial activity mechanism in gene expression level.

**Keywords:** *Actinobacteria*, *Microbacterium*, Genome analysis, Silver nanoparticles, SEM-EDX

## *ORAL PRESENTATION*

# Inhibition profile of the antiviral drug foscarnet to cholinesterase enzymes

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**Abstract**

One of the neurodegenerative disorders, Alzheimer's disease (AD), is today a serious global health concern. A complex disorder associated with aging, AD is characterized in elderly patients by progressive cerebral atrophy impairs cognition. Over the past few decades, certain AD medications have been developed, but sadly, they have limited ability to stop the progression of the illness or do not provide a cure. The cholinesterase enzymes (acetylcholinesterase; AChE and butyrylcholinesterase; BChE) inhibition, which are enzymes involved in the breakdown of acetylcholine (ACh) in the brain, is the standard approach for maintaining the levels of ACh. Herein, an active antiviral drug foscarnet was assayed as the inhibitor of AChE (EC 3.1.1.7) and BChE (EC 3.1.1.8) involved in these conditions. The AChE and BChE were lowly inhibited by foscarnet at micromolar concentrations with $K_I$s of 02.09 ± 0.18 µM and 9.29 ± 0.50 µM, respectively, compared with the reference drug tacrine ($K_I$ of 0.17 ± 0.01 µM for AChE and 0.13 ± 0.01 µM for BChE). Additionally, to learn more about the likely binding interactions and affinities for foscarnet at the active sites of AChE and BChE, molecular docking studies were carried out.

**Keywords:** Acetylcholinesterase, butyrylcholinesterase, inhibition

## *ORAL PRESENTATION*

# Optimization of ultrasound-assisted extraction of purple basil (*Ocimum basilicum. L.*) extracts by response surface methodology in pursuit of effective natural pH-indicator dyes

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**Abstract**

Increasing consumer awareness towards human health and environmental issues has manifested itself on the preference of natural colour pigments over synthetic pH-indicator dyes in food packaging applications. Purple basil (*Ocimum basilicum. L.*) has been reported to have high anthocyanin content, therefore it is a good candidate as a source of natural pH-indicator agent. This study was designed for the first time to optimize the ultrasound-assisted extraction (UAE) of dry purple basil by response surface methodology in terms of maximum total monomeric anthocyanin (TMA) content and pH-dependent increase in the green intensity (difference in absorbance ratios at pre-specified wavelengths between pH 1 and 10). Box-Behnken design with three levels and three factors was used. Three independent process variables including ethanol concentration (10-90% v/v), solvent-solid ratio (10-30 mL/g), and time (15-75 min) for two different solvent systems containing ethanol acidified with 0.1% (v/v) hydrochloric acid (HCl) or acetic acid (Ac) were studied. Optimum processing conditions were determined for single and multiple responses for the two solvent systems. The extracts obtained under different optimum UAE conditions were further characterized according to total phenolic content (TPC), percent polymeric colour (PPC), ABTS antioxidant activity and DPPH radical scavenging activity. As a result, 55.25% ethanol containing 0.1% Ac, 30 mL/g solvent-solid ratio and 39.24 min extraction time were determined as the optimum conditions based on the multiple response of TMA content and the increase green intensity with pH. Validation experiments verified that the corresponding model was successful in predicting both responses under the optimized conditions. The corresponding TMA, pH-dependent increase in green intensity, PPC, TPC, ABTS and DPPH values were determined as 4.83±0.18 mg cyanidin-3-glucoside equivalent, 2.29±0.05, 68.07±3.17, 28.79±1.54 mg gallic acid equivalent, 55.85±1.71 mg trolox equivalent, and 16.80±1.08 mg ascorbic acid equivalent per g dry weight, respectively for the extracts obtained under optimum conditions.

**Keywords:** anthocyanin, purple basil (*Ocimum basilicum. L.*), ultrasound-assisted extraction, response surface methodology

## *ORAL PRESENTATION*

# The removal efficiency of *Egeria densa* Planch. for zinc oxide nanoparticles (ZnO NP) in aquaeos media

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**Abstract**

There is a growing interest in the use and production of engineered nanomaterials which inevitably lead to an increase in the amount of nanoparticles (NP) released into environment. Several studies indicated the potential risks of NPs on the health of living organisms. Aquatic habitats are the final sink for various pollutants including nanomaterials, which generates a greater risk for aquatic biota. Therefore, the removal of NPs from wastewaters and natural waterbodies polluted with nanomaterials is crucial from an environmental standpoint. Although, several aquatic macrophytes have been proposed for the removal of heavy metals, pesticides, and macronutrients; nanoparticles seem to be neglected. Hence, in this study, we aimed to determine the removal capacity of *Egeria densa* Planch to remove zinc oxide nanoparticles (ZnO NPs) from aqueous media. For this purpose, plants were exposed to 3 different ZnO NP concentrations (1, 10 and 20 ppm) for 1, 4 and 7 days. The removal percentages and accumulated zinc levels in plant tissues were determined and compared among groups. Results indicated that the removal percentages were between %72.8 and 87.4; showing a gradual decrease with increasing ZnO NP concentration. In addition, our results also indicated that absorption, adsorption and precipitation might be responsible for the removal of ZnO NPs.

**Keywords:** *Pistia stratiotes*, *Egeria densa*, nanomaterial, zinc oxide nanoparticles, phytoremediation.

## *ORAL PRESENTATION*

# Fenolik bileşiklerde enkapsülasyon uygulamaları

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**Özet**

Fenolik bileşikler benzen halkasına bağlı bir veya birden fazla hidroksil ve fonksiyonel gruba sahip bitkilerin sekonder metabolitleridir. Polifenoller bitkinin hemen hemen her yerinde üretilen ve antioksidan, anti-inflamatuar, anti-viral etkilere sahip doğal antioksidanların en aktif üyeleridir. Fenolik bileşikler, insan ve hayvan sağlığı üzerindeki olumlu etkileri sebebiyle günlük diyette önemli bir yere sahiptir Ancak faydalı etkilerine karşın gıda proseslerine karşı dayanıklı değillerdir. Isı, ışık ve oksijenden etkilenerek yapılarında bozulmalar meydana gelebilmektedir. Ayrıca mide pH'sı ve çeşitli enzim ve besin bileşenlerinden de etkilenebilen fenolik bileşiklerin faydalarından yararlanabilmek için çeşitli mekanizmalar geliştirilmiştir. Enkapsülasyon teknolojisi de fenoliklerin korunması ve taşınması için kullanılabilecek en etkili mekanizmadır. Kapsülleme, biyoaktif bileşiklerin bir duvar malzemesi ile kaplandığı veya kabuklar ya da taşıyıcılar içinde tutulduğu bir tekniktir. Enkapsülasyon, aktif/çekirdek/öz materyal olarak adlandırılan bir maddenin (küçük katı partiküller, sıvı damlacıklar veya bir gaz) aktif materyali kapsül formunda kısmen veya tamamen izole eden, duvar/kaplama materyali veya kapsülleme ajanı adı verilen bir malzeme ile çevrelenmiş olduğu bir fiziksel kaplama işlemidir.
Enkapsülasyon uygulamalarında kullanılan tekniklerin başlıcaları; püskürterek kurutma, püskürtme soğutma, ekstraksiyon, koaservasyon, liyofilizasyon ve emülsifikasyon olup, uygulanacak prosese bağlı olarak, çekirdeği oluşturan aktif maddenin yapısına, mikro veya nanokapsül için uygulanmasına, elde edilmesi istenen parçacık boyutuna, çekirdek ve çekirdeği kaplayan duvarın fiziksel ve kimyasal özelliklerine, istenen serbest salım mekanizmasına, üretim ölçeğine ve maliyet hesaplamasına göre tercih edilmektedir. İnsan ve hayvan sağlığı üzerindeki olumlu etkileri sebebiyle günlük diyette önemli bir yere sahip fenolik bileşiklerin gıda proseslerinde ve sindirim esnasında yapılarındaki bozulmaları engelleyebilmek için farklı kapsülleme yöntemleri arasında, nanoemülsiyon ve mikroemülsiyon sistemlerinde en iyi koruma sağlanmıştır. Bu yöntemlerin emülsifikasyon, üstün ara yüz ve kullanılabilirlik, gıda özellikleri üzerinde en az istenmeyen etki, çok çeşitli doğal yüzey aktif madde inşası gibi üstünlükleri vardır.
Günümüzde, özellikle yararları birçok çalışma tarafından onaylanmış olan fenolik bileşiklerin kapsülleme uygulamaları göz önünde olan çalışma alanlardandır. Enkapsülasyon oluşumunda, birçok tekniğin uygulanması ve duvar malzemelerin fenolik bileşikler için fazla miktarda bulunması sebebiyle araştırma konusu olarak dikkat çekmektedir.

**Anahtar Kelimeler:** Fenolik bileşikler, Enkapsülasyon, Kaplama, Emülsiyon

## *ORAL PRESENTATION*

# Recombinant Production and Characterization of a Novel *N*-glycosidases (EndoBI-2) from *Bifidobacterium longum subsp. infantis* 157F

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**Abstract**

The gastrointestinal (GI) tract harbors several microorganisms, which contribute considerably to the maintenance of health and the progression of diseases. The survival of microorganisms that live in the GI tract is mainly dependent on their ability to utilize complex carbohydrates (glycans and polysaccharides) that are not digestible by the host organism. Human milk is the primary source of nutrition for a newborn before they can digest any other food. Glycosylated milk components such as lactoferrin, immunoglobulins, etc., are indigestible to the infant, and only certain gut microbes have the enzymes to release glycans from glycoproteins and utilize these compounds as the only carbon source. Glycosidases are the type of enzymes that are used for *N*-glycan release from glycoproteins. Commercially available glycosidases are inefficient at cleaving these glycans from proteins in their native state. To overcome this deficiency, efficient deglycosylation approaches are required to better understand the biological roles of *N*-glycans and their large-scale production. A novel enzyme, endo-*ß*-*N*-acetylglucosaminidase 2 (EndoBI-2) was isolated from *Bifidobacterium longum subsp. infantis* is a unique and effective enzyme for this process, but this enzyme isn't commercially available.
Within this study, the novel enzyme was produced and characterized recombinantly to understand the unique features of *N*-glycans. Comparative modeling approaches were used to investigate the structural properties and substrate selectivity of EndoBI-2. Furthermore, the optimum reaction/kinetic parameters of EndoBI-2 were investigated using glycoproteins and released bioactive glycans were characterized.
The kinetic and structural characterization parameters of EndoBI-2 suggest that this novel enzyme can facilitate the large-scale release of bioactive glycans from glycoprotein sources. Successful application of this novel enzyme and released bioactive glycans in the food industry will enable to development of functional foods for health and wellbeing.

**Keywords:** *N*-glycans, Endoglycosidases, Gut Microbiome, Bifidobacterium

**Acknowledgement:** The study was supported by The Scientific and Technological Research Council of Turkey 2210-A Program.

## *ORAL PRESENTATION*

# Majör depresif bozukluğu olan olgularda leptin reseptör gen polimorfizmlerinin belirlenmesi

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**Özet**

Depresyonun alt tiplerinden olan majör depresif bozukluk (MDB) tüm Dünya'da sıklıkla gözlenmektedir. Kadınlarda erkeklere oranla 2 kat daha fazla görülse de yaş ilerledikçe bu ayırım ortadan kalkmaktadır. Bu insidans farklılıkları yol açan sebepler arasında hormonlar, genetik duyarlılık, monoamin oksidaz (MAO) yüksekliği, tiroid hastalıkları ve menstürasyon döngüsünün yanı sıra çevresel ve sosyal etkilerin de katkısı büyüktür. Bunların dışında MDB'nin nedenlerinden biri obezite olarak gösterilmektedir. Obezitenin gelişiminde leptinin rol oynadığı araştırmalarla kanıtlanmıştır. Bu nedenle çalışmamızın amacı MDB olan olgularda leptin reseptör (LEPR) geninde meydana gelen 223 A/G polimorfizmini belirleyerek MBD ile ilişkisini göstermektir. Çalışmamızın örneklemini Tokat Gaziosmanpaşa Üniversitesi Tıp Fakültesi Hastanesi Psikiyatri AD'na başvurup MDB tanısı alan olgular(n=47) ile kontrol grubu (n=46) oluşturmaktadır. Demografik bilgileri ve onam formları aldığımız olguların EDTA'lı kan örneklerinden DNA izolasyonları gerçekleştirilmiştir. Gen polimorfizm çalışmaları için PCR-RFLP yöntemi ile kullanılmıştır. Sonuçların istatistiksel veri analizi WinPepi ile yapılmıştır. Çalışmamıza katılan MDB tanılı olguların 35'ü kadın (%74.5) ve yaş ortalamaları 38.3±13.9 iken, 12'si erkek (%25.5) ve yaş ortalamaları 41±18'dir. Kontrol grubunun ise 24'ü kadın (%) ve yaş ortalamaları 32.1±7.1 iken, 22'si erkek (%) ve yaş ortalamaları 46.8±13.6'dır. LEPR 223 A/G gen polimorfizm çalışmasının sonuçlarına göre MDB sahip olgularda 5 GG (kadın=5), 18 GA( kadın=13, erkek=5) ve 24 AA (kadın=17, erkek=7) genotipine sahip iken kontrol grubunda 4 GG (kadın=3, erkek=1), 23 (kadın=12, erkek=11) ve 19 AA (kadın=9, erkek=10) genotipine sahip olduğu bulunmuştur. Yaptığımız analizlerde LEPR 223 A/G polimorfizminin MDB tanılı olgular ile kontrol grup arasında istatistiksel açıdan anlamlı bir farkın olmadığı gözlenmiştir ($p>0.05$). Sonuç olarak obezite ile ilişkisinin olduğu gösterilen LEPR'ünde meydana gelen A/G değişimi MDB ile ilişkisinin olmadığı gösterilmiştir.

**Anahtar Kelimeler:** Leptin reseptörü, Majör depresyon, Gen polimorfizmi

## *ORAL PRESENTATION*

# *Salvia ceratophylla* (Lamiaceae), Mardin florası için yeni bir bitki kaydı

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**Özet**

Lamiaceae familyası kozmopolit bir dağılıma sahip olup, dünyada 236 cinse ait 7000'den fazla tür içermektedir. Türkiye'de ise Ballıbabagiller olarak adlandırılan Lamiaceae familyası 46 cinse ait 586 takson ile temsil edilmektedir. Bu familyanın taksonları ülkemizde ağırlıklı olarak Akdeniz bölgesinde yayılış göstermektedir. *Salvia* L. (Adaçayı), bu familyanın en büyük cinslerinden biridir. Cins, Türkiye Florasında 87 tür olarak tanımlanmaktadır. Son araştırmalara göre, *Salvia* cinsi 106 takson ve bu taksonlardan 58'i endemik olup, endemizm oranı % 51'dir. Latince kelime anlamı sağlığı ifade eden *Salvia* taksonları; otsu, çalı veya yarıçalı formunda tek yıllık veya çok yıllık bitkilerdir. Genellikle belirgin aromatik kokuya ve salgı tüylerine sahiplerdir. Çiçekleri beyaz, sarı, pembe, mavi veya mor renkli, iki dudaklıdır. Farklı habitatlarda ve yükseltilerde yayılış gösterirler. *Salvia* türleri gerek tıbbi gerekse de birçok türü baharat olarak kullanımlarından dolayı ekonomik önem taşır ve güzel görünümlü çiçekleri nedeniyle bahçe ve parklarda dekoratif süs bitkileri olarak yetiştirilmektedir.

Bu çalışmada, Mardin ilinin Mazıdağı, Savur ve Midyat ilçelerinden toplanan örneklere dayanarak *Salvia ceratophylla* L. türü, Mardin Florası için yeni kayıt olarak rapor edilmektedir. Türkiye Florası'nda, Güneydoğu Anadolu Bölgesinde Diyarbakır, Şanlıurfa ve Siirt illerinde yayılış gösterdiği belirtilmektedir. Toplanan çok sayıda örneklere dayalı olarak bu türün, deskripsiyonu yapılmıştır. Belirgin olarak, limon kokulu, çok yoğun salgılı, yapraklar pinnatifit, çiçek durumu sarımsı-yeşil, çiçekler 2-5 halka dizilişli, korolla beyaz veya sarımsı-beyaz ve nadiren üst dudak sarımsı özelliktedir. Ayrıca bu türün etnobotanik ve fitokimyasal özellikleri özetlenmiştir. Fitokimyasal içerik olarak, flavonoid, terponoid, uçucu yağ ve polifenolik bileşikler açısından zengin olduğu belirtilmektedir. Biyolojik aktivite çalışmaları sonucunda, antioksidan ve antimikrobiyal aktiviteye sahip olduğu ifade edilmektedir. Tür ülkemizde tıbbı olarak kullanımı az olsa da bilinmektedir. Türkçe bilimsel adı "tarakşalba" olmasına rağmen, genellikle ülkemizde adaçayı olarak adlandırılmaktadır. Bu bitki ülkemizde halsizlik, öksürük, gribal enfeksiyonlar ve ter kokusunu önleme gibi tıbbi amaçlarla kullanıldığı belirtilmektedir. Yurt dışında ise; kanser, mikrobiyal enfeksiyonlar ve idrar rahatsızlıklarının tedavisinde kullanıldığı ifade edilmektedir.

**Anahtar Kelimeler:** etnobotanik-fitokimyasal-Mardin- *Salvia ceratophylla*-yeni kayıt- taksonomi

## *ORAL PRESENTATION*

# Synthesis and Structural Characterization of New Substituted Pyrazolcarbaldehydes Derivatives

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**Abstract**

Pyrazole structure attracts attention because of its utility in drug design, which has proven to be potential chemotherapeutic and pharmacotherapeutic agents. The aims of the study were the synthesis and structural characterization of some new substituted pyrazole-carbaldehyde derivatives from different benzaldehydes and acetophenones. In the first phase we have synthesized some syntheses of substituted chalcone. In the second phase we synthesized substituted pyrazole-carbaldehyde derivatives. A total of 8 synthesis of chalcones were synthesized from benzaldehyde and various aromatic acetophenones, and 8 synthesis of pyrazole-carbaldehydes were obtained from chalcones. The obtained syntheses were analyzed by measuring melting points, recrystallizing them, performing chromatography, recording IK spectra and recording 1H-RBM spectra. Retention factor values ranged from 0.36 to 0.92. Melting point values ranged from 127ºC to 217ºC . With the interpretation of IR spectra, we confirmed the presence of functional groups, groups -NO2, C=O, C-N, C-F and C-Br. The results obtained from this study showed that the obtained pyrazole-carbaldehydes are the required pyrazolecarbaldehydes.

**Keywords:** Chalcones, acetophenones, synthesis, benzaldehyde, pyrazole-carbaldehyde

## *ORAL PRESENTATION*

# Computational Modelling of Abrocitinib Derivatives/Metabolites and Unveiling their Janus Kinase (JAK) Inhibitory Activities using Molecular Docking and *in silico* ADMET Studies

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**Abstract**

Janus kinase (JAK) is a cytoplasmic non-receptor tyrosine kinase family that consists of four members: JAK1, JAK2, JAK3, and TYK2. JAKs transmit cytokine signaling through the JAK-signal transducers and activators of transcription (JAK-STAT) pathway, which regulates the transcription of numerous genes implicated in immunological, inflammatory, and cancer diseases. Among these kinases, JAK1 and JAK2 have been implicated in a variety of inflammatory disorders, including atopic dermatitis (AD), asthma, allergic rhinitis, and inflammatory bowel disease. Therefore, several small molecules with JAK1 and/or JAK2 inhibitory activity have provided therapeutic benefits in the treatment of these aforementioned diseases. In this regard, Pfizer has developed the small molecule abrocitinib (PF-04965842), which is being investigated for the treatment of psoriasis and AD. It is well-known that abrocitinib, when administered orally, specifically inhibits JAK1 (**Figure 1**).

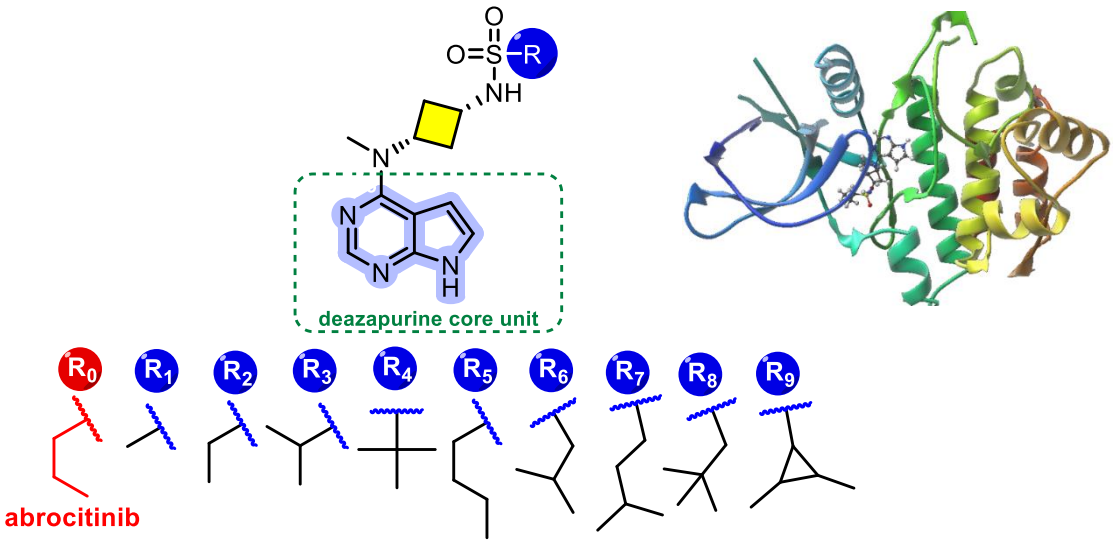


**Figure 1.** *(left)* Molecular structure of abrocitinib and a series of investigated abrocitinib derivatives *(right)* Binding pose of abrocitinib with JAK1 (PDB ID: 6BBU).

The primary purpose of this work was, therefore, to conduct an integrative computational investigation of experimentally determined abrocitinib metabolites (**Met**$_{1\text{-}7}$). Subsequently, we also focused to tailor the alkylsulfonyl scaffold (R) of the pristine abrocitinib and generating twenty new drug candidates (**Abr**$_{1\text{-}20}$). To this purpose, target molecules were rendered using GaussView 5.0.8, and geometry optimization studies were conducted using Gaussian 09 (DFT/B3LYP/6-31G(d,p)). Adsorption, distribution, metabolism, elimination, and potential toxicity (ADMET) (SwissADME, Petra-Osiris-Molinspiration (POM)) investigations were performed to evaluate their effectiveness and drug-likeness properties. Furthermore, all generated compounds (**Met**$_{1\text{-}7}$ and **Abr**$_{1\text{-}20}$) underwent molecular docking (SAMSON platform/2022-R2, OneAngstrom/AutoDock Vina Extension) to assess their potential inhibitory action against JAK1 (PDB ID: 6BBU) and JAK2 (PDB ID: 6BBV).

**Keywords:** Abrocitinib, Janus kinase, JAK1, JAK2, molecular docking, ADMET.

## *ORAL PRESENTATION*

# *Gundelia dersim* ekstrelerinin *in vitro* ve *in vivo* toksisitelerinin araştırılması

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**Özet**

İnsanlar tarafından tüketilen tıbbi bitkilerin çoğu, uzun zamandır geleneksel olarak kullanılmalarından dolayı güvenli olarak kabul edilmektedirler. Buna rağmen bitkiler, doğal toksik, mutajenik veya kanserojen bileşikler de üretebilirler. Bitkilerin yararlı biyolojik aktivitelerinin yanı sıra eğer genotoksik bir bileşeni varsa, DNA'ya müdahale ederek genetik bir hasara sebep olabilirler. Bu nedenle, bitkisel araştırmalarda birinci öncelik, bitkisel ürünlerin güvenilirliğinin belirlenmesidir. Tıbbi ve aromatik bitkiler sınıfında bulunan Asteraceae familyasına ait bitki ekstreleri ile yapılan çalışmalar farklı biyolojik aktivitelere sahip olduklarını göstermektedir. Bu familyaya ait olan *Gundelia* cinsinin çeşitli türlerinin de birçok hastalığın tedavisinde ve gıda maddesi olarak kullanıldığı bilinmektedir. *Gundelia dersim* bitkisinin ilaç hammaddesi olabilme özelliklerinin belirlenmesi ile ilgili literatürde yeterli çalışma bulunmamaktadır. Bu çalışmada *G. dersim* bitkisinin su, metanol ve etanol ekstrelerinin sağlıklı insan lenfosit hücreleri üzerindeki genotoksik ve antigenotoksik etkileri mikronükleus testi ile araştırılmış ve ekstrelerin *in vivo* toksisitesi *Drosophila melanogaster* üzerinde puparasyon, hayatta kalma ve eklosyon yüzdeleri hesaplanarak belirlenmiştir. Bu araştırma sonucunda *G. dersim* ekstreleriyle muamele edilen sağlıklı insan lenfosit hücreleri üzerinde önemli bir mikronükleus oluşumu gözlemlenmemiştir hatta $H_2O_2$ kaynaklı oluşan mikronükleusların bitki ekstreleri tarafından azaltıldığı görülmüştür. Bununla birlikte *G. dersim* ekstreleri *D. melanogaster*'in *in vivo* eklosyon sürecini etkilememiştir. Bu çalışmadan elde edilen sonuçlar doğrultusunda *Gundelia dersim* bitki ekstlerinin daha detaylı toksikolojik değerlendirmesi farklı model organizmalar üzerinde yapılarak literatüre sunulabilir.

**Anahtar Kelimeler:** *Gundelia dersim,* genotoksisite, mikronükleus, *Drosophila melanogaster*

## *ORAL PRESENTATION*

# Interaction of carbonic anhydrase isozymes I and II with antibiotic agent gatifloxacin

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**Abstract**

Metalloenzyme inhibition is a widely used technique for managing enzymatic activity in diverse biological systems with physiological relevance. The reversible hydration of carbon dioxide to bicarbonate and proton is catalyzed by carbonic anhydrases (CAs), known as metalloenzymes. Only twelve humans (*h*) isoenzymes are catalytically active and involved in significant activities, including pH balance, bone resorption, calcification, electrolyte secretions, gluconeogenesis, respiration, and tumorigenesis. In this study, the cytosolic isoform *h*CA I, which is distributed widely in erythrocytes and the gastrointestinal (GI) tract, was low-level inhibited by gatifloxacin, which is a fourth-generation fluoroquinolone, at micromolar concentration with $K_I$ of 13.07 ± 0.92 µM compared with the reference drug acetazolamide (AAZ, $K_I$ of 0.19 ± 0.01 µM). In the same direction, another isoform, *h*CA II, found mainly in the GI tract, eyes, kidney, bone osteoclasts, brain, lungs, erythrocytes, and testis, was also a weakly inhibited isoform by this drug, with $K_I$ of 11.93 ± 1.14 µM compared with AAZ ($K_I$ of 0.17 ± 0.01 µM). Additionally, the active sites of the *h*CA I and II isoforms were investigated using molecular docking experiments to determine the likely binding interactions and affinities for the gatifloxacin.

**Keywords:** Carbonic anhydrase, inhibition, molecular docking

## *ORAL PRESENTATION*

# Evaluation of albumin, protein and creatinine parameters in urine with total analytical error calculations

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**Abstract**

Total Analytical Error (TAE) has a significant place as a quality control indicator. TAE has two components: random error and systematic error. Repeatability is evaluated by internal quality control, %CV is obtained from internal quality control data and indicates random error. Moreover, bias is an expression of the distance of the analyte result from the actual value, bias% calculated from external quality control data and reflects systematic error. TAE is calculated from the bias% and CV% values with the computation formulated by Westgard. Allowable Total Error (TEa) is the maximum analytical error to be tolerated. The TAE should remain within the established TEa limit. In this study, we aimed to evaluate the TAE values of urinary albumin, protein, creatinine parameters according to Ricos criteria.
Urinary albumin, protein, creatinine analytes were measured in autoanalyzer (Roche, Cobas 8000 e602). TAE was calculated with the formula: TAE (%)= Bias(%) + 1.65*CV(%). Data from the RIQAS Monthly Human Urine external quality control program between October 2021 and October 2022 were analyzed retrospectively. Bias% was obtained with the formula: bias(%)= [(laboratory result-mean of peer group)/mean of peer group]*100. CV% values were calculated from September 2022 internal quality control results for each analyte using Roche Precinorm PUC, Roche Precipath PUC (Roche Diagnostics, Mannheim, Germany) control materials from the same lot.
The TAE values of urinary albumin, protein and creatinine were found to be within the TEa limits of Ricos. Additionally, the calculated %CV, %bias values did not exceed the desirable %CV and desirable %bias.
The calculation of TAE is a useful and easy analytical performance evaluation model.

**Keywords:** Total analytical error, total allowable error, analytical performance

## *ORAL PRESENTATION*

# Türkiye'de doğal olarak yayılış yapan Dar Yapraklı Dişbudak (*Fraxinus angustifolia* Vahl)'ın odun anatomik özelliklerinin yükseltiye bağlı değişimi

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**Özet**

Bu çalışmada Türkiye'de doğal olarak yayılış yapan Dar Yapraklı Dişbudak (*Fraxinus angustifolia* Vahl.) taksonlarının odun anatomik özelliklerinin yükseltiye bağlı değişimleri incelenmiştir. Odun örnekleri Türkiye'de 94 farklı lokaliteden ve 0-1600 m yükseltiler arasından doğal yayılışlı ağaçlardan alınmıştır. Hazırlanan preparatlar üzerinde trahe teğet ve radyal çapları, 1 $mm^2$ deki trahe sayıları, özışını yükseklik ve genişlikleri, 1 mm de ve 1 $mm^2$ de özışını sayıları, trahe hücre uzunluğu, lif uzunluğu, lif genişliği, lif lümen genişliği, lif çeper kalınlığı belirlenmiştir. Trahe hücre verileri kullanılarak vulnerabilite oranı (trahe teğet çapının birim alandaki sayısına bölümü) ve mezomorfi değeri (vulnerabilite oranı ile trahe hücre uzunluğunun çarpımı) hesaplanmıştır. Anatomik ölçüm ve sayımlar sonucu elde edilen verilerle yükseltiye bağlı korelasyon analizi yapılmıştır. Bu analiz sonucuna göre yükselti ile birim alandaki trahe ve özışını sayıları arasında pozitif ilişki, trahe (radyal ve teğet) çapları, lif ve trahe hücre uzunlukları, vulnerabilite oranı ve mezomorfi değeri arasında negatif ilişki bulunmuştur.

**Anahtar Kelimeler:** Odun anatomisi, Dar Yapraklı Dişbudak, Yükselti, Türkiye

## *ORAL PRESENTATION*

# Biosensors for on-site detection of pesticides

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**Abstract**

The rapid growth of the world's population is creating an increasing demand for food, agricultural products, and related resources. Regarding these demands, pesticides (insecticides, herbicides, fungicides, etc.) are being used by farmers to prevent pests and insects from damaging plants. On the one hand, they play an important part in current agricultural approaches since the extensive use of pesticides has undeniably enhanced food production. Pesticide residue accumulation on or in food could be extremely harmful to both the human body and the environment. Long-term pesticide residue exposure damages the human neurological system and immune system to varying degrees, eventually leading to a range of disorders and raising the risk of cancer. Thus, pesticide residue has become one of the most serious worldwide issues as a result of these dangers. All traditional pesticide detection and identification techniques have high reliability, selectivity, sensitivity, and low detection limits, applying them is time-consuming, costly, and labour-intensive. Many researchers have recently developed rapid-sensing techniques and portable devices for pesticide detection. Among them, biosensors, as compact analytical instruments (with a bio-recognition element and signal transducers), appear to be ideal for the on-site detection of pesticides. Biosensors' success can be attributed to their distinguishing benefits of fast or rapid analysis, high sensitivity, and no requirement for the specific skill of operation that standard analytical processes necessitate. Pesticide residue detection by on-site sensing technologies is thought to be the foundation of future pesticide mitigation. Although significant progress has been achieved in the use of biosensors, there is still a need for research development and refinement, particularly in translating the majority of previously published laboratory studies into portable on-site and implementable in the public domains. Herein, a detailed summary of the most recent research developments on sensing techniques and portable devices for the detection of pesticides is presented.

**Keywords:** pesticide detection, biosensors, on-site sensing, food safety.

## *ORAL PRESENTATION*

# Tıbbi Laboratuvardaki Biyolojik Risk Etmenlerin Değerlendirilmesi

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**Özet**

Çalışma ve Sosyal Güvenlik Bakanlığı tarafından hazırlanan biyolojik etkenle etkileşim sonrasında oluşabilecek risklerin önlenmesi ile ilgili yönetmelikte biyolojik etkenler "enfeksiyon, alerji veya zehirlenme gibi sonuçları olan, genetiği değişime uğramış olanlar da dâhil mikroorganizmalar, hücre kültürleri ile insan endoparazitleri" olarak tanımlanmıştır. Sağlık sektöründe bulunan çalışma ortam koşulları, risk ve tehlikeler dikkate alındığında biyolojik risk faktörlerine çalışanların sıklıkla maruz kaldığı görülmektedir. Tıbbi laboratuvarların çok tehlikeli işkollarında yer alması ile birlikte çalışma sürelerinin uzun olması ve bu laboratuvarlarda aralıksız hizmetin olması sebebiyle oluşabilecek tehlike ve risklere karşı çalışan sağlığının korunması büyük önem arz etmektedir. Bakanlığa göre biyolojik etkenler 4 risk grubunda incelenmektedir. İlgili mevzuatta gruplar insanda enfeksiyona neden olma ihtimallerine göre gruplandırılmıştır. Tıbbi laboratuvar çalışanları için başlıca mesleki riskler arasında enfeksiyon ve bulaşıcı hastalıklar gelmektedir. Çalışanların virüs, parazitler, mantarlar, riketsiya, bakteriyel mikroorganizmalar veya genetiği değiştirilmiş organizmalar gibi patojenik ajanlara maruziyeti bulunmaktadır. Tıbbi laboratuvar ortamlarında çalışanlar için bir çok risk içeren görev bulunmaktadır. Ayrıca etkileşimlerin neden olabileceği tehlikeli durumlar da mevcuttur. Biyolojik etkenler; solunum yolu, deri/mukoza yolu ve sindirim yolu ile vücuda etki ederek olumsuz sonuçlar doğurabilmektedir. Tıbbi laboratuvarda santrifüjleme, vorteksleme, kültür ekimi, kesici delici alet, pipet kullanımı, kontamine materyaller, enfeksiyöz materyallerin dökülmesi ve saçılması vb. diğer taraftan çalışanların güvensiz davranışları çalışma ortamında bulunan doğrudan ve dolaylı biyolojik tehlike unsurlarındandır. Bu derleme çalışmasında, tıbbi laboratuvar ortamında çalışanların kullanılan yötem, teknik ve ekipmanlar kaynaklı maruz kaldığı biyolojik riskler araştırılmıştır.

**Anahtar kelimeler:** Biyolojik Risk Etmenleri, Tıbbi Laboratuvar, İş Güvenliği, İş Sağlığı.

## *ORAL PRESENTATION*

# The effects of microwave drying on kefir biomass increase and fermentation kinetics

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**Abstract**

Drying is the process of removing water (dehydration) from solids in order to prevent microbial growth and chemical reactions that may cause food spoilage. Convective drying, vacuum drying, freeze drying, and microwave drying are among the most used methods for drying foods. In the drying of cultures containing live bacteria and yeast, it is important that the bacteria and/or yeast in the culture can become active again and fulfill their functions after the drying process. Kefir is a fermented dairy product with a slightly acidic/sour taste and distinctive aroma. It contains a special mixture of microflora symbiotically contained in a carbohydrate polysaccharide matrix. It contributes positively to human health due to its antimicrobial, antitumoral, antiviral, antimutagenic, and antioxidant effects. Kefir grains are starter cultures containing various lactic acid bacteria, acetic acid bacteria, and yeasts used in kefir production. Besides the production of kefir beverages, it is a valuable culture used in bread making, cheese production, ethanol production, and obtaining aroma compounds. In this study, the kefir grains were dried at different microwave powers, and the effect of microwave output power on drying, rehydration, and fermentation kinetics and biomass increase of kefir grains were examined. The results showed that the drying times decrease significantly with the increase of microwave power. It was seen that the initial drying rates are higher at high microwave powers and the drying process of kefir grains exhibits a decreasing rate of drying period with the decrease of moisture content at all microwave powers. The highest biomass increase over the 10 days fermentation with 24 hours periods was obtained for the kefir grains dried at 100 W.

**Keywords:** Drying, kefir, biomass increase, fermentation, kinetics

## *ORAL PRESENTATION*

# 1,2,4-TMB ve Toluenin transalkilasyonu ile ksilen üretimine Klinoptilolit/ZSM-5/Beta üçlü zeolit katalizörün etkisi.

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## Özet

Son yıllarda çevresel baskılardan dolayı benzindeki benzen ve aromatik oranının düşürülmesi benzine katılan petrokimya sanayi reforming ünitesinden elde edilen ağır reformatın bir kısmı kullanım dışı kalmış böylece arz fazlası oluşmuştur. Bu ağır reformatın içinde önemli oranda 1,2,4-Trimetil Benzen (1,2,4-TMB) bulunmakta ve değerlendirmesi önem arz etmektedir. Bu ürünün en iyi değerlendirilmesi tolüen ile trnasalkilasyona sokularak ticari değeri yüksek ksilenlerin (özellikle p-ksilen) üretilmesidir ve bu konu üzerine yoğun çalışmalar yapılmaktadır. Toluenin 1,2,4-trimetilbenzen (124-TMB) ile transalkilasyon işlemi, aktif ve stabil bir katalizör üzerinden ekonomik olarak ksilen üretmek için bu tür düşük değerli aromatikleri dönüştürmek için faydalıdır. Reforming ünitesinden alınan arz fazlası ağır reformat bileşeni 1,2,4-TMB'den ksilen gibi değerli aromatiklere dönüştürülmesi üzerine literatürde farklı zeolit katalizörler araştırılmıştır. Buradan elde edilen ksilenin verimini artırmak için transalkilasyon reaksiyonları Klinoptilolit-ZSM5-Beta üçlü zeolit katalizörünün nasıl bir sonuç verdiği araştırılmamıştır. Bu çalışmada, Klinoptilolit-ZSM-5-Beta üçlü zeolit katalizörü üzerinde 1,2,4-TMB ve Toluenin transalkilasyonu ile ksilen üretimi amaçlanmıştır. Bu amaç doğrultusunda Klinoptilolit-ZSM-5-Beta üçlü zeolitinde 1:1:1 oranında alınarak bir katalizör hazırlanmış ve sabit yataklı sürekli bir sistemde 1,2,4-TMB ile toluenein transalkilasyonunda kullanılmıştır. Sonuç olarak, deneysel tansalkilasyon reaksiyonu sonucunda elde edilen sıvı ürünlerin GC-MS (Gaz Kromatografisi-Kütle Spektrometrisi) analizleri yapılmış, ürünlerin zeolit oranı, sıcaklık ve boşluk hızı ile değişimi incelenmiştir.

**Anahtar Kelimeler:** 1,2,4-TMB, transalkilasyon, p-ksilen, alkil aromatikler, zeolit katalizörler.

## *ORAL PRESENTATION*

# Investigation of the effect of new generation antioxidant combination (Carvacrol and Rosmarinic acid) in chronic wounds with different application methods

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**Abstract**

The process of restoring tissue integrity following tissue destruction is defined as wound healing. Delayed wound healing or the formation of non-healing wounds as a result of the wound healing process being affected by factors such as diabetes and increased oxidative stress adversely affect human life. In this study, we aimed to reveal whether these components have a synergistic effect on the oxidative events occurring during the healing process of diabetic wounds by using Carvacrol and Rosmarinic acid, which have natural antioxidant activities, with both topical and intraperitoneal applications. This experiments were performed with 72 male Wistar-albino diabetic rats with streptozotocin-induced and they were divided into five groups: control group, untreated, carbopol 974P treatment group, topical treatment group, and intraperitoneal treatment group. Excisional skin wounds were made with full thickness punch biopsy (6 mm) in all animals except the control group. In the treated groups, wounds were treated by topical application with 2% carbopol 974P gel containing Carvacrol and Rosmarinic acid (10 mg/kg) and other treated groups were treared intraperitoneally with Carvacrol and Rosmarinic acid (10 mg/kg). In the groups treated with 2% carbopol 974P, the wounds were treated topically with an equal amount of carbopol gel. Afterwards, animals were sacrificed on the 3rd and 7th days of wound healing, and the levels of malondialdehyde (MDA), glutathione (GSH) were measured spectrophotometrically in the wound tissues. As a result, the treated and untreated groups were compared, it was determined that the applications of Carvacrol and Rosmarinic acid in rats (on the 3rd and 7th days) decreased the MDA levels of the scar tissue compared to the untreated group ($p<0,05$). It has been determined that both topical and systemic applications of carvacrol and rosmarinic acid increase the antioxidant capacity of the wound tissue and reduce the lipid peroxidation.

**Keywords**: Oxidative stress, Diabetic wounds, Carvacrol, Rosmarinic acid, Glutathione

**Acknowledgement:** This research was supported by Gazi University Scientific Research Projects (BAP), Ankara, Turkey (Project Number: FYL-2022-7703)

## *ORAL PRESENTATION*

# The Factors Affecting Kidney of Fish

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**Abstract**

Nowdays, total fisheries and aquaculture productions have been quiet developed within 40 years on the World. They have reached approximently 214 million in 2020. Water is an important component of fish production and water resources have been contaminated. Gradually, reservoirs, streams, rivers, lakes, seas and oceans are influenced by antropogenic activities on the Earth. Pollution increases in all bodies of water resources. Water pollution causes pathological changes in fish. These exposures are depended on concentrations and time duration. Species may be affected with viseral orgas and internal functions by lethal exposure. The functions and structures could be influenced by chronical exposures. It affects individual resistance, reproduction, growth performance, condition factors, etc (Duft et al., 2003; Hampel et al., 2008). The chemicals in water could affect viseral organs (liver, kidney, spleen, intestines, gonads). Particularly, kidney is vital organ for fish. Pollution cause some important damages on the fish kidney. Some xenobiotic (industrial wastes, sewages, heavy metals, pesticids, PCB, etc) could contaminate aquatic products, included fish. Pollution effects could be seen with some histological alternations (hypertrophy, hyperemy, vacuolization, necrosis). The great avantages are to use histological alternation as biomarkers for environmental monitoring. These kinds of markers have allowed examining water quality in this way (Thophon et al., 2003; Gernhofer et al., 2001). In this study, it was given more detail information about the mechanism, functions of kidney and interaction between kidney and pollution factors in water. It was indicated some previous studies were done about it.

**Key Words:** Fish, Kidney, Pollution, Heavy metal, Pesticids

## *ORAL PRESENTATION*

# Some Phytochemical Properties of *Spiraea japonica* var. *fortunei*

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**Abstract**

*Spiraea japonica* var. *fortunei* is a perennial plant widely distributed throughout Eastern Asia. It has been used in Chinese traditional medicine for various purposes. In the current study, total phenolic content, antioxidant activity, and flavonoid contents of *Spiraea japonica* var. *fortunei* were determined. For this purpose, a comparative study was performed using methanol and ethanol extracts from both leaves and the flowers of the plants. The total phenolic content was determined using the Folin Ciocalteau method, and flavonoid content with the Aluminum chloride colorimetric method. The total phenolic contents of the ethanol and methanol extracts of the flowers were 11.08±0.13 and 19.33±1.24 mg GAE(Gallic Acid Equivalent)/g dry matter, respectively. On the other hand, total phenolic contents were significantly higher in the leaves, which were determined as 18.13±1.61 and 25.64±0.32 mg GAE/g dry matter in the ethanol and methanol extracts, respectively. A similar trend has been observed with the flavonoid contents which was almost doubled in the leaves (11.62±0.74 mg QE/g dry matter) compared to the flowers (5.67±0.66 mg QE/g dry matter). Antioxidant activity was determined as 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity and it was found that there was no significant difference in the antioxidant properties of flowers and leaves either using ethanol or methanol.

**Keywords:** *Spiraea japonica* var. *fortunei*, plant extract, total phenolics, DPPH, flavonoids

## *ORAL PRESENTATION*

# Effect of uropathogenic *E. coli* on the progress of renal cell cancer

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**Abstract**

Studies have shown that the risk of infection increases with the collapse of the immune system in cancer cases, while the death and development of various cancers are affected by infectious agents. Uropathogenic *Escherichia coli* is a pathogen that is effective in the vast majority of urinary tract infections and infectious complications, including cystitis and pyelonephritis. This bacterium causes critical conditions that can result in acute kidney failure in both healthy individuals and kidney transplant patients. Kidney cancer accounts for approximately 3% of all adult malignancies and is the twelfth most common cancer in the world. History of urinary tract infection is accepted as an independent risk factor for the development of bladder cancer, and such a history may also play a role in the development of renal cell carcinoma. However, to date, limited data have been reported on whether a history of urinary tract infection increases the risk of developing renal cancer and how it affects the development of cancer. The GSE162250 dataset was used in our study. Using this dataset, differentially expressed genes were detected as a result of the treatment of renal cancer cells with Uropathogenic *Escherichia coli*. The data were analyzed in two groups: renal cancer cells and Uropathogenic Escherichia coli-treated renal cancer cells using various statistical analyses (DESeq2, hierarchical clustering, network analysis, and pathway analysis) after normalizing with the RMA method. Eight genes with highly differential gene expression detected in renal cancer cells treated with uropathogenic *Escherichia coli* were examined within the scope of the study. Within the scope of the congress, a detailed discussion will be made of how the results obtained in this study and the determined genes affect the kidney cancer of Uropathogenic *Escherichia coli* infections.

**Anahtar Kelimeler:** Uropathogenic *Escherichia coli*, renal cell carcinoma, gene biomarkers, transcriptomic analysis

## *ORAL PRESENTATION*

# Farklı üzengi kemik protezlerinin modellenerek ses iletim performanslarının mevcut ticari protezlere göre değerlendirilmesi

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**Özet**

Orta kulakta yer alan çekiç, örs ve üzengi kemikçikleri sesin iç kulağa aktarılmasını sağlayan önemli yapı taşlarıdır. Kronik orta kulak iltihabı, kolesteatoma varlığı, otoskleroz, akut ya da timpanosklerosiz gibi enflamatuar süreçler orta kulak kemikçiklerinde hasar meydana getirerek sesin iç kulağa iletilmesinde sorun oluşturmaktadır. Sözü edilen hastalıklarda operasyonla kemikçik zincire müdahale edilmekte ve çoğunlukla üzengi kemiği alınarak yerine protez yerleştirilmektedir. Protez üretiminde, malzeme seçimi, modelin kişiye uygunluğu, mekanik yapı gibi birçok faktör rol oynamaktadır. Anatomik olarak örs ile üzengi kemikçikleri arası mesafenin her bireyde farklılık göstermesi, üzengi taban alanına belli bir çapta delik açılarak protezin yerleştirilmesi, belirlenen protezin operasyon esnasında yerleştirilme kolaylığı gibi birçok faktör yeni tür protezlerin modellenmesi ihtiyacını ortaya çıkarmıştır. Bu nedenle çalışmamızda, farklı üzengi kemik protezlerinin modellenmesi ve ses iletim performanslarının mevcut ticari protezlere göre değerlendirilmesi amaçlanmıştır. Çalışmada protez tasarımları, modellenmeleri ve analizleri Solidworks ve Ansys programları kullanılarak gerçekleştirilmiştir. Çalışma kapsamında üç farklı çap ve uzunluk belirlenerek üç ayrı protez modelinde bu çap ve uzunlukların değerlendirilmesi yapılmıştır. Ayrıca hazırlanan her bir protez modeli için dört farklı malzeme seçilerek gerçek kulak modeli ile birlikte Ansys programında harmonik analizleri yapılmıştır. 100 Hz ile 10 kHz aralığındaki frekans değerlerinde çalışılmıştır. Analizde modellenen kulağın timpanik membran kısmından anlık ses basınç seviyesi 90 dB olan yaklaşık 0.632 Pa'lık bir basınç uygulanarak üzengi taban alanının yer değiştirmesi ölçülmüştür. Ansys programında gerçekleştirilen harmonik analiz yöntemlerinden frekans tepkisi ve hız değerlerine karşılık gelen genlik ile sesin iletim hızının grafikleri oluşturulmuştur. Bu çalışmada tasarlanan tüm üzengi kemik protezinin taban alanında titreşim sonucu oluşacak etkilerin frekans yanıtı ile sesin iletimindeki hız dağılımları gerçek kulak modeli ile kıyaslanarak mevcut ticari protezlerle işitme üzerindeki performansları değerlendirilmiştir. Çalışmanın sonucunda, her bir protez modelinin farklı çap ve uzunluk değeri ile farklı malzeme özelliklerinde uygunluğu görülmüştür. Ayrıca üzengi kemiği yerine yerleştirilen protezin işitmede kabul edilebilir olduğu sonucuna varılmıştır.

**Anahtar Kelimeler:** Orta Kulak, Protez, Biyomalzeme, ANSYS, SolidWorks, Harmonik Analiz

## *ORAL PRESENTATION*

# Investigation of Total Phenolics and Flavonoid Contents of *Polygala major* Using Different Solvents

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**Abstract**

*Polygala major* is a xero-xeromesophile, subthermophilic and oligotrophic species, as well as being a common species in meadows and shrubs starting from the steppe. In this study, the presence of polyphenols and flavonoids in the *Polygala major* was investigated. For this purpose, three different solvents (ethanol, methanol, and acetone) were used for the extraction of both the leaves and the flowers. Total phenolic contents of the plant were varied between 30.42 and 41.74 mg GAE/g sample, and the highest content was detected in the leaf extract of ethanol. On the other hand, the highest flavonoid was obtained with the leaf extract of acetone. The total phenolic and flavonoid contents of the leaf were higher than the flower in all the conditions, which indicated that the leaves of the plant is richer in phytochemicals than the flowers. Furthermore, the presence of polyphenols and flavonoids were also confirmed by using UV-VIS and FTIR.

**Keywords:** *Polygala major*, plant extract, total phenolics, flavonoids

## *ORAL PRESENTATION*

# Use of kaolin clay as cleanup adsorbent and determination of organochlorine pesticides in kiwi by GC-ECD

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**Abstract**

Pesticides which accelerate the growth of agricultural products and protecting them from diseases and pests are natural or synthetic substances. Due to their unconscious and excessive use, one of the pollutants and chemicals that threaten public health is pesticides. There is a need for accurate, fast and reliable analytical methods for pesticide determinations in very complex sample matrix such as food samples. Improper sample preparation methods can cause losses in the analyte as well as an increase in the analyte peaks and which may cause incorrect quantification. Hence, sample preparation steps have a great importance.
A cleanup procedure based on kaolin clay followed by GC-ECD method for the simultaneous determination of organochlorine pesticides was developed. Firstly, the type and volume of extraction solvent were optimized. Then the performances of kaolin, silicagel, florisil and commercial SPE cartridge were compared in the cleanup procedure. It has been observed that 0.4 g kaolin adsorbent with 15 mL of acetonitrile extraction solvent is as effective and efficient as SPE cartridge for the removal and separation of interfering substances in the sample. The method was validated on kiwi spiked at the concentration of 15, 50 and 90 μg $kg^{-1}$ with five replicates. The recoveries of pesticides ranged from 70% to 120%, with relative standard deviations lower than 20%. Good linearity ($R^2 \geq 0.996$) was obtained at the range of 10–100 μg/L for all pesticides in the selected matrice. The method was successfully applied to the routine monitoring of pesticide residues in market samples.

**Keywords:** Pesticide residue analysis, organochlorine pesticides, clean-up, adsorbent, kaolin, GC-ECD.

**Acknowledgements:** This study was supported by Yildiz Technical University Scientific Research Coordination Unit (Project No. FYL-2022-4872).

## *ORAL PRESENTATION*

# Effect of Plasticizers on Soluplus® filaments Produced with Hot Melt Extrusion Technique

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**Abstract**

Soluplus® is an amphiphilic polymer which exhibits excellent solubilizing properties for BCS class II drugs [1]. Fused Deposition Modelling (FDM) is one of the most common and economic 3D printing techniques for pharmaceutical research studies. In the FDM method, a mixture of active pharmaceutical ingredients and excipients are extruded with hot-melt extrusion (HME) technology to obtain filaments appropriate for 3D printing. HME technologies can also enhance the solubility and dissolution of poorly soluble drugs [2]. The aim of this study is to obtain a suitable filament for printing in FDM. For this purpose, Soluplus® filaments were obtained using HME technology. The effects of five different plasticizers (Triacetin, Tween® 80, Plucare® E 300, Polyethyleneglycol 1000 and Polyethyleneglycol 4000) in different ratios were investigated to obtain Soluplus® filaments. The temperature required to obtain the filaments ranged from 110 to 130 °C. The extruder speed was set to 15 rpm for all filaments. When Polyethyleneglycol 4000 was used as plasticizer, filament could not be obtained. All other plasticizers promoted filament formation. While the Soluplus® filament obtained without the use of plasticizers was brown, the filaments obtained using the plasticizer were transparent. All obtained filaments were evaluated in terms of diameter (mm), breaking force (g) and flexibility (mm). While the diameters of the filaments were required to be 1.75 ± 0.1 mm for printing in FDM, the diameters of the obtained filaments varied between 1.51-2.12 mm. Triacetin was found to be more effective than other plasticizers for a suitable filament.

**Acknowledgments**

This study was supported by the Gazi University, BAP Council (Project Number: TDK-2022-7536). A. Yılmaz was supported by scholarships from the CoHE 100/2000 PhD Scholarship Program and by TUBITAK 2211/A Domestic PhD Scholarship Program.

**Keywords:** Hot melt extrusion, Soluplus®, Plasticizers, Triacetin

**References**

1. Shamma, R.N. and M. Basha, Soluplus®: a novel polymeric solubilizer for optimization of carvedilol solid dispersions: formulation design and effect of method of preparation. Powder technology, 2013. **237**: p. 406-414.
2. Saydam, M. and S. Takka, Improving the dissolution of a water-insoluble orphan drug through a fused deposition modelling 3-Dimensional printing technology approach. Eur J Pharm Sci, 2020. **152**: p. 105426.

## *ORAL PRESENTATION*

# *cis/trans*-Diklorodispirosiklotrifosfazen türevlerinin sentezi, spektroskopik ve kristallografik özellikleri, biyolojik aktivite çalışmaları

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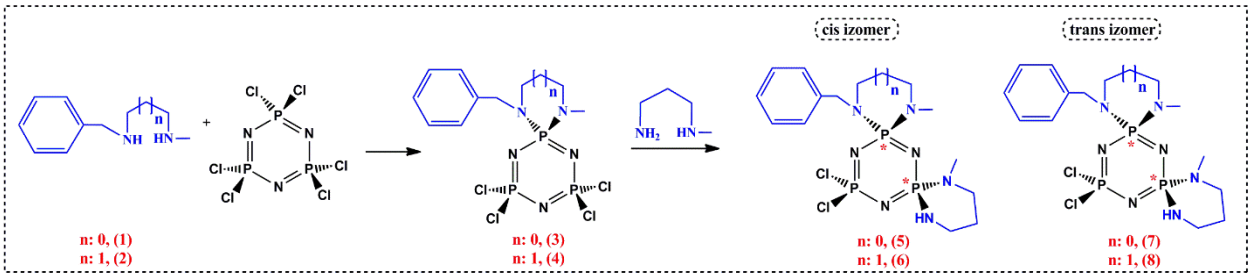
## Özet

Fosfazen bileşikleri ($-X_2P=N-)_n$ (X: F,CI, Br, organik gruplar…), fosfor ve azot atomlarının birbirlerine bağlanması ve bu birimlerin tekrar etmesi ile oluşan düz zincirli, halkalı veya polimerik yapıda bulunabilen heterosiklik türevlerdir. Özellikle halkalı fosfazen sınıfında yer alan hekzaklorosiklotrifosfazen ($N_3P_3Cl_6$; trimer; **HCCP**) çok yönlü reaktivite, termal kararlılık ve özellikle biyo-uyumluluk gösterdiğinden biyolojik olarak etkin kiral inorganik-organik hibrit bileşiklerin sentezinde öncü bileşik olarak kullanılmaktadır [1-3]. Seçilen sübstitüente bağlı olarak kimyasal ve biyolojik açıdan farklı özellikler sergileyen fosfazenler sentezlenebilmektedir [4-7].
Bu çalışmada **HCCP**, hibrit bileşiklerin sentezlenmesinde başlangıç molekülü olarak kullanılmıştır. Çalışmada ilk olarak iki farklı benzildiamin ile trimerin nükleofilik sübstitüsyon reaksiyonu sonucunda monospirosiklotrifosfazenler sentezlenmiştir. Bunun için öncelikle N-metil-1,2-diaminoetan ve N-metil-1,3-diaminopropan ile benzaldehitin etkileştirilmesinden monobenzildiaminler (**1** ve **2**) elde edilmiştir. Daha sonra **1** ve **2** bileşiklerinin trimer ile reaksiyonundan tetrakloromonospirofosfazenler (**3** ve **4**) sentezlenmiştir. Monospirofosfazenlerin, N-metil-1,3-diaminopropan ile 1:1 stokiyometrik orandaki tepkimelerinden simetrik olmayan dispirofosfazenler (**5**-**8**) hazırlanmıştır. Dispirofosfazenlerin, spiro halkalarındaki N-$\mathbf{CH_3}$ gruplarına bağlı olarak cis/trans geometrik izomerleri halinde oluştuğu tespit edilmiştir. Sentezlenen yeni bileşiklerin yapıları MS ve tek boyutlu NMR spektroskopi teknikleri ($^1$H, $^{13}$C, $^{31}$P NMR) ile bulunmuştur. Uygun tek kristali elde edilen iki adet dispirofosfazen türevinin kristal yapısı X-ışını kırınımmetre yöntemi ile aydınlatılmıştır. Ayrıca, bileşiklerin antimikrobiyal aktiviteleri agar kuyucuk yöntemi ile ve plasmid-DNA ile etkileşimleri ise agaroz jel elektroforez metodu ile incelenmiştir. Antimikrobiyal aktivite sonuçlarından (MİK değerleri) yola çıkılarak tüm bileşiklerin *P. Vulgaris* ve *P. Aeruginosa* bakterilerine karşı referans ilaçlardan daha etkin olduğu bulunmuş ve **6** bileşiğinin ise *C. Albicans* mantarına karşı referans ilaç ketokonazolden daha etkin olduğu belirlenmiştir.




**Anahtar kelimeler:** Dispirofosfazenler, trimer, spektroskopi, kristallografi, biyolojik aktivite.

**Bu çalışma, 221Z311 numaralı Tubitak projesi ile desteklenmektedir.**

## Referanslar

**1**. İşcan Ö, Cemaloğlu R., Asmafiliz N., Zeyrek C. T., Kılıç Z., Açık L, Aydın B., Türk M., Hökelek T., *Molecular Diversity*, **26,** 1077–1100, 2021.
**2**. Kuzey N. G., Cemaloğlu R., Özgür M., Asmafiliz N., Kılıç Z., Aydın B., Açık L., Hökelek T., *Research on Chemical Intermediates*, **47**, 3933–3962, 2021.
**3**. Cemaloğlu R., Berberoğlu İ., Asmafiliz N., Kılıç Z., Hökelek T., *Journal of. Molecular Structure*, **1269**, 133866, 2022.
**4**. Kwon N., Kim K. H., Park S., Cho Y., Park E. Y., Lim J., Çetindere S., Tümay S. O., Kim W. J., Li X., Nam K. T., Kim C., Yeşilot S., Yoon J. *Biosensors and Bioelectronics*, **216**, 114612, 2022.
**5**. Usri S. N. K., Jamain Z., Makmud M. Z. H. *Polymers*, **13(17)**, 2916, 2021.
**6**. Beytur A., Tekin Ç., Çalışkan E., Tekin S., Koran K., Görgülü A. O., Sandal S. *Bioorganic Chemistry*, **127**, 105997, 2022.
**7**. Asmafiliz N., Civan M., Özben A., Kılıç Z., Ramazanoğlu N., Açık L., Hökelek T. *Applied Organometallic Chemistry*, **32(4)**, e4223, 2018.

## *ORAL PRESENTATION*

# Covid-19 tanısı alan hastalarda IL-10 gen polimorfizminin etkisi

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**Özet**

Amaç: 2019'dan bu yana tüm dünyada yaygınlaşan COVID-19'a SARS-CoV-2 virüsü neden olmaktadır. SARS-CoV-2 virüsüne bağlı ARDS, sitokin fırtınalarına, çoklu organ yetmezliğine, sepsise ve tromboza neden olabilmektedir. Sitokin fırtınasında, TNF-alfa;, IL-1beta;, IL-6, IL-10, IL-12, IL-15, IL-18, IL-23, IL-27, IFN-alfa, IFN-beta gibi sitokinler, yüksek miktarda ve aniden ortama salınmakta ve bu durum morbidite ve mortalite de artışa neden olmaktadır. Bu çalışmada sitokin fırtınası sırasında ortama salınan interlökinlerden IL-10 1082 ile polimorfizminin klinik üzerindeki etkisini ağır klinik bulgularla ve ayakta geçiren grup üzerinde çalışılmıştır.
Bu amaçla çalışmaya Tokat Devlet Hastanesine başvuran covid-19 (RT-PCR sonucu + gelen) tanısı alan toplam 94 hasta dahil edildi. Hasta grupları 42 hafif seyirli, 38 yoğun bakım ihtiyacı olan ve 14 orta seyirli servis hastası olarak gruplandırıldı. Çalışma gruplarından alınan kan örneklerinden DNA izolasyonu yapılarak Arms Polimeraz Zincir Reaksiyonu (PZR) ile gen polimorfizminin allel tipleri belirlendi. Verilerin istatistiksel analizi için, SPSS 16.0 paket programı kullanıldı. Covid-19 hasta ve kontrol gruplarında IL10 1082 A/G gen polimorfizminin dağılımı chi-square testi ile karşılaştırıldı. p değeri ≤ 0,05 olarak tespit edildiği için sonuçlar istatistiksel olarak anlamlı kabul edildi.
Çalışma sonuçlarımıza göre IL10 1082 A/G gen polimorfizmi ile covid-19 tanısı alan hasta gruplarında hastalık şiddeti açısından aralarında genotip dağılımı ve allel sıklığı bakımından istatistiksel olarak anlamlı bulunmuştur(p=0,000). Bu sonuçlar ışığında; IL10 1082 A/G gen polimorfizminin covid-19 hastalık şiddetinde güçlü bir etkisi olduğu tespit edilmiştir. Bununla birlikte elde edilen sonuçların daha büyük örneklem grupları ile desteklenmesi gerekmektedir.

**Anahtar kelimeler:** COVID-19, IL-10, gen polimorfizmi

## *ORAL PRESENTATION*

# Preparation of new generation materials to be used in the polishing of precious metals and examination of their properties

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**Abstract**

Jewellery is the art of producing all kinds of jewelry or ornaments by processing precious metals and stones and transforming these products into works that are used as investment tools after processing. When it comes to jewelry, gold comes to mind first. However, in the jewelry sector, other precious metals such as silver and non-mineral raw materials or their complex forms are used as raw materials other than gold. The jewelery industry is a sector that has been developing since ancient times and is advancing with modern production techniques. Today, the formation adventure of a jewelry takes place in four main steps. These; designing the product, modeling, manufacturing (melting, casting, molding… etc.), polishing. The polishing process, which has a very important place in the jewelry sector, is done with many techniques. Today; polishing, masking and polishing are made with abrasive material and polishing liquid placed in the drum, which is expensive. In the early days, maskal machines were used for this purpose. Maskal machines are vibration-based mechanical polishers that contain symmetrical (flat, oval) balls. However, the long application time of this method has led to the development of different techniques. Today, drum machines, which are generally costly, are used. In both techniques (mascal, drum) it constitutes the first step of the polishing process.




In this study; the spongy combination of the walnut shell, which has a special porous structure, provides gold, silver, platinum, copper, etc., thanks to the organic compounds it contains. The most important point here is to reveal the porous structure of the walnut shell. In this, the sintering process is very important. Within the scope of the study, this material was first prepared correctly. The second important point is that a chemical paste was prepared in order to increase the effect of this mechanical friction and to obtain the blue glow on the surface. In this way, the local heat increase caused by mechanical friction is distributed on the surface and an equal saturation is obtained. Analytical and spectroscopic characterization of the prepared walnut shell and chemical paste was carried out with various techniques. In particular, the polishing effects on 22 and 18 carat gold were investigated.

**Keywords:** Walnut, polishing, jewellery

## *ORAL PRESENTATION*

# Acid treated halloysite nanotube/carbon composites as an adsorbent for the removal of heavy metals from wastewater

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**Abstract**

Nowadays, water pollution is seen as one of the global problems. The uncontrolled release of chemicals to water resources has become an important problem for both the environment and human health due to the rapid industrialization. Heavy metals, one of the major concern for environment, leads to contamination in water, soil and air cause toxic effects when they exceed certain limit values. They can also cause health problems by affecting the psychological structure even at low concentrations. Clays are one of the adsorbents used to remove heavy metals from water. In this study, acid treated halloysite nanotube/carbon (HNT/C) composites were prepared with two different carbon sources (fructose and carboxy methylcellulose) by using hydrothermal process, and used to remove Cu(II), Pb(II), Zn(II) ions from the water. These adsorbents were characterized by FTIR, XRD and elemental analysis. Also, the relationship between the amount of adsorbent and the metal concentration was examined, and it can be said that increasing the amount of adsorbent increases the metal adsorption efficiency. It has been concluded that the prepared composite materials are cheap, effective and environmentally friendly adsorbents for metal removal.

**Keywords:** water pollution, low-cost adsorbents, heavy metal removal, adsorption, wastewater treatment

## *ORAL PRESENTATION*

# Isotherm, kinetic and thermodynamic studies of malachite green adsorption using peanut husk adsorbent

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**Abstract**

In this study, adsorbent prepared from peanut husk as an agro-waste was used for removal of malachite green (MG) from aqueous solutions by batch adsorption process. Effects of initial dye concentration, solution temperature and contact time on adsorption were studied. It was seen that the removal of malachite green increased with increasing initial dye concentrations (10, 30, 50 and 70 mg/L), three solution temperatures (298 K, 308 K, 328 K) and contact time (from 5 to 300 min). Removal percentage of 50 mg/L initial MG dye adsorption on peanut husk adsorbent increases from 61.23 (%), 66.24 (%) and 75.64 (%) for 298 K, 308 K and 328 K, respectively. Adsorption isotherms were analyzed by Langmuir, Freundlich and Temkin models at different temperatures, and Freundlich model shows more suitable results for removal of malachite green. Adsorption kinetics were analyzed by pseudo first order (PFO), pseudo second order (PSO) and intraparticle diffusion (IPD) models for different temperatures and initial dye concentrations. Kinetic studies was best fit the PSO model which kinetic constants were higher than PFO and IPD models. Moreover, the thermodynamics parameters of adsorption were determined. It was found that the values of Gibbs free energy (ΔG) were negative and enthalpy (ΔH) and entropy (ΔS) values were found to be positive for this removal process. Thermodynamic results were shown that separation process have spontaneous nature and these values suggest feasibility of adsorption. The results of this study showed that prepared adsorbent which peanut husk waste could be employed as effective and low-cost materials for the removal of dyes from aqueous solution.

**Keywords:** Adsorption, malachite green, peanut husk, isotherm, kinetic, thermodynamic

## *ORAL PRESENTATION*

# Investigation of Anti-Corrosive Properties of CrNi Alloy on Carbon Steel Surface

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**Abstract**

Steel is an important alloy, that has been used for various purposes since the beginning of industrual history and cannot be abandoned even today due to the superior properties of the metal industry, which is one of the trivets of industries and states [1]. The dependence of industries and states on metals inevitably brings along the problem of corrosion. Corrosion, which can be summarized as the wear of metals, causes serious financial and technical damage to industries, and states every year. For this reason, many researchers are developing methods such as electrochemical coatings to reduce the damage caused by corrosion. It has been previously reported in the primary literature that the CrNi alloy, which consists of elements whose passive layers are very durable, is quite durable in the chloride environment [2], [3]. In this study, the anti-corrosive resistance of the CrNi alloy which coated on the carbon steel (CS) electrode surface, were investigated in artificial seawater [4] .

Chromium-glycine bath was used as coating solution which containing 0.40 M $CrCl_3$, 0.40 M Glycine, 0.20 M $NiCl_2$, 0.50 M $NH_4Cl$, 0.15 M $H_3BO_3$ and 0.50 M NaCl [5]. The bath was prepared exactly according to the instructions in the report by Hasegawa et al., 2014. The bath pH was adjusted to 1 with 1% $NH_4OH$ aqueous solution. With this bath, freshly sanded CS electrode was coated with 10 µm thickness chrome-nickel (CrNi) alloy at 250 mA/cm$^2$ constant current density at room conditions via chronopotentiometry technique. The metallic characterization of the coated and uncoated electrodes was carried out by linear sweep voltammetry technique. Corrosion performances have been monitored with open circuit potential – time curves, anodic polarization curves and A.C. impedance spectroscopy in artificial seawater solution. Morphological and elemental investigation of electrodes was carried out with scanning electron microscope.

As a result of the study, CrNi alloy was successfully plated on the CS electrode under the specified conditions. According the SEM and EDX evaluations, it was observed that the surface was covered with homogeneous layers and the alloy consisted mainly of chromium. According to the corrosion evaluations, it was observed that the alloy coated electrode had higher polarization resistance and lower corrosion current than the bare CS electrode.

**Acknowledgements:** This research is supported by the Hatay Mustafa Kemal University Coordinatorship of Scientific Research Projects with Project No: 21.D.009.

**Keywords:** CrNi, alloy, electroplating, corrosion

**References**

[1] Oxford Economics, “The Role of Steel Manufacturing in the Global Economy: A Report for the World Steel Association,” 2019. [Online]. Available: https://www.worldsteel.org/en/dam/jcr:fdf44918-de3b-455b-9083-f770afa4a214/OE%2520Executive%2520Summary.pdf.
[2] K.-L. Lin, C.-J. Hsu, I.-M. Hsu, and J.-T. Chang, “Electroplating of Ni-Cr on steel with pulse plating,” *J. Mater. Eng. Perform.*, vol. 1, no. 3, pp. 359–361, Jun. 1992, doi: 10.1007/BF02652390.
[3] L. Xu, Z. Gong, J. Tang, Q. He, N. He, and J. Du, “Ni-Cr alloy electrodepositing technology on Fe substrate and coating performance,” *J. Cent. South Univ. Technol.*, vol. 14, no. 2, pp. 181–185, Apr. 2007, doi: 10.1007/s11771-007-0036-x.
[4] D. R. KESTER, I. W. DUEDALL, D. N. CONNORS, and R. M. PYTKOWICZ, “PREPARATION OF ARTIFICIAL SEAWATER,” *Limnol. Oceanogr.*, vol. 12, no. 1, pp. 176–179, Apr. 1967, doi: 10.4319/lo.1967.12.1.0176.
[5] M. Hasegawa *et al.*, “The electrodeposition of FeCrNi stainless steel: microstructural changes induced by anode reactions,” *Phys. Chem. Chem. Phys.*, vol. 16, no. 47, pp. 26375–26384, 2014, doi: 10.1039/C4CP03744H.

## *ORAL PRESENTATION*

# UV ile başlatılan çift emülsiyon polimerizasyonu kullanılarak poliHIPE partiküllerinin hazırlanması

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**Özet**

Bio-türetilmiş monomerlerin polimer malzeme hazırlama proseslerine adaptasyonu yenilenebilir kaynakların kullanımı açısından oldukça önemlidir. Terpenler bu amaç doğrultusunda en çok araştırılan türlerdendir. Bunun en önemli nedeni ise yapılarında iki veya daha fazla sayıda konjuge doymamış grup içermeleridir. Ancak sterik engelleme veya düşük reaktivite sebebiyle yüksek molekül ağırlıklı homo- ve/veya kopolimerler elde edilememesi pek çok terpen türevinin en büyük dezavantajlarındandır. Bilim insanları tarafından en çok araştırılan terpen türevi *β*-mirsen'dir. Bu doğrultuda, kontrollü ve serbest radikal polimerizasyon veya iyonik polimerizasyon gibi yöntemler ile *β*-mirsen esaslı polimerlerin sentezi gerçekleştirilmiştir. Yapılan bu çalışmalarda en sık kullanılan proses ise emülsiyon polimerizasyonudur. Bununla birlikte, yüksek iç fazlı emülsiyon (High Internal Phase Emulsion, HIPE) kalıplama yaklaşımı da son yıllarda *β*-mirsen esaslı makrogözenekli monolitlerin sentezinde sıklıkla tercih edilmektedir.
HIPE, iç faz hacim oranı toplam emülsiyon hacminin en az %74'ünü oluşturan konsantre bir emülsiyon sistemidir. Bir HIPE'nin sürekli fazının polimerleştirilmesi ile hazırlanan gözenekli polimerler poliHIPE olarak adlandırılır. PoliHIPE'ler, monolit, film, mikroküre (boncuk) veya çubuk formunda olabilir. Mikroküre veya boncuk formundaki poliHIPE'ler, HIPE'lerin ikincil bir ortamda dağıtılması ile elde edilen çift emülsiyon oluşturma tekniği ile sentezlenir. Ancak, bu yaklaşım emülsiyon kararlılığının neden olduğu sınırlamalar nedeni ile pek tercih edilmez. Bu nedenle literatürde rapor edilen poliHIPE boncukları veya mikroküreleri sınırlıdır. Terpen-esaslı poliHIPE mikroküreleri ise bilgimiz dahilinde literatürde mevcut değildir.
Bu çalışmada, *β*-mirsen ve akrilat komonomerleri varlığında hazırlanan yağ-içinde-su (yağ/su) türündeki HIPE'ler öncelikle 60°C'de serbest radikal polimerizasyon ile polimerleştirilerek poliHIPE monolitleri sentezlenmiştir. Ardından, benzer formülasyonlara sahip olan HIPE'ler çift emülsiyon tekniği ile poliHIPE partiküllerinin sentezinde kullanılmıştır. Bu amaçla UV-ile başlatılmış serbest radikal polimerizasyon kullanılmıştır. Elde edilen poliHIPE monolitlerinin ve partiküllerinin kimyasal yapısı FTIR ile, gözenek morfolojisi SEM ile ve spesifik yüzey alanları $N_2$ adsorpsiyon/desorpsiyon izotermlerine BET denklemi uygulanarak belirlenmiştir.
Bu proje Yalova Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmektedir (Proje No: 2021/YL/0008).

**Anahtar Kelimeler:** Yüksek iç fazlı emülsiyon, çift emülsiyon polimerizasyonu, terpen, *β*-mirsen.

## *ORAL PRESENTATION*

# Antibacterial Effect of Zinc Phthalocyanine Bound $TiO_2$

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**Abstract**

Different solutions are sought for antibiotic resistance of bacteria, which is one of the important problems of today[1,2]. With recent studies, it has been shown that nanomaterials are used in many drug treatment. In this study, the effects of Zinc Phthalocyanine (ZnPc)/$TiO_2$ *on Staphylococcus aureus (S.aureus)* were investigated. It has been shown to be lethal at low concentrations. Light irradiation of Znpc/TiOs increased the lethality against *S.aureus* bacteria by a non-negligible amount. In the experiment to investigate the antibacterial effect, a 8h growth curve at 600 nm was measured after treatment of *S. aureus* with ZnPc/$TiO_2$ and $TiO_2$. It showed 60% antibacterial effect for ZnPc/$TiO_2$ *S.aureus.* In this curve, bacterial growth in the logarithmic phase was inhibited in a short time. It was observed that the bacteria entered the stationary phase over time with ZnPc/$TiO_2$ and $TiO_2$. The resulting scanning electron microscope (SEM) images showed that *S. aureus* were damaged by ZnPc/$TiO_2$. The results showed that ZnPc/$TiO_2$ could be used in antibacterial studies and also paved the way for in vivo experiments.

**Keywords:** Zinc Phthalocyanine, $TiO_2$, Antibacterial Effect

**References**

1. Brown, E. D., & Wright, G. D. (2016). Antibacterial drug discovery in the resistance era. *Nature*, *529*(7586), 336–343. https://doi.org/10.1038/nature17042
2. Ahmad, M., & Khan, A. U. (2019). Global economic impact of antibiotic resistance: A review. *Journal of Global Antimicrobial Resistance*, *19*, 313–316. https://doi.org/10.1016/j.jgar.2019.05.024

## *ORAL PRESENTATION*

# Photothermal antibacterial effect of seleno chevrel phase structures

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**Abstract**

Transition metal dichalcogenides are used as antibacterial agents by causing physical bacterial damage due to their morphological structure[1]. When TMDs are used together with different plasmonic metals, their antibacterial effects increase by gaining photothermal activity[2]. It has been reported that the antibacterial effect of TMDs in 1T phase structure with metallic properties is more than TMDs in 2H phase structure[3]. TMDs, which are unstable in 1T phase structure, form 1T Chevrel phase structure by the introduction of an extra metal into their structure. In our study, due to the cubic crystal structure of the $Cu_2Mo_6Se_8$ seleno chevrel phase and the advanced optoelectronic properties brought by the bimetallic structure, it has created an intense antibacterial effect by showing both physical bacterial damage and photothermal effect. [4]. $Cu_2Mo_6Se_8$ seleno chevrel phase synthesized by solid state method were investigated for photothermal antibacterial effect on *Staphylococcus aureus* bacteria with 808 nm NIR light irradiation.

**Keywords**:Chevrel phase, transition metal dicalcogenide, photothermal activity

**References**

[1] B. Li, Y. Luo, Y. Zheng, X. Liu, L. Tan, S. Wu, Two-dimensional antibacterial materials, Progress in Materials Science, (2022) 100976.
[2] Y. Cai, L. Wang, H. Hu, W. Bing, L. Tian, J. Zhao, A synergistic antibacterial platform: Combining mechanical and photothermal effects based on van-mos2–au nanocomposites, Nanotechnology, 32 (2020) 085102.
[3] T.I. Kim, J. Kim, I.-J. Park, K.-O. Cho, S.-Y. Choi, Chemically exfoliated 1T-phase transition metal dichalcogenide nanosheets for transparent antibacterial applications, 2D Materials, 6 (2019) 025025.
[4] J. Strachan, A.F. Masters, T. Maschmeyer, The Catalytic Nature of Chevrel Phases (MxMo6S8) in Review, Materials Research Bulletin, 139 (2021) 111286.

## *ORAL PRESENTATION*

# First Record of Invasive *Plotosus lineatus* (Thunberg, 1787) in Erdemli, Mersin Bay-Northeastern Mediterranean

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**Abstract**

The striped eel catfish, *Plotosus lineatus* (Thunberg, 1787) is an alien species invaded the Mediterranean waters upon the opening of Suez Canal. This species has been included in the 10 dangerous alien species by risk assessment evaluation reports since its first record in 2002. The invasion of the species has reached to Turkish marine waters by 2016 and the first record was from Çevlik-Samandağ in Iskenderun bay. This article is an evidence of ongoing invasion of *Plotosus lineatus* in the northeastern Mediterranean and reports four new locations for the presence of the species in Turkish marine waters. The specimens were caught in (1) Madenli-Arsuz, (2) Karaağaç-Arsuz ve (3) Payas in Iskenderun Bay and (4) Erdemli in Mersin Bay. The occurrence of the species in Mersin bay is an evidence of ongoing westward invasion. The calculations revealed that this species invades approximately 47.33 km of Turkish marine waters per year. Total invasion rate in the eastern Mediterranean is about 41.3 km/year. Calculation of invasion speed suggests that invasion has started at least 5-6 year before 2002. Hence, the striped eel catfish must have entered the Mediterranean around the second half of 1990's. The rate of invasion (152 km/year) in the southern Mediterranean from Suez Canal to Tunisia was calculated to be much higher than both the calculations for Turkish waters and the eastern Mediterranean. This species can adapt a wide range of habitat and establishes successful populations. This study also provides evidence for the stable population establishment of *Plotosus lineatus* in the coastal region of Turkish marine waters. Therefore, *Plotosus lineatus* invasion does not have only adverse effects on the marine biodiversity but also has socio-economic consequences and influences the fisheries and coastal tourism. Further studies should be urgently conducted on monitoring, population structure and dynamics, and genetics of the species.

**Keywords:** *Plotosus lineatus*, The striped eel catfish, First record, Mersin; Invasive alien species, Mediterranean

## *ORAL PRESENTATION*

# Transfersomes for topical ocular administration: Devolopment and in vitro characterization

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**Abstract**

Cytomegalovirus is a common virus that is usually asymptomatic in healthy people but can cause retinitis in people with compromised immune systems. The disease is treated with intravitreal ganciclovir (GCV) administrations. Side effects such as infection, bleeding, tearing of intraocular tissues and increased intraocular pressure may occur due to intravitreal injection or implant use [1]. The aim of study is to develop controlled release transfersome formulations that can be applied topically. 7 formulations were prepared. The effect of phosphatidylcholine:surfactant ratio or phosphatidylcholine type on formulations was investigated. GCV loaded transfersomes was prepared by ethanol injection method. The entrapment efficiency was determined according to the indirect method. The amount of GCV was evaluated by HPLC. The particle size (PS), zeta potential (ZP), and polydispersity index (PdI) of the transfersomes were determined using a Zetasizer Nano ZS. In vitro release of GCV from transfersomes was conducted by dialysis method using Franz diffusion cell. PS of transfersomes was determined between 236.6-2009.3 nm; ZP was between -23.93 and -43.1 mV. Low PS (262.7± 35.1nm) and low PdI (0.387± 0.019) value; high ZP (-28.3± 2.707) and high encapsulation efficiency (%47.5) value were obtained with T9 formulation. T9 formulation released 90.4% of the drug in 96 hours and provided controlled release. Nanocarriers must have optimum particle size (100-300nm) and (-) zeta potential to pass the sclera and reach the retina [2]. Accordingly, T9 was chosen as the optimum formulation. Also, the result of the release study is promising for prolonging the dose intervals.

**Keywords:** Transfersomes; ganciclovir; cytomegalovirus retinitis.

[1] Port A. D., Orlin A., Kiss S., Patel S., D'Amico D. J., Gupta M. P. 2017. "Cytomegalovirus Retinitis: A Review", J of Ocular Pharmacology and Therapeutics, 33(4), 224-234.
[2] Xu, Q., Boylan, N. J., Suk, J. S., Wang, Y.-Y., Nance, E. A., Yang, J.-C., McDonnell, P. J., Cone, R. A., Duh, E. J., Hanes, J. 2013. "Nanoparticle Diffusion in, and Microrheology of, The Bovine Vitreous Ex Vivo", J of Controlled Release", 167(1), 76–84.

## *ORAL PRESENTATION*

# Degradation of a herbicide using LaZnFe layered double hydroxide: Synthesis and characterization

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**Abstract**

Recently, concern about the continued and excessive use of herbicides has increased rapidly due to their potential environmental effects. Advanced oxidation processes (AOPs) are effective methods for converting target pollutants into less or no toxic products by generating reactive oxygen species (ROSs). Owing to their high stability, wide pH range, and low cost, peroxydisulfate (PDS)-based AOPs have become increasingly significant in recent years compared to the other AOPs. To produce sulfate radicals, PDS can be activated using various methods, including heating, alkalinity, ultraviolet radiation (UV), ultrasonic irradiation (US), and catalyst. Moreover, US can increase the generation of free radicals by providing active cavitation bubbles. Layered doubled hydroxides (LDHs), a type of metal-based catalyst, have attracted interest in recent years due to their unique structure and compositional flexibility. In the present study, LaZnFe LDH was synthesized and characterized using various analyses. The high crystallinity of the catalyst was confirmed by the XRD analysis. The surface morphology was evaluated with SEM analysis. The simultaneous presence of Zn, Fe, Cl, and La elements was well confirmed by EDS analysis illustrating the successful synthesis of La-doped ZnFe LDH nanocomposite. More active sites for the sonocatalytic process were provided by the high specific surface area (110.93 $m^2$ $g^{-1}$) of LaZnFe LDH. The effectiveness of the as-synthesized catalyst was tested in the degradation of metribuzin as a toxic herbicide, mention the name. Owing to the synergistic effect of the combined system, the degradation efficiency of the US+PDS process increased from 22.2% to 79.1% with the addition of LaZnFe LDH. Consequently, this study demonstrates that US+PDS+LaZnFe LDH system has potential application prospects in the degradation of herbicides.

**Keywords:** Herbicide degradation; Environmental remediation; Nanocatalysts; Sonocatalysis; Peroxydisulfate activation.

## *ORAL PRESENTATION*

# Orexin-A mediates the protective effects of central neuropeptide-S against 6-OHDA-induced motor disorders in rats

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**Abstract**

Parkinson's disease (PD) is characterized by progressive loss of the dopaminergic neurons in the substantia nigra (SN). The typical motor symptoms of PD include bradykinesia, tremor, muscle rigidity, and loss of postural reflexes. Recent studies have revealed the protective effects of central neuropeptide-S (NPS) treatment on 6-hydroxydopamine (6-OHDA)-induced motor dysfunction in rodents. Moreover, the expression of NPSR has been detected in nigral dopaminergic cells. Likewise, protective effects and potential therapeutic applications of orexins were demonstrated in both cellular and animal models of PD. The present study was aimed to investigate whether orexin-a (OXA) mediates the NPS-induced motor response alterations in parkinsonian rats. Adult male Sprague Dawley rats (280–300 g) were placed in a stereotaxic apparatus, the experimental Parkinson's model was created by injecting 6-OHDA unilaterally, into the medial forebrain bundle using 12 µg of 6-OHDA in 3µL vehicle (saline) and then a 26G intracerebroventricular cannula was implanted into the right lateral ventricle for chronic central injections. Following the injection of 6-OHDA, the OXA receptor antagonist SB-334867, NPS, and/or vehicle was daily administered through an intracerebroventricular (icv) cannula for 7 days. The OXA receptor antagonist was administered 30 min prior to the NPS injections. To assess motor performance, open field test and catalepsy test were performed 7 days after the central 6-OHDA injection. All data were analyzed with the non-parametric Kruskal–Wallis test followed by Dunn's post hoc test. All protocols were approved by the Animal Ethical Committee of Akdeniz University. Central administration of NPS improved the 6-OHDA-induced motor impairments, but the effects were blocked by pretreatment of the OXA receptor antagonist SB-334867, suggesting an OXA-dependent mechanism. These results suggest that the orexinergic system mediates the protective effect of central NPS on 6-OHDA-induced motor deficits.

**Keywords:** Orexin-A, Neuropeptide-S, Parkinson's Disease on 6-OHDA-induced motor function

## *ORAL PRESENTATION*

# Synthesis, Characterization and Fluorescence Properties of New Fluorescent Substituted Phenanthroimidazole Compound

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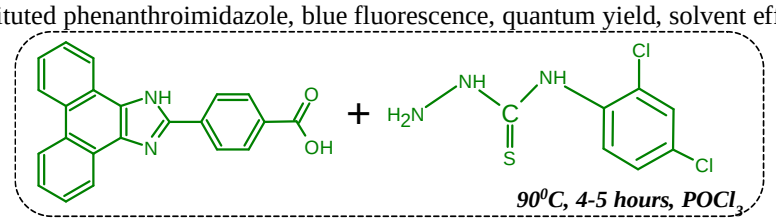
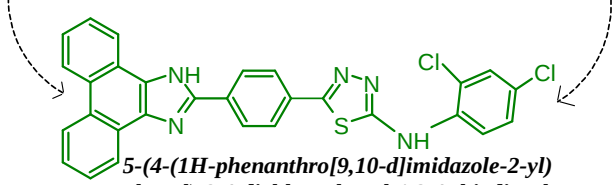
**Abstract**

Phenanthroimidazole and its derivatives among fluorescent organic molecules have received tremendous attention owing to application in DSSCs, bioimaging sensor and display technology based on OLEDs. For this reason, researches on the solution or solid state fluorescence properties of phenanthroimidazole molecules are gaining importance day by day. In this work, *-diCl* bearing phenanthroimidazole compound was synthesized and purification for investigation of fluorescence properties. Its structure analyses were used NMR, ATR-IR and mass spectroscopies (ESI). The fluorescence properties of the new compound were determined by UV-Vis and fluorescence spectroscopy in steady state. The fluorescence quantum yield was found in solution using Parker & Rees method. The variation of absorption and emission peaks were studied in the presence of solvents with different polarities. Hypsochromic or bathochromic shift was determined that showed depending on the solvent of the synthesis compound. Thanks to the fluorescence properties of the synthesized compound, it will take its place among organic fluorescent molecules that can be used in different technologies such as diodes, sensors, OLEDs, OFETs and solar cells.

**Keywords:** *-diCl* substituted phenanthroimidazole, blue fluorescence, quantum yield, solvent effect




***5-(4-(1H-phenanthro[9,10-d]imidazole-2-yl) phenyl)-2,4-dichlorophenyl-1,3,4-thiadiazol-2-amine***



## *ORAL PRESENTATION*

# Detection of the probiotic potential of human originated *Lactobacillus reuteri*

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**Abstract**

According to clinical research intestinal probiotics are live microorganisms that provide health benefits when ingested in sufficient quantities and their main functions are related to modulating and maintaining the health of the intestinal tract. Probiotics have a number of novel applications because of their special ability to produce bioactive molecules including vitamins, bacteriocins, 3-indoleacrylic acid, Indole-3-aldehyde, short-chain fatty acids, exopolysaccharides, peptides, and proteins. These substances are essential in giving the host a variety of health advantages. Although Lactobacillus species are commonly used as probiotics, only limited data are available for the probiotic properties of human originated *Lactobacillus reuteri*. This study aims to characterize human originated *L. reuteri* for its probiotic potential according to the international guidelines for the evaluation of probiotics. In the present study, the probiotic potential of *L. reuteri* isolated from healthy human was evaluated. The probiotic properties of *L. reuteri* were tested for aspects of antimicrobial activity, antibiotic susceptibility, adhesion and aggregation ability, bile salt tolerance and low pH resistance. While clear zones of inhibition test results indicated that the cell free supernatant and cell lysate of *L. reuteri* antagonistic activity against three pathogenic bacteria, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Salmonella typhimurium*, the antibiotic test results showed that *L. reuteri* was sensitive for Erythromycin, but resistance for Neomycin and Kanamycin. On the other hand, *L. reuteri* showed a strong ability to adhere to pathogenic bacteria as well as self-binding because it has a high sedimentation rate. Furthermore, *L. reuteri* showed a high survival rate under acidic and bile salt containing environment that means gastrointestinal tract conditions. Our results show that such properties of the human originated *L. reuteri* may be used as probiotics in the healthcare sector and various products.

**Keywords:** Probiotics, *Lactobacillus reuteri*, human health

## *ORAL PRESENTATION*

# Importance of heterocyclic amines in meat and meat products

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**Abstract**

Different chemical reactions take place among the food components with heat treatments which also cause the formation of new compounds. It is stated that the Maillard reaction which occurs during the heat treatment, has an important role in the emergence of these newly formed compounds, some of which are considered as harmful compounds for human beings. Heterocyclic amines are among these harmful compounds and can be formed during heat treatment and cooking in meat and meat products. Many of the heterocyclic amines are reported to be carcinogenic and/or mutagenic. In 1993, 2-amino-3-methylimidazo quinoline (IQ) was classified as a type 2A (Probable) carcinogen for humans by the International Agency for Research on Cancer (IARC), while 2-amino-3,4-dimethylimidazo[4,5-*f*]quinolone (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-6-methyldipyrido[1,2-*α*:3′,2′-*d*]imidazole (Glu-P-1), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) were classified as type 2B (possible) carcinogens. Heterocyclic amines are divided into two groups as aminoimidazoazarenes (AIAs) and aminocarbolines (ACs) according to their molecular structures and formation pathways. AIAs are known as thermal heterocyclic amines and are formed in the reaction of amino acids, creatine/creatinine and hexoses during heat treatment at temperatures below 300°C (150-300°C). Alternatively ACs are known as pyrolytic heterocyclic amines and are formed as a result of the pyrolysis of proteins and amino acids at temperatures above 300°C. The effects of physical factors such as meat type, applied temperature, time, heat treatment equipment and method, pH and water activity, and chemical factors such as carbohydrates, free amino acids and creatine on the formation and concentrations of heterocyclic amines have been reported. Although the formation of heterocyclic amines during processing or cooking of meat and meat products is mostly associated with high temperature applications such as frying, roasting and grilling, it is stated that they are also formed and accumulated in sausages processed at lower temperatures.

**Keywords:** Heterocyclic amines, Meat, Sausage, Heat treatment

## *ORAL PRESENTATION*

# Synthesis of Some New Thiazoline-Thiazole Substituted Compounds

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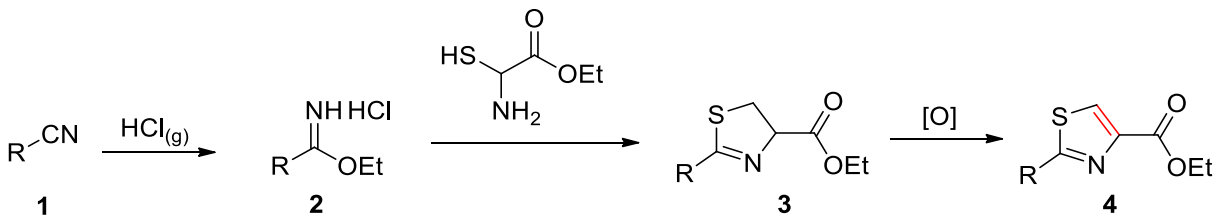
**Abstract**

Organic compounds having strong odors have been known to use not only fragrance but also flavor purposes. There are many odor compounds exist such as aldehydes, alcohols, ketones, esters and heterocyclic ones. Among them, heterocyclic compounds seem to be promising for development of new odorant molecules due to their limited usage in perfumery and aroma industry. Specifically, thiazole and thiazolines as a members of heterocyclic compounds have been known for their olfactive properties. The development of new odorant molecules bearing these units are still desiring and challenging. On the basis of above consideration, in this study, we focused on the synthesis of new thiazolines and thiazoles starting with diverse range of nitriles. This synthetic method covers three sequential steps: formation of imino esters, their cyclizations with (*L*)-cysteine methyl ester hydrochloride and oxidation of thiazolines. The structures of title compounds were identified by the means of IR, NMR and mass measurements. Besides, both thiazoline and thiazole derivatives was subjected to olfactory evaluation by an expert nose.

**Keywords:** Fragrance, thiazoline, thiazole, odor




**Scheme:** Synthetic pathway for the synthesis of both thiazoline and thiazole derivatives

**REFERENCES**

1-Surburg H., & Panten J. Common Fragrance and Flovor Materials, Sixth Edition. 2016.
2-Sell C.S. Fundamentals of Fragrance Chemistry. 2019.
3-Meyers A. I., & Tavares F. Oxidation of Oxazolines and Thiazolines to Oxazoles and Thiazoles. Application of the Kharasch-Sosnovsky Reaction. J. Org. Chem. 1996, 61, 8207-8215.
4-Emtenas H., Alderin L., & Almqvist F. An Enantioselective Ketene-Imine Cycloaddition Method for Synthesis of Substituted Ring-Fused 2-Pyridinones. J. Org. Chem. 2001, 66, 6756-6761.
5-Yue H., Haifeng G., Shang L., Jinyi X., Xiaoming W., & Hequan Y. Oxidation of 4-carboxylate Thiazolines to 4-carboxylate Thiazoles by Molecular Oxygen. Tetrahedron Letters 2010, 51 (13), 1751–1753.

## *ORAL PRESENTATION*

# Nutrition in Arthritis

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**Abstract**

Arthritis is defined as a degenerative joint disease that has different forms, primarily rheumatoid arthritis, osteoarthritis, gouty arthritis, and involves most of the cartilage and surrounding tissues. It is an autoimmune disease characterized by painful joints, joint stiffness and limitation of movement, and intense inflammatory responses. Factors such as cigarette consumption, infectious agents, environmental pollution and chronic stress have been found to trigger intense inflammatory responses in arthritis. Apart from these factors, the role of nutrition has been suggested by increasing evidence recently. In some studies, fruit, vegetable or meat consuming has been associated with diet and disease status, but no definitive conclusion has been reached. Arthritis has been observed that the prevalence is lower in those who eat the Mediterranean diet. It is thought that some other diet types also have a relieving effect on the symptoms of the disease. Vegetarian diet, gluten-free diet, Mediterranean diet, elimination diet or intermittent fasting diet are some of these diets. Genetics is also thought to have an effect on arthritis. Genetic and environmental factors are thought to play an important role in the pathophysiology and occurrence of the disease. The data identify diet as an environmental factor in the development of arthritis, with diet increasing the inflammatory response in genetically predisposed individuals. Recently, it has been suggested that obesity, diabetes and metabolic syndrome are important risk factors for the development of arthritis. Obesity is a modifiable risk factor for arthritis. Arthritis treatment is limited. In general, there is a treatment that reduces the symptoms related to the patient's symptoms or if there is a serious dysfunction in the joint, there are treatment options with total joint replacement. There is a call for a change in a system that focuses on teaching patients with arthritis to self-manage their condition based on their symptoms. Increased inflammation and impaired immune system regulation, which are among the basic pathophysiological mechanisms in the formation of arthritis, play an important role in the development of comorbidities. It is known that many comorbidities accompany arthritis. Some of these comorbidities are cardiovascular diseases, malignancies, infections, and osteoporosis. In addition, comorbidities can significantly increase the risk of mortality. In this study, the mechanisms and nutritional approaches in the formation of arthritis will be examined in the light of the current literature.

**Keywords:** Arthritis, Mediterranean diet, Elimination diet, Obesity, Inflammatory response

## *ORAL PRESENTATION*

# Purification of methylal/methanol mixtures by pervaporation process using ZIF-8 doped PEI membrane

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## Abstract

In this study, pervaporation process is evaluated for the separation of methylal/methanol mixtures. Polyetherimide (PEI) polymer was chosen for the membrane preparation due to the affinity of the methanol. ZIF-8 was used as additive for the membrane. ZIF-8 doped PEI membrane was synthesized and characterized. Membranes were characterized by using Fourier-transform infrared spectroscopy (FTIR), Thermogravimetric Analysis (TGA) and Scanning electron microscope (SEM). Separation performance of membranes were investigated in different operation temperatures, different feed methanol concentrations and different ZIF-8 loading ratios. Optimum operation conditions were determined as 20 °C of operation temperature and 6 wt. % of feed methanol concentration and 0.5 wt.% ZIF-8 loading ratio. Flux and methanol selectivity values were 0.57 kg/m$^2$h and 8169, respectively.

**Keywords:** Membrane, Methylal, Pervaporation, Polyetherimide

## *ORAL PRESENTATION*

# Synthesis of Some Terpenoid-Based Carbamates

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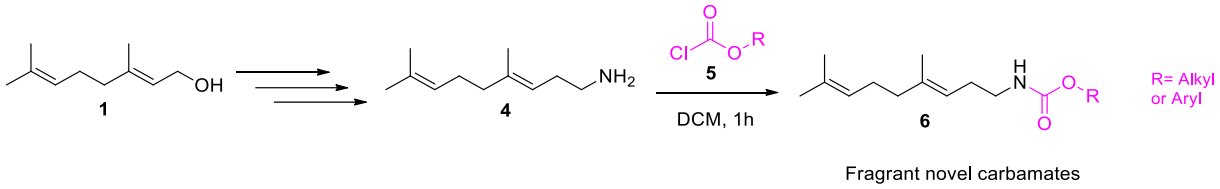
## Abstract

Terpenes are compounds which can be supplied by natural and synthetic ways and they have been utilized in the flavor and pharmaceutical industry, especially for the production of fragrances for many years. Carbamates have also been studied in fragrance chemistry since 2004. In this regard, in the current study, firstly homogeranylamine **4** was synthesized in moderate to good yields starting from geraniol **1** over a three-step transformation and then, novel homogeranyl carbamates **6** were prepared efficiently using homogeranyl amine **4** and a variety of chloroformates **5**. Structures of carbamates **6** were fully characterized by means of IR, NMR and Mass analyses and besides, they were tested for their fragrance properties and cosmetic value by the expert perfumers.




**Keywords:** Perfume, carbamate, terpenoids, chloroformates, odor.

## References

1. Breitmaier, E. (2006). Terpenes: Flavors, Fragrances, Pharmaca, Pheromones (1st ed.). Wiley-VCH.
2. F. Flachsmann, J.P. Bachmann, Int. Pat. Appl. (to Givaudan) (2003), WO 2004089880
3. Adachi, M., Zhang, Y., Leimkuhler, C., Sun, B., LaTour, J. V., & Kahne, D. E. (2006). *Journal of the American Chemical Society*, *128*(43), 14012–14013.
4. Cimino, G., Gavagnin, M., Sodano, G., Spinella, A., Strazzullo, G., Schmitz, F. J., & Yalamanchili, G. (1987). *The Journal of Organic Chemistry*, *52*(11), 2301–2303.
5. Gómez-Bengoa, E., García, J. M., Jiménez, S., Lapuerta, I., Mielgo, A., Odriozola, J. M., Otazo, I., Razkin, J., Urruzuno, I., Vera, S., Oiarbide, M., & Palomo, C. (2013). *Chemical Science*, *4*(8), 3198.
6. Mori, K., & Funaki, Y. (1985). *Tetrahedron*, *41*(12), 2369–2377.
7. Shi, H., Hu, W., & Sun, Y. (2004). *Journal of Chemical Research*, *2004*(10), 708–709.
8. Weber, J. C., & Paquette, L. A. (1988). *The Journal of Organic Chemistry*, *53*(22), 5315–5320.

## *ORAL PRESENTATION*

# Effect of TAD mutations on phosphorylation of p53 by DNA-PK

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**Abstract**

The DNA-PK enzyme phosphorylates the Ser15 and Ser37 residues in the TAD domain of p53. Mainly, the Ser15 phosphorylation event prevents p53 from interacting with MDM2 and stabilizes it. It has been reported that Phe19Arg and Leu22Gln-Trp23Ser mutations located close to Ser15 cause tumorigenesis in mouse models. These mutations are also known to reduce p53-MDM2 interaction. In this study, to determine the effect of Phe19Ala and Leu22Gln-Trp23Ser mutations on Ser15 phosphorylation by DNA-PK, these mutations were generated using site-directed mutagenesis. Then the phosphorylation was monitored by western blot using specific antibodies for Ser15P. The Ser15 phosphorylation rate was around 3.7 times slower in the Phe19Ala mutant and 2.7 times slower in the Leu22Gln-Trp23Ser mutant variant compared to the wild type. The data obtained in this study indicate that the steric effect of phenylalanine is needed for effective Ser15 phosphorylation. Additionally, the Leu22Gln-Trp23Ser mutant, which was reported to reduce the interaction between MDM2 and p53, also reduced Ser15 phosphorylation, suggesting that Leu22 and Trp23 are also required for efficient Ser15 phosphorylation.

**Keywords:** p53, DNA-PK, Site-Directed Mutagenesis, Ser Phosphorylation.

## *ORAL PRESENTATION*

# Bazı Flavonoidlerin COX-2 Enzimi Üzerine Olan Etkisinin *İn Silico* Olarak İncelenmesi

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**Özet**

Siklooksijenaz, vücutta ağrı ve inflamasyona neden olan prostaglandinlerin sentesinden sorumlu bir enzimdir. Kullanılan non-steroidal antiinflamatuar ilaçlar (NSAİİ) bu siklooksijenaz enzimini inhibe ederek etkinlik gösterir. Siklooksijenaz enziminin siklooksijenaz-1 (COX-1) ve siklooksijenaz-2 (COX-2) olmak üzere iki izoformu vardır. Bu izoformlardan biri olan COX-2 enzimi birçok kanser türünde aktivitesi oldukça arttığı tespit edilmiştir. Bu sebep ile birçok kanser türünde COX-2 enzimi önemli bir hedef haline gelmiştir. Bu çalışmamızda, antienflamasyon ve antikanser etkileri açısından son yıllarda araştırma konusu olan bazı flavonoidlerin COX-2 ile bağlanma potansiyelleri *in siliko* olarak incelenmiştir. Bu incelemeler için seçilen moleküller PubChem veri tabanından indirilerek COX-2 enzimi (PDB ID:5KIR) ile *i*GEMDOC ve Autodock Vina Chimera 1.15, Discovery Studio yazılımları kullanılarak moleküler kenetleme çalışması gerçekleştirilmiştir. Bu çalışmaların ışığında, Naringenin ve Eupatorin molekülünün (-9.5kcal/mol, -7.3kcal/mol sırası ile) en iyi bağlanmayı yaptığı tespit edilmiştir. Bu moleküllerin enflamasyonda ve kanserde terapötik amaçla kullanımı açısından umut vaad etmektedir. Bununla beraber in siliko, in vitro ve in vivo açısından daha detaylı ve ileri çalışmalara ihtiyaç duyulmaktadır.

**Anahtar Kelimeler:** (Flavonoidler, *COX-2*, Enflamasyon, Kanser, *İn silico*, )

## *ORAL PRESENTATION*

# PEO/PVA/Nanokitin nanokompozit hidrojellerinin yara örtü malzemesi olarak kullanılmak üzere *in vitro* ve *in vivo* toksisitelerinin araştırılması

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**Özet**

Diyabetik ülser yaraları başta olmak üzere kronik yaralarda kullanılmak üzere sentezlenen nanokompozit hidrojel yapısındaki yara örtü malzemeleri, yara bölgesini nemli tutarak yara iyileşmesinde migrasyon ve proliferasyonu desteklemeleri ve yapısal olarak standart hidrojellerden mekanik olarak daha fazla sağlamlılık göstermeleriyle yara örtü malzemeleri arasında üstünlük sağlamaktadırlar. Polietilen oksit (PEO) ve polivinil alkol (PVA) gibi hidrofilik yapıdaki sentetik polimerler kullanılarak sentezlenecek hidrojelin yapısına kitin gibi doğal polimerlerin de ilavesi hidrojelin mekanik sağlamlılığını artırıp, doğal polimerin yara iyileşmesindeki ekstraselüler matriks tabakasını taklit edebilme özelliğini desteklemektedir. Yara iyileşmesi sırasında aktif olarak prolifere olan fibroblast hücrelerinin yara örtü malzemelerine verdiği yanıt *in vitro* sitotoksisite çalışmalarında önemlidir. Bu amaçla bu çalışmada %1, %2.5 ve %5 oranlarında nanokitin ilavesiyle hazırlanan PEO ve PVA polimerlerinden oluşan hidrojellerin insan deri fibroblast hücreleri üzerindeki sitotoksik etkisi indirekt ve direkt yöntemlerle MTT ve MTS testleriyle araştırılmıştır. Ayrıca *in vitro* deneylerde anlamlı sonuçlar gösteren kompozitlerin *in vivo* toksisitesi *Drosophila melanogaster* üzerinde yapılan puparasyon, pupadan çıkma ve canlılık oranından oluşan gelişim testleri ile larval sürünme ve yetişkin tırmanma testinden oluşan davranış testleriyle incelenmiştir. *In vitro* sitotoksisite sonuçlarına göre hem direkt hem de indirekt testte nanokompozitlerin sitotoksik etki oluşturmadığı görülmüştür. *In vivo Drosophila* sonuçlarına göre ise %5 kitin ilaveli PEO/PVA'nın 100µg/mL dozları gelişim testlerinde larva-pupa-yetişkine dönme oranlarını, sürünme testinde larva hızını ve tırmanma testinde yetişkin sinek tırmanma sayısını azaltarak *Drosophila* üzerinde toksik bir etki oluşturmuştur. Bu çalışmadan elde edilen sonuçların farklı hücre hatları ve farklı model organizmalarda tekrarlanmasının hidrojelin güvenilirliğini artıracağı ve klinikte kullanımı için bir ön çalışma niteliği taşıyacağı düşünülmüştür.

**Anahtar Kelimeler:** Polietilen oksit, polivinil alkol, insan dermal fibroblast, nanokompozit hidrojel, *Drosophila melanogaster*

## *ORAL PRESENTATION*

# A Novel Approach to Precipitation of Heavy Metals from Industrial Effluents using immobilizing alkaline phosphatase with lactoferrin

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**Abstract**

In the mining, tannery, electrical, and electroplating sectors, heavy metals are employed. When the amounts in the effluents approach threshold values, these heavy metals' extremely poisonous, carcinogenic, and mutagenic properties can cause major health concerns in living beings. Heavy metal clean up via enzyme precipitation is a cutting-edge, economical, and environmentally beneficial technique. Magnetic nanoparticles (MNPs) were modified by Lactoferrin (LA). Alkaline phosphatase (Alk) has been immobilized on MNPs using two distinct methods after the functionalization procedure. Cross-linked enzyme aggregates were created in the first method. Alkaline phosphatase cross-linked enzyme fine aggregates with magnetic nanoparticles that have been modified by lactoferrin (Alk/LA/MNPs-CLEAs) were initially created for this purpose, and then BSA was utilized as a co-feeder to create the Alk/BSA/LA/MNPs-CLEAs. The Alk was essentially immobilized on the LA/MNPs surface in the second method. Scanning electron microscopy (SEM), X-ray diffraction (XRD), vibrating sample magnetometer (VSM), Fourier transforms infrared spectroscopy (FTIR), and zeta potential measurements have all been used to examine the characteristics of MNPs and their derivatives. Alk/LA/MNPs, Alk/LA/MNPs-CLEAs, and Alk/BSA/LA/MNPs-CLEAs all had improved Alk activity and stability. Additionally, the immobilized enzyme was easily removed from the reaction media using an external magnet and then reused several times. According to the research, LA/MNPs are a unique binder and support system for Alk, and Alk immobilized on this system can play a crucial role. Toxic heavy metals from different industrial effluents, such as those from the tannery, electroplating, and dye industries, can be remedied using an innovative, environmentally friendly technique called enzyme-mediated precipitation of heavy metals.

**Keywords:** lactoferrin, CLEAs, heavy metals, Alkaline phosphatase

## *ORAL PRESENTATION*

# Recurrent Ketamine Exposure Impaired Spatial Memory and Increased Stretched-attend Posture in Juvenile Male Rat

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**Abstract**

Ketamine is commonly used anesthetic agents in pediatric population due to some clinical advantages. But contradictory results have been reported in the literature regarding negative or positive effects on developing brain. The present study aimed to investigate the short-term effect of recurrent and different doses ketamine exposure on spatial memory, anxiety-like behaviors and locomotor activity in juvenile rats.32 Wistar Albino male rats were randomised into four groups [(n=8), Control, 5, 20 and 50 mg/kg ketamine group]. Ketamine was administered 3 days, 3 doses a day by 3 hours intervals intraperitoneally (i.p). After 10 days, behavior parameters were evaluated by an Open Field Test, Elevated Plus Maze Test and Light Dark Box Test. Statistical analysis was performed One- Way Anova, and followed by Tukey Tests. Significance value was admitted $p<0.05$. There was no difference between groups in total rearing number and total distance travelled as locomotor activity. Transfer latency time incerased significantly in 50 mg/kg ketamine group vs control group ($p < 0.001$). Anxiety- like behavior decreased in only 20 mg/kg Ketamine group particularly light dark box parameters ($p<0.05$). Stretched attend posture increased significantly in 50 mg/kg group ($p<0.01$). Unssuported rearing behavior decreased in 5 and 50 mg/ketamine group vs control ($p<0.05$). Our study revealed that 50 mg/kg ketamine had shown detrimental effect on effect on spatial memory, exploratory behavior and incresed anxiety like behaviors such as streched attand posture. Despite of the 50 mg/kg group, 20 mg/kg had anxiolytic effect on juvenile rats. Doses of the ketamine must be considered in pediatric group whose are critical for neurogenesis. Further behavioral and histopathological researches are needed to elucidate the dose effects relation in pediatric groups for ketamine.

**Keyword:** Ketamine, Rat behaviours, Spatial memory

## *ORAL PRESENTATION*

# Spinosad causes oxidative stress in the rat prefrontal cortex

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**Abstract**

Spinosad is an insecticide depend on chemical compounds. Although the main purpose is insecticidal, it has been shown to cause damage in small mammals (aka: rat, mice). Our aim is to investigate of spinosad on the different brain regions. Methods: 15 Adult Male Wistar albino rats were divided into three groups of 5 rats each; first group (Group 1) served as a control, second group (Group 2) received a low sub-chronic dose of spinosad (SPD) that is equal to 9 mg, and third group (Group 3) received a lethal dose of SPD that is equal to 37,5 mg. Prefrontal cortex and striatum analyzed by terms of oxidative stress parameters. There was no significant difference between the groups in terms of total antioxidant status, total oxidant status and oxidative stress index (OSI) in 9mg spinosad group vs control group ($p>0.05$). Total oxidant status increased after the administration of high dose Spinosad (37,5 mg/kg) particularly prefrontal cortex and adversely affect OSI ($p<0.05$). Prefrontal cortex may be more vulnerable to toxicity due to vascularization, gray/white matter ratio and anatomical location. Prolonged exposure to spinosad may cause a number of neurological disorders. Further studies are needed for the negative changes that oxidative stress can cause in the cell.

**Keywords:** Prefrontal cortex, Rat, Spinosad

## *ORAL PRESENTATION*

# Genotoxic effects of $Fe_2O_3$ nano- and microparticles in human lymphocytes by the chromosomal abnormalities and sister chromatid exchanges

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**Abstract**

The place of inorganic nanoparticles has been expanding in the field of nanotechnology, which attracts great interest for a long time, especially in the medical world. After the term of theranostic, modifications to the insoluble particles were developed rapidly to monitor the prognosis of diseases. For such applications, iron (III) oxide ($Fe_2O_3$) particles have an important place with their robust structure and easy detectability. Yet, there is an insufficient amount of observation and many controversies about the potential genotoxicity of these particles. In this ongoing *in vitro* research, the results of two genotoxicity tests, chromosomal abnormalities (CAs) and sister chromatid exchanges (SCEs), were evaluated to determine the genotoxic effect of $Fe_2O_3$ nanoparticles (NPs) and microparticles (MPs) with 125, 250, 500, and 750 µg/mL doses and control groups (Sterile distilled water for negative, mitomycin-C (MMC) for positive) for 24 and 48-hours on human lymphocytes. While the CA test is widely used to detect the effect of clastogenic and aneugenic agents on chromosomes, the SCE test is applied to detect chromosome regions that have undergone DNA repair based on recombination, i.e., breaks in DNA. Compared to the control groups, results showed a significant increase in CAs for 48 hours of NP treatment. Again, significant elevation was determined in the number of SCEs for all the treatment groups. The higher degree of exchanges seems for 24-hours treatments of both particles. On the other hand, the mitotic index presented a dose-dependent decrease in NPs treated groups. The present results revealed that NPs appear to induce more adverse effects compared to MPs on chromosomal level at long exposure term *in vitro*. Properties of the NPs can be cited as the causes of these results. The interaction of NPs with cells increases due to reasons such as nano-size and increase in surface/volume ratios.

**Keywords:** Iron (III) Oxide, Nanoparticles, Microparticles, Genotoxicity, Chromosomal abnormalities, Sister chromatid exchanges.

## *ORAL PRESENTATION*

# *Diospyros kaki* ekstraktlarının *Leishmania infantum* üzerindeki etkinliğinin incelenmesi

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**Özet**

Leismaniasis, enfekte dişi kum sineklerinin ısırmasıyla insanlara bulaşan, protozoa grubunda yer alan 20'den fazla leismania parazitlerinin neden olduğu zoonotik bir hastalık türüdür. Bu hastalık, yoksulluk ve yetersiz beslenmenin yoğun olarak görüldüğü tropikal bölgelerde yaygındır. Leishmaniasis, klinik semptomlar temel alınarak Kutanöz Leishmaniasis, Mukokutanöz Leishmaniasis ve Viseral Leishmaniasis şeklinde sınıflandırılmıştır.
*Leishmania infantum,* Viseral Leishmaniasis etkenidir. Kala-azar olarak da bilinen Viseral Leishmaniasis, iç organlarda gelişir ve düzensiz ateş nöbetleri, kilo kaybı, dalak ve karaciğerde büyüme ve anemi şeklinde kendini gösterir. Hastalık tedavi edilmezse, esas olarak immünosupresyon ve ikincil enfeksiyonlar nedeniyle yüksek bir ölüm oranına sahiptir.
Hastalığın tedavisinde kullanılan ilaçların yetersizliği ve mevcut ilaçların da yüksek toksisiteye neden olması, yeni ve etkili tedavi yöntemlerine gereksinimi arttırmıştır. Bu sebeplerle düşük toksiste ve hızlı iyileşme sağlayabilecek bitkisel kaynaklı ilaç çalışmalarının başarıyı arttırdığı anlaşılmıştır.
Bu bilgiler ışığında; ilk aşamada antioksidan, antibakteriyel, antitümör ve antidiyabetik etkileri ile bilinen *Diospyros kaki*'den ekstraktlar elde edilmiştir. Sonrasında ekstraktların antioksidan özellikleri incelenmiştir. Son olarak ekstraktların önce sağlıklı memeli hücresi(L929) üzerinde biyouyumluluğu araştırılmış, sonra ise Viseral Leishmaniasis etkeni olan *Leishmania infantum* üzerinde *a*ntileishmanial etkinliği incelenmiştir.
Yapılan literatür araştırmalarında *Diospyros kaki* üzerine çalışmalar olmasına rağmen antileishmanial etkisinin uygulandığı herhangi bir çalışma bulunmamaktadır. Bu çalışma ile Viseral Leishmaniasis'in iyileştirilmesinde kullanılabilecek ilaçların üretimi için bitkisel bir kaynak tespiti amaçlanmıştır.

**Anahtar Kelimeler:** *Leishmania infantum,* Viseral Leishmaniasis, Antileishmanial Etkinlik, *Diospyros kaki,*

## *ORAL PRESENTATION*

# Determination of antioxidant and enzyme inhibitory activity of *Cistus creticus* L. on α-amylase, α-glucosidase, and pancreatic lipase, and phytochemical analysis by using HPLC-DAD

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**Abstract**

*Cistus creticus* is a member of Cistaceae, that is used for the treatment of abdominal pains, burns, diabetes mellitus, diarrhea, rheumatism, snakebites, stomach ailments, wounds, and urethral inflammation traditionally in Turkey. Accordingly, the inhibitory activity of hydroalcoholic extract of C. creticus herba on diabetes mellitus related enzymes, α-amylase, α-glucosidase, and pancreatic lipase, was analyzed in the study. The inhibitory activities of the extract on α-amylase, α-glucosidase, and pancreatic lipase were found 86.50 ± 0.90 %, 99.49 ± 0.44 %, and 51.55 ± 1.46 % respectively at 200 µg/mL final concentration. The antioxidant activity of the extract was tested by using ferric ion reducing power (FRAP), DMPD radical scavenging, and metal chelating activities. Moreover, the phytochemical analysis of the extract was conducted by using HPLC-DAD. The amount of the gallic acid, luteolin-7-O-glucoside, and cinnamic acid was found 0.031 ± 0.001, 0.007 ± 0.00, and 0.005 ± 0.001 g/100g respectively.

**Keywords:** Cistus creticus, amylase, glucosidase, lipase, antioxidant, HPLC

## *ORAL PRESENTATION*

# Screening of WRKY transcription factor and its role in seed germination of *Pistacia chinensis*

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**Abstract**

WRKY, an important transcription factor family, is widely distributed in the plant kingdom. Many reports focused on analysis of phylogenetic relationship and biological function of WRKY protein at the whole genome level in different plant species. However, little is known about WRKY proteins in the genome of *Pistacia* chinensis and their roles in seed germination. In this study, we identified 74 WRKY proteins from the genome of *Pistacia* species using bioinformatics approaches. They were classified into three main groups (Group I–III) according to the classification method of *Arabidopsis thaliana*. The motifs, domains and structure of the identified WRKY proteins were also predicted and analyzed. During seed germination of *Pistacia* chinensis, the seeds at 0 HAI (0 h after imbibing), 40 HAI and 84 HAI were sampled to sequencing to analyze the role of WRKY proteins in germination. Different expressed WRKY genes (12) were identified: EVM0005356, EVM0005870, EVM0008757, EVM0010461, EVM0015295, EVM0015311, EVM0022572, EVM0022903, EVM0026451, EVM0028131, EVM0028271 and EVM0029702. Combined with the promoter prediction results, our analysis indicated that these WRKY genes may regulate ABA and auxin signal transduction pathway to affect seed germination. These results could provide valuable information for *Pistacia* chinensis cultivation and production.

**Keywords:** WRKY, *Pistacia chinensis*, Seed germination, Regulation

## *ORAL PRESENTATION*

# Fabrication of hemp seed oil-doped gelatin/sodium alginate nanofibers using the Box-Behnken experimental design

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**Abstract**

Hemp seed oil is a nutritious oil containing over 80% polyunsaturated fatty acids obtained by cold pressing from the seeds of the Cannabis Sativa L. plant. It contains omega-6 fatty acids such as linoleic acid (LA, 54%), gamma-linolenic acid (GLA, 3%) and omega-3 fatty acids (alpha-linoleic acid (ALA, 17%) and stearidonic acid (2%). The hemp seed oil shows pharmacological activity due to the benefits it provides. The antibacterial property of the oil is proven against gram-negative bacteria *Escherichia coli* (E.Coli) and gram-positive bacteria *Staphylococcus auerus* (S.Aureus) according to the disc diffusion method. Due to this antibacterial feature, hemp seed oil is expected to provide an antibacterial wound healing environment and accelerate healing thanks to the components it contains. Therefore, in the present study, it was aimed to investigate the use of hemp seed oil in a wound dressing system. For this purpose, it was tried to obtain a wound dressing system with nanofiber structure with the most suitable fiber diameter by encapsulating hemp seed oil into gelatin/sodium alginate nanofibers by electrospinning method. The antibacterial activity test results showed that the antibacterial activity decreased with the decrease in the oil concentration. Furthermore, three-level, three-variable Box-Behnken design-based response surface methodology was employed as an optimization tool to select the most appropriate parameter settings required to obtain the desired fiber diameter. Accordingly, the diameter of the nanofibers obtained from the solutions prepared at different component concentrations based on the experimental design was investigated by using scanning electron microscopy (SEM) analysis and a second-order polynomial equation was developed to estimate the fiber diameter. It was found that the experimental data and the developed models were in good agreement. The effectiveness of the nanofiber wound dressing material fabricated under optimum conditions based on the design results will be supported by future characterization tests.

**Keywords:** Hemp seed oil, Box-Behnken, electrospinning, gelatin, sodium alginate

## *ORAL PRESENTATION*

# Fabrication of antibacterial chitosan/silk fibroin/ZnO biocomposite hydrogel sponges for wound dressing applications

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**Abstract**

Wound infections are still problematic in many cases and demand new alternatives for current treatment strategies. In recent years, biomaterials-based wound dressings have received much attention due to their potentials and many studies have been performed based on them. The biopolymer based chitosan wound dressing such as porous sponges, hydrogels, membranes encapsulated with drugs serves better in wound healing and exudate management.
Silk fibroin(SF) has been used in wound dressing applications in combination with polymers, due to its wide availability, biodegradability, water absorption capacity and biocompatibility. SF can also enhance the adhesion, spread and proliferation of epidermal cells and fibroblasts, thereby accelerating wound healing. Accordingly, in this study, we fabricated chitosan/silk fibroin/zinc oxide (CS/SF/ZnO) hydrogel sponges containing different concentrations of an antimicrobial ZnO nanoparticles and model drug (tetracycline hydrochloride,TCH) to develop a suitable material with potential application as high performance wound dressing with controlled drug release. For that, the physicochemical, thermal, morphological and biological characterizations of the biocomposite hydrogel sponges were evaluated, as well as the release assessment of TCH and the determination of the mechanism of release through the application of mathematical models to kinetic data. This biocomposite sponges in all compositions showed desirable swelling behavior at pH 7.4 and pH 5.5 . To develop the potential application of CS/SF/ZnO biocomposite dressings, the antibacterial potentials, as well as the wound healing activities, were investigated *in vitro*. From the antibacterial activity results, it was found that drug-free ZnO NPs-integrated biocomposite hydrogel sponges showed antibacterial potential against Klebsiella pneumonia and the TCH-loaded hydrogel sponges showed enhanced antibacterial activity against Staphylococcus aureus. Overall, these results indicated that CS/SF/ZnO biocomposite hydrogel sponges could be a suitable candidate for wound healing. ZnO nanoparticles in the matrix have synergistic effect with TCH and result in more effective antibacterial property.

**Keywords:** biocomposite hydogel sponge, antibacterial activity, drug release, wound healing, ZnO nanoparticle

## *ORAL PRESENTATION*

# Magnetic Nanoparticles and Their Applications In Analytical Chemistry

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**Abstract**

Nanoparticles, one of the most important researches of recent years, have made rapid progress by opening up to applications in a wide area. Nanoparticles generally have unique physical and chemical properties in the size range of 1-100 nm due to their small size. Magnetic nanoparticles perform best with their supermagnetic properties (10-20 nm in size), making them particularly suitable when seeking rapid response due to applied magnetic fields. In this study, research has been done on the installation of magnetic nanoparticles in analytical chemistry and their analytical applications were investigated. Various sensor applications, biosensors, sensors used in food analysis, MNPs used in electrochemical, immuno and aptasensor applications, separation, extraction and immunoassay applications are mentioned. With the use of MNPs in analytical applications that have been researched in detail, the most used MNP types have been examined. Since the properties of MNPs are highly dependent on their particle properties, their size, synthesis and preparation must be well designed to obtain adequate size-dependent particles with physicochemical properties. In MNP synthesis methods, a total of seven important synthesis methods, namely thermal decomposition, co-precipitation, microemulsion, sol-gel, co-condensation, oil phase and water phase, one-pot synthesis, were examined. The critical points such as how the synthesis methods are applied, their advantages and disadvantages are mentioned. Then, MNP coating methods and the kind of materials they are coated with were examined. Finally, characterization studies of MNPs are examined and compared carefully.
While they are involved in many applications such as sensor applications, detection systems, detection of food contaminants, immunological tests and verifications, it is obvious that the areas of use will expand. The aim of this study is to have information about the types, synthesis methods, coatings and characterizations of MNPs used in analytical applications that are already found in literature and the novelties introduced by the researches in concept of this study.

**Keywords:** Magnetic nanoparticles, analytical chemistry, application

## *ORAL PRESENTATION*

# Immune-related genes as gene biomarkers for some FDA-approved drugs in acute myeloid leukemia

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**Abstract**

Acute myeloid leukemia is the most common acute leukemia in adults. Acute myeloid leukemia is a biologically complex and molecularly and clinically heterogeneous disease, and its incidence is increasing as the population ages. Outcome in patients with acute myeloid leukemia ranges from death within a few days of beginning treatment to likely cure. The major reason patients are not cured is resistance to treatment, often manifested as relapse from remission, rather than, even in older patients, treatment-related mortality, whose incidence is decreasing. Several new drugs have been approved by the FDA and still, their role has been discussed in treatment. The aim of the study is to use a combination of cytokines to be effective in better predicting the chemotherapeutic response of a gene combination rather than a gene. It was aimed to determine whether the protein expression profile resembles the genes whose expression has been determined as an effective biomarker for certain drugs. In the literature study, we identified 12 genes that are important for the immune system. Then, acute myeloid leukemia resistance profiles were determined by making combinations with 5 drugs approved by the FDA. A total of 21 drugs were identified, which are among the drugs accepted both in the Cancer Genome Project data and in the FDA. The combination of the identified immune genes and the drug sensitivity profile were examined. In this study, the prediction combination formula of these genes will be discussed in detail.

**Keywords:** acute myeloid leukemia, gene biomarkers, drug resistance

## *ORAL PRESENTATION*

# Marine Benthic Diatoms from the Sensitive and Protected Area of Yumurtalık Lagoon

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**Abstract**

Diatoms are important components of marine waters consisting of an essential source for primary production and a high rate of photosynthesis. Benthic diatom composition can be very diverse in coastal areas. Diatom biodiversity is also used to determine water quality. Therefore, it became crucial to reveal the diatom flora of the coasts, lagoon and marine wetlands. Lagoons are fragile and important areas which are easily affected by environmental changes. Recently, diatoms are used to reveal the status of lagoons, due to their presence throughout the years. Yumurtalık Lagoon located in the Eastern Mediterranean Sea coasts of Adana province is an important wetland for the area. The area is at the entrance of Iskenderun Gulf which is exposed to high salinity and high water temperature throughout the year. The objective of this study was to reveal the diatom community of Yumurtalık Lagoon. Diatom samples were collected in April 2015 from the submerged stones and soft bottoms of the shallow water. Additionally, physicochemical parameters were measured in the sampling area. Diatom flora was determined with light microscopy (LM). According to the results, a total of 45 species belonging to 19 genera were observed. The highest relative abundance was observed in *Navicula* (29%), *Achnanthes* (16%) and *Nitzchia* (15%) respectively. Among the species diversity, *Navicula* was represented with the highest number of species (7), followed by *Nitzschia* (6) and *Amphora* and *Cocconeis* (5). The results showed that pollutant-tolerant *Navicula* and *Nitzschia* were abundant genera. The diatom flora of Yumurtalık Lagoon gives an insight into the diverse community structure of marine benthic diatoms and contributes to the knowledge of diatom flora of the Turkish coastal waters.

**Keywords:** Benthic diatoms, biodiversity, lagoons, taxonomy, Turkey.

## *ORAL PRESENTATION*

# Karışık Reaktif Sistemlerin Kullanılması İle Kömür Yüzdürülmesinin İncelenmesi

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**Özet**

Hidrofobik kömür parçacıklarının hidrofilik gang minerallerinden köpüklü yüzdürme ile seçici olarak ayrılması 1920'li yıllara dayanmaktadır. Bundan önce, köpüklü flotasyonun ticari uygulamaları, yalnızca boyutu 100 um'den daha ince olan cevherler ve minerallerle sınırlı durumdaydı. Kömür yüzdürmede, 500 µm'den daha ince partiküller, doğal yüzebilirlikleri ve yüksek hacimli yanıcı maddeye sahip olmalarından dolayı yaygın olarak kullanılmakla birlikte, bu durum yaklaşık %80 kadar yüksek yanıcı geri kazanımlarla ile sonuçlanmaktadır. Bu bağlamda iyileştirmeye yönelik yeni reaktifler, havalandırma modu, kabarcık dağılımı ve hücre tasarımlarında modifikasyonlar gibi alanlar bilim dünyası tarafından güncel araştırma konusu olarak incelenmektedir. Kömür yüzdürme işleminin ince parçacıkları ayırmadaki etkinliği, birincil olarak seçilen reaktif türünden etkilenmektedir. Temiz kömürde minimum kül ile karbonlu malzemenin daha iyi geri kazanılması için yapılan çabalar şüphesiz ki sonsuzdur. Günümüzde kömür hazırlama endüstrisi, sürdürülebilirlik için temiz kömür geri kazanımını geliştirmek adına çeşitli yollar üzerinden oldukça büyük çabalar sarf etmektedir. Bu çabalardan biri, yıllar içerisinde önemli ilerleme gösteren ancak reaktif adsorpsiyon mekanizmaları hakkında daha az anlaşılan karışık reaktiflerin kullanılması olmuştur. Karışık toplayıcılar, karışık yüzey aktif maddeler ve emülsifikasyon kullanımı, yağlı toplayıcıların yüzey aktif maddelerle birleştirilmesi ve bireysel reaktiflerin kullanılmasına yönelik geleneksel yaklaşıma göre oldukça büyük avantajlar göstermiştir. Yüksek ayırma verimlerinin temeli; kömür yüzeyleri üzerine hızlı ve seçici bir şekilde adsorbe edilme kapasitesinden kaynaklanabilmektedir. Reaktif adsorpsiyonu için artan fırsat, esas olarak, özellikle oksitlenmiş veya düşük dereceli kömürlerde, yağ ve su arasındaki azaltılmış arayüzey geriliminin kolaylaştırılmasından kaynaklanmaktadır. Mevcut konjüktürde yüzebilirliği arttırmak adına deneysel ve moleküler dinamik simülasyon yaklaşımları benimsenerek yeni yöntemler keşfedilmeye çalışmakta ve reaktif adsorbsiyonları da tartışılmaktadır.

**Anahtar kelimeler:** Filotasyon, Kömür, Yüzey Kimyası.

## *ORAL PRESENTATION*

# Koordinasyon Polimerlerinin Tasarımında Organik Köprü Ligantların Etkisi

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**Özet**

Bir merkezi atomun, ligant adı verilen değişik sayıda atom veya atom gruplarınca koordine edilmesi ile oluşan bileşiğe koordinasyon bileşiği veya kompleks adı verilir. Koordinasyon polimerleri, bir metal iyonu ile organik ligantlardan oluşan birimin, bir, iki ya da üç boyutlu olarak birbirini tekrar etmesiyle oluşan bileşikler olarak tanımlanabilir. Son yıllarda koordinasyon polimerleri üzerine yapılan çalışmalarda muazzam bir artış olduğu görülmektedir. Bunun nedeni, hiç kuşkusuz bu bileşiklerin gaz adsorbsiyonu, moleküler/iyonik ayırma, optik, elektrik, manyetik özellikleri, kataliz gibi potansiyel uygulama alanlarına sahip olmasıdır [1-3]. Literatürde koordinasyon polimerinin sentezi ve karakterizasyonu üzerine yayımlanan çok sayıda çalışma bulunması, koordinasyon kimyacılarına istenilen uygulama alanlarına sahip yeni bileşiklerin tasarımı için uygun stratejiler oluşturma şansı vermektedir. Koordinasyon polimerlerinin tasarımında, sentez yöntemi, çözelti pH'ı, metal-ligand oranı, reaksiyon sıcaklığı ve süresi, çözücü(ler) gibi birçok faktör etkilidir. Bunların yanı sıra metal iyonu ve organik köprü ligant seçimi hayati rol oynamaktadır [4,5]. Bu çalışmada, organik köprü ligantların koordinasyon polimerleri tasarımı üzerine etkisi; boşluk etkisi, sübsitüent etkisi ve konumsal izometrik etki başlıkları altında incelenmektedir [6]. Ayrıca organik köprü ligantların fiziksel ve kimyasal özelliklerinin (ligant boyutu, esnekliği, donör atomun pozisyonu, çifte bağ ve/veya subsitüent grubun eklenmesi/çıkarılması vb.) koordinasyon polimerlerinin boyutu, çeşitliliği ve olası uygulamaları üzerine etkisi tartışılmaktadır.

**Anahtar Kelimeler**: Koordinasyon Polimerleri, Koordinasyon Polimerlerinin Tasarımı, Organik Köprü Ligantlar

**Kaynaklar**

[1] Y. Güçlü, H. Erer, H. Demiral, C. Altintas, S. Keskin, N. Tumanov, B.-L. Su, F. Semerci, ACS Appl. Mater. Interfaces 13 (28) (2021) 33188–33198
[2] A. Sun, Y. Yang, Y. Liu, L. Ding, P. Duan, W. Yang, Q. Pan, , Cryst. Growth Des. 21 (9) (2021) 4971–4978.
[3] Wilmer, C. E., Farha, O. K., Bae, Y. S., Hupp, J. T., & Snurr, R. Q. Energy & Environmental Science, 5(12), (2012), 9849-9856.
[4] Maity D K, Halder A, Ghosh S, Ghoshal D, Cryst. Growth Des. 16 (9) (2016) 4793-4804.
[5] Tan, J. C., & Cheetham, A. K. Chemical Society Reviews 40(2), (2011), 1059-1080.
[6] Du, M., Li, C. P., Liu, C. S., & Fang, S. M. Coordination Chemistry Reviews, 257(7-8), (2013), 1282-1305.

## *ORAL PRESENTATION*

# The significance and potential environmental impacts of jarosite mineral in mining residues

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**Abstract**

Jarosite [$KFe_3(SO_4)_2(OH)_6$] is an iron-hydroxy sulfate mineral commonly found in acidic, sulfate-rich environments and in mining and ore processing residues as secondary mineral particularly formed as a result of hydrolysis and oxidation of sulfide minerals e.g., pyrite, chalcopyrite, enargite, stibnite. Hence, the presence of jarosite in mining sites may be a good indicator for understanding past acid mine drainage (AMD) formations. X-ray diffraction (XRD), transmission electron microscopy (TEM) or scanning electron microscopy (SEM) techniques are used for the detection of jarosite. The peaks related to jarosite group minerals can be scanned by the XRD technique, while the detection of crusty and powdery jarosite from SEM images is not easy due to their relatively low reflectance (22-25%). However, the pseudocubic jarosite crystals can be clearly observed by SEM and TEM procedures. To determine jarosite quantitatively, appropriate chemical methods including specific leaching methods using ammonium oxalate or hydrochloric acid should be applied depending on the rock mineralogy. On the other hand, contributions of jarosite to acid generation have not been taken into consideration in most of scientific and technical research leading to underestimation in acid production potential of mining residues. Hence, the jarosite acidity should be calculated by multiplying jarosite-S% by the stoichiometric calculation factor of 23.44 (if paste pH<6.3) or 46.88 (if paste pH>6.3), and subsequently added to reactive sulfur acidity. Lastly, jarosite group minerals consisting of ammoniojarosite, argentojarosite, natrojarosite, and plumbojarosite, in which ammonium, silver, sodium, and lead, respectively substitute for potassium are able to scavenge and role as sinks for potentially toxic cations such as $As^{+3}$, $NH_4^+$ and $Pb^{2+}$, and therefore resulting in the reduction of mobilization of toxic elements to aquatic systems.

**Keywords:** Mining wastes, Acid mine drainage, Jarosite detection, Jarosite acidity, Scavenge effect

## ORAL PRESENTATION

# Türkiye'de satılan süt ve süt ürünlerinin besin değeri: Organik ve konvansiyonel besinler arasında fark var mı?

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**Özet**

Organik ürünlerin pazarı her geçen gün hızla büyümektedir. Bu durumun, tüketicilerin geleneksel ürünlere göre organik ürünlerin daha iyi bir beslenme profiline sahip sağlıklı besinler olduğunu düşünmeleri ile ilişkilendirilmektedir (1). Organik pazarı hızla büyüyen süt ve süt ürünleri beslenme planlarının önemli bir kısmını oluşturmakla birlikte bütün yaş grupları için önemli fonksiyonlara sahiptir (2). Bu nedenle bu çalışmada Türkiye'de satılan organik (O) ve konvansiyonel (K) süt ve süt ürünlerinin besin değeri incelenmiştir. Toplamda 36 besinin incelenmiş ve süt, yoğurt, peynir ve tereyağı-sürülebilir yağlar olmak üzere dört alt grupta değerlendirilmiştir. Besin değeri incelemesinde ürünlerin etiket bilgileri dikkate alınmıştır. Ürün seçimi, aynı markanın tüm potansiyel ürün çiftlerine (O ve K) odaklanılarak gerçekleştirilmiştir. Paketli olmayan, organik ürün logosu bulunmayan, besin değeri ve içindekiler bilgisi açık bir şekilde verilmeyen ürünler çalışmaya dahil edilmemiştir. Enerji ve besin ögesi değerleri 100 g veya 100 mL üzerinden hesaplanmıştır. Süt, tereyağı ve sürülebilir yağ örneklerinde enerji ve makro besin ögeleri arasında benzerlik bulunurken, kalsiyum değerinin K sütte daha yüksek olduğu görülmüştür. Organik yoğurdun enerji, makro ve mikro besin ögeleri K yoğurttan daha yüksekken, O peynirin kalsiyum değeri K peynirlerden daha yüksek bulunmuştur (Tablo 1). Ayrıca organik ürünlerin logoları incelenmiş ve ürünlerin %80'inin "organik" logosunun paketin ön tarafında, %15'inin paketin yan tarafında, %5'inin ise paketin arkasında olduğu tespit edilmiştir.

**Tablo 1.** Ürün gruplarına göre besin değerinin karşılaştırılması

| Ürün grubu | Enerji (kcal) | CHO (g) | Şeker (g) | Protein (g) | Yağ (g) | Doymuş yağ (g) | Kalsiyum (mg) | Tuz (g) |
|---|---|---|---|---|---|---|---|---|
| **Süt-K** | 58.0 | 4.6 | 4.6 | 3.0 | 3.1 | 2.0 | 116.7 | - |
| **Süt-O** | 56.8 | 4.9 | 5.2 | 3.0 | 3.1 | 1.9 | 113.3 | 0.1 |
| **Yoğurt-K** | 61.0 | 6.2 | 3.0 | 4.0 | 2.3 | 1.5 | 165.0 | - |
| **Yoğurt-O** | 93.2 | 10.3 | 6.0 | 4.8 | 4.0 | 2.8 | 157.5 | 0.2 |
| **Peynir-K** | 226.4 | 3.0 | 2.5 | 14.9 | 17.2 | 8.8 | 471.3 | 1.5 |
| **Peynir-O** | 233.1 | 2.9 | 2.1 | 15.6 | 17.8 | 10.7 | 579.0 | 1.5 |
| **Tereyağı-sürülebilir yağlar-K** | 733.0 | 0.5 | 1.0 | 0.6 | 81.0 | 50.5 | - | 0.1 |
| **Tereyağı-sürülebilir yağlar-O** | 720.0 | 0.6 | 0.6 | 0.6 | 80.0 | 41.5 | - | 0.1 |

*CHO: karbonhidrat; O: organik; K: konvansiyonel

Bu çalışmada O ve K süt ve süt ürünleri kıyaslanmış ve ürün grubuna bağlı olarak besin ögelerinin farklılık gösterebileceği belirlenmiştir. Gelecekte yapılacak çalışmaların daha fazla ürün grubunu ve üretim ile ilgili ulaşılabilir bilgileri ele alarak planlanması önerilmektedir.

**Anahtar Kelimeler:** organik besin, süt, yoğurt, besin değeri, kalsiyum

**Kaynaklar**

1. Crinnion WJ. Organic foods contain higher levels of certain nutrients, lower levels of pesticides, and may provide health benefits for the consumer. Altern Med Rev. 2010 Apr;15(1):4-12. PMID: 20359265.
2. FMI. Organic Milk Market Outlook. 2022.

## *ORAL PRESENTATION*

# TURP (TURkiye bütünleşik tuzlu su fitoplankton indeksi)

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**Özet**

Bu çalışma Akdeniz, Ege, Marmara ve Karadeniz'in kıyısal sularında 2017-2020 yılları arasında ilkbahar, yaz ve sonbahar dönemlerinde, toplam 77 istasyondan biyolojik izleme tebliğine uygun şekilde alınmış fitoplankton komünitesi üzerine yapılmıştır. Denizel fitoplankton verilerinden elde edilen ekometrik indeks denemelerinin çevresel parametrelerle olan uygunluğunun test edilmesi için bir yapay sinir ağı yöntemi olan ANFIS metodu uygulanmıştır. Sonuç olarak ülkemize özgü ve kıyısal fitoplanktonun ekolojik durumunu başarılı bir şekilde ortaya koyan "Türkiye Bütünleşik Tuzlu Su Fitoplankton İndeksi (TURP)" ilk defa tasarlanmıştır. Bu indeks kıyısal sularda fitoplankton komünitesinin çevresel parametrelere olan ilişkilerini farklı yönlerden yansıtabilen alt metrik indekslere sahiptir ve bu alt metrik indekslerden elde edilen ve ekolojik kalite oranı (EKO) değerlerinin ortalaması olarak tanımlanan bütünleşik bir indeks tipidir. Fitoplankton biyokütle ve biyoçeşitlilik indeksleri, fonksiyonel çeşitlilik indeksleri, ağırlıklı ortalama indeksleri, Ellenberg katsayısı indeksleri ve saprobik sistem ana grupları altında toplanan alt metrik indeksler için tipe özgü EKO değerleri hesaplanmıştır.

**Anahtar Kelimeler:** Fitoplankton, WFD, EKO, Türkiye, TURP

**Teşekkür:** Bu çalışma T.C. Tarım ve Orman Bakanlığı Su Yönetimi Genel Müdürlüğü uhdesinde yürütülen "Türkiye'de Referans İzleme Ağının Kurulması Projesi'' kapsamında elde edilen veriler kullanılarak hazırlanmıştır.

## *ORAL PRESENTATION*

# Peptides with the "xxFWxFWxxLxx" motif may interact with MDM2 more efficiently than peptides with the "xxFxxxWxxLxx" motif

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**Abstract**

The p53 protein is encoded by the TP53 gene, one of the most mutated genes in human cancer. p53 activates several genes involved in genomic stability, cell cycle, apoptosis, DNA repair, etc. p53 level inside the cells regulated by MDM2. MDM2 interacts with the TAD domain of p53 resulting in its degradation by the proteasome. Residues, Leu14, Phe19, Leu22, Trp23, and Leu26 in p53 are involved in interaction with MDM2. Disrupting the interaction between the MDM2 and p53 is an approach in cancer therapy. The few 12-mer peptide inhibitors designed and experimentally validated for this purpose are p53 mimics containing residues Phe19, Trp23, and Leu26 ($^{17}$xxFxxxWxxLxx$^{29}$) that interact directly with MDM2. In this study, 12-mer peptides were designed by modifying experimentally validated peptides to improve their MDM2 interaction efficiency. Structure predictions for the designed peptides were generated using the PEP-FOLD3 peptide structure prediction server. The interactions of the designed peptides with MDM2 were predicted using the HPEPDOCK blind docking server and compared with the experimentally validated peptides. Peptides having additional tryptophan in 20th and phenylalanine in the 23rd positions showed stronger interaction potential with MDM2 relative to the original "xxFxxWxxLxx" sequences. Among all the tested peptides "xxFWxFWxxLxx" motif was more efficient than the "xxFxxxWxxLxx" motif. The results obtained in this study suggest that a more effective p53-MDM2 inhibition could be achieved by altering two residues in the 12-mer inhibitor peptides.

**Keywords:** p53, MDM2, Peptide Motif.

## *ORAL PRESENTATION*

# The syntheses and structural characterizations of various cyclotriphosphazenium salts

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**Abstract**

Hexachlorocyclotriphosphazene ($N_3P_3Cl_6$, trimer, HCCP) is mostly used as the starting compound among the cyclophosphazene derivatives. Therefore, HCCP has been widely used in the design of new substituted cyclotriphosphazene derivatives, especially in the field of phosphazene chemistry. The properties of cyclic phosphazenes vary according to the inorganic PN units as well as the nature of the substituents bonded to the P-atoms. Partially and fully substituted organocyclophosphazene and polymeric phosphazene derivatives have received great attention in technology and medicine due to their chemical, spectral, chiral and bioactivity properties. On the other hand, fully amino and alkylamino substituted cyclotriphosphazenes are used as potential phosphazene bases. In recent years, articles on phosphazenium salts containing bulky organic acids and their biological activities have been published. Since the solubility in biological fluids is very important in biological studies, investigation of antimicrobial and cytotoxic activities of phosphazenium salts may be very important and useful for future studies. In this study, cyclotriphosphazenium salts were obtained as a result of the reactions of fully amino substituted cyclotriphosphazene bases and aliphatic carboxylic acids for this purpose. Their structures were elucidated by elemental analysis, FTIR, $^{1}H$, $^{13}C$, and $^{31}P$ NMR techniques.

**Keywords:** Cyclotriphosphazenes, salts, spectroscopy

## *ORAL PRESENTATION*

# Off-Pump Ve On-Pump Yapılan Koroner Bypass Cerrahisinin Post Operatif Değerlerinin Karşılaştırılması

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**Özet**

Kardiyopulmoner bypass (KPB) cerrahisi koroner arter hastalığı olan hastalar için köklü bir tedavi yöntemi haline gelmiştir. Bununla birlikte, Koroner arter bypass cerrahisi ile ilişkili bazı morbiditeden Kardiyopulmoner bypassın sorumlu olabileceğini gösteren kanıtlar mevcuttur. Ekstrakorporal devre tarafından başlatılan sistemik inflamatuar reaksiyon, kanda mekanik travma, çeşitli immünolojik kaskadların (kompleman, sitokinler) aktivasyonu, bozulmuş hemostaz ve bozulmuş nörolojik, renal ve gastrointestinal fonksiyon ile sonuçlanmaktadır.Ayrıca, aort kanülasyonu, kros klempleme ve kardiyopulmoner byypass, mikroembolizasyon ve makroembolizasyon, ardından nörolojik hasar ve global miyokardiyal iskemi/reperfüzyon hasarı dahil olmak üzere diğer uç organ hasarı ile sonuçlanabilmektedir.Bu nedenle, Kardiyopulmoner bypasstan kaçınılabilirse Koroner arter cerrahisinin cerrahisinin daha güvenli olacağı öne sürülmüştür.Yeni kardiyak stabilizasyon cihazlarının da geliştirilmesi, atan kalp üzerinde güvenli ve tekrarlanabilir koroner anastomozların oluşturulmasına olanak sağlamış bulunmaktadır.Bununla birlikte riskli vakalara yönelik seçim yanlılığı bir sorun olmaya devam etmiştir.Bugüne kadar yayınlanan büyük randomize çalışmalar çelişki göstermiştir, bazıları hastanede kalış süresinin ve miyokardiyal enzim salınımının azaldığını gösterirken, bazıları atriyal fibrilasyon insidansının, hastanede kalış süresi ve atan kalp üzerinde gerçekleştirilen koroner bypass cerrahisi ile kan ürünü kullanımının azaldığını göstermiştir.Çalışmada Kardiyopulmoner bypass cerrahisi geçiren hastaların off-pump ve on-pump olmaları göz önüne alınarak post operatif değerleri incelenerek cerrahinin güvenliği ve etkinliği karşılaştırılacaktır.

**Anahtar kelimeler:** Kardiyopulmoner Bypass, off-pump ,on-pump,Koroner bypass cerrahisi**,**

## *ORAL PRESENTATION*

# A new Schiff-base chemosensor for detection of metal ions in aqueous solution

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**Abstract**

Metal ions pollution is a serious problem because of its potential to harm the environment and human health even at low concentrations. Therefore, it is quite important to develop an effective analytical method for the sensitive and selective detection of trace amounts of metal ions. To date, many methods, including atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, chromatography and ion selective electrodes have been applied to detect metal ions. However,these methods often require time-consuming analysis,complicated procedures, large sample volumes, expensive instruments, or special skills. Recently, the synthesis of efficient chemosensors to easily recognize and quantify metal ions is an important topic in environmental sciences. Schiff base molecules are attractive for chemosensor applications because they are easy to synthesize, exhibit an intense color change after coordination with metal ions, and have high sensitivity and selectivity at low cost.

In this study, a new, simple and sensitive colorimetric chemosensor was synthesized for the detection of metal ions and characterized by $^{1}$H-NMR, FTIR and mass spectroscopy. The sensing properties of the synthesized chemosensor were investigated by using UV–Visible absorption spectroscopy. The results showed that the chemosensor sensitively and selectively detects $Hg^{2+}$ ions in weakly acidic or neutral conditions. The detection limit, association constant and binding stoichiometry were examined by UV–Visible titration data, Benesi-Hildebrand equation and Job's plot.

**Keywords:** Schiff-base, heavy metal, chemosensor, colorimetric detection

## *ORAL PRESENTATION*

# Antimicrobial and antibiofilm activities of turmeric (*Curcuma longa* L.) rhizome extracts against Gram-positive/negative bacteria and yeast strains

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**Abstract**

Antimicrobial resistance is an important problem for the human health and it is known that the microorganisms are less susceptible to antimicrobial agents in biofilm structures. *Curcuma longa* L. rhizomes have been an important source for medicinal purposes of a wide range. Its antimicrobial potential has been studied over a long time mainly covering medium polarity solvents. Therefore, the goals of this study were determined as comparatively investigating the antimicrobial activities of four types of *Curcuma longa* extracts (hexane, dichloromethane, ethanol and water) within full range of apolar to polar and investigating the alteration levels on biofilm production of bacteria (*Staphylococcus aureus* and *Escherichia coli* ATCC strains) and fungi (*Candida albicans* ATCC strain) in the presence of the extracts. Inhibition zone diameters and minimum inhibitory concentration (MIC) values were determined by disk diffusion test and microdilution method. Alteration in the biofilm production levels were determined by spectrophotometric microplate method using crystal violet staining. All the experiments were performed in triplicate, and the statistical analyses were performed. According to the results, mean inhibition zone diameters with hexane extract were 11, 12 and 13 mm against *S. aureus*, *E. coli* and *C. albicans*, respectively. MIC values of hexane extracts were 64 µg/mL against *S. aureus*, *E. coli* and *C. albicans*, as the MIC values of dichloromethane extracts were found to be 64 µg/mL against *E. coli* and *C. albicans*. Biofilm production levels of *S. aureus*, *E. coli* and *C. albicans* were inhibited significantly in the presence of hexane extract at MIC values at a range of 30.6%, 22.5% and 30.8%, respectively. Additionally, the biofilm production levels of *S. aureus*, *E. coli* and *C. albicans* were also inhibited in the presence of water extract. In conclusion, antimicrobial potential of apolar extracts of turmeric rhizomes were identified. Besides well-known phenolic components, apolar compounds in turmeric rhizomes deserve to be involved in further research. Targeting of biofilm structures may have important role in the control of infectious diseases. Further antimicrobial activity, microscopic scanning and gene expression experiments were planned to investigate the effects of the apolar extracts on different standard strains and clinical isolates.

**Keywords:** *Curcuma longa*, antimicrobial activity, antibiofilm activity, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans.*

## *ORAL PRESENTATION*

# Canine oral papillomatosis

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**Abstract**

Canine oral papillomas, caused by papillomavirus, are a common skin condition. An-1,5-year-old male, mixed breed dog was brought for necropsy to the to the Department of Pathology. At necropsy, there were masses around the mouth, palate, gums, tongue and tongue root, lower and upper jaw, epiglottis and around the nose. These samples were fixed in 10% neutral buffered formalin, embedded in paraffin, cut at 5µm, and stained with hematoxylin-eosin (H&E) for histopathological analysis. Macroscopically, many masses with a cauliflower-shaped, elastic consistency, homogeneous cross-sectional surface and gray-white color, protruding from the surface, measuring up to 5 cm from the size of a pin tip were seen. Microscopically, it was noticed that the epidermis, especially numerous, uniform appearance, stratum spinosum layer cells with vesicular nuclei were excessively increased. It was also recorded that hydropic degeneration was seen in places and finger-like extensions were observed in some areas towards the dermis. Canine oral papillomatosis was diagnosed based on macroscopic and microscopic diagnoses. In cases of papillomatosis, it can turn into a malignant form depending on its location and it can be lethal, as seen in this case.

**Keywords:** Dog, Oral, Papillomatosis, Pathology

## *ORAL PRESENTATION*

# Pharmacogenetics in the treatment of depression

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**Abstract**

Major depression is a psychiatric disease with a high prevalence that restricts the psychosocial functions of individuals and reduces their quality of life. Today, it is accepted that many factors play a role in the etiology of the disease. Pharmacogenetics is a science that investigates the role of genes in the responses of individuals to drugs. The purpose of pharmacogenetics is to predict the patient's response to a particular drug, thereby providing an individualized optimum treatment. Treatment of this disease is important because of the recurrent death and suicidal ideation in patients with depression. However, it has been shown in some cases that the response of depressed patients to antidepressants is insufficient and that it differs between individuals. It has been understood that genetic factors play an important role in both treatment response and frequency of side effects in major depressive disorder. In the light of this information, the concept of individualized treatment has been introduced in the treatment of depression in recent years. For this purpose, genes determining the pharmacokinetic and pharmacodynamic properties of drugs used in antidepressant treatment were investigated, the possible polymorphism results in these genes were evaluated and the relationship between candidate gene and treatment response was reported. Studies have led to the emergence of dosage recommendations for drugs used during treatment. As a result, pharmacogenetics plays an important role in antidepressant treatment and it is considered appropriate to target individualized therapy as much as possible in the treatment of patients.

**Keywords:** Antidepressants, pharmacogenetics, major depressive disorder, polymorphism

## *ORAL PRESENTATION*

# Experimental epilepsy models and histopathological changes in the central nervous system

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**Abstract**

Epilepsy, a chronic neurological condition characterized by the recurrence of unprovoked seizures affecting about 65 million people worldwide, is a serious disease that needs to be treated as it increases the risk of death. Experimental studies are needed to elucidate the essential neuronal dysfunctions underlying epilepsy and to examine the pathological changes caused by the disease, select antiepileptic drugs, and develop new treatments. Human tissues except biopsy or autopsy materials cannot be used in research due to ethical restrictions. For this reason, preclinical studies are carried out by generating seizures similar to spontaneous and recurrent seizures in epilepsy by various methods in experimental animals. Experimental epilepsy models can be classified into three groups according to the model formation: (a) models created with convulsant chemicals or electrical stimulation, (b) reflex epilepsy models triggered by auditory or visual stimuli, and (c) idiopathic models using genetically epilepsy-prone animals. Since the seizures induced in these models can be simple partial, complex partial, generalized tonic-clonic, generalized absence, and status epilepticus, it should be considered which method produces which type of epilepsy when deciding on the experimental model. In addition, the age of onset, etiology of the disease, phenotype and EEG pattern of the seizure, and acute and long-term results of the disease should also be regarded in model selection. Different seizure inducers can cause distinct changes in cognitive and behavioral functions. Moreover, histopathologically, varying degrees of edema, neuronal pyknosis and apoptosis, a decrease in the number of neurons, and a reduction in hippocampal volume can be observed in the brain regions such as the amygdala, hippocampus, thalamus, neocortex, and olfactory cortex. This review summarizes the widely used experimental epilepsy models and mentions their similarities and differences in terms of cognitive and behavioral functions and histopathology.

**Key words:** Epilepsy, seizures, experimental animal models, histopathology.

## *ORAL PRESENTATION*

# Efficacy of sugar beet pulp as a bio-adsorbent in removing anionic and cationic dyes from aqueous solutions

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**Abstract**

The aim of this study is to increase the usage areas of various value-added products, such as sugar beet pulp, as feedstock. The potential benefits of beet pulp should be highlighted rather than being sold as feed. In this context, adsorption was investigated to determine the dye removal efficiency in wastewater to contribute to the cyclical use of economically, socially and environmentally beneficial resources. Synthetic dyes are widely used in many fields, such as textile, paper, leather tanning, and paint manufacturing industries. Dyes can be classified as cationic (basic dyes), anionic (direct, acid and reactive dyes) and non-ionic (disperse dyes) according to their charge when dissolved in an aqueous medium. In an aqueous environment, dyes can cause skin allergies, cancer, mutation, decreased light penetration and decreased photosynthetic activity. Adsorption is the most widely used method for wastewater removal. Considering the importance of aquatic life, the adsorption method in waste removal still maintains its popularity. As a result of this study, over 90% efficiency was achieved in the removal of anionic and cationic dyes such as Congo Red (CR), Crystal Violet (CV), Methylene Blue (MB), Malachite Green (MG), Reactive blue 4 (RB4), Rhodamine B (Rhod B), Thioflavin T (TFT). Results, the bio-economic gain and added value of sugar beet pulp, which is supplied from the Van/Erciş region and used as raw material, is stated and the data obtained show that it is quite effective in removing dyes from wastewater.

**Keywords :** Adsorption, anionic dye, cationic dye, sugar beet pulp.

## *ORAL PRESENTATION*

# Synthesis, Enzyme Inhibition Profile and Molecular Docking Studies of Uracil Derived Benzylic Amines

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**Abstract**

Pyrimidines are one of the most common heterocyclic compounds found in biologically active natural products.[1] One of RNA's four nucleobases, uracil, is a typical and naturally occurring pyrimidine derivative. Due to its synthetic accessibility and broad spectrum of biological activities including anti-cancer, antiviral, antibacterial, and antioxidant properties, uracil derivatives are crucial structures in drug development studies.[2-4]
Here in we report the synthesis of a series of novel uracil derived benzylic amines via a simple reductive amination procedure with moderate to good yields (30-84% yields). Excess sodium borohydride was used to reduce *in situ* produced 5-(arylidene)-6-aminouracils synthesized by the condensation reaction of 5,6-diamino-1,3-dimethyluracil with substituted salicylaldehydes. All of the compounds were characterized using FT-IR,$^{1}$H NMR,$^{13}$C NMR spectroscopy and elemental analysis. Novel uracil-appended benzylic amines were tested for their ability to inhibit a variety of metabolic enzymes, including acetyl cholinesterase (AChE) and the isoenzymes of human carbonic anhydrase I and II (hCA I and II), which are linked to a number of common diseases, including Alzheimer's disease (AD), epilepsy, diabetes, and glaucoma. Molecular docking simulations were also conducted to explain the binding interactions of compounds with AChE.

**Keywords:** Benzylic amines, uracil, enzyme inhibition; acetylcholinesterase, carbonic anhydrase.

REFERENCES:
1) Lagoja, I. (**2005**), Pyrimidine as Constituent of Natural Biologically Active Compounds. Chemistry & Biodiversity, 2: 1-50.
2) Aleksandra, P. Dariusz C. (**2015**), In search of uracil derivatives as bioactive agents. Uracils and fused uracils: Synthesis, biological activity and applications. European Journal of Medicinal Chemistry, 97: 582-611.
3) Sanduja M, Gupta G, Virmani T. (**2020**) Recent advancements in Uracil and 5-Fluorouracil hybrids as potential anticancer agents: A review. J Appl Pharm Sci, 10(02): 129–146.
4) Rakesh K, Neerja S, Mahendra N, Holly A. S, and D. Lorne J. T, (**2001**), Synthesis and Antiviral Activity of Novel Acyclic Nucleoside Analogues of 5-(1-Azido-2-haloethyl)uracils *Journal of Medicinal Chemistry 44* (24), 4225-4229

## *ORAL PRESENTATION*

# Enhancement of Biogas Production from Poultry Manure Using Microwave Assisted Acid Pretreatment

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**Abstract-** This study focused on the effects of microwave-assisted acid pretreatment on biogas production from poultry manure (with 6,9 ± 0,2 pH and 41% VS). To understand the impact of microwaves combined with acid pretreatment on the disruption of biomass structure, the poultry manure was pretreated with 1M sulfuric acid solution at room temperature for 10 minutes (pH 4). Microwave irradiation (frequency of 2.45 GHz) at 600 W for 4 minutes was applied. The Biochemical Methane Potential (BMP) tests were carried out in 100 mL serum bottles sealed with butyl rubber stoppers at mesophilic conditions (35 °C) for a hydraulic retention time (HRT) of 22 days. According to the experimental results, pretreatments with acid, microwave, and combined acid-microwave pretreatment achieved 16%, 33%, and 41% COD solubilization respectively. The BMP test with raw poultry manure also attained a methane yield of 55 mL CH4/ g VS. The specific methane potential (SMP) was increased by 4% after acid pretreatment. It increased by 9% after microwave pretreatment compared to untreated biomass while methane yield was further increased by about 15% by applying microwave-assisted acid pretreatments. This study showed that microwave-assisted acid pretreatment is a promising technology for enhanced biogas production.

**Keywords-** biogas; anaerobic digestion; poultry manure, microwave assisted acid pretreatment.

## *ORAL PRESENTATION*

### Investigation of the role of $H_2S$ in the hepatoprotective effects of sildenafil and osthole

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**Abstract**
High-calorie diet, alcohol, and multiple drug use increase reactive oxygen species (ROS) and cause liver damage. ROT plays a key role in the initiation and progression of liver diseases. Antioxidant therapies have beneficial effects in the management of liver diseases. Hydrogen sulfide ($H_2S$) is a gasotransmitter involved in the regulation of vascular function, oxidative stress, glucose and lipid metabolism, many hepatic functions, including mitochondrial functions. Since $H_2S$ plays role in the pathogenesis/treatment of liver diseases, it is considered a promising therapeutic target. Sildenafil is used in the treatment of pulmonary hypertension and erectile dysfunction. Osthole is a natural coumarin with antioxidant, anti-inflammatory and smooth muscle relaxant effects. Sildenafil/osthole exert their antioxidant and hepatoprotective effects by increasing antioxidants (GSH, catalase, SOD, GPx, GR), regulating Keap1/Nrf2 pathway which are common mechanisms with $H_2S$. While the sildenafil-$H_2S$ relationship has been recently reported in some tissue except the liver, the relationship between osthole and $H_2S$ has not been investigated yet. The contribution of $H_2S$ to the hepatoprotective and antioxidant effects of sildenafil and osthole have not been investigated. In our project, for the first time the effects of sildenafil/osthole on endogenous $H_2S$ production in the presence/absence of oxidative stress were investigated by $H_2S$ microsensor, and the role of $H_2S$ in their antioxidant effects by chemiluminescence (luminol, lucigenin). Sildenafil/osthole increased L-cysteine-induced $H_2S$ synthesis in healthy liver and reversed the pyrogallol-induced reduction in $H_2S$ production. Sildenafil/osthole decreased the pyrogallol-induced increase in ROT levels, these effects were inhibited in the presence of the $H_2S$ synthesis inhibitor aminooxyacetic acid. These results indicate that sildenafil/osthole have antioxidant and hepatoprotective effects by increasing $H_2S$ synthesis. Our study is a guide for investigating the effects of sildenafil/osthole in the treatment of pathologies (diabetes, hypertension, etc.) in which $H_2S$ synthesis is decreased.

**Keywords:** Liver, oxidative stress, hydrogen sulfide, osthole, sildenafil

## *ORAL PRESENTATION*

# Development of optical based nanosensors for the determination of adenosine

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**Abstract**

The concentration levels of nucleotides and their analogues in urine and plasma are important. Adenosine is one of the main nucleosides that has biomarker properties with its concentration levels in urine and plasma. In this study, methacrylic acid (MAA) as monomer and adenosine nucleoside as template molecule were imprinted as a polymeric film on the surface plasmon resonance (SPR) sensor chip surface using the molecularly imprinted polymer (MIP) technique. The surface of the adenosine-imprinted SPR chip was characterized by taking atomic force microscopy, ellipsometry and contact angle measurements. Kinetic interactions were investigated with SPR nanosensors with adenosine selective binding sites. pH 7.4 value was determined in phosphate buffer by scanning pH with adenosine imprinted nanosensors. Kinetic analyzes were performed using pH 7.4 phosphate buffer at different adenosine concentrations in the range of 0.5-400 nM. Guanosine and cytidine nucleosides were used as competitor molecules to determine the selectivity of the adenosine nanosensor. Since the adenosine imprinted SPR chip is intended to be used in realistic medical analysis and research, a nanosensor that can detect adenosine from artificial urine and artificial plasma samples in real time has been designed. As a result of the studies, the limit of detection (LOD) value in aqueous solutions in the linear range of 0.1 nM - 100.0 nM was determined as 0.018 nM, the limit of detection value in artificial plasma was determined as 0.015 nM, and the limit of detection in artificial urine was determined as 0.013 nM. Otherwise, the limit of determination (LOQ) values for aqueous solution, artificial plasma and artificial urine were calculated as 0.061 nM, 0.052 nM, and 0.046 nM, respectively. Finally, reusability and shelf life of adenosine imprinted nanosensors were determined.

**Keywords:** Adenosine, Molecularly Imprinted Polymers, Surface Modification, SPR, Nanosensors

## *ORAL PRESENTATION*

# The pharmacological treatment of obesity

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**Abstract**

Obesity is defined as excessive fat accumulation that causes a risk to health. Obesity is related to many diseases including hypertension, diabetes, stroke, myocardial infarction, chronic obstructive pulmonary disease, increased coagulation, osteoarthritis, and cancer. It depends on environmental factors such as socioeconomic status, age, and gender; genetic factors, and the person's mental health. Obesity is a global problem that has increased over time from the past to the present and will probably continue to increase in the future. Also, it may be or be the sole cause of mortality. It has been reported that the prevalence of obesity in Turkey is the highest among European countries. Because the increase in the prevalence of obesity and its association with various diseases has led to the development of pharmacological and non-pharmacological treatments for obesity. This study aimed to review the pharmacological treatment of obesity in light of current guidelines and recent literature.

**Keywords:** Obesity, pharmacological treatment of obesity, non-pharmacological treatments for obesity

## *ORAL PRESENTATION*

# Sığır *Staphylococcus aureus* Kaynaklı Mastitis Enfeksiyonunda *bta-miR-23a-3p*'nin Rolü

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**Özet**

Subklinik mastitis, süt üretimi ve kalitesi üzerindeki olumsuz etkisinden dolayı süt ineği yetiştiriciliğinde ekonomik kayıplara sebep olan önemli bir hastalıktır. Bu ekonomik kayıplarda *Staphylococcus aureus* (*S.aureus*) önemli rol oynamaktadır. Bu çalışmada ineklerde subklinik mastitis enfeksiyonlarında *S. aureus* patojenine ve sığır türüne özgü doğal (innate) immun sistemle ilişkili hedef genler biyoinformatik analizler aracılığıyla belirlenmiştir. Belirlenen hub genlere ait hedef miRNA'ların saptanmasında ve moleküler yolaklarının belirlenmesinde en sık güncellenen ve kullanılan algoritmalar olan TargetScan, miRIAD, mirWalk, miRTARBase, miRmap ve miRnet veri tabanları kullanılmıştır. Elde edilen biyoinformatik analiz sonuçlarına göre, *S. aureus* patojenine özgü immun sistemle ilişkili hedef gen *MYD88* ve bu genin hedef miRNA'sı *bta-miR-23a-3p* olarak belirlenmiştir. Belirlenen *bta-miR-23a-3p*'nin sağlıklı ve mastitisli sütlerde ekspresyonunun doğrulanması amacıyla 38 adet sağmal hayvandan alınan süt örnekleri kullanılmıştır. Sağlıklı ve subklinik mastitisli hayvanların belirlenmesi için; California Mastitis Test ile (CMT) (+) ve CMT (-) hayvanlar tespit edilmiştir; CMT (+) ve CMT (-) hayvanlarda somatik hücre sayımı yapılmıştır; CMT (+) ve somatik hücre sayısı yüksek hayvanlarda Baird-Parker besi yerine ekim ile *S.aureus* patojeninin kesin tanısı konulmuştur. Bu analizler sonucu 6 adet sağlıklı 6 adet subklinik mastitisli süt örneğinden total RNA izolasyonu yapılmıştır. Elde edilen miRNA'ların ekspresyonu eş zamanlı PCR (qPCR) analizi ile değerlendirilmiştir. Sonuç olarak *bta-miR-23a-3p*'nin sağlıklı ve mastitisli süt örneklerindeki ekspresyonu için farklılık bulunmamıştır. Konuyla ilgili daha ayrıntılı araştırmaların yapılması gerektiği düşünülmektedir. Berna Kaya, 1919B012107210 proje numaralı 2209 mali desteği için TÜBİTAK'a teşekkür eder.

**Anahtar Kelimeler:** Subklinik mastitis, inek, süt, biyoinformatik, miRNA, qPCR

## *ORAL PRESENTATION*

# A Novel Actinobacterium, *Arthrobacter* sp. H14-L1, Synthesizing Silver Nanoparticles

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**Abstract**

Actinobacteria constitute one of the essential microbial groups in biotechnological applications. Most members of this group can synthesize a diverse array of bioactive metabolites and enzymes with potential applications in medicine, agriculture, and industry. These bacteria also have importance for the biological synthesis of nanoparticles such as silver nanoparticles. *Arthrobacter* sp. H14-L1, a psychrotolerant actinobacterium isolated from the Antarctica in a previous study, was shown to synthesize silver nanoparticles. The physicochemical characterization of the synthesized nanoparticles was achieved by UV-vis and SEM-EDX analyses. The synthesized silver nanoparticles gave a peak between 400 nm and 450 nm with a maximum at 419 nm resulting from surface plasmon resonance. The average nanoparticle size was determined as 58-68 nm. In addition, whole genome analysis of *Arthrobacter* sp. H14-L1 was conducted to reveal possible metabolites contributing to the synthesis of silver nanoparticles. The genome annotation performed on the RAST server (https://rast.nmpdr.org/) revealed that strain H14-L1 encodes 50 RNA-coding and 3834 protein-coding sequences, including nitrate reductase gene, which might be responsible for the reduction of silver ions to silver nanoparticles. The strain also has genes encoding resistance to antibiotics and toxic compounds, such as fluoroquinolones and mercury. The secondary metabolite biosynthetic gene cluster analysis conducted on the antiSMASH server (https://antismash.secondarymetabolites.org/#!/start) showed that strain H14-L1 has four gene clusters encoding for a terpene, type-III polyketide, betalactone and ribosomally synthesized-posttranslationally modified peptide. The phylogenomic analysis conducted on the TYGS server (https://tygs.dsmz.de/) revealed that strain H14-L1 is a novel actinobacterium within the genus *Arthrobacter*. Considering the significance of novel actinobacteria isolated from extreme habitats, strain H14-L1 may have unique chemical capability for biotechnological applications. In future studies, a transcriptomic analysis will be conducted for *Klebsiella pneumoniae* exposed to silver nanoparticles synthesized by *Arthrobacter* sp. H14-L1 to reveal the pharmaceutical potential of these nanoparticles.

**Keywords:** Actinobacteria, *Arthrobacter*, Genome analysis, Silver nanoparticles, SEM-EDX

## *ORAL PRESENTATION*

# An amperometric horseradish peroxidase inhibition biosensor for the determination of heavy metals

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**Abstract**

Pollution from heavy metals is a critical problem that can seriously threaten the environment and human health; even trace levels of these metals are highly toxic [1]. Thus, highly selective, sensitive, rapid, and accurate heavy metal detection is crucial [1,2]. Different analytical methods have been reported for analyzing these metals [3]. Among them, electrochemical methods have many advantages, such as low-cost, easy operation, small-sized devices, short-time analysis, high sensitivity, and selectivity. In recent years, inhibition biosensors have been designed to assay heavy metals; the enzyme inhibition biosensors' principle is based on the measurement of enzymatic activity in the absence and presence of the inhibitor [4].

In this work, a novel horseradish peroxidase (HRP) inhibition biosensor was developed to determine heavy metal ions. Metal oxide nanoparticles (indium tin oxide) were used in combination with the mediator (hexaammineruthenium (III) chloride). The composite film facilitated the electron transfer due to their synergistic effect between them. Electron transfer characteristics of the electrodes were examined using cyclic voltammetry and electrochemical impedance spectroscopy. The surface morphology of the electrodes was also studied by scanning electron microscopy. Various experimental parameters, such as pH, working potential, and enzyme loading, were investigated in detail. The developed inhibition biosensor showed good analytical performance, such as fast response, good operational stability, high reproducibility, good selectivity and sensitivity, and low detection limit. The detection of $Pb^{2+}$, $Ni^{2+}$, and $Cd^{2+}$ in tap water was successfully carried out to demonstrate the analytical applicability of the sensor. The results from both methods showed that the presented biosensor could be used as a promising analytical approach for monitoring trace metals in different water samples. The obtained results from the proposed method agreed with the atomic absorption spectrometry (AAS) results.

**Keywords:** inhibition biosensor, metal oxide nanoparticles, mediator, heavy metals.

**References**

[1] Joseph L., Jun B.M., Flora J.R., Park C.M., Yoon Y. *Chemosphere,* (2019) 229:142-159.
[2] Arjomandi M., Shirkhanloo H. *Analytical Methods in Environmental Chemistry Journal* (2019) 2(03):97-126.
[3] Yuan X., Chapman R.L., Wu Z. *Phytochemical analysis* (2011) 22(3):189-198.
[4] Gong C., Fan Y., Zhao H. *Talanta* (2022) 240:123145.

## *ORAL PRESENTATION*

# Potential inhibitory effects of some pyrazole carbohydrazide derivatives on the fatty acid amide hydrolase (FAAH) enzyme

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**Abstract**

FAAH (Fatty Acid Amide Hydrolase) is a membrane enzyme, a member of the serine hydrolase family, which is responsible for the hydrolysis of fatty acid amides such as endogenous cannabinoid (eCB) anandamide (AEA). [1, 2]. Anandamide is generated on-demand during physiological or pathophysiological events and steers analgesic and anti-inflammatory effects through its activating action on cannabinoid receptors. However, anandamide is a short-lived lipid transmitter and is rapidly metabolized into arachidonic acid (AA) and ethanolamine by the action of FAAH, which diminishes its levels at the receptor site as well as its associated beneficial biological effects. Therefore, inhibition of FAAH has quickly been recognized as an attractive research field in an effort to strengthen and extend the biological functions of endogenous FAAs like anandamide with the aim of providing in vivo anti-inflammatory and pain-relieving effects. In the recent literature, urea, carbamate and amide bearing aryl/heteroaryl groups have been widely studied as FAAH inhibitors [3]. Recently, carbohydrazide hydrazone-based I and II (Fig. 1) have been identified as FAAH inhibitors [4,5]. Inspired by compounds reported in the literature, this presentation aimed to identify potential FAAH inhibitors that could serve as new chemical libraries suitable for further development. With a fluorometric-based enzyme analysis used for the evaluation of biological activities, it was observed that five compounds with pyrazole hydrazone structure had 50% inhibition at 3 μM concentrations. Based on these results, it has been demonstrated that derivatives containing pyrazole hydrazone have the potential to develop FAAH inhibitors. Here, the synthetic approach, biological data and structure analysis will be discussed.

**Keywords:** FAAH, inflammation, arylhydrazones, heterocycles.

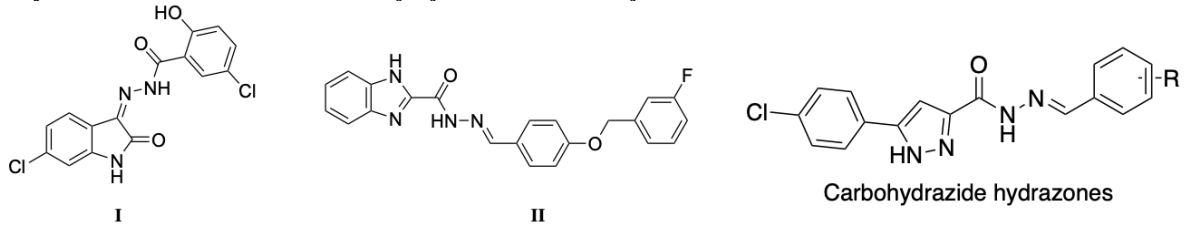


Figure 1

**References**

**[1]** Van Egmond, N., Straub, V. M., van der Stelt, M. (2021). Targeting Endocannabinoid Signaling: FAAH and MAG Lipase Inhibitors. Annual Review of Pharmacology and Toxicology, 61, 441-46.

**[2]** Mechoulam, R., Parker, L. A. (2013). The Endocannabinoid System and the Annual Review of Psychology, 64(1), 21-47.

**[3]** Rati Kailash Prasad T. (2020). A perspective review on fatty acid amide hydrolase (FAAH) inhibitors as potential therapeutic agents. European Journal of Medicinal Chemistry, 188, 111953.

**[4]** Shang, Y., Hao, Q., Jiang, K., He, M., Wang, J. (2020). Discovery of heterocyclic carbohydrazide derivatives as novel selective fatty acid amide hydrolase inhibitors: design, synthesis and anti-neuroinflammatory evaluation. Bioorganic Medicinal Chemistry Letters, 30(10), 127118.

**[5]** Jaiswal, S., Ayyannan, S. R. (2022). Discovery of Isatin-Based Carbohydrazones as Potential Dual Inhibitors of Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase. ChemMedChem

## *ORAL PRESENTATION*

# Kanatlı hayvan beslemede açai meyve ekstraktı (*Euterpe oleracea* fruıt extract) kullanımı: yumurta tavuklarında performans ve yumurta kalitesi

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**Özet**

Yumurta tavuğu karmalarına Açai Meyve Ekstraktı (AE) ilavesinin performans ve yumurta kalitesi üzerine etkilerini değerlendirmek amacıyla 300 adet 22 haftalık yaşta Lohmann Kahverengi yumurta tavuğu tesadüf parselleri deneme desenine göre 4 tekerrürlü 3 muamele grubuna ayrılmıştır. Deneme süresince (2 hafta ön deneme+8 hafta) kontrol grubu (K) standart karma ile beslenirken (%17 HP ve 2750 kcal/kg ME) 2. ve 3. grupların karmalarına sırasıyla %0,65 (AE1) ve %1,3 (AE2) oranlarında AE ilave edilmiştir. Yumurta verimi ve yem tüketimi AE2 grubunda, yumurta ağırlığı ve kütlesi ise AE1 grubunda diğer gruplardan yüksek bulunmuştur ($P<0.05$). Yemden yararlanma oranı AE1 ve K gruplarında AE2 grubundan düşük tespit edilmiştir ($P<0.05$). Yumurta kalite kriterleri muamelelerden etkilenmemiştir ($P>0.05$). Çalışma sonucunda elde edilen bulgular, yumurta tavuğu karmalarına Açai Meyve Ekstraktı ilavesinin yapısında bulunan biyoaktif bileşenlerden dolayı performansta iyileşme sağladığını göstermiştir.

**Anahtar kelimeler:** açai, yumurta verimi, yem katkı maddesi, yumurta tavuğu besleme

## *ORAL PRESENTATION*

# Development of 3D Printed Bovine Serum Albumin Loaded Alginate-Based Hydrogel Dressing for Wound-Healing Application

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**Abstract**

Wound healing is a process that depends on various conditions and requires a suitable environment. Hydrogels can provide mechanical support and humid environment for wounds and are widely used in the biomedical and drug delivery areas. Alginate is widely used in wound care due to its good biocompatibility and high fluid absorption capacity. 3D printing technology allows to produce wound dressings suitable for the shape and depth of the wound. The aim of this study is to provide controlled release of a drug from 3D printed wound dressings. In this study, BSA was chosen as the model drug. Ink formulations of 5% concentration were prepared using different alginate types (CR 8133, Manucol, CR 8223) for the 3D printing process. Surface tension and viscosity measurements of the inks were carried out and the printability of inks were evaluated. Before to 3D printing, release studies were carried out by directly crosslinking ink formulations containing BSA without 3D printing to determine the release properties of polymers. According to the results of the release and characterization studies, CR8223 based ink formulation was chosen as the optimum. Then, 3D printing process was carried out with optimum ink formulations. After 3D printing, the formulations were crosslinked with $CaCl_2$ solution for 15(F1) and 30(F2) minutes to evaluate the effect of the crosslinking time on the release profiles. In vitro release studies were carried out with F1 and F2 formulation and as a result of these studies, BSA release from F1 and F2 formulations reached 80% at 80 and 105 hours, respectively It was observed that the release of BSA was slower in the F2 formulation compared to F1 formulation. It was concluded that the cross-linking time has an effect on the release profile and controlled release can be achieved at the wound site with 3D-printed alginate-based dressings.

**Keywords:** alginate; bovine serum albumin; hydrogel dressing; 3D printing; wound healing.

## *ORAL PRESENTATION*

# Havuç posasının silaj olarak değerlendirilmesi

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**Özet**

Bu çalışmada havuç posasına (HP) adsorbant olarak (%80 havuç posası+%20 adsorbant) buğday samanı (HP+B), fiğ-yulaf kuru otu (HP+FY), mısır sap-samanı (HP+M), yonca kuru otu (HP+Y) ve arpa samanı (HP+A) ilavesi ile elde edilen silajların kimyasal kompozisyonu ve nispi yem değerini (NYD) belirlemek amacıyla 5 tekerrürlü 5 farklı silaj hazırlanmıştır. Silaj kavanozları 60 gün sonra açılarak fiziksel ve kimyasal değerlendirmeleri yapılmıştır. Kuru madde düzeyi bakımından en yüksek grup HP+A silajı olurken ($P<0,001$), ham protein düzeyi bakımından HP+Y ve HP+FY grupları ($P<0,001$), ADF ve NDF bakımından ise HP+Y bulunmuştur ($P<0,001$). NYD ve Fleig skor değerlendirmesi olarak gruplar arasında farklılık bulunmazken havuç posası silajlarının tümü "yüksek kalite" NYD bareminde değerler alırken Fleig skor değerlendirmesi yapılan tüm silaj gruplarının "pekiyi" kalitede olduğu saptanmıştır. Çalışma sonucunda elde edilen veriler doğrultusunda havuç posası silajının ruminant hayvan beslemede alternatif yem kaynağı olarak kullanılabilir olduğu görülmüştür.

**Anahtar Kelimeler:** Silaj, Havuç posası, İn-vitro, Alternatif kaba yem

## *ORAL PRESENTATION*

# Electrochemical determination of hesperetin at poly(luminol) modified graphene oxide electrodes

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**Abstract**

Hesperetin (3′,5,7-trihydroxy-4-methoxy flavonone) is a flavonoid found in citrus fruits in nature and frequently used in some herbal medicines [1,2]. Hesperetin exhibits anti-adipogenic, anti-inflammatory, anti-oxidative activity. In addition, hesperetin has been shown to reduce plasma cholesterol, antiplatelet activity, inhibit vascular formation and prevent cardiovascular diseases. Hesperetin is of great interest in cancer prevention. For the determination of hesperetin can be used techniques such as liquid chromatography, HPLC, UHPLC-UV, HPLC-ECD, LC-MSMS [1,2,3]. These techniques involve the complexity of real samples, multiple solvent extraction techniques, or expensive equipment for analysis. Therefore, electrochemical methods have attracted great interest due to their rapid response, low cost, easy handling, high sensitivity, and selectivity [1]. Electrochemical tools for the determination of hesperetin are limited in the literature. In this study, poly(luminol) was obtained by polymerizing luminol monomer on the electrode surface by cyclic voltammetry. In the experimental method, parameters such as monomer concentration, number of cycles, scanning rate, and pH medium were studied. Linear sweep voltammetry (LSV) and amperometric methods were used for the determination of hesperetin. The limit of detection (LOD) for hesperetin was calculated as $2x10^{-8}$ M by amperometric method. The determination of hesperetin in orange peels was determined sensitively.

**Keywords:** Hesperetin, poly(luminol), voltammetry, amperometry.

References

1. J. Wu, L. Wang, Q. Wang, L. Zou, B. Ye, The novel voltammetric method for determination of hesperetin based on a sensitive electrochemical sensor, *Talanta* 150 (2016) 61-70.
2. M. Aswar, P. Kute, S. Mahajan, U. Mahajan, G. Nerurkar, U. Aswar, *Pharmacol. Biochem. Behav*., 124 (2014) 101-107.
3. S.H. Moon, J.H. Lee, K. Kim, Y. Park, S. Nah, D.U. Ahn, H. Paik, *Int. J. Environ. Res. Public Health*, 10 (2013) 5459-5469.

## *ORAL PRESENTATION*

# Biochemical parameters in metabolic syndrome

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**Abstract**

Metabolic syndrome (MetS) is defined as a cluster of risk factors, such as insulin resistance, abdominal obesity, glucose intolerance, dyslipidemia and hypertension. MetS is also associated with cardiovascular diseases. The prevalence of MetS is increasing in the world and also in Turkey. MetS is seen in one of every four men and one of every three women in Turkey. This situation has made the MetS an important public health problem. Different diagnostic criteria for MetS are reported by the International Diabetes Federation (IDF), the World Health Organization (WHO), the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATPIII), the Turkish Endocrinology Metabolism Society and the Metabolic Syndrome Society, with slight differences between each. Accurate analysis of biochemical parameters in individuals is important in terms of early diagnosis, treatment and prevention of MetS. For this purpose, clinical studies in humans and animal models of MetS were examined. Animal models are divided into two main groups, genetic models and diet-induced models. Genetic models include db/db mice, ob/ob mice, Zucker diabetic fatty rats and Otsuka Long-Evans Tokushima Fatty rats, Goto-Kakizaki rats. Diet-induced models, are formed by increasing the carbohydrate and fat ratios of the nutritional contents (1). There are common biomarkers in the criteria used to diagnose MetS. According to literature, total cholesterol, LDL, TG, fasting blood sugar, serum insulin levels and HOMA-IR value are increased in MetS. On the other hand, HDL value is decreased in MetS (2,3,4,5,6,7,8,9). It was reported that some macro and trace elements are associated with MetS. In the animal models, it has been reported that there are changes in the antioxidant enzyme activities and the levels of various elements in MetS, and these changes may play an important role in understanding the pathogenesis of MetS (9).

**Keywords:** Metabolic syndrome, animal model, biochemical parameters

**References:**

1. Panchal SK, Brown L 2011. Rodent models for metabolic syndrome research. Journal of Biomedicine and Biotechnology, 351982.
2. Berberoğlu HT 2018. Serum ferritin ve inflamasyon belirteçlerinin metabolik sendrom ve insülin direnci ile ilişkisi. Yüksek Lisans Tezi. Gazi Üniversitesi Sağlık Bilimleri Enstitüsü, Ankara/Türkiye.
3. Dolapoğlu N, Yüreklı BPŞ, Eker MÇ, Elbı H 2021. Ötimik Bipolar Hastalarda Serum Agouti-İlişkili Peptid Düzeyleri ve Metabolik Sendrom Arasındaki İlişki. Noro-Psikyatri Arsivi, 58(1), 16-20.
4. Bozkuş Y, Mousa U, Kirnap NG, İyidir ÖT, Ramazanova L, Aslı NAR, Tütüncü NB 2020. Kadınlarda metabolik sendromun alternatif prediktörleri. Pamukkale Tıp Dergisi, 13(2), 341-349.
5. Yılmaz SK, Özçiçek F, Mertoğlu C 2022. Yetişkin bireylerde metabolik sendromun belirlenmesinde obeziteyle ilişkili geleneksel ve yeni indekslerin karşılaştırılması. Cukurova Medical Journal, 47(1), 62-70.
6. Ovali MA 2020. Melatoninin metabolik sendrom modeli oluşturulmuş sıçanlarda kardiyak iyon kanalları üzerine etkileri. Doktora Tezi. Trakya Üniversitesi Sağlık Bilimleri Enstitüsü, Edirne/Türkiye.
7. Karaman ME 2020. Metabolik sendrom oluşturulan ratlarda aerobik ve anaerobik egzersizlerin betatrofin ve kartonektin düzeylerine etkisi. Doktora Tezi. Fırat Üniversitesi Sağlık Bilimleri Enstitüsü, Elazığ/Türkiye.
8. Alptekin Ö, Tükel SS, Turan B, Kuyucu Y 2022. Alterations in Antioxidant Defence Systems and Metal Profiles in Liver of Rats with Metabolic Syndrome Induced with High-Sucrose Diet. Journal of the Turkish Chemical Society Section A: Chemistry, 9(1), 13-2
9. Tükel HC, Alptekin Ö, Turan B, Delilbaşı E 2015. Effects of metabolic syndrome on masseter muscle of male Wistar rats. European Journal of Oral Sciences, 123(6), 432-438.

## *ORAL PRESENTATION*

# Pyridine-PEG functionalized graphene nucleants for protein crystallization

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**Abstract**

Structural characterization of proteins is crucial in protein-based applications. Graphene and its derivatives are used as nucleants because their layered structure provides nucleation sites. Furthermore, polyethylene glycol(PEG) is another nucleating agent for protein crystallization. In this study, PEG-modified graphene nanostructures bearing positive charges are prepared as novel nucleants, and their nucleation ability is assessed. Pyridine functionalized graphene oxide (RGO-Pyr) is functionalized with three different PEG chains (PEG550, PEG200, PEG5000) and octadecane (RGO-Pyr-OD). Protein crystallization is performed using the hanging drop vapor diffusion method, and crystallization of lysozyme as model protein is monitored by a polarized light microscope. Protein crystals with good diffraction quality are obtained within 24 hours with all graphene nucleants except PEG2000 whereas control experiments with no graphene showed no crystals within the same time period.

**Keywords:** Protein, crystallization, nucleant, pyridine, graphene

## *ORAL PRESENTATION*

# Urease immobilization for removing urea

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**Abstract**

Immobilized urease has high usage potential for the food industry to remove urea in a bioreactor, fertilizer wastewater effluents from foods and beverages, for bio-analytical applications in biosensors, and for biomedical purposes to remove the urea from the blood in the dialysate regeneration system for artificial kidneys. For instance, the REDY system (Recirculation of DialYsate) purifies the dialysate effluent and permits dialysate regeneration (1,2). The continuous regeneration of the dialysate is necessary for wearable artificial kidney devices where the sorbent-based dialysate regeneration system is essential to restrict the dialysate volume (3). In this study, Jack bean urease was immobilized onto an inorganic carrier modified with polyethyleneimine by the ionic adsorption method in this study. The binding efficiency of urease onto the carrier is 98%. The Vmax value of urease did not change significantly after immobilization. The performance of immobilized urease was tested with recirculation packed bed reactor at different flow rates (5 mL/min and 10 mL/min). At the end of 7 h, 73% of amount of urea was removed at 5 mL/min flow rate.

**Keywords:** Urease, Immobilization, Recirculation packed bed reactor

**References:**

1. Alptekin O. Characteristics of immobilized urease onto modified zirconium (IV) oxide via glutaraldehyde: kinetic, stability, and operational stabilities in bioreactors. Chemical Papers (2022) 76:749–761
2. Agar JW (2010) Review: understanding sorbent dialysis systems. Nephrology (carlton) 15:406–411. https:// doi. org/ 10. 1111/j. 1440- 1797. 2010. 01321.x
3. Bazaev NA, Bizyukov IO, Dorofeeva NI, Putrya BM (2016) Removal of urea from spent dialysate using an enzymatic method. Biomed Eng 50:138–214. https:// doi. org/ 10. 1007/ s10527- 016- 9605-3

## *ORAL PRESENTATION*

# The effects of selective inhibition of IKKbeta as a key regulator of the NF-kappaB pathway in prostate cancer cells

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**Abstract**

Nuclear Factor kappa B (NF-kappaB) transcription factors regulate the expression of hundreds of genes that control inflammation and cellular survival. A special focus has been placed on the NFkappaB signaling pathway because of its critical role in human cancer initiation, progression, metastasis, and resistance to treatment [1, 2]. Therefore, the NF-kappaB signaling pathway is an important target for the development of novel anti-tumor agents. This study aimed to investigate the inhibitory activities of ML120B, a beta-carboline derivative, and IKK2 Inhibitor VI, a thiophene-carboxamide derivative, on NF-kappaB signaling in androgen-dependent human and mouse prostate cancer cell lines LNCaP and MycCaP, respectively. The effect of compounds on cell viability was assessed by MTT assay. Cell migration was assayed using in vitro wound-healing assay. The expression levels of total and phosphorylated IkappaBalpha and IKKs in TNF-stimulated cells were evaluated by Western blot and IKK kinase assay. As result, ML120B and IKK2 Inhibitor VI inhibited the viability and migration of the prostate cancer cells in a concentration and time-dependent manner. The $IC_{50}$ values of ML120B for LNCaP and MycCaP cells were calculated to be 23.7 microM and 19.4 microM, respectively. The $IC_{50}$ values of IKK2 Inhibitor VI for LNCaP and MycCaP cells were calculated to be 17.2 microM and 12.1 microM, respectively. IKK2 Inhibitor VI and ML120B inhibited TNFalpha-induced IkappaBalpha phosphorylation in prostate cancer cells. The expression level of IKKalpha did not change in the presence of ML120B. These results support the evaluation of the inhibitors as IKKbeta-specific anti-tumor agents for the treatment of prostate cancer.

This study was supported by TUBITAK (BIDEB-2214 Programme).

References

1. Karin, M., Nature, 2006, 441 (7092): 431-436.
2. Staal, J. and R. Beyaert, Cells, 2018, 7(9).

**Keywords:** NFkappaB, prostate cancer, IKKbeta, ML120B, IKK2 Inhibitor VI.

## *ORAL PRESENTATION*

# Determination of acute toxicity (Lc50) of rimsulfuron for common carp (*Cyprinus carpio* Linnaeus, 1758)

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**Abstract**

For the acute toxicity (Lc50) of commercial rimsulfuron (RAMSES®), 360 fish with an approximate weight of 30 g (grams) were obtained from a carp production company and their average weight was 35.05 ±0.6 g after 15 days of adaptation. These fish weighing were subjected to the acute toxicity test. For the acute toxicity test, 18 aquariums of 45x28x80 cm dimensions filled with 80 liters of water were prepared in triplicate. The aquariums were rested for at least 96 hours, dechlorinated by aeration with an air motor, and filled with UV-filtered company water before being taken into the experimental environment. In this study with a trial period of 96 hours, after measuring the height and weight of the adapted fish, a total of 180 fish, 10 fish in each aquarium, were placed. Pre-calculated doses (10, 20, 40, 160 mg/L) were applied once a day from the stock solution for 4 days and 50% of the water was renewed with rested water once in 24 hours. During the experiment, the fish were not fed and the dead fish were counted daily. The 96-hour Lc50 value of rimsulfuron for common carp (*Cyprinus carpio*) was found to be 34 mg/L as a result of probit analysis. It has been determined that this value is quite low when compared to the acute toxic values of rimsulfuron, which is used analytically in the experiments in the literature that is the toxic effect value of this substance for carp fish is high even though it contains 25% rimsulfuron. This is due to the analytical use of the active ingredient rimsulfuron in other studies. Studies in the literature now reveal the importance of investigating the effects of commercial products on fish rather than analytical standards.

**Keywords:** Rimsulfuron, Herbicide, Acute toxicity, Common carp, *Cyprinus carpio*

**Acknowledgments**: We would like to thank Sinan AKSUOĞLU, Hayriye AKSUOĞLU and Umut TURUPLU. This abstract is derived from a part of his doctoral study titled “The Effect of Sub-lethal Concentrations of Rimsulfuron (Herbicide) on Blood Parameters in Carp (*Cyprinus carpio*, Linnaeus, 1758) and Determination of DNA Damage ". Rıdvan Erdem KANAT is PhD student of YÖK 100/2000 project.

## *ORAL PRESENTATION*

# Preparation of *Helichrysum plicatum* DC. subsp. *plicatum* Extracts by Different Extraction Methods and Quantitative Analysis of Phenolic Compounds

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**Abstract**

From the existence of humanity to the present day, plants have had a great place in human life. The role of plants in traditional folk medicine is very important and plants have been used to treat diseases for many years. *Helichrysum* species have also been used in traditional medical practices for nearly 2000 years. *Helichrysum plicatum* subsp. *plicatum* is a plant that is widely used among the population in our country and is known by many names such as "ölmez çiçek, altın otu, arı çiçeği, herdemtaze, sarılık çiçeği, bozoğlan and sarı solmaz"[1]. The *H.plicatum* plant is used in stomach ailments, kidney disorders, especially kidney stones reduction, urinary tract disorders and wound burns treatment. As a result of the some studies, *Helichrysum* species have been proven to have antioxidant, anti-inflammatory, antiviral, diuretic, antispasmodic, antimicrobial and anticarcinogenic effects. *Helichrysum plicatum* subsp. *plicatum* plant is a plant which have rich flavonoid content[2]. Considering the literature studies, it is thought that the compounds responsible for biological activities may be flavanoids contained in the plant. Based on this idea, in this study it is aimed to prepare the extracts of the *Helichrysum plicatum* subsp. *plicatum* plant in different conditions and conduct quantitative analysis of the phenolic components. For this purpose, *Helichrysum plicatum* subsp.*plicatum* plant, which was collected and dried from Basyayla in Karaman, Ermenek region and then it was dusted by using laboratory-type blender. After this step, the extracts were prepared by different extraction methods such as the soxhlet extraction, under the reflux condenser in an ultrasonic water bath extraction and under the reflux condenser in the water bath extraction. 100% ethanol, 50:50% ethanol-water and 100% water were used as solvents respectively. Quantitative analysis of the phenolic components of the prepared extracts were determined by LC-HRMS.

**Keywords:** Extraction methods, LC-HR-MS, Phenolic content.

**References**

1. Demirezer, Ö., *FFD Monografları Bitkiler ve Etkileri*. Akademisyen Kitabevi: Ankara, 2017.
2. Cubukcu B., M. A. H., Bingöl S., Yüksel V.,Damadyan B. , Yukarı Fırat Bölgesinde Yetişen Helichrsyum Türlerinin Flavanoid Bileşikleri Yönünden İncelenmesi. In *Fırat Havzası Tıbbi ve Endüstriyel Bitkiler Sempozyumu Bildiriler Kitabı*, Elazığ, 1986; pp 179-187.

## *ORAL PRESENTATION*

# Muğla ili köyceğiz ilçesi portakal bahçelerinde akdeniz meyve sineği [*Ceratitis capitata* Wied. (Diptera: Tephritidae)]'nin popülasyon yoğunluğu ve zarar oranının belirlenmesi

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**Özet**

Bu çalışma, 2016 yılı Mayıs-Aralık ayları arasında Köyceğiz'in Toparlar, Zaferler, Döğüşbelen, Hamitköy, Ulucami, Yangı, Eski Köyceğiz, Kavakarası ve Beyobası mahallelerinde bulunan portakal bahçelerindeki Ceratitis capitata'nın poplasyon yoğunluğu ve zarar oranı tespit etmek amacıyla gerçekleştirilmiştir. Araştırma alanında mayıs ayından itibaren feromon tuzaklar kullanılarak örnekleme yapılmıştır. Tuzaklar Nisan-Haziran ayları arasında haftada bir, Temmuz-Kasım ayları arasında haftada en az iki defa olmak üzere kontrol edilmiştir. Arazi çalışmaları boyunca toplam 9318 adet C. capitata ergin bireyi yakalanmıştır. İlk erginler 18 Mayıs 2016 tarihinde, son erginler ise 13 Aralık 2016 tarihinde gözlemlenmiştir. En fazla ergin, ekim ayında Yangı'da yakalanmıştır (1564 birey). Portakal üzerinde akdeniz meyve sineği'nin zarar oranı en yüksek % 22,8 ile Yangı'da; en düşük ise % 4,4 ile Döğüşbelen'de tespit edilmiştir.

**Anahtar Kelimeler:** Akdeniz Meyve Sineği, Popülasyon, Zarar Oranı, Köyceğiz

## *ORAL PRESENTATION*

# 4-Aminotetrahidrokinolin Türevlerinin Sentezi ve Asetilkolinesteraz Enzimi Üzerine İnhibisyon Etkilerinin İncelenmesi

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**Özet**

Asetilkolinesteraz enzimin inhibitörleri Alzheimer hastalığının (AH) tedavisi için kullanılan en etkili ilaçlardandır. Bu çalışmada Alzheimer hastalığının tedavisinde kullanılabileceği düşünülen 4-Aminotetrahidrokinolin türevlerinin sentezi gerçekleştirildi. Sentezlenen 4-Aminotetrahidrokinolin türevlerinin spektrofotometrik olarak Asetilkolinesteraz enzimi (AChE) üzerindeki inhibisyon etkileri araştırılması amaçlanmıştır. Moleküllerin sentezi için Povarov reaksiyon yöntemi kullanıldı. Sentezlenen moleküllerin karekterizasyonları $^{13}C$ NMR, $^{1}H$, LC-MS-MS ve IR de yapıldı. Sonraki aşamada bu moleküllerin asetilkolinesteraz enzimi üzerine inhibisyon etkileri incelendi. Standart inhibitör olarak 9-Amino-1,2,3,4-tetrahydroacridine hydrochloride hydrate (Takrin) kullanıldı. Yapılan çalışma sonucunda sentezi daha önce yayınlanmış olan dört ve ilk kez sentezlenen dört olmak üzere toplam 8 farklı 4-aminotetrahidrokinolin türevinin inhibisyon değerleri hesaplandı. Kinetik çalışma olarak hem $IC_{50}$ hem de Ki değerleri hesaplandı. Çalışma sonucunda $IC_{50}$ değerleri 0,22 µM-0,36 µM aralığında olduğu bulundu. Moleküller için Lineweaver-burk grafikleri çizilerek sırasıyla ortalama $K_i$ sabitleri hesaplandı ve elde edilen verilerin 0,50 µM ile 1,13 µM aralığında olduğu görüldü. Çalışmanın son basamağında Takrin molekülünün $IC_{50}$ değeri 0.770 µM ve $K_i$ değeri ise 0.880 µM olarak hesaplandı. Ayrıca DTNB ve AChI substratları için Vmax ve $K_M$ değerleri sırasıyla 0,106 EÜ/ml, 0,096 EÜ/ml ve 1.637 µM, 1.021 µM olarak hesaplandı. Veriler değerlendirildiğinde çalışılan organik moleküllerin tamamının standart inhibitöre benzer ölçüde inhibisyon etkisi gösterdiği gözlendi.

**Anahtar Kelimeler:** Asetilkolinesteraz**,** Takrin, Alzheimer, inhibisyon**,** 4-aminotetrahidrokinolin

## *ORAL PRESENTATION*

# Synthesis, characterization and in silico studies of a schiff base ligand

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**Abstract**

Ligands are key ingredients in pharmaceutical, biological and chemical synthesis. These ligands are also important compounds for the preparation of metal complexes. Transition metal complex chemistry has been extensively studied to synthesize metal complexes with ONS donor ligands due to the reactivity of polydentate centers. In this context, ONS donor ligands have been chosen in most research studies because of their versatile properties, transition metals Co, Ni, Cu, and Zn, secondly Pd and Ru, and thirdly Pt, to prepare metal complexes of Schiff bases. In the last few years, it has been known that Schiff base complexes with ONS donor ligands are used in both homogeneous and heterogeneous catalysis in catalytic processes in various chemical reactions [1]. In addition, the chemical affinities and influence of some metals on the biological activity of ONS donor ligands have led to a significant increase in the study of their coordination behavior. In addition, the development of new chemotherapeutic Schiff base ligands and their metal complexes has attracted the attention of researchers [2]. In this study, a Schiff base ligand was synthesized in absolute ethyl alcohol at 60 $^{o}$C under the catalysis of p-toluenesulfonic acid and then complexes of this ligand were prepared with using M(II) acetates. Instrumental analysis methods such as IR, $^{1}$H-NMR, $^{13}$C-NMR, UV-Vis, XRD, SEM, magnetic susceptibility, and thermogravimetric analysis were used to elucidate the structures of the compounds [3]. To elucidate the mechanism of action of the synthesized compounds, some descriptive molecular properties were calculated using the Schrödinger Suite computer-aided molecular modeling program, the active region where the coupling would take place with the Receptor Grid Generation module was determined, and the interactions of the possible drug molecule candidates synthesized with the Structure-Based Drug Design (SBDD) method with the target receptor region were calculated. Activity studies were performed according to the molecular coupling scores between the synthesized complexes and AChE (PDB ID: 4EY7) [4].

**Keywords:** Molecular Modelling, Schiff Base, Transition Metal Complex

**References**

[1] Gupta, K. C., & Sutar, A. K. (2008), Catalytic activities of Schiff base transition metal complexes. Coordination Chemistry Reviews, 252(12-14), 1420-1450.
[2] Ali, A. Q., Teoh, S. G., Eltayeb, N. E., Ahamed, M. B. K., & Majid, A. A. (2014), Synthesis of copper (II) complexes of isatin thiosemicarbazone derivatives: In vitro anti-cancer, DNA binding, and cleavage activities. Polyhedron, 74, 6-15.
[3] Abdel-Latif, S. A., Hassib, H. B., & Issa, Y. M. (2007), Studies on some salicylaldehyde Schiff base derivatives and their complexes with Cr (III), Mn (II), Fe (III), Ni (II) and Cu (II). Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 67(3-4), 950-957.
[4] Almarhoon, Z. M., Al-Onazi, W. A., Alothman, A. A., Al-Mohaimeed, A. M., & Al-Farraj, E. S. (2019), Synthesis, DNA binding, and molecular docking studies of dimethylaminobenzaldehyde-based bioactive schiff bases. Journal of chemistry, 2019.

## *ORAL PRESENTATION*

# The long-range cation-π interactions between ionizing alpha radiation and DNA nucleotides

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**Abstract**

Ionizing alpha radiation ($He^{2+}$) is known to adversely affect human DNA, but the biochemical reasoning is not clear yet. Relatedly, the present computational study was conducted investigating the effects of ionizing alpha radiation onto the Watson-Crick type DNA base pairs (nucleotides) Adenine-Thymine (AT') and Guanine-Cytosine (GC'). The long-range cation ($He^{2+}$)−π interactions were modeled for this purpose. A hybrid DFT functional of M06-2X was used with 6-31G(d,p) and 6-311G(d) basis sets at unrestricted level. The results showed that alpha radiation severely changed the considered base pairs' hydrogen bond lengths and their interaction enthalpies and Gibbs free energies, however, the more drastic changes were observed in GC' rather than AT'. This observation was also supported by frontier molecular orbital analyses performed. GC' was more favored to form $He^{2+}$ complexes (oxidize) than AT' and consequently these complexes had more exothermic interaction energies (formed more spontaneously) than that of AT'. It could be highlighted that the molecular modeling proposed in this study would contribute to the elucidation of the uncertainty in this field.

**Keywords:** DNA base pairs, alpha radiation, interaction enthalpy, interaction Gibbs free energy, M06-2X.

## *ORAL PRESENTATION*

# Synthesis, characterization and docking studies of a Schiff base ligand and its metal complexes

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**Abstract**

Imines, first reported by Hugo Schiff in 1864, are compounds in the azomethine group (-C=N-) structure and are important intermediates in the organic chemistry and chemical industry. Moreover, these compounds represent an important class of organic compounds, especially in the medical and pharmaceutical fields. There are various methods used to prepare imines, which are versatile components, the classical method involving the condensation of aldehydes and amines is widely used [1]. There are other approaches in the literature such as dimerization, oxidation, or dehydrogenation of primary amines [2]. Examples of using imines as versatile compounds include asymmetric synthesis of α-aminonitriles, preparation of secondary amines by hydrogenation, cycloaddition reactions, and chelation reactions. For this reason, it has great importance to add new syntheses to imines, which have many uses. In this study, a new ligand was synthesized from the reaction of a salicylaldehyde derivative and aminophenol, and its complexes were prepared from the reaction of this ligand with M(II) acetates. Instrumental analysis methods such as IR, $^{1}$H-NMR, $^{13}$C-NMR, UV-Vis, magnetic susceptibility, and thermogravimetric analysis were used to elucidate the structures of the compounds [3]. To elucidate the mechanism of action of the synthesized compounds, some descriptive molecular properties were calculated using the Schrödinger Suite computer-aided molecular modeling program, and the active region where the coupling would take place with the Receptor Grid Generation module was determined [4].

**Keywords:** Kompleks, Ligand, Moleküler Modelleme

**References**

[1] Huang, H., Huang, J., Liu, Y. M., He, H. Y., Cao, Y., & Fan, K. N. (2012). Graphite oxide as an efficient and durable metal-free catalyst for aerobic oxidative coupling of amines to imines. Green Chemistry, 14(4), 930-934.
[2] Kim, J. W., Yamaguchi, K., & Mizuno, N. (2008). Heterogeneously catalyzed efficient oxygenation of primary amines to amides by a supported ruthenium hydroxide catalyst. Angewandte Chemie International Edition, 47(48), 9249-9251.
[3] Almarhoon, Z. M., Al-Onazi, W. A., Alothman, A. A., Al-Mohaimeed, A. M., & Al-Farraj, E. S. (2019), Synthesis, DNA binding, and molecular docking studies of dimethylaminobenzaldehyde-based bioactive schiff bases. Journal of chemistry, 2019.
[4] Meenukutty, M. S., Mohan, A. P., Vidya, V. G., & Kumar, V. V. (2022), Synthesis, characterization, DFT analysis and docking studies OFA novel schiff base using 5-bromo salicylaldehyde and β-alanine. *Heliyon*, e09600.

## *ORAL PRESENTATION*

# Elma ( *Malus domestica*) Bitkisinde Polen Çimlenmesi Üzerine Herbisit Glufosinate ammonium' un Etkisi

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**Özet**

Herbisitler ,tarımsal ekosistemlerde en yaygın olarak uygulanan pestisitlerdir. Herbisitler arasında, Glifosinate geniş spektrumlu sistemik etkilere sahip organofosfor bileşikleridir . Suda Çözünen Konsantre Herbisit olarak yabani otların ortadan kaldırılmasında kullanılır Sonuç olarak, hem fiziko-kimyasal özellikleri hem de yoğun kullanımları nedeniyle, bu bileşikler topraklarda ve sularda yoğun bir şekilde izlenmiştir. Ağaçlarda direkt olarak kullanılmasa dahi ekosistemde bulunan pestisitlerin polenlere ulaşabildiği gözlenmiştir. Çiçekli bitkilerde tozlaşma, temel bir ekosistem hizmeti ve gıda güvenliğini sürdürmek için hayati bir süreç olarak kabul edilmektedir. Tozlaşma içerisinde polen çimlenme oranı başarılı tozlaşmanın ön koşullarından biridir. Çimlenme testleri polen numunelerinin yapay bir ortamda çimlenerek polen yararlılığının değerlendirildiği en iyi in vitro göstergesi olarak kabul edilmiştir.
Bu çalışmada elmanın oluşumunu sağlayan polen çimlenmesi değişik miktarlarda herbisit ile muamele edilerek sonuçları değerlendirilmiştir. Araştırma 1 L su içerisine ilave edilerek hazırlanan herbisitin prospektüste verilen kullanım oranı ile başlamış ve katlanarak artan kimyasal miktarları ile deneme grupları oluşturulmuştur. Çimlendirme için standart bir kültür ortamı hazırlanmış, uygun şartlarda toplanan polenler hazırlanan ortama yayılmış ve petri kapları parafilm ile kapatılmıştır. Daha sonra petri kapları, 24 ° C'de inkübatörde tutularak süre sonunda optik bir mikroskop ile polen sayımı yapılarak ve çimlenme yüzdesi belirlenmiştir.
Elde edilen sonuçlara göre sistemik pestisitler arasında bir herbisit olarak yer alan Glufosinate ammonium' un olası maruziyeti ile elma polenlerinin değişik seviyelerde etkilendiği bulunmuştur. Ayrıca herbisitin elma polenlerinin çimlenmesini tamamen engelleyen 1 L su içerisindeki miktar belirlenmiştir. Ele alınan polenin çimlenme aktivasyonunun herbiste olan cevabı meyve yetiştiricilerine, sunni çimlendirme çalışmalarına, polen çimlenmesine bağlı uygulamalara ve tarımsal araştırmalara tavsiyeler bildirecektir.

**Anahtar Kelimeler:** Herbisit, Polen, *Malus domestica*

## *ORAL PRESENTATION*

# Investigation of photo/electrochemical water splitting for hydrogen evolution by bio-based C-doped graphitic carbon nitride

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**Abstract**

Banana fiber (bio-base and carbon dopant), known as a versatile biomass, was mixed with melamine (precursor) to produce a bio-based C-doped graphitic carbon nitride (g-$C_3N_4$) by pyrolysis method. The electrochemical measurements suggested that the bio-based C-doped g-$C_3N_4$ exhibits a superior photoinduced electron-hole pairs separation efficiency due to C doping significantly promoting excellent conductivity and electron redistribution in the sample. The addressed of C doping in the g-$C_3N_4$ significantly exhibits excellent photocatalytic hydrogen production which was a 30.2 improvement over bulk g-$C_3N_4$.

**Keywords:** Bio-based carbon, graphitic carbon nitride, hydrogen evolution, photo/electrochemical water splitting.

## *ORAL PRESENTATION*

# Investigation of antimicrobial effects of *O. ficus-indica, A.dealbata* and *E. japonica* on ventilator-associated pneumonic bacteria.

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**Abstract**

Pneumonia is a serious lower respiratory tract infection that causes an inflammatory response of one or both of the air sacs in the lung, causing a specific, phlegmatic cough, fever, chills, and shortness of breath. Medicinal plants have antibacterial, anticancer, and anti-inflammatory therapeutic effects as they contain important secondary metabolites. The estimation of antimicrobial activity of spring plants from Çukurova University campus on some gram-negative/positive pathogenic microorganisms was determined by the agar well diffusion obtained with method. In the study, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* isolates of ventilator-associated pneumonia (VAP) growing patients receiving mechanical ventilation treatment in Çukurova University Faculty of Medicine Reanimation unit were used. The plants extracts were prepared two different solutions (water(W) and ethyl alcohol(EA)), (*Acacia dealbata*(flowers), *Opuntia ficus-indica* (root), *Eriobotrya japonica* (unripe fruit)). As a result of the analysis, *O. ficus-indica* (EA extraction, 23 and 19 mm zone diameter, respectively) in two of the three isolates of *Pseudomonas aeruginosa* bacteria and *A.dealbata* (EA extraction, 20 mm zone diameter) showed the most antagonistic effect in the other *Pseudomonas* isolate. Two of the three isolates of *A. baumannii* showed the most antagonistic effect in *E. japonica* extracts with a zone diameter of 25 mm (EA) and 14 mm (W), respectively, and other acinetobacter isolate the *A.dealbata* (W) extract (28 mm zone diameter) showed the most antagonistic effect. *E. japonica* extracts (34 mm (W) and 11 mm (EA) zone diameter, respectively) showed the most antagonistic effect in two of the three isolates of *K. pneumoniae* bacteria, and in the other klebsiella isolate *A.dealbata* (W) extract (28 mm zone diameter) showed the most antagonistic effect. In *Staphylococcus aureus* pathogen, EA extract of *O. ficus-indica* plant showed maximum antagonistic effect in one isolate (25 mm zone diameter). As a result of the analysis, it was determined that *O. ficus-indica*, *A.dealbata* and *E. japonica* plants showed the most antimicrobial effect on pathogenic pneumonia bacteria. These plants can be used as an auxiliary source to alleviate the effects of the disease in the field of complementary medicine.

**Key words:** Ventilator-associated pneumonic isolate, Antimicrobial activity, *O. ficus-indica, A.dealbata, E. japonica*

## *ORAL PRESENTATION*

# Physiological responses of *Lemna gibba* to Phosmet toxicity

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**Abstract**

Pesticides are one of the chemicals that adversely affect the environment and human health. It may have effects on non-target organisms due to its wide range of uses. Therefore, in this study, we determined the toxic effects of different concentrations of Phosmet insecticide on *Lemna gibba*, an aquatic macrophyte. The study was carried out in a climate cabinet under controlled conditions. It was determined that the photosynthetic pigment and total carbohydrate content of *L. gibba* decreased with increasing Phosmet concentrations. Similarly, there were decreases in total phenolic contents with increasing Phosmet concentrations. In contrast, increases in malondialdehyde and hydrogen peroxide content showed that Phosmet toxicity caused oxidative stress in *L. gibba* tissues.

**Keywords:** *Lemna gibba*, Phosmet, Toxicity, Oxidative stress

## *ORAL PRESENTATION*

# Determination of bioactive compounds from pine rosin in Mediterranean location: Kaburgediği, Mavisilifke, Karabucak

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**Abstract**

Rosins have a large number of chemical ingredients in their chemical composition. Rosin is obtained by distilling pine tree resin. Pine trees have been used by humans for centuries for various activities throughout history. Biosynthesis of pines is carried out by epithelial cells and is found inside the cells of trees, in channels called schizogen or in special vesicles. When the tree is injured, the resin covers the wound, preventing water loss and protecting the plant against parasites and disease-causing microorganisms.
This study aims to evaluate the volatile oil components from rosins of three locations in Mediterranean region by headspace gas chromatography/ mass spectroscopy (headspace/GC-MS) analysis. GC Oven temperature program: After standing at 60 oC for 6 minutes, the temperature was increased to 230 °C by 2 °C per minute. The analysis was terminated by waiting for 30 minutes at 230 °C. Flow rate: Helium 1.2 mL / min. NIST, Wiley 9, Redlip, Mainlip, WinRI libraries were used to identify the peaks obtained from the analysis.
The Clevenger method was used to detect the bioactive components of essential oils. As a result of the analysis, it has been documented by headspace/GC-MS analysis that Pinene was the highest with a value of 61.04%, myrecene was the second with a value of 13,56%, following %4.41 of terpinolene, and limonene was an essential component of rosins has been determined by 4.11%. The compounds, 2 α-Pinene, camphene, Myrcene, Limonene, 2α-terpınolene, 1,4 Cyclohexadiene, Delta-3 Carene, Caryophyllene, Sabinene, 1,4 Methanoazulene, Humulene, 1,4 Cyclohexadiene, Butane (CAS), 1 α-Pinene, Trimethyl 9 methylene.

**Key words:** Pine rosin, Bioactive component, Mediterranean location

## *ORAL PRESENTATION*

# Removal of Al, Fe ve Mn ions using multi-wall carbon nanotubes in Denizli pond in Gebze district of Kocaeli province

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**Abstract**

Heavy metals are among the most important pollutants in water due to their strong toxicity to plants, animals and humans. Because they tend to be biological, metals accumulate in food chains and cannot be degraded or destroyed. Many methods are used to remove heavy metals from water. In this study, multi-walled carbon nanotubes were used to eliminate the seasonally detected aluminum (Al), iron (Fe), and manganese (Mn) in the Denizli pond. Experiments were conducted after samples were generated using ultrapure water from the principal Al, Fe, and Mn standards. Experiment results show that multi-walled carbon nanotubes do not fulfill the existing commercially accessible Al, Fe, and manganese element requirements. However, a minor amount of absorption was noticed; based on this, studies were performed on the standard samples, and the absorption quantity was shown to be independent of the number of carbon nanotubes. Samples obtained from Denizli pond at different periods were evaluated in the laboratory environment using multi-walled carbon nanotubes to determine the quantity of absorption in these experiments. The efficiency of removing aluminum, iron, and manganese from waters using carbon nanotubes was determined based on the results. According to the results of this study, the highest removal efficiency for Al, Fe and Mn in pond samples was observed in August samples compared to January and March.

**Keyword:** Carbon nanotubes, Aluminum, Iron, Manganese, Heavy metal removal, Drinking water treatment.

## *ORAL PRESENTATION*

# Apoferritin Stabilized Cobalt Ferrite Nanoparticles as Methotrexate Carriers For Anticancer Drug Delivery: pH Dependent Disassemble and Reassemble Encapsulation

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**Abstract**

Apoferritin is a natural cage-like protein made up by 24 identical subunits, serving as nanocarriers of several molecules for diagnosis and therapy purposes (1,2). Apoferritin nanocages have intrinsic properties such as its small structure with an inner diameter of 8 nm and outer diameter of 12 nm (3) and self-targeting, and robust structure for harsh conditions and for modification of its outer surface with targeting molecules or drugs (4). Apoferritin can reversibly disassemble into its subunits and reassemble to its intact structure based on the pH. Disassembling of apoferritin begins below pH: ~3 and above pH: ~10 (5). Encapsulation of small molecules into its inner cavity is successfully achieved by disassembling way (6). Reassembling of apoferritin into its spherical structure occurs at pH: ~7 (7). In the study, magnetic $CoFe_2O_4$ nanoparticles were synthesized by coprecipitation method and stabilized by tetramethylammoniumhydroxide (TMAOH) at ph: 12 with a size of 6.5 ± 0.62 nm. $CoFe_2O_4$ nanoparticles were then encapsulated in cavity of apoferritin by the disassembly/reassembly route. In the reassembly step at pH around ~9, methotrexate axe anticancer drug were also added to system in order to load drug molecules through hydrophobic interactions in the inter-subunits and hydrophobic channels of apoferrritin. The characterization of nanoparticles was performed by elemental (ICP-MS), microscopic (STEM), magnetic (VSM) measurements and the existence of methotrexate were confirmed through HPLC analysis. The cytotoxicity of the nanocarrier was evaluated by XTT test using normal (MCF-10A) and cancer (MDA-MB-231) cells. Nanoparticles displayed more toxicity on cancer cells than normal cells. A stable and biocompatible platform was prepared for magnetic targeted drug delivery for cancer therapy that would reduce toxicity and improve the efficiency of drugs.

**Keywords:** Apoferritin, Cobalt ferrite ($CoFe_2O_4$) nanoparticles, Drug delivery, Disassemble-reassemble method, Methotraxe.

**References**

1. Zhao, G.; Abdallah, F.B.; Arosio, P.; Levi, S.; Chandler, C.J.; et al., Biochemistry, 42 (2003) 3142.
2. Chasteen, N.D.; Harrison, P.M., Journal of Structural Biology, 126 (1999) 182-194.
3. Kim, J.W.; Choi, S.H.; Lillehei, P.T.; Chu, S.H.; King, G.C.; et al., Chem. Comm., 32 (2005) 4101.
4. Uchida, M.; Flenniken, M.L.; Allen, M.; Willits, D.A.; Crowley, B.E.,; et al., J. Am. Chem. Soc., 128 (2006) 16626.
5. Valero, E.; Tambalo, S.; Marzola, P.; Ortega-Munoz, M.; Lopez-Jaramillo, F.J.; et al., JACS, 133 (2010) 4888.
6. Makino, A.; Harada, H.; Okada, T.; Kimura, H.; Amano, H.; et al., Nanomedicine: Nanotechnology, Biology, and Medicine,7 (2011) 638.
7. Aime, S.; Frullano, L.; Crich, S.G., Angew. Chem. Int. Ed., 41 (2002) 1017.

## *ORAL PRESENTATION*

# Possible effects of medicine plant extracts on growth of probiotic microorganisms : *Lb. reuteri, B. clausii*

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**Abstract**

For centuries, medicine or aromatic plants have been used in the treatment of diseases as well as being used as food preservation and flavor enhancer. Synthetic products have been used extensively for many years. Today, the orientation toward natural products, and reducing or eliminating synthetic additives in foods is a current worldwide demand. In both uses, the antibacterial effect is mainly targeted. However, increasing the probiotic properties in foods and gut microbiota is another important consumer demand. Therefore, it is important how the probiotic flora is affected by the consumption of medicinal and aromatic products. The aim of the study is to determine the positive or negative effects of plant products on probiotics growth. Attention is drawn to researching the effects of medicinal products in a broad perspective in order to protect and increase probiotics. The effects of natural products was plant, vinegar plant essential oil on some probiotic microorganism invastegated by the microwell dilution assay methods. Natural products, *Ecballium elaterium, Rheum ribes, Allium sativum, Sesamum indicum* and two probiotic bacteria (*Bacillus clausii, Lactobacillus reuteri)* were tested. *Lactobacillus reuteri* were more sensitive than the bacillus clausii against plant extracts. It was concluded that the high level of *E. elaterium*, *R. ribes*, *A. sativum*, *S. indicum* can negatively influence the intestinal microflora. The lower and upper (0.03125, 0.5) concentrations of the *E. elaterium* was no inhibition effect on *Lb. reuteri*, while moderate concentrations showed an inhibitory effect in the range of 25 to 28%. *Rheum ribes* concentrations suppressed *Lb. reuteri* growth by more than 50%, but the highest concentration increased two fold *B. clausii* growth, while lower concentrations did not affect it, while 0.25 suppressed it by 62%. *A. sativum*, and *S. indicum* oil showed an inhibitory effect of 50% or more on both probiotics.
According to the results of the analysis, both of *E. elaterium* and *R. ribes* showed a positive effect on *B. clausii* and increased it, while it had a negative effect on *L. reuteri* , *A. sativum* and *S. indicum* extracts showed negative effects on both probiotics.

**Keywords:** Plant extract, Gut microbiota, Possible effects, Probiotics

## *ORAL PRESENTATION*

# Biyoaktif doku iskelesi üretimi ve antimikrobiyal aktivitesinin değerlendirilmesi

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**Özet**

İlaç taşıma sistemleri, ilaçların suda çözünürlüğünü ve bu yolla biyoyararlanımını arttırarak, onların istenen bölgelerde daha uzun sürelerde ve belli dozlarda etki etmesini sağlamak için doku mühendisliği alanında sıkça tercih edilmektedir. Günümüzde çeşitli ilaç taşıyıcı sistemler üzerinde çok sayıda çalışma yapılmış olsa da hali hazırda yeni sistemlerin kullanımı da araştırılmaya devam edilmektedir. Yara iyileşmesi prosesi zaman alan ve bu süre içinde enfeksiyonlara açık kalan bir süreçtir. Bu nedenle yara iyileşmesine yardımcı olan ve antibakteriyel ajanların yüklenmesiyle oluşturulan yenilikçi doku iskelelerinin kullanımı giderek önem kazanmaktadır. Bu çalışmada, yara iyileşmesinde kullanılmak üzere polimerik yapıda bir doku iskelesi tasarlanması amaçlanmıştır. Tasarlanan polimerik üç boyutlu doku iskelesine, damlatma yolu ile antimikrobiyal bir ajan yüklenmiştir. Elde edilen yapı fizikokimyasal ve antibakteriyel özellikleri ile biyouyumluluğu ve hücre tutunması açılarından karakterize edilmiştir. Elde edilen sonuçlar göz önüne alındığında oluşturulan doku iskelesinin ilaç taşıma sistemi olarak yara iyileşmesi ve doku mühendisliği uygulamalarında kullanılmaya uygun bir aday olduğu değerlendirilmiştir.

**Anahtar Kelimeler:** doku iskelesi, yara örtüsü, yara iyileşmesi, proliferasyon, antimikrobiyal aktivite

## *ORAL PRESENTATION*

# Stripping voltammetry as an effective tool for meeting the needs of green analysis chemistry

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**Abstract**

The development of industry is highly dependent on the reliable and fast delivery of the results of analytical determinations of hundreds of thousands of substances. As the number of newly synthesized organic and inorganic chemical substances increases exponentially, with the number doubling between 2009 and 2015[1], the need for new and environmentally friendly analytical procedures continues to increase. To respond to this challenge, green analytical methods that offer (i) reduction of the consumption of chemical reagents, (ii) reduction of the volume of vapor and gas emissions, as well as minimization of the load of wastewater and solid waste, (iii) elimination of highly toxic reagents, and (iv) reduction of energy consumption should be developed[2]. The article provides evidence that stripping voltammetry can be considered as a green-chemistry-friendly analytical method. Examples of voltammetric determinations employing sensors prepared using nontoxic materials (synthetic and of natural origin) and those made of waste materials containing precious metals, of compounds important for human health, modern industrial production, and advanced technologies are presented. The accomplished tasks included: (i) assessment of the antioxidant properties of plant extracts containing polyphenols using a natural glassy carbon paste electrode ($GC_n$/CPE), (ii) determination of chlorogenic acid in green coffee contained in a dietary supplement using $GC_n$/CPE, (iii) determination of capsaicin in piri-piri pepper using $GC_n$/CPE, (iv) determination of quercetin using an Au-DVD-based electrode, (v) determination of ascorbic acid using Ag-DVD-based electrode, (vi) determination of azo dyes and quinine in soft drinks using bismuth film electrode, and determination of critical elements (indium and cerium) using glassy carbon (Ce) and $GC_n$/CPE (In) electrodes. Additionally, apart from capsaicin extraction, the determination of organic compounds did not require the use of organic solvents and the samples did not need to undergo any pre-treatment prior to testing which significantly simplified and shortened the course of analytical determination.

**Keywords:** green analytical chemistry, stripping voltammetry, carbon paste electrodes

Research was carried out using the ‘Financing of research carried out as part of the preparation of a doctoral dissertation at the Faculty of Materials Science and Ceramics at AGH University of Science and Technology’.

1. *https://www.cas.org/cas-data/cas-registry*. [cited 2022 20.10.2022].
2. Galuszka, A., Z. Migaszewski, and J. Namiesnik, *The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices.* Trac-Trends in Analytical Chemistry, 2013. **50**: p. 78-84.

## *ORAL PRESENTATION*

# Phytochemical study on endemic *Polygonum sivasicum* Kit Tan &Yıldız

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**Abstract**

Oxidative stress, an imbalance between the production and accumulation of reactive oxygen species (ROS), has been related to the onset of metabolic diseases. Therefore, plants with high antioxidant capacity have been a subject of interest. *Polygonum* species have been widely used for the treatment of common ailments such as diabetes, cardiovascular diseases, liver problems and kidney problems both in traditional Chinese medicine and Turkish folk medicine (Baytop, 1999; Teka et. al., 2021; Xiang et al., 2011). Herein, an endemic *Polygonum sivasicum* Kit Tan & Yıldız was investigated for its antioxidant capacity, fatty acid profile, and phenolic compound content.

Aerial parts of *P. sivasicum* were collected from Sivas and dried under optimum conditions. Hexane, ethyl acetate, chloroform, and methanol extracts were obtained by maceration of ground *P. sivasicum*. The water extract of the plant was obtained by infusion. A cooked plant extract was also included in the antioxidant activity tests. Antioxidant capacities of all obtained extracts were evaluated by $ABTS^{+}$• scavenging, DPPH• scavenging, CUPRAC, $\beta$-carotene-linoleic acid and ferrous ion-chelating ability activities.Total flavonoid and phenolic contents were determined as quercetin and pyrocatechol equivalents. The fatty acids of *P. sivasicum* were studied by GC and GC-MSD. Major compounds were found to be $\alpha$-linolenic acid (21.14 %), palmitic acid (18.76 %), and 8,11-octadecadienoic acid (17.24 %). In addition, the phenolic content of the methanol extract was analyzed by LC-MS. The results showed that the aerial parts of *P. sivasicum* have rich phenolic compounds and hyperoside (226.75 mg/L), rutin (219.37 mg/L), and chlorogenic acid (165.33 mg/L) were the major phenolic compounds.

**Keywords:** *Polygonum sivasicum,* antioxidant activity, phenolics, GC-MS, LC-MS.

**References**

Baytop, T. 1999. Therapy With Medicinal Plants in Turkey Past and Present. İstanbul: Nobel Tıp Kitab Evleri.
Teka, T., Wang, L., Gao, J., Mou, J., Pan, G., Yu, H., Gao, X., Han, L. 2021. "*Polygonum multiflorum*: Recent updates on newly isolated compounds, potential hepatotoxic compounds and their mechanisms, Journal of Ethnopharmacology, 271, 17, 113864.
Xiang, M., Su, H., Hu, J., & Yan, Y. (2011). Isolation, identification and determination of methyl caffeate, ethyl caffeate and other phenolic compounds from *Polygonum* amplexicaule var. sinense. Journal of Medicinal Plants Research, 5(9), 1685–1691.

## *ORAL PRESENTATION*

# Quantitative Determination of the Phenolic Compounds in Different Organs of *Arctium minus* (Hill) Bernh. by LC-MS using Response Survey Methodology

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**Abstract**

Species belonging to the Asteraceae family are constantly being studied because of the distribution of this family in the world and because they contain substances with potential activity. *Arctium minus* (Hill) Bernh. is a species belonging to the *Arctium* genus of the Asteraceae family. Herein, it was aimed to comparatively investigate the antioxidant capacities and phenolic profiles of the different organs of *A. minus*.




In this study, *A. minus* (lesser burdock) was collected from Kars province in Turkey. The plant was dried for extraction and separated into leaves (A), flowers (B), and branches (C). These organs were extracted with methanol by ultrasonic extraction methods using the response surface methodology (RSM). Temperature, extraction time, and ultrasound power were selected as independent variables to produce the most potent extracts that contain phenolics. All the obtained extracts were subjected to four different antioxidant tests: ABTS+• scavenging, DPPH• scavenging, CUPRAC, and ferrous ion-chelating ability activities. The selected extracts which have the highest antioxidant activity of the different organs of the A. minus were analyzed by LC-MS. The major phenolic compounds of the leaves were identified as chlorogenic acid (8492.85 mg/kg crude extract), hyperoside (620.28 mg/kg crude extract), and quercitrin (446.43 mg/kg crude extract). Moreover, the major phenolic components of the flower and branch parts were determined as chlorogenic acid and vanillic acid. In addition, among the three different organs, flower parts were found to contain more arctiin and arctigenin.

**Keywords:** *Arctium minus*, Response surface methodology (RSM), Phenolic compounds, Ultrasonic extraction.

**Acknowledgments:** This work was supported by the Research Fund of Bezmialem Vakif University, Project number 20211222.

## *ORAL PRESENTATION*

# *Paronychia dudleyi* Chaudhri taksonunun kromozom sayısının tespiti

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**Özet**

Caryophyllaceae familyası içerisinde yer alan *Paronychia* Mill. yaklaşık 110 türe sahip bir cinstir. Cinsin tek yıllık veya çok yıllık üyeleri, Güney Afrika ve Güney Asya dışında tüm dünyada yayılış gösterir. Bu çalışmada, *Paronychia dudleyi* taksonunun diploid kromozom sayısının tespit edilmesi amaçlanmıştır. *Paronychia dudleyi*, gövdesi tabanda dallanmış, 2 ile 15 cm boylanabilen, yatık-yükselici, çok yıllık bir bitkidir. Taksonun kromozom sayısını tespit etmek amacıyla, doğal yaşam alanlarından toplanan bitki tohumları çimlendirme işlemine tabi tutulmuştur. Daha sonra çimlenen tohumlara sırasıyla; ön işlem, fiksasyon, hidroliz, boyama ve preparat hazırlama aşamalarından oluşan sitogenetik prosedür uygulanmıştır. *Paronychia dudleyi* taksonunun kromozom sayısı $2n = 4x = 36$ olarak belirlenmiş ve kromozomların detaylı ölçümleri yapılmıştır. Taksonun diploid kromozom sayısı ile ilgili bu ilk raporun, *Paronychia* sitotaksonomisine önemli katkı sağlayacağını düşünmekteyiz.

**Anahtar Kelimeler:** *Paronychia dudleyi*, Kromozom, Sitogenetik

## *ORAL PRESENTATION*

# Spectrofluorimetric Determination of Montelukast Sodium Based on Molecularly Imprinted Solid-Phase Extraction

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**Abstract**

The molecular imprinting technique is a highly determinative recognition technology. Molecularly imprinted polymers (MIPs) can be applied to the cleanup, pre-concentration, and selective determination of analytes as the high-affinity adsorbent of solid-phase extraction (SPE). In recent years, a new type of SPE has formed, molecularly imprinted polymer solid-phase extraction (MISPE), and has been widely applied to the extraction in different areas. A simple, sensitive, and accurate spectrofluorimetric method has been developed for the determination of Montelukast Sodium in aqueous media. 2-(dimethylamino)ethyl methacrylate, ethylene glycol dimethacrylate, n,n-azobisisobutyronitrile (AIBN), and DMSO were used as a functional monomer, cross-linker, initiator, and porogen, respectively. Thanks to the selectivity of the MISPE technique and the sensitivity of the fluorescent measurement, montelukast sodium has been applied and analyzed quantitatively in aqueous media successfully.

**Keywords:** MISPE, Spectrofluorimetry, Molecularly imprinted polymers

## *ORAL PRESENTATION*

# Micromorphological and anatomical variations of *Eranthis hyemalis* populations in Turkey

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**Abstract**

The genus Eranthis is represented by 9 species in the world, while only *Eranthis hyemalis* (L.) Salisb. (Ranunculaceae) naturally spreads in Turkey. It grows naturally in open areas at an altitude of 1300-1800 m in Anatolia. It is a tuberous herbaceous plant that grows 10-12 cm tall and upright. The flowering period lasts from late January to May, depending on ecological conditions. *E.hyemalis* is one of our tuberous plants with medicinal and economic value. Toxic effects on pets have been reported due to the cardiotonic glycosides in the content of *E. hyemalis*. It has also been shown to have antiviral activity.

Micromorphological analyses with scanning electron microscopy and light microscopy have also identified differences that will contribute significantly to the taxonomy of this genus. Seed shape in the genus *Eranthis* is elliptical or globose circular. Irregular wrinkled seed coat with polygonal to irregular cell wall decoration has been observed in the genus *Eranthis*. It's leaves with stomata only on the lower surface are hypostomatous. The genus has anomocytic (meaning irregular celled) stomata have guard cells that are surrounded by cells that have the same size, shape and arrangement as the rest of the epidermis cells.

According to the results of this study, new taxa can be defined for science by examining other characters according to the level of variation.

**Keywords:** *Eranthis,* Ranunculaceae, Anatomy, SEM, Turkey

## *ORAL PRESENTATION*

# Investigation of the effect of different culture conditions on recombinant protein production

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## Abstract

After the COVID-19 pandemic, vaccine production technologies have become the focus of attention of researchers. As a matter of fact, recombinant protein-based antigen production, which is one of them, has taken its place in the first place. Proteins obtained by recombinant DNA technology are used in many industrial areas, especially vaccine applications, due to their reliability. Therefore, it is very important to produce targeted recombinant proteins in large quantities. This study, for the high amounts production of Omp25 protein, which is used as a vaccine candidate against brucellosis, in laboratory conditions, is aimed to reveal the effects of conditions that are the pre-culturing process, inoculation in LB or TB media, denatured or native purification, culturing with/without IPTG. All the results were analyzed by SDS-PAGE, confirmed Western Blot, and the total protein amounts were measured Bradford method. According to the results, Omp25 protein could not be obtained under native purification conditions in both cultures without induction, but it was observed under denatured conditions. This result can be explained that the protein in the cell is either misfolded or incorporated into the membrane. The amount of protein appears to be much higher in the presence of the inducer in both media inoculated with the starter pre-culture compared to the overnight pre-culture; 8.79 mg and 39.4 mg from 1 L culture, respectively. Additionally, as expected, the addition of IPTG increased the amount of protein, approximately one-and-a-half-fold for LB and about three-fold for TB. Finally, it was observed that TB medium provided higher protein production than LB, which can be explained by the presence of glycerol and high yeast extract in the medium. Although our study contains results that will attract the attention of vaccine industry, it should be kept in mind that all process should always be optimized depending on the structure of the targeted protein and thus the production amount can be further increased.

**Keywords:** Recombinant protein, protein amount, culture condition, Omp25

## *ORAL PRESENTATION*

# Examination of the chemical composition of animal feed and its influence on the quality of milk

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**Abstract**

Knowledge of the chemical composition of animal feed is important for the normal health of dairy cows and high production of quality milk. An examination of the chemical composition in % of animal feed was made: fodder crop - alfalfa hay, straw - which is a by-product of wheat and two types of fodder mixes (KMK1 and KMK2). Nutrients were examined: protein, crude fiber, fat, moisture, ash and their total %, as well as nitrogen-free extractive substances (BEM) in 3 samples of each food from 3 different farms (A, B, C). The highest total value (%) for all nutrient compounds was obtained in alfalfa of farm A =65.91%, and in the other two farms the total value is equal to 65.22%. The lowest total value was obtained in straw used in farm A = 53.78%. The total value of BEM in all three farms is around 34.78%.Namely was obtained in KMK2 used in farm A=38.54%, in KMK1 from farm B=39.13% and KMK1 from farm C=42.15 %, while the average value of BEM in KMK 2 of farm A = 61.33%, KMK1 in farm B=60.87% and KMK1 from farm C=57.49%. Analyses of chemical composition of the milk (dry matter, fat, protein, lactose and density) concluded that the values from the three farms is similar, the milk from farm B has the highest value dry matter was 8.22% and fat 3, 65%, and the milk from farm C is dominated by proteins with 3.20% and a density of 28.56%. It concluded that the feed mixture KMK1 and KMK2 used for dairy cows contain at least 18-22% protein and it is ideally balanced feed in order to obtain optimal energy and higher milk production with a daily production of over 30 liters. The use of alfalfa and straw enrich the animal feed with proteins and necessary substances for good milk quality.

**Keywords:** animal feed, concentrates, fodder mixture, alfalfa hay, wheat straw, chemical compounds

## *ORAL PRESENTATION*

# Rhein promotes ER stress-mediated apoptosis in pancreatic cancer cell line

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**Abstract**

Pancreatic cancer is a highly lethal malignancy characterized by close parallelism between incidence and mortality, asymptomatic clinical development, and poor prognosis. Rhein is one of the effective anthraquinone components extracted from polygonaceae. In recent studies, it has been determined that Rhein has an anticarcinogenic effect by regulating apoptosis on human breast, colon, lung and glioma cancer cell lines. In our study, we aimed to investigate the anticarcinogenic effects of Rhein on the endoplasmic reticulum stress-mediated apoptosis pathways in the PANC-1 pancreatic cancer cell line. We evaluated the cytotoxic effect of Rhein on PANC-1 cells by using the XTT proliferation assay. Rhein was treated to PANC-1 cells in a dose (5-10-20-30-40-50-75-100 µM) and time (for 24 and 48 hours) dependent manner. Based on cell viability calculated for each concentration and time, the IC50 (half maximal inhibitory concentration) dose was determined as 22.5 µM at 48 hours. To evaluate the effects of Rhein on ER stress-mediated apoptosis by RT-qPCR, RNA isolation was performed from both dose and control wells. The cDNA of each sample was synthesized from the isolated RNAs. The expression profile of target genes associated with ER stress and apoptosis was determined by RT-qPCR and the changes were calculated with "RT²Profiler™PCRArrayDataAnalysis". According to the results obtained, it was determined that TNF-a, FADD gene expression decreased by 1.44, 4.86 times, and CASP3, CASP7, CASP8, CASP9, CYCS, BAX, PERK, ERN1,CHOP,ATF4,ATF6,ASK1,TRAF2,EIF2A,GADD34,BIP,XBP1 increased by 1.64, 1.04, 1.35, 2.19, 1.92, 5.28, 12.04, 8.06, 4.72, 3.18, 2.2, 1.17, 8.11, 1.22, 45.25, 5.17, 5.46 times, respectively, in dose group PANC-1 cells. Taken together, our findings revealed that Rhein promotes apoptosis in PANC-1 cells by regulating ER stress signaling pathways. These results may contribute to the understanding of Rhein's roles in apoptosis and to the development of new therapeutic approaches for the prevention and treatment of pancreatic cancer.

**Keywords:** Rhein, pancreatic cancer, ER stress, apoptosis, Natural compound.

## *ORAL PRESENTATION*

# Kaya kartalında şiddetli miks enfeksiyon ve peritonitis olgusu

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**Özet**

Bu olguda, yaralanma, iştahsızlık ve şiddetli solunum yetmezliği sonucu Bingöl Doğa Koruma ve Milli Parklar Şube Müdürlüğü tarafından, Bingöl Üniversitesi Hayvan Hastanesine getirilen bir kaya kartalının (Aquila Chrysaetos Chrysaetos) yapılan klinik ve hematolojik muayenesinde; şiddetli iştahsızlık, hırıltılı solunum, halsizlik ve uzun süre önce silahlı yaralanma geçirdiği tespit edildi. Ağız içerisinde herhangi bir lezyon olmadan gelen kötü kokunun tespiti amacıyla kursağın sondalanması yapıldı. Elde edilen kursak içeriğinin de aynı şekilde pis koktuğu ve pH'sının 6 düzeyinde olduğu belirlendi. Tam kan muayenesi için hayvanın kanat venasından alınan kanda, şiddetli lökositoz (WBC:171,5x$10^3$), lenfositoz (lenfosit:%59,4), granülositopeni (granülosit:%13,1) tablosu ile eritrosit (RBC:1,56x$10^6$) ve trombosit (PLT:34x$10^3$) sayıları ve % hematokrit (PCV:19,8) değerinde düşüş saptandı. Ayrıca, bazı biyokimyasal parametrelerde [ALP:84 (U/L)-ALT:51,1 (U/L)-AST:333,7 (U/L)] artış olurken, bazılarında azalma [TP:4,27 (g/dL)-ALB:1,74 (g/dL)], diğer bir kısmının [Ca:4,27 (mmol/L)-P:1,74 (mmol/L)] da normal seviyelerde olduğu tespit edildi. Tüm bunlar vakanın bir kronik enfeksiyon sürecinde olduğunu göstermiştir. Peritonitis, pnömoni ve kursak ekşimesi tanısı konulan kartal, yapılan yoğun medikal tedaviye rağmen kurtarılamamıştır. Sonrasında yapılan nekropside abdominal sahada lokal nekroz odakları ile yaygın peritonitis, pneumoni ve proventriculus dahil sindirim sisteminde yangılara rastlanmıştır. Yine karın bölgesi çevresiyle özellikle karinada peteşiyel kanamalar ve nekrozlara rastlanmıştır. Göğüs ve karın bölgesinin tamamında sarı-kahverenginde mukopürülant sıvıyla dolu olduğu görülmüştür. Bu nesli tükenmekte olan avcı kuşlarda uzun süreli silahlı yaralanmaya ve yüksek enfeksiyon tablosuna rağmen hayatta kalabildiği için vaktinde yapılacak tıbbi desteklerle yaşamlarını sürdürebileceklerini göstermesi adına ortaya konulmuş bir rapordur.

**Anahtar Kelimeler:** Kartal, yaralanma, pnömoni, peritonitis, kursak ekşimesi

**Kaynaklar**

Can R, Keçeci H 1996. Kafes Kuşlarının Hastalıklarında Teşhis ve Tedavi Yöntemleri. Derleme, Veteriner Hekimler Derneği Dergisi. Aralık-Cilt 67, sayı 4, 45-56.

Franson JC, Russell RE 2014. Lead and eagles: demographic and pathological characteristics of poisoning, and exposure levels associated with other causes of mortality. *Ecotoxicology*, *23*(9), 1722-1731.

Ganz K, Jenni L, Madry MM, Kraemer T, Jenny H, Jenny D 2018. Acute and chronic lead exposure in four avian scavenger species in Switzerland. *Archives of Environmental Contamination and Toxicology*, *75*(4), 566-575.

Glawischnig W, Netzer M, Schöpf K 2011. Case report: Two golden eagles (Aquila chrysaetos chrysaetos) crashed into the roof of a house in Austria. *Veterinary Medicine Austria*, *98*, 133-135.

Glutz Von Blotzheım UN 2001. Handbuch der Vögel Mitteleuropas. Aula-Verlag, Wiesbaden, S. 637-675.

Madry MM, Kraemer T, Kupper J, Naegeli H, Jenny H, Jenni L, Jenny D 2015. Excessive lead burden among golden eagles in the Swiss Alps. Environ Res Lett 10:034003. https ://doi.org/10.1088/1748-9326/10/3/03400 3

Nazifi S, Nabinejad A, Sepehrimanesh M, Poorbaghi SL, Farshneshani F, Rahsepar M 2008. Haematology and serum biochemistry of golden eagle (Aquila chrysaetos) in Iran. Comp Clin Pathol. 17:197–201 DOI 10.1007/s00580-008-0720-8

Stauber E, Finch N, Talcott PA, Gay JM 2010. Lead poisoning of bald (Haliaeetus leucocephalus) and golden (Aquila chrysaetos) eagles in the US inland Pacific Northwest region—An 18-year retrospective study: 1991–2008. *Journal of Avian Medicine and Surgery*, *24*(4), 279-287.

## *ORAL PRESENTATION*

# Rifaksimin yüklü misellerin üretilmesi ve biyolojik aktivitelerinin değerlendirilmesi

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**Özet**

Rifamisin grubunun yarı sentetik bir türevi olan rifaksimin, suda çözünürlüğü oldukça sınırlı, gram pozitif ve gram negatif bakterilerde etkili olduğu kanıtlanmış geniş spektrumlu bir antibiyotiktir. Rifaksimin halihazırda farklı ticari isimlerle kullanılıyor olsa da düşük çözünürlüğü, oral kullanımda biyoyararlanımını % 0,4'ten daha aza indirmektedir [1]. Bu çalışmada, rifaksiminin metoksi poli(etilen glikol) – poli(ε-kaprolakton) (mPEG-PCL) misellere yüklenerek çözünürlüğünün ve dolayısıyla biyoyararlanımının arttırılması amaçlanmıştır. Çalışma kapsamında rifaksimin yüklü mPEG-PCL miseller nanoçöktürme yöntemiyle [2] üretilmiş ve karakterize edilmiştir. Misellerin üretiminde boyut, PDI, reaksiyon verimi, enkapsülasyon etkinliği ve ilaç yükleme kapasiteleri incelenerek sentez parametrelerinin optimizasyonu sağlanmıştır. Üretilen misellerin antioksidan aktiviteleri değerlendirilmiştir. Elde edilen sonuçlar, optimum oranlarda üretilen rifaksimin yüklü mPEG-PCL misellerin, etkenin çözünürlüğünü arttırarak daha yüksek antioksidan etki sağladığını göstermiştir.

**Anahtar Kelimeler:** Antioksidan aktivite, Çözünürlük, Misel, mPEG-PCL, Rifaksimin.

**Teşekkür:** Çalışma, TÜBİTAK 2210-C Yurt İçi Öncelikli Alanlar Yüksek Lisans Burs Programı ile desteklenmektedir.

**Referanslar:**

**[1]** Pentikis, H. S., Connolly, M., Trapnell, C. B., Forbes, W. P., Bettenhausen, D. K. 2007. "The Effect of Multiple-Dose, Oral Rifaximin on the Pharmacokinetics of Intravenous and Oral Midazolam in Healthy Volunteers". Pharmacotherapy, 27(10), 1361–1369.

**[2]** Kheiri Manjili, H., Ghasemi, P., Malvandi, H., Mousavi, M. S., Attari, E., Danafar, H. 2017. "Pharmacokinetics and in vivo delivery of curcumin by copolymeric mPEG-PCL micelles". European Journal of Pharmaceutics and Biopharmaceutics, 116, 17–30.

## *ORAL PRESENTATION*

# Comparison of Two Different Propolis Samples Obtained from Artvin in terms of Phenolic Content and Antioxidant Activity

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**Abstract**

Propolis is a natural product that honeybees collect from various plants. The compounds in the content of propolis and responsible for antioxidant activity are polyphenols and flavonoids. This study aimed to compare the phenolic content and antioxidant activity of aqueous and phosphate-buffered saline (PBS) extracts of two different propolis samples obtained from Ağıllar Village of Artvin province. By preparing 25 mg/mL aqueous and PBS extracts of two different propolis samples, the total polyphenol content was determined according to the modified Folin-Ciocalteu method, and the total flavonoid content was determined according to the Aluminum chloride colorimetric method. In addition, the ferric ($Fe^{3+}$) reducing antioxidant power was determined. As the result of the analyses the total polyphenol content of aqueous propolis extract numbered 1 and 2 from Artvin province was respectively, 9.12 ± 0.61 mg Gallic acid/g propolis and 10.62 ± 1.85 mg Gallic acid/g propolis, the total flavonoid content was respectively, 0.93, ± 0.06 mg Quercetin/g propolis and 0.92 ± 0.10 mg Quercetin/g propolis, ferric reducing antioxidant power was respectively, 6.80 ± 0.20 mg Trolox/g propolis and 3.09 ± 0.34 mg Trolox/g propolis. The total polyphenol content of propolis extract with PBS numbered 1 and 2 from Artvin province was respectively, 9.65 ± 0.35 mg Gallic acid/g propolis and 10.03 ± 1.47 mg Gallic acid/g propolis, the total flavonoid content was respectively, 2.24 ± 0.09 mg Quercetin/g propolis and 1.45 ± 0.30 mg Quercetin/g propolis, and ferric reducing antioxidant power was respectively, 7.31 ± 1.09 mg Trolox/g propolis and 3.54 ± 0.11 mg Trolox/g propolis. The aqueous and PBS extracts of two different propolis samples obtained from Ağıllar Village of Artvin province were found to be close to each other in terms of the amount of polyphenolic and flavonoid content and antioxidant activity. It was concluded that different propolis samples from the same region exhibit similar phenolic content and antioxidant activity.

**Keywords:** Propolis, Polyphenol, Flavonoid, Antioxidant, Artvin

## *ORAL PRESENTATION*

# Metal Oxide-Paladium Nanoparticles Modified Graphene Oxide Catalyst for Oxidation of Formaldehyde

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**Abstract**

In parallel with population growth, industrialization and technological developments, energy requirement is increasing day by day. Fuel cells are one of the environmentally friendly and clean energy sources that many researchers are focused on the improvement of this technology [1-3]. Direct fuel cells based on the electrooxidation of simple organic molecules such as alcohol, urea, formic acid, and formaldehyde have been reported as clean energy generation technologies. The electrooxidation of formaldehyde possesses many advantages. For instance, formaldehyde is used as a keystone for the insight of partial methanol oxidation in direct methanol fuel cells (DMFC). Formate ions ($HCOO^-$) that are produced during formaldehyde electrooxidation have the potential to become a prominent candidate for replacing fossil fuels in direct formate fuel cells to generate clean energy [4].

Graphene oxide is frequently used as an electrode material in electrochemical studies due to its large surface area and ability to increase the electron transfer rate in many studies. Metal-metal oxide nanoparticles show higher conductivity and catalytic effect compared to bulk metal atoms with the synergistic effect they create. Gold, silver, copper and palladium metals, ruthenium, cobalt and nickel metal oxide films are widely used examples of catalyst materials [1-3].

In this study, composite surfaces were electrochemically prepared for catalyzing the oxidation of formaldehyde. Electrodes were prepared by the deposition of graphene oxide, metal oxide (MeOx), and metal nanoparticles on bare GCE. 0.01 mol $L^{-1}$ formaldehyde oxidation peak current was found as 1.5 µA, 8.32 µA, 23.49 µA and 46.39 µA on bare GCE, Pd/NiOx/GO/GCE, Pd/CuOx/GO/GCE, Pd/CoOx/GO/GCE, respectively. Among all, Pd/CoOx/GO/GCE was found as an optimum surface for formaldehyde oxidation in terms of both peak current and potential. In consequence, it is thought that the obtained composite catalyst will be a good alternative for future fuel cell studies.

**Key Words:** Formaldehyde, paladium nanoparticles, metal oxide, graphene oxide

References:

[1] Koçak, S., Aslışen, B., Koçak, Ç.C. Determination of hydrazine at a platinum nanoparticle and poly(bromocresol purple) modified carbon nanotube electrode. Analytical Letters. 2016, 49(7), 990–1003.
[2] Koçak, Ç.C., Altın, A., Aslışen, B., Koçak, S. Electrochemical preparation and characterization of gold and platinum nanoparticles modified poly(taurine) film electrode and ıts application to hydrazine determination. International Journal of Electrochemical Science. 2016, 11, 233–249.
[3] Koçak, S., Aslişen, B. Hydrazine oxidation at gold nanoparticles and poly(bromocresol purple) carbon nanotube modified glassy carbon electrode. Sensors and Actuators B: Chemical. 2014, 196, 610–618.
[4] Elghamry,I, Al-Jendan,S., Saleh,M.M., and Abdelsalam ,E. Bimetallic nickel/manganese phosphate–carbon nanofiber electrocatalyst for the oxidation of formaldehyde in alkaline medium. RSC Advances, 2022, 12,20656.

## *ORAL PRESENTATION*

# Investigation of Cholinesterase Inhibitory Properties and Molecular Docking Studies of Pyridine-Based Compounds Carrying Triazole Group

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**Abstract**

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by deficiencies in the cholinergic system and accumulation of beta amyloid (Aβ) in the form of neurofibrillary tangles and amyloid plaques. Since the cholinergic system plays an important role in the regulation of learning and memory processes, it is targeted for the design of anti-Alzheimer's drugs. Cholinesterase inhibitors directly increase cholinergic transmission by inhibiting the enzyme acetylcholinesterase (AChE), which hydrolyzes acetylcholine. It has also been shown that both acetylcholinesterase and butrylcholinesterase (BuChE) play an important role in Aβ-aggregation in the early stages of senile plaque formation. If so, AChE and BuChE inhibition have been documented as critical targets for effective management of AD, with an increase in acetylcholine availability and a decrease in Aβ accumulation in brain regions. 1,2,3-triazole groups have advantages in binding biological molecular targets such as being stable against metabolic degradation, being able to bind hydrogen and increasing the solubility of the compound. Synthetic compounds containing 1,2,3-triazole units have diverse biological activities and are indispensable as starting compounds for many heterocyclic syntheses. Molecular docking is a technique that predicts the preferred orientation, affinity and interaction of a ligand at the binding site of a protein. Preferred orientation information can be used to estimate the strength of binding affinity between a drug target and ligand molecule using scoring functions.

**Keywords:** 1,2,3-Triazole, Cholinesterase, Molecule Docking.

## *ORAL PRESENTATION*

# Histochemical Effects of Spinosad on Rat Liver

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**Abstract**

A bioinsecticide called spinosad is commonly used for control of insect pests. This insecticide affects both target organisms (insects) and non-target organisms (indirectly human and other vertebrates). While spinosad neutralizes insects, humans are also affected by this substance if there is accidental contamination. Therefore, rats in the mammal group, including humans, were used as model in experiments. The rats used in the experiments were divided into 14 groups. Two were determined as the control group, six were determined as the spinosad applied group, and six were determined as the Spinosad + Vitamin E applied group. Spinosad applied rats were then sacrificed under ether anesthesia after 24 h, 72 h, and 1 week, and liver tissues were collected from these rats. Tissue samples were examined in a light microscope with Hematoxylin-Eosin staining and in a fluorescence microscope with DAPI staining. This study showed that spinosad has detrimental impacts on the liver cellular structure.

**Keywords:** Liver, Spinosad, Insecticide

## *ORAL PRESENTATION*

# Effect of pretreatment in carrot chips produced by drying: Energy consumption and physico-chemical properties

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**Abstract**

Carrot is a healthy product that can be consumed in all seasons due to its nutritional values and satiating properties. Besides being used fresh, carrots are also consumed as chips. In the production of chips, the most widely used method in the world is frying in oil. However, this method is not a highly recommended method in terms of health due to the change in the biochemical structures of the oils. An alternative to this method is the drying process. In this study, dipping pretreatments in 25% salt, 25% vinegar, 25% (salt + vinegar), 10% *Pyracantha coccinea* (Fire thorn) and 10% *Malus floribunda* (Japanese apple) solutions were used. The effects of applied carrot chips on drying model, effective diffusion, rehydration, color, shrinkage rate and energy consumption properties were investigated. Among the thin-layer drying models, Wang-Sing predicted the best and Rational 1-parameter 2 models the weakest. It was determined that the effective diffusion values varied between 2.96-9.12x$10^{-9}$ $m^2$ $s^{-1}$. The highest rehydration capacity was found to be 82.88% and 82.13% in the samples dipped in 25% vinegar and 10% Malus floribunda solutions respectively. The maximum % volumetric change was determined as 92.60% in the samples dipped in 10% *Pyracantha coccinea* solution. In terms of chroma and color change properties, it was determined that the samples that were dried after dipping in 25% salt solution were closest to the fresh samples. Optimum specific moisture absorption rate (SMER) and specific energy consumption (SEC) were determined during the drying process of the samples dipped in 25% vinegar solution. The highest steam energy and energy efficiency values were determined in the drying process after dipping in 25% vinegar solution. In terms of the data obtained in this study, a pre-treatment of dipping in 25% vinegar solution is recommended as an alternative to dipping in 10% *Pyracantha coccinea* (fire thorn) water.

**Keywords:** Carrot chips, Drying Process, Dipping Pretreatment, Energy Analysis, Quality

## *ORAL PRESENTATION*

# Methanolic extract of Fabaceae species, a source of natural antioxidants

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**Abstract:**

The current work involves the quantification and identification of antioxidant compounds by LC-MS analysis and the evaluation of the antioxidant activity of methanolic extracts by DPPH test.
LC-MS analysis identified 15 flavonoid and phenolic bioactive compounds that exhibited high antioxidant activity.
Rutin and fisetin were detected as the main flavonoids at concentrations of 1728.5567 mg/mL and 944.0435 mg/mL, respectively. And 4-hydroxybenzoic acid is a phenolic compound with a concentration of 1243.0717 mg/mL.
The DPPH test used to evaluate antioxidant activity showed that the MeOH extract has significant antioxidant activity with IC50 = 0.042 ± 0.003 mg/mL.
The results showed that the methanol extract was rich in high concentrations of phenolics and flavonoids with interesting antioxidant activities. We can think of this plant as a source of antioxidants.

**Keywords:** phenolic compounds, flavonic compounds, antioxidant activity , LC-MS.

## *ORAL PRESENTATION*

# Biochemical Effects of Certain Alifatic Side Chain Amino Acids on *Saccharomyces cerevisiae* L.

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**Abstract**

In this study, the effects of hydrogen peroxide ($H_2O_2$) added to *Saccharomyces cerevisiae* culture on aliphatic chained amino acids, lipophilic vitamins, fatty acids composition, malondialdehyde (MDA), glutathione (GSH) and oxidized glutathione (GSSG) were investigated. Amino acids are the building blocks and monomers of proteins that make up 50% or more of the cell structure. Amino acids, which are included in the structure of proteins, can have different functions alone. Amino acids greatly support hormone production, muscle structure, nervous system, functioning of vital organs, and cellular functioning in the body. Protein synthesis does not occur as a result of a deficiency or insufficient level of amino acids in the body and causes fatigue, irritability, hormonal imbalance and depression.

The yeast Saccharomyces cerevisiae generally exhibits a budding morphology. Within the scope of the experiment, Control, $H_2O_2$, leucine, valine, and their $H_2O_2$ added groups were used. Leucine and valine amino acids were added to the cell culture media as 0.5 g/L and H2O2 0.5 ml/L.

According to our findings, although total protein and MDA levels decreased in leucine and valine groups compared to the control group. It was determined that it increased in the groups to which $H_2O_2$ was added. GSH levels were also decreased in groups given $H_2O_2$. It was determined that palmitic acid level of fatty acids decreased in leucine and valine groups and $H_2O_2$ added groups compared to the control group. While the amounts of stearic, oleic and linoleic acid increased, only the amount of linoleic acid increased in the groups given $H_2O_2$. An increase was observed in the amounts of stearic acid and linoleic acid in the groups given $H_2O_2$. Although the amount of α-tocopherol decreased in the leucine and valine groups compared to the control group, an increase was observed in the levels of ergosterol and stigmasterol.

As a result, it was observed that adding amino acids to the culture medium of *Saccharomyces cerevisiae* was not effective in antioxidant defense against $H_2O_2$ toxicity and had a negative or positive effect on enzymes in lipid biosynthesis and other metabolic pathways.

**Keywords:** Aliphatic Amino acids , Antioxidant enzymes, Total protein, *Saccharomyces cerevisiae*, Fatty acids, Lipophilic vitamins, Hydrogen peroxide

References: 1. Weijs, P. J. M., Cynober, L., DeLegge, M., Kreymann, G., Wernerman, J. & Wolfe, R. R. (2014) Proteins and amino acids are fundamental to optimal nutrition support in critically ill patients, Crit Care. 18. Volume Editor: Prof. Dr. Volker F.Wendisch. Institute of Molecular Microbiology and Biotechnology WestfalianWilhelms University Münster Corrensstr. 3 48149Münster Germany e-mail: wendisch@uni-muenster.de
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## *ORAL PRESENTATION*

# Biochemical Effects of Certain Aromatic Side Chain Amino Acids on *Saccharomyces cerevisiae* L.

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**Abstract**

In this study, the effects of hydrogen peroxide ($H_2O_2$) added to *Saccharomyces cerevisiae* culture on aromatic chained amino acids, lipophilic vitamins, fatty acids composition, malondialdehyde (MDA), glutathione (GSH) and oxidized glutathione (GSSG) were investigated. Amino acids are the building blocks and monomers of proteins that make up 50% or more of the cell structure. Amino acids, which are included in the structure of proteins, can have different functions alone. Amino acids greatly support hormone production, muscle structure, nervous system, functioning of vital organs, and cellular functioning in the body. Protein synthesis does not occur as a result of a deficiency or insufficient level of amino acids in the body and causes fatigue, irritability, hormonal imbalance and depression.
The yeast *Saccharomyces cerevisiae* generally exhibits a budding morphology. Within the scope of the experiment, Control, $H_2O_2$, phenylalanine and tryptophan, and their $H_2O_2$ added groups were used.
According to our findings, although total protein and MDA levels decreased in phenylalanine and tryptophan groups compared to the control group. It was determined that it increased in the groups to which $H_2O_2$ was added. GSH levels were also decreased in groups given $H_2O_2$, it was observed that the MDA and GSH results of the groups to which, phenylalanine and tryptophan were added groups. It was determined that palmitic acid level decreased in phenylalanine and tryptophan groups and $H_2O_2$ added groups compared to the control group. While the amounts of stearic, oleic and linoleic acid increased, only the amount of linoleic acid increased in the groups given $H_2O_2$. An increase was observed in the amounts of stearic acid and linoleic acid in the groups given $H_2O_2$. Although the amount of α-tocopherol decreased in the phenylalanine and tryptophan groups compared to the control group, an increase was observed in the levels of ergosterol and stigmasterol. It was found that the change of ergosterol and stigmasterol in the groups given phenylalanine and tryptophan was similar. In addition, it was observed that the amount of α-tocopherol increased in the phenylalanine and tryptophan groups given $H_2O_2$ compared to the control group.
As a result, it was observed that adding amino acids to the culture medium of *Saccharomyces cerevisiae* was not effective in antioxidant defense against $H_2O_2$ toxicity and had a negative or positive effect on enzymes in lipid biosynthesis and other metabolic pathways.

**Keywords:** Aromatic Amino acids (phenylalanine and tryptophan), Total protein, *Saccharomyces cerevisiae*, Fatty acids, Lipophilic vitamins, Hydrogen peroxide

**References :** 1. Weijs, P. J. M., Cynober, L., DeLegge, M., Kreymann, G., Wernerman, J. & Wolfe, R. R. (2014) Proteins and amino acids are fundamental to optimal nutrition support in critically ill patients, Crit Care. 18.
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## *ORAL PRESENTATION*

# Synthesis of A New Carbazole-Based Bismaleimide and Poly(Bismaleimide)

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**Abstract**

In recent years, increasing demands in the field of optoelectronics have attracted a lot of attention in academic studies for the development of new optic polymers with thermal stability, reliability, tunable optical properties (refractive index, transmittance ext.), discoloration, compatibility with substrates [1]. Bismaleimide monomers are a new class of thermoset polyimides and have been important in the advanced composite and electronics industries due to their excellent properties such as thermal stability, mechanical strength, low moisture absorption, good chemical and corrosion resistance, and dielectric performance. To design new bismaleimides, the structure among amine compounds can be tailored with the functional groups to meet various abilities such as high melting point, chemical stability, fire resistance, photochemical behavior, and electrical performance, etc. [2, 3]. However, despite all these examples, there are only a few studies in which a bismaleimide monomer has been introduced and its optical properties have been investigated.
In this study, Carbazol-Bismaleimide (Cz-BisMI) was synthesized as a new bismaleimide compound based on a bismaleimide monomer to be used in the application of optoelectronic materials, as a first time in the literature. Carbazole has received great attention in optoelectronic applications due to the unique electroluminescent and photorefractive properties [4]. Then, a new poly(bismaleimide): P(Cz-BisMI)s containing carbazole units was prepared by the self-polymerization process of Cz-BisMI. The optical and thermal properties of Cz-BisMI and P(Cz-BisMI)s were investigated.

**Keywords:** Bismaleimide, poly(bismaleimide), carbazole, thermal properties, optical properties.

**References**

[1] Iredale, R.J.; Ian Hamerton, C.W. Modern advances in bismaleimide resin technology: A 21st centuryperspective on the chemistry of addition polyimides. Prog. Poly. Sci. 2017, 69, 1–21.
[2] Airinei, A.; Fifere, N.; Homocianu, M.; Gaina, C.; Gaina, V.; Simionescu, B.C. Optical properties of some new azo photoisomerizable bismaleimide derivatives. Int. J. Mol. Sci. 2011, 12, 6176-6193.
[3] Liu, Y.L.; Liu, Y.L.; Jeng, R.J.; Chiu, Y-S. Triphenylphosphine oxide-based bismaleimide and poly(bismaleimide): synthesis, characterization, and properties. J. Polym. Sci. Part A Polym. Chem. 2001, 39, 1716-1725.
[4] McGrath, J.E.; Rasmussen, L.; Shultz, A.R.; Shobha, H.K.; Sankarapandian, M.; Glass, T., Long, T.E.; Pasquale, A.J. Novel carbazole phenoxy-based methacrylates to produce high-refractive index polymers, Polymer 2006, 47, 4042–4057.

## *ORAL PRESENTATION*

# Investigation of Caspase Pathway and Possible Stress Mechanisms of Curcumin Analog GO-Y030 on Head and Neck Squamous Cell Carcinoma

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**Abstract**

Anticancer agent research on head and neck tumors and tumor microenvironment related tissues has gained importance in recent years. Growth suppressive evidence on cancer cell lines regarding the anticancer therapeutic potential of diarylpentanoids is available in the literature. The anticancer activity of GO-Y030, one of the diarylpentanoids, on breast, pancreatic and colorectal cancers is remarkable. In this study, the caspase and ER stress pathway of the curcumin analog GO-Y0302, which is a diarylpentanoid derivative, on the Head and Neck Squamous Cell Carcinoma (HNSCC) cell line was investigated. MTT test was applied to the cells to investigate the effective dose of GO-Y0302; different doses of 160, 80, 60, 40, 20, 10, 5, 2.5 and 1 µM were evaluated in different times (24 h, 48 h, 72 h). The effective dose was found to be 10 µM administered at the 48th hour after measurements at a wavelength of 570 nm. Total RNA was isolated by the TRIzol method and then cDNA was synthesized with a commercial cDNA synthesis kit. Gene expression levels were analyzed by RT-qPCR. Fold changes were calculated by the $2^{-\Delta\Delta CT}$ method. In conclusion, there were differences in the expression levels of Casp2, Casp3, Casp6 Casp9, Casp12, EDEM1, CHOP and GRP78 genes in HNSCC cells treated with GO-Y030, compared to the untreated group and HEK293 control cells. Diarylpentanoids can have a strong anticancer effect, so in the future, investigating different diarylpentanoids in head and neck cancers will have an important place in the fight against cancer.

**Keywords:** GO-Y0302, HNSCC, HEK293, Caspase.

## *ORAL PRESENTATION*

# Point-of-Care Applications Based on Biodecorated Metallic Nanoparticles for Diagnosis

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**Abstract**

Metal nanoparticles are metallic nanosized structures in the size range of 1-100 nm. These entities can be pure metals or compounds like oxides, hydroxides, sulfides, chlorides, etc. These particles show superior optical, physicochemical, and electrochemical properties. Due to their wide variety of properties, metal multifunctional gold, iron, zinc, platin, and silver nanoparticles, are frequently used in fields such as medical diagnosis and treatment, biomedical imaging, cancer treatment, and biosensor applications. The advantages of their small size, easy functionality, and biocompatibility allow the development of different biorecognition applications. Metallic nanoparticles can have sharp colors, fluorescence properties, and magnetic properties which makes them suitable for obtaining the electrochemical or optic signal. In addition, paramagnetic nanoparticles can capture the analyte and carry it and concentrate directly on the detector for detection with the help of an applied magnetic field. In this way, it is possible to increase the chance of receiving high-precision, stable electrochemical or optical signals. The diversity of metallic nanoparticles and molecular recognition elements can adapt to surface modification of different test devices including electrochemical detection and optical detection with mobile phones. Thus, this study presents the strategies and methodologies to prepare biofunctional metallic nanoparticles with desired decoration from laboratory tests to mobile phone detection systems.

**Keywords:** metallic nanoparticle, biosensor, point-of-care, diagnosis.

## *ORAL PRESENTATION*

# Effect Of Kefir Supplementation On Feacal Microbiota In Balbc Male Mice

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**Abstract**

In this study, the identification and characterization of kefir from traditional and commercial kefir were performed and its' effects on the feacal microbiota of the 4-week male BALBc mice were analyzed by conducting seven sets of experiments with different amounts of kefir.
The experimental protocol was performed in compliance with the institutional guidelines for the use and care of animals established by the Yeditepe University Laboratory Animal Care Committee (YUDHEK No:736). Thirty-five 4-week old BALBc male mice type were obtained from Yeditepe University Animal Labs. The mice were divided into seven groups (n=5) consisting of standard diet control, traditional kefir diet which was differentiated by the amount of kefir they were given, 0.05 mL(n=5), 0.1 mL(n=5), and 0.2 mL(n=5), and commercial kefir diet which was differentiated by the amount of kefir they were given, 0.05 mL(n=5), 0.1 mL(n=5), and 0.2 mL(n=5). The kefir product was administered daily by oral gavage.
This study demonstrates that identical genotypes of *Enterococcus faecalis*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactococcuslactis*, *Lactobacillus plantarum*, and *Streptococcus thermophilus* were found in traditional kefir-fed mice feces in seven cases.
Milk kefir is a functional food with great potential. Numerous observed physiological effects support the health-promoting benefits of kefir. However, different study designs of clinical trials on kefir cause difficulties in drawing clear conclusions. In this context, further research on kefir is needed, with the focus on the interaction between microbiota and host, and mechanisms of action and detoxification, which may positively affect human and animal well-being.

**Keywords:** Kefir supplementation, Microbiota

## *ORAL PRESENTATION*

# Surface molecularly imprinted bacterial cellulose to controlled anti-cancer drug release

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**Abstract**

In this study, we prepared molecularly imprinted bacterial cellulose to develop drug carrier system to show high loading capacity and controlled drug release profile for anti-cancer drug release. Molecular imprinting technique was utilized for the preparation of anti-cancer drug-imprinted bacterial cellulose. Recently, control drug release technology has drawn the attention of scientists, and biomedical fields. The aim of this system is to increase the efficacy of drug treatment. Bacterial celluloses are used as a broad bio-polymer in nature. Bacterial cellulose has a lot of advantages such as porosity, biocompatibility, water-holding capacity, and outstanding mechanical properties.
Thin drug imprinted film was developed onto the bacterial cellulose nanofibers by surface imprinting method using metal ion coordination interactions with N-methacryloyl–(L)-histidinemethylester (MAH) and copper ions. The imprinted bacterial cellulose was characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR), and swelling degree measurements. Cytotoxicity of imprinted bacterial cellulose was studied using mouse fibroblast cell line L929.
The in-vitro drug release tests were shown to evaluate the release performance of the result at bacterial cellulose at 37 °C. Finally, kinetic models were applied to the drug release data.

**Keywords:** Drug imprinted, molecular imprinting technology, bacterial cellulose, anti-cancer drug, drug release.

## *ORAL PRESENTATION*

# Prediction of the heat capacity for aromatic aldehydes to Tetrahymena pyriformis using QSPR approach

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**Abstract**

The primary role that characterized the Quantitative Structure-Property Relationship (QSPR) method is to relate the molecular structure of natural and synthetic chemicals with their physical and chemical properties. By the employment of the physico- chemical molecular descriptors for a purpose to use realistic molecular quantities ,in addition to represent the molecular structure . In this study, the correlation between the heat capacity (CV J/molK) of 77 aromatic aldehydes with their molecular structures was established. This thermodynamic property is taken from HF level using the ab initio 6-31G basis sets from the program package GAUSSIAN 09. The best obtained model had three molecular descriptors and statistics of, $R^2$ = 0. 94,44, F = 299,98, and SE = 1,2881, this model was developed by applying multiple linear regression analysis using the ordinary least square regression method and genetic algorithm- variable subset selection. The reliability of the proposed model was evaluated by using the leave- one- out cross- validation evaluation method ($Q^2$ = 0.9357and SPRESS=1,3358).

**Key Words** : aromatic aldehydes, external validation, QSPR, Molecular descriptors, Multiple linear regression.

## *ORAL PRESENTATION*

# Ladin meşcereleri üst topraklarında derinliğe göre Cmic ve Nmic 'in değişimi

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**Özet**

Mikrobiyal canlılar/biyokütle, karasal ekosistemlerde toprak karbonu ve besin döngüsünde çok önemli düzenleyici rol oynamaktadır. Bu çalışmada saf doğu ladini (*Picea orientalis* L.) ormanı altındaki toprakların özellikle organik C, toplam N, ayrışma oranı (Corg/Ntoplam), mikrobiyal biyokütle C (Cmic) ve N (Nmic) gibi karakteristiklerinin toprak derinliği ile değişimi araştırılmıştır. Araştırma sahası Doğu Karadeniz bölgesi Trabzon ili Vakfıkebir ve Tonya İlçeleri sınırlarında bulunan Fol Deresi Mikro Havzası'nda yer almaktadır. Çalışma alanında alanı temsilen, rastgele 10 adet örnek nokta belirlenmiş ve bu noktalardan farklı iki derinlik kademesinden (0–15 cm ve 15–30 cm derinlik) toprak örneklemesi yapılmıştır. Alandan toprakların bazı fiziksel ve kimyasal analizleri için her derinlik kademesinden 10'ar örnek olacak şekilde toplamda 20 adet toprak örneği alınmıştır. Mikrobiyal analizler için de her iki derinlik kademesinden toplamda 20 adet toprak örneği alınmıştır. Toprakların mikrobiyal biyokütle C ve N içerikleri kloroform-fumigasyon-ekstraksiyon metoduyla bulunmuştur. Çalışma sonunda toprak derinliğine göre, toprakların fiziksel özelliklerinden % kum ve toz içerikleri istatistiki olarak birbirinden farklılık ($P < 0,05$) göstermiştir. Benzer olarak kimyasal özelliklerden organik C, toplam N ve ayrışma oranı (Corg/Ntoplam) değerlerinin her iki derinlik kademesine göre istatistiksel olarak birbirinden farklı ($P < 0,05$) olduğu ortaya çıkmıştır. Toprakların mikrobiyal biyokütle C içerikleri, 0-15 cm derinlik kademesinde 437,40–1315,20 µg g-1 ve 15-30 cm derinlik kademesinde 194,20–578,20 µg g-1 arasında değişim gösterdiği tespit edilmiştir. Toprakların mikrobiyal biyokütle N içerikleri, 0–15 cm derinlik kademesinde ortalama 88,08 ± 8,08 µg g-1 ve 15–30 cm derinlik kademesinde ortalama 41,17 ± 4,32 µg g-1'dır. Toprakların hem Cmic hem de Nmic içeriklerinin toprak derinliğine göre istatistiksel farklılık ($P < 0,05$) gösterdiği ortaya çıkmıştır. Ayrıca toprakların organik C ve toplam N içeriği ile Cmic ve Nmic arasında pozitif ve anlamlı bir korelasyonun olduğu belirlenmiştir. Çalışmada elde edilen sonuçlar, toprak derinliğine (derinliğin artmasıyla) göre değişen toprak özelliklerinin mikrobiyal biyokütle C ve N içerikleri üzerinde etkili olduğunu göstermektedir.

**Anahtar Kelimeler:** Doğu ladini, karasal ekosistem, mikrobiyal gösterge, toprak organik karbonu, toprak özelliği

## *ORAL PRESENTATION*

# Ceviz kabuğunun antioksidan kapasitesi ve üreaz enzimi üzerine etkisinin incelenmesi

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**Özet**

Fenolik bileşikler, tüm bitki türlerinin farklı kısımlarında bulunan ikincil metabolitlerdir. Ceviz (*Juglans regia* L.) Juglandaceae ailesine ait olup ekonomik etkisinin yanında biyolojik açıdan da oldukça önemli bir ağaç türüdür. Ceviz kabuğu, ürünün işlenmesi sırasında açığa çıkan, antioksidan özelliklere sahip doğal bileşikler içeren bir atıktır. Mevcut çalışmada ceviz kabuğu ekstraktlarının antioksidan özellikleri ve üreaz aktivitesine olan etkisi araştırılmıştır. Folin-Ciocalteu yöntemiyle ceviz kabuğunun metanolik ekstraktlarının toplam fenolik içerikleri eşdeğer gallik asit cinsinden belirlenmiştir. Numunedeki toplam fenolik madde miktarı 18.75±0.68 mg GAE $g^{-1}$ olarak tespit edilmiştir. Antioksidan aktivitesinin belirlenmesi amacıyla DPPH ve ABTS radikal temizleme kapasiteleri incelenmiş ve sonuçlar askorbik asit standardıyla karşılaştırmalı olarak $SC_{50}$ şeklinde verilmiştir. DPPH· serbest radikali yakalama aktivitesi tayini Mishra ve ark. metodunun modifiye edilmesi ile gerçekleştirilmiştir. Numune ve askorbik asidin DPPH radikali üzerindeki $SC_{50}$ değerleri sırasıyla 35.14±1.1 ve 11.2±0.3 µg.$ml^{-1}$ olarak bulunmuştur. $ABTS^{\cdot+}$ radikal katyon yakalama aktivitesi tayini Arnao ve ark. metodunun modifiye edilmesiyle belirlenmiştir. Örnek ve standardın ABTS üzerindeki $SC_{50}$ değerleri sırasıyla 44.29±1.4 ve 15.28±0.4 µg.$ml^{-1}$ olarak bulunmuştur. Üreaz enzim inhibisyonu Weatherburn metoduyla incelenmiş ve standart olarak kullanılan tiyoüre ile numunenin $IC_{50}$ değerleri sırasıyla 20.57±0.9 ve 98.45±4.1 µg.$ml^{-1}$ olarak bulunmuştur. Mevcut çalışmada elde edilen sonuçlarla biyoatık olan ceviz kabuğunun antioksidan etkinliği göz önünde bulundurularak ilaç ve gıda sanayisinin farklı alanlarında kullanım potansiyeli olabileceğini göstermektedir.

**Anahtar Kelimeler:** *Juglans regia* L, Toplam Fenol, DPPH, ABTS, Üreaz

## *ORAL PRESENTATION*

# Antifungal activity of *Ferula* genus

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**Abstract**

The genus Ferula comprises nearly 180 species. Among them, 130 species are common to the Mediterranean Basin and Central Asia. Species of this genus are known for their uses in traditional medicine, as tranquilizers, antispasmodics and antihysterics. Extracts from the Ferula species studied revealed the presence of biologically active secondary metabolites. The study consists to compare three methods of extraction by evaluating antifungal activity aerial part of *Ferula* species. The extraction rates are determined from three extraction methods, namely maceration,Infusion and decoction antifungal activity was determinated by the pathogenic fungus: Fusarium Oxysporum f.sp, on a PDA medium.The results obtained showed that the methanolic extract produced larger zones of inhibition than the other extracts.These results can be explained by the difference in composition between the two extracts, methanol allowing better extraction of less polar compounds such as terpene derivatives or flavonoids. This indicates that the antifungal activity also depends on several factors such as the type of solvent used for the extraction.

**Keywords:** antifungal activity,PDA, Fusarium Oxysporum f.sp.

## *ORAL PRESENTATION*

# Esnek ve kendi kendini iyileştirebilen hidrojel sentezi

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**Özet**

Kendi kendini iyileştirme, biyolojik hasarların iyileşmesini taklit eden bir kavramdır. Kendi kendini iyileştirme tekniği, malzemelerin moleküler yapısında ya da dokusunda meydana gelen hasarlı kısımlarını kendiliğinden onarabilecek şekilde kullanım ömrünü uzatmasıdır. Bu teknik, bir kesiğin iyileşmesi, kırık bir kemiğin kendini onarması gibi doğal iyileşme süreçlerinden alınan ilhamla ortaya çıkmıştır ve bilim insanları bu süreci malzemelere uygulayarak o malzemelerin kullanım ömürlerini uzatabileceklerini düşünmüşlerdir. Bu düşünceyle kendi kendini iyileştirme tekniği birçok mühendislik uygulamalarına dahil edilmiştir. Kendi kendini iyileştirebilen malzemelerin mekanik ve yapısal özelliklerindeki iyileşmelerin yanında, korozyon önleyicilik, süperhidrofobiklik, çizilmezlik, antibakteriyellik, iletkenlik özelliklerinde de malzemelerin kendi kendilerini iyileştirebilmeleri üzerine çalışmalar yapılmaktadır. Kendi kendini iyileştirebilen materyallere olan ilgi her gün artmaktadır, kendi kendini iyileştirme yeteneğine sahip malzemeler kullanılarak üretilen cihazlar, dayanıklılıkları ve uzun ömürlü olması nedeniyle dikkat çekmektedir.
Yapılan çalışma kapsamında kendi kendini iyileştirebilen hidrojeller sentezlenmiştir. Çalışmada akrilik asit, polivinil alkol monomerleri başlangıç maddesi olarak kullanılmıştır. Bu monomerler, çeşitli başlatıcı (amonyum persülfat) ve çapraz bağ ajanları (etilen glikol, boraks) ile polimerleştirilerek kendi kendini iyileştiren hidrojeller üretilmiştir. Üretilen hidrojellerin özelliklerine monomerin, çapraz bağ ajanı çeşidinin, miktarı etkisi incelenmiştir. Sentezlenen hidrojellere kesme-iyileştirme testleri yapılmıştır. Bu amaçla sentezlenen hidrojeller kesilerek yeniden birleştirilmiştir ve tekrarlanan hasar-iyileşme döngülerinden sonra da orijinal özelliklerini %80 koruduğu görülmüştür. Sentezlenen hidrojellerin hem orijinal hallerinin hem de hasar-iyileşme döngülerinden sonraki hallerinin karakterizasyon analizleri yapılacaktır. Böylece sentezlenen hidrojellerin kendi kendini iyileştirme kapasitesi detaylı olarak yorumlanacaktır.

**Anahtar Kelimeler**: Kendi kendini iyileştirme, Kendi kendini iyileştirebilen malzemeler, Hidrojel

## *ORAL PRESENTATION*

# Adipoz doku kökenli mezenkimal kök hücrelerde TLR4 sinyalinin metabolik aktivite ve rejeneratif kapasitesi üzerine etkilerinin değerlendirilmesi

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**Özet**

TLR4 reseptörünün lipopolisakkaritler ile uyarılması sonucunda proinflamatuar sitokinler salgılanır ve immün sistemi hücrelerinde proinflamatuvar yanıt başlar. Güncel çalışmalar TLR4 sinyalinin immün sistem hücreleri üzerindeki etkileri üzerinde yoğunlaşmıştır. TLR4 reseptörü bulunduran mezenkimal kök hücrelerin de immünregülasyona etkisi olduğu bilinmektedir ve fakat diğer hücresel mekanizmalar üzerindeki etkileri bilinmemektedir. Bu çalışmada mezenkimal kök hücrelerin TLR4 reseptörünün aktivasyonunun ve inhibisyonunun metabolik aktivite ve rejeneratif kapasitesi üzerindeki etkisi araştırılmıştır. Adipoz doku kökenli mezenkimal kök hücreler karakterizasyonu yapıldıktan sonra pasaj numarası beşi geçemeyecek şekilde çoğaltılarak deneylerde kullanılmıştır. Metabolik aktivite 0.1 µg/ml , 1 µg/ml ve 10 µg/ml LPS ve TLR4 inhibitörü LPS-RS ile dozlarında 0, 24, 48, 72 ve 96 saat boyunca metabolik aktivitesi MTT testi ile değerlendirilmiştir. Rejeneratif kapasitesi ise MMP9, MMP2 ,TIMP1 ve VEGFR2 genlerinin ekspresyonları ve koloni oluşturma testi ile değerlendirilmiştir. 0.1 µg/ml LPS dozunda 0, 24 ve 48. Saatlerde proliferasyonu indüklemiştir. 10 µg/ml LPS-RS 0, 24 ve 48. Saatlerde proliferasyonu inhibe etmiştir. 4 saat 0.1 µg/ml LPS indüksiyonunda MMP9 ekspresyonu 1.7, TIMP1 ekspresyonu 2.3, MMP2 ekspresyonu 1.8 ve VEGFR2 geni 1.4 kat artmıştır. 4 saat 10 µg/ml LPS-RS uygulamasında ise MMP9 ekspresyonu 0.8, TIMP1 ekspresyonu 0.3, MMP2 ekspresyonu 0.5 ve VEGFR2 geni 0.7 kat azaltmıştır. On dört gün boyunca LPS ve LPS-RS ile inkübasyondan sonra koloni oluşturma kapasitelerini belirlemek için kristal viyolet ile boyandıktan sonra 595 nmdeki absorbansları kaydedilmiştir. Kontrol grubunun ortalaması 0.095, LPS grubunda 0.131 ve LPS-RS grubunda 0.081 olarak bulunmuştur. Sonuç olarak TLR4 reseptörünün hem metabolik ativite üzerinde hem de rejeneratif kapasite üzerinde olumlu etkileri bulunmuştur. Klinikte yaygın olarak kullanılan mezenkimal kök hücrelerin verimini arttırmak için bir strateji olarak değerlendirilebilir.

**Anahtar Kelimeler:** TLR4, Kök hücre, rejenerasyon, LPS

## *ORAL PRESENTATION*

# Determination of vitamin C amounts in frequently consumed fruits by HPLC method

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**Abstract**

In order to maintain a healthy life, macronutrients such as proteins, fats, and carbohydrates are required, as well as micronutrients such as vitamins. Since most of the vitamins cannot be made by the body, they must be taken with food. Today, it is seen that there is an increase in the occurrence of many diseases, especially cancer. Therefore, a healthy diet is very important. Especially vitamin C is an essential antioxidant vitamin that strengthens our immune system. With this study, it was aimed to investigate the amount of vitamin C in fruits by stating the vital importance of vitamin C. Tangerine, orange, apple, grapefruit, hawthorn, quince, lemon, pumpkin, pear, banana, grape, and persimmon fruits were used as fruit samples in our study. Standard vitamin C was used for the calculation and comparison of results. Amounts of vitamin C in samples using a high-performance liquid chromatographer and 3.7 mM $KH_2PO_4$ (pH: 4, with $H_3PO_4$), flow rate: 0.4 mL/min, wavelength: Inertsil ODS-4 (5 µm, 4.6×150 mm) at 245 nm The amount of vitamin C (ascorbic acid) was determined using a columnar column. As a result of the study, it was determined that lemon fruit (80.49 ± 3.84 µg/g wet weight) contained the highest amount of vitamin C, and pumpkin fruit (11.63 ± 2.25 µg/g wet weight) had the lowest vitamin C content. Thanks to this study, it is thought that it will contribute to the awareness of the public about both fruits rich in vitamin C and healthy nutrition.

**Keywords:** Vitamin C (Ascorbic acid), Fruits, HPLC.

## *ORAL PRESENTATION*

# Hayvanlarda C-Reaktif Protein

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**Özet**

Akut faz proteinler (AFP) sistemik yangısal cevaba (travmatik, enfektif ve yangısal durumlara) yanıt olarak karaciğerde üretilen proteinlerdir. Farklı fonksiyon ve özelliğe sahiptirler. Günümüzde hayvanlarda teşhiste kullanılan akut faz proteinler arasında haptoglobin (Hp), serum amiloid-A (SAA), C-reaktif protein (CRP), alfa 1 asid glikoprotein (α1-AGP), fibrinojen (Fb), seruloplazmin (Cp), transferin vb. maddeler yer almaktadır. Sağlıklı hayvanlarda önemsiz düzeyde bulunurlarken yangı sırasında artmakta hatta yangı indikatörü olarak rol oynamaktadırlar. Hayvanlarda yapılan araştırmalar CRP'nin viral, fungal, bakteriyel, paraziter hastalıklarda, tümöral olgular hatta kanserde düzeylerinin arttığı ve serum CRP konsantrasyonları ile hastalığın durumu ve tedaviye cevabı arasında önemli korelasyon olduğunu belirtmektedir. Viral hastalıklarda CRP'nin orta düzeyde arttığı, bakteriyel hastalıklarda ise çok daha yüksek seviyelerde olduğu belirtilmektedir. Bu yüzden bakteriyel ve viral hastalıkların ayrımında kullanılabileceği bilgiler arasındadır. Günümüz de yapılan araştırmalar gösteriyor ki akut faz proteinleri veteriner hekimlik alanında hastalıkların teşhis ve prognozunda kullanılmaktadır. Özellikle son zamanlarda yapılan araştırmalar veteriner hekimlik alanında AFP'lerin kullanım alanlarının önemli olduğunu bildirmektedir. Klinik önemi olan bu proteinler hayvan türlerine göre değişiklik gösterdiğinden (kan konsantrasyonları ve önem dereceleri) her hayvan türü için ayrı ayrı değerlendirilmesi gerekmektedir. Bu alanda daha çok araştırma yapılması bu proteinlerin veteriner hekimlik alanında önemini artıracağı açıktır.

**Anahtar Kelimeler:** Akut Faz proteinleri, C- Reaktif protein, Kan

## *ORAL PRESENTATION*

# Veteriner Hekimlikte Serum Biyokimya Testleri ve Önemi

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**Özet**

Serum biyokimyası, serumun kimyasal analizini ifade etmektedir. Analizlerde serum tercih etmenin avantajları vardır. Bunlar arasında daha az hücre kontaminasyonu, bazı parametreler için daha iyi sonuç alınması, antikoagülanların yokluğu, numuneyi serum protein elektroforezi için kullanma imkanı bulunmaktadır. Serumda; proteinler, enzimler, lipidler, hormonlar vb. dahil olmak üzere birçok madde bulunmaktadır. Bu maddelerin analizi vücuttaki organ, dokular ve hayvanın metabolik durumu hakkında bilgi vermektedir. Genel olarak bakıldığında proteinlerden albümin; karaciğer, böbrekler ve sindirim sistemi hakkında, Globülin ise inflamasyon, antikor üretimi ile ilişkilidir. Artan globulin seviyesi bazı kanserler, enfeksiyöz ve bağışıklık ile ilgili hastalıklar ile ilişkilendirilebilir. Karaciğer enzimlerinden olan alanin aminotransferaz (ALT) ve alkalin fosfataz (ALP) spesifik olarak karaciğer hasarı olduğu durumlarda belirgin oranda değişmektedir. Diğer enzimlerden Kreatinin kinaz (CK), kas hasarını değerlendirmek için en sık ölçülen enzimdir. Aspartat aminotransferaz (AST) genellikle kas aktivitesi, ayrıca travma ve kas iltihabı ile ilişkilidir. Bilirubindeki artışlar, artan kırmızı kan hücresi yıkımı veya karaciğerden safra akışının azalması ile ilişkilidir. Böbrek fonksiyonunu değerlendirmek için en yaygın kullanılan kan üre nitrojeni veya BUN, protein yıkım ürünüdür. Üredeki artışlar dehidratasyon, gastrointestinal kanama, kalp hastalığı veya böbrek hastalığını, azalmalar ise aşırı hidrasyon, karaciğer yetmezliği veya protein alımı azalması ile ilişkilidir. Kreatinin seviyesindeki artış, böbrek fonksiyon yetersizliğini ifade eder. Pankreas enzimlerinden, amilaz ve lipazda ki artışlar genellikle pankreas yangıları bunun yanısıra böbrek veya bağırsak hastalığı ile bazı ilaçlar kullanıldığında olmaktadır. Kanda bulunan elektrolitler potasyum, klorür, sodyum ve bikarbonattır. Elektrolitler kan ve doku sıvılarını dengeli durumda tutmaya yardımcı olur. Elektrolitlerdeki bozukluklara genellikle kusma, ishal ve böbrek hastalığı neden olur ve birçok metabolik bozukluğa eşlik ederler. Kolesterol; karaciğerde yağ metabolizmasının bir parçası olarak üretilir. Kolesteroldeki artışlar hormonal ve metabolik hastalıklar, karaciğer hastalığı ve ciddi böbrek hastalığı ile ilişkili olabilir. Özet ile biyokimyasal analiz sonuçları toplu olarak değerlendirildiğinde hastalıklar hakkında önemli bilgiler elde edilebilmektedir.

**Anahtar Kelime:** Kan, Serum, Biyokimya

## *ORAL PRESENTATION*

# *In Vivo* and *in Vitro* Genotoxic Evaluation of Lithium Used in the Treatment of Bipolar Disorder by the Comet Assay

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**Abstract**

Bipolar disorder is a psychological disease that is seen in 3% of the population. Lithium is most commonly used for the treatment of bipolar disorder. It is quite important that lithium, which is indispensable in the treatment, should cause any toxic effects. Some studies have been done to find out lithium's toxic effect. It is determined that a high level of lithium caused biochemical side effects, chromosomal disorder, teratogenic effect and inhibits DNA synthesis. In this study, it is investigated whether lithium causes genotoxic effect (DNA breaks) and it is analyzed using Comet Assay method *in vivo* and *in vitro*. 32 rats were used for *in vivo* studies, blood was collected from 6 volunteers for *in vitro* studies. Comet Assay was performed in blood samples collected from rats in the experimental groups and human lymphocyte culture cells. And also, biochemical and histological experiments were carried out on the samples taken from rats, and cytogenetic experiments were performed on cells taken from cultured lymphocyte cells. Statistical analysis of the data obtained was performed using SPSS 25.0 program. The analysis showed that normal dose of lithium causes neither genotoxic damage nor biochemical and histological side effects in rats. It has been observed that toxic doses of lithium cause some side effects biochemically and histologically, but do not create DNA breaks. Similarly, DNA breaks were not seen in normal and high doses of lithium in the cultured cells but cytogenetic abnormalities were seen in toxic doses of lithium. As a result, it was found that toxic doses of lithium do not cause DNA breaks at a level detectable with Comet Assay, but may lead to cytogenetic anomalies and biochemical and histological side effects.

**Keywords:** Bipolar Disorder, Comet Assay, DNA Break, Genotoxicity, Lithium

## *ORAL PRESENTATION*

# Spreading status of 'Candidatus Phytoplasma' in tomato and grapevine plants in Western Marmara region of Turkey

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**Abstract**

Phytoplasmas are belongs class mollicutes and insect-transmitted plant pathogens. They infect more than 1000 plant species including many important crops and are associated with devastating yield losses worldwide. The increasing threat of phytoplasma related diseases has also started in Turkey with climate change. The lack of successful in vitro cultures, gene delivery and mutagenesis systems have made phytoplasmas one of the least characterized plant pathogens. The aim of this study, detect and diagnose phytoplasmas and determine spreading state of disease in agricultural areas in western marmara region by using conventional PCR and real-time PCR also developt universal detection tools for phytoplasmas. Plant samples were collected from field 2021 summer from Western Marmara Region of Turkey. DNA were extracted by CTAB protocol. Nested PCR and real time PCR assays performed with phytoplasma specific universal primer pairs. Positive samples were subjected to sanger sequencing. Initial analysis showed that phytoplasmas are spreading in tomato and grapvine plants in Western Marmara region of Turkey. Determining the spreading status of phytoplasmas will supports the researchs aiming to plant protection and prevent crop losses due to infection in Turkey.

**Keywords:** Phytoplasma, phytopathology, detection, nested PCR

## *ORAL PRESENTATION*

# Cytotoxic and Antiproliferative Potentials of Ag(I)-BPET on Breast and Ovarian Cancer Cells

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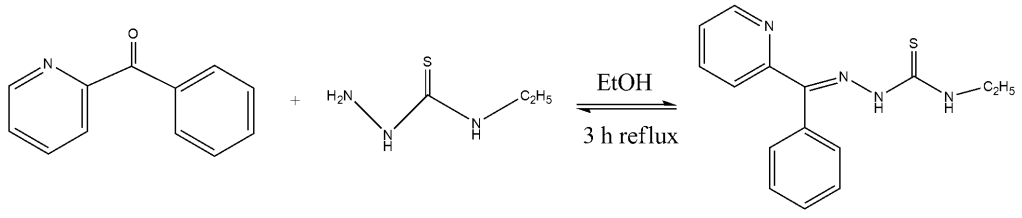
**Abstract**

Thiosemicarbazones' metal complexes play an important role in Inorganic Medicinal Chemistry due to their chelating ability [1]. In this study, 2-benzoylpyridine N(4)-ethyl thiosemicarbazone (BPET) [2] and its Ag(I) complex were synthesized. Their potential anticancer abilities on triple negative breast cancer (MDA-MB-231) and ovarian cancer (A2780) cells were compared to healthy fibroblast (L929) cells. Cytotoxic effects of the compounds were assessed by MTT assay. Survival rates and proliferative abilities of the treated cells were evaluated by colony forming assays. Our findings showed that, Ag(I)-BPET complex had higher cytotoxic and antiproliferative properties compare to BPET ligand against MDA-MB-231 and A2780 cells. Proportionally, the viabilities (%) of the cancer cells were affected more than healthy fibroblast cells. According to the results, it can be debated that BPET and its Ag(I) complex have hopeful anticancer potential due to their strong antiproliferative and cytotoxic effects against these aggressive gynecological cancer cells.

**Keywords:** Thiosemicarbazone, Silver complex, Breast cancer, Ovarian cancer, Colony forming, MTT assay.




2-benzoylpyridine — 4-ethyl-3-thiosemicarbazide — 2-benzoylpyridine N(4)-ethyl thiosemicarbazone (BPET)

**Figure 1**. Synthesis of BPET.

**References**

[1] Ferziz, K.O., Wardel, S.M.S.V., Wardell, J.L., Louro, S.R.W., Beraldo, H., Copper(II) complexes with 2-pyridineformamide-derived thiosemicarbazones: Spectral studies and toxicity against Artemia salina, Spectrochimica Acta Part A 73 (2009) 140–145.

[2] Akgemci, E.G., Bingol, H., Atalay, T., Ersoz, M., Effect of N(4)-substituent groups on transfer of 2-benzoylpyridine thiosemicarbazone derivates at the water/1,2-dichloroethane interface, Electrochimica Acta 53 (2007) 673–679.

## *ORAL PRESENTATION*

# Copper(II) complex of thiosemicarbazone: Synthesis and interaction with DNA/BSA

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**Abstract**

DNA is the primary target molecule for many small molecules, such as drugs, dyes and drug actives, that carries genetic information vital to biological development [1]. In particular, rapid and reliable determination of the interactions of anticancer drug molecules with DNA is very important for drug development studies [2]. Thiosemicarbazones are highly promising compounds in both biology and chemistry that can be extensively studied in vivo and in vitro due to their pharmacological activities. It has been confirmed by researchers that their biological activities increase as a result of their coordination with metals. The pharmacological properties of many metal-based anticancer drugs have been recognized for their DNA and protein binding properties. Studies of the interaction of metal complexes with DNA indicate potential biological activity, which depends on the mode and affinity of binding with DNA. In this study, ECCAT (N-Ethy1-3-carbazole carboxaldehyde-3-thiosemicarbazone) and its copper (II) complex have been synthesized and characterized by $^{1}$H-NMR, $^{13}$C-NMR, FT-IR, and UV-Vis spectroscopic methods. The binding efficiency of ECCAT and Cu(ECCAT)$_2$ with ct-DNA and BSA was investigated by UV–Vis and fluorescence spectroscopic methods. ct-DNA binding results clearly show that the Cu(ECCAT)$_2$ strongly binds with ct-DNA, which is approximately 2.3 times higher than that of ECCAT, having an intercalative binding mode. Similarly, Cu(ECCAT)$_2$ has better BSA binding ability compared to ECCAT.

**Keywords:** Thiosemicarbazone, copper complex, DNA/BSA binding

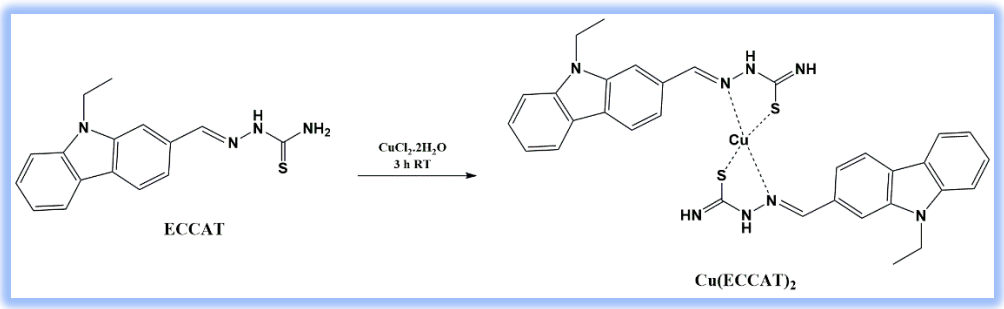


**Figure 1.** Schematic representation of ECCAT and Cu(ECCAT)$_2$

**References**

[1] CE Unsworth, CC Kuo, A Kuzmin, S Khalid, D Saha, Appl. Mater. Interfaces, 12 (38) (2020) 43180–43190.
[2] Y Figueroa-DePaz, K Resendiz-Acevedo, SG Davila-Manzanilla, JC García-Ramos, L Ortiz-Frade, J Serment-Guerrero, L Ruiz-Azuara, J. Inorg. Biochem. 231 (2022) 111772.
[3] L Zarei, Z Asadi, E Samolova, M Dusek, Z Amirghofran, Inorg. Chim. Acta 509 (2020) 119674.

## *ORAL PRESENTATION*

# Bingöl ili bal arılarında (*Apis mellifera*) *Varroa spp.* izolatlarının morfometrik analizi ve moleküler karakterizasyonu

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**Özet**

Ektoparazit olarak yaşamını devam ettiren ve bal arısı akarı olarak bilinen *Varroa* türleri arıcılık sektörünün dünya çapındaki en önemli sorunlarından birisidir. Aynı zamanda dünyadaki birçok bal arısı kolonisinin kaybından sorumlu olan Koloni Çöküş Bozukluğuna katkıda bulunan önemli bir faktör olarak da kabul edilmektedir. Bu çalışma ile Bingöl ili ve ilçelerindeki bal arılarında (*Apis mellifera*) *Varroa spp.*'nin morfometrik analizi ve moleküler karakterizasyonunun belirlenmesi amaçlanmıştır.

Bingöl ili ve ilçelerindeki toplamda 41 farklı arılık ziyaret edilerek 2440 arı kolonisi Varroasis yönünden tesadüfi örnekleme yöntemine göre incelenerek akarlar elde edildi. Her bir arılığı temsilen birer adet ergin *Varroa* akarı alınarak tür düzeyinde morfometrik analizi gerçekleştirildi. Toplamda 41 ergin *Varroa spp*. izolatının altı farklı morfometrik parametresine (vücut uzunluğu, vücut genişliği, genital kalkan uzunluğu, genital kalkan genişliği, anal kalkan uzunluğu ve anal kalkan genişliği) ait uzunluk ölçümleri stereo mikroskop altında gerçekleştirildi. Genetik çeşitliliğin belirlenmesi amacıyla toplamda 82 adet *Varroa* izolatının gDNA izolasyonu yapıldı, mt-CO1 gen bölgesi (376 bp) spesifik primerler kullanılarak PZR ile çoğaltılıp DNA dizi analizi gerçekleştirildi.

Morfometrik analizler sonucunda arılıklar arasında önemli farklılıklar olmaksızın ergin *Varroa spp*. izolatlarının altı farklı morfolojik parametresinin uzunluklarına ait ortalama standart sapma değerleri (±SD) vücut genişliği ve vücut uzunluğu sırasıyla 1710,56±56,83 µm ve 1151,53±39,74 µm olarak belirlendi. Bu veriler bütün izolatların morfometrik olarak *Varroa destructor* olduğunu göstermiştir. Sekans sonuçlarının BLAST analizleri sonucunda tüm izolatlara ait sekans örneklerinin referans sekanslar ile karşılaştırıldığında, *Varroa destructor*'un Kore (K) haplotipi ile benzer olduğu belirlendi. Haplotip analizi neticesinde de *Varroa* akarlarının altı (Hap01-Hap06) haplotipi tespit edildi. Sekansların oluşturduğu haplotip gruplar kendi aralarında düşük nükleotid çeşitlilik sergilediği ve 1-3 arası nükleotid farklılığı veya nokta mutasyon ile birbirinden ayrıldığı ortaya konuldu.

**Anahtar Kelimeler:** Bal Arısı, *Varroa*, PZR, Sekans, Haplotip.

## *ORAL PRESENTATION*

# Altı farklı *Galanthus elwesii* Bitki Ekstresinin *Drosophila melanogaster'* de Larval Mortalite Üzerine Etkisi

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**Özet**

Alternatif tedavi ya da geleneksel tıpta bitkilerle yapılan iyileştirme yöntemleri oldukça önemli bir yeri kapsamaktadır. Bununla birlikte kimyasal ilaçlarla yapılan tedavilerin yan etkileri de gün geçtikçe daha da fazla ortaya çıkmaktadır. Bu nedenle bitkilerin içerik analizleri ve *in vivo* şartlarda etkinliklerinin tespiti oldukça önemli bir hale gelmiştir. Bu bitkilerden birisi olan Kardelen, *Galanthus elwesii* Hook. f. (Amarylidaceae) bitkisinin toprak üstü ve toprak altı kısımları, içerdikleri önemli alkoloidler sayesinde tıbbi açıdan çok önemli yere sahiptir.
Bu çalışmada, kardelen bitkisinin olası toksik veya antitoksik etkisinin *in vivo* şartlarda *Drosophila melonagaster* (meyve sineği) larvalarında araştırılması amaçlanmıştır. Bu amaçla kardelen bitkisinin toprak üstü ve toprak altı kısımlarının su, metanol ve etanol olmak üzere toplam altı ekstresi hazırlanmıştır. *D. melonagaster* Oregon R yabanıl soyunun 72±4 saatlik larvaları bu ekstraktlarla hazırlanmış farklı konsantrasyonlardaki (1 mg/mL; 0,5 mg/mL; 0,25 mg/mL ve 0,125 mg/mL) besinlerle akut olarak beslenmiştir.
Elde edilen verilerden kontrol grubuna göre; konsantrasyon artışına bağlı olarak ergine dönüşen larva sayısında artış, ölüm oranlarında önemli sayıda düşüşün olduğu tespit edilmiştir. Özellikle en etkili sonuçlar toprak altı metanol ekstrelerinde gözlenmiştir. Bu çalışmanın devam uygulamalarında ekstraktların ömür uzunluğu üzerine etkilerine bakılacak, antioksidan etkinlikler araştırılacak ve bitki içerik analizleri yapılacaktır.
Ayrıca çalışmamız, kardelen bitkisi ile *D. melanogaster* model organizması üzerinde yapılan ilk ve tek çalışmadır.

**Anahtar Kelimeler:** *Galanthus elwesii*, Ekstraksiyon, Larval Mortalite, *Drosophila melanogaster*

## *ORAL PRESENTATION*

# Investigation of *Phlomis bruguieri* Desf. (Lamiaceae) from a biofunctional perspective: its antioxidant potential, anticholinesterase, antityrosinase activities

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**Abstract**

The Lamiaceae family is a very large family consisting of about 7200 species, including many well-known, economically and medicinally important plants. Lamiaceae family is the third largest family in Turkey. The family consists of 45 genus and 574 species, 256 of which are endemic in Turkey. It has been noted in many studies that species belonging to the genus have various biological activities and a wide variety of phytochemicals. In the present study, some biological abilities of *Phlomis bruguieri* Desf. such as total phenolic and flavonoid content, antioxidant potential, anticholinesterase, antityrosinase activity were investigated. Ethanol extracts of root and aerial parts of *P. bruguieri* were used in the analyses. It was found that the total phenolic content of the root (55.80±00µg PEs/mg extract) has a higher value than the aerial parts. In terms of total flavonoid content, it was found that the aerial part (51.19±0.70 µg QEs/mg extract) had a higher value than the root. It was determined that the root extract had a better antioxidant potential than the aerial extract in all of the antioxidant activities performed by DPPH free radical scavenging ($IC_{50}$: 33.62±0.57µg/mL), ABTS cation radical scavenging ($IC_{50}$: 17.28±0.36 µg/mL) and CUPRAC methods ($A_{0.5}$: 20.06±0.09 µg/mL). Acetylcholinesterase and butyrylcholinesterase inhibitions associated with Alzheimer's disease were investigated by Ellman method. Extracts of both root and aerial parts of *P. bruguieri* was not active against acetylcholinesterase enzyme, however, the root extract showed activity (Inhibition $IC_{50}$: 316.82±5.27 µg/mL) against butyrylcholinesterase enzyme. Root ($IC_{50}$: 586.14±5.34 µg/mL) and aerial parts ($IC_{50}$: 158.96±1.04 µg/mL) extract of the *P. bruguieri* were found to have an antityrosinase activity. Hence, the results highlighted that *P. bruguieri* has an important potential in pharmacological targets due to its total phenolic and flavonoid content, antioxidant capacity, antibutyrylcholinesterase and antityrosinase enzymes activity.

**Keywords:** Anticholinesterase, antioxidant, antityrosinase, *P. bruguieri*

## *ORAL PRESENTATION*

# Aspir yağının *Drosophila melanogaster* larvalarında $H_2O_2$ ile indüklenen stres koşullarında antitoksik etkisi

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**Özet**

Yağlar temel gıda bileşenlerimizden biri olmak üzere beslenmemizde önemli yer tutar. Uzunca bir dönem yağlı beslenmeden korkulmuş olsa da bu yanlış anlaşılmadan ibarettir. Önemli olan hangi yağ kaynağını tükettiğimizdir. Doymamış yağ asitlerinin sağlığımız üzerindeki olumlu etkileri kanıtlanmıştır, bu sebeple doymamış yağ asitlerinden zengin bitkisel yağlar beslenmemizde önemli yer tutar.
Aspir (*Carthamus tinctorius*) en eski kültür bitkilerindendir. Değerli bir yağ ve enerji bitkisidir. Farklı yöntemlerle tohumdan ayrıştırılan aspir yağı, birçok bitkisel yağa göre üstün özelliklere sahip olup %90-93 oranında doymamış yağ asidi içermektedir.
Bu çalışma ile aspir yağının toksik, antitoksik veya antioksidan etkilerinin belirlenmesi amaçlanmıştır. Bu amaçla beslenme ve toksisite çalışmalarında sıkça kullanılan *Drosophila melanogaster* (meyve sineği) model organizması Oregon R yabanıl soyunun 72±4 saatlik larvaları kullanılmıştır. Yapılan ön çalışmalarla uygulama dozları %0,3125; %0,625; %1,25 ve %2,5 olarak belirlenmiştir. Ayrıca Distile su, askorbik asit ve $H_2O_2$ kontrol grupları oluşturularak ayrı ayrı ve birlikte kullanımın larvalar üzerindeki toksik ya da antitoksik etkinliği araştırılmıştır. Çalışmamız aspir yağı ile *D. melanogaster* üzere yapılan ilk ve tek çalışmadır. Tüm deney setleri üç kez tekrar edilmiştir.
Sonuç olarak Aspir yağının kullanılan konsantrasyonlarda herhangi bir toksik etki yaratmadığı aksine $H_2O_2$ ile kullanıldığında toksik etkiyi azaltıcı etkinlik gösterdiği tespit edilmiştir. Aspir yağının ömür uzunluğu ve antioksidan etkinliği üzerine çalışmalarımız devam etmektedir. Ayrıca aspir yağının içerik analizinin yapılması da planlanmaktadır.

**Anahtar kelimeler:** Aspir yağı, *Drosophila melanogaster,* Antitoksik etki, Larval mortalite,

## *ORAL PRESENTATION*

# Total antioxidant and oxidant status of *Dryopteris raddeana* (Fomin) Fomin

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**Abstract**

Plants are very important among natural products with antioxidant properties. Many studies have shown that plants are important sources of antioxidants. Plants contain many biologically active compounds. These bioactive compounds are responsible for many different activities. In this study, total antioxidant status (TAS), total oxidant status (TOS) and Oxidative stress index of *Dryopteris raddeana* (Fomin) Fomin were determined. TAS, TOS and OSI values Rel Assay kits were used. The ethanol extract of the plant was extracted in a soxhlet apparatus. According to the results obtained, the TAS value of *D. raddeana* was determined as 4.838±0.100, TOS value as 12.208±0.186, and OSI value as 0.253±0.009. In conclusion, in our study, it was determined that *D. raddeana* has antioxidant potential.

**Keywords:**Antioxidant, Fern, medicinal plants, oxidant.

## *ORAL PRESENTATION*

# Otofaji ve retrograd sinyal aktivasyonunun CYC1 geni transkripsiyonuna etkilerinin incelenmesi

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**Özet**

Mitokondri ökaryotik hücrelerde çok çeşitli metabolik olayların gerçekleştirildiği ve kontrol edildiği yarı otonom bir organeldir. Ökaryotik hücrelerde mitokondrinin hem kalitesi ve hem de sayısı sıkı şekilde kontrol edilir. Mitokondri sayısı bölünme ve mitokondrilerin birleşmesi ile dengelenir. İnsanda mitokondri genomundan çok az sayıda protein kodlanır. Mitokondri proteomu yakın zamanda tamamlanmıştır ve ortalama 1100-1400 protein içerir. Mitokondri işlevi için gerekli olan ve nükleustan kodlanan mitokondriyel proteinlerden birisi de sitokrom C1'dir. Bu protein S. cerevisiae nükleusundan CYC1 geni tarafından kodlanır. Sitokrom C1 elektron transport zincirinde yer alır. Mitokondriyel proteinlerin ekspresyonu sıkı şekilde retrograd kontrol mekanizma olarak tanımlanan mekanizma ile kontrol edilir. Retrograd kontrol ökaryotlarda organeller arası koordinasyonun proteomik ve transkriptomik seviyede koordinasyonunu sağlar. Bu çalışmada CYC1 geni transkripsiyonunun mitokondri işlevine bağlı olarak retrograd sinyali ve ayrıca otofaji sinyalleri ile kontrol edilip edilmediği incelendi. CYC1 geni transkripsiyonunun tayininde maya ekspresyon vektörüne klonlanan LacZ gen füzyonu kullanıldı. Normal, standart *S. cerevisae* laboratuvar suşunda yapılan analizlerde CYC1 geni transkripsiyonunun genel otofaji sinyaline yanıt olarak en az 2-kat baskılandığını görüldü. ERAD sinyal yolağının aktivasyonunun da CYC1 transkripsiyonunu 3 kat kadar baskıladığı bulundu. Bu çalışmalara ek olarak, selektif otofajinin de CYC1 transkripsiyonunu önemli seviyede baskıladığı görüldü. Elde edilen sonuçlar CYC1 geni transkripsiyonunun retrograd sinyali ve otofaji aktivasyonunun CYC1 geni transkripsiyonunu CYC1 geni aktivatörü olan HAP4 aracılığı ile kontrol ettiğini göstermektedir.

**Anahtar Kelimeler:** Otofaji, Sitokrom C1, CYC1 geni, Transkripsiyon, *S. cerevisiae*.

Bu araştırma Bursa Uludağ Üniversitesi BAP birimi tarafından desteklenmektedir (Proje no: FHIZ-2022-846)

## *ORAL PRESENTATION*

# Spectrophotometric Determination of Rhodamine B in Various Samples

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**Abstract**

Nowadays, synthetic dyes are frequently used in cosmetics and food products to increase their stability and quality [1]. Rhodamine B, shown in Figure 1, is a basic and cationic, water-soluble xanthene fluorescent dye [2,3]. It has four aromatic benzene rings and a color index of 45,100. IUPAC name; [9-(2-carboxyphenyl)-6-diethylamino-3-xanthenilidene]-diethylammonium chloride [4]. Rhodamine B is a cheap, toxic, and harmful colorant and is used in many industries such as food, cosmetics, plastics and leather [1]. The quantitative determination of this dye, which is toxic to human health, is an important issue. The UV-Vis spectrophotometer is an important device due to its simplicity and low cost compared to other instrumental devices. There are limitations to the determination of rhodamine B by UV-Vis spectrophotometer due to low quantitation limits in the sample. In order to avoid these limitations, sample pretreatments such as solvent extraction, cloud point extraction, solid phase extraction are applied to the samples [5]. In this study, rhodamine B will be determined in various food, paint, cosmetics and beverage samples by UV-Vis spectrophotometer.

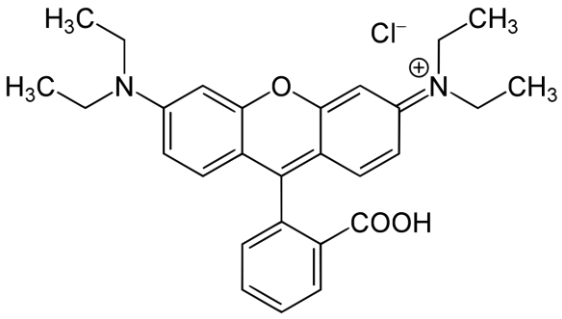


**Fig 1.** Molecular structure of Rhodamine B.

**Keywords:** Rhodamine B, UV-Vis spectrophotometer, synthetic dye, extraction.

**References:**

[1] A.R. Bagheri, M. Ghaedi, Synthesis of chitosan based molecularly imprinted polymer for pipette-tip solid phase extraction of Rhodamine B from chili powder samples, Int J Biol Macromol. 139 (2019) 40–48. https://doi.org/10.1016/j.ijbiomac.2019.07.196.
[2] E. Yilmaz, M. Soylak, A novel and simple deep eutectic solvent based liquid phase microextraction method for rhodamine B in cosmetic products and water samples prior to its spectrophotometric determination, Spectrochim Acta A Mol Biomol Spectrosc. 202 (2018) 81–86. https://doi.org/10.1016/j.saa.2018.04.073.
[3] N. Pourreza, S. Rastegarzadeh, A. Larki, Micelle-mediated cloud point extraction and spectrophotometric determination of rhodamine B using Triton X-100, Talanta. 77 (2008) 733–736. https://doi.org/10.1016/j.talanta.2008.07.031.
[4] A.T. Bişgin, Y. Sürme, M. Uçan, İ. Narin, Simultaneous Preconcentration and Determination of Rhodamine B and Brilliant Blue, Iran J Sci Technol Trans A Sci. 44 (2020) 695–705. https://doi.org/10.1007/s40995-020-00892-6.
[5] M. Soylak, Y.E. Unsal, E. Yilmaz, M. Tuzen, Determination of rhodamine B in soft drink, waste water and lipstick samples after solid phase extraction, Food and Chemical Toxicology. 49 (2011) 1796–1799. https://doi.org/10.1016/j.fct.2011.04.030.

## *ORAL PRESENTATION*

# Tubing Diameter Dependency of Microwave-Fluidic Nanocomposite Synthesis

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**Abstract**

Polymer-metal nanocomposites are preferred over pure polymers due to their improved chemical and physical properties. Microwave-fluidic synthesis (MWFS) of the NCs using a horizontally coiled flow reactor is a novel method with advantages including tuning nanoparticle (NP) size distribution, ease of methodology, rapid processing, low reaction temperatures, high purity, and yield [1]. In the MWFS, the size and distribution of NPs can be tuned by microwave power, irradiation time, flow rate, concentration and type of reactor [2, 3].
As shown in Figure 1 and listed in Table 1, the effect of reactor tubing diameter on silver nanoparticle properties in a polyurethane matrix was studied at a constant residence time and the varying microwave powers.

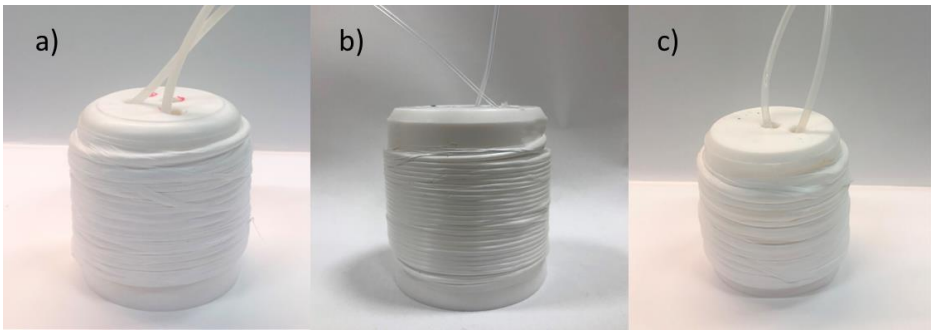


Figure 2. Images of Reactor A (a), Reactor B (b) and Reactor C (c)

Table 1. Specifications of Reactor A, Reactor B and Reactor C

| Reactor | Material | OD (mm) | WT (mm) | ID (mm) |
|---|---|---|---|---|
| Reactor A | PTFE | 3.2 | 0.6 | 2.0 |
| Reactor B | PFA | 1.6 | 0.4 | 0.8 |
| Reactor C | PTFE | 3.2 | 0.4 | 2.4 |

**Keywords:** Microwave-fluidic synthesis, polymer nanocomposites, silver nanoparticles, tubing size, reactor type

**References**

1. Saleem, Q., et al., *Microwave-Promoted Continuous Flow Systems in Nanoparticle Synthesis—A Perspective.* ACS Sustainable Chemistry & Engineering, 2021. **9**(30): p. 9988-10015.
2. Torabfam, M., et al., *A microwave-powered continuous fluidic system for polymer nanocomposite manufacturing: a proof-of-concept study.* Green Chemistry, 2022. **24**(7): p. 2812-2824.
3. Saleem, Q., et al., *Microwave-promoted continuous flow synthesis of thermoplastic polyurethane–silver nanocomposites and their antimicrobial performance.* Reaction Chemistry & Engineering, 2022. **7**(7): p. 1510-1524.

## *ORAL PRESENTATION*

# Ratlarda Imatinib ile Oluşturulan Dalak Toksisitesi Üzerine Apilarnilin Etkilerinin Araştırılması

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**Özet**

İmatinib (İMA), günümüzde kanserle mücadelede sıklıkla kullanılan fakat çeşitli organlarda toksik etkiler oluşturabilen önemli bir kemoterapötik ilaçtır. Apilarnil (APL), erkek arı larvalarının olgunlaşmadan önceki 3-7 günlük larva dönemidir. Hem yumurta hem de larva gövdesinde bulunan besleyici bileşiklerin toplamından dolayı yüksek biyolojik aktiviteye ve önemli antioksidan etkilere sahiptir. Bu çalışma, erkek arı larvası apilarnilin (APL) sıçanlarda imatinib (İMA)' in neden olduğu dalak toksisitesi üzerine etkilerini araştırmak için yapılmıştır.
35 adet wistar albino erkek sıçanlar her grupta 7 sıçan olacak şekilde rastgele 5 gruba ayrıldı. Grup 1 (Kontrol): Sıçanlara 14 gün boyunca gavaj ile mide içerisine 0.5 ml serum fizyolojik verildi. Grup 2 (APL): 14 gün boyunca gavaj ile 400 mg/kg v.a apilarnil serum fizyolojikte çözdürüldü ve uygulandı. Grup 3 (İMA): 14 gün boyunca 100 mg/kg v.a imatinib serum fizyolojikte çözdürüldü ve gavaj ile verildi. Grup 4 (İMA+APL 200): 14 gün boyunca 100 mg/kg v.a imatinib uygulamasına ek olarak, 200 mg/kg APL gavaj ile verildi. Grup 5 (İMA+APL 400): 14 gün 100 mg/kg v.a İMA uygulamasıyla birlikte 400 mg/kg v.a APL gavaj ile uygulandı. Son uygulamalardan 24 saat sonra tüm sıçanlar hafif sevofluran anestezisi altında dekapite edildi ve dalak dokuları çıkarıldı.
Dalak dokusunda İMA grubunda malondialdehit (MDA) seviyelerinde artış, süperoksit dismutaz (SOD) ve glutatyon (GSH) seviyelerinde ise kontrol grubuna göre azalma belirlendi ( $p<0.05$). Tedavi için verilen her iki APL dozunun (200 ve 400 mg/kg), İMA uygulanan ratlarda antioksidan parametrelerde artışa sebep olduğu ve MDA düzeylerini düşürdüğü saptandı ($p<0.05$).
Elde edilen veriler ışığında bu çalışmanın, ilaca bağlı dalak toksisitesinde APL kullanımının yararlı etkiler doğurduğu ve bunları destekler biyolojik kanıtlar ortaya koyduğu tespit edilmiştir.

**Anahtar Kelimeler:** Dalak Toksisitesi, İmatinib, Apilarnil, Oksidatif Stres.

## *ORAL PRESENTATION*

# Evaluation of Oxidative Stress: İmpact of Bromelain in Hyperlipidemic Wistar rats

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**Abstract**

The mechanism of action of Bromelain, a bioactive compound, against changes in oxidative stress parameters in rats with hyperlipidemia model was investigated. For the treatment groups, 18 male Wistar rats were used and three classes were randomly generated. These; A: Healthy Control (SK) Group (No application was made. Group receiving physiological water [2.5 mL/kg, intraperitoneal (i.p)]), B: Tyloxapol (TYX) Group (Physiological water (2.5 mL/kg) , Tylaxopol (400 mg/kg, i.p) group 30 minutes after administration) and C: Tyloxapol + Bromelain (TYX+BRO) Group (Bromelain (250 mg/kg, i.p) 30 minutes before Tyloxapol (400 mg/kg, i.p) administration) kg, o.d) receiving group). One day after the experimental inductions, kidney tissue was collected, homogenized and centrifuged, and MDA, SOD and CAT parameters were measured with commercial kits. TYX caused oxidative stress by increasing MDA levels and decreasing SOD and CAT levels in kidney tissue. With the BRO application, it was determined that the MDA, SOD and CAT levels approached the SK group data and the damages were eliminated.
As a result of the study, it was determined that Bromelain had antioxidant properties by showing a positive effect in Tyloxopol-induced hyperlipidemic rats.

**Keywords:** Bromelain, Hyperlipidemia, Oxidative stress, Tyloxopol, Kidney

## *ORAL PRESENTATION*

# Pathological Effect of Bromelain in a Hyperlipidemic Rat Model

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**Abstract**

In our study, the effect of Bromelain on the histopathological changes in the kidney tissue of hyperlipidemic rats induced by an artificial surfactant, Tyloxopol, was evaluated. For the analysis, 18 Wistar rats (Rattus norvecigus, male) weighing 250±30 g were used. Experimental animals were randomly divided into three groups (n=6) and caged. Group A: Healthy control, Group B; Hyperlipidemic control group (a single dose of Tyloxapol (400 mg/kg, i.p) administered 24 hours before bromelain administration) and Group C: Tyloxapol + Bromelain group (bromelain (250 mg/kg, daily for 18 days) orally for 18 days after 24 hours of bromelain administration) ) were classified as the given group. For histopathological examinations, kidney tissues taken from rats 24 hours after the applications were taken into the pathological process and made ready for microscopic examination. While no pathological findings were observed in the healthy control group in the imaging process, pathological changes such as tubular dilatation, congestion, bleeding, mononuclear cell infiltration, apoptosis and necrosis in the cells were observed in the hyperlipidemia group. The pathological findings that emerged with the application of bromelain were reduced by the effect of bromelain.
As a result of the examinations, it has been clearly stated that bromelain has a protective effect against the damage to the kidney tissue caused by hyperlipidemia.

**Keywords:** Bromelain, Tyloxopol, Hyperlipidemia, Histopathology, Kidney

## *ORAL PRESENTATION*

# Evaluation of pomegranate peel extract and its thermosensitive gel formulation for the treatment of oral infections

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**Abstract**

Oral infections are often seen due to many bacteria, viruses, and fungi in the oral cavity. For the treatment of these infections, various methods have been developed for the administration of drugs. One of these methods is the use of thermosensitive gels. With thermosensitive gels, which are liquid at room temperature and gel at intraoral temperature, the drug can be administered easily and can stay in the application site for a longer time. On the other hand, pomegranate peels are rich in phenols and have been shown to have antibacterial, antifungal, and antiviral activity with their high polyphenol content. Its extract has the potential to be a remarkable alternative in the treatment of oral infections. In this study, it was aimed to obtain the extract of pomegranate peel and to investigate the antimicrobial activity of the thermosensitive gel formulation prepared with the obtained extract. For this purpose, the ethanol extract of the pomegranate peel was obtained, and the gel form were prepared, then the antibacterial and anti-fungal activity of these products was scanned. Thermosensitive gel formulation of pomegranate peel extract was prepared with poloxamer 407, and it was determined that even though there was a partial decrease in the effectiveness against the solution group, the antimicrobial activity was also provided via thermosensitive gel formulation. The results of this study agreed that the suggested gel formulation obtained in this study can be used to support oral health.

**Keywords:** Pomegranate peel, extraction, thermosensitive gel, oral infections

Acknowledgment: This study was supported by Bilecik Seyh Edebali University Scientific Research Project Coordination (Project number: 2022-01.BŞEÜ.12-02).

## *ORAL PRESENTATION*

# Trace Lead Determination in Urine Samples by Using Flame And Furnace Atomic Absorption Spectrometry After Its Preconcentration With Dispersive Liquid Phase Microextraction

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**Abstract**

Lead is a toxic element that is widely used in the industry and is taken into the body mainly through the air (gasoline, cigarette, etc.), water, food and drinks, dust, pellets, in utero transmission from mother to baby, medicines and cosmetic products, and contact with skin. Lead is of clinical importance due to its effects on blood and nerve cells. The amount of lead in biological samples is very low. Therefore, reliable high-precision techniques are needed for the determination of this element. The most common methods used for the determination of lead are atomic absorption spectrometry (AAS). Atomization in AAS can be done with a flame (flame atomic absorption spectrometer - FAAS) or an electrothermal (graphite furnace atomic absorption spectrometer - GFAAS) source.
In these studies, dispersive liquid-liquid microextraction methods were developed to preconcentrate lead after complexing with dithizone and determined by flame and furnace atomic absorption spectrometry.
These methods are more environmentally friendly because they use fewer organic solvents than traditional extraction methods.
The detection limits of the developed methods determined from the calibration graphs were found to be 1.7 µg L-1 for FAAS (linear range: 3.4 – 250 µg L-1) and 0.1 ng mL-1 for GFAAS (linear range: 0.2 – 4.0 ng mL-1) under the optimum conditions. The precision and accuracy of the developed method provided satisfactory lead recovery results between 98-107% for FAAS and 94.6-112% for GFAAS and a low relative standard deviation for urine samples.

**Keywords:** Lead, Flame atomic absorption spectrometer, Graphite furnace atomic absorption spectrometer, Dispersive liquid phase microextraction, Urine.

## *ORAL PRESENTATION*

# Nanoflowers in the determination of different pesticides and their removal from wastewater

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**Abstract**

Determination of pesticides at low levels in different matrices has become increasingly important due to their worldwide use and negative health effects. Pesticide residues have contaminated the environment such as air, soil, and water causing serious problems such as urological, respiratory/neurological diseases and several cancer types, by accumulating after entering the human body through respiration, contact and digestion. Since determination of pesticides at trace levels is vital, different sample preparation strategies based on liquid-liquid microextraction and solid phase microextraction were developed for separation/preconcentration of the target analyte(s) from the matrix. The enrichment of the target analyte not only reduces the detection limits, but also eliminates the interference effects from the matrix. Recently, nanoparticles have been played an important role in the determination of pesticides at trace levels and their removal from different matrices. Nanoflowers, a member of the nanoparticle class, are innovative adsorbents known for having a flower or petal structure. In addition to its large surface area, it attracts attention in pesticide determination and removal with its high adsorption capacities. In this study, the effects of different nanoflower structures on pesticide determination and removal were investigated.

**Keywords:** Extraction, nanoparticles, nanoflowers, pesticide.

## *ORAL PRESENTATION*

# Sensitive and Accurate Determination of Urinary Lead at Trace Levels by Slotted Quartz Tube-Flame Atomic Absorption Spectrometry

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**Abstract**

Some heavy metals such as lead can accumulate in the metabolism of living organisms and show toxic effects if they exceed a certain concentration. This makes it important to develop an accurate, reliable, easy, and less costly analysis method for the determination of trace amounts of lead in biological fluids or tissues. Flame Atomic Absorption Spectrophotometry (FAAS) device, determination limits are insufficient for the determination of trace levels of metals. For this purpose, the slotted quartz tube technique (SQT) was used to improve the analytical performance of the AAS system. After the precursorisation, determinations were carried out by increasing the interaction between light and atoms with the help of a slotted quartz tube placed in the flame head of the flame atomic absorption spectrometer with the help of an apparatus. In order to determine the optimum conditions, important analytical parameters such as pH of the medium, buffer amount, shaker type and duration, temperature and duration, eluent volume, and matrix (interference) effect were screened and optimized. Analytical variables such as the limit of detection (LOD), the limit of quantification (LOQ), and the linear operating range of the method were determined under the optimized conditions. Under the optimum conditions, the detection power of FAAS was increased 305-fold using DLLME-SQT-FAAS. The detection limit for the DLLME-SQT-FAAS system was 0.5 µg L-1. The applicability of the developed method was confirmed by spiking measurements in real urine samples. The percentage recovery values for these were 98.2% and 107.0%, respectively.

**Keywords:** Slotted quartz tube technique, Lead, Flame atomic absorption spectrometer, Dispersive liquid phase microextraction, Urine.

## *ORAL PRESENTATION*

# Ardahan İli Covid-19 dönemi Kreatin Kinaz değerlerinin retrospektif değerlendirilmesi

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**Özet**

Kreatin kinaz enzimi dimerik yapıda bulunan bir enzimdir. Bu enzim klinikte miyozit tayini amacıyla kullanılmaktadır. Kreatin kinaz enzimi ve miyozit ilişkisi miyokard enfarktüs açısından da önem arz etmektedir. Bu çalışmanın amacı kardiyolojik tanısı olmayan bireylerde Covid-19 dönemi öncesi ve sonrasında kreatin kinaz değerleri değişiminin araştırılmasıdır.

Çalışmanın örneklemini ülkemizde ilk Covid-19 vakasının görüldüğü dönem olan Mart 2020 öncesi ve sonrasında Ardahan ili devlet hastanesine gelen, kardiyak tanı konulmamış ancak her iki dönemde de kreatin kinaz testi yaptırmış kişiler oluşturmaktadır. Bu kişiler arasından rastgele 47 kişi seçilmiş olup kreatin kinaz değerleri her iki dönem için istatistiksel olarak karşılaştırılmıştır.

Covid-19 öncesi ve sonrası kreatin kinaz enzimi seviyeleri incelenen bireylerin 29'unda (%61,7) Covid-19 öncesine göre kreatin kinaz değerlerinde artış olduğu gözlemlendi. Tüm örneklemin ortalaması her iki dönem için istatistiksel olarak karşılaştırıldığında Covid-19 sonrası dönem sonuçlarının daha yüksek olduğu belirlendi ($P<0,05$)

Sonuç olarak Covid-19'un hayatımıza girmesiyle beraber viral enfeksiyon kaynaklı, kullanılan ilaçların yan etkileri ve hastalık dönemi stresinin sağlıklı bireyleri etkilediği kanaatine ulaştık. Bu sebepten dolayı risk grubunda olmayan bireylerin dahi Covid-19 dönemi sonrasında kardiyolojik olarak düzenli kontrollerinin sağlanması önem arz etmektedir.

**Anahtar Kelimeler:** Kreatin kinaz, Covid-19, Kardiyak belirteç, Retrospektif, Ardahan

## *ORAL PRESENTATION*

# Ardahan ilinde Covid-19 dönemi öncesi ve sonrasında hastaların ALT-AST değerlerinin retrospektif karşılaştırılması

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**Özet**

Karaciğer fonksiyon testleri olarak da bilinen alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) enzimleri karaciğer hasarı hakkında bilgi vermektedir. Bu çalışmanın amacı Covid-19 dönemi öncesinde ve sonrasında bireylerin ALT ve AST değerlerinin değişiminin araştırılmasıdır.

Çalışmanın örneklemini Covid-19 vakasının ülkemizde ilk olarak gözlemlendiği tarih olan Mart 2020 öncesinde ve sonrasında Ardahan devlet hastanesine başvuran ayrıca her iki başvurusunda da ALT-AST düzeyi ölçümü yapılmış bireyler oluşturmaktadır. Bu bireyler arasından rastgele 40 yaş üstü 96 örnek seçilmiş olup bu örneklerin ALT ve AST verileri Covid-19 öncesi ve sonrası dönem değerleri istatistiksel olarak karşılaştırılmıştır.

Covid-19 öncesi ve sonrası ALT ve AST enzim değerleri incelenen 68 bireyin (%70,8) Covid-19 öncesi döneme göre ALT ve AST seviyelerinde artış olduğu belirlendi. İki dönem için istatistiksel olarak tüm örneklerin ortalaması kıyaslandığında Covid-19 sonrasındaki dönemde ALT ve AST sonuçlarının Covid-19 öncesi döneme göre daha yüksek seviyede olduğu gözlemlendi ($P<0,05$)

Sonuç olarak insan hayatında yeni bir dönem olan Covid-19 pandemi etkisini kaybetse de devam etmektedir. Bu etkiler hastalık dönemi stresi, kullanılan ilaçların yan etkileri ve Covid-19 enfeksiyonunun bıraktığı kalıcı hasarlardır. Bu sonuçlardan dolayı Covid-19 dönemi sonrasında karaciğer kontrollerinin yapılması ve sağlıklı yaşama yönelim büyük önem arz etmektedir.

**Anahtar Kelimeler:** ALT, AST, Covid-19, Karaciğer hasarı, Retrospektif, Ardahan

## *ORAL PRESENTATION*

# Exposure to oxidative stress may increase mastitis susceptibility by affecting activation of the NF-κB/MAPK signaling pathway in dairy cows

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**Abstract**

This review study aimed to explain how exposure to oxidative stress affects mastitis susceptibility by altering the activation of the nuclear factor-kappa B (NF-κB) / mitogen-activated protein kinase (MAPK) signaling pathway in dairy cows. One of the most important production problems of dairy cows is mastitis. Mastitis, which is defined as the inflammation of the mammary gland against various factors, mostly occurs during the early lactation period in cows. It appears in clinical or subclinical forms and its major cause is bacteria. It is of great economic importance to keep its incidence to a minimum and to recover the animals with mastitis as quickly as possible. It is stated that the main initiative in combating mastitis is a successful management strategy in terms of udder health and keeping the redox balance of cows at an optimum level. Oxidative stress occurs when the redox balance is disturbed due to various reasons, that is, when antioxidants decrease and oxidant substances increase. Oxisan substances can affect the body's immune function in many ways. In the case of oxidative stress, the secretion of some inflammatory cytokines (such as TNF-α, IL-1β, and IL-6) due to ROS accumulation can be stimulated, and these cytokines accumulate. This situation may result in further activate the Nuclear factor-Kappa B NF-κB/MAPK signaling pathway. In other words, cytokines released by oxidative damaged cells increase NF-κB/MAPK pathway activation and cause an expand the inflammatory response, which may predispose to the formation of mastitis. In conclusion, the NF-κB/MAPK signaling pathway normally has important regulatory effects on inflammation. However, oxidative stress causes significant changes in NF-κB/MAPK signaling pathway activation and thus can promote the formation of mastitis in dairy cows. Moreover, it is thought that activation of NF-κB/MAPK signaling pathways can be regulated by antioxidant administrations.

**Keywords:** Cow; mastitis; NF-κB/MAPK; oxidative stress.

## *ORAL PRESENTATION*

# Investigation of the protective effect of Quercetin supplementation against Cyclophosphamide-induced ovarian toxicity in mice

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**Abatract**

The aim of this study was to investigate the protective effect of quercetin supplementation against cyclophosphamide-induced ovarian toxicity in mice. The study was conducted on a total of 24 healthy mice, and the animals were assigned to four groups: control (C; n=6), sham (S; n=6), cyclophosphamide (CP; n=6) and cyclophosphamide + quercetin (CPQ; n=6) groups. While no application was made to the C group, dimethyl sulfoxide (DMSO) was administered orally at a volume of 0.5 ml/day for 14 days in the S group. Mice in the CP and CPQ groups received cyclophosphamide at a dose of 200mg/kg intraperitoinally twice with one week intervals. In addition, mice in the CPQ group were administered orally at a dose of 50 mg/kg/day of quercetin dissolved in a volume of 0.5 ml of DMSO for 14 days. Serum total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) levels, ovarian follicle counts and, Bcl-2-associated-X-protein (Bax) and caspase 3 expression levels of ovarian were evaluated. TAS levels decreased ($p<0.05$) in the CP group compared to the other groups, but TOS ($p<0.05$) and OSI ($p<0.01$) levels increased. While the number of primordial follicles decreased significantly in the CP ($p<0.01$) and CPQ ($p<0.05$) groups compared to the C and S groups, it increased in the CPQ group compared to the CP group ($p<0.01$). The number of pirimear ($p<0.05$), secondary and antral ($p<0.01$) follicles decreased in the CP group compared to the other groups, however the number of atretic follicles increased significantly ($p<0.01$). Bax expression level increased in CP group according to C and S groups ($p<0.01$), while it decreased in the CPQ group compared to the CP group ($p<0.05$). Caspase 3 expression level was significantly higher in CP group compared to the other groups ($P<0.01$). In conclusion, cyclosphamide led to pathological consequences in oxidative status and the ovary. However, treatment with quercetin had positive effects on oxidative stress status and ovarian histodynamics in mice with Cyclophosphamide-induced ovarian toxicity.

**Keywords:** Cyclophosphamide; mice; ovarian toxicity; oxidative stress; Quercetin.

**Acknowledgement:** Ethical approval for this study was obtained from Experimental Animal Ethics Committee of Adana Veterinary Control Institute (Approval no: 2022-3/310).

## *ORAL PRESENTATION*

# MCF-7 meme kanseri hücre dizisinde Paklitaksel ile Oleuropein kombinasyonunun SOD, GSH ve lipid peroksidasyonu üzerine olan etkisi

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**Özet**

Meme kanseri, kadınlar arasında yaygın olarak görülen ve tüm dünyayı ilgilendiren bir sağlık sorunu haline gelmiştir. Paklitaksel (PTX), meme, yumurtalık, akciğer gibi çeşitli kanser türlerinin tedavisinde kullanılan bir kemoterapötik ajandır. Oleuropein (OLE), zeytin ve zeytin ürünlerinde farklı konsantrasyonlarda bulunan antioksidan, antikanser, antidiyabetik etkilere sahip olduğu bilinen bir fenolik bileşiktir. Ancak OLE ve PTX kombinasyonunun MCF-7 hücreleri üzerindeki etkisini gösteren bir çalışma bulunmamaktadır. Bu çalışma OLE, PTX ve kombinasyonlarının MCF-7 meme kanseri hücre hattı üzerindeki oksidatif strese olan etkilerini ve PTX'in hücreler üzerinde oluşabilecek olası toksik etki dozunu azaltmayı amaçlamaktadır. Bu amaç doğrultusunda xCELLigence RTCA yöntemi ile OLE ve PTX'in sitotoksik etkileri belirlenmiştir. Ölçüm sonucunda $IC_{50}$ dozlarının sırasıyla 230 µM ve 7,5 µM olduğu saptanmıştır. Elde edilen $IC_{50}$ dozlarından farklı yüzdelerde kombinasyon oranları oluşturulmuştur. CompuSyn programı kullanılarak sinerjik ve antagonistik etki durumları belirlenmiştir. %10 (23 µM OLE+0,75 µM PTX), %20 (46 µM OLE+1,5 µM PTX), %30 (69 µM OLE+ 2,25 µM PTX) ve %40 (92 µM OLE+ 3 µM PTX) kombinasyon oranlarının sinerjik etkili olduğu saptanmıştır. Daha sonra OLE, PTX $IC_{50}$ dozları ve sinerjik etkili kombinasyon gruplarında oksidatif stres belirteçleri olan glutatyon (GSH) ve malondialdehit (MDA) düzeylerinin ve süperoksit dismutaz (SOD) aktivitesinin ölçümü yapılmıştır. Kombinasyon gruplarında (%10, %20, %30, %40) SOD aktivitesi kontrole göre (0,733±0,09) sırasıyla 1,132±0,13; 1,542±0,29; 1,445±0,22; 1,219±0,15; GSH düzeyi kontrole göre (10,413±1,43) sırasıyla 5,123±1,24; 6,679±2,57; 6,255±1,71; 5,128±2,26; MDA düzeyi kontrole göre (0,625±0,12) sırasıyla 3,682±0,39; 2,708±0,45; 1,875±0,12; 3,165±0,20 olarak ölçülmüştür. Sonuç olarak, OLE ve PTX'in kombinasyon halinde uygulanmasının PTX toksik dozunu düşürdüğü saptanmıştır. Tek başına OLE ve PTX $IC_{50}$ dozu uygulanan deney gruplarına göre kombinasyon gruplarındaki SOD aktivitesinin arttığı ve GSH düzeyinin düştüğü saptanmıştır. Ayrıca kombinasyon gruplarının MDA düzeyini arttırdığı saptanmıştır.

**Anahtar Kelimeler:** Meme kanseri, Oleuropein, Paklitaksel, Oksidatif stres

Bu çalışma Anadolu Üniversitesi BAP komisyonu tarafından 2010S105 no.lu proje kapsamında desteklenmiştir.

## *ORAL PRESENTATION*

# Morphological Investigation of Zinc Coated Working Electrode Obtained From Choline Chloride Based Non-Aqueous Solvent with Inorganic and Organic Additives

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**Abstract**

Zinc coating plays an important role in different industries which demanding resistance to corrosion. Obtaining zinc on the particular substrates has examined to enhance the performance of the final material not only in terms of corrosion resistance but also for alloying or esthetical purposes. While there are diverse techniques to coat zinc, such as physical vapor deposition, immersion coating, electroplating process maintains its forefront position due to low cost and ease of operation. Aqueous solvents based on cyanide, sulfate or acidic solutions are mainly prepared for electrochemical deposition because of the comparatively electronegative nature of zinc among other metals. Zinc electroplating process generally requires more electronegative potentials than -1V (vs reference electrode in three electrode system), and therefore shows a tendency of hydrogen evolution which may later result in hydrogen embrittlement in the final deposition. In the last 20 years, various types of non-aqueous solvents have gained attention to eliminate both the drawbacks of aqueous solutions and produce relatively green solutions. One of the literature dominated non-aqueous solvents is termed as deep eutectic solvents, which are accepted with their low toxicity, vapour pressure, cost, non-flammability, and ease of preparation. In this research, cyclic voltammetry data were merged with a high resolution optical microscope images to investigate the effect of different additives both in the class of organic and inorganic on zinc reduction performed in non-aqueous chloride based solvents.

**Keywords:** Cyclic voltammetry, electrodeposition, additive-morphology effect

## *ORAL PRESENTATION*

# Türk kömürü sıvılaştırma sürecinde demineralize edilmiş kömürlerin kinetik mekanizmasının kalman filtreleme metodu yardımıyla modellenmesi

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**Özet**

Bu çalışmada sıvılaştırma sırasında kömürün ağırlık kaybı için reaksiyon hızı parametrelerinin hesaplanmasını sağlayan matematiksel bir model formüle edilmiştir. Mikrodalga enerjisi ile kömür sıvılaştırma verimlerinin incelendiği deneysel çalışmalardan elde edilen veriler model algoritmasına girdi teşkil etmiştir. Demineralize edilmiş kömürlerin Mikrodalga enerjisi kullanılarak sıvılaştırılma verimlerinin incelendiği deneysel çalışmaların kinetik modelinin belirlenmesi bu çalışmanın temel önceliği olarak belirlenmiştir. Bu nedenle bilgisayar programı yardımı ile oluşturulan algoritmanın deneysel sonuçlar ile uyumluluğunun belirlenmesi amacıyla MatLab tabanlı olarak geliştirilen ve Kalman Filtreleme yöntemi kullanılarak oluşturulan algoritma ile önerilen dört farklı kinetik mekanizmanın deneysel çalışmaları ne kadar uyumladığı incelenmiştir. Yapılan çalışmalarda deneysel sonuçlardan elde edilen veriler ile uyumlu en yakın kinetik model çıkarılmıştır. Programdan elde edilen verilerde en uyumlu kinetik model için reaksiyon hız sabitleri de belirlenmiştir. Program kapsamında deneysel veriler ve algoritma sonuçlarının karşılaştırılması ile oluşturulan algoritmanın kömür sıvılaştırma mekanizmasını belirlediği ispatlanmıştır. Deneysel çalışmalardan elde edilen verilerin değerlendirmesinde kömür sıvılaştırma mekanizmasının uyum sağladığı model göz önüne alındığında, sürecin çoklu paralel, bağımsız, tersinir ve tersinmez reaksiyonlar eşliğinde, pseudo birinci dereceden reaksiyonlar aracılığıyla ilerlediği kabul edilmiştir.

**Anahtar Kelimeler:** Demineralize kömür, kömür sıvılaştırma, kömür sıvılaştırma modelleme, kinetik model, Kalman Filtre Yöntemi

## *ORAL PRESENTATION*

# Alzheimer hastalığının tedavisine yönelik bitkisel içerikli ilaç etken madde bağlı grafen oksit nanomalzeme sentezi

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**Özet**

Alzheimer hastalığı beyin hücrelerine zarar veren ve demans olarak da adlandırılan kalıcı hafıza kaybına yol açan ilerleyici bir nörodejeneratif hastalıktır. Günümüzde Alzheimer hastalığı tedavisinde kullanılan geleneksel farmakolojik ilişkili ilişkili çeşitli yan etkiler mevcuttur. Bu bağlamda bitkisel içerikli ürünler daha iyi alternatif sağlamakta ve daha az istenmeyen yan etki oluşturmaları nedeniyle önemli bir tercih sebebi sayılmaktadır. Huperzin A (Hup A) Huperzia serrata bitkisinden türetilen doğal bir asetilkolinesteraz (AChE) inhibitörüdür. Son yıllarda yapılan çalışmalarda Hup A'nın çok yönlü farmakolojik etkileri olduğu gösterilmiştir. Bu çalışmada, Alzheimer hastalığı tedavisinde potansiyel olarak kullanılması öngörülen bitkisel içerikli ilaç etken madde bağlı grafen oksit nanomalzeme sentezi ve optimizasyon koşullarının belirlenmesi amaçlanmaktadır. Çalışmamızda, fonksiyonel grupların çeşitliliği ve bolluğu, konjugasyon sistemi, geniş yüzey alanı, düşük sitotoksik etkisi ve düşük maliyet özellikleriyle ilaç yükleme ve dağıtımında önemli bir potansiyele yol açan grafen oksit (GO) nanomalzemesi, modifiye edilmiş Hummers metodu ile sentezlenmiştir. Sentezlenen grafen oksit türevlendirilip histidin (His) ile graftlama yapılmıştır. GO graft - His nanomalzemenin karakterizasyonu Fourier Dönüşümlü Kızılötesi Spektrometre (FTIR) ve Taramalı Elektron Mikroskop – Enerji Dispersiv Spektrum (SEM-EDS) analiz yöntemleri ile gerçekleştirilmiştir Hazırlanan GO graft – His ve ilaç etken madde Huperzine A Ultraviyole Spektrofotometre ile 313 nm'de bağlama çalışmaları gerçekleştirilmiştir. Çalışma konsantrasyon, zaman, pH ve sıcaklık parametreleri ile optimize edilmiştir. Karakterizasyon çalışmaları sonucunda GO nanomalzemelerinin tabakalı halindeki grafen oksit yapısının literatürle uyumlu katman ve dağınık halde üst üste binmiş GO yapısının olduğu ve GO-His yapısının oluştuğu gözlenmiştir. Hup A'nın nanomalzemeye optimum adsorpsiyon koşulları incelendiğinde; 37℃'de, 60 dakika süresinde, pH 7.4'te, 0.5 mg/mL Hup A konsantrasyonunda maksimum adsorbsiyon miktarı 158.4175 mg/g olarak bulunmuştur. Bu çalışma ileri aşamada planlanan ve Alzheimer hastalığının tedavisine yönelik terapötik yaklaşımlarla daha kolay uygulanabilecek ilaçların üretilmesi için yapılan çalışmalara taban oluşturacaktır.

**Anahtar Kelimeler:** Alzheimer, Grafen Oksit, Huperzin A, Hummers Metodu,

## *ORAL PRESENTATION*

# Novel carbonic anhydrase enzyme inhibitors; design, synthesis, and prediction of biological activities of indole derivatives

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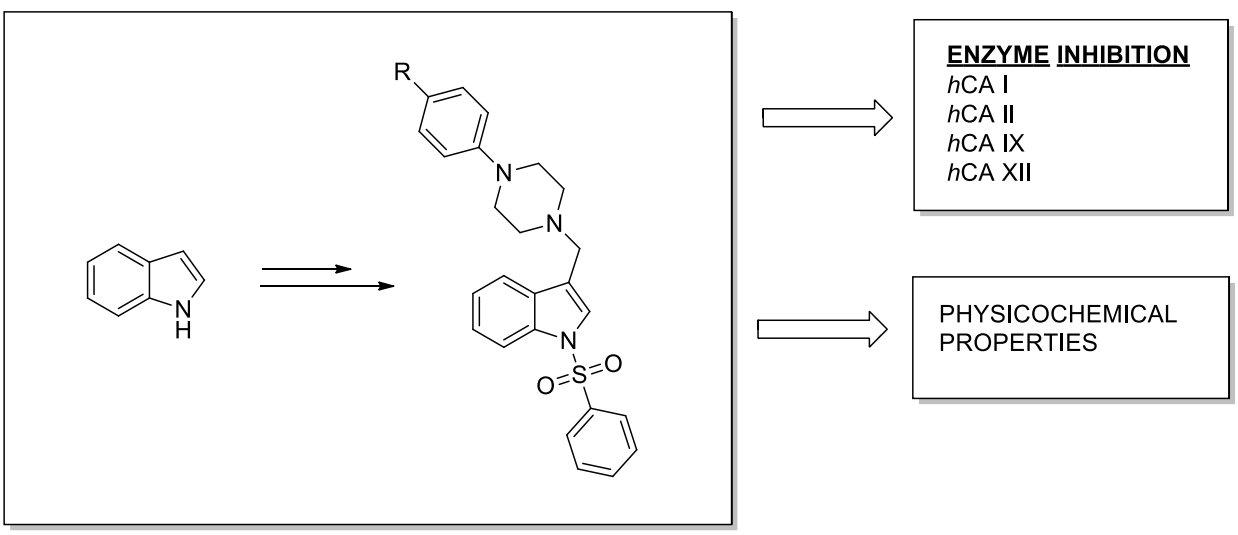
**Abstract**

Cancer is one of the most researched diseases in the world for its treatment. Many different pathways play a role in stopping cancer and killing cancerous cells. Carbonic Anhydrase (CA) enzyme is one of the important enzyme family responsible for various biological processes in living things. Although there are many isoforms of this enzyme family, CA IX and CA XII isoforms are effective on tumors. They are responsible for regulating the tumor microenvironment and adjusting the pH of the tumor environment. In addition, the CA IX isoform is over-expressed in solid tumors, and conversely, low-expressed in healthy tissues. For this reason, these two isoforms are becoming an important target in cancer treatment. Reduction (inhibition) of CA IX/XII expression affects the pH of the tumor environment, resulting in reduced tumor cell survival and proliferation. The aim of this study is to design, synthesize and prediction of the biological activities of CA IX and XII inhibitors. In new targeted molecules, the indole molecule will be used as the main structure. Position 1 of the indole structure will be derivatized with the sulfonyl group responsible for the activity in many CA inhibitors. Then, the carbonic anhydrase enzyme inhibition effect will be evaluated by binding different piperazine derivatives to the 3 positions.

**Keywords:** Carbonic anhydrase enzyme, cancer, indole, piperazine.



## *ORAL PRESENTATION*

# Effect of Aluminum on Biodegradable Zinc-Silver Alloys

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**Abstract**

Zinc (Zn) alloys have been a frequently researched area for biomaterial production due to their high biocompatibility and biodegradability properties. Zn is not suitable for use in this field due to its low mechanical and biocompatibility properties. Therefore, it is possible to control the mechanical properties, biodegradation behaviors and cell biocompatibility at the targeted values by alloying. In this study, Zn-Ag-Al alloy system is studied for urological applications. After Zn-based biodegradable implants are applied, the need for a secondary surgery will degrade spontaneously in the body. While some of the degradation products can be excreted by urinary tract excretion, some of them can be metabolized in the body. In this way, it will benefit the patient both economically and psychologically.

In this study, the Zn-Ag-xAl alloy system will be studied and the alloys will be produced by melting under vacuum and then homogenization. The microstructure and hardness of the alloys will be characterized by in vitro biodegradation behavior and cell biocompatibility studies. In this context, the microstructure, mechanical properties, degradation behavior of the alloys in artificial body fluids under in vitro conditions and cell biocompatibility were studied.

**Keywords:** Zinc, Biodegradability, Stent, Biocompatibility

## *ORAL PRESENTATION*

# Assessment of Lydersen Method in Estimation of Critical Properties of Various Alcohols

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**Abstract**

Critical properties of chemical compounds are essential in designing and operating chemical industrial operations. Obtainment of these properties experimentally requires extreme conditions that can be unattainable. The applicability of such experiments is limited due to the high consumption of time, and energy. Over the past years, many scientists have worked on developing methods to predict critical properties without the need for experimental work. In terms of simplicity, one of the best methods developed is known as the Lydersen method. Lydersen method uses the group contribution method to predict critical properties depending on the functional groups contained in each compound. However, some concerns about the accuracy of this method arose from the growing demand for highly accurate data. In this study, the performance of the Lydersen method was investigated by evaluating the group contributions of 60 alcohol samples. The results were compared to the experimental critical properties. (RMSE, $R^2$) pairs for the estimation of critical temperature, critical pressure, and critical volume by using the Lydersen method were calculated as (13.3030, 0.9635), (4.8234, 0.8381), and (19.0390, 0.9757), respectively. The results clearly show that while the Lydersen method provides appropriate estimates for the critical properties, there is still room for improvement, and advanced modeling applications for specific material groups should be investigated further in order to obtain more satisfactory critical property estimations.

**Keywords:** Critical properties, Lydersen method, Group contribution method.

## *ORAL PRESENTATION*

# Biomass-lignite gasification; analysis of liquid by-products

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**Abstract**

The previous step of gasification can be considered as pyrolysis, and the biomass liquid obtained from pyrolysis is also very valuable in terms of raw material and energy content, and it is very important to clarify the content of these liquids. In this study, the structure of the liquid by-products obtained from the gasification of the lignite and biomass mixture at 700 °C was characterized. In addition, pyrolysis conversion amount, liquid solid and gaseous product amount were also calculated in the experiments. 5 g of biomass-lignite mixture (four different mixture) was carried out at 700 ºC, 400 ºC/min heating rate and 100 $cm^3$/min Argon (Ar) flow rate. The characterization results of the biomass liquid obtained from these experiments was achieved. The highest liquid product yield was determined as 45% by weight, and it was acquired at 700 °C temperature, 100 $cm^3$/min Ar, flow rate, 1:1 biomass-lignite mixture and 400 °C/min heating rate. According to the data obtained from the elemental analysis results of liquid products, the H/C molar ratios of the pyrolysis liquids from two separate experiments are higher than the other liquid products with 0.56 and 0.54. The fact that the H/C molar ratio is closer to 1 has a significant positive effect on the potential of using liquid products as fuel. When the GC (gas chromatography) results in the experiments are examined; guiasol, cresol, furfural and ethyl pyruvate compounds seem to come to the fore. Related substances are chemicals and synthesis intermediates used extensively in the pharmaceutical industry, cosmetics, industrial personal care products, and the food industry. The present functional group data (ester, ketone, aldehyde, alcohol, methyl, phenol, carboxylic acid, aromatic) in the FT-IR results of the pyrolysis liquids obtained from the experiments are compatible with the GC results.

**Keywords :** Pyrolysis, Gasification, Liquid by-product

## *ORAL PRESENTATION*

# Kolşisin'in spektroskopik olarak DNA ile etkileşimi ve enzim inhibisyon aktivitesi

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**Özet**

Kolşisin, Colchicum Autumnale'den elde edilen eski bir bitkisel ilaçtır. Kolşisin binlerce yıldır çeşitli rahatsızlıkları tedavi etmek için kullanılmıştır. İlk olarak ailesel Akdeniz ateşi ve gut tedavisinde kullanılmıştır. Bir anti-inflamatuar ajan olarak benzersiz etkinliğine dayanarak, kolşisin, koroner arter hastalığı, ateroskleroz, tekrarlayan perikardit, vasküler restenoz, kalp yetmezliği ve miyokard enfarktüsü dahil olmak üzere kardiyovasküler hastalıkların tedavisinde kullanılmıştır. Daha yakın zamanlarda, kolşisin ayrıca COVID-19'un kardiyovasküler komplikasyonlarını hafifletmede terapötik etkinlik göstermiştir. Yaygın olarak bilinen kullanımlarına ek olarak kolşisin, geniş anti-inflamatuar etkisinden dolayı çok çeşitli diğer koşullarda potansiyel faydalara sahiptir. DNA molekülünün Watson-Crick tarafından çift sarmal yapısının aydınlatılmasından bu yana ilaç tasarım çalışmaları hız kazanmıştır. Antikanser ve antibakteriyel ilaçların DNA ile yaptığı kovalent ve kovalent olmayan etkileşimlerle yeni ilaçlar geliştirilerek insan sağlığı daha refah düzeye ulaştırılmıştır. Bu çalışmada Kolşisin FSdsDNA ile etkileşimi, Etidyum bromür ile yarışmalı reaksiyonu UV-Vis spektroskopisi ve fotolüminesans spektroskopisi ile ayrı ayrı incelenmiştir. Kolşisin FSdsDNA molekülüne bağlanma sabiti (Kb) hesaplanmıştır. Ayrıca çalışmada kolşisinin enzim inhibisyon aktivitesi incelenmiştir.

**Anahtar Kelimeler:** Kolşisin, İlaç-DNA etkileşimleri, Asetilkolinesteraz (AChE)

## *ORAL PRESENTATION*

# Synthesis and characterization of hemoglobin-loaded poly(lactic acid) nanospheres for artificial blood

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**Abstract**

Blood transfusion, which is frequently applied to hospitalized patients, is a very risky procedure due to limited blood stocks, problems in transfusion, and infection. For this reason, it is of great importance to develop artificial oxygen carrier systems (artificial blood) that can be used in emergency situations by using polymeric materials that are known to be compatible with living organisms and that can fulfill the oxygen-carrying function of blood. Poly(lactic acid) is an economical polymer that can be used in artificial blood production due to its high compatibility with the human body and its biodegradable feature. In the study, hemoglobin encapsulated poly(lactic acid) nanospheres were synthesized using the double emulsion solvent evaporation method. The synthesized hemoglobin loaded poly(lactic acid) nanospheres were analyzed with Zetasizer (Malvern) and the values of these nanospheres were found as 217.9 nm Z-average, 0.054 polydisperse index (PDI) and 258.1 count rate (kpcs). As a result of electron microscopy (Zeiss Ultra Plus) analysis of hemoglobin loaded poly(lactic acid) nanospheres, it was determined that they were spherical and below 1 µm. Therefore, the $P_{50}$ value of the nanospheres was calculated between 55-60 mmHg in the oxygen saturation experiments performed in the blood's oxygen transport in-vitro environment. As a result of the study, it is thought that hemoglobin loaded poly(lactic acid) nanospheres can be used in oxygen transport in terms of size, shape and oxygen saturation.

**Keywords:** Artificial blood, Poly(lactic acid), Hemoglobin, Nanosphere.

## *ORAL PRESENTATION*

# Histopathological Investigation Of Atrazine Toxicity In The Mantle Tissues Of *Physa acuta* Draparnaud, *1805*

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**Abstract**

Based on levels of atrazine accumulations, the study aims to determine histopathological changes taking place on mantle tissues in the treatment of atrazine in sublethal doses (0.3, 3, and 30 µg/L) and in different time points (7, 14 and 21 days) to *Physa acuta*, a freshwater snail. As an experimental material, samples of *Physa acuta* were adapted to the laboratory conditions for 15 days. After that, snails were divided to 4 groups; Group I: control (no atrazine treatment) Group II: 0.3 µg/L, Group III: 3 µg/L, Group IV: 30 µg/L atrazine treatment. All groups, except Group I (control), were exposed to sublethal doses of atrazine for 21 days. Snails from control and experimental groups were dissected on Day 7, Day 14 and Day 21 to determine histopathological changes. Histopathological changes were examined under light and electron microscopes and representative pictures were taken. Histopathologic changes in mantle were found to increase with dose and duration as a result of exposure of the *Physa acuta* specimens to atrazine sublethal concentrations (0.3, 3, and 30 µg/L) atrazine for 21 days. After examining the mantle tissue of the specimens at the end of the process with optical microscope, lesions like swelling and desquamation in the epithelium, atrophy in muscle fibrils, increase in lipid vacuoles, in pigment cells, in mucous cells and and necrosis; were observed. Several changes in the mantle epithelium can be also noticed under electron microscopic observation, including an initial increase of Mitochondria followed by a decrease and eventual loss of these organelles.

**Keywords:** *Physa acuta*, atrazine, mantle, histopathology, snail, tem.

Acknowledgement: We thank Scientific Research Project Council (FEN.18.005), Dicle University for financial support.
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## *ORAL PRESENTATION*

# Düşük sıcaklık uygulamaları için faz değiştiren malzemelerin hazırlanması ve ısıl özelliklerinin incelenmesi

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**Özet**

Enerjinin ihtiyaca göre depolanarak kullanılması kaynakların etkin kullanılması noktasında önem arz etmektedir. Bir sisteme enerji depolama teknolojilerinin entegre edilmesiyle sistemin enerji verimliliği iyileştirilebilir ve hatta yenilenebilir enerji kaynaklı uygulamalarda süreksizlikten ileri gelen olumsuz etkiler azaltılabilir. Son yıllarda, dikkat çeken enerji depolama teknolojilerinden biri de ısıl enerji depolamadır. Isıl enerji depolama gizli, duyulur ve termokimyasal yöntem kullanılarak gerçekleştirilebilir. Enerjinin gizli ısı formunda depolanmasını sağlamada kullanılan yeni nesil enerji malzemelerine faz değiştiren malzemeler adı verilmektedir. Bu yöntem düşük, orta ve yüksek sıcaklık uygulamaları için malzemenin belirli sıcaklıktaki faz geçişi süresince enerjinin depolanması/salınması prensibine dayanmaktadır. Bu çalışmada, düşük sıcaklık enerji uygulamalarında kullanılmak üzere kaprik asit esaslı yeni kompozit faz değiştiren malzemelerin tek adımda impregnasyon yöntemi ile hazırlanması gerçekleştirilmiştir. Üretilen faz değiştiren malzemelerin kimyasal, ısıl ve morfolojik özellikleri FTIR, DSC ve SEM analizleri ile belirlenmiştir. Elde edilen bulgulara göre, üretilen şekilce kararlı kompozit faz değiştiren malzemesinin 31.14 $^{o}$C pik erime sıcaklığı ve sahip olduğu erime gizli ısısı (36,18 kJ/kg) ile farklı düşük sıcaklık uygulamaları için kullanılabileceği değerlendirilmektedir.

**Anahtar Kelimeler:** Enerji, Gizli Isı, Faz Değiştiren Malzemeler, Enerji Depolama

Teşekkür: Bu çalışmanın gerçekleştirilmesinde yüksek lisans tez projesi desteği (Proje No: 2020/YL/0021) sağlayan Yalova Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi'ne teşekkür ederiz.

## *ORAL PRESENTATION*

# Synthesis of Bis-Thiourea Derivatives and Their Acid Dissociation Constants

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**Abstract**

Thiourea derivatives have been widely used in many scientific disciplines as chemosensors in analytical chemistry [1,2] and as pharmacophores [3] or intermediate compounds for the synthesis of various pharmacophore groups such as thiazole and thiohydantoin [4,5] in medicinal chemistry. In this study, new bis-thiourea derivatives were synthesized and their structures were characterized using $^{1}H$ NMR, $^{13}C$ NMR, FT-IR, and HRMS techniques. The acid dissociation constants of the bis-thiourea products were determined potentiometrically in DMSO : water hydro-organic solvent system at an ionic strength of 0.1 M NaCl at 25.0 ± 0.1 °C.

**Keywords:** Acid dissociation constants, Bis-thiourea, Potentiometry

**Acknowledgments:** We are thankful to Mersin University (project grant 2021-1-TP2-4217) for financial support.

**References**

[1] Al-Saidi, H. M., Khan, S. *Crit. Rev. Anal. Chem*., 2022, DOI: 10.1080/10408347.2022.2063017.
[2] Nural, Y., Karasu, E., Keleş, E., Aydıner, B., Seferoğlu, N., Efeoğlu, Ç., Şahin, E., Seferoğlu, Z. *Dyes Pigm*., 198, 110006, 2022.
[3] Efeoglu, C., Yetkin, D., Nural, Y., Ece, A., Seferoğlu, Z., Ayaz, F. *J. Mol. Struct*., 1264, 133284, 2022.
[4] Nural, Y., Gemili, M., Ulger, M., Sari, H., De Coen, L. M., Sahin, E. *Bioorg. Med. Chem. Lett*., 28, 942-946, 2018.
[5] Erşen, D., Ülger, M., Mangelinckx, S., Gemili, M., Şahin, E., Nural, Y., *Med. Chem. Res*., 26, 2152-2160, 2017.

## *ORAL PRESENTATION*

# Çerezlik kabakta (*Cucurbita pepo* L.) ZYMV'ye dayanıklı hatların belirlenmesi ve geliştirilmesi

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**Özet**

Kabakgil yetiştiriciliğinde virüs hastalıkları büyük önem arz etmektedir. Kabak Sarı Mozayik Virüsü (*Zucchini yellow mosaic virüs*-ZYMV*)* kabaklarda büyük kayıplara neden olan önemli bir virüs hastalığıdır. Yapılmış olan bu çalışmada Kayseri'nin farklı ilçelerinden toplanan 96 adet çerezlik kabak genotipi kullanılmıştır. Kullanılan genotipler s5 kademesine kadar kendileme yapılarak saflaştırılmış genotiplerdir. Çerezlik kabak genotiplerine 4-5 gerçek yapraklı aşamada, ZYMV izolatı mekanik bulaştırma yöntemi kullanılarak inoküle edilmiştir. Çalışma kabak genotiplerinin ZYMV'ye hassasiyetlerinin serolojik ve moleküler yöntemlerle saptanması ve tanımlanması amacıyla yürütülmüştür. Hastalığa karşı kabak bitkisinin oluşturduğu simptomolojik tepkileri 1 ve 5 skalasına göre değerlendirilmiştir. Bazı genotiplerin 5 skalasına sahip olarak daha hassas, bazı genotipler ise 3 skalasına sahip olarak daha tolerant olduğu gözlenmiştir. 1 ve 5 skalasına göre simptom gösteren genotiplerden 40 adeti seçilip RT-PCR ve DAS-ELİSA yöntemi ile testlenmiş ve dayanıklı veya duyarlı genotipleri tespit edilmiştir. Simptomolojik gözlemler, serolojik ve moleküler analizler sonucunda örneklerin büyük çoğunluğu duyarlı olarak bulunmuştur. Bu çalışma, yapılacak olan melezleme çalışmalarıyla ZYMV' ye dayanıklı çerezlik kabak ıslahı için temel bir çalışma niteliğindedir.

**Anahtar Kelimeler:** *Cucurbita pepo*, Çerezlik Kabak, RT-PCR, DAS-ELİSA

## *ORAL PRESENTATION*

# Designing gRNA and ssODN sequences for establishment of R124C mutation in *TGFBI* gene using CRISPR/Cas9 technology

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**Abstract**

The CRISPR/Cas9 (Clustered regularly interspaced palindromic repeats/CRISPR-associated nuclease-9) a genome editing tool developed with engineering approaches. This technology is used for various research purposes such as gene therapy, study of gene functions, creation of transgenic animal and cell models and disease modelling. Transforming growth factor beta-induced (*TGFBI*) gene encodes TGFBI protein, which is an extracellular matrix element. It plays an important role in maintaining the structural integrity and transparency of the cornea by interacting with different types of collagens and integrins. Up to date, 74 different mutations have been identified in *TGFBI* gene. Among these mutations, R124C changes located in the 4th exon were reported as hot spot mutation. The c.417C>T transition in the *TGFBI* gene leads to the conversion of arginine to cysteine (R124C) which has been associated with lattice corneal dystrophy type 1 (LCD1). Due to the difficulties in obtaining and culturing primary corneal cells, the identification of corneal epithelial cells, cellular mechanisms and their roles in the pathogenesis of the disease cannot be understood and effective drug trials cannot be conducted. Therefore, we aimed to model LCD1 at cellular level using CRISPR/Cas9 genome editing technology. In this study, we performed the necessary cloning and validation steps for modelling. Guide RNA (gRNA) and single-stranded oligonucleotide (ssODN) template were designed to generate the R124C mutation in the *TGFBI* gene using the https://www.benchling.com/ online software program and received commercially. Designed gRNA pairs were oligoduplexed and then cloned into the CRISPR/Cas9 vector (79145, Addgene). Following the cloning step, vectors were transformed into DH5alpha bacteria, an E.coli variant. After transformation, colony PCR and sequence analysis were performed. Successfully cloned gRNAs were confirmed according to the results of the sequence analysis. By this way, CRISPR/Cas9 constructs ready for transfection into eukaryotic cells have been created.

**Keywords:** CRISPR/Cas9, genome editing, R124C mutation, lattice corneal dystrophy modelling

## *ORAL PRESENTATION*

# Mersin Erdemli Gölünün Su Canlılığının İncelenmesi

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**Özet**

Protozoalar tek hücreli ve ökaryotik mikroorganizmalardır. Yeryüzünde 30.000 kadar türe sahiptirler. Hem karada, hem de sularda yaşarlar. Protozoaların hemen hemen tamamı hareketlidir. Protozoaların büyük bir kısmı aerobik veya geçici aerobik solunum yapan mikroorganizmalardır. Serbest çözünmüş O2 bulunan ortamda yaşarlar ve heterotrofturlar. Mersin Erdemli'den alınan su örnekleri mikroskop altında incelendi. Nostoc, Cymbella, Pinnularia, Bacillaria, Microcystis, Aphanizomenon, Oscillatoria, Pediastrum, Scenedesmus, Ceratıum canlıları gözlemlendi.

**Anahtar Kelimeler:** Protozoa, su, mikroorganizma, ekoloji

## *ORAL PRESENTATION*

# Recovery of ammonium and phosphate from an aqueous solution by modified activated carbon and reusing as a slow-release fertilizer

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**Abstract**

A magnesium-modified biochar adsorbent (MgBC) was prepared by an immersion pyrolysis integrated method for the simultaneous removal and recovery of ammonium ($NH_4^+$) and phosphate ($PO_4^{3-}$) from aqueous solution to reuse them as a slow-release fertilizer. Under the optimized conditions (dosage 0.6 g/L; initial $NH_4^+$ and $PO_4^{3-}$ concentrations of 120 and 60 mg/L), the maximum $NH_4^+$ and $PO_4^{3-}$adsorption capacities of MgBC were 59.95 mg g -1 and 98.6 mg $g^{-1}$ at initial pH 8.0. Self-regulating alkaline MgBC maintained the pH value suitable for struvite. The adsorption equilibrium was quickly reached within 60 min. The pseudo-second-order kinetic model well described the adsorption kinetics. In contrast, adsorption isotherm results demonstrated that the Langmuir model fitted the experimental data better than the Freundlich adsorption model. The intra-particle diffusion model showed that struvite formation, electrostatic attraction and physical adsorption through pore diffusion affected $NH_4^+$ adsorption. X-ray diffraction characterizations suggested that under the optimal parameters, the NP-laden MgBC produced high-purity struvite. MgO and $Mg(OH)_2$ particles on the biochar surface were the main adsorption sites for struvite, and that a precipitation mechanism controlled the adsorption. Moreover, the as prepared MgBC had high removal efficiency for $NH_4^+$ and $PO_4^{3-}$, and is an excellent $NH_4^+$ and $PO_4^{3-}$ carrier material. The NP-laden MgBC in the soil planted with rice significantly promoted crop growth. These results suggested that MgBC is a promising adsorbent, that can simultaneously reclaim $NH_4^+$ and $PO_4^{3-}$ from water, alleviate eutrophication and reduce $NH_4^+$ and $PO_4^{3-}$ removal pressure in water. In addition, the NP-laden MgBC could be cycled back to soils as an effective slow-release NP-fertilizer, allowing recycling and utilization of $NH_4^+$ and $PO_4^{3-}$ resources. This study was provided within the scope of FBA-2021-3768 project supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination unit.

**Keywords:** Magnesium-modified, Reclaim, Ammonium, Phosphate, Slow release fertilizer

## *ORAL PRESENTATION*

# Evaluation of the genotoxic and antigenotoxic effect of boric acid against mitomycin-c in human lymphocytes

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**Abstract**

Mitomycin C (MMC) is an alkylating chemotherapy agent used for the treatment of several cancers. Boron is a naturally occurring essential element. Boric acid, borax, sodium perborate, ulexite, and colemanite are among the common boron compounds used as food additives and anti-cancer agents. Boric acid (BA-$H_3BO_3$) is widely used in medicine as well as in industrial, cosmetics, and agricultural applications. Due to its interesting biological functions, BA is considered a crucial micronutrient. It can increase antioxidant defense *in vivo*. Therefore, this study aimed to investigate the genotoxic and antigenotoxic potential of BA against MMC-induced genotoxicity in human peripheral lymphocytes (HPLs) *in vitro* using the micronucleus-MN test. The lymphocytes were incubated with either different concentrations of BA (0.25, 0.5, 1, and 2.5 µg/mL) alone or simultaneously with MMC (0.20 µg/mL). Single treatment of MMC significantly increased the frequency of micronucleated binucleate cells (MNBC) compared to the negative control. While all the concentrations of BA increased the formation of MNBCs compared to the negative control, none of them were significant. On the contrary, BA attenuated the frequency of MNBCs induced by MMC in the simultaneous treatment at all concentrations. The ameliorative effect was significant at 0.5 µg/mL BA+MMC treatment ($P<0.01$). Nuclear division index (NDI), Cytokinesis block proliferation index (CBPI), and the frequency of nucleoplasmic bridges (NPBs) did not significantly change following MMC and MMC+BA treatments. On the contrary, BA treatments significantly decreased the formation of nuclear buds (NBUDs) induced by MMC alone at the three highest concentrations. The results demonstrate that BA seems to have antigenotoxic potential against MMC-induced genomic damage in HPLs *in vitro*. Besides, it has no genotoxic potential in single treatments. However, further investigations need to be conducted to conclude the antigenotoxic effects of BA against MMC.

**Keywords:** Boric acid, Mitomycin-C, Genotoxicity, Antigenotoxicity, Micronucleus assay, Human lymphocytes

## *ORAL PRESENTATION*

# *Xanthoparmelia orientalis* mikobiyont özütlerinin *Pseudomonas aeruginosa las* quorum sensing sistemi üzerine inhibisyon etkileri

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**Özet**

*P. aeruginosa*'nın patojenitesini çevresindeki organizmalar tarafından algılanabilecek küçük ve yayılabilirmolekülleri üretmesine dayalı mekanizmanın "salt çoğunluk algılama (quorum sensing, QS)" ile düzenlendiği bilinmektedir. **P. aeruginosa**'da ve birçok Gram negatif bakteride bu moleküllerin açil homoserin laktonlar (AHL) olduğu bilinmektedir. Bununla birlikte virülans genlerinin bu düzenlenmiş ekspresyonunun konağın savunmasına karşıseçici bir avantaj sağladığı düşünülmektedir; nitekim bunun da organizmanın patogenezi için önemli olduğu belirtilmektedir. **P. aeruginosa'nın**, LasR, LasI (**las** sistemi). RhIR, RhlI (**rhl** sistemi) ve pqs sistemi adlı LuxRI homologlarını içeren 3 temel salt çoğunluk algılama mekanizmasına sahip olduğu bilinmektedir. *P. aeruginosa*'nın QS sinyal mekanizmasını engelleyerek tedaviye yardımcı olabilecek yeni ilaç moleküllerinin geliştirilmesi ile gereksiz ilaç kullanımının önüne geçilebileceği düşünülmektedir. Likenlerden elde edilen sekonder metabolitlerin QS inhibitörü özelliklerine sahip olduğu literatürde yer almaktadır. Bu çalışmada farklı bölgelerden toplanmış *Xanthoparmelia orientalis* liken oluşturan fungi kültürlerinden elde edilen aseton özütlerinin *P. aeruginosa* QS sistemleri üzerine inhibisyon etkileri incelenmiştir. Bu amaçla PAO1 referans suşu ile *lasB-gfp* raportör suşlarına 30, 15 ve 7,5 µg/ml konsantrasyonlarında özüt uygulaması gerçekleştirilmiştir. M9 minimal besi yeri kullanılarak mikroplaka kuyucuklarına özütler seri dilüsyon ile dağıtılmış ve bakteri son konsantrasyonları OD600nm: 0.1 olacak şekilde düzenlenmiştir. Büyüme ve GFP ekspresyon analizleri multimod mikroplaka okuyucu ile 12-20 saat boyunca her 15 dakikada bir absorbans ve floresans ölçümü alınarak yapılmıştır. GFP ekspresyonunun floresans miktarı 485 nm eksitasyon ve 535 nm emisyon dalga boyları ile ölçülmüştür. Sonuçlar ile *X. orientalis* mikobiyontlarının *las* QS sistemi üzerinde de inhibisyon etkileri belirlenmiştir. En yüksek inhibisyon 30 µg/ml konsantrasyonlarında gözlenmiştir. Elde edilen sonuçların likenlerin özgün sekonder metabolitleri ile antivirulans yaklaşım kapsamında önemini tekrar ortaya koyduğu düşünülmektedir.

**Anahtar Kelimeler:** *Pseudomonas aeruginosa*, *Xanthoparmelia orientalis*, quorum sensing

## *ORAL PRESENTATION*

# Synthesis of new indeno-quinoxaline-based asymmetric bis-hydrazone derivatives and investigation of various ion-sensing properties

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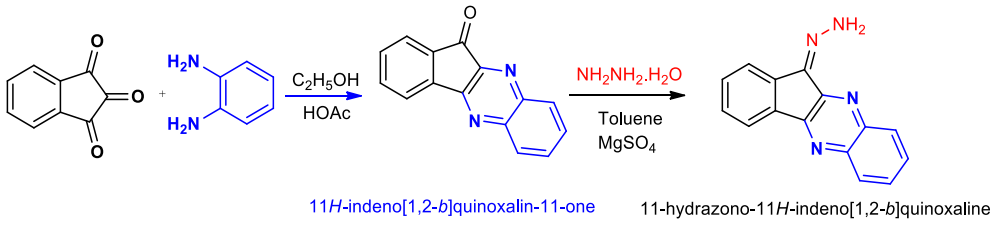
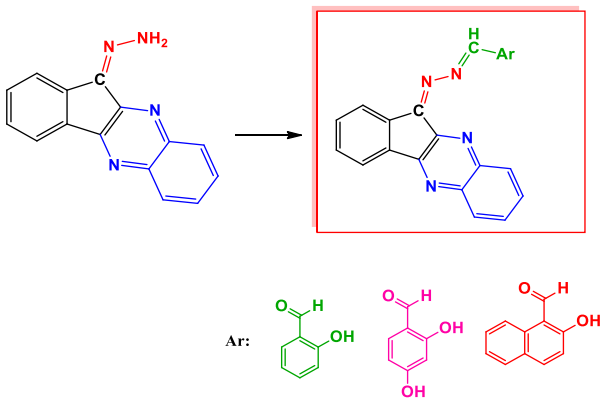
**Abstract**

The development of colorimetric sensors as a solution to the problems in selectivity and the slow response of commonly used sensors in the determination of anions and cations has been an important area of interest in recent years. Studies on this subject have focused on the development of rapid change detectors with high selectivity towards the structures to be analyzed.

In this study, new indeno-quinoxaline-based asymmetric bis-hydrazone derivatives were synthesized and their structures were elucidated spectroscopically. The interactions of both biologically important anions (tetrabutylammonium salts) and cations (various transition metal salts) with this sensor, which can be clearly observed with the naked eye, were also investigated by UV-Vis absorption spectroscopic method trials.




**Keywords:** indeno-quinoxaline, asymmetric bis-hydrazone derivatives, ion sensors, colorimetric analysis.

This work was supported by the Scientific Research Coordination Unit of Çanakkale Onsekiz Mart University, [Project No: **2022-3834 ]**.

## *ORAL PRESENTATION*

# Genotoksisite Tahmininde Kullanılan Güncel Yöntem: *in silico* Metotlar

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**Özet**

Genotoksisite, ilaçlar, gıda katkı maddeleri, evlerde ve sanayilerde kullanılan kimyasallar, elektromanyetik alan ve nanomateryaller gibi çeşitli fiziksel ve kimyasal ajanların incelenmesi açısından önemli bir toksikolojik son noktadır. Kanser gibi canlıların sağlığını tehdit eden ya da hayat kalitesini düşüren ksenobiyotiklerin incelenmesi ve güvenilirliğinin sağlanması açısından genotoksikolojik testler önem arz etmektedir.
Günümüze kadar genotoksisite çalışmaları daha çok *in vitro* ve *in vivo* çalışmalar üzerinden gerçekleştirilmekteydi. Ancak *in vitro* çalışmalar her ne kadar *in vivo* çalışmalara göre daha kısa sürede ve daha az emekle yapılsa da, *in silico* çalışmaların, hem *in vitro* hem de *in vivo* çalışmalara göre daha kısa sürede ve emekle yapılması ve daha ucuz olması, günümüzde artık genotoksisite tahminleri için *in silico* yöntemlerin de konusunda uzman kişiler tarafından değerlendirilmesine neden olmuştur. Hatta son yıllarda yapılan genotoksisite çalışmalarında araştırmacılar öncelikle *in silico* testleri kullanmakta, daha sonra bu çalışmaları *in vitro* ve *in vivo* çalışmalarla devam ettirmektedirler.
Günümüze kadar kimyasalların moleküler yapıları, fizikokimyasal özellikleri, toksisiteleri gibi çeşitli özellikleri ve bu konuda yapılmış olan çalışmaların sonuçlarından alınan veriler toplanmış ve *in silico* testler için çeşitli modeller oluşturulmuştur. *In silico* yöntemlerin güvenilirliğinin arttırılması açısından bu bilgiler toplanmaya devam etmekte ve her geçen gün yeni modeller bu testlere ilave edilmektedir.
Günümüzde genotoksisite tahmininde (Q)SAR, VEGA-Hub, Toxtree, Leadscope gibi çeşitli ücretli ve ücretsiz programlar kullanılmaktadır. Bu çalışmanın amacı günümüzde bilgisayar tabanlı genotoksisite tahmininde en sık kullanılan programlar ve bunların dayandığı modeller hakkında bilgi vermektir.

**Anahtar Kelimeler:** Genetik toksikoloji, *in silico*, mutajenite, karsinojenite

## *ORAL PRESENTATION*

# Pediatrik Nadir Tümörlerde Yeni Nesil Dizileme ile Pankanser Panelin Rolü

Tekincan Aktas, Deniz Kızmazoğlu, Safiye Aktas, Nur Olgun

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**Özet**

Giriş : Moleküler yöntemler kanser tanı ve tedavisinde artan ivme ile önem kazanmaktadır. Yeni nesil dizileme (NGS) yöntemi kısa sürede çok genin durumunu inceleme olanağı sağlamıştır. Solid tümör dokusunda somatik olarak çalışılmaktadır. Ayrıca gereklilik durumunda periferik kan mononükleer hücrelerinde germline çalışma yapılabilmektedir. Bu çalışmanın amacı nadir görülen , ayırıcı tanı sorunu yaşanan ve agresif gidişli pediatrik tümörlerde NGS ile araştırılan pankanser panelin önemini araştırmaktır.
Gereç Yöntem: 2019-2022 yılları arasında Dokuz Eylül Üniversitesi Temel Onkoloji Anabilim Dalında Tanı zorluğu yaşanan pediatrik kanserlerde uygulanan NGS pankanser panel olguları çalışmaya alınmıştır. Yeni nesil sekanslama için Illumina MiniSeq cihazı kullanıldı. Pan Kanser Panel, 405 gen celemics kiti kullanıldı. Parafin tümör dokusu örnekleri gerekli olgularda periferik kan MN hücreleri çalışıldı. Örnekler üretici talimatları doğrultusunda çalışıldı. DNA izolasyonu sonrası kalite ve kantite değerlendirildi. İstenen miktarda DNA elde edildikten sonra NGS cihazı için kütüphane hazırlama aşamasına geçildi. Bu amaçla Hedef Yakalama (Target Capture) temelli NGS kiti kullanıldı. Biyoinformatik analiz için Genomize şirketi ile çalışıldı Raw reads (FASTQ), Alignment/Gene mapping (BAM), Variant calling (VCF) Tek nükleotid değişiklikleri, Delesyonlar, İnsersiyonlar araştırıldı. Mikrosatellit instabilite düzeyleri değerlendirildi. Tümor mutation burden değerlendirildi.
Bulgular: Moleküler incelemeye alınan 180 olgudan 9 nadir pediatrik kanser olgusunda pankanser panel çalışıldı. Böbrek tümörü Anaplastik sarkom olgusunda DICER-1 mutasyonu varlığı tanıya katkı sağlamıştır. Böbreğin malign tümör olmayan kistik alanları ile karşılaştırıldığında eklenen DNA onarım gen ilişkili mutasyonların ve DİCER ! deki ek mutasyonların karsinogeneze katkısı açıklanmıştır. Overde juvenil granülosa hücreli tümör olgusunda DICER1 mutasyon saptanması genetik yatkınlık açısından klinik katkı sağlamıştır. Anevrizmal kemik kisti ve telenjiektatik osteosarkom ayırıcı tanısında mutasyonları önemi sorgulanmıştır.
Sonuç: Yeni nesil dizileme ile pankanser panel analizi (SNV,CNV, MSI ve TMB içeren) çalışılması pediatrik kanserlerdeyararlı bulunmuştur. Tanıya ulaşmada, yeni tedavi hedefi belirlemede moleküler patogenezi anlamada, ailesel kanser yatkınlık ve genetik ilişkili sendromları ön görmede belirgin katkı sağlamıştır. İmmunoterapi kararında tümör mutasyon yükünün önemi henüz kanıtlanmamıştır.

**Anahtar Kelimeler:** Yeni Nesil Dizileme, Pediatrik Kanser, Pankanser Panel

## *ORAL PRESENTATION*

# Design and *in silico* evaluation of novel small molecule inhibitors of class I histone deacetylase enzymes

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**Abstract**
Histone deacetylases (HDACs) catalyze the removal of the acetyl group from lysine residues in the N-terminal tails of histones and non-histone proteins. HDACs are overexpressed in cancer cells and have been identified as remarkable drug targets, and their inhibition shows promising results in cancer and related disease therapies. Four HDAC inhibitors, vorinostat, romidepsin, belinostat, and panobinostat have been approved by the Food and Drug Administration (FDA). Class I HDAC inhibitors can be characterized by a very well-known pharmacophore that contains a zinc-binding group, linker, and cap group, and HDAC inhibitors are very useful in the development of multiple target ligands, too. Using computational tools in drug design is an inevitable part of today's drug discovery studies because it saves time and cost. In this study, a novel compound that has an N-acyl hydrazone (NAH) scaffold was designed for targeting the $Zn^{2+}$ binding site of the class I HDAC enzyme. For this purpose, molecular docking studies and molecular dynamics (MD) simulations followed by MM-PBSA (Molecular Mechanics-Poisson-Boltzmann Surface Area) were applied. Results have shown that the designed molecule could be used as a lead structure for designing new compounds for class I HDAC enzyme inhibition.

**Keywords:** Class I HDAC enzyme, inhibitor design, molecular docking, MD, MM-PBSA

**Acknowledgments**: The author would like to thank Specialist Hüseyin Güner for molecular dynamics (MD) simulation studies.

## *ORAL PRESENTATION*

# Nor-wogonin (5, 7, 8-trihydroxyflavone) Has Anticancer and Apoptotic Effect in Human Prostate Cancer Cells

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## Abstract

Natural products have been studied to provide alternative treatments against human diseases as they have various medicinal properties. One of these natural products is substances in the class of flavonoids. These bioactive molecules have antioxidant, anti-inflammatory and antitumor activities. The number of studies focusing on these molecules is increasing to discover new therapeutic agents against diseases such as cancer. We aimed to determine in-vitro the anticancer and apoptotic effects of Nor-wogonin flavone, which is one of these bioactive molecules and has various biological properties, on human prostate cancer (PC-3) cells.
Nor-wogonin concentrations of 10, 20, 40, 80 µM were prepared and applied to human prostate cancer cells for 24 hours. The anticancer effect of flavone was determined by MTT 3-(4,5-dimethylthiazol-2-yl)-diphenyl tetrazolium bromide) method, and its effect on pro-poptotic and anti-apoptotic genes was determined by Real-Time PCR analysis.
According to the obtained data, Nor-wogonin applied to PC-3 cells decreased in-vitro cell viability due to increasing concentration ($p<0.05$) and the IC50 value was calculated as 57.29 µM. In addition, it was determined that Nor-wogonin directed PC-3 cells to apoptosis by acting on various anti-apoptotic and pro-apoptotic gene expressions.

**Keywords:** Prostate cancer, Nor-wogonin, Anticancer effect, Apoptosis

## *ORAL PRESENTATION*

# Preparation of L-histidine and D-histidine imprinted cryogels for chiral separation

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**Abstract**

Each amino acid can be found in D or L form. Only L-amino acids participate in the synthesis of vegetable and animal proteins. D-amino acids are usually found in the cell walls of bacteria. Although D-amino acids are not found in plant and animal proteins, it has been observed that the D-form of amino acids is also present in case of bacterial contamination in some food products such as canned food and fruit juice. In addition, D-amino acids have been found in the human body in some infections. In the presented study, L-Histidine and D-Histidine imprinted p(HEMA-MAH) cryogels for chiral separation were prepared by free radical polymerization at -12 °C. The prepared cryogels were characterized by swelling tests, SEM and FTIR analysis. Adsorption studies were carried out in a continuous system. 1 M NaCl was used as the desorption agent. In addition, it was observed that there was no significant decrease in adsorption capacities for both cryogels as a result of repeated adsorption-desorption processes.

**Keywords:** Cryogel, L-Histidine, D-Histidine

## *ORAL PRESENTATION*

# Distribution of interleukin-37 and its receptor in bovine fetal liver

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**Abstract**

The liver is one of the largest organs in the body and plays a central role in metabolic homeostasis, nutrient processing and coordinating detoxification. It also consists of parenchymal (hepatocytes) and non-parenchymal (Kupffer cells, sinusoidal endothelial cells, fat-storing cells, cholangiocytes, and other immune cell types) cells that communicate with each other through cytokines in response to inflammatory stimuli and function to maintain homeostasis in the liver. This study was designed to determine the localization and possible physiological effects of interleukin-37 (IL-37) and its receptor in the bovine fetal liver. In the study, 27 healthy fetuses obtained from private slaughterhouses were used. The ages of the fetuses were calculated and the fetuses were divided into three groups consisting of 3-month periods. Liver samples were taken from each group and sections were stained with immunohistochemistry method following routine histological procedures. As a result of immunohistochemical staining, it was observed that IL-37 was expressed at different concentrations in hepatocytes, vascular endothelium and smooth muscle cells, and its receptor was also expressed in hepatocytes and especially in the cells of the hematopoietic islet in the second period. In conclusion, it was thought that IL-37 may have a role in the proliferation and differentiation of endothelial and smooth muscle cells in the vessels of the fetal liver during pregnancy, and its receptor in the maturation of erythropoietic cells. In addition, the predominance of IL-37 and its receptor, especially in hepatocytes, suggests that it may have important physiological effects on metabolic events in the liver.

**Keywords:** Bovine, liver, interleukin-37

## *ORAL PRESENTATION*

# Microwave-assisted extraction of essential oil from *Lavandula angustifolia* and its insecticidal activity on stored product pest, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

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**Abstract**

Synthetic insecticides have been used for many years to control important agricultural pests. However, increasing public concern about the potential negative environmental impacts associated with the massive use of conventional insecticides has led to the investigation of alternative methods for pest control. Essential oils from aromatic plants could be an alternative for pest control due to their diverse biological activities, biodegradability, and low impact on non-target organisms and the environment. In this study, the essential oil of *Lavandula angustifolia* was extracted by a solvent-free microwave extraction method and chemical composition was determined by GC-MS. In addition, fumigant toxicity of essential oil against the larval and adult stages of *Tenebrio molitor* was evaluated. Five different concentrations (5, 10, 15, 20, 25 µl) of the essential oil were used for the toxicity tests. Mortality was observed on larvae below 10% even at the highest concentration 72 hours after treatment. However, %40 mortality was observed on adult stage at the same conditions. Also, $LC_{50}$ values for larvae and adult were determine as 30.83 and 57.19 µl, respectively using probit analysis. The findings indicate that essential oil from *Lavandula angustifolia* appears to be a promising biocontrol agent for use against *T. molitor*.

**Keywords:** Essential oil, lavender, *Tenebrio molitor*, fumigant toxicity,

## *ORAL PRESENTATION*

# Complementary Approaches in Unexplained Intellectual Disability/Developmental Delay: A Single Center Experience

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**Abstract**

Introduction: Global developmental delay (GDD), intellectual disability (ID) and autism spectrum disorder (ASD) are mainly evaluated under the Neurodevelopmental disorder framework. In this study, we aimed to determine the genetic diagnosis yield using step-by-step genetic analysis by performing CMA, CES and WES analyzes in 38 patients with unexplained ID/DD and/or ASD.
Methods: In 38 cases (27 male, 11 female) with unexplained ID/DD and/or ASD, CMA, CES and WES analysis were applied, respectively.
Results: We found a diagnostic rate of only CMA analysis as 18.4% presenting 7 pathogenic and likely pathogenic CNVs. The rate of patients diagnosed with CES/WES methods was 35.4%. When all pathogenic and likely pathogenic variants were evaluated, the diagnosis rate was 47.4%. We identified eight novel variants, TUBA1A (c.787C>G), TMEM63A (c.334-2A>G), YY1AP1 (c.2051_2052del), ABCA13 (c.12064C>T), ABCA13 (c.13187G>A), USP9X (c.1189T>C), ANKRD17 (c.328_330dup) and GRIA4 (c.17G>A) genes.
Discussion: Using complementary approach to genetic analysis (CMA, CES and WES), our total diagnostic rate including pathogenic and likely pathogenic variants was 47.4%. The combined use of genetic analysis methods in unexplained ID/DD and/or ASD cases has contributed significantly to diagnosis rates. Also, we presented detailed clinical characteristics to improve genotype-phenotype correlation in the literature for rare and novel variants.

**Keywords:** Global developmental delay, intellectual disability, CMA, CES,WES

## *ORAL PRESENTATION*

# Use of Simulation Applications in Medicine

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**Abstract**

Simulation applications have been widely used in recent years, especially in medical research, because they are practical, low-cost and effective methods. ANSYS is a simulation software used for the solution of structural and heat transfer engineering analysis. Its solutions include static analysis, dynamic analysis, transmission and fluid flow dependent structures. First of all, it started to be used for testing prototype products in a virtual environment before they are produced, and it is currently used in many fields, including in the field of health. Although the program has many modules, ANSYS Fluent, one of its modules, is a Computational Fluid Dynamics (CFD) software. With the Ansys Fluent module, you can have information on topics such as heat transfer, internal flow analysis, external flow analysis, wind tunnel calculations and blood flow dynamics. The aim of this research is to determine the usage areas of the ANSYS simulation program in the health field in the literature and to determine the application areas. Studies published in Web of Science, Google Scholar and Pubmed academic databases between 25.05.2010 - 25.05.2022 were searched with the keywords "ANSYS", "fluids dynamics" and "medical applications". Aortic valve diseases, which are frequently seen among cardiovascular defects, cause deterioration of blood flow. A Fluid-structure interaction modeling methodology for aortic valve hemodynamics using ANSYS is presented in the literature. In another study, an airway model was aimed by simulating different inspiratory flow rates and the accumulation of inhaled particles with varying diameters with the Ansys-Fluent program. As a result, in the field of medicine, physical values can be obtained by simulating the blood in the vein thanks to this module, and its effects can be examined by simulating different diseases. In addition, it is thought that it can help in the early diagnosis of diseases with the contribution of imaging techniques.

**Keywords:** Medical Simulation Applications, Simulation, Fluids Dynamics

## *ORAL PRESENTATION*

# Behavior of a stainless-steel Austen-ferritic in chloride environments.

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**Abstract**

Studies on Ferrite/Austenite stainless steels are still in progress because of the difficulties related to their two-phase structure. Since it is from the relative concentration of the phases; their distribution and their individual physico-chemical properties determine the mechanical characteristics of two-phase systems. Therefore, the relationships between the phases are still poorly known. The improvement of the mechanical and physical properties of these materials, is conditioned by a better knowledge of their structures and connections, and one must pass obligatorily by the implementation of adapted experimental methods. In this study we propose to better known in the first stage the aspect of the residual stresses. In this study, it is better to know, at first, the aspect of the residual stresses which are important in the use of materials because they affect significantly the conditions of their operation. The determination of these residual stresses by the X-ray diffraction method has become a well-controlled technique, but its application to the study of two-phase materials remains more or less difficult. In the present work, we want to make the distinction and the behavior of an austenitic-ferritic in a chlorinated environment since it is in this type of environment that the majority of the cracks of stainless steel in service occur. The development of the nuclear industry has also revealed other forms of stainless-steel cracking even in neutral environments, with very little solute content, or even in water that is considered pure.
For the distinction of the behavior of these types of steel, we presented the studied material, with the indication of its characteristics; the areas of its use and the problems associated with the preparation of samples for testing. Then we studied the methods of carrying out the manipulations and the ways used to measure the phase dosage, the radio crystallographic elastic constant and the residual stresses.

**Keywords:** Austenitic-ferritic; stainless steels; volume fraction; residual stresses; elastic constants; ferric phase; austenitic phase.

## *ORAL PRESENTATION*

# Importance Of Clonality Analysis In CVID Cases

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**Abstract**

Common variable immunodeficiency (CVID) is characterized by low serum immunoglobulin A levels and recurrent bacterial infections. Generally, in cases with CVID with T cell dysfunction; lymphoproliferative, autoimmune diseases and hematological cytopenias are observed.
In this study, blood and bone marrow biopsy taken during the follow-up process in a female patient who was diagnosed with lymphoma accompanying the development of CVID. It was aimed to evaluate the results of T cell and B cell clonality analysis obtained from the samples.
Genomic DNA isolations were performed from tissue and bone marrow samples taken from the case with CVID between 2007 and 2014. T-cell and B-cell gene clonalities from DNA samples were determined by PCR and gel electrophoresis.
It has been reported that the risk of misdiagnosis increases in case of detection of lymphoma accompanying immunodeficiency. For this reason, it was concluded that a comprehensive evaluation including T cell and B cell gene clonality analysis, which is one of the molecular methods, should be performed in addition to extensive histopathological examination by following the biopsy samples to be taken from the cases.

**Keywords:** Clonality, immunodeficiency, CVID

## *ORAL PRESENTATION*

# Ultrasensitive detection of tert-butylhydroquinone in edible oils with molecularly imprinted electrochemical sensor

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**Abstract**

Precisely detection of food preservatives is very important for maintaining food safety and quality. Therefore, in this study, a molecularly imprinted electrochemical sensor for the selective, rapid, simple and ultrasensitive detection of tert-butylhydroquinone (TBHQ) as food preservative was designed. To construct this sensor, multiwalled carbon nanotubes (MWCNTs), and gold nanoparticles (GNPs) were modified on the glassy carbon electrode (GCE) surface. o-Phenylenediamine (o-PDA) and TBHQ was used as the modification monomer and template molecule respectively. The structural characterization and the surface properties of the developed sensor were evaluated with using various methods. Under optimized conditions the fabricated MIP sensor has excellent selectivity and stability Also, the fabricated MIP/MWCNT/GNP/GCE electrode was applied to the detection of TBHQ in edible oil samples.

**Keywords:** Food preservative; Multi-walled carbon nanotube; Molecularly imprinted electrochemical sensor; Tert-butylhydroquinone; Gold nanoparticles

## *ORAL PRESENTATION*

# Chemical composition antileishmanial and antioxidant activities of essential oils of Algerian *Cymbopogon shoenanthus* Spreng

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**Abstract**

This work aims to study the chemical composition and the antioxidant, antimicrobial and antileishmanial activities of the essential oils of a local medicinal plant *Cymbopogon shoenanthus*. Chemical composition of essential oil was identified by GC/MS analysis. DPPH and FRAP were used for the estimation of the antioxidant activity. Antimicrobial activity was evaluated using well diffusion method. The phytochemical analysis of *Cymbopogon schoenanthus* essential oil showed that 24 compounds were identified. Piperitone and Elemol were the major compounds (53.21±0,42% and 10.87%, respectively). It exhibited a potential antioxidant effect with (45.73±0.59 mg/ml and 76.77±0.73 µg AAE/mg by DPPH and FRAP assays) as well as high antimicrobial properties. In addition, this essential oil showed good antileishmanial potential against *L. infantum* and *L. major* promastigotes ($IC_{50}$= 66.43±0.88 µg/mL and 80.44±1.08 µg/mL, respectively) with low toxicity on macrophage Raw264.7. Considering these results, *Cymbopogon Schoenanthus* essential oils may have a potential therapeutic application for the treatment of bacterial and leishmanial infections.

**Key Words:** *Cymbopogon schoenanthus*, Poaceae, Essential oil, GC-MS, Antioxidant, Antimicrobial, Antileishmanaial

## *ORAL PRESENTATION*

# Genetic factors influencing resistance to gastrointestinal parasitic infections in small ruminants

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**Abstract**

Parasitic infestation is a significant health problem influencing animal species worldwide and decreases productivity by reducing milk production, weight gain, working capacity, or skin integrity. The main way to control animals from endo/ectoparasites is chemotherapy. In 2022, antiparasitic drug production was approximately 11,5 million units in Turkey. The amount of usage of these drugs is seemed to be extremely high, so this consumption puts a huge financial burden on breeders. In addition to the cost of drugs, the increased resistance to antiparasitic drugs interrupts the treatments. Thus, genetic resistance to infection or infestations could be a functional and profitable method against infections. Moreover, reducing the drug requirement is also important in terms of organic livestock breeding. The immunity against parasitic infections gains by innate and acquired (humoral and cell-mediated) in general. However, some variation was observed between the breeds, such as adapted (climatic) sheep and goat breeds are declared to be more resistant than the exotic breeds. Not only between the breeds but also the individual variations against the parasitic infections are known. The genetic variations of host immune response have been well documented in sheep and goat breeds. The differences in defense between the breeds have been studied in gastrointestinal parasites such as *Haemonchus, Trichostrongylus, Echinococcus, Trichuriasis,* or *Monezia* spp. frequently. The QTL studies for nematode resistance in small ruminants focus on candidate genes that focus on fecal egg counts, body weight, and blood parameters such as IgA, IgGI, and eosinophil. The QTL studies showed that the largest amount of QTLs on Oar3 informed to be significantly associated with resistance to gastrointestinal nematodes. Moreover, the *MHC class II locus DRB1, IFN-γ, IL-2,4,13, TLRs (TLR-2, TLR-4, and TLR-9), CLRs* genes were found to be a significant effect on gastrointestinal nematodes in goats by the effects of early inflammatory responses, according to the literature. The *NOS2, CSF, TLR4,* and *CD86* genes play a key role in immune responses and are significant in parasite-antigen recognition by immune response cells such as T-cells. As a result, producing genetically resistant animals with low FEC should be a useful and profitable target for animal breeding. Thus, the effects of genetic factors should be studied in further studies to improve the management strategies for immune protection of small ruminant breeding. In this respect, the aim of the study was reviewed to highlight the genetic factors affecting parasitic infections in sheep and goats

**Keywords:** gastrointestinal parasites, infection, goat, sheep, genetic resistance

## *ORAL PRESENTATION*

# Hematopoezde Yeni Bir Transkripsiyon Faktörü

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**Özet**

Hematopoez sürecinde fizyolojik kontrolün ortadan kalkması ya da hasara uğraması hücrelerin kontrolsüzce çoğalmasına ve tümör dokusu oluşturmasına sebep olmaktadır. Grubumuzun önceki çalışmalarında, farklı tür hematolojik kanserlerde Tbx3 transkripsiyon faktörünün yüksek ifade düzeyleri gösterilmiştir. Ayrıca, birçok kanser türünde Tbx3 artmış düzeyde bulunmaktadır. Bu bulgular, Tbx3'ün kontrolsüzce çoğalmaya sebep olan hücresel farklılaşmada rol oynayabileceğini göstermektedir. Kanser çalışmalarında Tbx3 ifadesi olmasına rağmen, fizyolojik şartlarda Tbx3'ün hematopoezde bulunup bulunmadığı henüz bilinmemektedir. Bu sebeple, bu çalışmada, hematopoez sırasında oluşan farklılaşma basamaklarında Tbx3 protein düzeyinin saptanması, dolayısıyla Tbx3'ün bir fizyolojik gelişim süreci olan hematopoez boyunca yer aldığı basamakların belirlenmesi amaçlandı. Bu amaçla, Akdeniz Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulundan (Karar no: 20.09.2017/554) izin alındıktan sonra, çalışmaya gönüllü 20 gebe dâhil edildi. Doğum sonrası tıbbi atık olan kordon kanı alınarak, hematopoietik kök hücre ve progenitör hücre izolasyonu GMP (Good Manufacturer Practice) laboratuvarında gerçekleştirildi. Kültür ortamında progenitör hücrelerin myeloid seriye farklılaşması sağlandı. Protein ekspresyon düzeyleri flow sitometri yöntemi ile gösterildi. Hematopoietik kök hücrelerde Tbx3 protein düzeyi ile myeloid serideki protein düzeyi karşılaştırıldı. Her iki seviye arasındaki Tbx3 protein düzeyleri arasında istatistiksel olarak anlamlı fark görülmedi. Bu çalışma ile hematopoezde Tbx3 protein varlığı, kordon kanından elde edilen hematopoietik kök hücrelerde ve farklılaşma sonrası myeloid seri hücrelerinde ilk kez gösterildi.

**Anahtar kelimler:** hematopoez; hematopoietik kök hücre; kordon kanı; Tbx3; transkripsiyon faktörü

## *ORAL PRESENTATION*

# Metal Oksit Nanopartiküllerin Genotoksik Etkilerinin Modellenmesi

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**Özet**

Nanomalzemeleri teknolojik olarak avantajlı kılan benzersiz özellikleri aynı zamanda insan sağlığı açısından bir takım yeni risklerin ortaya çıkmasına neden olabilmektedir. Literatürdeki göstergeler farklı nano-formların kendilerine özgü toksikolojik özellikler gösterebildiğini doğrulamaktadır. Nanoparçacıklar küçük boyutları sebebiyle deri ve benzeri biyolojik membranlardan kolayca geçerek hücrelere nüfuz edebilir ve kan yardımıyla taşınarak hayati organlara ulaşabilirler. Yapılan araştırmalar vücuda öncelikli olarak inhalasyon yoluyla girebilen nanoparçacıkların akciğerde hasara yol açabildiğini ortaya koymaktadır. Bu çalışmada metal oksit nanoparçacıklarının genotoksik potensiyellerini kontrol eden yapısal özellikler kantitatif yapı-etki ilişkileri analiz yöntemiyle belirlenmiştir. Literatürdeki farklı çalışmalardan oplanan genotoksisite verileri 9 farklı metal oksit nanoparçacığa (CuO, ZnO, NiO, SiO2, TiO2, CeO2, Fe2O3, Fe3O4 and Co3O4) iki farklı konsantrasyonda (20 µg/cm2 ve 40 µg/cm2) ve 4 saat süreyle maruz kalan akciğer epitel hücrelerinde (A549) meydana gelen DNA hasarının alkali COMET testiyle ölçümünü içermektedir. Sınıflama ve regresyon temelli veri analiz metodları kullanılarak metal oksitlerin yapısal ve fizikokimyasal özelliklerini tanımlayan 24 farklı deneysel/teorik nitelik ile genotoksik potansiyel arasındaki matematiksel ilişki incelenmiştir. Sonuçlar 3 metal oksit nanoparçacığın (CuO, NiO ve CeO2) göreceli olarak daha yüksek genotoksisite potansiyeline sahip olduğunu ve bu durumun 4 farklı yapısal özellikle (zeta potansiyel, elektronegativite, Fermi enerjisi ve çekirdek elektronların değerlik elektronlara oranı) ilişkili olabileceğini işaret etmiştir. Bu tür kantitatif yapı-etki ilişki analizleri ve bilgisayar-destekli yaklaşımlar kısıtlı nanotoksisite verisinin kullanımını en üst düzeye çıkartmak, tehlike riski yüksek nanoparçacıkları tespit edip detaylı toksisite çalışmalarını önceliklendirmek ve dizayn aşamasında güvenliği arttıtılmış yeni nanomalzemeler geliştirmek için oldukça önemlidir.

**Anahtar Kelimeler:** genotoksisite, nanoparçacıklar, nanotoksikoloji, hesaplamalı biyoloji, hesaplamalı kimya, QSAR

## *ORAL PRESENTATION*

# *Aloe vera* Bitkisinden Yeşil Sentez Gümüş Nanopartiküllerin Klinik Patojenlere Antimikrobiyal Etkisi

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**Özet**

Bakteriyel enfeksiyonlar klinikte ciddi sağlık sorunu haline gelmiştir. Özellikle hastane ortamlarındaki çoklu antibiyotik direncine sahip patojen mikroorganizmalar büyük morbidite ve mortaliteye neden olmaktadır. Bu nedenle etkili antimikrobiyal ajanların geliştirilmesine ihtiyaç duyulmaktadır. Bakterilerin bütün antibiyotik direnç mekanizmalarını kullanıyor olması araştırmaları nano tıpa yönlendirmiştir. Bitkiden hazırlanan (yeşil sentez) nanopartiküller antibiyotik direnci konusunda iyimser etkinlik göstermektedir. Ayrıca bitkilerden ortaya çıkan metabolitler doğal kimyasal fabrikalar olarak kabul edilmektedir. Bugüne kadar antimikrobiyal ajanlar olarak farklı metaller ve metal oksitler kullanılmıştır. Son birkaç yılda nano biyoteknoloji alanındaki gelişmeler, yeni antibakteriyel ajanlardan biri olarak ortaya çıkan çeşitli metal oksit nanomalzemelerinin geliştirilmesine de yol açmıştır.
Buna dayanarak çalışmamızda Aloe vera bitkisinden yeşil sentez yoluyla elde ettiğimiz gümüş nanopartiküllerin antimikrobiyal etkisini araştırmaktayız. Özellikle hastane enfeksiyonlarıyla öne çıkan çoklu antibiyotik direncine sahip Pseudomonas aureginosa izolatları üzerinde çalışmamızı sürdürmekteyiz. Patojenlerimiz VİTEK2 cihazı sonuçlarına göre çalışmaya dahil edilmiştir. İzolatlarımızın farklı konsantrasyonlarda Aloe vera gümüş nanopartiküllerinin eklendiği minimum inhibitör konsantrasyon testi ile antibakteriyel etkisi değerlendirilmiştir. Farklı bakteri suşlarına karşı aktif antimikrobiyal ajanlar olan gümüş nanopartiküller dirençli suşlarımız için iyimser sonuçlar vermiştir. Sonuçlarımızın tıbbi alanda umut verici antimikrobiyal aktiviteye sahip olduğu ortaya çıkmaktadır. Bitkisel ürünlerin uygulanmasıyla bakteri yayılımının azalması, ulaşılabilir olması, düşük maliyetli, uygulanması kolay ve çevre dostu olması gümüş nanopartiküllerin hayatımızdaki yerini arttıracaktır.

**Anahtar Kelimeler:** Yeşil sentez, gümüş nanopartiküller, *Aloe vera*

## *ORAL PRESENTATION*

# AS1411: an important G-quadruplex aptamer with excellent therapeutic applications

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**Abstract**

G-quadruplex DNA structures are secondary DNA structures and are frequently seen in regions containing TTAGGG repeats such as telomere regions of the chromosome. Bioinformatics studies show that these structures can be formed in vivo [1, 2]. These structures are formed by stacking planar tetrads formed by hydrogen bonds of guanine base. The presence of monocharged metal cations is essential for the stabilization of these structures. As1411 is one of the important aptamers forming the G-quadruplex. This structure consists of 26 bases. It shows high target selectivity to a protein called nucleolin, which is expressed over the surface of cancer cells. According to some recent studies, this g-quadruplex has unique cellular internalization ability [3]. On the other hand, more than 30 animal studies demonstrate that it has cancer-targeting activity. In the present study, the most common and most current therapeutic applications of AS1411 are summarized and the importance of this structure is emphasized.

**Keywords:** AS1411, G-quadruplex, nucleolin

1. Bartas, M., Brázda, V., Karlický, V., Červeň, J., & Pečinka, P. (2018). Bioinformatics analyses and in vitro evidence for five and six stacked G-quadruplex forming sequences. Biochimie, 150, 70-75.
2. Monsen, R. C., Trent, J. O., & Chaires, J. B. (2022). G-quadruplex DNA: A Longer Story. Accounts of Chemical Research, 4649-4659.
3. Bates, P. J., Reyes-Reyes, E. M., Malik, M. T., Murphy, E. M., O'toole, M. G., & Trent, J. O. (2017). G-quadruplex oligonucleotide AS1411 as a cancer-targeting agent: Uses and mechanisms. Biochimica et Biophysica Acta (BBA)-General Subjects, 1861(5), 1414-1428.

## *ORAL PRESENTATION*

# The up-to-date analytical/molecular and instrumental techniques for clarifying the drug-DNA interaction

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**Abstract**

In addition to carrying genetic information, DNA is a very important drug target for cancer treatment because of the active role it plays in the cell. Ligands that can interact with DNA are considered as potential anticancer drugs. Clarifying the interaction of drugs targeting DNA with these structures in vitro is the most important step in the development of targeted drugs. Techniques used for drug-DNA interactions include spectroscopic, chromatographic, and electroanalytical techniques [1-4]. Although each technique has its own advantages and disadvantages, the cumulative interpretation of the data obtained from them is very important for elucidating the mechanism. In this study, the most up-to-date techniques such as circular dichroism, NMR, and FRET melting techniques used for this purpose are discussed with recent examples.

**Keywords:** Circular dichroism, NMR, FRET

1. Rauf, S., Gooding, J. J., Akhtar, K., Ghauri, M. A., Rahman, M., Anwar, M. A., & Khalid, A. M. (2005). Electrochemical approach of anticancer drugs–DNA interaction. Journal of Pharmaceutical and Biomedical Analysis, 37(2), 205-217.
2. Nimal, R., Unal, D. N., Erkmen, C., Bozal-Palabiyik, B., Siddiq, M., Eren, G., ... & Uslu, B. (2022). Development of the electrochemical, spectroscopic and molecular docking approaches toward the investigation of interaction between DNA and anti-leukemic drug azacytidine. Bioelectrochemistry, 146, 108135..
3. Venugopal, S., Sharma, V., Mehra, A., Singh, I., & Singh, G. (2022). DNA intercalators as anticancer agents. Chemical Biology & Drug Design, 100(4), 580-598.
4. Sirajuddin, M., Ali, S., & Badshah, A. (2013). Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltametry. Journal of Photochemistry and Photobiology B: Biology, 124, 1-19.

## *ORAL PRESENTATION*

# Effect of rosemary extract on *Lemna gibba* under salt stress

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**Abstract**

This study was carried out to determine the effect of ethanolic extract of rosemary (*Rosmarinus officinalis*) (RE) on *Lemna gibba*, an aquatic free-floating plant, which was subjected to salt stress in a climate cabinet under controlled conditions. *L. gibba* was collected from natural water bodies in Gaziantep (Turkey). Plants were acclimated for two weeks in containers containing 10% nutrient solution. The following treatments were applied to healthy growing plants: 0 (without treatment), 10 mg/L RE, 100 mg/ RE, 1000 mg/L RE, 50 mM NaCl, 10 mg/L RE+50 mM NaCl, 100 mg/ RE + 50 mM NaCl, 1000 mg/L RE + 50 mM NaCl. As a result of the applications, reductions in photosynthetic pigment contents were determined. The highest reductions compared to controls were 39.28% ($p<0.001$) for chlorophyll-a, 37.50% ($p<0.01$) for chlorophyll a+b and 49.42% ($p<0.001$), for carotenoid at 50 mM NaCl. The highest reductions in chlorophyll b were determined with 50.28% at 1000 mg/L RE + 50 mM NaCl. Total carbohydrate contents increased only in RE applications compared to control. However, the lowest carbohydrate contents were found in 50 mM NaCl application. Hydrogen peroxide content generally increased insignificantly in only RE applications, but increased significantly in NaCl and RE+NaCl applications. Besides, decreases in total phenolic contents were determined. According to the results obtained, it can be said that the role of RE application in reducing stress on the salt-treated *L. gibba* is partial.

**Keywords:** *Lemna gibba*, rosemary extract (RE), NaCl stress, physiological response

# 1.2. FULL-TEXT PAPERS

## *ORAL PRESENTATION*

# Ankirin tekrar dizileri kanser araştırmaları için potansiyel bir hedef olabilir mi?

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**Özet**
Dizileme teknolojisindeki gelişmeler sayesinde, çok sayıda organizmanın genomu eksiksiz bir şekilde belirlenebilmiştir. Genom veri tabanlarına bakıldığında, özellikle ökaryotlarda tekrar dizilerinin yaygın olduğu görülmektedir. İnsan genomu tarafından kodlanan proteinlerin yaklaşık %20'si, genellikle tekrar alanları olarak adlandırılan tandem dizilerde meydana gelen, 30-40 amino asitlik çoklu tekrarlanan birimler içerir. Tekrar birimleri tüm organizmalardan yaygın olarak görülse de, ökaryotlarda, prokaryot ve arkelerde daha fazla tekrar birimi vardır.
Ankrinler; integral zar proteinlerini, zardaki hücre iskeleti yapıları olan spektrin-aktin kompleksine bağlamakta görevlidir. Ankirin molekülleri çoğu formda yaygın olarak bulunur ve sayısız fizyolojik süreçte yer alır. Hücre iskeletinin bütünlüğü, hücre döngüsünün kontrolü, transkripsiyonel düzenleme, hücre sinyalizasyonu, gelişim, farklılaşma, apoptoz, inflamatuar yanıt gibi birçok fizyolojik süreçte etkin rol oynamaktadır. Birkaç fonksiyonel membran proteinine de bağlanan ankirin proteinleri, hücresel bütünlüğün korunmasını sağlar. Bunun yanı sıra, sitozolik ankirinler hücre sinyal iletiminde görevlidirler. Ankirin tekrarları içeren kofaktör (ANCO) proteinler, hücre içindeki hem yardımcı aktivatörler hem de yardımcı baskılayıcılarla etkileşime girerler. Memeli genomları 3 farklı ankrin polipeptidi (ankirin-r, ankirin-b, ankirin-g) üç farklı gen tarafından kodlanır (*Ank1, Ank2,* ve *Ank3*). Ankyrin-B, kardiyovasküler hastalıkların çeşitli formlarıyla ilişkilidir. Ankyrin-R, eritrositleri etkileyen nadir hastalıklardan olan kalıtsal sferositozda önemli rol oynar. Ankirin B proteinini kodlayan *Ank2*'nin kolorektal ve tiroid kanserlerinde ekspresyonunun azaldığı belirlenmiştir. Benzer şekilde, meme kanserinde de *Ank2*'nin ekspresyonunun azaldığı gösterilmiştir. Ayrıca *Ank2*'nin meme kanserinde teşhis, prognoz ve ilaç araştırması için potansiyel biyobelirteç olduğu düşünülmektedir.
*Ank1* ekspresyonunun olmaması; bazı hücrelerde morfolojik değişiklikleri ve in vitro tümörogenezi indükler. Buna ek olarak *Ank1* pankreas adenokarsinomlarının çoğunda DNA hipometilasyonu ile bağlantılı olarak aşırı eksprese edilmektedir.
Ankirinlerin transkripsiyonel düzenlemesi ve tümör sürecindeki kesin rolü hakkında daha fazla çalışmaya ihtiyaç vardır. Ayrıca ankrinler, çeşitli kanser türleri için önemli bir terapötik hedef olabilir.
**Anahtar kelimler:** Ankirin, tekrar dizileri, karsinogenez

**Giriş**
Proteinler, benzersiz kimliklerini, yapısal çok yönlülük ve karmaşıklıklarını içerdikleri amino asitlerin sayısı ve düzeninden alır. Farklı proteinler, benzer amino asit parçalarından oluşabilir. Motif olarak adlandırılan bu kısa amino asit parçaları, özellikle ökaryotlarda proteinlerin önemli bileşenleri olarak kabul edilir ve diğer moleküllere veya proteinlere bağlanır. Tek bir protein, aynı motifin birden fazla tekrarı da dahil olmak üzere birçok motif içerebilir. Protein tekrarları, çeşitli uzunluklarda ve işlevlerde çok tekrarlı düzenekler oluşturan düzenli ikincil yapılara sahiptir. Genel olarak, bu tekrarlama, proteine biyolojik işlevi için gerekli, büyütülebilir bir bağlanma yüzey alanı sağlar (Islam, Nagampalli, Fatima, & Ashraf, 2018).
Tekrarlayan amino asit dizileri içeren proteinler son on yılda büyük ilgi görmüştür. Dizileme teknolojisindeki gelişmeler sayesinde çok sayıda organizmanın genomları eksiksiz olarak tanımlanmıştır. Bu sayede iç tekrar dizilerine sahip olan proteinlerin özellikle ökaryotlarda yaygın olduğu belirlenmiştir. İnsan genomunda kodlanan tüm proteinlerin yaklaşık yüzde 20'si tekrar eden birimler içerir. İmmünoglobulinlerden sonra, tekrar proteinleri de en fazla bulunan doğal protein sınıfını oluşturmaktadır (Galpern, Freiberger, & Ferreiro, 2020). Tekrar proteinleri yaşayan tüm formlarda yaygın olarak dağılmış olsa da, ökaryotik genomlar, prokaryotik ve arke genomlarına nazaran, daha fazla iç tekrarları olan protein kodlar (Kumar & Balbach, 2021).
Bugüne kadar 20'den fazla tekrar protein sınıfı tanımlanmıştır; bunlar arasında en yaygın olanları lösin bakımından zengin tekrar proteinleri, ankirin (ANK) proteinleri, armadillo/HEAT tekrar proteinleri ve tetratrikopeptit tekrar proteinleridir (Islam et al., 2018; Main, Jackson, & Regan, 2003). Tekrar protein aileleri arasında, ANK tekrar proteinleri, bakteri, arke, ökaryot ve viral genomlarda bol miktarda bulunan en büyük aileyi oluşturur (Kumar & Balbach, 2021).
Ankirin tekrarları, ilk olarak mayalardaki iki hücre döngüsü düzenleyicisinde (Cdc10 ve Swi6) ve daha sonra da hücre iskeleti proteini olan ankirinde keşfedilen ~33 rezidülü bir protein motifidir. Ankirin tekrarları; siklin bağımlı kinaz (CDK) inhibitörleri, transkripsiyonel ve gelişimsel düzenleyiciler, hücre iskeleti proteinleri ve virüslerden insana kadar çeşitli organizmalarda bulunan toksinlerin de dahil olduğu farklı fonksiyonlara sahip proteinlerde bulunur. Birçok ANK tekrar proteini yalnızca ANK tekrarlarından oluşurken, diğerleri farklı diziler arasına eklenen tekrarları içerir. Bir proteindeki ardışık kopyaların sayısı değişkendir, ancak dörtten az ardışık tekrarı olan proteinler nadirdir (Tang, Fersht, & Itzhaki, 2003).
Ankirin tekrarları, zara bağlı proteinler (Notch membran reseptörü vb.), sitozolik proteinler (INK4 ailesinin sikline bağımlı protein kinaz inhibitörleri vb.) veya çekirdekteki proteinler (IκB α: nükleer faktör κB'nin inhibitör alt birimi, vd.) gibi protein-protein etkileşimlerine aracılık etme işlevini paylaşan çeşitli proteinlerin bir parçasıdır (Kumar & Balbach, 2021). ANK proteinlerinin işlev bozukluğu, insanlarda birçok patoloji ile ilişkilendirilmiştir (Kang, Xie, & Zhao, 2019).

**ANK Yapısı**
Doğada en yaygın olarak bulunan protein motiflerinden biri olan Ankirin tekrarı protein-protein etkileşimlerine aracılık etme işlevi görür. Her ankirin tekrarı bir sarmal-dönüş-sarmal (helix−turn−helix) konformasyonu sergiler ve bu tür tandem tekrarlarının dizileri, nispeten esnek döngülere sahip sarmal-dönüş-sarmal demetleri oluşturmak için neredeyse doğrusal bir şekilde paketlenir. Bir ankirin tekrar proteininin global yapısı, esas olarak tekrar içi ve tekrar arası hidrofobik ve hidrojen bağı etkileşimleri içerir (J. Li, Mahajan, & Tsai, 2006).
Ankirin tekrarı, komşu ankirin tekrarına bir anti-paralel β-loop aracılığıyla bağlanan, bir çift antiparalel α-heliks ile karakterizedir. β-loop'un ilk β-dalı tekrarın C-terminaline ve ikinci β-dalı ankirinin N-terminaline katılır. Ankirin tekrarlarında sol yönlü bir bükülme vardır ve tandem tekrar yığını hafifçe kavislidir, içbükey ve dışbükey yüzler oluşturur. Çoğu protein, 2-7 tekrarda tandem sarmal dizileri içerir ve tek bir proteinde 33'e kadar tekrar bulundu. Bir ankirin tekrarının konsensüs dizisi 33 kalıntı içerir ve bu motiften sapmalar tipik olarak ilgili tekrarın termodinamik stabilitesini azaltır (Kumar & Balbach, 2021).

**ANK Tekrarları ve Kanser İlişkili Moleküller**

**Notch Reseptör Ankirin Tekrar Alanı (Nank)**
ANK tekrarları içeren bir diğer protein ailesi de Notch reseptörleridir. Tüm Notch reseptörleri, ankyrin tekrarları içerir ve reseptörler arasındaki en yüksek homoloji derecesi ankirin tekrarlarında bulunur (Allenspach, Maillard, Aster, Pear, & therapy, 2002).Notch reseptörü, plazma zarında bulunan 300 kDa'lık tek geçişli bir transmembran reseptör proteinidir. Notch'un hücre içi alanı (NICD), zar proksimal RAM bölgesi, yedi ankirin tekrarı alanı, iki parçalı bir nükleer lokalizasyon sekansı (NLS) ve ve bir C-terminali PEST bozunma motifinden oluşur (Kopan & Ilagan, 2009).
Yüksek organizmalarda, Notch'e yanıt veren gen, Notch tarafından düzenlenen ankirin tekrar proteinini (NRARP) kodlar. Bu protein, Notch yanıtlarının negatif bir geri besleme düzenleyicisi olarak işlev görür. Notch bağımlı T hücre akut lenfoblastik lösemi (T-ALL) hücre hatlarında büyümenin NRARP tarafından inhibe edilebildiği gösterilmiştir (Jarrett et al., 2019).

Hücrenin kaderi ile ilgili kararlar sıklıkla Notch sinyal yolu tarafından yönlendirilir. Bu yol yüksek oranda korunur ve yetişkin organizmada gelişim ve kök hücre homeostazisi sırasında hücre farklılaşmasını düzenler. Hipoaktif Notch sinyali gibi yanlış düzenlemeler, erken gelişim sırasında bazı doku malformasyonlarına yol açabilir ve hiperaktif Notch sinyali, çocuklarda T hücreli akut lenfoblastik lösemiye yol açabilir (Kumar & Balbach, 2021).
Notch sinyali farklılaşma, hayatta kalma ve/veya çoğalma üzerindeki etkileri nedeniyle birçok doku ve hücre tipinin normal gelişiminde önemli bir rol oynar. Farklılaşma, hayatta kalma ve/veya proliferasyonun düzenlenmesindeki bozulmalar malign transformasyonun altında yatan önemli mekanizmalar olduğundan, patofizyolojik Notch sinyalleri potansiyel olarak kanser gelişimine birçok farklı yolla katkıda bulunur (Allenspach et al., 2002).

**IκBα Ankirin Tekrar Alanı**

Nükleer faktör kappa B (NF-κB), birçok gen ve virüsün ekspresyonunu kontrol eden bir pleiotropik transkripsiyon faktörüdür. Bugüne kadar, tümör oluşumu ve metastaz gelişimindeki rolü için iyi bilinmektedir (Bours, Dejardin, Goujon-Letawe, Merville, & Castronovo, 1994). Ökaryotik NF-κB nükleer transkripsiyon faktörü ailesi, hücrenin, hücre büyümesinin ve gelişiminin inflamatuar ve immün yanıtlarında yer alan genlerin ekspresyonunu düzenler. Otoimmünite, kronik inflamasyon ve çeşitli kanserler, NF-κB sinyal yollarının bozulmuş aktivasyonu ile ilişkilendirilmiştir (Basseres & Baldwin, 2006).
Transkripsiyon faktörü NF-κB'nin biyolojik aktivitesi, üç IκB proteini (IκBa, IκBβ ve IκBε) tarafından diferansiyel olarak kontrol edilir. IκB ailesinin üyeleri, kanonik IκB proteinleri (IκBa, IκBβ ve IkBε), NF-κB öncü proteinleri (p100 ve p105) ve nükleer IκB'lerdir. Yapısal olarak, IκB proteinleri bir N-terminal sinyal alma alanı, bir merkezi ANK alanı ve bir C-terminal prolin-glutamat-serin- treonin bakımından zengin sekansı içerir. ANK alanı, her biri bir β-döngüsü ve iki antiparalel α-heliks içeren altı 33 rezidülü ankirin tekrarından oluşur. IκBa, içbükey ve dışbükey bir yüzeye sahip uzun, fıçı benzeri bir yapıya katlanır. IκBa'nın sadece NFκB'ye bağlandığında tamamen katlandığı gösterilmiş olup IκB'ler, ANK alanı aracılığıyla NF-κB ile etkileşime girmektedir (Kumar & Balbach, 2021).
IκB proteinleri dinlenme hücrelerinde NF-κB 'nin sitoplazmik ayrılmasından sorumludur, buna ek olarak çekirdekte NF-κB ile birleşirler, burada NF-κB DNA bağlanmasını inhibe ederler ve NF-κB 'nin sitoplazmaya taşınmasını teşvik ederler, böylece transkripsiyonu sonlandırırlar (Simeonidis, Stauber, Chen, Hendrickson, & Thanos, 1999).
IκB transkripsiyon düzenlemesinde yer alır, metabolizmaya ve inflamatuar yanıtlara aracılık eder. IκB ayrıca IKKa'nın inhibisyonu olarak kanser hücrelerinde apoptozu indükleyebilir, östrojenlerin insan meme kanseri MCF-7 hücreleri üzerindeki etkilerini anti-apoptotikten proapoptotik hale getirebilir (Xiao, Ghosh, & X, 2005).

**CDK Inhibisyonu**

Sikline bağımlı kinazlar (CDK'ler) ökaryotik hücre döngüsünü kontrol eder. CDK4/siklin D, CDK6/siklin D, CDK2/siklin E ve CDK2/siklin A, G1 ilerlemesini ve hücre döngüsünün S fazına girişi düzenler. CDK aktivitesi, farklı sinyallere yanıt olarak hücre döngüsü durmasının indüklenmesine yardımcı olan CDK inhibitörleri (CDKI'ler) tarafından ayrıca düzenlenir (Kumar & Balbach, 2021).
İki farklı CDKI sınıfı tanımlanmıştır. İlk sınıfta Cip/Kip ailesinin üyeleri arasında yer alan p21Cip1,WAF−1, p27Kip1 ve p57Kip2 bulunur. Bunlar, tüm G1- ve S-faz CDK'lerini inhibe eder ve p53- ve TGF-β aracılı hücre döngüsü durmasında önemlidir. İkinci sınıf ise, p16INK4a, p15INK4b, p18INK4c ve p19INK4d dahil olmak üzere kinaz 4 (INK4) ailesinin inhibitörünün üyeleridir. Bunlar, CDK4 ve CDK6'ya özgüdür ve siklin D'nin varlığında veya yokluğunda bağlanabilir. İfade düzeyindeki değişiklikler veya siklin D1, CDK4, CDKI ve pRB'deki mutasyonlar, kanserde güçlü bir şekilde rol oynar (Hirai et al., 1995; Kumar & Balbach, 2021; Sherr & Roberts, 1995).
p16INK4a, p15INK4b, p18INK4c ve p19INK4d'den oluşan INK4 ailesinin üyeleri, Cdk4 veya Cdk6'ya bağlanarak ve siklin D'nin etkisini engelleyerek hücre döngüsünün ilerlemesini bloke eder. Bu INK4 proteinleri, birkaç ankirin tekrarının hakim olduğu benzer bir yapıya sahiptir (Cánepa et al., 2007).
INK4 proteinleri, çeşitli kanser türlerinde mutasyonlar tarafından yaygın olarak silinebilir veya etkisiz hale getirilir ve bunlar bilinen veya aday tümör baskılayıcılar olarak tanımlanır. Hücre döngüsünün G1 fazında hücreleri durdurma kapasitelerinin yanı sıra, artan sayıda hücresel sürece katıldıkları gösterilmiştir. Temel fizyolojik ve patolojik süreçlerde ortaya çıkan rolleri göz önüne alındığında, INK4 ailesi üyelerinin hücre döngüsü düzenlemesi dışında, yaşlanma, apoptoz, DNA onarımı ve çok aşamalı onkogenezde rolü olduğu belirlenmiştir (Cánepa et al., 2007).
P16 (P16INK4A ve MTS1 olarak da bilinir), neredeyse tüm insan kanser türlerinde p16INK4A (veya CDKN2A) geninin genetik inaktivasyonunun prevalansı nedeniyle bir tümör baskılayıcı olarak kabul edilir. p16 geninin delesyon, metilasyon ve nokta mutasyon yoluyla genetik inaktivasyonu tüm insan kanserlerinin yaklaşık %50'sinde bulunurken, P16'nın hem mRNA hem de protein seviyelerinde aşırı ekspresyonu nöroblastom, servikal, yumurtalık ve meme kanserleri, prostat tümörleri ve ağız kanserleri dahil olmak üzere birçok kanser için kötü prognoz ile ilişkilidir. Bununla birlikte, onkogenler, DNA hasar yanıtı veya yaşlanma tarafından indüklenen yüksek p16 ekspresyonu seviyesi hücresel yaşlanmayı tetikleyebilir ve hızlandırabilir (J. Li, Poi, & Tsai, 2011).

**Gankyrin**

Gankyrin (PSMD10 veya p28GANK olarak da bilinir), yedi tekrar içeren başka bir hücre döngüsü ile ilgili ankirin tekrar proteinidir. İnsan kromozomu Xq22.3'te lokalizedir ve evrim boyunca yüksek oranda korunmuştur (H. Li, Zhang, Zhen, Yang, & Feng, 2018).
Gankyrinin ankirin tekrarları, protein-protein etkileşimlerinde yer alan fonksiyonel alandır. Gankyrin, çeşitli neoplazmaların karsinogenezinde, metastazında ve prognozunda kilit rol oynar. Gankyrin normal dokularda zayıf veya negatif olarak ifade edilir, ancak çeşitli kanserlerde yüksek oranda ifade edilir. Gankyrin ekspresyonu, sağlıklı hasta dokularına kıyasla çok çeşitli karsinomlarda önemli ölçüde yüksektir ve ekspresyonu, tümör hastalarının kötü prognozu ile ilişkilidir (C. Wang & Cheng, 2017).
Gankyrin, hepatokarsinogenezde ve hepatoselüler karsinomlarda erken dönemde aşırı eksprese edilen güçlü hücre döngüsü ve apoptotik özelliklere sahip yeni bir onkoprotein olarak tanımlanmaktadır. Gankyrin, retinoblastoma proteininin (pRb) CDK4 tarafından fosforilasyonunu düzenler ve RING ubiquitin ligaz MDM2 tarafından p53'ün her yerde bulunmasını arttırır. Gankyrin, 26S proteazomunun bir bileşenidir ve sadece p53'ün MDM2 aracılığıyla her yerde bulunma oranını kontrol etmekle kalmaz aynı zamanda her yerde bulunan p53'ü bozunma için 26S proteazomuna ileten bir adaptör protein görevi de görebilir (Dawson, Higashitsuji, Wilkinson, Fujita, & Mayer, 2006).
Gankyrinin aşırı ekspresyonu, hücre döngüsü sürecini, hücre büyümesini, apoptozu ve diğerlerini kontrol eden birkaç sinyal yolunu düzenleyerek tümörün başlaması ve ilerlemesinde rol oynar. Aksine, Gankyrinin aşağı regülasyonu, hücre büyümesini, çoğalmasını ve metastazı önemli ölçüde engeller. Bu nedenle, Gankyrin, tümör tedavisi için potansiyel bir hedef gibi görünmektedir (H. Li et al., 2018).

**Tankirazlar**

Poli(ADP-riboz) polimerazlar (PARP'ler), çeşitli hücresel ve moleküler işlemlerde yer alan büyük bir protein ailesidir. PARP'ler, poli(ADP-ribosil)asyon (PARsilasyon) dahil olmak üzere translasyon sonrası modifikasyon yoluyla donör NAD+'dan hedef proteinlere ADP-riboz moleküllerini aktarır. PARsilasyon, DNA hasar onarımı, hücresel stres sinyali, gen transkripsiyonu ve yaşlanma dahil olmak üzere çok sayıda hücresel süreci düzenler (Haikarainen, Krauss, & Lehtio, 2014; Kim, 2018).
Tankirazlar, Wnt sinyali, telomer bakımı ve mitoz regülasyonu dahil olmak üzere çeşitli hücresel süreçleri düzenleyen çok işlevli poli(ADP-riboz) polimerazlardır. Tankirazlar, hedef proteinlerle etkileşime girer ve poli(ADP-ribosil)asyon yoluyla etkileşimlerini ve stabilitelerini düzenler. PARP ailesine ait iki tankiraz proteini vardır; tankiraz 1 (TNKS1; PARP5A ve ARTD5 olarak da bilinir) ve tankiraz 2 (TANK2; PARP5B ve ARTD6 olarak da bilinir). TNKS1'in ANK bölgesi 24 ankirin tekrarından oluşur, TANK2 ANK alanı ise TNKS1 ile %83 özdeştir ve protein-protein etkileşimlerinde yer alır (Kim, 2018).
Tankiraz proteinleri, telomer bakımı ve mitoz düzenlenmesindeki rollerine ek olarak, AXIN (Wnt yolundaki anahtar efektör) (Clevers, 2006), fosfataz ve tensin homoloğu (PTEN; önemli bir tümör baskılayıcıdır ve bir tankiraz bağlayıcı proteindir) (N. Li et al., 2015) ve anjiyomotin (negatif onkogenik YAP düzenleyicisi) (W. Wang, Huang, & Chen, 2011) gibi tümör baskılayıcıları düzenler.
Bu nedenle, tankirazlar kanser tedavisi için etkili hedefler olabilir. Tankiraz inhibitörleri, Wnt, AKT, evet ile ilişkili protein, telomer bakımı ve mitoz düzenlemesi dahil olmak üzere kontrolsüz çoğalmayı destekleyen çeşitli kanserojen yolları etkileyebilir (Seimiya, Muramatsu, Ohishi, & Tsuruo, 2005).
Son zamanlarda, tankirazların işlevi ve mekanizmasının yeni yönleri rapor edilmiş ve bir dizi tankiraz inhibitörü tanımlanmıştır. Tankiraz inhibitörleri ile geleneksel kemoterapi ajanlarının bir kombinasyonu, sinerjistik antikanser etkilere sahip olabilir. Bu nedenle, kansere ve diğer tankiraza bağlı hastalıklara karşı yeni terapötik stratejiler sağlayan daha gelişmiş ve iyileştirilmiş tankiraz inhibitörlerinin geliştirilmesi beklenmektedir (Quackenbush et al., 2016).

**KANK1/ ROCK**

KN motifi ve ankirin tekrar alanları 1 geni (KANK1) kromozom 9p24'te bulunur ve KANK N-terminali (KN) motifi, merkezi sarmal-sarmal alanları ve C-terminali ankirin tekrarlarından oluşur. Özellikle, KANK1 proteini, sırasıyla sarmal bobin ve ankirin tekrar alanları aracılığıyla diğer ilişkili proteinlerle etkileşime girer. KANK1, hücre iskeleti proteinlerinin üretim hızını düzenleyerek ve aktin polimerizasyonunu kontrol ederek hücre iskeletinin korunmasında önemli bir role sahiptir. KANK1, proliferasyon, adezyon, hücre farklılaşmaları ve apoptoz gibi çeşitli hücresel fonksiyonlarda yer aldığı kabul edilen Rho ile ilişkili kinaz (ROCK) yolunun aşağı regülasyonunda önemli bir rol oynar. Bu, KANK1'in β-katenin ile birlikte çekirdekteki dağılımını düzenlemeyi ve transkripsiyonunu yoğunlaştırmayı, dolayısıyla kanser gelişimini etkilemesini sağlar. Önemli olarak, birkaç in vivo çalışma, ROCK yolu ile tümör hücresi metastazı arasında bir bağlantıya sahip olduğunu belirlemiş ve birçok insan kanserinde rolü olduğunu göstermiştir (Kariri et al., 2020).

**Ankirin tekrarları içeren kofaktör proteinleri (ANCO)**

Ankirin tekrarları içeren kofaktör proteinleri, sikline bağımlı kinaz inhibitörleri, transkripsiyon faktörleri ve hücre iskeleti düzenleyicileri dahil olmak üzere hem ankirin tekrarı hem de transkripsiyonel baskılama/aktivasyon alanları içerir (Zhang et al., 2004). ANCO proteinleri, histon deasetilazların (HDAC'ler) alınması yoluyla nükleer reseptörlerin transkripsiyonel aktivitesini inhibe edebilir (Bai et al., 2013).

ANCO-1 olarak da adlandırılan ankirin tekrar alanı 11 (ANKRD11), ankirin tekrarları içeren kofaktör ailesinin bir üyesidir. ANKRD11 gen lokusu, 16q24.3 meme kanseri heterozigotluk kaybı bölgesinde bulunur. İnsan kromozomu 16q'nun heterozigotluk kaybı, tüm meme tümörlerinin en az yarısında meydana gelmektedir. Bu nedenle ANKRD11 konumu nedeniyle meme kanserinde aday bir tümör baskılayıcı gen olarak seçilmiştir (Powell et al., 2002).

Son zamanlarda yapılan araştırmalarda ayrıca ANKRD11'in bir p53 koaktivatörü olduğunu ve meme kanserinde p53 ile düzenleyici bir geri besleme döngüsüne dahil olduğunu gösterdi. Bunların tümü, ANKRD11'in varsayılan bir tümör baskılayıcı gen olduğunu gösterilmiştir. p53'ün bir transkripsiyon faktörü olarak hareket etme yeteneği, tümör baskılayıcı bir protein işlevi için kritik öneme sahiptir. ANKRD11'in, p53'ün transkripsiyonel aktivitesini arttıran yeni bir p53 etkileşimli protein olarak tanımlanmıştır. ANKRD11 ekspresyonunun meme kanseri hücre dizilerinde aşağı regüle edildiği gösterilmiştir (Neilsen et al., 2008).

Ankirin tekrar alanı 12 (ANKRD12), 224 kDa'lık bir nükleer proteini kodlar ve N-terminali ankirin tekrarları bölgesinde ve C-terminali ortak aktivatör etkileşim alanında bulunur. Kolorektal kanser tümör dokularında ve normal komşu mukozada ANKRD12 mRNA ekspresyonunu ve prognozla olan potansiyel ilişkisinin araştırtıldığı bir çalışmada, ANKRD12 mRNA'nın kolorektal kanser tümör dokularında aşağı regüle edildiği ve düşük ANKRD12 ekspresyonunun karaciğer metastazı ve kolorektal kanser hastalarının zayıf sağkalımı ile ilişkili olduğunu ortaya koyulmuştur (Bai et al., 2013).

**Sonuç**

Ankirin tekrarları protein-protein etkileşimlerinde önemli rol oynayan tekrar dizileridir. Bu tekrar dizilerine sahip proteinler arasında, hücre döngüsü düzenleyiciler, onkogenik proteinler ve tümör süpresörler gibi karsinogenezde önemli role sahip proteinler ile bunların düzenleyicileri yer alır. Bu nedenle ankirin tekrar proteinleri kanser oluşumu, tanılaması ve tedavisi için önemli hedefler olabilirler.

**Kaynaklar**

Allenspach, E. J., Maillard, I., Aster, J. C., Pear, W. S. J. C. b., & therapy. (2002). Notch signaling in cancer. *1*(5), 466-476.
Bai, R., Li, D., Shi, Z., Fang, X., Ge, W., Zheng, S. J. J. o. E., & Research, C. C. (2013). Clinical significance of Ankyrin repeat domain 12 expression in colorectal cancer. *32*(1), 1-5.
Basseres, D., & Baldwin, A. J. O. (2006). Nuclear factor-κB and inhibitor of κB kinase pathways in oncogenic initiation and progression. *25*(51), 6817-6830.
Bours, V., Dejardin, E., Goujon-Letawe, F., Merville, M.-P., & Castronovo, V. J. B. p. (1994). The NF-κB transcription factor and cancer: high expression of NF-κB-and IκB-related proteins in tumor cell lines. *47*(1), 145-149.
Cánepa, E. T., Scassa, M. E., Ceruti, J. M., Marazita, M. C., Carcagno, A. L., Sirkin, P. F., & Ogara, M. F. J. I. l. (2007). INK4 proteins, a family of mammalian CDK inhibitors with novel biological functions. *59*(7), 419-426.
Clevers, H. J. C. (2006). Wnt/β-catenin signaling in development and disease. *127*(3), 469-480.
Dawson, S., Higashitsuji, H., Wilkinson, A. J., Fujita, J., & Mayer, R. J. J. T. i. c. b. (2006). Gankyrin: a new oncoprotein and regulator of pRb and p53. *16*(5), 229-233.
Galpern, E. A., Freiberger, M. I., & Ferreiro, D. U. J. P. o. (2020). Large Ankyrin repeat proteins are formed with similar and energetically favorable units. *15*(6), e0233865.
Haikarainen, T., Krauss, S., & Lehtio, L. J. C. p. d. (2014). Tankyrases: structure, function and therapeutic implications in cancer. *20*(41), 6472-6488.
Hirai, H., Roussel, M. F., Kato, J., Ashmun, R. A., Sherr, C. J. J. M., & biology, c. (1995). Novel INK4 proteins, p19 and p18, are specific inhibitors of the cyclin D-dependent kinases CDK4 and CDK6. *15*(5), 2672-2681.
Islam, Z., Nagampalli, R. S. K., Fatima, M. T., & Ashraf, G. M. J. I. j. o. b. m. (2018). New paradigm in ankyrin repeats: Beyond protein-protein interaction module. *109*, 1164-1173.
Jarrett, S. M., Seegar, T. C., Andrews, M., Adelmant, G., Marto, J. A., Aster, J. C., & Blacklow, S. C. J. S. s. (2019). Extension of the Notch intracellular domain ankyrin repeat stack by NRARP promotes feedback inhibition of Notch signaling. *12*(606), eaay2369.
Kang, Y., Xie, H., & Zhao, C. J. G. (2019). Ankrd45 is a novel ankyrin repeat protein required for cell proliferation. *10*(6), 462.
Kariri, Y. A., Joseph, C., Kurozumi, S., Toss, M. S., Alsaleem, M., Raafat, S., . . . treatment. (2020). Prognostic significance of KN motif and ankyrin repeat domains 1 (KANK1) in invasive breast cancer. *179*(2), 349-357.
Kim, M. K. J. O. L. (2018). Novel insight into the function of tankyrase. *16*(6), 6895-6902.
Kopan, R., & Ilagan, M. X. G. J. C. (2009). The canonical Notch signaling pathway: unfolding the activation mechanism. *137*(2), 216-233.
Kumar, A., & Balbach, J. J. B. (2021). Folding and stability of ankyrin repeats control biological protein function. *11*(6), 840.
Li, H., Zhang, J., Zhen, C., Yang, B., & Feng, L. J. A. J. o. T. R. (2018). Gankyrin as a potential target for tumor therapy: evidence and perspectives. *10*(7), 1949.
Li, J., Mahajan, A., & Tsai, M.-D. J. B. (2006). Ankyrin repeat: a unique motif mediating protein− protein interactions. *45*(51), 15168-15178.
Li, J., Poi, M. J., & Tsai, M.-D. J. B. (2011). Regulatory mechanisms of tumor suppressor P16INK4A and their relevance to cancer. *50*(25), 5566-5582.
Li, N., Zhang, Y., Han, X., Liang, K., Wang, J., Feng, L., . . . development. (2015). Poly-ADP ribosylation of PTEN by tankyrases promotes PTEN degradation and tumor growth. *29*(2), 157-170.
Main, E. R., Jackson, S. E., & Regan, L. J. C. o. i. s. b. (2003). The folding and design of repeat proteins: reaching a consensus. *13*(4), 482-489.
Neilsen, P. M., Cheney, K. M., Li, C.-W., Chen, J. D., Cawrse, J. E., Schulz, R. B., . . . Callen, D. F. J. J. o. c. s. (2008). Identification of ANKRD11 as a p53 coactivator. *121*(21), 3541-3552.
Powell, J. A., Gardner, A. E., Bais, A. J., Hinze, S. J., Baker, E., Whitmore, S., . . . Doggett, N. A. J. G. (2002). Sequencing, transcript identification, and quantitative gene expression profiling in the breast cancer loss of heterozygosity region 16q24. 3 reveal three potential tumor-suppressor genes. *80*(3), 303-310.
Quackenbush, K. S., Bagby, S., Tai, W. M., Messersmith, W. A., Schreiber, A., Greene, J., . . . Pitts, T. M. J. O. (2016). The novel tankyrase inhibitor (AZ1366) enhances irinotecan activity in tumors that exhibit elevated tankyrase and irinotecan resistance. *7*(19), 28273.
Seimiya, H., Muramatsu, Y., Ohishi, T., & Tsuruo, T. J. C. c. (2005). Tankyrase 1 as a target for telomere-directed molecular cancer therapeutics. *7*(1), 25-37.
Sherr, C. J., & Roberts, J. M. (1995). Inhibitors of mammalian G1 cyclin-dependent kinases. *9*(10), 1149-1163.
Simeonidis, S., Stauber, D., Chen, G., Hendrickson, W. A., & Thanos, D. J. P. o. t. N. A. o. S. (1999). Mechanisms by which IκB proteins control NF-κB activity. *96*(1), 49-54.
Tang, K. S., Fersht, A. R., & Itzhaki, L. S. J. S. (2003). Sequential unfolding of ankyrin repeats in tumor suppressor p16. *11*(1), 67-73.
Wang, C., & Cheng, L. J. I. N. D. (2017). Gankyrin as a potential therapeutic target for cancer. *35*(5), 655-661.
Wang, W., Huang, J., & Chen, J. J. J. o. B. C. (2011). Angiomotin-like proteins associate with and negatively regulate YAP1. *286*(6), 4364-4370.
Xiao, C., Ghosh, S. J. M. o. l. a., & X, i. r. (2005). NF-κB, an evolutionarily conserved mediator of immune and inflammatory responses. 41-45.
Zhang, A., Yeung, P. L., Li, C.-W., Tsai, S.-C., Dinh, G. K., Wu, X., . . . Chen, J. D. J. J. o. B. C. (2004). Identification of a novel family of ankyrin repeats containing cofactors for p160 nuclear receptor coactivators. *279*(32), 33799-33805.

## *ORAL PRESENTATION*

### Farklı bezelye (*Pisum sativum* L.) genotiplerinin yem verimleri ve bazı kalite özellikleri

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**Özet**

Araştırma, iki farklı lokasyona (Edirne ve Tekirdağ) ait ekolojik koşullarda kışlık ekilen bezelye genotiplerinin yem verimleri ile bazı kalite özelliklerinin belirlenmesi amacıyla yapılmıştır. Çalışmalar, Edirne ili Kemerler Köyü ve Tekirdağ ili Malkara ilçesindeki çiftçi arazilerinde susuz koşullarda, 2020-2021 yetiştirme sezonunda kışlık olarak yürütülmüştür. Sıra arası 25 cm ve 5 m uzunlukta 6 sıradan oluşan parsellere metrekarede 100 bitki olacak şekilde ekim yapılmıştır. Ekimle birlikte dekara 5 kg saf azot ve fosfor gelecek şekilde gübreleme yapılmıştır. Bezelye genotiplerinin doğal konumdaki bitki boyu, bitki boyu, bitkide dal sayısı, sap çapı, yeşil ot verimi, kuru ot verimi, ham selüloz, ham protein oranı, ADF, NDF, Ca, Mg, P ve K oranları saptanmıştır. En yüksek doğal konumdaki bitki boyu Edirne lokasyonunda Töre çeşidinde (107.75cm) ölçülürken, en uzun bitki boyu ise Taşkent çeşidinde (188.43 cm) Edirne lokasyonunda ölçülmüştür. Bitkide dal sayısı en fazla 3.95 adet ile Edirne lokasyonunda P15 genotipinde bulunurken, sap çapı en fazla (3.85 mm) Malkara lokasyonunda Töre, Edirne lokasyonunda da Ateş çeşidinde ölçülmüştür. En yüksek yeşil ot verimi Edirne lokasyonunda P10 genotipinde (6519.00 kg/da), en düşük kuru ot verimleri ise P15 (987.25 kg/da), P11 (1028.50 kg/da) ve P10 (1121.75 kg/da) genotiplerinde tespit edilmiştir.

**Anahtar Kelimeler:** Bezelye, genotip, kalite özellikleri, yem verimi

### Forage yield and some quality traits of different pea (*Pisum sativum* L.) genotypes

**Abstract**

The aim of this investigation was to determine forage yield and some quality traits of different pea genotypes. The study was conducted during 2020-2021 at Kemerler village of the Edirne province and Malkara-Tekirdag province in Thrace, Türkiye. The 100 seed per square meter was sown in plots of 6 rows, with a spacing of 25 cm and 5 m in length. A basal fertilizer containing 50 kg $ha^{-1}$ nitrogen and phosphorus were incorporated into the soil at the time of land preparation. The natural plant height, plant height, number of branches per plant, stem diameter, green fodder yield, hay yield, crude cellulose, crude protein, NDF, ADF, Ca, Mg, K and P ratios were determined. The highest natural plant height (107.75 cm) was measured for cultivar Töre at the Edirne location. The maximum plant height was found for cultivar Taşkent (188.43 cm) at the same location. The highest number of branches per plant (3.95 piece) was observed for P15 genotype grown at Edirne compared to those grown at Malkara-Tekirdag. The maximum stem diameter was measured for cultivar 'Töre' (3.85 mm) at the both locations. Higher green fodder yield (65.19 t $ha^{-1}$) was found for P10 genotype grown at Edirne. The lowest hay yield was determined in P15 (9.87 t $ha^{-1}$), P11 (10.28 t $ha^{-1}$) and P10 (11.21 t $ha^{-1}$) genotypes.

**Keywords:** Forage yield, genotype, pea, quality properties

## GİRİŞ

Küresel ısınma ile yaşanan pandemi ve son günlerde global olarak yaşanan gıda arzındaki sorunlar ve buna bağlı gıda fiyatlarının artması sonucunda, bitkisel üretim ve hayvancılığın önemi daha da ön plana çıkmış bulunmaktadır. Yurdumuzda da hayvancılık işletmelerinin giderlerinin büyük kısmını oluşturan kaba yem üretiminin yetersizliği sonucunda oluşan kaba yem tedariki konusundaki yaşanan zorluklar da hayvansal ürün fiyatlarının artmasına neden olan ana sebeplerin en büyüğü olarak karşımıza çıkmaktadır.

İstatistikler incelendiğinde, 1962 yılında ülke nüfusumuz 28.23 milyon iken bu nüfusa karşılık gelen büyük baş hayvan birimi (BBHB) 13.47 milyon adettir (Düzgüneş ve ark. 1965). Türkiye İstatistik Kurumu 2021 yılı

verilerine göre ise büyükbaş hayvan varlığı 18 036 117 adet, küçükbaş hayvan varlığı 57 519 204 adet olsa da geçen 60 yıla göre ülkemizin nüfus artışına paralel olarak hayvan sayımız artmadığı gibi hayvancılık yapan çiftçi sayısı azalmış ve azalmaya devam etmektedir. Ayrıca, günümüzde tarım ile uğraşan çiftçilerin büyük çoğunluğu hayvancılık yapmadığı gibi hayvancılık yapan çiftçi ve işletmelerinde kaba ot ihtiyacını yeteri kadar üretecek arazilerinin olmaması, hayvancılık ile buna bağlı olan hayvansal üretimi sınırlandıran ve azaltan faktörlerin başında gelmektedir. Tarımsal desteklemeler planlanırken, tarım ve hayvancılığı bir arada yürüten ve toprakların organik madde miktarını artıracak şekilde ekim nöbeti uygulayan çiftçilerimize kat sayılı bir destek sistemi getirilerek, hayvancılık sorunu ile birlikte toprak koruma ve ıslahı, yetiştiriciliğinden kaçınılan bitkilerin üretimi konularıyla, bu faaliyetlerle bağlantılı birçok sorun da çözüme kavuşturulabilir. Bu şekilde, geniş arazi varlığına rağmen sadece bitkisel üretim yapan ve üretimden kalan hasat artıklarını (saman vb.) kendi hayvanlarına yedirmeden satan çiftçilerin hayvan bakmaları da teşvik edilmiş olacaktır.

Yurdumuzda tahıl hasadının büyük çoğunluğu haziran ve temmuz aylarında yapılmaktadır. Hayvancılığın yoğun olduğu Trakya yöresi gibi buğday (*Triticum* spp.)-ayçiçeği (*Helianthus annuus* L.) ekim nöbeti uygulanan bölgelerde buğday hasadından sonra ayçiçeği ekimine kadar yaklaşık 9 ay boş kalan bu tarım alanlarında buğday hasadı sonrasında kışlık ekilebilecek olan bezelye (*Pisum sativum* L.), Macar fiği (*Vicia pannonica* Crantz.), tüylü fiğ (*V. villosa* Roth.), koca fiğ (*V. narbonensis* L.), mavi çemen otu/mavi taş yoncası/mavi üçgül (*Trigonella melilotus-caerulea* (L.) Asch. & Graebn. / *Melilotus caeruleus* (L.) Desr. / *Trifolium caeruleum* L., baharat ve yem bitkisi olarak) (Arslan ve ark., 2016) ve mürdümük (*Lathyrus sativus* L.) gibi yetiştiriciliği kolay olan tek yıllık baklagil yem bitkileri nisan-mayıs aylarında biçilerek ucuz ve kaliteli kaba yem üretiminde artış sağlanarak kaba yem açığının bir kısmını da karşılanmış olacaktır. Ayrıca yörede yaygın olan çeltik (*Oryza sativa* L.) yetiştiriciliği yapılan alanlarda da çeltik hasadı sonrasında bir sonraki yıl mayıs ayında tekrar çeltik ekilecek tarlaların boş kalmaması için mürdümük ekimi teşvik edilerek kaba yem üretimi daha da artırılabileceği gibi bu alanlardaki topraklarının ıslahına da katkı sağlanabilecektir. Son yıllarda; Trakya yöresi ile pamuk (*Gossypium hirsutum* L.)-pamuk ekim nöbetinin uygulandığı Harran ve Çukurova yörelerimiz başta olmak üzere ülkemizin birçok bölgesinde yetiştiricilerin yalın bezelye, bezelye-ekmeklik buğday (*Triticum aestivum* L.), tritikale (× *Triticale* Hurst) veya yulaf (*Avena sativa* L.) gibi tahıl türleri ile kuru ot veya silaj üretimi amacıyla karışım ekim yaptıkları ve bezelye ekim alanlarında her geçen yıl artışın olduğu gözlenmektedir.

Farklı bezelye genotiplerinin Trakya yöresinin iki lokasyonundaki ot verimleri ile bazı kalite özelliklerinin belirlenmesi amacıyla yürütülen bu araştırma, tarım sektörüne öneri sunabileceği gibi bezelye ile ilgili daha sonra yapılacak olan bilimsel çalışmalara da kaynak oluşturabilecektir.

## MATERYAL VE METOT

Farklı iki lokasyonda (Tekirdağ ili Malkara ilçesi ve Edirne Merkez ilçe) 2020-2021 üretim sezonunda (Kasım-Haziran) çiftçi koşullarında kışlık ekilerek yürütülen araştırmada, farklı tohum şirketlerinin geliştirdikleri P10, P11, P15, MB95 bezelye genotipleri ile Töre, Ateş ve Taşkent bezelye çeşitleri materyal olarak kullanılmıştır. Edirne lokasyonun toprak özellikleri killi-tınlı tekstüre sahip, organik madde bakımından iyi (%3.25), tuzluluk olmayan (% 0.03), kireç bakımından zengin (% 20.42), pH'ı (7.63) hafif alkali yapıdadır. Malkara lokasyonundaki toprak yapısı aynı tekstürde, organik madde bakımından iyi (% 2.54), tuzluluk olmayan (% 0.03), kireç bakımından orta kireçli (% 7.60) olup pH'ı (% 7.78) hafif alkali yapıdadır. Araştırmanın yürütüldüğü dönemde Edirne ili Merkez ilçe ve Tekirdağ ili Malkara ilçesinin sıcaklık ortalamaları sırasıyla 11.4 °C ve 11.9 °C ve toplam yağış miktarları da sırasıyla 332.6 mm ve 369 mm'dir.

Tesadüf blokları deneme desenine göre 4 tekrarlamalı olarak kurulan denemede her parsel 5 m uzunluğundaki, sıra arası açıklıkları 25 cm olan altı sıradan oluşmuştur. Parsel hazırlığı sırasında 20-20-0 kompoze gübre kullanılarak dekara 5 kg saf azot ve fosfor uygulanmıştır. Tüm genotiplerin ekimleri $m^2$'de 100 bitki (Yıldırım ve ark., 2005) olacak şekilde elle yapılmıştır. Ot verimine doğrudan etkili olan doğal konumdaki bitki boyu (cm), bitki boyu (cm), bitkide dal sayısı ve sap çapı (mm) Tekeli ve Ates (2003) ile Tenikecier ve Ates (2021)'in belirttikleri yöntemlerle belirlenmiştir. Her parselde 2.5 $m^2$'lik alan tam çiçeklenme döneminde toprak seviyesinden biçilip tartılmış ve dekara çevrilerek yeşil ot verimi (kg/da) bulunmuştur. Daha sonra yaklaşık 1 kg yeşil ot örneği gölgede çevrilerek kurutulmuş ve kuru ot verimi (kg/da) hesaplanmıştır (Servet ve Ate, 2004). Kuru örnekler 0.5 mm elek açıklığında öğütülmüş ve kimyasal analizler (ham protein, ham selüloz, asit deterjanda çözünmeyen lif (ADF), nötr deterjanda çözünmeyen lif (NDF), kalsiyum (Ca), potasyum (K), magnezyum (Mg) ve fosfor (P) oranları) yapılmıştır. Ham protein (%) ve ham selüloz oranları (%) AOAC (2019)'a göre, NDF ve ADF oranları (%) ise Van Soest ve ark. (1991)'nın belirttikleri yöntem kullanılarak belirlenmiştir. Ca, K, Mg oranları (%) fleymfotometrede, P oranı (%) ise vanadamolibdofosforik sarı renk yöntemine göre spektrofotometrede okunmuştur (AOAC, 2019). Tüm analizler iki paralel yapılmıştır.

Elde edilen verilerin varyans analizleri TARIST (Açıkgöz ve ark., 1993) paket programı ile yapılmıştır. Bulunan sonuçların ortalama değerleri arasındaki farkların istatistikî anlamda önemlilikleri MSTAT-C (1989) istatistik programı kullanılarak belirlenmiştir.

## BULGULAR ve TARTIŞMA

Araştırmadan elde edilen sonuçlar tablo 1, 2, 3, ve 4'te verilmiştir.

En yüksek doğal konumdaki bitki boyu 81.24 cm ile Edirne lokasyonunda ölçülmüştür. Genotipler arasında ise en yüksek doğal konumdaki bitki boyu Töre çeşidinden (102.46 cm) elde edilmiş, genotip x lokasyon interaksiyonunda aynı çeşidin doğal konumdaki bitki boyu (107.75 cm) Edirne lokasyonunda en yüksek saptanmıştır. Araştırmada, bitki boyu bakımından genotip, lokasyon ve genotip x lokasyon interaksiyonu arasındaki farklılıklar istatistiki olarak %1 düzeyinde önemli bulunmuştur. Bitki boyu değerleri 59.62-188.42 cm arasında değişmiş ve en uzun bitki boyu Edirne lokasyonunda Taşkent çeşidinden (188.43 cm) elde edilmiştir. Açıkgöz ve ark. (2001) bezelyenin 39-189 cm arasında bitki boyuna sahip olduğunu belirtirken; Guy (2002) 60-75 cm arasında, Tekeli ve Ateş (2011) 50-100 cm arasında, Ateş ve Tekeli (2017) ise bitki boyunu ortalama 135.3 cm belirlemişlerdir. Bilgili ve ark. (2010) bezelyede en yüksek bitki boyunu Doğankent lokasyonunda yapraklı genotipler olan Gölyazı (185 cm) ve P98 (176.9 cm) ile yarı yapraklı Ulubatlı (172.9 cm) genotipinde saptamışlardır. Belirlenen bitki boyu değerleri araştırıcıların bulguları ile uyumludur. Lokasyonlar arasında bitkide dal sayısı bakımından fark bulunmamıştır (P>0.05). Genotip ve genotip x lokasyon interaksiyonu %5 düzeyinde önemlidir. Bitkide dal sayısı en fazla Edirne lokasyonunda P15 genotipinde (3.95 adet) bulunurken, aynı genotipin dal sayısı (3.83 adet) genotip ortalamaları arasında en fazladır. Tekeli ve Ates (2003), Servet ve Ate (2004), Gündüz (2013), Sayar ve Han (2016), Ateş ve Tekeli (2017), Tan ve Kadıoğlu (2018) ile Temel ve Keskin (2018) dal sayısını sırasıyla 3.6-5.5, 3.4-6.2, 2.1-7.25, 1.44-1.46, 4-6, 2-3.7 ve 2.1-3.3 adet/bitki arasında tespit etmişlerdir. Dal sayısına ait sonuçlar, Sayar ve Han (2016)'ın belirledikleri değerlerden yüksek, Ateş ve Tekeli (2017)'nin bulgularından düşük iken diğer araştırıcılarla benzerlik göstermektedir. Genotipler arasında ise sap çapı en fazla Töre (3.70 mm) ve Ateş (3.66 mm) çeşitleri ile P15 genotipinde (3.57 mm) saptanmıştır. Tekeli ve Ateş (2006) bezelyenin sap çapını 3-4 mm belirtirlerken; Sayar ve Anlarsal (2008), bezelyenin ana sap çapının 2.08-3.47 mm arasında değiştiğini ölçmüşlerdir. Tenikecier ve Ates (2021) bezelyede sap çapını ortalama 4.1 mm bulmuşlardır. Sonuçlar, bu araştırıcıların bulgularıyla yakınlık göstermektedir. Genotip (P<0.05) ile genotip x lokasyon interaksiyonun (P<0.01) yeşil ot verimine etkisi istatistiki olarak önemli bulunurken; lokasyonlar arasındaki yeşil ot verimleri önemsizdir (P>0.05). Genotipler incelendiğinde, en düşük yeşil ot verimi 4726 kg/da ile P15 genotipinde bulunmuştur.

**Tablo 1.** Farklı bezelye genotiplerinin iki lokasyondaki bazı morfolojik özellikleri

| | | Lokasyonlar | | |
|---|---|---|---|---|
| | **Genotipler** | **Malkara** | **Edirne** | **Ortalama** |
| | | **Genotip (G) x Lokasyon (L) İnteraksiyonu** | | |
| **Doğal Konumdaki Bitki Boyu (cm)** | **P10** | 46.12ı | 66.37f | 56.25c |
| | **P11** | 47.75hı | 49.80h | 48.77e |
| | **P15** | 50.40h | 55.85g | 53.12d |
| | **Töre** | 97.17c | 107.75a | 102.46a |
| | **Ateş** | 91.95e | 99.27b | 95.61b |
| | **Taşkent** | 92.85e | 95.90cd | 94.37b |
| | **MB95** | 93.67de | 93.77de | 93.72b |
| | **Ortalama** | 74.27b | 81.24a | |
| | **LSD** | L: 4.001* G: 3.107** G x L: 2.778** | | |
| **Bitki Boyu (cm)** | **P10** | 61.82gh | 66.25fgh | 64.03c |
| | **P11** | 59.62h | 70.77fg | 65.20c |
| | **P15** | 60.55h | 71.20f | 65.87c |
| | **Töre** | 141.47de | 165.92b | 153.70b |
| | **Ateş** | 135.80e | 158.10bc | 146.95b |
| | **Taşkent** | 141.90de | 188.42a | 165.16a |
| | **MB95** | 149.07cd | 159.97b | 154.52b |
| | **Ortalama** | 107.17b | 125.80a | |

| | | | | |
|---|---|---|---|---|
| | **LSD** | L: 10.079** G: 10.116** G x L: 9.048** | | |
| **Bitkide Dal Sayısı (adet)** | | | | |
| | **P10** | 3.65b | 3.02d | 3.33b |
| | **P11** | 3.45c | 3.35c | 3.40b |
| | **P15** | 3.72b | 3.95a | 3.83a |
| | **Töre** | 2.32f | 2.35f | 2.33d |
| | **Ateş** | 2.35f | 2.35f | 2.35d |
| | **Taşkent** | 2.65e | 2.65e | 2.65c |
| | **MB95** | 2.57e | 2.57e | 2.57c |
| | **Ortalama** | 2.961 | 2,893 | |
| | **LSD** | G: 0.187** G x L: 0.167** | | |
| **Sap Çapı (mm)** | | | | |
| | **P10** | 3.10g | 3.32ef | 3.21cd |
| | **P11** | 3.05g | 3.30ef | 3.17d |
| | **P15** | 3.47cd | 3.67b | 3.57a |
| | **Töre** | 3.85a | 3.55bc | 3.70a |
| | **Ateş** | 3.47cd | 3.85a | 3.66a |
| | **Taşkent** | 3.40def | 3.40def | 3.40b |
| | **MB95** | 3.27 f | 3.42cde | 3.35bc |
| | **Ortalama** | 3.37 | 3.50 | |
| | **LSD** | G: 0.161** G x L: 0.143** | | |

*: P<0.05, ***: P<0.01

**Tablo 2.** Farklı bezelye genotiplerinin iki lokasyondaki ot verimleri ile ham protein ve selüloz oranları

| | **Lokasyonlar** | | |
|---|---|---|---|
| **Genotipler** | **Malkara** | **Edirne** | **Ortalama** |
| | **Genotip (G) x Lokasyon (L) İnteraksiyonu** | | |
| **Yeşil Ot Verimi (kg/da)** | | | |
| **P10** | 4954.50d | 6519.00a | 5736.75a |
| **P11** | 4347.00e | 6090.00ab | 5218.50ab |
| **P15** | 4112.00e | 5340.00cd | 4726.00b |
| **Töre** | 5815.00bc | 5655.00bc | 5735.00a |
| **Ateş** | 5852.00bc | 5444.00cd | 5648.00a |
| **Taşkent** | 5387.50cd | 5516.00c | 5451.75a |
| **MB95** | 5698.00bc | 5391.00cd | 5544.50a |
| **Ortalama** | 5166.57 | 5707.85 | |
| **LSD** | G: 576.82* G x L: 515.95** | | |
| **Kuru Ot Verimi (kg/da)** | | | |
| **P10** | 974.25 | 1269.25 | 1121.75b |
| **P11** | 882.00 | 1175.00 | 1028.50b |
| **P15** | 863.50 | 1111.00 | 987.25b |
| **Töre** | 1369.25 | 1497.75 | 1433.50a |
| **Ateş** | 1332.75 | 1445.25 | 1389.00a |
| **Taşkent** | 1275.75 | 1540.50 | 1408.12a |
| **MB95** | 1268.75 | 1399.00 | 1333.87a |
| **Ortalama** | 1138.03 | 1348.25 | |
| **LSD** | G: 142.960** | | |
| **Ham Protein (%)** | | | |
| **P10** | 17.67h | 18.24ef | 17.96d |
| **P11** | 17.74h | 18.40cd | 18.07d |
| **P15** | 17.48ı | 18.10g | 17.79e |
| **Töre** | 18.36def | 19.75a | 19.05b |

| | | | | |
|---|---|---|---|---|
| | **Ateş** | 18.82b | 19.79a | 19.30a |
| | **Taşkent** | 18.52c | 19.67a | 19.09b |
| | **MB95** | 18.23fg | 18.38de | 18.30c |
| | **Ortalama** | 18.12b | 18.90a | |
| | **LSD** | L: 0.267** G: 0.154** G x L: 0.137** | | |
| **Ham Selüloz (%)** | | | | |
| | **P10** | 23.18 | 23.19 | 23.18 |
| | **P11** | 23.26 | 23.21 | 23.24 |
| | **P15** | 23.23 | 23.22 | 23.22 |
| | **Töre** | 23.35 | 23.14 | 23.24 |
| | **Ateş** | 23.22 | 23.19 | 23.21 |
| | **Taşkent** | 23.13 | 23.22 | 23.17 |
| | **MB95** | 23.23 | 23.22 | 23.22 |
| | **Ortalama** | 23.18 | 23.19 | |
| | **LSD** | Önemli değil (ÖD) | | |

*: P<0.05, ***: P<0.01, ÖD: P>0.05

**Tablo 3.** Farklı bezelye genotiplerinin iki lokasyondaki NDF, ADF, Ca ve Mg oranları

| | **Lokasyonlar** | | |
|---|---|---|---|
| **Genotipler** | **Malkara** | **Edirne** | **Ortalama** |
| | **Genotip (G) x Lokasyon (L) İnteraksiyonu** | | |
| **P10** | 37.87 | 37.81 | 37.84 |
| **P11** | 38.00 | 37.86 | 37.93 |
| **P15** | 38.06 | 38.03 | 38.05 |
| **Töre** | 38.13 | 37.81 | 37.97 |
| **Ateş** | 37.90 | 38.12 | 38.01 |
| **Taşkent** | 38.02 | 38.07 | 38.05 |
| **MB95** | 37.77 | 38.00 | 37.89 |
| **Ortalama** | 37.96 | 37.96 | |
| **LSD** | Önemli değil (ÖD) | | |
| **ADF (%)** | | | |
| **P10** | 28.65 | 28.98 | 28.81 |
| **P11** | 28.94 | 28.98 | 28.96 |
| **P15** | 28.91 | 28.98 | 28.94 |
| **Töre** | 28.92 | 28.99 | 28.95 |
| **Ateş** | 28.77 | 28.93 | 28.85 |
| **Taşkent** | 28.98 | 29.00 | 28.99 |
| **MB95** | 28.97 | 28.96 | 28.97 |
| **Ortalama** | 28.88 | 28.97 | |
| **LSD** | ÖD | | |
| **Ca (%)** | | | |
| **P10** | 1.54 | 1.55 | 1.54 |
| **P11** | 1.55 | 1.55 | 1.55 |
| **P15** | 1.55 | 1.54 | 1.55 |
| **Töre** | 1.55 | 1.55 | 1.55 |
| **Ateş** | 1.55 | 1.55 | 1.55 |
| **Taşkent** | 1.56 | 1.54 | 1.55 |
| **MB95** | 1.55 | 1.55 | 1.55 |
| **Ortalama** | 1.55 | 1.55 | |
| **LSD** | ÖD | | |
| **M g (** | | | |

| | | | | |
|---|---|---|---|---|
| | **P10** | 0.40 | 0.40 | 0.40 |
| | **P11** | 0.41 | 0.41 | 0.41 |
| | **P15** | 0.41 | 0.39 | 0.40 |
| | **Töre** | 0.41 | 0.40 | 0.40 |
| | **Ateş** | 0.40 | 0.41 | 0.40 |
| | **Taşkent** | 0.41 | 0.40 | 0.40 |
| | **MB95** | 0.40 | 0.40 | 0.40 |
| | **Ortalama** | 0.40 | 0.40 | |
| | **LSD** | ÖD | | |

ÖD: P>0.05

**Tablo 4.** Farklı bezelye genotiplerinin iki lokasyondaki K ve P oranları

| | **Lokasyonlar** | | | |
|---|---|---|---|---|
| | **Genotipler** | **Malkara** | **Edirne** | **Ortalama** |
| | **Genotip (G) x Lokasyon (L) İnteraksiyonu** | | | |
| **K (%)** | **P10** | 1.69 | 1.71 | 1.70 |
| | **P11** | 1.69 | 1.70 | 1.69 |
| | **P15** | 1.69 | 1.70 | 1.70 |
| | **Töre** | 1.69 | 1.72 | 1.70 |
| | **Ateş** | 1.69 | 1.70 | 1.70 |
| | **Taşkent** | 1.70 | 1.70 | 1.70 |
| | **MB95** | 1.68 | 1.69 | 1.68 |
| | **Ortalama** | 1.69 | 1.70 | |
| | **LSD** | Önemli değil (ÖD) | | |
| **P (%)** | **P10** | 0.31 | 0.31 | 0.31 |
| | **P11** | 0.32 | 0.31 | 0.31 |
| | **P15** | 0.31 | 0.31 | 0.31 |
| | **Töre** | 0.31 | 0.33 | 0.32 |
| | **Ateş** | 0.30 | 0.32 | 0.31 |
| | **Taşkent** | 0.31 | 0.31 | 0.31 |
| | **MB95** | 0.31 | 0.31 | 0.31 |
| | **Ortalama** | 0.31 | 0.31 | |
| | **LSD** | ÖD | | |

ÖD: P>0.05

Genotip x lokasyon interaksiyonunda ise en düşük yeşil ot verimi Malkara lokasyonunda 4112 kg/da ile P15 genotipi ile 4347 kg/da ile P11 genotipinde tespit edilmiştir (Tablo 2). En düşük kuru ot verimleri ise P15 (987.25 kg/da), P11 (1028.50 kg/da) ve P10 (1121.75 kg/da) genotiplerinde bulunmuştur. İnteraksiyonların önemli çıkması beklenmekteyse de tekrarlamalar arasındaki farkın fazla olması nedeniyle istatistiki olarak önemli çıkmamıştır. Açıkgöz ve ark. (2001) bezelyeden 236-901 kg/da arasında kuru madde verimi alınabileceğini ifade ederlerken, Tekeli ve Ateş (2011) bezelyenin 500-1400 kg/da arasında değişen kuru ot verimine sahip olduğunu belirtmektedir.

Genotip, lokasyon ve genotip x lokasyon interaksiyonunun ham protein oranı üzerine etkileri istatiksel olarak önemli bulunmuştur (P<0.01, Tablo 2). En yüksek ham protein oranı Edirne lokasyonunda Töre, Ateş ve Taşkent çeşitlerinde (%19.67-19.79) tespit edilirken, en düşük ham protein oranı Malkara lokasyonunda (%17.48) saptanmıştır. Genotip, lokasyon ve genotip x lokasyon interaksiyonun ham selüloz, ADF, NDF, Ca, Mg, K ve P oranlarına etkisi istatistiki olarak önemli bulunmamıştır (P>0.05, Tablo 2, 3 ve 4). Ham selüloz oranı %23.13-23.55 arasında, NDF oranı %37.77-38.13 arasında, ADF oranı%28.65-29.00 arasında, Ca oranı %1.54-1.56 arasında, Mg oranı %0.39-0.41 arasında, P oranı %0.30-0.33 arasında ve K oranı %1.68-1.72 arasında değişmiştir. Ham protein ve selüloz oranlarına ait bulgular, Tekeli ve Ates (2003), Timurağaoğlu ark. (2004) ile Tekeli ve Ateş (2007)'in sonuçları ile benzerlik gösterirken; NDF ve ADF oranlarına ait değerler Tekeli ve Ateş (2007)'in sonuçlarından düşüktür. Ates ve ark. (2020) bezelyede Mg ve P oranlarını sırasıyla

%0.39-0.44 ve %0.26-0.34 aralığında belirlemiştir. Çalışmamız bu araştırmacıların sonuçları ile paralellik göstermektedir.

## SONUÇ

Yüksek yeşil ot verimi ve bazı kalite özellikleri bakımından P10 genotipi yeşil ot üretimi amacıyla, kuru ot verimi yüksek ve bazı kalite özellikleri iyi olan MB95 genotipi ümitvar genotipler olup bu genotiplerin farklı lokasyonlardaki çalışmaları sürdürüldükten sonra çeşit tescili mümkün olabilecektir.

## TEŞEKKÜR

Bu makale Tekirdağ Namık Kemal Üniversitesi Fen Bilimleri Enstitüsü tarafından 2022 yılında kabul edilmiş olan Mert BAŞ'a ait Yüksek Lisans Tezinin bir bölümü olup sağladığı destek ve katkılardan dolayı adı geçen enstitüye teşekkür ederiz.

## KAYNAKLAR

Açıkgöz E, Uzun A, Bilgili U, Sincik M 2001. Bezelye (*Pisum sativum* L.) çeşitleri arasında yapılan melezlemelerle geliştirilen hatların verim ve bazı kalite özellikleri. Türkiye 4. Tarla Bitkileri Kongresi, 73-77 s. Tekirdağ.

Açıkgöz N, Akkaş E, Moghaddam A, Özcan K 1993. TARİST, Pc'ler İçin Türkçe İstatistik Paketi. Ulusal Ekonometri ve İstatistik Sempozyumu, İzmir.

AOAC 2019. Association of Official Analytical Chemists. The 21st Ed., Maryland, USA.

Arslan B, Culpan E, Ateş E. 2016. A New Alternative Species as a Medicinal, Aromatic and Forage Crop: Blue Melilot (*Melilotus caeruleus* (L.) Desr.). Journal of Agricultural Faculty of Uludag University, 30 (Special Issue): 392-394.

Ates E, Tenikecier, HS, Ozkan U 2020. The dry matter yield, α-tocopherol, β-carotene and some mineral contents in fodder pea (*Pisum arvense* L.) varieties at different growth stages. Comptes rendus de l'Académie bulgare des Sciences, 73: 579-586.

Ateş E, Tekeli AS 2017. Farklı taban gübresi uygulamalarının yem bezelyesi (*Pisum arvense* L.)'nin ot verimi ve kalitesine etkisi. KSÜ Doğa Bilimleri Dergisi, 20: 13-16.

Bilgili U, Uzun A, Sincik M, Yavuz M, Aydinoglu B, Cakmakci S, Geren H, Avcioglu R, Nizam I, Tekeli AS, Gul I, Anlarsal E, Yucel C, Avci M, Acar Z, Ayan I, Üstün A, Acikgoz E 2010. Forage yield and lodging traits in peas (*Pisum sativum* L.) with different leaf types. Turkish Journal of Field Crops, 15: 50-53.

Düzgüneş O, Bakır Ö, Aksoy S, Akyüz Ö, Alınoğlu N 1965. Mer'alarımızla ilgili problemler ve çözüm önerileri. T.M.M.O.B. Ziraat Mühendisleri Odası Yayınları, Sıra No: 21, s.11.

Guy S 2002. Evaluation of wheat and pea varieties under direct and conventional seeding in Washington, Idaho Oregon. Steep III Progress Report. Pacific Northwest Conservation Tillage System Information Source. USA

Gündüz H. 2013. Kuzeydoğu Anadolu Bölgesi popülâsyonundan seçilen yem bezelyesi hatlarının bazı morfolojik ve tarımsal özellikleri (Yüksek Lisans Tezi), Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum.

MSTAT-C 1989. Mstat-C, A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments. Michigan State University, ABD.

Sayar MS, Anlarsal AE 2008. Diyarbakır ekolojik koşullarında bazı yem bezelyesi (*Pisum arvense* L.) hat ve çeşitlerinin verim ve verim öğelerinin belirlenmesi üzerine bir araştırma. Çukurova Üniversitesi Fen Bilimleri Enstitüsü, 17: 78- 88.

Sayar MS, Han Y 2016. Forage yield performance of forage pea (*Pisum sativum* spp. *arvense* L.) genotypes and assessments using GGE Biplot analysis. Journal of Agricultural Science and Technology, 18:1621-1634

Servet A, Ate E 2004. Determination of some agricultural characters in field pea (*Pisum arvense* L.) lines at Tekirdağ (Turkey) ecological conditions. Cuban Journal of Agricultural Science, 38: 313-316.

Tan M, Kadıoğlu S 2018. Erzurum şartlarında farklı tarihlerde kışlık ekilen yem bezelyesi çeşitlerinin verim ve bazı özellikleri. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 27: 25-32.

Tekeli AS, Ates E 2003. Yield and its components in field pea (*Pisum arvense* L.) lines. Journal of Central European Agriculture, 4: 313-318.

Tekeli AS, Ateş E 2006. Baklagil Yem Bitkileri. Trakya Üniversitesi Tekirdağ Ziraat Fak. Yayın No.293, Ders Kitabı No.42, s.153.

Tekeli AS, Ateş E 2007. Farklı biçim dönemlerinin yem bezelyesi (*Pisum arvense* L.)-buğday (*Triticum aestivum* L.) karışımının yem verimi ve kalitesi ile tetani oranına etkileri. Türkiye VII. Tarla Bitkileri Kongresi. 25-27 Haziran 2007 Erzurum, s. 106-109.

Tekeli AS Ateş E 2011. Baklagil Yem Bitkileri (Yenilenmiş II. Baskı). Sevil Grafik Tasarım ve Cilt Evi, Tekirdağ.

Temel S, Keskin B 2018. Iğdır koşullarında farklı yem bezelyesi çeşitleri (*Pisum sativum* ssp. *arvense* L.) ve ekim zamanlarının bazı ot verim özelliklerine etkisi. I. International Iğdır Congress on Multidisciplinary Studies, November 6-8, Iğdır, Türkiye, s. 315-325.

Tenikecier HS, Ates E 2021. Yield, some cell wall component and mineral contents of fodder pea (*Pisum sativum* ssp. *arvense* L. Poir) forage as influenced by cultivar, growth stages and phosphorus application. Journal of Elementology, 26: 319-332.

Timurağaoğlu KA, Genç A, Altınok S 2004. Ankara koşullarında yem bezelyesi hatlarında yem ve tane verimleri. Tarım Bilimleri Dergisi, 10: 457-461.

Van Soest PJ, Robertson JB, Lewis BA 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. Jornal of Dairy Science, 74: 3583-3597.

Yıldırım B, Togay N, Togay Y, Dogan Y, Tamkoç A 2005. Determining agronomic properties of some pea genotypes. Research Journal of Agriculture and Biological Sciences, 1: 315-319.

## *ORAL PRESENTATION*

# Nanostructured ZnO/CuO composite thin films with capping agent of HDTMA-Br for future electronics

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**Abstract**

In this work, composite thin films of zinc oxide/copper oxide (ZnO/CuO) capped with the surfactant hexadecyltrimethyl Ammonium Bromide (HDTMA-Br) were synthesized on glass slides using a simple and inexpensive SILAR technique. The different concentrations of HDTMA-Br (1.0%, and 2.0%) were used to study the influence of the surfactant on the structural, morphological and optical properties of the ZnO/CuO composite thin films. The as-prepared samples were characterized by X-ray diffraction (XRD), scanning probe microscopy (SPM), scanning emission microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), and UV–Vis spectrometry. The results show that the HDTMABr molecules bind strongly to ZnO/CuO surface via their head groups ($Br^-$). The crystallite size of the composite films changed from 11.08 to 9.17 nm. The optical band gap and transmittance of the films changed significantly with the HDTMA-Br capping as a result of the crystallinity quality. The obtained results show that addition of the HDTMA-Br to the ZnO/CuO composite samples is responsible for regulating the physical properties of optoelectronic devices.

**Keywords:** SILAR, ZnO/CuO, HDTMA-Br, Surfactant

## INTRODUCTION

Nanostructured metal-oxide semiconductors (MO) such as nickel oxide (NiO), zinc oxide (ZnO), cadmium oxide (CdO), copper oxide (CuO), titanium dioxide ($TiO_2$), tungsten oxide ($WO_3$) represents one of the most important groups of beneficial equipment due to their richness in practical use and the possibilities of adding value for continuous performance and quality development (Mishra et. al., 2021). MO have an exptensive range of applications in various fields such as solar cells, catalysis, supercapacitors, gas sensors, and thin-film transistors, thanks to their perfect chemical, electronic and optical properties (Zhang et. al.,2006; Shen et. al., 2009; Sadri et. al., 2014; Miller et. al., 2014; Zhou et. al., 2019). Among these oxides, CuO and ZnO are used in many fields, as they can be used in electrical engineering, optoelectronics, sensor technology, catalysis, as photocatalysts and in solar cells (Sathishkumar et. al., 201). CuO is a p-type semiconductor with a bandgap of 1.2 eV that exhibits photovoltaic, field-emitting, photoconductive, storage devices, and gas sensing properties (Moraless et, al., 2005; Zhu et. al., 2012; Rashad et. al., 2013; Sone et. al., 2020). ZnO is an n-type semiconductor with a bandgap of 3.37 eV, which offers a broad application potential for photocatalysts, luminescence, gas and biosensors, piezoelectric materials, and solar cells (Xu et. al., 2009; Taşdemir et. al., 2021).

Recently, MO composites have potential importance in many technological applications such as gas and humidity sensors, solar cells, photovoltaic devices, battery materials, detectors and fuel cells due to their unique structural, electrical, optical and thermal properties. In paticular, ZnO/CuO composites have recently attracted much interest due to their potential use as photocatalysts, for photovoltaic applications, gas and humidity sensors, and solar cells (Wei et. al., 2010; Sathishkumar et. al., 2011; Jung and Yong, 2011; Şahin and Tolga, 2021; Safdar et. al., 2022).

Hexadecyltrimethylammonium bromide (HDTMA-Br) is an important cationic surfactant with a long chain structure, compared to other surfactants, of 16 carbons attached to the ammonium head group with three methyl groups. It has been widely used for metastable modifications of metal oxides due to the electrostatic interaction between metal oxide materials and surfactant molecules (Wang et. al., 2009). Therefore, the electrostatic interactions between the head group of the HDTMA-Br and the ZnO/CuO composite material can help to confine the particles to nanosized (Varghese et al., 2017; Ganesan et al., 2017).

In this study, we investigated the impact of surfactants on the structural, morphological and optical properties of ZnO/CuO nanostructured composite thin films using a surfactant HDTMA-Br as a capping agent. The effects on the main physical parameters of ZnO/CuO composite thin films were investigated as a function of the concentration of the surfactant HDTMA-Br.

## MATERIALS AND METHODS

The nanostructured ZnO/CuO, 1.0% HDTMA-Br: ZnO/CuO and 2.0% HDTMA-Br: ZnO/CuO composite films were deposited by SILAR method. For the ZnO/CuO composite thin films, aqueous solutions were first mixed with 50 ml of 0.1 M zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) and 50 ml of 0.1 M copper (II) chloride dehydrate ($CuCl_2{\cdot}2H_2O$). To obtain a homogeneous solution from the obtained mixture, it was stirred for a few minutes at room temperature in a magnetic stirrer. Ammonium hydroxide ($NH_4OH$) was then added to adjust the pH (~10.0) of the aqueous solution. Soda-lime glass substrates were first immersed in the SILAR solution heated at 85 °C, and then in DI water at the same temperature, with an immersion time of 20 seconds (see Figure 1). In order to achieve a good quality of the ZnO/CuO nanocomposite thin film, the deposition cycle in this process was set to 12 (Fig. 1.). Finally, the acquired composite films were air annealed at 190 °C for 90 minutes, based on previous research results (Altinay et. al.,2022, Dinc et. al.,2020). To synthesize 1.0 % and 2.0 % HDTMA-Br-capped zinc oxide/copper oxide (ZnO/CuO) composite thin films, 1.0 % and 2.0 % HDTMA-Br were added to the $Zn(CH_3COO)_2{\cdot}2H_2O$ and $CuCl_2{\cdot}2H_2O$ precursor solution, respectively and the procedure was repeated.

The crystallite structure and phase composition of the synthesized thin films were determined using an X-ray diffraction (XRD) diffractometer (Rigaku SmartLab, 40 kV, 30 mA). The morphology and structure of the films were characterized by scanning electron microscopy (SEM). The absorption and transmittance spectra of the films were evaluated using a UV–Visible spectrometer (Thermo Scientific Genesys 10S UV–Vis., range 190 - 1100 nm). Surface topography measurements of the ZnO/CuO composite films were performed using the scanning probe microscopy (SPM).




**Figure 1.** The scheme of the SILAR method for the deposition of ZnO/CuO thin films.

## RESULTS and DISCUSSIONS

Figure 2a and 2b show the XRD patterns of nanostructured ZnO/CuO composite thin films capped with 0, 1.0% and 2.0% HDTMA-Br and ZnO and CuO with ICDD card number of uncapped ZnO/CuO thin films, respectively. The XRD results show the presence of diffraction peaks of the polycrystalline hexagonal wurtzite structure of ZnO with space group P63mc and the monoclinic tenorite structure of CuO with space phase 15: C12/c1. As can be seen in Figure 2, no peaks related to impurity were observed in the XRD pattern. Moreover, the thin films showed well-crystallized diffraction peaks.

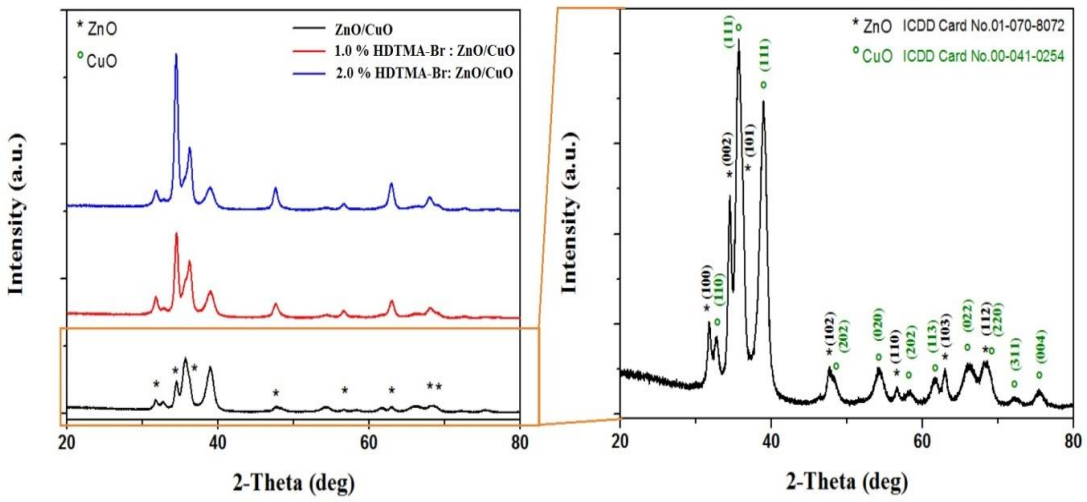


**Figure 2. (a)** XRD pattern of nanostructured ZnO/CuO composite thin films, and **(b)** ICDD card no. 01-070-8072 and 00-041-0254 of ZnO and CuO, respectively.

**Table 1.** The relative peak intensity and calculated average crystallite size values of the ZnO/CuO composite films at different HDTMA-Br concentrations.

| Sample Name | Relative Peak Intensity (a.u.) | | | | Crystallite size (nm) |
|---|---|---|---|---|---|
| | ZnO | | CuO | | |
| | (100) | (002) | ($\bar{1}$11) | (111) | |
| ZnO/CuO | 3270 | 11580 | 22930 | 18780 | 11.08 |
| 1.0 % HDTMA-Br: ZnO/CuO | 7530 | 38900 | 14060 | 11320 | 10.42 |
| 2.0 % HDTMA-Br : ZnO/CuO | 6120 | 77550 | 26070 | 8950 | 9.17 |

Table 1 shows that the relative peak intensity and the calculated average crystallite size values agree well with the values obtained from the ICDD card number for ZnO and CuO (Tewari and Bhattacharjee, 2011; Daoudi et al., 2019). The average crystallite size of the thin films is estimated according to the following Debye-Scherrer formula (Tasdemir et al., 2021):

$$D = \frac{K\lambda}{\beta cos\theta} \quad (1)$$

where the $K$ is a constant (0.94), $\lambda$ is the X-ray wavelength (1.5406 A$^o$ for Cu Kα), $\beta$ is the full width half maximum (FWHM) of the peak relating to $2\theta$. As can be seen from the Table 1, an increase in the relative peak intensity of the (002) plane was observed for ZnO with the addition of HDTMA-Br, while a decrease in the peak intensity of the (111) plane was observed for CuO. This decrease and increase in peak intensity is associated with a decrease and increase in lattice strain and average crystallite size, respectively (Varghese et. al., 2017). The average crystallite size of ZnO/CuO thin films decreases with the increase of HDTMA-Br concentration. This decrease in average crystallite size indicates that the increase in peak intensity of some plane with HDTMA-Br concentration does not affect the overall structure of ZnO/CuO composite films.

The FT-IR spectrum of ZnO/CuO thin films with and without HDTMA-Br is shown in Figure 3. By examining the transmittance spectra, Zn-O and Cu-O vibrational bands, hydroxyl OH and C-O bands can be seen. Moreover, the peak at 1422 cm$^{-1}$ in the thin films with 1.0 % and 2.0 % HDTMA-Br is due to C-H bending vibration. HDTMA-Br is due to the vibrations of the aromatic rings. In particular, in the pure HDTMA-Br spectrum, the bands in the 1400-1500 cm$^{-1}$ region are caused by C-H bending vibrations (Su etc., 2014). The FTIR spectrum of the HDTMA-Br agreed well with previous work (Kasilingam et al., 2014; Su et. al., 2014; Varghese et. al., 2017). The appearance of some bands in thin films with HDTMA-Br is evidence that the surfactant acts sufficiently on the surface of the ZnO /CuO nanoparticles.

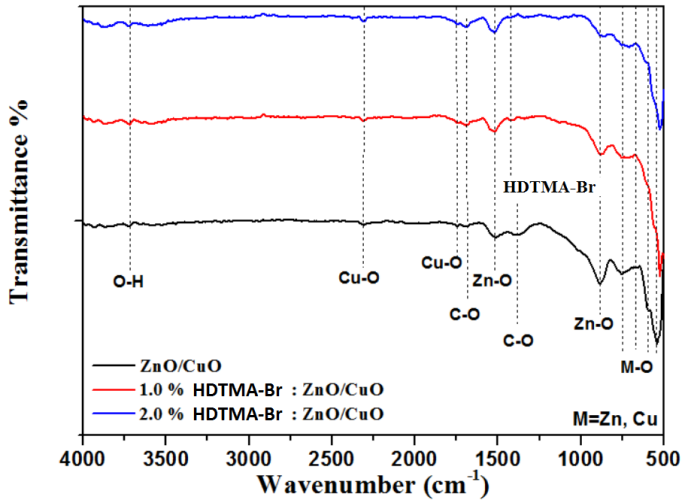


**Figure 3.** FT-IR spectrum of ZnO/CuO, 1.0 % HDTMA-Br : ZnO/CuO and, 2.0 % HDTMA-Br : ZnO/CuO composite thin films.

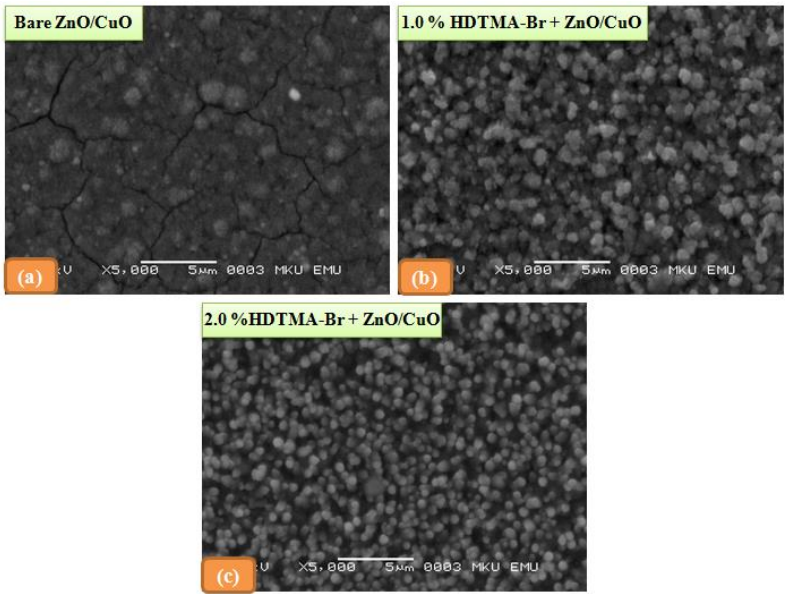


**Figure 4.** SEM images of **(a)** ZnO/CuO, **(b)** 1.0 % HDTMA-Br: ZnO/CuO and, **(c)** 2.0 % HDTMA-Br: ZnO/CuO composite thin films. Images are with 5000x magnification and 5 µm resolution.

SEM measurement of the surface morphology of ZnO/CuO thin films was performed. Fig. 4 shows the SEM images of ZnO/CuO and 1.0 % and 2.0 % HDTMA-Br capped ZnO/CuO thin films. It can be clearly seen from the images of SEM that due to the increase of HDTMA-Br additive content on the ZnO/CuO thin film, spherical grains are observed to be distributed approximately homogeneously on the surface. The size of these grains decreased with increasing HDTMA-Br concentration. The results are in good agreement with the XRD results.

Figure 5 also shows the SPM images (20 µm × 20 µm scan area) of thin films in 2D and 3D. While the grain size of the uncapped ZnO/CuO thin films varied irregularly, the grain size of the HDTMA-Br capped ZnO/CuO thin films decreased with increasing concentration, and their shape was almost uniform and homogeneously distributed. The obtained results are in good agreement with SEM and XRD measurements.

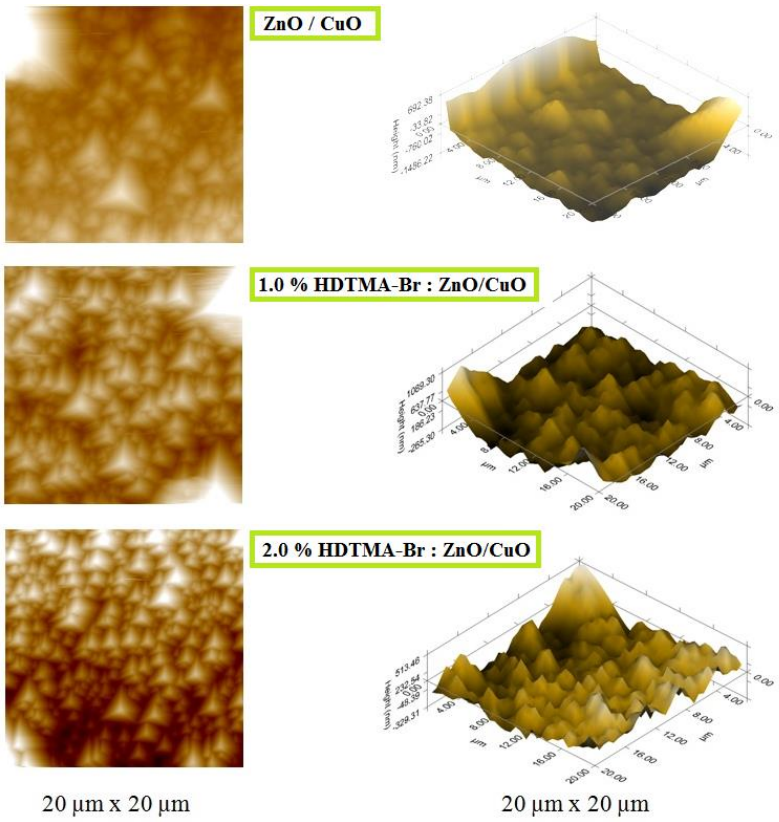


**Figure 5.** SPM images of ZnO/CuO, 1.0 % HDTMA-Br : ZnO/CuO and, 2.0% HDTMA-Br : ZnO/CuO composite thin films in 2-D and 3-D.

Figure 6 shows that the average roughness ($R_a$) and root mean square roughness ($R_q$) of the ZnO/CuO films change significantly with the addition of HDTMA-Br. Consistent SEM images (Fig. 4) show a smoother surface due to the increased HDTMA-Br concentration ratio. This result indicates that the surface roughness of the bare ZnO/CuO composite thin film is higher than that the ZnO/CuO thin films capped with HDTMA-Br. All the obtained findings are in perfect agreement with the results of SEM and XRD investigations.

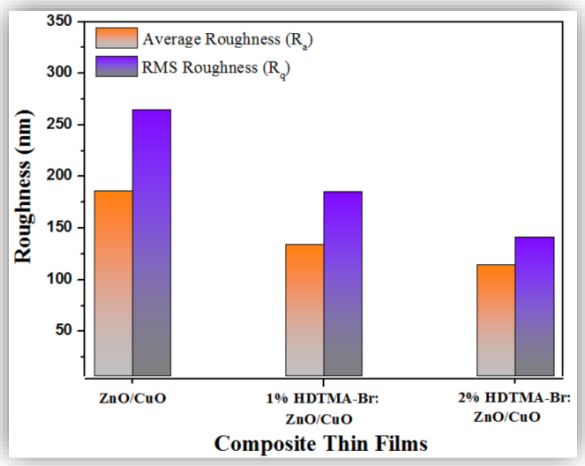


**Figure 6.** Average ($R_a$) and RMS ($R_q$) roughness of ZnO/CuO, 1.0 % HDTMA-Br : ZnO/CuO and, 2.0% HDTMA-Br : ZnO/CuO composite thin films.

The optical bandgaps of the thin films have been determined using the Tauc's plot fomula (Swain et. al., 2003); (Fig. 7(a))

$$\alpha h\nu = A\left(h\nu - E_g\right)^n \quad (2)$$

where, n is the photon's frequency (n=1/2 for direct allowed transitions), $\alpha$ is the absorption coefficient, $E_g$ is the optical bandgap energy and A is the absorbance. Fig. 7(a) shows that the optical bandgap energy of ZnO/CuO samples with bare, 1.0 % and 2.0 % HDTMA-Br are 1.76, 1.80 and 1.86 eV, respectively. This tendency toward lower wavelengths is primarily due to the quantum size effect in the nanostructured ZnO/CuO thin films with smaller grain size (Varghese et. al.,2017). Figure 7(b) shows the UV–Vis transmittance spectra

of ZnO/CuO films with and without HDTMA-Br. It can be seen that the 2.0% HDTMA-Br:ZnO/CuO film has the highest transmission. The light transmittance increased with the addition of HDTMA-Br. This change in transmittance can be attributed to the recombination of electrons in the conduction band with holes in the valence band. Consequently, this may affect the crystalline properties of the structure (Kumar, 2013)

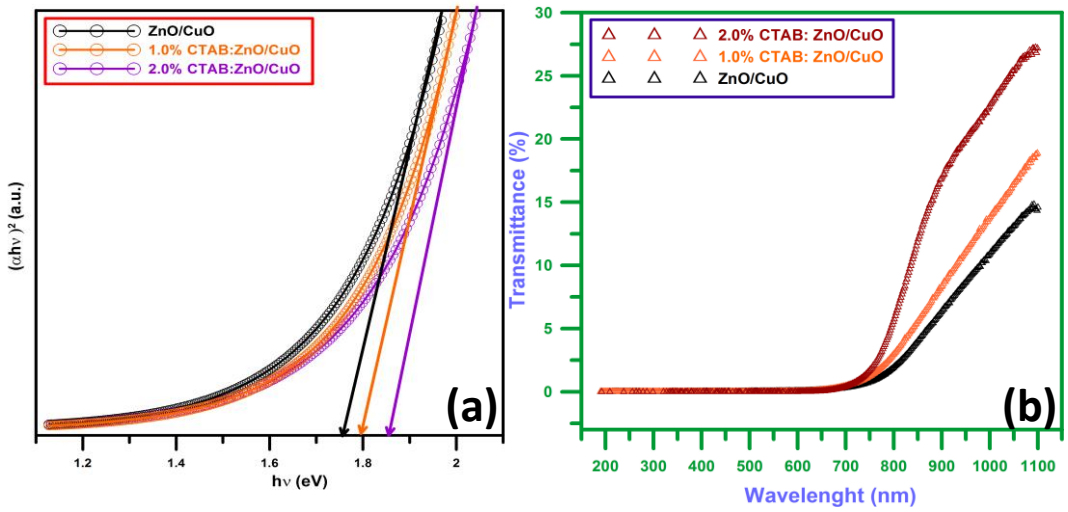


**Figure 7. (a)** Touch plots of $(\alpha h\upsilon)^2$ versus photon energy of ZnO/CuO thin films. **(b)** UV-Vis **t**ransmittance spectra of ZnO/CuO, 1.0% HDTMA-Br : ZnO/CuO and, 2.0% HDTMA-Br : ZnO/CuO composite thin films

## CONCLUSION

ZnO/CuO thin films with nanocomposite structure were prepared by SILAR method, which is an easy and low-cost. The effect of surfactant (HDTMA-Br) on the crystal structure, surface morphology, surface topography, molecular band structure and optical properties of ZnO and CuO nanostructures was investigated. The results show that, the behavior of ZnO/CuO thin films changes with the addition of HDTMA-Br. XRD, SEM and SPM results show that the addition of the surfactant to the ZnO/CuO thin films significantly improves the crystalline properties, surface morphology and topography of the formed structure. The lowest transmittance of ~14 % was obtained for the bare ZnO/CuO composite films. We concluded that by capping nanostructured ZnO/CuO composite films with a surfactant (HDTMA-Br), important parameters (such as crystallite size, light transmittance, surface morphology and topography, optical bandgap) of the structure can be improved, adjusted and controlled.

## REFERENCES

Altinay Y, Gökoğlan E, Yener Ç, Ünlü G & Şahin B 2022. SILAR processing and characterization of bare and graphene oxide (GO) and reduced graphene oxide (rGO)-doped CuO thin films. Applied Physics A, 128: 784.

Daoudi O, Qachaou Y, Raidou A, Nouneh K, Lharch M, Fahoume M 2019. Study of the physical properties of CuO thin films grown by modified SILAR method for solar cells applications. Superlattices and Microstructures,127: 93-99.

Dinc S, Şahin B, Kaya T 2020. Improved sensing response of nanostructured CuO thin films towards sweat rate monitoring: Effect of Cr doping. Materials Science in Semiconductor Processing 98: 1–8.

Donna V, Tom C and Chandar N K 2017. Effect of CTAB on structural and optical properties of CuO nanoparticles prepared by coprecipitation route. IOP Conf. Series: Materials Science and Engineering 263: 022002.

Ganesan KP, Anandhan N, Dharuman V, Sami P, Panneerselvam R, Marimuthu T 2017. Electrochemically modified crystal orientation, surface morphology and optical properties using CTAB on $Cu_2O$ thin films. Results in Physics 7: 82-86.

Jung S and Yong K 2011. Fabrication of CuO–ZnO nanowires on a stainless steel mesh for highly efficient photocatalytic applications. Chemical Communications, 47: 2643-2645.

Kumar V, Singh N, Mehra RM, Kapoor A, Purohit LP, Swart HC 2013. Role of film thickness on the properties of ZnO thin films grown by sol-gel method., Thin Solid Films, 539:161–165.

Miller DR, Akbar SA, Morris PA 2014.Nanoscale metal oxide-based heterojunctions for gas sensing: a review. Sens. Actuators B Chem., 204 :250-272.

Mishra S K, Tripathi UK, Awasthi RR, Shukla RK, Kumar I, Naik R M, Mishra DP 2021. CTAB mediated synthesis of ZnO nanoparticles: Structural, optical and enhanced blue-green optical emission. Materials Today: Proceedings, 46 (6): 2229-2234.

Morales J, Sánchez L, Martín F, Ramos-Barrado J R and Sánchez M 2005. Use of low temperature nanostructured CuO thin films deposited by spray-pyrolysis in lithium cells. Thin Solid Films 474 133–40.

Rashad M, Rüsing M, Berth G, Lischka K, and Pawlis A 2013. CuO and Co3O4 Nanoparticles: Synthesis, Characterizations, and Raman Spectroscopy. Journal of Nanomaterials, 82:82

Sadri F, Ramazani A, Massoudi A, Khoobi M, Tarasi R, Shafiee A, Azizkhani V, Dolatyari L, Joo SW 2014.Green oxidation of alcohols by using hydrogen peroxide in water in the presence of magnetic Fe3O4 nanoparticles as recoverable catalyst. Green Chem. Lett. Rev., 7:257-264.

Safdar H, Aydın R, Şahin B 2022. Syntheses, structural evolution, electrical and optoelectronic characterization of ZnO/CuO composite films doped with transition metal $Mn^{2+}$ ions. Ceramics International, 48:26678-26688.

Sathishkumar P, Sweena R, Wu JJ and Anandan S 2011. Synthesis of CuO–ZnO nanophotocatalyst for visible light assisted degradation of a textile dye in aqueous solution. Chemical Engineering Journal, 171:136-140.

Shen G , Chen P-C, Ryu K, Zhou C 2009.Devices and chemical sensing applications of metal oxide nanowires. J. Mater. Chem., 19:828-839.

Sone BT, Diallo A, Fuku XG, Gurib-Fakim A , Maaza M 2020.Biosynthesized CuO nano-platelets: physical properties & enhanced thermal conductivity nanofluidics. Arab. J. Chem., 13:160-170.

Su D G, Yang C, and Zhu J-J 2014. Fabrication of Gold Nanorods with Tunable Longitudinal Surface Plasmon Resonance Peaks by Reductive. Langmuir, 31:817-823.

Swain BP, Patil SB, Kumbhar A, Dusane RO 2003. Revisiting the B-factor variation in a-SiC: H deposited by HWCVD. Thin Solid Films, 430(1–2):186–188.

Şahin B, Kaya T 2021. Facile preparation and characterization of nanostructured ZnO/CuO composite thin film for sweat concentration sensing applications. Materials Science in Semiconductor Processing, 121:105428.

Taşdemir A, Aydin R, Akkaya A, Akman N, Altınay Y, Çetin H, Şahin B, Uzun A, Ayyıldız E 2021, A green approach for the preparation of nanostructured zinc oxide: Characterization and promising antibacterial behaviour, Ceramics International, 47: 19362-19373.

Tewari S and Bhattacharjee A 2011. Structural, electrical and optical studies on spray-deposited aluminium-doped ZnO thin films. Pramana, 76 (1):153.

Wang S, Xu H, Qian L, Jia X, Wang J, Liu Y, Tang W 2009. CTAB-assisted synthesis and photocatalytic property of CuO hollow microspheres. Journal of Solid State Chemistry, 182 (5): 1088-1093.

Wei S, Chen Y, Ma Y and Shao Z 2010. Fabrication of CuO/ZnO composite films with cathodic co-electrodeposition and their photocatalytic performance. Journal of Molecular Catalysis A: Chemical, 331:112-116.

Xu L, Hu Y-L , Pelligra C, Chen C-H, Jin L, Huang H, Sithambaram S, Aindow M, Joesten R, Suib SL 2009. ZnO with different morphologies synthesized by solvothermal methods for enhanced photocatalytic activity. Chem. Mater., 21 :2875-2885.

Zhang J, Liu J, Peng Q, Wang X, Li Y 2006. Nearly monodisperse Cu2O and CuO nanospheres: preparation and applications for sensitive gas sensors. Chem. Mater., 18:867-871.

Zhou Z, Lan C, Wei R, Ho JC 2019.Transparent metal-oxide nanowires and their applications in harsh electronics. J. Mater. Chem. C, 7:202-217.

Zhu G, Xu H, Xiao Y, Liu Y, Yuan A, Shen X 2012. Facile fabrication and enhanced sensing properties of hierarchically porous CuO architectures. ACS Appl. Mater. Interfaces, 4 : 744-751.

## *ORAL PRESENTATION*

# Epidemic and risk status of the rosemary powdery mildew (*Golovinomyces biocellatus*) in terms of plant growth food and medicinal use in Şanlıurfa of Turkey

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## Abstract

Rosemary **(***Rosmarinus officinalis*) leaves are consumed as medicinal, aromatic and food both in Turkish and other human communities of the world. The disease of powdery mildew was detected on this plant recently in Şanlıurfa district. Mould materials of the pathogen may be consume and harmful in human healthy. It is considered that to be known of active periods of this plant disease may be beneficial. Rosemary leaves in 5 different areas of Şanlıurfa city centre was investigated for two years. Visual and microscopic examinations were performed on the obtained samples. The epidemic position of the pathogen was determined by measuring the conidial densities on the infected leaves at intervals of 15 days. It was observed that the pathogen produce noticeable symptoms between 15 February and 15 March, and macroscopic symptoms between February and early April. Epidemic of the disease arisen between early February and early March. It formed a striking bell curve and exhibited a polynomial distribution with a consistency of 97%. It was concluded that powdery mildew on rosemary is especially active in early spring in closed and shaded areas in Sanlıurfa climatic conditions, and consuming diseased leaves during these periods may pose a health risk.

**Key Words***:* Rosemary medicinal powdery mildew epidemic risk

## INTRODUCTION

The use of plants or their extracts in the treatment of human diseases is quite old (Geven, 2021). Due to the undesirable side effects of chemical drugs, the resistance of disease agents to them, especially antibiotics and their negative effects on the environment (Haghparast et al., 2020; Drugwatch, 2022), the tendency towards herbal medicine is increasing. In this area, medicinal and aromatic plants come to the fore, and rosemary is one of them.

Rosemary, *Rosmarinus officinalis*, Labiatae family, is a greenish, sunny-loving and perennial plant that can grow 50-100 cm in its bushy form. Regions with Mediterranean climate, sunny and semi-shaded areas constitute the main growing areas of the plant. However, it is also easily grown in more terrestrial climates such as Şanlıurfa district. Rosemary plant is traditionally cultivated for food, spice and therapeutic purposes both in Şanlıurfa and vicinity ın Turkey. As well as open field cultivations, gardens of houses and workplaces, and large pots constitute other cultivation areas. It is also used as a hedge plant besides growing in parks, gardens and rock gardens as a favourite landscape and ornamental plant with its long blooming ability and always green properties (Atamov et al., 2004). Since ancient times, rosemary has been described as an herb that is used in the treatment of various diseases. Recently it has been reported that studies on rosemary extracts on experimental animals have anti-depressive properties (Al Mukhtar et al., 2013). The pharmacological properties of this plant (Al-Sereiti et al., 1999), anti-inflammatory and antinociceptive effects (Takaki et al., 2008), anti-proliferative and antioxidant effects (Cheung & Tai, 2007) and anti ulcerogenic effects have been reported (Dias et al., 2000). Despite many therapeutic features in the field of medicine and many uses in food, there are also parasitic factors that adversely affect the development of rosemary and infect it at different levels. It has been reported that *Golovinomyces biocellatus*, which is an obligat parasit exhibits significant mould infestation on leaves and shoots (Ashrafi, 2010; Park et al., 2009), is an important one, while the local and complete wilt of the root due to root and stem base infection is caused by *Phytophthora citrophthora, Rhizoctonia solani* and *Fusarium oxysporum* species (Ashrafi et al., 2010).

The aim of this study is to determine the disease epidemic and critical periods of the powdery mildew, which may be essential for control in the rosemary, which has important usage area of food and medical field. It was

also done by the agent to draw attention to the possible adverse effects of external mould materials such as mycelium, conidiophore and conidia produced on the host, to the food and drug properties of the plant

## MATERIAL AND METHODS

Rosemary leaf and shoot specimens exhibiting ash similar signs constituted the main materials of study. The identification of the powdery mildew species was made according to the morphological structures briefly conidiophore and conidial size, sequence, pattern, etc. in sexual and asexual forms of the agent (Braun, 1987; Kavak, 2010). Visual indicators were made on the basis of whether or not the active powdery mildew layer consisted of mycelium, conidiophore and conidia on the leaves was visually confirmed. The microscopic specification is based on whether or not conidia or conidial chains are identified from microscopic preparations obtained from diseased leaf specimens identified in the sampling areas. This was done with a binocular microscope in leaves, either with the presence of active conidial chains or in the form of at least one chain in the light microscope, or by the presence of at least 3-4 conidia.

Conidial density of powdery mildew was determined by determining the concentrations of conidia in the samples in the sampling areas and time periods shown in Table 1. The conidial spore densities in the samples were determined by determining the number of conidial in one leaf (conidial number in ml water) in 1 ml of purified water. For this, a random leaf was obtained from the sampling areas defined by the code numbers in Table 1 and then placed in 1 ml of purified water in the Eppendorf tube and shaken slowly for 20 seconds**.** From this, 0.01 ml from suspension water were removed and left on Thoma slice, and then counts were made to determine the conidial number in 1 ml suspension. The conidial numbers determined in the samples at different time periods were grouped by Duncan's multiple comparison test (Duncan, 1995). Regression analyse was also performed on same data. Statistical calculations were performed by using of SPSS 10.0 software (SPSS, 1999).

## RESULTS AND DISCUSSION

According to the this study conducted during two years in Sanliurfa climatic conditions, powdery mildew on rosemary was important, but observed within short time periods throughout the year. Infected leaves and shoots including dust layers, composed of conidia and mycelia, did reflect the disease very well (Figure 1). The sexual form of the disease agent has not been observed. However, the asexual forms including spore clusters, conidial chains, surface design and germination forms of conidia were found to be quite evident in the diagnosis of *Golovinomyces biocellatus* (Figures 1, 2, 3) as previously described by Braun (1987). This obligate parasite has also been reported in rosemary and other some close plants (Liberato & Cunnington, 2007; Cho & Park, 2014), considering that it is becoming more and more important. In this study it is observed that infected areas mostly concentrated in the lower and inner sections of the plants. Also infected plants mostly observed in the semi-shaded areas.



**Figure 1.** Leaf clusters of rosemary with dense powdery mildew from *Golovinomyces biocellatus*



**Figure 2.** Conidial chain and spores of *Golovinomyces biocellatus*



**Figure 3.** A germinated conidial spore of *Golovinomyces biocellatus*

Visual and microscopic examinations of the two-year sampling areas showed that the powdery mildew of the specimens produced visible signs between February 15 and March 15, but could also be found in microscopic measurements until the beginning of February and April. No visual and microscopic findings were found outside of these two-year determinations. Based on these results, it may be concluded that this pathogen is effective at the cool climatic conditions. Also its life cycle display differences from powdery mildews that adapted to hot climatic conditions as reported by Kavak (2004), Kavak (2011).

The conidial numbers in the samples obtained in five different time periods, which is the active phase, determined in two years were statistically 2 different groups**.** According to average of two years, conidial concentrations of single leaf from diseased specimens were estimated to be 12.5 / ml, 137 / ml, 139 / ml, 121 / ml, 7.5 / ml between February 1 and April 1 at 15-day intervals (Table 1). These results can also explain the possible epidemic period of this pathogen at its life cycle.

**Table 1.** The activity of powdery mildew on rosemary plant

| Symptomatic status of examples in determined time period | | | | | | | |
|---|---|---|---|---|---|---|---|
| Years | 2013/2014 | 2013/2014 | 2013/2014 | 2013/2014 | 2013/2014 | 2013/2014 | 2013/2014 |
| **Months | 15 January | 1 February | 15 February | 1 March | 15 March | 1 April | 15 April |
| Visual symptom | Abs/ Abs | Abs / Abs | Prs/ Prs | Prs/ Prs | Prs/ Prs | Abs / Abs | Abs / Abs |
| Microscopic symptom | Abs / Abs | Prs/ Abs | Prs/ Prs | Prs/ Prs | Prs/ Prs | Prs/Abs | Abs / Abs |
| Conidial numbers in sampled suspension | | | | | | | |
| Sample cod (recurrent) | | | | | | | |
| *OK-1 | - | 22/- | 150/121 | 148/157 | 134/94 | 18/- | - |
| *OK-2 | - | 28/- | 136/135 | 155/128 | 129/106 | - | - |
| *AP-3 | - | 34/- | 142/116 | 164/149 | 133/108 | 27/- | - |
| *KP-4 | - | - | 115/127 | 130/106 | 125/79 | - | - |
| *DP-5 | - | 43/- | 178/155 | 147/104 | 167/135 | 30/- | - |
| Average | - | 25/- | 144/130 | 149/129 | 138/104 | 15/- | - |
| St error | | 7.23/0 | 10.24/6.82 | 5.59/10.81 | 7.09/9.23 | 6.43/0 | |
| St deviation | | 10.38/0 | 18.83/21.14 | 9.57/28.63 | 19.27/28.9 | 6.57/0 | |
| Statistical group | | a/- | b/b | b/b | b/a | a/- | |

***** = Explain recurrent and also the place, the sample was take. OK-1 = Osmanbey Campus, AP = Abide Park, KP = Kasaptaşı Park, DP = Dergâh Park **=Explain the month the sample was take. Abs=Absent, Prs=Present

The disease showed a minimum at the beginning of February, a maximum between February 15 and March 15 and a minimum at the beginning of April. The epidemic course of the disease within this time period is best characterized by a polynomial distribution (Figure 4) with a consistency of 97% and a formulation of $ax^2+bx+c$ as described by Snedecor & Cochran (1989). This curve displays one of the best samples of the short time plant disease epidemics as described by Agrios (1997)

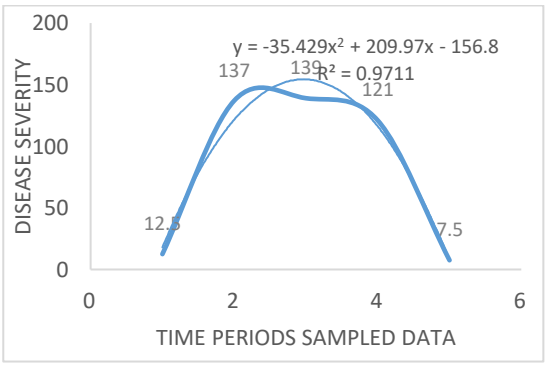


**Figure 4**. Active development curve of rosemary powdery mildew in Şanlıurfa climatic conditions.

Considering Table 1 results, it was estimated that no chemical struggle was necessary during active periods observed between Fabruray and March. However, it is thought that the use of infected plant leaves in food and medicinal products in these months may be formed certain risks, and accordingly a clean material selection must be made. Because this obligate pathogen performs the parasitic effect by entering its haustoria to the cytoplasm of epidermis cells of leaves and other tissues. Accordingly, the related tissue cells initially lose their chlorophyll and change to chlorosis. Then lose all their function and become necrosis. It is believed that plant leaves that have been dried by an obligate parasite are harvested with healthy leaves and if used for their intended purpose, may reduce or adversely affect food, treatment and pharmacological values. On the other hand, it is estimated that mould materials produced on the surface of the leaves and tissues as ashy-dust forms can change their biochemical contents if they are contaminated with food and pharmacological purpose. Because due to a different living group from the plant, the biochemical contents of this fungi structure also display the differences.

On the other side, it is not yet known whether these structures contain toxic compounds, which we call the mould materials. Because some mould species externally produced on mainly seeds and some plant tissues, produce secondary metabolite compounds called mycotoxins, which have toxicity at different levels. These have been reported to result in deaths in humans and animals as well as transient and permanent damages (Hussein & Brasel, 2001; Liew & Redzwan, 2018). For example aflatoxin is one of them, and it has been reported that different doses of aflatoxin, the most important of mycotoxins, increase the malondialdehyde ratios in blood plasma when given to quail pups (Dinç et al., 2006). Also, it has been reported that some off-toxin derivatives have biological activities in teratogenic, mutagenic and carcinogenic types, as well as acute toxicity in high organisms (Michelle & Michael, 1995; Sarma et al., 2017). As in these examples, some of the mycotoxins produced after apparent fungal infestation of some fungi species in plant and plant products can also be produced by *G. biocellatus*, which causes infestation by mould structures, and may lead to adverse biological consequences described above.

## CONCLUSION

In the Şanlıurfa region, powdery mildew disease on rosemary plant occurs in a period of 2-2.5 months between February and April. This time period is very early and has different climatic requirements than many other powdery mildews that are effective in hot climatic conditions. On the other hand, the use of rosemary plants for food or medicinal purposes may be carrying a risk in the process where powdery mildew is active in this area. Therefore, it is recommended to take care to harvest plant materials intended for consumption in these periods from disease-free plants.

## REFERENCES

Agrios GN 1997. Plant Disease Epidemiology 153-171. In: Plant Pathology. Fourth Edition. Academic press. Inc. p 619.

Al-Sereiti MR, Abu-Amer KM, Sen P 1999. Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. Indian Journal of Experimental Biology, 37: 124-130.

Ashrafi SJ, Rastegar MF, Saremi H 2010. Rosemary wilting disease and its management by soil solarisation technique in Iran. African Journal of Biotechnology, 9: 7048-7057.

Atamov V, Akan H, Kaya ÖF, Polat T, Ağdemir H 2004. Şanlıurfanın Egzotik Ağaç ve Çalıları, 64 s

Braun, U. (1987). A Monograph of the Erysiphales (Powdery Mildews). Beihefte zur Nova Hedwigia, 89: 1-700.

Cheung S, Tai J 2007. Anti-proliferative and antioxidant properties of Rosmarinus officinalis. Oncology Reports, 17: 1525-1531

Cho SE, Park JH 2014. First Report of Powdery Mildew Caused by *Golovinomyces biocellatus* on *Agastache rugosa* in Korea. Plant Disease, 98: 1278.

Dias PC, Foglio MA, Possenti A, Carvalho JE 2000. Antiulcerogenic activity of crude hydroalcoholic extract of Rosmarinus officinalis L. Journal of Ethnopharmacology, 69: 57-62.

Dinç E, Altıntaş L, Kürşat Y 2006. Effects of Aflatoxin and various adsorbents ob plasma malondialdehyde levels in Quails. Bulletin of Veterinary Institute Pulawy, 50: 585-588.

Drugwatch 2022. Drug Side effects and medical Device Complications. https://www.drugwatch.com/side-effects/

Duncan DB 1995. Multiple Range and Multiple F-Tests. Biometrics, (11), pp. 1-42

Entisar J, Mukhtar, AI, Selman SM, Sahib Z, Naji H 2013. Antidepressant-Like Effect of Rosmarinus officinalis Extract in Male Mice. Medical Journal of Babylon, 10: 803-808.

Geven F 2021. Tıbbi Bitkilerin Tarihçesi.
https://acikders.ankara.edu.tr/pluginfile.php/63155/mod_resource/content/0/1.Ders

Haghparast P, Pondrom M, Ray SD 2020. Chapter 6 Sedatives and hypnotics, pages 67-79. In Side Effects of Drugs Annual. Elsevier

Hussein HS, Brasel JM 2001. "Toxicity, metabolism, and impact of mycotoxins on humans and animals" Toxicology, 167: 101–134.

Kavak H 2004. Epidemic outbreaks of powdery mildew caused by *Leveillula taurica* on *Capparis spinosa* in Turkey. Plant Pathology, 53: 809.

Kavak H 2011. Two new records of powdery mildews with their effectiveness on three ornamentals in Turkey. African Journal of Agricultural Research, 6 : 1076-1079.

Liberato JR Cunnington H 2007. Powdery mildew on Mentha in Australia. Australasian Plant Disease Notes. 2: 83-86

Liew WPP, Redzwan SM 2018. Mycotoxin: Its Impact on Gut Health and Microbiota. Frontiers in Cellular and Infection Microbiology, 8: 60

Michelle McL, Michael FD 1995. Cellular interactions and metabolism of aflatoxin: An update. Pharmacology and Therapeutics, 65: 163-192.

Park, MJ, Han JG, Shin HD 2009. First Korean report of rosemary powdery mildew caused by *Golovinomyces biocellatus*. New Disease Reports, 19: 60.

Sarma UP, Bhetaria PJ, Devi P, Varma A 2017. Aflatoxins: Implications on Health. Indian Journal of Clinical Biochemistry, 32: 124-133

Snedecor GW, Cochran WG 1989. Nonlinear relations: In Statical Methohods (Eight edition) Iowa state University press, Ames Iowa

SPSS 1999. Statistical Package for the Social Sciences, Release 10.0. SPSS Inc. Illinois, Chicago, USA

Takaki I, Bersani-Amado LE, Vendroscolo A, Sartoretto SM, Diniz SP, Bersani-Amado CA 2008. Anti-inflammatory and antinociceptive effects of Rosmarinus officinalis L. essential oil in experimental animal models. Journal of Medicinal Food, 11: 741-746.

## *ORAL PRESENTATION*

# The influence of the extraction process on the antioxidant activity of *Boletus edulis* wild mushroom

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## Abstract

The aim of this research was to prepare aqueous, ethanolic and methanolic extracts from *Boletus edulis* mushroom, to determine the antioxidant activity as well as to prove which type of extraction is the most suitable for this mushroom species. Aqueous extract was characterized with the highest ($p<0.05$) total phenols content, 4.72%, compared to both alcoholic extracts. From the point of total flavonoids content, it can be seen that ethanolic extract had higher ($p<0.05$) content (2.91%) compared to the methanolic (2.65%) and aqueous (2.39%) extract. The ability for capturing DPPH radicals at the highest tested concentration of 10 mg/mL was as follows: aqueous extract > ethanolic extract > methanolic extract, or: 56.37% > 53.69% > 51.45%. Alcoholic extracts showed better ability to chelate iron ions: ethanolic extract > methanolic extract > aqueous extract, or: 48.89% > 47.37% > 45.61%. Finding new natural sources that have antioxidant potential is of particular importance for replacing synthetic antioxidants used in industrial conditions. It also increases the possibility of creating new, functional products in the food industry. In the Republic of North Macedonia, until now there is no data on the antioxidant activity of this type of mushroom. So the extracts from *Boletus edulis* mushroom can be used as a completely replacement for the synthetic additives in the food industry.

**Keywords:** Mushroom, extraction, antioxidant activity, bioactive compounds.

## INTRODUCTION

Mushrooms are food with highly valued nutritional properties (Chang and Miles, 2004). The low caloric value is especially important, which comes from the high water content (80%–90%), from 26 to 35 kcal for every 100 g. In addition, they are a good source of protein with an amino acid composition, more similar to animal proteins than to plant proteins, making them an ideal supplement to a vegetarian diet (Guillamón et al., 2010).

*Boletus* is a genus of mushrooms, comprised of over 100 species. The genus *Boletus* was originally broadly defined and described by Fries in 1821, essentially containing all fungi with pores. Almost all of these fungi grow in symbiotic association with trees, forming mycorrhizas that aid the uptake of mineral nutrients from soil (Binder and Hibbett, 2006).

*Boletus edulis*, known as the king mushroom, or porcini, is an edible mushroom that grows in forests throughout Romania. It is a basidiomycete fungus that contains a low amount of fat but is rich in minerals, fibers, and proteins. Ergosterol is also present in its composition (Vamanu and Nita, 2013). The fruit bodies have a high antioxidant capacity and significant alkaloid content (Ribeiroa et al., 2008; Vamanu and Nita, 2013).

The mushrooms have components that can contribute to human wellness and mitigate threats and assaults that render the human body vulnerable to several life-threatening diseases including cardiovascular ailments, cancer, metabolitic (diabetes and obesity), and neurodegenerative disorders (Vikineswary and Chang, 2013).

It is generally accepted that mushroom extracts contain multiple components, each of which may have its own biological or medicinal effects. Mushroom triterpenoids and some of the proteins have been referenced as imune-system modulators. It was previously shown that phenolic compounds act as potent antioxidants (Liu et al., 2013; Sokovic et al., 2018).

Bioactive compounds of the medicinal mushrooms prove their use as a source of functional food. It was demonstrated that mushrooms could be an excellent source of a wide range of interesting molecules with pharmacological activity (antioxidant, antibacterial, antifungal, and antiproliferative) properties or even with antitumor potential (Sokovic et al., 2018).

The aim of this research was to prepare different extracts from *Boletus edulis* mushroom (aqueous, ethanolic and methanolic extracts), to determine the antioxidant activity as well as to prove which type of extraction is the most suitable for this mushroom species.

## MATERIALS AND METHODS

In this research, as a work material *Boletus edulis* mushroom species was used collected from the territory of Skopska Crna Gora in the Republic of North Macedonia. The collected fresh mushrooms were chopped into thin slices. The mushroom pieces were dried in a chamber dryer with hot air at a temperature of 40 ºC for 6–7 h. Dried mushrooms were first ground to a fine powder and then, extracted in three ways, with water, ethyl and methyl alcohol as extragens.

### Preparation of aqueous extract

Aqueous extract was prepared by Sławińska et al. (2013) and Ribeiro et al. (2015) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with about 200 mL of distilled water, and after that was extracted on a boiling water bath for 1 h.

### Preparation of ethanolic extract

Ethanol extract was prepared by Vidović et al. (2011) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with 100 mL of 50% ethanol and extract was covered for 40 minutes on an ultrasonic bath at 45 °C.

### Preparation of methanolic extracts

Methanolic extract was prepared by Barros et al. (2007) 10 g of dried and finely powdered mushroom sample was poured with 150 mL of 80% methanol and was extracted for 24 hours at room temperature on a magnetic stirrer at 100 rpm. The sample was then filtered through filter paper. After that the samples were evaporated in a vacuum evaporator at temperatures lower than 45 ºC. The samples were then additionally dried in a stream of warm air (40 ºC) to a constant mass.

### Determination of total phenolic compounds

The content of total phenols was determined by Singleton et al. (1999) method, adapted for microplates. The method is based on the reaction of phenol with Folin-Ciolcateu reagent where a colored complex is formed. The phenol content was calculated on the basis of the calibration curve (concentration-dependent absorbance function) of a standard gallic acid solution.
Equivalent gallic acid (GAE)/g dry matter values were obtained according to the following formula:

$$\text{mg eq. GAE/gd.m.} = \text{read concentration GAE (µg/mL)} / \text{working concentration} \times 1000$$

The results are expressed as the mean of the three measurements.

### Determination of total flavonoid content

The flavonoid content was determined by a modified method of Chang et al. (2002), adapted for microplates. The principle of the method is based on the properties of flavonoids and flavoglycosides to give appropriate complexes with metal ions, and the Al-complex is especially important. It is a simple method based on the construction of a colored complex, whose absorption maximum is 430 nm.
The flavonoid content was calculated based on the calibration curve (concentration-dependent absorption function) of a standard quercetin solution.

Equivalent quercetin (QE)/g dry matter values were obtained according to the following formula:

mg eq. QE/g d.m. = read concentration QE (µg/mL) / working concentration x 1000

The results are expressed as the mean of the three measurements.

**Determination of antioxidant potential**

***Ability to capture DPPH radicals***

The ability to capture DPPH radicals was determined by Brand-Williams et al. (1995) method.

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100\%$$

The radical scavenging capacity of the samples was calculated as $IC_{50}$ values (inhibitory concentration of extract reducing the absorbance of DPPH solution by 50%) by regression analysis:

$$IC_{50}\ (mg/mL) = (50 - b)/a^*$$
(*a – slope; b – intercept)

BHT was used as positive controls. The results are expressed as the mean of the three measurements.

***Ability to chelate iron ions***

The chelating ability of iron was determined by Dinis et al. (1994) method. The chelating ability of iron ions is calculated by the formula:

$$\text{Ability to chelate iron } \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100\%$$

The ability to chelate iron ions of the samples was calculated as $IC_{50}$ values by regression analysis:

$$IC_{50}\ (mg/mL) = (50 - b)/a^*$$
(*a – slope; b – intercept)

Citric acid was used as positive controls. The results are expressed as the mean of the three measurements.

**Statistical analysis**

The obtained results were statistically processed using the software package SPSS 20. To determine the statistical significant differences of the obtained results ANOVA test was used, *post hoc* Tukey's test ($p$ = 0.05).

**RESULTS**

From data presented in Table 1, can be seen that aqueous extract was characteristic with the highest ($p$<0.05) total phenols content, 4.72%, compared to both alcoholic extracts (3.28% for the ethanolic extract and 3.07% for the methanolic extract). From the point of total flavonoids content, it can be seen that ethanolic extract had higher ($p$<0.05) content (2.91%) compared to the methanolic (2.65%) and aqueous (2.39%) extract.

According to data presented in Figure 1, can be seen that, generally, all of the tested extracts were competitive with the BHT, as positive control, at all of the tested concentrations. Nevertheless, aqueous extract showed the highest antioxidant activity ($p$<0.05) in terms of the ability to capture free DPPH radicals, which was 56.37% at the concentration of 10 mg/mL, compared to the others extracts. The ability for capturing DPPH radicals at the highest tested concentration of 10 mg/mL was as follows: aqueous extract > ethanolic extract > methanolic extract, or: 56.37% > 53.69% > 51.45%.

**Table 1.** Total phenols and flavonoids content in *Boletus edulis* extracts

| ***Boletus edulis* mushroom extracts** | **n** | **Total phenols (%)** | **Total flavonoids (%)** |
|---|---|---|---|
| | | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ |
| Aqueous extract | 3 | $4.72^{a} \pm 0.07$ | $2.39^{a} \pm 1.01$ |
| Ethanolic extract | 3 | $3.58^{b} \pm 0.36$ | $2.91^{b} \pm 0.62$ |
| Methanolic extract | 3 | $3.07^{c} \pm 0.50$ | $2.65^{c} \pm 0.19$ |

a, b, c - values of different extracts marked with different letters, have a statistically significant difference, ANOVA, *post hoc* Tukey's test ($p<0.05$).

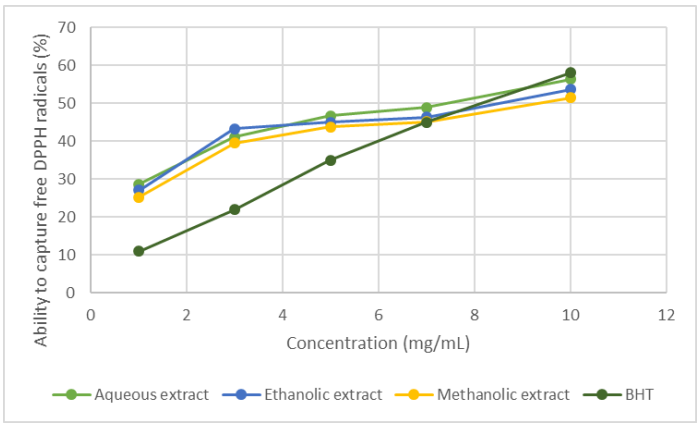


**Figure 1.** Ability of *B. edulis* extracts to capture DPPH radicals

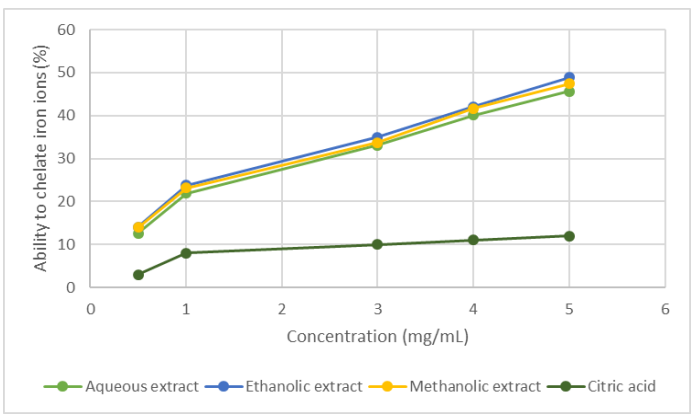


**Figure 2.** Ability of *B. edulis* extracts to chelate iron ions

On the other hand, Figure 2 showed that the ethanolic extract had the highest ($p<0.05$) ability for chelating iron ions (48.89%), compared to the others extracts. In this case, alcoholic extracts showed better antioxidant activity: ethanolic extract > methanolic extract > aqueous extract, or: 48.89% > 47.37% > 45.61%. Moreover, all of the tested extracts showed higher ($p<0.05$) ability to chelate iron ions compared to the citric acid at all of the tested concentrations.

**Table 2.** $IC_{50}$ values of tested extracts

| ***Boletus edulis* mushroom extracts** | **n** | **$IC_{50}$ (mg/mL)** | |
|---|---|---|---|
| | | **DPPH** | **Chelating $Fe^{3+}$ ions** |
| | | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ |
| Aqueous extract | 3 | $4.92^{a} \pm 0,05$ | $3.67^{a} \pm 0,09$ |
| Ethanolic extract | 3 | $6.85^{b} \pm 0,03$ | $2.81^{b} \pm 0,10$ |
| Methanolic extract | 3 | $8.01^{c} \pm 0,03$ | $2.93^{b} \pm 0,16$ |

a, b, c - values of different extracts marked with different letters, have a statistically significant difference, ANOVA, *post hoc* Tukey's test ($p<0.05$).

Antioxidant activity of the extracts was followed by $IC_{50}$ values (Table 2), so in terms of DPPH test, aqueous extract was characterized with the lowest ($p<0.05$) $IC_{50}$ value (4.92 mg/mL), while from the point of chelating iron ions, the lowest ($p<0.05$) $IC_{50}$ values was obtained for ethanolic extract (2.89 mg/mL).

## DISCUSSION

The content of phenols and flavonoids is thought to greatly contribute to the antioxidant activity of mushroom extracts. In general, the presence of phenols is associated with the ability of mushrooms to chelate metals, inhibit lipid peroxidation, and capture free radicals, while the presence of flavonoids correlates with the antioxidant, anti-inflammatory, antiviral, and anticancer activity of mushrooms (Stojanova et al., 2020).

The results for total phenolic compounds are in accordance to those from Vidovic et al. (2010), where authors found that the content of total phenols (41.82±0.08 mg GAE/g of dry extract) was higher for *B. edulis* than that for *B. auranticus* ethanolic extract. On the other hand, Vamanu and Nita (2013) concluded that *B. edulis* aqueous extract had 17.22 mg/g total phenolic content, ethanolic extract had 21.32 mg/g total phenolic content, while methanolic extract had 18.96 mg/g total phenolic content. The total flavonoid content has been the highest in the ethanolic extract (116.64 mg/g).

Antioxidant potential of methanolic extract of *B. aereus*, revealed a good reducing power ($EC_{50}$ 0.75 mg/mL), scavenging of DPPH radicals ($EC_{50}$ 3.29 mg/mL), inhibition of β-carotene bleaching ($EC_{50}$ 2.89 mg/mL), and lipid peroxidation inhibition ($EC_{50}$ 0.33 mg/mL) (Stojković et al., 2015).

Kosanic et al. (2012) reported the acetone extract of the highly valued and endangered species *B. edulis*, originating from Serbia was more effective than α-tocopherol, BHA, and BHT.

The scavenging antioxidant activities of aqueous and ethanolic extract increased with the increase of concentrations (Liu et al., 2013), as in the case of this study.

It has been reported that the antioxidant activity has a positive correlation with the reducing power, prevention of chain initiation, radical scavenging and so on (Zhang et al., 2011). Vamanu and Nita (2013) pointed out that for scavenging ability on DPPH radicals, various extracts were effective in the order of ethanolic > hot water > cold water > methanolic extracts.

Ruiz-Rodriguez (1998) and Soler-Rivas (2009) pointed out that the results concerning the antioxidant properties of many mushroom species varied from author to author because of different environmental conditions, cultivation methodologies, developmental stage, and genetic variation within strains.

In addition, water as a polar extraction agent probably proved to be better due to the higher value of most of the chemical components that are thought to be responsible for antioxidant activity (e.g., total phenols) than in ethanolic extracts of examined mushroom strains. Moreover, ethanolic extracts have a higher content of flavonoids, which is considered to contribute to the antioxidant activity of the extracts (Stojanova et al., 2020).

Finding new natural sources that have antioxidant potential is of particular importance for replacing synthetic antioxidants used in industrial conditions. It also increases the possibility of creating new, functional products in the food industry. In the Republic of North Macedonia, until now there is no data on the antioxidant activity of this type of mushroom.

## CONCLUSION

Based on the results, can be concluded that aqueous extract had higher content of total phenols, while both alcoholic extracts had higher total flavonoids content. The results for the antioxidant activity was in a correlation with the total phenolic and flavonoid content. So, the aqueous extract showed the highest ability to capture free DPPH radicals, while this extract was characterized with the highest phenolic content. Furthermore, ethanolic extracts showed the highest ability for chelating iron ions, which is in accordance to the highest presence of flavonoids in this extract. Nevertheless, can be seen that antioxidant potential is in a correlation with the $IC_{50}$ values.

According to that, can be seen that both, water and ethyl alcohol are suitable for the mushroom extraction, depending on the bioactive component that should be extracted. Water, as a polar extragent, was better for total phenolic extraction, while ethanol, as a less polar extragent, was more efficient in the case of total flavonoid extraction. However, in both cases better results were obtained compared to the synthetic antioxidants (BHT and citric acid). So the extracts from *Boletus edulis* mushroom can be used as a completely replacement for the synthetic additives in the food industry. Further research will be focused on creating new foodstuffs as a functional food enriched with *Boletus edulis* mushroom extracts.

## REFERENCES

Barros L, Baptista P, Estevinho L, Ferreira ICFR 2007. Effect of fruiting body maturity stage on chemical composition and antimicrobial activity of *Lactarius* sp. Mushrooms. Journal of Agriculture and Food Chemistry 55: 8766-8771.

Brand-Williams W, Cuvelier ME, Berset C 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28: 25–30.

Binder M, Hibbett DS 2006. Molecular systematics and biological diversification of Boletales. Mycologia 98: 971–981.

Chang ST, Miles PG 2004. Mushrooms – Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact - 2nd ed., CRC Press.

Chang CC, Yang MH, Wen HM, Chern JC 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis., 10: 178–182. https:// doi.org/10.38212/2224-6614.2748

Dinis TCP, Madeira VMC, Almeida L M 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics, 315: 161–169.

Guillamón E, García-Lafuente A, Lozano M, D´Arrigo M, Rostagno MA, Villares A, Martínez JA 2010. Edible mushrooms: Role in the prevention of cardiovascular diseases. Fitoterapia, 81: 715–723. https://doi.org/10.1016/j.fitote.2010.06.005

Kosanic M, Rankovic B, Dašic M 2012. Mushrooms as possible antioxidant and antimicrobial agents. Heal. Serv. Iran. J. Pharm. Res. 11: 1095–1102.

Liu J, Jia L, Kan J, Jin C 2013. *In vitro* and *in vivo* antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). Food and Chemical Toxicology, 51: 310-316.

Ribeiro B, Lopes R, Andrade PB 2008. Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes, Food Chemistry, 110: 47–56.

Ribeiro A, Ruphuy G, Lopes JC, Dias MM, Barros L, Barreiro F, Ferreira ICFR 2015. Spray-drying microencapsulation of synergistic antioxidant mushroom extracts and their use as functional food ingredients. Food Chemistry, 188: 612–618. https://doi. org/10.1016/j.foodchem.2015.05.061.

Ruiz-Rodriguez A, Santoyo S, Soler-Rivas C 2009. Antioxidant properties of edible mushrooms. Functional Plant Science and Biotechnology 3(1): 92–102.

Sławińska A, Radzki W, Kalbarczyk J 2013. Antioxidant activities and polyphenolics content of Flammulina velutipes mushroom extracts. Kerla Polonica, De Gruyter, 59(3): 26–36. https://doi. org/10.2478/hepo-2013-0014.

Singleton VL, Orthofer R, Lamuela-Raventos RM 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciolcateu reagent. Methods in Enzymology, 299: 152–178. https://doi.org/10.1016/S0076-6879(99)99017

Soler-Rivas C, Jolivet S, Yuksel D, Arpin N, Olivier JM, Wichers HJ 1998. Analysis of *Agaricus bisporus* tyrosinase activation and phenolics utilization during Pseudomonas tolaasii- or tolaasin-induced discolouration. Mycological Research 102: 1497–502. doi:10.1017/ s0953756298006583

Sokovic M, Glamoclija J, Ciric A, Petrovic J, Stojkovic D 2018. Mushrooms as Sources of Therapeutic Foods. Therapeutic Foods, Springer, Chapter 5: 141-175. http://dx.doi.org/10.1016/B978-0-12-811517-6.00005-2

Stojanova M, Pantić M, Karadelev M, Čuleva B, Nikšić M 2020. Antioxidant potential of extracts of three mushroom species collected from the Republic of North Macedonia. Journal of Food Processing and Preservation, 00:e15155. https:// doi.org/10.1111/jfpp.15155

Stojkovic DS, Reis FS, Ćiric A, Barros L, Glamoclija J, Ferreira ICFR, Sokovic M 2015. Boletus aereus growing wild in Serbia: chemical profile, in vitro biological activities, inactivation and growth control of food-poisoning bacteria in meat. J. Food Sci. Technol. 52: 7385–7392.

Vamanu E, Nita S 2012. Compounds, Anthocyanin, and Tocopherol Content in Various Extracts from the Wild Edible Boletus edulis Mushroom. BioMed Research International Volume 2013, Article ID 313905, http://dx.doi.org/10.1155/2013/313905

Vidović S, Zoran-Zeković Z, Mujić I, Lepojević Ž, Radojković M, Živković J 2011. The antioxidant properties of polypore mushroom Daedaleopsis confragosa. Central European Journal of Biology, 6(4): 575–582. https://doi.org/10.2478/s11535-011-0029-5.

Vidović SS, Mujić IO, Zeković ZP, Lepojević ZD, Tumbas VT, Mujić AI 2010. Antioxidant Properties of Selected *Boletus* Mushrooms. *Food Biophysics* 5: 49–58. https://doi.org/10.1007/s11483-009-9143-6

Vikineswary S, Chang ST 2013. Edible and medicinal mushrooms for sub-health intervention and prevention of lifestyle diseases. Technol. Trends: 33–43.

Zhang A, Xiao N, He P, Sun P 2011. Chemical analysis and antioxidant activity in vitro of polysaccharides extracted from *Boletus edulis*. International Journal of Biological Macromolecules 49, 1092–1095. doi:10.1016/j.ijbiomac.2011.09.005

*ORAL PRESENTATION*

# Drying of Pomegranate Seeds Using Infrared Radiation

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## Abstract

Drying kinetics of pomegranate seeds was researched in this study. The initial moisture content of pomegranate seeds was defined as 78.74% using the AOAC method. The drying process was carried out at 62, 74, 88 and 104 W power values of the infrared dryer. The drying process was maintained until the moisture content of the product decreased to 20% on wet basis. Drying took place in the falling-rate period and constant-rate period was not beheld. It was determined that the drying rate and drying times were directly dependent on the infrared drying power. It was observed that with the increase of infrared power drying rate also increased and as a result, drying times was shortened. The mathematical modeling of the drying process was carried out using four different thin-layer drying models (Henderson & Pabis, Page, Midilli & Kucuk, Vega-Galvez). The model that best represents the drying process was determined by looking at the $R^2$, P, $\chi^2$ and RMSE values of the models. Midilli & Kucuk model was determined as the most suitable thin-layer drying model for the drying process. Effective diffusion coefficients ($D_{eff}$) were calculated by using Fick's second equation. The values of $D_{eff}$ depending on the different infrared powers varied between $8.76\times10^{-10}$ and $1.96\times10^{-9}$ $m^2/s$. The activation energy value was calculated as 2.1 kW/kg. The color parameters of pomegranate seeds were investigated for different infrared drying powers. Experimental studies were showed that, with the increase of infrared power, L and a values decreased, b values increased.

**Keywords:** Activation Energy, Effective Moisture Diffusivity, Drying, Pomegranate Seeds.

## INTRODUCTION

Pomegranate (*Punica granatum L.*) is a fruit of the Punicaceae family (Süfer and Palazoğlu, 2019). In its scientific name, Punica comes from the scientific name of the apple, Pomum, while granatum means that it is granular. The cultivation of pomegranate dates back to 4000 BC and is the oldest known edible fruit. It is believed that the origin of the pomegranate fruit is Persia and its surroundings. From there it spread to Ancient Egypt, Iraq and Greece (Silva et al., 2013). Pomegranate is grown in every region in Turkey but it is mostly grown in the Mediterranean, Aegean and South-eastern Anatolia (Özgüven and Yılmaz, 2000). The pomegranate fruit consists of seeds and peel, and the edible part constitutes half of the total weight (Allahdad et al., 2019). Pomegranate seeds generally contain water, polysaccharides, vitamins, minerals, and polyphenols (Bchir et al., 2012). In addition to these components, pomegranate also contains some components that are very beneficial for human health. These components are tannins, anthocyanins, organic acids and phenolic compounds (Calín-Sánchez et al., 2013). Having these components has led to the use of pomegranate as a medicinal material for years. As a result of the studies, it was concluded that the beneficial components contained in the pomegranate fruit have positive effects in the treatment of diseases such as dental diseases, infertility, alzheimer and obesity (Banihani et al., 2013). However, fruits and vegetables are classified as very perishable foods due to their 80-90% water content. Drying is a preservation method that extends the time period in which foods can be consumed healthily by removing the water in the food (Orsat et al., 2006).

The drying is a food preservation method that has been used since ancient times. The drying process can be defined as the removal of excess water by giving heat to the material. With the drying process, not only the shelf life of the food is extended, but also the costs required to store or transport the food are reduced as the weight of the food is reduced. Drying is frequently used in industrial food preservation (Onwude et al., 2016). Freeze drying, hot air-drying, infrared drying, vacuum drying, sun drying and microwave drying are different drying methods (Alp and Bulantekin, 2021). Each of these methods has different advantages and disadvantages according to their application areas. Sun drying is a method frequently used in areas with long sun exposure

times. However, this method is highly dependent on weather conditions and the dried product is susceptible to contamination. High quality final products can be obtained by freeze drying method. However, both the investment and operating costs of the freeze drying method are quite high. For this reason, the freeze drying method cannot be applied to every product. The hot air-drying method is the most used technique industrially. However, this method also has high waiting times and energy costs (Toğrul, 2006; Shukla, 2011). Infrared drying method is a new technology that is becoming increasingly popular with its short drying times, low energy costs and high-quality final product (Riadh et al., 2015). When infrared energy hits the product to be dried, the energy directly penetrates the product and spreads to its inner layers. The water molecules, activated by the penetration of energy, heat up and evaporate. Since infrared energy is absorbed directly by the product to be dried and is not used for ambient heating, drying times are lower in this method (Toğrul, 2005; Adak et al., 2017).

Some studies in the literature on the drying of pomegranate fruit are listed below: Kingsly and Singh (2007) was dried pomegranate seeds with the convective drying method and modelled the drying process using four particular thin-layer drying models. Doymaz (2012) dried pomegranate seeds using infrared drying method. In the study, ten various thin-layer drying models were studied and Page, Midilli et al. and Weibull drying models were reported to be compatible with the drying process. Calín-Sánchez et al. (2013) were dried pomegranate seeds using a combination of freeze drying, hot air-drying, vacuum-microwave drying and combined vacuum-microwave with hot air-drying methods. In their study, they investigated the effect of different drying methods on the total phenolic compound amount and antioxidant capacity of pomegranate seeds. Alaei and Chayjan (2013) dried pomegranate seeds in a vacuum assisted infrared dryer. The drying kinetics and color values of pomegranate seeds were examined for different drying temperatures and pressure values, and the activation energy and effective diffusion coefficient values were calculated. Horuz and Maskan (2015) dried pomegranate seeds with hot air and microwave drying methods. As a result of the mathematical modeling, it was reported that Page thin-layer drying model was the most compatible model for drying pomegranate seeds. Mazandarani et al. (2017) were dried pomegranate seeds with microwave, hot air drying and sun drying methods. According to $R^2$, $\chi^2$, RMSE and MBE values, Midilli et al. thin-layer drying model was determined as the model most compatible with the drying process. Briki et al. (2019) were dried pomegranate seeds with traditional drying method, convective drying method and infrared drying methods and compared these two methods with each other. Allahdad et al. (2019) were investigated the impact of ultrasound osmotic dehydration as a pre-treatment on convective drying of pomegranate seeds. Adetoro et al. (2020) were investigated the phytochemical content and antioxidant content of pomegranate seeds dried by hot air drying and freeze drying methods under long-term storage conditions. Hamid et al. (2020) were dried pomegranate seeds with sun drying, in sun tunnel dryer, in mechanical cabinet and in oven. In this study, the effects of different drying methods on the phenolic compounds and antioxidant capacity of pomegranate seeds were investigated. Ozay Arancıoğlu et al. (2022) were examined the impact of different drying methods on the bioactive components of pomegranate seeds. It has been reported that freeze-dried pomegranate seeds have the highest bioactive component content.

In this study, pomegranate seeds were dried using infrared drying method at different infrared powers. The drying kinetics of pomegranate was examined and the most suitable thin-layer drying model was determined. The colour parameters of pomegranate seeds dried at different infrared powers were investigated.

## MATERIALS AND METHODS

### Materials

Pomegranate was purchased from a supermarket in Maltepe, Istanbul. The beginning moisture content of pomegranate seeds was determined by the AOAC method. The initial moisture content of pomegranate was calculated as on wet basis 78.74%. Pomegranate was stored under refrigerator conditions (4°C) until the experiments.

### Drying Process

The drying was fulfilled in a moisture analyser with 250W lamp power (Snijders Moisture Balance, Snijders b.v., Tilburg, Holland). The moisture analyser consists of a control panel, a 250-watt infrared lamp and a pot. Pomegranate seeds were weighed approximately 60 grams and placed in the pot. Infrared drying process was carried out of 62 to 104 W power range. The drying process was continued until the moisture content of pomegranate seeds decreased to 20% (wet basis). The loss of weight of the samples was followed at 10 minute intervals using a digital balance (model BB3000, Mettler-Toledo AG, Grefensee, Switzerland). Dried pomegranate seeds were cooled in laboratory conditions for about 15 minutes and packed in polyethylene bags.

## Mathematical Modelling

Using the experimental data, mathematical modeling of the drying process was made. For this, four well-known thin-layer drying models in Table 1 were used.

**Table 1.** Thin-layer drying models used

| Model no | Model name | Model equation[1] |
|---|---|---|
| 1 | Henderson & Pabis | $MR = a \exp(-kt)$ |
| 2 | Page | $MR = \exp(-kt^n)$ |
| 3 | Midilli & Kucuk | $MR = a\exp(-kt^n) + bt$ |
| 4 | Vega-Galvez | $MR = \exp(n + kt)$ |

[1] a, b, c, k and n: Drying constants and coefficients

Moisture content (MC) and moisture rate (MR) of pomegranate seeds were calculated by the Eqs. 1 and 2, respectively.

$$MC = \frac{W_i - W_d}{W_d} \quad (1)$$

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (2)$$

where MC is the moisture content (kg water/kg dry matter), $W_i$ is the weight of sample (kg), and $W_d$ is the dry matter content of sample (kg), $M_0$ is initial moisture content, $M_e$ is the equilibrium moisture content and $M_t$ is moisture content at t (kg water/kg dry matter) and t is drying time (min). Eq. 2 is usually simplified and used as $MR=M_t/M_0$. Drying rates (DR) were obtained by the following equation.

$$DR = \frac{M_t - M_{t+\Delta t}}{\Delta t} \quad (3)$$

where $M_{t+\Delta t}$ is moisture content at $t+\Delta t$ (kg water/kg dry matter), and t is time (min).

## Data Analysis

The suitability of the experimental data to the models was investigated by statistical analysis. The $R^2$ values were determined with the Statistica (StatSoft, Inc., Tulsa, OK) computer program. Relative percent error (P), reduced chi-square ($\chi^2$) and root-mean-square error (RMSE) values were calculated using Eqs. 4, 5 and 6, respectively. The model with the highest $R^2$ and lowest P, $\chi^2$ and RMSE values was determined to choose the most suitable model to drying process.

$$P = \frac{100}{N}\sum_{i=1}^{N}\frac{|MR_{exp,i} - MR_{pre,i}|}{MR_{exp,i}} \quad (4)$$

$$\chi^2 = \frac{\sum_{i=1}^{N}(MR_{exp,i} - MR_{pre,i})^2}{N - z} \quad (5)$$

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}(MR_{pre,i} - MR_{exp,i})^2\right]^{1/2} \quad (6)$$

Where $MR_{exp}$ and $MR_{pre}$ are experimental and predicted moisture ratios, respectively. N is number of trials and z is number of constants.

## Computation of Effective Moisture Diffusivity and Activation Energy

The diffusion equation of Fick's second law is used to calculate the effective diffusion coefficient ($D_{eff}$) of agricultural products. The diffusion of water during the drying process of agricultural crops can be expressed by Fick's following diffusion equation (Eq. 7).

$$MR = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{(2n+1)^2\pi^2 D_{eff} t}{4L^2}\right) \quad (7)$$

where $D_{eff}$ effective diffusion coefficient ($m^2/s$), L is half thickness of the slab (m), n is positive integers and t is time (min). Since drying is a long-continued process, the other terms of the series can be neglected and only the first term is used in the calculations. Eq. 8 is obtained by substituting the first term (n=0) in Eq. 7.

$$MR = \frac{8}{\pi^2}\exp\left(-\frac{\pi^2 D_{eff} t}{4L^2}\right) \quad (8)$$

The effective diffusion coefficient ($D_{eff}$) can be estimated by the slope of line of the ln(MR) versus time (t) graph.

The connection through effective moisture diffusion coefficient and infrared power can be obtained by modifying the Arrhenius equation. The activation energy value can be estimated using the modified Arrhenius equation shown as Eq. 9.

$$D_{eff} = D_0 \exp\left(-\frac{E_a m}{p}\right) \quad (9)$$

where $D_0$ is the pre-exponential factor of Arrhenius equation ($m^2/s$), $E_a$ is the activation energy (W/kg), p is the infrared power level (W), and m is the sample weight (kg).

### Color Measurements

Color of foods is an important parameter in determining their quality. After all drying conditions, the color parameters of the pomegranate seeds were measured by hand colorimeter (PCE-CSM 1; PCE Instruments UK Ltd., Southampton Hampshire, United Kingdom). Six measurements were taken for each infrared power value and the results were given as an average. The parameters measured in the colorimeter were L, a and b values. L, a and b terms measure brightness, green/redness, and blue/yellowness, respectively. The L term is scaled from 0 to 100. On the other hand, a and b terms are scaled between -60 and +60.

## RESULTS and DISCUSSION

### Drying Curves

Moisture content values of pomegranate seeds were plotted against drying time. Moisture content-drying time graph was given in Figure 1.

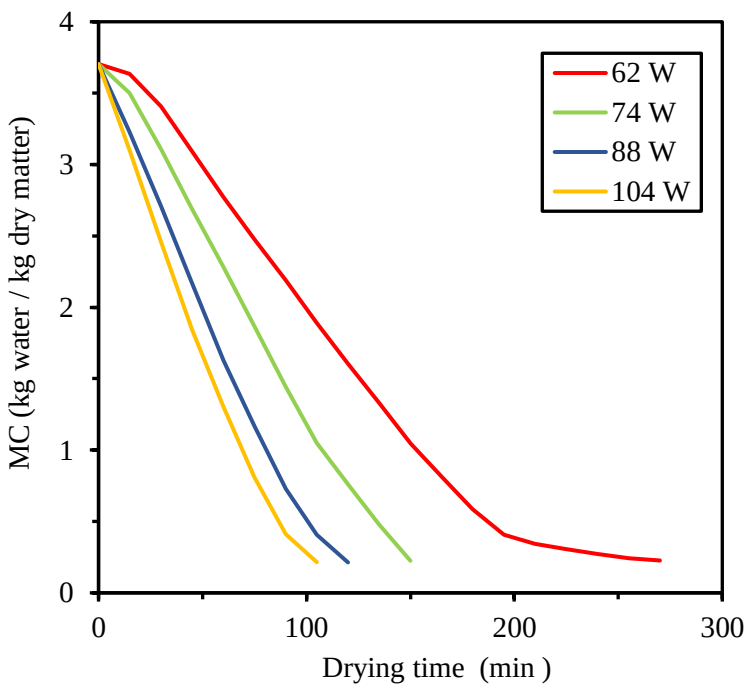


**Figure 1.** Drying Curve of Pomegranate Seeds at Different Infrared Power

It is observed that in infrared drying, an increase in the power level resulted in a decrease at drying time where the required time to reach the final moisture content at the power levels of 62, 74, 88 and 104 W, were about 270, 150, 120 and 105 min, respectively. The average drying rates increased 2.57 times as infrared power increased from 62 W to 104 W. In fact, at higher infrared powers, more heat is generated within the material leading to more vapour pressure differences between the centre and surface of the product and consequently, increases the drying rate. There are some studies in the literature proving that the drying time is shortened with the increase of infrared power. Some of these studies for the other agricultural products can be given as example: hot pepper (Saengrayap et al., 2015), strawberry (Adak et al., 2017), wormwood (Beigi, 2018) and apricot (Satorabi et al., 2021).

**Drying Rate**

The variation of the drying rate with drying time was plotted and shown in Figure 2. The drying rate showed different distribution curves for various infrared powers. The drying rate rised with the rise in infrared power. The highest drying rate was recorded at 104 W infrared power. The drying process occurred in the falling-rate period and a constant-rate period was not observed search out.

Similar studies on drying rate have been done before. The results of drying rate in the study of red pepper slices (Nasıroğlu and Kocabıyık, 2007), carrot slices (Kocabıyık and Tezer, 2009) and onion (Mongpraneet et al., 2002) support our study. In studies on infrared drying of agricultural crops, it has been noted that increasing the infrared drying power shortens the drying time. In addition, it is noteworthy that no constant-rate period was search out in any of the studies and the drying process took place in a falling-rate period.

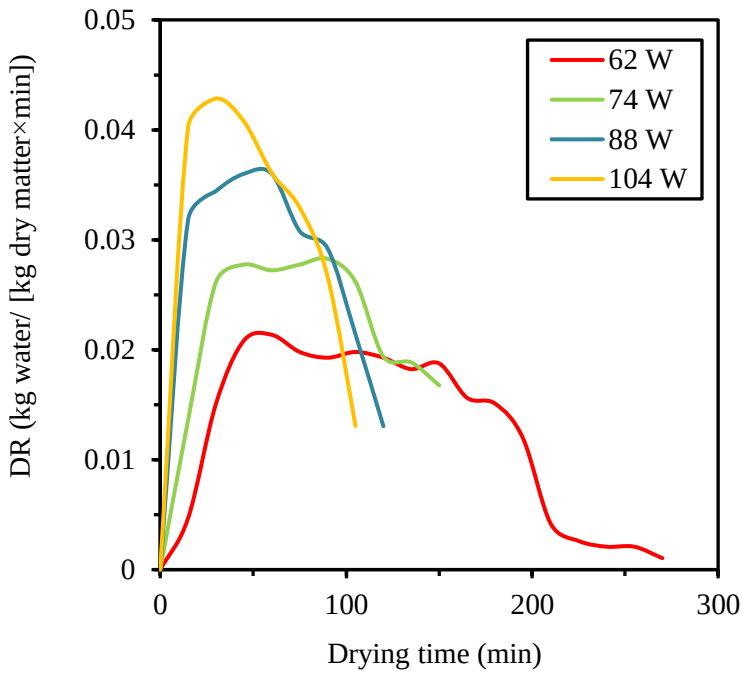


**Figure 2.** Variation of drying rate with drying time

**Evaluation of Drying Models**

Moisture content values of pomegranate seeds were converted into moisture content values using Eq. 2 for mathematical modelling. The compatibility of the moisture ratios obtained during the experimental study was compared with the thin-layer drying models stated in Table 1 by performing non-linear regression analysis. High $R^2$ and low P, $\chi^2$ and RMSE values indicate that the equation is suitable for the drying process. The $R^2$, P, $\chi^2$ and RMSE values acquired in consequence of the statistical analysis are demonstrated in Table 2.

**Table 2.** Result of the statistical analysis

| IR power (W) | Model name | $R^2$ | P | $\chi^2$ | RMSE |
|---|---|---|---|---|---|
| 62 | Henderson & Pabis | 0.9578 | 34.1347 | 0.005210 | 0.267635 |
| | Page | 0.9981 | 8.8457 | 0.000223 | 0.049406 |
| | Midilli & Kucuk | **0.9984** | **6.5555** | **0.000214** | **0.048420** |
| | Vega-Galvez | 0.9578 | 34.1332 | 0.005210 | 0.267633 |
| 74 | Henderson & Pabis | 0.9461 | 33.4566 | 0.006590 | 0.216174 |
| | Page | 0.9975 | 7.4995 | 0.000294 | 0.041869 |
| | Midilli & Kucuk | 0.9946 | 10.3805 | 0.000840 | 0.066251 |
| | Vega-Galvez | 0.9461 | 33.4572 | 0.006590 | 0.216175 |
| 88 | Henderson & Pabis | 0.9605 | 33.5563 | 0.005192 | 0.170927 |
| | Page | 0.9968 | 8.5757 | 0.000416 | 0.045776 |
| | Midilli & Kucuk | **0.9994** | **3.9170** | **0.000102** | **0.019991** |
| | Vega-Galvez | 0.9605 | 33.5567 | 0.005192 | 0.170927 |
| 104 | Henderson & Pabis | 0.9676 | 32.6969 | 0.004468 | 0.152482 |
| | Page | 0.9968 | 9.1871 | 0.000431 | 0.043019 |
| | Midilli & Kucuk | **0.9993** | **4.8262** | **0.000126** | **0.019397** |
| | Vega-Galvez | 0.9676 | 32.6994 | 0.004468 | 0.152480 |

As can be clearly seen from Table 2, the thin-layer drying model that best represents the drying processes was determined as Midilli & Kucuk for drying process at different infrared powers. The $R^2$, P, $\chi^2$ and RMSE values in the non-linear regression analysis for the drying process performed at different infrared powers varied between 0.9461-0.9994, 3.9170-34.1347, 0.000102-0.006590 and 0.019397-0.267635, respectively. In addition to this Figure 3 hold a candle to the empirical data with the data estimated according to the Midilli & Kucuk thin-layer drying model for different infrared powers.

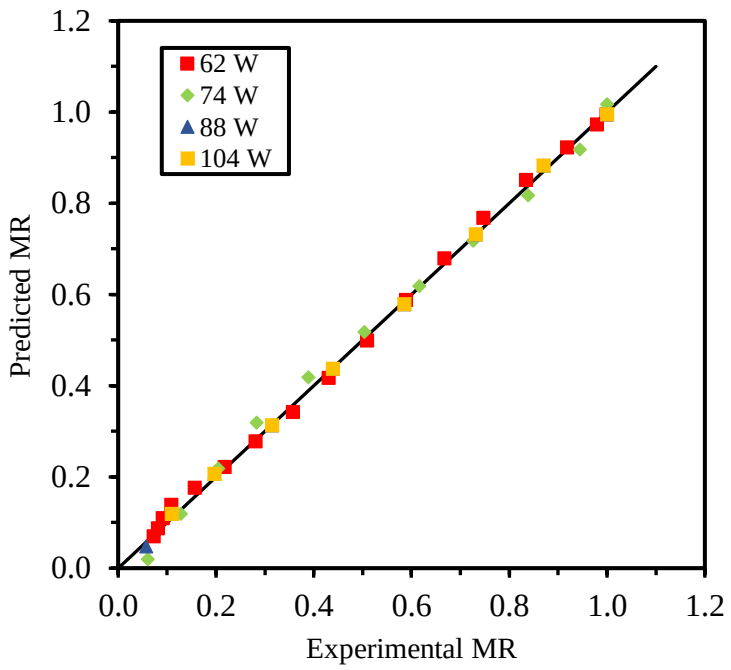


**Figure 3**. Comparison of experimental and predicted moisture ratio values using Midilli & Kucuk drying model

As can be seen in Figure 3, the estimated data is take part on the beeline. This proves that the Midilli & Kucuk thin layer drying model is compatible with the experimental data. Similarly, Alaei and Chayjan (2014) reported that the most compatible model with the drying kinetics of pomegranate and nectarine were Midilli & Kucuk and Aghbashlo et al. thin-layer drying models. In another study by Kayran and Doymaz (2015), apricot halves was dried and the most compatible thin-layer drying model was recorded as Midilli & Kucuk as in our study.

**Effective Moisture Diffusivity and Activation Energy**

The effective moisture diffusion coefficients ($D_{eff}$) were calculated using Eq. 8 for different infrared power values. The effective moisture diffusion coefficient values for drying experiments performed at different infrared powers varied between $8.76\times10^{-10}$ and $1.96\times10^{-9}$ $m^2/s$. Increasing the infrared power has been the main factor increasing the $D_{eff}$ values. The calculated $D_{eff}$ values were shown in Figure 4 for each infrared power value. Similar $D_{eff}$ values were calculated as a result of drying some agricultural products with infrared drying method by some authors. The effective diffusion coefficient was reported to be in the range of $0.71\text{-}2.86\times10^{-9}$ for pumpkin slices (Ghaboos et al., 2016), $2.55\text{-}6.82\times10^{-9}$ for jackfruit (Wu et al., 2021), and for sweet potato slices $1.31\text{-}3.66\times10^{-10}$ $m^2/s$ (Doymaz, 2011).

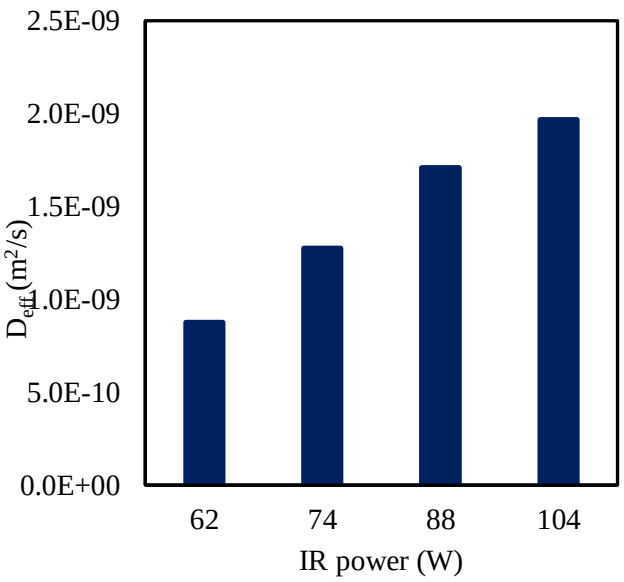


**Figure 4.** The $D_{eff}$ values at varied infrared power values

The activation energy ($E_a$) was calculated using the modified Arrhenius equation. Arrhenius-type relationship between effective moisture diffusivity and infrared power graph was drawn by using Eq. 9 (Fig. 5). $E_a$ values were estimated by using the slope of the line in the graph as 2.10 kW/kg.

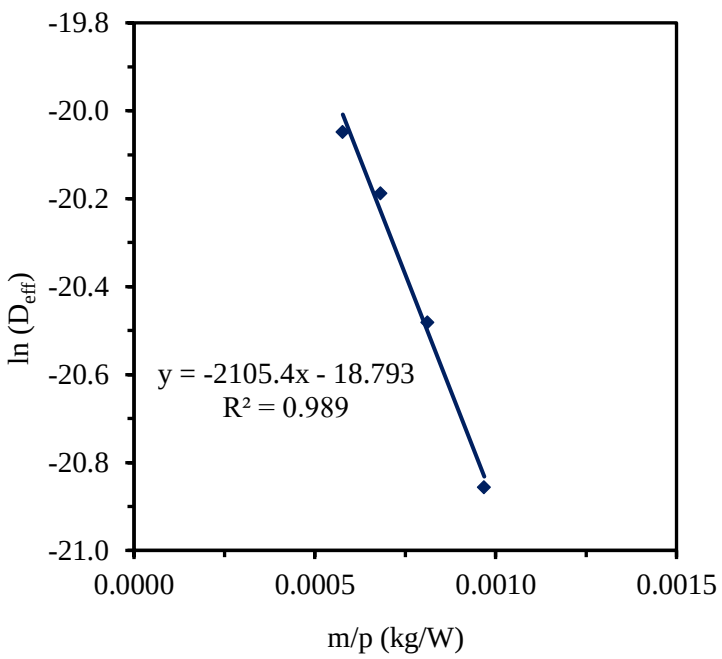


**Figure 5.** Arrhenius-type relationship between effective moisture diffusivity and infrared power

**Color Parameters**

L, a and b color parameters of pomegranate seeds were quantify for each infrared power value and were shown in Figure 6.

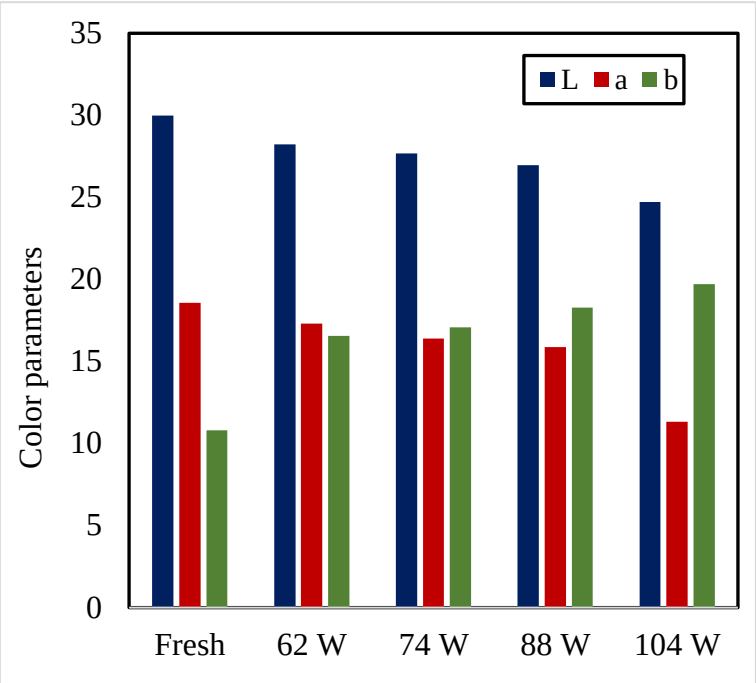


**Figure 6.** L, a and b values measured for fresh and dried pomegranate seeds at different infrared powers

It was noted that the L, a and b values varied for fresh and different infrared drying powers in the range of 24.70-29.99, 11.33-18.56 and 10.82-19.71, respectively. As can be clearly seen from the graph, the color parameters were directly affected by the infrared drying power. While the increase in infrared drying power decreased the brightness (L) and redness (a) values, it increased the yellowness (b) values. The color values of fresh pomegranate seeds were preserved at most in the drying made with 62 W infrared power.

## CONCLUSION

In the present study, pomegranate seeds were dried in infrared dryer at different power values. It has been observed that the infrared power value is the main parameter affecting the drying process. It was clearly determined that pomegranate seeds dried at higher infrared powers dried faster. The mathematical modeling of the drying process was carried out and the most suitable model for the drying process was determined as the Midilli & Kucuk thin-layer drying model. The $D_{eff}$ values of the drying process were calculated and varied between $8.76\times10^{-10}$ and $1.96\times10^{-9}$ $m^2/s$. The activation energy was calculated using the modified Arrhenius equation and was determined as 2.10 kW/kg. It has been noted that the color values of pomegranate seeds are directly affected by the infrared drying power. It was determined that pomegranate seed samples dried at low infrared power had color parameters closer to fresh pomegranate samples.

## REFERENCES

Adak N, Heybeli N and Ertekin C 2017. Infrared drying of strawberry. Food Chemistry, 219: 109-116.

Adetoro AO, Opara UL and Fawole OA 2020. Effect of hot-air and freeze-drying on the quality attributes of dried pomegranate (*Punica granatum* L.) arils during long-term cold storage of whole fruit. Agriculture, 10: 493-509.

Alaeı B and Chayjan RA 2015. Drying characteristics of pomegranate arils under near infrared-vacuum conditions. Journal of Food Processing and Preservation, 39: 469-479.

Allahdad Z, Nasiri M, Varidi M and Varidi MJ 2019. Effect of sonication on osmotic dehydration and subsequent air-drying of pomegranate arils. Journal of Food Engineering, 244: 202-211.

Alp D and Bulantekin Ö 2021. The microbiological quality of various foods dried by applying different drying methods: a review. European Food Research and Technology, 247: 1333-1343.

Banihani S, Swedan S and Alguraan Z 2013. Pomegranate and type 2 diabetes. Nutrition Research, 33: 341-348.

Beigi M 2018. Effect of infrared drying power on dehydration characteristics, energy consumption, and quality attributes of common wormwood (*Artemisia absinthium L.*) leaves. Journal of Agricultural Science and Technology, 20: 709-718.

Bchir B, Besbes S, Karoui R, Attia H, Paquot M and Blecker C 2012. Effect of air-drying conditions on physico-chemical properties of osmotically pre-treated pomegranate seeds. Food Bioprocess Technology, 5: 1840-1852.

Briki S, Zitouni B, Bechaa B and Amiali M 2019. Comparison of convective and infrared heating as means of drying pomegranate arils (*Punica granatum L.*). Heat and Mass Transfer, 55: 3189-3199.

Calín-Sánchez A, Figiel A, Hernández F, Melgarejo P, Lech K and Carbonell-Barrachina AA 2013. Chemical composition, antioxidant capacity, and sensory quality of pomegranate (*Punica granatum L.*) arils and rind as affected by drying method. Food Bioprocess Technology, 6: 1644-1654.

Doymaz İ 2011. Infrared drying of sweet potato (*Ipomoea batatas L.*) slices. Journal of Food Science Technology, 49: 760-766.

Doymaz İ 2012. Drying of pomegranate seeds using infrared radiation. Food Science and Biotechnology, 21: 1269-1275.

Ghaboos SHH, Ardabili SMS, Kashaninejad M, Asadi G and Aalami M 2016. Combined infrared-vacuum drying of pumpkin slices. Journal of Food Science Technology, 53: 2380-2388.

Hamid, Thakur NS, Thakur A and Kumar P 2020. Effect of different drying modes on phenolics and antioxidant potential of different parts of wild pomegranate fruits. Scientia Horticulturae, 274: 109656.

Horuz E and Maskan M 2015. Hot air and microwave drying of pomegranate (*Punica granatum L.*) arils. Journal of Food Science Technology, 52: 285-293.

Kayran S and Doymaz İ 2017. Infrared drying and effective moisture diffusivity of apricot halves: influence of pretreatment and infrared power. Journal of Food Processing and Preservation, 41: e12827.

Kingsly ARP and Singh DB 2007. Drying kinetics of pomegranate arils. Journal of Food Engineering, 79:741-744.

Kocabiyik H and Tezer D 2009. Drying of carrot slices using infrared radiation. International Journal of Food Science and Technology, 44: 953-959.

Mazandarani Z, Aghajani N, Daraei Garmakhany A, Bani Ardalan MJ and Nouri M 2017. Mathematical Modeling of Thin Layer Drying of Pomegranate (*Punica granatum L.*) Arils: Various Drying Methods. Journal of Agricultural Science and Technology, 19: 1527-1537.

Mongpraneet S, Abe T and Tsurusaki T 2002. Accelerated drying of welsh onion by far infrared radiation

under vacuum conditions. Journal of Food Engineering, 55: 147-156.

Nasiroglu S and Kocabiyik H 2007. Thin-layer infrared radiation drying of red pepper slices. Journal of Food Process Engineering, 32: 1-16.

Onwude DI, Hashim N, Janius RB, Nawi NM and Abdan K 2016. Modeling the thin-layer drying of fruits and vegetables: a review. Institute of Food Technologists, 15: 599-618.

Orsat V, Changrue V and Raghavan GSV 2006. Microwave drying of fruits and vegetables. An International Journal for Reviews in Postharvest Biology and Technology, 6: 1-7.

Ozay-Arancioglu I, Bekiroglu H, Karadag A, Saroglu O, Tekin-Çakmak ZH and Karasu S 2021. Effect of different drying methods on the bioactive, microstructural, and in-vitro bioaccessibility of bioactive compounds of the pomegranate arils. Food Science and Technology, 42: e06221.

Özgüven AI and Yılmaz C 2000. Pomegranate growing in Turkey. Options Méditerranéennes, 42: 41-48.

Riadh MH, Ahmad SAB, Marhaban MH and Soh AC 2015. Drying Technology, 33: 322-335.

Saengrayap R, Tansakul A and Mittal GS 2015. Effect of far-infrared radiation assisted microwave-vacuum drying on drying characteristics and quality of red chilli. Journal of Food Science Technology, 52: 2610-2621.

Satorabi M, Salehi F and Rasouli M 2021. The influence of xanthan and balangu seed gums coats on the kinetics of infrared drying of apricot slices: ga-ann and anfis modeling. International Journal of Fruit Science, 21:468-480.

Shukla S 2011. Freeze drying process: a review. International Journal of Pharmaceutical Sciences and Research, 2: 3061-3068.

Süfer Ö and Palazoğlu TK 2019. A study on hot-air drying of pomegranate. Journal of Thermal Analysis and Calorimetry, 137: 1981-1990.

Teixeira da Silva JA, Rana TS, Narzary D, Verma N, Meshram DT, Ranade SA 2013. Pomegranate biology and biotechnology: A review. Scientia Horticulturae, 160: 85-107.

Toğrul H 2005. Simple modeling of infrared drying of fresh apple slices. Journal of Food Engineering, 71:311-323.

Toğrul H 2006. Suitable drying model for infrared drying of carrot. Journal of Food Engineering, 77:610-619.

Wu B, Guo X, Guo Y, Ma H and Zhou C 2021. Enhancing jackfruit infrared drying by combining ultrasound treatments: Effect on drying characteristics, quality properties and microstructure. Food Chemistry, 358: 129845.

## *ORAL PRESENTATION*

# Experimental Study on Hot Air Drying of Pomegranate Seeds

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## Abstract

Pomegranate seeds were dried by hot air drying method. Cabinet dryer was operated at 60, 65, 70, 75 and 80°C air temperatures. The drying process was continued until the humidity level decreased to 20% on wet basis. The drying curves show that the drying process takes place in the falling rate drying period. The data obtained from the drying process were modeled by using four different thin-layer drying models (Logarithmic, Aghbashlo et al., Parabolic and Vega-Galvez). The compatibility of the models was determined by high $R^2$ and low P (relative percent error), $\chi^2$ (reduced chi-square) and RMSE (root mean square error) values. Considering the drying data the most suitable thin-layer drying model was determined as Aghbashlo et al. model. The effective diffusion coefficients were calculated according to Fick's diffusion model. Diffusion coefficients for different temperatures varied between $5.67\times10^{-10}$ and $1.38\times10^{-9}$ $m^2/s$. Activation energy values were calculated depending on the diffusion coefficients as 39.96 kJ/mol.

**Keywords:** Activation Energy, Effective Moisture Diffusivity, Drying, Mathematical Modelling, Pomegranate Seeds.

## INTRODUCTION

Pomegranate (*Punica granatum* L.) is a granular fruit usually grown in the Mediterranean climate. The pomegranate tree, which is between 3.5 and 4.8 meters in size, can live up to two hundred years. The pomegranate fruit, which is believed to bring abundance and fertility since ancient times, has also been used for medicinal purposes (Jurenka, 2008). Although it is believed that its homeland is Iran and its surroundings, there are different opinions on this issue. Today, the countries with the highest pomegranate fruit production capacity are Iran, USA, China, Japan, India and Russia (Akbarpour et al., 2009). Pomegranate fruit can be consumed fresh, but also as jam, juice or marmalade. Pomegranate fruit contains water, carbohydrate, protein, fat, mineral and vitamin content, as well as high polyphenolic compounds. Pomegranate fruit, which contains a high rate of beneficial compounds, also draws attention with its antioxidant content. Today, with the increasing importance of healthy nutrition, this kind of fruits and vegetables have started to attract more attention and take place in diet lists. Pomegranate shows antidiabetic, antimicrobial and anti-inflammatory properties thanks to its flavonoid content (Singh et al., 2018). However, it is impossible for the pomegranate fruit, which has a water content of around 78%, to remain intact for a long time (Dahham et al., 2010). High water content increases the rate of spoilage reactions and shortens the shelf life of foods. In order for these nutritionally important foods to last longer, a preservation method such as drying should be applied.

Due to the high water content of foods, some microbiological reactions occur in them. Drying is a food preservation method that has been practiced since ancient times. Storage and transportation costs are also reduced due to the weight reduction in foods with the drying process. By reducing the humidity in foods below a critical level, the reproduction of microorganisms is prevented (Kaveh et al., 2021). The drying process can be applied in two different ways as natural and artificial. Sun drying is a natural drying type and has many disadvantages such as being unprotected against contamination, microorganisms and insects. Convective drying, microwave drying, infrared drying and freeze drying are artificial drying types. Although each method has advantages/disadvantages, the hot air drying method is the most frequently used drying method in industry (Delfiya et al., 2021). There are a few study on the drying of pomegranate fruit in the literature.

Nikbakht et al. (2014), dried pomegranate seeds in a microwave assisted hot air dryer at different temperatures and drying air values and investigated their energy efficiency. Kingsly and Singh (2007), dried pomegranate seeds at different temperatures with the hot air drying method. As a result of the mathematical modeling, the

most suitable thin layer drying model was suggested as Page. Dak and Pareek (2014), dried pomegranate seeds in a vacuum assisted microwave dryer. The effective diffusion coefficient was calculated for different microwave powers and varied between $5.18\times10^{-11}$ and $6.58\times10^{-10}$ $m^2/s$. Cano-Lamadrid et al. (2017), were dried pomegranate seeds by convective pre-drying and vacuum assisted microwave post-drying method. In order to preserve the antioxidant capacity and color values of pomegranate seeds, they was applied osmotic dehydration with concentrated fruit juices before drying. Karaaslan et al. (2014), investigated the anthocyanin, phenolic compound and antioxidant capacity of dried pomegranate seeds by applying different temperatures and pre-treatments. Eissa (2021), dried pomegranate seeds using a combination of infrared and hot air drying methods. The rehydration capacity of the dried samples has been reported to be between 0.55 and 1.16 for different infrared power levels.

In this study, pomegranate seeds will be dried with hot air drying method at different drying air temperatures. The drying kinetics of pomegranate seeds will be examined and mathematical modeling of the drying process will be made. The most suitable thin layer drying model for the drying process will be determined. Effective diffusion coefficient and activation energy values will be calculated.

## MATERIALS AND METHODS

### Materials

Pomegranate samples (sour) was obtained from a supermarket in Maltepe, Istanbul. The beginning moisture content of pomegranate seeds was defined by the AOAC method by taking the average of four trials. The beginning moisture content of pomegranate seeds was calculated as %77.42 on wet basis.

### Drying Procedure

The drying process was performed in a cabin dryer at Yıldız Technical University, Department of Chemical Engineering. The cabinet dryer consists of control panel, fan, electric heater and air filter. First, the temperature value of the cabinet dryer was adjusted to ensure that the dryer reaches steady state conditions. Then, some pre-treatments were applied to the pomegranate. The pomegranate was first washed with cold and clean water. Then the outer shells were peeled off. The pomegranate seeds were separated and taken into a different container. Approximately 60 grams of pomegranate seeds samples was weighed for all test sets and uniformly sprawl out in a sphere basket in a single layer. Drying experiments were carried out at 60, 65, 70, 75 and 80℃. The relative humidity (5-14%) and air velocity (2 m/s) were kept constant.

All drying experiments were maintained up to the moisture content of the pomegranate seeds decreased to 20% on a wet basis. The moisture losses were observed at 15 min intervals by a digital balance (Mettler, model BB3000, Mettler-Toledo AG, Grefensee, Switzerland). Drying experiments were replicated five times and the results were given as average. After the drying process was completed, the product was cooled for about 10 minutes under laboratory conditions and packaged in polyethylene packs.

### Mathematical Modelling

The moisture content (M) of pomegranate seeds was counted up by the following equation:

$$M = \frac{W_i - W_d}{W_d} \quad (1)$$

where M is the moisture content (kg water/kg dry matter), $W_i$ is the weight of sample (kg), and $W_d$ is the dry matter content of sample (kg).

The drying rate (DR) of pomegranate seeds at a specific time period was counted up as follows:

$$DR = \frac{M_{t1} - M_{t2}}{t_2 - t_1} \quad (2)$$

where $t_1$ and $t_2$ are drying times (min); and $M_{t1}$ and $M_{t2}$ are the moisture contents (on dry basis) at times $t_1$ and $t_2$, respectively.

Four different thin-layer models were used to defining the drying characteristic of pomegranate seeds. The models were resumed in Table 1. Moisture ratio (MR) value was counted up with the Eq. 3.

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (3)$$

where $M_0$ is initial moisture content, $M_e$ is the equilibrium moisture content and $M_t$ is moisture content at t (kg water/kg dry matter). And t is drying time (min). The equation represented by Eq. 3 is usually simplified and used a $MR=M_t/M_0$.

**Data Analysis**

The compatibility of the thin-layer drying models in Table 1 is measured by the values of coefficient of determination ($R^2$), relative percent error (P), reduced chi-square ($\chi^2$) and root-mean-square error (RMSE). The $R^2$ values were determined with the Statistica (StatSoft, Inc., Tulsa, OK) computer program. The P, $\chi^2$ and RMSE values were calculated by Eq. 4, 5 and 6, respectively.

$$P = \frac{100}{N}\sum_{i=1}^{N}\frac{\left|MR_{exp,i}-MR_{pre,i}\right|}{MR_{exp,i}} \quad (4)$$

$$\chi^2 = \frac{\sum_{i=1}^{N}(MR_{exp,i}-MR_{pre,i})^2}{N-z} \quad (5)$$

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}(MR_{pre,i}-MR_{exp,i})^2\right]^{1/2} \quad (6)$$

where $MR_{exp}$ and $MR_{pre}$ are experimental and predicted moisture ratios, respectively. N is number of trials and z is number of constants. High $R^2$ and low P, $\chi^2$ and RMSE values show that the model is more compatible to experimental datas observed.

**Table 1.** Thin-layer drying models used

| Model no | Model name | Model equation[1] |
|---|---|---|
| 1 | Logarithmic | $MR = a\exp(-kt) + c$ |
| 2 | Parabolic | $MR = a + bt + ct^2$ |
| 3 | Aghbashlo et al. | $MR = \exp\left(-\left(\frac{at}{1+bt}\right)\right)$ |
| 4 | Vega-Galvez | $MR = \exp(n + kt)$ |

[1] a, b, c, k and n: Drying constants and coefficients

**Computation of Effective Moisture Diffusivity and Activation Energy**

The effective diffusion coefficient ($D_{eff}$) was caunted up by Fick's second law of diffusion equation. This law is generally appropriate in describing the diffusion of water that takes place in the drying of agricultural crops. Following equation (Eq. 7) gives the solution of the diffusion model for slab geometry:

$$MR = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{(2n+1)^2\pi^2 D_{eff}t}{4L^2}\right) \quad (7)$$

where $D_{eff}$ effective diffusion coefficient ($m^2/s$), L is half thickness of the slab (m), n is positive integers and t is time (min). Since drying is a long-continued process, the other terms of the series can be neglected and only the first term is used in the calculations. Equation 8 is obtained by substituting the first term (n=0) in Eq. 7.

$$MR = \frac{8}{\pi^2}exp\left(-\frac{\pi^2 D_{eff}t}{4L^2}\right) \quad (8)$$

Take the logarithm of both sides of the Eq. 8.

$$\ln(MR) = \ln\left(\frac{8}{\pi^2}\right) - \frac{\pi^2 D_{eff}t}{4L^2} \quad (9)$$

The slope of the line obtained by plotting the graph of ln(MR) versus time (t) can be expressed by the Eq. 10.

$$slope = \frac{\pi^2 D_{eff}}{4L^2} \quad (10)$$

The relationship between the effective diffusion coefficient and temperature is expressed by the Arrhenius equation.

$$D_{eff} = D_0\exp\left(-\frac{E_a}{R(T+273.15)}\right) \quad (11)$$

where $D_0$ is pre-exponential factor ($m^2/s$), $E_a$ is activation energy (kJ/mol), R is the universal gas constant (kJ/mol K) and T is temperature (°C).

## RESULTS AND DISCUSSION

### Drying Curves

The variation of moisture content with time graph for the drying process performed at five different temperatures (60, 65, 70, 75 and 80°C) is shown in Figure 1.

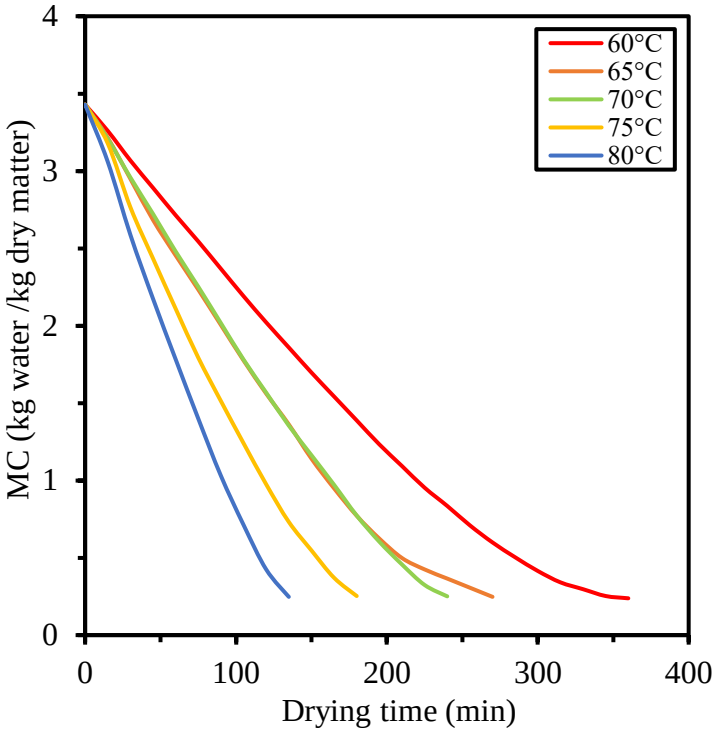


**Figure 1.** Temperature effect on pomegranate seeds dried

As seen in Fig. 1, the drying time was shortened by increasing the temperature of the drying air. Drying times were recorded as 360, 270, 240, 180 and 135 minutes for pomegranate seeds dried at 60, 65, 70, 75 and 80°C, respectively. The average drying rate was rose 2.66 times by rising the temperature from 60°C to 80°C. With the rise in temperature, the steam pressure of the moisture in the food also increases and it evaporates faster. Süfer and Palazoğlu (2019) also dried pomegranate seeds with the convective drying method. Drying times for the drying processes performed at 55, 65 and 75°C were reported as 660, 240 and 150 minutes, respectively. Their study supports our study. In addition, there are also other studies indicating that the drying duration is shortened by rising the temperature in the drying processes for different agricultural products. Similar results regarding the effect of temperature have been obtained recently for apricot cubes (Polat and İzli, 2022) and ginger slices (Pei et al., 2022).

### Drying Rate

The characteristics of the drying rate (DR) versus drying time curves were shown in Fig. 2. Drying rate curves can be characterized as a falling-rate period. A constant drying rate period was not observed in the drying curves. It can be clearly seen in the curves that the drying rate rised with the increase in temperature.

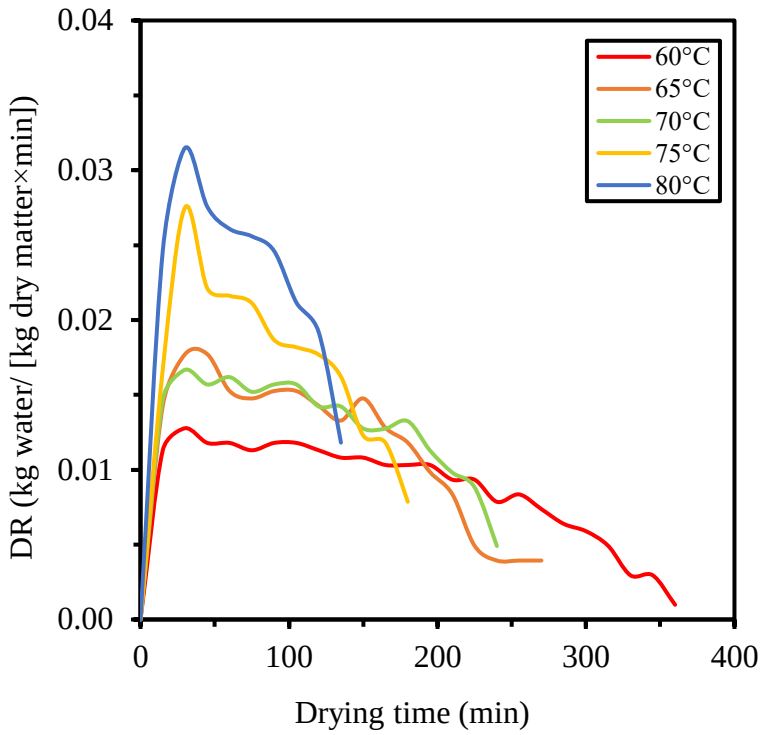


**Figure 2.** Drying rate as a function of time at different drying temperature

As can be seen in Figure 2, the highest drying rate was obtained after the heating period at 80°C. Similar results regarding drying rate were obtained for pomegranate seeds (Kingsly and Singh, 2007) and for carrot slices in a different study (Doymaz, 2017).

**Evaluation of Drying Models**

The choosing of the most fit model for the drying process was made by non-linear regression analysis. Regression analysis was carried out for four different thin-layer drying models shown in Table 1. The choosing of the most suitable model was made according to the $R^2$, P, $\chi^2$ and RMSE statistical analyses. The model with high $R^2$ and low P, $\chi^2$ and RMSE values was chosen as the most suitable thin-layer model. The results of the statistical analysis were summarized in Table 2. The most compatible thin-layer drying model with the drying process was chosen as Aghbashlo et al. with the highest $R^2$ and lowest P, $\chi^2$ and RMSE values. The $R^2$, P, $\chi^2$ and RMSE values for of Aghbashlo et al. thin-layer drying model varied between 0.9986-0.9997, 1.17-5.54, 0.000008-0.000136 and 0.008649-0.039711, respectively. Fig. 3, contrasts the empirical data with the estimated data for the Aghbashlo et al. thin-layer drying model. The fact that the empirical data obtained and the estimated data are on the same line proves the compatibility of the model with the drying process.

**Table 2.** Statistical values of thin-layer drying of pomegranate seeds at different air temperature

| T (°C) | Model name | $R^2$ | P | $\chi^2$ | RMSE |
|---|---|---|---|---|---|
| 60 | Logaritmic | 0.997516 | 6.9847 | 0.000249 | 0.062768 |
| | **Aghbashlo et al.** | **0.999565** | **3.2368** | **0.000041** | **0.022471** |
| | Parabolic | 0.998569 | 5.0683 | 0.000143 | 0.048667 |
| | Vega-Galvez | 0.973954 | 22.3329 | 0.002499 | 0.215015 |
| 65 | Logaritmic | 0.996178 | 8.4117 | 0.000408 | 0.069399 |
| | **Aghbashlo et al.** | **0.998630** | **5.5425** | **0.000136** | **0.039711** |
| | Parabolic | 0.997873 | 5.7557 | 0.000227 | 0.052288 |
| | Vega-Galvez | 0.976897 | 19.6944 | 0.002325 | 0.180372 |
| 70 | Logaritmic | 0.998647 | 3.56541 | 0.000107 | 0.030076 |
| | **Aghbashlo et al.** | **0.999747** | **1.1750** | **0.000008** | **0.008649** |
| | Parabolic | 0.998949 | 3.0917 | 0.000079 | 0.025606 |
| | Vega-Galvez | 0.966280 | 22.6304 | 0.003174 | 0.190863 |
| 75 | Logaritmic | 0.998647 | 3.7701 | 0.000171 | 0.031937 |
| | **Aghbashlo et al.** | **0.999747** | **1.1847** | **0.000062** | **0.013008** |
| | Parabolic | 0.998949 | 2.7670 | 0.000114 | 0.025192 |
| | Vega-Galvez | 0.966280 | 21.1550 | 0.002835 | 0.155642 |
| 80 | Logaritmic | 0.998647 | 4.9930 | 0.000182 | 0.031708 |
| | **Aghbashlo et al.** | **0.999747** | **1.3397** | **0.000029** | **0.010181** |
| | Parabolic | 0.998949 | 4.2084 | 0.000141 | 0.027643 |
| | Vega-Galvez | 0.966280 | 25.5613 | 0.003981 | 0.159312 |

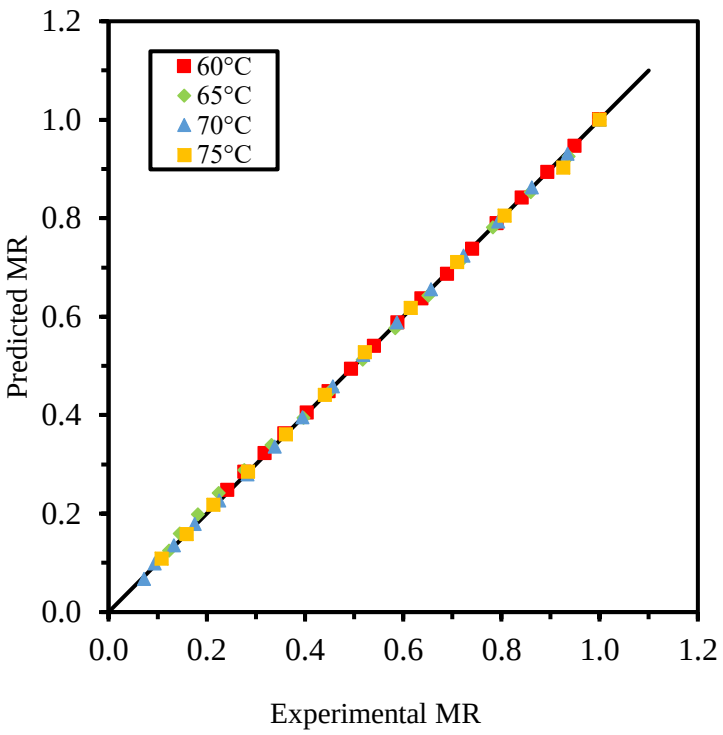


**Figure 3.** Comparison of experimental and predicted moisture ratio values using Aghbashlo et al. model

### Effective Moisture Diffusivity and Activation Energy

The effective moisture diffusion coefficient ($D_{eff}$) values were counted up for different temperature by using Eq. 10. The calculated $D_{eff}$ values were given in Figure 4. The $D_{eff}$ values varied between $5.66\times10^{-10}$ and $1.38\times10^{-9}$ $m^2/s$ at different drying air temperatures. The rise in drying temperature was the primary reason that increased the $D_{eff}$ value.

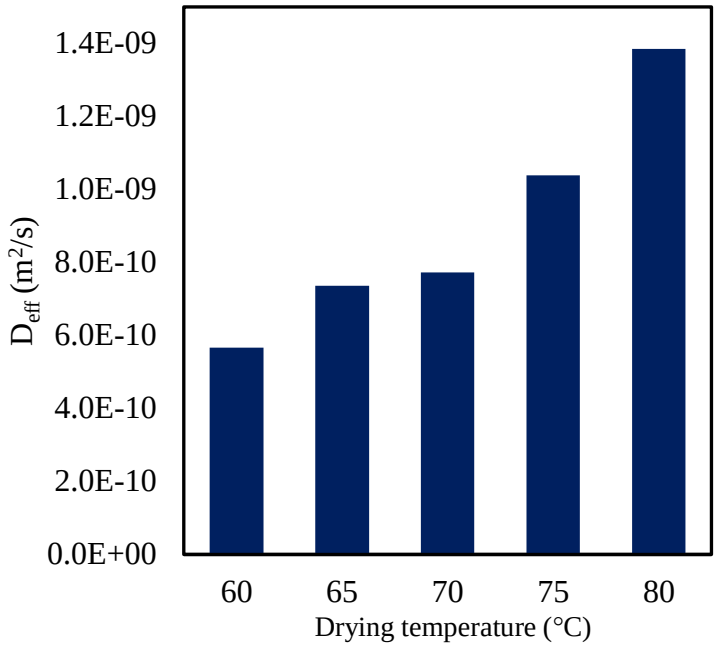


**Figure 4.** Effective moisture diffusion coefficients variation depending on drying air temperature

Adetoro et al. (2020) dried pomegranate seeds with the hot air drying method. They reported that the effective moisture diffusion coefficient varied between $4.81\times10^{-9}$ and $1.11\times10^{-8}$ $m^2/s$. In addition, Doymaz (2012) dried pomegranate seeds with the hot air drying method and calculated the effective moisture diffusion coefficients in the range of $9.373\times10^{-11}$ and $3.429\times10^{-10}$ $m^2/s$. These studies in the literature are support our study.

Activation energy ($E_a$) value were calculated with Equation 11, using slope of the lines in Figure 5. The $E_a$ values calculated as 35.92 kJ/mol. In the literature, there are activation energy values calculated for drying different agricultural products: for pomegranate aril 49.7 kJ/mol (Doymaz, 2012), for jambu fruit 16.61 kJ/mol (Gomes et al., 2018), for tomato 33.32 kJ/mol (Taheri-Garavand et al., 2011) and for red pepper 41.95 kJ/mol (Gupta et al., 2002).

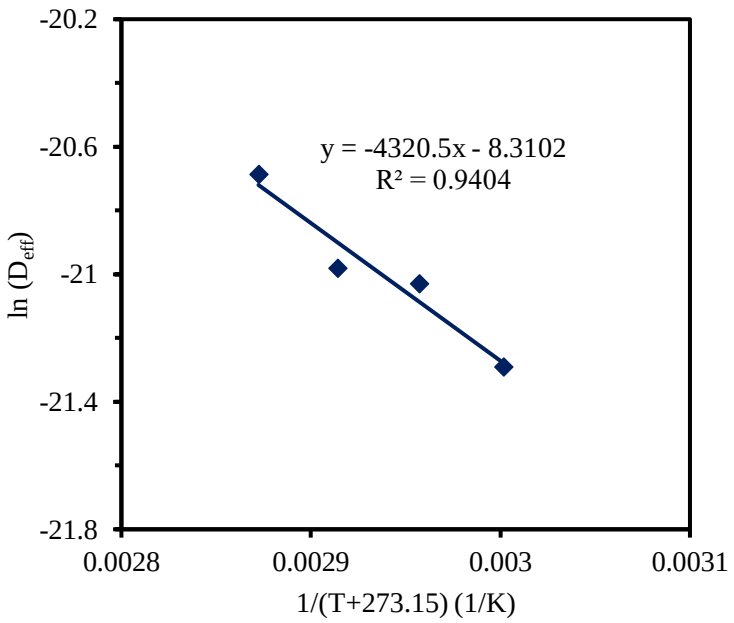


**Figure 5.** Arrhenius type relationship between effective moisture diffusivity and drying air temperature

## CONCLUSION

Pomegranate seeds were dried in a cabinet dryer at different temperatures to find out the influence of temperature on the drying process. The rise in drying air temperature accelerated the drying process by increasing the vapor pressure of the moisture in the food. This is all to say, by increasing the drying temperature from 60°C to 80°C, the drying time was shortened by 62.5%. The thin-layer drying model that best represents the drying process has been determined as Aghbashlo et al. model. In addition, the effective moisture diffusion coefficient was calculated and it was determined that it was between $5.66\times10^{-10}$ and $1.38\times10^{-9}$ $m^2/s$ at different drying temperatures. The $E_a$ was counted up as 35.92 kJ/mol using the Arrhenius equation.

## REFERENCES

Adetoro AO, Tsige AA, Opara UL and Fawole OA 2020. Mathematical modelling of blanch-assisted drying of pomegranate (*Punica granatum*) arils in a hot-air drier. Processes, 8: 611-629.

Akbarpour V, Hemmati K and Sharifani M 2009. Physical and Chemical properties of pomegranate (Punica granatum L.) fruit in maturation stage. American-Eurasian Journal of Agricultural & Environmental Sciences, 6: 411-416.

Cano-Lamadrid M, Lech K, Michalska A, Wasilewska M, Figiel A, Wojdyło A and Carbonell-Barrachina AA 2017. Influence of osmotic dehydration pre-treatment and combined drying method on physico-chemical and sensory properties of pomegranate arils, cultivar Mollar de Elche. Food Chemistry, 232: 306-315.

Dahham SS, Ali MN, Tabassum H and Khan M 2010. Studies on antibacterial and antifungal activity of pomegranate (Punica granatum L.). American-Eurasian Journal of Agricultural & Environmental Sciences, 9:273-281.

Dak M and Pareek NK 2014. Effective moisture diffusivity of pomegranate arils under going microwave-vacuum drying. Journal of Food Engineering, 122: 117-121.

Delfiya DSA, Prashob K, Murali S, Alfiya PV, Samuel MP and Pandiselvam R 2021. Drying kinetics of food materials in infrared radiation drying: A review. Journal of Food Process Engineering, 45: e13810.

Doymaz İ 2012. Prediction of drying characteristics of pomegranate arils. Food Analytical Methods, 5: 841-848.

Doymaz İ 2017. Drying kinetics, rehydration and colour characteristics of convective hot-air drying of carrot slices. Heat and Mass Transfer, 53: 25-35.

Eissa AS 2021. Development and evaluation of pomegranate seeds drying unit using infrared / hot air dryer. Misr Journal of Agricultural Engineering, 38:49-64.

Gomes FP, Resende O, Sousa EP, Oliveira DEC and Araújo Neto FR 2018. Drying kinetics of crushed mass of 'jambu': Effective diffusivity and activation energy. Revista Brasileira de Engenharia Agrícola e Ambiental, 22: 499-505.

Gupta P, Ahmed J, Shivhare US and Raghavan GSV 2002. Drying characteristics of red chilli. Drying Technology, 20: 1975-1987.

Jurenka J 2008. Therapeutic applications of pomegranate (Punica granatum L.): A review. Alternative Medicine Review, 13: 128-144.

Karaaslan M, Yılmaz FM, Cesur Ö, Vardin H, Ikinci A and Dalgıç AC 2014. Drying kinetics and thermal degradation of phenolic compounds and anthocyanins in pomegranate arils dried under vacuum conditions .
International Journal of Food Science and Technology, 49: 595-605.

Kaveh M, Abbaspour-Gilandeh Y and Nowacka M 2021. Optimisation of microwave-rotary drying process and quality parameters of terebinth. Biosystems Engineering, 208: 113-130.

Kingsly ARP and Singh DB 2007. Drying kinetics of pomegranate arils. Journal of Food Engineering, 79: 741-744.

Nikbakht AM, Motevali A and Minaei S 2014. Energy and exergy investigation of microwave assisted thin-layer drying of pomegranate arils using artificial neural networks and response surface methodology. Journal of the Saudi Society of Agricultural Sciences, 13: 81-91.

Pei Y, Li Z, Song C, Li J, Xu W and Zhu G 2022. Analysis and modelling of temperature and moisture gradient for ginger slices in hot air drying. Journal of Food Engineering, 323: 111009.

Polat A and Izli N 2022. Determination of drying kinetics and quality parameters for drying apricot cubes with electrohydrodynamic, hot air and combined electrohydrodynamic-hot air drying methods. Drying Technology, 40: 527-542.

Singh B, Singh JP, Kaur A and Singh N 2018. Phenolic compounds as beneficial phytochemicals in pomegranate (*Punica granatum L.*) peel: A review. Food Chemistry, 261: 75-86.

Süfer Ö and Palazoğlu TK 2019. A study on hot-air drying of pomegranate. Journal of Thermal Analysis and Calorimetry, 137: 1981-1990.

Taheri-Garavand A, Rafiee S and Keyhani A 2011. Effective moisture diffusivity and activation energy of tomato in thin layer dryer during hot air drying. International Transaction Journal of Engineering, 2: 239-248.

*ORAL PRESENTATION*

# Low Temperature Effects on Some Physiological Parameters in Red Capia Peppers (*Capsicum annuum* L.) Grown in Greenhouses

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## Abstract

Pepper (Capsicum annuum L.) is one of the most common greenhouses grown vegetables is located on the Mediterranean coast of Turkey. Pepper seedlings are more sensitive to temperature drops, especially during reproductive periods and, they are stressed below 15 degrees. Sudden low temperatures in vegetable cultivation are an important problem that restricts production and causes yield loss. The Mediterranean region, which includes Turkey, is the region where climate change is felt most deeply. Sudden and short-term temperature fluctuations negatively affect plants. For this reason, especially in this study, the changes in pepper seedlings grown in greenhouses as a result of the sudden temperature drop in the Mediterranean region of Turkey in March 2022 were investigated in real time. Pepper seedlings in greenhouse conditions where the day temperature is 15 degrees and the night temperature is 5 degrees were examined at 24, 48, 72 hour intervals. In addition, the plants were subjected to a 24-hour recovery period (26/18 day/night degrees). Under low temperature conditions, metabolic events such as changes in membrane structure and water loss are observed. In the study, determine Malondialdehite (MDA) contentent ,water status analyzes (LWP-MPa, RWC-%) and growth parameters measurements (fresh weight-g), were carried out time depended. The healing process was followed in the same way. Cold stress was carried out during the flowering period. There was an increase in MDA and LWP values at 72 hours compared to the control, and a significant decrease in RWC and weight. In the recovery result, there was no significant increase in weight while MDA, LWP and RWC values approached the control. Rapid intervention/support in the first 72 hours in greenhouses under low temperature stress can save plants.

**Keywords:** *Capsicum annum*, Cold stress, Recovery

## INTRODUCTION

Drought due to high temperature, frost due to low temperature are the main stress factors affecting the survival and development of plants. Suboptimal temperatures affect growth and development with biochemical, morphological and anatomical changes that affect the physiology of the whole plant (Berry and Bjorkman 1980). Hot-cold weather events, winter storms, frost events significantly damage the agricultural production patterns and economies of the countries. Sudden weather events due to climate change threatens agricultural production (Bhutia et al., 2018). Agriculture is an important sector of the Turkey economy. [MGM, 2018-2020].

In general, plants encounter two types of low temperature stress; chilling and freezing. Chilling stress is when plants are exposed to a low temparuture above 0 °C.Freezing stress is when plants are exposed to a low temparuture below 0 °C (Thomashow et al., 2010]. Chilling stress; it can affect growth and development in different ways and rates depending on the severity and duration of the stress, the genotype of the plant exposed to stress and the development period. Chilling stress can have negative consequences in the flowering period, fruit setting and seed formation of the plant by directly affecting the yield and the continuation of the plant's generation (Mock and Eberhart, 1972). When plants are exposed to low temperature stress, changes occur in their cell walls and membranes, imbalances in water structures occur and photosynthesis slows down.
Red capia pepper is a fruit that plays an important role in the agricultural sector of Mediterranean countries and has a high economic value. Due to being a valuable nutrient, it is among the most cultivated plant species in greenhouses in the world. Red capia pepper very sensitive to cold. Since the pepper is a plant that has an important role in open and greenhouse cultivation, it is also adversely affected by cold stress. In particular, product losses can reach serious levels due to adverse conditions in which the heating is insufficient and the sudden and short-term coldness is experienced. However, the most important and surest way to eliminate these negativities will be the rapid support and interventions to be made in these periods.

Especially in this study, the changes in pepper seedlings grown in greenhouses as a result of the sudden temperature drop in the Mediterranean region of Turkey in March 2022 were investigated in real time. We aimed to reveal the changes caused by low temperature stress effects on some physiological parameters in red capia peppers (Capsicum annuum L.) grown in greenhouses.

## MATERIALS AND METHODS

### Plant Material and Low Temperature Treatment

*Capsicum annuum* L. seeds were obtained from Antalya/Turkey. Plants were grown in greenhouse conditions in March 2022, Mersin/Turkey. Pepper seedlings in greenhouse conditions where the day temperature is 15 degrees and the night temperature is 5 degrees were examined at 24, 48, 72 hour intervals. In addition, the plants were subjected to a 24-hour recovery period (26/18 day/night degrees). Pepper seedlings were grown in greenhouses in pots (Figure 1).



Figure 1. *Capsicum annuum* plants exposed to low temperature stress in greenhouse.

### Growth Analysis

In order to determine the changes that cold stress may cause in plant biomass, 5 seedlings were taken randomly from plants belonging to each group. Shoot fresh weights were weighed (Morel et al., 2010). Growth parameter measurements (fresh weight-g), were carried out time depended.

### Water Status Analysis

Leaf water potential (LWP) and relative water content (RWC) analyzes were performed to determine the changes that may occur in plant-water status over time under cold stress conditions (Figurer ). Leaf water potential (LWP, Ψleaf) was measured by using the PMS Instrument Model 1000 pressure chamber and the values were calculated as MPa (Scoffoni et al., 2018). The leaf relative water content (RWC) was determined according to Smart and Bingham (1974). 5 seedlings were taken from the control and cold stress groups.




Figure 2. Measurement of leaf water statuses.

**Malondialdehyde (MDA) Content Measurement**

Lipid peroxidation was determined by measuring the malondialdehyde (MDA) content, according to Ohkawa et al. (1979), with some modification [11]. Fresh leaf tissue (0.2 g) was homogenized in 1 mL (5%) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at room temperature for 15 min at 16,000g. The supernatant was transferred to the tubes by taking equal volumes of 0.5% thiobarbituric acid (TBA) and 20% TCA solutions. The tubes were incubated at 96 °C for 25 min. Then, the tubes were transferred to ice bath and centrifuged at 12,000g for 5 min. The supernatant was measured at 532 and 600 nm. 0.5% TBA in 20% TCA solution was used as blank sample. The MDA content was calculated using the extinction coefficient.

**Electrolyte Leakage Measurement**

In order to determine the ion leakage of plants under cold stress, 4 leaf discs with a diameter of 1 cm were taken and placed in the tubes and 30 ml of water was added, shaken in a shaker for 4 hours at 100RPM at 25 degrees. The ion leakage in each sample was determined with the help of an EC meter (EC600 model, Extech Instruments A Flir Company) and accepted as the first measurement (EC1). Leaf discs in the same solution, ıt was kept at 70 degrees for 24 hours and ion leakage was determined at room temperature and was accepted as the final measurement (EC2).The percentage of electrolyte leakage was calculated using the following formula: Electrolyte leakage% = (EC1/EC2) × 100 (Arora et al, 1998, Gülen et al, 2007, Yasemin et al, 2017).

**Statistical Analysis**

Stress treatment was carried out in a completely randomized experimental design. Treatments had four replications with five plants each. Data were subjected to ANOVA and the means were separated using the LSD multiple range test at $p<0.05$. All the statistical analyses were performed using the JMP13 Software package. The reliability of the experiment was determined by calculating the coefficients of variation.

## RESULTS and DISCUSSION

In the study, low temperature exposure in pepper seedlings grown in pots in greenhouses occurred exactly during flowering period.The reproductive organs are most susceptible to low temperatures, and cold can affect flower induction, pollen production and germination time. In particular, it can prevent the transformation from flower to fruit and cause sterility in the plant (Zinn et al., 2010). In the study, it is seen in figure 3 that as the duration of cold stress increases, the flowers are affected negatively.




Figure 3. Effect of time-dependent low temperature stress on flowers.




Figures 4. Effect of time-dependent low temperature on development of pepper plants.

Low temperature negatively affected development and growth in pepper plants (Figure 4). Low temperature stress decreased the shoot fresh weight of *Capsicum annuum* plants compared to the control groups (Table 1, Figure 4 and 5 ). Pepper originating from subtropical areas is a warm season vegetable, and its optimal growth temperature ranges from 21 °C to 28 °C. Pepper plants lack mechanisms for cold acclimatization, making them very sensitive to low temperature (Korkmaz et al. 2010). Exposed to cold stress, pepper exhibit the reduction of nutriture and metabolic imbalances (Mercado et al., 1997).

Table 1. Effects of low temperature on MDA, RWC, LWP, weight and EC in *Capsicum annuum*. The values (mean and standard deviation) followed by different letters are significantly different at least significant difference test (p<0,05). 0

| LT (time) | MDA () | LWP (MPa) | RWC (%) | Weight (g) | EC (%) |
|---|---|---|---|---|---|
| 0 | 0.924±0.08c | -0.517±0.08a | 89±0.9a | 15.3±0.6a | 32.6±0.4a |
| 24h | 1.143±0.01b | -0.700±0.0c | 90±0.1a | 10.6±0.4b | 26.9±1.8c |
| 48h | 1.059±0.06b | -0.800±0.0d | 82±3.7b | 9.6±0.5c | 30.6±1.5ab |
| 72h | 1.542±0.05a | -1.067±0.06e | 78±1.6c | 8.4±0.4d | 31.6±0.2ab |
| Recovery | 0.918±0.05c | -0.617±0.03b | 90±0.6a | 9.9±0.2c | 28.1±3.8bc |
| LSD | 0.104*** | 0.083*** | 3.394*** | 0.801*** | 3.593* |
| VK | 5.11 | 6.04 | 2.2 | 4.1 | 6.6 |

*p<0.05 ***p<0.001

LWP and RWC decreased under cold stress in red capia peppers. The decreases were found to be statistically significant for all groups (Table 2, Figure 5). The decrease in leaf water content increased depending on stres-time, so the greatest decrease was experienced under prolonged cold application conditions. At 72 hours, the water status showed the lowest value compared to the control. LWP and RWC was improve during the recovery period. The most common visible symptom of low temperature stress in plant tissues is dehydration leading to wilting. Wilting that occurs during and/or after low temperature stress is attributed to a primary mechanism involving loss of membrane properties or transition of membranes from a normal fluid state to a restricted, less fluid, semi-crystalline state (Wright, 1974, Korkmaz et al., 2010).

MDA is a very sensitivity analysis index of oxidative damage and a reliable measure of cold tolerance. Lipid composition also negatively influenced by cold stress through ROS induced lipid peroxidation in chili genotypes (Nandagopal and Girija Shanmugam 2022). Under cold stress, malondialdehyde content (MDA) was increas leaves of red capia peppers and decreased significantly during the recovery period. When the changes due to cold exposure time were examined, it was determined that the changes in 72 hours condition were more evident (Table 2, Figure 5). It was observed that the ion leakage (EC) showed a sudden decrease in the first 24 hours at the beginning of the cold stress and reached a value close to control again at the 72nd hour. Electrolyte leakage reflects the extent of cell membrane injury.

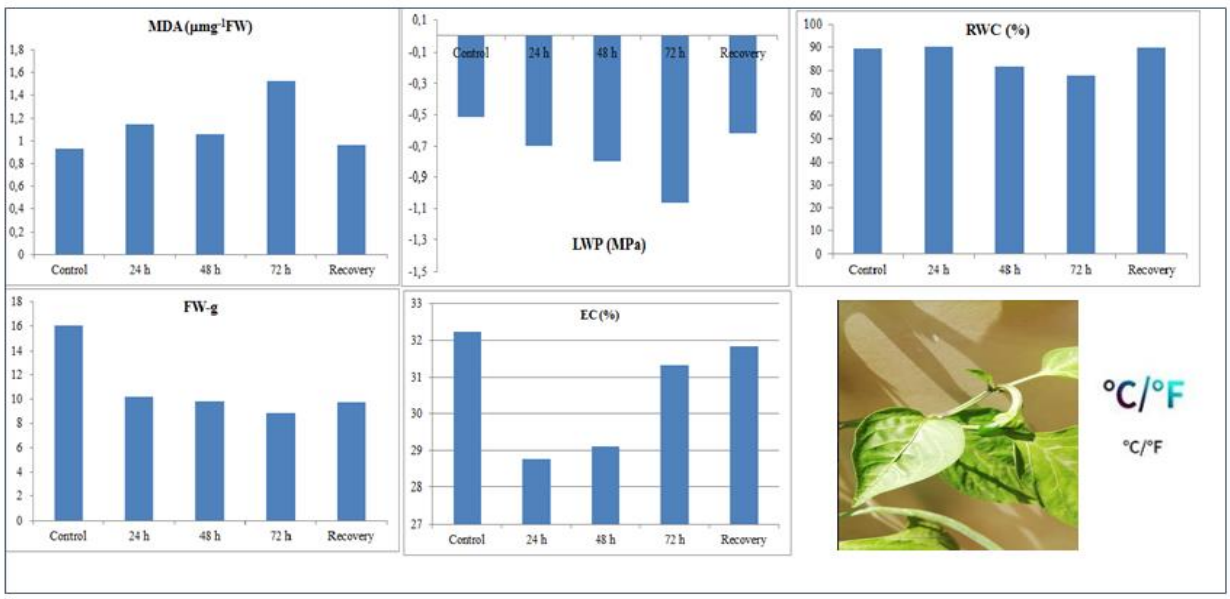


Figure5. Effects of low temperature on MDA, RWC, LWP, weight (g) and EC in *Capsicum annuum*.

## CONCLUSION

In this study, morphological and physiological changes occurring under low temperature stress conditions in red capia pepper seedlings grown in greenhouse conditions were investigated in real time depending on time. Considering all the measurements together, low temperature stress decreased the growth and development of red capia pepper over time. This study supports the conclusion of how important time is in the intervention of cold stress, especially in greenhouse conditions.

## ACKNOWLEDGEMENTS

I thank the Dr. Sara Yasemin, Siirt University, for contributions to statistical analyses.

## REFERENCES

Arora R, Pitchay DS, and Bearce BC (1998). Water-stress induced heat tolerance in geranium leaf tissues: A possible linkage through stress proteins?. Physiol. Plant., 103: 24-34

Berry, J., & Bjorkman, O. (1980). *Photosynthetic Response and Adaptation to Temperature in Higher Plants. Annual Review of Plant Physiology, 31(1), 491–543.* doi:10.1146/annurev.pp.31.060180

Bhutia, K.; Khanna, V.; Meetei, T.; Bhutia, N. Effects of climate change on growth and development of chilli. Agrotechnology **2018**.

Christine Scoffoni, Caetano Albuquerque, Hervé Cochard, Thomas N. Buckley, Leila R. Fletcher, Marissa A. Caringella, Megan Bartlett, Craig R. Brodersen, Steven Jansen, Andrew J. McElrone, Lawren Sack, The Causes of Leaf Hydraulic Vulnerability and Its Influence on Gas Exchange in *Arabidopsis thaliana* , *Plant Physiology*, Volume 178, Issue 4, December 2018, Pages 1584–1601.

Gülen H, Turhan E, İpek A, Köksal N, Cansev A, Kesici M, and Eriş A (2007). Bazı çilek çeşitlerinde yüksek sıcaklığa toleransın belirlenmesi. Türkiye V. Ulusal Bahçe Bitkileri Kongresi, 4-7 September, Cilt:1 s: 32-36. Erzurum, Turkey

Jaya Ganesh Thiruvengadam Nandagopal, Girija Shanmugam,"Screening and differential oxidative stress responses of hot pepper (Capsicum annuum L.) genotypes under cold stress,"South African Journal of Botany,"2022.

Korkmaz, A.; Korkmaz, Y.; Demirkıran, A.R. Enhancing chilling stress tolerance of pepper seedlings by exogenous application of 5-aminolevulinic acid. *Environ. Exp. Bot.* **2010**, *67*, 495–501.

Mercado, J.; Reid, M.S.; Valpuesta, V.; Quesada, M.A. Metabolic Changes and Susceptibility to Chilling Stress in Capsicum annuum Plants Grown at Suboptimal Temperature. *Funct. Plant Biol.* **1997**, *24*, 759–767.

MGM, (2018). 2017 Yılı Afet Değerlendirilmesi. [Erhan, A., Kılıç, G., Çamalan, G. ve ark. (der.)]Ankara.

MGM, (2019). Meteorolojik Karakterli Doğal Afetler: Orman Yangınları Meteorolojik Erken Uyarı Sistemi (MEUS). https://www.mgm.gov.tr/arastirma/dogalafetler.

Mock J J and Eberhart S A. 1972. Cold tolerance in adapted maize populations. Crop Science 12: 466–9

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2), 351–358. doi:10.1016/0003-2697(79)90738-3 9)

Smart, R.E. and Bingham, G.E. (1974) Rapid Estimates of Relative Water Content. Plant Physiology, 53, 258-260.https://doi.org/10.1104/pp.53.2.258

Thomashow, M.F. Molecular basis of plant cold acclimation: Insights gained from studying the CBF cold response pathway. *Plant Physiol.* **2010**, *154*, 571–577.

Wright, M., 1974. The effect of chilling on ethylene production, membrane permeability, and water loss of leaves of Phaseolus vulgaris. Planta 120, 63–69

Yasemin, S. (2017). "Growth and physiological responses of 'Chrysanthemum paludosum' under salinity stress". Journal of Biological and Environmental Sciences, 11(32), 59-66." *Scientia Horticulturae* 123 (2010): 402-411.

Zinn KE, Tunc-Ozdemir M, Harper JF. Temperature stress and plant sexual reproduction: uncovering the weakest links. J Exp Bot. 2010 Apr;61(7):1959-68. doi: 10.1093/jxb/erq053. Epub 2010 Mar 29. PMID: 20351019; PMCID: PMC2917059.

## *ORAL PRESENTATION*

# Chemical content and antioxidant activity analyses of okra (*Abelmoschus esculentus* L.) seed methanol extract grown in Manisa, Türkiye

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## Abstract

*Abelmoschus esculentus* L. (also known as *Hibiscus esculentus*) is an annual plant belonging to the Malvaceae family. This plant, which has local names such as ladies finger, okra, bhindi, and gumbo, is grown in Africa, Asia, Southern Europe, and America. Okra is a multipurpose crop due to the varied use of its leaves, buds, flowers, pods, stems, and seeds. Okras are a rich source of oil, proteins, carbohydrates, fibers, vitamin C, minerals, and antioxidants. Especially its seeds are used in turkey, as a substitute for coffee. In this study, we determined volatile organic compounds in methanol extract of okra seeds by gas chromatography-mass spectrometry (GC-MS). According to the results, pyrazine methyl-, parabanic acid, 2-furanmethanol, 1,2-cyclopentanedione, germacrene d, 9-octadecenoic acid, methyl ester, (e)- and vitamin E were obtained. The antioxidant capacity was determined via evaluating nitric oxide (NO) and 1,1- diphenyl-2-picrylhydrazyl (DPPH) scavenging activities (%) of okra seeds. It was observed that scavenging activities increased in parallel with increasing seed extract concentration. The IC50 values of the activity were calculated as 9.20 and 21.09 mg/mL for NO and DPPH scavenging activities (%), respectively. Macro and micro element content of okra seeds was determined by inductively coupled plasma-mass spectrometry (ICP-MS). According to element analysis results, potassium (K) and magnesium (Mg) contents were determined higher than other elements. The amount of K and Mg were found to be 12.22 and 3.32 mg/g, respectively. Zinc (Zn) and copper (Cu) results were determined as 7.5 and 40.57 µg/g, respectively. The biochemical, mineral content and antioxidant capacity of the seeds of the okra plant were determined and compared with other studies in the literature. According to the results, it can be concluded that okra seeds can be used as a good food source for both humans and animals.

**Keywords:** Okra, GC-MS, ICP-MS, volatile organic compound, vitamin E, antioxidant activity.

## INTRODUCTION

Okra (*Abelmoschus esculentus* L.) a flowering plant member of the **malvaceae** family with multiple nutrition, has been considered as a natural feed supplement for improving growth performance (Ashour et al., 2020). Okra is widely consumed as a fresh vegetable in both temperate and tropical countries. The fruit have nice taste and is rich in polysaccharides, polyphenols, flavonoids, tannins, sterols and triterpenes 8 Adelakun et al., 2009). However, several studies have reported that okra is also useful for its seeds. In Turkey, the seeds of mature okra are roasted ground and used as coffee substitute (Çalisir et a., 2005).

Seeds are the part with larger protein content and in okra pods average crude protein value is 10–25 % on dry weight base. Okra contains other relevant classes of constituents as polysaccharides, tocopherols, fatty acids, chlorophylls, and important fraction is the mucilage (Liu et al., 2021). It has a wide range of active medicinal constituents like vitamins, polyphenolic compounds, polysaccharides, and flavonoids. These phytoconstituents have potent antidiabetic, antihyperlipidemic, neuroprotective, chemoprotective, and radical scavenging properties. This plant's lipophilic and hydrophilic antioxidant components allow it to neutralize free radicals in both lipid and aqueous phases. The flavonoids as well as the vitamin C present in this plant safeguard against the oxidation of low-density lipoproteins in a synergistic way (Xia et al., 2015). Different stages of okra plant were given in figure 1.




**Figure 1.** Different stages of okra (https://veggiesinfo.com/okra/).

## MATERIALS AND METHODS

### Extraction Process

Okra seeds were obtained from a local herbal market in Manisa, Turkey. Before the extraction process, okra seeds were washed in pure water and dried at room temperature. For analysis, the seeds were pulverized in high-speed plant mill and prepared for extraction. 5 g of powdered seed samples were extracted in 40 ml of 99.5% methanol in ultrasonic bath for 30 minutes then left at room temperature. The final extraction concentrate was then adjusted to 50 mg / mL. DPPH and NO scavenging activities in percentage and GC-MS analyzes were determined using this extraction. ICP-MS analyzes were determined using powdered seed samples.

### Antioxidant Test

### DPPH and NO Scavenging Activity

The antioxidant activity of the extracts was tested using the DPPH radical with minor modifications (Zhang et al., 2011). A 2.10−4 M DPPH solution was prepared in methanol for this method. Receive 0.5 mL of the sample and 4 mL the DPPH solution was added. The mixture was shaken and kept at room temperature for 60 min in the dark. Eighty percent methanol was used as a blind and samples were read at 517 nm by spectrophotometer (TECAN Infinite M200 microplate reader). The results were calculated according to the following equation.

DPPH scavenging activity % = (1- Absorbance $_{sample}$ / Absorbance $_{blank}$) x 100

Nitric oxide scavenging activity was determined according to the following method (Marcocci et al., 1994). Four milliliters of each of the extracts prepared in different concentrations was taken and 1 mL of sodium nitroprusside solution (25 mM) was added to the mixture and incubated for 2 h at 37°C. After incubation, 0.5 mL of the solutions was removed and mixed with 0.3 mL of Griess reagent (5% $H_3PO_4$ in 1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the formed chromophore was read against the blind at 570 nm on the spectrophotometer (TECAN Infinite M200 Microplate reader). The results were calculated according to the following equation.

NO scavenging activity % = (1- Absorbance $_{blank}$ - Absorbance $_{sample}$/ Absorbance $_{blank}$) x 10

### Volatile organic component analysis by gas chromatography-mass spectrometry (GC-MS)

Volatile organic molecules in the extract were qualitatively analyzed in electron ionization (EI) mode with Agilent Technology 7890A Gas Chromatography (GC) Mass spectrometer (MS). Chromatographic column Agilent HP-5 MS, capillary column (30 mx0.25 mm, film thickness of 0.25 mm). The furnace temperature was started at 40°C, followed by standing for 5 min, then at 5°C min-1 at 280°C and held for 5 min. Helium gas (99.999%) was used as the carrier gas. The constant flow rate is 1.5 mL min-1 and the injector temperature

is 250°C. The extract was injected in splitless mode with 1 mL. Interpretation of the mass spectrum was performed according to the National Institute of Standards and Technology (NIST) database.

**ICP-MS Metal Analyzes**

Microwave digestion was performed before metal analysis. 0.5 g of seed samples were taken and 10 mL of nitric acid was added. The microwave device (CEM, Mars 6, NC, USA) was degraded at 200 °C temperature, 15 min ramp, 20 min hold and 20 min cooling program. After microwave pretreatment, macro- and micronutrient contents (Na, Mg, Al, K, Ca, Mn, Fe 56, Fe 57, Cu, Zn) of samples were investigated using ICP-MS (7700x Agilent, CA, USA). Calibration graphs were drawn and nutrient content analysis was done for each sample.

## RESULTS and DISCUSSION

**DPPH and NO Scavenging Activity**

The antioxidant capacity was determined via evaluating nitric oxide (NO) and 1,1- diphenyl-2-picrylhydrazyl (DPPH) scavenging activities (%) of okra seeds. The IC50 values of the activity were calculated as 9.20 and 21.09 mg/mL for NO and DPPH scavenging activities (%), respectively. It was observed that scavenging activities increased in parallel with increasing seed extract concentration (figure 2)

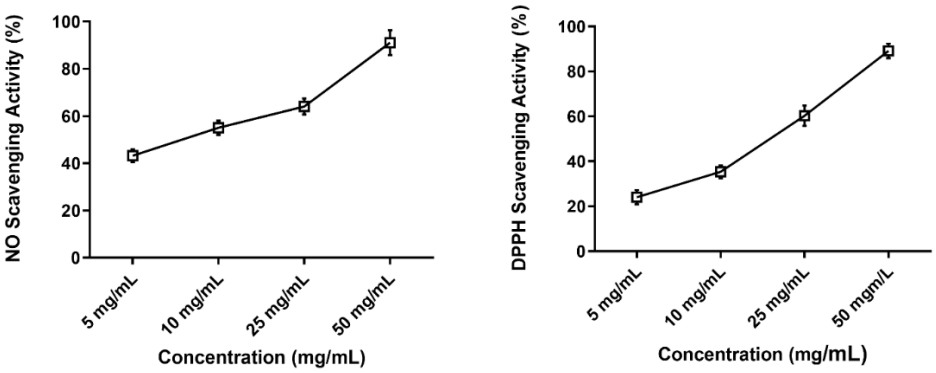


**Figure 2.** NO and DPPH Scavenging Activity (%) results.

**Volatile organic compound analysis reults by gas chromatography-mass spectrometry (GC-MS)**

In this study, we determined volatile organic compounds in methanol extract of okra seeds by gas chromatography-mass spectrometry (GC-MS). According to the results, pyrazine methyl-, parabanic acid, 2-furanmethanol, 1,2-cyclopentanedione, Germacrene D, 9-octadecenoic acid, methyl ester, (e)- and vitamin E were obtained (Table 1).

The pods of A. esculentus contain majorly esters of fatty acids with simpler alcohols (such as methyl-, ethyl-n-propyl-, isopropyl-) which are applicable in the production of soap, emollients and lubricants for cosmetic purposes and metallic soaps, respectively. They are also used as emulsifiers, texturizing, anti-foam and stabilizing agents fatty acids are also converted via their methyl esters, to fatty alcohols and amines, which are precursors to lubricants, surfactants and detergents (David ert al., 2011; Ramsden et al., 2015). Compounds with more phenolic compounds have been reported to have antioxidant activity (Sofowora, 2008; Boligon et al., 2014).

GC-MS Counts and acquisition time (min) of okra seed extraction were given in figure 3. Vitamin e; GC-MS counts vs. acquisition time (min) and counts vs. mass-to-charge (m/z) of okra seed extraction were given in figure 4.

**Table 1.** Volatile organic compound analysis reults

| *Cpd* | *Name* | *RT* | *Formula* | *Score* | *Library* | *CAS* |
|---|---|---|---|---|---|---|
| *1* | trans-.beta.-ıonon-5,6-epoxıde | 1,458 | C13H20O2 | 83,37 | Wiley7Nist05.L | 23267-57-4 |
| *2* | Methanamine, N-methyl- | 2,025 | C2H7N | 83,04 | Wiley7Nist05.L | 124-40-3 |
| *3* | Acetic acid, hydroxy-, methyl ester | 2,365 | C3H6O3 | 79,72 | Wiley7Nist05.L | 96-35-5 |
| *4* | Methane, oxybis- | 3,408 | C2H6O | 86,65 | Wiley7Nist05.L | 115-10-6 |
| *5* | Methylamine, N,N-dimethyl- | 3,585 | C3H9N | 86,42 | NIST11.L | 75-50-3 |
| *6* | Acetic acid | 3,893 | C2H4O2 | 97,58 | Wiley7Nist05.L | 64-19-7 |
| *7* | 2-Propanone, 1-hydroxy- | 4,282 | C3H6O2 | 93,11 | Wiley7Nist05.L | 116-09-6 |
| *8* | 4-(benzoyloxy)-2H-pyran-3-one | 4,459 | C12H10O4 | 72,81 | Wiley7Nist05.L | 89890-53-9 |
| *9* | Acetic acid, propyl ester | 4,87 | C5H10O2 | 81,49 | Wiley7Nist05.L | 109-60-4 |
| *10* | Methanamine, N-hydroxy-N-methyl- | 5,256 | C2H7NO | 81,34 | NIST11.L | 5725-96-2 |
| *11* | Propanoic acid, 2-oxo-, methyl ester | 5,658 | C4H6O3 | 88,19 | Wiley7Nist05.L | 600-22-6 |
| *12* | Pyrazine, methyl- | 6,247 | C5H6N2 | 96,19 | Wiley7Nist05.L | 109-08-0 |
| *13* | 2-Furancarboxaldehyde | 6,465 | C5H4O2 | 96,89 | Wiley7Nist05.L | 98-01-1 |
| *14* | 2-Furanmethanol | 7,049 | C5H6O2 | 91,66 | Wiley7Nist05.L | 98-00-0 |
| *15* | 2(3H)-Furanone, dihydro- | 8,613 | C4H6O2 | 87,23 | Wiley7Nist05.L | 96-48-0 |
| *16* | 1,2-Cyclopentanedione | 8,928 | C5H6O2 | 93,47 | NIST11.L | 3008-40-0 |
| *17* | Neopentane | 9,556 | C5H12 | 83 | NIST11.L | 463-82-1 |
| *18* | 1,2-Ethanediol, monoacetate | 9,708 | C4H8O3 | 81,78 | Wiley7Nist05.L | 542-59-6 |
| *19* | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | 10,543 | C6H8O4 | 80,34 | Wiley7Nist05.L | 10230-62-3 |
| *20* | (+)-(S)-2-Hydroxy-3,3-dimethyl-.gamma.-butyrolactone | 12,237 | C6H10O3 | 88,58 | Wiley7Nist05.L | 5405-40-3 |
| *21* | Parabanic acid | 12,35 | C3H2N2O3 | 85,58 | Wiley7Nist05.L | 120-89-8 |
| *22* | Butanoic acid, 2-methyl-, 2-methylbutyl ester | 14,334 | C10H20O2 | 96,68 | Wiley7Nist05.L | 2445-78-5 |
| *23* | 2-acetyl-2-hydroxy-.gamma.-butyrolactone | 15,226 | C6H8O4 | 84,79 | Wiley7Nist05.L | 135366-64-2 |
| *24* | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 15,473 | C6H8O4 | 93,49 | NIST11.L | 28564-83-2 |
| *25* | 5-Hydroxymethylfurfural | 17,943 | C6H6O3 | 95,85 | NIST11.L | 67-47-0 |
| *26* | Heptanoic acid, 3-methylbutyl ester | 21,287 | C12H24O2 | 93,53 | NIST11.L | 109-25-1 |
| *27* | 2-methylbutyl octanoate | 23,817 | C13H26O2 | 87,46 | Wiley7Nist05.L | 0-00-0 |
| *28* | Germacrene D | 24,731 | C15H24 | 76,32 | Wiley7Nist05.L | 23986-74-5 |
| *29* | Ethanone, 1-(3,4-dimethoxyphenyl)- | 26,667 | C10H12O3 | 81,08 | NIST11.L | 1131-62-0 |
| *30* | Hexadecanoic acid, methyl ester | 34,332 | C17H34O2 | 91,85 | NIST11.L | 112-39-0 |
| *31* | 9,12-Octadecadienoic acid, methyl ester, (E,E)- | 37,528 | C19H34O2 | 94,9 | NIST11.L | 2566-97-4 |
| *32* | 9-Octadecenoic acid, methyl ester, (E)- | 37,638 | C19H36O2 | 86,67 | Wiley7Nist05.L | 1937-62-8 |
| *33* | Palmitic acid .beta.-monoglyceride | 44,44 | C19H38O4 | 90,23 | Wiley7Nist05.L | 23470-00-0 |
| *34* | Octadecanoic acid, 2,3-dihydroxypropyl ester | 47,533 | C21H42O4 | 93,49 | NIST11.L | 123-94-4 |
| *35* | 13-Docosenamide, (Z)- | 48,428 | C22H43NO | 83,45 | Wiley7Nist05.L | 112-84-5 |
| *36* | 1-Octadecanol | 51,698 | C18H38O | 82,68 | Wiley7Nist05.L | 112-92-5 |
| *37* | beta.-Tocopherol | 52,459 | C28H48O2 | 93,13 | NIST11.L | 148-03-8 |
| *38* | **Vitamin e** | 53,98 | C29H50O2 | 92,65 | Wiley7Nist05.L | 59-02-9 |

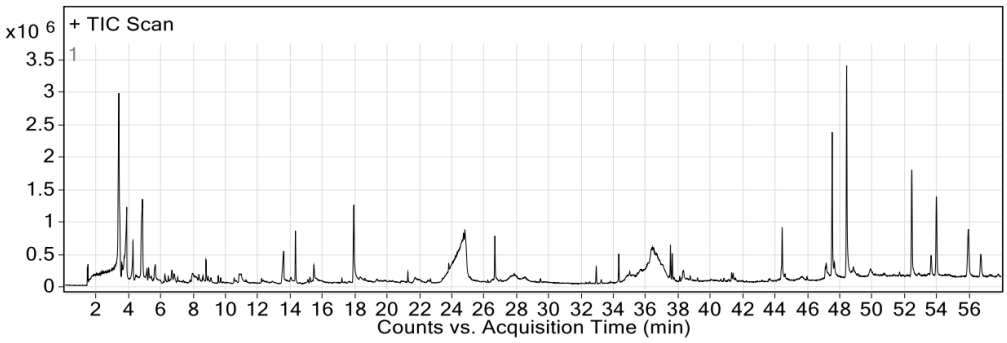


**Figure 3**. GC-MS Counts and acquisition time (min) of okra seed extraction.

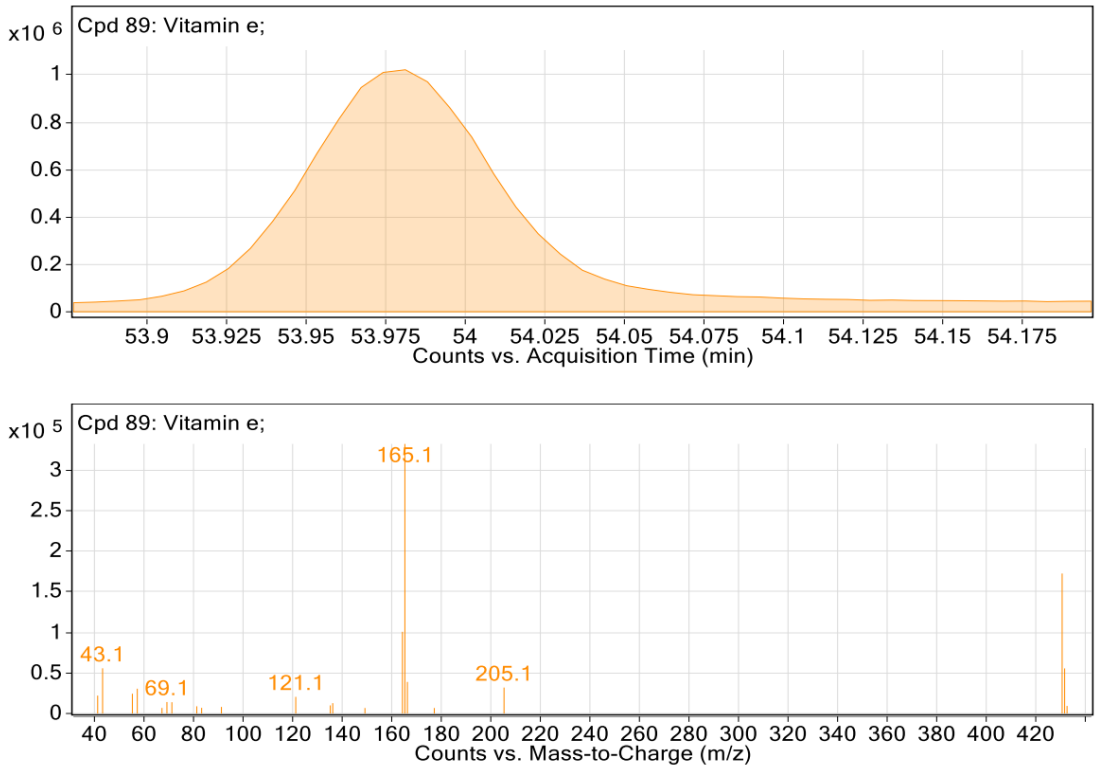


**Figure 4**. Vitamin e; GC-MS counts vs. acquisition time (min) and counts vs. mass-to-charge (m/z) of okra seed extraction.

Vitamin E is one of the best quenchers for singlet oxygen, and can act as a chain-breaking anti- oxidant. Furthermore, singlet oxygen is very powerfully quenched by carotenoids, especially b-carotene. In the case of phenolic compounds, the ability of the phenolics to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups, that allow them to act as reducing agents, hydrogen-donating antioxidants, oxygen quenchers (Rice-Evans and Miller, 1996).

## ICP-MS macro-micro element content

Macro and micro element content of okra seeds was determined by inductively coupled plasma-mass spectrometry (ICP-MS). According to element analysis results, potassium (K) and magnesium (Mg) contents were determined higher than other elements. The amount of K and Mg were found to be 12.22 and 3.32 mg/g, respectively. Zinc (Zn) and copper (Cu) results were determined as 7.5 and 40.57 µg/g, respectively (Table 2). Okra is a popular health food due to its high fiber, vitamin C, folate and mineral contents such as calcium, potassium, sodium, iron and magnesium Kumar et al., 2013; Adelakun et al., 2010)

**Table 2.** ICP-MS metal content results (n:3, **p<0.05, *** p<0.01)

| *Metal name* | *Unit* | *Count* |
|---|---|---|
| ***Na*** | µg/g | 67.15±0.23*** |
| ***Mg*** | mg/g | 3.328±0.22*** |
| ***Al*** | µg/g | 4.580±0.18*** |
| ***K*** | mg/g | 12.2234±0.21*** |
| ***Ca*** | mg/g | 0.686±0.19*** |
| ***Mn*** | µg/g | 14.64±0.20*** |
| ***Fe 56*** | µg/g | 34.85±0.21*** |
| ***Fe 57*** | µg/g | 38.13±0.18*** |
| ***Cu*** | µg/g | 7.550±0.17** |
| ***Zn*** | µg/g | 40.57±0.19*** |

## CONCLUSION

The biochemical, mineral content and antioxidant capacity of okra seeds (*Abelmoschus esculentus* L.) grown in Manisa, Türkiye were determined and compared with other studies in the literature. It can be said that okra seeds act as a good radical scavenger due to their high antioxidant activity values. In addition, as a result of GC-MS analysis, 38 valuable volatile components were obtained. According to the results, okra seeds can be used as a good food source for both humans and animals.

## EFERENCES

Adelakun OE, Oyelade OJ, Ade-Omowaye BI, Adeyemi IA, Van de Venter M 2009. Chemical composition and the antioxidative properties of Nigerian Okra Seed (*Abelmoschus esculentus Moench*) Flour. Food and Chemical Toxicology, 47(6): 1123–1126.

Adelakun, OE, Ade-Omowaye BIO, Adeyemi IA. and Venter M 2010. Functional properties and mineral contents of a Nigerian okra seed (*Abelmoschus esculentus Moench*) flour as influenced by pretreatments. J. Food Technol, 8: 39-45.

Ashour EA, Bin-Jumah M, Abou Sayed-Ahmed ET, Osman AO, Taha AE, Momenah MA, Allam AA, Swelum AA, Abd El-Hack ME 2020. Effects of dried okra fruit (*Abelmoschus esculentus* L.) powder on growth, carcass characteristics, blood indices, and meat quality of stored broiler meat. Poultry. Sci. 99: 3060–3069.

Boligon AA, Machado MM and Athayde ML 2014. Technical evaluation of antioxidant activity. Med. Chem, 4: 517-522.

Çalisir S, Özcan M, Haciseferogullari H, Yildiz UM 2005. A study on some physico-chemical properties of Turkey okra (*Hibiscus esculenta* L.) seeds. Journal of Food Engineering 68: 73 – 78.

David S, Zelda P, and Fulton GK 2011. Gas Chromatography and Mass Spectrometry: A Practical Guide. Academic Press, UK. 25.

https://veggiesinfo.com/okra/.

Kumar DS, Tony DE, Kumar AP, Kumar KA, Rao DBS, Nadendla R 2013. A review on: *Abelmoschus esculentus* (Okra). Int. Res. J. Pharm. Applied Sci, 3: 129-132.

Liu Y, Qi JY, Luo JY, Qin W, Luo QY, Zhang Q, Chen H 2021. Okra in Food Field: Nutritional Value, Health Benefits and Effects of Processing Methods on Quality. Food Reviews International, 37(1):67–90.

Marcocci I, Marguire JJ, Droy-Lefaiz, MT, Packer L 1994. Thenitricoxide scavenging property of Ginkgo biloba extract. Biochemical and Biophysical Research Communications, 201: 748-755.

Ramsden CE, Zamora D, Makriyannis A, Wood JT, Mann JD, Faurot KR, MacIntosh BA, Majchrzak-Hong SF, Gross JR, Courville AB, Davis JM, Hibbeln JR 2015. Diet-induced changes in n-3-and n-6- derived endocannabinoids and reductions in headache pain and psychological distress. J. Pain, 16: 707-716.

Rice-Evans CA, Miller NJ 1996 Antioxidant activities of flavonoids as bioactive components of food. Transactions and Biochemical Society, 24(3), 790–795.

Sofowora A 2008. Medicinal Plant and Traditional Medicine in African. 2nd Edn., Spectrum Books Ltd., Ibadan, Nigeria, pp: 112.

Xia F, Zhong Y, Li M, Chang Q, Liao Y, Liu X, Pan R 2015. Antioxidant and anti-fatigue constituents of okra. Nutrients, 7 (10): pp. 8846-8858.

Zhang GW, He L, Hu MM 2011. Optimized ultrasonic-assisted extraction of flavonoids from *Prunella vulgaris* L. and evaluation of antioxidant activities in vitro. Innovative Food Science and Emerging,12(1): 18-25.

*ORAL PRESENTATION*

# The effects of different drip irrigation levels and irrigation practices on yield and water use efficiency of cotton

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## Abstract

This study was conducted with May-505 cotton variety in the Aydın plain conditions during 2021. The purpose of this study is to evaluate different water levels with different irrigation programs in terms of seed cotton yield and water use efficiencies. The field trial, which was designed as two factors and three replications, was designed according to the randomized complete block trial pattern. Four different irrigation levels (100%, 67%, 33% and 0%) and two different irrigation approaches (gravimetric and pan evaporation) were investigated in the study. The applications of water significantly affected seed cotton yield and water use efficiency values. The highest irrigation water was given to the full irrigation (100%) subject in each irrigation approach. Seasonal water use values in the parcels varied between 216 (0%) and 691 (100%) mm during the production period. The average seed cotton yield varied from 2067 – 5115 kg $ha^{-1}$. The highest average seed cotton yield was obtained from $C_1$ (100%) gravimetric treatment as averaging 5115 kg $ha^{-1}$. It was determined gravimetric approach performed higher yields than pan evaporation application. Average water use efficiency (WUE) values varied between 0.715 and 1.025 kg $m^{-3}$. Yield response factor (ky) was found to be 0.88 for gravimetric and 0.80 for pan evaporation applications. In conclusion, the easier pan evaporation approach with 100% treatment ($D_1$) is suggested to farmers for cotton production in the parts of the Aydın plain conditions within the semi-arid climate zone, while water resources are sufficient. Else, IL-67% treatment with a gravimetric approach can be used for a deficit irrigation strategy.

**Keywords:** Cotton, drip irrigation, irrigation levels, water use efficiency, Aydın.

## INTRODUCTION

Aegean Region is one of the most important agricultural and industrial region in west part of Turkey. All cotton production areas of this region receive inadequate amounts or inadequate distribution of rainfall. Decreasing ground water supplies and the high cost of energy also affect production of irrigated cotton. Turkey must take urgent action to solve the water-shortage problem for important agricultural crops including cotton, wheat, and other crops in coming years. Nowadays limited availability of irrigation water requires fundamental changes in irrigation management and urges the application of water saving methods. Under such conditions, different irrigation systems such as drip irrigation, sprinkler irrigation, subsurface drip irrigation (SDI), and low energy precision applicators (LEPA) should be considered to provide growers with high irrigation efficiency. Especially, the use of drip irrigation techniques is inevitable in the near future because of the salinity problem caused by traditional irrigation methods (Simsek et al., 2004). Thus, this creates the need for continuous improvement in irrigation practices, especially in the cotton production of the Aegean region. Turkey's seed cotton production meets approximately 44% of the needs of its domestic market. Turkey is the 11th cotton producer in the world. In 2018/2019, seed cotton area and crop production of Turkey was 508.000 ha and 988.000 t, respectively. Average cotton lint yield is about 1944 kg $ha^{-1}$ (Anonymous, 2019a). Reduction in underground water sources as a result of climate change and increase in industrial and domestic water consumption, led to a reduction in the available water for agricultural production. In addition to this, the effects of global warming are more and more being felt, and one of the most important of these is drought. This has a negative effect on crop production. Cotton is irrigated by the surface irrigation method in Turkey although sprinkler and drip irrigation have been suggested as a means of supplying most types of crops with frequent and uniform applications of water, since it is adaptable over a wide range of topographic and soil conditions (Cetin and Bilgel, 2002; Dagdelen et al., 2006). They also reported that cotton yield and water use efficiency was higher in drip irrigation than furrow and sprinkler irrigation. Mateos et al. (1991) reported that water use

efficiency was 30% higher with drip irrigation treatments, indicating a definitive advantage of this method under conditions of limited water supply. Water availability is a major concern in cotton production. Yazar et al. (2002) studied the effect of irrigation methods, irrigation frequency and pan coefficients under Southeast Anatolia conditions of Turkey. Highest seed cotton yield of 5850 kg $ha^{-1}$ was obtained from the full irrigation treatment (100 %) in trickle-irrigated plots with 6-day intervals. In another study, the highest yield was found to be 4220 kg $ha^{-1}$ with 5-day irrigation interval and seasonal water consumption was 511 mm under drip irrigated cotton in Çukurova plain-Turkey (Ertek and Kanber, 2001). Efficient use of irrigation water is becoming increasingly important and alternative water application methods such as drip and sprinkler, may contribute substantially toward making the best use of water for agriculture and improving irrigation efficiency (Sezen et al., 2004). A number of previous studies reported the advantage of drip irrigation methods in terms of high WUE and cotton yield (Mateos et al., 1991; Ertek and Kanber, 2001; Çetin and Bilgel, 2002; Yazar et al., 2002; Karam et al., 2006; Ibragimov et al., 2007). Basal et al. (2009), in a study to determine the effect of five different doses of water in a drip irrigation system on water use efficiency, yield, yield components and fibre quality characteristics, found that when the dose of water was reduced from 100% to 75%, water use efficiency rose from 0.62 to 0.71 kg $m^{-3}$. It was also found that raw cotton yield, the number of bolls and the weight of cotton per boll fell in parallel with the reduction in irrigation level. Dagdelen et al. (2009) conducted field trials in the Aegean region in 2004-2005 to determine the effect of various levels of water using the drip irrigation method on water use efficiency and fibre quality parameters. They reported variations of 256-753 mm in average seasonal plant water consumption, 2550-5760 kg $ha^{-1}$ in average cotton yield, and 0.76-0.98 kg $m^{-3}$ in water use efficiency. Hussein et al. (2011), in a study conducted on cotton under Syrian conditions to determine the effect of different irrigation doses on water use efficiency, cotton yield and fibre quality, found variations of 408-773 mm in crop water consumption and 2909-5090 kg $ha^{-1}$ in average cotton yield. Also, in each of the years in which the study was conducted, the highest water use efficiency value, 0.71 kg $m^{-3}$, was obtained from a water level of D180. Erten and Dagdelen (2020), conducted a study to determine the effects of 0%, 25%, 50% 75% and 100% irrigation levels on the yield of cotton using drip irrigation under Aydın-Turkey conditions. The researchers found average WUE and IWUE values varied between 0.747-1.120 and 0.972-2.503 kg $m^{-3}$ respectively.

Cotton is an excellent candidate for irrigation management experiments. Besides different drip irrigation levels, with proper management such as different irrigation approaches should be studied by the researchers. Limited availability of irrigation water requires fundamental changes in irrigation management or urges the application of water saving methods especially for strategic crops with high water consumption. The objectives of this study were; to determine the effects of irrigation approaches and irrigation levels on the seed cotton yield and water use efficiency under drip irrigation system, and to choose the most suitable irrigation schedule for cotton cultivar under semi-arid climatic conditions.

## MATERIALS AND METHODS

Field experiments was carried out at the Agricultural Research Station of Aydın Adnan Menderes University, Aydin-Turkey at 37° 51’ N latitude, 27°51’ E longitude and 56 m altitude during the 2021 growing season. Climate in this region is semi-arid with total annual precipitation of 657 mm. Average seasonal rainfall is 657 mm, with 90% of the rain occurring between November and March. Typical Mediterranean climate prevails in the experimental area. Table 1 summarizes the monthly mean climatic data compared with the long-term mean climatic data for Aydın (Anonymous, 2019b). The growing season temperatures were typical of long term-means (1970-2020) for Aydın located in the western Aegean region of Turkey.

**Table 1.** Weather conditions prevailed during the experiments compared to the long- run at the experimental site

| 1970-2020 | | | | |
|---|---|---|---|---|
| **Month** | **Temperature (ºC)** | **Relative Humidity (%)** | **Rainfall (mm)** | **Evaporation (mm)** |
| **May** | 21 | 56.9 | 35.6 | 161.3 |
| **June** | 26 | 49.2 | 16.6 | 222.1 |
| **July** | 28.6 | 48.6 | 7.5 | 257.5 |
| **August** | 27.6 | 52.9 | 5.3 | 231.6 |
| **September** | 23.3 | 55.9 | 15.1 | 161.9 |
| **2021** | | | | |
| **Month** | **Temperature (ºC)** | **Relative Humidity (%)** | **Rainfall (mm)** | **Evaporation (mm)** |
| **May** | 22.3 | 57.6 | 3.3 | 149.9 |
| **June** | 25.2 | 56.0 | 1.9 | 185.6 |
| **July** | 29.6 | 51.4 | 0.1 | 248.5 |
| **August** | 28.9 | 50.0 | 0.0 | 228.7 |
| **September** | 23.8 | 55.8 | 0.0 | 154.6 |

The soil texture, bulk density, field capacity, wilting point, and available water holding capacity values of each 30 cm layer of 0 - 120 cm soil depth in the experimental area are given in Table 2. The soil series in the research area was Büyük Menderes Basin developed on aluvial materials (Aksoy et al., 1998). The soil of the experimental site is classified as Entisols and Fluvisols-Regosols silty-clay-loam with relatively high water holding capacity. For the experiment area, water content at field capacity varied from 18.4 to 23.1% and wilting point varied from 7.2 to 10.1% on dry weight basis. Research area soils contain high percentages of sand (49.7-68.2%), followed by silt (19.2-32.0%) and clay (13.6-17.5%) and could be classified as Sandy-loam. The dry soil bulk densities ranged from 1.35 to 1.52 g cm-3 throughout the 1.2 m deep profile.

**Table 2**. Some physical characteristics of experimental site soils

| **Soil depth (cm)** | **Soil texture** | **Bulk density (g $cm^{-3}$)** | **Field capacity (%)*** | **Wilting point (%)*** | **Available water holding capacity (mm)** |
|---|---|---|---|---|---|
| 0-30 | Sandy-Loam | 1.35 | 23.1 | 10.1 | 52.6 |
| 30-60 | Sandy-Loam | 1.45 | 22.9 | 9.4 | 58.7 |
| 60-90 | Sandy-Loam | 1.52 | 18.4 | 7.3 | 50.7 |
| 90-120 | Sandy-Loam | 1.50 | 20.3 | 7.2 | 58.9 |
| 0-120 | | | | | 221.0 |

*on dry weight basis

The May 505 cotton variety was planted on 27 May 2021, with 0.70 × 0.20 m spacing. Before starting the field experiment, 60 kg $ha^{-1}$ compound fertilizer (containing 15% pure N, 15% P, and 15% K) was applied to the planting area The required remaining portion of nitrogen was followed by 82 kg $ha^{-1}$ as ammonium nitrate 33% before first irrigation.

The study, prepared as a randomized complete block design with three replicates; four different irrigation levels (100, 67, 33, and 0%) and two different irrigation program techniques (gravimetric and pan evaporation) were investigated. There is 3 m space between each of the trial plots, and four cotton rows with 0.7 m intervals and 5 m lengths have been created within the plot. In the pan evaporation method, irrigation water was applied to $D_1$ (control), $D_2$, $D_3$, and $D_4$ subjects, respectively 100%, 67%, 33%, and 0% of the 7-day cumulative pan evaporation amounts measured from the class-A pan. When 50% of the moisture content of 0 – 90 m root zone in the soil in the parcels irrigated by the gravimetric method was consumed, irrigation water was applied to $C_1$ (control), $C_2$, $C_3$, and $C_4$ subjects, respectively 100%, 67%, 33% and 0% of the water needed.

Equation (1 and 2) was used to calculate the irrigation water amount for two approaches;

$$V = P * A * Epan * WL \quad (1)$$

$$I = (FC - AW)/100 * \gamma_t * D$$

$$V = I * A * WL \quad (2)$$

Where V is the volume of irrigation water (L), P wetting percentage (taken as 100 % for row crops), A is plot area ($m^2$), Epan is the amount of cumulative evaporation during a seven-day irrigation interval (mm), WL represents irrigation levels (0.33, 0.67 and 1.00), FC field capacity (mm), AW available water in the soil within 90 cm depth before irrigation applications (mm), $\gamma_t$ bulk density (g $cm^{-3}$) and D effective root zone (mm). Class A pan, used to measure the evaporation, was placed next to the plots in the meteorology.

A drip irrigation system was designated for the experiment. Irrigation water was used from a deep well located near the experimental site. The control unit consisted of screen filter with 10 l $s^{-1}$ capacity, control valves, manometers mounted on the inlet and outlet of each unit. Distribution lines consisted of PVC pipe manifolds for each plot. The diameters of the laterals were 16 mm PE and each lateral irrigated one plant row. The inline emitters were used with a discharge rate of 4 L $h^{-1}$ above 10 m operating pressure. In the system, emitter and the lateral spacing were chosen as 0.20 and 0.70 m, respectively.

Crop water consumption under varying irrigation regimes was calculated using the soil water balance equation as (Heerman, 1985);

$$ET = R + I - D \pm \Delta W \quad (3)$$

Where, ET is the water use (mm), R is the rainfall (mm), I is the depth of irrigation (mm), D is the depth of drainage (mm), and ΔW is the change of soil water storage in the measured soil depth.

WUE was calculated as yield (kg $ha^{-1}$) divided by seasonal water use (mm). IWUE was determined as yield (kg $ha^{-1}$) per unit irrigation water applied (mm) (Howell et al., 1990). Regression analysis was used to evaluate the water use-yield relationships derived from seasonal crop water use and yield data obtained from the experiment. Seasonal values of the yield response factor ($k_y$), which represent the relationship between relative yield reduction [1-(Ya/Ym)] and relative evapotranspiration deficit [1-(ETa/ETm)], were determined using equation 2 given by Doorenbos and Kassam (1986):

$$1 - (Y_a/Y_m) = k_y(1 - ET_a/ET_m) \quad (4)$$

Where, ETa and ETm are the actual and maximum seasonal crop water use values (mm), respectively, and Ya and Ym are the corresponding actual and maximum yields (kg $ha^{-1}$).

Seed cotton yield was determined by hand harvesting in each plot on 16 November 2021. In order to determine the differences between irrigation treatments, the data relating to seed cotton yield was subjected to variance analysis. The Least Significant Differences (LSD) test was used for comparing and ranking the treatments. Differences were declared significant at $P < 0.05$. Variance analysis and LSD tests were carried out with the use of the TARİST program, which was developed for this purpose (Acikgoz et al., 1994).

## RESULTS and DISCUSSION

The total irrigation water amounts applied, seasonal water use and water use efficiency values (WUE, IWUE) were presented in Table 3. The first irrigation was applied on July 29, and irrigations were lasted on September 8, in 2021, respectively. Seasonal amount of irrigation water applied for different drip treatment ranged from 169 to 627 mm in growing season. Irrigation water was applied seven times to the treatments over the growing season (Table 3).

**Table 3.** Seed cotton yield and water use efficiency values as influenced by seed applications and irrigation levels

| Irrigation application methods | Irrigation Levels | Seed cotton yield (kg $ha^{-1}$) | Irrigation water applied (mm) | Water use (mm) | Water use efficiency (WUE) (kg $m^{-3}$) | Irrigation water use efficiency (IWUE) (kg $m^{-3}$) |
|---|---|---|---|---|---|---|
| Gravimetric | $C_1$-100% | 5115 | 513.0 | 569.0 | 0.898 | 0.997 |
| | $C_2$-67% | 4295 | 343.0 | 459.0 | 0.935 | 1.252 |
| | $C_3$-33% | 3768 | 169.0 | 380.0 | 0.991 | 2.229 |
| | $C_4$-0% | 2215 | - | 216.0 | 1.025 | - |
| Pan evaporation | $D_1$-100% | 4942 | 627.0 | 691.0 | 0.715 | 0.788 |
| | $D_2$-67% | 4115 | 420.0 | 540.0 | 0.762 | 0.979 |
| | $D_3$-33% | 3552 | 207.0 | 415.0 | 0.855 | 1.715 |
| | $D_4$-0% | 2067 | - | 217.0 | 0.952 | - |

Seasonal plant water use values varied in connection with the irrigation water applied to the treatments and the amount of moisture at planting and harvest. At the same time, although it has a great effect on plant water consumption, there was no rain on the experimental area during the growing season. Water use values increased with increasing irrigation levels in each irrigation approaches.

Seasonal water use varied from 691 mm $D_1$ treatment (pan evaporation) to 217 mm in $D_4$ treatment (rain-fed) plots in the production period and 569 mm in $C_1$ (gravimetric) treatment to 216 mm in $C_4$ treatment (rain-fed) plots. This was followed by $D_2$ and $C_2$ treatments, 540 and 459 mm in the growing season, respectively (Table 3). The highest seasonal water use values were obtained from treatments $D_1$ and $C_1$ control treatments (100%) as 691 mm and 569 mm, respectively. Seasonal water use in the full irrigation treatment $S_1$, was in agreement with results obtained by Erten and Dagdelen (2020) in the Aydın plain with the drip system and who determined water use values as 800 mm. Seasonal water use of cotton under the same region has been reported as 899 mm by Sezgin et al. (2001); as 855-882 mm by Dagdelen et al. (2006) under furrow irrigation system; as 265-753 mm by Dagdelen et al. (2009) and as 268-754 mm by Basal et al. (2009) under drip irrigation system. Once the results of this study are compared with those of furrow irrigation studies at the same region, it is clear that drip irrigation systems are able to save substantial amount of water. Under drip irrigation applications, seasonal water use of cotton was obtained by Unlu et al. (2011) as 287-584 mm in Adana conditions; as 410-725 mm by Colaizzi et al. (2005) in the High Texas Plains. On the other hand, Ibragimov et al. (2007) found that seasonal water use in cotton varied between 432 and 739 mm depending on irrigation regimes in Uzbekistan conditions by using drip and furrow irrigation methods. In another study, Karam et al. (2006) applied a total of 738 mm irrigation water amount to drip irrigated cotton in the Bekaa Valley of Lebanon. The results observed in this research were in agreement with the others given above.

The response of seed cotton yield to different irrigation treatments are given in Table 4. Data obtained from study showed that seed cotton yield was significantly affected by irrigation levels. No interactions between irrigation application methods (App.) and irrigation levels (IL) were observed for any investigated parameters in year. Irrigation application methods (App) had no significant effect on seed cotton yield. The gravimetric method resulted in higher yield than pan evaporation applications. Seed cotton yield was found to increase with irrigation water levels. Examining these results from the point of view of irrigation levels (IL), four groups formed in year. The first group consisted of the 100% treatments where no water restriction had been applied in the whole growing season, treatments in which water had been applied at the 67 % level were second, and treatments which had received water at the 33 % level formed the third group. Especially, as the irrigation level increased, seed cotton yield were increased in all applications. The highest average yield was obtained from IL-100 treatment as 5029.1 kg $ha^{-1}$, followed by IL-67 treatment as 4086.1 kg $ha^{-1}$. The lowest yield was obtained from IL-0 treatment as 2141 kg $ha^{-1}$. The reduction rate was 42.5% between the highest and lowest seed cotton yield. When the amount of applied water through drip irrigation was reduced 33% (IL-67) the decrease in yield was about 18.7% in growing year. A significant decline in seed cotton yield under deficit irrigation treatment is reported in many previous researches.

**Table 4.** Seed cotton yield (kg $ha^{-1}$) as influenced by irrigation applications and irrigation levels

| | | |
|---|---|---|
| **Irrigation application methods (App.)** | Gravimetric<br>Pan evaporation | 3789.3<br>3669.5 |
| **F value (App.)** | | ns |
| **LSD $_{\%5}$** | | |
| **Irrigation levels (IL)** | IL-100%<br>IL-67%<br>IL-33%<br>IL-0% | 5029.1a<br>4086.1b<br>3660.8c<br>2141.6d |
| **F value (IL)** | | ** |
| **LSD $_{\%5}$** | | 204.515 |
| **App. x IL** | | ns |

*P< 0.05; **P< 0.01; ns: not significant

In a column values with a common letter are not significantly differ from one another using $LSD_{\%5}$

According to the results of a study conducted on drip irrigated cotton in Aydın area, the highest cotton yield was achieved from a treatment in which 100 % of the amount of evaporation from a class A-pan was applied at 8-day irrigation interval Dagdelen et al. (2005). On the other hand, the highest seed cotton yield (5870 kg $ha^{-1}$) was reported in the Harran plain from the full irrigation treatment (100%) with 6-day irrigation interval using drip irrigation method (Yazar et al., 2002). The average seed cotton yield was obtained as 5760 kg $ha^{-1}$ under drip irrigated treatment in western Turkey (Dagdelen et al., 2009). Another Aydın plain conditions the highest average raw cotton yield was obtained from $S_1$ treatment (Carisma-V1) as averaging 6300 kg $ha^{-1}$. It

was determined Carisma (V1) cultivar performed higher yields than Candia (V2) and Gloria (V3) (Dagdelen et al. 2019). Similar results were obtained by Erten and Dagdelen (2020) as 5985 kg ha$^{-1}$ at the same conditions. The results observed in this research were in agreement with the others given above. In evaluations conducted previously, it has been found that irrigation level have significant effect on seed cotton yield. It has been concluded that the most proper irrigation programme suggested for achieving highest cotton yield would be using the delinted seed applications under water abundant conditions in which the crop water requirements were fully met by IL-100 treatment (treatment $D_1$).

In order to evaluate the effects of water use on seed cotton yield regression analysis was conducted. There was a significant second order polynomial relationships were found between seasonal water use and seed cotton yield in irrigation treatments (Fig 1). Polynomial relationships of water use and seed cotton yield for drip irrigated cotton were given by (Yazar et al., 2002; Dagdelen et al., 2009; Unlu et al., 2011; Erten and Dagdelen, 2020).

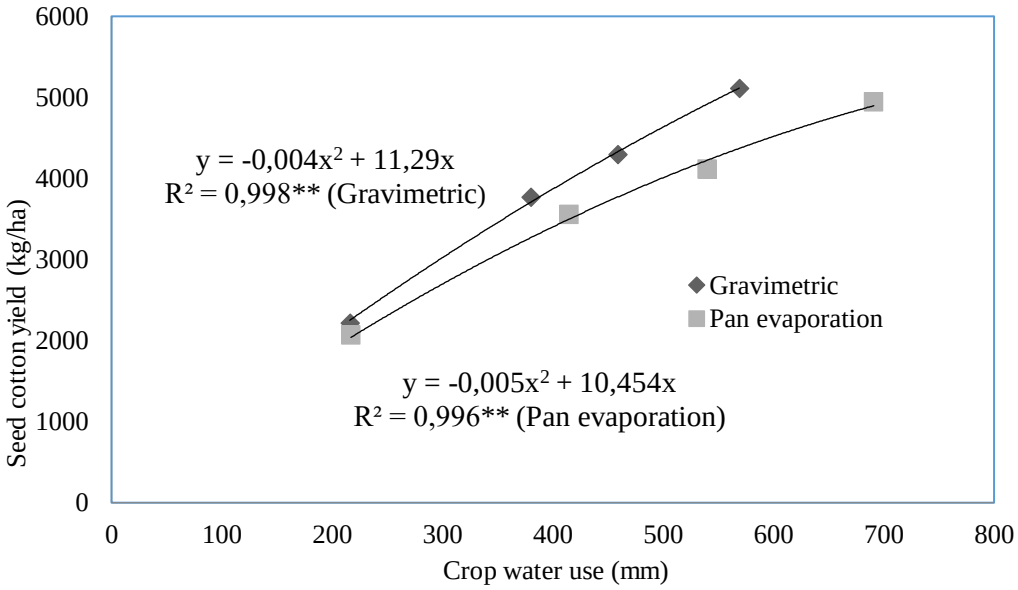


**Figure 1.** The relationship between water use and seed cotton yield

The $k_y$ factor which represents the slope of the relationship between relative ET and relative yield, was determined the methods of Stewart et al. (1977) (Fig 2). Yield response factor ($k_y$) was found to be 0.88 for gravimetric and 0.80 for pan evaporation applications. The average $k_y$ for the whole growing season were found to be 0.84 by Doorenbos and Kassam (1986), 0.89 by Yazar et al. (2002) and 0.78 by Dagdelen et al. (2009) in Aydın conditions.

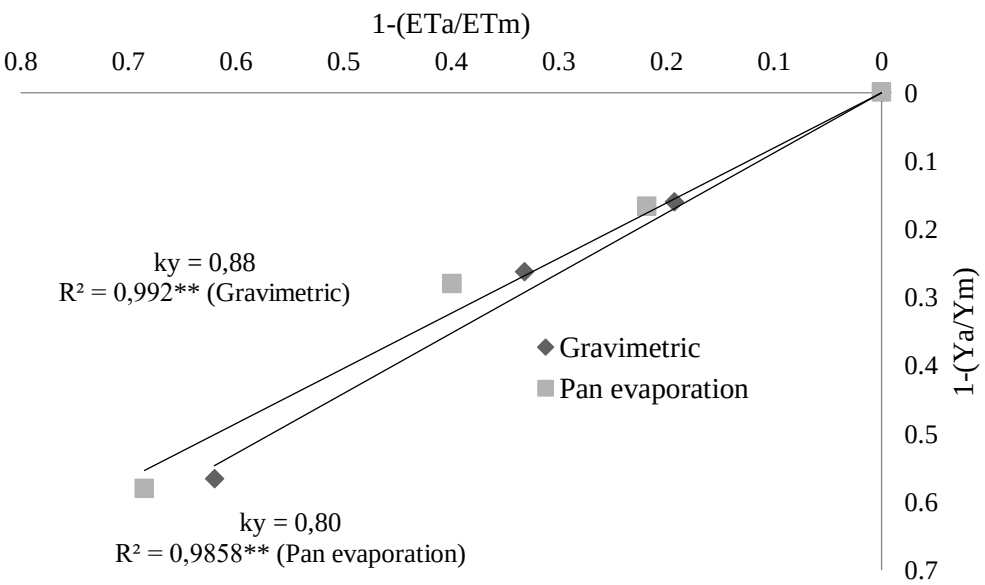


**Figure 2:** Yield response factor ,$k_y$, of cotton for different irrigation approaches

Water use and irrigation water use efficiencies (WUE, IWUE) values are listed in Table 4 for growing season. As the amount of water applied increased, WUE and IWUE decreased. In general, the IWUE values were higher than those of WUE in all treatments. This could be attributed to water used from soil storage. Water

use efficiency (WUE) varied from 0.715 kg $m^{-3}$ to 1.025 kg $m^{-3}$ among treatments. The highest WUE was obtained as 1.025 from $C_4$ (gravimetric-for IL-00) treatments and the lowest WUE was observed 0.898 kg $m^{-3}$ from $C_1$ (gravimetric for IL-100) treatments. Treatment IL-33% from all applications ($C_3$ and $D_3$) used to water more efficiently. Thus, when water was restricted under these conditions, a reduction of 26.3% and 28.1% were seen in seed cotton yield. Table 4 shows a comparison of the WUE and IWUE values obtained from our study and the water efficiency values reported by other researchers. The table shows that both WUE and IWUE values were similar to the findings of other researchers.

**Table 4.** The comparison of WUE and IWUE values for different research

| Sources | Irrigation system | WUE (kg $m^{-3}$) | IWUE (kg $m^{-3}$) |
|---|---|---|---|
| Our research | Drip | 0.71-1.02 | 0.78-2.22 |
| Ertek and Kanber (2001) | Drip | 0.58-0.62 | 0.75-0.94 |
| Yazar et al. (2002) | Drip | 0.50-0.74 | 0.60-0.81 |
| Yazar et al. (2002) | Lepa | 0.55-0.67 | 0.58-0.77 |
| Karam et al. (2006) | Drip | 0.80-1.30 | - |
| Ibragimov et al. (2007) | Drip | 0.63-0.88 | 0.82-1.12 |
| Dagdelen et al. (2009) | Drip | 0.77-0.96 | 0.82-1.44 |
| Basal et al. (2009) | Drip | 0.62-0.85 | 0.66-1.57 |
| Akcay and Dagdelen (2017) | Drip | 0.83-1.26 | 1.05-1.96 |
| Dagdelen et al. (2019) | Drip | 0.73-1.13 | 0.91-2.23 |
| Erten and Dagdelen (2020) | Drip | 0.74-1.12 | 0.97-2.50 |

## CONCLUSION

Results obtained in the experiment were in line with the literature and showed significant decrease in cotton yield with water deficit. Yield response factor and polynomial relationship produced in this study can be used as a guide by researchers and farmers to plan accordingly during dry seasons and drought events. Further economic analysis can be made by evaluating water use efficiency results to find an optimum spot for water use and cotton yield.

Since the effect on cotton yield between gravimetric method and pan evaporation method was not significant, the easy to use pan evaporation method can be recommended for farmers. On the other hand, results obtained from the gravimetric applications, with IL-67 treatment could be used as a good basis for reduced drip irrigation strategy development in semi-arid regions under water shortage.

## ACKNOWLEDGEMENTS

This paper includes part of the results obtained from a research project funded by the Aydın Adnan Menderes University Scientific Research Coordination Services (BAP Project Number: 21004, Aydın/Turkey).

## REFERENCES

Açıkgöz N, Aktas ME, Mokhaddam AF, Özcan K 1994. Tarist an agrostatistical package programme for personel computer. Ege Üniv. Fac. of Agriculture, Field Crops Symposium, İzmir, Turkey (in Turkish with English abstract).

Akçay S, Dağdelen N 2017. Water productivity and fiber quality parameters of deficit irrigated cotton in a semi-arid environment. Fresenius Environmental Bulletin, 26(11): 6500-6507.

Aksoy E, Aydın G, Seferoğlu S 1998. The important characteristics and classification of soils of the land of Agricultural Faculty, Adnan Menderes University. In: First Agricultural Conference in Aegean Region, 7–11 September, Aydın, Turkey (in Turkish with English abstract).

Anonymous 2019a. Cotton report. T.C. Ministry of Customs and Trade, Ankara, TURKEY, pp. 2-3.

Anonymous 2019b. Climatic report of Aydın Province. State Meteorological Organization Publications, Aydın, Turkey.

Basal H, Dağdelen N, Ünay A, Yılmaz E 2009. Effects of deficit drip irrigation ratios on cotton (*Gossypium hirsutum L.*) yield and fiber quality. Journal of Agronomy & Crop Science, 195(1): 19-29.

Çetin Ö, Bilgel L 2002. Effects of different irrigation methods on shedding and yield of cotton. Agricultural Water Management, 54: 1-15.

Colaizzi PD, Evett SR, Howell TA 2005. Cotton production with SDI, LEPA and spray irrigation in a thermally limited climate. In: Proceedings of the Conference on Emerging Irrigation Technology. 6-8 November 2005. The Irrigation Association, Phoenix, Arizona, (CD ROM), 15-30.

Dağdelen N, Basal H, Yılmaz E, Gürbüz T, Akçay S 2009. Different drip irrigation regimes affect cotton yield, water use efficiency and fiber quality in western Turkey. Agricultural Water Management, 96(1): 111-120.

Dağdelen N, Gürbüz T, Tunalı SP 2019. Response of different cotton cultivars to water stress on water-yield relations under drip irrigation conditions in Aydın Plain. Derim, 36(1): 64-72 (in Turkish with English abstract).

Dağdelen N, Yılmaz E, Sezgin F, Gürbüz T 2006. Water-yield relation and water use efficiency of cotton (*Gossypium hirsutum L.*) and second crop corn (*Zea mays L.*) in Western Turkey. Agricultural Water Management, 82: 63-85.

Dağdelen N, Yılmaz E, Sezgin F, Gürbüz T, Akçay S 2005. Effects of different trickle irrigation regimes on cotton (*Gossypium hirsutum L.*) yield in Western Turkey. Pakistan of Biological Sciences, 8(10): 1387–1391.

Doorenbos J, Kassam AH, 1986. Yield response to water. Irrigation and Drainage, FAO, ROME, Paper: 33, pp. 257.

Ertek A, Kanber R 2001. Effects of different irrigation programs on the growth of cotton under drip irrigation. Turkish Journal of Agriculture and Forestry, 25: 415-425 (in Turkish with English abstract).

Erten E, Dağdelen N 2020. Yield and water relations of drip irrigated cotton under various irrigation levels. Journal of Scientific and Engineering Research, 7(2): 204-211.

Heerman DF 1985. ET in irrigation management. In: Proceedings of the National Conference on Advances in Evapotranspiration. 16-17 December 1985, Transactions of the ASAE, 323-334.

Howell TA, Cuence RH, Solomon KH 1990. Crop yield response. In: Management of Farm Irrigation Systems. (G.J. Hoffman, et al), ASAE, St. Joseph, MI, 93-122.

Hussein F, Janat M, Yakoub A 2011. Assessment of yield and water use efficiency of drip-irrigated cotton (*Gossypium hirsutum L.*) as affected by deficit irrigation. Turkish Journal of Agriculture and Forestry, 35: 611-621.

Ibragimov N, Evett SR, Esanbekov Y, Kamilov BS, Mirzaev L, Lamers JPA 2007. Water use efficiency of irrigated cotton in Uzbekistan under drip and furrow irrigation. Agric. Water Manag., 90: 112-120.

Karam F, Lahoud R, Masaad R, Daccache A, Mounzer O, Rouphael Y 2006. Water use and lint yield response of drip irrigated cotton to length of irrigation season. Agricultural Water Management, 85: 287-295.

Mateos l, Berengena J, Orgaz F, Fereres E 1991. A comparison between drip and furrow irrigation in cotton at two levels of water supply. Agric. Water Manag., 19: 313-324.

Sezen SM, Yazar A, Canbolat M, Eker S, Çelikel G, 2004. Effect of drip irrigation management on yield and quality of field grown green beans. Agric. Water Manag., 54: 189-203.

Sezgin F, Yılmaz E, Dağdelen N, Bas S 2001. Effect of different irrigation methods and water supply level application on water-yield relations in cotton growing. In: Proceedings of the Third National Hydrology Congress. 27-29 June 2001, 9 Eylül Univ., Izmir, Turkey (in Turkish with English abstract).

Simsek M, Kacıra M, Tonkaz T 2004. The effects of different drip irrigation regimes on watermelon (*Citrullus lanatus*) yield and yield components under semi-arid climatic conditions. J. Agric. Res, 55: 1149-1157.

Stewart JL, Danielson RE, Hanks RJ, Jackson EB, Hagan RM, Pruitt WO, Franklin WT, Riley JP 1977. Optimizing crop production through control of water and salinity levels in the soil. Utah Water Lab, PRWG151-1, Logan, USA, p 191.

Ünlü M, Kanber R, Koç DL, Tekin S, Kapur B 2011. Effects of deficit irrigation on the yield and yield components of drip irrigated cotton in Mediterranean Environment. Agricultural Water Management, 98: 597-605.

Yazar A, Sezen SM, Sesveren S 2002. LEPA and trickle irrigation of cotton in the Southeast Anatolia Project (GAP) area in Turkey. Agricultural Water Management, 54(3):189-203.

## *ORAL PRESENTATION*

# Production of SS316L/HA Functionally Graded Material For Biomedical Applications Using Powder Metallurgy

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**Abstract**

In this research, SS316L/HA functionally graded material (FGM) was fabricated by powder metallurgy technique for biomedical applications and its microstructure, corrosion resistance, mechanical, and bioactivity properties were investigated. The FGM layers consisted of 316L and three different composites containing 3, 5, and 7 wt. % hydroxyapatite (HA). Experimental results revealed that FGM was successfully produced by the powder metallurgy technique without cracks and the hardness of the layers increased with the HA content. Electrochemical tests were performed in Ringer's and Hank's physiological fluids and results showed that samples tested in Ringer's solution were more susceptible to pitting corrosion as compared to tested in Hank's solution. Bioactivity tests were carried out in simulated body fluid (SBF) for 7 and 14 days and at the end of both periods, apatite layer consisting of spherical crystallites was observed on the sample surfaces.

**Keywords:** Functionally graded materials, SS316L, Hydroxyapatite, Powder metallurgy, Biomaterials.

## INTRODUCTION

In recent years, the performance of biomedical implants has become a growing subject of research. The performance of implants may be significantly improved by functionally graded material (FGM), which mimics bone structure. Due to their graded structure, functionally graded materials (FGMs) present special advantages for the development of advanced biomaterials. Metal-ceramic FGMs are attracting much attention as biomaterials. They are a good option for hard tissue applications like orthopedic implants since they can simulate bone. One of these FGMs that attracted the greatest attention from researchers is the combination of 316L stainless steel with other ceramics. The literature has reported on the following investigations for biological applications: 316L/HA/Calcium Silicate (Oshkour et al.,2015), 316L/CNT/HA (Hussain et al. 2015), $ZrO_2$/316L (Mishina et.al.,2008) and 3YPSZ/316L (Yoshida et al., 2005) 316L is extensively used as a biomaterial, however for long-term use, it needs to have enhanced biocompatibility. This improvement can be attributed to HA-reinforced SS316L-based FGM due to excellent bioactivity and biocompatibility.

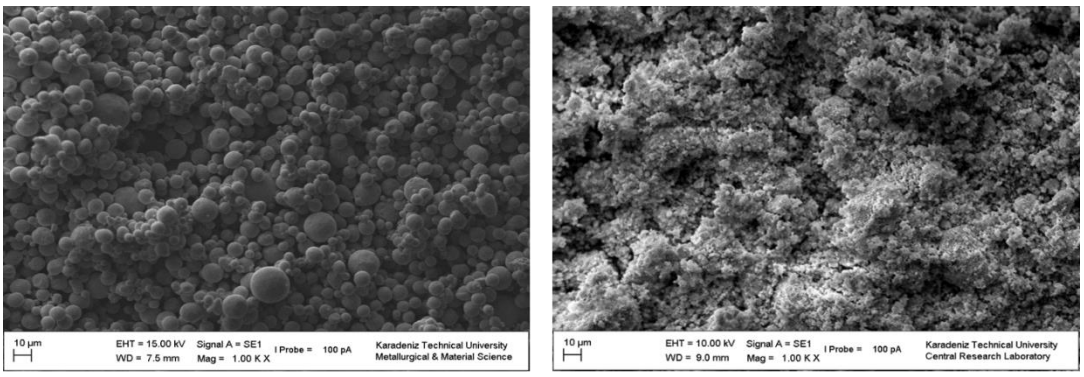
This study's major aim was to investigate the microstructure, biological, and electrochemical properties of functionally graded SS316L/HA biomaterials. The samples were produced utilizing the powder metallurgy method, which makes it simple to regulate the composition, microstructure, and shape formation of FGM over a broad range (Übeyli et al.,2014). The HA ratio of the FGM layers was changed up to 7 wt. % with a 2wt. % gradient. FGMs were submerged in SBF for 7 and 14 days to evaluate their in vitro performance, while corrosion experiments were carried out using Ringer's and Hank's solution.

## MATERIALS AND METHODS

As starting materials SS316L (average particle size of 15.8 μm) and HA powders (average particle size of 7.75 μm) were provided from Anhui Fitech Materials and Sigma Aldrich respectively. The chemical composition of SS316L powder is given in Table 1, and the morphology of as-received powders is presented in Figure.1.

**Table 1.** Chemical Compositions (wt.%) of as-recevied SS316L.

| Element | C | S | P | Si | Mn | Mo | Ni | Cr | Fe |
|---|---|---|---|---|---|---|---|---|---|
| **Content** | 0.005 | 0.001 | 0.010 | 0.950 | 0.743 | 2.595 | 11.742 | 17.667 | Bal. |




(a) (b)

**Figure. 1** Morphology of as received SS316L and HA powders

To produce the 316L/ HA composite powders, 316L powders containing 3,5, and 7 wt. % of HA particles were milled with tungsten carbide balls (in diameter10 mm) in a tungsten carbide vial using a Fritsch Pulverısette 7. The powder mixture was milled for 5h at a rotation speed of 200 rpm, the ball-to-powder weight ratio was 10:1. The milled powders were stacked in the die, pressed at 600 MPA pressure and then sintered in argon atmosphere at a temperature of 1200 $^{O}$C for 150 min with 10 °C/min heating rate. The schematic representation of the FGM consisting of 4 distinct layers is shown in the Figure 2.

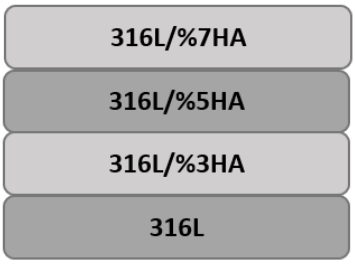



**Figure. 2** Schematic presentation of SS316L/HA FGM

The microhardness of each layer was determined by Vickers Hardness Tester (INNOVATEST) with a load of 200 gf and a dwell time of 15 s. Scanning electron microscopy (SEM, Zeiss Evo LS10) were used to characterize the microstructures of FGM. The corrosion behavior of 316L/HA was investigated using Gamry potentiostat/galvanostat/ZRA (Reference 3000). Potentiodynamic polarization measurements were carried out at 37 °C in the Ringer's and Hank's solution. Simulated body fluid (SBF) prepared in accordance with Kokubo's procedure was used to investigate the in vitro bioactivity of FGM (Kokubo et al.,2006). The samples were stored at 36.5 $^{O}$C for 7 and 14 days after being soaked in SBF.

## RESULTS and DISCUSSION

### Microstructure of FGM

The cut section of 316L/HA FGM successfully fabricated by the powder metallurgy process is shown in Figure 3 without any fractures or voids. The structure is made of 316L and three composite layers with, respectively, 3, 5, and 7 weight percents of HA. Despite the reinforcement and matrix having different thermal expansion coefficients, the layers were well-bonded and no interlayer cracks were found. The microstructure suggests that the network interface layer has a good mechanical bond.

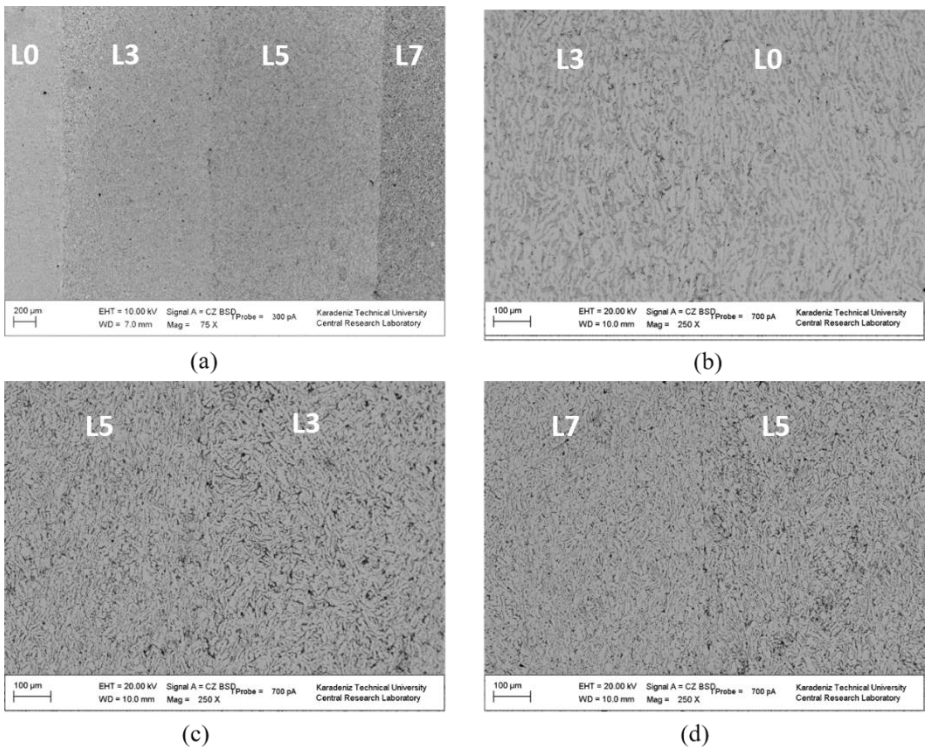



**Figure 3.** Microstructure of SS316L/HA FGM

## Hardness of FGM

The microhardness value for each layer of the FGM sample is shown in Figure 4 as a function of HA concentration. It is clear that the hardness of the gradient layers increased with increasing HA content, with the L7 layer reaching a maximum hardness of 271 HV. The stress shielding effect is decreased by the hardness gradation (Akmal et al., 2005). The elimination of natural bone stress by an implant, in accordance with the stress shielding concept, results in bone loss surrounding both orthopedic and dental implants (Mehrali et al., 2013). The FGM design, which must be built with the optimum shape and material, is thus an important factor in minimizing the stress shielding effect.

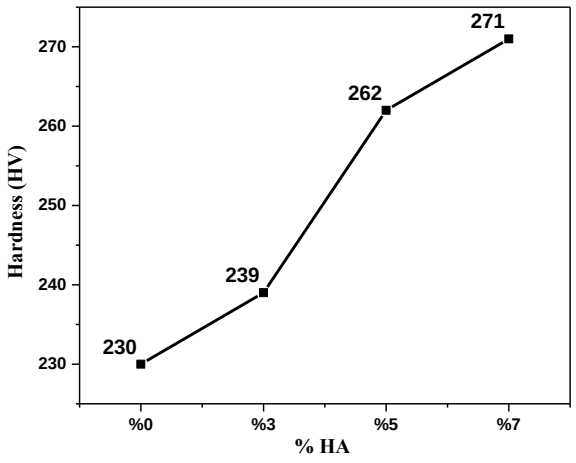



**Figure 4.** Hardness of SS316L/HA FGM

## Corrosion Evaluation

The Tafel curves from the potentiodynamic polarization test in Ringer and Hank's solution at 37 °C are shown in Figure 5. Table 2 lists the corrosion parameters including corrosion potential (Ecorr), current density (Icorr),

and corrosion rate derived from the curves. As can be shown, FGM exhibits parallel characteristics in terms of corrosion potentials (Ecorr) and current densities (Icorr) in both Ringer's and Hank's solutions. Hank's solution has a higher corrosion potential for FGM compared to Ringer's solution. In Hank's solution, the sample's Ecorr was -614 mV, while in Ringer's solution, it was -695 mV. Correspondingly, it was found that corrosion rates of FGM were 0.842 and 0.692 mm/y in Ringer's and Hank's solutions, respectively. The greater corrosion rate could be caused by a higher concentration of chloride ions in Ringer's solution.

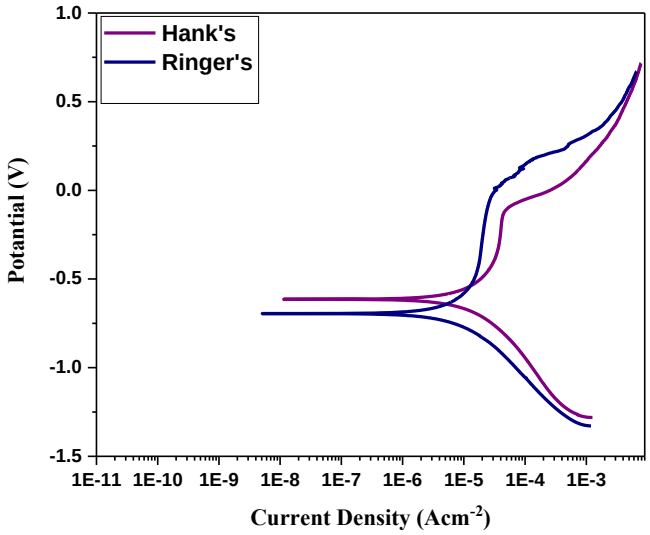


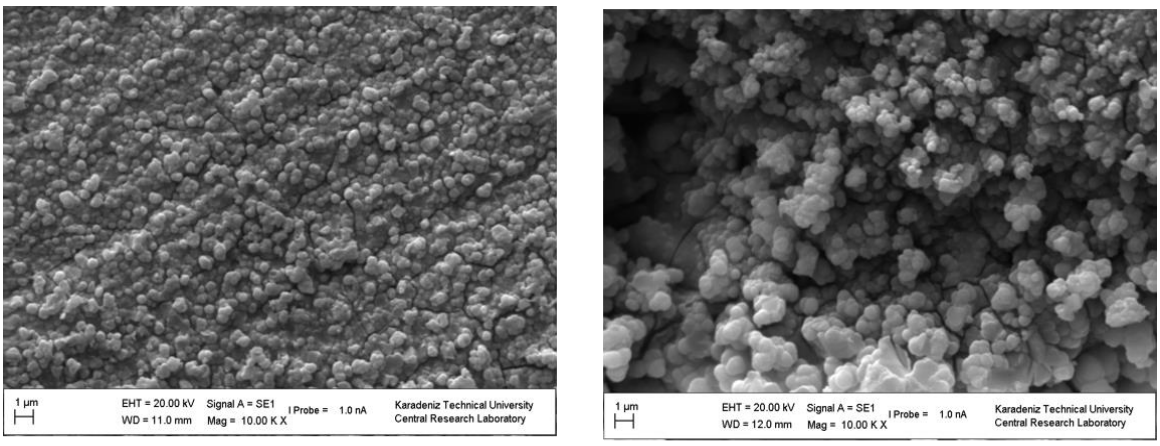

**Figure 5.** Potentiodynamic polarization curves of SS316L/HA FGM

**Table 2.** Corrosion Parameters

| Solution | $E_{corr}$ mV | $I_{corr}$ µA/cm$^2$ | Corrosion Rate mm/y |
|---|---|---|---|
| **Ringer's** | -695 | 11,6 | 0,842 |
| **Hank's** | -614 | 8,93 | 0,692 |

**Bioactivity**

FGM was submerged in a simulated bodily fluid (SBF) solution for 7 and 14 days to determine their potential to produce apatite. It has been discovered that hydroxyapatite that resembles bone has a strong affinity to active bone cells and good osteoconductivity. It promotes in cell attraction and osteoblasts' production of new bone tissue. This is why the formation of a bone-like hydroxyapatite layer on the implant's surface is necessary for osseointegration between the implant and the living bone tissue (Kumar et al.,2012). Figure 6 provided the morphology of FGM after immersion in SBF. At the end of 7 and 14 days, the surface of FGM is covered with a spherical apatite layer which indicates a strong bond between FGM and bone, which will aid osseointegration and implant fixation (Fan et al.,2009).




(a) (b)

**Figure 6.** Morphology of FGM after immersion in SBF a) 7days b) 14 days

## CONCLUSION

In this study, the microstructure, electrochemical and biological properties of functionally graded material produced by powder metallurgy method, consisting of 316L stainless steel matrix and wt. 3%, 5%, and 7% hydroxyapatite-reinforced composite layers were investigated. While the gradual structure of FDM is clearly seen in SEM analysis, there is no separation or crack between the layers. The hardness of the FDM layers increased proportionally with the increasing amount of reinforcement. The hardness values of the L0-L3-L5-L7 layers were found to be 230-239-262-271 HV. As a result of electrochemical experiments in Hank's and Ringer's physiological fluids, the corrosion rate of the sample in Hank's solution was lower. Apatite formation was observed in both FDM submerged in SBF for 7 and 14 days.

## ACKNOWLEDGEMENTS

This work was supported by Karadeniz Technical University Scientific Research Projects Coordination Unit. Project Number: FDK-2019-8440.

## REFERENCES

Akmal, M., Khalid, F. A., & Hussain, M. A. (2015). Interfacial diffusion reaction and mechanical characterization of 316L stainless steel-hydroxyapatite functionally graded materials for joint prostheses. Ceramics International, 41(10), 14458–14467.

Ataollahi Oshkour, A., Pramanik, S., Mehrali, M., Yau, Y. H., Tarlochan, F., & Abu Osman, N. A. (2015). Mechanical and physical behavior of newly developed functionally graded materials and composites of stainless steel 316L with calcium silicate and hydroxyapatite. Journal of the Mechanical Behavior of Biomedical Materials, 49: 321–331.

Fan, X., Chen, J., Zou, J. peng, Wan, Q., Zhou, Z. cheng, & Ruan, J. ming. (2009). Bone-like apatite formation on HA/316L stainless steel composite surface in simulated body fluid. Transactions of Nonferrous Metals Society of China (English Edition), 19(2), 347–352.

Hussain, M. A., Maqbool, A., Khalid, F. A., Farooq, M. U., Abidi, I. H., Bakhsh, N., Amin, W., & Kim, J. Y. (2015). Improved sinterability of hydroxyapatite functionally graded materials strengthened with SS316L and CNTs fabricated by pressureless sintering. Ceramics International, 41(8), 10125–10132.

Kokubo, T., & Takadama, H. (2006). How useful is SBF in predicting in vivo bone bioactivity? Biomaterials, 27(15), 2907–2915.

Madhan Kumar, A., & Rajendran, N. (2012). Influence of zirconia nanoparticles on the surface and electrochemical behaviour of polypyrrole nanocomposite coated 316L SS in simulated body fluid. Surface and Coatings Technology, 213, 155–166.

Mehrali, M., Shirazi, F. S., Mehrali, M., Metselaar, H. S. C., Kadri, N. A. Bin, & Osman, N. A. A. (2013). Dental implants from functionally graded materials. Journal of Biomedical Materials Research - Part A,

101(10), 3046–3057.

Mishina, H., Inumaru, Y., & Kaitoku, K. (2008). Fabrication of $ZrO_2$/AISI316L functionally graded materials for joint prostheses. Materials Science and Engineering A, 475(1–2), 141–147.

Übeyli, M., Balci, E., Sarikan, B., Öztaş, M. K., Camuşcu, N., Yildirim, R. O., & Keleş, Ö. (2014). The ballistic performance of SiC-AA7075 functionally graded composite produced by powder metallurgy. Materials and Design, 56, 31–36.

Yoshida, K., Mishina, H., Sasaki, S., Morita, M., & Mabuchi, K. (2005). Development of 3Y-PSZ/AISI 316L composites for joint prostheses. Nippon Kinzoku Gakkaishi/Journal of the Japan Institute of Metals, 69(8), 793–800.

## *ORAL PRESENTATION*

# Changes in activity of antioxidant enzymes in zebrafish muscle after chronically exposed to fluoride

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**Abstract**

Fluoride is frequently used in drinking water, ready-to-eat foods, medicines, cosmetics and especially in tooth cleaning products. The aim of this study is to investigate the effect of fluoride on antioxidant enzymes in muscle tissue in a zebrafish model. In the study, adult zebrafish were exposed to fluoride at different concentrations (1.5 ppm, 5 ppm and 100 ppm) for 6 weeks. Muscle tissues of the fish were dissected after chronic exposure. SOD, CAT and GPx enzyme activities were measured in the tissues. The data obtained showed a statistically significant decrease in SOD and CAT activities depending on the dose increase of fluoride. However, it was observed a statistically significant increase at the highest dose in GPx activity. The results showed that chronic exposure to fluoride affects the antioxidant enzyme system in zebrafish muscle tissue.

**Keywords:** Oxidative stress, Sodium fluoride, Zebrafish.

## INTRODUCTION

Fluoride is an inorganic and monoatomic anion. It occurs naturally in water, air and soil. It has been reported that fluoride has effects such as neurotoxic, genotoxic and oxidative damage (Bhowmik and Chattopadhyay 2019; Kabir et al. 2019). Fluoride can be taken into the body from various sources (drinking water, food, drugs and cosmetic products). Especially toothpastes and oral care products are sources of fluoride and increase the fluoride concentration in the body (Kabir et al. 2019).

There is a critical balance in the formation of free radicals of oxygen under normal physiological conditions. Living creatures have a network of antioxidant defense systems that are used to protect and neutralize themselves against free radical toxicity (Halliwell and Gutteridge 1989, Ravi Kiran and Aruna 2010). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are prominent factors in this system. Disruption of the oxidant-antioxidant balance creates oxidative stress that causes molecular and cellular tissue damage in the living (Klaunig and Kamendulis 2004, Ravi Kiran and Aruna 2010).

This study investigated the changes in the activities of muscle antioxidant enzymes (SOD, CAT and GPX) in zebrafish chronically exposed to fluoride.

## MATERIALS AND METHODS

### Animals and treatment

In the experiment, 6 months old and healthy AB genotype zebra fish obtained from Atatürk University Fisheries Faculty Fisheries Experimental Research Unit were used. A total of 4 groups were formed in the study: three treatment groups (1.5 mg/L, 5 mg/L and 100 mg/L NaF) and a control group. Fish were exposed to fluoride for 6 weeks.

### Experimental protocol

Muscle tissues of zebrafish chronically exposed to fluoride were dissected. Tissues were weighed, washed with PBS and homogenized 1:5 in phosphate buffer (50 mM, pH:7.4, containing 10mM EDTA). It was then centrifuged at 10000 g for 30 min at 4°C. Supernatants were used for enzyme analysis.

SOD activity assay was performed according to the colorimetric assay described by Sun et al. (1998). One unit of SOD is given as the amount of superoxide dismutase required to inhibit the reduction of nitroblue tetrazolium by 50% under assay conditions.

CAT activity assay was carried out according to the method described by Aebi (1984). One unit of CAT activity was calculated as the amount of CAT needed to convert 1 µmol $H_2O_2$ per minute.

GPx activity assay was performed according to the method described by Beutler (1975). One unit of GPX activity was calculated as the amount of GPx required to oxidize 1 µmol of NADPH per minute.

**Statistical analysis**

Statistical analysis of the data was performed using GraphPad Prism Ver 5.0 (Graph Pad Software Inc, San Diego, CA, USA). All data were carried out using one-way analysis of variance (ANOVA) with Tukey's post hoc test. The $p<0.05$ level was considered as significant.

## RESULTS

After fluoride treatment, SOD, CAT and GPx levels were evaluated in muscle tissue of zebrafish. When compared to the control group, SOD activity was reduced with a significant level at the fluoride concentrates of 5 ppm and 100 ppm as exhibited in Figure 1 ($P < 0.01$). After treatment with fluoride of 1.5 ppm, no statistically significant change was observed in sod activity ($P > 0.05$).

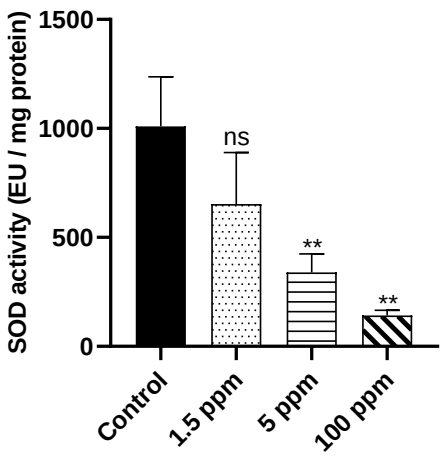


**Figure 1.** SOD activity in muscle tissue of the zebrafish after chronically exposed to fluoride (SOD: superoxide dismutase; ns: not significant $P > 0.05$; *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$)

Values obtained for CAT activity showed no differences at the group of 1.5 ppm (Figure 2). There was a statistically significant decrease in the activity at groups of 5 ppm and 100 ppm compared to the control group ($P < 0.05$ and $P < 0.001$, respectively).

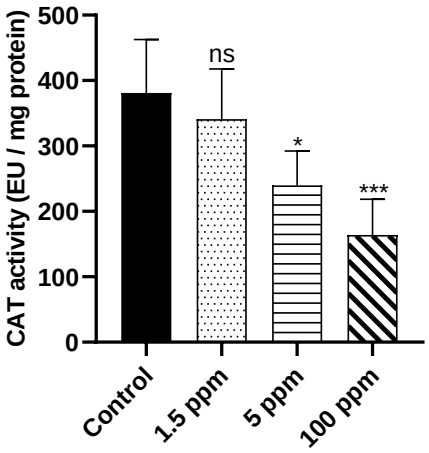


**Figure 2.** CAT activity in muscle tissue of the zebrafish after chronically exposed to fluoride (CAT: catalase; ns: not significant $P > 0.05$; *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$)

According to the GPx activity results given in Figure 3, while there was a significant increase in the 100 ppm group ($P < 0.001$), no statistically significant change was observed in the other groups ($P > 0.05$).

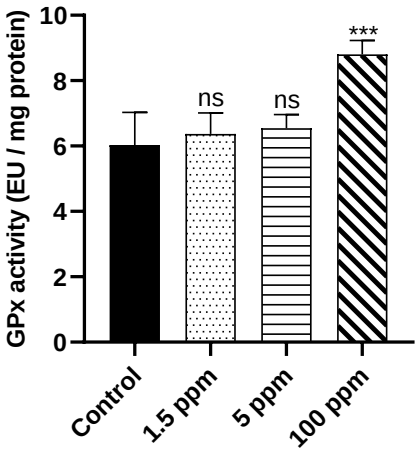


**Figure 3.** GPx activity in muscle tissue of the zebrafish after chronically exposed to fluoride (GPx: glutathione peroxidase; ns: not significant $P > 0.05$; *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$ )

## DISCUSSION

SOD, CAT and GPx enzymes play a role in the antioxidant defense system in the organisms. SOD catalyzes the production of $H_2O_2$ by binding reactive oxygen species with water and is the first enzymatic defense against superoxide anion. CAT catalyzes the decomposition of $H_2O_2$ into water and oxygen and protects the cell from the harmful effects of $H_2O_2$ and the hydroxyl radical. GPx catalyzes the reaction of hydroperoxides with reduced GSH. Thus, the reduction product of glutathione disulfide and hydroperoxide is formed. Changes in the activity levels of antioxidant enzymes are associated with oxidative stress (Yadav et al. 2015). One of the important properties of SOD and GPx enzymes is their inducibility under oxidative stress conditions. It has been reported that this induction may be an important adaptation to pollutant-induced stress (Zhang et al. 2004). In a study, the effect of fluoride on the soft tissues of rabbits exposed to 50, 100 and 200 ppm concentrations of NaF were observed for 90 days. It was found that lipid peroxides, SOD and CAT activities were decreased in kidney tissues (Ranjan et al. 2009). In addition, reductions in SOD, CAT and GPx activities were observed in rats exposed to 25 ppm fluoride for 8 and 16 weeks (Shanthakumari et al. 2004). In another study, zebrafish were exposed to 15 ppm NaF for 30 and 90 days. It has been reported that fluoride significantly changes the antioxidant enzyme activities (GST, CAT, SOD) in the liver (Mukhopadhyay et al. 2015). In our work, a decrease in SOD and CAT activity was observed in zebrafish muscle for 6 weeks at the treatment groups, compared to the control group. The decrease was found to be statistically significant for exposure to 5 ppm and 100 ppm fluoride, indicating that SOD and CAT activity of muscle was inhibited by the fluoride

intake. A statistically significant increase in GPx activity was observed only in 100 ppm group. As Tunçsoy et al. (2017) stated, this may be due to the overuse of CAT and SOD after the exposure, resulting in an increase in GPx activity for greater protection against hydroperoxides as their levels decrease. Fluoride has been associated with oxidative stress by stimulating the production of free radicals and inhibiting the action of antioxidant enzymes (Ranjan et al. 2009).

## CONCLUSION

In conclusion, fluoride showed a clear effect on the activities of three antioxidant enzymes (SOD, CAT and GPx) in zebrafish muscles. The present result indicates that chronic exposure to fluoride may affect the antioxidant enzyme network.

## ACKNOWLEDGEMENTS

This work was supported by Research Fund of the Ataturk University. Project Number: FBA-2020-8668.

## REFERENCES

Aebi H 1984. Catalase in vitro. Methods Enzymology, 105: 121-126.

Beutler E 1975. Red cell metabolism, A Manual of Biochemical Methods Grune and Starton, New York, 160 pp.

Bhowmik AD, Chattopadhyay A 2019. A review on fluoride induced organotoxicity and genotoxicity in mammals and zebrafish. Nucleus, 62: 177-185.

Halliwell B, Gutteridge JMC 1989. Free radicals in biology and medicine, 2nd, Oxford: Clarendon Press, pp 139-187.

Kabir H, Gupta AK and Tripathy S 2019. Fluoride and human health: Systematic appraisal of sources, exposures, metabolism, and toxicity. Critical Reviews in Environmental Science and Technology, 1-78.

Klaunig JE, Kamendulis LM 2004. The role of oxidative stress in carcinogenesis. Annual Review of Pharmacology and Toxicology, 44: 239-267.

Mukhopadhyay D, Srivastava R, Chattopadhyay A 2015. Sodium fluoride generates ROS and alters transcription of genes for xenobiotic metabolizing enzymes in adult zebrafish (Danio rerio) liver: expression pattern of Nrf2/Keap1 (INrf2). Toxicology Mechanisms and Methods, 25(5): 364-373.

Ranjan R, Swarup D, Patra RC 2009. Oxidative stress indices in erythrocytes, liver, and kidneys of fluoride-exposed rabbits. Fluoride, 42 (2): 88.

Ravi Kiran T, Aruna HK 2010. Antioxidant enzyme activities and markers of oxidative stress in the life cycle of earthworm, Eudrilus eugeniae. Italian Journal of Zoology, 77(2): 144-148.

Shanthakumari D, Srinivasalu S, Subramanian S 2004. Effect of fluoride intoxication on lipidperoxidation and antioxidant status in experimental rats. Toxicology, 204 (2-3): 219-28.

Sun Y, Oberley LW, Li Y 1988. A simple method for clinical assay of superoxide dismutase. Clinical Chemistry, 34(3): 497-500.

Tunçsoy M, Duran S, Ay Ö, Cicik B, Erdem C 2017. Effects of copper oxide nanoparticles on antioxidant enzyme activities and on tissue accumulation of Oreochromis niloticus. Bulletin of environmental contamination and toxicology, 99(3): 360-364.

Yadav SS., Kumar R., Khare P, Tripathi M 2015. Oxidative stress biomarkers in the freshwater fish, Heteropneustes fossilis (Bloch) exposed to sodium fluoride: antioxidant defense and role of ascorbic acid. Toxicology international, 22(1): 71.

Zhang J, Shen H, Wang X, Wu J, Xue Y 2004. Effects of chronic exposure of 2, 4-dichlorophenol on the antioxidant system in liver of freshwater fish Carassius auratus. Chemosphere, 55(2): 167-174.

## ORAL PRESENTATION

### *Rhodotorula Mucilaginosa* BT59 mayası ile tek hücre yağı (THY) biyosentezi ve karakterizasyonu

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**Özet**

Oleaginous mayalar alternative yağ kaynağı olarak Kabul edilen tek hücre yağlarını üretmektedir. Tek hücre yağları (THY) fonksiyonel bir yağ kaynağı olarak popülerlik kazanmaktadır. THY, gıda, biyoyakıt ve oleokimya sektörleri için hammadde olarak kullanılma potansiyeline sahiptir. *Rhodotorula mucilaginosa* BT59, bozadan izole edilerek potansiyel oleaginous özellikleri araştırılmıştır. Karbon kaynağı olarak glukoz kullanışmış ve *R. mucilaginosa*'nın lipid sentezi araştırılmıştır. %4 glukoz, %10 inokulum ve 200 rpm karıştırma hızında biyomas verimi 12.28 $gL^{-1}$ olarak tespit edilmiştir. THY oranı ise kuru ağırlık üzerinden %12,06 bulunmuştur. Sentezlenen THY'nın serbest yağ asitliği, peroksit sayısı, dien ve trien (K232 ve K270) değerleri, toplam fenolik madde, antioksidan aktivite, antimikrobiyal aktivite ve yağ aside kompozisyonu analiz edilmiştir. Antimikrobiyel aktivite *Escherichia coli* O157:H7 ATCC 33150, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 7644, *Salmonella Enteritidis* ATCC 13076 patojenlerine karşı test edilmiştir. Serbest asitlik %0,33, peroksit sayısı 10,53 meq O2/kg yağ, dien ve trien değerleri 0,17 ve 0,18 olarak bulunmuştur. THY'nın toplam fenolik madde miktarı ve antioksidan aktivite değerleri [1,1-diphenyl-2-picrylhydrazyl (DPPH) ve Trolox eşdeğeri (TEAC)] sırasıyla 1523±16.26 g GAE $kg^{-1}$, 116.17±2.82 mg $L^{-1}$, ve 12.11±0.17 µmol Trolox $kg^{-1}$ olarak tespit edilmiştir. THY *Salmonella Enteritidis* ATCC 13076 patojenine karşı 8.09±0.03mm zon çapında antimikrobiyel aktivite göstermiştir. *R. mucilaginosa* BT59'den üretilen THY'nın yağ asidi bileşiminin temel olarak %16,75 palmitik asit ($C_{16:0}$), %4,52 stearik asit ($C_{18:0}$), %60,05 oleik asit ($C_{18:1}$), %15,68 linoleik asit ($C_{18:2}$) ve %2,96 linolenik asitten ($C_{18:3}$) oluştuğu tespit edilmiştir. THY yağ aside kompozisyonunun esansiyel yağ asitlerinden olan oleic asit için önemli bir kaynak olduğu, aynı zamanda yüksek oranda linoleic asit içerdiği görülmüştür.

**Anahtar kelimeler:** Tek hücre yağı, *Rhodotorula Mucilaginosa*, oleik asit, microbiyel yağlar

### Biosynthesis and characterization of single-cell oil (SCO) from *Rhodotorula Mucilaginosa* BT59

**Abstrat**

Oleaginous yeasts produce single-cell oils, which are considered an alternative oil source. Single-cell oils (SCO) are currently gaining popularity as a source of functional oils. SCO has the potential to be used as a raw material for the food, biofuel, and oleochemical sectors. *Rhodotorula mucilaginosa* BT59 was isolated from a traditional fermented beverage called Boza and considered for its potential oleaginous properties. Using glucose as a carbon source, lipid synthesis by *R. mucilaginosa* was investigated. The biomass yield was 12.28 $gL^{-1}$ with 4% glucose, 10% inoculum, and a mixing speed of 200 rpm. SCO content was found to be 12.06% of dry cell weight. The free fatty acid content, peroxide value, diene and triene (K232 and K270) values, total phenol content (TPC), antioxidant activity, antimicrobial activity, and fatty acid composition of SCO were analyzed. Antimicrobial activity was determined by disc diffusion method against four different pathogens (*Escherichia coli* O157:H7 ATCC 33150, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 7644, *Salmonella Enteritidis* ATCC 13076). The free fatty acid content, peroxide value, diene and triene (K232 and K270) values were found to be 0.33%, 10.53 meq O2/kg oil, 0.17, and 0.18, respectively. The TPC and antioxidant capacity [1,1-diphenyl-2-picrylhydrazyl (DPPH) values and Trolox equivalent antioxidant capacity (TEAC)] of the SCO were found to be 1523±16.26 g GAE $kg^{-1}$, 116.17±2.82 mg $L^{-1}$, and 12.11±0.17 µmol Trolox $kg^{-1}$, respectively. SCO exhibited an antibacterial effect only against *Salmonella Enteritidis* ATCC 13076, with an inhibition zone of 8.09±0.03mm. The fatty acids of SCO from *R. mucilaginosa* BT59 were mainly composed of palmitic acid 16.75 % ($C_{16:0}$), stearic acid 4.52% ($C_{18:0}$), oleic acid 60.05% ($C_{18:1}$), linoleic acid 15.68% ($C_{18:2}$), and linolenic acid 2.96% ($C_{18:3}$). The fatty acid content of SCO demonstrated that it is a significant source of oleic acid, an essential fatty acid, as well as a high concentration of linoleic acid.

**Keywords:** Single-cell oil, *Rhodotorula Mucilaginosa,* oleic acid, microbial oils

## GİRİŞ

Tek HücreYağları (Single Cell Oils) başlıca maya, küf, alg ve bakteriler tarafından üretilen yenilebilir yağlar olarak tanımlanmaktadır. Tek hücre yağları, triaçilgliserollerden oluşan hücre içi depolama lipidleri olarak da bilinmektedir (Fakas ve ark., 2009). Tek hücre yağları, aynı anda fazla karbon kaynağı ile birlikte, örneğin azot veya fosfor gibi besin sınırlamaları altında sabit büyüme fazında kuru biyokütle başına %20 ila %80 arasında lipid biriktirebilen yağlı mikroorganizmalar tarafından üretilmektedir. Bakteri, maya, mikroalg veya mantar türleri dahil olmak üzere yağlı mikroorganizmaya bağlı olarak, tek hücre yağlarının yağ asidi profilini değiştirebilmekte ve bu da onları çeşitli endüstriyel uygulamalar için oldukça uygun hale getirmektedir (Ratledge, 2010).

ω-3 ve ω-6 sınıfının (örneğin, a-linolenik asit, linoleik asit) çoklu doymamış yağ asitleri (PUFA), biyofonksiyonların sürdürülmesi için esastır. İnsanlar bu esansiyel yağ asitlerini sentezleyemedikleri için farklı besin kaynaklarından almaları gerekmektedir. Çoklu doymamış yağ asitlerinin (PUFA) geleneksel kaynakları, üretimi çeşitli doğal kısıtlamalara (balık popülasyonlarının azaltılması) büyük oranda maruz kalan balık yağları olmuştur (Fakas ve ark., 2009). Bunun yanında, istenmeyen kokuları ve düşük stabilite göstermeleri ile mutajenik bileşikler ve ağır metaller gibi kirleticileri barındırabilmesinden dolayı balık yağlarının gıda katkı maddesi olarak kullanımına ilişkin önemli kısıtlamalar olduğu bilinmektedir. Aksine, mikrobiyal çoklu doymamış yağ asitleri üretimi sürdürülebilirdir ve iklimsel veya mevsimsel değişikliklerden etkilenmemektedir. Mantarlar ve mikroalgler dahil olmak üzere yağlı mikroorganizmalar tarafından üretilen yağlar yüksek saflığa sahip olmasının yanında, nispeten yüksek büyüme oranlarıyla birçok çeşitli substratlar üzerinde büyüyebilmektedir (Bellou ve ark., 2016).

Mikrobiyal yağlar, yağların yağ asidi bileşimlerine bağlı olarak çeşitli uygulamalarda kullanılabildiği yapılan çalışmalar ile ortaya konulmuştur. Triaçilgliseroller bakımından zengin mikrobiyal yağlar, biyodizel veya kakao yağı ikamelerinin üretimi için kullanılabilmektedir (Ahmad ve ark., 2019). Mikrobiyal kaynaklı yağ üretimi konusunda son zamanlarda yapılan araştırma girişimleri, çeşitli yenilenebilir kaynaklar üzerinde fermantasyon sırasında yağlı mikroorganizmalar tarafından biriken mikrobiyal yağı kullanarak biyodizel üretimine odaklanmış oldukları görülmektedir (Koutinas ve ark., 2014).

Maya, küf, bakteri ve alg gibi mikroorganizmaların uygun koşullar sağlandığı zaman yağ üretebilecekleri bilinmektedir ve bu şekilde ticari yağ üretimi olanakları üzerine çok sayıda araştırmalar yapılmıştır. Yağların fermantasyonu yoluyla ticari olarak üretilmesi yoğun bir şekilde 1. ve 2. dünya savaşları esnasında Almanya'da gündeme gelmiştir. Bu konudaki ilk önemli büyük ölçüde çalışma 1914-1918 yılları arasında, Almanya'da Linder adında bir araştırmacı tarafından *Endomyces vernalis* kullanılarak yapılmış ve bu çalışma HESSE (1949) ve WOODBİNE (1959) tarafından tekrar edilmiştir. Fakat bu sıralarda ciddi çalışmalar yapılmasına rağmen işlemin ekonomik fayda sağlayacağı hiç düşünülmemiştir (Denli ve Tekin, 2000).

1970'lerden sonra mikrobiyal yağ üretimi, endüstriyel kirliliğin giderek artması enerji maliyetlerinin yükselmesi ve endüstri atıklarının fermantasyonu yoluyla özellikle gıda endüstrisinde kullanılabileceğinin farkedilmesiyle ilgi artmıştır (Denli ve Tekin, 2000).

Bebek maması için mikrobiyal tek hücre yağı kullanılmadan önce, kakao yağı yetersiz olduğu için ilk olarak 1980'lerde kakao yağı ikamelerinin hazırlanması için keşfedilmiştir (Ward ve Singh, 2005). Elde edilen yağ, kakao yağına fazlasıyla benzeyen 24:21:30 (%, w/w) oranında palmitik asit (16:0), stearik asit ve oleik asit yağ asitlerini içermektedir (28:35: 35; %, w/w). Bununla birlikte, kakao yağının dünya fiyatı 8000$/tondan 2500$/tona düştüğünde sürecin rekabet edemez hale gelmesi kaçınılmaz olmuştur (Ochsenreither ve ark., 2016).

Bu kapsamda lipid hayvansal, bitkisel ve mikrobiyal kaynaklardan elde edilmektedir. Dünya yağ gereksiniminin %80'i tarımsal ürünlerden sağlamaktadır. Hayvansal kaynaklar ve su ürünleri ise artakalan kısmı sağlamak için birer kaynaktır. Mikroorganizmalardan lipid eldesi hayvansal ve bitkisel kaynaklara alternatif olarak geliştirilmektedir. Mikrobiyal kaynak olarak mayalar, mantarlar, küfler ve mikroalgler hücre içi depolama bileşikleri olarak lipid biriktirebilme yeteneğine sahiptir.

Bu çalışmada daha önce Oleaginous özellikleri incelenmemiş ve yeni bir kaynak olarak bozadan izole edilmiş olan *Rhodotorula mucilaginosa* (BT59) mayasının tek hücre yağı üretim kabiliyeti araştırılmıştır. Üretilen tek hücre yağının karakterizasyonu ile serbest yağ asitliği, peroksit sayısı, dien ve trien (K232 ve K270) değerleri, toplam fenolik madde, antioksidan aktivite, antimikrobiyal aktivite ve yağ aside kompozisyonu tespit edilmiştir.

## MATERYAL VE METOT

### Materyal:

Tek hücre yağı üretiminde önceki çalışmalarımızda (Tortum ve ark., 2021) bozadan izole edilen ve PCR'da tanımlaması yapılan kırmızı maya *Rhodotorula mucilaginosa* (BT59) kullanılmıştır.

### Metod:

### Mikroorganizma ve Kültür Ortamı

*Rhodotorula mucilaginosa* (BT59) 4 ˚C'de 10 g $L^{-1}$ yeast ekstrakt, 10 g $L^{-1}$ pepton, 20 g $L^{-1}$ glukoz, 20 g $L^{-1}$ agar içeren Yeast Extract Peptone Dextrose (YEPD) besiyerinde aktifleştirilmiş ve kullanım süresince yatık agarda muhafaza edilmiştir (Wu ve ark., 2011).

### İnkübasyon

İnkübasyon süresi 5 gün sonunda taze kültür olarak hazırlanan maya platin öze ile alınarak içerisinde 100 ml inokulüm ortamı bulunan erlenmayere aktarılmıştır. Bu kültür, 25 ˚C'de 200 rpm fermentasyon şartlarında 24 saat inkübasyona bırakılmıştır. Maya hücreleri %2 glikoz, %0,1 $KH_2PO_4$, %0,05 yeast extract ve %0,02 $(NH_4)_2SO_4$ içeren ortamda 24 saat boyunca 200 rpm'de çalkalamalı inkübatör kullanılarak yeniden aktifleştirilmiştir. Lipit üretimi için $KH_2PO_4$ (8 g/L), $MgSO_4.7H_2O$ (0,5 g/L), karbon kaynağı (glukoz) ve azot kaynağı içeren ortam kullanılmış ve 200 rpm/dakikada çalkalama hızında kültür ilave edilerek inkübasyona bırakılmıştır (Liu ve ark., 2009).

### Tek hücre yağı ekstraksiyonu ve yağ oranının belirlenmesi

Fermentasyon sonrası elde edilen biyomas 15 dk boyunca 7500 rpm'de santrifüj yoluyla toplanmıştır. Daha sonra lipitler, oda sıcaklığında 48 saat boyunca 10 ml kloroform-metanol (2:1, h/h) ile ekstrakte edilmiştir. Nihai homojenat süzülemüş, 10 mL %0,9 NaCl çözeltisi ile yıkanmıştır. Daha sonra 2000 rpm'de 5 dakika santrifüjlenerek, yağ içeren kloroform fazı elde edilmiştir. Kloroform, vakumlu rotary evaporatör ile uçurulmuş ve lipid içeriği aşağıdaki denkleme göre hesaplanmıştır (Folch ve ark., 1957).

$$\boldsymbol{Lipit\ verimi} = \frac{\boldsymbol{Kuru\ ağırlık\ (g)}}{\boldsymbol{Hacim\ (L)}}$$

### Biyomas verimi

İnkübasyon süreleri sonunda kültür ortamından 50 ml alınarak 5000 rpm'de 15 dk süre ile santrifüj edilmiştir. Elde edilen pelet kısmı iki kez saf su ile yıkandıktan sonra sabit ağırlığa gelinceye kadar kurutulmuştur. Kurutulan biyomas g/L olarak hesaplanmıştır (Alakraa,2014).

### Serbest Yağ Asitliği Analizi

Tek hücre yağının serbest yağ asidi miktarı % oleik asit cininden AOCS Ca 5a-40 metoduna göre belirlenmiştir. Örneğin serbest yağ asidi miktarı aşağıdaki formüle göre hesaplanmıştır (AOCS, 2003).

$$\boldsymbol{Oleik\ asit}\ \% = mL\ 0{,}1N\ NaOH \times 0{,}05$$

### Peroksit Analizi

Yağ örneklerinin peroksit değeri AQCS Cd 8-53(AOCS, 2003) metoduna göre belirlenmiştir. Örneğin peroksit değeri aşağıdaki formüle göre hesaplanmıştır.

$$\textbf{Peroksit değeri}\left(miliekiivalent\frac{O2}{kg}yağ\right) = S \times N \times 1000/m$$

S: Harcanan sodyum tiyosülfat miktarı (ml)

N: Sodyum tiyosülfat için ayarlı normalite

m: Tartılan örnek miktarı (g)

**Metil Esterlerinin Elde Edilmesi**

Yağ asitlerinin GC-FID ile tanımlanabilmeleri için, serbest yağ asidi hallerinden uçucu türevleri haline getirilmeleri gerekmektedir. Bu nedenle yağ ekstraktları transesterifikasyon işlemi ile metil esterlerine çevrilmiştir. Türevlendirme işlemi için, 100 mg yağ ekstraktı 10 ml hekzanda tamamen çözünene kadar oda sıcaklığında vortekslenmiştir. Sonra çözeltinin üzerine 0,5 ml 2 N KOH (ve MeOH) ilave edilerek ve 30 sn boyunca vorteks cihazı ile karıştırılmıştır. Üst faz berraklaşana kadar karanlıkta 1-2 saat bekletilmiştir. Bekleme sonrasında oluşan ikili fazdan; üst faz (hekzan) ayrılarak GC vialine aktarılmıştır (Paquot, 2013).

**GC-FID Analizi**

Yağ asitlerinin uçucu türevlerinin analizi, SHIMADZU 2010 Gaz Kromatografisi (GC) cihazı ile gerçekleştirilmiştir. Analiz için gaz kromatografisi cihazı alev iyonizasyon detektörü (FID) ile birlikte kullanılmıştır. Yağ asitlerinin analizinde, TR-CN100 (0,25mmx100mx0,2mm) kapiler kolon kullanılmıştır. İnlet sıcaklığı 250°C'ye ayarlanmıştır. Taşıyıcı gaz olarak Helyum kullanılarak, akış hızı (He) 30 ml/dk olarak belirlenmiştir. Fırın sıcaklık programı 100°C'den başlayarak 240°C'ye 3°C/dk hızla çıkarılmış, 10 dk 240°C'de bekletilmek üzere toplam 60 dk olarak uygulanmıştır (Paquot, 2013).

**Toplam Fenolik Madde tayini**

Toplam fenolik madde miktarı tek hücre yağının %80 metanol ile ekstrakte edilmesinin ardından bir spektrofotometrede (Shimadzu 02910, Japonya) Folin-Ciocalteu reaksiyonu kullanılarak değerlendirilmiştir. Kısaca, örneğin 100 µL'sine 500 µL Folin-Ciocalteu reaktifi eklenmiş, 2 dakika sonra 1 mL 75 g $L^{-1}$ $Na_2CO_3$ ve ardından 9,4 mL distile su eklenmiştir. 60 dakikalık karanlık inkübasyonun ardından 760 nm'de absorbans ölçülmüştür. Standart eğri için gallik asit kullanıldı ve sonuçlar mg galaktik asit eşdeğerleri (GAE)/g olarak verilmiştir (Cemeroğlu, 2007).

**Toplam Antioksidan Aktivite Tayini**

Antioksidan aktivite, 2,2'-azino-bis-3-etilbenzotiazolin-6-sülfonik asit (ABTS) ve 1,1-difenil-2-pikrilhidrazil (DPPH) reaktifleri kullanılarak değerlendirilmiştir. Numunenin metanol ekstraktı, 600 µL DPPH metanolik solüsyonuna (1 mM) ilave edilmiştir. Karışım karanlıkta 30 dakika inkübe edilmiş ve 517 nm'de absorbans ölçülmüştür. Radikal süpürme aktivitesi, IC50 (ug/mL) olarak ifade edilmiştir.

ABTS testi, stok ABTS için, PBS içindeki 7 mmol$L^{-1}$ ABTS, 2.45 mmol $L^{-1}$ potasyum persülfat çözeltisi ($K_2S_2O_8$) ile reaksiyona sokulmuştur. Daha sonra karanlıkta, oda sıcaklığında 15-16 saat bekletilmiştir. Stok solüsyonu 0.700±0.05'lik bir nihai absorbansa seyreltmek için PBS kullanılmıştır. Trolox eşdeğeri antioksidan kapasite (TEAC) değerlerini hesaplamak için bir Trolox kalibrasyon eğrisi kullanılmıştır.

**Antimikrobiyal Aktivite Tayini**

Tek hücre yağının patojenik test bakterileri üzerindeki inhibe edici etkisi, bir Agar Well Difusion Assay (AWDA) kullanılarak belirlenmiştir. Mueller-Hinton agar, patojenik bakterileri 18 saat boyunca aktifleştirmek için kullanılmıştır. 0,5 McFarland standardı (1,5 x $10^6$ cfu $mL^{-1}$) hücre konsantrasyonu, inkübasyonun ardından kültür ortamının seyreltilmesiyle elde edilmiştir. 18 saat boyunca üreyen test patojenik bakteri kültürleri, antibakteriyel aktivite için seçilmiş ve MHA besiyerine yayılmıştır. Ardından THY 20µL olarak steril diskler üzerine edilmiş ve petriler 35°C'de 48 saat inkübe edilmiştir. Ortaya çıkan zone çağları, bir kumpas kullanılarak mm cinsinden ölçülmüştür (Kittisakulnam ve ark., 2017).

## BULGULAR VE TARTIŞMA

Lipit üretim ortamında 72 saatlik inkübasyonun ardından, toplanan maya biyokütlesi 12,28 g/L ve tek hücre yağ içeriği kuru hücre ağırlığının % 12,06 olarak tespit edilmiştir. Ekstre edilen lipitlerdeki yağ asitleri transmetile edildikten ve GC ile analiz edildikten sonra, Tablo 1'deki sonuçlar, *R. mucilaginosa* BT59 maya türünden elde edilen yağ asitlerinin %85,8'inden fazlasının $C_{16:0}$ ve $C_{18:2}$, özellikle $C_{18:1}$ olduğunu göstermektedir (% 60,05). Bu, *R. mucilaginosa* BT59'dan elde edilen lipidlerin, biyodizel üretimi için iyi bir yağ hammaddesi olduğu anlamına gelmektedir. *R. mucilaginosa* BT59'dan elde edilen lipidler ayrıca esas

olarak 16 ve 18 karbon atomlu uzun zincirli yağ asitleri içermektedir. Yapılan bir çalışmada yağlı maya *C. curvatus'tan* elde edilen lipitlerin yağ asitleri esas olarak oleik ($C_{18:1}$), palmitik ($C_{16:0}$) ve stearik ($C_{18:0}$) olduğu belirtilmiştir (Hassan ve ark.,1996). Bu, *R. mucilaginosa* BT59'un yağ asidi bileşiminin diğer yağlı mayalarınkine benzer olduğunu göstermektedir. Bunun yanısıra mikrobiyal yağlar, yağların yağ asidi bileşimlerine bağlı olarak çeşitli uygulamalarda kullanılabildiği yapılan çalışmalar ile ortaya konulmuştur. Triaçilgliseroller bakımından zengin mikrobiyal yağlar, biyodizel veya kakao yağı ikamelerinin üretimi için de kullanılabilmektedir (Ahmad ve ark. 2019). Bu çalışma sonucunda da mikrobiyal yağların TAG'lerinin, biyodizel üretiminde kullanılan bitkisel yağların TAG'leriyle karşılaştırıldığında benzerlik görülmüştür.

Zengin antioksidan özelliklere sahip gıdaların rolü, kardiyovasküler ve kanser bozukluklarına karşı sağlığa yararlı etkileri olduğu için insanların ilgisini çekmektedir (Kamiloğlu ve ark., 2016). Yapılan bu çalışmada *Rhodotorula mucilaginosa* (BT59) izolatından ekstrakte edilen tek hücre yağının fenolik bileşik içeriği 1523±16,26 GAE $kg^{-1}$, antioksidan kapasitesi [1,1-diphenyl-2-picrylhydrazyl (DPPH) ve Trolox eşdeğeri antioksidan kapasitesi sırasıyla 116,17±2,82 mg $L^{-1}$ ve 12,11±0,17 µmol Trolox $kg^{-1}$ bulunmuştur. Sonuçlar, THY'nın güçlü bir antioksidan aktivite sergilediğini (Tablo 2) göstermiştir. Bu bağlamda THY, gıda formülasyonlarında fonksiyonel bir gıda bileşeni olarak kullanılabilme potansiyeli taşımaktadır.

Bitkisel yağların bozunma derecelerinin tespit edilmesi amacıyla UV-Vısıble spektrofotometrede 232 ve 270 nm‟deki absorbans ölçümleri yapılmaktadır (Arslan, 2009). Elde ettiğimiz tek hücre yağının başlangıç fazında $K_{232}$ ve $K_{270}$ nm'deki absorbans ölçümleri yapılmış olup sonuçlar Tablo 2'de görüldüğü gibi sırasıyla 0,17 ve 0,18 ppb bulunmuştur. Bitkisel ve hayvansal yağlarda özellikle kızartma ortamında zayıf nükleofil olan su moleküllerinin, triaçilgliserollerin ester zincirlerine atak yapması sonucu oluşan serbest yağ asitlerinin miktarının tayini yapılması elde edilen yağlar için kalite parametresidir (Arslan, 2009). Bu çalışmada da bitkisel ve hayvansal yağlara altarnatif olarak değer görmeye başlayan elde ettiğimiz mikrobiyal yağın serbest yağ asitliği ve peroksit değerleri Tablo 2'de gösterildiği gibi sırasıyla %0,33±0,02 ve 10,53±3,05 meq $O_2$/kg'dır.

Tablo 3'te elde edilen tek hücre yağının antibakteriyel özellikleri incelendiğinde THY'nın analiz edilen patojenler içerisinde yalnızca *Salmonella Enteritidis'*e karşı etkili olduğu tespit edilmiştir.

**Tablo 1.** *Rhodotorula mucilaginosa* (BT59) izolatından ekstrakte edilen tek hücre yağının yağ asitleri kompozisyonu

| Yağ asidi çeşidi | Miktar (%) |
|---|---|
| C16:0 | 16,75 |
| C18:0 | 4,52 |
| C18:1 | 60,05 |
| C18:2 | 15,68 |
| C18:3 | 2,96 |

**Tablo 2.** *Rhodotorula mucilaginosa* (BT59) izolatından ekstrakte edilen tek hücre yağının karakterizasyonu

| Yapılan analizler | Tek hücre yağı |
|---|---|
| Serbest yağ asitliği (%) | 0,33±0,02 |
| Peroksit değeri (meq $O_2$/kg) | 10,53±3,05 |
| Dien ve trien değerleri ($K_{232}$ ve $K_{270}$ sırasıyla) | 0,17-0,18±0,01 |
| Fenolik bileşikler (GAE $kg^{-1}$) | 1523±16,26±32,86 |
| Antioksidan (DPPH) (mg $L^{-1}$) | 116,17±2,82±10,34 |
| Antioksidan (µmol Trolox $kg^{-1}$) | 12,11±0,17±1,89 |

**Tablo 3.** *Rhodotorula mucilaginosa* (BT59) izolatından ekstrakte edilen tek hücre yağının antibakteriyal ektisi

| Patojen bakteriler | Zone çapı (mm) |
|---|---|
| *Escherichia coli* O157:H7 ATCC 33150 | *T.e |
| *Staphylococcus aureus* ATCC 25923 | *T.e |
| *Listeria monocytogenes* ATCC 7644 | *T.e |
| *Salmonella Enteritidis* ATCC 13076 | 8,09±0.03 |

*T.e: tespit edilemedi

## SONUÇLAR

*Rhodotorula mucilaginosa* (BT59) izolatı ile üretilen tek hücre yağı verimi fermentasyon koşullarının optimize edilmesi ile yüksek seviyelere çekilebilir. Üretilen tek hücre yağının önemli bir esansiyel yağ asidi kaynağı olduğu ortaya konmuştur. Aynı zamanda yüksek fenolik içeriği ve antioksidan aktvitesi ile fonksiyonel bir yağ

üretilmiştir. Mikrobiyal yağ üretiminin maliyeti şu anda bitkisel yağdan daha yüksektir, ancak mikrobiyal yağ üretim süreçlerinin tekno-ekonomisini büyük ölçüde iyileştirmek için birçok yöntem vardır. Şimdiye kadar, tek hücreli yağ üretimi için en yaygın kullanılan karbon kaynağı glukozdur. Bu nedenle tek hücreli yağ üretiminde maliyetin düşürülmesi için glukoz yerine düşük maliyetli bir hammadde kullanılması ile farklı özellikle ve düşük maliyette yağ üretimi sağlanabilir. Sonuç olarak, mevcut mikroorganizmaların çeşitliliği, metabolik aşamalarının takibi, düşük maliyetli substratların kullanılması ve reaksiyonun yan ürünlerinin geri dönüşüm olasılığı da dahil olmak üzere süreç hakkında sağlam bir bilgi birikimi, mikroorganizmaların geliştirilmesi için üzerinde durup takip edilmesi gereken önemli parametrelerdir.

## KAYNAKÇA

Koutinas A. A., Chatzifragkou A., Kopsahelis N., Papanikolaou S., Kookos I. K. 2014. Design and techno-economic evaluation of microbial oil production as a renewable resource for biodiesel and oleochemical production. Fuel, 116, 566-577. 2014.

Alakraa, F. 2014. Rhodosporidium Toruloidesy 27012 Mayasından Karotenoid ve Tek Hücre Yağı Üretimi ve Optimizasyonu (Doctoral dissertation, Fen Bilimleri Enstitüsü).

Arslan, F. 2009 Ülkemizde üretilen pamuk yağlarinin rafinasyonun iyileştirilmesiyle kullanim verimliliklerinin arttırılması (Yüksek Lisans Tezi), Selçuk Üniversitesi Fen Bilimleri Enstitüsü Konya.

Cemeroğlu, B. 2007. Gıda analizleri. *Gıda Teknolojisi Derneği Yayınları*, *34*, 168-171.

C. Ratledge, 2010 "Single cell oils for the 21st century." *Single cell oils*. AOCS Press, 3-26.

Ahmad F. B., Zhang Z., Doherty W. O., O'Hara I. M. 2019 The prospect of microbial oil production and applications from oil palm biomass. Biochemical Engineering Journal, 143, 9-23.

Folch, J., Lees, M., Sloane Stanley, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J biol Chem, 226(1), 497-509.

Garzón, G. A., Wrolstad, R. E. 2009. Major anthocyanins and antioxidant activity of Nasturtium flowers (Tropaeolum majus). *Food Chemistry*, *114*(1), 44-49.

Hassan, M., Blanc, P. J., Granger, L. M., Pareilleux, A., Goma, G. 1996. Influence of nitrogen and iron limitations on lipid production by Cryptococcus curvatus grown in batch and fed-batch culture. *Process Biochemistry*, *31*(4), 355-361.

Kamiloglu, S., Toydemir, G., Boyacioglu, D., Beekwilder, J., Hall, R. D., & Capanoglu, E. 2016. A review on the effect of drying on antioxidant potential of fruits and vegetables. Critical reviews in food science and nutrition, 56(sup1), S110-S129. Kittisakulnam, S., D. Saetae, W. Suntornsuk. 2017. Antioxidant and antibacterial activities of spices traditionally used in fermented meat products. J. Food Process. Preserv. 41 (4):e13004.

Liu, H., Zhao, X., Wang, F., Li, Y., Jiang, X., Ye, M., Zou, H. 2009. Comparative proteomic analysis of Rhodosporidium toruloides during lipid accumulation. Yeast, 26(10), 553-566.

Li, Y., Zhao, Z. K., Bai, F. 2007. High-density cultivation of oleaginous yeast Rhodosporidium toruloides Y4 in fed-batch culture. *Enzyme and microbial technology*, *41*(3), 312-317.

Ward O. P., Singh A., 2005. Omega-3/6 fatty acids: alternative sources of production. *Process biochemistry*, *40*(12), 3627-3652.

Ochsenreither K., Glück C., Stressler T., Fischer, L., Syldatk, C. 2016. Production strategies and applications of microbial single cell oils. *Frontiers in microbiology*, *7*, 1539.

Paquot, C. 2013. Standard methods for the analysis of oils, fats and derivatives. Elsevier.

Bellou, S., Triantaphyllidou, I. E., Aggeli, D., Elazzazy, A. M., Baeshen, M. N., Aggelis, G. 2016. Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. *Current opinion in Biotechnology*, *37*, 24-35.

Sharma, R., Ghoshal, G. 2020. Optimization of carotenoids production by Rhodotorula mucilaginosa (MTCC-1403) using agro-industrial waste in bioreactor: A statistical approach. *Biotechnology Reports*, *25*, e00407.

Fakas, S., Papanikolaou, S., Galiotou-Panayotou, M., Komaitis, M., Aggelis, G. 2009. Biochemistry and biotechnology of single cell oil. *New horizons in Biotechnology*, 38-60.

Denli Y., Tekin A 2000 Oil Production and Microorganisms (Turkish with English Abstract). *Gıda Dergisi; Cilt: 25 Sayı: 4*.

Wu, S., Zhao, X., Shen, H., Wang, Q.and Zhao, Z. K. 2011. Microbial lipid production by Rhodosporidium toruloides under sulfate-limited conditions. Bioresource technology, 102(2), 1803-1807.

## *ORAL PRESENTATION*

# Investigation of Rapamycin on PON1 enzyme levels in colon cancer cell line

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## Abstract

PON1 is a calcium-dependent ester hydrolase and is involved in the metabolism of biologically active lipids. PON1 enzyme activity is associated with various types of cancer and decreases in patients with colorectal cancer (CRC). One of the important oncogenic signaling pathways, PI3K/Akt/mTOR plays a key role in the development of CRC. Rapamycin (RAPA), an inhibitor of mTOR signaling pathway, is known to act an active role in preventing the growth and development of CRC cells. The increased amount of reactive oxygen species (ROS) seen in CRC cells provides the activation of cancer signaling pathways, giving cancer cells a survival advantage and also activates the antioxidant system. ROS plays a role in the development of lipid peroxidation related to PON1. In this study our aim is to observe RAPA's effects on PON1 enzyme levels. In our study, WST-1 cytotoxicity test was performed to find the appropriate dose of Rapamycin, which is a PI3K/Akt/mTOR inhibitor, for the CRC cell line HT-29, and the effective dose for the cells was determined as 46.97 µM and applied to the HT-29 cell line. After inhibitor application, PON1 enzyme levels were measured at 340 nm by using Epoch™ Microplate Spectrophotometer. According to our results, PON1 activity was increased when Rapamycin was applied to CRC cells ($p \leq 0.05$). As a result of this study, when using RAPA, the increased in the PON1 enzyme activity may have an important factor in the activating of antioxidant system.

**Keywords:** Colon cancer, mTOR, Paraoxonase1, Rapamycin

## INTRODUCTION

Paraoxonase (PON1) is a calcium dependent ester hydrolase that scavenges free radicals. It counteracts lipid peroxidation caused by free radicals and plays a role in lipid metabolism (Sehitogulları et.al., 2014), Also, it contributes to the development of cancer by playing a role in the balance between antioxidants and oxidants (Fang et.al., 2009; Khandrika et.al., 2009). Besides, PON1 activity changes significantly in various cancer types (Medina-Díaz, et.al., 2022). Oncogenic signal transduction pathways that regulate cell growth and differentiation, apoptosis and angiogenesis modulates PON1 (Bacchetti et.al., 2019). The phosphatidylinositol-3-kinase (PI3K)/Protein Kinase B/ The mammalian target of rapamycin (PI3K/AKT/mTOR) pathway, plays a critical role in the regulation of many essential cell processes, including protein synthesis, cell growth, metabolism, survival, catabolism and autophagy (Popova et.al., 2021). mTOR is a protein kinase involved in the PI3K/Akt pathway. It is the main regulator of cell proliferation and has a critical role in the development of cancer (Hua et.al., 2019; Chiarini et.al., 2015; Saxton et.al., 2017) One of the the most well-known of mTOR inhibitors is rapamycin (RAPA) that shows its effect by binding to the catalytic region of mTOR. For this reason, rapamycin and its analogues have become important tools in the treatment of cancer (Ballouet et.al., 2008) Since PON1 is modulated by cancer signaling pathways, it can be thought inhibitors of this pathway may affect enzyme levels. Considering the relationship between PON1 enzyme and signaling pathways, we aimed to investigate the effects of mTOR inhibition with RAPA on PON1 enzyme in colorectal cancer cell HT29.

## MATERIALS AND METHODS

### Cell Culture

In this study, we used HT29 colorectal cancer cell line due to PON1 is upregulated colorectal cancer (Afsar et.al., 2015). Human colorectal cancer cells HT29 were obtained from ATCC (HTB-38TM) and cultured in DMEM High Glucose Medium (Biowest, L0102) supplemented with 10% heat-inactivated FBS (Capricorn, FBS-HI-11A) at 37°C in a 5% CO2 humidified atmosphere. RAPA was purchased from Cayman Chemical, and prepared as stock solutions by dissolving them in DMSO at a concentration of 300 µM. Then HT29 cells were plated into 6-well plate (0.3 × 106 cells/well) and treated with different concentrations of RAPA (0, 2.5, 5, 10, 25, 50, 100, 200) for 24h and 48h.

### Cell viability assay (WST-1) and $IC_{50}$ measurement

WST-1 assay was performed to determine the appropriate dose of RAPA on the HT-29 cells. Firstly, HT29 cells were seeded into 96-well plates and incubated. After incubation, RAPA was prepared at concentrations of 0, 2.5, 5, 10, 25, 50, 100, 200 µM and was applied to the cells, respectively. The measurement was performed at 24h and 48h. Then, 10 µL of WST-1 solution (Cayman Chemical, 10008883) was added to each well and incubated at 37°C for 2-4 hours. After incubation, the appropriate inhibitors' concentration for the cells were determined by measuring at 450 nm on a Epoch Microplate Reader (Winooski, VT, USA). The inhibitor doses ($IC_{50}$), which inhibited 50% of the cells were calculated with Graphpad Prism 9.1.0 programme.

### Cell Lysate Preparation

To prepare the cell lysate, HT-29 colorectal cells were washed with phosphate buffered saline (PBS) and then the cells were lysed with lysis buffer containing 50 mM Tris, 150 µM NaCl, 1% NP-40 (Intron Biotechnology, IBS-BN015) and proteinase inhibitor factor (Intron Biotechnology, PIC001). After lysis, the cells were centrifuged (+4ºC, 17000 g, 15 min). Then, the supernatant was aliquoted and washed twice with PBS.

### Measurement of PON1 activity

PON1 activity towards paraoxon as a substrate was quantified spectrophotometrically by the method described by Gan et. al. (1991). The reaction was followed for 2 min. at 37 °C by monitoring the appearance of p-nitrophenol at 412 nm on a Biotek (Winooski, VT, USA). The final substrate concentration during enzyme assay was 2 mM, and all rates were determined in triplicate and corrected for the non-enzymatic hydrolysis. A molar extinction coefficient ($\epsilon$) of 17.100 $M^{-1}$ $cm^{-1}$ for p-nitrophenol at pH 8.0 in 100 mMTris–base buffer was used for the calculation. One unit of PON1 activity is defined as 1 µmol of p-nitrophenol formed per minute under the above assay conditions (Arslan et.al. 2012). Enzyme units were calculated from the absorbance change **(**Bilen et.al., 2013).

### Statistical analysis

Statistical analysis were performed in GraphPad Prism 9.1.0 program. All experiments were repeated three times. Student's t-test was performed PON enzyme activity. Statistical significance level was accepted as $p \leq 0.05$.

## RESULTS

### Cell viability assay

RAPA was applied to HT-29 cells at doses of 0, 2.5, 5, 10, 25, 50, 100 and 200 µM at the 24th and 48th hours and the cytotoxic dose for the cells was determined. According to the WST-1 results, the viability % at 0, 2.5, 5, 10, 25, 50, 100 and 200 µM concentrations for HT-29 cells treated with RAPA for 24 h at concentrations of 0, 2.5, 5, 10, 25, 50, 100, and 200 µM were 100%, 100%, 99%, 92%, 73%, 39%, 28%, , 20%, respectively. The viability % at 0, 2.5, 5, 10, 25, 50, 100 and 200 µM concentrations for HT-29 cells treated with RAPA for 48h hours at concentrations of 0, 2.5, 5, 10, 25, 50, 100, and 200 µM were 100%, 66%, 66%, 59%, 48%, 27%, 19%, 13%, respectively The $IC_{50}$ value for RAPA is found to be 46.97 µM at 24h (Figure 1).

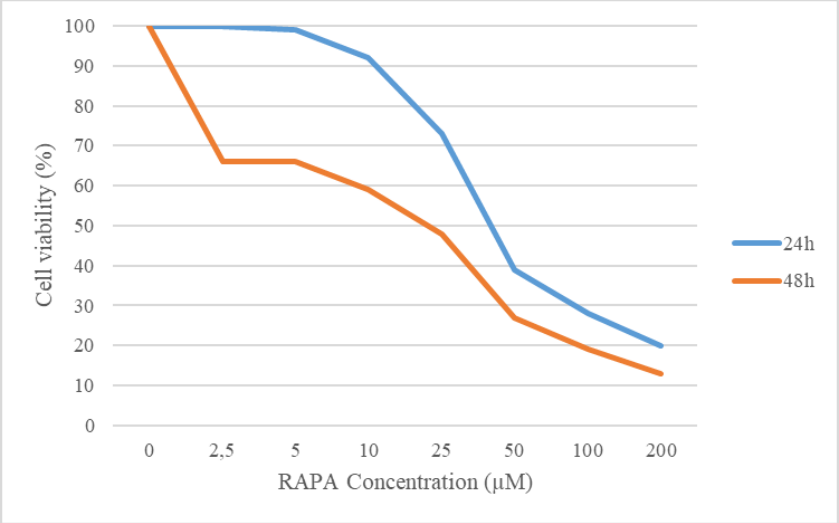


**Figure 1.** % cell viability of the HT29 cells treated with RAPA at 24h and 48h.

### Measurement of PON1 Activity

To determine the effect of RAPA on PON1 in HT-29 cells, enzyme activities were measured spectrophotometrically. PON enzyme levels were increased significantly in HT-29 cells treated with RAPA, compared to the non-treated group ($p \leq 0.05$) (Figure 2).

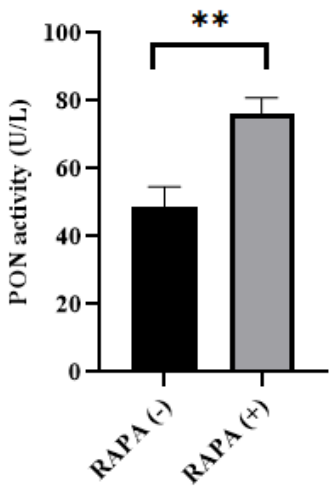


**Figure 2.** PON1 enzyme levels in HT29 cells untreated group and treated with RAPA.

## DISCUSSION

PON1 plays a crucial role in cancer. However, there is limited information about the relation between PON1 and oncogenic signaling pathways. This currrent study investigated the effect of RAPA, a well known inhibitor for mTOR, on PON1 enzyme levels in HT29 colorectal cancer cells. Alim et. al. (2016) examined effects of some anticancer agents on PON1 enzyme levels and the group found that these agents reduce PON enzyme activity. Thompson et.al. (2022) found that doxorubucin (a chemotherapeutic agent) increases PON1 activity in breast cancer. RAPA is a anticancer agent and inhibits mTOR activity. In this study we found higher PON1 enzyme levels in HT29 cells treated with RAPA.

## CONCLUSION

This present study was designed to determine the effect of RAPA on PON1 enzyme levels in colorectal cancer cells. Our study has shown that,when using mTOR inhibitor, RAPA, PON1 enzyme levels increased significantly. The findings suggest that ongogenic pathways are related with PON1 enzyme and inhibitors of these pathways could be good candidates for PON1 activation. PON1 may be a prominent player fighting against colorectal cancer.

## ACKNOWLEDGEMENTS

There is no conflict of interest

## REFERENCES

Alim Z, Beydemir Ş. 2016. Some Anticancer Agents Act on Human Serum Paraoxonase-1 to Reduce Its Activity. Chem Biol Drug Des.,88(2):188-96.

Afsar CU, Gunaldı M, Okuturlar Y, Gedikbası A, Tiken EE, Kahraman S, Karaca F, Ercolak V, Karabulut M. 2015. Paraoxonase-1 and arylesterase activities in patients with colorectal cancer. Int J Clin Exp Med., 8(11):21599-604.

Arslan M, Gencer N, Arslan O, Guler OO. 2012. In vitro efficacy of some cattle drugs on bovine serum paraoxonase 1 (PON1) activity. J Enzyme Inhib Med Chem.,27(5):722-9.

Bacchetti T, Ferretti G, Sahebkar A. 2019. The role of paraoxonase in cancer. In Seminars in câncer biology, Academic Press.,72-86.

Ballou LM, Lin RZ. 2008. Rapamycin and mTOR kinase inhibitors. J Chem Biol.,1(1-4):27-36.

Bilen Ç, Beyaztaş S, Arslan O, Güler ÖÖ. 2013. Investigation of heavy metal effects on immobilized paraoxanase by glutaraldehyde. J Enzyme Inhib Med Chem.,28(3):440-6.

Chiarini F, Evangelisti C, McCubrey JA, Martelli AM. 2015. Current treatment strategies for inhibiting mTOR in cancer. Trends Pharmacol Sci,36(2):124-35.

Fang J, Seki T, Maeda H. 2009.Therapeutic strategies by modulating oxygen stress in cancer and inflammation. Adv Drug Deliv Rev.,61(4):290-302.

Gan KN, Smolen A, Eckerson HW, La Du BN. 1991.Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. Drug Metab Dispos.,19(1):100-6.

Hua H, Kong Q, Zhang H, Wang J, Luo T, Jiang Y. 2019. Targeting mTOR for cancer therapy. J Hematol Oncol.,12(1):71.

Khandrika L, Kumar B, Koul S, Maroni P, Koul HK. 2009. Oxidative stress in prostate cancer. Cancer Lett, 282(2):125-36.

Medina-Díaz IM, Ponce-Ruíz N, Rojas-García AE, Zambrano-Zargoza JF, Bernal-Hernández YY, González-Arias CA, Barrón-Vivanco BS, Herrera-Moreno JF. 2022. The Relationship between Cancer and Paraoxonase 1. Antioxidants (Basel),11(4):697.

Popova NV, Jücker M. 2021.The Role of mTOR Signaling as a Therapeutic Target in Cancer. Int J Mol Sci.,22(4):1743.

Saxton RA, Sabatini DM. 2017. mTOR Signaling in Growth, Metabolism, and Disease.Cell,169(2):361-371.

Sehitogulları A, Aslan M, Sayır F, Kahraman A, Demir H. 2014. Serum paraoxonase-1 enzyme activities and oxidative stress levels in patients with esophageal squamous cell carcinoma. Redox Rep.,19(5):199-205.

Thompson EW, Demissei BG, Smith AM, Brahmbhatt P, Wang J, Clark A, DeMichele A, Narayan V, Shah P, Sun L, Lefebvre B, Fradley MG, Carver JR, Tang WHW, Ky B. 2021. Paraoxonase-1 Activity in Breast Cancer Patients Treated With Doxorubicin With or Without Trastuzumab. JACC Basic Transl Sci.,7(1):1-10.

## *ORAL PRESENTATION*

# Organik tarım teknikleri ile yetiştirilmiş *Mentha piper*ita ve *Origanum onites*'den hazırlanan kombinasyonların antidiyabetik potansiyelinin değerlendirilmesi

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**Özet**

*Mentha piperita* L. ve *Origanum onites* L. geleneksel olarak çeşitli hastalıkların tedavisinde kullanılan Lamiaceae familyasına ait türlerdir. Fas'ta *M. piperita*'nın, Türkiye'de ise *O. onites*'den elde edilen uçucu yağın geleneksel olarak diyabet tedavisinde kullanıldığı bildirilmektedir. Daha önceki çalışmalarımızda organik tarım ile yetiştirilmiş *M. piperita* ve *O. onites* türlerinin toprak üstü kısımlarından infüzyon tekniği ile hazırlanan ekstrelerin güçlü antidiyabetik etki potansiyeline sahip oldukları *in vitro* enzim sistemleri kullanılarak tespit edilmiştir. Bu sebeple bu iki türü ihtiva eden farklı tipte ekstre kombinasyonları hazırlanmıştır. Organik tarım yöntemi ile yetiştirilmiş *M. piperita* ve *O. onites* türlerini ihtiva eden bu karışımların (Kombinasyon A ve kombinasyon B) $\alpha$-glukozidaz ve $\alpha$-amilaz enzimleri üzerindeki inhibisyonları değerlendirilmiştir. Yapılan çalışma sonucunda 2 mg/ml konsantrasyonda α-glukozidaz enzimini, kombinasyon A %73.33 ± 0.63; kombinasyon B ise %59.23 ± 1.51 değerinde bir oranla inhibe ettiği tespit edilmiştir. Aynı konsantrasyonda $\alpha$-amilaz enzimini, kombinasyon A %30.02 ± 0.53 kombinasyon B ise %31.72 ± 1.54 oranında inhibe etmiştir. Tüm sonuçlar göz önüne alındığında $\alpha$-amilaz enzimi için 2 mg/ml konsantrasyonda, ekstreleri tek başları yerine iki farklı kombinasyonda kullanmanın daha etkili olacağı sonucuna varılmıştır. Ayrıca *M. piperita* ekstresinin (%91.43 ± 0.90) tek başına $\alpha$-glukosidaz enzimi üzerinde daha etkili olduğu, çünkü her iki kombinasyonun da tüm konsantrasyonlarda $\alpha$-glukosidaz enzimi üzerinde zayıf inhibitör etkiye sahip olduğu sonucuna varıldı.
**Anahtar Kelimeler:** Enzim inhibisyonu, $\alpha$-glukozidaz, $\alpha$-amilaz, organik tarım, *Mentha piperita*, *Origanum onites*

# Evaluation of the antidiabetic potential of combinations prepared from *Mentha piperita* and *Origanum onites* grown with organic farming techniques

**Abstract**

*Mentha piperita* L. and *Origanum onites* L. are species belonging to the Lamiaceae family traditionally used in the treatment of various diseases. It is reported that the essential oil obtained from *M. piperita* in Morocco and *O. onites* in Turkey is traditionally used in the treatment of diabetes. In our previous studies, it was determined by using *in vitro* enzyme systems that the extracts prepared by infusion technique from the aerial parts of *M. piperita* and *O. onites* species grown with organic farming have strong antidiabetic effect potential. For this reason, different types of extract combinations containing these two species have been prepared. The inhibition of these mixtures (Combination A and combination B) containing *M. piperita* and *O. onites* species grown by organic farming method on $\alpha$-glucosidase and $\alpha$-amylase enzymes were evaluated. As a result of the study, $\alpha$-glucosidase enzyme at 2 mg/ml concentration, combination A 73.33% ± 0.63%; combination B inhibited with a rate of 59.23 ± 1.51 %. At the same concentration, combination A inhibited the $\alpha$-amylase enzyme by 30.02% ± 0.53 and combination B by 31.72% ± 1.54%. Considering all the results, it was concluded that for the $\alpha$-amylase enzyme, it would be more effective to use the extracts in two different combinations instead of alone at a concentration of 2 mg/ml. It was also concluded that *M. piperita* extract (91.43 ± 0.90%) alone was more effective on $\alpha$-glucosidase enzyme because both combinations had weak inhibitory effect on $\alpha$-glucosidase enzyme at all concentrations.

**Keywords:** Enzyme inhibition, $\alpha$-glucosidase, $\alpha$-amylase, organic farming, *Mentha piperita*, *Origanum onites*

# GİRİŞ

Diyabet, insülin sekresyonundaki ya da insülin etkisindeki veya her ikisindeki kusurlardan kaynaklanan hiperglisemi ile karakterize metabolik bir hastalıktır. Kronik hiperglisemi; başta gözler, böbrekler, sinirler, kardiyovasküler sistem de dahil olmak üzere çeşitli organ ve sistemlerde ciddi hasarlara ve işlev bozukluklarına neden olabilmektedir (American Diabetes Association, 2010). Diyabet tedavisindeki terapötik yaklaşımlardan birisi, tokluk hiperglisemisini azaltmaktır. Bu terapötik yaklaşım, $\alpha$-glukozidaz ve $\alpha$-amilaz gibi sindirim sisteminde karbonhidratı hidrolize edebilen enzimleri inhibe ederek, glukoz emilimini geciktirmek suretiyle yapılabilir (Bhandari ve ark., 2008).

Organik tarım, insan sağlığına ve çevreye zarar vermeyen, üretiminde kimyasal girdi kullanılmadan üretimden tüketime kadar her aşaması kontrollü olarak gerçekleştirilen, sertifikalı tarımsal üretim biçimi olarak tanımlanmaktadır. Aynı zamanda doğal dengeyi koruyarak hava ve su gibi yaşamsal kaynakların ve doğal hayatın korunmasını amaçlayan bir üretim yöntemi olarak ifade edilmektedir. (Tarım ve Orman Bakanlığı, 2016). Tıbbi ve aromatik bitkilerin yetiştirilmesinde organik tarım tekniklerinin kullanımının yaygınlaştırılması önemli bir husus olarak karşımıza çıkmaktadır.

Literatürde çalışmada kullanılan türlere dair tedaviye yönelik geleneksel kullanımlarının olduğu belirtilmektedir. *Mentha piperita* L.'nin, Fas geleneksel tıbbında diyabetin tedavisi için yaygın olarak kullanıldığı ifade edilmiştir. Bununla beraber *Origanum onites* L.'den elde edilen uçucu yağın, Türkiye'de şeker hastalığı da dahil olmak üzere çeşitli hastalıkların tedavisinde kullanılan geleneksel bir halk ilacı olduğu bildirilmektedir (Hamad Al-Mijalli ve ark., 2022; Lermioglu ve ark., 1997). Diyabet üzerinde etkili olan yeni ilaç moleküllerinin araştırılmasında, doğal kaynaklar önemli bir yere sahiptir. Bu hedef doğrultusunda; *M. piperita* ve *O. onites*'in antidiyabetik potansiyeli $\alpha$-amilaz ve $\alpha$-glukozidaz enzimleri üzerindeki inhibisyonlarından yola çıkarak tarafımızca daha önceki çalışmalarımızda değerlendirilmiştir. *M. piperita*'nın $\alpha$-glukozidaz enzimi üzerinde göstermiş olduğu yüksek inhibisyon ve *O. onites*'in ise hem $\alpha$-amilaz hem de $\alpha$-glukozidaz enzimi üzerinde göstermiş olduğu inhibisyondan hareketle bu türlerin farklı şekillerde hazırlanan kombinasyonlarının antidiyabetik etki potansiyelinin değerlendirilmesi bu çalışmanın temelini oluşturmaktadır. Bu çalışma, organik tarım ile yetiştirilmiş olan *M. piperita* ve *O. onites* türleri kullanılarak farklı tekniklerle hazırlanmış kombinasyonlarının antidiyabetik etki potansiyelinin değerlendirildiği ilk çalışmadır.

# MATERYAL VE METOT

## Bitki Materyali

Organik tarım teknikleriyle yetiştirilen *M. piperita* ve *O. onites* türleri Beyşehir Yolu, 3. km Akyokuş Mevki, Konya'dan Nisan 2021'de temin edilmiştir (Sertifika no: TR-OT-014-İ-197/01, Temmuz Organik Çiftliği). Bu türler Organik Tarım Kanunu ve Türkiye Cumhuriyeti Yönetmeliğine uygun olarak üretilmiş olup, Tarım Bakanlığı tarafından yetkilendirilmiş Nissert tarafından sertifikalandırılmıştır.

## Ekstraksiyon

A kodlu kombinasyonda her birinden 5 gram alınan *M. piperita* ve *O. onites* türlerinin toprak üstü kısımlarının üzerine 200 ml sıcak su eklenerek bu türler beraber ekstraksiyona bırakılmıştır. Daha sonra bu ekstre süzülmüş ve bu işlem 3 kere tekrar edilmiştir. Elde edilen süzüntüler birleştirildikten sonra dondurularak kurutulmuştur. Hazırlanan bu kuru ekstre kombinasyonu üzerinden farklı konsantrasyonda ekstreler hazırlanmıştır.
B kodlu kombinasyonda ise 10 gram *M. piperita ve 10 gram O. onites* türlerinin toprak üstü kısımları üzerine 200 ml sıcak su eklenerek ayrı ayrı ekstraksiyon işlemi yapılmıştır. Daha sonra bu ekstreler süzülmüş ve bu işlem 3 kere tekrar edilmiştir. Elde edilen süzüntüler kendi türleri özelinde birleştirildikten sonra dondurularak kurutulmuştur. Bu işlemlerin ardından bu kombinasyondan hareketle farklı konsantrasyonda ekstreler hazırlanırken %50 oranında *M. piperita* kuru ekstresi ve %50 oranında *O. onites* kuru ekstresi kullanılarak dilüsyon işlemleri gerçekleştirilmiştir.

## Kullanılan Kimyasal Maddeler

Ekstraksiyon aşamasında ve enzim inhibisyonu ile aktivite değerlendirme çalışmalarında kullanılan kimyasal maddeler yüksek saflıkta olup Sigma-Aldrich firmasından temin edilmiştir.

### Enzim Deneyleri

### *α*-Glukozidaz Enzim İnhibisyon Aktivitesi Yöntemi

*α*-Glukozidaz tip IV enzimi fosfat tampon çözeltisi (0.5 M, pH 6.5) ierisinde çözülmüştür. Farklı tekniklerle hazırlanan A ve B kodlu kombinasyonlardan hareketle %80'lik metanol çözeltisi kullanarak farklı konsantrasyonlarda (2, 1 ve 0.5 mg/ml) ekstreler hazırlanmıştır. Enzim çözeltisi ve ekstreler, 37°C'de 15 dakika süreyle 96 kuyucuklu bir mikroplakta ön inkübasyona bırakılmıştır. Bu işlemin ardından kuyucuklara 20 mM *p*-nitrofenil-*α*-D-glukopiranozit çözeltisi (PNG) ilave edilmiştir. Mikroplaktaki karışım, 37 °C'de 35 dakika süreyle inkübe edildikten sonra, PNG'nin *α*-glukozidaz tarafından hidrolizi nedeniyle 405 nm'de absorbsiyondaki artış, bir ELISA mikroplak okuyucusunda ölçülmüştür. Akarboz (Bayer, Türkiye) bu deneyde referans madde olarak kullanılmıştır. Deneyler 3'lü tekrarlar şeklinde yapılmıştır. İnhibisyon yüzdeleri ise belirtilen formül kullanılarak hesaplanmıştır. İnhibisyon (%) = [1-(Absorbans $_{(örnek)}$ /Absorbans $_{(kontrol)}$] × 100 (Orhan ve ark., 2017).

### *α*-Amilaz Enzim İnhibisyon Aktivitesi Yöntemi

*α*-Amilaz tip I-A (EC 3.2.1.1, Sigma) tampon çözeltisi içerisinde çözülmüştür. Fosfat tampon çözeltisi (pH 6.9) içerisinde hazırlanmış patates nişastası (%2.5, a/h) deneyde substrat çözeltisi olarak kullanılmıştır. Farklı tekniklerle hazırlanan A ve B kodlu kombinasyonlardan hareketle %80'lik metanol çözeltisi kullanarak farklı konsantrasyonlarda (2, 1 ve 0.5 mg/ml) ekstreler hazırlanmıştır. Enzim çözeltisi ilave edildikten sonra karışımlar oda sıcaklığında 5 dakika süreyle inkübe edilmiştir ve içerisine substrat çözeltisi ilave edilmiştir. Daha sonra karışımlar 37°C'de 15 dakika inkübasyona bırakılmıştır. Karışımlara 3,5-dinitrosalisilik asit (DNS) renk reaktif çözeltisi (96 mM DNS, 2 M NaOH içinde 5.31 M sodyum potasyum tartarat) ilave edildikten sonra, mikroplak içindeki karışım 80°C'lik etüve konulmuştur. 40 dakika sonra bu karışıma distile su ilave edilmiştir. Karışımların absorbansları ise 540 nm'de ELISA mikroplak okuyucusunda okunmuştur. Deneyde akarboz referans madde olarak kullanılmıştır. Standart maltoz kalibrasyon grafiği hazırlandıktan sonra üretilen maltoz miktarı, standart maltoz kalibrasyon grafiği (y = 1,0295x-0,1242 ve $r^2$ = 0.9923) ve elde edilen net absorbans kullanılarak hesaplanmıştır. Deneyler 3'lü tekrarlar şeklinde yapılmıştır (Orhan ve ark., 2017).

## BULGULAR ve TARTIŞMA

Tarafımızca daha önceden *M. piperita* ve *O. onites* türlerinden hareketle 0.5, 1 ve 2 mg/ml olmak üzere 3 farklı konsantrasyonda hazırlanan ekstrelerin *α*-amilaz ve *α*-glukozidaz enzimi üzerindeki inhibisyon değerleri şu şekilde bulunmuştur. *M. piperita α*-glukozidaz enzimi üzerinde 0.5; 1 ve 2 mg/ml konsantrasyonlarda sırasıyla %47.56 ± 5.01; %76.32 ± 2.62; %91.43 ± 0.90 inhibisyon oranına neden olurken; *α*-amilaz enzimi üzerinde herhangi bir inhibisyon oluşturamamıştır. *O. onites* ise *α*-glukozidaz enzimini 0.5, 1 ve 2 mg/ml konsantrasyonlarda sırasıyla %25.45 ± 4.26; %46.86 ± 4.90; %70.18 ± 2.02 oranlarında inhibe etmiş ancak *α*-amilaz enzimi üzerinde 0.5, 1 ve 2 mg/ml konsantrasyonlarda sırasıyla %18.53 ± 3.39; %24.90 ± 1.40; %21.88 ± 4.65 oranında inhibitör etki olşuturabilmiştir. Yapılan bu deneyde referans madde olan akarboz *α*-glukozidaz enzimini 2 mg/ml konsantrasyonda %99.35 ± 0.22 oranında inhibe ederken; *α*-amilaz enzimini aynı konsantrasyonda %95.24 ± 2.60 oranında inhibe etmiştir.

**Tablo 1.** Kombinasyon A ve B'nin *α*-glukozidaz. *α*-amilaz enzimi üzerindeki inhibisyon sonuçları

| **Kombinasyon** | | ***α*-Glukozidaz (% İnhibisyon ± SS)** | ***α*-Amilaz (% İnhibisyon ± SS)** |
|---|---|---|---|
| **Kombinasyon** | **Konsantrasyonlar (mg/ml)** | | |
| Kombinasyon A | 0.5 | 6.80 ± 4.15$^{*}$ | 12.12 ± 2.47$^{**}$ |
| | 1 | 43.41 ± 2.01$^{***}$ | 27.70 ± 0.92$^{***}$ |
| | 2 | 73.33 ± 0.63$^{***}$ | 30.02 ± 0.53$^{***}$ |
| Kombinasyon B | 0.5 | 12.44 ± 1.08$^{**}$ | 14.88 ± 2.89$^{**}$ |
| | 1 | 37.82 ± 1.47$^{***}$ | 19.60 ± 0.63$^{**}$ |
| | 2 | 59.23 ± 1.51$^{***}$ | 31.72 ± 1.54$^{***}$ |
| Referans (Akarboz) | 0.5 | 99.26 ± 0.20$^{***}$ | 93.60 ± 1.30$^{***}$ |
| | 1 | 99.27 ± 0.20$^{***}$ | 98.10 ± 0.21$^{***}$ |
| | 2 | 99.62 ± 0.19$^{***}$ | 97.17 ± 2.00$^{***}$ |

SS: Standart Sapma, *p<0.05, **p<0.01, ***p<0.001

Farklı tipte hazırlanan kombinasyonların *α*-glukozidaz ve *α*-amilaz enzimi üzerindeki etkileri değerlendirildiğinde *α*-glukozidaz enzimini 1 ve 2 mg/ml konsantrasyonlarda kombinasyon A sırasıyla %43.41

± 2.01 ve % 73.33 ± 0.63 değerleri ile kombinasyon B ise %37.82 ± 1.47 ve %59.23 ± 1.51 değerleri ile inhibe etmiştir. Bu sonuçlar doğrultusunda kombinasyon A'nın daha yüksek etkiye sahip olduğu düşünülmektedir. *α*-Amilaz enzimi üzerinde ise kombinasyonlar arasında belirgin bir farklılık olmayıp yaklaşık olarak benzer değerlerde inhibisyon değerleri elde edilmiştir. Farklı tekniklerle hazırlanan kombinasyonların enzimler üzerindeki inhibisyon değerleri daha önceden sadece tek bir tür üzerinden hazırlanan ekstreler ile mukayese edildiğinde *M. piperita*'dan hareketle hazırlanan ekstrenin *α*-glukozidaz enzimi üzerindeki inhibisyon değeri; farklı şekillerde hazırlanan her iki kombinasyon ile mukayese edildiğinde bu kombinasyonların daha zayıf enzim inhibisyonuna neden olduğu görülmüştür. Ancak *M. piperita* ekstresinin *α*-amilaz enzimini inhibe edemediği görülürken her iki kombinasyonun da *α*-amilaz enzimi üzerinde inhibitör etkilere neden olduğu sonucuna varılmıştır. *O. onites* ekstresi *α*-glukozidaz enzimi kombinasyon A ile benzer inhbisyon oranları ile inhibe ederken; kombinasyon B tek başına hazırlanan ekstreye kıyasla daha zayıf bir inhibitör etki göstermiştir. *O. onites* ekstresinin *α*-amilaz enzimi üzerindeki inhibisyonu kombinasyonlar ile mukayese edildiğinde 2 mg/ml konsantrayonda her iki kombinasyonda da inhibisyon değerinde bir artış olduğu tespit edilmiştir.

Tüm bu sonuçlar göz önüne alındığında *α*-glukozidaz enzimi inhibisyonu odaklı bir çalışma yürütüldüğünde *M. piperita* ekstresinin daha etkili olduğu ancak *O. onites* ile bir kombinasyon yapılacak ise bunun kombinasyon A şeklinde olması gerektiği sonucuna varılmıştır. Bununla beraber *α*-amilaz enzimi inhibisyonu odaklı bir çalşma yürültüğünde ise özellikle *M. piperita* ekstresi hazırlamak yerine *O. onites* ile beraber kombinasyon şeklinde bir ekstre hazırlamanın daha etkili olacağı öngörülmüştür.

*M. piperita* ve *O. onites* türlerinden ayrı ayrı hazırlamış olduğumuz ekstrelerin *α*-glukozidaz enzimine karşı göstermiş olduğu güçlü inhibitör etkiden ötürü bu iki türden farklı tipte kombinasyonlar oluşturulmuştur. Gholamhoseinian ve arkadaşları (2008) tarafından gerçekleştirilen çalışmada *M. piperita*'nın yaprak sulu ekstresinin 5 mg/ml konsantrasyonda *α*-glukozidaz enzimini %90 ± 2 oranında inhibe ettiği ve Ekin ve arkadaşları (2019) tarafından gerçekleştirilen çalışmada *O. onites*'in toprak üstü kısmının %80'lik etanol ekstresinin *α*-glukozidaz enzimini 2 mg/ml konsantrasyonda %77.39 ± 0.76 ve aynı konsantrasyonda *α*-amilaz enzimini % 3.16 ± 0.99 oranında inhibe ettiği bildirilmiştir. Bizim çalışmamızın sonuçları da daha önceki yapılmış çalışmalarını sonuçlarını destekler niteliktedir (Gholamhoseinian ve ark., 2008; Ekin ve ark., 2019). Yapılan araştırmalar sonucunda literatürde bu iki türden hareketle hazırlanmış bir kombinasyonun antidiyabetik etkinliğinin incelendiğine dair bir çalışmaya rastlanamamıştır. Bu da çalışmanın orijinalliğini ortaya çıkarmaktadır.

## SONUÇ

Tüm bu sonuçlar dahilinde organik tarım yöntemi ile yetiştirilmiş *M. piperita* ve *O. onites* türlerinden hareketle antidiyabetik etki potansiyeli olan bu iki türü ihtiva eden kombinasyon hazırlanmak istendiğinde özellikle *α*-amilaz enzimi için bu yaklaşımın uygun olacağı sonucuna varılmıştır. Benzer şekilde *O. onites* türünün kombinasyon A tekniği ile *M. piperita* ile olan kombinasyonunda *α*-glukozidaz enziminin inhibisyonunda az da olsa bir artış olduğunu göstermiştir. Tüm bunların yanında *M. piperita* ekstresinin *α*-glukozidaz enzimini kombinasyonlardan daha güçlü bir şekilde inhibe edeceği ortaya çıkmıştır.

## TEŞEKKÜR

Çalışma TÜBİTAK 4004 'Doğadan İlham Aldım Hammaddemi Kendim Yaptım.' Projesi ile desteklenmiştir. Organik tarım ile yetiştirilmiş bitkilerinin teminini sağladığı için Temmuz Organik Çiftliği'ne teşekkür ederiz.

## KAYNAKLAR

American Diabetes Association 2010. Diagnosis and classification of diabetes mellitus. Diabetes Care, 33 Suppl 1(Suppl 1): S62–S69.

Bhandari MR, Jong-Anurakkun N, Hong G, Kawabata J 2008. *α*-Glucosidase and *α*-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*. Haw.). Food Chemistry, 106(1): 247-252.

Gholamhoseinian HA, Falah H, Sharıfıfar F, Mırtaj AS. The inhibitory effect of some Iranian plants extracts on the alpha glucosidase 2008. Iranian Journal of Basic Medical Sciences. 11(1): 1-9.

Ekin H N, Orhan D D, Orhan İ E, Orhan N, Aslan M 2019. Evaluation of enzyme inhibitory and antioxidant activity of some Lamiaceae plants. Journal of Research in Pharmacy, 23(4): 749-758.

Hamad Al-Mijalli S, ELsharkawy ER, Abdallah EM, Hamed M, El Omari N, Mahmud S, Merae Alshahrani M, Naceiri Mrabti H, Bouyahya A 2022. Determination of Volatile Compounds of *Mentha piperita* and Lavandula multifida and Investigation of Their Antibacterial, Antioxidant, and Antidiabetic Properties. Evidence-Based Complementary and Alternative Medicine, 2022.

Lermioglu F, Bagci S, Onderoglu S, Ortac R, Tugrul, L 1997. Evaluation of the long-term effects of oleum origani on the toxicity induced by administration of streptozotocin in rats. The Journal of pharmacy and pharmacology, 49(11): 1157-1161.

Orhan N., Deliorman Orhan D, Gökbulut A, Aslan M, Ergun F 2017. Comparative analysis of chemical profile, antioxidant, *in-vitro* and *in-vivo* antidiabetic activities of *Juniperus foetidissima* Willd. and *Juniperus sabina* L. Iranian Journal of Pharmaceutical Research, 16(Special Issue): 64-74.

Tarım ve Orman Bakanlığı 2016. Organik Tarım Ulusal Eylem Planı 2013-2016, Organik tarım nedir. Erişim linki: https://www.tarimorman.gov.tr/Konular/Bitkisel-Uretim/Organik-Tarim/Genel-Bilgiler. [07.09.2022]

## *ORAL PRESENTATION*

# The phenolic compounds, *in vitro* antioxidant activity, and carbonic anhydrase inhibition of *Vaccinium myrtillus* L.

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**Abstract**

Bilberry (*Vaccinium myrtillus* L.) fruit has various biological activities due to its high compounds. The object of this research was to investigate phenolic compounds, antioxidant activity, total phenolic content (TPC), and inhibitory effect on the carbonic anhydrase (CA) enzyme in naturally grown bilberry of juice and pulp extract in Gümüşhane, Turkey. The phenolic composition of juice and pulp extract by HPLC-DAD was determined. The antioxidant activity was evaluated using the methods of 2,2-diphenyl-1-picrylhydrazil (DPPH•) radical scavenging assay and ferric reducing/antioxidant power (FRAP), while the TPC was evaluated according to the Folin–Ciocalteu method. Also, the inhibition effect of fruit juice on CA was determined by the esterase activity of CA. The main phenolic component in the juice and pulp extract was chlorogenic acid (233.515 mg/L extract and 153.100 mg/L extract, respectively), and various levels of phenolics have been identified, including gallic acid, protocatechuic acid, and rutin. The pulp extract was found the richest in phenolic content (526.19±1.09 GAE, µg/mL) compared to juice and showed the highest DPPH• and FRAP activities ($SC_{50}$: 0.184±0.002 µg/mL, 525.19±2.51 µM TEAC). The CA inhibition potential ($IC_{50}$: 0.0154 mg/mL) of bilberry juice was indicated for the first time in this research. Consequently, it was revealed that bilberry juice and pulp extract have significant antioxidant and CA inhibition potential. It can be used as a natural antioxidant source and enzyme inhibitor for the food and pharmaceutical industry.

**Keywords:** *Vaccinium myrtillus* L., bilberry, phenolic, HPLC-DAD, antioxidant activity, carbonic anhydrase inhibition.

## INTRODUCTION

*Vaccinium myrtillus* L. (bilberry) rich in anthocyanins, one of the excellent phytochemicals in natural products, belongs to the Ericaceae family. Similar in flavor and appearance, *V. myrtillus* is about three times smaller than *V. corymbosum* (blueberry) and is known as the European blueberry or bilberry. Increased consumption of bilberries which are naturally rich in phenolic compounds has been found to be associated with the prevention of cardiovascular diseases, diabetes, diseases caused by aging, obesity, and cancer (Pires et al., 2020). Researchers have documented a high correlation between the high phenolic content of bilberries and their antioxidant activity (Brasanac-Vukanovic et al., 2018; Ciulca et al., 2021; Urbonaviciene et al., 2022).

The main phenolic compounds of bilberries are phenolic acids (gallic, chlorogenic, protocatechuic, caffeic, sinapic, *p*-coumaric, syringic, cinnamic, ellagic, and ferulic acid), flavonoids (quercetin, isoquercetin, rutin, myricetin, naringin, hesperidin, neohesperidin, and kaempferol), flavonols (catechin, epicatechin, and epigallocatechin), stilbene (*trans*-resveratrol), tannins and anthocyanins (cyanidin, delphinidin, peonidin, petunidin, and malvidin) (Može et al., 2011; Antolak et al., 2016; Değirmencioğlu et al., 2017; Brasanac-Vukanovic et al., 2018; Okan et al., 2019; Georgescu et al., 2022). Phenolic compounds in fruits not only act as a potential source of antioxidants, but they can also be natural enzyme inhibitors.

According to the literature, bilberry fruit has some enzyme inhibition activities such as cholinesterase (Borowiec et al., 2014; Okan et al., 2019; Borowiec and Szwajgier, 2020), xanthine oxidase (Okan et al., 2019), urease (Okan et al., 2019), α-glucosidase (Ji et al., 2021), α-amylase (Ji et al., 2021), and some antioxidant enzymes (Borowiec et al., 2022). Although different enzyme activities of bilberries have been reported, the inhibitory potential of the carbonic anhydrase (CA) enzyme has not been studied. Therefore, the evaluation of the CA inhibition activity of bilberry, which is a good research prospect for CA inhibition, was investigated

for the first time with this study. CA (carbonate hydrolyase E.C.4.2.1.1) has been the target enzyme to discover drugs used in the treatment of many diseases in animals and especially in humans. There are many biological processes (respiration, pH and $CO_2$ homeostasis, tumor formation, gluconeogenesis, bone resorption, lipogenesis, ureagenesis, etc.) in which the enzyme is involved. Overactivity of CA enzymes causes glaucoma, atherosclerosis, tumor formation, obesity, and some other human diseases (Supuran and Scozzafava, 2007). Currently, drugs such as acetazolamide, methazolamide, and dichlorphenamide are the best-known synthetic drugs for the treatment of glaucoma. However, some side effects of synthetic drugs can be seen. Therefore, studies on the inhibition of CAs in drug design are increasing day by day (Supuran, 2008). There is a need to develop natural and potent CA inhibitors with slight or nonexistent side effects in the treatment of diseases caused by the CA enzyme.

The chemical compositions and bioactivity (antioxidant, enzyme inhibition, etc.) of fruits are affected by many factors such as season, climate, geography, or experimental conditions. Therefore, the target of this research was to evaluate the phenolic compounds, total phenolic contents, antioxidant activity, and the inhibitory effect of wild bilberry fruit (*Vaccinium myrtillus* L.) against CA *in vitro*. In the present study, the phenolic compounds of bilberry were found by High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD). The antioxidant activities of fruit and pulp extract were investigated to determine using 2,2-diphenyl-1-picrylhydrazil (DPPH•) radical scavenging assay and ferric reducing/antioxidant power (FRAP) assay and total phenolic content (TPC) was investigated by Folin-Ciocalteu method. The inhibition effect of the bilberry juice on *in vitro* bovine carbonic anhydrase (bCA) esterase activity was also studied.

## MATERIALS AND METHODS

### Chemicals and fruit material

Folin Ciocalteu reagent, anhydrous iron (III) chloride ($FeCl_3$), sodium hydroxide (NaOH), and all phenolic standards were obtained from Sigma Aldrich. HPLC-grade methanol, hydrochloric acid, and acetic acid were purchased from Merck. Bovine carbonic anhydrase (bCA), *p*-nitrophenyl acetate (*p*-NPA), acetazolamide, tris (hidroksimetil)-aminomethane, acetone, and $H_2SO_4$ were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Wild bilberry fruit (*V. myrtillus* L.) was collected from the Büyükoba plateau in Karaçukur village, Özkürtün district of Gümüşhane province in Turkey, in the summer of 2020. After the collected fruits were washed with distilled water, they were crushed by hand to obtain juices. The fruit pulp of bilberry was obtained after juice separation. The fruit pulp was dried at 40 ºC and 100% methanol extract was prepared. Approximately 10 g of powdered sample was extracted by mixing with 100 mL of methanol for two hours at 600 rpm at 40°C. The juice and pulp extract were used for antioxidant tests and phenolic analysis. Only juice was used for *in vitro* carbonic anhydrase inhibition. The samples were kept at -18 °C until analyzed.

### Phenolic analysis by HPLC-DAD

HPLC-DAD separation was performed by reverse phase column (Fortis, $C_{18}$ column, 150 mm x4.6 mm, 5µ) under the following gradient program: 0-10 min (5% B), 10-20 min (10% B), 20-25 min (25% B), 25-28 min (38% B), 28-35 min (43% B), 35-37 min (50% B), 37-39 min (90% B), 39-43 min (20% B). The mobile phase solutions (2% acetic acid-ultra-pure water in reservoir A and 50-50% acetonitrile-ultra-pure water including 0.5% acetic acid in reservoir B) were performed with a flow rate of 0.7 mL/min. A 20 µL injection volume and 25°C column temperature were used. 18 phenolic standards (benzoic, caffeic acid, chlorogenic, ferulic, gallic, protocatechuic, *p*-OH benzoic, *p*-coumaric, syringic, rosmarinic, vanillic acids, catechin, epicatechin, protocatechuic aldehyde, syringaldehyde, rutin, vanillin, and quercetin) were used by comparison at 260, 280, 308, and 324 nm wavelengths.

### Total phenolic content (TPC)

TPCs of Bilberry juice and pulp extract were evaluated with the modified Slinkard and Singleton (1977) method. Samples were diluted 1:10 with distilled water and methanol, respectively. In brief, a 50 µL sample, 2.5 mL distilled water, and 250 µL 0.2 N Folin- Ciocalteu reagent were mixed and vortexed. After 3 min incubation at room temperature, 750 µL 7.5% Na2CO3 was added, mixed, and incubated for 2 h. The experiment was performed in three parallels. Finally, absorbances were spectrophotometrically measured at 765 nm. TPC values were expressed in microgram catechin (CE) and gallic acid (GAE) equivalents per mL sample by using a calibration curve for catechin and gallic acid (62.5−1000 µg/mL).

### Biological activity assays

### DPPH• radical scavenging assay

DPPH• assay was measured with the method described by Brand-Williams et al. (1995). Briefly, each 0.75 mL sample was mixed with a freshly prepared 0.1 mM 0.75 mL DPPH• solution with methanol. After a 50 min incubation, the absorbance of the mixtures was read at 517 nm against a control using a spectrophotometer. Butylated hydroxytoluene (BHT) and Trolox were used as positive controls to compare the test results. The results were expressed as $SC_{50}$ (50% scavenging of DPPH•) values (µg/mL). A low $SC_{50}$ value indicates high radical scavenging potential.

### Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was used with several modifications as described by Benzie and Strain (1999). Firstly, bilberry juice and pulp extract were diluted 1:100 with distilled water and methanol, respectively. Then, 50 µL of each sample was mixed with 1.5 mL of FRAP reagent (20 mM acetate buffer, 2,4,6-Tris (2-pyridyl) -s-triazine (TPTZ) and 20 mM $Fe_3Cl_3.3H_2O$ at a ratio of 10: 1: 1) and incubated for 20 min. The absorbance was measured at 595 nm against a control. Trolox (62.5−1000 µM) was used to draw the calibration graph and antioxidant activity was expressed as micromolar Trolox Equivalent Antioxidant Capacity (µM TEAC).

### *In vitro* CA inhibition assay

CA inhibition assay of bilberry juice was analyzed according to a method previously described with minor modifications (Verpoorte et al., 1967). Briefly, bCA was dissolved in pure water (0.1 mg/mL), while 3 mM of *p*-NPA was selected as a substrate. Next, 50 µL of bilberry juice was mixed with 550 µL 0.05 M Tris-$SO_4$ buffer (pH 7.4), 150 µL 0.1 mg/mL bCA enzyme solution, 750 µL 3 mM of *p*-NPA and incubated at room temperature for 30 min. After 30 min, the absorbance was measured at 348nm. The same mixture was prepared separately without enzyme and sample, and measurements were obtained with two positive controls. All assays were tested in triplicate. The results were expressed as $IC_{50}$ (mg/mL).

## RESULTS and DISCUSSION

### Phenolic compounds by HPLC-DAD

HPLC-DAD analysis results are given in Table 1. The most abundant phenolic acid identified in the juice (233.515 µg/L extract) and pulp extract (153.100 µg/L extract) is chlorogenic acid. The phenolic compounds present in the samples, but in lower amounts compared to chlorogenic acid, were gallic acid, protocatechuic acid, and rutin. Rutin was not detected in the juice, while this flavonoid was detected in pulp extract (83.865 µg/L extract).

Analysis of the individual phenolic acids, stilbene, and anthocyanins in bilberry fruit has been reported in several studies (Može et al., 2011; Antolak et al., 2016; Değirmencioğlu et al., 2017; Brasanac-Vukanovic et al., 2018; Čanadanović-Brunet et al., 2019; Okan et al., 2019; Georgescu et al., 2022). Previous research has revealed that the main phenolic in bilberry fruit is chlorogenic acid (Može et al., 2011; Brasanac-Vukanovic et al., 2018; Okan et al., 2019). One research has reported that syringic acid is considered a major phenolic acid in the bilberry fresh and dried fruit and leaf extracts grown in Erdek and Kapıdağ regions of Turkey climate conditions (Değirmencioğlu et al., 2017). Nevertheless, in this study high syringic acid levels in the juice and pulp extract were not determined. In the literature, rutin was not present in bilberry fruits and leaves extracts from Montenegro (Brasanac-Vukanovic et al., 2018), while it was detected for the first time in Slovenian fruits (Može et al., 2011). In another study, significant levels of protocatechuic and gallic acids were detected in bilberry pomace (pulp), as well as the predominant flavonoid rutin (Čanadanović-Brunet et al., 2019). Literature data reveal that the different contents of phenolic compounds in bilberry fruits depend on environmental factors, variations in genotypes, growing conditions, and mostly on the extraction procedure or solvent polarity used in the extraction (Xiaoyon and Luming, 2014; Hidalgo and Almajano, 2017; Ciulca et al., 2021; Urbonaviciene et al., 2022).

**Table 1**. Analysis of Phenolic Compounds of *V. myrtillus* L. by HPLC-DAD

| Phenolic compounds | Concentration (µg/L extract) | |
|---|---|---|
| | Juice | Pulp extract |
| **Gallic acid** | 1.433 | 4.521 |
| **Protocatechuic acid** | 6.435 | 6.756 |
| **Chlorogenic acid** | 233.515 | 153.100 |
| **Rutin** | N.D. | 83.865 |

N.D.: Not Detected

**Total phenolic content and antioxidant activity**

The *in vitro* antioxidant activities of bilberry juice and pulp extract were performed by DPPH• and FRAP while the TPC was determined by the Folin-Ciocalteu method. For all methods, the best value was seen in pulp methanol extract given as TPC 526.19±1.09 µg/mL GAE, DPPH• 0.184±0.002 µg/mL $SC_{50}$, and FRAP 525.19±2.51 µM TEAC, respectively (Table 2). The highest antioxidant activity was detected in pulp extract. The juice and pulp extract showed higher and more effective antioxidant activity than standard antioxidants (BHT and Trolox). The high phenolic content and strong antioxidant activity of bilberries show that this fruit has great potential in the food industry.

Different results have been reported in antioxidant and total phenolic studies on bilberries all over the world. The antioxidant potential was evaluated using two *in vitro* DPPH• and FRAP assays. The fruit extracts had $IC_{50}$ values in the range of 50.82-105.46 µg/mL, FRAP values 0.0003-0.0014 mmol/g, and TPC values 53.95-69.32 mg GAE/g (Brasanac-Vukanovic et al., 2018). One study reported that the TPC, DPPH•, and FRAP values of bilberry collected from the Black Sea region in northern Turkey were 2.06±0.01 mg GAE/g sample, 2.45±0.01 mg/mL $SC_{50}$, and 30.00±0.08 µmol $FeSO_4.7H_2O$/g DW, respectively (Okan et al., 2019). Ciulca et al. (2021) analyzed bilberries from Romania and reported an antioxidant activity between 67.67 and 85.72% (DPPH•) and a TPC between 315.20 and 432.40 mg GAE/100 g FW. In another study, the TPC of the naturally grown bilberries gathered from different sites in Northern Europe ranged from 452 to 902 mg/100 g FW while the FRAP values ranged from 35.1 to 57.7 µmol TE/g FW (Urbonaviciene et al., 2022). The TPC and antioxidant activities of the examined samples were consistent with previously reported findings. For instance, among six selected small dark berries (Borowiec et al., 2022) and eight types of berries harvested from two different geographical regions in the same year (Georgescu et al., 2022), bilberry was at the forefront in terms of high polyphenolic content and antioxidant activity. Differences between antioxidant activity determination assays may be due to the mechanism of action or differences in reaction conditions. Additionally, differences such as geographical region, fruit maturity, climate, genotype, storage conditions, and experimental conditions may be related to similar factors that may affect the antioxidant activities of the extracts.

**Table 2.** Total Phenolic Content and Antioxidant Activities of *V. myrtillus* L. and Standards*

| Samples | TPC (GAE, µg/mL) | DPPH• ($SC_{50}$, µg/mL) | FRAP (µM, TEAC) |
|---|---|---|---|
| **Juice** | 321.67±1.80 | 0.421±0.003 | 200.00±2.22 |
| **Pulp methanol extract** | 526.19±1.09 | 0.184±0.002 | 525.19±2.51 |
| **BHT** | N.D | 11.586±0.094 | N.D |
| **Trolox** | N.D | 2.824±0.009 | N.D |

*: Each value represents the average of three repetitions. N.D: Not Detected

**CA inhibition effect**

The discovery of new and natural enzyme inhibitors has become important in recent years. For the first time, the inhibitory effect of bilberry juice on the esterase activity of CA was investigated *in vitro*. Specifically, the $IC_{50}$ was calculated to evaluate the inhibitory activity of bilberry juice on CA. The lower the $IC_{50}$, the higher the inhibitory ability of the juice. According to the results, the $IC_{50}$ value (half-inhibitory concentration) of bilberry fruit juice related to CA inhibition was 0.0154 mg/mL. According to this result, bilberry juice had an effective inhibitory activity on CA. Also, it was obvious that bilberry juice inhibited CA activity in a concentration-dependent manner (Figure 1). Although the inhibition potential of bilberry extracts on certain enzymes has been reported in the literature, no studies have been conducted on the CA inhibitory activity of bilberry juice. Borowiec et al. (2014) had shown that bilberries and polyphenols from bilberries are a considerable source of cholinesterase inhibitors. Okan et al. (2019) reported that the acetylcholine esterase ($IC_{50}$: 3.89±0.03 mg/mL), xanthine oxidase ($IC_{50}$: 2.77±0.03 mg/mL) and urease ($IC_{50}$: 2.13±0.03 mg/mL)

inhibition activities of methanol bilberry extract. On the other hand, Ji et al. (2021) reported anthocyanin-rich bilberry extract against α-glucosidase and α-amylase inhibition with the $IC_{50}$ value of 0.31 ± 0.02 and 4.06 ± 0.12 mg/mL), respectively.

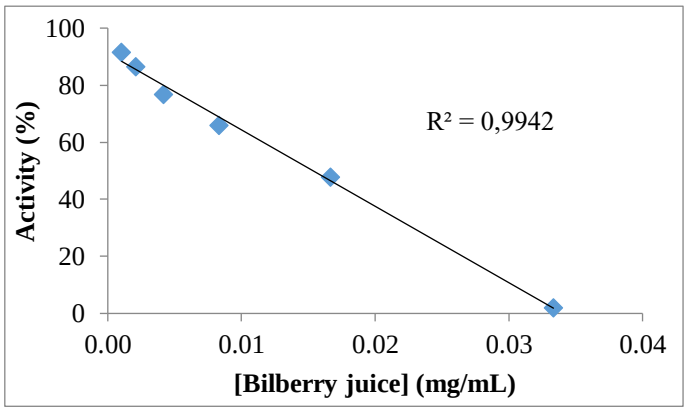


**Figure 1.** CA Activity (%) - Bilberry Juice Concentration (mg/mL) Graph

## CONCLUSION

In this study, the phenolic compounds, total phenolic content, antioxidant activity, and inhibition effect on the CA enzyme of the bilberry juice and pulp methanol extract were evaluated. As a result, it was observed that bilberry fruit exhibited remarkable antioxidant activity and enzyme inhibition potential with high phenolic content. These findings show that bilberry juice and pulp extract should be considered potential sources of antioxidants and enzyme inhibitors and can be used for added value in food formulations.

## REFERENCES

Antolak H, Czyzowska A, Kregiel D 2016. Black currant (*Ribes nigrum* L.) and bilberry (*Vaccinium myrtillus* L.) fruit juices inhibit adhesion of *Asaia* spp. BioMed Research International, 2016: 3671306.

Benzie IFF, Strain JJ 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology, 299: 15-27.

Borowiec K, Szwajgier D, Targoński Z, Demchuk OM, Cybulska J, Czernecki T, Malik A 2014. Cholinesterase inhibitors isolated from bilberry fruit. Journal of Functional Foods, 11: 313-321.

Borowiec K, Szwajgier D 2020. Effect of processing on antioxidant and anticholinesterase activities of bilberry (*Vaccinium myrtillus* L.) juice. Acta Scientiarum Polonorum, Hortorum Cultus**,** 19: 113-123.

Borowiec K, Stachniuk A, Szwajgier D, Trzpil A 2022. Polyphenols composition and the biological effects of six selected small dark fruits. Food Chemistry, 391:133281.

Brand-Williams W, Cuvelier ME, Berset C 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28: 25-30.

Brasanac-Vukanovic S, Mutic J, Stankovic DM, Arsic I, Blagojevic N, Vukasinovic-Pesic V, Tadic VM 2018. Wild bilberry (*Vaccinium myrtillus* L., Ericaceae) from Montenegro as a source of antioxidants for use in the production of nutraceuticals. Molecules, 23: 1864.

Ciulca S, Roma G, Alexa E, Radulov I, Cocan I, Madosa E, Ciulca A 2021. Variation of polyphenol content and antioxidant activity in some bilberry (*Vaccinium myrtillus* L.) populations from Romania. Agronomy, 11: 2557.

Čanadanović-Brunet J, Tumbas Šaponjac V, Stajčić S, Ćetković G, Čanadanović V, Ćebović TN, Vulić JJ 2019. Polyphenolic composition, antiradical and hepatoprotective activities of bilberry and blackberry pomace extracts. Journal of Berry Research, 9: 349-362.

Değirmencioğlu N, Gürbüz O, KE Gözde, Irkin R 2017. Influence of hot air drying on phenolic compounds and antioxidant capacity of blueberry (*Vaccinium myrtillus*) fruit and leaf. Journal of Applied Botany and Food Quality 90: 115-125.

Hidalgo GI, Almajano MP 2017. Red Fruits: Extraction of antioxidants, phenolic content, and radical scavenging determination: A review. Antioxidants (Basel), 6: 7.

Ji Y, Liu D, Jin Y, Zhao J, Zhao J, Li H, Li L, Zhang H, Wang H 2021. In vitro and in vivo inhibitory effect of anthocyanin-rich bilberry extract on α-glucosidase and α-amylase. LWT–Food Science and Technology, 145: 11484.

Georgescu C, Frum A, Virchea LI, Sumacheva A, Shamtsyan M, Gligor FG, Olah NK, Mathe E, Mironescu M 2022. Geographic variability of berry phytochemicals with antioxidant and antimicrobial properties. Molecules, 27: 4986.

Okan OT, Serencam H, Baltas N, Can Z 2019. Some edible forest fruits their in vitro Antioxidant activities, phenolic compounds and some enzyme inhibition effects. Fresenius Environmental Bulletin, 28: 6090-6098.

Može S, Polak T, Gašperlin L, Koron D, Vanzo A, Poklar Ulrih N, Abram V 2011. Phenolics in Slovenian bilberries (*Vaccinium myrtillus* L.) and blueberries (*Vaccinium corymbosum* L.). Journal of Agricultural and Food Chemistry, 59: 6998-7004.

Musilová J, Franková H, Lidiková J, Vollmannová A, Bojňanská T, Jurítková J 2022. The content of bioactive substances and their antioxidant effects in the European blueberry (*Vaccinium myrtillus* L.) influenced by different ways of their processing. Journal of Food Processing and Preservation, 46: e16549

Pires TCSP, Caleja C, Santos-Buelga C, Barros L, Ferreira ICFR 2020. *Vaccinium myrtillus* L. Fruits as a novel source of phenolic compounds with health benefits and industrial applications-a review. Current Pharmaceutical Design, 26: 1917-1928.

Slinkard K, Singleton VL 1977. Total phenol analysis: automation and comparison with manual methods. American journal of enology and viticulture, 28: 49-55.

Supuran CT 2008. Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. Nature Reviews Drug Discovery, 7: 168-181.

Supuran CT, Scozzafava A 2007. Carbonic anhydrases as targets for medicinal chemistry. Bioorganic & Medicinal Chemistry, 15: 4336-4350.

Urbonaviciene D, Bobinaite R, Viskelis P, Bobinas C, Petruskevicius A, Klavins L, Viskelis J 2022. Geographic variability of biologically active compounds, antioxidant activity and physico-chemical properties in wild bilberries (*Vaccinium myrtillus* L.). Antioxidants, 11: 588.

Verpoorte JA, Mehta S, Edsall JT, 1967. Esterase activities of human carbonic anhydrases B and C. Journal of Biological Chemistry, 242: 4221-4229.

Xiaoyon S, Luming C 2014. Phenolic constituents, antimicrobial and antioxidant properties of blueberry leaves (V5). Journal of Food and Nutrition Research 2: 973-979.

## ORAL PRESENTATION

# The comparison of the effects of water and oil repellent processes on modal fibre based fabrics

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## Abstract

In this study, it was aimed to enhance the water and oil repellency performance of the knitted fabrics made from modal fibres that are originally in hydrophilic structure and determine the optimum process conditions to obtain washing durable performance and acceptable physical properties. For this aim, fluoroalkyloligosiloxane as a water and oil repellent agent, β-cyclodextrin (β-CD) as an auxiliary agent, 1,2,3,4-Butanetetracarboxylacid (BTCA) as a crosslinking agent and sodiumhypophosphite (SHP) as a catalyst were applied in different processes. The water and oil repellency performance properties of the modal fibre based fabrics were compared before washing and after washing cycles of 1, 5 and 10. In order to determine the effects of the treatments on physical properties of the samples, bursting strength, thickness, whiteness and yellowness values were measured. The excellent washing durable water and oil repellent performance properties were achieved by the application of 10% fluoroalkyloligosiloxane, 10% β-CD, 10% BTCA and 5% SHP. The effects of this treatment on physical properties of modal fibre based fabrics were also found to be acceptable.

**Keywords:** Modal fibre, water repellency, oil repellency, cyclodextrin, crosslinking

## INTRODUCTION

In textile finishing processes, moisture, water and dye sorption capabilities of the textile fibres are very important factors. These sorption properties of textile fibres have been depended on the parameters like chemical structure, degree of polymerization, molecular mass, degree of crystallinity and molecular orientation. After wetting, the equilibrium between moisture adsorption and water adsorption has been occurred and the fibres begin to swell. When the degree of crystallinity is low and amorphous regions are heavy in the fibre structure, the fibres have easily been wetted and swelled and these fibres have been called as hydrophilic (Peršin et al., 2002). Although the natural and regenerated cellulosic fibres used in textile applications are generally in hydrophilic structure, this can be changed to hydrophobic or oleophobic structure to use these textile materials for different purposes. But it is important for these fabrics to allow evaporation and provide effective cooling while having a hydrophobic or oleophobic surface (Wright et al., 2020).

Modal fibre is a kind of regenerated cellulosic fibre. When compared to the viscose fibres that are the first generation of regenerated cellulosic fibres, modal fibres are known to have higher wet strength, higher comfort and higher performance properties (Gnanapriya and Moses, 2015, Basit et al., 2018, Basit et al., 2019, Sayyed et al., 2019). Modal fibres possess also soft, smooth and shiny surface properties. In addition, their hydrophilic characters due to the hydroxyl groups in the anhydroglucose units enable ideal moisture management (Gnanapriya and Moses, 2015, Basit et al., 2019, Sayyed et al., 2019, Moriam et al., 2021).

Cyclodextrins are cyclic oligosaccharides degraded from starch and composed of six, seven or eight α-D-glucose units, linked by α-1,4-glycosidic bonds. β-cyclodextrin which has seven α-D-glucose units is the most preferred one for the textile applications due to be biocompatible, biodegradable, quite cheap and available in large quantities (Azizi et al., 2019, Bezerra et al., 2020, El-sayed et al., 2021). Cyclodextrins can form inclusion complexes with essential oils, antibacterial agents and organic compounds. Moreover, they improve the chemical stability of these agents by preventing oxidation and by controlling the release rate (Lis et al., 2018). For textile industry, the mainly used applications of cyclodextrins are dyeing, printing, finishing and wastewater treatment processes (Bezerra et al., 2020, El-sayed et al., 2021). But, using cyclodextrins with water and oil repellent agents in order to attain washing durable performance has not been studied yet. In addition, there are still limited studies about the finishing treatments of modal fibre based fabrics. So, in this study, water and oil repellency performance against repetitive washing cycles and some physical properties of modal fibre based fabrics were compared by applying different process alternatives.

## MATERIALS AND METHODS

### Materials

In this study, modal fabric with a weight of 150 g/m$^2$ knitted from 100% ring modal yarn was used. The yarn number of modal fabric was Ne 28/1. Fluoroalkyloligosiloxane (Dynasylan F 8815) with a density of 1058 g/cm3 was supplied from Evonik. β-cyclodextrin (Kleptose β-CD) with a molecular weight of 1135 g/mole was supplied from Roquette. 1,2,3,4-Butanetetracarboxylacid (BTCA) with a molecular weight of 234.16 g/mole and sodiumhypophosphite (SHP) with a molecular weight of 87,98 g/mole were supplied from Sigma Aldrich.

### Methods

### Application Processes

The chemical concentrations of each treatment were given in Table 1.

**Table 1.** The chemical concentrations of the treatments

| Chemicals | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 |
|---|---|---|---|---|
| **First Step** | | | | |
| Fluoroalkyloligosiloxane (g) | 10 | | | |
| β-cyclodextrin (g) | | 10 | 10 | 10 |
| BTCA (g) | | | 5 | 10 |
| SHP (g) | | | 2.5 | 5 |
| Water (ml) | 100 | 100 | 100 | 100 |
| **Second Step** | | | | |
| Fluoroalkyloligosiloxane (g) | | 10 | 10 | 10 |
| Water (ml) | | 100 | 100 | 100 |

The chemical solutions were prepared according to the Table 1, mixed in the magnetic stirrer and applied to the modal fabrics by padding machine (Proser/Y002). For Treatment 1, fluoroalkyloligosiloxane was applied in one step, while in the other treatments fluoroalkyloligosiloxane was applied in the second step. After padding, the modal fabrics were dried at 100°C for 5 min. and cured at 150°C for 5 min. by stenter (Proser/Y003). For two step processes, the second step was applied after curing. So, in the first step, β-CD was fixed on the fabrics and in the second step inclusion complexes with fluoroalkyloligosiloxane were formed.

### Washing Processes

Washing processes were performed at 40°C for 30 min. with a liquor ratio of 1:50 according to the ISO 105 C01 standard. For washing, non-phosphated detergent solution was prepared in the concentration of 5 g/L and Gyrowash machine was used. Then, the samples were rinsed with cold water for 10 min. and dried at the room temperature. After drying, the samples were ironed at 190°C for 10 sec. The washing processes were repeated for 5 and 10 cycles.

### Water Repellency Test

Spray test method was used to determine water repellency. In the spray test method, the sample was placed to the frame and 250 ml pure water was poured from perforated funnel (150 mm high and 45° slope) to the surface of the sample. Then, the wetting degree was determined according to the ISO standard scale score (100 the best, 0 the worst). In addition, the add-on (%) weight gains after wetting were also calculated.

### Oil Repellency Test

Oil repellency test was performed according to the AATCC 118-2002 standard method. In this method, eight types of oils with different surface tensions were dropped to the samples respectively from high tension to low tension. The test was continued until oil drop being absorbed by the surface instead of remaining in a spherical form on the surface. When the oil drop was absorbed, the previous test oil number was accepted as the final result.

### Physical Tests

The bursting strength values of the samples were determined according to ISO 13938-2 standard by James Heal-TruBurst test machine. Each sample was measured four times and average values were calculated.

The thickness values of the samples were measured by Louis Schopper Automatic Micrometer. Each sample was measured four times and average values were calculated.

The whiteness index (Stensby) and the yellowness index (E 313) values were measured by spectrophotometer (Data Color 600TM). Each sample was measured four times and average values were calculated.

## RESULTS and DISCUSSION

### Water Repellency Test Results

Water repellency test results are given in Table 2.

**Table 2.** Water repellency test results

| | Before Washing | | After 1 Washing | | After 5 Washing | | After 10 Washing | |
|---|---|---|---|---|---|---|---|---|
| | Spray Test Score | Add-On (%) | Spray Test Score | Add-On (%) | Spray Test Score | Add-On (%) | Spray Test Score | Add-On (%) |
| **Untreated** | 0 | 157.78 | - | - | - | - | - | - |
| **Treatment 1** | 100 | 3.40 | 90 | 9.53 | 90 | 30.31 | 70 | 63.43 |
| **Treatment 2** | 90 | 14.64 | 90 | 15.33 | 90 | 23.52 | 80 | 40.90 |
| **Treatment 3** | 90 | 10.81 | 90 | 19.60 | 90 | 23.12 | 80 | 51.27 |
| **Treatment 4** | 90 | 9.51 | 90 | 14.19 | 90 | 24.44 | 80 | 26.11 |

According to the spray test results, the score of untreated modal sample was found as 0 that meant this fabric was hydrophilic and completely wetted. On the other hand, treated modal fabrics showed quite excellent scores and their surfaces turned to hydrophobic structures. After first and fifth washing cycle there was no significant change, but after tenth washing, the hydrophobic performances decreased. The modal fabric treated without β-CD, BTCA and SHP showed lower performance compared to others.

Add-on (%) values that are the weight gains of the samples after wetting showed that untreated sample was quite hydrophilic (157.78%) and the treatments enabled very well performance in terms of water repellency since all the values under 15% before washing. After washing cycles, add-on values increased, but in the samples treated with β-CD, this increase was more controlled than the sample treated with only fluoroalkyloligosiloxane (Treatment 1). After ten washing cycles, the best washing stability was achieved with the Treatment 4. According to the results, it was understood that β-CD enhanced the stability and BTCA and SHP had positive contributions. In terms of the concentrations of BTCA and SHP, it was found that minimum 10% and 5% should be applied respectively to obtain excellent washing durable water repellent performance.

### Oil Repellency Test Results

Oil repellency test results are given in Table 3.

**Table 3.** Oil repellency test results

| | Before Washing | After 1 Washing | After 5 Washing | After 10 Washing |
|---|---|---|---|---|
| | Oil Repellency Test Score | Oil Repellency Test Score | Oil Repellency Test Score | Oil Repellency Test Score |
| **Untreated** | 0 | - | - | - |
| **Treatment 1** | 4 | 4 | 4 | 3 |
| **Treatment 2** | 6 | 5 | 5 | 4 |
| **Treatment 3** | 6 | 5 | 5 | 5 |
| **Treatment 4** | 6 | 5 | 5 | 5 |

According to the oil repellency test results, the samples treated with β-CD showed better performance than the sample treated with only fluoroalkyloligosiloxane (Treatment 1). The effects of BTCA and SHP were not significant until the tenth washing. After tenth washing, it can be said that the addition of BTCA and SHP with β-CD enabled improved oil repellency performance for 1 score point. On the other hand, the fact that the concentrations of BTCA and SHP were not the same did not cause any change between Treatment 3 and 4.

**Physical Test Results**

The physical test results (bursting strength, thickness, whiteness and yellowness) are given in Table 4.

**Table 4.** The physical test results

| | Bursting Strength (kPa) | Thickness (mm) | Whiteness (Stensby) | Yellowness (E313) |
|---|---|---|---|---|
| **Untreated** | 206.1 | 0.34 | 76.28 | 8.51 |
| **Treatment 1** | 185.3 | 0.36 | 70.21 | 10.95 |
| **Treatment 2** | 190.6 | 0.38 | 72.37 | 9.88 |
| **Treatment 3** | 176.1 | 0.39 | 71.44 | 10.55 |
| **Treatment 4** | 171.9 | 0.40 | 70.10 | 11.33 |

The physical tests were also applied to the samples in order to determine the effects of the treatments on mechanical and physical properties of the fabrics. For this purpose, bursting strength, thickness, whiteness and yellowness values were measured.

According to the bursting strength results, the treatments without BTCA and SHP addition did not cause significant decrease, while the crosslinking reaction of BTCA and SHP clearly decreased the bursting strength. On the other hand, since the increase of the concentrations of BTCA and SHP twice did not alter the bursting strength values much, it can be said that the concentrations of 10% BTCA and 5% SHP were also acceptable.

Thickness results showed that the thickness values increased by the increase in the concentrations of the applied chemicals. The maximum thickness value was in the sample treated by Treatment 4.

Whiteness and yellowness index values showed that treatments caused a decrease in whiteness and an increase in yellowness. On the other hand, since the values of all the treatments were quite close, the effect of the addition of β-CD, BTCA and SHP was found to be negligible.

## CONCLUSION

Water and oil repellent treatments with fluoroalkyloligosiloxane, β-CD, BTCA and SHP were applied to modal fibre based fabrics in different combinations and concentrations in this study. The water and oil repellency performance of the samples before washing and after first, fifth and tenth washing cycles were determined, compared and discussed. Moreover, the mechanical and physical properties like bursting strength, thickness, whiteness and yellowness were also measured. According to the results, Treatment 4 (10% fluoroalkyloligosiloxane, 10% β-CD, 10% BTCA and 5% SHP) was found to be the favorable method both for excellent washing durable water and oil repellent performance and physical properties.

## REFERENCES

Azizi N, Ben Abdelkader M, Chevalier Y, Majdoub M 2019. New β-cyclodextrin-based microcapsules for textiles uses. Fibers and Polymers, 20(4): 683-689.

Basit A, Latif W, Baig SA, Rehman A, Hashim M, Rehman MZU 2018. The mechanical and comfort properties of viscose with cotton and regenerated fibers blended woven fabrics. Materials Science, 24(2): 230-235.

Basit A, Latif W, Ashraf M, Rehman A, Iqbal K, Maqsood HS, Jabbar A, Baig SA 2019. Comparison of mechanical and thermal comfort properties of tencel blended with regenerated fibers and cotton woven fabrics. Autex Research Journal, 19(1): 80-85.

Bezerra FM, Lis MJ, Firmino HB, Dias da Silva JG, Curto Valle RDCS, Borges Valle JA, Scacchetti FAP, Tessaro AL 2020. The role of β-cyclodextrin in the textile industry-review. Molecules, 25(16): 3624.

El-Sayed EA, Othman H, Hassabo AG 2021. Cyclodextrin usage in textile industry. Journal of Textiles, Coloration and Polymer Science, 18(2): 111-119.

Gnanapriya K, Moses J 2015. A study on modal fibre based on the absorption characteristics. SOJ Materials Science & Engineering, 3(2): 1-4.

Lis MJ, García Carmona Ó, García Carmona C, Maestá Bezerra F 2018. Inclusion complexes of citronella oil with β-cyclodextrin for controlled release in biofunctional textiles. Polymers, 10(12): 1324.

Moriam K, Rissanen M, Sawada D, Altgen M, Johansson LS, Evtyugin DV, Guizani C, Hummel M, Sixta H 2021. Hydrophobization of the man-made cellulosic fibers by incorporating plant-derived hydrophobic compounds. ACS Sustainable Chemistry & Engineering, 9(13): 4915-4925.

Peršin Z, Stana-Kleinschek K, Kreže T 2002. Hydrophilic/hydrophobic characteristics of different cellulose fibres monitored by tensiometry. Croatica Chemica Acta, 75(1): 271-280.

Sayyed AJ, Deshmukh NA, Pinjari DV 2019. A critical review of manufacturing processes used in regenerated cellulosic fibres: viscose, cellulose acetate, cuprammonium, LiCl/DMAc, ionic liquids, and NMMO based lyocell. Cellulose, 26(5): 2913-2940.

Wright T, Mahmud-Ali A, Bechtold T 2020. Surface coated cellulose fibres as a biobased alternative to functional synthetic fibres. Journal of Cleaner Production, 275: 123857.

## *ORAL PRESENTATION*

# Investigation of Alternative Use of White Pitahaya: Natural Feed Additive in Aquaculture

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**Abstract**

With the increasing world population, the need for food is also increasing. Aquaculture has an important role in meeting the growing food needs. Although there are many difficulties in aquaculture, production continues rapidly. Among these challenges are infectious diseases. The most preferred treatment method to combat these diseases is the addition of antibiotics to the feed. In this treatment method, the formation of a resistance gene causes a negative effect on the human food chain. In our study, in order to determine the potential of using plant extracts with natural antimicrobial activity instead of antibiotics, the antimicrobial activity of methanol extracts obtained from white pitahaya against fish pathogens was determined. Disc diffusion and micro-dilution assay methods were used to determined the antimicrobial activity of white pitahaya extracts. The highest inhibition zone diameter was determined as 12.42 mm against *Vibrio anguillarum* A4 in white pitahaya fruit methanol extract, while the lowest inhibition zone diameter was determined as 9.18 mm against *Lactococcus garvieae* in white pitahaya fruit methanol extract. While the minimum inhibition concentration (MIC) values were determined in the range of 40-80 µg/µl, the lowest minimum bactericidal concentration (MBC) was found to be 40 µg/µl against *Aeromonas hydrophila* ATCC 19570, in white pitahaya peel methanol extract. As a result of our study, it was reported that white pitahaya methanol extracts have the potential to be used as a feed additives or natural antimicrobial substances in aquaculture.

**Keywords:** White pitahaya, Fish pathogens, Extracts, Antimicrobial activity, Natural additive

## INTRODUCTION

One of the cheapest and most promising sources of animal protein is fish; people can easily digest 93.7% of fish oil and 93.2% of its protein (Olusola et al., 2013). Aquaculture is a natural resource for the food industry. The aquaculture industry has an important place among the rapidly growing industries in many developed and developing countries. In recent years, there has been a fourfold growth in industrial aquaculture around the world. (Techaoei, 2022; Cabello, 2006). Therefore, aquaculture is a developing industry that requires constant research. (Alicia et al., 2005). Despite many challenges in the aquaculture environment, production continues to increase. Among the challenges limiting production, infectious disease causes billions of dollars in losses each year (Assefa and Abunna, 2018).

The widespread and effective use of antibiotics for the prevention of bacterial infections in aquaculture has led to the development of serious health problems in other animals and humans. The intensive use of antibiotics with a wide range of effects, including the inability of antibiotics to biodegrade, allows them to stay on the water for a long time (Manage, 2018). The use of antibiotics in fish feed causes health problems in aquaculture. This increases the likelihood of residual antibiotics in fish and fish products (Cabello, 2006). The result the emergence of antibiotic-resistant bacteria in the environment, the increase in antibiotic resistance in fish pathogens, and the transfer of these resistance determinants to bacteria and then to land animals turn into human pathogens with the change of bacterial flora both on land and in water (Manage, 2018; Cabello, 2006). Therefore, alternatives that will not harm the environment or minimize this harm have priority in solving the health problems of aquatic creatures. The use of plants can provide an environmentally friendly solution in aquaculture. Scientific evidence is growing on the beneficial effects of supplements enriched with plant extracts or natural compounds on fish health and the prevention of disease outbreaks (Reverter et al., 2014).

Potential antimicrobial activity of plant extracts in addition to the digestibility and availability of nutrients can increase immunity in fish as well as various culture, thus can cause an increase in feed conversion efficiency

and protein synthesis (Talpur and Ikhwanuddin, 2013). Pitahaya (*Hylocereus* spp.) is the fruit of the tropical cactus, known as the tropical and subtropical regions of Central and South America with its homeland Mexico. Pitahaya, which has spread as an ornamental plant, has been consumed as fresh fruit in recent years (Gunasena et al., 2007). In our country, pitahaya is grown in the Mediterranean region, mainly in Mersin, Antalya and partly in Adana (Seday and Sanal, 2017). Pitahaya is known to have a very rich content of antioxidants effect on many diseases such as arthritis, atherosclerosis, cancer, heart disease, inflammation, and brain dysfunction (Susanti et al., 2012).

The aim of the study is primarily to determine the potential of using plant extract products that can be used as natural additives instead of products containing chemical additives. In our study, the potential for use of extracts from white pitahaya as a feed additive in aquaculture was determined in vitro.

## MATERIAL-METHOD

### Plant Material

The white pitahaya fruit was purchased from Antalya-Turkey in September 2021 (Figure 1).




**Figure 1.** The greenhouse of Pitahaya in Antalya (A), White pitahaya fruit (B-C)

### Preparation of Extracts

The pitahaya fruit samples were washed with distilled water. Then, the fruits and their peels were air-dried separately at room temperature. Dried fruit samples were ground with Waring blender. In extraction, 10 g powder from white pitahaya fruit and peel were extracted with 30 ml of methanol (96%) using a sonication device (Hielscher) on ice in 2 repetitions in 10 minutes every day (2 days) each and then the solvent was evaporated. White pitahaya fruit and peel extracts were dissolved in dimethyl sulfoxide (DMSO) and then sterilized with a 0.45 µm filter. The extracts were stored at 4°C until used.

### Determination of Antimicrobial Activity

Disc diffusion and micro-dilution assay methods were used to determine antimicrobial activity of methanol extracts from white pitahaya fruit and peel.

### Disc Diffusion Assay

Fish pathogens (*A. hydrophila* ATCC 19570, *L. garvieae*, *Yersinia ruckeri*, *V. anguillarum* A4) were used. *A. hydrophila* ATCC 19570 was cultured at 37ºC in Nutrient Broth (NB) media for 24 hours. *L. garvieae*, *Y. ruckeri* and *V. anguillarum* A4 were cultured at 25ºC in Tryptic Soy Broth (TSB) and Tryptic Soy Broth/Sodium Chloride (TSB/NaCl) media for 24 hours. The test microorganisms were washed twice with saline solution and their concentration was adjusted to 0.5 Mcfarland. 100 µl of prepared microbial suspension (0.5 Mcfarland) was spread onto agar media. Sterile discs (diameter of 6 mm) were placed on the solid agar and 20 µl (4000 µg/disc) of white pitahaya fruit and peel extracts were then dropped onto the discs in triplicates. The petri dishes were incubated at appropriate temperatures for 24 hours. After incubation, the inhibition zones around the disc were measured with a caliper and recorded.

### Micro-dilution Assay

The minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined using the micro-dilution assay. The test microorganisms adjusted to 0.5 McFarland were

added to each tube containing extract and media. The tubes containing the mixture were incubated at appropriate temperatures for 24 h. After incubation, the concentration of the extract in the non-growth tube was recorded as MIC values. Then, the samples from the tubes were inoculated onto solid media using spot-dropping method and the plates were incubated at appropriate temperatures for 24 h. After the incubation, the extract concentrations that prevent bacterial growth on the solid media were evaluated as MBC values.

## RESULTS AND DISCUSSION

Disc diffusion assay results of methanol extracts obtained from white pitahaya fruit and peel are given in Table 1. Petri images of the disc diffusion assay results of white pitahaya extracts are given in Figure 2.

**Table 1.** Inhibition zone diameter of the extracts from white pitahaya (mm ± SD)

| Test microorganisms | Extracts | |
|---|---|---|
| | Inhibition zone diameter (mm) | |
| | WPFM | WPPM |
| ***Aeromonas hydrophila* ATCC 19570** | 10.82±1.58 | 11.51±0.48 |
| ***Lactococcus garvieae*** | 9.18±0.73 | 10.34±0.93 |
| ***Yersinia ruckeri*** | 10.65±1.08 | 9.82±0.21 |
| ***Vibrio anguillarum* A4** | 12.42±0.74 | 12.14±1.59 |

*WPFM: White Pitahaya Fruit Methanol extract, *WPPM: White Pitahaya Peel Methanol extract

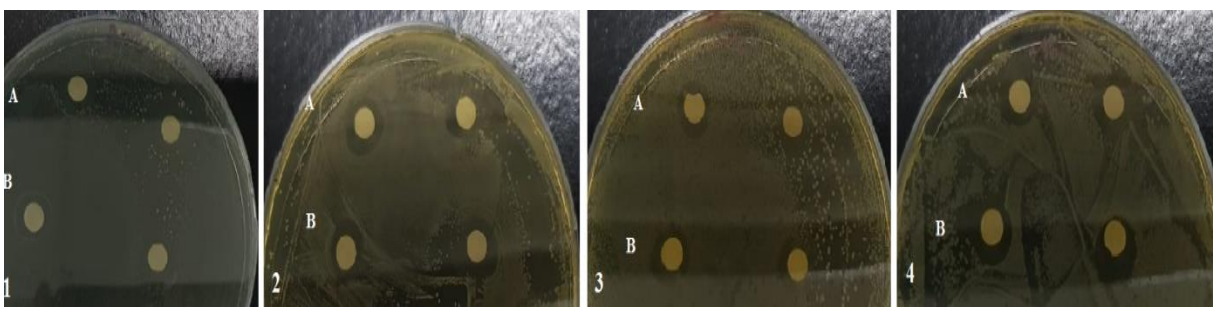


**Figure 2.** The disc diffusion assay results

**A:** White pitahaya peel methanol extract, **B:** White pitahaya fruit methanol extract
**1:** *A. hydrophila* ATCC 19570, **2:** *L. garvieae*, **3:** *Y. ruckeri*, **4:** *V. anguillarum* A4

In our study, the highest inhibition zone diameter was found to be 12.42 mm against *V. anguillarum* A4, while in a study conducted in Bangladesh, the inhibition zone diameter of pitahaya aqueous and methanol extracts was found to be 13.00 mm and 9.70 mm (Tahera et al., 2014). In the same study, *Aeromonas* spp. while the inhibition zone diameter against was not determined, it was determined as 10.82 mm in fruit methanol extract and 11.51 mm in peel methanol extract in our study (Tahera et al., 2014).

The micro-dilution test results of the extracts are presented in Table 2. While MIC values vary according to bacteria and extracts, MBC values of the extracts were determined as 80 µg/µl for all test microorganisms.

**Table 2.** Micro-dilution assay results of the white pitahaya extracts

| Test microorganisms | Extracts | | | |
|---|---|---|---|---|
| | WPFM | WPPM | WPFM | WPPM |
| | MIC (µg/µl) | | MBC (µg/µl) | |
| ***Aeromonas hydrophila* ATCC 19570** | 40 | 40 | 80 | 40 |
| ***Lactococcus garvieae*** | 40 | 40 | 80 | 80 |
| ***Yersinia ruckeri*** | 40 | 40 | 80 | 80 |
| ***Vibrio anguillarum* A4** | 80 | 80 | 80 | 80 |

*WPFM: White Pitahaya Fruit Methanol extract, *WPPM: White Pitahaya Peel Methanol extract

In our study, the MIC value was determined as 40 µg/µl against all test microorganisms except *V. anguillarum* A4. In a study conducted by Hendra et al., the MIC value of n-hexane and ethyl acetate extracts obtained from red pitahaya peel against *Vibrio alginolyticus* shows a 72.6-74.2% change (Hendra et al., 2019). In their studies

Nurmahani et al., determined the antimicrobial activities of ethanol, chloroform, hexane extracts obtained from white pitahaya and red pitahaya peel by disc diffusion and micro-dilution assay methods. The result, they determined that the extracts exhibit good antimicrobial activity (Nurmahani et al., 2012).

The result of our study, we have determined that white pitahaya extracts have antimicrobial activity against some fish pathogens. Considering the literature studies, more studies are needed to determine the antimicrobial activity of pitahaya against fish pathogens.

## CONCLUSIONS

Pitahaya is an interesting type of fruit that has gained popularity in our country in recent years. Although studies on pitahaya are limited, it is known that it is a fruit rich in nutritional value. Against the growing demand for food, interest in aquaculture has increased. Intensive production conditions are required to supply this increasing demand. One of the disadvantages of intensive production conditions is bacterial infections. The disadvantages of the use of antibiotics in feed are known in the fight against these infections. In our study, it has been reported that white pitahaya methanol extracts have the potential to be used as natural feed additives in aquaculture.

## REFERENCES

Alicia E, Toranzo T, Beatriz Magarin˜ OS, Romalde JL 2005. A review of the main bacterial fish diseases in mariculture systems. *Aquaculture*, 246, 37– 61.

Assefa A, & Abunna F 2018. Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary Medicine International*, *2018*. https://doi.org/10.1155/2018/5432497

Cabello F C 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology*, *8*(7), 1137–1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Gunasena HPM, Pushpakumara DKNG, & Kariyawasam M 2007. Dragon Fruit *Hylocereus undatus* (Haw.) Britton and Rose. *Underutilized Fruit Trees in Sri Lanka*, *1*, 110-141.

Hendra R, Masdeatresa L, Abdulah R, & Haryani Y 2019, November. Antibacterial Activity of Red Dragon Peel (*Hylocereus polyrhizus*) Pigment. *In Journal of Physics*: Conference Series (Vol. 1351, No. 1, p. 012042). IOP Publishing.

Manage PM 2018. Heavy use of antibiotics in aquaculture; emerging human and animal health problems–a review. *Sri Lanka Journal of Aquatic Sciences 23*(1), 13-27. http://doi.org/10.4038/sljas.v23i1.7543

Nurmahani M, Osman A, Hamid AA, Ghazali FM, and Dek MP 2012. Antibacterial property of *Hylocereus polyrhizus* and *Hylocereus undatus* peel extracts. *International Food Research Journal.* 19(1):77.

Olusola S, Emikpe BO, & Olaifa FE 2013. The potentials of medicinal plant extracts as bio -antimicrobials in aquaculture. *International Journal of Medicinal and Aromatic Plants, 3*, 404-412.

Reverter M, Bontemps N, Lecchini D, Banaigs B, & Sasal P 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture*, 433, 50-61.

Susanti E, Budi Utomo S, Syukri Y, Redjeki T 2012. Pytochemical screening and analysis polyphenolic antioxidant of methanolic extract of white dragon fruit (*Hylocereus undatus*). *Indonesian Journal of Pharmacy*. 23(1):60-64.

Tahera J, Feroz F, Senjuti JD, Das KK, & Noor R 2014. Demonstration of Anti-Bacterial Activity of commonly available Fruit Extracts in Dhaka, Bangladesh. *American Journal of Microbiological Research*, 2, 68-73.

Talpur AD, & Ikhwanuddin M 2013. Azadirachta indica (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, Lates calcarifer challenged with Vibrio harveyi. *Fish & Shellfish Immunology*, 34(1), 254-264.

Techaoei S 2022. Time-kill kinetics and antimicrobial activities of Thai medical plant extracts against fish pathogenic bacteria. *Journal of Advanced Pharmaceutical Technology & Research*, *13*(1), 25–29. https://doi.org/10.4103/japtr.japtr_241_21

Seday Ü, Sanal D 2017. Pitaya yetiştiriciliği ve Türkiye'de üretim potansiyeli. Alata Bahçe Kültürleri Araştırma Enstitüsü Meyvecilik,50-52.

## *ORAL PRESENTATION*

# Tuning the Photophysical Properties of Aza-Boron Dipyridylmethene (DIPYR) with Replacement of F with Cl and Br

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**Abstract**

Boron dipyridylmethenes (DIPYRs), analogs of both acene and BODIPY molecules, are intensely fluorescent organic structures built around a conjugated polycyclic system. Replacing the meso-position carbon of dipyridylmethane dyes with nitrogen creates a structure with good photophysical properties for dipyridylamine analogs.



**Figure 1:** Optimized geometries of the DIPYR and azaDIPYR dye molecules calculated at B3LYP/6-31+G(d) level

Whether the DIPYR derivatives, identified to have a wide range of absorption, will lose or not the energy absorbed by the internal relaxation mechanisms is determined by calculating the triplet state energies. If the triplet state energies ($T_1$) are close to the first excited singlet state energies ($S_1$) (<400 meV), the probability of intersystem crossing (ISC) increases.

In this study, we have calculated the $S_1$-$T_1$ and $S_1$-$T_2$ energy differences of several azaDIPYR derivatives by employing TD-DFT methods. We applied -0.44 eV singlet state energy correction for correcting the error originating from the B3LYP methodology. The calculations indicated that a hypsochromic shift occurred in the azaDIPYR structure formed replacement of meta-carbon atom with nitrogen in the DIPYR structure. Moreover, the replacement of fluorine atoms with Cl and Br causes a bathochromic shift in the photophysical properties of azaDIPYR.

**Keywords:** DIPYR, azaDIPYR , TD-DFT, B3LYP.

## INTRODUCTION

In recent years, studies on light sources consuming lower power than the existing sources have shifted from inorganic technologies to organic technologies (Gupta and Singh, 2005; Okur and et. al., 2009; Aydoğan and at. al., 2010; Yakuphanoğlu and Okur, 2010; Yüksel and et al., 2013). White OLEDs, a developing and promising technology, draw attention in lighting technology due to their thin structure and high efficiency, consuming less energy than fluorescent lamps and LEDs. The use of OLEDs in the screens of electronic devices has gained great importance due to their bright color images, low-power wide-viewing angle, and their ability to be applied on flexible surfaces (Güllü and et al., 2010).

The efficiency of OLEDs varies depending on the materials used and the device structure. Therefore, the synthesis of new molecules for OLED applications is gaining importance.

Like the BODIPY organic dyes, aza-dipyrromethene (aza-BODIPY) complexes are considered active fluorophores. The most important feature of aza-BODIPY is their ability to absorb electromagnetic radiation

in the near infrared (NIR) region. Boron dipyridylmethene (DIPYR) dyes are a class of intensely fluorescent pyridine-based chromophores that are structural analogues of both acenes and BODIPYs (Golden et al., 2017). It has become the focus of attention (Peumans et al. 2003) to obtain high-efficiency blue fluorescence in OLEDs. It is difficult to shift the emission color of BODIPY to blue and these compounds have intrinsically low triplet energies. Therefore, DIPYR derivatives have become a good alternative for use in WOLEDs as blue fluorescent additives.

It is predicted that the DIPYR structure can improve photoluminescence efficiency while preserving emission lifetime and bandwidths as a result of benzanulation, thus creating a potential as blue fluorescent additives (Kondo et al. 2019). Thus, through a simple conversion from methane to imine, the green-emitting DIPYR molecule is converted to blue-lighting azaDIPYR (aD) (Tadle et al. 2021). The same working group argued that azaDIPYR derivatives could be used as blue fluorescent additives due to their minimal overlap between HOMO and LUMO and low $S_1$-$T_1$ energy gaps. In addition, the excited state $S_1$-$T_1$ energies for azaDIPYR emitting blue light were given at the B3LYP/6-311G** level by applying -0.44 eV singlet energy correction.

The strong electron withdrawing nature of Fluorine atoms provides an advantage for the design of n-type materials. For example, perfluoropentacene exhibits n-type organic semiconductor properties because both HOMO and LUMO energies are stabilized by fluorine groups (Hiszpanski et al., 2015). The effectiveness of halogens other than fluorine on azaDIPYR has not been fully investigated. Fluorination, chlorination and bromination on organic semiconductors need to be well investigated. Moreover, examples for systematic comparison of the properties of halogenated semiconductors and unsubstituted semiconductors are very limited.

In this study, we calculated the singlet and triplet excited states of the azaDIPYR derivatives obtained from replacing the F atoms with Cl and Br atoms by employing TD-DFT methods.

## MATERIALS and METHODS

Ground state ($S_0$) geometries of all compounds were optimized with the hybrid B3LYP functional with the 6-31+G(d) basis set. That all the optimized structures being a minimum on the molecular potential energy surface was characterized by harmonic vibration analysis at the level of optimization.

The electronic transitions, molecular orbital energies and the UV/Vis spectra were calculated by the time-dependent density functional theory (TD-DFT) at B3LYP/6-31G+(d,p) level in the gas phase. Likewise, the triplet (T) and singlet excited states (S) of the compounds were relaxed using TD-DFT to obtain their minimum energy geometries.

Whether the DIPYR derivatives will lose their absorbed energy by radiation or internal damping mechanisms is determined by calculating their triplet excited state energies. If the triplet excited state energies ($T_1$) are close to the singlet excited state energies ($S_1$) (<400 meV), the probability of intersystem crossing (ISC) increases. Therefore, the energy differences $S_1$-$T_1$ and $S_1$-$T_2$ will be calculated in this study.

Throughout the study, all DFT and TD-DFT calculations were performed with GAUSSIAN 09 (Frish et al., 2009) and Gauss View 5.0 were used for visualization of molecular geometries and orbital images.

## RESULTS and DISCUSSION

The ground state geometries of the DIPYR and azaDIPYR molecules are depicted in Figure 2. The excitation wavelengths, oscillator strengths, transition characters and transition coefficients of DIPYR and azaDIPYR molecules calculated at B3LYP/6-31+G(d,p) level are given in the Table 1.

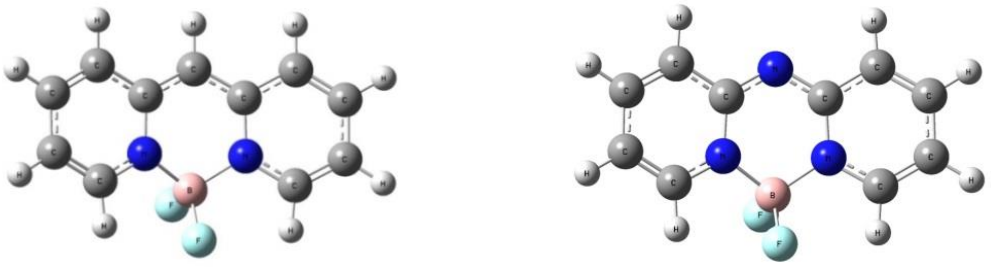
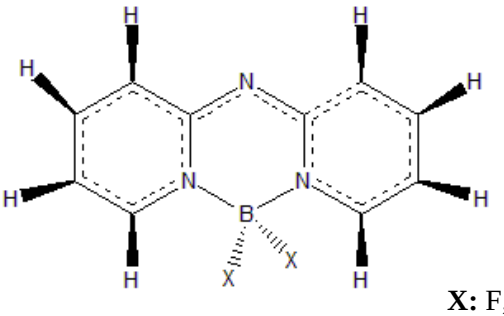


**Figure 2:** Optimized geometries of the DIPYR and azaDIPYR dye molecules calculated at B3LYP/6-31+G(d) level in gas phase

**Table 1:** Excitation wavelengths (λ, nm), oscillator strength (f) of DIPYR and azaDIPYR molecules calculated at B3LYP/6-31+G(d,p) level

| | λ (nm) | f | Transition Character | Transition Coefficient |
|---|---|---|---|---|
| **DIPYR** | 405 | 0.2660 | H → L | 0.68 |
| **azaDIPYR** | 355 | 0.3218 | H → L | 0.70 |

As seen from the table 1, the excitation wavelengths of the DIPYR and azaDIPYR dye molecules predicted by the TD-DFT calculations are 405 and 355 nm, respectively. These values show that the replacement of the meta-carbon atom in the DIPYR structure with a nitrogen atom causes a hypsochromic shift in the azaDIPYR molecule.




**X:** F, Cl , Br

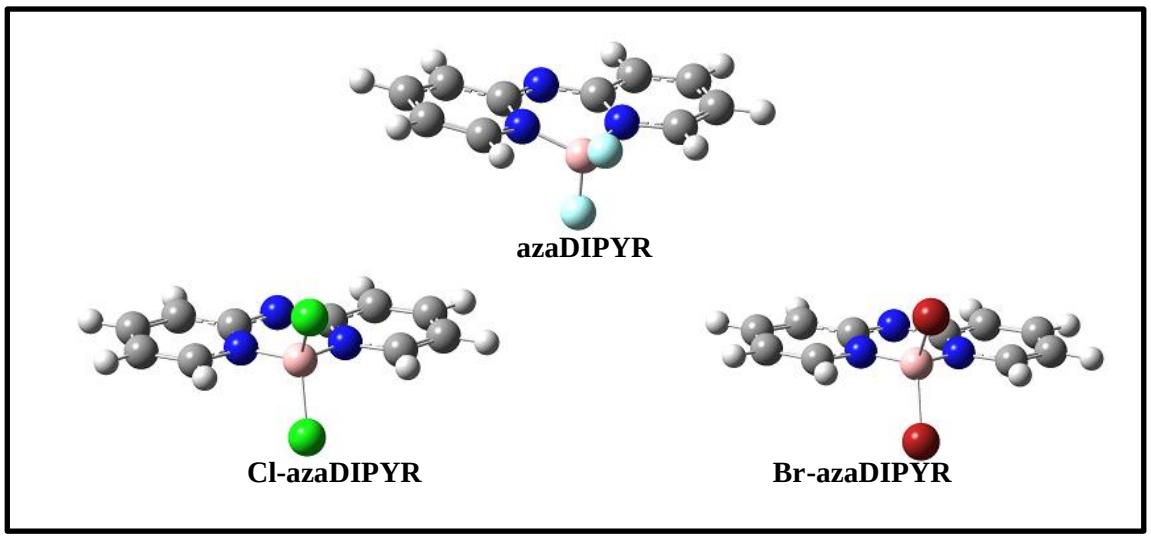



**Figure 3:** The geometries of the azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules optimized at TD-B3LYP/6-31+G(d) level.

In this study, the $BF_2$ moiety of the azaDIPYR molecule was replaced with $BCl_2$ and $BBr_2$ to scrutinize the effect of halogens upon excitation. Figure 3 depicts the excited state geometries of the azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules optimized at the TD-B3LYP/6-31+G(d) level. As seen from the Figure 3, while the $BF_2$ part in the excited-state azaDIPYR geometry is out of the molecular plane, replacing fluorine atoms with Cl and Br atoms causes the boron atom to remain in the molecular plane. Excitation wavelengths (λ, nm), oscillator strengths (f), transition characters and transition coefficients of azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules calculated at the TD-B3LYP/6-31+G(d,p) level are given in Table 2. The excitation wavelengths of azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules are predicted as 344, 356 and 363 nm, respectively. The excitation wavelengths of Cl-azaDIPYR and Br-azaDIPYR shift 12 and 19 nm to red region of the spectrum, respectively. The replacement of F atoms with Cl and Br atoms causes a bathochromic shift in the phosphorescent properties of these molecules relative to the parent azaDIPYR molecule.

**Table 2:** Excitation wavelengths (λ, nm), oscillator strengths (f), transition characters and transition coefficients of azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules calculated at the TD-B3LYP/6-31+G(d,p) level

| Molecule | λ (nm) | f | Transition Character | Transition Coefficient |
|---|---|---|---|---|
| azaDIPYR | 344 | 0.3200 | H → L | 0.69 |
| Cl-azaDIPYR | 356 | 0.2735 | H → L | 0.70 |
| Br-azaDIPYR | 363 | 0.2304 | H → L | 0.70 |

**Table 3:** Electronic properties of the $S_0 \rightarrow S_n,T_n$ transitions of the azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules calculated at the TD-B3LYP/6-31G+(d,p) level

| Molecules | State | Transition | % | $\lambda_{abs}$ (nm) | $E_{abs}$ (eV) | $\lambda_{em}$ (nm) | $E_{em}$ (eV) | *f* |
|---|---|---|---|---|---|---|---|---|
| azaDIPYR | $S_1$ | H=>L | 69 | 344 | 3.60 | 364 | 3.40 | 0.3002 |
| | $S_2$ | H=>L+1 | 70 | 328 | 3.78 | 341 | 3.64 | 0.0426 |
| | $S_3$ | H=>L+2 | 68 | 276 | 4.49 | 279 | 4.40 | 0.3193 |
| | $T_1$ | H=>L | 70 | 466 | 2.66 | 508 | 2.44 | 0.0000 |
| | $T_2$ | H=>L+1 | 67 | 438 | 2.83 | 461 | 2.69 | 0.0000 |
| Cl-azaDIPYR | $S_1$ | H=>L | 69 | 356 | 3.48 | 378 | 3.28 | 0.2736 |
| | $S_2$ | H=>L+1 | 69 | 336 | 3.69 | 351 | 3.53 | 0.0580 |
| | $S_3$ | H=>L+2 | 67 | 274 | 4.51 | 278 | 4.46 | 0.2081 |
| | $T_1$ | H=>L | 70 | 482 | 2.57 | 525 | 2.36 | 0.0000 |
| | $T_2$ | H=>L+1 | 67 | 451 | 2.75 | 478 | 2.60 | 0.0000 |
| Br-azaDIPYR | $S_1$ | H=>L | 70 | 363 | 3.42 | 386 | 3.21 | 0.2304 |
| | $S_2$ | H=>L+1 | 69 | 342 | 3.62 | 356 | 3.48 | 0.0634 |
| | $S_3$ | H=>L+2 | 70 | 319 | 3.88 | 332 | 3.73 | 0.0139 |
| | $T_1$ | H=>L | 70 | 489 | 2.55 | 530 | 2.34 | 0.0000 |
| | $T_2$ | H=>L+1 | 67 | 457 | 2.71 | 481 | 2.58 | 0.0000 |

Table 3 shows the electronic properties of $S_0 \rightarrow S_n,T_n$ transitions of the azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules calculated at the TD-B3LYP/6-31G+(d,p) level. $\lambda_{abs}$ and $E_{abs}$ indicate absorption wavelength and vertical excitation energies, respectively, while $\lambda_{em}$ and $E_{em}$ refers to the emission wavelength and emission energies, respectively. Momeni and Brown showed that the B3LYP/6-31G+(d,p) level calculations 0.44 eV overestimate the energies compared with experimental values for BODIPYs, cyanins and other similar pseudoaromatic dyes. Since azaDIPYR is a species included in that category, we applied -0.44 eV energy correction to each energy obtained at the B3LYP/6-31G+(d,p) level. In other words, all energy values given in Table 3 are the singlet corrected energy values. Figure 4 depicts the excited electronic states of the azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules. As seen from this figure, $S_1 \rightarrow T_2$ energy differences of the azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules are 0.77, 0.73 and 0.70 eV, respectively. The TD-DFT calculations revealed that as the halogen size increases in these molecules the $S_1 \rightarrow T_2$ energy differences decreases. This brings about the fact that increasing size of halogen connected to

boron atom in azaDIPYR derivatives increases the probability of spin forbidden intersystem crossing (ISC). In addition, the emission wavelengths are 466, 525 and 530 nm for azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules, respectively. Consequently, the phosphorescence radiation of these molecules shift from the blue to the blue-green region as the size of halogen increases.

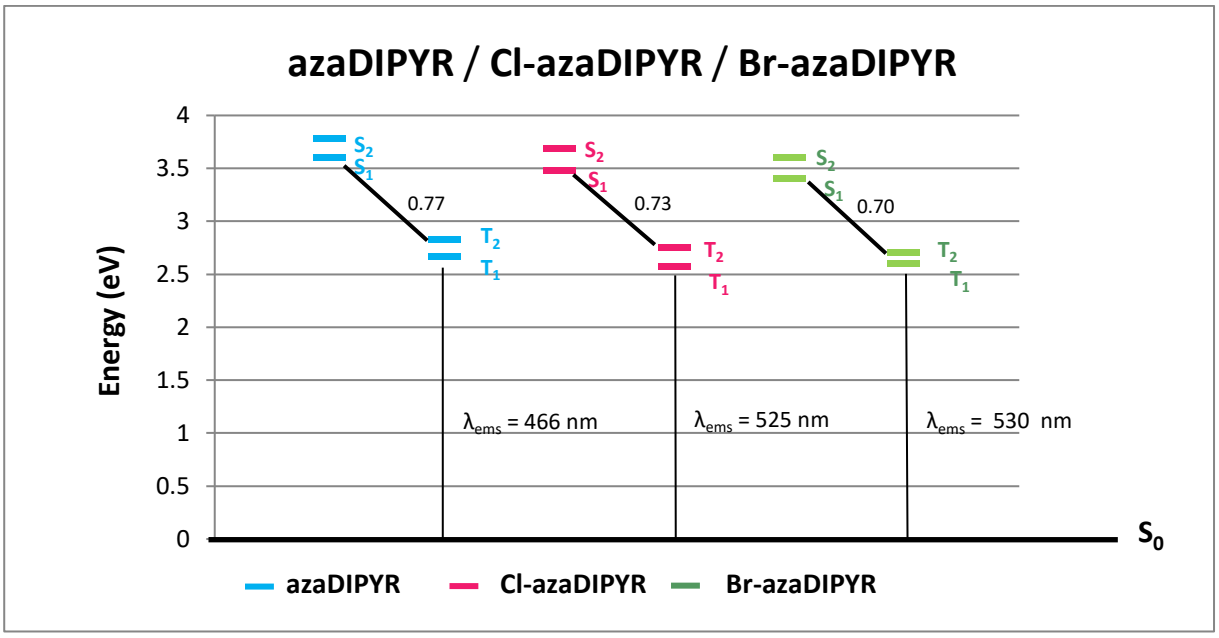



**Figure 4:** Excited states electronic structures for azaDIPYR, Cl-azaDIPYR and Br-azaDIPYR molecules

## CONCLUSION

In this study, we have calculated the $S_1$-$T_1$ and $S_1$-$T_2$ energy differences of several azaDIPYR derivatives by employing TD-DFT methods and the following results were obtained throughout the study. While the $BF_2$ part in the excited state azaDIPYR geometry was out of the molecular plane, the replacement of fluorine atoms with Cl and Br atoms caused the B atom to remain in the molecular plane. The calculations showed that a hypsochromic shift occurs in the azaDIPYR molecule resulting from replacing the meta-carbon atom of the DIPYR structure with a nitrogen atom. Replacement of fluorine atoms with Cl and Br causes a bathochromic shift in the phosphorescent properties of these molecules relative to the azaDPIYR molecule. Thus, the replacements cause shifts of the phosphorescent radiation from the blue to the blue-green boundary. The probability of ISC increases as the $S_1$-$T_2$ energy differences decrease from azaDIPYR to Cl-azaDIPYR and Br-azaDIPYR. With all this knowledge, a new molecule with the desired absorption wavelength might be synthesized so a more efficient dye might be designed.

## ACKNOWLEDGEMENTS

We would like to thanks for financial support received from The Scientific and Technological Research Council of Turkey (2211-C National Priority Fields Doctoral Scholarship Program). The numerical calculations reported in this paper were fully performed at TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources).

## REFERENCES

Aydoğan Ş, İncekara Ü, Deniz AR, Türüt A 2010. Extraction of electronic parameters of Schottky Diode Based on An Organic Orcein. Microelectronic Engineering, 87(12): 2525-2530.

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, et al. 2009. Gaussian 09, Revision D.01.Wallingford CT:Gaussian, Inc.

Golden, JH, Estergreen, L, Porter, T, Tadle, AC, Daniel Sylvinson, MR, Facendola, JW, Kubiak, CP, Bradforth, SE and Thompson, ME 2018. "Symmetry-Breaking Charge Transfer in Boron Dipyridylmethene (DIPYR) Dimers", ACS Appl. Energy Mater, 1, 1083−1095.

Golden, JH, Facendola, JW, Daniel Sylvinson, MR, Baez, CQ, Djurovich, PI and Thompson, ME 2017. "Boron Dipyridylmethene (DIPYR) Dyes: Shedding Light on Pyridine-Based Chromophores", The Journal of Organic Chemistry, 82, 7215−7222.

Gupta RK, Singh RA 2005. Fabrication and Characteristics of Schottky Diode Based on Composite Organic Semiconductors. Composites Science and Technology, 65(3-4): 677-681.

Güllü Ö, Asubay S, Aydoğan Ş, Türüt A 2010. Electrical Characterization of The Al/new fuchsin/n-Si Organic-modified Device. Physica E: Low-dimensional Systems and Nanostructures, 42(5): 1411-1416.

Hiszpanski AM, Saathoff JD, Shaw L, et al. 2015. Halogenation of a nonplanar molecular semiconductor to tune energy levels and bandgaps for electron transport Chem. Mater., 27, pp. 5813-5819.

Kohn, W and Sham, LJ 1965. Self-Consistent Equations Including Exchange and Correlation Effects, Physical Review, 140(4A), A1133-A1138.

Kondo Y, Yoshiura K, Kitera S, Nishi H, Oda S, Gotoh H, Sasada Y, Yanai M, Hatakeyama T 2019. Nat. Photonics, 13, 678.

Momeni MR, Brown AJ 2015. Chem. Theory Comput., 11, 2619−2632.

Okur S, Yakuphanoglu F, Ozsoz M, Kadayifcilar PK 2009. Electrical and Interface properties of Au/DNA/n-Si Organic on Inorganic Structures. Microelectronic Engineering, 86(11): 2305-2311.

Peumans P, Yakimov A, Forrest SR 2003. J. Appl. Phys., 93, 3693.

Tadle AC, El Roz AK, Chan Ho Soh, Ravinson DSM, Djurovich PI, Forrest SR, and Thompson ME 2021. Adv. Funct. Mater. 31, 2101175.

Yüksel ÖF, Tuğluoğlu N, Şafak H, Kuş M 2013. The Modification of Schottky Barrier Height of Au/p-Si Schottky Devices by Perylene-diimide. Journal of Applied Physics, 113(4): 044507.

*ORAL PRESENTATION*

# A theoretical study on hydrazone ligand: molecular properties, NBO and FMO analyses and the investigation of interaction with some heavy metals

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**Abstract**

Hydrazone compounds are obtained from the condensation reactions of mono or dihydrazides with aldehydes or ketones. Hydrazone compounds containing the group -O=C-NH-N=C- have donor atoms such as O and N. These compounds can coordinate to metal ions as monodentate, bidentate, tridentate, or polydentate ligands depending on the other donor atoms in the appropriate position in their structure. Therefore, hydrazones are extensively used as ligands in the determination of various metal ions and the synthesis of metal complexes. In addition, hydrazone ligands and metal complexes are used in many fields such as analytical chemistry, biochemistry, industrial chemistry, and pharmaceutical chemistry due to their different structural properties. In this study, the Density Functional Theory (DFT) calculations in B3LYP/6-311G (d,p) level were performed to get an insight into molecular properties and ligand behavior of N'-(3-ethoxy-2-hydroxybenzylidene)isobutyrohydrazide ligand whose X-ray crystal structure was reported by our working group. The molecular structure of this ligand was optimized in the ground state. It was seen that the obtained values for bond parameters were in agreement with the X-ray diffraction results. The possible coordination modes of the hydrazone ligand with metal ions were estimated using the Natural Population Analysis (NPA) charges obtained from Natural Bond Orbital (NBO) analysis. For this ligand, the energy of frontier molecular orbitals (HOMO and LUMO) and the reactivity descriptors such as electronic affinity, ionization energy, electronegativity, chemical hardness, chemical potential, and electrophilicity index were calculated in gas, water, and ethanol phases. The interaction of this ligand with some heavy metals such as Zn, Cd, Hg, and Sn was investigated by using the values of Energy Lowering (ΔE) and Charge Transfer (ΔN).

**Keywords:** Hydrazone ligand, Metal-ligand interaction, DFT

## INTRODUCTION

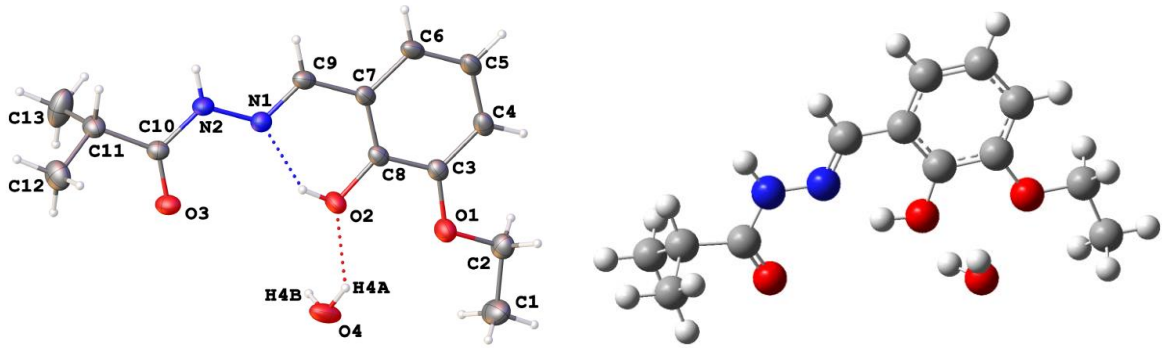
Hydrazones are compounds that have attracted much attention due to their chemical reactivity since 1894 (Elassar, 2007). Hydrazones, which are the products of condensation formed by aldehydes or ketones with hydrazides, are a class of Schiff Base compounds.

In coordination chemistry, some of the molecules with ligand character are hydrazone compounds. Hydrazones containing the azomethine group (-C = N-) are good nitrogen donor ligands. The presence of a second functional group as close as possible to the azomethine group and having a displaceable hydrogen atom (preferably the hydroxyl group) allows these compounds to form highly stable 4, 5, or 6-ring complexes. In addition, in acylhydrazones, the keto hydrazone group can form mono-, di- or polynuclear complexes with metal ions in the form of keto amide or enol imine (Gup and Giziroğlu, 2006; Qian et al., 2016; Sheng et. al., 2016).

Hydrazones are compounds that are widely used in various fields such as organic, analytical, industrial chemistry, and medicine due to their chemical properties, coordination capabilities, and pharmacological applications (Babahan et al., 2006; Gup and Giziroğlu, 2006). Hydrazones and their complexes with antifungal, antibacterial, anti-viral, anti-convulsant, anti-inflammatory, anti-malarial, analgesic, anti-thrombocyte, anti-tuberculosis, and anti-cancer activity are in the composition of drugs used in the treatment of diseases such as tumor, schizophrenia, tuberculosis, leprosy and mental disorders (Buu-Hoï et al., 1953; Zülfikaroğlu et al., 2020).

Hydrazones with donor atoms such as nitrogen and oxygen have unique chemical reactivity. As a ligand, they can easily coordinate to the metal ions to form stable metal complexes (Zülfikaroğlu et al., 2020; Zülfikaroğlu et al., 2021). In our previous works, N'-(3-ethoxy-2-hydroxybenzylidene)isobutyrohydrazide ligand containing hydrazone group (Figure 1), and its Ni(II), Cu(II) and Co(II) metal complexes were synthesized and characterized by various spectroscopic techniques (Zülfikaroğlu et al., 2021). In the present work, the

interaction of this ligand with halides of Zn (II), Cd(II), Hg(II), Sn(II), and Sn (IV) was investigated by computational chemistry methods.




(a) (b)

**Figure 1.** (a) X-ray (Zülfikaroğlu et al., 2021) and (b) optimized structures of the ligand (red= oxygen; blue = nitrogen; gray = carbon; white = hydrogen)

## MATERIALS AND METHODS

All theoretical calculations of the ligand were performed with the Gaussian 09 program (Frisch et al., 2009). By using X-ray diffraction data, the molecular structures of the ligand in the gas, water, and ethanol phases (in the ground state) were optimized using the Density Functional Theory (DFT) method at B3LYP/6-311G (d,p) level (Becke, 1998; Becke, 1993; Kohn et al., 1996). The Natural Population Analysis (NPA) charges in all three phases were obtained at the same level using NBO 3.1 program (Glendening et al., 1998) as implemented in Gaussian 09 package. Based on the energy values of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), the various reactivity descriptors of the ligand such as ionization potential ($I= -E_{HOMO}$), electron affinity ($A = -E_{LUMO}$), energy gap ($\Delta E= E_{LUMO}-E_{HOMO}$), electronegativity ($\chi= (I+A)/2$), chemical hardness ($\eta= (I-A)/2$), chemical potential ($\mu= -\chi$), chemical softness ($S= 1/2\eta$) electrophilicity index ($\omega= \chi^2/2\eta$) were calculated in water, ethanol and water phases (Gazguez, 2008; Geerlings et al., 2003; Parr and Pearson, 1983). To investigate metal-ligand interactions, the halides of Zn (II), Cd(II), Hg(II), Sn(II) and Sn (IV) were optimized in water using the B3LYP/LANL2DZ method, and their reactivity parameters were calculated. The values of Charge Transfer ($\Delta N= (\chi_A - \chi_B)/2(\eta_A+ \eta_B)$) and Energy Lowering ($\Delta E= - (\chi_A - \chi_B)^2 /4(\eta_A+ \eta_B)$) for the interaction of the ligand (donör) with the metal halides (acceptor) in water were computed (Parr and Pearson, 1983).

## RESULTS and DISCUSSION

*Optimization of the Molecular Structure of the Ligand*

The molecular structure of the ligand was optimized by using DFT/B3LYP with 6-311G (d,p) basis set in the gas phase and solvent phases (water and ethanol) in the ground state. The selected bond parameters (bond lengths and angles) of the optimized molecular structure are given with the experimental data in Table 1. Its optimized molecular geometry is shown in Figure 1.

**Table 1.** Selected bond lengths (Å) and angles (°) for the ligand

| *Bond length* | *X-Ray* | *B3LYP/6-311G (d,p) Gas* | *B3LYP/6-311G (d,p) Water* | *B3LYP/6-311G (d,p) Ethanol* |
|---|---|---|---|---|
| O2—C8 | 1.357 | 1.34596 | 1.35494 | 1.35451 |
| N1—C9 | 1.283 | 1.28489 | 1.28634 | 1.28628 |
| N1—N2 | 1.370 | 1.35618 | 1.35806 | 1.35796 |
| C10—N2 | 1.351 | 1.38702 | 1.37657 | 1.37702 |
| C10—O3 | 1.228 | 1.21089 | 1.22044 | 1.22002 |
| O1—C3 | 1.367 | 1.35745 | 1.36002 | 1.35990 |
| O1—C2 | 1.426 | 1.43410 | 1.43779 | 1.43751 |
| C10—C11 | 1.508 | 1.52876 | 1.52555 | 1.52568 |
| C3—C8 | 1.399 | 1.41544 | 1.41215 | 1.41230 |
| C7—C9 | 1.452 | 1.45214 | 1.45488 | 1.45481 |
| *Bond angles* | *X-Ray* | *B3LYP/6-311G (d,p) Gas* | *B3LYP/6-311G (d,p) Water* | *B3LYP/6-311G (d,p) Ethanol* |
| C3—O1—C2 | 117.88 | 119.23113 | 119.25074 | 119.26921 |
| C10—N2—N1 | 119.03 | 120.45377 | 120.43983 | 120.44625 |
| O2—C8—C7 | 122.72 | 123.58370 | 122.96680 | 122.99981 |
| O2—C8—C3 | 116.94 | 117.03579 | 117.24130 | 117.22766 |
| C9—N1—N2 | 119.22 | 119.49565 | 119.29906 | 119.29891 |
| O3—C10—N2 | 121.83 | 122.62168 | 122.45263 | 122.46042 |
| O3—C10—C11 | 123.3 | 123.24058 | 123.09046 | 123.09768 |
| C7—C9—N1 | 119.58 | 121.48097 | 120.72542 | 120.75839 |
| O1—C3—C8 | 115.21 | 114.68123 | 114.61576 | 114.60311 |
| O1—C3—C4 | 125.23 | 125.48975 | 125.60338 | 125.60614 |

To compare the bond parameters calculated with experimental data, the regression values ($R^2$) were obtained from the compliance graphs drawn using the experimental and theoretical calculation results (Figure 2).

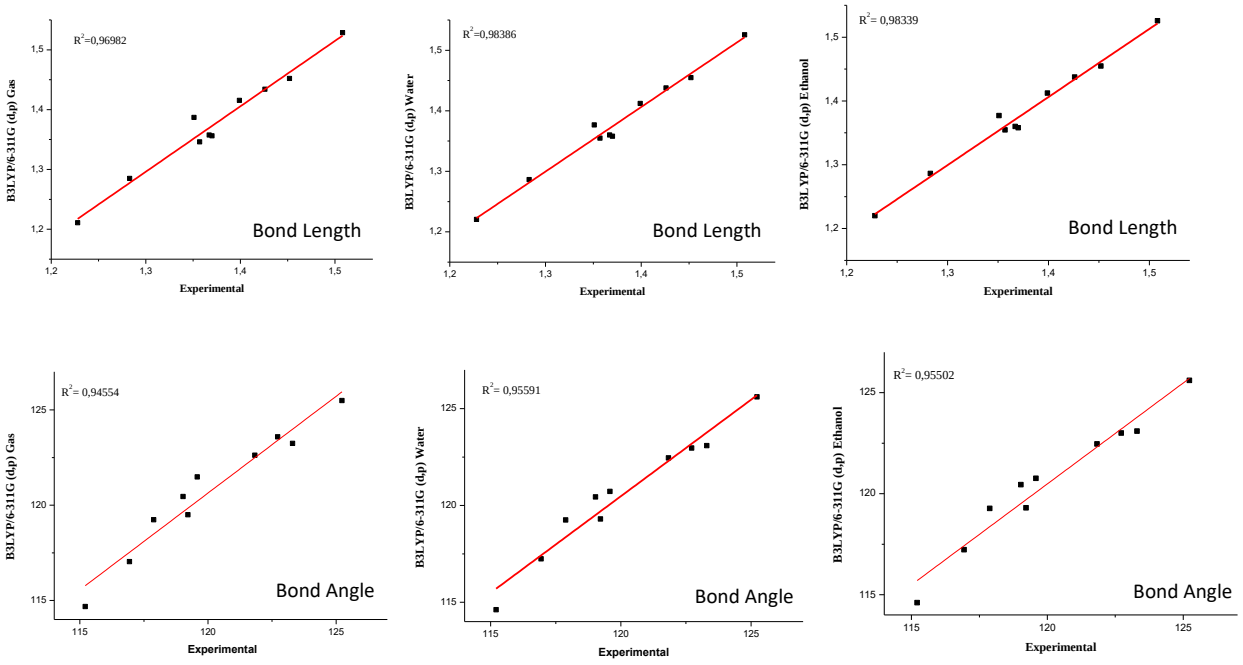



**Figure 2.** The compliance graphs of bond parameters of the ligand

As seen in Figure 2, the correlations were linear, and $R^2$ values were in the range from 0.945 to 0.994 for all phases. All results indicated that the bond parameters calculated in gas, water, and ethanol phases were in generally good agreement with the XRD values.

*NPA Charges*

NPA atomic charges are a helpful tool to investigate the possible coordination modes of a molecule to metal. To examine the ligand behavior of the title molecule, NPA charges on all atoms of the ligand in gas, water, and ethanol phases were calculated by natural bond orbital analysis (NBO), and the atomic charges are listed in Table 2.

**Table 2.** NPA atomic charges (a.u)

| Atom | Gas phase | Water phase | Ethanol phase |
|---|---|---|---|
| O2 | -0.68596 | -0.71186 | -0.71064 |
| H2 | 0.51144 | 0.50907 | 0.50930 |
| O3 | -0.59819 | -0.65837 | -0.65592 |
| O1 | -0.54392 | -0.55539 | -0.55482 |
| N2 | -0.42637 | -0.41375 | -0.41428 |
| H2A | 0.36642 | 0.39595 | 0.39485 |
| N1 | -0.31124 | -0.32785 | -0.32699 |
| C1 | -0.59132 | -0.58544 | -0.58550 |
| H1A | 0.19816 | 0.19767 | 0.19781 |
| H1B | 0.19546 | 0.20723 | 0.20680 |
| H1C | 0.23156 | 0.20211 | 0.20270 |
| C2 | -0.01395 | -0.01746 | -0.01742 |
| H2B | 0.15955 | 0.17170 | 0.17138 |
| H2C | 0.16343 | 0.17194 | 0.17150 |
| C3 | 0.29168 | 0.28309 | 0.28333 |
| C4 | -0.26471 | -0.26652 | -0.26669 |
| H4 | 0.21170 | 0.22284 | 0.22243 |
| C5 | -0.21270 | -0.21602 | -0.21605 |
| H5 | 0.20396 | 0.21369 | 0.21334 |
| C6 | -0.19392 | -0.19461 | -0.19478 |
| H6 | 0.19851 | 0.21351 | 0.21294 |
| C7 | -0.16849 | -0.16577 | -0.16583 |
| C8 | 0.34493 | 0.33027 | 0.33088 |
| C9 | 0.08912 | 0.10024 | 0.09955 |
| H9 | 0.15995 | 0.18859 | 0.18748 |
| C10 | 0.70007 | 0.71055 | 0.71022 |
| C11 | -0.27599 | -0.27528 | -0.27530 |
| H11 | 0.18184 | 0.20047 | 0.19970 |
| C12 | -0.56156 | -0.56301 | -0.56300 |
| H12A | 0.20891 | 0.19870 | 0.19919 |
| H12B | 0.19664 | 0.20250 | 0.20224 |
| H12C | 0.19605 | 0.20034 | 0.20016 |
| C13 | -0.56249 | -0.56302 | -0.56299 |
| H13A | 0.19620 | 0.20033 | 0.20017 |
| H13B | 0.19612 | 0.20253 | 0.20226 |
| H13C | 0.20998 | 0.19871 | 0.19921 |

As seen in Table 2, the oxygen and nitrogen atoms of the ligand exhibit negative charges. The O1, O2, O3, N1, and N2 atoms in which the negative charge is delocalized can serve as coordinating sites to metal ions and thus the ligand can act as a multi-dentate ligand through the oxygen and nitrogen atoms.

*Reactivity Descriptors*

Reactivity parameters such as ionization potential ($I= -E_{HOMO}$), electron affinity ($A = -E_{LUMO}$), energy gap ($\Delta E= E_{LUMO}-E_{HOMO}$), electronegativity ($\chi= (I+A)/2$), chemical hardness ($\eta= (I-A)/2$), chemical potential ($\mu= -\chi$), chemical softness ($S= 1/2\eta$) and electrophilicity index ($\omega= \chi^2/2\eta$) are important tools to widely use in describing the stability and reactivity of molecules. The reactivity parameters for the title ligand were calculated at DFT/B3LYP/6311G(d,p) level in gas, water, and ethanol to analyze its reactivity. The results are given in Table 3. The results indicated that the electron-donating tendency of the molecule was very similar in both the water and ethanol phases.

**Table 3.** The reactivity descriptors for the ligand in gas, water, and ethanol

| Reactivity descriptors | Gas | Water ($\mathcal{E}$=78.39) | Ethanol ($\mathcal{E}$=24.55) |
|---|---|---|---|
| $E_{HOMO}$ (eV) | -5.8743 | -6.0376 | -6.0319 |
| $E_{LUMO}$ (eV) | -1.7464 | -1.77742 | -1.77525 |
| $E_{total}$ (eV) | | -986.8176 | -986.8164 |
| ΔE (eV) (HOMO-LUMO) | 4.127909 | 4.260154 | 4.256617 |
| I (eV) | 5.8743 | 6.0376 | 6.0319 |
| A (eV) | 1.7464 | 1.7774 | 1.7752 |
| χ (eV) | 3.8104 | 3.9075 | 3.9036 |
| μ (eV) | -3.8104 | -3.9075 | -3.9036 |
| η (eV) | 2.0640 | 2.1301 | 2.1283 |
| S (eV) | 179.3738 | 173.8056 | 173.9500 |
| ω (eV) | 3.5172 | 3.5840 | 3.5798 |

*Metal (acceptor) - Ligand (donor) interaction*

The strength of interaction between metal (acceptor) and ligand (donor) can be estimated by quantum chemical parameters such as Energy Lowering (ΔE = −(χA – χB)2 /4 (ηA + ηB)) and Charge Transfer (ΔN = (χA – χB)/2 (ηA + ηB)) (Boukli-Hacene et al., 2014). In this study, the values of ΔN and ΔE parameters for the interaction of the ligand (donor) with halides of Zn (II), Cd(II), Hg(II), Sn(II) and Sn (IV) (acceptor) were calculated. The results are reported with the values of χ and η of these metal salts in Table 8. The metal bond strength between the interaction of metal halides (acceptor) and the ligand (donor) increases as the energy lowering (ΔE) decreases and the charge transfer (ΔN) increases (Boukli-Hacene et al., 2014).

Where the acceptor is $MX_2$ (M = Zn, Cd, Hg, Sn; X = Cl, Br, I), the most stable complexes are formed by iodide halogen because the value of ΔN is highest and the value of ΔE is lowest. the bond strength of the ligand with metal (II) chlorides based on the values ΔN and ΔE is in the following order: $SnCl_2 \approx SnBr_2 > SnI_2 > ZnCl_2 \approx ZnBr_2 \approx ZnI_2 \approx CdCl_2 \approx CdBr_2 \approx CdI_2 \approx HgCl_2 \approx HgBr_2 \approx HgI_2$. The acceptor strength of $SnX_4$ halides (X = Cl, Br, I) is in the following order:

$SnCl_4 > SnBr_4 > SnI_4$

Among all heavy metal halides, the most stable complex is formed with the ligand and $SnCl_4$ (ΔE = -0,50535 eV and ΔN = 0.331813 eV), and the least stable complex is formed with ligand and $CdI_2$ (ΔE = -0,00281 eV and ΔN = 0,024782 eV).

**Table 4.** Interaction of the ligand with $SnCl_4$, $SnBr_4$, $SnI_4$, $SnCl_2$, $SnBr_2$, $SnI_2$, $ZnCl_2$, $ZnBr_2$, $ZnI_2$, $CdCl_2$, $CdBr_2$, $CdI_2$, $HgCl_2$, $HgBr_2$, $HgI_2$

| Acceptor | $\eta_A$ | $\chi_A$ | ΔN | ΔE |
|---|---|---|---|---|
| $ZnCl_2$ | 3.087677 | 4.4977716 | 0.056564 | -0.01669 |
| $ZnBr_2$ | 2.783046 | 4.4350494 | 0.053688 | -0.01416 |
| $ZnI_2$ | 2.418821 | 4.3345033 | 0.046935 | -0.01002 |
| $CdCl_2$ | 2.958967 | 4.2803526 | 0.036633 | -0.00683 |
| $CdBr_2$ | 2.697874 | 4.250284 | 0.0355 | -0.00608 |
| $CdI_2$ | 2.449842 | 4.1344995 | 0.024782 | -0.00281 |
| $HgCl_2$ | 2.733249 | 4.4726011 | 0.058098 | -0.01642 |
| $HgBr_2$ | 2.522496 | 4.4281105 | 0.055948 | -0.01456 |
| $HgI_2$ | 2.152557 | 4.3776333 | 0.054888 | -0.0129 |
| $SnCl_2$ | 2.338819 | 5.4422792 | 0.171717 | -0.13177 |
| $SnBr_2$ | 2.10589 | 5.3560191 | 0.170978 | -0.12383 |
| $SnI_2$ | 1.853096 | 5.1875805 | 0.160685 | -0.10284 |
| $SnCl_4$ | 2.459774 | 6.9534641 | 0.331813 | -0.50535 |
| $SnBr_4$ | 1.977316 | 6.5586267 | 0.322724 | -0.42779 |
| $SnI_4$ | 1.600574 | 6.0964412 | 0.293371 | -0.32109 |

## CONCLUSION

In this study, the interaction of N'-(3-ethoxy-2-hydroxybenzylidene)isobutyrohydrazide ligand containing hydrazone group with some heavy metals was investigated by computational chemistry methods based on quantum chemistry. The molecular structure of the ligand was optimized using the Density Functional Theory (DFT) at B3LYP/6-311G(d, p) level in the gas, water, and ethanol phases. The results indicated that the calculated bond parameters of the molecular structure were compatible with the experimental data in all phases. The NPA atomic charges were obtained from NBO analysis to predict the possible electron-donating centers on the ligand which would likely interact with metal ions. It was determined that the ligand may coordinate with oxygen and nitrogen atoms to metal ions. By using FMO values of the molecule in the gas, water, and ethanol phases, the reactivity descriptors such as ionization potential, electron affinity, energy gap, electronegativity, chemical hardness, chemical potential, chemical softness, and electrophilicity index were been calculated at B3LYP/6-311G(d,p) level. The results showed that the chemical reactivity of the compound was very similar in all the phases. Finally, the interaction of the ligand with halides of Zn (II), Cd(II), Hg(II), Sn(II), and Sn (IV) was investigated with quantum chemical parameters such as the energy lowering $\Delta E$ and charge transfer $\Delta N$. From the values of the calculated $\Delta E$ and $\Delta N$ parameters, it was concluded that the ligand can form stable complexes with $SnCl_4$ and $SnBr_4$. Therefore, this ligand can be used as a chelating ligand in the detection, determination, and extraction of metal ions, especially Sn(IV) ion in water phase.

## REFERENCES

Babahan İ, Anıl H, Sarıkavaklı N 2006. Synthesis And Extraction Properties of Trioxime with Hydrazone Side and its Metal Complexes. Journal of The Faculty of Science, 29: 32-45.

Becke AD 1993. Density-functional thermochemistry. III. The role of exact Exchange. The Journal of Chemical Physics, 98(7): 5648-5652.

Becke AD 1988. Density-functional exchange-energy approximation with correct asymptotic behavior. Physical Review A, 38: 3098-3010.

Boukli-Hacene F, Merad M, Ghalem S, Soufi W 2014. DFT study of the interaction of Cu(II), Zn(II), Sn(II) with carbohydrates in aqueous solution. Journal of Chemistry and Chemical Engineering, 8: 1009-1017.

Buu-Hoï N.P, Xuong ND, Nam NH, Binon F, Royer R 1953. Tuberculostatic hydrazides and their derivatives. Journal of the Chemical Society, 1358-1364.

Elassar AA-Z 2007. Chemistry of Carbofunctionaly Substituted Hydrazones. Arkivoc, ii: 272-315.

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, et al. 2009. Gaussian 09, Revision C.01, Gaussian, Inc., Wallingford, CT.

Gázquez JL 2008. Perspectives on the Density Functional Theory of Chemical Reactivity. Journal of the Mexican Chemical Society, 52(1): 3-10.

Geerlings P, Proft FD, Langenaeker W 2003. Conceptual density functional theory. Chemical Reviews, 103: 1793–1874.

Glendening ED, Reed AE, Carpenter JE, Weinhold F 1998. NBO Version 3.1, TCI, University of Wisconsin, Madison.

Gup R, Giziroğlu E 2006. Metal complexes and solvent extraction properties isonitrosoacetophenone 2-aminobenzoylhydrazone. Spectrochimica Acta Part, A65: 719-726.

Kohn W, Becke AD, Parr RG 1996. Density Functional Theory of Electronic Structure. The Journal of Physical Chemistry, 100: 12974-12980.

Parr RG, Pearson RG 1983. Absolute hardness: companion parameter to absolute electronegativity. Journal of the American Chemical Society, 105: 7512–7516.

Qian S, Han X, Ye YT 2016. Vanadium(V) Complexes Derived from N'-(3-Bromo-2-hydroxybenzylidene)-2-methylbenzohydrazide and N'-(3-Bromo-2-hydroxybenzylidene)-2-methoxybenzohydrazide: Syntheses and Structures. Synthesis and Reactıvity İn İnorganic Metal-Organic and Nano-Metal Chemistry, 46(8): 1124-1128.

Sheng G.H, Han X, Ye YT 2016. Synthesis, Characterization, and Crystal Structures of New Oxovanadium(V) Complexes With Mixed Nicotinohydrazone and 8-Hydroxyquinoline Ligands. Syntheses and Structures. Synthesis and Reactıvity İn İnorganic Metal-Organic and Nano-Metal Chemistry, 46(8): 1129-1132.

Zülfikaroğlu A, Yüksektepe Ataol Ç, Çelikoğlu E, Çelikoğlu U, İdil Ö 2020. New Cu(II), Co(III) and Ni(II) metal complexes based on ONO donor tridentate hydrazone: Synthesis, structural characterization, and investigation of some biological properties. Journal of Molecular Structure, 1199: 127012.

Zülfikaroğlu A, Taş M, Vural H, Çelikoğlu E, İdil Ö 2021. Synthesis, structural characterization, DNA cleavage studies, antimicrobial activities, and time-killing kinetics of tetranuclear Cu (II) with partial cubane Cu4O4 cores and mononuclear Co (II) and Ni (II) complexes of a new acylhydrazone ligand. Applied Organometallic Chemistry, 35:e6218.

## *ORAL PRESENTATION*

# Coagulation of cheese whey effluent with chitosan

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## Abstract

In this study, the coagulation method with chitosan was applied to the cheese whey effluent obtained from a dairy company under laboratory conditions, and color, turbidity and total solids (TS) removal efficiencies were investigated. Within the scope of experimental studies, jar test experiments consisting of rapid mixing, slow mixing and settling processes were carried out with samples added with different concentrations of chitosan. Box-Wilson statistical experimental design method was used to determine the optimum chitosan concentration and precipitation times at which maximum color, turbidity and TS removal efficiencies were obtained. As a result of the experimental studies, the optimum chitosan concentration and settling time were found to be 10 mg/l and 90 minutes, respectively. Color, turbidity and TS removal efficiencies obtained under these conditions were 82%, 85% and 45%, respectively.

**Keywords:** Dairy Industry, Cheese Whey Effluent, Chitosan, Box-Wilson Experimental Design Method.

## INTRODUCTION

Cheese whey is a green-yellowish liquid that results from the precipitation and removal of milk casein in cheese making processes. It is considered the most important pollutant in dairy wastewater, not only because of the high organic load, but also for the volume produced. The amount of whey produced is related to the productivity of the cheese (Siso, 1996).

When the main physicochemical properties of cheese whey are examined, the basic parameters of whey show a biological oxygen demand in the range of 27-60 g/l and a chemical oxygen demand in the range of 50-102 g/l. When we compare the organic load of domestic wastewater with the organic load of whey, we can conclude that the load is equivalent to the pollution load of one hundred times the volume of common domestic wastewater. Total suspended solids have values in the range of 1.3–22.0 g/l. Normally, this effluent has a low pH values (Carvalho et.al., 2013)

Since whey contains high amount of organic matter, the treatment of such wastewater has gained importance. For this purpose, biological treatment technologies are generally used. However, in recent years, studies on the chemical treatability of milk and dairy industry wastewater have also been carried out. Among the different physicochemical processes available, coagulation–flocculation is likely the most simple and economical system. The existence of proteins (mainly casein with an isoelectric point of 4.6) and further pH increase involve the precipitation of these species. In fact a higher COD removal has been reported by using $FeSO_4$ at pH 8.5 than $FeCl_3$ utilized at pH 4.5 (Rivas et al., 2010). Rivas et al. (2010), found a similar optimum reagent dose in both systems (0.25 g/l). However a better BOD removal was obtained by using Fe (II) instead of Fe (III). Both systems showed acceptable phosphorus and turbidity removals.

Protein precipitation with coagulant agents like sodium polyphosphate, sodium hexametaphosphate, iron salts and polyelectrolytes are effective methods in protein content removal but inefficient in terms of protein recovery due to contamination by the coagulant. Chitosan is suitable for use in cheese whey effluent for this purpose (Prazeres et.al., 2012).
In this study, coagulation of cheese whey effluent with chitosan was applied. Box-Wilson statistical experimental design method was used to determine the optimum chitosan concentration and precipitation times at which maximum color, turbidity and TS removal efficiencies were obtained.

## MATERIALS AND METHODS

### Cheese Whey Composition

The cheese whey effluent used in this study was taken from Ege University Food Engineering Department, Izmir-Turkey, and kept in dark at 4°C. The average composition of cheese whey used in this study is shown in Table 1.

**Table 1.** Characterization of cheese whey effluent

| Parameter | Unit | Value |
|---|---|---|
| pH | - | 4.7 |
| COD | mg/l | 63500 |
| SS | mg/l | 2513 |
| TOC | mg/l | 22150 |
| Oil and Grease | mg/l | 1500 |

### Coagulation and Flocculation Experiments

Experiments on cheese whey coagulation and flocculation were performed using Jar-test equipment (The Arm field Flocculation Test Unit). Desired concentrations of chitosan was added to a beaker each containing 1-L volume of cheese whey effluents. The mixture was stirred at high velocity (200 rpm) for 3 min, then the velocity was decreased to 40 rpm, and this slow stirring was maintained for 30 min. Subsequently, the stirring was stopped, and different settling times (30-90 min) were applied. Following the settling time, samples were collected from clear supernatant using a syringe and color, turbidity, total solid analysis were done

### Box–Wilson Experimental Design Method

A Box–Wilson statistical experimental design method was used to determine the effects of chitosan concentration and settling time on coagulation efficiency. The Box–Wilson design is a response surface methodology, which is an empirical modelling technique, devoted to the evaluation of the relationship of a set of controlled experimental factors and observed results. Basically, this optimization process involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model, and predicting the response and checking the adequacy of the model (Akdemir and Aygan, 2019).

Two important operating parameters; chitosan concentration and settling time were chosen as the independent variables and designated as $X_1$ and $X_2$, respectively. Chitosan concentration ($X_1$) was varied between 10-100 mg/l 4, while settling time ($X_2$) was ranged from 60 to 90 minutes. Color, turbidity and TS removal efficiencies after coagulation-flocculation-sedimentation process were chosen as system responses. The experimental works consist of four axial (A), four factorial (F) and center (C) points. The center point was repeated three times for the estimating experimental error.

The color, turbidity and TS removal efficiencies (Y) were correlated with the other independent parameters ($X_1$, $X_2$, $X_3$) using Eq. (1).

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} x_1^2 + b_{22} x_2^2 \quad (1)$$

where Y: the predicted response function, $b_0$: the constant, $b_1$, $b_2$: the linear coefficients, $b_{12}$: the cross product coefficient, and $b_{11}$, $b_{22}$: the quadratic coefficients.

The coefficients of the response functions were determined by using the experimental data and the Statistica 5.0 computer program for regression analysis. Experimental conditions determined by the Box–Wilson statistical design are presented in Table 2.

**Table 2.** Experimental Data Points Used in Box–Wilson Statistical Design

| Experiment Number | Chitosan concentration (mg/l) | Settling time (min) |
|---|---|---|
| Axial points | | |
| A1 | 100 | 75 |
| A2 | 10 | 75 |
| A3 | 55 | 90 |
| A4 | 55 | 60 |
| Factorial points | | |
| F1 | 23 | 96 |
| F2 | 87 | 96 |
| F3 | 23 | 84 |
| F4 | 87 | 84 |
| Center point | | |
| C | 55 | 75 |

## RESULTS and DISCUSSION

### Box–Wilson Experimental Design Method Results

Experimental data was used for determination of the response function coefficients. The estimated coefficients of the response functions are presented in Table 3. As it can be seen from Table 3, response function predictions were in good agreement with the experimental data.

**Table 3.** Coefficients of the response functions

| | Color removal | Turbidity removal | TS removal |
|---|---|---|---|
| $b_0$ | 31.4856 | 69.33136 | 21.94975 |
| $b_1$ | 0.12211 | -2.55438 | 0.00906 |
| $b_2$ | -6.34075 | -6.91262 | -2.02121 |
| $b_{12}$ | -0.01185 | 0.01667 | 0.000741 |
| $b_{11}$ | 0.00549 | 0.00898 | -0.00101 |
| $b_{22}$ | 0.04278 | 0.04083 | 0.01311 |
| $R^2$ | 0.918 | 0.995 | 0.855 |

### Variation of Color Removal

In the first part of the experimental studies, the variation of the color removal efficiency was investigated in jar test experiments, where different chitosan concentrations and different settling times were applied. The results are given in Figure 1. For the 10 mg/l chitosan concentration where the highest color removal was obtained, the color removal efficiency, which was 82% in the 60-minute settling time, decreased to 69% by increasing the time to 75 minutes, and then efficiency increased again with the increasing time.

When the change in color removal efficieny with chitosan concentration was examined from Figure 1, it can be seen that similar results were obtained. With the increase in chitosan concentration, first a decrease and then an increase in color removal efficiency was observed. In 60 minutes settling time, the color removal efficiency was 82% for 10 mg/l chitosan concentration, 65% for 60 mg/l chitosan concentration, and 80% for 100 mg/l chitosan concentration.

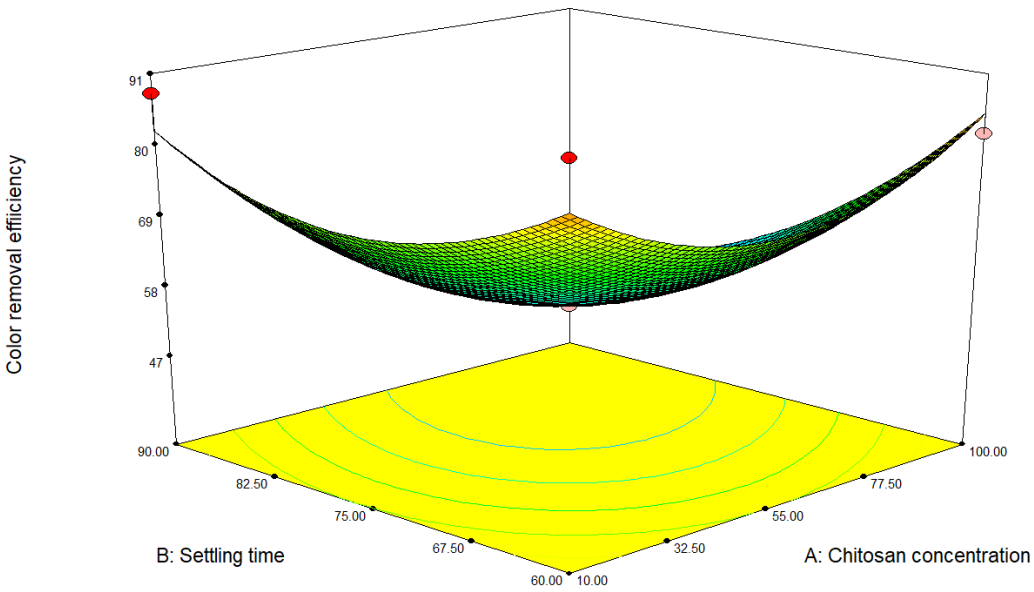


**Figure 1.** Variation of color removal efficieny with chitosan concentration and settling time

**Variation of Turbidity Removal**

In the second part of the study, the change of turbidity removal efficiency after coagulation of cheese whey effluent with chitosan was investigated and the results are given in Figure 2. For the 60 min settling time, the increase in chitosan concentration significantly reduced the turbidity removal efficiency. While the efficiency obtained at 10 mg/l chitosan concentration was 85%, it decreased to 35% at 100 mg/l chitosan concentration.

The increase in settling time also decreased the turbidity removal efficiency. For 10 mg/l chitosan concentration, the efficiency was 85% during the 60-minute settling time, while it decreased to 70% when the time was increased to 90 minutes. This decrease is a smaller decrease relative to the change in removal efficiency with chitosan concentration.

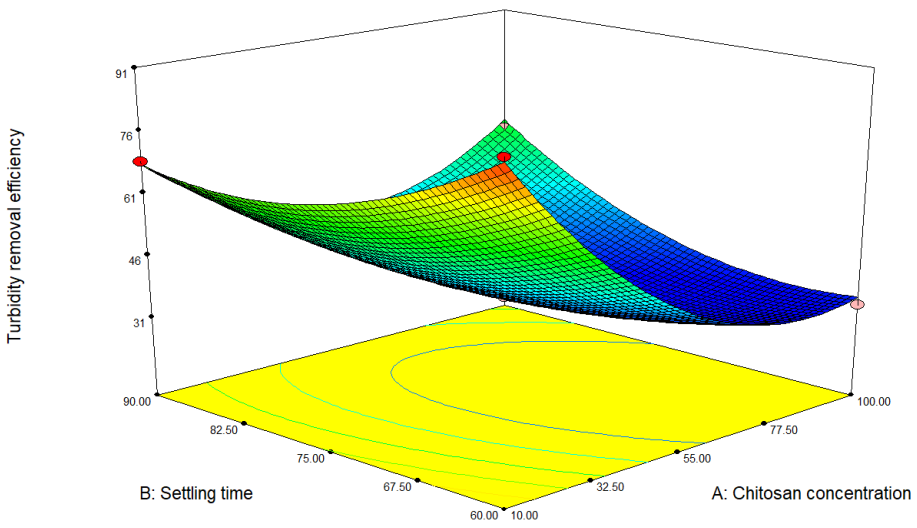


**Figure 2.** Variation of turbidity removal with chitosan concentration and settling time

## Variation of Total Solid (TS) Removal

Finally, the effect of changes in chitosan concentration and settling time on TS removal efficiency was investigated within the scope of the paper and given in Figure 3. The change in settling time has no significant effect on the TS removal efficiency. For the 10 mg/l chitosan concentration, the efficiency obtained at all settling times studied within the scope of the paper is 45%. The increase in chitosan concentration decreased the TS removal efficiency. For 60 minutes settling time, 45% TS removal efficiency was obtained at 10 mg/l chitosan concentration and 39% at 100 mg/l chitosan concentration.

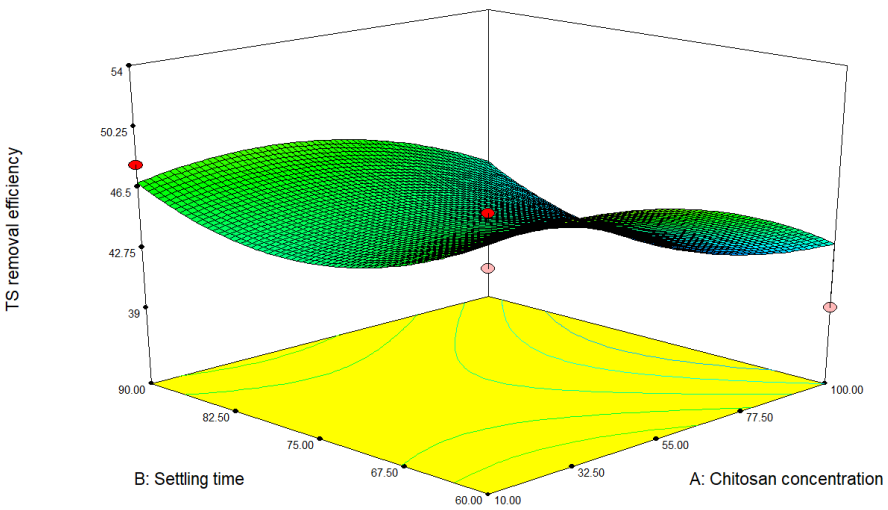


**Figure 3.** Variation of TS removal efficieny with chitosan concentration and settling time

## CONCLUSION

Color, turbidity and TS removal from cheese whey effluent by chitosan coagulation was investigated in this study. The Box–Wilson statistical experiment design was used to determine the optimization of parameters such as chitosan concentration and settling time on removal efficiencies. In the Box–Wilson statistical experiment design, response function coefficients were determined by regression analysis of the experimental data and predicted results obtained from the response functions were in good agreement with the experimental results indicating the reliability of the methodology used.

The experimental results indicated that, the optimum chitosan concentration was found to be 10 mg/l and the optimum settling time was found to be 60 minute. In these conditions, obtained color, turbidity and TS removal efficiencies were 82%, 85% and 45%, respectively.

## REFERENCES

Akdemir EO, Aygan E 2019. Pretreatment of olive mill wastewater by ultrafiltration process using chitosan, Desalination and Water Treatment, 142: 49-55.

Carvalho F, Prazeres AR, Rivas J 2013. Cheese whey wastewater: Characterization and treatment. Science of The Total Environment, 445–446: 385-396.

Prazeres AR, Carvalho F, Rivas J 2012. Cheese whey management: A review. Journal of Environmental Management, 110: 48-68.

Rivas J, Prazeres AR, Carvalho F, Beltrán F 2010. Treatment of cheese whey wastewater: Combined coagulation–flocculation and aerobic biodegradation. Journal of Agricultural and Food Chemistry, 58 (13): 7871-7877.

Siso MIG 1996. The biotechnological utilization of cheese whey: A review. Bioresource Technology, 57 (1): 1-11.

## *ORAL PRESENTATION*

# Sonochemical synthesis of cobalt aluminates: Effect of CTAB as a capping agent

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## Abstract

Cobalt aluminate powders were synthesized by a sonochemical route. CTAB (N-Cetyl-N,N,N-trimethylammonium bromide) was handled in the production of $CoAl_2O_4$ nanoparticles as capping agent. In the colour analyses, consistent results were achieved in terms of the use of ultrasound when compared with the literature reported. Also, a decline was observed in the b* value analysis of the capping agent containing synthesis in comparison with the synthesis without any additives. In the X-Ray Diffraction (XRD) results, the crystal configuration of the synthesized samples was identified as single-phase Co-spinel structure. The Scanning Electron Microscope (SEM) morphologies of the prepared powders indicated that the positive effect of capping agent on the manufacture of nano-sized particles. The particle size distribution of the CTAB capped powder was found in the range of 74 – 88 nm.

**Keywords:** Capping agent, Characterization, Cobalt aluminate, Sonochemical synthesis

## INTRODUCTION

Spinel structures, which can be identified with the general formula $XY_2O_4$, have arisen great interest with their distinctive optical features and crystal structure (Baseri et al., 2020; Gurugubelli et al., 2021). Among these spinel structures, cobalt aluminates ($CoAl_2O_4$) stands out owing to its superior properties such as perfect colour performance, abrasion resistance, chemical and thermal stability (Tan et al., 2021). There are a number of different techniques for the synthesis of cobalt aluminates: solid-state, sol-gel, hydrothermal, co-precipitation, combustion. (Yoneda et al., 2019; Rajabi et al., 2019; El-Said Bakeer, 2020; Gurugubelli et al., 2021; Ferraz et al., 2021). Among these procedures, there have been various studies conducted with the hydrothermal method (Chen et al., 2004; Chang et al., 2018; El-Said Bakeer, 2020). However, on account of some unfavourable conditions such as agglomeration formation in the synthesized nanoparticles and poor colour quality brought about by conventional hydrothermal method, the ultrasonic effect was taken into account in the production. In this respect, the ultrasonic effect contributes to enhance the uniformity of nanoparticle dispersion and effective contact between the particles in a sonochemical process. Furthermore, with the use of ultrasonic application in fabrication of chemical compounds, the reaction time was reduced whereas the contact of raw materials was increased. This situation makes the sonochemical synthesis of cobalt aluminate powders more efficient (Kim et al., 2012; Kaplan et al., 2015; Dumanli and Derun, 2022).

Capping agents can play an active role in controlling particle size and shape by modifying the spinel structure (Naseri et al., 2011; Phan and Nguyen, 2017). In this research, it is aimed to synthesize cobalt aluminate nanoparticles by ultrasonic-assisted hydrothermal method by using the capping agent of CTAB (N-Cetyl-N,N,N-trimethylammonium bromide) added capping agent. The prepared powders were characterized utilizing the techniques of colour analysis, X-Ray Diffraction (XRD) and Scanning Electron Microscope (SEM).

## MATERIALS AND METHODS

Aluminum and cobalt sources of aluminium chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) and cobalt (II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) were purchased from Sigma-Aldrich chemicals. Sodium hydroxide (NaOH) was retrieved from Merck chemicals and used to adjust the pH of the solution.

In the synthesis stage of cobalt aluminate samples, the determined amounts of cobalt and aluminium sources were dissolved in 50 ml of water and sonicated for 30-minute period by adding 1 ml of a capping agent. During this ultrasonic treatment, the pH of mixture was set to 13 by making use of 3 M NaOH solution. The pink-purple mixture was poured to a hydrothermal reactor and the solution was hold on at the reaction temperature of 230 °C for 24 hours. Once the reaction was completed, the blue-colored solution was filtrated and dried. The experimental parameters for cobalt aluminate synthesis are given in Table 1.

**Table 1.** Experimental parameters for cobalt aluminate synthesis

| Sample | Amount of capping agent (mL) | Co/Al Molar Ratio | Ultrasonic Effect Time (min) | Reaction Time (h) | Temperature (°C) |
|---|---|---|---|---|---|
| Without capping agent | – | 0.5 | 30 | 24 | 230 |
| With capping agent | 1 | 0.5 | 30 | 24 | 230 |

The resulting samples were conducted to colorimetric analysis by utilizing the PCE CSM 1 model colorimeter. The crystal structure of the prepared powder samples was determined by employing a PANalytical Xpert Pro X-Ray Diffractometer in the 2θ range of 10 - 90° and at the operating conditions of 40 mA and 45 kV. The morphological analyses of the synthesized samples were carried out in the SEM of ZEISS EVO LS 10 model at the magnification value of 50 and 75 kX.

## RESULTS and DISCUSSION

### Colour Analyses Results

Colour performance of powders can be beneficial for the qualitative identification of materials such as Co-spinel. Colorimetric analysis results of the synthesized samples in this study are given in Table 2. The color performance of the powders produced is evaluated according to colorimetric parameters. When interpreting these colorimetric parameters, a high $L^*$ value ranging from 0 to 100 means that the powder has a brighter colour. Moreover, the value a* stands for the color axis between red (+) and green (-), whereas the value b* stands for the color axis between yellow (+) and blue (-). The bright blue colour is sighted, when the conversion to the cobalt aluminate spinel structure is complemented. In this way, a lower value for the parameter b* is associated with blue color formation in the powder (D'Ippolito et al., 2012; Senberber and Ozdemir, 2020).

**Table 2.** Colorimetric analyses results of the synthesized samples

| Sample | L* | a* | b* |
|---|---|---|---|
| Without capping agent (Dumanli and Derun, 2022) | 30.29 | -15.67 | -23.58 |
| With capping agent | 26.73 | -20.54 | -14.23 |

The comparison of the obtained results with the published literature with regard to b* values is presented in Table 3. According to the Table 3, minor differences were seen between the b* values. It can be interpreted as the suggested method can be an alternative for the cobalt aluminate fabrication.

**Table 3.** Comparison of the obtained results with the published literature with regard to b* values

| Technique | b* value | Reference |
|---|---|---|
| Hydrothermal | -23.28 | (Zhong et al., 2015) |
| Hydrothermal | -17.20 | (Chang et al., 2018) |
| Microwave hydrothermal processing | -11.72 | (Obata et al., 2011) |
| Organic ligand assisted hydrothermal | -15.62 | (Rangappa et al., 2007) |
| Ultrasonic-assisted hydrothermal | -23.58<br>-14.23 | (Dumanli and Derun, 2022)<br>This work |

## XRD Results

In accordance with the XRD analysis, the fabricated powders were defined as Spinel with the chemical formula of $CoAl_2O_4$ and both of the powders matched as powder diffraction file number of 01-082-2251. As it can be observed in Figure 1, the characteristic peaks (h k l [$d_{spacing}$]) were achieved at the $2\theta$ of 19.07° (1 1 1 [4.65 Å]), 31.08° (2 2 0 [2.88 Å]), 36.63° (3 1 1 [2.45Å]), 44.55° (4 0 0 [2.03Å]), 55.33° (4 2 2 [1.66Å]), 58.96° (5 1 1 [1.57Å]), and 64.83° (4 4 0 [1.44Å]).

When sonochemical synthesis is compared with conventional hydrothermal synthesis, it can be deduced that there is no negative effect on the powder crystal structure. XRD patterns of the synthesized $CoAl_2O_4$ powders can be seen in Figure 1.

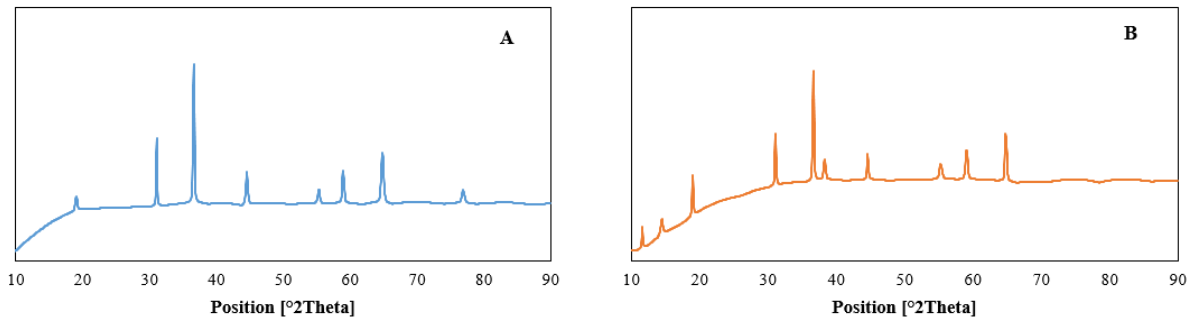


**Figure 1.** XRD patterns of the synthesized $CoAl_2O_4$ powders
(A: without capping agent; B: with capping agent)

## SEM Results

The surface morphologies of the fabricated $CoAl_2O_4$ powders are shown in Figure 2. When the SEM images of the samples are examined, angular and orbicular shaped distributions of the particles can be observed. In the images resulting from both synthesis methods, conglomeration is viewed in the structure and it is thought that this situation arises due to the hydrothermal synthesis circumstances (Dumanli and Derun, 2022). Also, uniform distribution does not exist because of the conglomerated particles. While sonochemical synthesis provides a reduction in particle size compared to the traditional hydrothermal method, the contribution of the capping agent included to the structure also led to a decrement in particle size and the size distribution was reached in the range of 74-88 nm.

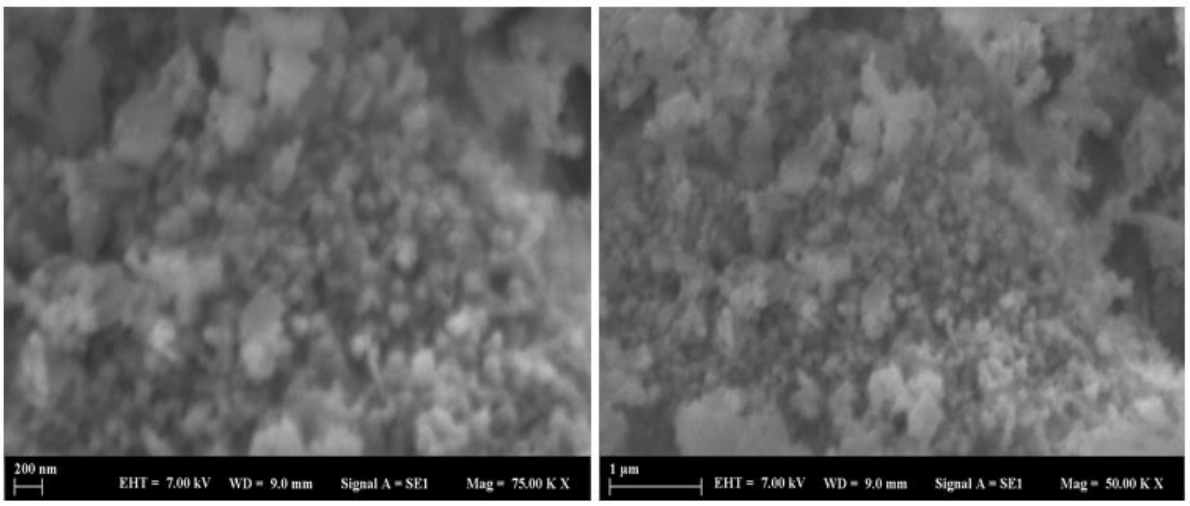


**Figure 2.** The surface morphologies of the fabricated $CoAl_2O_4$ powders (a) without capping agent and (b) with capping agent

## CONCLUSION

Spinel structures, which have industrial importance with their functionality, can be fabricated by different methods. In this research, in addition to the traditional hydrothermal synthesis method of cobalt aluminate of the spinel structures, the contribution of ultrasound and capping agent CTAB was investigated. The ultrasonic effect on the reaction medium allowed the synthesis of nanoparticles in smaller particle size without distorting the crystal structure of the powder. With respect to the XRD analysis results, the single-phase Co-spinel structure was achieved with the characteristic peaks obtained. In the colorimetric analysis results, although consistent results were acquired in terms of brightness, the b* value, which symbolizes the cobalt aluminate blue performance of the synthesized powder, decreased in the CTAB capped powder produced. SEM results revealed that sonochemical synthesis and CTAB addition reduced the particle size in the samples obtained, but they comprises agglomerated particles that could be caused by the hydrothermal method. The obtained results have exhibited that the use of capping agents will improve the fabricated compounds characteristic and they can be suitable to use with the novel technology of ultrasound.

## ACKNOWLEDGEMENTS

This research was supported by the Coordination Unit for Scientific Research Projects, Nisantasi University (project no BAP00009).

## REFERENCES

Baseri J, Naghizadeh R, Rezaie HR, Golestanifard F, Golmohammad M 2020. A comparative study on citrate sol-gel and combustion synthesis methods of $CoAl_2O_4$ spinel. International Journal of Applied Ceramic Technology, 17: 2709-2715.

Chang Y, Feng T, Wu C, Chen Y, Ke K, Liu Y, Wang H, Dong S 2018. Controlled synthesis of blue spherical $CoAl_2O_4$ pigment powder in Pickering emulsion assisted with a hydrothermal process. Advanced Powder Technology, 29: 1222-1229.

Chen ZZ, Shi EW, Li WJ, Zheng YQ, Zhuang JY, Xiao B, Tang LA 2004. Preparation of nanosized cobalt aluminate powders by a hydrothermal method. Materials Science and Engineering: B, 107: 217-223.

D'Ippolito V, Andreozzi GB, Bosi F, Hålenius U 2012. Blue spinel crystals in the $MgAl_2O_4$-$CoAl_2O_4$ series: Part I. Flux growth and chemical characterization. American Mineralogist, 97: 1828-1833.

Dumanli FTS, Derun EM 2022. A comparative study of ultrasonic-assisted methods to synthesise spinel ($CoAl_2O_4$) nanoparticles. Ceramics International, 48: 19047-19055.

El-Said Bakeer D 2020. Investigation on optical, dielectric, and magnetic properties of $CoAl_{2-x}Fe_xO_4$ nanoparticles. Journal of Superconductivity and Novel Magnetism, 33: 1789-1801.

Ferraz ISB, Castro TJ, Mantilla J, Coaquira JAH, Garg VK., Oliveira AC, Jr AF, Morais PC, da Silva SW 2021. Structural, optical and magnetic properties of CoAlxFe2-xO4 nanoparticles prepared by combustion reaction method. Journal of Alloys and Compounds, 887: 1-9.

Gao Y, Chang H, Wu Q, Wang H, Pang Y, Liu F, Zhu H, Yun Y 2017. Optical properties and magnetic properties of antisite-disordered $Ni_{1-x}Co_xCr_2O_4$ spinels. Transactions of Nonferrous Metals Society of China, 27: 863-867.

Gurugubelli TR, Babu B, Yoo K 2021. Structural, optical, and magnetic properties of cobalt-doped $ZnAl_2O_4$ nanosheets prepared by hydrothermal synthesis. Energies, 14: 1-9.

Kaplan A, Kipcak AS, Senberber FT, Derun EM, Piskin S 2015. A low-temperature, environment-friendly approach to the synthesis of magnesium borates using magnesium waste scraps. Main Group Metal Chemistry, 38: 99-110.

Kim JH, Son BR, Yoon DH, Hwang KT, Noh HG, Cho WS Kim US 2012. Characterization of blue $CoAl_2O_4$ nano-pigment synthesized by ultrasonic hydrothermal method. Ceramics International, 38: 5707-5712.

Naseri MG, Saion EB, Ahangar HA, Hashim M, Shaari AH 2011. Simple preparation and characterization of nickel ferrite nanocrystals by a thermal treatment method. Powder Technology, 212: 80-88.

Obata S, Kato M, Yokoyama H, Iwata Y, Kikumoto M, Sakurada O 2011. Synthesis of nano $CoAl_2O_4$ pigment for ink-jet printing to decorate porcelain. Journal of the Ceramic Society of Japan, 119: 208-213.

Phan CM, Nguyen HM 2017. Role of capping agent in wet synthesis of nanoparticles. The Journal of Physical Chemistry A, 121: 3213-3219.

Rajabi M, Kharaziyan P, Montazeri-Pour M 2019. Microwave-assisted processing of cobalt aluminate blue nano-ceramic pigment using sol–gel method. Journal of the Australian Ceramic Society, 55: 219-227.

Rangappa D, Naka T, Kondo A, Ishii M, Kobayashi T, Adschiri T 2007. Transparent $CoAl_2O_4$ hybrid nano pigment by organic ligand-assisted supercritical water. Journal of the American Chemical Society, 129: 11061-11066.

Senberber FT, Ozdemir OD 2020. Effect of synthesis parameters on the color performance of blue $CoAl_2O_4$ ceramic pigment. Russian Journal of Inorganic Chemistry, 65: 2020-2027.

Tan L, Wang Q, Cheng Z, Hu Z, Liu K, Wang Y, Chang Q 2021. Research on the low-temperature synthesis of cobalt aluminum spinel type blue pigments. Journal of Alloys and Compounds, 864: 1-14.

Yoneda M, Gotoh K, Nakanishi M, Fujii T, Konishi Y, Nomura T 2019. Solid-state synthesis and characterization of cobalt blue core-shell pigment particles. Journal of the American Ceramic Society, 102: 3468-3476.

Zhong X, Lu X, Cao C, Chen Y, Shen H 2015. Diethanolamine controlled hydrothermal synthesis of submicron spherical $CoAl_2O_4$ pigments. Chinese Journal of Materials Research, 29: 542-548.

## *ORAL PRESENTATION*

# Antimicrobial peptides from *Hermetia illucens*: An Alternative to Antibiotics in Animals

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## Abstract

Antimicrobial peptides (AMPs) are a type of effector molecule used by the innate host immune system to protect against pathogen invasion. Most AMPs do not cause bacteria to acquire resistance, they can be utilized as an alternative to antibiotics, helping to build a new generation of antimicrobials. Because of their vast variety, insects are potential prolific producers of new AMPs. *Hermetia illucens* (The black soldier fly; BSF) is a scavenger and can survive in the presence of many bacteria which makes BSF among the most potent resource for AMP detection. The discovery, functional analysis, and medicinal development of AMPs from *Hermetia illucens* to replace antibiotics is in its early phases. In this mini-review, general information on AMPs of BSF has been addressed and the mechanics of their synthesis and interactions, as well as their potential medicinal effects in animal nutrition, is discussed.

**Keywords:** *Hermetia illucens*, antimicrobial peptides, an alternative to antibiotics, insects, black soldier fly.

**Discipline:** Department of animal nutrition and nutritional diseases

## Introduction

The growing global population and improved living standards in developing countries are associated with increased consumption and demand for a quality protein of animal origin (Kim et al., 2019). Antibiotics' discovery ushered in a golden age, livestock personals used them in animal ration irrationally to ensure a high yield of animal-derived protein sources and enhance production performance (Nhung et al., 2016). On the other hand, antibiotics in animal diets resulted in a drop in meat quality and the spread of medicine resistance among bacteria (Thakur and Panda, 2017). Eventually, humans will run out of antibiotics to treat infections, posing a threat to global public health (López-Gálvez et al., 2021). Many countries have banned the use of antibiotics in livestock due to negative consequences (Jones et al., 2017; Phillips, 2007; Bedford, 2000). As a result, extensive research is being conducted to identify safe antibiotic alternatives (El-Hack et al., 2020; Agnolucci et al., 2020).

Insects can be considered a sustainable source of animal feeds due to their nutritional profile (Makkar, 2018). Insects have a higher feed conversion efficiency than production animals and are better at utilizing the nutrients in their diet (Oonincx et al., 2019). Insects as feed, on the other hand, can help to reduce the environmental impact of vertebrate meat production indirectly (Makkar et al., 2014). *Hermetia illucens*, the black soldier fly (BSF) is a warm-weather (Spranghers et al., 2017) and harmless insect (Čičková et al., 2015) that is colonized quickly by rotten waste materials such as kitchen garbage (Green and Popa, 2012) or food scraps, manure (Lalander et al., 2013), and straw (Liu et al., 2021). BSF can survive in harsh environments, implying that it has an innate immune system capable of producing peptides (Choi et al., 2018) that protect against microorganisms (Elhag et al., 2017; Feng et al., 2020). Defensin-like peptide 4 (DLP4) was newly discovered in BSF larvae immune tissues and extracted. Methicillin-resistant *Staphylococcus aureus* infested mice were given 3-7.5 mg/kg of DLP4 resulting in an 80-100% of survival rate (Li et al., 2017). The DLP4 is a potential new nominee antimicrobial peptide (AMP) to counter MRSA infections, according to this discovery. BSF AMPs have been used as potent research material for decades and can replace antibiotics in poultry feed while the horizontal transmission of resistance genes in the environment also blocked to some extent (Chung and Khanum, 2017).

## Antimicrobial Peptide Diversity in Insects

The classification of identified AMPs is based on a secondary structure, which is very diverse and difficult to categorize. Insects account for 90% of all animals on planet Earth; whereas AMPs obtained from them account

for only about 10% of the over 2830 AMPs mentioned in the database of Antimicrobial Peptides (Li et al., 2017), implying that more AMPs could be found in insects (Yi et al., 2014).
In 1974, antibacterial AMPs were discovered in the hemolymph of immunized *Cecropia moth* pupae, *Hyalophora cecropia*, and in 1980, the *cecropin* type was purified from the hemolymph of immunized *Cecropia* moth pupae, *Hyalophora cecropia* which is another insect AMP (Hultmark et al., 1980). More than 150 AMPs have since been identified after being extracted and purified from insects (Yi et al., 2014). Insect AMPs are divided into four categories based on their structural features: (1) structural peptides with α-helical structure, (2) peptides high in glycine, (3) peptides high in proline, and arginine, and (4) peptides with a lot of cysteines (Yi et al., 2014). Most active AMPs have 20–50 residues and are produced by limited proteolysis from inactive precursor proteins (Wang and Lai, 2010). In different insect species, the biological properties of AMPs from the same classes or subclasses differ.
Cecropin A, for example, is an antibacterial and antifungal compound found in *Anopheles gambiae*; Cecropin A from silk moths, on the other hand, has only antibacterial activity and structural differences are the reason for this variability (Bulet et al., 2004). AMPs participate in wound healing and angiogenesis by acting as selective immunoregulators, as well as enhancing innate immune reactions as antimicrobial effects (Żyłowska et al., 2011).
In recent years, progress has been made to obtain AMPs from *Hermetia illucens* through the isolation and identification protocol (Elhag et al., 2017; Lee et al., 2020; Shin and Park, 2019). Defensin and cecropin AMPs were studied in greater depth than other classes of AMPs obtained from *Hermetia illucens* because of structural similarity or unique sequences. The most common AMPs in insects are defensins, which contain six conserved cysteines (Čeřovský and Bém, 2014). Park and Yoe (2017) investigated *cecropin* a new family of AMPs as an alternative to ampicillin from *Hermetia illucens* and its peptide *cecropin*-like peptide 1 (CLP1), which contains the N-terminal helix, inhibited *Escherichia coli* 50 times better activity than ampicillin. This finding suggested that *cecropin's* N-terminal primary structure is crucial for antibacterial activity. Various undiscovered structures having antimicrobial properties similar to *Hermetia illucens* in host defense systems need to be investigated as researchers consider the AMPs to have useful applications in the future (Shin and Park, 2019).

**Antimicrobial Peptides of BSF and their application in livestock**

The AMPs that are produced in BSF larvae have some beneficial properties, like better thermal stability, lower molecular weight, broad antibacterial spectra, and unique mode of action are just a few examples. A substantial antibacterial impact on drug-resistant microorganisms has been observed (Xia et al., 2021). As a part of insect immunity, AMPs prevent dangerous infections from invading the insect host (Harlystiarini et al., 2019). They are produced in the insect adipose cell, which is like the vertebrate liver, and then released in the hemolymph (Reichhart et al., 1992; Park et al., 2014). Presently, 57 genes coding for recognized AMPs has been discovered in BSFL larvae and adults (Moretta et al., 2020). Out of the 57 discovered genes, 53 have been discovered from the larvae stage, with 26 encoding for defensin AMPs; this is the maximum number documented for any insect (Vogel et al., 2018). Only the invasive harlequin ladybird has a comparable number of known AMP genes (52) (Vilcinskas et al., 2013). The BSFL is the first fly larvae with known AMP action towards *Helicobacter pylori*. Four AMPs with similar action were discovered in BSFL hemolymph (Alvarez et al., 2019). Their action was equivalent to metronidazole, a popular antibacterial used to cure *Helicobacter pylori* and other illnesses. Specific immunological recognition functions of BSF AMPs have also been demonstrated. Certain AMPs have an impact only on prokaryotic and sick eukaryotic cells, and these qualities can decrease the negative effects of therapeutic therapy (Driemeyer, 2016).

*In vitro* effective antibacterial activity has been established utilizing hemolymph isolates against bacteria *Helicobacter pylori*, *Escherichia coli*, *Salmonella* spp. (gram-negative bacteria), *Staphylococcus aureus*, *Bacillus subtilis* (gram-positive bacteria) and *Candida albicans* (yeast) (Choi et al., 2012; Vogel et al., 2018; Harlystiarini et al., 2019). Hemolymph removal can be accomplished in a series of processes. The acidic methanol extraction technique for extracting tiny compounds while inactivating and aggregating bigger molecules and lipid isolation using chloroform and ethyl acetate (Park et al., 2014; Vogel et al., 2018). The hemolymph of BSFL isolated with methanol, when matched to chloroform-isolate, displayed better antibacterial efficiency (Choi et al., 2012; Park et al., 2014; Harlystiarini et al., 2019). Methanol-isolated hemolymph inhibited gram-negative bacteria, gram-positive bacteria, and yeast at a lesser dosage 12.5 or 25 mg/mL vs >100 mg/mL (Park et al., 2014). Furthermore, Choi et al. (2012) found that methanol-isolated hemolymph was more efficient after 12 hours against notorious bacteria, and yeast than chloroform-isolated hemolymph. Differential reactions of hemolymph isolate to chloroform and methanol support the notion that the antimicrobial effect is derived from short peptides, most likely AMPs. Although the hemolymph component seems to be best for identifying antimicrobial activity *in vitro*, research also demonstrates that BSFL-derived components display comparable effectiveness without additional fractionation. Furthermore, when gastrointestinal processing was replicated, activity was still preserved. For instance, *C. perfringens*

proliferation was greatly suppressed by BSFL protein meal breakdown (Dong et al., 2021a). Digested BSFL protein meal and chitin-rich powder were evaluated against *C. difficile* and greatly lowered the detrimental effect of *C. difficile* toxin A on layers of cell mucosa (Dong et al., 2021b). These findings support the notion that the components generated by a commercial insect (AMP) could bestow antibacterial action and improve immune responses against pathogens' challenges when offered as part of an overall nutritional plan. *In vivo* AMP function has been reported by Lee et al. (2018) in broiler chicks, inclusion of BSFL (1, 2, and 3 %) in the whole diet reflected in a spike in the amount of CD4+ cells, elevated lysozyme activity and enhanced chick life expectancy against *Salmonella Gallinarum* (67, 75, and 85 %, respectively) vs chicks fed no BSFL (50 % survival). Moreover, Lei et al. (2019) conducted a trial in Beagle dogs by serving 1 and 2 % of defatted BSFL meal for six weeks before being exposed to *E. coli* lipopolysaccharide showed higher antioxidative and anti-inflammatory capability than control dogs provided no BSFL diet. A study found that using prepupa meal to substitute an adequate percentage of soy, fish, and plasma protein meal had no negative impacts on growth performance, serum indices, diarrhea rate, or nutritional digestibility in weaned piglets given BSF larva food (Biasato et al., 2019). Moreover, when BSF larvae nourished on kitchen garbage were utilized to substitute fish and soy meal, the nutritional digestibility, growth performance, and immunological capability of weaned piglets were increased (Spranghers et al., 2018).

BSFL might potentially influence bacterial populations in their feedstuffs (substrate) (Bessa et al., 2020), this has consequences for the utilization of polluted feedstuffs that would otherwise end up in the garbage. For example, BSFL grown in cattle dung, chicken dung, and "fecal waste" have been shown to reduce *Salmonella* spp. (Bessa et al., 2020). It is crucial to remember that AMP production and effectiveness are organism and food-specific and may be affected by other variables such as larval age and raising frequency (Kong et al., 2019; De Smet et al., 2021). The continuous and verifiable antibacterial activity of different BSFL fractions calls for more research into active ingredients and particular mechanisms of action. To present, five types of AMPs from BSFL hemolymph have been recognized: 1) cecropins, 2) attacins, 3) defensins, 4) knottin-like proteins, and 5) diptericins. Gram-negative bacteria-binding proteins, Peptidoglycan recognition proteins, and phenoloxidases are three kinds of bacterial recognition proteins (Vogel et al., 2018).

**Antimicrobial Peptides' Mechanisms of Action**

The biological mechanism by which AMPs exert their effects is linked to the damage to the bacterial cell membrane. Cationic structure, present in most AMPs, binds to anionic lipopolysaccharides (LPS), teichoic acids, and lipoteichoic acids and forms ion channels or transmembrane pores resulting in termination of the cell membrane integrity allowing cell contents to leak out and kill the cells (Epand and Vogel, 1999). Membrane lipids are primarily targeted by the action of the AMPs, mechanically binding AMPs to lipids results in pore formation leading to bacterial membrane disruption (Jozefiak and Engberg, 2017). There are four different types of membrane lysis mechanisms including toroidal pore, carpet-like pore, barrel-stave pore, and unstructured ring pore. AMPs envelop the cell membrane in a carpet-like mode, according to the second model. A higher concentration of AMPs is required in this mechanism to lyse the cell membrane (Jozefiak and Engberg, 2017).

Sato and Feix (2006) for example, showed that continuous accumulation of cecropin-type AMPs at the bacterial lipid bilayer leads to the formation of a "carpet-like" peptide structure on the membrane surface. The membranes are dissolved by the detergent-like lytic properties of this "carpet-like" structure. In the barrel stave model, peptides combine with the cell envelope and enter the hydrophobic core of the phospholipid bilayer, resulting in leakage of intracellular substances and a decrease in membrane potential. Ceratotoxins (Bessin et al., 2004) and amphotericin B (Yamamoto et al., 2019) are examples of AMPs that damage membranes through this mechanism, resulting in cell death. The fourth mechanism involves cell envelope damage caused by the formation of "unstructured ring pores," in which AMPs form a ring-like structure that can attach to the membrane at various angles (Duclohier, 2002).

Some AMPs can cross cell membranes spontaneously, interact with intracellular molecules, and disrupt intracellular metabolic processes in addition to causing membrane damage. Furthermore, DNA could be a target. The N-terminal region of AMP lactoferricin has been found to bind to specific DNA domains, allowing it to regulate transcription (He and Furmanski, 1995).

The permeabilization or disruption of the cell envelope occurs when high concentrations of AMPs interact with biological membranes, resulting in an antimicrobial effect. Due to interactions with intracellular small molecules, low concentrations of AMPs can affect the microbial activity or viability (Nicolas, 2009).

## Conclusion

BSF larvae have a wide range of prospective uses for development and can be exploited as a resource due to their large biomass, rapid reproduction, adult harmlessness, extensive feeding, and fast conversion and absorption rates. The BSF is a revolutionary waste resource treatment technique that requires minimal energy and creates a lot of value while being environmentally friendly. Few investigations have shown that BSF-derived AMPs have antibacterial properties. AMPs' modes of action in insects have developed over hundreds of years, while AMPs themselves have stayed largely constant, implying that the possibility of bacterial resistance to BSF-derived AMPs is minimal. Therefore, BSF-derived AMPs might be viable antibiotic alternatives in the cattle sector, which is critical given the growing worldwide problem of antibiotic resistance. Resistance and pathogenic activity of AMPs derived from BSF is a novel topic and has not been studied in detail, except for some studies on it.

## Future Directions

Antibiotic overuse in several situations contributes to unsolved scientific issues related to drug tolerance in the environment because of antibiotic pollution. The detection and classification of AMPs from BSF have lately been expedited because of these agents' extraordinary superiority. Consequently, more study is needed into the possible use of these AMPs as antibiotic substitutes. Identification of pathogen receptors, different immunological induction techniques, mode of action against dangerous bacteria, and investigation of AMPs from BSF as an antibiotic substitute should be focused on in the future.

## References

Agnolucci M, Daghio M, Mannelli F, Secci G, Cristani C, Palla M, Giannerini F, Giovannetti M and Buccioni A 2020. Use of chitosan and tannins as alternatives to antibiotics to control mold growth on PDO Pecorino Toscano cheese rind. Food Microbiology, 92: 103598.

Alvarez D, Wilkinson KA, Treilhou M, Téné N, Castillo D and Sauvain M 2019. Prospecting peptides isolated from black soldier fly (Diptera: Stratiomyidae) with antimicrobial activity against Helicobacter pylori (Campylobacterales: Helicobacteraceae). Journal of Insect Science, 19: 17.

Bedford M 2000. Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. World's Poultry Science Journal, 56: 347-365.

Bessa LW, Pieterse E, Marais J and Hoffman LC 2020. Why for feed and not for human consumption? The black soldier fly larvae. Comprehensive Reviews in Food Science and Food Safety, 19: 2747-2763.

Bessin Y, Saint N, Marri L, Marchini D and Molle G 2004. Antibacterial activity and pore-forming properties of ceratotoxins: a mechanism of action based on the barrel stave model. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1667: 148-156.

Biasato I, Renna M, Gai F, Dabbou S, Meneguz M, Perona G, Martinez S, Lajusticia ACB, Bergagna S and Sardi L 2019. Partially defatted black soldier fly larva meal inclusion in piglet diets: effects on the growth performance, nutrient digestibility, blood profile, gut morphology and histological features. Journal of animal science and biotechnology, 10: 1-11.

Bulet P, Stöcklin R and Menin L 2004. Anti-microbial peptides: from invertebrates to vertebrates. Immunological reviews, 198: 169-184.

Čeřovský V and Bém R 2014. Lucifensins, the insect defensins of biomedical importance: the story behind maggot therapy. Pharmaceuticals, 7: 251-264.

Choi WH, Choi HJ, Goo TW and Quan FS 2018. Novel antibacterial peptides induced by probiotics in Hermetia illucens (Diptera: Stratiomyidae) larvae. Entomological Research, 48: 237-247.

Choi WH, Yun JH, Chu JP and Chu KB 2012. Antibacterial effect of extracts of H ermetia illucens (D iptera: S tratiomyidae) larvae against G ram-negative bacteria. Entomological research, 42: 219-226.

Chung PY and Khanum R 2017. Antimicrobial peptides as potential anti-biofilm agents against multidrug-resistant bacteria. Journal of microbiology, immunology and infection, 50: 405-410.

Čičková H, Newton GL, Lacy RC and Kozánek M 2015. The use of fly larvae for organic waste treatment. Waste management, 35: 68-80.

De Smet J, Vandeweyer D, Van Moll L, Lachi D and Van Campenhout L 2021. Dynamics of Salmonella inoculated during rearing of black soldier fly larvae (Hermetia illucens). Food Research International, 149: 110692.

Dong L, Ariëns RM, America AH, Paul A, Veldkamp T, Mes JJ, Wichers HJ and Govers C 2021a. Clostridium perfringens suppressing activity in black soldier fly protein preparations. LWT, 149: 111806.

Dong L, Tomassen MM, Ariëns RM, Oosterink E, Wichers HJ, Veldkamp T, Mes JJ and Govers C 2021b. Clostridioides difficile toxin A-mediated Caco-2 cell barrier damage was attenuated by insect-derived fractions and corresponded to increased gene transcription of cell junctional and proliferation proteins. Food & Function, 12: 9248-9260.

Driemeyer H 2016.Evaluation of black soldier fly (Hermetia illucens) larvae as an alternative protein source in pig creep diets in relation to production, blood and manure microbiology parameters. Stellenbosch: Stellenbosch University.

Duclohier H 2002. How do channel-and pore-forming helical peptides interact with lipid membranes and how does this account for their antimicrobial activity? Mini reviews in medicinal chemistry, 2: 331-342.

El-Hack A, Mohamed E, Alagawany M, Shaheen H, Samak D, Othman SI, Allam AA, Taha AE, Khafaga AF and Arif M 2020. Ginger and its derivatives as promising alternatives to antibiotics in poultry feed. Animals, 10: 452.

Elhag O, Zhou D, Song Q, Soomro AA, Cai M, Zheng L, Yu Z and Zhang J 2017. Screening, expression, purification and functional characterization of novel antimicrobial peptide genes from Hermetia illucens (L.). PloS one, 12: e0169582.

Epand RM and Vogel HJ 1999. Diversity of antimicrobial peptides and their mechanisms of action. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1462: 11-28.

Feng M, Fei S, Xia J, Labropoulou V, Swevers L and Sun J 2020. Antimicrobial peptides as potential antiviral factors in insect antiviral immune response. Frontiers in immunology: 2030.

Green TR and Popa R 2012. Enhanced ammonia content in compost leachate processed by black soldier fly larvae. Applied biochemistry and biotechnology, 166: 1381-1387.

Harlystiarini H, Mutia R, Wibawan IWT and Astuti DA 2019. In vitro antibacterial activity of black soldier fly (Hermetia Illucens) larva extracts against gram-negative bacteria. Buletin Peternakan, 43: 125-129.

He J and Furmanski P 1995. Sequence specificity and transcriptional activation in the binding of lactoferrin to DNA. Nature, 373: 721-724.

Hultmark D, STEINER H, RASMUSON T and BOMAN HG 1980. Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of Hyalophora cecropia. European Journal of Biochemistry, 106: 7-16.

Jones HE, O'Connell White K, Norman WV, Guilbert E, Lichtenberg ES and Paul M 2017. First trimester medication abortion practice in the United States and Canada. PloS one, 12: e0186487.

Jozefiak A and Engberg RM 2017. Insect proteins as a potential source of antimicrobial peptides in livestock production. A review. Journal of Animal and Feed Sciences, 26: 87-99.

Kim SW, Less JF, Wang L, Yan T, Kiron V, Kaushik SJ and Lei XG 2019. Meeting global feed protein demand: challenge, opportunity, and strategy. Annual Review of Animal Biosciences, 7: 221-243.

Kong H, Dong C, Jing W, Zheng M, Tian Z, Hou Q, Wang C, Cheng Y, Zhang L and Jiang X 2019. Transcriptomic insight into antimicrobial peptide factors involved in the prophylactic immunity of crowded Mythimna separata larvae. Developmental & Comparative Immunology, 98: 34-41.

Lalander C, Diener S, Magri ME, Zurbrügg C, Lindström A and Vinnerås B 2013. Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens)—From a hygiene aspect. Science of the Total Environment, 458: 312-318.

Lee J, Kim Y-M, Park Y-K, Yang Y-C, Jung B-G and Lee B-J 2018. Black soldier fly (Hermetia illucens) larvae enhances immune activities and increases survivability of broiler chicks against experimental infection of Salmonella Gallinarum. Journal of Veterinary Medical Science, 80: 736-740.

Lee K-S, Yun E-Y and Goo T-W 2020. Antimicrobial activity of an extract of Hermetia illucens larvae immunized with Lactobacillus casei against Salmonella species. Insects, 11: 704.

Lei X, Kim T, Park J and Kim I 2019. Evaluation of supplementation of defatted black soldier fly (Hermetia illucens) larvae meal in beagle dogs. Annals of animal science, 19: 767-777.

Li Z, Mao R, Teng D, Hao Y, Chen H, Wang X, Wang X, Yang N and Wang J 2017. Antibacterial and immunomodulatory activities of insect defensins-DLP2 and DLP4 against multidrug-resistant Staphylococcus aureus. Scientific reports, 7: 1-16.

Liu C, Wang C, Yao H and Chapman SJ 2021. Pretreatment is an important method for increasing the conversion efficiency of rice straw by black soldier fly larvae based on the function of gut microorganisms. Science of The Total Environment, 762: 144118.

López-Gálvez G, López-Alonso M, Pechova A, Mayo B, Dierick N and Gropp J 2021. Alternatives to antibiotics and trace elements (copper and zinc) to improve gut health and zootechnical parameters in piglets: A review. Animal Feed Science and Technology, 271: 114727.

Makkar H 2018. Feed demand landscape and implications of food-not feed strategy for food security and climate change. Animal, 12: 1744-1754.

Makkar HP, Tran G, Heuzé V and Ankers P 2014. State-of-the-art on use of insects as animal feed. Animal feed science and technology, 197: 1-33.

Moretta A, Salvia R, Scieuzo C, Di Somma A, Vogel H, Pucci P, Sgambato A, Wolff M and Falabella P 2020. A bioinformatic study of antimicrobial peptides identified in the Black Soldier Fly (BSF) Hermetia illucens (Diptera: Stratiomyidae). Scientific reports, 10: 1-14.

Nhung NT, Cuong NV, Thwaites G and Carrique-Mas J 2016. Antimicrobial usage and antimicrobial resistance in animal production in Southeast Asia: a review. Antibiotics, 5: 37.

Nicolas P 2009. Multifunctional host defense peptides: intracellular-targeting antimicrobial peptides. The FEBS journal, 276: 6483-6496.

Oonincx DG, van Broekhoven S, van Huis A and van Loon JJ 2019. Correction: feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLoS One, 14: e0222043.

Park SI, Chang BS and Yoe SM 2014. Detection of antimicrobial substances from larvae of the black soldier fly, H ermetia illucens (D iptera: S tratiomyidae). Entomological Research, 44: 58-64.

Park SI and Yoe SM 2017. A novel cecropin-like peptide from black soldier fly, Hermetia illucens: Isolation, structural and functional characterization. Entomological Research, 47: 115-124.

Phillips I 2007. Withdrawal of growth-promoting antibiotics in Europe and its effects in relation to human health. International journal of antimicrobial agents, 30: 101-107.

Reichhart J-M, Meister M, Dimarcq J-L, Zachary D, Hoffmann D, Ruiz C, Richards G and Hoffmann J 1992. Insect immunity: developmental and inducible activity of the Drosophila diptericin promoter. The EMBO journal, 11: 1469-1477.

Sato H and Feix JB 2006. Peptide–membrane interactions and mechanisms of membrane destruction by amphipathic α-helical antimicrobial peptides. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1758: 1245-1256.

Shin HS and Park S-I 2019. Novel attacin from Hermetia illucens: cDNA cloning, characterization, and antibacterial properties. Preparative Biochemistry and Biotechnology, 49: 279-285.

Spranghers T, Michiels J, Vrancx J, Ovyn A, Eeckhout M, De Clercq P and De Smet S 2018. Gut antimicrobial effects and nutritional value of black soldier fly (Hermetia illucens L.) prepupae for weaned piglets. Animal Feed Science and Technology, 235: 33-42.

Spranghers T, Noyez A, Schildermans K and De Clercq P 2017. Cold hardiness of the black soldier fly (Diptera: Stratiomyidae). Journal of economic entomology, 110: 1501-1507.

Thakur SD and Panda A 2017. Rational use of antimicrobials in animal production: a prerequisite to stem the tide of antimicrobial resistance. Current Science: 1846-1857.

Vilcinskas A, Mukherjee K and Vogel H 2013. Expansion of the antimicrobial peptide repertoire in the invasive ladybird Harmonia axyridis. Proceedings of the Royal Society B: Biological Sciences, 280: 20122113.

Vogel H, MŘller A, Heckel DG, Gutzeit H and Vilcinskas A 2018. Nutritional immunology: diversification and diet-dependent expression of antimicrobial peptides in the black soldier fly Hermetia illucens. Developmental & Comparative Immunology, 78: 141-148.

Wang Y-P and Lai R 2010. Insect antimicrobial peptides: structures, properties and gene regulation.

Xia J, Ge C and Yao H 2021. Antimicrobial peptides from black soldier fly (Hermetia illucens) as potential antimicrobial factors representing an alternative to antibiotics in livestock farming. Animals, 11: 1937.

Yamamoto T, Umegawa Y, Yamagami M, Suzuki T, Tsuchikawa H, Hanashima S, Matsumori N and Murata M 2019. The perpendicular orientation of amphotericin B methyl ester in hydrated lipid bilayers supports the barrel-stave model. Biochemistry, 58: 2282-2291.

Yi H-Y, Chowdhury M, Huang Y-D and Yu X-Q 2014. Insect antimicrobial peptides and their applications. Applied microbiology and biotechnology, 98: 5807-5822.

Żyłowska M, Wyszyńska A and Jagusztyn-Krynicka EK 2011. Antimicrobial peptides–defensins. Post. Mikrobiol, 50: 223-234.

*ORAL PRESENTATION*

# Gıda güvenliği ve büyük veri (big data)

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## Özet

Gıda güvenliği, gıda kaynaklı hastalıkları önleyerek, gıdaların işlenmesi ve depolanmasını ele alan bilimsel bir disiplindir. Gıda kaynaklı hastalıkların sağlık, ekonomik ve sosyal açıdan etkileri nedeniyle gıda güvenliği ile ilgili yapılan çalışmaların önemi giderek artmaktadır. Günümüzde gelişen ileri teknolojik uygulamalar sayesinde gıda güvenliği konusunda elde edilen verilerin doğru sonuçlarla hızlı bir şekilde analiz edilmesi sağlanmaktadır. Akıllı telefonlar, sosyal medya, nesnelerin interneti ve sosyal medyada üretilen veriler, yapılandırılmış veya yapılandırılmamış biçimde büyük miktarlarda veri akışı oluşturmaktadır. Gıda tedarik zincirinde bu verileri toplayan ve analiz eden büyük veri teknolojilerinin uygulama alanları günümüzde sınırlı sayıda da olsa gelişmekte ve kullanılmaktadır. Bu tür teknolojiler, veri toplama, depolama, işleme ve bilgi çıkarmada yeni yaklaşımlar öne sürmektedir. Bu derleme, gıda güvenliğinde büyük veri uygulamalarına ait son gelişmelere genel bir bakış sunmaktadır. İncelenen çalışmalar, büyük verinin gıda güvenliğinde uygulanabilirliğini ve tüm gıda tedarik zincirini etkilediğini göstermektedir. Ayrıca büyük veri analizi, gıda tedarik zinciri aktörlerini gerçek zamanlı kararlar almada, destekleme, izleme ve örnekleme stratejilerini tasarlamak için oldukça avantajlı sonuçlar elde edilebileceğini ortaya koymaktadır.
**Anahtar Kelimeler:** Büyük veri, Gıda güvenliği, Gıda tedarik zinciri, Tarımsal uygulamalar.

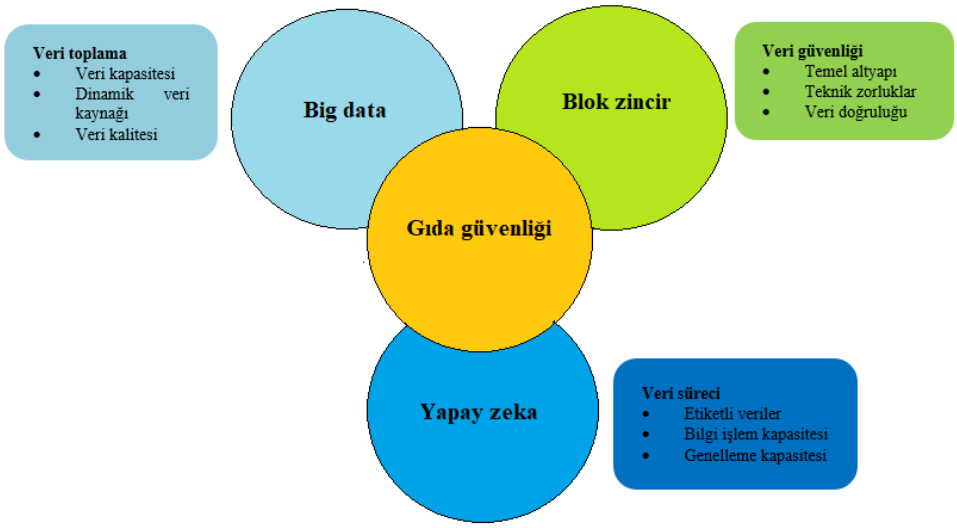
## Food safety and big data

**Abstract**

Food safety is a scientific discipline that deals with handling and storing food to prevent foodborne illnesses. Due to the health, economic and social effects of foodborne diseases, the importance of studies on food safety is increasing. Thanks to the advanced technological applications developed today, it is possible to quickly analyse the data on food safety with accurate results. The data produced in smartphones, social media, the internet of things, and social media creates large amounts of data flow in a structured or unstructured form. The application areas of big data technologies that collect and analyse this data in the food supply chain are developing and used today, albeit in limited numbers. Such technologies suggest new approaches to data collection, storage, processing, and information extraction. This review provides an overview of the latest developments in big data applications in food safety. The studies reviewed show the applicability of big data in food safety and its impact on the entire food supply chain. In addition, big data analysis reveals that very advantageous results can be obtained for designing strategies for supporting, monitoring, and sampling food supply chain actors in making real-time decisions.
**Keywords:** Big data, Food safety, Food supply chain, Agricultural applications.

## 1. GİRİŞ

Gıda güvenliği, üretimden tüketime kadar geçen süreçte gıdalardan kaynaklanabilecek riskleri önlemek, gıdanın kalitesini korumak ve tüm insanların sağlıklı ve aktif bir yaşam için güvenli, besleyici ve yeterli gıdaya sahip olmalarını sağlamaktır. Günümüzde dünyanın her yerinde artan gıda talebi, gıda sevkiyatı ve gıda işleme süreçlerinde uygulanan işlemler gıda güvenliği risklerini artırmaktadır. Küresel iklim değişikliklerinin giderek artması tarım uygulamalarını ve gıda üretimini etkilemektedir. Bu olumsuz etkileri en aza indirmek, gıda sahtekarlığını azaltmak, gıda güvenliğini, şeffaflığı ve izlenebilirliği sağlamak amacıyla gıda ve tarım sektörlerinde ileri teknolojilerin kullanımı giderek artmaktadır (Rowley, 2018). Bu teknolojilerin kullanımında birçok zorluk bulunmasına rağmen genellikle birçok araştırma alanında yaygın olarak büyük veri (big data), yapay zeka (AI) ve blok zinciri uygulamaları kullanılmaktadır (Şekil 1).




**Şekil 1.** Gıda güvenliği için büyük veri, yapay zeka (AI) ve blok zinciri uygulamaları ve zorlukları (Zhou ve ark., 2022).

## 2. BÜYÜK VERİ (BİG DATA)

Dünya Sağlık Örgütü (WHO) tarafından büyük veri (BD), hızla toplanan ve büyük miktarlardaki karmaşık veriler olarak tanımlanmaktadır (Ward ve Barker, 2013). Büyük verilerin depolanması terabaytlara ($10^{12}$ bayt), petabaytlara ($10^{15}$ bayt) ve hatta zettabaytlara ($10^{21}$ bayt) ulaşabilmekte olup, yüksek hacim, yüksek hız ve yüksek çeşitlilik gerektiren üç önemli özelliğe sahiptir (Katal ve ark., 2013). Geniş bir veri kaynağı dizisine dayanan büyük veri, seçilen örnekler ve belirli değişkenler hakkında değil, popülasyonlar ve geniş değişkenler hakkında ayrıntılı bilgi sunmaktadır (George ve ark., 2016). Gerçek zamanlı büyük verileri toplamak, depolamak ve analiz etmek için kullanılan teknolojiler sürekli bir evrim içindedir (Benjelloun ve ark., 2015). Bu nedenle, bulut bilişimdeki gelişmeler, bu verilerden bilgi eldesi, gizli kalıpları ortaya çıkarmak, anlamak ve büyük veri analizinin sonuçlarını kolayca paylaşmak için geniş fırsatlar sunmaktadır (Chen ve Zhang, 2014).

Dünya çapında 25 milyardan fazla iletişim sensörü, bilgisayar, akıllı telefon ve diğer cihazlar, 2025 yılına kadar 75 milyar cihaza ulaşması beklenen verilerini Nesnelerin İnterneti (IoT) aracılığıyla paylaşmaktadır (WHO, 2019). Tarımsal gıda güvenliği alanı söz konusu olduğunda, gıda işleme endüstrileri, gıda tüketim anketleri, hastane kayıtları ve jeo-uzamsal ve çevresel değişikliklerden gibi çok çeşitli kaynaklardan ve bölgelerden veriler toplanmaktadır (Marvin ve ark., 2017; WHO, 2019). Bu tür verilerin karmaşıklığı, gıda güvenliğinin artan karmaşıklığını yansıtmaktadır. Bu durum aynı zamanda gelişmiş analitik araçlar (örneğin, web kazıma, veri madenciliği, bilimsel, endüstriyel ve devlet veri tabanlarından metin çıkarma), gelişmiş kontrol önlemleri ve gıda güvenliğiyle ilgili kararlar için Büyük Veri ve Karar Destek Sistemleri (DSS) gibi büyük veri depolama ve karar verme teknolojilerinin kullanımını gerektirmektedir (WHO, 2019; Jin ve ark., 2020).

### 2.1. Veri Kaynakları ve Veri Toplama

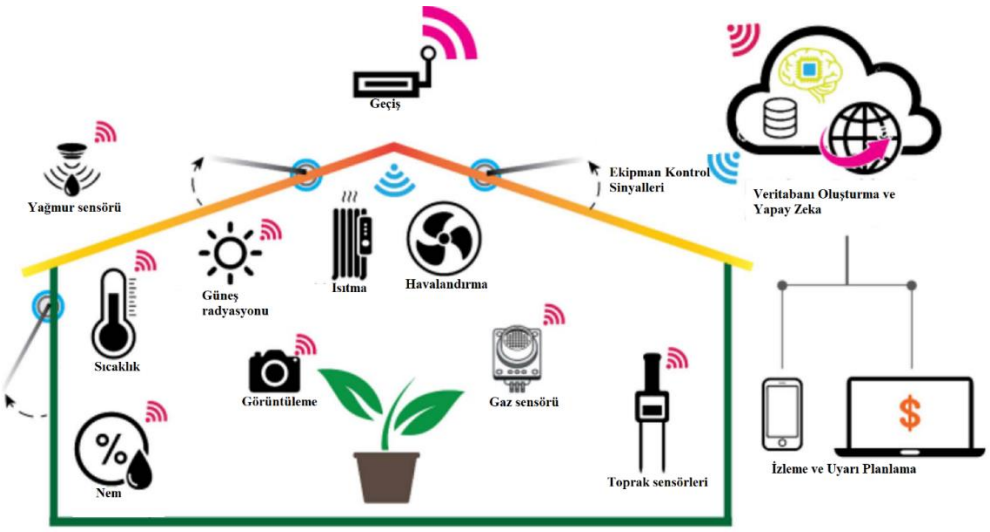
Çevrimiçi veri tabanları, internet, omik profilleme, sensörler, akıllı telefonlar ve sosyal medya gibi farklı veri kaynakları gıda güvenliği ile ilgili veri elde etmenin ana kanallarıdır (Marvin ve ark., 2017). Ayrıca gıda güvenliği ile ilgili verilerin toplanması için akıllı gözetim sistemlerinde yeni teknolojiler uygulanmaktadır. Bu tür teknolojilere örnek olarak video izleme (Subudhi ve ark., 2019), Nesnelerin İnterneti (IoT) teknolojisini kullanan sensörler ile taşınabilir cihazlar (Pal ve Kant, 2019), Coğrafi Bilgi Sistemi (CBS), uydu görüntüleri (Strawn ve ark., 2015) ve blok zinciri teknolojisi verilebilir (George ve ark., 2019) (Tablo 1).

**Tablo 1.** Gıda Güvenliği uygulamaları için büyük veri toplama seçenekleri (Marr, 2015; Tsai ve ark., 2016)

| Veri tipi | Büyük Veri yakalama mekanizması | Örnek Büyük Veri kaynağı | Büyük Veri Analitiği yaklaşımı | Büyük Veri Hedefi |
|---|---|---|---|---|
| Yapılandırılmış | İşlemsel (iş süreci/zincir etkileşimleri yoluyla) | Gıda tedarik zinciri, Ekonomi ve Pazar verileri | Sınıflandırma | Ölçüm |
| Yarı Yapılı | Derlenmiş (mevcut veri tabanlarından ve veri kümelerinden) | 3PL şirketleri, Devlet Kuruluşları, Düzenleyiciler, İklim tahminleri, Güvenlik açığı analizi | Sıralı Desenler Birliktelik Kuralları | |
| Yapılandırılmamış | *Deneysel (gıda güvenliği stratejisi ve politika modellerinden) *Sensörlerden saptanan *Kullanıcı tarafından oluşturulan (tüketicilerden veya gıda güvenliğinden doğrudan etkilenenlerden) | Tarla sensörü verileri Hava raporu Tüketici talepleri Sağlık/Beslenme indeksleri | Kümeleme/Veri Madenciliği Tahmine dayalı analitik | Deneme |

Sosyal medya, potansiyel bir gıda güvenliği verisi kaynağı olarak oldukça fazla ilgi görmektedir. Facebook, Twitter, YouTube gibi sosyal medya platformları, gıda güvenliği ile ilgili tartışmaları, görüşleri veya çevrimiçi anketleri toplamak için kullanılabilir (Soon, 2020b). Web madenciliği, sosyal medya verilerini toplamak için yaygın olarak kullanılır. Müşterilerin duygu ve düşüncelerini analiz ederek, sosyal medya verileri, kamu bilincini artırmak, kamu algılarını anlamak ve gıda güvenliği yönetiminde müşteri davranışlarını geliştirmek için verimli ve değerli bir yoldur (Yuan ve ark., 2020). Uydu görüntülerine ait veriler, mahsul büyümesini tespit etmek, hasat zamanını tahmin etmek ve tarım izleme sistemlerini iyileştirmek için kullanılabilir. Böylece tarımsal ürünlerin kalitesinin iyileştirilmesine yardımcı olur.

IoT, sensörlerin, cihazların, makinelerin ve bilgi işlem cihazlarının internet veya bir iletişim ortamı (örneğin Wi-Fi, Bluetooth ve RFID) aracılığıyla birbirine bağlanmasıdır. Gıda güvenliği konusundaki IoT çalışmalarının çoğunluğunu et, soğuk zincir ürünleri ve tarım ürünlerine ait uygulamalar ile ürünlerin sıcaklık, nem ve konumunu izlemek için kullanılan sensörlere ait araştırmalar oluşturmaktadır (Şekil 2).




**Şekil 2.** Sera yetiştirme ortamlarının IoT tabanlı izlenmesi ve kontrolü. Sera ortamı, çeşitli IoT tabanlı sensörler kullanılarak izlenir ve otomatik kontrol, tahrikli motorlar kullanılarak ısıtma, havalandırma veya pencerelerin açılması yoluyla uygulanır (Misra ve ark., 2022).

Gıda güvenliğinde CBS uygulamaları geniş çapta araştırılmıştır. Veri yakalama, işleme, entegrasyon, sorgulama, modelleme, analiz ve görselleştirme için güçlü CBS araçları geliştirilmektedir. CBS tabanlı cihazlardan elde edilen veriler, çoklu mekânsal özelliklerin analizine, eşzamanlı grafik görüntülemeye, farklı haritaların doğru bir şekilde örtüşmesine ve veri entegrasyonuna izin verir (Beni ve ark., 2011). Ayrıca, jeo-uzamsal verilerin toprak, bitki ve gıda araştırmalarından elde edilen verilerle entegrasyonu, hastalığın kökenini izlemeye ve hastalıkların yayılmasını önlemeye yardımcı olmaktadır (Beni ve ark., 2011).

İnternet veri alımı ve metin madenciliği yoluyla, gıda güvenliğini ve gıda sahtekarlığının erken uyarısını sağlamada dünya çapında çeşitli bilgi sistemleri geliştirilmiştir. Örneğin, bir Japon grubu, Google web sayfalarından anahtar kelime araması yoluyla gıda güvenliği tehlikesiyle ilgili bir veri tabanı oluşturmuştur (Maeda ve ark., 2005). Singapur Ulusal Çevre Ajansı, internetten ilgili gıda güvenliği içeriklerini kullanarak Singapur'da ortaya çıkan gıda güvenliği sorunlarını proaktif olarak izlemede bir Gıda Güvenliği Bilgi Sistemi (FoodSIS) kullanmıştır (Kate ve ark., 2014). Çin'deki gıda güvenliği konularının verimli bir şekilde değerlendirilmesine yardımcı olmak için medya ve hükümet Web sitelerinden gelen gıda güvenliği haberlerine dayalı olarak 2016 yılında gıda güvenliği bilgileri veri tabanı geliştirmiştir (Chen ve ark., 2016).

### 2.2. Veri Analizi

Veri analizi, veri işlemenin temelidir. Gıda güvenliğinde büyük veriden bilgi elde etmek için çeşitli yöntemler kullanılmış olup bunlar; içerik analizi, ekonometrik analiz, öneri sistemi ve makine öğrenimidir.

#### 2.2.1. İçerik analizi

İçerik analizi, nitel verileri (yani kelimeler, temalar, metin) araştırma yöntemidir. Temel gıda güvenliği gerçeklerini açıklığa kavuşturmak veya test etmek, gizli detayları ortaya çıkarmak ve eğilimleri tahmin etmek için uygulanır. Gıda güvenliği olayları (Liu ve ark., 2015), gıda sahtekarlığı vakaları (Tahkapaa ve ark., 2015), gıda noktası kontrolü ve numune inceleme verileri (Li ve ark., 2020) gibi çeşitli gıda güvenliği verileri üzerinde içerik analizi gerçekleştirilmektedir.

#### 2.2.2. Ekonometrik analiz

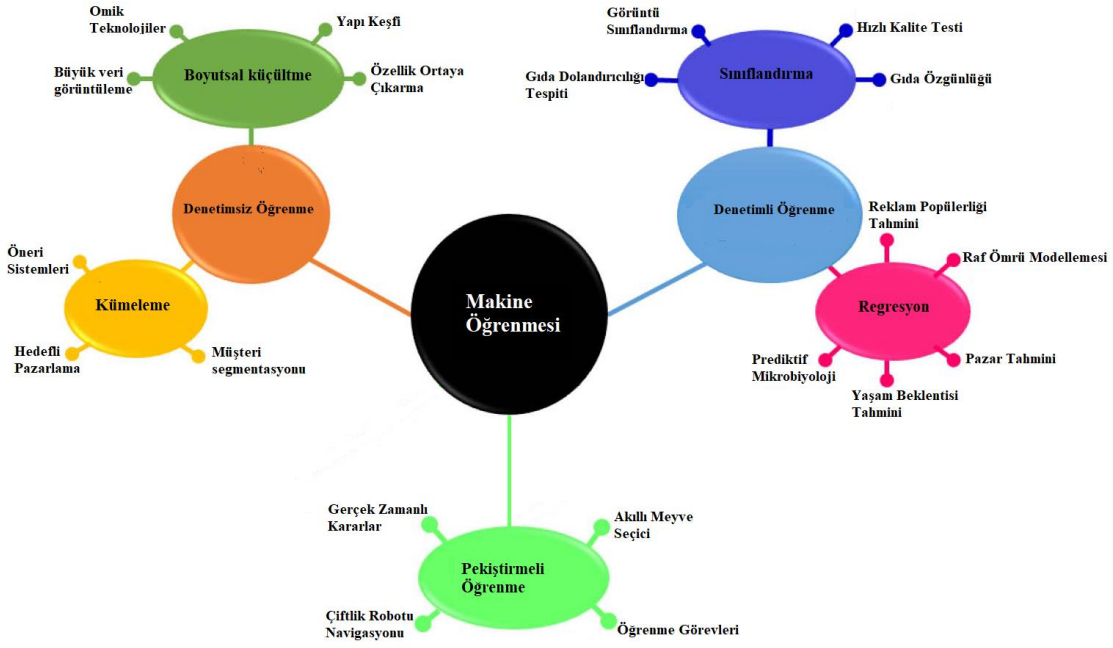
Ekonometrik analiz, gıda reddi raporlarına, gizli ticaret korumacılığı veya ithalat reddetme riskinin gıda güvenliği üzerindeki etkisi gibi sınır denetimi vakalarına dayalı olarak uluslararası gıda ticaretinde gıda güvenliği konularını incelemek için yaygın olarak kullanılmaktadır. Ayrıca, ithalat reddinin (yani gıda güvenliği sorunu) gelişmekte olan ülkelerin itibarı ve ekonomisi üzerindeki etkisini araştırmak için de ekonometrik analizin kullanıldığı çalışmalar da bulunmaktadır (Jouanjean ve ark., 2015; Beestermoller ve ark., 2018).

#### 2.2.3. Öneri sistemi

Öneri sistemi, tüketicilerin tercihlerini, ilgi alanlarını veya davranışlarını filtreleyerek tavsiyeleri hedeflemek için e-ticarette yaygın olarak kullanılmaktadır. Ancak bu sistemin gıda güvenliğindeki uygulamalarına ait çalışmalar sınırlı sayıdadır. Örneğin Singh ve ark. (2018), sığır eti tedarik zincirini kullanarak gıda güvenliği ve kalitesi ile ilgili konular hakkında bilgilendirmede sosyal medya ve büyük veri analitiğine dayalı bir öneri sistemi kullanmıştır.

#### 2.2.4. Makine öğrenimi (ML)

ML, gıda güvenliğinde kullanılan analiz yöntemlerinden biridir (Şekil 3). ML araçları, algoritmalar ve girdi verilerinden öğrenme yoluyla, karmaşık gıda güvenliği sorunlarıyla başa çıkmada belirlemek, tahmin etmek ve kararlar almak için yüksek doğrulukta modeller oluşturabilmektedir (Pollard ve ark., 2019). Gıda güvenliğinde en yaygın kullanılan ML yöntemleri, Bayes ağları (BN'ler) (Soon, 2020a), Sinir Ağları (NN'ler) (Zheng ve ark., 2020), Rastgele Ormanlar (RF'ler) ve Karar Ağaçları'dır (DT'ler) (Chang ve ark., 2020). Örneğin, Bouzembrak ve Marvin (2016), Gıda ve Yem için Hızlı Uyarı Sistemi (RASFF)'ni kullanarak gıda dolandırıcılık türünü tahmin etmek için bir BN modeli önermiştir. Chang ve ark. (2020), büyük verilerden (gıda elektronik faturalarından) değer çıkarmak için RF ve DT yöntemleri kullanılarak geliştirilen bir gıda güvenliği alarm sistemi belirlemiştir.




**Şekil 3.** Tarımsal gıda alanındaki Makine Öğrenmesi ve uygulamaları (Misra ve ark., 2022).

## 3. TARIMDA BÜYÜK VERİ

Tarım alanlarındaki azalma, su kirliliği, iklim değişikliği, sosyokültürel gelişme (örneğin, et proteini diyet tercihi), hükümet politikaları ve piyasa dalgalanmaları, toplum tarafından yeterli, güvenli ve besleyici gıdaya erişimde sorunlara neden olmaktadır (Gebbers ve Adamchuk, 2010). Günümüzde bu sorunlarla mücadelede tarımsal uygulamaların biyoteknoloji (Rahman ve ark., 2013), uzaktan algılama (Bastiaanssen ve ark., 2000), bulut bilişim (Hashem ve ark., 2015), ve Nesnelerin İnterneti (IoT) (Weber ve Weber, 2010) gibi dijital teknolojiler tarafından desteklenmesi "akıllı tarım" kavramını ortaya çıkarmıştır (Tyagi, 2016). Bununla birlikte tarla düzeyinde mahsul/çiftlik yönetimi için yeni bilgi ve iletişim teknolojilerinin oluşturulması, hassas tarım kavramını genişleterek, mevcut sistemi oluşan şartlara ve konum farklılıklarına göre karar verme sürecini geliştirmiştir (Kamilaris ve ark., 2016).

Tarımda büyük veri hem mikro (hava tahmini gibi) (Bendre ve ark., 2015) hem de makro ölçekte (örneğin çiftçilerin iş sağlığının izlenmesi) (Maugard ve ark., 2018) birçok uygulama alanı bulmaktadır. Büyük veri analitiğinin en büyük avantajı ise çiftçilerin, politikacıların ve tarımsal gıda tedarik zincirlerinde yer alan diğer aktörlerin kararlarını gerçek zamanlı bilgilere dayandırarak hem karar verme kapasitesini hem de karar verme performansını artırmasıdır (Lioutas ve ark., 2019).

Fiziksel nesnelerin birbirleriyle veya daha büyük sistemlerle bağlantılı olduğu iletişim ağını oluşturan IoT, tarımsal üretim ve tedarik zincirinin her aşamasında veri toplamaya yardımcı olmaktadır (Kim ve Laskowski, 2018) (Şekil 4). Blok zincir ise aynı anda birden fazla bilgisayar ağı üzerinde çalışan dağıtılmış bir veri tabanıdır. Dijital işlemlerin kaydını kriptografik olarak tutar, güvenli bir veri tabanında depolar ve bir ağın üyeleri arasında paylaşır. Gıda üretimi ve tedarik zincirlerindeki blok zinciri, çiftlikten son tüketiciye kadar gıda güvenliğini sağlamada şeffaflığı ve kontrolü artırabilir (Tablo 2). Ek olarak, çiftçiler IoT platformunun ve hassas çiftçiliğin benimsenmesi yoluyla mahsul sağlığını izlemek için blok zinciri benimseyebilirler (Jin ve ark., 2020).




**Şekil 4.** Tarımsal gıda endüstrisinde IoT uygulaması (Misra ve ark., 2022).

**Tablo 2.** Gıda güvenliğinde blok zinciri uygulamaları

| Gıda türü | Uygulamalar | Referans |
|---|---|---|
| Bitkisel kaynaklar | Pestisit gibi işlemlerin kaydı | Verdouw ve ark., 2013; Leng ve ark., 2018 |
| Hayvan yemleri | Çevre izleme, antibiyotik izleme | Garnero ve ark., 2018; Fernández-Cisnal ve ark., 2018 |
| Güvenlik kontrolü | İzleme sistemi | Coward, 2016; Tian, 2016 |

Tarım-gıda güvenliği ve kalitesi ile ilgili verileri toplamak ve bunların analizi için Wang (2019), büyük veri füzyon modeli kullanmıştır. Çalışmada, geliştirilen bu yeni modelin geleneksel modellerden daha avantajlı olduğu belirlenmiştir. Kamble ve ark. (2020), IoT, büyük veri ve blok zincirin füzyon uygulamalarının veri odaklı sürdürülebilir bir tarım-gıda tedarik zinciri haline gelebileceğini bildirmiştir. Ayrıca büyük veri uygulamaları ile çevresel faktörler arasında patojenlerin veya kirleticilerin varlığı değerlendirebilmektedir (Armbruster ve MacDonell, 2015). İklim değişikliği sonuçlarından ve çiftlik düzeyinde mahsul koşullarının izlenmesinden elde edilen veriler sayesinde ürün gıda-tedarik zincirine girmeden önce aflatoksin açısından yüksek potansiyel riskleri belirlenebilmektedir (Battilani ve ark., 2016). Bununla birlikte akıllı tarımdaki büyük veri uygulamalarının sosyo-ekonomik zorlukları da bulunmaktadır. Yukarıda bahsedilen çalışmalara göre, büyük veri uygulamalarının çoğu, hedeflenen kullanıcılar tarafından tam olarak benimsenmemiş olup tarımla ilgili riskleri yeterince ele alınmadıkları için gelişme aşamasında olup sınırlı bir kapsama sahiptir (Wolfert ve ark., 2017).

## 4. GIDA TEDARİK ZİNCİRLERİNDE (FSC) BÜYÜK VERİ ANALİTİĞİ (BDA)

Günümüzde artan küreselleşme, dünya nüfusunda devam eden bir genişleme ve teknolojilerdeki yeniliklerle, gıda tedarik zinciri daha karmaşık ve savunmasız hale gelmiştir (Mahroof ve ark., 2021). Meydana gelen sorunların çözümünde gıda güvenliği risk değerlendiricileri, büyük veri ve karar destek sistemleri (DSS) gibi web tabanlı teknolojilere artan bir ilgi göstermektedir (Power ve Kaparthi, 2002). DSS, bilgi ve yönetim sistemlerinin belirli bir alandaki problemi keşfetmek ve analiz etmek için veri ve model bilgisini kullanan bilgisayar tabanlı bir karar verme sistemidir (Goswami ve Barua, 2007). Büyük veri ve DSS, teknolojiyi kullanarak yapılandırılmamış ve yarı yapılandırılmış karmaşık sorunları çözmek ve gıda güvenliği risk değerlendirmesinde yeni veriye dayalı yaklaşımları daha da geliştirmek için bir geçit görevi görmektedir (Jin ve ark., 2020) (Tablo 3).

**Tablo 3.** Gıda zincirinde büyük veri (Ji ve ark., 2017)

| Etki Alanları | | Büyük verinin kaynağı | Değer Yaratma Faaliyetleri | Zorluklar |
|---|---|---|---|---|
| Gıda Tedarik Zinciri | Gıda Tüketimi | Perakendeciler<br>Tedarikçiler<br>Devlet<br>Üçüncü kişi veri sağlayıcıları<br>Müşteriler | Geliştirilmiş ürün tasarımı ve üretimi<br>Etkili depolama işlemleri<br>Hedefli pazarlama ve satış<br>Fiyat şeffaflığı sunmak<br>Satış sonrası hizmetlerin geliştirilmesi | Gizlilik kaygısı<br>Uyuşmazlık<br>Teknik zorluklar |
| | Gıda Lojistiği | Devlet<br>Ulaşım Operatörleri<br>Bireyler<br>Üçüncü kişi veri sağlayıcıları | Teknoloji yatırımları ve inovasyon<br>Daha akıllı ve daha hızlı karar verme<br>Müşteriler için en uygun deneyimi sunmak | Maliyet ve gizlilik kaygıları<br>Teknik zorluklar<br>Mevcut verilerin kapsamı ve kalitesi |

FSC, aksamaların etkilerine karşı savunmasızdır. Bu nedenle görünürlük, şeffaflık (Kumar ve Ganguly, 2021) ve veri bütünlüğü gibi özellikler FSC'de son derece önemli ve kullanışlıdır (Rejeb ve ark., 2020). Bu özellikleri sağlamak için FSC'de BDA'nın uygulanması gereklidir (Tasnim, 2020). BDA, sürdürülebilir tedarik zinciri operasyonlarını sağlamak için verimli veri toplama ve kayıt işlemi sağlamaktadır (Kamilaris ve ark., 2017).

Kaynak israfının en aza indirilmesi, gıda tedarik zincirinde oldukça önemli bir konudur. BD'nin görselleştirme yetenekleri, gıda tedarik zincirlerinin izlenebilirliğini ve temel iş süreçlerinin görünürlüğünü artırabilir ve bu sayede gıda israfını azaltabilir. Li ve Wang (2017), gıda tedarik zincirinin her aşamasında sıcaklığı ölçen ve kalite kontrollerini sağlayarak tüketime uygun olmayan (örneğin, raf ömründeki azalma, bozulma riski) gıda ürünlerini belirleyen BD tabanlı bir sistem geliştirmiştir. Bu artırılmış kontrol sayesinde, perakendecilerin ve üreticilerin tedarik zincirindeki gıda kaybı ve israfı önlenebilmiştir. BD araçlarının bir başka yararı da şeffaflıktır. Ürünler tedarik zincirinden geçtiğinde, gıda ürünlerinin mevcut raf ömürlerine göre fiyatlandırılmasında da atıkla ilgili etkili kararlar alınabilmektedir (Li ve Wang, 2017). İklim değişikliğinin süt tedarik zincirindeki etkisini değerlendirmek amacıyla kimyasal kirletici özellikleri, mikrobiyal büyüme koşulları ve hava durumu raporları (van der Spiegel ve ark., 2012) için bilgi gereklidir. BD tarafından desteklenen DSS ile süt tedarik zincirinde önleyici tedbirler uygulanabilir, elde edilen veriler gerçek zamanlı tüm taraflara iletilir ve hızlı bir şekilde çözüm için analiz edilebilir (Marvin ve ark., 2017).

Blok zincir uygulamaları gelişmiş izleme özellikleri, hatalı/bozuk gıdaların geri çağrılmalarında hızlı yanıt verilmesini sağlar. Ayrıca iyileştirilmiş tedarik zinciri şeffaflığı, dolandırıcılığı, yasa dışı faaliyetleri caydırır ve maliyeti düşürür (Kamath, 2018). Gıda tedarik zincirinin herhangi bir noktasında belirli bir gıdada yer alan hammaddelerin ve bileşenlerin kökenlerinin izlenmesine yardımcı olur. Örneğin *E.coli* salgını sonrasında tedarik zinciri şeffaflığını oluşturmak amacıyla tüm süreç boyunca kontamine gıdaları izlemede blok zincir uygulamıştır (Tan ve ark., 2018). Bir başka çalışmada, satın alınan hindileri takip etmek için pazara ilk giren ürünün ilk çıkmasını sağlamada blok zinciri tabanlı bir çözüm kullanılmıştır (Bumblauskas ve ark., 2020). Kumar ve Iyengar (2017), gıda sahtekarlığıyla mücadelede tam izlenebilirliği sağlamak amacıyla blok zinciri kullanan bir sistem uygulaması önermiştir.

Beni ve ark. (2011) tarafından yürütülen bir çalışmada, gıda güvenliği risklerinin değerlendirilmesini destekleyen CBS uygulamalarına ilişkin bir anket örneği bulunmaktadır. Çalışmada, Arena simülasyon aracılığıyla güvenlik açıklarını belirlemek ve gıda sistemlerinin biyolojik ajanlarla kontaminasyonuyla ilişkili riskleri ölçmek için CBS tabanlı bir yaklaşım geliştirilmiştir. Benzer şekilde CBS, Kanada gıda dağıtım sistemlerinde gıda güvenliği risklerinin mekansal-zamansal değerlendirmesi (Hashemi Beni ve ark., 2012), mahsullerin bölgesel bazda kontaminasyonunu tahmin etmek (Beni ve ark., 2011) ve Kaliforniya'da yetiştirilen marul ve ıspanakta meydana gelen *Escherichia coli* O157:H7 salgınlarını izlemek için kullanılmıştır (Cooley ve ark., 2007). Yapılan diğer örnek çalışmalara ise Tablo 4'de yer verilmiştir.

**Tablo 4.** Gıda endüstrisi ile ilgili incelemeler

| Kapsam | İlgili bağlam | Referans |
|---|---|---|
| Gıda güvenliğindeki büyük verilerin durumlarına genel bir bakış ve gelecekteki gelişmeler ve fırsatlar için çeşitli örnekler | Büyük veri | Marvin ve ark., 2017 |
| Gıda güvenliğinde büyük veri uygulamalarına ait güncel çalışmalar | Büyük veri | Jin ve ark., 2020 |
| IoT ve Büyük verilerin tarım tedarik zinciri modernizasyonundaki rolü, gıda endüstrisinde sosyal medya, gıda kalitesinin değerlendirilmesi ve gıda güvenliği | IoT, büyük veri ve yapay zeka | Misra ve ark., 2020 |
| Çiftçilere tarım ve gıda işlemede yardımcı olabilecek gelişmiş yapay zeka ve bilgisayarlı görü teknolojileri | AI ve bilgisayarlı görü | Kakani ve ark., 2020 |
| Gıda perakendeciliği için vizyon, metin, etkileşimli, analitik veya işlevsel tabanlı çözümler olarak kategorize edilen AI uygulamaları | Al | Friedlander ve Zoellner, 2020 |
| Meyve, sebze ve et gibi farklı gıda sistemlerinde akıllı telefon tabanlı görüntüleme sistemlerine ait güncel araştırmalar | Bilgisayarlı görü | Amani ve ark., 2020 |
| Gıda izlenebilirliğini geliştirmede, iş birliğini güçlendirmede, operasyonel verimliliği sağlamada ve gıda işlem süreçlerini basitleştirmede blok zincir teknolojisinin gıda tedarik zincirine faydaları | Blok zincir | Rejeb ve ark., 2020 |
| Blok zinciri teknolojisini ve geleneksel tedarik zinciri sistemine kıyasla gıda tedarik zincirinin güvenliğindeki uygulamaları | Blok zincir | Tse ve ark., 2017 |

## 5. MÜCADELELER

Gıda güvenliğinde büyük veri araçlarından yararlanabileceği açık olsa da sağladığı faydalardan yararlanmak için ele alınması gereken bir dizi zorluk bulunmaktadır. Gıda tedarik zinciri boyunca üretilen verilerle ilgili en büyük zorluklar, veri adaleti (bulunabilirlik, erişilebilirlik, birlikte çalışabilirlik ve yeniden kullanılabilirlik), veri kalitesi ve standardizasyon eksikliği ile ilgilidir. Bununla birlikte büyük veri sorunlarının ele alınması zorlu ve zaman alıcıdır. Makul sürede başarılı veri işleme ve analiz sağlamak için büyük bir hesaplama altyapısı gereklidir. Bulut bilişim birçok kuruluş tarafından bir çözüm olarak benimsenmiş olsa da bulut bilişim teknolojisi kullanılarak gıda güvenliğinde büyük veri üzerine yapılan araştırmalar henüz başlangıç aşamasındadır. Ölçeklenebilirlik, kullanılabilirlik, veri bütünlüğü, güvenlik, gizlilik ve yasal konular gibi çeşitli araştırma zorlukları tam olarak ele alınmamıştır.

Gıda güvenliğinde IoT teknolojisinin kullanımının sınırlı olarak alınmasına neden olabilecek zorluklardan biri, bugün IoT cihazları tarafından üretilen verilerin standartlaştırılmış iletişim protokollerinin olmaması nedeniyle yorumlanmasının, iletilmesinin ve paylaşılmasının zor olabilmesidir (Bouzembrak ve ark., 2019). Ek olarak, yetersiz donanım ve yazılım güvenliği gibi gıda güvenliğinde IoT uygulamalarında çeşitli sorunlar vardır. Gıda tedarik zincirindeki herhangi bir güvensiz IoT düğümü, tüm IoT sisteminin güvenliği için savunmasız bir nokta olabilir. Blok zincir teknolojisinin gıda güvenliğinde uygulanması umut verici, güvenli ve şeffaf olmasına rağmen karmaşıklığı nedeniyle hala olgunlaşmamış ve uygulanması zordur. Şu anda, gıda güvenliğindeki uygulaması izlenebilirlik ile sınırlıdır. Ancak veri bütünlüğü ve yönetim sistemleri gibi konulara hala daha fazla dikkat edilmesi gerekmektedir (Jin ve ark., 2020).

## 6. SONUÇ

Dünya çapında gıda güvenliği ile ilgili doğrudan ve dolaylı olarak büyük miktarlarda veri üretilmektedir. Elde edilen bu verilerle gıda güvenliğini öngörmek, izlemek ve kontrol etmek için ileri teknolojik uygulamalara ihtiyaç duyulmaktadır. Bu nedenle, veri karmaşıklığı göz önünde bulundurularak gıda güvenliğinde büyük veri analitiğinin etkin bir şekilde kullanılmasını sağlamada, büyük miktarda veri toplamak ve yönetmek için gelişmiş büyük veri analitik araçları gereklidir. Ancak büyük verinin tüm potansiyellerinden yararlanmak için bu yazıda açıklanan teknolojik zorlukların ve engellerin çoğu ele alınmalıdır. Özellikle tarımda büyük veri uygulamalarına ait araştırmalara hala ihtiyaç bulunmaktadır. Bu alanda daha fazla araştırmacıya uygulama, yenilik yapma ve çiftçilere bilgi aktarımı sağlamak için platformlar oluşturulmalıdır. Ayrıca, robot teknolojisi kullanımının geliştirilmesi, gıda kontrollerinde önemli bir rol oynayabilir, analiz ve planlamada artan makine yardımı, otonom bir siber-fiziksel döngüye dönüşmesine imkân sağlayabilir.

## KAYNAKLAR

Amani H, Badak-Kerti K, Mousavi Khaneghah A 2020. Current progress in the utilization of smartphone-based imaging for quality assessment of food products: a review. Crit Rev Food Sci Nutr, 1-13.

Armbruster W, MacDonell M 2015. Big data for big problems-climate change, water availability, and food safety. In Proceedings of EnviroInfo and ICT for sustainability ISSN 2352-538X.

Bastiaanssen W, Molden D, Makin I 2000. Remote sensing for irrigated agriculture: examples from research and possible applications. Agric. Water Manage, 46 (2): 137-155.

Battilani P, Toscano P, Van der Fels-Klerx HJ, Moretti A, Camardo Leggieri M, Brera C, Rortais A, Goumperis T, Robinson T 2016. Aflatoxin B1 contamination in maise in Europe increases due to climate change. Scientific Reports, 6: 24328.

Beestermoller M, Disdier AC, Fontagne L 2018. Impact of European food safety border inspections on agri-food exports: Evidence from Chinese firms. China Economic Review, 48: 66-82.

Bendre MR, Thool RC, Thool VR 2015. Big data in precision agriculture: weather forecasting for future farming. In: 2015 1st International Conference on Next Generation Computing Technologies (NGCT). IEEE, 744-750.

Beni LH, Villeneuve S, Leblanc DI, Delaquis P 2011. A GIS-based approach in support of an assessment of food safety risks. Transactions in GIS, 15(suppl. 1): 95-108.

Benjelloun FZ, Lahcen AA, Belfkih S 2015. An overview of big data opportunities, applications and tools. In: 2015 Intelligent Systems and Computer Vision (ISCV). IEEE.

Bouzembrak Y, Kluche M, Gavai A, Marvin HJP 2019. Internet of Things in food safety: Literature review and a bibliometric analysis. Trends in Food Science & Technology, 94:54-64.

Bouzembrak Y, Marvin HJP 2016. Prediction of food fraud type using data from Rapid Alert System for Food and Feed (RASFF) and Bayesian network modelling. Food Control, 61: 180-187.

Bumblauskas D, Mann A, Dugan B, Rittmer J 2020. A blockchain use case in food distribution: Do you know where your food has been? International Journal of Information Management, 52: 102008.

Chang WT, Yeh YP, Wu HY, Lin YF, Dinh TS, Lian I 2020. An automated alarm system for food safety by using electronic invoices. PLoS ONE, 15.

Chen, C.P., Zhang, C.Y., 2014. Data-intensive applications, challenges, techniques and technologies: a survey on big data. Inf. Sci. 275, 314–347.

Chen S, Huang D, Nong W, Kwan HS 2016. Development of a food safety information database for Greater China. Food Control, 65: 54-62.

Cooley M, Carychao D, Crawford-Miksza L, Jay MT, Myers C, Rose C, Keys C, Farrar J, Mandrell RE 2007. Incidence and tracking of *Escherichia coli* O157: H7 in a major produce production region in California. PloS One, 2(11): e1159.

Coward J 2016. Meet the visionary who brought blockchain to the industrial IoT. London, UK: IOT World News, 14.

Fernández-Cisnal R, García-Sevillano MA, García-Barrera T, Gómez-Ariza JL, Abril N 2018. Metabolomic alterations and oxidative stress are associated with environmental pollution in Procambarus clarkii. Aquat Toxicol, 205: 76-88.

Friedlander A, Zoellner C 2020. Artificial intelligence opportunities to improve food safety at retail. Food Protect Trends, 40: 272-278.

Garnero PL, Monferran MV, González GA, Griboff J, de Los Ángeles BM 2018. Assessment of exposure to metals, As and Se in water and sediment of a freshwater reservoir and their bioaccumulation in fish species of different feeding and habitat preferences. Ecotoxicol Environ Saf, 163: 492-501.

Gebbers R, Adamchuk V 2010. Precision agriculture and food security. Science, 327 (5967): 828-831.

George RV, Harsh HO, Ray P, Babu AK 2019. Food quality traceability prototype for restaurants using blockchain and food quality data index. Journal of Cleaner Production, 240: 118021.

George G, Osinga EC, Lavie D, Scott BA 2016. Big data and data science methods for management research. Acad. Manag. J., 59 (5): 1493-1507.

Goswami R, Barua P 2007. Web-Based decision support system: Concept and issues. Business Information Systems: Concepts, Methodologies, Tools and Applications, 300-314.

Hashem IAT, Yaqoob I, Anuar NB, Mokhtar S, Gani A, Khan SU 2015. The rise of "big data" on cloud computing: review and open research issues. Inform. Syst., 47: 98-115.

Hashemi Beni L, Villeneuve S, LeBlanc DI, Cote K, Fazil A, Otten A, McKellar R, Delaquis P 2012. Spatio-temporal assessment of food safety risks in Canadian food distribution systems using GIS. Spatial and Spatio-Temporal Epidemiology, 3(3): 215-223.

Ji G, Hu L, Tan HK 2017. A study on decision-making of food supply chain based on big data. J Syst Sci Syst Eng, 26(2):183-198.

Jin C, Bouzembrak Y, Zhou J, Liang Q, van den Bulk LM, Gavai A, Liu N, van den Heuvel LJ, Hoenderdaal W, Marvin HJP 2020. Big Data in food safety- A review. Current Opinion in Food Science, 36: 24-32.

Jouanjean MA, Maur JC, Shepherd B 2015. Reputation matters: Spillover effects for developing countries in the enforcement of US food safety measures. Food Policy, 55: 81-91.

Kakani V, Nguyen VH, Kumar BP, Kim H, Pasupuleti VR 2020. A critical review on computer vision and artificial intelligence in food industry. J Agric Food Res, 2: 100033.

Kamath R 2018. Food traceability on blockchain: Walmart's pork and mango pilots with IBM. The Journal of the British Blockchain Association, 1(1): 3712.

Kamble SS, Gunasekaran A, Gawankar SA 2020. Achieving sustainable performance in a data-driven agriculture supply chain: a review for research and applications. Int. J. Prod. Econ., 219: 179-194.

Kamilaris A, Gao F, Prenafeta-Boldú FX, Ali MI 2016. Agri-IoT: A Semantic framework for internet of things-enabled smart farming applications. Reston, VA, USA.

Kamilaris A, Kartakoullis A, Prenafeta-Boldu F 2017. A review on the practice of Big Data analysis in agriculture. Computers and Electronics in Agriculture, 143: 23-37.

Katal A, Wazid M, Goudar RH 2013. Big data: issues, challenges, tools and good practices. In: 2013 sixth international conference on contemporary computing (IC3). New York City, USA: IEEE, 404-409.

Kate K, Chaudhari S, Prapanca A, Kalagnanam J 2014. Food-SIS: A text mining system to improve the state of food safety in Singapore. Proceedings of the 20th ACM SIGKDD International Conference on Knowledge Discovery and Data Mining, New York, NY: ACM, 1709-1718.

Kim HM, Laskowski M 2018. Agriculture on the blockchain: Sustainable solutions for food, farmers, and financing. Supply Chain Revolution, Barrow Books. USA: SSRN Electronic Journal.

Kumar MV, Iyengar N 2017. A framework for Blockchain technology in rice supply chain management. Adv. Sci. Technol. Lett, 146: 125-130.

Kumar N, Ganguly KK 2021. External diffusion of B2B e-procurement and firm financial performance: role of information transparency and supply chain coordination. Journal of Enterprise Information Management, 34: 1037-1060.

Leng K, Bi Y, Jing L, Fu HC, Van Nieuwenhuyse I 2018. Research on agricultural supply chain system with double chain architecture based on blockchain technology. Future Generat Comput Syst, 86: 641-649.

Li D, Wang X 2017. Dynamic supply chain decisions based on networked sensor data: an application in the chilled food retail chain. Int J Prod Res, 55: 5127-5141.

Li D, Zang M, Li X, Zhang K, Zhang Z, Wang S 2020. A study on the food fraud of national food safety and sample inspection of China. Food Control, 116: 107306.

Lioutas ED, Charatsari C, La Rocca G, De Rosa M 2019. Key questions on the use of big data in farming: an activity theory approach. NJAS-Wageningen J. Life Sci., 100297.

Liu Y, Liu F, Zhang J, Gao J 2015. Insights into the nature of food safety issues in Beijing through content analysis of an Internet database of food safety incidents in China. Food Control, 51: 206-211.

Maeda Y, Kurita N, Ikeda S 2005. An early warning support system for food safety risks. Proceedings of the Annual Conference of the Japanese Society for Artificial Intelligence. Berlin, Springer, 446-457

Mahroof KA, Omar A, Rana NP, Sivarajah S, Weerakkody V 2021. Drone as a service (DaaS) in promoting cleaner agricultural production and circular economy for ethical sustainable supply chain development. Journal of Cleaner Production, 287:125522.

Marr B 2015. Big Data. John Wiley and Sons, Chichester: UK.

Marvin HJ, Janssen EM, Bouzembrak Y, Hendriksen PJ, Staats M 2017. Big data in food safety: an overview. Crit Rev Food Sci Nutr, 57: 2286-2295.

Maugard C, Bosson-Rieutort D, François O, Bonneterre V 2018. 1154 Big data and occupational health surveillance: use of French medico-administrative databases for hypothesis generation regarding occupational risks in agriculture. Occup. Environ. Med. 75: A121-A122.

Misra N, Dixit Y, Al-Mallahi A, Bhullar MS, Upadhyay R, Martynenko A 2020. IoT, big data and artificial intelligence in agriculture and food industry. IEEE Internet Things J, 1-19.

Misra NN, Dixit Y, Al-Mallahi A, Bhullar MS, Upadhyay R, Martynenko A 2022. IoT, big data, and artificial intelligence in agriculture and food Industry. IEEE Internet of Things Journal, 9: 6305-6324.

Pal A, Kant K 2019. Using blockchain for provenance and traceability in internet of things-integrated food logistics. Computer, 52: 94-98.

Pollard S, Namazi H, Khaksar R 2019. Big data applications in food safety and quality. In Encyclopedia of Food Chemistry. Edited by Melton L, Shahidi F, Varelis P. Academic Press, 356-363.

Power DJ, Kaparthi S 2002. Building Web-based decision support systems. Studies in Informatics and Control, 11(4): 291-302.

Rahman H, Sudheer Pamidimarri D, Valarmathi R, Raveendran M 2013. Omics: applications in biomedical, agriculture and environmental sciences. s.l. CRC PressI Llc, ISBN: 9781466562813.

Rejeb A, Keogh JG, Zailani S, Treiblmaier H, Rejeb K 2020. Blockchain technology in the food industry: a review of potentials, challenges and future research directions. Logistics, 4: 27.

Rowley MJ 2018. How blockchain is supporting climate action. https://newsroom.cisco.com/feature-content?articleId=1941325.

Singh A, Shukla N, Mishra N 2018. Social media data analytics to improve supply chain management in food industries. Transportation Research Part E: Logistics and Transportation Review, 114: 398-415.

Soon JM 2020a. Application of bayesian network modelling to predict food fraud products from China. Food Control, 107232.

Soon JM 2020b. Consumers' awareness and trust toward food safety news on social media in Malaysia. Journal of Food Protection, 83: 452-459.

Strawn LK, Brown EW, David JRD, Den Bakker HC, Vangay P, Yiannas F, Wiedmann M 2015. Big Data in food safety and quality. Food Technology, 69.

Subudhi BN, Rout DK, Ghosh A 2019. Big Data analytics for video surveillance. Multimedia Tools and Applications, 78: 26129-26162.

Tahkapaa S, Maijala R, Korkeala H, Nevas M 2015. Patterns of food frauds and adulterations reported in the EU rapid alert system for food and feed and in Finland. Food Control, 47: 175-184.

Tan B, Yan J, Chen S, Liu X 2018. The impact of blockchain on food supply chain: The case of Walmart. Smart Blockchain, 167-177.

Tasnim Z 2020. Disruption in global food supply chain (FSC) due to Covid-19 pandemic and impact of digitization through block chain technology in FSC management. European Journal of Business and Management, 12: 73-84.

Tian F 2016. An agri-food supply chain traceability system for China based on RFID & blockchain technology. In: 2016 13th international conference on service systems and service management (ICSSSM). New York City, USA: IEEE, 1-6.

Tsai CW, Lai CF, Chao HC, Vasilakos AV 2015. Big data analytics: a survey. J. Big Data, 2 (21): 1-32.

Tse D, Zhang B, Yang Y, Cheng C, Mu H 2017. Blockchain application in food supply information security. In: 2017 IEEE international conference on industrial engineering and engineering management (IEEM). New York City, USA: IEEE, 1357-1361.

Tyagi AC 2016. Towards a second green revolution. Irrigation Drainage, 65 (4): 388-389.

van der Spiegel M, van der Fels-Klerx HJ, Marvin HJP 2012. Effects of climate change on food safety hazards in the dairy production chain. Food Research International, 46(1): 201-208.

Verdouw C, Sundmaeker H, Meyer F, Wolfert J, Verhoosel J 2013. Smart agri-food logistics: requirements for the future internet. In: Dynamics in logistics. Cham, Switzerland, Springer, 247-257.

Wang K 2019. Design of agricultural product quality and safety big data fusion model based on blockchain technology. Advanced Hybrid Information Processing, 216-225.

Ward JS, Barker A 2013. Undefined by data: a survey of big data definitions. Computer Science, 1-2.

Weber RH, Weber R 2010. Internet of Things. ISBN 978-3-642-11709-1, Springer, New York, NY.

WHO 2019. https://www.who.int/docs/default-source/resources/digitalization-food -safety-and-trade-en.pdf?sfvrsn=a11a03b8_2.

Wolfert S, Ge L, Verdouw C, Bogaardt MJ 2017. Big data in smart farming - a review. Agric. Syst., 153: 69-80.

Yuan J, Lu Y, Cao X, Cui H 2020. Regulating wildlife conservation and food safety to prevent human exposure to novel virus. Ecosystem Health and Sustainability, 6: 1741325

Zheng C, Song Y, Ma Y 2020. Public opinion prediction model of food safety events network based on BP neural network. IOP Conference Series: Materials Science and Engineering, 719: 012078.

Zhou Q, Zhang H, Wang S 2022. Artificial intelligence, big data, and blockchain in food safety. Int. J. Food Eng., 18(1): 1-14.

## *ORAL PRESENTATION*

### Ağrı İli Merkezindeki İçme Sularında K, Mg ve Co Düzeylerinin Belirlenmesi

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**Özet**

İntrasellüler sıvıların en önemli elektrolitleri olan K (potasyum) ve Mg (magnezyum) hücrenin kimyasal işlevlerini sürdürebilmesi için gereken sıvı ortamı sağlamada görev alırlar. Co (kobalt) ise İz elementlerdendir ve B12 vitamininin yapısında bulunur. Ayrıca organizmada, demirin kullanımı ve tiroid hormonlarının sentezinde rol oynar. Bu çalışmanın amacı, Ağrı ilinde yer alan merkez mahallelerden toplanan içme sularında K, Mg ve Co düzeyinin belirlenmesidir. Bu amaçla, 2018 yılında Ağrı ili merkez mahallelerinde bulunan 50 farklı lokasyondan alınan su numuneleri daha önceden %1'lik $HNO_3$ çözeltisiyle yıkanmış 15 ml'lik falkon tüplere alındı, alınan numunelere %65'lik $HNO_3$ çözeltisi ilave edilerek analiz gününe kadar 4 $^oC$ de muhafaza edildi. İçme suyu numunelerinin analizleri AAS cihazı ile yapıldı. İçme suyu numunesi toplanan 13 mahalle arasında K ve Co düzeyleri açısından istatistiksel olarak ($p<0.05$) fark bulunmamıştır. En yüksek K değeri Mehmet Akif Ersoy Mahallesi, en düşük K değeri Cumhuriyet mahallesinden toplanan numunelerde belirlenmiştir. K seviyeleri 1.67 mg/L ile 4.43 mg/L arasındadır. K için TSE'de belirtilen müsaade edilen maksimum değer (12 mg/L) limitini aşan su yoktur. Bu çalışmada tespit edilen Co seviyeleri 0.002 mg/L ile 0.19 mg/L arasındadır. En yüksek Co düzeyi Sıtkiye mahallesi, en düşük Co düzeyi ise kazım Karabekir mahallesinden toplanan numunelerde tespit edilmiştir. Mg düzeyi yönünden Murat mahallesinden toplanan içme suyu numunelerinde Mg düzeyi diğer mahallere göre daha yüksektir. Magnezyum miktarları 3,15 mg/L ile 4,34 mg/L değerleri arasında değişmektedir. Magnezyum için TSE'de belirtilen müsaade edilecek maksimum değer (30 mg/L) limitini aşan su yoktur.

**Anahtar Kelimeler:** İçme Suyu, Ağrı ili, K, Mg, Co

### Determination of K, Mg and Co Levels in Drinking Water in The Center of Ağrı Provvince

**Abstract**

K (potassium) and Mg (magnesium), the most important electrolytes of intracellular fluids, take part in providing the necessary fluid environment for the cell to maintain its chemical functions. Co (cobalt) is one of the trace elements and is found in the structure of vitamin B12. It also plays a role in the use of iron and the synthesis of thyroid hormones in the organism. The aim of this study is to determine the levels of K, Mg and Co in drinking water collected from central neighborhoods in Ağrı. For this purpose, water samples taken from 50 different locations in the central neighborhoods of Ağrı province in 2018 were taken into 15 ml falcon tubes that were previously washed with 1% $HNO_3$ solution, 65% $HNO_3$ solution was added to the samples taken and kept at 4 $^oC$ until the day of analysis. Analysis of drinking water samples was done with AAS device. There was no statistically ($p<0.05$) difference in terms of K and Co levels among the 13 neighborhoods where drinking water samples were collected in Ağrı province. The highest K value was determined in Mehmet Akif Ersoy District, and the lowest K value was determined in the samples collected from Cumhuriyet district. K levels range from 1.67 mg/L to 4.43 mg/L. There is no water exceeding the maximum allowable value (12 mg/L) limit specified in TSE for K. Co levels detected in this study are between 0.002 mg/L and 0.19 mg/L. The highest Co level was found in Sıtkiye neighborhood and the lowest Co level was determined in the samples collected from Kazım Karabekir district. In terms of Mg level, the Mg level is higher in the drinking water samples collected from Murat district compared to other districts. Magnesium amounts vary between 3.15 mg/L and 4.34 mg/L. There is no water exceeding the maximum permissible value (30 mg/L) specified in TSE for magnesium.

**Keywords:** Drinking Water, Ağrı province, K, Mg, Co

## GİRİŞ

Su yaşamın temel yapı taşlarından birisidir. Su, bir besin maddesi olmasının yanında, içerisinde yer alan mineral ve bileşiklerle vücudumuzdaki her türlü biyokimyasal reaksiyonların gerçekleşmesinde önemli derecede etkin rol oynamaktadır. Vücudumuzun pH dengesinin korunmasından başlayarak, fizyolojik olayları gerçekleşmesi, besinlerin ve artık maddelerin ilgili yerlere taşınmasına kadar pek çok görevi bulunmaktadır (Akın ve Akın, 2007). Suyun kirlenmesinde en önemli etkenlerin başında suyun çözücü özelliği gelmektedir. Bu çözücü özelliği çözünen maddenin molekül yapısına bağlıdır. Su; alkol, şeker, üre gibi organik maddeleri çok iyi çözerken yağları ve bazı hidrokarbonları çözmez. Su, hidrolojik döngüde karşılaştığı bazı maddeleri çözerken sahip olduğu bazı özellikleri de değişir. Bu durum su kirliliğine neden olmaktadır (Solak ve Doğan, 2000). İçme suyunda belli seviyenin üzerinde olması istenmeyen kimyasallar kirletici adıyla bilinir. Kirleticiler, İnorganik, organik, pestisit ve dezenfektan olarak sınıflandırılabilirler (Van, 2009). Bu kirleticiler arasında inorganik sınıfta bulunan ve sağlık açısından ciddi tehdit oluşturan ağır metaller ve yüksek mineral düzeyleri, içme suyundaki konsantrasyonları bakımından izlenmesi gereken en önemlileridir. Bu minerallerden intrasellüler sıvıların en önemli elektrolitleri olan K (potasyum) ve Mg (magnezyum) hücrenin kimyasal işlevlerini sürdürebilmesi için gereken sıvı ortamı sağlamada görev alırlar. Potasyum (K) hücre içi ortamın en önemli katyonudur. Erişkin bir insanda yaklaşık 3500-4000 mmol olan toplam vücut potasyumunun %98'i hücre içinde bulunur. Özellikle iskelet kasları, karaciğer, eritrositler ve kemikler potasyumdan zengin dokulardır. Hücre içindeki yüksek K konsantrasyonu hücre metabolizması ve büyümesi, hücre bölünmesi, optimal enzim fonksiyonu, DNA sentezi, volüm düzenlenmesi ve asit-baz dengesi gibi normal hücre fonksiyonlarının devamı için gereklidir (Aygencel, 2018).

Magnezyum iyonu (Mg), insan vücudunda en çok bulunan iki değerli hücre içi katyondur, hücre ve potasyumdan sonra ikinci katyon (K)' dur. Mg atom ağırlığı 24.305 g/mol'dür ve atom numarası 12'dir. Mg, sayısız birçok biyolojik süreçte çok önemli bir role sahiptir. Oksidatif fosforilasyon, enerji üretimi, glikoliz, protein ve nükleik asit sentezi önemli biyolojik süreçlerdir (Saris et al., 2000). Mg, adenozin trifosfatın mitokondriyal sentezinde ayrıca hücresel homeostaz ve organ işleyişi için anahtar bir role sahiptir. Böylece Mg, enzim substratı, yapısal ve membran fonksiyonları dahil olmak üzere çeşitli temel hücresel aktiviteleri ve metabolik yolları kontrol etmede fizyolojik rol oynar (Barbagallo, and Dominguez,2007; Reddy et al., 2018). Mg bir kofaktördür. 600'den fazla enzimatik reaksiyon ve protein kinazların aktivitesi için gereklidir, glikolitik enzimler, tüm fosforilasyon süreçleri ve ATP'yi içeren tüm reaksiyonlar için gereklidir (Barbagallo, and Dominguez,2007; Reddy et al., 2018). Mg bakımından zengin sulardan Mg tüketimi, alternatif bir Mg kaynağı olarak dikkate alınabilir (Galan et al., 2002). İçme suyu, özellikle mineral bakımından zengin daha sert su, mineral bakımından zengin olabilir. Mg alımı, oral takviyelere olası bir alternatifi temsil eder, ancak Ca/Mg sudaki oranı rol oynayabilir. Mg'un içme suyundaki biyoyararlanımı, su ile karşılaştırıldığında genellikle daha yüksektir.

Kobalt (Co), insanlar için gerekli (mineral) bir mikro besindir. Bununla birlikte, tarihsel olarak, insanlarda bariz beslenme ile Co eksikliği meydana gelmemiştir (Ott, 1984). Co kırmızı kan hücrelerinin üretilmesi için gerekli olan B12 vitaminin yapısında bulunur, bu vitamin bütün hayvanlarda büyüme ve eritropoiezis için gereklidir (Bayşu Sözbilir ve Bayşu, 2008; Korkmaz, 2012). Ruminantlar, B12 vitaminini sindirim kanallarında sentezlerler fakat sentezleyebilmeleri için dışarıdan kobalt almaları gerekmektedir (Bayşu Sözbilir ve Bayşu, 2008). Kobalt, kaynak sularında çok çözünmeyen CoS (kobalt sülfür) olarak bulunmaktadır. İnsanlar soludukları hava, içme suları ve kobalt içeren yiyecekler yiyerek kobalta maruz kalabilirler. Toprak ve su ile bitkilerde yüksek konsantrasyonlarda birikebilir, bitki ve hayvansal besinler aracılığıyla da insanlara geçebilir. Kobalt, insan sağlığı için gerekli olan B12 vitamininin bir parçası olduğu için insanlar için faydalı olabilmektedir. Kırmızı kan hücrelerinin üretimini uyarır, hamile kadınlarda ve anemi tedavisinde kullanılmaktadır. Ancak buna rağmen yüksek konsantrasyonları insan sağlığı için zararlı olmaktadır. Soluk alma yoluyla havadan alınan kobaltın yüksek konsantrasyonları astım, zatürre gibi akciğer rahatsızlıklarına neden olabilir. Kobaltın yüksek konsantrasyonları kusma, bulantı, kalp sorunları, Tiroit hasarı gibi sorunlara neden olabilmektedir (Bayşu Sözbilir ve Bayşu, 2008; Atasoy et al., 2011). Bu çalışmanın amacı, Ağrı ilinde yer alan merkez mahallelerden toplanan içme sularında K, Mg ve Co düzeyinin belirlenmesi olmuştur.

## 2. MATERYAL VE METOT

### 2.1.Materyal

Çalışma materyalini 2018 yılında Ağrı ili Abide Mahallesi, Alparslan Mahallesi, Cumhuriyet Mahallesi, Fevzi Çakmak Mahallesi, Fırat Mahallesi, Gazi Mahallesi, Hürriyet Mahallesi, Kazım Karabekir Mahallesi, Leylek Pınar Mahallesi, Mehmet Akif Ersoy Mahallesi, Murat Mahallesi, Sıtkiye Mahallesi, Yavuz Mahallesi alınan toplam 50 adet su numunesi oluşturmaktadır. Araziden toplanan su numuneleri önceden hazırlanmış seyreltik %1'lik HNO3 çözeltisiyle yıkanıp kurutulmuş 15 ml'lik falkon tüplere alındı. Alınan su örneklerinin üzerine kaynakta %65'lik HNO3 çözeltisinden ilave edilmiştir. Böylece ortam asitlendirilerek alınan su örnekleri içerisinde bulunan mikroorganizmaların canlılık faaliyetlerine son verilmiş ve ortamda bulunan metallerin

başka formlar haline geçmeleri engellenmiştir. Alınan su numuneleri analiz yapılıncaya kadar 4 °C de muhafaza edilmiştir.

**2.2. Metot**

Su numunelerinin metal analizleri Van Yüzüncü Yıl Üniversitesi Merkez Laboratuvarında bulunan Thermo Scientific İCE-3000 Atomik Absorbsiyon Spektrofotometresi cihazı ile yapılmıştır. Analiz işlemine önce her metal için ayrı ayrı 1 ppm, 0.1 ppm 0.01 ppm ve 0.001 ppm konsantrasyonlarında standart çözeltiler hazırlanarak başlanmıştır. Her metal için farklı konsantrasyonlarda hazırlanan standart çözeltiler AAS cihazında analiz edilerek cihazın kendi standart grafiklerin çizmesi sağlanmıştır. Standart grafikler her metal için %95- 99 hassaslıkta çizilerek elde edilen analiz sonuçlarının hassas ve anlamlı olması hedeflenmiştir. Bu grafikler yardımı ile okumalar gerçekleştirilmiştir.

**İstatistik Analiz**

Üzerinde durulan özellikler için Tanımlayıcı İstatistikler; Medyan, Ortalama, Standart Sapma, Minimum ve Maksimum değer olarak ifade edilmiştir. Bu özellikler bakımından grupları karşılaştırmada Kruskal Wallis testi kullanılmıştır. Farklı grupları belirlemede Dunnet çoklu karşılaştırma testi kullanılmıştır. Hesaplamalarda istatistik anlamlılık düzeyi %5 olarak alınmış ve hesaplamalar için SPSS (ver: 21) istatistik paket programı kullanılmıştır.

## BULGULAR

Ağrı'da su numunelerinin alındığı noktalar ile numunelere ait K, Mg ve Co Düzeylerinin konsantrasyon değerleri, her parametre için tespit edilen maksimum ve minimum değerler, hesaplanan ortalama ve standart sapma değerleri, Tablo 1'te gösterildi.

**Tablo 1. İçme suyu numunelerinde tespit edilen K, Mg ve Co düzeyleri**

| Numunenin alındığı yer | K | | | Mg | | | Co | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ort± Std. Sap. | Min. | Max. | Ort± Std. Sap. | Min. | Max. | Ort± Std. Sap. | Min. | Max. |
| Abide Mahallesi | 2.35±0.02 | 2.34 | 2.37 | bc3.60±0.02$^{bc}$ | 3.59 | 3.62 | 0.0024±0.003 | 0.002 | 0.003 |
| Alparslan Mahallesi | 1.74±0.53 | 1.42 | 2.36 | AB 4.07±0.39$^{ab}$ | 3.62 | 4.30 | 0.0025±0.003 | 0.001 | 0.005 |
| Cumhuriyet Mahallesi | 1.67±0.46 | 1.42 | 2.36 | AB 4.04±0,53$^{ab}$ | 3.25 | 4.31 | 0.0033±0.004 | 0.001 | 0.006 |
| Fevzi Çakmak Mahallesi | 2.36±0.11 | 2.24 | 2.43 | C 3.15±0.02$^{c}$ | 3.13 | 3.17 | 0.0049±0.005 | 0.001 | 0.009 |
| Fırat Mahallesi | 2.30±0.05 | 2.23 | 2.37 | C 3.16±0.01$^{c}$ | 3.14 | 3.17 | 0.0066±0.003 | 0.004 | 0.009 |
| Gazi Mahallesi | 2.37±0.25 | 2.24 | 2.82 | C 3.18±0.01$^{c}$ | 3.17 | 3.19 | 0.0098±0.002 | 0.009 | 0.011 |
| Hürriyet Mahallesi | 2.37±0.57 | 1.40 | 3.00 | C 3.45±0.48$^{c}$ | 3.13 | 4.33 | 0.0084±0.002 | 0.007 | 0.010 |
| Kazım Karabekir Mahallesi | 2.23±0.04 | 2.19 | 2.27 | C 3.15±0.06$^{c}$ | 3.12 | 3.22 | 0.0020±0.002 | 0.001 | 0.003 |
| Leylek Pınar Mahallesi | 2.32±0.07 | 2.24 | 2.38 | C 3.15±0.01$^{c}$ | 3.13 | 3.15 | 0.0090±0.001 | 0.009 | 0.009 |
| Mehmet Akif Ersoy Mahallesi | 4.43±5.23 | 2.05 | 15.12 | BC 3.59±0.24$^{c}$ | 3.14 | 3.80 | 0.0041±0.003 | 0.002 | 0.006 |
| Murat Mahallesi | 2.08±1.02 | 1.43 | 3.26 | A 4.34±0.01$^{a}$ | 4.33 | 4.35 | 0.0085±0.002 | 0.009 | 0.009 |
| Sıtkiye Mahallesi | 2.16±0.15 | 1.99 | 2.25 | BC 3.64±0.70$^{bc}$ | 3.22 | 4.45 | 0.0192±0.001 | 0.019 | 0.019 |
| Yavuz Mahallesi | 1.80±0.93 | 0.73 | 2.40 | C 3.23±0.44$^{c}$ | 2.82 | 3.70 | 0.0100±0.001 | 0.010 | 0.010 |

## TARTIŞMA

Bu çalışmada Ağrı ilinde yer alan 13 mahalleden ceşme suları alınarak sulardaki ağır metal düzeyleri atomik absorbsiyon cihazı ile araştırılmıştır. İçme suyu numunesi toplanan 13 mahalle arasında K ve Co düzeyleri açısından istatistiksel olarak ($p<0.05$) fark bulunmamıştır. En yüksek K değeri Mehmet Akif Ersoy Mahallesi, en düşük K değeri Cumhuriyet mahallesinden toplanan numunelerde belirlenmiştir. K seviyeleri 1.67 mg/L ile 4.43 mg/L arasındadır. K için TSE'de belirtilen müsaade edilen maksimum değer (12 mg/L) limitini aşan su yoktur. Bu çalışmada tespit edilen Co seviyeleri 0.002 mg/L ile 0.19 mg/L arasındadır. En yüksek Co düzeyi Sıtkiye mahallesi, en düşük Co düzeyi ise kazım Karabekir mahallesinden toplanan numunelerde tespit edilmiştir. Mg düzeyi yönünden Murat mahallesinden toplanan içme suyu numunelerinde Mg düzeyi diğer mahallere göre daha yüksektir. Magnezyum miktarları 3,15 mg/L ile 4,34 mg/L değerleri arasında değişmektedir. Magnezyum için TSE'de belirtilen müsaade edilecek maksimum değer (30 mg/L) limitini aşan su yoktur. Atasoy ve ark.'nın (2011) Van Şehir Merkezindeki İçme suyunda yaptıkları çalışmada, su numunelerindeki kobalt seviyelerini 0.007-0.014 mg/L ve Magnezyum seviyelerini 6.78-6,81 mg/L olarak bulmuşlardır. Nehir, yağmur ve yeraltı sularıyla Magnezyum sonuç olarak sulara karışır. Suda yüksek seviyeler (> 0.1mg/L) lezzet değişiklikleri yaratır ve ayrıca müshil etkisi vardır (Atasoy, 2011). Soylak ve ark. (2002) Yozgat bölgesi içme suyunda magnezyumu 0.17- 1.19 µg/l olarak bulmuşlardır. Manisa ilinde yapılan başka

bir çalışmada Gediz nehri su örneklerinde Co konsantrasyonu ölçümleri yapılmış sonuçlar su kalite standartlarına göre III. kalite su olarak değerlendirilmiştir (Aksoy, 2005). Mardin ilinde içme sularında yapılan başka bir çalışmada numunelerdeki Co konsantrasyonunun 3 numunede su kalite standartlarına göre II. kalite kalan numunelerde ise III. kalite su tanımına uyduğu bildirilmiştir (Yaşar ve ark., 2016). Van ilinde yapılan bir çalışmada Co için yapılan analizler sonucunda numunelerden elde edilen ortalama değer 0,190±0,020 ppm olarak bulunmuştur. Çalışmada içme ve kuyu sularının ölçülen Co konsantrasyonları açısından su kalite sınıflandırılmasına göre III. kalite sular sınıfında yer aldığı bildirilmiştir (Alemdar et al., 2007). Potasyum, duyarlı kişilerde bazı sağlık etkilerine neden olabilse de, İçme suyundan potasyum alımı, sağlığın olumsuz etkilendiği seviyenin oldukça altındadır. Ağrı İli Merkezindeki içme sularında K, Mg Ve Co düzeylerinin tespitinde elde edilen sonuçlar tüm su numunelerindeki K, Mg ve Co içeriğinin izin verilen sınırlar içinde olduğunu göstermektedir (TSE 2005; WHO, 2011; USEPA, 2012).
Bazı ağır metaller ve mineraller vücutta eser miktarda bulunur. Bunların fazlalığı gibi eksikliği de sorunlara yol açabilir. Sularda bulunan ağır metallerin ve yüksek konsantrasyondaki minerallerin zararlı veya zararsız olmasına kanaat getirmek için ağır metalin ve yüksek konsantrasyondaki mineralin miktarına, cinsine dikkat edilmesi gerekir. Dolayısıyla kaliteli su, hem hastalıkları önlemek hem de yaşam kalitesini artırmak için vazgeçilmez bir özellikte olmalıdır ve bunun için gereken tedbirler alınmalıdır.

## KAYNAKLAR

Akın M, Akın G 2007. Suyun önemi, Türkiye'de su potansiyeli, su havzaları ve su kirliliği. Ankara Üni. Dil ve Tarih-Çoğrafya Fak. Der, 47(2): 105-118.

Aksoy G, 2005. Gediz nehri ağzında su, sediment ve planktondaki ağır metal düzeylerinin ölçülmesi.
Celal Bayar Üniversitesi Fen Bilimleri Enstitüsü Hidrobiyoloji Yüksek Lisans Programı, Yüksek lisans tezi, Manisa.

Alemdar S, Ağaoğlu S, Alişarlı M, Dede S 2007. Van bölgesi su kaynaklarında ağır metal kirlilik düzeyleri. Vet. Bil. Derg, 23, 1:19-29

Anonim,2004,https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=7221&MevzuatTur=7&MevzuatTertip=5

Atasoy N, Mercan U, Alacabey İ, Kul AR. 2011.Van şehir merkezindeki içme ve musluk suyunda bulunan ağır metaller ve bazı makro element seviyeleri. Hacettepe J. Biol. & Chem, 39 (4), 391-396

Aygencel G 2018. Potasyum Metabolizması Bozuklukları.Yoğun Bakım Dergisi, 12(1):31-42

Barbagallo M, Dominguez L 2007. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance.
Arch. Biochem. Biophys, 458, 40-47.

Bayşu Sözbilir N, Bayşu, N 2008. Biyokimya, Güneş tıp kitapevleri, Ankara.

Galan P, Arnaud MJ, Czernichow S, Delabroise AM, Preziosi P, Bertrais S, Franchisseur C, Maurel, Favie A, Hercberg S 2002. Contribution of mineral waters to dietary calcium and magnesium intake in a French adult population. J. Am. Diet. Assoc, 102, 1658-1662.

Kahraman ÜC 2007. Konya garnizon birliklerindeki kuyu suları ile şehir şebeke sularının su kalitesi ve ağır metaller yönünden karşılaştırılması. Selçuk Üniversitesi Sağlık Bilimleri Enstitüsü Besin Hijyeni ve Teknolojisi Anabilim Dalı, Yüksek Lisans Tezi, Konya.

Korkmaz S 2012. Kobalt (II) Q (Florobenzoat) N.N'- Dietilnikotinamid'in Fare (Mus musculus) Karacigeri Üzerine Histopatolojik Etkileri. Yüksek Lisans Tezi, Kafkas Üniv. Fen Bilimleri Enstitüsü.

Ott W H, Rickes EL, Wood TR 1948. J. Biol. Chem, 174, 1047.

USEPA (US Environmental Protection Agency) 2012. Ground water and drinking water. http://www.water.epa. gov/drink/index.cfm. Accessed 01/15/2011.

Salam MA, Al-zhrani G, Kosa SA 2014. Journal of Industrial and Engineering Chemistry Removal of heavy metal ions from aqueous solution by multi-walled carbon nanotubes modified with 8-hydroxyquinoline : Kinetic study,‖ J. Ind. Eng. Chem, 20, 2, 572-580,

Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A 2000. Magnesium. An update on physiological, clinical and analytical aspects. Clin. Chim. Acta, 294, 1-26.

Solak M, Doğan M 2000. Suyun özellikleri ve suyun kirliliği. Su Kimyası. Erciyes Üni. Yayınları, pp.1-155, Kayseri.

Soylak M, Aydın FA, Saraçoğlu S, Elçi L, Doğan M 2002. Chemical Analysis of Drinking Water Samples from Yozgat-Turkey, Polish Journal of Environmental Studies, 11-151.

Tekinşen OC, Yalçın S 1990. Su hijyeni ve Muayenesi. Selçuk Üniv. Aksaray MYO Ders Notları, Teksir No:2, Aksaray,

TSE, (2005). İnsani Tüketim Amaçlı Sular. Türk İçme Suyu Standartları TS 266 sayılı standart -Türk Standartları Enstitüsü –Ankara.

Reddy ST, Soman SS, Yee J 2018. Magnesium Balance and Measurement. Adv. Chronic Kidney Dis, 25, 224-22

Van FXR 2009. Leeuwen Food and Chemical Toxicology, 38, 51.

Yaşar S, Üstek MA, Bengü AŞ, Mis L 2016. Mardin bölgesi içme sularında ağır metal düzeylerinin araştırılması. Cumhuriyet Üniv. Sağ. Bil. Enst. Derg, (1)2, 63-71

WHO 2011. Guidelines for Drinking-Water Quality, 4th edt., World Health Organization, WHO Library Cataloguing-in-Publication Data, ISBN 978 92 4 1548151.

*ORAL PRESENTATION*

**Hafif ve katlanabilir bir insan taşıyıcı tasarımı ve üretimi**

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**Özet**
Bu bildiride, havalimanları, alışveriş merkezleri vb. kısa mesafeli varış noktalarına kolaylıkla ulaşmak için kullanılan akıllı bir insan taşıyıcının tasarım ve üretim prosedürleri sunulmaktadır. Bu taşıyıcının emsallerine göre özellikleri, hafifliği ve boyutu ile daha taşınabilir olması ve daha güvenli sürüş sağlamasıdır. Hafif, katlanabilir tekerlekler ve direksiyon çubuğu özelliğine sahip olması ve uygun ebatta olması, kullanıcıların laptop çantası içinde taşımasına olanak sağlar. Bu özelliklerle birlikte, akıllıca kaçınmak için sabit ve hareketli engelleri algılayabilir.
**Anahtar Kelimeler:** Elektrikli scooter, Elektrikle çalışan kaykay, Katlanabilir scooter ve kaykaylar.

**Design and manufacture of a lightweight and foldable human transporter**

**Abstract**
Designing and manufacturing procedures of an intelligent human carrier that utilized to reaching out short distance destination points such as airports, shopping malls, etc. with ease, is presented in this paper. The specialties of this carrier comparing to its equals, are being more portable with light weight and size, and ensuring safer ride. Have the feature of light weight, foldable wheels and steering bar, and suitable size allows users to carry it around in a laptop bag. Along with these specifications, it can detect the fixed and moving obstacles, to avoid them intelligently.
**Keywords:** Electric powered scooter, Electric powered skateboard, Foldable scooter, and skateboards.

## INTRODUCTION

In last couple years, our lives are affected by modern technology and its applications in different areas, especially in transportation. Utilization of electric powered motors comes with the benefits of faster, safer and more environment friendly option in transportation and the developments reveal the age of e-scooters. Nowadays e-scooters and variations can be found in almost every part of the city and this age of e-scooters is referred as a revolution in the transportation area that induce a decrease in the number of people who are using car. More than that helps people who are not able to walk such distances. Recently, electric scooters have become very common means of transportation, since they take up less space than the cars and motorcycles, hence can minimize the time loss caused by traffic, and use renewable energy sources.

First, a comprehensive field survey on the subject including the market products [1-8] and the patents [9-12] was implemented to study their main specifications and outcomes and consequently to benefit from their advantages. Also in this regard, some related performance and safety standards [13-14] published by ISO and IEC were studied to understand how we can protect the user and the environment from the harmful conditions. Nitro Scooter is one of the biggest e-scooter companies in Europe, which produces scooters based on the patents and with strictly controlled quality. The product that intersects with our project is Nitro Plus E9 electric scooter [1]. Nitro Plus E9 is dust and water resistant. It has very short charge time, which is an important parameter in the electric vehicles. Another market product designed by Eco Rider Scooter is its E4-max model [2]. This product has a portable design with the appearance of science and technology sense. This model is preferable comparing to the other products because portability is very crucial feature for the e-scooters. VIP scooter, which is manufactured in Turkey, is our other reviewed products. One of the biggest advantages of this scooter is that it can be controlled from a phone via Bluetooth. In addition, similar scooters on the market reach 25 km/h, while the VIP scooter reaches 35 km/h [3]. The most distinctive feature of the x350-pro model electric scooter produced by Citycoco company is that its steering mechanism is foldable [4]. Thanks to this feature, it saves space and gains an advantage over its competitors. Also, this model has a smaller battery compared to its competitors in the market, which is an advantage in terms of weight and fast charging, but as a disadvantage in terms of scooter operation time. Electro wind company is a turkey company, we investigated its Dualtron Thunder model [5]. This product has a 5.400 W brushless DC dual upgraded Hub motor which

can accelerate to 80 Km/h. Other advantage is that it has a 60V 35Ah battery with a usage time of 20 hours. The disadvantage of this product is its heavy weight (42.8 kg) comparing to the market products. Another reviewed product is Dualtron X2 [6]. It has a high performance 42Ah battery which can work up to 24 hours. Like the above-mentioned product, its disadvantage is that it is very heavy, with a weight of 68.5 kg. The Vsett 10 model can travel longer distances and has less payload than the other reviewed models [7]. It has also fast charging capability. Scorpion model of the Yakasuma brand, has the payload same as the other models [8]. Its motor power is lower than the other scooters, also it is categorized as a low-weight model in the segment (32 Kg). The first investigated patent is a foldable electric scooter [9]. A primary objective of the invention is to provide a configuration that employs three simple actions to facilitate rapid folding away and realizes formation of a portable scooter. Prior to usage, the electric scooter can be folded away and thus form a reduced size baggage-like form which can be carried, or auxiliary wheels configured on the scooter can be utilized to enable a user to pull along the scooter onto a rapid transit system or facilitate transport storage thereof. Another objective of the present invention is providing a structure with two auxiliary wheels respectively configured on outer sides of a front wheel of a body member, and a pull rod configured on a front edge of a cover member. The second patent presented in [10], is a classic electric scooter which comprises a deck configured to support a rider, a front wheel and a rear wheel, a steering column comprising a steering tube and a handlebar assembly. The steering column configured to steer the scooter by controlling a direction of the front wheel, and a foot brake, wherein the foot brake is configured to apply a braking force to the rear wheel when the foot brake is pressed down. Third patent [11] is invented by Decathlon. It is an invention relates to a vehicle with a folding system and is included in the field of application of foldable scooter type vehicles. The fourth patent [12] is an electric powered scooter having a chassis with a tubular frame extend in between a front steerable wheel and a rear electrically powered wheel. A platform for the standing support of the scooter rider is mounted from frame mounted brackets to define between the upper portion of the tubular frame and the lower portion of the platform a spatial interval. The scooter design has the powering electric batteries mounted under the platform giving the scooter having a low center of gravity with optimal riding profile.

In this paper, the design and production procedures of an electric powered scooter is presented. Comparing to its equals, the specialties of this scooter are being more portable with light weight and size and ensuring safer ride. Its lightweight feature along with foldable wheels and steering bar, and suitable size allows users to carry it around in a laptop bag. In addition to these specifications, it can detect the fixed and moving obstacles, and avoid them automatically.

## SYSTEM DESING

As mentioned above, the specialties of this scooter are light weight, proper size, foldable wheels, and foldable steering bar. Also, it should be able to detect the fixed and moving obstacles. Hence the design requirements are defined as below restrictions:

It will weigh maximum 4 kilograms.
The platform size is 17 inches.
Carrying capacity is between 70-100 kilograms.
It travels with speed of 10 km/h.
It detects the obstacles.

Based on these specifications and functionalities which the transporter should have and perform, a proper system was designed.

### Traction system design

To design a proper traction system suitable for desired moving of the transporter, the below mentioned calculations were done to find out the needed rotational speed, torque, and power of the traction system.

Needed rotational speed:

$$v_{max} = 10\ Km/h \qquad (1)$$

$$\omega = \frac{v_{max}}{2\pi r} = \frac{10\times\frac{1000}{60}}{2\times 3.14\times 0.0415} = 642 rpm \qquad (2)$$

Here “r” is motor wheel radius which will be determined based on the selected motor, but for now we assume it 0.0415 m. Since we want to fold the wheel under the plate, so it should have a small radius.

Needed torque and power

$$m = 100\ Kg \quad (3)$$

$$F_N = m \times g = 100 * 9.81 = 981\ N \quad (4)$$

$$F_F = \mu \times F_N = 0.2 \times 981 = 196\ N \quad (5)$$

Here, since the scooter will be used in different road conditions, $\mu = 0.2$ has been considered and applied as an average value for the friction coefficient.

The needed torque and power are achieved as:

$$\tau = F_F \times r = 196 \times 0.041 = 8\ N.m \quad (6)$$

$$P = \frac{\omega \times \tau}{9.55} = \frac{642 \times 8}{9.55} = 530\ W \quad (7)$$

Therefore, we should employ a motor with rotational speed of 642 rpm, a torque of 4 N.m, and a power of 265 W per motor. To this aim, we employ 2 BLDC (Brushless DC) Hub motors as the rear wheels of the transporter.

**Structure design, analysis, and simulation**

Based on the desired mechanical requirements and limitations of the transporter a proper structure was designed. This structure includes a platform, a steering mechanism or telescopic handlebar, a free rotating wheel, and foldable mechanisms for all the rear and front wheels as well as the handlebar. To meet the lightweight property, the material of the platform was selected based on needed requirements. All the design procedures and drawings of the mechanical structure were performed in SOLIDWORKS software.

To enable the user to fold the wheels and handling bar comfortably and carry it in his bag, the critical problem is designing a proper mechanism to fold, unfold and its locking mechanism. Among some locking systems, like hinges and pin, umbrella locking mechanisms, the most suitable one is selected and used for our need. Fixed hinges and pin system were designed to fold the rear and front wheels under the plate. The lower cost and easy production procedure, compared to other lock systems, were the main reason to choose this mechanism.

Also, a telescopic handling bar with pin mechanism for locking, was designed to fold the handling bar on the plate. The handlebar consists of three interlocking 35 cm circular profile pipes to construct 100 cm as the height of the handlebar. The rotational movement of the handlebar is provided by a bearing that is placed inside the platform. To connect the handlebar to the front free rotating wheel a proper knuckle joint is designed.

In the below illustrated figures 1-6, the drawings of the foldable front wheel, the telescopic handlebar, the knuckle joint, the platform, the pin, and 3-dimensional drawing of the designed transporter have been displayed.

In the second phase, to investigate whether the designed structure meet the desired mechanical requirements and limitations, some analyses were performed. One main requirement is platform size and weight, so to select a proper platform material, stress and strain analyses were implemented in NX software. Also, the weight and price parameters were taken to account to choose a proper material. These analyses led to select Aluminium with 3 mm thickness as the platform material, among Steel, Aluminium, Copper, Titanium, Epoxy, Waspaloy, Polycarbonate, and hard Polyurethane materials.

Then the performance of the mechanical structure components including the folding of the front and rear wheels, folding of the telescopic handlebar, as well as moving of the whole structure were simulated and analysed in SOLIDWORKS environment.

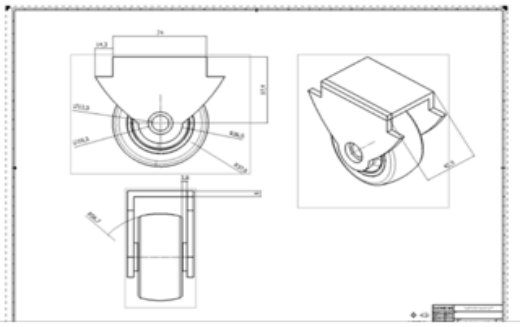

**Figure 1.** Drawing of the Foldable Front Wheel

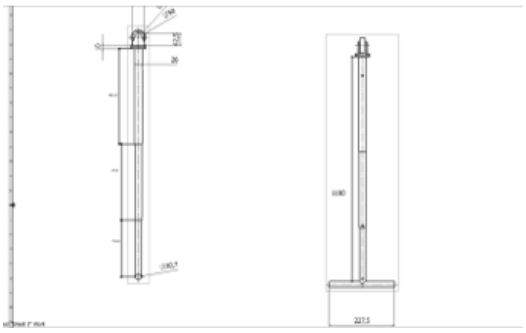

**Figure 2.** Drawing of the  Telescopic Handlebar

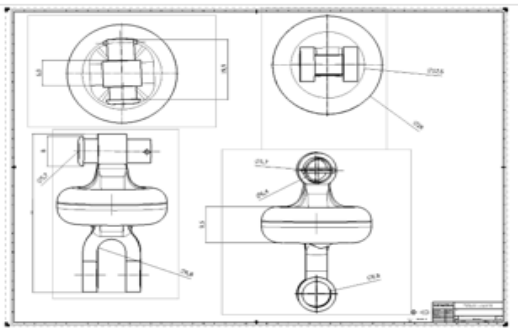

**Figure 3.** Drawing of the Knuckle Joint

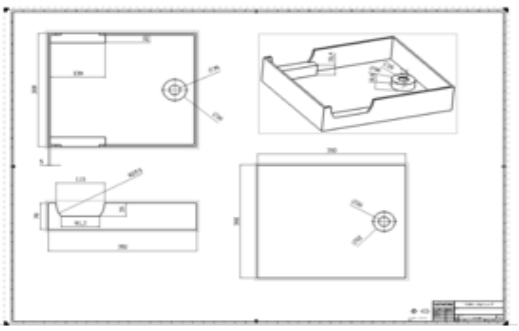

**Figure 4.** Drawing of the Platform

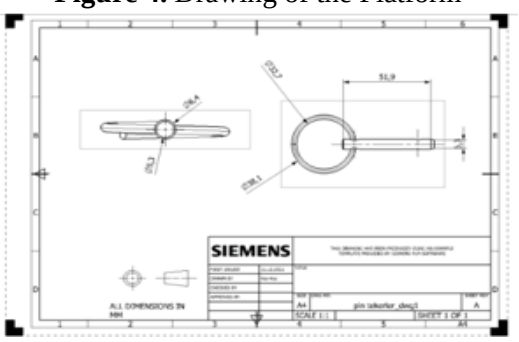


**Figure 5.** Drawing of the Pins



**Figure 6.** 3-D Drawing of the Transporter

**Electrical and control system design and simulation**

The electrical system is including 2 hub motors and their drivers, a battery, a micro-controller board, an electric gas pedal and 4 ultrasonic sensors.

Based on the calculations in the traction system design, the needed torque and power was achieved as 7.4 N.m and 350 W per motor, and the needed rotational speed as 650 rpm. To this aim, we employed 2 BLDC Hub motors which cover these specifications, as the rear wheels of the transporter.

The travelling speed is given by the user through an accelerating hand pedal which will be mounted on the handlebar. This reference signal is converted to PWM signal by the microcontroller and sent to motor drivers to control the speed of the transporter.

Four ultrasonic sensors will be mounted on the front and corner sides of the platform to detect any fixed and moving obstacles. The measured distance by each sensor is transferred to the microcontroller board to employ in the navigation control system.

The microcontroller board (Arduino MEGA 2560) was selected based on the number of analog or digital inputs and outputs. 8 of them are echo input and output trigger pins of the ultrasonic sensors, 1 of them is to read input data from accelerator pedal, 2 of them are outputs to control the motor drivers with PWM signals.

The needed power to drive the system is calculated based on the motors continuous currents (11 A). The system power is supplied by a 24 V 10 Ah Li-Ion battery. Hence, based on the below calculation, we can consider roughly 30 minutes as the operation time of the system.

$$\frac{10\ Ah}{2\times 11\ A} \approx 30\ min \qquad (8)$$

Lastly a navigation control system was designed, employing a simple PID controller in MATLAB Simulink environment. Here the error signal, which is the input of the controller, is defined as the difference between the given reference speed and the measured speed captured by the motor driver's hall sensor. The navigation control architecture also includes an obstacle avoidance algorithm. In this algorithm, when each of four sensors sees an object with a distance between 100 – 50 cm, the controller decreases linearly the reference speed signal and sends it to the motor drivers. If the distance is smaller than 50 cm, the control input sent to the motor drivers will be zero, which means the motors should stop. The performance of the navigation control system was simulated and studied in MATLAB Simulink environment. This simulation includes the speed control motion of the differential drive structure, along with the behavior of the obstacle avoidance algorithm.

## SYSTEM IMPLEMENTATION

After production of the platform and the handling bar, the mechanical parts including the folding mechanisms on the front and rear wheels and on the bottom side of the handling bar were assembled. Then 2 hub motors on rear side, and a free rotating wheel on the front side were installed. The motors drivers along with the micro-controller board were mounted under the platform, and 4 ultrasonic sensors were installed on the front and corners of the platform. The gas pedal was also installed properly on the handling bar. Lastly the data and power-supply cabling between the electronic components were performed. Figure 6 illustrates the manufactured prototype.



**Figure 7.** Manufactured Transporter

It is worth mentioning that a weight analysis was implemented to calculate the total weight of the manufactured prototype, as below, which shows 1.250 Kg heavier than the primary weight limitation. The main reason is motor's high weight.

Total weight = Plate weight + Hub motors weight + Battery weight + other components weight = 1.800 + 1.950 + 0.300 + 1.20 = 5.250 Kg

## TESTS AND RESULTS

The integrated prototype system was tested practically to evaluate whether it meets the design requirements or not. Different problems were encountered during and after the assembly. Some of these were design errors and some were manufacturing errors.

One main design flaw is balance problem of the platform. When a person rides the scooter, a kind of bending problem is observed on the platform which causes the balancing problem. The solution to this problem was determined in the following manner. The platform material and shape should be changed such a way it is more rigid and durable against bending and unbalancing problems. Prototype overweight problem can also be solved by these changes. Overweight problem may be solved by displacing of the rear wheels and front wheel. In other words, there should be just one motorized wheel in the front side, and two free rotating wheels in the back side. Another problem was faced while producing the structure of the folding mechanisms. The lower surface of the powered rear wheels is not completely in contact with the ground. The reason is that the produced folding mechanism which is installed under the platform and the wheels are mounted on it, is not completely placed perpendicular to the platform. This problem can solve by changing of the folding mechanism structure.

## CONCLUSION

The purpose of this project was to manufacture a mechatronic product to provide easy travel in short distances. Among the classic transportation vehicles, electric scooters have become very common means of transportation. There are several reasons why electric scooters are such a popular means of transportation; renewable energy sources such as electricity are more efficient than energy sources such as gasoline, do not take up as much space as cars and motorcycles, and minimize the time loss caused by traffic. The specialties of this scooter comparing to its equals, are being more portable with light weight and size, and ensuring safer ride by detecting the fixed and moving obstacles around itself. To realize this aim, the followed steps were design a proper system, analyzes, calculations, material selection and implementation of the system. While selecting all the materials and components to be used in production, it was attempted to choose the lowest cost products after careful research.

## ACKNOWLEDGEMENT

We would like to appreciate Prof. Dr. Bülent Ertan, head of the Mechatronics Eng. Dept., for his guidance and insight throughout the work. This project was supported by Directorate of ARGEDA Technology Transfer Office of Atilim University, under the Grant ATÜ-LAP-2122-09.

**REFERENCES**

Hepsiburada Ana Sayfa. (n.d.) from https://www.hepsiburada.com/cool-wheels-2-tekerlekli-katlanabilir-scooter-8-yas-pembe-fr58352-pm-HB00000KA829-yorumlari. [Accessed: 15-Jul-2022]

10inch two wheel foldable electric scooter EcoRider E4-7. Shenzhen EcoRid. (2020, May 21) from https://www.ecoriderscooter.com/foldable-electric-scooter/10inch-two-wheel-foldable-electric-scooter-ecorider-e4-7.html. [Accessed: 15-Jul-2022]

VIP scooter e-scooter Electrical Scooter. Vip Scooter e-scooter Electrical Scooter. (n.d.) from https://www.vipscooter.net/product/vip-e-scooter-siyah/ [Accessed: 19-Jul-2022]

https://www.hepsiburada.com/citycoco-350w-amortisorlu-x350-pro-elektrikli-scooter-pm-HB00000YBWFK?ds_rl=1294343&wt_gl=cpc.6810.dsa.nelk.spor-spor-branslari&isFashion=true&ds_rl=1294343&gclsrc=ds&gclsrc=ds [Accessed: 28-Jun-2022]

Dualtron Thunder. (n.d.) from https://www.escooter.com.tr/dualtron-thunder [Accessed: 15-Jul-2022]

Dualtron x 2. (n.d.) from https://www.escooter.com.tr/dualtron/dualtron-X-II. [Accessed: 15-Jul-2022]

Vsett 10+. (n.d.) from https://www.escooter.com.tr/vsett [Accessed: 15-Jul-2022]

Electrical Scooter. Yakasuma Scorpion Electrical Scooter. (n.d.) from https://www.escooter.com.tr/elektirikli-scooter-scorpion [Accessed: 15-Jul-2022]

Lee, S.-Y. (n.d.). US7077229B2 - folding and portable electric scooter. Google Patents from https://patents.google.com/patent/US7077229B2/en. [Accessed: 21-Agu-2022]

Lovley, J. B., Klingl, J. C., Groenhuyzen, M., & Edlauer, K. (n.d.). US9994278B2 - Scooter Assemblies. Google Patents from https://patents.google.com/patent/US9994278B2/en. [Accessed: 22-Agu-2022]

Ferret, Patrick. Katlanır sistemli scooter tipi bir araç. Patent. Türkpatent, 2016 02428.EP2736796B1,2016-03-21. https://portal.turkpatent.gov.tr/anonim/arastirma/patent/detayli [Accessed: 16-Oct-2022]

Patmont, S. J. (n.d.). US5775452A - Electric Scooter. Google Patents from https://patents.google.com/patent/US5775452A/en. [Accessed: 23-Agu-2022]

“Standards,” ISO, 23-Jun-2020. https://www.iso.org/standards.html. [Accessed: 19-Jan-2022].

“TC 125,” IEC. https://www.iec.ch/dyn/www/f?p=103%3A7%3A0%3A%3A%3A%3AFSP_ORG_ID%2CFSP_LANG_ID%3A23165%2C25. [Accessed: 19-Jan-2022].

## *ORAL PRESENTATION*

### Determination of microbial impact on fading of inks with time: Ink-biogram

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**Abstract**

The question of how the ink is affected by the environment in which it is found is of great importance for forensic document investigators, especially in clarifying the issues related to ink age determination from document forgery crimes. The physical conditions in which the document is stored and how the ink is affected by these physical conditions are important factors affecting the examinations. Papers offered for writing on the market absorb ultraviolet light if kept under sunlight, and therefore, the different physical conditions of the inks change the examination results. For this reason, the document investigator should examine the different physical conditions in which the document may have been found, while making an ink comparison. In addition to light, many external and environmental factors such as temperature, humidity, amount of radiation, air contaminants from pollution such as sulfur dioxide and nitrous oxide, oxygen, concentration of pigments, surface properties, adsorption/diffusion properties of the paper-solvent system affect the molecular transformation/fading of ink pigments.The structure and amount of other additives added against microbial growth in the ink vary from manufacturer to manufacturer. In the literature, no study has been found on how the biological factors (microorganisms) in the archive environment where the documents are stored affect the speed of fading in ink pigments and which paths they will follow. Based on this gap in the literature, first of all, in order to examine the effect of microorganisms, that may be present in the environment under the storage conditions of the document, on the change of pigments in the composition of the ballpoint pen ink over time, we have determined mold, fungus and bacterial species that may be present in the archive environment and on the document through culture research. After the preliminary research; microbiological agents of *Aspergillus flavus, Aspergillus fumigatus, Penicillium sp., Cladosporium sp., Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Mycobacterium lentiflavum* were included in our study and pure strains were obtained. Samples of ink-paper discs contaminated with these factors in a controlled manner, were prepared. To determine whether discs containing different volumes of ink give the same response to the same amount of microbial agents; The sizes of the inhibition zones, formed at the end of the ink-biograms performed with paper discs, containing different volumes of ink, in media where microbial agents prepared at 0.5 McFarland concentration were passaged separately were observed. It was determined that the responses of different amounts of inks to the same microbial agent at the same concentration were almost the same. In the observation made on how the ink samples, which are kept for certain periods after application to the paper, respond to microbial factors over time; ink-paper disc samples were kept randomly at room conditions for up to 6 months without direct exposure to sunlight. These samples were inoculated on appropriate media where microorganism suspensions (prepared at 0.5 McFarland concentration) were passaged at 0th, 3th, and 6th months after drafting, and the diameters of the inhibition zones around the discs incubated under appropriate conditions were examined step by step. The word “ink-biogram” used here for the first time for the macro and micro images on the incubated petri dishes used in our study, in which the effect of microorganisms on the ink was investigated, refers to the ink-microorganism interaction. As a result of the examination, differences were observed between the inhibition zone diameters of the ink-biograms regarding different inks for the same microorganism. Differences in stained zone diameters may be due to diffusion into the agar at different amounts and diameters due to differences in ink formulations. This suggests that, in addition to the methyl violet molecule with antimicrobial effect, a formulation that allows this molecule to diffuse more or less through paper surface and depth also has an effect in the interaction of the ink on the paper with the microorganisms.

**Keywords:** Document Analysis, Ink-biogram, Microorganism, Inhibition Zones, Methyl Violet

## INTRODUCTION

Paper, pen and ink are the elements that make up the document. Pens are classified as ballpoint pens and non-ballpoint pens according to their ink structure (Bügler et al., 2005). In a study published by M. Calcerrada et al. in 2015, it is stated that forensic document analysis studies around the world focus especially on ballpoint pen inks (Calcerrada & García-Ruiz, 2015).

Ink is a chemical compound whose formulation is kept secret by manufacturers (Brunelle & Reed, 1985). Ink is a chemical compound whose formulation is kept secret by manufacturers (Brunelle R.L. & Crawford K. R., 2003; Bügler et al., 2005). Apart from these, manufacturers add two chemical markers (marks) to the ink, of which the name and quantity is known only by the manufacturer; 1) one of these do not change over the years and 2) the other change every year (Cengiz, 2013). Although these signs are generally composed of rare earth elements, the extent of their participation is kept secret by the manufacturer.

In the structure of ballpoint pen ink; There are dyes called crystal violet (CV), methyl violet (MV) and Victorian blue (VB). CV and MV dyes from the methylene blue family fade when exposed to light, exhibiting an unstable structure and losing their methyl groups (Andrasko, 2001; Brezová et al., 2004). As a result of this fading, the color of the text on the document becomes lighter. In this fading reaction, CV is converted to MV and MV to tetramethylpararosaniline (TPR) through hydrogen bonding instead of the cleaved methyl ($-CH_3$) groups. The intensity and duration of the light which the document has been exposed to, the presence of oxygen in the environment, the physical and chemical properties of the paper significantly affect the fading due to light. Victorian blue (VB) is more stable. Depending on the intensity of the light exposed, it can lose one $-CH_3$ group over time and turn into a photodegradation product VB-$CH_3$ molecule.

When we look at the literature, the fading of dyes, the evaporation of solvents, and the hardening and polymerization of resins have been studied in detail by researchers (Brunelle & Reed, 1985; Weyermann C., 2005; Weyermann et al., 2011). However, it is understood that physicochemical transformations of ink components have been studied in our country and in the world, but how the factors, especially biological factors, affect this transformation has not been investigated in detail (Grim et al., 2001; Weyermann et al., 2011). In a study by Steflinger K. (2010), it was reported that fungi cause decay on the paper surfaces of documents stored for a long time in the archive/museum environment, through their enzymatic effects, therefore, it is necessary to prevent fungal contamination by microbiological monitoring taking necessary measures in these environments (Sterflinger, 2010). No study has been found in the literature, on how the biological factors (microorganisms) in the archive environment where the documents are stored, affect the speed of fading in ink dyestuffs and which paths they will follow. So we aimed to examine the effect of microorganisms that may be present in the environment under the storage conditions of the document on the change of dyestuffs in the composition of the ink over time.

## MATERIAL AND METHOD

Our study was carried out using the devices and equipment in the Forensic Sciences Laboratory of the Institute of Forensic Medicine and Forensic Sciences, at IU-C. American Type Culture Collection (ATCC) standard strains for the microbiological agents of *Aspergillus flavus, Aspergillus fumigatus, Penicillium sp., Cladosporium sp., Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Mycobacterium lentiflavum* were obtained from I.U., Capa Tuberculosis Laboratory of Basic Medicine Microbiology. Tryptic Soy Broth (Gelous Agar) (Merck), Blood Agar (Merck) and Malt Extract Agar (Merck) media were used for the analysis. Before each study, the media were carefully prepared fresh in the biosafety cabinet, taking into account the risk of contamination. In our study, blue and black ballpoint pen inks from 4 different manufacturers randomly selected from the market [Pensan MV-Pen Vision (S1: black; M3: blue), Faber Castell Speedx10 (S2: black; M1: blue), Art Ballpoint Pen (S3: black; M4:blue), Schneider slider Edge XB (S4:black; M2:blue)] and A4 copy paper (Office International Copier Bond Dough 80g/m2 210x297mm) were used. In our study, the step in which the media were contaminated with strains and the biogram step that we made with paper-ink discs, which we called "inkbiogram", were carried out in separate biosafety cabinets as a precaution for any possible contamination risk that could adversely affect the reliability of the study.

**Preliminary Research**

At this stage of the research, in order to examine the effect of microorganisms that can be found in the storage conditions of any document on the fading of dyestuffs present in the composition of ballpoint pen ink, over time; a culture study was carried out in terms of microorganisms to determine the types of mold, fungus and bacteria that may be present in the archive environment and on the document, The prepared media were first left in a random archive environment without air circulation and in a drawer in the archive. Afterwards, 1 cm of randomly selected paper samples, which were written with blue ballpoint pen and black ballpoint pen, known to be old and worn out, were cut 1 cm from the writing part and inoculated in 3 types of media. In another examination step where the modeling method was used; each of the 1) cut paper with the drawn line at the top and 2) cut paper with the drawn line at the bottom, 3) 2 µL of direct ink itself and 4) 5 mm diameter blank piece of paper as a negative control, were inoculated into 3 different types of media separately. One petri dish from each medium was also subjected to the incubation process without inoculation, for negative control purposes.

Macroscopic and microscopic examinations were performed for the identification of breeding colonies (Figures 1 and 2). Microfungi grown in Petri plates were transferred to slanted malt media and stored as a stock culture in the refrigerator at +4 ºC.

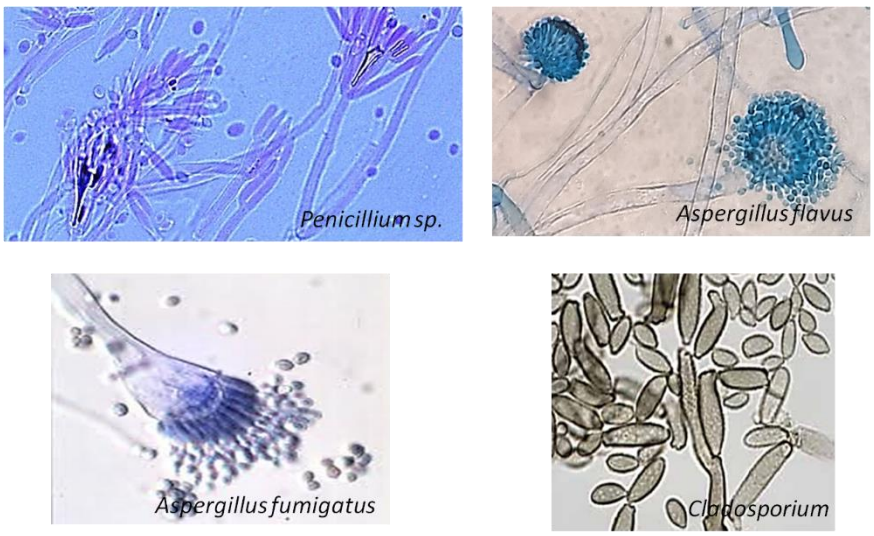


**Figure 1.** Microscopic image of some molds and fungi grown in media

In accordance with the findings of the studies in the literature, *Aspergillus flavus, Aspergillus fumigatus, Penicillium sp., Cladosporium sp., Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Mycobacterium lentiflavum species*, which are among the microorganisms that we determined in our preliminary experiments and reported to be frequently found in the archive environment, were included in our study (Karbowska-Berent et al., 2011).

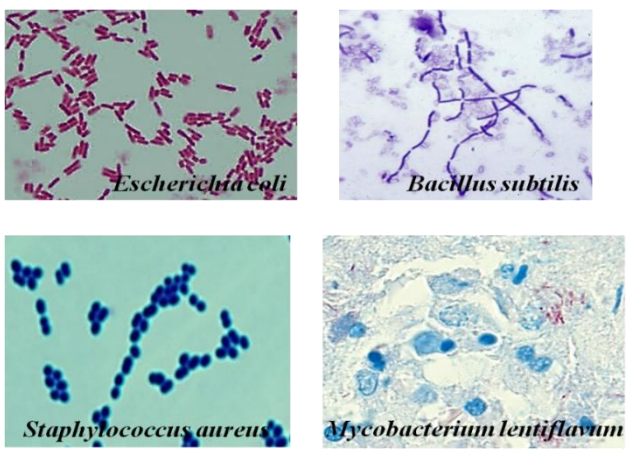


**Figure 2.** Microscopic image of bacteria grown in media

**Formation of Inkbiograms (Ink-Paper Discs) on Media Contaminated by Passaging of Microorganism Strains**

In the second stage of our study, 2 µL volumes of the 4 randomly selected blue and black ballpoint pen inks from the market were dripped and diffused on the front and back surfaces of UV sterilized 5 mm diameter paper punches, on the sterilized surface (with 10% zefirol) inside the biosafety cabinet. The paper punches were dried in the cabinet for 30 minutes (Figure 3). To fix the number of microorganisms included in the study, each of the strains was individually suspended by the 0.5 McFarland method before passage into media. These suspensions were passaged onto plates of malt and agarose gels using the "Smear Culture Method (parallel inoculation)" (Figure 4).

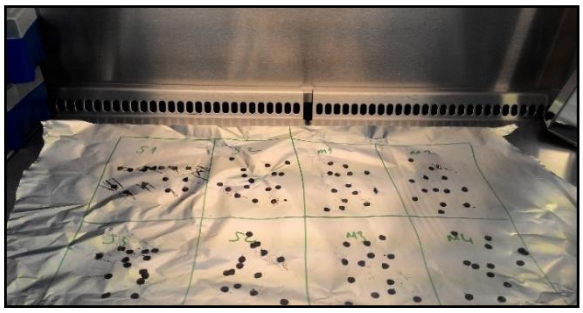

**Figure 3.** Preparation of ink-paper discs (ink-biogram)

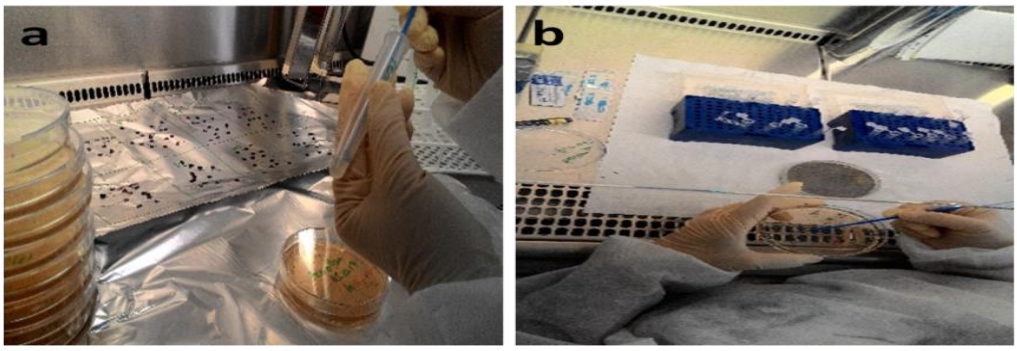


**Figure 4.** (a) Retrieval of suspended Penicillium sp. with the help of sterile loop for inoculation, (b) inoculation for inkbiogram research

For negative control purposes; two types of samples including empty media types without passage and inoculation, and sterile paper discs untreated with ink were used. For positive control purposes; the studied mold, fungus and bacterial species were passaged into malt and agarose media. All petri dishes were closed after passing through a bunsen flame. The inoculated agar media were incubated at 37°C in an oven for 24 hours, and the malt media were incubated at 25-27°C at room temperature (dark environment) for 7-10 days.

**Investigation of the Effect of Discs Containing Different Volumes of Ink on Inhibition Zones**

Microorganism suspensions of S. aureus, representing the bacteria class, and A. fumigatus, representing the mold fungus class, prepared at 0.5 McFarland concentration, were passaged into malt and agar. 80 discs were prepared through applying 1.0 µL, 1.5 µL, 2.0 µL, 2.5 µL and 3.0 µL from each ink type to the front and back sides of 5 mm punches [8 different inks x 5 different volumes x 2 types of microorganisms]. Discs containing the same volume of ink were placed in eight mediums with a distance of 35-40 mm from a disc center to the other disc center. After incubation, the presence of inhibition zones around the discs was observed. By measuring the diameters of the inhibition zones, discs of the same inks containing different volumes of ink were compared.

**Investigation of the Effect of Time on Inhibition Zones**

Ink-paper disc samples, which are kept randomly in an environment where the temperature is 23°C (±2) and the relative humidity is 55% (±8), without direct exposure to sunlight for up to 6 months, were inoculated at 0th, 3th, and 6th months to the suitable media where microorganism suspensions prepared at 0.5 McFarland

concentration were passaged (Figure 5). Blank samples, negative and positive controls were used for each monthly measurement. The diameters of the zones of inhibition observed after incubation were measured and compared versus time (Fig. 6).



**Figure 5.** Ink-paper discs kept for 0-6 months

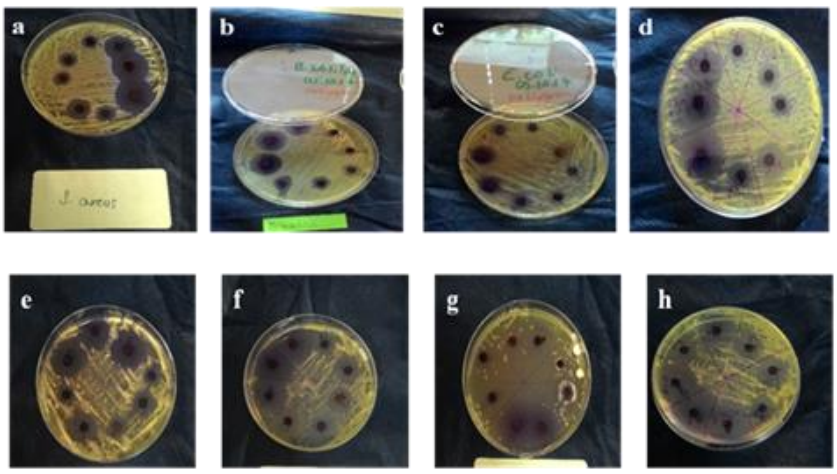


**Figure 6.** Examples of 3 month inhibition zones in inkbiograms *(a) S. aureus, (b) B. subtilis, (c)E. coli, (d)M. lentiflavum, (e) A. flavus, (f) A. fumigatus, (g) Penicillium sp., (h) Cladosporium sp.*

## RESULTS AND DISCUSSION

The structure and amount of other additives added against microbial growth in the ink vary among manufacturers. As a result of our investigations, differences were observed between the inhibition diameters of different ink-paper discs against the same microorganism. During the investigation of the effect of the discs containing different volumes of ink on the inhibition zones; when the sizes of the inhibition zones were measured, it was determined that the responses of different amounts of the same ink against the microbial agent which was passaged at a certain concentration were almost the same.

When we look at the effect of time on the inhibition zones in order to examine the microbial effect on the molecular transformation of the ink pigments in the paper-ink discs contaminated with microorganisms over time; significant changes were observed in the inhibition zone sizes versus time at 0th, 3rd and 6th months regarding the contaminated and uncontaminated paper-ink discs kept under the same conditions.

Light intensity or exposure to sunlight in the environment where documents are stored affects the ink on them (Weyermann & Spengler, 2008). Paper fibers exposed to sunlight gradually lose their durability. The speed of this destruction changes with light intensity, temperature and humidity of the environment. There are some studies in the literature on how relative humidity, natural or artificial light, temperature, wavelength of light affect the fading of ink and the photochemical degradation of the paper (Ezcurra et al., 2010; Weyermann C., 2005). It is recommended that the relative humidity of the environment should be around 55-65% (Adcock, 1999). However, in terms of biological risk, 70% is the limit value and the relative humidity in the environment should not exceed 65%. According to the principles of the International Federation of Library Associations and Institutions (IFLA), it has been stated that the rate of chemical degradation on documents doubles with every 10$^{o}$C increase in temperature. Temperature accelerates drying in low relative humidity, and mold growth in high relative humidity. Therefore, the ambient temperature where the document is stored should not exceed 20$^{o}$C.

## CONCLUSION

Many physical (storage, transportation, use, light), chemical (atmospheric pollution, structure of paper and ink) and biological (presence of microorganisms, insects and mice) factors accelerate the degradation/fading process of the ink. The control of environmental conditions is very important in stopping or slowing down the destruction caused by chemical deterioration in the paper. Regulation of storage conditions is an important step in stopping or slowing down chemical deterioration (Binark, 1988). The organisms that cause biological deterioration of archival materials are rodents, microorganisms and insects. In total, fungi, insects, heat, sunlight, humidity, dust and dirt destroy the paper in the archive environment and deteriorate its properties. In addition, acidic and other gases in the air, harmful chemicals added during production destroy the paper and the ink on it.

In our study, although the MV ratio in the inks was approximately the same in the total area, differences were observed between the inhibition diameters of the inkbiograms against the same microorganism, since the structure and amount of other additives added against microbial growth in the ink varied among the manufacturers. Although it is anticipated that the differences in zone diameters may be due to deviations in repeatability that may result from the very low pipetting volume (varying between 1-3 µL) when pipetting ink onto the paper disc; when it is observed that the zone diameters in the inkbiograms obtained after adding incremental volumes of the same ink on the paper discs are approximately the same for all volumes in this range, it was thought that there might be another reason for these differences. Considering that there will be an increasing amount of antimicrobial effective MV in increasing volumes of ink, it was concluded that an increase or decrease in the amount of MV cannot cause such an effect; and the differences in the diameters of the dyed zones were attributed to diffusion into the agar at different amounts and diameters due to differences in ink formulations. In order to obtain more detailed findings, it is necessary to conduct research on samples kept in archive or room environment in longer periods. The use of the word "inkbiogram" for macro and micro images on incubated petri dishes, which is used in this study where the effect of microorganisms on the ink is investigated, is suggested to be used in the scientific literature on the ink-microorganism interaction, and this term was used for the first time in this study.

## REFERENCES

Adcock, E. (1999). *IFLA Principles for the care and handling of library material*.

Andrasko, J. (2001). HPLC Analysis of Ballpoint Pen Inks Stored at Different Light Conditions. *Journal of Forensic Sciences*, *46*(1), 14907J. https://doi.org/10.1520/JFS14907J

Binark, İ. (1988). *Arşiv Malzemesini Tahrib Eden Unsurlar, Bunlara Karşı Korunma Metodları ve Arşiv Malzemesinin Restorasyonu*. FSM. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/http://acikerisim.fsm.edu.tr/xmlui/bitstream/handle/11352/269/Binark.pdf?sequence=1

Brezová, V., Pigošová, J., Havlínová, B., Dvoranová, D. & Ďurovič, M. (2004). EPR study of photochemical transformations of triarylmethane dyes. *Dyes and Pigments*, *61*(2), 177–198. https://doi.org/10.1016/j.dyepig.2003.10.012

Brunelle, R. L. & Reed, R. W. (1985). *Forensic examination of ink and paper*. Charles C. Thomas.

Brunelle R.L. & Crawford K. R. (2003). *Advances in the Forensic Analysis and Dating of Writing Ink*. Charles C Thomas Publisher Ltd., Springfield, Illinois.

Bügler, J. H., Buchner, H. & Dallmayer, A. (2005). Characterization of Ballpoint Pen Inks by Thermal Desorption and Gas Chromatography-Mass Spectrometry. *Journal of Forensic Sciences*, *50*(5), 1–6. https://doi.org/10.1520/JFS2004486

Calcerrada, M. & García-Ruiz, C. (2015). Analysis of questioned documents: A review. *Analytica Chimica Acta*, *853*, 143–166. https://doi.org/10.1016/j.aca.2014.10.057

Cengiz, S. (2013). *Adli Belge İncelemelerinde İmza ve Yazının Yaş Tayini*.

Ezcurra, M., Góngora, J. M. G., Maguregui, I. & Alonso, R. (2010). Analytical methods for dating modern writing instrument inks on paper. *Forensic Science International*, *197*(1–3), 1–20. https://doi.org/10.1016/j.forsciint.2009.11.013

Grim, D. M., Siegel, J. & Allison, J. (2001). Evaluation of Desorption/Ionization Mass Spectrometric Methods in the Forensic Applications of the Analysis of Inks on Paper. *Journal of Forensic Sciences*, *46*(6), 15164J. https://doi.org/10.1520/JFS15164J

Karbowska-Berent, J., Górny, R. L., Strzelczyk, A. B. & Wlazło, A. (2011). Airborne and dust borne microorganisms in selected Polish libraries and archives. *Building and Environment*, *46*(10), 1872–1879. https://doi.org/10.1016/j.buildenv.2011.03.007

Sterflinger, K. (2010). Fungi: Their role in deterioration of cultural heritage. *Fungal Biology Reviews*, *24*(1–2), 47–55. https://doi.org/10.1016/j.fbr.2010.03.003

Weyermann C. (2005). *Mass Spectrometric Investigation of the Aging Processes of Ballpoint Ink for the Examination of Questioned Documents*. Justus-Liebig University.

Weyermann, C., Almog, J., Bügler, J. & Cantu, A. A. (2011). Minimum requirements for application of ink dating methods based on solvent analysis in casework. *Forensic Science International*, *210*(1–3), 52–62. https://doi.org/10.1016/j.forsciint.2011.01.034

Weyermann, C. & Spengler, B. (2008). The potential of artificial aging for modelling of natural aging processes of ballpoint ink. *Forensic Science International*, *180*(1), 23–31. https://doi.org/10.1016/j.forsciint.2008.06.012

## *ORAL PRESENTATION*

# MEMS-based biosensor design and analysis for melanoma detection

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## Abstract

Melanoma is a life-threatening malignant skin cancer. It occurs as a result of excessive and uncontrolled proliferation of cells called melanocytes that give the skin its color. One of the first steps to be taken for the treatment of melanoma, which takes a long time to detect, is to make an accurate and effective diagnosis. When the literature was examined, it was seen that the interaction between MITF, which is known to provide high sensitivity and specificity among melanoma biomarkers, and D5 antibody, the human monoclonal antibody of MITF, was detected only by immunohistochemical methods. However, the measurement and analysis of specific binding strengths between antigen-antibody are very important for better understanding the biological functions of tumor markers, developing methods to be used in the diagnosis and treatment of disease, accurate and rapid diagnosis, and drug and vaccine design for the treatment of diseases.
This work presents the design and simulation of the MEMS micro console sensor for rapid detection of melanoma in a given blood sample. The surface of the micro-console is coated with D5 monoclonal antibodies (anti-MITF) known to be specific to the MITF antigen. When a blood sample containing MITF antigen (from a patient with melanoma) is added, biochemical reactions occur between the MITF antigen and the D5 antibody due to the affinity they have for each other and an antigen-antibody complex is formed. This complex creates stress and tension on the console. COMSOL Multi Physics software was used in micro console design and simulation. Current melanoma diagnosis methods include radiological imaging techniques, serological methods and ABCD scale. MEMs sensors are needed for rapid, early and accurate diagnosis of melanoma due to the disadvantages of these methods in addition to their inability to respond accurately and quickly in the disease staging process. When the patient's blood sample is placed on the micro-console, the antigen-antibody complex will cause the console to bend and the adsorption molecules will generate a surface tension on the micro-console. This tension is created due to the interactions between the molecules and the micro cantilever surface. By using this designed MEMS micro console bio sensor, we provide a much faster and more accurate diagnosis of melanoma compared to current diagnosis methods.
**Keywords:** Melanoma, MEMS, Micro console, Antigen, Antibody

## INTRODUCTION

The skin, which can lose many important functions due to injury, genetics, or chronic diseases, is the largest organ of the human body. Among all skin diseases, melanoma, a type of malignant skin cancer, is one of the most severe diseases that can lead to death due to high metastasis and mortality (Chaturvedi et al., 2020). Representing the most aggressive and deadliest form of skin cancer, melanoma accounts for approximately 75% of skin cancer-related deaths (Jain and Pais,2015; Andor et al.,2016), and has a prevalence of 80% due to its high resistance to radiotherapy and chemotherapy. known to have a death rate. Early detection of melanoma is very important to increase the early survival rate. Individuals can usually notice the progression of the disease by self-examination, which usually coincides with the later stages of the disease. Serological methods such as ELISA and PCR and medical imaging methods used in the diagnosis of melanoma have limitations such as the need for large amounts of samples, the long detection process, high cost, and the need for qualified personnel and expensive laboratory equipment. Micro-electro-mechanical system (MEMS) technology has revolutionized the analysis of different types of diseases. Microcantilever beams are a type of MEMS device used in a variety of physical, chemical, and biological applications (Fanse, 2021). MEMS has been used as a microscale biosensor in various biological processes (Basu et al., 2020; Hagan et al.,2002; Mahalik, 2006).
MEMS microcantilevers are biosensors with one end fixed and the other free for displacement. The displacement is due to the stress caused by the interaction of antigen and antibody on the sensitive surface of the cantilever. The cantilever beam deviates after immobilizing the specific antibody on the upper surface of the cantilever beam to interact with the antigen. By binding specific antibodies to the upper surface of the beam, the cantilever surface becomes sensitive. The cantilever is used because its structure, sensitivities, and

performances in detecting bio-molecular elements are outstanding. Given the difficult conditions in melanoma diagnosis and staging, there is an urgent need to develop alternative, simpler, and less costly techniques for diagnosing melanoma. In this article, a MEMS microcantilever sensor was designed and simulated, which offers a fast and accurate method for the detection of melanoma. Both V shape and Rectangular microcantilevers were investigated in the study.

## MATERIALS AND METHODS

### Antigen-Antibody Selection

Microphthalmia Transcription Factor (MITF) is known to have high sensitivity (approximately 100%) and specificity for metastatic melanoma. The molecular weights of MITF antigen and human D5 monoclonal antibody known to be specific to MITF antigen are 59 kDa and 52 kDa, respectively. The sum of the total molecular weight (111 kDa) of the MITF antigen and D5 antibodies is approximately 18.4315 x $10^{-23}$ kg.

### Micro Cantilever Design Structure and Working Principle

The 3D design of the microcantilever biosensor model was made using COMSOL Multiphysics. MEMS-based models can be designed, modeled, and simulated using COMSOL software. COMSOL software provides a platform to perform analysis on different physics flows. COMSOL simulation includes selecting physics, defining geometry and materials, tuning physics, networking, and analysis of results. Structural mechanics are selected based on the defined mode of the microcantilever.

In this study, both V shape and Rectangular microcantilevers were investigated. The model has three layers, the substrate, the upper electrode, and the lower electrode. Silicon Nitride ($Si_3N_4$) material, which is a polysilicon layer, was preferred as the substrate material in the design. The polysilicon layer helps to avoid non-specific interactions. Gold material was used to immobilize antibodies on the upper surface of the cantilever and to perform detection. Thus, with the Surface Plasmon resonance, the microcantilever helps detect beam deflection. The total surface tension produced on the microcantilever is proportional to the number of analyte molecules attached to the gold layer. The absorption of biomolecules onto the gold surface creates surface tension on the surface of the cantilever beam. The stress is produced due to the antigen-antibody reaction on the cantilever surface.

Young's modulus, spring constant, Poisson's ratio, stress, etc. Many factors are taken into account, such as Since the thickness of the beam is very small, rotational inertia and shear deformation can be neglected.

Adsorption molecules create surface tension on the microcantilever. This stress is created due to the interactions between the molecules and the microcantilever surface. The relationship between surface tension (σ) due to analyte adsorption and beam deflection (d) can be explained by Stoney's equation (1).

$$d = \frac{3\sigma(1-\vartheta)}{E}\left(\frac{L}{t}\right)^2 \quad (1)$$

Here, E: Young's modulus, V: Poisson ratio, L: length of the microcantilever, and t: the thickness of the microcantilever.

Young's modulus and Poisson's ratio depend on the material, the surface tension changes depending on the amount of analyte placed on the sensor, and the deflection of the beam changes with changes in the length and thickness of the microcantilever beam. Deviation can be calculated for various lengths by making the width constant and vice versa. The induced mass can be detected by the shift in frequency in dynamic mode. The resonance frequency shift can be found by equation (2)

$$f = \frac{1}{2\pi}\sqrt{\frac{E}{\rho}}\frac{t}{L^2} \quad (2)$$

Here f: fundamental resonance frequency, E: Young's modulus, ρ: density of the material.

Thus, the resonant frequency of the V-shaped console is obtained in equation 3.

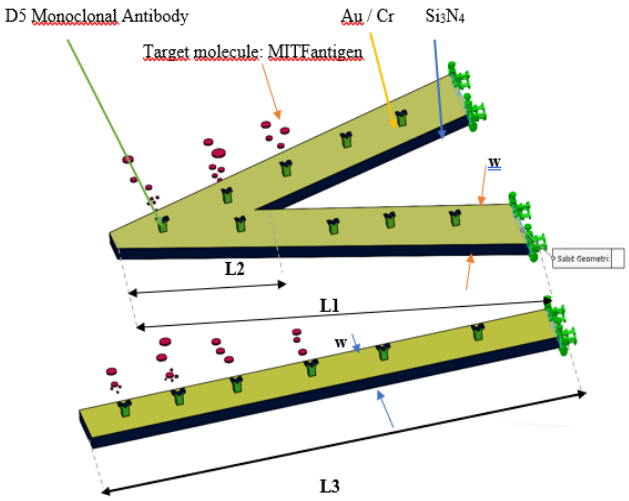
$$f(W(x)) = \frac{\sqrt{210}}{\pi L_1^{\,2}}\sqrt{\frac{\frac{E_1H_1^3}{12}+\frac{2E_2H_2^3}{3}+\frac{E_2H_2H_1^2}{2}+E_2H_1H_2^2}{\rho_1H_1+2\rho_2H_2}}\,g(u,v) \quad (3)$$

A cantilever array consisting of a triangular beam with fixed lengths of 80 µm, width of 60 µm, and thickness of 0.2 µm was constructed. Si3N4 material is used for microcantilever beams.

It is assumed that the surface of each cantilever is functionalized with 100 molecules of D5 antibody. A pressure equivalent to 100 molecules of D5 antibody weight (4.5052 x $10^{-11}$ Pa) was entered as input and deviations were observed. Again, a total pressure (9.6142 x $10^{-11}$ Pa) equivalent to the weight of 100 antigen molecules and 100 antibody molecules was applied. Antigens are assumed to interact with all antibodies of the cantilever. The displacement will therefore be the same as the bias caused by the pressure equivalent of 100 antigens and 100 antibodies. V-shaped and Rectangular micro console data are given in Figure 1.

**Boundary Conditions and Materials Properties**

A 3D model structure was designed in COMSOL Multiphysics software. Solid mechanics are added as physics. Self-frequency is provided for proper analysis after the selection of mechanism physics. The geometry is created in the model builder window. The structural unit is kept on the micrometer (μm) scale. Then the build is finally built using the build all option in the block window. V-shaped biosensor drawing with fixed lengths of 80 μm, width of 60 μm, and thickness of 0.2 μm was made using drawing equipment from the Comsol program. $Si_3N_4$ material is used in V Shaped Cantilever. The material properties of this material such as Density, Young Modulus, and Poisson Ratio are shown in Table 1. The designed $Si_3N_4$-defined V-shaped cantilever surface is defined as 50 nm Gold and 9 nm PDMS layer. The height of the D5 antibody is 8.5 nm, and since the antibody parameters are close to PDMS on this biosensor, 9 nm high antibody equivalent material is defined as Layer. The structural properties of Gold and PDMS materials are given in Table 2.




a

| | |
|---|---|
| L1 | 80 μm |
| L2 | 40 μm |
| L3 | 210 μm |
| $T_{konsol}$ | 0,2 μm |
| $T_{gold}$ | 50 nm |
| $T_{antibody}$ | 9 nm |
| w | 20 μm |
| $P_{gold}$ | 2 x $10^{-7}$ N/$m^2$ |
| $P_{antibody}$ | 4.5052 x $10^{-11}$ N/$m^2$ |

b

**Figure 1**. a) Representation of the designed V shape Micro cantilever and Rectangular micro cantilever b) Data of the cantilever

**Table 1.** Characteristics of Si3N4 building materials in the cantilever

| **Property** | **Value** |
|---|---|
| Density | 3100[kg/m^3] |
| Young's modulus | 250e9[Pa] |
| Poisson's ratio | 0.23 |
| Electrical conductivity | 0[S/m] |
| Coefficient of thermal expansion | 2.3e-6[1/K] |
| Heat capacity at constant pressure | 700[J/(kg*K)] |
| Relative permittivity | 9.7 |
| Thermal conductivity | 20[W/(m*K)] |

**Table 2.** Properties of Gold and PDMS building materials in the cantilever

| Property | Gold / Value | PDMS/ Value |
|---|---|---|
| Electrical conductivity | 45.6e6[S/m] | - |
| Coefficient of thermal expansion | 14.2e-6[1/K] | 9e-4[1/K] |
| Heat capacity at constant pressure | 129[J/(kg*K)] | 1460[J/(kg*K)] |
| Density | 19300[kg/m^3] | 970[kg/m^3] |
| Thermal conductivity | 317[W/(m*K)] | 0.16[W/(m*K)] |
| Young's modulus | 70e9[Pa] | 750[kPa] |
| Poisson's ratio | 0.44 | 0.49 |

In the solid modeling of the drawn cantilever, "Isotropic" was chosen as the linear elastic material as the solid material model. Material properties were determined as Young's modulus, density, and Poisson's ratio. In the V-shape cantilever component definition process, the center of mass was selected and the entire surface of the cantilever was selected as the boundary system. In line with this selection, the deformation mode on the z-axis was examined by using deformed configurations as the frame. ScanAsyst-Fluid probes combine the low spring constant of a Silicon Nitride cantilever for force control with the sharpness of a silicon tip and have a tip to provide high-resolution imaging in liquid. The frequency of this probe ranges between 150 kHz and 200 kHz. The results of the designed micro cantilever will be compared with this probe frequency. The geometry drawing of the V shape and Rectangular micro console designed using COMSOL Multiphysics software is given in Figure 2.

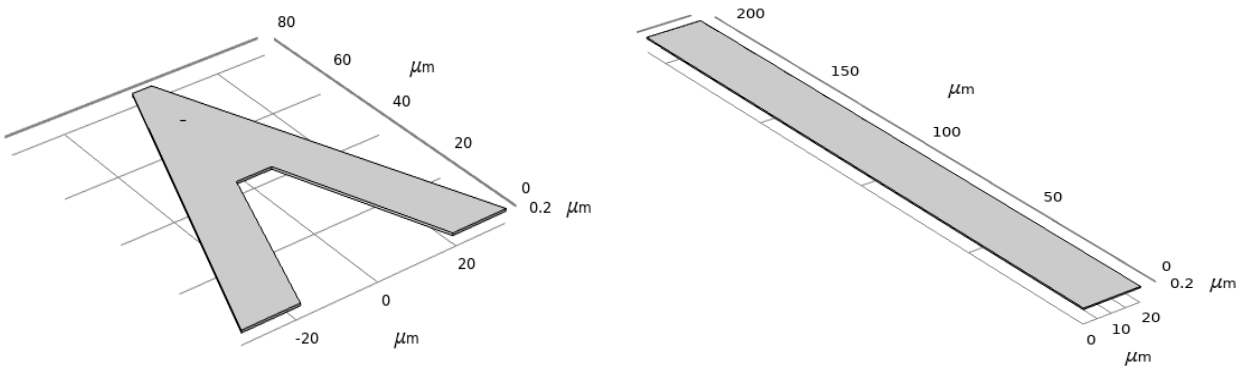



**Figure 2.** Geometry drawing of the designed V shape and Rectangular microcantilever using COMSOL Multiphysics software

## RESULTS and DISCUSSION

This present work demonstrates the finite element method to achieve optimum performance of the cantilever array sensor for MITF antigen-based Melanoma detection by optimizing the materials and geometric size of the cantilever. COMSOL Multiphysics 5.5, a commercial finite element analysis tool for MEMS, was used to develop cantilever-based array sensors.

A V-shaped cantilever with fixed lengths of 80 µm, a width of 60 µm, and a thickness of 0.2 µm and a Rectangular cantilever with a length of 210 µm, a width of 20 µm, and a thickness of 0.2 µm have been arrayed. It is assumed that the surface of each cantilever is functionalized with 100 molecules of D5 antibody. A pressure equivalent to 100 molecules of MITF antibody weight (4.50512x $10^{-11}$ Pa) was entered as input and deviations were observed. (Molecular weight of MITF antigen = 59 kDa and molecular weight of D5 antigen = 52 kDa). Again, a total pressure (9.6176 x $10^{-11}$ Pa) equivalent to the weight of 100 antigen molecules and 100 antibody molecules MITF+D5=>MITFD5 was applied.

F=m*a

$M_{D5}$= 52kDa*1.6605 x $10^{-24}$ = 8.6346 x $10^{-23}$ kg

$M_{MITF}$= 59kDa*1.6605 x $10^{-24}$ = 9.79695 x $10^{-23}$kg

$F_{D5}$= 8.6346 x $10^{-23}$ kg * 9.81 = 8.4705 x $10^{-22}$ N

$F_{MITF}$= 9.79695 x $10^{-23}$ kg *9.81= 9.6108 x $10^{-22}$ N

P= F /A

A (cantliver alanı) = 188 x $10^{-11}$ $m^2$

$P_{D5}$= $F_{D5}$ / Alan = 8.4705 x $10^{-22}$ / 188 x $10^{-11}$ = 4.5055 x $10^{-13}$ $N/m^2$

$P_{MITF}$= $F_{MITF}$ / Alan =9.6108 x $10^{-22}$ / 188 x $10^{-11}$ = 5.1121 x $10^{-13}$ $N/m^2$

$F_{D5}+F_{MITF}$ = 1.80813 x $10^{-21}$ N

$P_{D5}+P_{MITF}$= 9.6176 x $10^{-13}$ $N/m^2$ = Pressure of 1 antibody and antigen immobilization

Since there are 100 antigen and antibody assumptions;
100 x 9.6176 x $10^{-13}$ $N/m^2$ = 9,6176 x $10^{-11}$
Antigens are assumed to interact with all antibodies of the cantilever. The displacement will therefore be the same as the bias caused by the pressure equivalent of 100 antigens and 100 antibodies. Based on this applied pressure, a resonance frequency of 155.91 kHz was observed in the V shape Micro cantilever and 16,517 kHz in the Rectangular microcantilever. The Bruker ScanAsyst-Fluid probes we take as reference is in the 150 kHz-200 kHz range. The accuracy was determined as 155.91 kHz in our simulation result and 156.92 kHz in the theoretical calculation. The Rectangular Micro cantilever is based on the Bruker MLCT-B probe. This probe has a resonant frequency in the range of 10-20 kHz. As a result of the simulation, 16,517 kHz and 17,514 kHz resonance frequencies were found in the theoretical calculation. Displacement and Input force for different Cantilever types are given in Table 3. Graphical representation of frequency calculation of V-shaped and Rectangular micro console using Comsol is in Figure 3, Displacement data as a result of Gold, antibody, and antigen+Antibody immunization on a V-Shaped and Rectangular Console, respectively, are given in Figure 4.

**Table 3.** Displacement and Input force for different Cantilever types

| Cantilever type | Displacement (um) | Eigen Frequency(kHz) |
|---|---|---|
| **V Shaped** | 4.49512* $10^{-4}$ | 1.5591 kHz |
| **Rectangular Shape** | 3.54648 *$10^{-9}$ | 16.517 kHz |

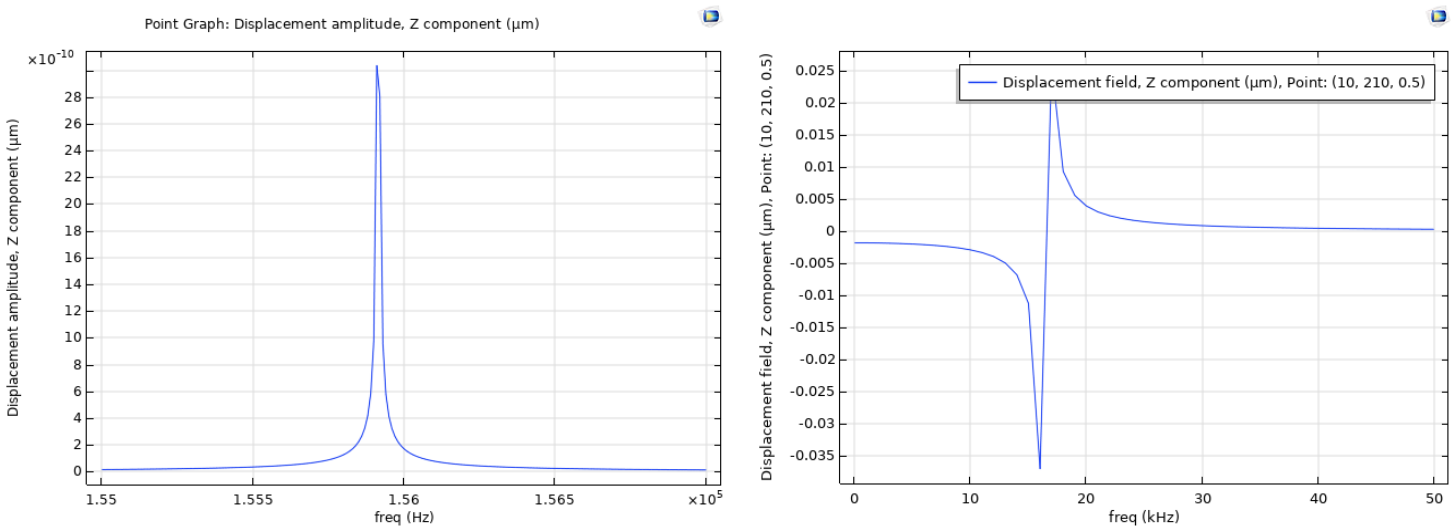



**Figure 3.** Graphical representation of frequency calculation of V-shaped (left: f=1.5591 kHz) and Rectangular (right: f=16.517 kHz) microcantilever using Comsol 5.5

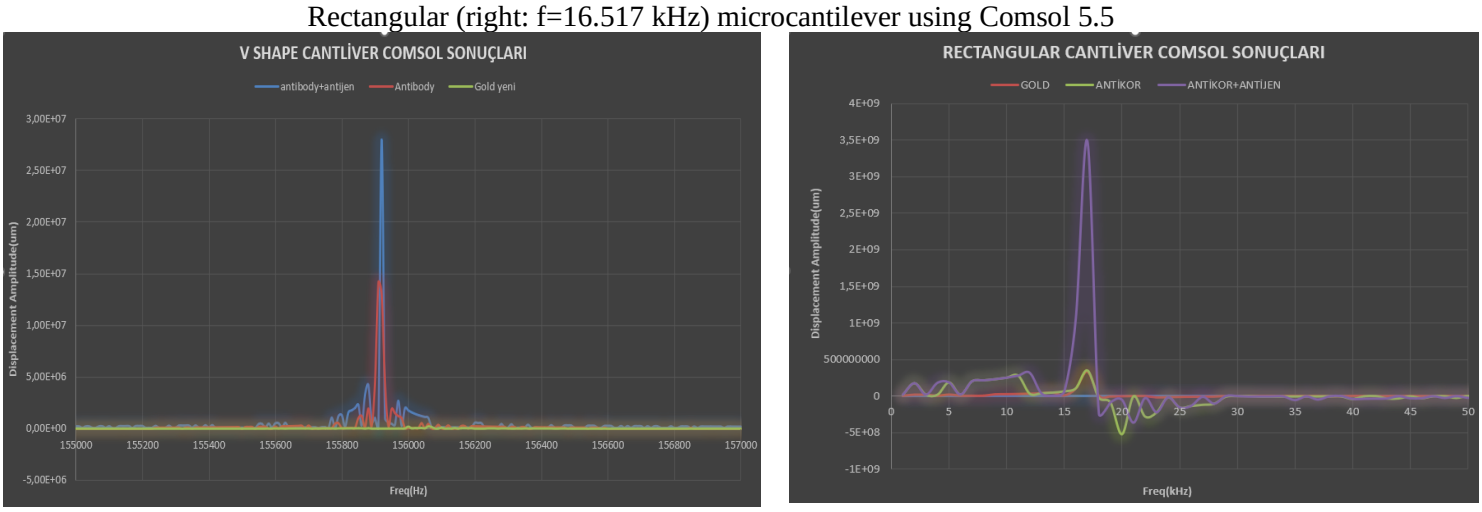



**Figure 4.** Displacement data as a result of Gold, antibody, and antigen+Antibody immunization applied on a V-Shaped (left) and Rectangular (right) Cantilever, respectively.

## CONCLUSION

Melanoma, which represents the most aggressive and deadliest form of skin cancer, is responsible for approximately 75% of skin cancer-related deaths (Jain and Pise, 2015) and its prevalence is 80% due to its high resistance. It is known that early diagnosis and effective treatment are critical for long-term survival. The five-year survival rate for early-stage malignant skin cancers is 99%, and the survival rate after metastasis is as low as 15% (Somwanshi et al.,2020). Early diagnosis of melanoma is very important to increase the early survival rate. Serological methods such as ELISA and PCR and medical imaging methods used in the diagnosis of melanoma have limitations such as the need for large amounts of samples, long detection process, high cost, need for qualified personnel and expensive laboratory equipment. Studies on the analysis, diagnosis and treatment of different disease types using micro-electro-mechanical system (MEMS) technology have been the subject of many studies in recent years (Kaufman and Mehnert,2016). Given the difficult conditions in melanoma diagnosis and staging, there is an urgent need to develop alternative, simpler and less costly techniques for melanoma diagnosis.

In this research, the MEMS microcantilever biosensor for rapid detection of melanoma was designed and simulated in COMSOL Multiphysics. The model is designed with the dimensions and materials of the V-shaped cantilever with 80 µm length, 60 µm width, and 0.2 µm thickness, and the Rectangular cantilever with 210 µm length, 20 µm width, and 0.2 µm thickness. After the simulation, displacement analysis was performed.

Unlike existing traditional diagnostic methods, this sensor has made the detection of melanoma very easy and accurate. This concludes our micro cantilever work and its application on the biosensor, a device for biosensing. The overall goal of this device is to detect a variety of bioanalytics for disease identification, making it a portable platform for biological detection. These MEMS sensors have advantages such as low production cost due to less material, easy integration, greater portability, robustness, and low power consumption.

## REFERENCES

Andor, N., Graham, T. A., Jansen, M., Xia, L. C., Aktipis, C. A., Petritsch, C., … Maley, C. C. (2016). Pan-cancer analysis of the extent and consequences of intratumor heterogeneity. Nature Medicine, 22(1), 105–113.

Basu, A. K., Basu, A., & Bhattacharya, S. (2020). Micro/nano fabricated cantilever based biosensor platform: A review and recent progress. Enzyme and Microbial Technology, 139, 109558.

Chaturvedi, S. S., Gupta, K., & Prasad, P. S. (2020). Skin lesion analyser: an efficient seven-way multi-class skin cancer classification using MobileNet. In International Conference on Advanced Machine Learning Technologies and Applications (pp. 165-176). Springer, Singapore.

Fanse, T. S. (2021). Design and Modification of MEMS Based Micro Cantilever. arXiv preprint arXiv:2111.01890.

Jain, S., & Pise, N. (2015). Computer aided melanoma skin cancer detection using image processing. Procedia Computer Science, 48, 735-740.

Somwanshi, D., Chaturvedi, A., & Mudgal, P. (2020). ABCD features extraction-based melanoma detection and classification. In International Conference on Artificial Intelligence: Advances and Applications 2019 (pp. 327-335). Springer, Singapore.

Kaufman, H. L., & Mehnert, J. M. (Eds.). (2016). Melanoma. Springer.

Hagan, M. F., Majumdar, A., & Chakraborty, A. K. (2002). Nanomechanical forces generated by surface grafted DNA. The Journal of Physical Chemistry B, 106(39), 10163-10173.

Mahalik, N. P. (2006). Micromanufacturing and nanotechnology (pp. 178-180). Berlin: Springer.

## *ORAL PRESENTATION*

### Bal ile polifenol oksidaz enziminin inhibisyonu

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**Özet**

Polifenol oksidaz enzimi (PPO) aktif merkezinde bakır bulunduran ve fenolik bileşiklerin *o*-kinonlara dönüşümünü katalize ederek enzimatik esmerleşmeye neden olan bir enzimdir. Enzimatik esmerleşme reaksiyonları gıda kalitesinde, görünümünde olumsuz değişime neden olduğu, besin değerini düşürdüğü, gıdanın pazarlanabilirliğinin ve raf ömrünün azalmasına neden olarak ülkenin ekonomik durumunu etkileyebileceği için çoğu gıda prosesinde istenmemektedir. İstenmeyen enzimatik esmerleşme reaksiyonlarının önlenmesi için çeşitli kimyasallar veya doğal içerikli maddeler kullanılmaktadır. İnsan sağlığına zararlı olabilecek kimyasal maddelerin yerine doğal alternatif esmerleşme önleyici maddelerin kullanılması daha avantajlıdır. Doğal inhibitörlerin araştırılmasına yönelik çok sayıda çalışma yapılmıştır. Çalışılan bu doğal inhibitörlerden biri de baldır. Bal eski zamanlardan beri dünya çapında beğeni kazanmış mevcut tek konsantre şeker formu olarak kullanılmaktadır. Geleneksel olarak gıdalarda tatlandırıcı olarak kullanılır. Aynı zamanda bir gıda koruyucusu olarak da işlev görmektedir. Ballar, koruyucu olarak işlev gördüğü bilinen askorbik asit, α-tokoferol, flavonoidler ve diğer fenolik bileşikler, katalaz, glukoz oksidaz ve peroksidaz gibi enzimleri de içermektedir. Bal, gıda prosesinde PPO enziminin neden olduğu enzimatik esmerleşmenin olumsuz etkilerini azaltmak için doğal bir inhibitör olarak kullanılma potansiyeline sahiptir.

**Anahtar Kelimeler:** Bal, İnhibisyon, Polifenol oksidaz enzimi.

### Polyphenol oxidase enzyme inhibition with honey

**Abstract**

Polyphenol oxidase enzyme (PPO) is an enzyme that contains copper in its active center and causes enzymatic browning by catalyzing the conversion of phenolic compounds to *o*-quinones. The enzymatic browning reactions, which cause negative changes in food quality, and appearance, reduce the nutritional value, marketability, and shelf life of the food, thereby affecting the economic situation of the country, are undesirable in most food processes. Various chemicals or natural ingredients are used to prevent enzymatic browning reactions. It is more advantageous to use natural alternative anti-browning agents instead of chemical substances that may be harmful to human health. Numerous studies have been done to investigate natural inhibitors. One of these natural inhibitors that have been studied is also honey. Honey, which is the only available concentrated form of sugar has gained an appreciation and has been used worldwide since ancient times. It is traditionally used as a flavoring in foods. It also functions as a food protective. Honey is known to function as a protective that contains ascorbic acid, α-tocopherol, flavonoids, and other phenolic compounds, enzymes such as catalase, glucose oxidase, and peroxidase. Honey has the potential to be used as a natural inhibitor in food processing to reduce the negative effects of enzymatic browning caused by the PPO enzyme.

**Keywords:** Honey, Inhibition, Polyphenol oxidase enzyme.

## GİRİŞ

Polifenol oksidaz enzimi (PPO) oksidoredüktaz grubunda bulunan ve aktif merkezinde bakır içeren bir enzimdir. Polifenol oksidaz enzimi; kresolaz, katekolaz, katekol oksidaz, tirozinaz, monofenol oksidaz, *o*-difenol oksidaz ve fenolaz olarak da bilinmektedir (Mayer ve Harel, 1979; Yavuz, 2019). Polifenol oksidaz enzimleri substrat spesifikliklerine ve etki mekanizmalarına göre başlıca üç sınıfa ayrılır. Bunlar; katekolaz (EC 1.10.3.1), lakkaz (EC 1.10.3.2) ve tirozinaz (EC 1.14.18.1) olarak bilinmektedir (Güray, 2009; Millioğlu, 2019). Doğada yaygın olarak bulunan, hayvanlarda melanizasyondan ve bitkilerde esmerleşmeden sorumlu

olan enzimdir (Demir ve ark., 2012). PPO, polifenol substratları ve monofenollerin *o*-difenollere hidroksilasyonunu ve *o*-difenollerin *o*-kinonlara oksidasyonunu katalize etmektedir (Demir ve ark., 2012; Liao ve ark., 2021; Zhou ve ark., 2023). *O*-kinonlar, melanin üretmek için proteinler ve amino asitler tarafından nükleofilik saldırılara karşı savunmasız olan oldukça reaktif bileşiklerdir (Liao ve ark., 2021). Bu sebeple kinonlar, amino asitler, proteinler veya diğer kinonlar tarafından kahverengi, kırmızı ve siyah pigmentlere polimerize edilmektedir (Demir ve ark., 2012; Zhou ve ark. 2023). Sebze, meyve ve tahıllarda doğal olarak bulunan polifenol oksidaz enziminin sebep olduğu oksidasyon sonucunda enzimatik esmerleşme reaksiyonları meydana gelmektedir (Yang ve ark., 2001; Mathewson, 2000; Çelik, 2018). Bitki hücrelerinde, genellikle PPO'ların organellerin zarında (plastidler, mitokondri, peroksizomlar, vb.) fenolik bileşiklerin ise vakuolde bulunduğu belirtilmektedir. Kesme ve soyma sonucunda hücrelerin parçalanması, hücresel bölümlerin bozulması, fenolik bileşiklerin serbest kalmasına yol açarak PPO'nun fenolik bileşikler ve oksijen ile karşılaşmasına sebep olmaktadır. Bu sebeple kesilen yüzey esmerleşmektedir (Fan, 2022). Elma, patates, muz ve avokado gibi meyve ve sebzelerin hazırlanması ve saklanması sırasında esmerleşme sık görülen bir durumdur (Zawawi ve ark., 2022). Dokularda istenmeyen renk bozulmasına yol açan enzimatik esmerleşme, gıda kalitesini (özellikle görünümünü) olumsuz etkilediği ve dolayısıyla tüketicinin kabul edebilirliğini azalttığı için gıda endüstrisinde önemli bir endişe kaynağıdır (Jiang ve Penner, 2022). Enzimatik esmerleşme gıdanın besin değerini düşürmekte, raf ömrünü kısaltmakta gıdanın pazarlanabilirliğini azaltmaktadır (Lim ve Wong, 2018). Hasat ve hasat sonrası, nakliye, depolama, dağıtım ve haşlama gibi işlemler sırasında görülen enzimatik esmerleşme hem üreticiler hem de endüstri için ciddi bir sorundur (González ve ark., 2020). Bu işlemler sırasında ortaya çıkan enzimatik esmerleşmenin meyve ve sebzelerin %50'den fazlasının kaybına neden olduğuna inanılmaktadır (Hoo ve ark., 2022; Zawawi ve ark., 2022). Enzimatik esmerleşme reaksiyonlarının verdiği kayıplar gıda israfına da neden olmaktadır (Hoo ve ark., 2022; Jiang ve Penner, 2022). Bu durum ülkenin ekonomik durumunu etkileyebileceği için çoğu gıda prosesinde istenmemektedir (Lim ve Wong, 2018). İstenmeyen bu süreç, endüstride kimyasal ve/veya fiziksel ajanlar kullanılarak kontrol edilmektedir. Ancak bu yöntemlerin son ürünlerin duyusal ve besinsel kalitesinin değişmesi gibi önemli dezavantajları vardır (González ve ark., 2020). Yüksek performansa sahip olmaları ve düşük maliyetleri nedeniyle kimyasal inhibitörler daha çok tercih edilmektedir (Vhangani ve Van Wyk., 2021 ). Ancak bu yöntemin gıda ürünlerindeki zararlı kimyasal kalıntılar bırakması nedeniyle potansiyel sağlık etkileri bulunmaktadır (Hamdan ve ark., 2022). Enzimatik esmerleşme reaksiyonlarının önlenmesi için çeşitli kimyasal inhibitörlerin yanı sıra doğal içerikli maddeler de kullanılmaktadır. İnsan sağlığına zararlı olabilecek kimyasal maddelerin yerine doğal alternatif esmerleşme önleyici maddelerin kullanılması daha avantajlıdır (Lim ve Wong, 2018). Doğal bileşiklerin kullanıldığı yöntemler sağlık açısından da büyük önem taşımaktadır (Aksoy ve Öztekin, 2021). Bu sebeplerle doğal inhibitörlerin araştırılmasına yönelik çok sayıda çalışma yapılmıştır (Tinello ve ark., 2018; Firdous ve ark., 2020.; Lim ve Wong, 2018; Legcharoen ve ark., 2020; Vhangani ve Van, Wyk, 2021 ). Doğal inhibtörler, meyve ve sebze ürünlerinde güvenliği sağlamak, kaliteyi korumak ve raf ömrünü uzatmak için koruyucu olarak kullanılabilmektedirler. Doğal koruyucular olarak kullanılan bitki özütlerinin düşük toksisite, düşük maliyet, biyolojik olarak parçalanabilirlik, yenilenebilirlik vb. gibi sağladığı faydalar da bulunmaktadır (Hamdan ve ark., 2022). Ayrıca bu doğal maddeler polifenoller, flavonoidler, terpenler, alkoloidler ve diğer biyoaktif bileşikler bakımından da zengindirler (Zhang ve ark., 2022). Soğan, ananas, limon, longan meyvesi, olgunlaşmamış üzüm, domates kabuğu vb. gibi doğal inhibitörlerin PPO aktivitelerini engellemede etkili olduğu belirtilmektedir (Moon ve ark., 2020; Hoo ve ark., 2022). İnhibisyon etkisi olduğu öne sürülen bu doğal inhibitörlerden biri de baldır (Redondo ve ark., 2016). Bal, eski zamanlardan beri dünya çapında beğeni kazanmış mevcut tek konsantre şeker formu olarak kullanılmaktadır (FAO 1996). Geleneksel olarak gıdalarda tatlandırıcı ve tedavi edici olarak kullanılır. Aynı zamanda bir gıda koruyucusu olarak da işlev görmektedir. Ballar, koruyucu olarak işlev gördüğü bilinen askorbik asit, α-tokoferol, flavonoidler ve diğer fenolik bileşikler, katalaz, glukoz oksidaz ve peroksidaz gibi enzimleri de içermektedir (Ioyrish, 1974; Crane, 1975; Ferreres ve ark., 1993; Vela ve ark., 2007). Balın, gıda prosesinde PPO enziminin neden olduğu enzimatik esmerleşmenin olumsuz etkilerini azaltmak için doğal bir inhibitör olarak kullanılma potansiyeline sahip olduğu bildirilmektedir (Chen ve ark., 2000). Bu derlemede meyve ve sebze ürünlerinde polifenol oksidaz enziminin etkisi ile meydana gelen enzimatik esmerleşme reaksiyonlarının bal özütleri ile inhibisyonuna yönelik yapılan çalışmalar Tablo 1-2'de özetlenmiştir.

## MATERYAL VE METOT

Bu derlemede polifenol oksidaz enziminin ve polifenol oksidaz kaynaklı enzimatik esmerleşmenin farklı bal özütleriyle inhibisyonu üzerine bugüne kadar yapılmış olan bazı çalışmalarda yer alan veriler kullanılmıştır.

## BULGULAR VE TARTIŞMA

Literatürde, polifenol oksidaz enziminin ve polifenol oksidaz kaynaklı enzimatik esmerleşmenin farklı bal özütleriyle inhibisyonu üzerine çalışmalar bulunmaktadır. Bu çalışmalarda; meyve ve sebze örneklerinin esmerleşmesinin engellenmesinde, bal çeşitleri, balların antioksidan içerikleri ve ballardan saflaştırılan peptidlerin kullanılmasının esmerleşme üzerine etkisine yönelik bilgiler özetlenmiştir. Polifenol oksidaz enziminin ve enzimatik esmerleşme reaksiyonlarının bal özütleri ile inhibisyonuna yönelik yapılan çalışmalar Tablo 1-2'de özetlenmiştir.

**Tablo 1.** Bal ile polifenol oksidaz enziminin inhibisyonu üzerine yapılan çalışmalar

| Bal Türü | Etken madde /Derişim | Substrat | İnhibisyon | $IC_{50}$ ve $K_i$ | PPO Kaynağı | Referans |
|---|---|---|---|---|---|---|
| Ticari yonca karışımlı çiçek balı | %4 bal özütü ve baldan saflaştırılan 600 Da'luk peptit | Epikateşin | Nonkompetitif | Ki = %0.731 | Mantar PPO | Oszmianski ve Lee, 1990. |
| Baldan elde edilen fenolikler | t-sinnamik asit | Kateşin | Nonkompetitif | Ki=0.014mM | Mantar PPO | Martyniuk, 1994. |
| | *p*-kumarik asit | | | Ki=0.026mM | | |
| [1]Akasya, yakıotu, soya, yonca, tupelo, noel meyvesi, karabuğday çiçek balları | Bal derişimleri, toplam reaksiyon derişiminin %0.045-0.27 arasında çalışılmıştır. | Katekol | Tüm örneklerde en iyi inhibisyon etkisini 37.6-45.7 PPO aktivitesiyle Noel meyvesi balı göstermiştir. | - | Red Delicious elma, d'Anjou armudu, Idaho patates ve tatlı patates PPO | Chen ve ark., 2000. |
| [2]Karışık çiçek balı | %5 ve %3 bal özütü | Katekol | Nonkompetitif | - | Mantar PPO | Ates ve ark., 2001. |
| | Baldan elde edilen 0.6 kDa'luk peptit | | %38.9 inhibisyon | | | |
| [2] Sivas balı | | | İnhibisyon yok | | | |
| Çiçek balı | Balın %50-90 derişimleri ve saf bal kullanılmıştır. | - | Nonkompetitif PPO aktivitesi; %50'lik bal için 1.697U/mg, saf bal için 689U/mg'dır. | - | Elma suyu PPO | Gacche ve ark., 2009. |
| Palo fierro balı (Çöl demir otu) | Flavonoid açısından zengin; 0.87, 1.94, 3.5g/L derişimli bal özütü | Klorojenik asit | Nonkompetitif | $IC_{50}$=1.17g/L $K_i$=0.8 g/L | Elma PPO | de la Rosa ve ark., 2011. |
| Bal | %0.2 derişimli bal | Pirokatekol | %56 inhibisyon etkisi | - | Sapodilla PPO (*Achras sapota*) | Cortez ve ark., 2013. |
| Manuka Balı | Bal özütü | Pirokatekol | Kompetitif | - | Zencefil PPO (*Zingiber officinale* Roscoe) | Lim ve Wong, 2018. |
| | | 4-metilkatekol | Nonkompetitif | | | |
| Manuka Balı | Bal özütü(%1-%5) | | Bal özütüyle (%41.39–%48.0) inhibisyon | - | Tatlı patates PPO | Lim ve ark., 2019. |
| | | Pirokatekol | Unkompetitif | $IC_{50}$=10.10 | | |
| | | 4-metilkatekol | Nonkompetitif | $IC_{50}$=5.91 | | |

[1] Akasya (*Robinia pseudoacacia*), Yakıotu (*Epilobium angustifolium*), Soya fasulyesi (*Glycine max*), Yonca (*Melilotus* spp.), Tupelo (*Nyssa aquatica*), Hawaiian Noel meyvesi (*Schinus terebinthifloiius*), Kara buğday (*Fagopyrum esculentum*).
[2] Atatürk Orman Çiftliği'nden temin edilen karışık çiçek balı ve Sivas Bölgesi'nden elde edilen bal

**Tablo 2.** Bal ile enzimatik esmerleşmenin engellenmesi üzerine yapılan çalışmalar

| Bal Türü | Etken Madde /Derişim | İnhibisyon Etkisi | Esmerleşen Kaynak | Referans |
|---|---|---|---|---|
| Ticari yonca karışımlı çiçek balı | %10 bal özütü | Balla muamele edilen dilimlerde en düşük esmerleşme oranı, %62 inhibisyon. | Elma dilimi | Oszmianski ve Lee, 1990. |
| | Üzüm suyu ile karıştırılan bal | Kontrole göre daha az esmerleşme olmuştur. | Üzüm suyu | |
| Akasya, yakıotu, soya, yonca, tupelo, noel meyvesi, Karabuğday çiçek balları | Bal derişimi toplam homojenatın %0,5-4 arasındadır. | Homojenatta esmerleşme %2-45 azalmış; esmerleşmenin engellenmesinde 12.07-8.53 esmerleşme birimi ile en etkili Noel meyvesi balıdır. | Red Delicious elma, d'Anjou armudu, Idaho patates ve tatlı Patates homojenatı | Chen ve ark., 2000. |
| On üç faklı bal belirlenmiş. koyu renkli kır çiçeği balı ve orta koyu renkli ahududu balı kullanılmış | %10 ve %25 derişimleri belirlenmiş, %10'luk derişim tercih edilmiştir. | 14 günlük depolamada kır çiçeği balı özütüne daldırılan dilimler daha az; ahududu balına daldırılan dilimler daha hızlı esmerleşmiştir. | Gala elma dilimleri (*Malus domestica* cv. Royal Gala) | Jeon ve Zhao, 2005. |
| İspanya'nın farklı bölgelerinden biberiye, funda, okaliptüs, ekiyum (engerekotu) gibi toplam 36 multifloral bal | %0.5-%4 arasında beş farklı derişimde bal | Salgı ballarında, nektar (ekyum, biberiye ve funda) ballarına göre daha iyi esmerleşme inhibisyonu görülmüştür. | Golden Delicious elma suyu ve homojenatı | Vela ve ark., 2007. |
| Çiçek balı | Balın farklı (%5-10) derişimleri | Tüm derişimler ile muamele edilen elma suyunun kontrole göre esmerleşme hızı azalmış, yüksek bal derişimlerinde elma suyu daha geç esmerleşmiştir. | Elma suyu | Gacche ve ark., 2009. |
| [3] On altı farklı bal | Fenolik ve antioksidan içeriği zengin ballar | Ihlamur balı %85 ile en yüksek inhibisyon oranını göstermiştir. | Red Delicious elma suyu | Chang ve ark., 2011. |
| Palo fierro (Çöl demir otu) balı | Flavonoid açısından zengin; 0.87, 1.94, 3.5g/L derişimli bal özütü | Tüm derişimlerde enzimatik esmerleşme engellemiştir. | Elma suyu | de la Rosa ve ark., 2011. |
| [4] Altmış altı örnekten oluşan on üç farklı bal grubu | Toplam homojenatta bal derişimi 20-60 g/L | Enzimatik esmerleşme %21-69 değişen oranlarda azalmıştır. | Lahana homojenatı (*Brassica oleracea* L. Capitata) | Nyawali ve ark., 2015. |
| Geven, ılgın, kişniş, dereotu, maydonoz yonca, hünnap, portakal çiçeği balları | %5 derişimli bal | Balların inhibisyon aktivitesi homojenatlarda %7.4-39.6, elma suyunda %9.2-59.8 bulunmuş; portakal çiçeği balı homojenat ve ve elma suyu için en iyi inhibisyonu göstermiştir. | Golden Delicious elma suyu ve homojenatı | Khalafi ve ark., 2016. |
| Bal | %0, %5, %10, %15, %20, %25 derişimli ballar | Dilimler farklı derişimli bal özütlerine daldırılmış, %25 bal özütüne daldırılanlar daha az esmerleşmiştir. | 'Nam Dok Mai' Mango dilimleri | Supapvanich ve Boonyaritthongchai, 2016 |
| Manuka balı | %1'lik bal özütü | Bal özütüne daldırılan dilimler kontrole göre az esmerleşmiştir. | Tatlı patates dilimleri | Lim ve ark., 2019. |

[3] Ihlamur (*Tilia tuan*), Çin kurt üzümü/Goji berry (*Lycium barbarum*), Geven türü (*Astragalus sinicus* L.), Ayçiçeği (*Helianthus annus* L.), Liçi (*Litchi chinensis*), Yeni dünya (*Eriobotrya japonica*), Beşparmak Ağacı (*Vitex negundo* var. Heterophylla), Portakal (*Citrus reticulata* Banco), Yonca (*Medicago sativa* L.), Kodonopsis/ Ginseng (*Radix codonopsis*), Schisandra (*Fructus schisandrae*), Yalancı akasya (*Robinia pseudoacacia* L.), Geven türü (*Astragalus adsurgens*), Leonuri (*Herba leonuri*), Ejderha gözü/Longan (*Dimocarpus longans* Lour.) ve Hünnap (*Zizyphus jujuba* Mill.).

[4] Zambiya'nın farklı coğrafi bölgelerinden alınan bal örnekleri

## TARTIŞMA

Literatürde, polifenol oksidaz enziminin ve polifenol oksidaz kaynaklı enzimatik esmerleşmenin farklı bal özütleriyle inhibisyonu üzerine çalışmalar bulunmaktadır. Bu çalışmalarda genellikle meyve ve sebze örneklerinin esmerleşmesine sebep olan polifenol oksidaz enziminin inhibe edilmesinde; bal çeşitlerinin, ballardan saflaştırılan peptidlerin, ballarda bulunan bazı bileşiklerin ve antioksidan içeriklerin etkili olabileceği belirtilmiştir. İncelenen çalışmalardan elde edilen veriler Tablo 1-2'de özetlenmiştir.

Meyve ve sebzelerin esmerleşme süreci, oksijen ve fenolik bileşikler gibi reaktantların uzaklaştırılmasıyla veya PPO inhibitörleri kullanılması ile önlenebilmektedir (Cortez ve ark., 2013). Meyve ve sebzelerdeki enzimatik esmerleşmeyi baskılamada antioksidan ve şelatlama ajanları gibi kimyasal bazlı yöntemler kullanmaktır, ancak bu yöntemler gıda ürünlerindeki zararlı kimyasal kalıntılar nedeniyle potansiyel sağlık etkilerine sahiptir (Hamdan ve ark., 2022). Bu sebeple günümüzde tüketiciler sentetik gıda katkı maddelerinin olası tehlikeleri konusunda endişe duymaktadır. Tüketicilerin bilinçli tutumu nedeniyle, doğal ve güvenli esmerleşme önleyici maddelerin araştırılması teşvik edilmiştir (Chaisakdanugull ve ark., 2007). Bal bitkisel ürünlerde esmerleşme önleyici doğal inhibitör olarak kullanılmaktadır (Oszmianski ve Lee. 1990; Lim ve ark., 2019). Balın, sağlığı geliştirici, antioksidan ve antimikrobiyal aktivitelere sahip peptitler, fenolik asitler ve flavonoidler dahil olmak üzere çeşitli biyoaktif bileşikler içerdiğinden, meyve ve sebzelerde doğal koruyucu ve esmerleşme önleyici olarak kullanılma potansiyelinin olduğu belirtilmiştir (de la Rosa ve ark., 2011). Proteinlerin, peptitlerin ve amino asitlerin, PPO'nun aktif bölgesindeki temel bakırı şelatlayarak ve $Cu^{2+}$ ile stabil kompleksler oluşturarak PPO aktivitesini etkileyebildiği rapor edilmiştir (Oszmianski ve Lee. 1990). Oszmianski ve Lee (1990) tarafından balın elma dilimlerinde, üzüm suyunda ve model sistemlerde enzimatik esmerleşmeyi engelleyebildiğini, balın bu inhibitör etkisinden sorumlu olan bileşiğin, yaklaşık 600 Da moleküler kütleye sahip küçük bir peptit olduğu belirtilmiştir. Benzer sonuçlar bir başka çalışmada mantardan elde edilen PPO'ın inhibe edilmesinden sorumlu olan bileşiğin 0.6 kDa ağırlığında bir peptit olduğu rapor edilmiştir (Ateş ve ark., 2001). Buna karşılık bir başka çalışmada baldaki PPO inhibitörlerinin t-sinnamik ve *p*-kumarik asit olduğu ve bu moleküllerin mantar PPO'sunu güçlü bir şekilde inhibe ettiği ifade edilmiştir (Martyniuk, 1994). Balın sadece polifenollerin enzimatik oksidasyonunu engellemekle kalmadığı, aynı zamanda *o*-kinonların bir kısmını orijinal polifenollere dönüştürdüğü belirtilmiştir (Oszmianski ve Lee. 1990; Jeon ve Zhao, 2005). Balın PPO aktivitesini inhibe eden ve *o*-kinon oluşumunu baskılayan bazı bileşikler içerdiği bildirilmiştir. Bu tip reaksiyonun, *o*-kinonları hızla polifenollere çeviren askorbik asit ile meydana geldiği düşünülmektedir (Oszmianski ve Lee. 1990). Balda askorbik asit (Vitamin C), riboflavin ve flavonoidler gibi çeşitli indirgeyici ajanların bulunduğu belirtilmiştir. Askorbik asitin, PPO tarafından üretilen kinonlardan daha düşük bir redoks potansiyeline sahip olması sebebiyle kinonlar melanin pigmentleri oluşturmak için reaksiyona girmeden önce oksitlendiği ve kinonların ana dihidroksifenollerine indirgendiği rapor edilmiştir (Gacche ve ark., 2009). Ayrıca baldaki fenolik bileşiklerin, çeşitli reaktif oksijen türlerini etkin bir şekilde inaktive etme potansiyeline sahip olması beklenmektedir. Baldaki bu fenolik bileşiklerin antioksidan ve oksidatif esmerleşme reaksiyonları için substrat olma şeklinde ikili role sahip olduğu belirtilmektedir. Balın yapısındaki enzimlerin oksijenin uzaklaştırılmasını teşvik ederek bir antioksidan olarak hareket edebileceği de belirtilmiştir (Jeon ve Zhao, 2005). Balın PPO'yu inhibe etme mekanizması kullanılan balın çeşidine, PPO'nun kaynağına ve kullanılan substratlara bağlı olarak değişmektedir (Lim ve ark., 2018). Farklı çiçek kaynaklarından elde edilen balların, meyve ve sebze homojenatlarının esmerleşmesini farklı derecede inhibe ettiği bildirilmiştir. Çiçek kaynaklarının, balların benzersiz antioksidan dağılımını etkilediği buna bağlı olarak da oksidatif reaksiyonlarda farklı şekilde koruma gerçekleştirdiği hipotezi öne sürülmüştür (Chen ve ark., 2000). Balların antioksidatif kapasitesinin balın rengiyle güçlü bir şekilde ilişkili olduğu, bal ne kadar koyu renkli ise antioksidan kapasitesinin de o kadar yüksek olduğu araştırmacılar tarafından bildirilmiştir (Jeon ve Zhao, 2005). Balın renginin açık ve koyu olmasının muhtemelen balın enzimatik esmerleşmeyi azaltma gücünü gösterebileceğini düşündürmektedir (Nyawali ve ark., 2015). Tüm bu bahsedilen özellikleri nedeni ile balların meyve ve sebze proseslerinde enzimatik esmerleşmenin engellenmesinde kullanılabileceği, lezzeti arttırmanın yanı sıra görünümün iyileştirilmesine ve besleyici değerin korunmasına da katkıda bulunabileceğine dikkat çekilmektedir.

## SONUÇ

İncelenen çalışmalardan meyve ve sebzelerde meydana gelen enzimatik esmerleşme reaksiyonlarının engellenerek besin değeri ve lezzetin korunmasını sağlayacak, ekonomik kayıpların önüne geçebilecek çevre dostu doğal inhibitörlerden birinin de bal olduğu görülmektedir. Balların yapısında bulunan fenolik ve antioksidan içerikler ile bazı peptid yapıları enzimatik esmerleşmenin engellenmesinde etkili olabilmektedir. Balların yapısındaki bu bileşiklerin sağlık yararlarının olması sebebiyle insan sağlığı için dezavantajları bulunan kimyasal katkı maddelerinin yerine alternatif olacağı düşünülmektedir.

## KAYNAKLAR

Aksoy M, Öztekin A 2021.Using of group-based selected flavonoids as alternative inhibitors for potato polyphenol oxidase. J Food Biochem.45(4):e13700.

Ateş S, Pekyardımcı S, Çökmüş, C 2001. Partial characterization of a peptide from honey that inhibits mushroom polyphenol oxidase. Joumal of Food Biochemistry 25,127-137.

Chaisakdanugull C, Theerakulkait C, Wrolstad RE 2007. Pineapple juice and its fractions in enzymatic browning inhibition of banana [Musa (AAA group) Gros Michel]. J Agric Food Chem.55(10):4252-4257.

Crane E 1975. In Honey, a Comprehensive Survey; Crane Russak and Co.: New York.

Chang X, Wang J, Yang S, Chen S, Song Y. 2011. Antioxidative, antibrowning and antibacterial activities of sixteen floral honeys, Food Function, 2, 541–546.

Chen L, Mehta A, Berenbaum M, Zangerl AR, Engeseth NJ. 2000. Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. J Agric Food Chem.;48(10):4997-5000.

Cortez J, Méndez C, Duch E, Vargas M, Pereira S 2013. Purification and partial characterization of polyphenol oxidase from Sapodilla Plum (*Achras sapota*). Food and Nutrition Sciences, Vol. 4 No. 7, pp. 727-734.

Çelik E 2018. Eğirdir (Isparta)'de Yetiştirilen Elmalardan Polifenol Oksidaz Enziminin Saflaştırılması, Karakterizasyonu ve Polifenol Oksidaz Enziminin Saflaştırılması için Alternatif Bir Yöntem Geliştirilmesi, Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi Isparta, 59 s.

de la Rosa LA, Alvarez-Parrılla E, Moyers-Montoya E, Vıllegas-Ochoa M, Ayala-Zavala JF, Hernández J, Ruız-Cruz S, González-Aguılar GA 2011. Mechanism for the inhibition of apple juice enzymatic browning by Palo Fierro (desert ironweed) honey extract and other natural compounds, LWT - Food Science and Technology 44, 269-276.

Demir D, Gençer N, Arslan O, Genç H, Zengin M 2012. In vitro inhibition of polyphenol oxidase by some new diarylureas, Journal of Enzyme Inhibition and Medicinal Chemistry, 27(1), 125-131.

Fan X, 2022 Chemical inhibition of polyphenol oxidase and cut surface browning of fresh-cut apples. Critical Reviews in Food Science and Nutrition, 1-15. (doi: 10.1080/10408398.2022.2061413).

FAO. 1996. Value-Added Products from Beekeeping; FAO Agricultural Services Bulletin; FAO: Rome, Italy.

Ferreres F, Garciaviguera C, Tomaslorente F, Tomasbarberan FA 1993. Hesperetin C a marker of the floral origin of citrus honey. J. Sci. Food Agric. 61, 121-123.

Firdous A, Ring E, Elumalai P 2020. Effects of green tea- and amla extracts on quality and melanosis of Indian white prawn (Fenneropenaeus indicus, Milne Edwards, 1837) during chilled storage, Aquaculture and Fisheries, https://doi.org/10.1016/j.aaf.2020.09.003.

Gacche RN, Shinde BT, Dhole NA, Pund MM, Jadhav AD 2009. Evaluation of floral honey for inhibition of polyphenol oxidase-mediated browning, antioxidant and antimicrobial activities. Journal of food biochemistry, 33(5), 693-706.

González MN, Massa GA, Andersson M, Turesson H, Olsson N, Fält AS, Storani L, Décima Oneto CA, Hofvander P, Feingold SE 2020. Reduced Enzymatic Browning in Potato Tubers by Specific Editing of a Polyphenol Oxidase Gene via Ribonucleoprotein Complexes Delivery of the CRISPR/Cas9 System. Front Plant Sci.;10:1649.

Güray M Z 2009. Partial Purification and Characterization of Polyphenol Oxidase from Thermophilic Bacillus sp. İzmir Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, İzmir, 54s.

Hamdan N, Lee CH, Wong SL, Fauzi CENCA, Zamri NMA, Lee TH 2022.Prevention of Enzymatic Browning by Natural Extracts and Genome-Editing: A Review on Recent Progress. Molecules.27(3):1101

Hoo NJHS, Siew ZZ, Lim WY, Wong CW 2022. Inhibitory effect of natural anti-browning agents on polyphenol oxidase from pearl brinjal (Solanum melongena var. serpentinum L.). Journal of Food Processing and Preservation, 00, e16963. https://doi.org/10.1111/jfpp.16963

Ioyrish N 1974. In Bees and People; MIR Publishers: Moscow, Russia.

Jeon M, Zhao Y 2005. Honey in combination with vacuum impregnation to prevent enzymatic browning of fresh-cut apples. International Journal of Food Sciences and Nutrition, 56(3): 165-176.

Jiang S, Penner MH 2022. The effect of p-coumaric acid on browning ınhibition in potato polyphenol oxidase-catalyzed reaction mixtures. Foods.;11(4):577.

Khalafi R, Goli SAH, Behjatian M 2016. Characterization and classification of several monofloral

Iranian honeys based on physicochemical properties and antioxidant activity. International Journal of Food Properties, 19:1065–1079.

Legcharoen T, Kubglomsong S, Theerakulkaıt C 2020. Inhibitory effect of rice bran protein and its fractions on enzymatic browning in potato puree. Agr. Nat. Resour., 54, 301–308.

Liao T, Zhou L, Liu J, Zou L, Dai T, Liu W, 2021. Inhibitory mechanism of salicylic acid on polyphenol oxidase: A cooperation between acidification and binding effects, Food Chemistry, Volume 348, 129100.
(https://doi.org/10.1016/j.foodchem.2021.129100)

Lim WY, Wong CW 2018. Inhibitory effect of chemical and natural anti-browning agents on polyphenol oxidase from ginger (*Zingiber officinale* Roscoe). J Food Sci Technol., 55(8), 3001–3007.

Lim WY, Cheun CF, Wong CW 2019. Inhibition of Enzymatic Browning in Sweet Potato (*Ipomoea batatas* (L.)) with Chemical and Natural Anti-Browning Agents. J. Food Process. Preserv. 43, e14195.

Martyniuk S 1994. Some inhibitors of polyphenoloxidase in honey. Pszczelinicze Zesz. Nauk. 38, 67-73.

Mathewson PR 2000. Enzymes. Eagen Press Handbook Series, pp. 37-38.

Mayer A, Harel M E 1979. Polyphenol oxidases in plants. Phytochemistry, 18(2), 193-215.

Millioğlu Ö 2019. Arap Kızı Yerel Elma Çeşidinde Polifenol Oksidaz Enziminin Saflaştırılması, Karakterizasyonu, Bazı Killerle İmmobilizasyonu Ve İnhibisyon Etkilerinin İncelenmesi. Isparta Uygulamalı Bilimler Üniversitesi Lisansüstü Eğitim Enstitüsü, Yüksek Lisans Tezi, Isparta, 82s.

Moon KM, Kwon EB, Lee B, Kim, CY 2020. Recent trends in controlling the enzymatic browning of fruit and vegetable products. Molecules, 25(12), 2754.

Nyawali B, Chungu D, Chisha-Kasumu E, Vinya, R, Chileshe F, Ng'andwe P 2015.Enzymatic browning reduction in white cabbage (Brassica oleracea) using honey: Does honey color matter?. LWT-Food Science and Technology, 61, 2.

Oszmianskii J, Lee CY 1990. Inhibition of Polyphenol Oxidase Activity and Browning by Honey, J. Agric. Food Chem. 38, 1892-1895

Redondo D, Venturini, ME, Oria R, Arias E 2016. Inhibitory effect of microwaved thinned nectarine extracts on polyphenol oxidase activity. Food Chemistry, 197, 603–610.

Supapvanich S, Boonyaritthongchai P 2016. Visual appearance maintenance of fresh-cut 'Nam Dok Mai' mango fruit by honey dip. International Food Research Journal, 23: 389-394.

Tinello F, Mihaylova D, Lante A 2018. Effect of Dipping Pre-treatment with Unripe Grape Juice on Dried "Golden Delicious" Apple Slices. Food Bioprocess Technol 11, 2275–2285.

Vela L, de Lorenzo C, Pérez RA 2007. Antioxidant capacity of Spanish honeys and its correlation with polyphenol content and other physicochemical properties. Journal of the Science of Food and Agriculture, 87(6), 1069–1075.

Vhangani LN, Van Wyk J 2021. Heated plant extracts as natural inhibitors of enzymatic browning: A case of the maillard rection. Journal of Food Biochemistry, 45(2), e13611.

Yang C P, Fujita S, Kohno K, Kusubayashi A, Ashrafuzzaman M D, Hayashi N 2001. Partial purification and characterization of polyphenol oxidase from banana (*Musa sapientum* L.) peel. Journal of Agricultural and Food Chemistry, 49, 1446-1449.

Yavuz T 2019. Ödemiş (İzmir) İlçesinde Yetiştirilen Marabel Ve Marfona Patates Çeşitlerindeki Polifenol Oksidaz Enziminin Saflaştırılması Ve Karakterizasyonu, Yüksek Lisans Tezi, Isparta Uygulamalı Bilimler Üniversitesi Lisansüstü Eğitim Enstitüsü Tarımsal Biyoteknoloji Anabilim Dalı, Isparta, s70

Zawawi NAF, Hazmi NAM, How MS, Kantono K, Silva FVM, Sulaiman A 2022. Thermal, high pressure, and ultrasound ınactivation of various fruit cultivars' polyphenol oxidase: kinetic ınactivation models and estimation of treatment energy requirement. Applied Sciences.; 12(4):1864

Zhang X, Meng W, Chen Y Peng Y 2022. Browning inhibition of plant extracts on fresh-cut fruits and vegetables—A review. Journal of Food Processing and Preservation, 46, e16532

Zhou H, Bie S, Li J, Yuan L, Zhou L 2023. Comparison on inhibitory effect and mechanism of inhibitors on sPPO and mPPO purified from 'Lijiang snow' peach by combining multispectroscopic analysis, molecular docking and molecular dynamics simulation. Food Chemistry 400,134048 (https://doi.org/10.1016/j.foodchem.2022.134048)

## *ORAL PRESENTATION*

# Esansiyel yağların *Candida albicans* üzerine etkileri

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## Özet

*Candida spp*. insanlarda pamukçuk, vulvovajinal kandidiyazis, candida özofajiti ve deri kandidiyazisi gibi birçok enfeksiyondan sorumlu, fırsatçı özelliğe sahip ve oldukça yaygın olarak gözlemlenen bir mantardır. En önemli türleri *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis* ve *Candida krusei*'dir. Mantar enfeksiyonlarının tedavisinde kullanılan antifungal ilaçlara (azoller, polienler, ekinokandinler ve flusitozin gibi) karşı oluşan mikroorganizma direnci ve bu ilaçların konakçı dokularda yüksek toksisiteye sebep olmasından dolayı alternatif tedavi yöntemlerine yönelim olmuştur. Bu alternatif yollardan bir tanesi bitkilerin yaprak, çiçek, meyve, tohum, kök ve kabuklarından çeşitli yöntemlerle elde edilen esansiyel yağların kullanılmasıdır. Yapılan bazı çalışmalarda esansiyel yağların Candida türlerine karşı etkin olduğu bulunmuştur. Bu derlemenin amacı, bazı esansiyel yağların başlıca *Candida albicans* olmak üzere Candida türleri üzerindeki antifungal etkilerinin araştırılmasıdır.
**Anahtar Kelimeler:** *Candida albicans*, insanlardaki etkileri, esansiyel yağlar

**The effects of essential oils on *Candida albicans***

**Abstract**

*Candida spp.* is an opportunistic and very common fungus responsible for many infections such as thrush, vulvovaginal candidiasis, candida esophagitis and skin candidiasis in humans. The most important species are *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis* and *Candida krusei*. Due to the resistance of microorganisms to antifungal drugs (such as azoles, polyenes, echinocandins and flucytosine) used in the treatment of fungal infections and the high toxicity of these drugs in the host tissues, there has been a trend towards alternative treatment methods. One of these alternative ways is to use essential oils obtained from leaves, flowers, fruits, seeds, roots and bark of plants by various methods. In some studies, essential oils have been found to be effective against Candida species. The purpose of this review is to investigate the antifungal effects of some essential oils on Candida species, mainly *Candida albicans*.
**Keywords:** *Candida albicans*, effects in humans, essential oils

## Giriş

Candida türleri insanlarda pamukçuk, candida özofajiti, vulvovajinal kandidiyazis ve deri kandisiyazisi gibi mantar enfeksiyonlarına neden olmaktadır (Seyedmousavı ve ark., 2015). Bu cinsin, klinik öneme sahip en az 30 tür içerdiği bilinmektedir (Pfüller ve ark., 2011; Silva ve ark., 2012). Candida türlerinin vücutta kolonize olduğu bölgeler bir sonraki Candida türleri için uygun ortamlardır. Candida türlerinin sağlıklı insanların %60'ından fazlasının deri ve bağırsak florasında doğal olarak bulunabildiği bilinmektedir. Konak immun sisteminde meydana gelen bozukluklara bağlı olarak invaziv kandidiyazis gözlemlenebilir. İnvaziv enfeksiyonların çoğundan sorumlu olan türler *Candida albicans, C. glabrata, C. tropicalis, C. parapsilosis ve C. krusei*'dir (Kullberg ve Arendrup., 2015; McCarty ve Pappas., 2016).

Tablo 1: Kanada ve Amerika Birleşik Devletleri'nde (A.B.D.) kandidiyazisten sorumlu bazı Candida türlerinin yayılımı (%)

| ***Candida Türleri*** | **A.B.D** | **Kanada** |
|---|---|---|
| ***Candida albicans*** | 44 | 42 |
| ***Candida glabrata*** | 25 | 27 |
| ***Candida parapsilosis*** | 18 | 16 |
| ***Candida tropicalis*** | 9 | 9 |
| **Diğer türler** | 4 | 6 |

(Lyon ve ark., 2010; Lockhart, 2014'den modifiye edilmiştir.)

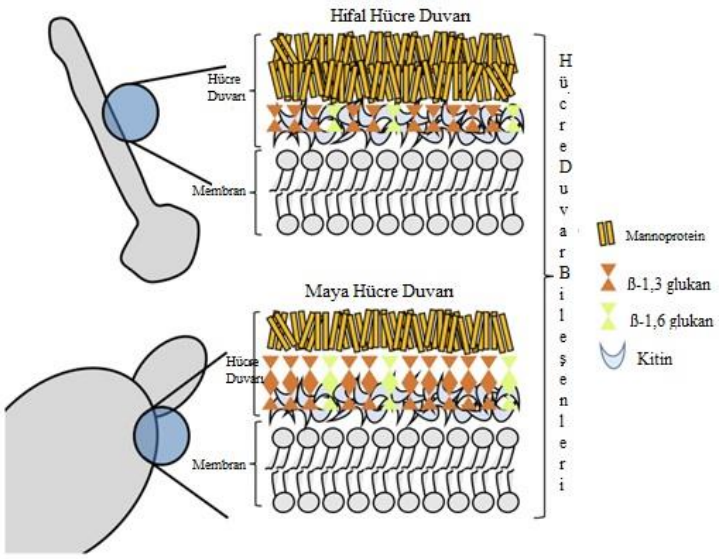
Kanada ve A.B.D'de kandidiyazisten sorumlu Candida türlerinin yayılımı (%) incelendiğinde *C. albicans, C. glabtara* ve *C. parapsilosis*'in en yaygın türler olduğu görülmektedir (Tablo 1). Türkiye'de kandidiyazisten sorumlu Candida türleri incelendiğinde ise en yaygın türlerin sırasıyla *C. parapsilosis (%48) C. albicans* (%33), *C. glabrata* (%8) ve *C. tropicalis* (%7) olduğu bildirilmiştir (Er ve ark., 2021). Dünyanın bazı bölgelerinde daha önce nadiren görülmüş olan *C. auris*'in de önemli bir patojen olduğu bildirilmiştir (Chowdhary ve ark., 2017). Yapılan bazı çalışmalarda ise Candida familyasında bulunan *C. albicans* türünün insanlardaki kandidiyazisin oluşumunda en sık (%45–50) izole edilen tür olduğu bildirilmiştir (Del Palacio ve ark., 2009). Fırsatçı özelliğe sahip olan *C. albicans* AIDS hastaları, kemoterapi alan ve bağışıklığı baskılanmış hastalarda ciddi enfeksiyonlar oluşturabilmektedir. *C.albicans* genel olarak mukozal ve sistemik enfeksiyonlara neden olur. Mukozal enfeksiyonlarda genellikle orofaringeal ve vajinal mukoza olumsuz etkilenir. Vajinal kandidiyazisin kadınlarda oldukça yaygın görüldüğü ve yaklaşık %75 kadarının yaşamları boyunca en az bir kez bu enfeksiyona yakalandığı bildirilmiştir. Sistemik enfeksiyonların ise *C. albicans*'ın kan dolaşımına ve ana organlara yayılmasıyla oluştuğu ve genellikle ölümcül olduğu rapor edilmiştir (Calderone, 2002).

*C. albicans*'ta morfogenez, tek hücreli maya formundan filamentli forma (yalancı hif veya hif) geçiş olarak tanımlanır ve virulans için gereklidir (Khan ve ark., 2010). Maya, yalancı hif ve hif gibi çeşitli morfolojik formlarda büyüyebilir (Sudbery ve ark., 2004). Bu mantar türü 37ºC'nin altındaki sıcaklıkta, asit pH'da ve serum bulunmayan bir ortamda maya formunda ürerken, 37ºC sıcaklık, nötr pH ve serum bulunan ortamda hif şeklinde ürer. *C. albicans*'ın hif oluşturabilme yeteneği, bu mantara dokulara penetrasyon ve fagositozdan kaçabilme yeteneği kazandıran önemli bir virulans faktörüdür (Arıkan, 2003). Bir diğer önemli virulans faktörü ise biyofilm oluşturabilme özelliğidir. Biyofilm oluşturan Candida türleri, antifungal ilaçların biyofilm matriksine iyi penetre olamamasından dolayı antifungal direnç ve kronik enfeksiyon gibi istenmeyen durumlara yol açabilir. *C. albicans* sitozolik pH'sını regüle ederek aşırı asidik (pH <2) ve aşırı alkali (pH> 10) ortamlarda yaşayabilme özelliğine sahiptir (Rabaste ve ark., 1995; Tournu ve ark., 2017). Glikoz bu mantar türlerinde hücresel homeostazın sürdürebilmesi için iyi bir enerji kaynağıdır (Lorenz, 2013). *C.albicans* polisakkarit hidroliz yolu, glukoneogenez gibi çeşitli yollardan glikoz elde edebilir. Ayrıca glikolitik yol ve TCA döngüsü yoluyla enerji kazanmak için glikozu metabolize edebilir (Barelle ve ark., 2006; Lorenz, 2013).

**Hücre duvarı özellikleri**

Mantar hücre duvarı, plazma zarının dışında bulunan ve hücrenin çevreyle ilişkisine aracı olan kısmıdır. Bu yapının hücre içeriğini koruma, hücreye sertlik verme ve hücresel yapıyı tanımlama özellikleri vardır. Mantarların hücre duvarındaki ana maddeler, glukanlar, kitin ve mannoproteinlerdir. Bunlara ek olarak hücre duvarı, adezinler ve çok sayıda bulunan reseptörler sayesinde dış çevre ile etkileşimi sağlar (Ponton, 2008).

Mantar hücre duvarının en önemli yapısal polisakkariti olan glukanlar, hücre duvarının yaklaşık %50-60'ını oluşturur. β-1,6, β-1,4, α-1,3 ve α-1,4 bağlantılı glukanlar olmasına rağmen, glukanın çoğu polimeri 1,3 bağlantı glikoz ünitesinden (%65-90) oluşur. β-1,3-D-glukan, bu yapının diğer bileşenlerinin kovalent olarak bağlı olduğu duvarın en önemli yapısal bileşenidir. β-1,3-D-glukan, plazma zarında bulunan glukan sentaz adı verilen bir enzim kompleksi tarafından sentezlenir (Douglas ve ark., 1994; Ponton, 2008).




Şekil 1: *Candida albicans* hücre duvarının yapısal organizasyonu ve bileşimi (Garcia-Rubio ve ark., 2020)

*C. albicans* hücre duvarı iki katmanlı bir yapıdır. Mannoproteinler hücre duvarında biri iç diğeri dış yüzeye yakın iki tabaka oluşturacak şekilde yerleşirler. Hücre duvarının ana unsuru, hücre duvarının sağlamlığından ve şeklinden sorumlu olan β-glukan-kitin iskeletinden oluşmuştur. Kitin hücre duvarının iç tabakasında bulunur (Şekil 1).

Mantar hücre duvarı yapısındaki bozulmaların, hücre büyümesi ve morfolojisi üzerinde hücre ölümüyle sonuçlanacak derecede ciddi etkileri olabilmektedir. Bu sebeple hücre duvarı antifungal tedavide iyi bir hedef olabileceği bildirilmiştir (Heitman, 2005).

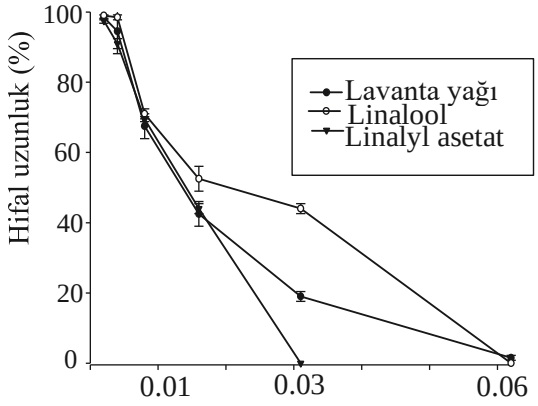
Mantar enfeksiyonlarının tedavisinde kullanılan antifungal ilaçlar, ergosterol biyosentezini inhibe eden azoller (klotrimazol, flukonazol, itrakonazol, ketokonazol), mantar hücre zarında ergosterol ile birleşen polienler (amfoterisin B, nistatin), β-1,3-glikoz biyosentezini inhibe eden ekinokandinler (caspofungin, anidulafungin, micafungin) ve DNA sentezini inhibe eden flusitozin gibi birkaç ana tiple sınırlıdır (Zavrel ve White, 2015). Azol grubu antifungaller, mantar hücrelerinin büyüme inhibisyonunu uyararak fungistatik bir etki gösterir (Di Bonaventura ve ark., 2004). Ancak, sıklıkla konakçı dokular için yüksek toksisite ve ilaç direncine sebep olabilirler (Arikan ve Rex 2007).

İlaca dirençli izolatların sayısının sürekli artışı ile birlikte, tedavide kullanılan ilaçların yüksek konsantrasyonlarda olması ve tedavinin uzun sürmesine bağlı olarak çıkan yan etkiler (Cleveland ve ark., 2015), mantar enfeksiyonlarını hala ciddi bir klinik sorun haline getirmektedir (Raut ve Karuppayil 2014).

Antifungallerin bu olumsuz etkilerinden dolayı alternatif tedavilere yönelim olmuştur. Bu alternatif yollardan birisi antifungal özellik gösteren esansiyel yağların kullanılmasıdır. Tıp literatüründe, bitkiler ve bu bitkilerden elde edilen esansiyel yağların alternatif tıpta birçok hastalığın tedavisinde yaygın bir şekilde kullanıldığı bildirilmiştir (Lang ve Buchbauer, 2012). Aromatik bitkilerden elde edilen uçucu özellikte, organik bileşikleri içeren ve güçlü kokulara sahip ikincil metabolitleri bulunduran (Bakkali ve ark., 2008) esansiyel yağların ve çeşitli bitkilerden elde edilen bitki ekstratlarının memeli hücrelerdeki toksisitesinin oldukça düşük olması nedeniyle çeşitli hastalıkların tedavisinde kullanımları oldukça yaygınlaşmıştır (Chauhan ve Kang, 2014).

Esansiyel yağlar bitkilerin yaprak, çiçek, meyve, tohum, kök ve kabuklarından çeşitli yöntemlerle elde edilir (Baser ve Demitri 2007; Bakkali ve ark., 2008). Bileşimleri genel olarak bitkinin yetiştiği coğrafi koşullar, iklim, toprağın bileşimi, bitkinin beslenme faktörleri ile büyümesi esnasındaki stres durumları gibi birçok faktöre bağlıdır (Copetta ve ark., 2006; Raut ve Karuppayil 2014).

Yapılan bazı çalışmalarda esansiyel yağların Candida türlerine karşı etkin olduğu bulunmuştur. Kekikten elde edilen esansiyel yağların *C. albicans*'ın *in vitro* koşullarda gelişimini inhibe ettiği bildirilmiştir (Cosentino ve ark., 1999; Manohar ve ark., 2001; Giordani ve ark., 2004). Geyikotu ve çay ağacından elde edilen esansiyel yağların da Candida türlerinin inhibisyonunda etkili olduğu gösterilmiştir (Oliva ve ark., 2003; Farag ve ark., 2004; Giordani ve ark., 2004; Tampieri ve ark., 2005). Lavanta yağı ile yapılan bir çalışmada ise lavanta yağının *C. albicans* üzerinde inhibe edici etkisinin olduğu ve ayrıca hif uzamasını da engellediği belirlenmiştir (D'auria ve ark., 2005).




Şekil 2: Farklı konsantrasyonlardaki lavanta yağı ve bileşenlerinin *C. albicans*'ın hifal uzunluğuna etkisi (D'auria ve ark., 2005)
Şekil 2'de lavanta yağı ve ana bileşenleri olan linalool ve linalyl asetatın *C. albicans*'ın hif uzunluğuna etkisi gösterilmiştir. En etkili maddenin lavanta yağında bulunan linalyl asetat bileşeni olduğu bildirilmiştir.

Tablo2: Bazı esansiyel yağların kaynakları, ana bileşenleri ve Candida spp. üzerine etkileri

| **Esansiyel yağlar** | **Bitki kökeni** | **Ana bileşenler** | **Etkisi** | **Kaynaklar** |
|---|---|---|---|---|
| Kekik | *Origanum vulgare*<br>*Thymus vulgaris* | Carvacrol<br>Thymol | Fungisidal etki | Giordani ve ark., (2004) |
| Çay ağacı | *Melaleuca alternifolia* | Terpinen-4-ol<br>1,8-Cineole | İyi<br>Düşük | Farag ve ark., (2004) |
| | *Melaleuca armillaris* | Terpinen-4-ol<br>1-8-Cineole | Orta | Farag ve ark., (2004) |
| Nane | *Mentha x piperita* | Menthol<br>Menthone<br>1,8-Cineole | Güçlü | Tampieri ve ark., (2005) |
| | | Linalool<br>Carvone | Orta | Duarte ve ark., (2005) |
| Acı kekik | *Tymbra capitata* | Carvacrol | Fungisidal etki | Salgueiro veark., (2004) |
| Tarçın | *Cinnamomum camphora* | Linalool | Orta | Tampieri ve ark., (2005) |
| | *Cinnamomum verum* | Eugenol | Güçlü | Tampieri ve ark., (2005) |
| Karanfil | *Syzygium aromaticum* | Eugenol | Güçlü | Giordani ve ark., (2004) |
| Geyik otu | *Satureja montana L.* | Carvacrol<br>*γ*-terpinene<br>*p*-cymene | Güçlü | Marin ve ark., (2012) |

*****Oliveira ve ark., 2009'dan modifiye edilmiştir.

**Esansiyel yağların *C. albicans* üzerindeki başlıca etki mekanizmaları**

Esansiyel yağların, *C. albicans* üzerine başlıca etkileri hücre membran bütünlüğünün bozulması, hücre duvar bütünlüğünün bozulması, fungal mitokondri metabolizmasının bozulması ve H+-ATPaz inhibisyonunu sağlayarak gerçekleşmektedir.

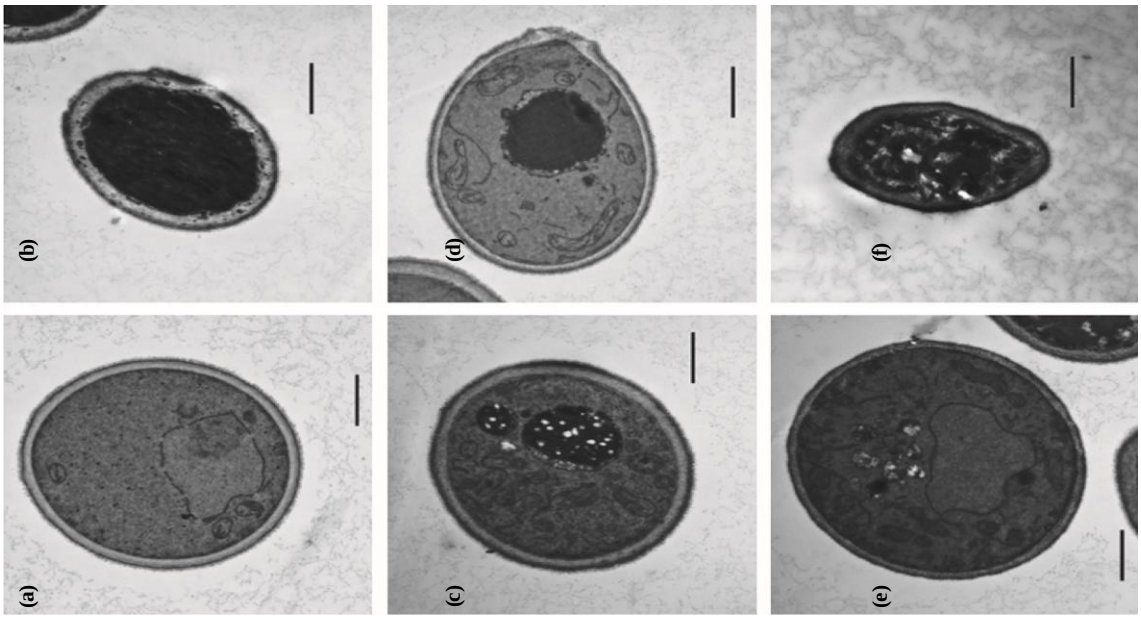
1*)* Hücre Membranının Bütünlüğünün Bozulması: Ergosterol, yalnızca mantarların hücre zarında bulunan, uygun büyümeleri ve işlevleri için önemli olan benzersiz bir steroldür. Bu nedenle, seviyesini etkileyen bileşikler antifungal aktiviteye sahip olabilir. Timol ile muamele edilen Candida hücre zarlarında ergosterol düşüşü gözlendiği, bu durumun membran bütünlüğünün bozulmasına, membranla ilişkili enzim bozukluklarına, kapsamlı hasara ve bunun sonucunda hücre ölümüne neden olduğu bildirilmiştir (Mota ve ark., 2012). Ek olarak çay ağacındaki esansiyel yağların bir kısmının ergosterol biyosentezini bozarak mantarın membran bütünlüğünün bozulmasına neden olduğu bildirilmiştir (Freires ve ark., 2014).

2) ⁺Hücre duvarı bütünlüğünün bozulması: β-glukan, β-glukan sentaz enzimi tarafından sentezlenen ve hücre duvar bütünlüğünü sağlayan önemli bir bileşiktir. Tarçından elde edilen esansiyel yağların β-glukan sentaz

enzimini inhibe ederek β-glukan oluşumunu engellediği ve bu sayede hücre duvarı bütünlüğünün bozulmasını sağladığı bildirilmiştir (Bang ve ark., 2000).

3*)* Fungal Mitokondri Metabolizmasının Bozulması: Dere otu, kafur ağacı, pelin otu ve kekikten elde edilen bazı esansiyel yağların ATP biyosentezinde rol alan mitokondrial dehidrojenaz enzimini inhibe ederek etki gösterdiği rapor edilmiştir (Bakkali ve ark., 2006).

4) $H^+$-ATPaz inhibisyonu: Mantar fizyolojisinde önemli rolü olan $H^+$-ATPaz enzimi sayesinde hücre içi pH'nın optimum seviyede tutulması ve mantar hücresinin büyümesini sağlanmaktadır. Yapılan bir çalışmada timol ve karvakrol gibi esansiyel yağların bu enzimi inhibe edip hücre içi asitliği arttırarak hücrenin ölümüne neden olduğu bildirilmiştir (Ahmad ve ark., 2013).




Şekil 3: Elektron mikroskobunda *C. albicans* hücrelerinin işlem görmemiş (a), clotrimazole (0.125 µg $ml^1$) (b), kekik esansiyel yağı (%0.0156 (c), %0.062 (d), geyikotu esansiyel yağı (%0.062 (e), %0.25 (f)) kullanılmış görünümü (Bona ve ark., 2016).

Yapılan çalışmalarda işlem görmemiş *C. albicans* hücrelerinde, bozulmamış hücre duvarları ve plazma zarlarını içeren normal bir yapı (Şekil 3a), Clotrimazole kullanılan hücrelerde, plazma zarı ve hücre içi organellerle birlikte hücre duvarının bozulduğu ve ardından kalınlaşmalar olduğu (Şekil 3b), kekik esansiyel yağı kullanılan hücrelerde, organeller ve membranın düzensiz olduğu (Şekil 3c, d), geyik otu esansiyel yağı kullanılan hücrelerde, hücre organellerinin tamamen bozulduğu ve son olarak hücrelerin küçüldüğü bildirilmiştir (Şekil 3e, f.). Bona ve ark., (2016) *C. albicans*'a karşı genel olarak kekik, geyik otu ve nane esansiyel yağlarının etkin olduğunu, nane yağının test edilen suşlarda Candida gelişimini %80 engellediği ayrıca kekik ve geyik otu yağlarının ise %100'ünde etkili olduğunu bildirmişlerdir.

Sonuç olarak Candida türlerine karşı gelişen ilaç direnci ve ilaçların konakçı dokularda oluşturduğu toksisite göz önüne alındığında çeşitli esansiyel yağlarla yapılan çalışmalar bu yağların alternatif bir tedavi yöntemi olarak kullanılabiliceğini göstermektedir.

## Kaynaklar

Ahmad A, Khan A, Manzoor N 2013. Reversal of efflux mediated antifungal resistance underlies synergistic activity of two monoterpenes with fluconazole. European Journal of Pharmaceutical Sciences, 48: 80-86.

Arıkan S 2003. Mantarlarda Pleomorfizm. 3. Ulusal Mantar Hastalıkları ve Klinik Mikoloji Kongresi, Tutanaklar. Türk Mikrobiyoloji Cemiyeti Dergisi, 46: 77-86.

Arıkan A, Rex JH 2007. Antifungal agents. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (eds), Manual of Clinical Microbiology. Washington D.C., USA: ASM Press, pp: 1949-1960.

Bakkali F, Averbeck S, Averbeck D, Zhiri A, Baudouxc D, Idaomar M 2006. Antigenotoxic effects of three essential oils in diploid yeast (Saccharomyces cerevisiae) after treatments with UVC radiation, 8-MOP plus UVA and MMS. Mutation Research, 606(1-2): 27-38.

Bakkali F, Idaomar M, Averbeck S, Averbeck D 2008. Biological effects of essential oils – a review. Food and Chemical Toxicology, 46: 446-475.

Bang KH, Lee DW, Park HM, Rhee YH 2000. Inhibition of Fungal Cell Wall Synthesizing Enzymes by trans-Cinnamaldehyde. Bioscience, Biotechnology, and Biochemistry, 64(5): 1061-1063.

Barelle CJ, Priest CL, Maccallum DM, Gow NA, Odds FC, Brown AJ 2006. Niche-specific regulation of central metabolic pathways in a fungal pathogen. Cellular Microbiology, 8: 961-971.

Baser KHC, Demitri F 2007. Chemistry of essential oils. In: Berger RG (ed), Flavours and Fragrances-Chemistry, Bioprocessing and Sustainability. Berlin: Springer Press, pp: 43-86.

Bona E, Cantamessa S, Pavan M, Novello G, Massa N, Rocchetti A, Berta G, Gamalero E 2016. Sensitivity of Candida albicans to essential oils: are they an alternative to antifungal agents?. Journal of Applied Microbiology, 121(6): 1530-1545.

Calderone RA 2002. Candida and Candidiasis. Washington DC: ASM Press.

Chauhan AK, Kang SC 2014. Thymol disrupts the membrane integrity of Salmonella ser. typhimurium invitro and recovers infected macrophages from oxidative stress in an ex vivo model. Research in Microbiology, 165: 559-565.

Chowdhary A, Sharma C, Meis JF 2017. Candida auris: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathogens, 13(5): e1006290.

Cleveland AA, Harrison LH, Farley MM, Hollick R, Stein B, Chiller TM, Lockhart SR, Park BJ 2015. Declining incidence of candidemia and the shifting epidemiology of Candida resistance in two US metropolitan areas, 2008-2013: results from population based surveillance. PLoS One, 10: 1-12.

Copetta A, Lingua G, Berta G 2006. Effect of three AM fungi on growth, distribution of glandular hairs, and essential oil production in Ocimum basilicum L. var Genovese. Mycorrhiza, 16: 485-494.

Cosentino S, Tuberoso C, Pisano B, Satta M, Mascia V, Arzedi E, Palmas F 1999. In vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. Letters in Applied Microbiology, 29: 130-135.

D'auria FD, Tecca M, Strippoli V, Salvatore G, Battinelli L, Mazzanti G 2005. Antifungal activity of Lavandula angustifolia essential oil against Candida albicans yeast and mycelial form. Medical Mycology, 43(5): 391-396.

Del Palacio A, Villar J, Alhambra A 2009. Epidemiology of invasive candidiasis in pediatric and adult populations. Revista Iberoamericana de Micología, 26: 2-7.

Di Bonaventura G, Spedicato I, Picciani C, D'Antonio DA, Piccolomini R 2004. In vitro pharmacodynamic characteristic of amphotericin B, caspofungin, fluconazole, and voriconazole against bloodstream isolates of infrequent Candida species from patiens with hematologic malignancies. Antimicrobial Agents and Chemotherapy, 48: 4453-4456.

Douglas CM, Foor F, Marrinan JA, Morin N, Nielsen JB, Dahl, AM 1994. The Saccharomyces cerevisiae FKS1 (ETG1) gene encodes an integral membrane protein which is a subunit of 1,3-beta-D-glucan synthase. Proceedings of the National Academy of Sciences, 91: 12907-12911.

Duarte MC, Figueira GM, Sartoratto A, Rehder VL, Delarmelina C 2005.Anti-Candida activity of Brazilian medicinal plants. Journal of Ethnopharmacology, 97: 305-11.

Er H, Yılmaz NÖ, Derici YK, Hancı S, Çopur ŞS 2021. Kandidemi Etkenlerinin Tür Dağılımı ve Duyarlılıkları: Hastanemizde Ampirik Antifungal Tedavi Politikası Değiştirilmeli mi? Türk Mikrobiyoloji Cemiyeti Dergisi, 51(2): 150-155.

Farag RS, Shalaby AS, El-Baroty GA, Ibrahim NA, Ali MA, Hassan EM, 2004. Chemical and biological evaluation of the essential oils of different Melaleuca species. Phytotherapy Research, 18: 30-35.

Freires de Almeida I, Murata RM, Furletti VF, Sartoratto A, Matias de Alencar S, Figueira GM, de Oliveira Rodrigues JA, Duarte MCT, Rosalen PL 2014. Coriandrum sativum L. (Coriander) essential oil: Antifungal activity and mode of action on Candida spp. and molecular targets affected in human whole-genome expression. PLoS One, 9: e99086.

Garcia-Rubio R, de Oliveira HC, Rivera J, Trevijano-Contador N 2020. The fungal cell wall: Candida, Cryptococcus, and Aspergillus species. Frontiers in Microbiology, 10: 2993.

Giordani R, Regli P, Kaloustian J, Mikaeil C, Abou L, Portugal H 2004. Antifungal effect of various essential oils against Candida albicans. Potentiation of antifungal action of amphotericin B by essential oil from Thymus vulgaris. Phytotherapy Research, 18: 990-995.

Heitman J 2005. Cell biology. A fungal Achilles' heel. Science, 309: 2175-2176.

Khan MSA, Ahmad I, Aqil F, Owais M, Shahid M, Musarrat J 2010. Virulence and pathogenicity of fungal pathogens with special reference to Candida albicans. In: Combating fungal infections. Berlin, Heidelberg: Springer, pp: 21-45.

Kullberg BJ, Arendrup MC 2015. Invasive candidiasis. The New England Journal of Medicine, 373: 1445-1456.

Lang G, Buchbauer G 2012. A review on recent research results, 2008–2010: on essential oils as antimicrobials and antifungals. Flavour and Fragrance Journal, 27: 13-39.

Lockhart SR 2014. Current epidemiology of Candida infection. Clinical Microbiology, 36(17): 131-136.

Lorenz MC 2013. Carbon catabolite control in Candida albicans: new wrinkles in metabolism. MBio, 4(1): e00034-13.

Lyon GM, Karatela S, Sunay S, Adiri Y 2010. Antifungal susceptibility testing of Candida isolates from the Candida surveillance study. Journal of Clinical Microbiology, 48(4): 1270-1275.

Manohar V, Ingram C, Gray J, Talpur NA, Echard BW, Bagchi D, Preuss HJ 2001. Antifungal activities of origanum oil against Candida albicans. Molecular and Cellular Biochemistry, 228: 111-117.

Marin M, Novacovic M, Tesevic V, Vuckovic I, Milojevic N, Vukovic-Gacic B, Marin PD 2012. Antioxidative, antibacterial and antifungal activity of the essential oil of wild-growing Satureja montana L. from Dalmatia, Croatia. Flavour and Fragrance Journal, 27: 216-223.

McCarty TP, Pappas PG 2016. Invasive candidiasis. Infectious Disease Clinics, 30(1): 103-124.

Mota KSDL, Pereira FDO, De Oliveira WA, Lima IO, Lima EDO 2012. Antifungal activity of Thymus vulgaris L. essential oil and its constituent phytochemicals against Rhizopus oryzae: interaction with ergosterol. Molecules, 17(12): 14418-14433.

Oliva B, Piccirilli E, Ceddia T, Pontieri E, Aureli P, Ferrini AM 2003. Antimycotic activity of Melaleuca alternifolia essential oil and its major components. Letters in Applied Microbiology, 37: 185-187.

Oliveira AP, Salgueiro L, Oliveira RP, Oliveira JM, Vaz CP, Queiroz JA, Rodrigues AG, 2009. Anti-Candida Activity of Essential Oils. Medicinal Chemistry, 9: 1292-1305.

Pfüller R, Gräser Y, Erhard M, Groenewald M 2011. A novel flucytosine-resistant yeast species, Candida pseudoaaseri, causes disease in a cancer patient. Journal of Clinical Microbiology, 49(12): 4195-4202.

Ponton J 2008. The fungal cell wall and the mechanism of action of anidulafungin. Revista Iberoamericana de Micología, 25: 78-82.

Rabaste F, Sancelme M, Delort AM, Blais J, Bolard J 1995. Intracellular pH of Candida albicans blastospores as measured by laser microspectrofluorimetry and 31P-NMR. Biochimica et Biophysica Acta, 1268(1): 41-49.

Raut JS, Karuppayil SM 2014. A status review on the medicinal properties of essential oils. Industrial Crops and Products, 62: 250-264.

Salgueiro LR, Pinto E, Goncalves MJ, Pina-Vaz C, Cavaleiro C, Rodrigues AG, Palmeira A, Tavares C, Costa-de Oliveira S, Martinez-de-Oliveira J 2004. Chemical composition and antifungal activity of the essential oil of Thymbra capitata. Planta Medica, 70: 572-5.

Seyedmousavı S, İlkit M, Durdu M, Ergin Ç, Polat S, Melchers W, Verweıj P, 2015. Candida ve Kandidoz: Epidemiyoloji, tanı, tedavi, antifungal ilaç direnci ve konağın genetik yatkınlığında güncel durum. Türk Mikrobiyoloji Cemiyeti Dergisi. 45(1): 1-11.

Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J, 2012. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiology Reviews, 36: 288-305.

Sudbery P, Gow N, Berman J 2004. "The Distinct Morphogenic States of Candida albicans," Trends in Microbiology, 12(7): 317-324.

Tampieri MP, Galuppi R, Macchioni F, Carelle MS, Cioni PL Morelli I 2005. The inhibition of Candida albicans by selected essential oils and their major components. Mycopathologia, 159: 339-345.

Tournu H, Luna-Tapia A, Peters BM, Palmer GE 2017. In vivo indicators of cytoplasmic, vacuolar, and extracellular pH using pHluorin2 in Candida albicans. Msphere, 2(4): e00276-17.

Zavrel M, White TC 2015. Medically important fungi respond to azole drugs: an update. Future of Microbiology, 10: 1355-1373.

## ORAL PRESENTATION

# Antifungal effect of thyme essential oil on *Aspergillus flavus*

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## Abstract

*Aspergillus flavus* is a mycotoxigenic fungus that can produce B aflatoxins. It is the most commonly reported food-borne fungus and one of the most common species found on stored goods, particularly grains, due to its ability to flourish in conditions with little water activity and high temperatures. It has been discovered that the ingredients of essential oils and the essential oils themselves are powerful antifungal agents. The potent antifungal compounds isolated from thyme essential oil are thymol, carvacrol, linalool, citral, geraniol, and 1,8-cineole. Numerous studies have been done on their use as food preservatives. The antifungal effect of thyme essential oil on *A. flavus* was investigated. The agar dilution method was used to evaluate the fungal growth inhibitory effect of the thyme oil. *A. flavus* was inoculated in Sabouraud Dextrose Agar. The thyme oil was added to the agar plates at the concentrations of 0, 31.25, 62.50, 125, 250, 500, and 1000 ppm and then kept at 27 °C for seven days. The agar dilution method has some advantages over the other methods, such as disc and well diffusion tests; since they give inconsistent and not reproducible results because of water-insoluble essential oils. The results showed that thyme oil had an antifungal effect against *A. flavus*. The minimal inhibitory concentration of thyme oil was 1000 ppm.

**Keywords:** *Aspergillus flavus*, Antifungal activity, Thyme oil

## INTRODUCTION

*Aspergillus* species are the most common fungi that can produce mycotoxins in food and feed materials, which have an important place in terms of economy (Perrone et al., 2007). Mycotoxins, that is, secondary metabolites, are mostly produced by *Aspergillus*, *Fusarium*, and *Penicillium* species. *Aspergillus spp.* is known as an active agent in decomposition processes, as they are animal and human pathogens and producers of metabolic products. They cause serious damage to products such as corn, peanuts, and cotton, which occupy an important place in the world economy, during the harvest and post-harvest periods (Navale et al., 2021).

*Aspergillus flavus* is one of the *Aspergillus* species, and corn, peanuts, and cotton are the main products affected by *A. flavus* (Beyki et al., 2014). These products have an important place in the food economy. *A. flavus* secretes aflatoxin (mycotoxin), a highly toxic secondary metabolite, and it causes food spoilage (Beyki, et al., 2014). In addition to producing aflatoxin, *A. flavus* causes various diseases in humans such as aspergillosis and onychomycosis. In addition, aflatoxins were identified as human liver carcinogens (da Silva et al., 2022).

Fresh fruits and vegetables deteriorate in a variety of ways before and after harvest. The majority of these deteriorations, which cause very important economic losses, are caused by fungal diseases. Due to fungal diseases, fruits and vegetables are damaged significantly, their quality is reduced, and their shelf life is generally shortened. Exposure of fruits and vegetables to deterioration caused by fungal pathogens can be attributed to reasons such as the high nutrient and water content of fruits and vegetables, the low pH level, and the decreased resistance of fruits and vegetables to internal spoilage after harvest. Damage to fruits and vegetables during harvest or post-harvest transportation and packaging causes a significant portion of post-harvest diseases. Today, synthetic fungicides are mainly used to control post-harvest diseases of fruits and vegetables around the world (Ağırman et al., 2019). The dangers of synthetic fungicides to human health and the environment are well documented. These negative consequences hurt animal health and the food economy. To eliminate these drawbacks, natural preservatives can be used. Natural preservatives are an effective way to eliminate negative aspects of living healthily and eating (Davies et al., 2021).

Essential oils found in many plant organs contain compounds related to various functions necessary for plant survival (Kohiyama et al., 2015). Essential oils are complex mixtures of volatile compounds synthesized by plants containing terpenes, terpenoids, aromatic, and aliphatic components, all of which are characterized by low molecular weight (Boukaew et al., 2017). About 35% of essential oils show antibacterial properties, while 65% show antifungal properties (Kohiyama et al., 2015).

Thyme (*Thymus vulgaris* L.), a plant species native to the Western Mediterranean region, and a member of the Lamiaceae family, is an aromatic/medicinal plant whose economic importance is increasing in temperate climates such as North America and Europe (Kohiyama et al., 2015).

There are various studies on the effect of thyme essential oil on *A. flavus*. In a study, thyme oil was shown to cause various modifications in the hyphae and conidia structures of *A. flavus*. Various changes were observed in the conidiophore properties related to the morphological structure of *A. flavus* in the samples analyzed by Scanning Electron Microscopy. Changes such as decreased cytoplasmic content and modifications of membrane integrity were also observed. These modifications caused by thyme essential oil may be related to interference caused by its components in cell wall synthesis affecting fungal growth and morphology, suggesting that thyme essential oil is responsible for degenerative changes (Kohiyama et al., 2015).

In another study, the effects of 14 essential oils against the toxigenic strain of *A. flavus* were tested. Among the tested oils, thyme oil was observed to have the highest antifungal effect. It inhibited the mycelial growth of *A. flavus* (Kumar et al., 2008).

In a recent study, the effect of various essential oils on *A. flavus* in cauliflower seeds was examined. Cauliflower seeds were inoculated with *A. flavus* isolated from bean seeds. Three different essential oils (thyme, lemongrass, and rosemary) were used and many properties (such as germination, seedling dry weight, and seedling height) were determined. It was observed that thyme and lemongrass essential oils had an antifungal effect against *A. flavus* in cauliflower seeds (Nakada-Freitas et al., 2022).

In one other study, the effect of thyme essential oil on *A. flavus* obtained from stored food products was examined. Among the stored food products are chickpeas (*Cicer arietinum*), corn (*Zea mays*), barley (*Hordeum vulgare*), and rice (*Oryzae sativa*). *Aspergillus* genus (with five species) was dominant in almost all of these products. It was noted that mycelium growth and aflatoxin B1 production decreased with increased thyme oil concentration. *Thymus vulgaris* (thyme) has shown fungal toxicity (Kumar et al., 2009).

The effect of 11 essential oils on *A. flavus* was examined in another study. The antifungal effect was determined by measuring the micelle diameter on PDA (Potato Dextrose Agar) plates. Cinnamon completely inhibited the growth of *A. flavus*, followed by the essential oils of thyme and lemongrass (Xiang et al., 2020).

The common aim of the studies is to show the potential of using various essential oils obtained from plants and showing antifungal effects instead of synthetic fungicides. Thus, the use of fungicides will be reduced and their negative effects on health will be minimized. The study aims to show the antifungal properties of thymol oil on *A. flavus* and to determine the dose of use. The difference between this study and the other studies was that the area occupied by *A. flavus* in the petri dish was measured using an image processing program.

## MATERIALS AND METHODS

### Essential Oil

Thyme oil used in the study was purchased from Arifoğlu company. Its density was measured using a precision balance. A stock solution was prepared with pure alcohol at a concentration of 50 mg/mL (50000 ppm).

### The strain

Thyme oil was used against MAM/MRC 200450 *Aspergillus flavus* strain purchased from TUBITAK MAM Mold collection. *A. flavus* was grown on plates containing "Potato Dextrose Agar (PDA)" and these plates were used in the study.

### Agar Dilution Method

*A. flavus* was tested with the "agar dilution method" on Sabouraud dextrose agar (SDA-40 g/L dextrose, 10 g/L peptone, 20 g/L agar) in appropriate culture medium. The media were prepared as 100 mL in 250 mL flasks (one flask for each thyme oil concentration) and autoclaved at 121 °C for 15 minutes. Thyme oil was added to the culture medium at a temperature of 40-45°C, with final concentrations of 1000, 500, 250, 125, 62.5 and 31.25 ppm from the stock solution, and then poured into sterile petri dishes with a diameter of 9 cm. Thyme oil-free plates were evaluated as 'Control'. After the medium solidified, a 6 mm diameter disc from a

seven-day old culture of *A. flavus* on PDA with a 'cork borer' was placed in the middle of each petri dish (Figure 1). The inoculated plates were kept in an oven at 27°C for seven days.

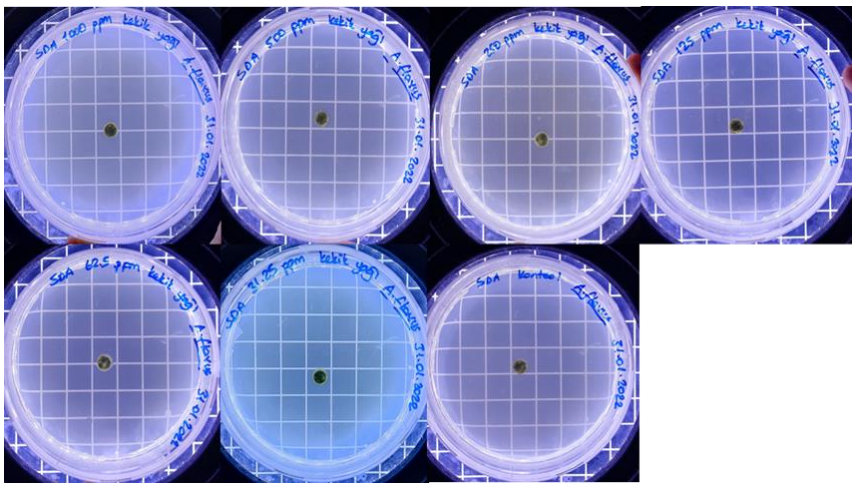

Figure 1. SDA plates containing 1000, 500, 250, 125, 62.5, 31.25 ppm thyme oil, respectively, and control (thyme oil-free) inoculated with *A. flavus*

The efficacy of thyme oil was evaluated by measuring the area occupied by the colonized *A. flavus* in the petri dish after seven days. The image processing program 'ImageJ' was used to calculate the area. Experiments were made with five replications and the results were given as the average of five replications.

## RESULTS AND DISCUSSION

The density of thyme oil was calculated as 0.965 g/mL. The antifungal activity of thyme oil on *A. flavus* was shown in Table 1, Figure 2 and Figure 3.

Table 1. Inhibition of growth in *A. flavus* by the effect of thyme oil

| **Concentration of thyme oil (ppm)** | **% Inhibition of growth** |
|---|---|
| Control | 0 |
| 31,25 | 15,33 |
| 62,5 | 13,84 |
| 125 | 18,63 |
| 250 | 17,57 |
| 500 | 43,77 |
| 1000 | 100 |

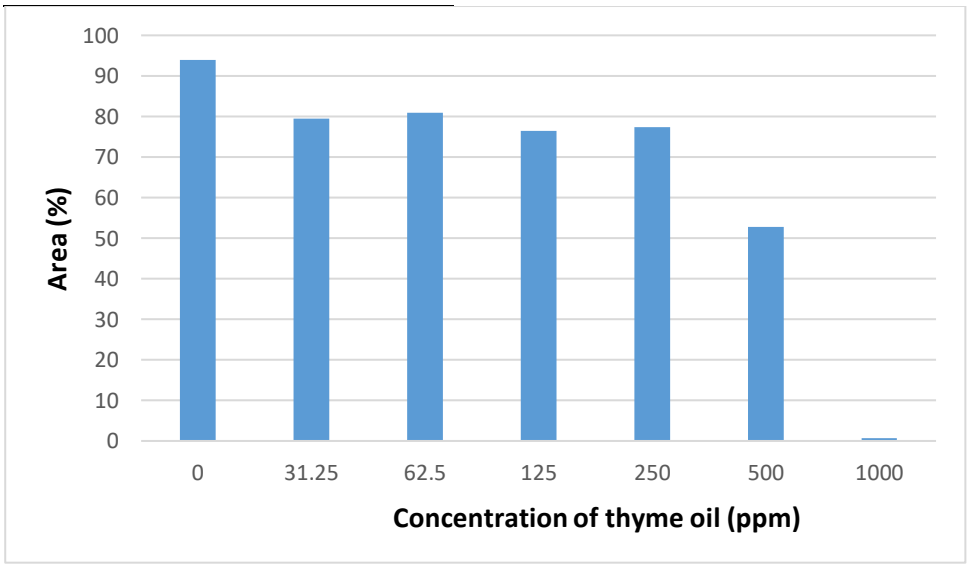


Figure 2. At different thyme oil concentrations, the area (%) occupied by *A. flavus* in the petri dishes after seven days. The area of the Petri dish was accepted as 100 %.

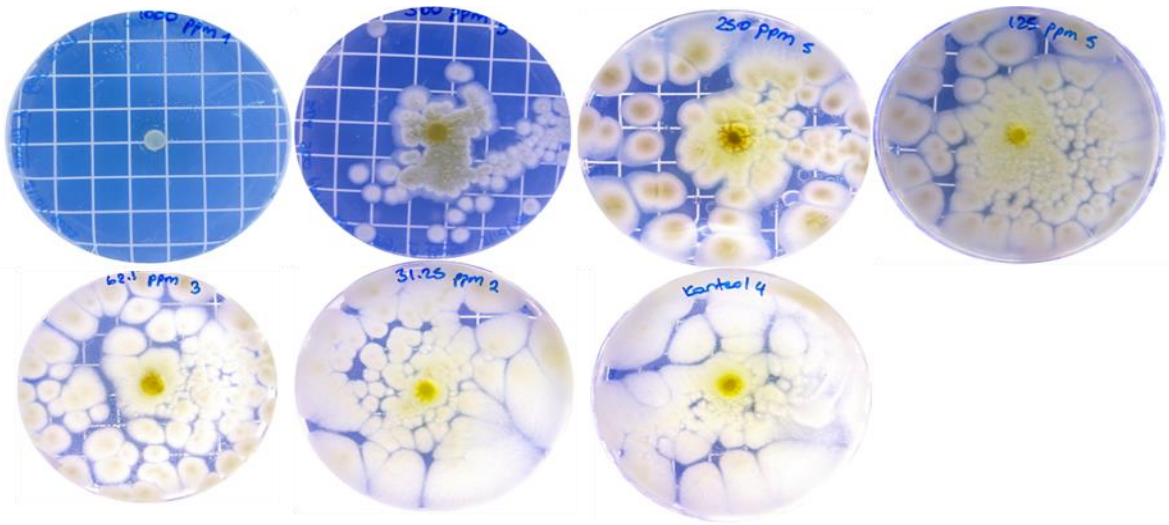


Figure 3. The appearance of *A. flavus* colonies at the end of seven days in the control SDA plates, which were inoculated with *A. flavus*, containing 1000, 500, 250, 125, 62.5, 31.25 ppm thyme oil, respectively. Photos taken from the back of the plate for a clear view of the area covered by the colonies.

Although thyme oil inhibited the growth of *A. flavus* by 18.63% at concentrations ranging from 31.25 to 250 ppm, growth inhibition increased up to 43.77% in plates containing 500 ppm. When the thyme oil concentration was 1000 ppm, 100% inhibition was observed. The minimal inhibitory concentration of thyme oil was 1000 ppm.The findings are consistent with the literature (Baratta et al., 1998; Bouchra et al., 2003).

Paster et al. (1995) demonstrated the antifungal activity of thyme oil at concentrations of 2 and 2.5 mL/L on the mycelium and spores of *A. niger*, *A. flavus* and *A. ochraceus*. Bouchra et al. (2003) showed that the antifungal activity of thyme oil on *A. niger* is much stronger than other essential oils such as rosemary or sage. It is well known that 'carvacrol' is the main component in thyme (Arnold et al., 2000; Veres et al., 2003) and the antifungal effect of thyme oil is thought to be due to this component (López-Malo et al., 2005; Soković et al., 2002).

The possible modes of action of essential oil components (phenolic and terpenes) have been studied differently, but their mechanisms have not been fully elucidated. Prindle and Wright (1977) stated that the effect of phenolic compounds is concentration dependent. At low concentrations, phenols affect the enzyme activity, especially of enzymes associated with energy production; At higher concentrations they cause protein denaturation. The effect of phenolic antioxidants on microbial growth and toxin production may be the result of macromolecules getting out of the cell by changing the microbial cell permeability of phenolic compounds. In addition, phenolic compounds can interact with membrane proteins and cause deformation in the structure and functionality of membrane proteins (Fung et al., 1977).

## CONCLUSION

The results show that thyme oil has antifungal activity against *A. flavus in vitro*. It is recommended to use thyme oil as an antifungal agent because it is not harmful to the environment when compared to other synthetic antifungal agents, and the risk of pathogens developing resistance to thyme oil is low.

## REFERENCES

Ağırman, B., Akalın, M. N., and Erten, H. (2019). Meyve Ve Sebzelerde Hasat Sonrasi Fungal Hastaliklari Antagonistik Mayalar İle Biyokontrolü. *Gida / the Journal of Food*, *44*, 31–49. https://doi.org/10.15237/gida.gd18082

Arnold, N., Bellomaria, B., and Valentini, G. (2000). Composition of the essential oil of three different species of origanum in the eastern mediterranean. *Journal of Essential Oil Research*, *12*(2), 192–196. https://doi.org/10.1080/10412905.2000.9699495

Baratta, M. T., Damien, H. J., Deans, S. G., Biondi, D. M., and Ruberto, G. (1998). Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. *Journal of Essential Oil Research*, *10*(6), 618–627. https://doi.org/10.1080/10412905.1998.9700989

Beyki, M., Zhaveh, S., Khalili, S. T., Rahmani-Cherati, T., Abollahi, A., Bayat, M., Tabatabaei, M., and Mohsenifar, A. (2014). Encapsulation of Mentha piperita essential oils in chitosan-cinnamic acid nanogel with enhanced antimicrobial activity against Aspergillus flavus. *Industrial Crops and Products*, *54*, 310–319. https://doi.org/10.1016/j.indcrop.2014.01.033

Bouchra, C., Achouri, M., Hassani, L. M. I., and Hmamouchi, M. (2003). Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against Botrytis cinerea Pers: Fr. *Journal of Ethnopharmacology*, *89*(1), 165–169. https://doi.org/10.1016/S0378-8741(03)00275-7

Boukaew, S., Prasertsan, P., and Sattayasamitsathit, S. (2017). Evaluation of antifungal activity of essential oils against aflatoxigenic Aspergillus flavus and their allelopathic activity from fumigation to protect maize seeds during storage. *Industrial Crops and Products*, *97*, 558–566. https://doi.org/10.1016/j.indcrop.2017.01.005

da Silva, J. V. B., de Oliveira, C. A. F., and Ramalho, L. N. Z. (2022). An overview of mycotoxins, their pathogenic effects, foods where they are found and their diagnostic biomarkers. *Food Science and Technology (Brazil)*, *42*, 1–9. https://doi.org/10.1590/fst.48520

Davies, C. R., Wohlgemuth, F., Young, T., Violet, J., Dickinson, M., Sanders, J. W., Vallieres, C., and Avery, S. V. (2021). Evolving challenges and strategies for fungal control in the food supply chain. *Fungal Biology Reviews*, *36*, 15–26. https://doi.org/10.1016/j.fbr.2021.01.003

Fung, D. Y. C., Taylor, S., and Kahan, J. (1977). Effects of Butylated Hydroxyanisole (Bha) and Butylated Hydroxytoluene (Bht) on Growth and Aflatoxin Production of Aspergillus Flavus. *Journal of Food Safety*, *1*(1), 39–51. https://doi.org/10.1111/j.1745-4565.1977.tb00258.x

Kohiyama, C. Y., Yamamoto Ribeiro, M. M., Mossini, S. A. G., Bando, E., Bomfim, N. D. S., Nerilo, S. B., Rocha, G. H. O., Grespan, R., Mikcha, J. M. G., and Machinski, M. (2015). Antifungal properties and inhibitory effects upon aflatoxin production of Thymus vulgaris L. by Aspergillus flavus Link. *Food Chemistry*, *173*, 1006–1010. https://doi.org/10.1016/j.foodchem.2014.10.135

Kumar, A., Shukla, R., Singh, P., Prasad, C. S., and Dubey, N. K. (2008). Assessment of Thymus vulgaris L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. *Innovative Food Science and Emerging Technologies*, *9*(4), 575–580. https://doi.org/10.1016/j.ifset.2007.12.005

Kumar, S. A., Chandrabhan, S., and Shriram, P. (2009). Isolation of Aspergillus flavus from stored food commodities and Thymus vulgaris (L.) essential oil used as a safe plant based preservative. *Pharmacognosy Magazine*, *5*(20), 343–349. https://doi.org/10.4103/0973-1296.58564

López-Malo, A., Alzamora, S. M., and Palou, E. (2005). Aspergillus flavus growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds. *International Journal of Food Microbiology*, *99*(2), 119–128. https://doi.org/10.1016/j.ijfoodmicro.2004.08.010

Nakada-Freitas, P. G., Santos, C. A., Magalhães, T. H., Bustamonte, S. S., Dos Santos, D. C., Cardoso, A. I. I., Amador, T. S., Lanna, N. de B., Bardiviesso, E. M., and Catão, H. C. R. (2022). Effect of thyme, lemongrass and rosemary essential oils on Aspergillus flavus in cauliflower seeds. *Horticultura Brasileira*, *40*(1), 71–75. https://doi.org/10.1590/s0102-0536-20220109

Navale, V., Vamkudoth, K. R., Ajmera, S., and Dhuri, V. (2021). Aspergillus derived mycotoxins in food and the environment: Prevalence, detection, and toxicity. *Toxicology Reports*, *8*, 1008–1030. https://doi.org/10.1016/j.toxrep.2021.04.013

Paster, N., Menasherov, M., Ravid, U., and Juven, B. (1995). Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. *Journal of Food Protection*, *58*(1), 70–75.

Perrone, G., Susca, A., Cozzi, G., Ehrlich, K., Varga, J., Frisvad, J. C., Meijer, M., Noonim, P., Mahakamchanakul, W., and Samson, R. A. (2007). Biodiversity of Aspergillus species in some important agricultural products. *Studies in Mycology*, *59*, 53–66. https://doi.org/10.3114/sim.2007.59.07

Prindle, R. F., and Wright, E. S. (1977). Phenolic compounds. In *In Disinfection, Sterilization and Preservation* (S.S. Block, pp. 115–118).

Soković, M., Tzakou, O., Pitarokili, D., and Couladis, M. (2002). Antifungal activities of selected aromatic plants growing wild in Greece. *Nahrung - Food*, *46*(5), 317–320. https://doi.org/10.1002/1521-3803(20020901)46:5<317::AID-FOOD317>3.0.CO;2-B

Veres, K., Varga, E., Dobos, A., Hajdu, Z., Mathe, I., Nemeth, E., and Szabo, K. (2003). Investigation of the Composition and Stability of the Essential Oils of Origanum vulgare ssp . *Chromatographia*, 95–98.

Xiang, F., Zhao, Q., Zhao, K., Pei, H., and Tao, F. (2020). The efficacy of composite essential oils against Aflatoxigenic fungus Aspergillus flavus in Maize. *Toxins*, *12*(9). https://doi.org/10.3390/toxins12090562

## *ORAL PRESENTATION*

# Evaluation of Combination of Unmanned Aerial Vehicles and Geographic Information Systems in Forest Road Planning

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**Abstract**

Forest road is important for forestry activities. Unmanned Aerial Vehicle (UAV) is very useful device and a revolutionary technology. As a result of the development of technology and the rapid spread of modern methods in forestry road design, innovative techniques offer a more efficient and more consistent decision-making environment. Considering the evolving software of UAV's and advantages of Geographic Information System (GIS) technology, the use of these two efficient technologies to process high-quality data and transform it into more useful information provides an important environment for planning in a shorter time and solving decision-making problems more effectively. The main subject of this study is to evaluate the combined use of GIS and UAV Systems, which constitute a platform that provides better added value in the forestry sector and other sectors (construction, agriculture, mining, etc.). A more logical and sustainable environment has been reached for planners, implementers and decision makers with the use of UAV's, which has become widespread since the beginning of the 2000s, and the more effective use of GIS techniques, as well as the high-precision data obtained. In this study, UAV's and UAV applications in the forestry sector were evaluated by referring to the sectors in which they are widely used.

**Keywords:** UAV, GIS, Forestry, Forest road, Road planning

## INTRODUCTION

One of the fundamental responsibilities of the state institutions in Turkey, as everywhere in the globe, is to ensure that public services are provided continuously and that they are continued within a continuity framework (Chen, 2008; Sonuvar, 2009). The Ministry of Agriculture and Forestry has been tasked with assuring the continuity of forestry services because our nation's woods are nearly entirely administered by the government. Forest roads stand out as the most fundamental amenity in this situation. Since ancient times, forest roads that are utilized to provide forestry and public services in forested areas have been significant infrastructure facilities (Acar, 2016). Forest roads are planned, built, and maintained in accordance with the guidelines of the General Directorate of Forestry (GDF) 292 numbered communiqué (Anonymous, 2008). A shift to a more productive era in the design and building of forest roads has taken place, particularly in the last twenty years as computer technologies and software have grown and spread with a quick acceleration and a practical evaluation opportunity is provided to plan makers. The information gathered from the ground can be transported to the computer environment by using global positioning systems effectively. Geographic Information Systems (GIS) have become increasingly necessary as a result of the developing world's extensive use of information systems, which has led to their quick development and wide adoption (Goodchild and Longley, 2021). The last century has seen an increase in the clarity and precision of planning and implementation studies due to the processing of data, its usefulness, and the availability of quality information (Geertman and Ritsema, 1995). High-accuracy forest road projects can be created with the aid of these methods, which work in conjunction with one another or complement one another (Matinnia et al., 2018).

A structure that supports planners, implementers, and decision-makers has emerged as a result of the rapid expansion of GIS and new technologies in the management of forests, which are a finite resource. With GIS, information can be accessed extremely quickly, but analysis and assessment processes can also be expressed with clarity (Picchio et al., 2018). The GIS environment, which is employed as a tool that directly influences productivity, continuously improves with new applications. With regard to the availability of information about the investigated places as well as the potential for continual update, it offers significant advantages to decision makers in terms of both space and time (Saaty, 2002; Buğday, 2018).

The demand for more exact and accurate geographical information has increased with the widespread usage of GIS software (Iglhaut et al., 2019). The accuracy of planning is improved by the environment, which enables

decision-makers to make more logical and efficient choices. Unmanned aerial vehicles (UAV), which are now evolving quickly, have assumed a crucial role in this regard. All of the data required for planning can be gathered with UAV devices with great precision and accuracy, and sent to the GIS environment (Kentsch et al., 2021). In this context, the combination of GIS and UAV technologies together offers great performance in terms of effectiveness and reason.

The use of UAV technology is widespread across numerous sectors. According to the geometric standards specified for the road project in the sectors, the national and international literature on construction (Riberio et al., 2021, Trubia et al., 2021), agriculture (Tsouros et al., 2019), mining (Ren et al., 2019), forestry (Buğday, 2018; Diez et al., 2021), etc., is used effectively in the calculation and determination of features like excavation-filling calculations (Ciritcioğlu and Buğday, 2022; Lee and Lee, 2022), alternative route evaluations (Salami et al., 2014; Çölkuşu and Buğday, 2022), current excavation area calculations (Remondino et al., 2011), mine reserve calculations (Dorobantu et al., 2018), the number of plants in the agricultural area, and their vitality (Raeva et al., 2019).

According to the GDF 292 "Forest Roads Planning, Construction and Maintenance" communiqué, forest roads are proposed (Anonymous, 2018). This communiqué outlines the routes and procedures to be used when planning forest roads for places that require them. The Communiqué states that the route is determined using certain slope values, taking into account factors including the starting and destination locations, the ground quality, the features of any existing forest stands, the distance to other forest roads, and positive cardinal points (forest village existence, forest facility, etc.). The regions that require an hydraulic structure and the areas that can serve as temporary storage sites are also identified in order for the planned forest road to constantly serve the general forestry activities.

In this study, studies from across the world that used UAV and GIS to develop forest roads are analyzed, and details on the project design and implementation phases are provided. The classification developed in the study is intended to bring the literature together.

## MATERIALS AND METHODS

In this study, search terms for forest road, UAV, and GIS (forestry-UAV, forestry-GIS, UAV-GIS, and forest road-GIS) from 2011 to 2021 were used in the Scopus database of 2022. The studies carried out by the countries according to the countries, years, fields of study and publication types are organized under four separate sections, in the order that the information about Turkey is included.

## RESULTS and DISCUSSION

The results of this study were provided under the headings below and were grouped using keywords into the groupings that emerged as a consequence of this study.

### 1. "UAV" with "forestry"

Information on 8 papers in 2011, 20 in 2012, 32 in 2013, 35 in 2014, 38 in 2015, 76 in 2016, 147 in 2017, 172 in 2018, 257 in 2019, 278 in 2020, and 291 in 2021 was discovered as a result of a search using the keywords "forestry" and "UAV" (Figure 1). It has been shown that the increasing trend has quickened as of 2015.

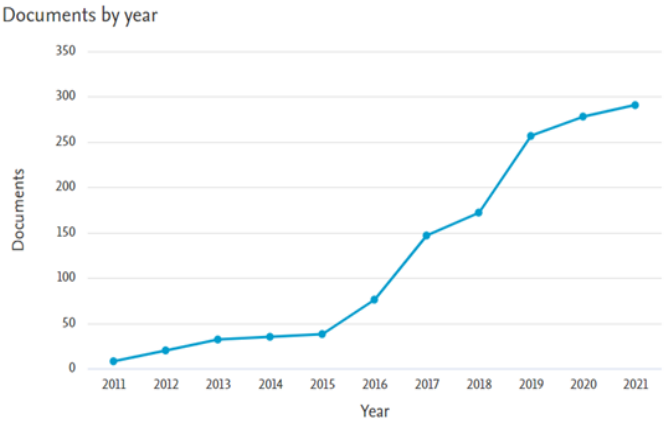


**Figure 1.** Trend of the studies by years

There are 815 articles, 490 conference papers, 29 reviews, 7 book chapters, 7 conference reviews, and 5 other publications (data paper, erratum, and letter) as a result of the search using the keywords "forestry" and "UAV." It has been discovered that it has primarily appeared in articles and conference papers (Figure 2).

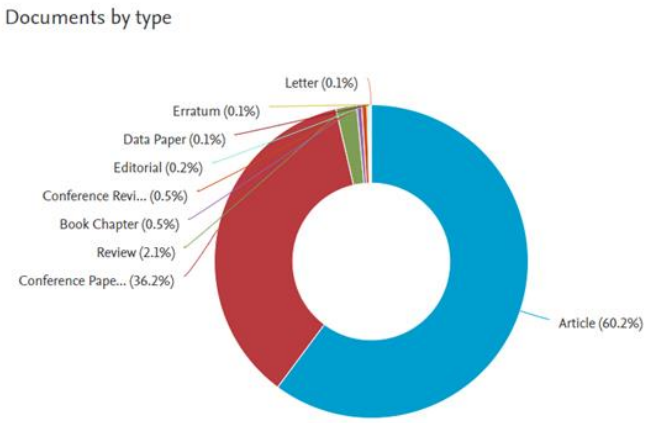


**Figure 2.** Type of publication

Earth and Planetary Sciences 555, Computer Science 484, Engineering 390, Agricultural and Biological Sciences 324, Physics and Astronomy 187, Environmental Science 143, Mathematics 142, Social Sciences 128, Materials Science 76, Biochemistry, Genetics and Molecular Biology 32 and other 131 documents were discovered as a result of the search using the keywords "forestry" and "UAV." It has been shown that the terms "forestry" and "UAV" are primarily used in scientific domains (Figure 3).

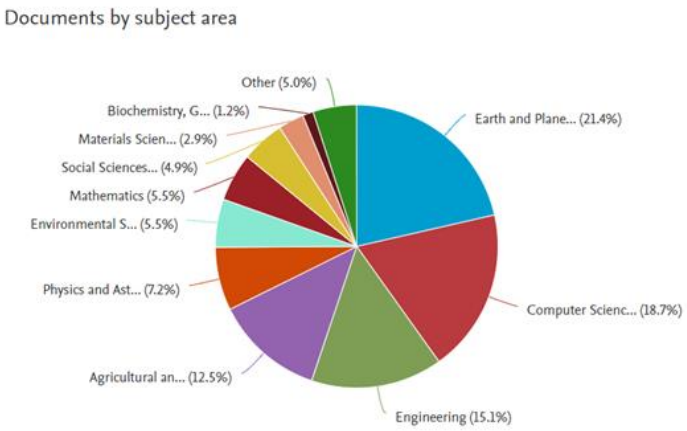


**Figure 3.** Type of subject area

As a result of the search using the terms "forestry" and "UAV," China earned the first spot in the list of the countries, followed by the United States and Germany. China accessed 342 documents through the Scopus interface, followed by the United States with 209, Germany with 87, Canada and Spain with 77, Brazil with 75, Australia and Italy with 66, the United Kingdom with 64, Finland with 58, and Turkey with 31 (Figure 4).

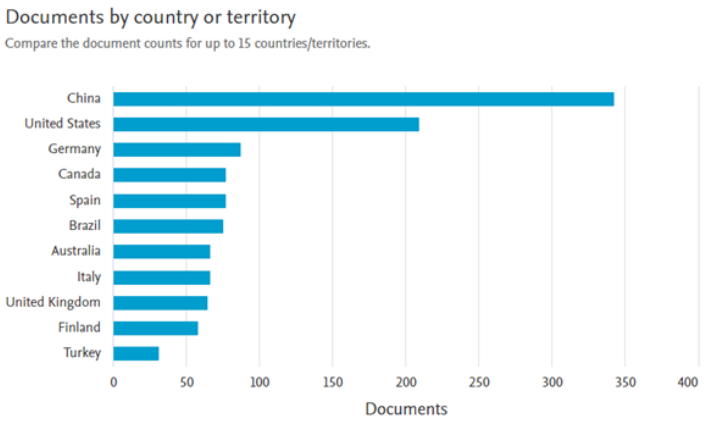


**Figure 4.** Distribution of the studies by country or territory

## 2. "GIS" with "forestry"

It has been discovered that 251 papers were published in 2011, 236 in 2012, 246 in 2013, 250 in 2014, 210 in 2015, 217 in 2016, 244 in 2017, 220 in 2018, 236 in 2019, 269 in 2020, and 230 in 2021 as a result of the search using the keywords "forestry" and "GIS." It has been noted that the figures generally trend upward and downward within a given range (Figure 5).

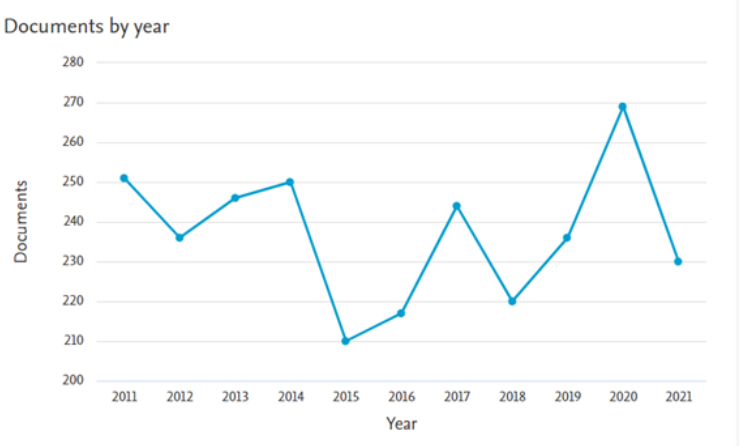


**Figure 5.** Trend of the studies by years

Article 1680, Conference Paper 816, Book Chapter 53, Review 39, Book 11, Conference Review 5, and 5 other items were found as a result of the search using the keywords "forestry" and "GIS." It has been determined that it is preferable to publish this information as an article and a conference paper (Figure 6).

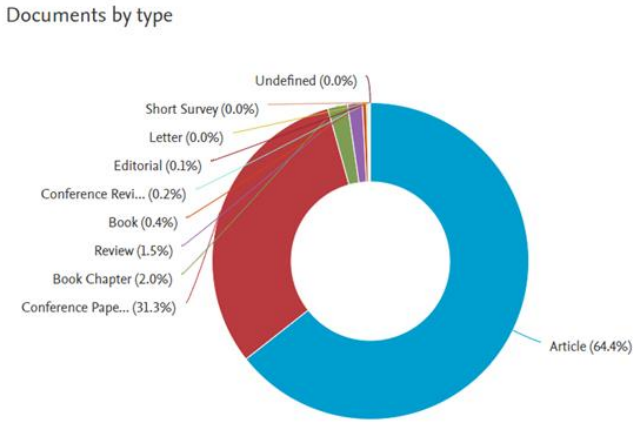


**Figure 6.** Type of publication

Environmental science 1150, agricultural and biological sciences 857, earth and planetary sciences 665, computer science 564, engineering 468, social sciences 378, energy 169, mathematics 154, physics and

astronomy 104, decision sciences 75, and 274 other documents were found as a result of the search using the keywords "forestry" and "GIS." These terms are typically used in studies in journals that are specialized to environmental science, it has been found (Figure 7).

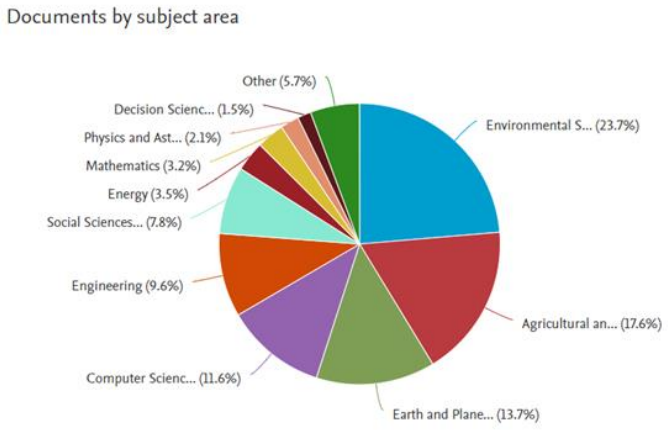


**Figure 7.** Type of subject area

Following a search using the terms "forestry" and "GIS," China came out on top in the ranking of nations, followed by the United States and India. Access has been made to papers from China (511), the United States (383), India (174), Italy (131), Germany (102), Australia (95), Indonesia (92), Iran (90), Turkey (90), Canada (85), and the Russian Federation (85). When our nation is considered in relation to Figure 8, it can be said that the number of publications is average (Figure 8).

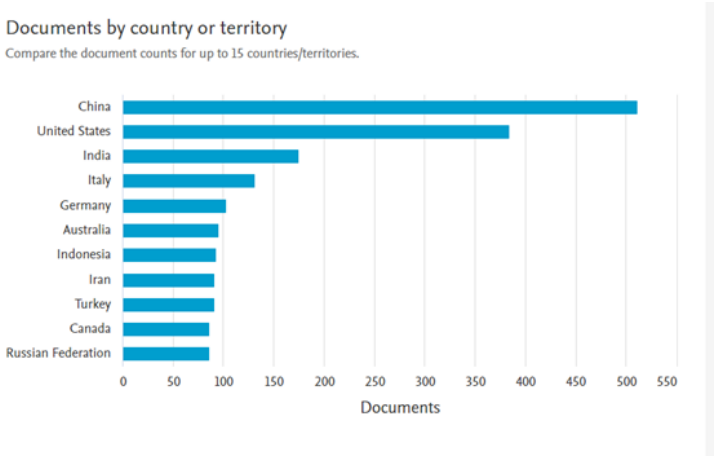


**Figure 8.** Distribution of the studies by country or territory

### 3. GIS and UAV

There were 20 papers in 2011, 12 in 2012, 26 in 2013, 18 in 2014, 30 in 2015, 63 in 2016, 58 in 2017, 77 in 2018, 84 in 2019, 112 in 2020, and 139 in 2021 as a result of the search using the keywords "UAV" and "GIS." It has been discovered that there is an upward tendency in the publications using these keywords, and that this trend has accelerated significantly since 2015 (Figure 9).

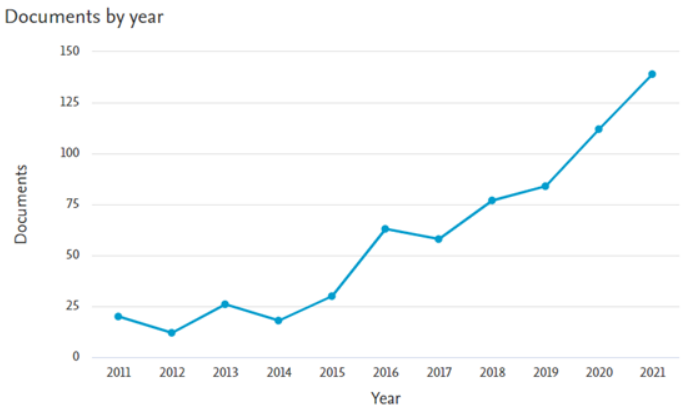


**Figure 9.** Trend of the studies by years

The Conference Paper 293, Article 280, Conference Review 32, Book Chapter 18, Review 9, Erratum 2, Abstract Report 1, Data Paper 1, Editorial 1, Letter 1 and Note 1 documents have been found as a result of the search using the keywords "UAV" and "GIS." These articles and conference papers are the most common formats for publications containing these keywords (Figure 10).

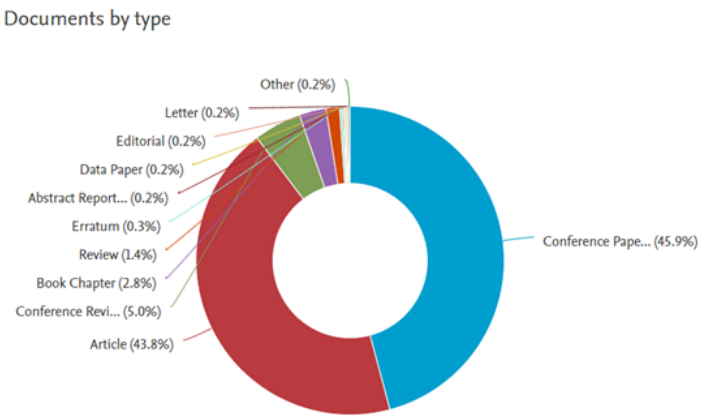


**Figure 10.** Type of publication

A search using the terms "UAV" and "GIS" turned up 233 documents in computer science, 226 in earth and planetary sciences, 186 in engineering, 165 in social sciences, 136 in environmental science, 73 in agricultural and biological sciences, 64 in physics and astronomy, 56 in mathematics, 43 in energy, and 39 in materials science, among 94 other publications. It has been observed that it is frequently utilized in both general mathematics and science (Figure 11).

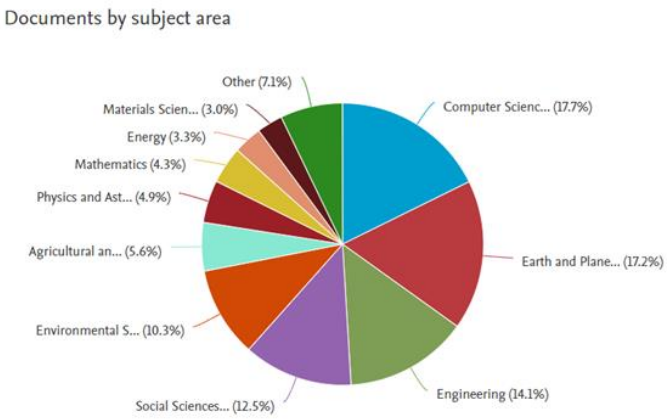


**Figure 11.** Type of subject area

According to a search utilizing the keywords "UAV" and "GIS," the top three countries were, in order, China, the United States, and Italy. There were found to be 102 publications in China, 68 in the United States, 58 in Italy, 31 in Japan, 30 in the Russian Federation, 24 in Germany, 24 in the United Kingdom, and 19 in Brazil. There were 8 documents released (Figure 12).

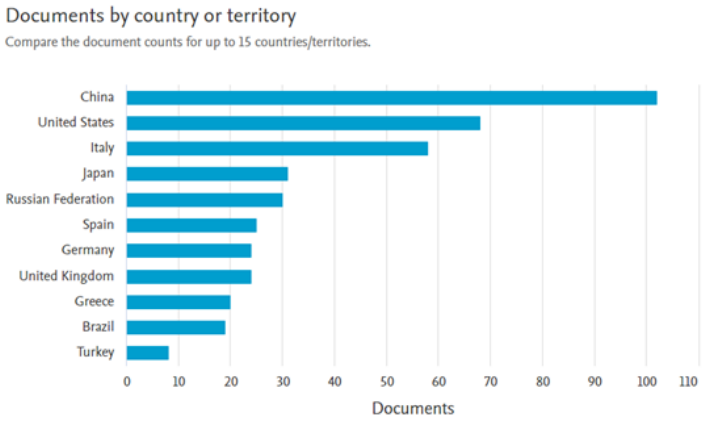


**Figure 12.** Distribution of the studies by country or territory

## 4. "GIS" and "forest road"

It was discovered that 9 papers were published in 2011, 10 in 2012, 15 in 2013, 17 in 2014, 10 in 2015, 8 in 2016, 15 in 2017, 13 in 2018, 13 in 2019, 11 in 2020, and 4 in 2021 as a result of the search using the keywords "Forest road" and "GIS." In general, it has been observed that the number of publications using these keywords has been declining over time (Figure 13).

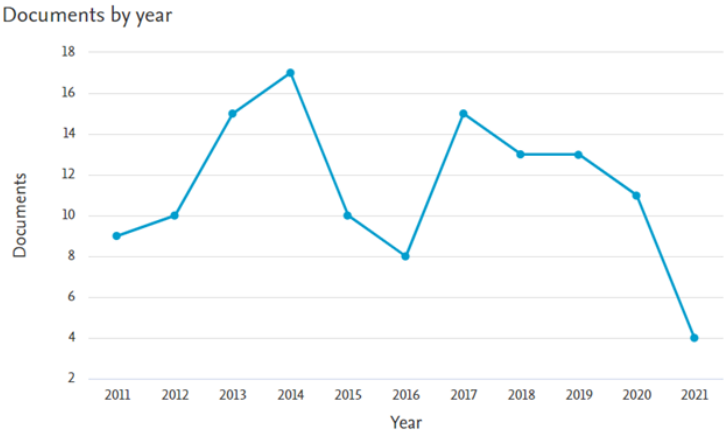


**Figure 13.** Trend of the studies by years

Using the search terms "Forest road" and "GIS," materials pertaining to Article 104, Conference Paper 17, Review 3, and Conference Review 1 were located. It has been shown that just articles and conference papers are employed, rather than a wide variety of publication tools (Figure 14).

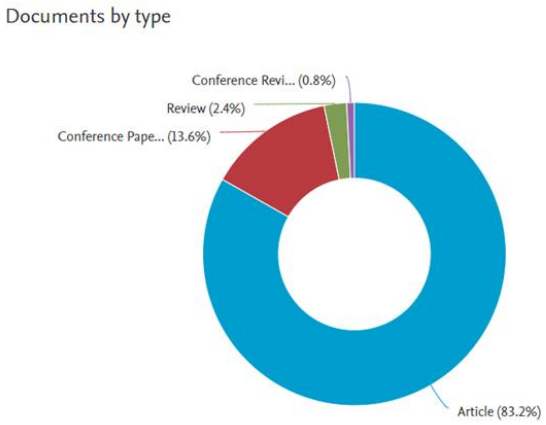


**Figure 14.** Type of publication

A total of 68 documents in the fields of agricultural and biological sciences, 44 in environmental science, 17 in the social sciences, 13 in computer science, 9 in mathematics, 6 in physics and astronomy, 4 in energy, and 21 in engineering have been found as a result of the search using the keywords "Forest road" and "GIS." These keywords are typically published based on Environmental Science, as has been seen (Figure 15).

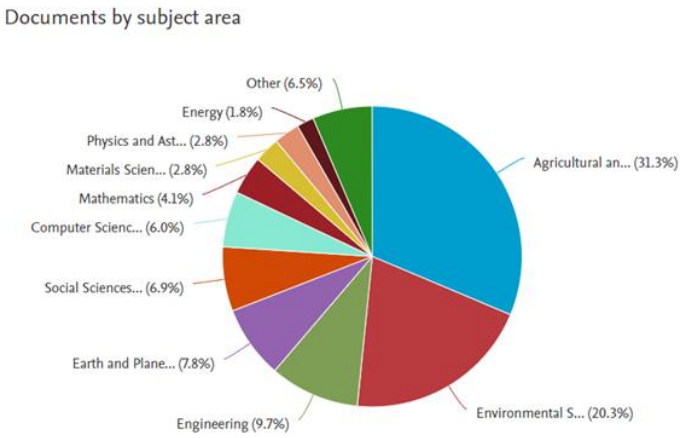


**Figure 15.** Type of subject area

Turkey came in second place behind Iran in the list of nations after a search using the terms "Forest road" and "GIS." The United States was ranked third. Iran 27, Turkey 18, the United States 13, Italy 10, Greece 9, Austria 5, Bosnia and Herzegovina 5, Poland 5, Slovakia 5, Canada 4, and Germany 4 documents were released, according to an order based on all publication numbers (Figure 16).

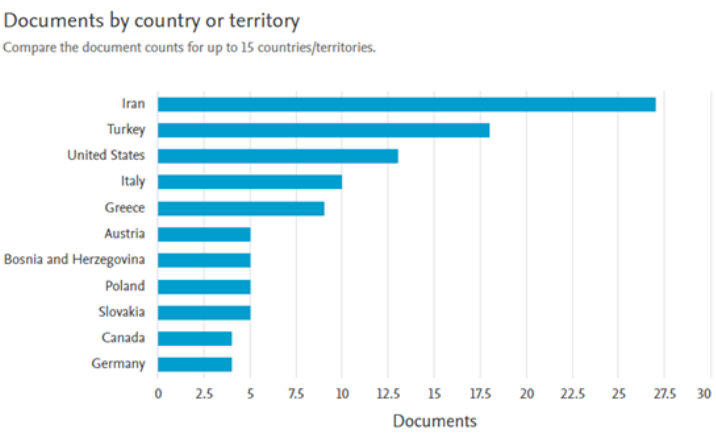


**Figure 16.** Distribution of the studies by country or territory

## CONCLUSION

In this study, articles published between 2011 and 2021 that were found using search terms related to forest roads, unmanned aerial vehicles, and geographic information systems in the Scopus environment of 2022 were identified. Additionally, evaluations were done in this study under four separate areas based on the nations, years, study fields, and publishing types. When revealing our nation's position in the international literature on forest road works, it can be said that our nation is in a favorable and developing position. Increasing the quantity and ability of combined UAV, GIS, and forest road planning studies would help planners and practitioners and foster a more logical environment for decision-making. By incorporating new approaches, technology, and methodologies into the planning of forest roads and forecasting studies for the coming years, it is anticipated that improvement will continue.

## REFERENCES

Acar, H. H. 2016. Environmental sensitive road planning and transportation techniques in forest engineering. Journal of the Faculty of Forestry Istanbul University, 66(2), 710-726.

Anonymous, 2008. Forest road planning, construction, and maintenance, No. 292 Notification, Ankara.

Buğday, E. 2018. Capabilities of using UAVs in Forest Road Construction Activities . European Journal of Forest Engineering , 4 (2) , 56-62 . DOI: 10.33904/ejfe.499784

Chen, B. 2008. Assesing interorganizational networks for public service delivery: A process-perceived effectiveness framework. Public Performance & Management Review, 31(3), 348-363.

Ciritcioğlu, M. G., & Buğday, E. 2022. Assessment of Unmanned Aerial Vehicle Use Opportunities in Forest Road Project Düzce Sample. Journal of Bartın Faculty of Forestry, 24(2), 247-257.

Çölkuşu, T. and Buğday, E. 2022. Planning Optimal Forest Road Network Using Unmanned Aerial Vehicle Eldivan Sample. 1st International Karatekin Science and Technology Conference Proceeding Book, 1-3 September 2022, ISBN: 978-605-82910-6-5, p:199-204.

Diez, Y., Kentsch, S., Fukuda, M., Caceres, M. L. L., Moritake, K., & Cabezas, M. 2021. Deep learning in forestry using uav-acquired rgb data: A practical review. Remote Sensing, 13(14), 2837.

Dorobantu, S., FILIP, L., & Dima, N. 2018. Modern methods for the calculation of stocks volumes existing in surface mining exploitations. Research Journal of Agricultural Science, 50(4), 127-136.

Geertman, S. C., & Ritsema Van Eck, J. R. 1995. GIS and models of accessibility potential: an application in planning. International journal of geographical information systems, 9(1), 67-80.

Goodchild, M. F., & Longley, P. A. 2021. Geographic information science. In Handbook of Regional Science (pp. 1597-1614). Berlin, Heidelberg: Springer Berlin Heidelberg.

Iglhaut, J., Cabo, C., Puliti, S., Piermattei, L., O'Connor, J., & Rosette, J. 2019. Structure from motion photogrammetry in forestry: A review. Current Forestry Reports, 5(3), 155-168.

Kentsch, S., Cabezas, M., Tomhave, L., Groß, J., Burkhard, B., Lopez Caceres, M. L., ... & Diez, Y. 2021. Analysis of UAV-acquired wetland orthomosaics using gis, computer vision, computational topology and deep learning. Sensors, 21(2), 471.

Lee, K., & Lee, W. H. 2022. Earthwork Volume Calculation, 3D Model Generation, and Comparative Evaluation Using Vertical and High-Oblique Images Acquired by Unmanned Aerial Vehicles. Aerospace, 9(10), 606.

Matinnia, B., Parsakhoo, A., Mohamadi, J., & Jouibary, S. S. 2018. Study of the LiDAR accuracy in mapping forest road alignments and estimating the earthwork volume. Journal of Forest Science, 64(11), 469-477.

Picchio, R., Pignatti, G., Marchi, E., Latterini, F., Benanchi, M., Foderi, C., ... & Verani, S. 2018. The application of two approaches using GIS technology implementation in forest road network planning in an Italian mountain setting. Forests, 9(5), 277.

Raeva, P. L., Šedina, J., & Dlesk, A. 2019. Monitoring of crop fields using multispectral and thermal imagery from UAV. European Journal of Remote Sensing, 52(sup1), 192-201.

Remondino, F., Barazzetti, L., Nex, F., Scaioni, M., & Sarazzi, D. 2011. UAV photogrammetry for mapping and 3d modeling–current status and future perspectives. International archives of the photogrammetry, remote sensing and spatial information sciences, 38(1), C22.

Ren, H., Zhao, Y., Xiao, W., & Hu, Z. 2019. A review of UAV monitoring in mining areas: Current status and future perspectives. International Journal of Coal Science & Technology, 6(3), 320-333.

Ribeiro, D., Santos, R., Cabral, R., & Shibasaki, A. 2021. Remote Inspection and Monitoring of Civil Engineering Structures Based on Unmanned Aerial Vehicles. In International Conference of the European Association on Quality Control of Bridges and Structures (pp. 695-703). Springer, Cham.

Saaty, T. L. 2008. Decision making with the analytic hierarchy process. International journal of services sciences, 1(1), 83-98.

Salamí, E., Barrado, C., & Pastor, E. 2014. UAV flight experiments applied to the remote sensing of vegetated areas. Remote Sensing, 6(11), 11051-11081.

Sonuvar, N. 2009. E-Devlet Kavramı ve Uygulamaları: Sosyal Güvenlik Kurumu Örneği. Yayımlanmamış Yüksek lisans Tezi). Ankara Üniversitesi Sosyal Bilimler Enstitüsü, (Msc Thesis).

Trubia, S., Curto, S., Severino, A., Arena, F., & Puleo, L. 2021. The use of UAVs for civil engineering infrastructures. In AIP Conference Proceedings (Vol. 2343, No. 1, p. 110012). AIP Publishing LLC.

Tsouros, D. C., Bibi, S., & Sarigiannidis, P. G. 2019. A review on UAV-based applications for precision agriculture. Information, 10(11), 349.

## *ORAL PRESENTATION*

### Polifenol oksidazın doğal inhibitör soğan ile inhibisyonu

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**Özet**

Bakır içeren bir enzim olan polifenol oksidaz (PPO), substrat spesifikliğine bağlı olarak katekol oksidaz, fenolaz, fenol oksidaz, *o*-difenol oksidaz ve tirozinaz olarak da bilinir. PPO, hasarlı meyve ve bitki dokularında enzimatik esmerleşme reaksiyonundan sorumlu ana enzimdir. Enzimatik esmerleşme genellikle belirli sebzeler ve meyvelerde hasattan sonra taşıma, işleme ve depolama sırasında meydana gelir ve gıda endüstrisinde önemli bir sorundur. Enzimatik esmerleşme reaksiyonu, gıdaların görünümünde, tadında, dokusunda ve besin değerinde değişikliklere neden olduğu için istenmeyen bir durumdur. Bu nedenle, enzimatik esmerleşmenin çeşitli bileşikler ve yaklaşımlarla kontrol edilmesi veya önlenmesi araştırmacılar tarafından çalışılmaktadır. PPO'nun aktivitesini engellemek için fiziksel ve kimyasal yöntemler geliştirilmiştir. Meyve ve sebze ürünlerinde PPO inhibitörleri olarak çeşitli doğal esmerleşme önleyici maddeler ve kimyasal bileşikler kullanılmaktadır, ancak esmerleşme reaksiyonlarını kontrol etmek için bazı kimyasal inhibitörlerin kullanılması zararlı olabilir. Tüketiciler, bu inhibitörler insan sağlığı için bir sorun olabileceğinden, yaygın olarak doğal kökenli bileşikleri kullanmayı tercih etmektedir. Bu nedenle, özellikle meyve ve sebzelerde doğal inhibitörlerin tanımlanması ve bunların PPO inhibisyonu için kullanılması çok önemlidir. Soğan, araştırmacılar tarafından incelenen bu doğal inhibitörlerden biridir. Bu konuda yapılan araştırmalar, taze ve ısıtılmış soğan özütlerinin enzimatik esmerleşme üzerinde iyi bir inhibitör etkiye sahip olduğunu bildirmiştir.

**Anahtar Kelimeler:** Enzimatik esmerleşme, İnhibisyon, Doğal inhibitör, Polifenol oksidaz, Soğan.

### Inhibition of polyphenol oxidase with natural inhibitor onion

**Abstract**

Polyphenol oxidase (PPO), which is a copper-containing enzyme is also known as catechol oxidase, phenolase, phenol oxidase, *o*-diphenol oxidase, and tyrosinase depending on its substrate specificity. PPO is the main enzyme responsible for the enzymatic browning reaction in damaged fruits and plant tissues. Enzymatic browning usually becomes in certain vegetables and fruits during handling, processing, and storage after harvest and it is major problem in the food industry. The enzymatic browning reaction is undesirable because it causes changes in the appearance, taste, texture, and nutritional value of foods. Therefore, controlling or preventing the enzymatic browning by several compounds and approaches have been studied by researchers. To inhibit the activity of PPO physical and chemical methods have been developed. Several natural anti-browning agents and chemical compounds have been used as PPO inhibitors in fruit and vegetable products, but the use of some chemical inhibitors to control browning reactions could be harmful. Consumers widely prefer to use compounds of natural origin because these inhibitors could be a problem for human health. Therefore, the identification of natural inhibitors specifically in the case of fruits and vegetables, and their use for PPO inhibition is very important. Onion is one of these natural inhibitors that has been studied by researchers. Studies on this subject have reported that fresh and heated onion extracts have a good inhibitory effect on enzymatic browning.

**Keywords:** Enzymatic browning, Inhibition, Natural inhibitor, Polyphenol oxidase, Onion.

## GİRİŞ

Polifenol oksidazlar, doğada yaygın olarak bulunan ve bakır içeren enzim grubudur. Substrat spesifikliklerine ve etki mekanizmalarına göre sınıflandırılan başlıca üç tip PPO vardır: tirozinaz (EC 1.14.18.1), katekolaz (EC 1.10.3.1) ve lakkaz (EC 1.10.3.2) (Demir ve ark., 2021). PPO enzimi, bitkiler aleminde bulunmasının yanında mikroorganizmalarda özellikle de funguslarda, kabuklu deniz hayvanlarında ve bazı hayvansal organlarda da bol miktarda bulunmaktadır. Ayrıca, bazı toprak türlerinde PPO varlığı ve aktivitesi bildirilmiştir (Çelik, 2018). Polifenol oksidaz, substrat spesifikliğine göre fenol oksidaz, fenolaz, tirozinaz, *o*-difenol oksidaz ve katekol oksidaz olarak da bilinmektedir (Lee ve ark., 2002). Polifenol oksidaz enzimi, hasarlı bitki dokularında ve meyvelerde esmerleşme reaksiyonundan sorumlu olan önemli bir enzimdir (Gnangui ve ark., 2010). Enzimatik esmerleşme, esas olarak, hasat ve işleme operasyonları sırasında meyve ve sebzeler zarar gördüğünde oksijen varlığında fenoller ve polifenol oksidazlar arasındaki ilişkiden kaynaklanmaktadır (Çınar ve Aksay, 2022). Bunun nedeni, mekanik hasarın, vakuollerden gelen fenolik bileşikler ile kloroplasttan gelen PPO arasındaki temasa izin vermesidir (Lim ve ark., 2020). Meyve ve sebzelerde yaygın olarak bulunan polifenol oksidaz enzimi, fenolik bileşiklerin oksidasyonunu katalizleyerek onları *o*-kinonlara yükseltgemekte ve oluşan bu *o*-kinonların polimerizasyonu sonucu esmerleşmeyi yapan kahverengi melanin pigmentlerinin oluşumuna yol açmaktadır. Meyvelerin ve sebzelerin depolanmasında, dilimleme, kesme, çarpma ve kabuk soyma gibi mekanik zedelenmelerden kaynaklanan pembeden siyaha kadar olan farklı tonlardaki renk değişimleri enzimatik esmerleşme olarak adlandırılmaktadır (Laurila ve ark., 1998). Gıda ürünlerinde mekanizmaya bağlı olarak esmerleşme reaksiyonu, genellikle enzimatik ve enzimatik olmayan esmerleşme olarak ikiye ayrılır. Herhangi bir enzim içermeyen enzimatik olmayan esmerleşme reaksiyonu, gıdadaki tek bir bileşiği veya birden fazla bileşeni içeren kimyasal bir reaksiyon yoluyla kahverengi bir madde üretir. Enzimatik olmayan esmerleşme reaksiyonları Maillard reaksiyonu, karamelizasyon ve askorbik asit oksidasyonunu içerir. Gıda ürünlerinde bu reaksiyonlar izole reaksiyonlar olarak değil çoğunlukla kombinasyon halinde gerçekleşir çünkü gıda kompleks bileşenlerden oluşur. Enzimatik olmayan esmerleşmenin aksine, enzimatik esmerleşme reaksiyonu gıdada bulunan polifenol oksidaz enziminin etkisini içerir (Moon ve ark., 2020). Esmerleşme reaksiyonu, gıdanın görünümünde, aromasında, tadında, dokusunda ve ayrıca besin değerinde değişikliklere neden olur (Lim ve ark., 2020). Esmerleşme, gıda kalitesini olumlu veya olumsuz yönde etkileyebilir. Siyah çay, kakao, kahve, ekmek, soya sosu, kurutulmuş hünnap ve kuru üzüm gibi diğer bazı gıdalarda eşsiz bir renk ve lezzet üretmek için önemlidir; ancak bu reaksiyon çoğu meyve ve sebze ürünü ile karides gibi deniz ürünleri için istenmeyen bir durumdur (Moon ve ark., 2020). PPO enzimi meyve ve sebzelerin renk, tat ve besin değerinde istenmeyen değişimlere yol açabileceği gibi pazarlanabilirlik değerinde kalite kayıplarına ve dolayısıyla ekonomik kayıplara neden olabilmektedir. Ürünlerin kalite optimizasyonu, gıda endüstrisi için çok önemli olan PPO aktivitesinin kontrolünü gerektirir (Çınar ve Aksay, 2022). Esmerleşme genellikle meyveler ve sebzelerde hasattan sonra taşıma, işleme ve depolama sırasında meydana gelir ve gıda endüstrisinde önemli bir sorundur. Gıdalarda PPO'nun neden olduğu esmerleşmeyi göz ardı etmek mümkün değildir, bu nedenle PPO'nun neden olduğu esmerleşmenin önlenmesi gıda endüstrisi için önemli bir konudur (Arzani ve ark., 2010). Tahıl ürünleri, meyveler ve sebzelerde PPO aracılı istenmeyen kahverengi renk değişikliği, ürünün raf ömrünü uzatmak ekonomik kayıpları azaltmak ve ürün kalitesini korumak için kontrolünü gerektiren muazzam ekonomik etkiye neden olur. İstenmeyen enzimatik esmerleşmeyi kontrol etmek için yaklaşımlar veya çeşitli mekanizmalar geliştirilmiştir. PPO'nun aktivitesini engellemek için fiziksel ve kimyasal yöntemler geliştirilmiştir (Jukanti, 2017). Meyve ve sebzelerde PPO aktivitesinin inhibisyonu için bugüne kadar çeşitli yöntemler kullanılmıştır. Isıl işlemler, oksijenin uzaklaştırılması, esmerleşmeyi önleyen maddelerin kullanımı ve kimyasalların eklenmesi en etkili yöntemler arasındadır. Gıda endüstrisinde, polifenol oksidazın ısıl işlemi kullanılarak enzimatik esmerleşmeden kaçınılsa da geleneksel ısıl işlemler renk değişimi, lezzet, besin ve vitamin kayıpları gibi olumsuz etkilere neden olmaktadır. Meyve ve sebzelerin vakumlanmaları veya suya, şuruba ve tuzlu suya daldırılmalarıyla enzimatik esmerleşmenin engellendiği bildirilmiştir. Vakumlanan paketlerin açılmasıyla oksijenin yeniden içeri girip enzimatik esmerleşme reaksiyonunun tekrar başlamasına neden olduğu için bu tür işlemler kesin sonuç vermemektedir. Enzimatik esmerleşme reaksiyonunun önlenmesi için yaygın bir yaklaşım, öncelikle enzim üzerinde etkili olan veya enzimatik kataliz substratları ve/veya ürünleri ile reaksiyona girerek pigment oluşumunu engelleyen esmerleşme karşıtı ajanların kullanılmasıdır. Ancak bu esmerleşme önleyici maddelerin gıda endüstrisinde kullanımı, toksisite gibi hususlar nedeniyle kısıtlanmaktadır (Arslan ve Doğan, 2005). Esmerleşme reaksiyonunu kontrol etmek için inhibitörler kullanılırken bazıları zararlı olabileceğinden, gıda güvenliği büyük bir endişe kaynağıdır. Bu nedenle, özellikle meyve ve sebzelerde doğal inhibitörlerin tanımlanması çok önemlidir (Jukanti, 2017). PPO ile esmerleşme, sülfitler, askorbik asit ve analoglarının yanı sıra sistein ilavesiyle önlenebilir. Sülfitler esmerleşmeyi önlemede çok etkili olsalar da insan sağlığı için bir sorun teşkil edebilirler. Buna göre, tüketiciler tarafından gıda bileşenleri olarak doğal maddelerle sentetik bileşiklerin ikame edilmesine yönelik artan bir talep vardır. Bu nedenle, doğal kökenli bileşikler, pazardaki tüketiciler tarafından geniş çapta kabul görecektir (Arzani ve ark., 2010). Yaşam tarzındaki değişiklikler ve

gıda ile sağlık arasındaki ilişkinin farkındalığı, işlenmiş gıdalarda daha güvenli ve toksik etkisi olmayan kimyasalların daha az eklenmesine yönelik bir talep ortaya çıkarmıştır (Arslan ve Doğan, 2005). Sülfitlere ve diğer sentetik katkı maddelerine alternatif inhibitörler bulmanın bir yolu olarak, enzimatik esmerleşmenin önlenmesinde bitki özütlerinin uygulanması üzerine çalışmalar yapılmıştır. Birçok yazar bitki özütleri, tarımsal gıda yan ürünleri ve atıklardan elde edilen bileşiklerin çeşitli gıda matrikslerinde polifenol oksidaza karşı inhibitör etkisi olduğunu kanıtlamıştır (Vhangani ve Van Wyk, 2021). Gıdalardaki enzimatik esmerleşme reaksiyonunu engellemek için kimyasal olmayan, toksik etki yaratmayan ve daha güvenli bitki özütlerinin kullanılmasına yönelinmiş ve birçok doğal inhibitör incelenmiştir. Soğan, araştırmacılar tarafından incelenen bu doğal inhibitörlerden biridir.

Soğan (*Allium cepa* L.) botanik olarak Liliaceae familyasına dahildir ve türleri Avrupa, Asya, Kuzey Amerika ve Afrika'da geniş bir enlem ve rakım aralığında bulunur (Griffiths ve ark., 2002). Soğan, yaklaşık 89 milyon ton olduğu tahmin edilen hasadı ile domatesten sonra dünya çapında en çok yetiştirilen ikinci üründür. Soğanın büyük rağbet görmesi çok yönlülüğü ile ilgilidir. Çiğ gıda olarak ve fırınlanmış, haşlanmış, kızartılmış, ızgaralanmış, kavrulmuş vb. gibi farklı pişmiş formlarda kullanılır. Son yıllarda, dünya çapında soğan üretimi en az %25 oranında artmıştır; bu durum soğanın sadece aromalı bir sebze veya baharatlı bir bileşen olarak kullanılmasına değil, aynı zamanda soğanın biyoaktif bitkisel besin kaynağı olarak kullanılmasına da bağlıdır (Woo ve ark., 2007). Soğan, insan sağlığına faydaları olduğu düşünülen iki kimyasal grup açısından zengindir. Bunlar flavonoidler ve alk(en)il sistein sülfoksitlerdir. Soğanda iki flavonoid alt grubu bulunur; bazı çeşitlere kırmızı/mor renk veren antosiyaninler ve diğer birçok çeşidin sarı ve kahverengi kabuklarından sorumlu olan kuersetin ve türevleri gibi flavanollerdir. Alk(en)il sistein sülfoksitler, alliinaz enzimi tarafından parçalandığında soğanın karakteristik kokusunu ve tadını oluşturan aroma öncüleridir. Sonraki ürünler, tiyosülfinatlar, tiyosülfonatlar, mono-, di- ve tri-sülfürleri içeren karmaşık bir bileşik karışımıdır. Soğandan elde edilen bileşiklerin antikanserojenik özellikler, antiplatelet aktivitesi, antitrombotik aktivite, antiastmatik ve antibiyotik etkileri içeren bir dizi sağlık yararına sahip olduğu bildirilmiştir (Griffiths ve ark., 2002). Soğanın antioksidan bileşikler açısından zengin olduğu ve tüketiminin oksidatif stresle ilişkili bazı hastalıkların önlenmesine katkıda bulunduğu bilinmektedir ve çok sayıda epidemiyolojik çalışma, düzenli soğan tüketiminin kardiyovasküler ve nörodejeneratif hastalıkların yanı sıra çeşitli kanser türlerinin görülme sıklığını azalttığını doğrulamıştır. Soğan kabuğu, başta kuersetin ve türevleri olmak üzere polifenolik antioksidanlar; flavonoidler grubuna ait glukozitler ve antidiyabetik, antioksidan, antienflamatuar, antitümör, antimikrobiyal ve enzim inhibe edici etkiler dahil olmak üzere çeşitli biyolojik aktivitelerle ilişkili önemli faydalı etkiler gösteren ferulik asit, gallik asit ve kaempferol bakımından zengindir. Soğan çeşitleriyle ilgili olarak, kuersetin ve türevleri, glukozitlerle birlikte, beyaz, sarı veya kırmızı renkten bağımsız olarak tüm soğan türlerinde baskın flavonollerdir; antosiyaninler ise esas olarak kırmızı soğanda bulunur ve taze ağırlıktaki toplam flavonoid içeriğinin yaklaşık %10'unu oluştururlar. Kuersetin ve glukozitleri gibi öne çıkan flavonoidlerin içeriği inci soğanlarda en yüksektir, bunu kırmızı, sarı ve beyaz soğan kabukları takip eder ve büyük ölçüde özütleme koşullarına bağlıdır (Chernukha ve ark., 2022). Soğanın taze ağırlığının %60-70'i sudur ve diğer en önemli bileşenleri organosülfür içeren bileşiklerdir. Soğan esas olarak sistein sülfoksitler içerir. Dokular doğrandığında, allinaz enzimi salınır ve sistein sülfoksitleri tiyosülfinatlara dönüştürür. Bu bileşikler reaktif, uçucu, koku yapıcı ve göz yaşartıcıdır. Soğanın besleyici bileşiklerine ek olarak, çeşitli organizmalara karşı antioksidan, antibakteriyel ve antifungal aktiviteleri kapsamlı bir şekilde araştırılmıştır (Woo ve ark., 2007). Soğan özütü sadece insan sağlığına yararlı etkiler sağlamakla kalmaz, aynı zamanda PPO'ya karşı esmerleşme üzerinde etkili bir inhibitördür (Wong ve Angle Lee, 2014).

Bu derleme çalışmasında polifenol oksidaz enziminin soğan ile inhibisyonu üzerine bugüne kadar yapılan çalışmaların değerlendirilmesi amaçlanmıştır.

## MATERYAL VE METOT

Bu derleme çalışmasında polifenol oksidaz enziminin soğan ile inhibisyonu üzerine bugüne kadar yapılmış olan çalışmalarda yer alan veriler kullanılmıştır.

## BULGULAR

Bu çalışmada soğanın polifenol oksidaz enziminin inhibisyonu üzerine günümüze kadar yapılan çalışmalar derlenmiş ve yapılan çalışmalar Tablo 1'de özet halinde verilmiştir.

**Tablo 1.** Polifenol oksidaz enziminin soğan (*Allium cepa*)'la inhibisyon çalışmaları*

| Bitki özütü | İnhibisyon etkisi/Substrat | PPO kaynağı | Kaynaklar |
|---|---|---|---|
| Soğan suyu | %50 konsantrasyonda %80 inhibisyon % 20 aktivite | Elma suyu | Hosoda ve Iwahashi, 2002 |
| 50 mM pH 6.8 fosfat tamponuyla soğan özütü | 3.1 mg/mL taze soğan özütü ile bağıl aktivite %44.4 ve 100ºC'de 10 dakika ısıtılmış 3.1 mg/mL soğan özütü ile bağıl aktivite %9.6; 1.55 mg/mL taze soğan özütü ile bağıl aktivite %78.6 ve 10 dakika 100ºC'de ısıtılmış 1.55 mg/mL soğan özütü ile bağıl aktivite %17.0, Substrat: katekol İnhibisyon çeşidi: yarışmasız inhibisyon | Patates | Lee ve ark., 2002 |
| Su ile soğan özütü | 60.0 mg/mL taze soğan ile %73.3 bağıl aktivite, 10 dakika 100ºC'de ısıtılmış 60.0 mg/mL soğan ile % 45.9 bağıl aktivite, Substrat: katekol | Armut | Kim ve ark., 2005 |
| 50 mM pH 6.8 fosfat tamponuyla soğan özütü | 60.0 mg/mL taze soğanla % 77.2 bağıl aktivite, 10 dakika 100ºC'de ısıtılmış 60.0 mg/mL soğanla %53.2 bağıl aktivite, Substrat: katekol İnhibisyon çeşidi: yarışmasız inhibisyon | Şeftali | Kim ve ark., 2007 |
| 50 mM pH 6.6 fosfat tamponuyla soğan özütü | 10 dakika 100ºC'de 3.1 mg/mL ısıtılmış soğan özütü taze soğan özütüne göre daha yüksek inhibisyon İnhibisyon çeşidi: yarışmasız inhibisyon | Muz (*Musa acuminata* cv. Jejudo) | Lee, 2007 |
| 50 mM pH 6.8 potasyum fosfat tamponu ile soğan özütü | 3.1 mg/mL taze soğanla katekole karşı %88.2 bağıl aktivite, 10 dakika 100ºC'de ısıtılmış 3.1 mg/mL soğanla %46.2 bağıl aktivite, L-DOPA, pirogallol, 4-metil katekol, resorsinol, (+)–kateşin ve hidrokinon subsratlarına karşı farklı oranlarda inhibisyon | Taro (*Colocasia antiquorum* var. Esculenta) | Lee ve ark., 2007 |
| İspanyol soğan çeşitleri - 'Figueres' ve 'Recas', Soğan yan ürünü suyu, püresi, küspesi | Katekole karşı: Soğan yan ürünü suyu: Dondurulmuş 'Figueres' %70.75 'Recas' %60.38; Pastörizasyon 'Figueres' %51.81 'Recas' %34.48; Sterilizasyon 'Figueres' % 63.51 'Recas' %40.36; Soğan yan ürünü püresi: Dondurulmuş 'Figueres' %65.49 'Recas' %42.92; Pastörizasyon 'Figueres' %67.18 'Recas' %46.50; Sterilizasyon 'Figueres' % 31.86 'Recas' %10.29; Soğan yan ürünü küspesi: Dondurulmuş 'Figueres' %44.18, 'Recas' %60.23; Pastörizasyon 'Figueres' %72.53, 'Recas' %86.08; Sterilizasyon 'Figueres' % 61.22, 'Recas' %27.32 bağıl aktivite | Avokado (*Persea americana* Miller var. *americana* 'Fuerte') | Roldán ve ark., 2008 |
| 100 mM pH 6,6 fosfat tamponuyla kırmızı soğanın özütü | 2.28 ve 0.97 mg protein içeren taze soğan özütüyle (yarışmasız inhibisyon); 30, 35, 40, 45, 50, 55, 60 ve 65 ºC sıcaklıklarda 5 ile 120 dakika süresince ısıtılmış soğan özütleri ile farklı inhibisyon oranları, Substrat: dopamin | Yenilebilir Yam bitkisi (*Dioscorea cayenensis-rotundata* cv. Longô) | Gnangui ve ark., 2010 |
| Su ile soğan özütü | 60.0 mg/mL taze soğan özütüyle %73.30 bağıl aktivite ve 10 dakika 100ºC'de ısıtılmış soğan özütüyle %45.90 bağıl aktivite, Substrat: katekol | Asya armudu (*P. serotina*) | Arzani ve ark., 2010 |
| Soğan özütü | 15 dakika 100ºC'de ısıtılmış soğan suyuyla % 54.2, pastörize lapa soğanla % 41.2 ve taze soğan suyuyla % 37.3 inhibisyon, Substrat: katekol | 'Birgah' patlıcanı | Barbagallo ve ark., 2012 |
| 0.1 M pH 7.5 fosfat tamponuyla kırmızı soğanın özütü | 100ºC'de 10 dakika 3 mg/mL ısıtılmış soğan özütüyle %47.00 inhibisyon (yarışmasız inhibisyon) ve taze soğan özütüyle %38.46 inhibisyon (yarışmasız inhibisyon), Substrat: katekol | Cassava (manyok) yaprağı (*Manihot esculenta*) | Wong ve Angle Lee, 2014 |
| Su ile küçük (Ekalot), kırmızı, beyaz, sarı ve yeşil soğanların özütleri | Küçük (Ekalot) soğan özütüyle (yarışmasız inhibisyon), kırmızı soğan özütüyle (yarışmasız inhibisyon), beyaz soğan özütüyle (yarışmasız inhibisyon), sarı soğan özütüyle (yarışmalı inhibisyon), yeşil soğan özütüyle (yarışmalı inhibisyon), Substrat: Dopamin | Yenilebilir Yam bitkisinin (*Dioscorea cayenensis-rotundata*) yumrusu | Yapi ve ark., 2015 |
| Soğanın kloroform, n-heksan, su ve etil asetat fraksiyonu | Soğanın kloroform fraksiyonuyla %52.97, n-heksan fraksiyonuyla %60.13, su fraksiyonuyla %47.09 ve etil asetat fraksiyonuyla %90.33, inhibisyon, | Ticari PPO enzimi | Yuniarti ve ark., 2018 |

| | | | |
|---|---|---|---|
| | Substrat: L-DOPA | | |
| Su ile kırmızı soğanın özütü | 10 mg/mL ısıtılmamış soğan özütüyle: 4-metil katekol için %14.02 inhibisyon (karışık tip inhibisyon), pirokatekol için %28.38 inhibisyon (karışık tip inhibisyon); 15 dakika 95ºC'de ısıtılmış 10 mg/mL soğan özütüyle: 4-metilkatekol için %15.89 inhibisyon, pirokatekol için %33.11 inhibisyon | Zencefil (*Zingeber officinale*) | Lim ve Wong, 2018 |
| Soğuk distile suyla özütlenip santrifüjlendikten sonra filtrelenen kırmızı soğan özütü | Taze soğan özütüyle 4-metilkatekol için %7.13 inhibisyon (yarışmalı inhibisyon), pirokatekol için %39.34 inhibisyon (yarı yarışmalı inhibisyon), 10 dakika 100ºC'de ısıtılan soğan özütüyle 4-metilkatekol için %17.58 inhibisyon (karışık tip inhibisyon), pirokatekol için %41.47 inhibisyon | Tatlı patates (*Ipomoea batatas*) | Lim ve ark., 2019 |
| Beyaz ve kırmızı soğan kabuğu | 100°C ve 115°C üzerinde ısıtma işlemi uygulanan beyaz ve kırmızı soğan kabuğuyla inhibisyon, beyaz soğan kabuğuyla 1:2 konsantrasyonda en iyi inhibisyon, kırmızı soğan kabuğuyla inhibisyon farklılık göstermiş, Substrat: katekol | Avokado (*Persea Americana Mill*) | Vega ve ark., 2020 |
| 0,05M pH 6,8 sodyum fosfat tamponuyla özütlenen santrifüjlenip süzülen kırmızı soğan özütü | Taze soğan özütü ile pirokatekol substratına karşı %65.10 inhibisyon, 4-metilkatekol substratına karşı %25.33 inhibisyon; 10 dakika 100ºC'de ısıtılan soğan özütü ile pirokatekol substratına karşı %68.51 inhibisyon, 4-metilkatekol substratına karşı %42.33 inhibisyon | Beyaz düğme mantarı (*Agaricus bisporus*) | Wong ve ark., 2021 |

*Demir ve Alan (2021)'dan modifiye edilmiştir.

Isıtılmış soğan özütünün taze soğandan daha güçlü bir inhibitör olduğu, ve soğan özütünün inhibitör etkisinin artan ısıtma sıcaklığı ve süresi ile arttığı rapor edilmiştir (Lee ve ark.,2002; Kim ve ark., 2005; Kim ve ark., 2007; Lee, 2007; Lee ve ark., 2007). Ayrıca, soğan özütüne hem glisin hem de glukoz ilave edilmesi, soğan özütünün polifenol oksidaza karşı inhibitör etkisini artırmıştır (Lee ve ark., 2002; Kim ve ark., 2007). Isıtılmış soğan özütü gıdalarda doğal bir esmerleşme önleyici olarak kullanılabilir (Lee ve ark.,2002).

## TARTIŞMA

Yapılan tüm çalışmalar incelendiğinde ısıtılmış soğan özütlerinin polifenol oksidaz enzimini taze soğan özütlerinden daha güçlü bir şekilde inhibe ettiği sonucuna varılmıştır. Özütlerin ısıtılması sırasında özütte farklı bileşenler oluşabilir. Isıtma işlemi ile soğan özütünün inhibitör etkisinin artması, Maillard reaksiyon ürünlerinin PPO'nun inhibitörleri olduğu ve soğan özütlerinin ısıtılmasıyla inhibitör etkideki artışın, ısıtma sırasında üretilen Maillard reaksiyon ürünleriyle sinerji etkisinden kaynaklanabileceği bildirilmiştir. Isıtılmış soğan özütü üzerindeki enzimatik esmerleşmenin engellenme mekanizmaları iyi anlaşılmamış olsa da, soğanın armut PPO aktivitesini etkili bir şekilde inhibe eden bazı bileşikler içerdiği bildirilmiştir. Soğan gibi *Allium* türlerinde tiyoller de dahil olmak üzere çeşitli uçucu sülfür bileşiklerinin mevcut olduğu bildirilmiştir. Tiyol bileşiklerinin PPO'yu inhibe ettiği için, soğandaki tiyol bileşiklerinin soğan özütünün inhibitör etkisinden sorumlu aktif bileşenler olabileceğini varsayılabilir. Soğan özütü diyaliz edildiğinde, armut PPO'suna karşı inhibitör etki tamamen ortadan kalktığı bildirilmiştir, bu da inhibitör etkisini düşük moleküllü bileşiklerin yaptığına işaret etmektedir (Kim ve ark., 2005). Isıtılmış soğanın patates PPO'su üzerindeki inhibitör etkisinin mekanizması, soğan özütüne glikoz, glisin veya her ikisinin eklenmesi ile de araştırılmıştır. Ne glikozun ne de glisinin tek başına patates PPO'su üzerinde herhangi bir etkiye sahip olmadığı bildirilmiştir. Ancak, soğan özütünün glisin ve glikoz varlığında kaynatılması soğanın inhibitör etkisini uyarmıştır. Bu durum, ısıtma sırasında üretilen enzimatik olmayan esmerleşme ürünlerinin, ısıtılmış soğan özütünün daha güçlü inhibitör etkisinden sorumlu olabileceğini düşündürmektedir. Çünkü, Maillard reaksiyon ürünlerinin PPO üzerinde inhibe edici bir etkiye sahip olduğu bildirilmiştir. Bununla birlikte, glikoz ve glisinin ısıtılması patates PPO'su üzerinde daha düşük bir inhibitör etki gösterdiği belirtilmiştir ve bu da ısıtılmış soğanın inhibitör etkisi için soğan bileşenlerinin gerekli olabileceğini düşündürmektedir. Soğanda bulunan hangi bileşenlerin esmerleşmeyi engellemekten sorumlu olduğunu belirlemek için daha fazla araştırmaya ihtiyaç vardır (Lee ve ark., 2002). Farklı renkte ve çeşitteki soğanların farklı çözücüler ile hazırlanmış özütleri ve çeşitli bileşenlerinin polifenol oksidaz enzimi üzerine inhibisyon etkileri araştırılmıştır. Yapılan çalışmalara bakıldığında, farklı özütleme yöntemlerinin kullanılması ve çözücü değişimiyle elde edilen soğan özütleri polifenol oksidaz inhibisyonunu farklı şekilde etkilemiştir. Özütlerin ısıtılarak veya taze olarak kullanılması da inhibisyonların farklı şekilde etkilenmesine neden olduğu görülmüştür. İnhibitör olarak kullanılan soğanların çeşit ve içeriği, çözücü, özütleme yöntemleri, özütün taze veya ısıtılmış olması gibi farklılıklar

sebebiyle elde edilen özüt içeriklerinin değişmesinden dolayı polifenol oksidaz enziminin güçlü veya zayıf olarak inhibe edildiği bildirilmiştir.

## SONUÇ

Soğan özütleri ile inhibisyon konusunda yapılan araştırmalar, soğan özütlerinin polifenol oksidaz enzim aktivitesi üzerinde iyi bir inhibitör etki gösterdiği için doğal bir inhibitör olma potansiyeline sahip olduğunu göstermiştir. Araştırma sonuçlarından, özellikle ısıtılmış soğan özütlerinin taze soğan özütlerine göre daha çok inhibisyon etkisinin olduğu görülmektedir. Yapılan çalışmalardan elde edilen sonuçlara bakılarak polifenol oksidaz enziminin inhibisyonunda insan sağlığına zararlı olabilecek maddelerin yerine soğan özütlerinin kullanılabileceği sonucuna varılmaktadır.

## KAYNAKLAR

Arslan O, Doğan S 2005. Inhibition of polyphenol oxidase obtained from various sources by 2,3-diaminopropionic acid. J. Sci. Food Agr., 85(9):1499-1504.

Arzani K, Khoshghalb H, Malakouti MJ, Barzegar M 2010. Prevention of enzymatic browning of Asian pear (*Pyrus serotina* Rehd.) by some anti-browning agents. Acta Horticulturae, 858: 273-278.

Barbagallo RN, Riggi E, Avola G, Patanè C 2012. Biopreservation of 'Birgah' eggplant from polyphenol oxidase activity assayed *in vitro* with onion (*Allium cepa* L.) by-products. Chemical Engineering Transactions, 27: 43-48.

Çelik E 2018. Eğirdir (Isparta)'de yetiştirilen elmalardan polifenol oksidaz enziminin saflaştırılması, karakterizasyonu ve polifenol oksidaz enziminin saflaştırılması için alternatif bir yöntem geliştirilmesi. Yüksek Lisans Tezi, Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Isparta.

Chernukha I, Kupaeva N, Kotenkova E, Khvostov D 2022. Differences in Antioxidant Potential of Allium cepa Husk of Red, Yellow, and White Varieties, Antioxidants. 11: 1243. https://doi.org/10.3390/antiox11071243

Çınar F, Aksay S 2022. Purification and characterization of polyphenol oxidase from myrtle berries (*Myrtus communis* L.). Journal of Food Measurement and Characterization, 16: 2282–2291. https://doi.org/10.1007/s11694-022-01350-0

Demir D, Alan Ü 2021. Polifenol Oksidaz Enziminin Farklı Bitkilerle İnhibisyonu Üzerine Çalışmalar. Ziraat, Orman ve Su Ürünlerinde Araştırma ve Değerlendirmeler, Gece Kitaplığı, Cilt II, 405-431.

Demir D, Eken C, Çelik E, Alkan N 2021. Effect of *Rhizoctonia* spp. on Polyphenol Oxidase Enzyme Activity in Alfalfa Seedling. Research Journal of Biotechnology, 16 (11): 47-50.

Gnangui SN, Niamke SL, Kouame LP 2010. Partial Characterization of a New Peptide from Ivorian Red Onion that Inhibits Polyphenol Oxidase and Enzymatic Browning of Edible Yam (Dioscorea cayenensis-rotundata cv Longbô). Chiang Mai J. Sci., 37(3): 464-475.

Griffiths G, Trueman L, Crowther T, Thomas B, Smith B 2002. Onions—A Global Benefit to Health. Phytotherapy Research, 16, 603–615. DOI: 10.1002/ptr.1222

Hosoda H, Iwahashi Y 2002. Inhibition of Browning of Apple Slice and Juice by Onion Juice. Journal of the Japanese Society for Horticultural Science, 71(3):452-454.

Jukanti A 2017. Polyphenol Oxidases(PPOs) in Plants. Springer, 978-981-10-5747-2. DOI 10.1007/978-981-10-5747-2

Kim CY, Kim MJ, Lee MY, Park I 2007. Inhibition of polyphenol oxidase and peach juice browning by onion extract. Food Science and Biotechnology,16(3): 421-425.

Kim MJ, Kim CY, Park I 2005. Prevention of enzymatic browning of pear by onion extract. Food Chemistry, 89(2): 181–184.

Laurila E, Kervinen R, Ahvenainen R 1998. The inhibition of enzymatic browning in minimally processed vegatables and fruits. Postharvest News and Information, 9(4): 53-66.

Lee MK 2007. Inhibitory effect of banana polyphenol oxidase during ripening of banana by onion extract and Maillard reaction products. Food Chem., 102: 146–149.

Lee MK, Kim YM, Kim NY, Kim GN, Kim, SH, Bang KS, Park I 2002. Prevention of Browning in Potato with a Heat-treated Onion Extract. Bioscience, Biotechnology, and Biochemistry, 66(4): 856-858. DOI: 10.1271/bbb.66.856

Lee MY, Lee MK, Park I 2007. Inhibitory effect of onion extract on polyphenol oxidase and enzymatic browning of taro (*Colocasia antiquorum* var. *esculenta*). Food Chemistry, 105: 528–532.

Lim WY, Cheng YW, Lian LB, Chan EWC, Wong CW 2020. Inhibitory effect of Malaysian coastal plants on banana (Musa acuminata colla ‘‘Lakatan’’), ginger (Zingiber officinale Roscoe) and sweet potato (Ipomoea batatas) polyphenol oxidase. J Food Sci Technol, https://doi.org/10.1007/s13197-020-04886-5

Lim WY, Wong CW 2018. Inhibitory effect of chemical and natural anti-browning agents on polyphenol oxidase from ginger (*Zingiber officinale* Roscoe). J. Food Sci. Techno., 55(8): 3001–3007.

Lim WY, Wong CW, Cheun CF 2019. Inhibition of enzymatic browning in sweet potato (*Ipomoea batatas* (L.)) with chemical and natural antibrowning agents. J. Food Process Preserv., 43(11):e14195.

Moon KM, Kwon E-B, Lee B, Kim CY 2020. Recent Trends in Controlling the Enzymatic Browning of Fruit and Vegetable Products. Molecules, 25: 2754. doi:10.3390/molecules25122754

Roldán E, Sánchez-Moreno C, Ancos B, Cano MP 2008. Characterisation of onion (*Allium cepa* L.) by-products as food ingredients with antioxidant and antibrowning properties. Food Chemistry, 108: 907–916.

Vega NA, Salazar SA, Bautistay LT, Muñoz GE 2020. Evaluation of the inhibitor effect of polifenol oxidaza enzyme in an avocado sauce (*persea americana*). Entre Ciencia e Ingeniería, 14(27):58-62.

Vhangani LN, Van Wyk J 2021. Heated plant extracts as natural inhibitors of enzymatic browning: A case of the Maillard reaction. J. Food Biochem., 00:e13611. https://doi.org/10.1111/jfbc.13611

Wong CW, Angel Lee PL 2014. Inhibitory effect of onion extract on cassava leaf (*Manihot esculenta* Crantz) polyphenol oxidase. International Food Research Journal, 21(2): 755-758.

Wong CW, Toh AYF, Lim WY 2021. Effect of Heated Onion Extract on White Button Mushroom (*Agaricus Bisporus*) Polyphenol Oxidase. Carpathian Journal of Food Science and Technology, 13(4): 16-23.

Woo KS, Hwang IG, Kim TM, Kim DJ, Hong JT, Jeong HS 2007. Changes in the Antioxidant Activity of Onion (*Allium cepa*) Extracts with Heat Treatment. Food Sci. Biotechnol., 16(5):828-831.

Yapi JC, Gnangui SN, Dabonné S, Kouamé LP 2015. Inhibitory effect of onions and garlic extract on the enzymatic browning of an edible yam (*Dioscorea cayenensis-rotundata* cv. Kponan) cultivated in Côte d'Ivoire. International Journal of Current Research and Academic Review, 3(1): 219-239.

Yuniarti T, Sukarno Yuliana ND, Budijanto S 2018. Inhibition of enzymatic browning by onion (*Allium cepa* L.): Investigation on inhibitory mechanism and identification of active compounds. Current Research in Nutrition and Food Science, 06(3): 770-780.

## *ORAL PRESENTATION*

# Glyphosate and microorganisms

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## Abstract

Glyphosate is a broad-spectrum herbicide used in many countries of the world. In Turkey, it is used in the fight against weeds in olive, citrus fruits, apple, vineyard and hazelnut orchards. Compared to many other herbicides, glyphosate has a relatively short half-life in soil and water (average approximate 30 days in temperate climates), mainly because of microbial degradation. However, there are growing concerns about glyphosate. It is reported that if not used wisely, it can cause off-target adverse effects in agroecosystems. It can prevent the synthesis of aromatic amino acids in fungi bacteria and higher plants by means of the inhibition of the enzyme 5-enolpyruvyl shikimic acid-3-phosphate synthase (EPSPS), triggering their death. However, the shikimate synthesis pathway also exists in microorganisms, and therefore glyphosate may also impair microbial growth and activity in sensitive species. Although a lot of studies have researched the effects of glyphosate on microorganisms, there are the many variable and contradictory results. The purpose of this review is to scan the publications on the effects of glyphosate on soil microorganisms, human and animal intestinal flora, and to present the methods used to examine the relationship between glyphosate and microorganisms, and different views coming up depending on these method difference.

**Keywords:** Glyphosate, Soil microorganism, Epsps enzyme, Herbicide, Sensitivity, Human gut microbiome

## INTRODUCTION

Microorganisms first appeared on the planet millions of years before plants and animals, and they are essential for supporting and preserving life on Earth. For the ecology of microorganisms, soil and related habitats are essential. The abundance and diversity of microorganisms in the soil is vital for soil formation, soil quality, plant yield and health. Soil bacteria and fungi play a vital role in soil functions associated with the distribution, transformation, availability and retention of soil components such as C, N and toxins (Bunemann et al., 2006). Through the symbiotic link between plants and mycorrhizal fungus, soil microorganisms also play a significant part in regulating the availability of phosphorus and other nutrients for crops. The diversity of soil microorganisms is enormous, and despite the intense interest in recent years, many of the microbial species are still unknown. (Torsvik and Ovreas, 2002).

Glyphosate, N-(phosphonomethyl) glycine, is a biocide with broad-spectrum activity and a crucial weed management agent in many agricultural systems. When glyphosate is applied in the field, it is absorbed by plant shoots and builds up in the phloem sap. The compound acts systemically and is transported to all metabolically active tissues, including seeds, nodules and roots within 24 hours. it inhibits the production of aromatic amino acids by interfering with the 5-enolpyruvyl shikimic acid-3-phosphate synthase (EPSPS) enzyme in the shikimate pathway found in plants, fungi, bacteria, archaea and protozoa groups. These amino acids help to produce the antibacterial phytoalexins and lignin that shield plants from infections (Duke et al., 2018). Therefore, the death of non-GM plants treated with glyphosate is accelerated by root pathogens. (Defarge et al., 2018).

Glyphosate has been declared safe for humans and other non-target organisms because the shikimate metabolic pathway inactivated by glyphosate is not present in vertebrates. However, microbiomes have a shikimate metabolic pathway and are made up of a wide range of commensals, beneficials, and pathogens. The health of all living things in the ecosystem is especially related to the transfer of microorganism groups in the food chain (van Bruggen et al., 2021). Glyphosate has a relatively short environmental half-life and is rapidly metabolized by many soil microbes. Blake and Pallett (2018) state that the average half-life of glyphosate in soil is about 30 days, and this period ranges from 5.7 to 40.9 days Transformation of glyphosate by microorganisms that degrade glyphosate can occur by two main degradation pathways, either to aminomethyl phosphonic acid (AMPA) or sarcosine (SAR) (Borggaard and Gimsing, 2008). The third pathway of GLP transformation is

conversion to N-acetyl derivatives. After glyphosate is applied to genetic modified soybeans, the metabolite N-acetyl-GLP is produced (FR, 2008). The US According to the US Environmental Protection Agency (EPA) (2008), its toxicological effects are similar to glyphosate.

From 2004 to 2008, global use of glyphosate reached approximately 600,000 tonnes, mainly due to the increase in cultivation sites devoted to glyphosate resistant crops (up to 75 million hectares) and crops planted for bioenergy production (Yin, 2011). (Yin, 2011). Studies of glyphosate effects on soil microflora using levels of glyphosate that can be found in agricultural soil have found minor effects that usually do not last long (Nguyen et al. 2016). In one study, glyphosate had only minor and transient effects on soil microbial communities, even when applied at 3 times the recommended field rates (Weaver et al. 2007). Duke et al (2020) concluded that the much lower levels of glyphosate that can be found in off-site soils will have no detectable or no effect on soil microflora. Research results show that the environmental impact of glyphosate is generally positive compared to other weed management methods. In addition to the studies that argue that glyphosate has positive effects on the environment, there are also the studies that reveal the existence of negative effects. According to reports, the use of glyphosate can have negative effects on non-target soil microorganisms or contaminate groundwater (Borggaard and Gimsing, 2008). Glyphosate has been linked to decreased earthworm activity, increased root colonization of *Fusarium* and other fungal diseases, and indirect change of the relationship between fungus and other microbes (Druille et al., 2015; Zaller et al., 2014).

In the last 50 years of the use of glyphosate, strains of several types of bacteria have emerged that can degrade the herbicide; these include potential animal/human pathogens. (Acosta-Cortés et al, 2019; Xu et al, 2019). Glyphosate can be detoxified by N-acetylation by various bacterial species, including *Bacillus cereus* (*B. cereus*) and *B. anthracis* (Acosta-Cortés et al., 2019). Therefore, prolonged use of glyphosate is likely to result in increased levels of *B. anthracis* (the causative agent of anthrax) in the environment. It has been reported that the microbial composition of various glyphosate-containing habitats such as soil, plant surfaces, and animal intestinal tracts may be affected differently due to differences in susceptibility to glyphosate among microorganisms (van Bruggen et al., 2018). Recent research has focused on the indirect effects of glyphosate on plant, animal and human health through its effects on the microbiomes. (Davoren and Schiestl, 2018).

Such divergent views on the results of glyphosate application are due to the use of different commercial formulations and different concentrations of glyphosate in the laboratory pot or field trials. In addition, the evaluation of effects of glyphosate on microbiomes through culture-based techniques, methods based on carbon and nitrogen mineralization in the soil, measurement of enzyme activity, lipid extraction, the use of molecular techniques, and the evaluation of data by in silico analysis also affect the results of glyphosate application.

The aim of this review is to review publications on the effects of glyphosate on soil microorganisms, human and animal intestinal flora, and to present the methods used to study the relationship between glyphosate and microorganisms and the different views that emerge due to differences in methods.

## IN VITRO RESEARCH

Yılmaz et al (1997) studied the effect of glyphosate herbicide at 10- 150 p.g/ml on the growth and lactic acid production of single and combined *Lactobacillus bulgaricus, Lactobacillus plantarum, Streptococcus thermophilus, Streptococcus lactis, Streptococcus diacetylactis, Pediococcus pentosaceus* bacteria. The results of this study revealed that while glyphosate generally stimulated the growth of *L. bulgaricus strains*, it had an inhibitory effect on the growth of other test bacteria. They also found that increasing concentrations of glyphosate (10-150 pg/ml) decreased acid production of both single and combined cultures. The microorganisms included in the experiment are especially important as they have starter culture characteristics and are also sensitive to glyphosate. It was shown that bacteria (*Klebsiella pneumoniae* and *Klebsiella variicola*) isolated from glyphosate-treated soil of Kahramanmaraş agricultural land could not degrade glyphosate in *in vitro* and moreover, glyphosate had toxic effects on these bacteria (Kurtoglu et al, 2020). Species like *Bacteroides vulgatus, Bifidobacterium adolescentis, Enterococcus faecalis, Staphylococcus aureus,* and *Lactobacillus buchneri* treated with Roundup UltraMax® were moderately to highly sensitive to glyphosate in *in vitro* tests (minimal inhibitory concentration (MIC) = 0. 075-0,600 mg/ml) whereas *Escherichia coli* and *Salmonella typhimurium* were relatively more resistant (MIC values = 1,200 and 5,000 mg/ml) (Priestman et al, 2005). *Bifidobacterium adolescentis* and *Enterococcus faecalis* are very important microorganisms for human intestinal microbiota. Especially Bifidobacterium *adolescentis* plays a key role in gamma aminobutyric acid (GABA) production in the mammalian intestine (Duranti et al., 2020).

In addition to sensitive bacteria, the presence of resistant bacteria has also been determined by *in vitro* experiments. Hadi et al. (2013) in their study on glyphosate (3 mM) degradation by *Ochrobactrum spp*. found that glyphosate was converted to AMPA within 60 hours. Some microbes use glyphosate as a nitrogen or carbon source, including *Agrobacterium radiobacter* SW9, *Achromobacter sp*. LW9, and *Ochrobactrum intermedium* Sq20 (Firdous et al., 2017). Furthermore, Zhan et al (2018) reported that *Arthrobacter sp*. strain GLP-1/Nit-1 can use glyphosate as a source of both nitrogen and phosphorus. *Aspergillus niger*, *Aspergillus oryzae* A-F02, *Penicillium chrysogenum*, and *Enterobacter cloacae* K7 have also been reported to breakdown glyphosate and use it as a source of carbon and phosphorus (Krzyśko-Lupicka et al., 1997, Klimek et al., 2001, Kryuchkova et al., 2014, Fu et al., 2017). In historical collections of *S. cerevisiae* gathered over the past century, glyphosate resistance was discovered, but only in yeasts isolated after the introduction of glyphosate (Barney et al., 2020). The majority of the glyphosate-resistant *S. cerevisiae* were isolated from agricultural fields.

In studies of Barney et al. (2020), different yeast species were gathered from 15 sites with known herbicide histories, including conventional farms, organic farms, reclaimed coal mines, state parks, national forests, and suburban settlements, in order to examine glyphosate resistance and its effect on non-target microorganisms. From 237 samples of plant, soil, spontaneous fermentation, nut, flower, fruit, fecal, and tree material samples, yeasts from 23 genera were isolated. *Saccharomyces*, *Candida*, *Metschnikowia*, *Kluyveromyces*, *Hanseniaspora*, and *Pichia* are other genera commonly found in the sampled environments. Less species diversity was found in the studied sites and only the *Saccharomyces* and *Pichia* species were isolated in the brewery. The study indicated that a conventional farm producing RoundUp Ready maize had the highest glyphosate resistance and the lowest phylogenetic diversity. Numerous herbicides, including a commercial glyphosate formulation, were sprayed on the mine, however *S. cerevisiae* did not exhibit strong glyphosate resistance. The mine had only been exposed to glyphosate for a year before the sample was isolated, in contrast to the typical farm. An example of anthropogenic selection of non-target organisms is glyphosate resistance.

## MEASUREMENT OF C AND N MINERALIZATION TO DETERMINE SOIL MICROBIAL ACTIVITY

Haney et al (2002) used the C and N mineralization method to measure microbial activity in their study by adding Roundup Ultra (234 mg kg1) to nine different soil samples. Mineralized C and N and soil microbial C and N had significant linear correlations. They reported that Roundup Ultra was rapidly degraded by soil microbes without adversely affecting microbial activity even at high application rates, regardless of soil type or organic matter content. Eser et al (2007) added the recommended field dose of glyphosate (480 g l-1) and 2 times this dose to olive tree (Olea europaea L., Oleaceae) soil grown in Çukurova University campus (Adana) under the influence of Mediterranean climate and used the carbon mineralization method to determine its effects on soil microbial activity. The recommended field dose of glyphosate and 2 times the recommended field dose of glyphosate significantly increased C mineralization after 30 days ($P < 0.001$) compared to the herbicide-free control. However, studies with 4 and 6 times the recommended field dose of glyphosate significantly reduced microbial activity. They reported that the recommended field doses of glyphosate isopropylamine herbicide increased microbial activity in olive tree soils, a Mediterranean region plant, but inhibitory effects occur due to the herbicide at higher doses.

In a study conducted by adding 2 and 4 times the recommended field dose of glyphosate to the soil of a citrus orchard in Osmaniye and Osmaniye Korkut University Campus, it was stated that all doses significantly increased carbon mineralization in citrus soil compared to control soils while the increase in campus soil was insignificant (Saglıker et al., 2018). The fact that glyphosate stimulates soil microbial activity in the presence of it suggests that soil microbes can utilise it as a source of carbon (Busse et al., 2001). According to Wardle and Parkinson (1990), glyphosate breakdown in soil increases microbial activity. According to Sprankle et al. (1975), glyphosate is rapidly broken down by soil microorganisms, presumably through co-metabolism. This results in an increase in microbial biomass and activity (Haney et al., 2000). The microbial population and activity of soil can alter when glyphosate is present (Busse et al., 2001).

## GENE-LEVEL SCREENING

After glyphosate was applied to EPSPS transgenic soybean plants at the recommended dose (3 L/ha), modifications in functional genes were also found in the rhizosphere. Although the microbial diversity in the rhizosphere of these plants was unaffected, glyphosate spraying had a deleterious impact on functional genes that support plant growth, such as those that fix nitrogen (Lu et al., 2018).

Barney et al. (2020) evaluated the worldwide alterations in gene expression of yeasts subjected to a glyphosate-based herbicide (GBH) to investigate the underlying genetic variations between susceptible and resistant strains of *Saccharomyces cerevisiae*. Following glyphosate application, copy numbers of 100 genes related to

cell wall proteins, mitochondria, and sterol transport showed increases. It is obvious from the transcriptome data that GBHs have an impact on a variety of cellular biological functions.

## FUNGAL BIOMASS DETERMINATION BY LIPID EXTRACTION

By extracting lipids from potting soil, Zaller (2014) investigated the effects of glyphosate on mycorrhizal fungus in soil. Due to their influence on plant nutrition supply and soil aggregation, mycorrhizal fungi are known as keystone species in temperate habitats. They symbiotically interact with more than 80% of vascular plant species (Siddiky et al., 2012). While neutral lipid fatty acid (NLFA) depicts fungus storage reserves including spores, vesicles, and propagules, soil phospholipid fatty acid (PLFA) represents live hyphal biomass (Olssan and Johansen2000). Additionally, the NLFA/PLFA ratio reveals fungal phenology, such as active colonization or stages of senescence. The study discovered that herbicides drastically decreased propagules, vesicles, and biomass of soil-based arbuscular mycorrhizal fungus.

## CULTURE-DEPENDENT METHODS

Two doses each of chlorpyrifos and glyphosate pesticides (recommended dose and twice the recommended dose) were added to the soil and the effects of herbicides on soil microorganisms were studied in a 2D setup (Türkmen et al., 2020). When the effects of glyphosate applications on microorganism counts in the setup were examined, it was observed that twice the recommended dose decreased the microorganism counts compared to the control application and these decreases were significant. In field trials, soil analysis using culture-based methods five days after glyphosate application showed that fungal biomass decreased significantly in soils treated with twice the recommended dose of glyphosate compared to the control (Vázquez et al., 2021). Urszula et al. (2021) used glyphosate Roundup 360 SL (3.12 mg per 1 kg dm soil) in pot trials using two different soil types. Glyphosate Roundup 360 SL was added to the selected pots as a spray at a dose of 3.12 mg per 1 kg dm soil. The results of the study showed that 240 hours after glyphosate application, the total number of bacteria decreased by about 26% and 13% in the first and second soil types, respectively. The application of glyphosate to the second soil type reduced the number of fungi by about 60% compared to the control sample. Despite their important role in ecosystems, studies testing the effects of high concentrations of glyphosate on arbuscular mycorrhizal fungi show that arbuscular mycorrhizal fungal spore germination and germ tube growth are inhibited (Malty et al 2006) or mycorrhizae are reduced in soil (Ronco et al 2008). Al ani et al. (2019) added herbicide (Glyset I.P.A, Glyphosate 48%) at 0, 50, 100 and 200 ppm to soil and incubated at 30 °C in the laboratory. The numbers of bacteria, fungi and actinomycetes were determined weekly for 7 consecutive weeks by cultural counting method. The results showed that the addition of pesticides significantly reduced the numbers of soil bacteria, fungi and actinomycetes.

## 16S rDNA DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)

In the microcosm study, 100 g of a soil-sand combination was deposited in each glass tube, along with two similar-sized barley seedlings, each measuring 25 mm in diameter and 200 mm in length. The glyphosate that was employed was an isopropylamine salt of glyphosate used in Monsanto's commercial RoundUp1 formulation, which contains 11.2% glyphosate. In the glyphosate treatment, each plant's leaves were given a daily dose of roughly 2.4 mL RoundUp1 (9.6 g L 1 glyphosate) using a brush. Glyphosate contamination of the soil indirectly was prevented. The system's bacterial community's 16S rRNA gene diversity fingerprint was ascertained using PCR-DGGE. The 16S rDNA gene PCR-DGGE fingerprint revealed that foliar spraying of glyphosate to vulnerable plants had some influence on the bacterial community structure, favoring specific members of the community. However, the findings also demonstrate that foliar glyphosate spray had no impact on overall bacterial abundance, community diversity, or richness (Imparato et al., 2016). Lu et al. (2018) sought to ascertain if glyphosate had an impact on the rhizosphere microbial population of soybean EPSPS-transgenic line ZUTS31 over the course of one growing season utilizing 16S rRNA gene amplicon sequencing and shotgun metagenome sequencing analyses. The findings of Lu et al (2018) match with those of Imparato et al (2016).

In recent years, there has been a focus on the impact of glyphosate not only on the soil microbiota but also on the animal and human microbiota of glyphosate wastes that are likely to be transported through the food chain. Mao et al (2018) applied glyphosate alone and Roundup (1.75 mg/kg bw/day), a commercial brand of glyphosate-containing herbicide, to the drinking water of F0 mouse mothers starting from day 6 of mice gestation until postnatal day 125. Multiple time points were used to gather animal waste from both F0 pregnant moms and F1 pups. The V3-V4 region of the 16S ribosomal RNA gene was used to analyze the diversity of the gut microbiota in 433 fecal samples. Microbiome profiling showed that only in F1 offspring did low-dose Roundup and glyphosate exposure result in significant and noticeable alterations in the overall bacterial composition. In particular, relative abundance for *Bacteriodetes (Prevotella)* was seen at PND31, which

corresponds to pre-pubertal age in humans, whereas *Firmicutes (Lactobacillus*) was decreased in F1 offspring exposed to both Roundup and glyphosate compared to controls. This study offers the first proof that early development, particularly before the onset of puberty, can be affected by exposure to the commonly used pesticide glyphosate at amounts considered safe.

In both lab and field settings, Motta et al. (2018) examined the oral and topical effects of different glyphosate doses on the gut microbiota and health of honey bees. By using 16S rRNA amplicon sequencing and qPCR studies to analyze the gut microbial composition, they discovered a substantial drop in the absolute abundance of *Bifidobacterium spp*. and *Lactobacillus* Firm-5 after the fifth day of treatment. Under each of these circumstances, the number of benevolent bacteria in the bee gut was dose-dependently impacted by the sucrose syrup or water-soluble formulation. Additionally, mark-recapture studies revealed that bees exposed to the formulation had a higher likelihood of disappearing from the colony when reintroduced. Although there was no noticeable impact on honeybees exposed to the formulation in field trials, challenge tests with the pathogen *Serratia marcescens* in a lab setting showed that exposed honeybees had higher mortality rates than control honeybees. In field studies, glyphosate was found in honey taken from exposed honeybees. This finding suggests that worker bees introduce xenobiotics into the colony, so extending exposure and raising the possibility of coming into contact with freshly emerging bees.

## IN SILICO ANALYSIS

With their study using 10,231 protein sequences describing 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzymes, 37 protein sequences predicted to be resistant to glyphosate, and 678 prokaryotic protein sequences, Leino et al. (2021) created the first bioinformatics method to evaluate the potential sensitivity to glyphosate. In 652 of the 678 prokaryotic genomes examined, the EPSPS protein was found. According to the study's findings, 54% of the species in the human gut microbiome are glyphosate-sensitive. 92% of fungi, 57% of bacteria, 82% of archaea, and 82% of bacteria contain EPSPSs in class I, meaning they may be sensitive to glyphosate.

## CONCLUSION

Glyphosate residues are increasing across the agri-food system (Benbrook, 2016, Bøhn and Millstone, 2019). We can suppose that persistent exposure to glyphosate residues causes the bacterial community to be dominated by resistant strains. Furthermore, glyphosate resistance may develop in some susceptible strains due to an accumulation of EPSPS gene mutations or the acquisition of a resistance gene via horizontal gene transfer. Change of Pro106 to Ser decreases sensitivity to glyphosate in both bacteria (*Escherichia coli* and *Salmonella typhimurium*) and plants (*Chloris virgata*) (Nandula, 2010; Heap and Duke, 2018). Although Pro106 doesn't directly interact with glyphosate at the molecular level, replacing it with other amino acids alters the active site's structure and prevents glyphosate from acting as intended (Sammons and Gaines, 2014). Through bacteriocins, many bacteria demonstrate antagonistic behavior (Juturu and Wu, 2018). They might affect how glyphosate interacts with bacteria. They may adversely influence its abilities to encourage plant growth. The buildup of glyphosate residues in soil can cause changes in the species composition of soil microorganisms on a quantitative and qualitative level, as well as internal modifications. These alterations may ultimately result in a decline in soil fertility and productivity. Future research should focus on identifying bacterial species that have evolved glyphosate resistance and monitoring the diversity of microbiomes for the sake of soil, human, and animal health.

## REFERENCES

Acosta-Cortés, A. G., Martinez-Ledezma, C., López-Chuken, U. J., Kaushik, G., Nimesh, S., and Villarreal-Chiu, J. F. (2019). Polyphosphate recovery by a native Bacillus cereus strain as a direct effect of glyphosate uptake. ISME J. 13, 1497–1505. doi:10.1038/s41396-019-0366-3

AL-Ani, M. A. M. et al. 2019. Effect of pesticides on soil microorganisms. Journal of Physics: Conf. Ser. 1294 072007

Barney, J. B., et all. 2020. The Yeast Atlas of Appalachia: Species and phenotypic diversity of herbicide resistance in wild yeast. Peer-reviewed version available at Diversity, 12, 139; doi:10.3390/d12040139

Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. Environ. Sci. Eur. 28, 3. doi:10.1186/s12302-016-0070-0

Bøhn, T., and Millstone, E. (2019). The Introduction of Thousands of Tonnes of Glyphosate in the food Chain-An Evaluation of Glyphosate Tolerant Soybeans. Foods 8, 669. doi:10.3390/foods8120669

Borggaard, O. K., Gimsing, A. L. (2008): Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: A review. – Pest Management Science 64(4): 441-456.

Bunemann, E.K., Schwenke, G.D., Van Zwıeten, L., Murphy, D.V. Kırkegaard, J.A., Mele, M. 2006. Impact of agricultural inputs on soil organisms : a review, Australian Journal of Soil Research, 44, 4, (1/4), 379-406.

Busse, M. D., Ratclıff, A. W., Shestak, C. J.,Powers, R. F. 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biology and Biochemistry, Oxford, v. 33, p. 1777-1789.

Davoren, M. J., and Schiestl, R. H. (2018). Glyphosate-based herbicides and cancer risk: a post-IARC decision review of potential mechanisms, policy and avenues of research. Carcinogenesis 39 (10), 1207–1215. doi:10.1093/carcin/bgy105

Defarge, N., Spiroux de Vendômois, J., and Séralini, G. E. (2018). Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides. Toxicol. Rep. 5, 156–163.

Druille, M., Cabello, M. N., García Parisi, P. A., Golluscio, R. A., and Omacini, M. (2015). Glyphosate vulnerability explains changes in root-symbionts propagules viability in pampean grasslands. Agric. Ecosyst. Environ. 202, 48–55. doi:10.1016/j.agee.2014.12.017

Duke, S. O. (2020): Glyphosate: Environmental fate and impact. – Weed Science 68(3): 201-207.

Duke, S. O., Rimando, A. M., Reddy, K. N., Cizdziel, J. V., Bellaloui, N., Shaw, D. R., Williams 2nd, M. M., Maul, J. E. (2018): Lack of transgene and glyphosate effects on yield, and mineral and amino acid content of glyphosate-resistant soybean. – Pest Management Science 74(5): 1166-1173.

Duranti et al. 2020. Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of GABA. Scientific Reports 10:14112.

Eser, f., Sağlıker, H.A., Darıcı, C. 2007. The Effects of Glyphosate Isopropylamine and Triflfluralin on the Carbon Mineralization of Olive Tree Soils. Turkish Journal of Agriculture and Forestry, 31, 297-302.

Firdous, S., Iqbal, S., Anwar, S. (2017a).Optimization and modeling of glyphosate biodegradation by a novel Comamonas odontotermitis P2 through response surface methodology‘‘, Pedosphere.

FR, 2008. Glyphosate; Pesticide Tolerances. Environmental Protection Agency. 40 C FR Part 180. Fed. Regist. 73, 73586–73592.

Fu, G. M., Chen, Y., Li, R. Y., Yuan, X. Q., Liu, C. M., Li, B., Wan, Y. (2017). Pathway and rate-limiting step of glyphosate degradation by Aspergillus oryzae A-F02. Preparative Biochemistry & Biotechnology, 47(8),782–788.

Hadi, F., Mousavi, A., Noghabi, K. A., Tabar, H. G., Salmanian, A. H. (2013). New bacterial strain of the genus Ochrobactrum with glyphosatedegrading activity. Journal of Environmental Science and Health, B 48(3), 208–213.

Haney R, Senseman S, Hons F. 2002. Effect of Roundup Ultra on micro-bial activity and biomass from selected soils. J Environ Qual. 31:730---5.

Haney, R.L.; Senseman, S.A.; Hons, F.M.; Zuberer, D.A. Effect of Glyphosate on Soil Microbial Activity and Biomass. Weed Sci. 2000, 48, 89–93.

Heap, I., Duke, S. O. (2018): Overview of glyphosate-resistant weeds worldwide. – Pest Management Science 74(5): 1040-1049.

Imparato Valentina, Santos Susana S., Johansen Anders, Geisen Stefan, and Winding Anne. 2016. “Stimulation of Bacteria and Protists in Rhizosphere of Glyphosate-Treated Barley.” Applied Soil Ecology

Juturu, W. ve Wu, J.C. (2018). Microbial production of bacteriocins: Latest research development and applications. Biotechnol Adv. 36,2187–2200.

Klimek, M., Lejczak, B., Kafarski, P., Forlani, G. (2001). Metabolism of the phosphonate herbicide glyphosate by a non-nitrate-utilizing strain of Penicillium chrysogenum. Pest Management Science, 57(9),815–821.

Kryuchkova, Y. V., Burygin, . GL., Gogoleva, N. E., Gogolev, Y. V., Chernyshova, M. P., Makarov, O. E., Fedorov, E. E., Turkovskaya, O. V. (2014). Isolation and characterization of a glyphosate-degrading rhizosphere strain, Enterobacter cloacae K7. Microbiological Research, 169(1),99–105.

Krzyśko-Lupicka, T., Strof, W., Kubś, K., Skorupa, M., Wieczorek, P., Lejczak, B., Kafarski, P. (1997). The ability of soil-borne fungi to degrade organophosphonate carbon-to-phosphorus bonds. Applied and Environmental Microbiology, 48(4):549–552.

Kurtoglu, C., Ceylan, F., Cömertpay, S., Akyol, İ. 2020. Glifosat uygulanmış topraktan izole edilen bakterilerin herbisit degradasyon potansiyellerinin belirlenmesi. Biological Diversity and Conservation. 13/2, 102-108.

Leino, L., Tall, T., Helander, M., Saloniemi, I., Saikkonen, K., Ruuskanen, S., et al. (2021). Classification of the glyphosate target enzyme (5-enolpyruvylshikimate- 3-phosphate synthase) for assessing sensitivity of organisms to the herbicide. J. Hazard. Mater. 408, 124556. doi:10.1016/j.jhazmat.2020.124556

Lu, G.H., et al., 2018. Impact of Glyphosate on the Rhizosphere Microbial Communities of An EPSPS-Transgenic Soybean Line ZUTS31 by Metagenome Sequencing. Current Genomics, 19: 36-49.

Malty, J. D. S., Siqueira, J. O. & Moreira, F. M. D. S. 2006. Effects of glyphosate on soybean symbiotic microorganisms, in culture media and in greenhouse. Pesquisa Agropecua´ria Brasileira 41, 285–291.

Mao, Q., Manservisi, F., Panzacchi, S., Mandrioli, D., Menghetti, I., Vornoli, A., et al. (2018). The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome. Environ. Health 17, 50. doi:10.1186/s12940-018-0394-xb

Motta, E. V. S., Raymann, K., and Moran, N. A. (2018). Glyphosate perturbs the gut microbiota of honey bees. Proc. Natl. Acad. Sci. USA 115 (41), 10305–10310. doi:10.1073/pnas.1803880115

Nandula, V. K. (2010): Glyphosate Resistance in Crops and Weeds: History, Development, and Management. – John Wiley & Sons: Hoboken, NJ, USA.

Nguyen, D.B., Rose, M.T., Rose, T.J., Morris, S.G., van Zwieten L., 2016. Impact of glyphosate on soil microbial biomass and respiration: a meta-analysis, Soil Biol. Biochem. 92, 50–57.

Olsson, P. A. & Johansen, A. 2000. Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycol Res 104, 429–434.

Priestman, M. A., Funke, T., Singh, I. M., Crupper, S. S., and Schönbrunn, E. (2005). 5-Enolpyruvylshikimate-3-phosphate synthase fromStaphylococcus aureusis insensitive to glyphosate. FEBS Lett. 579, 728–732. doi:10.1016/j.febslet.2004.12.057.

Ronco, M. G., Ruscitti, M. F., Arango, M. C. & Beltrano, J. 2008. Glyphosate and mycorrhization induce changes in plant growth and in root morphology and architecture in pepper plants (Capsicum annuum L.). J Hortic Sci Biotechnol 83, 497–505.

Sagliker, H.A. 2018. Carbon Mineralisation in Orange Grove Soils Treated with Different Doses of Glyphosate-Amine Salt. Journal of Environmental Protection and Ecology, 19(3): p. 1102- 1110.

Sammons, R. D., Gaines, T. A. (2014): Glyphosate resistance: state of knowledge. – Pest Management Science 70: 1367-1377.

Siddiky, M. R. K., Kohler, J., Cosme, M. & Rillig, M. C. 2012. Soil biota effects on soil structure: Interactions between arbuscular mycorrhizal fungal mycelium and collembola. Soil Biol. Biochem. 50, 33–39.

Sprankle, P., Meggit, W.F., Penner, D., 1975. Adsorption, mobility and microbial degradation of glyphosate in the soil. Weed Sci. 23, 229±234

Torsvik, V. and Ovreas, L. (2002) Microbial Diversity and Function in Soil: From Genes to Ecosystems. Current Opinion in Microbiology, 5, 240-245. http://dx.doi.org/10.1016/S1369-5274(02)00324-

Türkmen, C., Özkan, N., Özçelik, Y., Özçelik, D., Tiryaki, O. 2020. Effects of Pesticides (Chlorpyrifos, Glyphosate) Applied to Soil on Earthworm Behaviors and Some Soil Biological Parameters. Türk Tarım ve Doğa Bilimleri Dergisi 7(2): 301–307.

U.S. Environmental Protection Agency (EPA), 2008. EPA's 2008 Report on the Environment. National Center for Environmental Assessment. Washington, DC; EPA/600/R-07/045F.

Urszula W., Wołejko, E., Łozowicka, B., Jabło ´nska-Trypu´c , A. 2021. Microbial Diversity and P Content Changes after the Application of Sewage Sludge and Glyphosate to Soil. Minerals, 11, 1423. https://doi.org/10.3390/min11121423

van Bruggen AHC, Finckh MR, He M, Ritsema CJ, Harkes P, Knuth D and Geissen V (2021) Indirect Effects of the Herbicide Glyphosate on Plant, Animal and Human Health Through its Effects on Microbial Communities. Front. Environ. Sci. 9:763917. doi: 10.3389/fenvs.2021.763917 Blake ve Pallett (2018)

Vázquez, M.B., Moreno, M.V., Amodeo, M.R., Bianchinotti, M.V., 2021. Effects of glyphosate on soil fungal communities: A field study. Revista Argentina de Microbiología 53: 349---358.

Wardle D A and Parkinson D 1990 Effects of three herbicides on soil microbial biomass and activity. Plant and Soil 122, 21-28.

Weaver, M.A., Krutz, L.J., Zablotowicz, R.M., Reddy, K.N. 2007. Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil, Pest Manag. Sci. 63, 388–393.

Xu, B., Sun, Q.-J., Lan, J. C.-W., Chen, W.-M., Hsueh, C.-C., and Chen, B.-Y. 2019. Exploring the glyphosate-degrading characteristics of a newly isolated, highly adapted indigenous bacterial strain, Providencia rettgeri GDB 1. J. Biosci. Bioeng. 128 (1), 80–87.

Yılmaz, S., Aslım, B., Beyatlı, Y. 1997. Glifosat Herbisidinin Baz ı 'Laktik Asit Bakterilerinin Gelişimi ve Asit Üretimine Etkisi. Tarım Bilimleri Dergisi, 3 (3), 70-77.

Yin, G. (2011). Glyphosate: There is no substitute. Farm Chemicals International, 2011 (3), Available from http://www.farmchemicalsinternational.com/magazine/?storyid=2997

Zaller, J. G., Cantelmo, C., Santos, G. D., Muther, S., Gruber, E., Pallua, P., et al. (2018). Herbicides in vineyards reduce grapevine root mycorrhization and alter soil microorganisms and the nutrient composition in grapevine roots, leaves, xylem sap and grape juice. Environ. Sci. Pollut. Res. 25, 23215–23226. doi:10.1007/s11356-018-2422-3

Zaller, J.G., Heigl, F., Ruess, L., Grabmaier, A. 2014. Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. Scientific Reports, 4 : 5634. DOI: 10.1038/srep05634

Zhan, H., Feng, Y., Fan, X., Chen, S., 2018. Recent advances in glyphosate biodegradation. Appl. Microbiol. Biotechnol. 102, 5033–5043. https://doi.org/10.1007/s00253-018-9035-0.

## *ORAL PRESENTATION*

# Recombinant production of β-glucosidase enzyme for biotechnological applications and investigation of the anti-glucosidase effect of endemic plants

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## Abstract

Enzymes are biopolymers in protein structure formed by linking amino acids with peptide bonds. They are essential for the metabolic functions required by living organisms and quickly catalyze many biochemical and physiological reactions in cells. Enzymes have entered daily and economic life to be used for various purposes. β-glycosidases are in the glycosidases subgroup of the hydrolases class, and they have great functional importance and economic value for the textile, biofuel, food, medical and healthcare industries. Studies on the industrial use of β-glucosidases have gained intensity. Although there have been some initiatives on enzyme production in Turkey in the last few years, it is obvious that there are still essential steps to be taken when the diversity of enzymes is considered. In this study, it was aimed to produce β-glucosidase enzyme due to its use as an industrial catalyst in the hydrolysis of oligosaccharides and cellobiose, and because of its importance in health. Over-excessive β-glucosidase activity in the body causes glucose, monomer sugar in sphingolipids, to become free above the desired level, leading to an increase in blood glucose level. Inhibition of the glucosidase enzyme is one of the approaches to the treatment of diseases such as diabetes and breast cancer. The inhibitory potentials of the endemic *Allium kastambulense* and *Centaurea kilaea* plants in Turkey on the β-glycosidase enzyme were investigated for the first time. The β-glucosidase enzyme was produced by recombinant DNA techniques and checked its activity. The expression of the β-glucosidase was tested in various *E. coli* plyse strains, and the enzyme was successfully produced in *E. coli* plyse C41-4 strain. The $IC_{50}$ values of the herbal extracts on the enzyme activity were determined as 537.91 and 347.20 µg/mL, respectively. Although both plant extracts showed an inhibitory effect on enzyme activity compared to the control group, their inhibitory potential was weak. In summary, the recombinant production of the β-glycosidase enzyme, which has the potential to be used in various industrial areas, has been carried out.

**Keywords:** β-glucosidase, recombinant protein, enzyme inhibition

## INTRODUCTION

Enzymes are organic molecules that catalyze biochemical reactions in biological systems and are necessary for the continuity of the organism's life. Enzymes, produced in various cell types and have essential metabolic functions, have entered daily and economic life to be used for various purposes (Kıran et al., 2006). The highly specific biochemical transformations of enzymes produced by microbial cells and animal and plant tissues increase their use in various industrial processes. Fields of enzyme application should be categorized as technical applications such as pulp, paper, textile, and detergent industries, food fields such as dairy products, bakery, fruit juice, starch products, wine industry, animal nutrition, organic synthesis, cosmetic industry, and molecular studies (Kirk et al., 2002; Spök, 2006; Li et al., 2012; Gora et al., 2013; Porter et al, 2016; Singh et al., 2016).

β-glycosidases, which have great functional importance for biological systems, are included in the class of hydrolases. β-glycosidases play a role in biomass hydrolysis and in obtaining monomeric sugars from cellulose-based oligosaccharides by breaking β-glycoside bonds in polysaccharides. For this reason, they have the potential to be used in the production of paper, animal feed, textiles, biofuels, and various chemicals. Glucosidase enzymes are needed in various biotechnological processes, including ethanol production by cellulosic degradation and the synthesis of useful-glycosides. They are also involved in the production of aromatic compounds from glycosidic precursors and the extraction and release of flavor in fruit juice processing (Krisch et al., 2010; Srivastava et al., 2019).

β-glycosidases, with their various industrial uses, are essential in maintaining a healthy quality of life. The lysosomal β-glucosidase in the human body plays a vital role in the breakdown of glycosphingolipids. This

enzyme breaks down glycosylceramide, a glycolipid, into ceramide and glucose. A deficiency of lysosomal beta-glucosidase leads to Gaucher disease (GD), and glycosylceramide accumulates (Michelin et al., 2004; Mignot et al., 2013). GD is characterized as inherited due to a deficiency of the enzyme β-glycosidase. The enzyme, required for enzyme replacement therapy, which is one of the approaches for the treatment of the disease, is produced recombinantly in various organisms such as mammalian, insect, or plant cells, and then becomes stable and active in human cells. Therefore, human β-glycosidase has biotechnological and pharmacological importance due to its recombinant production and use in Gaucher disease sahiptir (Ketudat and Esen, 2010). In addition, β-glucosidase enzymes play a role in the catalysis of glucose units in the structure of glycoproteins, leading to the liberation of monomeric sugars. In this context, inhibiting the enzyme that breaks glycosidic bonds in the treatment approach of non-insulin-dependent diabetes mellitus (DM) is crucial. Therefore, inhibition of the β-glycosidase enzyme is one of the approaches for the treatment of type 2 DM (Krisch et al., 2010; Hengameh and Rajkumar, 2016; Gondokesumo et al., 2017; Mohammed et al., 2019).

In this study, the β-glucosidase enzyme, needed in different applications, was produced with recombinant DNA technology. In addition, the inhibitory potential of endemic plant extracts against the enzyme for the treatment of non-insulin-dependent DM was investigated for the first time.

## MATERIALS AND METHODS

BglB in pET29b(+) strain was used for the expression of 6xHis-tagged beta-glucosidase obtained from Addgene (http://n2t.net/addgene:55773). The plasmids were transferred to components *E. coli* BL21 (C41) PLysE cells. Transformed *E. coli* cells were allowed to grow in Luria-Bertani Broth (LB) medium containing the antibiotic kanamycin in an incubator shaker at 240 rpm at 37°C. It was induced with IPTG when the bacterial cell density (OD: 600 nm) reached 0.6. They were again incubated under the same conditions. The *E. coli* cells were then collected by centrifugation, and the pellet was stored at -20°C. The lysis buffer containing sodium phosphate and NaCI (pH=7.6, 100 mM) was added to suspend the cells embedded in ice. After pipetting of benzamidine and PMSF (100 mM), they were lysed with a sonicator. The lysed were centrifuged at +4°C, 30 000 rpm for 1 hour, and the obtained supernatant was applied to the column containing Ni-NTA agarose resin. The purification of beta-glucosidase protein was accomplished using imidazole. The obtained protein was analysed in SDS-PAGE gel electrophoresis.

The activity of the obtained enzyme and the inhibitory potential of the plants extracted with methanol-chloroform (4:1) were determined using β-D p-nitrophenyl glycoside (pNPG) substrate. The reaction mixture was formed by adding 125 µL, 100 mM sodium phosphate buffer (pH=7.0), 50 µL, 1 mM pNPG and 25 µL of enzyme solution. For the enzyme inhibition study, 25 µL of the plant extracts was added to the wells. It was incubated at 37°C for 30 minutes, and the amount of colored p-nitrophenol formed during the enzymatic hydrolysis of the substrate was measured at 410 nm in a microplate reader (Thermo Scientific Multiskan Go).

## RESULTS and DISCUSSION

Plasmid BglB in pET29b(+) carrying the gene encoding the beta-glucosidase protein is commercially available. After the transformation of the plasmid into *E. coli* BL21 (C41) cells, the expression of recombinant beta-glucosidase was carried out with IPTG. The expression of the β-glucosidase was tested in various *E. coli* plyse C41 strains (C41-1, C41-2, C41-3, C41-4, C41-5), and the enzyme was successfully produced in *E. coli* plyse C41-4 cells. They were sonicated to obtain cell lysate, and after the ultracentrifugation, the supernatant portion of the lysate was purified by affinity chromatography. Analysis of lyophilized β-glucosidase was performed with SDS-PAGE as seen in Figure 1. In recent years, β-glucosidases have received considerable attention due to their potential applications in various biotechnological processes. Therefore, various researchers have conducted studies to increase the production and production efficiency of β-glucosidase from different sources. Different methods have been developed to increase protein production; for example, it was stated that β-glucosidase enzyme production was increased by controlling the amount of acetic acid and light-induced expression for potential industrial applications (Zahoor et al., 2011; Shi et al., 2017; Chang et al., 2017).

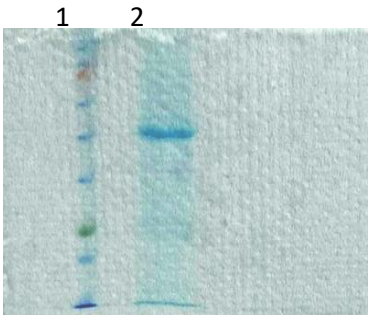


**Figure 1.** Analysis of the purified β-glucosidase protein in 12% SDS-PAGE (1: Marker, 2: Protein).

Enzyme activity and the inhibitory potential of the endemic *Allium kastambulense* and *Centaurea kilaea* plants were done using pNPG substrate. The $IC_{50}$ values of the herbal extracts on the enzyme activity were determined as 537.91 and 347.20 µg/mL, respectively (Figure 2). The inhibitory potency of plants endemic in Türkiye on β-glycosidase enzyme activity was investigated for the first time. Although both plant extracts showed an inhibitory effect on the enzyme activity compared to the control group, their inhibitory potential was weak. Although there are many studies to reveal the α-glucosidase enzyme inhibitory potentials of various substances, a limited number of studies have been reached on β-glucosidase enzyme inhibition. A study conducted on the inhibitory potential of *Hibiscus sabdariffa* L. declared that the inhibitory potency of Rosella calyx ethanolic-extract was comparable to the enzyme inhibitor acarbose, with its $IC_{50}$ value of 41.77 µg/mL (Gondokesumo et al., 2017). Another study stated that the β-glucosidase inhibitory potency of lichen (*Ramalina celastri*, *R. nervulosa*, and *R. pacifica*) metabolites have between 61.96 – 87.88 % at 20 µg $mL^{-1}$ in ethanolic extract (Verma et al., 2012). Compared to these studies, the current results possess that *A. kastambulense* and *C. kilaea* plant extracts have weak inhibitory potency. We assumed that the inhibitory property of the studied extracts might be related to the plant species and used extraction methods.

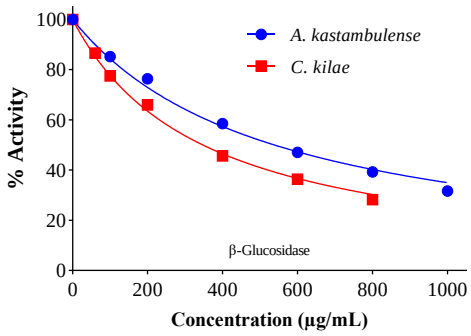


**Figure 2**: Inhibitory effects of plant extracts on β-glucosidase activity

## CONCLUSION

In summary, the recombinant production of the β-glycosidase enzyme, which has the potential to be used in various industrial areas, has been carried out. It was determined for the first time that both plant extracts had inhibitory activity against the enzyme, albeit weakly.

## ACKNOWLEDGEMENTS

This study is supported by TÜBİTAK 2209-A - Research Project Support Programme for Undergraduate Students. Project Number: 1919B012002495.

## REFERENCES

Chang F, Zhang X, Pan Y, Lu Y, Fang W, Fang Z, Xiao Y 2017. Light induced expression of β-Glucosidase in *Escherichia coli* with autolysis of cell. BMC Biotechnology, 17(74):1-11.

Gondokesumo ME, Kusuma HSW, Widowati W 2017. α-/β-Glucosidase and α-Amylase Inhibitory Activities of Roselle (*Hibiscus sabdariffa* L.) Ethanol Extract. Molecular and Cellular Biomedical Sciences, 1(1):34-40.

Gora A, Brezovsky J Damborsky J 2013. Gates of Enzymes. Chem. Rev, 113: 5871- 923.

Ketudat CJR, Esen A. 2010. β-Glucosidases. Cell Mol Life Sci. 67(20):389-405.

Kıran Ö, Çömlekçioğlu U, Dostbil N 2006. Bazı Mikrobiyal Enzimler ve Endüstrideki Kullanım Alanları. KSÜ. Fen ve Mühendislik Dergisi, 9(1).

Kirk O, Vedel T, Fuglsang C 2002. Industrial enzyme applications. Current Opinion in Biotechnology, 13:345–351.

Krisch J, Tako M, Papp T, Vagvolgyi C 2010. Characteristics and potential use of β-glucosidases from Zygomycetes. In: Mendez-Vilas A (ed) Current Research, Technology and Education, Topics in Applied Microbiology and Microbial Biotechnology, pp 891–896

Li S, Yang X, Yang S, Zhu M, Wang X 2012. Technology prospecting on enzymes: application, marketing and engineering. Computational and structural biotechnology journal, 2(3): 1-11.

Porter JL, Rusli RA, Ollis DL 2016. Directed Evolution of Enzymes for Industrial Biocatalysis. ChemBioChem, 17: 197 – 203.

Shi X, Xie J, Liao S, Wu T, Zhao LG, Ding G, Wang Z, Xiao W 2017. High-level expression of recombinant thermostable b-glucosidase in *Escherichia coli* by regulating acetic acid. Bioresource Technology 241: 795–801.

Singh G, Verma AK, Kumar V 2016. Catalytic properties, functional attributes and industrial applications of β-glucosidases. 3 Biotech, 6(3):1-14.

Spök A 2006. Safety regulations of food enzymes. Food Technol Biotech, 44 (2):197-209.

Srivastava N, Rathour R, Jha S, Pandey K, Srivastava M 2019. Microbial Beta Glucosidase Enzymes: Recent Advances in Biomass Conversation for Biofuels Application. Biomolecules.9(6):1-23.

Verma N, Behera BC, Sharma BO 2012. Glucosidase Inhibitory and Radical Scavenging Properties of Lichen Metabolites Salazinic Acid, Sekikaic Acid and Usnic Acid. Hacettepe J. Biol. & Chem., 40 (1):7–21

Zahoor S, Javed MM, Aftab MN, Haq I 2011. Cloning and expression of *β*-glucosidase gene from Bacillus licheniformis into E. coli BL 21 (DE3). Biologia 66(2): 213—220.

*ORAL PRESENTATION*

# Sübstitüe benzaldehit türevi schiff bazı bileşiklerinin antioksidan ve antimikrobiyal özelliklerinin araştırılması

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## Özet

Bu çalışmada 2-hidroksi-3-metilbenzaldehitten yola çıkarak elde edilen schiff bazı bileşiklerinin antioksidan ve antimikrobiyal aktiviteleri incelendi. 2-hidroksi-3-metilbenzaldehitin, metil 3-amino-4-metilbenzoat ve 2-klor-4-nitroanilin ile tepkimesi ile metil (E)-3-((2-hidroksi-3-metilbenzil)amino)-4-metilbenzoat (I), (E)-2-(((2-klor-4-nitrofenil)imino)metil)-6-metilfenol (II) bileşikleri elde edildi. Antioksidan aktivitesi incelenirken DPPH• radikal giderme, ABTS+• katyon radikal giderme metodları kullanıldı. Antimikrobiyal aktiviteleri ise Disk difüzyon ve Mikrodilüsyon metodları kullanılarak incelendi. Örneklerin antioksidan ve antimikrobiyal özelliklerine bakıldığında I bileşiğinin II bileşiğinden daha etkili olduğu belirlendi.

**Anahtar Kelimeler:** Schiff bazı, antioksidan, antimikrobiyal

**Investigation of antioxidant and antimicrobial properties of substituted benzaldehyde derivative Schiff base compounds.**

## Abstract

In this study, antioxidant and antimicrobial activities of schiff base compounds obtained from 2-hydroxy-3-methylbenzaldehyde were investigated. Reaction of 2-hydroxy-3-methylbenzaldehyde with methyl 3-amino-4-methylbenzoate and 2-chloro-4-nitroaniline to methyl (E)-3-((2-hydroxy-3-methylbenzyl)amino)-4-methylbenzoate Compounds (I), (E)-2-(((2-chloro-4-nitrophenyl)imino)methyl)-6-methylphenol (II) were obtained. While examining the antioxidant activity, DPPH• radical removal, ABTS+• cation radical removal methods were used. Antimicrobial activities were examined using Disk diffusion and Microdilution methods. When the antioxidant and antimicrobial properties of the samples were examined, it was determined that compound I was more effective than compound II.

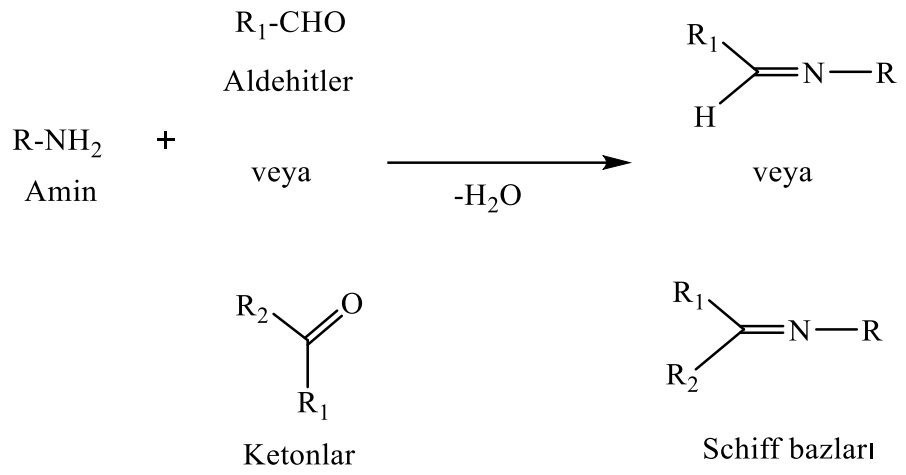
**Keywords:** Schiff base, antioxidant, antimicrobial

## GİRİŞ

Alman bilim insanı olan Hugo Schiff (1864-1915) (Şekil 1) bazı bazlar keşfetmiş ve onlara Schiff bazı adını vermiştir (Qin ve ark., 2013). Schiff bazları belirli koşullar altında bir birincil aminin karbonil (aldehitler veya ketonlar) ile tepkimesi ile elde edilir. Genel yapı $R_1R_2C=NR$ ($R\neq H$) olduğunda ana fonksiyonel grup imin veya azometin'dir (-C=N-) (Şekil 2) (Ghosh ve ark., 2019). Schiff bazları, antibakteriyel (Goszczyńska ve ark., 2015), antimikrobiyal (Jain ve Mishra, 2016), antifungal (Bharti ve ark., 2010), antioksidan (Bakır ve Lawag, 2020), antiviral (Kumar ve ark., 2010), anti-HIV (Sriram ve ark., 2006), antitüberküler (Pahlavani ve Kargar, 2021), antikanser (Liao ve ark., 2005), antiinflamatuvar ajanlar (Shehry ve ark., 2019) ve antikonvülsan (Beraldo ve ark., 2002) gibi geniş biyolojik aktivite özellikleri göstermektedir.

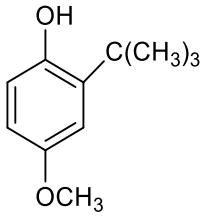


**Şekil 1.** Hugo Schiff, 24 Nisan 1915.




**Şekil 2.** Schiff bazlarının genel yapısı

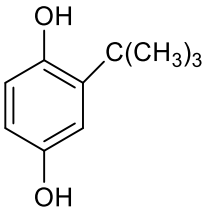
Antioksidanlar, serbest radikallerin oluşumunu engelleyerek veya mevcut radikalleri süpürerek hücrenin zarar görmesini engelleyen ve yapısında genellikle fenolik fonksiyonel grup taşıyan moleküllerdir (Kahkönen ve ark., 1999). Antioksidanların insan sağlığındaki yerini belirleyen en önemli faktörler, onların kimyasal yapıları, çözünürlükleri, yapı/aktivite ilişkileri ve doğal kaynaklardan elde edilebilmeleridir (Kaur ve Kapoor, 2001). Normal fizyolojik koşullarda hücreler, serbest radikal ürünleri ve peroksitler gibi moleküllerin neden olabileceği oksidatif hasara karşı, antioksidan savunma sistemleri tarafından korunur. Antioksidan savunma sistemleri enzimatik ve enzimatik olmayan iki kategoride incelenebilir (Rice-Evans ve ark., 1997).
Bu çalışmada kullanılan enzimatik olmayan antioksidanlar ise sentetik ve doğal olmak üzere iki sınıfa ayrılabilir. Sentetik antioksidan olan Bütillenmiş hidroksianisol (BHA), kimyasal olarak iki izomerin karışımıdır (3-tersiyer butil-4-hidroksianisol ve 2- tersiyer butil-4-hidroksianisol) ve beyaz mumsu parçacıklar halindedir. BHA (Şekil 3) özellikle uçucu yağların renk, tat ve kokularının korunmasında, bilhassa kısa zincirli yağ asitlerinin oksidasyonunu kontrol etmede etkilidir. Genellikle tahıl ve şekerli ürünlerde kullanılır.




Bütillenmis Hidroksianisol (BHA)

**Şekil 3.** BHA'nın kimyasal yapısı

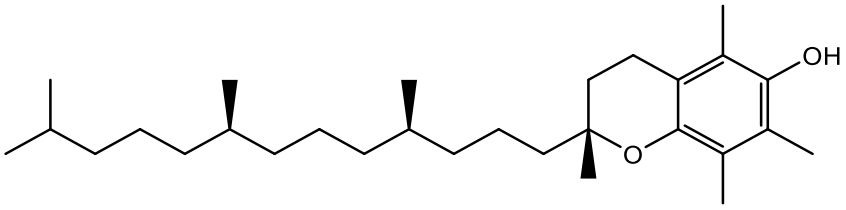
Sentetik antioksidan başka bir bileşik olan Tersiyer bütilhidrokinon (TBHQ), kızartma yağlarını oksidasyona karşı korumak için en iyi antioksidan olarak bilinmektedir. Kızartma işlemi bitmiş ürünleri de korumaktadır. Bej renkli bir toz olan TBHQ (Şekil 4), katı ve sıvı yağlarda çözünür. Diğer sentetik antioksidanların aksine bitkisel yağlar için en etkili sentetik antioksidandır. Yüksek sıcaklıklara dayanıklıdır (Keskin ve Erkmen, 1987).




Tersiyer Bütil Hidrokinon (TBHQ)

**Şekil 4.** TBHQ'nın kimyasal yapısı

Doğal antioksidan olan vitamin E (α-tokoferol), yüksek biyolojik aktivite göstermektedir. Yapısında bulunan fenolik hidroksil gruplu aromatik halka, vitaminin kimyasal olarak aktif kısmını oluşturur ve antioksidan özelliği bu gruptan kaynaklanır (Şekil 5)



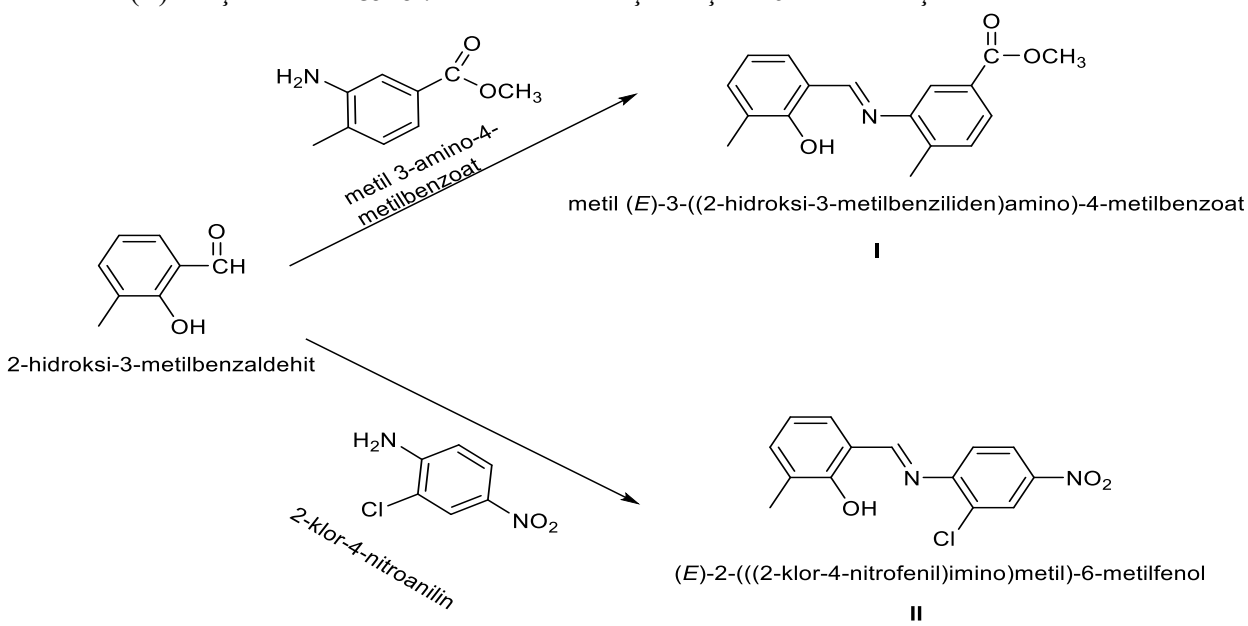

**Şekil 5.** E vitamininin (α-tokoferol) kimyasal yapısı

Bu çalışmada Antioksidan aktivitesi incelenirken DPPH• radikal giderme, ABTS+• katyon radikal giderme metodları kullanıldı. Antimikrobiyal aktiviteleri ise Disk difüzyon ve Mikrodilüsyon metodları kullanılarak incelendi.

## MATERYAL VE METOT

### Sentez

2-hidroksi-3-metilbenzaldehitin (0.02 mmol), metil 3-amino-4-metilbenzoat (0.02 mmol) ve 2-klor-4-nitroanilin (0.02 mmol) ile etil alkol içerisinde (20 mL) geri soğutucu altında altı saat kaynatılması ile metil (E)-3-((2-hidroksi-3-metilbenzil)amino)-4-metilbenzoat (I), (E)-2-(((2-klor-4-nitrofenil)imino)metil)-6-metilfenol (II) bileşikleri elde edildi. Sentezlenen bileşikler Şekil 6 'da verilmiştir.




**Şekil 6.** Sentezlenen Schiff bazlarının kimyasal yapısı

### Serbest Radikal Giderme Aktivitesi Tayini

Örneklerin etanol çözeltilerinin serbest radikal giderme aktivitesi DPPH˙ (1,1-difenil-2-pikril-hidrazil) metodu kullanılarak tayin edildi (Blois, 1958). 96 kuyucuklu plakada 150 □L farklı konsantrasyonlardaki örnekler veya standartlar (BHA, TBHQ ve E vitamini), 50 □L 0.1 mM DPPH (etanolde) homojen karıştırıldı. Örnekler oda sıcaklığında, karanlıkta 30 dk bekletildi. Her bir karışımın absorbans değerleri 517 nm' de BIOTEK (Epoch2) mikroplaka okuyucusu kullanılarak ölçüldü ve sonuçlar $IC_{50}$ (□g/mL) değerleri hesaplanarak belirlendi.

### ABTS˙⁺ Radikali Giderme Aktivitesi Tayini

Örneklerin etanol çözeltilerinin ABTS˙⁺ radikal giderme aktivitesi ABTS-$K_2SO_8$ (2,2′-azinobis-(3-etilbenzotiazolin-6-sulfonik asit)-potasyum persulfat) metodu kullanılarak tayin edildi (Dechayont ve ark., 2017; Re ve ark., 1999). 7 mM ABTS ve 2.45 mM $K_2SO_8$ (2:1) karıştırıldı ve 12-16 saat oda sıcaklığında, karanlıkta bekletildi. Karışımın absorbans değeri 0.700±0.020 olacak şekilde 1:33 (v/v) etanolle seyreltildi. 96

kuyucuklu plakada 20 □L farklı konsantrasyonlardaki örnekler veya standartlar (BHA, TBHQ ve E vitamini), 180 □L $ABTS^{\cdot+}$ çözeltisi homojen karıştırıldı. Her bir karışımın absorbans değeri 6 dk sonra 734 nm' de BIOTEK (Epoch2) mikroplaka okuyucusu kullanılarak ölçüldü ve sonuçlar $IC_{50}$ (□g/mL) değerleri hesaplanarak belirlendi.

**Antimikrobiyal Aktivite Yöntemleri**

Antimikrobiyal aktivitelerde gram negatif bakteriler olarak *Escherichia coli* (ATCC 25922), *Psedomonas aeruginosa* (ATCC 15442), *Klebsiella pneumoniae* (ATCC 10031), *Salmonella enterica* (ATCC 13311) ve gram pozitif bakteriler olarak ise *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (CCM 99), *Staphylococcus aureus* (ATCC 25213), *Lysteria monocytogenes* kullanıldı.

**Disk Difüzyon Metodu**

Örneklerin etanol çözeltilerinin antibakteriyel aktivitesi disk difüzyon metodu ile belirlendi (Reller ve ark., 2009). 5 mL steril serum fizyolojik (%0.85 NaCl) çözeltisine bir miktar bakteri eklendi ve McFarland 0.5 değerine ayarlandı. Steril petrilere 25 mL MHA (Muller Hinton Agar) ilave edildi. McFarland 0.5 değeri ayarlanan bakteri çözeltisi agar üzerine sürüldü. 50 □L örnekler ve antibiyotikler (1000 □g/mL) disklere emdirildi ve diskler agar üzerine yerleştirildi. 37 ºC' de 16-18 saat inkübasyona bırakıldı. İnhibisyon bölgeleri mm olarak belirlendi. Pozitif kontrol olarak amoksisilin ve tetrasiklin antibiyotikleri, negatif kontrol olarak ise ekstraktların ve antibiyotiklerin çözücüleri kullanıldı.

**Mikrodilüsyon Metodu**

Örneklerin etanol çözeltilerinin antibakteriyel aktivitesi mikrodilüsyon metodu ile belirlendi (Andrews, 2001). Katyonlu MHB için 100 mL MHB üzerine 400 □L $MgCl_2$ (2 mg/mL) ve 1 mL $CaCl_2$ (2 mg/mL) karıştırıldı. 5 mL steril serum fizyolojik (% 0.85 NaCl) çözeltisine bir miktar bakteri eklendi ve McFarland 0.5 değerine ayarlandı ve 1 mL alınarak 9 mL katyonlu MHB ile karıştırıldı. Steril 96-kuyucuklu plakadaki her bir kuyuya 100 □L katyonlu MHB (Mueller Hinton II Broth) ilave edildi. 100 □L örnekler (1024 □g/mL) ve antibiyotikler (amoksisilin (2048 □g/mL) ve tetrasiklin (1024 □g/mL)) ilk kuyuya eklendi, karıştırıldı ve 100 µL alınarak ikinci kuyuya eklendi. Bu işlem 12. kuyuya kadar devam etti ve son olarak 12. kuyudan alınan 100 □L çözelti atıldı. 5 □L bakteri çözeltisi bütün kuyulara eklendi. +4 ºC buzdolabında 2 saat bekletildi ve sonra 37 ºC' de 16-18 saat inkübasyona bırakıldı. Minimum inhibisyon konsantrasyonu (MİK) □g/mL olarak belirlendi.

**BULGULAR ve TARTIŞMA**

Örneklerin serbest radikal giderme aktivite sonuçları ve $ABTS^{\cdot+}$ radikal giderme aktivite sonuçları Tablo 1' de gösterildi.

**Tablo 1.** Antioksidan Aktivite Sonuçları

| Örnekler | Antioksidan Aktivite | |
|---|---|---|
| | Serbest Radikal Giderme Aktivitesi | $ABTS^{\cdot+}$ Radikali Giderme Aktivitesi |
| | **$IC_{50}$ (µg/mL)** | **$IC_{50}$ (µg/mL)** |
| I | 16.08±1.70 | 88.35±0.16 |
| II | 29.51±0.13 | 71.91±2.01 |
| BHA | 3.67±0.00 | 6.14±0.02 |
| TBHQ | 17.68±0.96 | 5.67±0.15 |
| EVİT | 2.34±0.17 | 16.00±0.12 |

Örneklerin aktiviteleri karşılaştırıldığında I örneğinin II örneğine göre daha etkili olduğu belirlendi.

Örneklerin antimikrobiyal aktivitesinin disk difüzyon metodu ve mikrodilüsyon metodu ile incelenmesinden elde edilen sonuçlar Tablo 2' ve Tablo 3'de gösterildi.

**Tablo 2.** Gram negatif bakteriler ile Antimikrobiyal Aktivite Sonuçları

| Antibakteriyel özellikler | Örnekler/ Antibiyotikler/ Kontrol | **Gram-negatif bakteri** | | | |
|---|---|---|---|---|---|
| | | *E. coli* (ATCC 25922) | *P. aeruginosa* (ATCC 15442) | *K. pneumoniae* (ATCC 10031) | *S. enterica* (ATCC 13311) |
| İnhibisyon alanı, mm | I | 7.9±0.0 | 6.4±0.0 | NA | 7.0±0.0 |
| | II | NA | 6.9±1.4 | NA | NA |
| | Amoksisilin | 23.0±0.0 | NA | NA | 15.0±0.0 |
| | Tetrasiklin | 18.9±0.8 | 14.1±0.7 | 23.0±0.0 | 16.8±0.4 |
| | Etanol | 10.1±0.0 | 10.1±0.0 | 11.4±0.0 | NA |
| | Kontrol | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 |
| | | | | | |
| Minimum İnhibitör Konsantrasyonu (MIK), µg/mL | I | 32 | 32 | 32 | 64 |
| | II | 32 | 32 | 32 | 32 |
| | Amoksisilin | 4 | 32 | >2048 | 32 |
| | Tetrasiklin | 1 | 8 | 8 | 8 |
| | Etanol | NA | NA | NA | NA |

NA: Aktivite yok
MIK (Minimum İnhibitör Konsantrasyonu**):** µg/mL

**Tablo 3.** Gram pozitif bakteriler ile Antimikrobiyal Aktivite Sonuçları

| Antibakteriyel özellikler | Örnekler/ Antibiyotikler/ Kontrol | **Gram-pozitif bakteri** | | | |
|---|---|---|---|---|---|
| | | *E. faecalis* (ATCC 29212) | *B. cereus* (CCM 99) | *S. aureus* (ATCC 25213) | *L. monocytogenes* |
| İnhibisyon alanı, mm | I | 7.9±0.0 | 7.9±0.0 | 7.5±2.1 | 11.0±0.0 |
| | II | 8.4±0.7 | NA | NA | 10.0±0.0 |
| | Amoksisilin | NA | 33.7±0.9 | NA | 13.4±0.1 |
| | Tetrasiklin | 20.0±0.0 | 22.0±0.3 | 15.6±0.0 | 15.8±0.8 |
| | Etanol | 11.0±0.6 | 7.1±0.0 | 9.1±0.0 | NA |
| | Kontrol | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 | 6.0±0.0 |
| | | | | | |
| Minimum İnhibitör Konsantrasyonu (MIK), µg/mL | I | 32 | 32 | 128 | 64 |
| | II | 32 | 32 | 32 | 64 |
| | Amoksisilin | >2048 | 128 | >2048 | 256 |
| | Tetrasiklin | 16 | <0.5 | 8 | 2 |
| | Etanol | NA | NA | NA | NA |

NA: Aktivite yok
MIK (Minimum İnhibitör Konsantrasyonu**):** µg/mL

Örneklerin antibakteriyel aktiviteleri antibiyotiklerle karşılaştırıldığında *P. aeruginosa* bakterisinde az etkili, *E. coli* ve *S. enterica* bakterisinde I örneği etkili, *E. faecalis* ve *L. monocytogenes* bakterisinde etkili, *B. cereus* ve *S. aureus* bakterisinde I örneği etkili olduğu belirlendi. Örneklerin antibakteriyel aktiviteleri antibiyotiklerle karşılaştırıldığında *E. coli* bakterisinde az etkili, *P. aeruginosa* bakterisinde amoksisilin antibiyotiğiyle aynı etkide fakat tetrasiklin antibiyotiğinden az etkili, *K. pneumoniae* bakterisinde amoksisilin antibiyotiğinden etkili fakat tetrasiklin antibiyotiğinden az etkili, *S. enterica* bakterisinde amoksisilin antibiyotiğiyle aynı etkide fakat tetrasiklin antibiyotiğinden az etkili, *E. faecalis* bakterisinde amoksisilin antibiyotiğinden etkili fakat tetrasiklin antibiyotiğinden az etkili, *B. cereus* bakterisinde amoksisilin antibiyotiğinden etkili fakat tetrasiklin antibiyotiğinden az etkili, *S. aureus* bakterisinde amoksisilin antibiyotiğinden etkili fakat tetrasiklin antibiyotiğinden az etkili ve *L. monocytogenes* bakterisinde amoksisilin antibiyotiğinden etkili fakat tetrasiklin antibiyotiğinden az etkili olduğu belirlendi.

## SONUÇ

DPPH• radikal giderme, ABTS+• katyon radikal giderme metodları kullanılarak incelen antioksidan aktivitesine bakıldığında I bileşiğinin II bileşiğinden daha etkili olduğu belirlendi. Disk difüzyon ve Mikrodilüsyon metodları kullanılarak incelenen antimikrobiyal aktivitelerinde de I bileşiğinin II bileşiğinden daha etkili olduğu gözlemlendi.

## KAYNAKLAR

Andrews JM, 2001. Determination of minimum inhibitory concentrations. Journal of antimicrobial Chemotherapy, 48:suppl_1, 5-16.

Bakır TK, Lawag JB, 2020. Preparation, characterization, antioxidant properties of novel Schif bases including 5-chloroisatin-thiocarbohydrazone. Res. Chem. Intermed., 46: 2541-2557.

Beraldo H, Sinisterra RD, Teixeira LR, Vieira RP, Doretto MC, 2002. An effective anticonvulsant prepared following a host–guest strategy that uses hydroxypropyl-□-cyclodextrin and benzaldehyde semicarbazone. Biochemical and Biophysical Research Communications, 296: 241-246.

Bharti S, Nath G, Tilak R, Singh S, 2010. Synthesis, anti-bacterial and anti-fungal activities of some novel Schiff bases containing 2,4-disubstituted thiazole ring. Eur. J. Med. Chem., 45: 651-660.

Blois MS, 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181:4617, 1199-1200.

Dechayont B, Ruamdee P, Poonnaimuang S, Mokmued K, Chunthorng-Orn J, 2017. Antioxidant and Antimicrobial Activities of Pogostemon cablin (Blanco) Benth. Journal of Botany, 2017:1-6.

Ghosh P, Dey SK, Ara MH, Karim KMdR, Islam ABMN, 2019. A Review on synthesis and versatile applications of some selected Schiff bases with their transition metal complexes. Egypt. J. Chem., 62 (Special Issue, Part 2): 523-547.

Goszczyńska A, Kwiecień H, Fijałkowski K, 2015. Synthesis and antibacterial activity of Schiff bases and amines derived from alkyl 2-(2-formyl-4-nitrophenoxy)alkanoates. Med. Chem. Res., 24: 3561-3577.

Kahkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala JTS, Heinonen M, 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry, 47: 3954–3962.

Kaur C, Kapoor HC, 2001. Antioxidants in Fruits and Vegetables—The Millennium's Health. International Journal of Food Science and Technology, 36: 703-725.

Keskin H, Erkmen G, 1987. Besin Kimyası c. 1, Güray Matbaacılık Tic. Ltd. Şti., İstanbul.

Kumar KS, Ganguly S, Veerasamy R, Clercq ED, 2010. Synthesis, antiviral activity and cytotoxicity evaluation of Schiff bases of some 2-phenyl quinazoline-4(3)H-ones. Eur. J. Med. Chem., 45: 5474-5479.

Liao YC, Yang S, Syu MJ, Xu Y, 2005. Development and validation of an HPLC–UV method for the analysis of methoxyamine using 4-(diethylamino)benzaldehyde as a derivatizing agent. Journal of Pharmaceutical and Biomedical Analysis, 39: 724-729.

Pahlavani E, Kargar H, 2021. Synthesis, Characterization, and Study of Anti-Tubercular and Anti-Microbial Activity of Isonicotinohydrazide Tridentate Schiff Base Ligands. Iran. J. Chem. Chem. Eng., 40:(1) 201-206.

Qin W, Long S, Panunzio M, Biondi S, 2013. Schiff Bases: A short survey on an evergreen chemistry tool. Molecules, 18(10): 12264-12289.

Rajendra KJ, Mishra AP, 2016. Microwave synthesis, spectral, thermal, 3D molecular modeling analysis and antimicrobial activities of some transition metal complexes of Schiff bases derived from 5-bromosalicylaldehyde. Journal of Saudi Chemical Society, 20: 127–137.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine, 26:9-10, 1231-1237.

Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ, 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases, 49:11, 1749-1755.

Rice-Evans CA, Miller NJ, Paganga G, 1997. Antioxidant properties of phenolic compounds. Elsevier Science Ltd., 2:(4) 152-159.

Shehry MFE, Ewies EF, Zayed EM, 2019. Synthesis of New Pyrazole Derivatives, Their Anti-Inflammatory and Analgesic Activities, and Molecular Docking Studies. Russian Journal of General Chemistry, 89:(3) 492-498.

Sriram D, Yogeeswari P, Myneedu NS, Saraswat V, 2006. Abacavir prodrugs: Microwave-assisted synthesis and their evaluation of anti-HIV activities. Bioorganic and Medicinal Chemistry Letters, 16: 2127-2129.

## *ORAL PRESENTATION*

# Biological activity study of the *Mentha pulegium's* Essential Oil "EO"as an aromatic and medicinal plant in Morocco

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## Abstract

The species of *Mentha pulegium* is widely used by the inhabitants of the Gharb region (Morocco) as a condiment, herbs and aromatics in culinary preparations. It is used for its therapeutic properties as a carminative, sedative, antispasmodic and for stomach ailments.
The main objective of this research is to study the biological activity of the essential oil "EO" of *Mentha pulegium* extracted by Clevenger type hydrodistillation, which revealed a yield of 1.65%. Gaz chromatography / Mass Spectrophotometry (GC/MS) which made it possible to identify 20 compounds presenting 98.91%, of which the majority compound is pulegone (76.35%) established the qualitative and quantitative study.
The evaluation of the antibacterial activity was carried out by different methods such as the disc method and the solid medium diffusion method. The values of the minimum inhibitory concentrations "MIC" have just confirmed the results of the disc method, which induces remarkable zones of inhibition. The six bacterial strains studied *Escherichia coli "EC", Klebsiella pneumoniae "KP", Vibrio parahaemolyticus "VP", Staphylococcus aureus "SA", Bacillus subtilis "BS"* and *Micrococcus luteus "ML"*, were inhibited at different concentrations. The "MIC" is about 1/500 for *EC* bacteria; *ML; VP; BS*, and about 1/250 for *SA* and 1/100 for *KP*. Therefore, the "EO" of *Mentha pulegium* is qualified as bactericidal at a concentration of 1/500 against the bacilli *E. coli*, *M. luteus*; *V. parahaemolyticus*; and *B. subtilis*. This strong biological activity of the essential oil of *Mentha pulegium* deserves to be exploited and confirmed by in vivo tests in order to develop a means of biological control based on natural substances against the microorganisms studied.

**Keywords**: Antibacterial, MIC, GC/MS, Hydrodistillation, EO, *Mentha pulegium*, Morocco.

## INTRODUCTION

All credit to antibiotics, there are several illnesses that seemed to be under control in time. Scientific and technological progress even suggested a promising eradication of many pathologies. The resistance developed more and more by microorganisms and the regular emergence of new infectious agents have belied this optimistic prognosis. For a long time, many resistance determinants have been described with the emergence of increasingly resistant bacteria. No known bacterial species and no antibiotic today escape the phenomenon of resistance and tolerance; it is a real biological problem that medicine will find difficult to eliminate. (Barchan, A et al., 2015)

Faced with the therapeutic limits, conventional antibiotics have pushed scientists to direct research towards new alternatives and above all the use of the active principles of plants (phenolic compounds, alkaloids, essential oils, etc.) as antibacterial agents.

Medicinal plants are considered as a valuable heritage for humanity, particularly for developing countries to ensure their primary health care and livelihoods (Tabuti & al., 2003). The latter have an important place in alternative medicine and play an important role in the national economy (Bellakhdar, 1997 and Scherrer & al., 2005).

However, although the aromatic and medicinal plant sector is more developed in Morocco than in other Maghreb countries, the existing potential is under-exploited and even often left fallow. Indeed, moroccan flora contains about 4200 spontaneous and cultivated species, including 400 with aromatic and medicinal potential, exploited for several purposes: traditional medicine, herbs, cosmetics, food preservation, export of raw plants and essential oils (ANPAM, 2017).

## MATERIAL AND METHODS

### 1. Extraction of essential oil

Mint (Mentha pelugium) essential oils were extracted by hydrodistillation using a Clevenger-type apparatus.

### 1.1. Principle

Hydrodistillation is the most commonly used method for the extraction of an essential oil (Meyer-Warnod, 1984). The process consists of immersing the plant material in a water bath; the whole is then brought to the boil, at an atmospheric pressure of 1 bar. Under the effect of temperature, the odorous molecules contained in the secretory glands of plants are released in the form of an azeotropic mixture. Although most of the constituents have boiling temperatures above 100°C, they are mechanically entrained with the water vapour. Cooling by condensation leads to the separation of the water-essential oil mixture by decantation. The "Clevenger" system, recommended by the European Pharmacopoeia (European Pharmacopoeia, 1997), allows the aqueous phase of the distillate to be recycled in the boiler by cohobage (Clevenger, 1928). Thus, water and volatile molecules are separated, by their density differences, in the essence into an aqueous phase (hydrosol) and a supernatant organic phase or essential oil (Meyer-Warnod., 1984).

### 1.2. Operating mode

To extract the essential oil of *Mentha pulegium*, the analysis consists of following the following steps:

Weigh a 100g sample of *Mentha pulegium* (leaves, flowers and stems) using an electronic scale;
Put the quantity of vegetable matter weighed in a balloon flask;
Add 1000 ml of distilled water;
Place the ball in the Clevenger;
Operate and set the Clevenger to a temperature of 100°C.
After about two hours, the essential oil was collected in opaque bottles.

### 2. Yield calculation

The yield of essential oils "EO" is defined as the ratio between the mass of essential oil obtained and the mass of dry plant material used (European Pharmacopoeia, 2000).

$$\textbf{EO content (\%) = (EO weight) / (Sample weight)×100}$$

### 3. Chromatographic analysis

The chromatographic analyzes were carried out by gas phase chromatography "**GPC**" with electronic pressure regulation, of the Perkin Elmer type.

The analysis of the essential oil of *Mentha pulegium*, is made on a capillary column of fused silica, length 60 cm, diameter 0.25 mm. The stationary phase is Macrogol 20000. The carrier gas used is helium with a flow rate of 1.5 ml/min. The column temperature is programmed automatically at 70°C for 15 min, then from 70 to 180°C in a time interval of 15-60 min. The temperatures of the injector and the detector keep a constant value of 220°C.

## 4. Evaluation of some chemical indices of the essential oil

To assess the quality of our essential oil, particularly with regard to its degradation over time during storage, the assays were carried out as follows:

### 4.1 Acid value

**Principle and purpose of the method**: The determination of the acid index of essential oils amounts to neutralizing the free acids of these with potassium hydroxide (KOH) or caustic potash.

The purpose of this method is to detect the acidity of the essential oil in order to determine its quality and subsequently exploit this result in the antibacterial activity.

**Protocol**: The technique consists of boiling 2.5 $cm^3$ of 5% ethanol in a water bath. Heating is used to remove dissolved gases. Next, 2.5 g of essential oil was added to 25 $cm^3$ of hot ethanol and the mixture was heated to a boil. A few drops of phenolphthalein indicator (1%) were added and titrated against 0.1 M KOH with constant stirring until a permanent pink color was obtained.

The acid index being expressed according to the indications of the AFNOR standard (1999).

$$\textbf{Acid number} = 56.1 \times M \times V / W$$

M = KOH concentration;
V = Security value;
56.1 = molecular weight of KOH;
W= Weight of the oil sample.

### 4.2. Determination of free fatty acid

To determine the free fatty acid, an amount of 2.0 g of "EO" was added to a 250 $cm^3$ Erlenmeyer flask, mixed with 100 cm3 of ethanol and 2 cm3 of phenolphthalein indicator. The mixture was stirred and titrated with 0.1 M NaOH solution, stirring continuously until reaching the end indicated by a light pink color, which persisted for 30 seconds. The free fatty acid is expressed by AFNOR.

$$\%FFA = V \times N \times 282 \times 100 \times W$$

% FFA = percentage of free fatty acid (g / 100g);
V = volume of NaOH (cm3);
N = molarity of NaOH;
282 = molecular weight of oleic acid (mg);
W = oil sample weight (mg).

## 5. In vitro evaluation of antimicrobial activity

The antimicrobial activity of essential oils has been evaluated against pathogenic strains, which are obtained at the Biotechnology Quality and Environment Laboratory, Department of Biology, Ibn Tofail University, Kenitra, 2019.

Two methods were performed to verify the antimicrobial activity in vitro. The disk diffusion method on agar medium to assess the activity in contact, and the method of dilution in solid medium for the determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) according to the method reported by Remmal et al., ( 1993) and Satranie et al., (2001).

### 5.1. Preparation of pre-cultures

The microbial strains are inoculated in streaks in a nutrient agar medium, after incubation for 18 hours at 37° C., a few well-isolated colonies are removed using a platinum loop. Then, the platinum loop is discharged into 10 ml of sterile physiological water at 0.9% and the whole is homogenized in bacterial suspension using a vortex. The inoculum is adjusted to 0.5 Mc Farland corresponding to an optical density of (0.08 to 0.10) at 625 nm.

### 5.2. The microbial strains used

For the determination of the antimicrobial activity of essential oils of Mentha pulegium, three *Gram-negative* bacterial strains; *Escherichia coli "EC", Klebsiella pneumoniae "KP"* and *Vibrio parahaemolyticus "VP",* and three *Gram-positive* bacterial strains, *Staphylococcus aureus "SA", Bacillus subtilis "BS", Micrococcus luteus "ML"* were chosen. These microorganisms are pathogenic and are known for their high antibiotic resistance and their invasive and toxic power in humans. They are frequently encountered in many infections in Morocco and cause therapeutic and clinical problems.

The bacterial strains are batches from the "American Type Culture Collection" (ATCC), they are maintained by sub-culturing on nutrient agar favorable to their growth for 24 hours in the dark at 37 ˚C.

### 5. 3: Disc Method

It involves preparing a Muller Hinton culture medium, on which the following operations must be planned:

- inoculate the strain to be tested, prepared beforehand in the form of an inoculum;

- Spread the whole box then soak the disc of Whatman paper with the essential oil of *Mentha pulegium*;

- Incubate the box in an oven at 37°C for 24 hours.

### 5.4. Dilution method (in solid medium)

The essential oil is emulsified by a 0.2% agar solution in order to disperse the compounds and improve their contact with the germs tested. Dilutions of the essential oil are prepared at 1/10, 1/25, 1/50, 1/100, 1/200, 1/300 and 1/500 in a 0.2% agar agar solution. In test tubes each containing 13.5 ml of Mueller Hinton medium, sterilized in the autoclave and cooled to 45° C., 1.5 ml of each of the dilutions are added aseptically to obtain the final concentrations of 1/100, 1/250, 1/500, 1/1,000, 1/2,000, 1/3,000 and 1/5,000 (v/v). The tubes are well shaken in order to disperse the essential oil well in the culture medium before pouring them into the Petri dishes. Controls, containing culture medium plus 0.2% agar solution alone, are also prepared.

Seeding is done by spreading a bacterial suspension from culture diluted in sterile physiological water to obtain an inoculum with an opacity equivalent to an optical density of 0.08 and 0.1 at 625 nm .The incubation takes place for 24 hours at 37°C.

## RESULTS AND DISCUSSION

### 1. Chemical Composition of Essential Oil

CG/MS analysis of the analyzed samples revealed 20 components (Figure 1), of which the dominant one is pulegone, its distribution is relatively high (76.35%), it is found in most essential oils of this species from different regions, in addition we can see the presence of Carvone (5.84%), Dihydrocarvone (5.09%), Octanol-3 (2.25%) (Figure 2), we can also notice the presence of other minority constituents which are not devoid of importance: p-Mentha-3,8-diene (1.95%), Pinocarvone (1.76%), and Limonene (1.41%) .

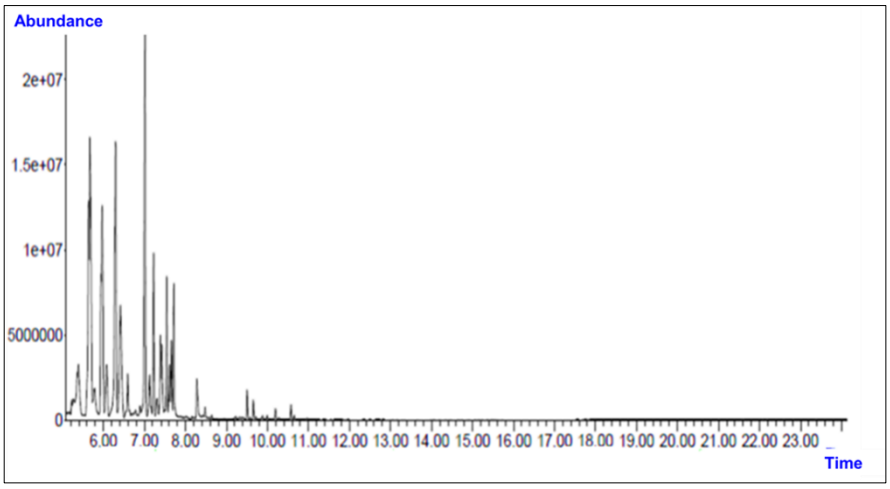


**Figure 1.** Chromatography profile of *Mentha pulegium* EO

These results are similar to most of the work already done in Morocco (Hmiri S.et al., 2011; Ouraini D., 2017; Chebli B., 2003 and Ouraini D., 2005). Similarly, work undertaken in Tunisia by Snoussi et al., (2009) and Hajlaoui et al., (2009) showed that **pulegone** is the main compound of *M. pulegium*, with concentrations of 44.27% and 61.11%. While the work of Mahboubi et al., (2008) in Iran as well as those of Derwich et al., (2010). In Morocco, these authors highlighted another chemotype whose major compounds are piperitone and piperitenone, with low levels of pulegone. In addition to pulegone at a concentration of 43.3% to 87.3%, Beghidji et al. (2007) found in different provenances of Algeria, a chemotype of M. pulegium characterized by its richness in monoterpenes (α and β-pinenes, camphene, sabinene, α-terpinene and myrcene).

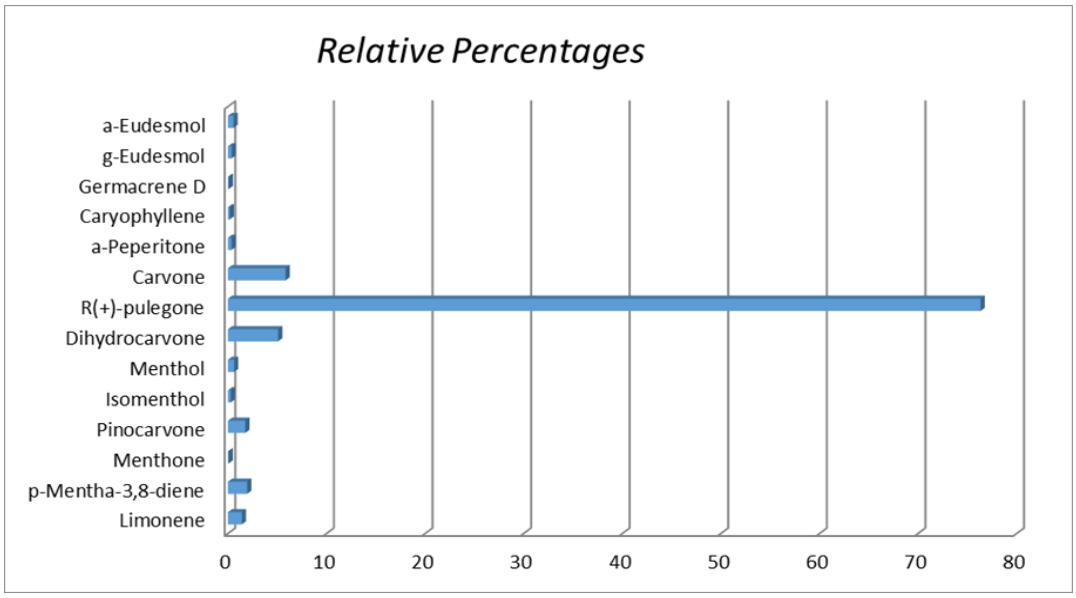


**Figure 2.** Distribution of some components of *Mentha pulegium* EO

## 2. Antimicrobial activity of essential oil

The antimicrobial activity of the essential oil of *Mentha pulegium* was evaluated against six Gram positive and negative bacteria, in the first place, the disc method is used to determine the activity of this oil which thereafter was evaluated by its *minimum inhibitory* (MIC) and bactericidal (MBC) concentration.

### 2.1. Disk method

The disk method showed an almost total positive response of EO, on the different microbial strains, expressed by a diameter of the inhibition zone between 10 and 17 mm. Constituting two groups, classified according to the average of the diameters in ascending order, of which the first group is composed of the strains; *Microccocus luteus*, *Staphyloccocus aureus*, and *Vibrion parahaemolytucus*, with an average of 15 mm. The second group is made up of strains; *Klebsellia pneumonia*, *Escherchia coli*, and *Bacillus subtilis*, with an average of 12 mm. The results of the inhibition diameters are shown below in Table 1**.** The results obtained revealed a greater virulence in the Gram(-) strains (average diameter, 13mm) than the Gram(+) strains (average diameter, 14.33mm).

**Table 1.** The diameters of the EO inhibition zones of Mentha pulegium (mm).

| Microbial Strains | E. coli | K. pneumoniae | Staph. aureus | B. subtilis | M. luteus | V. parahaemolyticus |
|---|---|---|---|---|---|---|
| Trial 1 | 15 | 13 | 13 | 10 | 17 | 12 |
| Trial 2 | 14 | 10 | 16 | 10 | 14 | 16 |
| Trial 3 | 10 | 12 | 14 | 12 | 16 | 15 |

In general, the action of essential oils takes place in three phases: 1/ Attack of the bacterial wall by the essential oil, causing an increase in permeability then the loss of cellular constituents. 2/ Acidification of the interior of the cell, blocking the production of cellular energy and the synthesis of structural components. 3/ Destruction of the genetic material, leading to the death of the bacteria.

*Mentha pulegium* EO is active against all bacterial strains with remarkable inhibition diameters, while this EO is then more active against Gram (+) than Gram (-). After verification of the existence of antimicrobial activity using the method of disk, the method of dilution in liquid medium is carried out for the strains whose zone of inhibition is large (>9 mm) (Yildirim A et al., 2001).

The MIC is then the concentration of the first cupule where a cloudiness is not observed with the naked eye and the inoculation of the contents of the cupules beyond the MIC, on agar medium is necessary to determine the nature of the activity antimicrobial (bactericidal or bacteriostatic) and deduce the CMB (Table 2).

**Table 2.** Results of MIC and CMB

| | C | MIC | CMB | MIC | CMB | MIC | CMB | MIC | MIC | MIC | MIC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration / Strains | | 1/100 | 1/100 | 1/250 | 1/250 | 1/500 | 1/500 | 1/1000 | 1/2000 | 1/3000 | 1/5000 |
| Escherichia coli | + | - | - | - | - | - | - | + | + | + | + |
| Klebsiella pneumoniae | + | - | - | + | | + | | + | + | + | + |
| *Vibrio parahaemolyticus* | + | - | - | - | - | - | - | + | + | + | + |
| Staphylococcus aureus | + | - | - | - | - | + | | + | + | + | + |
| Bacillus subtilis | + | - | - | - | - | - | - | + | + | + | + |
| Micrococcus luteus | + | - | - | - | - | - | - | + | + | + | + |

(-): inhibition; (+): growth/development; T (Control)

The MIC values confirm the results of the disc method, for *Mentha pulegium* EO that induced remarkable zones of inhibition. The four bacterial strains studied “EC”; “ML”; “VP” and “BS” were inhibited at a concentration of 1/500. However, *Staphylococcus aureus* was inhibited at a concentration of 1/250 and *Klebsiella pneumoniae* was inhibited at a concentration of 1/100.

Therefore, the EO of *Mentha pulegium* is qualified as **bactericidal** at a concentration of 1/500 against the bacillus Gram (-) "EC’’, “VP”; and Gram (+) ‘’ML, “BS". Indeed, several studies confirm the results obtained.

Essential oil of the same species in Iran exhibited antimicrobial activities against all bacteria tested in the 0.05% (v/v) dilution except for *P. aeruginosa*. The essential oil showed strong antimicrobial activity against S. aureus and *B. subtilis* (MIC, 0.00025% v/v). The MIC value for *E. coli* was 0.0005% (v/v) while for other types of EO, *K. pneumoniae* seemed to be classified as a microorganism with significant resistance (Oskuee et al., 2011).

The results obtained are in agreement with the literature according to which Gram (positive) bacteria exert the greatest sensitivity towards essential oils (Burt, 2004; Nedorostova, 2009).

## CONCLUSION

The extraction of the aerial part was carried out by Clevenger-type hydrodistillation. The essential oil content is 1.65%, this value varies according to the harvest season, the extraction process and even the drying and storage conditions.

The composition of the EO studied was analyzed by **CPG/MS**, the results obtained showed the presence of compounds which have very important role in microbial activities.

The assignment of an acid number for this essential oil is of major importance to help aroma therapists choose the most effective oil according to the sampling ecosystem.

The antimicrobial activity was evaluated by the disk method, the search for CMI, CMB of the essential oil of *Menta pulegium*, made it possible to qualify this essential oil as bactericidal against the strains tested "*Staphylococcus aureus*; *Escherichia coli; Micrococcus luteus; Klebsiella pneumoniae; Bacillus subtilis; Vibrio parahaemolyticus* »

This strong biological activity of *Mentha pulegium* EO is attributed to its richness in pulegone (76.35%), however, the existence of the phenomenon of synergy between majority and minority compounds cannot be overlooked.

These results can contribute to the valorization of *Mentha Pulegium* by the local production of its EO. The inhibiting effect of EO on bacterial development suggests the prospect of application in the food, cosmetics and pharmaceutical industries.

## REFERENCES

AFNOR. Norme NF ISO 1242. (1999). Huiles essentielles -- Détermination de l'indice d'acide.

Barchan, A., Bakkali, M., Arakrak, A., & Laglaoui, A. (2015). Effet antibactérien et anti-biofilm de trois espèces de Mentha *: Mentha spicata, Mentha pulegium et Mentha piperita. Phytothérapie, 14(2), 88–96.* doi:10.1007/s10298-015-0970-y

ANPAM, (2017) : Agence Nationale des Plantes Aromatiques et Médicinales, vision stratégique 2018-2022, Maroc.

Beghidji N., Bouslimani N., Benayache F., Benayache S., and Chalchat J.C.,(2007), Composition of the oils from *Mentha pulegium* grown in different areas of the East of Algeria, *Chem. Nat. Compd*., 43 (4), 481-483.

Bellakhdar, J. (1997) La Pharmacopée Marocaine, Tra-Ditionnelle. Médecine Arabe Ancienne Et Savoir Populaire— Ed. Le Fennec. Casablanca, Maroc. 129-533 .

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods-a review. Internat J Food Microbiol 94:223–53**;**

Chebli B., Achouri M., Idrissi Hassani L.M., And Hmamouchi M., (2003), Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers: Fr, *J. Ethnopharmacol.*, 89, 165-169.

Derwich E., Benziane Z., and Boukir A., (2010), GC/MS analysis and antibacterial activity of the essential oil of *Mentha pulegium* Grown in Morocco, *Research Journal of Agriculture and Biological Sciences*, 6(3), 191-198..

Hajlaoui H., Trabelsi N., Noumi E., Snoussi M., Fallah H., Ksouri R., and Bakhrouf A., (2009), Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine, *World J. Microbiol. Biotechnol.*,25, 2227-2238.

Hmiri S., Rahouti M., Habib Z. ; Satrani B., Ghanmi M., El Ajjouri M., (2011), évaluation du potentiel antifongique deshuiles essentielles de mentha pulegium et d'eucalyptus camal du lensis dans la lutte biologique contre les champignon responsables de la détérioration des pommesen conservation, Bulletin de la Société Royale des Sciences de Liège, Vol. 80, 2011, p.824 - 836

Mahboubi M. And Haghi G., (2008), Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil, *J. Ethno pharmacol*., 119, 325-327

Meyer-Warnod, B. (1984). Natural essential oils: extraction processes and applications to some major oils, Perfumer & Flavorist 9, 93-103.Remmal A, T-Elaraki A, Bouchikhi T, Rhyyour K, Ettayebi M, (1993). Improved Method For The Determination Of Microbial Activity Of Essential Oils In Agar Medium. J. Essent. OilRes., 5, 179-184

Nedorostova L, Kloucek P (2009) Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. Food Control 20:157–60

Oskuee R.K., Behravan J. et Ramezani M., (2011), Chemical composition, antimicrobial activity and antiviral activity of essential oil of *Carumcopticum* from Iran, Avicenna Journal of Phytomedicine, Vol. 1, No. 2, p: 83-90

Ouraini D., Agoumi A., Ismaili-Alaoui M., Alaoui K., Cherrah Y., Alaoui M.A. et Belabbas M.A., (2007), Activité antifongique de l'acide oléique et des huiles essentielles de *Thymus saturejoides*L. et *Menthapulegium*L., comparée aux antifongiques dans les dermatoses mycosiques, *Phytothérapie*, 1, 6-14.;

Pharmacopée Européenne. Recommandations Relatives Aux Critères De Qualité Des Huiles Essentielles. Agence Française De Sécurité Sanitaire Des Produits De Santé (Afssaps) Mai (2008)

Scherrer, A.M., Motti, R., Weckerle, C. S. (2005). Traditional Plant Use In The Areas Of Monte Vesole And Ascea, Cilento National Park (Campania, Southern Italy) — J. Ethnopharmacol. 97: 129-143

Snoussi M., Hajlaoui H., Noumi E., Usai D., Sechi L.A., Zanetti S., and Bakhrouf A., (2008), *In vitro* anti-*vibrio* spp. activity and chemical composition of some Tunisian plants, *World J. Microbiol. Biotechnol.* 24, 3071-3076.

Tabuti, J.R.S., Lye, K.A. &Dhillion, S.S. (2003) Tradi-Tional Herbal Drugs Of Bulamogi, Uganda: Plants, Useand Administration — J. Ethnopharmacol. 88: 19-44. .

Yildirim A., Mavi A. et Kara A. A., (2001), Determination of antioxidant and antimicrobial activities of *Rumexcrispus*L. extracts; Journal of Agricultural and Food Chemistry 49, p: 411-420.

## *ORAL PRESENTATION*

# Cardiotoxic Effect of Fenitrothione and Protective Role of Gallic Acid in Rats

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**Özet**

Fenitrotiyon(FNT) geniş spektrumlu bir organofosfat insektisittir. Pirinç, tahıl, meyve ve sebzelerdeki zararlıları kontrol altına almak için tarımda kullanılır. Gallik asit(GA) polifenollerin tanen grubu üyesidir, güçlü bir antioksidandır ve oksidatif strese etkili bir koruyuculuk sağlamaktadır. Yapısında katalaz, süperoksit dismutaz gibi enzimleri içeren antioksidan sistemi mevcuttur. Oksidatif strese karşı hücresel savunma sistemi, oksitlenmiş molekülleri indirgeyici maddelere dönüştüren glutatyon ve diğer enzimler tarafından kendiliğinden aktive edilir. Bu çalışmada, sıçanın kalp dokusunda fenitrotiyonun sebep olduğu hasarın üzerinde gallik asitin ne gibi bir koruyucu rol üstlendiği araştırılmıştır. Çalışmada deney hayvanları kullanımındaki etik standart protokollerine uyulmuştur ve Gazi Üniversitesi Hayvan Deneyleri Yerel Etik Kurulundan izin alınmıştır (G.Ü. ET-19.025). Deney hayvanları 4 gruba ayrılmıştır; kontrol grubu, gallik asit uygulanan grup, fenitrotiyon uygulanan grup ve gallik asit ile fenitrotiyon uygulanan grup. Kimyasallar deney hayvanlarına 28 gün boyunca günde bir kere olmak üzere gavaj yoluyla verilmiştir.. 4. hafta sonunda sıçan kalp dokularındaki malondialdehit (MDA) seviyeleri ve antioksidan aktiviteleri (SOD, CAT, GPx ve GST) çeşitli yöntemlerle spektrofotometrik olarak incelenmiştir. 4. Hafta sonunda MDA seviyeleri, antioksidan enzim (süperoksit dismutaz, katalaz, glutatyon peroksidaz, glutatyon-S-transferaz) aktiviteleri kontrol grubuyla karşılaştırılmıştır. Kontrol grubuyla gallik asit uygulanan gruplar arasında belirgin bir fark gözlenmemiştir. 4. hafta sonunda kontrol grubundaki sıçanlarla mukayese edildiğinde fenitrotiyon uygulanan grupta; SOD, CAT, GPx ve GST enzim aktivitelerinde düşüş yaşanırken MDA seviyelerinde artış yaşanmıştır. Bu çalışmada fenitrotiyonun sıçan kalp dokusunda oksidatif strese sebep olduğu gözlenirken, gallik asit uygulanmasının fenitrotiyon sebepli toksisiteyi azalttığı gözlenmiştir.

**Anahtar Kelimeler:** Fenitrotiyon, Gallik Asit, Kalp, Antioksidan, Oksidatif Stres

**Abstract**

Fenitrothion (FNT) is a broad-spectrum organophosphate insecticide. It is used in agriculture to control pests in rice, grains, fruits, vegetables. Gallic acid (GA), a member of the tannins group of polyphenols, is a powerful antioxidant and provides effective protection against oxidative damage. The body has its own antioxidant system consisting of enzymes such as catalase, superoxide dismutase. The cellular defense system against oxidative damage is intrinsically activated by glutathione and other enzymes that convert oxidized molecules into reducing agents. In this study, it was investigated how gallic acid had protective effects on the damaged rat heart tissue caused by fenitrothion. This experimental study was carried out by following appropriate protocols within ethical standards for the use of laboratory animals, with the approval of the Local Ethics Committee for Animal Experiments (G.Ü.ET-19.025). In the study, experimental animals were divided into 4 groups. These; control group, gallic acid-treated group, FNT treated-group and FNT plus gallic acid treated group. The chemicals were given to the rats by gavage once a day for 28 days. At the end of the 4th week, malondialdehyde (MDA) levels and antioxidant enzyme activities (SOD, CAT, GPx and GST) in rat heart tissues were investigated spectrophotometrically by various methods. At the end of 4th week, MDA levels, antioxidant enzyme [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST)] activities investigated compared to control group. No significant differences were observed between control and gallic acid treated groups. End of the fourth week, fenitrothion increased the levels of MDA while a decrease in SOD, CAT, GPx and GST enzyme activities were observed in comparison with the control group rats. In the study it is observed that fenitrothion caused oxidative stress in rat heart tissue, gallic acid treatment reduced fenitrothion induced toxicity.

**Key words:** Fenitrothion, Gallic acid, Heart, Antioxidants, Oxidative stress

## 1.INTRODUCTION

Organophosphorus compounds are widely used in agriculture, medicine and industry. Organophosphorus pesticides, in addition to their intended effects like control of insects or other pests, are sometimes found to affect nontarget organisms including humans (Cantelli-Forti et al., 1993). Organophosphates (OP) are the term that includes all insecticides containing phosphorus, Fenitrothion [O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphonothioate], which is a yellow-brown liquid with an unpleasant odour at room temperature. The OP insecticides act on the cholinergic nervous system by inhibiting the acetylcholinesterase as the primary target. (Casida and Durkin, 2013) Fenitrothion (FNT) [O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphonothioate] is a broad spectrum organophosphorus pesticide that distresses the nervous system by inhibiting acetyl cholinesterase activity (Sarikaya, 2004). That inhibition results in the accumulation of acetylcholine (Ach) at the neurone/neurone and neurone/muscle junctions or synapses, causing rapid twitching of voluntary muscles and finally paralysis (Afshar et al., 2008). Many pesticides in common use can produce some toxic and adverse effects on liver, kidney, thyroid gland and other biological systems (Poovala et al., 1999, Kovacic, 2003). One of the most important effects of FNT toxicity is that it causes cell damage by inducing oxidative stress (Galal et al., 2019) Oxidative stress, free radicals and antioxidant defense system is relevant. These toxic effects of pesticides occur through the generation of reactive oxygen species (ROS) that may be produced as a result of the FNT metabolism by cytochrome P450s or due to the high-energy consumption coupled with the inhibition of oxidative phosphorylation (Milatovic et al., 2006). The imbalance between the formation of ROS and mechanism of enzymatic and non-enzymatic antioxidants as a body defense system can lead to oxidative stress. It is reported to be the primary mechanism of organophosphate toxicity after prolonged exposure (Lukaszewicz-Hussain, 2010). Antioxidants have proved to be a good defense mechanism against free radical effects, which might be produced from contamination with pesticide and other toxic substances (Glodfarb, 1999, Valko et al., 2007). The body contains its own antioxidant system, made up by enzymes like catalase, super oxide dismutase and metal binding proteins. Cellular defense mechanism to oxidative damage is activated endogenously by glutathione and other enzymes which convert the oxidized molecules to their reduced form. The endogenous defense mechanism against oxidative damage is complemented by antioxidants. The aim of this study is to investigate the possible toxic effects of FNT which is an organophosphate insecticide, on rat heart tissue and the protective effects of gallic acid.

## MATERIAL AND METHOD

Permission was obtained from Gazi University Animal Experiments Local Ethics Committee for this study (G.U. ET-19.025).

### Experimental Animals

The experimental animals were 24 male Wistar rats and they are approximately 250-300 gr weight. They were kept in special cages (6 rats per cage) and fed by special lab diet and water.

### Groups and Treatments

Experimental animals were divided into 4 groups. These; control group (1ml/kg body weight distilled water), gallic acid-treated group (50 mg/kg b.w gallic acid), FNT treated-group (1/25 $LD_{50}$, 32 mg/kg bw) and FNT plus gallic acid treated group (50 mg/kg body weight gallic acid and 32 mg/kg b.w fenitrothion). The substances were administered to the rats by gavage once a day for 28 days.

### Measurement of Lipid Peroxidation and Antioxidant Enzyme Activities

On the purpose of determination of the MDA levels, heart tissue was incubated at 95 ºC with thiobarbituric acid for 60 minutes. A pink-coloured formation was observed from the reaction of thiobarbituric acid with MDA. MDA amounts were evaluated by spectrophotometer at 532 nano meter absorbance (Ohkawa et al., 1979).

For antioxidant enzyme activities, the method of Marklund and Marklund (1974) was used to determine the total amount of SOD in the heart tissues and absorbance was measured at 440 nm for 3 minutes. The method described by Aebi (1984) was used to determine CAT activity and absorbance was measured at 240 nm for 1 minute. The method of Pagline and Valentine (1987) was used for the determination of GPx and was measured in a spectrophotometer at 340 nm. For the analysis of GST activity, the method determined by Habig et al. (1974) was used and measured at 340 nm in a spectrophotometer.

**Statistical Analysis**

Obtained biochemical analyses were performed with SPSS IBM Statistics 22 version. One-way analysis of variance and Tukey tests were used and P<0.05 was accepted as significant.

## RESULTS

**Assessment of MDA Levels**

No significant differences were observed while analyzing parameters between control and gallic acid treated groups. MDA level in heart of FNT-treated rats showed increase in comparison with control group. In addition to this, MDA level in heart of FNT+GA treated rats showed decrease in comparison with FNT treated rats (Figure1).

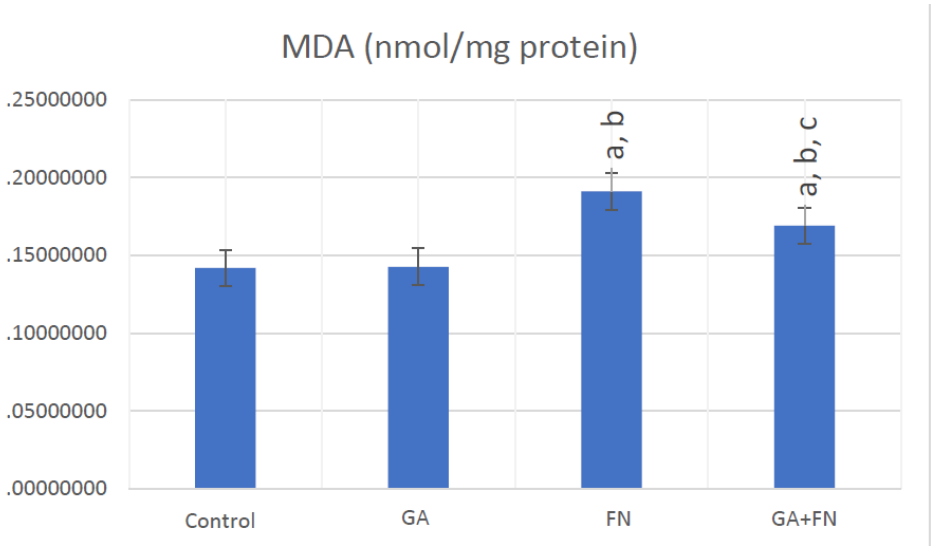


**Figure 1.** The effect of FNT and GA on the amount of MDA in the rat heart tissue. [a]Comparison of control group and other groups, comparison of [b]GA-treated group and other groups, comparison of [c]FNT-treated group and other groups. Mean ± standard deviation (P<0.05).

**Assessment of Antioxidant Enzyme Activities in Heart**

When comparing antioxidant enzyme activities between control group and GA-treated group, no significant differences were observed. SOD, CAT, GPx and GST enzyme activities in heart in FNT-treated rats decreased in comparison with the control group rats. Antioxidant enzyme activities increased in comparison with FNT+GA treated rats (Figure2-5).

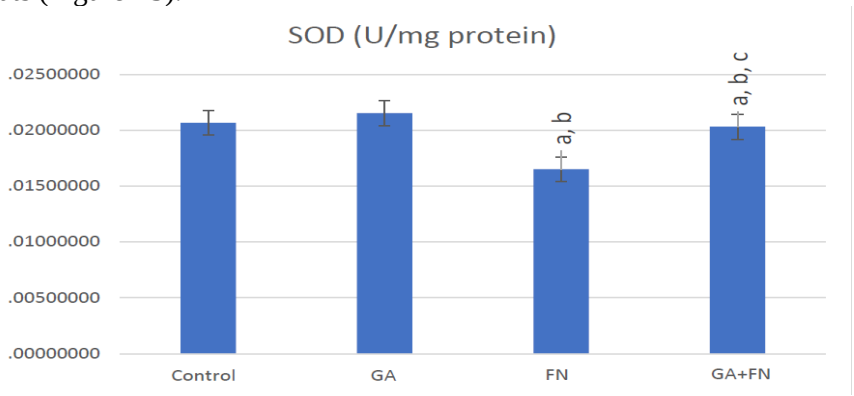


**Figure 2.** The effect of FNT and GA on the amount of SOD in the rat heart tissue. [a]Comparison of control group and other groups comparison. [b]GA-treated group and other groups comparison. [c]FNT-treated group and other groups comparasion. Mean ± standard deviation (P<0.05).

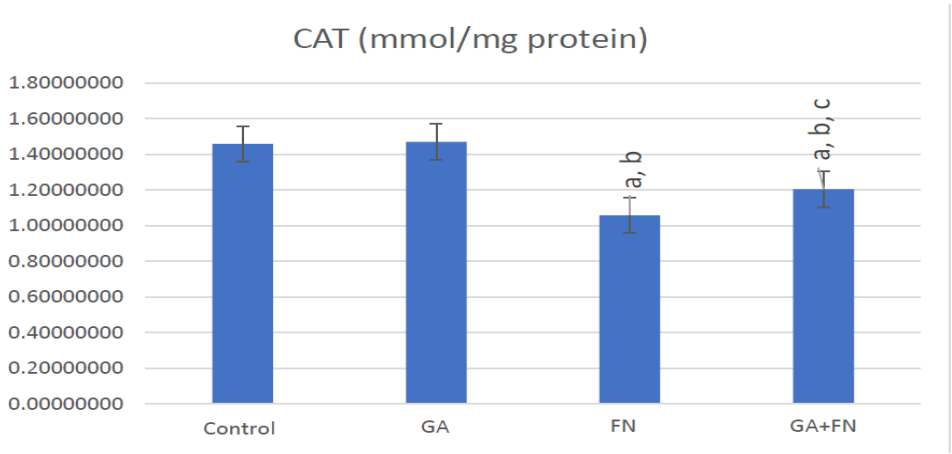


**Figure 3.** The effect of FNT and GA on the amount of CAT in the rat heart tissue. [a]Comparison of control group and other groups comparison. [b]GA-treated group and other groups comparison. [c]FNT-treated group and other groups comparasion. Mean ± standard deviation (P<0.05).

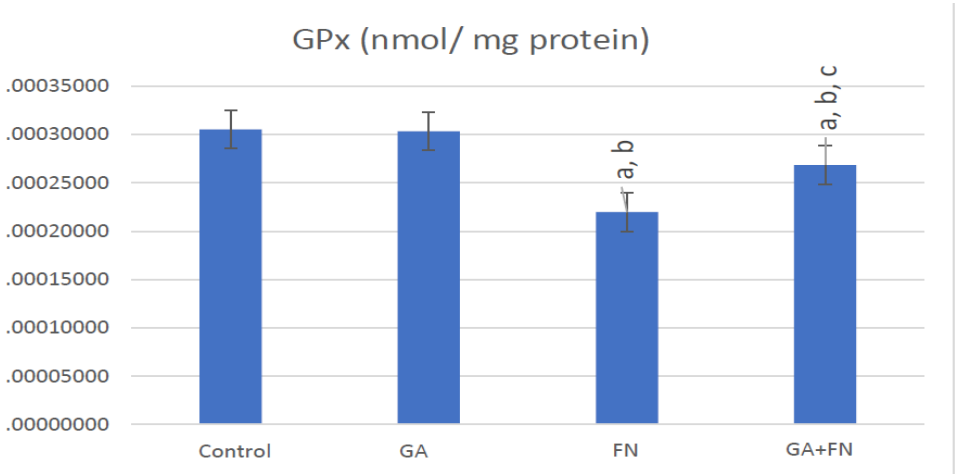


**Figure 4.** The effect of FNT and GA on the amount of GPx in the rat heart tissue. [a]Comparison of control group and other groups comparison. [b]GA-treated group and other groups comparison. [c]FNT-treated group and other groups comparasion. Mean ± standard deviation (P<0.05).

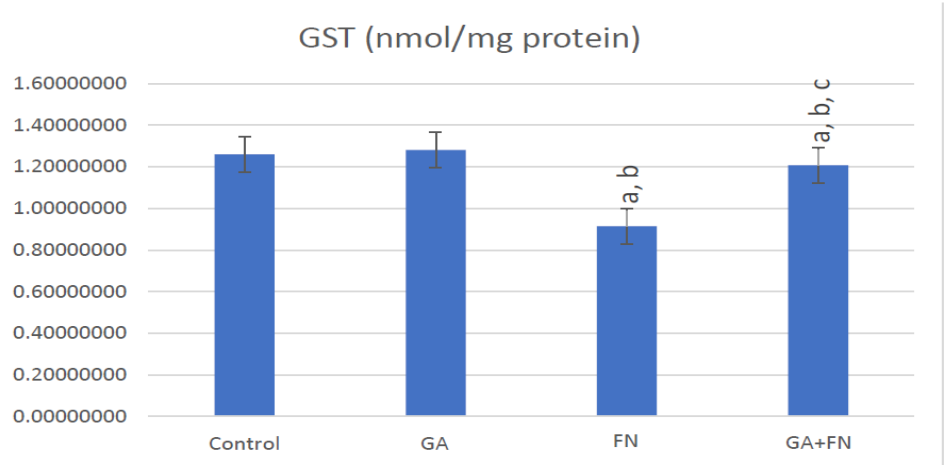


**Figure 5.** The effect of FNT and GA on the amount of GST in the rat heart tissue. [a]Comparison of control group and other groups comparison. [b]GA-treated group and other groups comparison. [c]FNT-treated group and other groups comparasion. Mean ± standard deviation (P<0.05).

## RESULTS AND DISCUSSION

A variety of methods are used in pest control, the most effective and most widely used method is chemical insecticides. Unconsciously used these chemical insecticides cause deterioration of ecological balance, these insecticides also constitute a serious threat for non- target organisms. It is stated that pesticides caused pathological changes in mammalian tissues (Kalender et al., 2004, 2005, 2007; Kalender et al., 2006; Uzunhisarcikli et al., 2007; Ogutcu et al., 2006, 2008; Uzun et al., 2009). It has been observed that pesticides cause an increase in the level of MDA, which is the end product of lipid peroxidation, an increase in free oxygen radicals and a decrease in antioxidant activities, resulting in oxidative stress (Banerjee et al. 1999). MDA, a major oxidation product of peroxidized polyunsaturated fatty acids, has been used to determine the degree of lipid peroxidation and as a biological marker of oxidative stress (Rael et al.,2004; Del rio et al., 2005). It was determined that lipid peroxidation increased in rats treated with FNT (Alam et al., 2019; Elhalwagy et al., 2008). In another study, it was determined that FNT applied to rats increased lipid peroxidation in brain tissue (El-Demerdash, 2011). In this study, an increase in the amount of MDA was observed in rat heart tissue as a result of FNT treatment. This can be considered as an indication of a damage in membranous structures in the cell. The cellular antioxidant system comprises of integral antioxidants and also different free radical scavenging antioxidant enzymes against oxidative injury. Among the antioxidant enzymes, SOD, GPx and GST are the first line of defense against oxidative injury. The inhibition of antioxidant system may cause the accumulation of $H_2O_2$ or products of its decomposition (Halli-well, 1994). SOD quenches $O_2^-$ into the corresponding $H_2O_2$ and $H_2O$ (Fridovich, 1972). It has been also reported that organophosphate pesticides caused a decrease in GPx activity both in vivo and in vitro (Altuntas et al., 2003; Verma and Srivastava, 2003). SOD and CAT have the most effective antioxidant enzyme defense mechanism against eliminating superoxide anion radical and reducing hydrogen peroxide concentration (Patlevic et al., 2016). There are many studies on antioxidants preventing/reducing pesticide toxicity (Kalender et al., 2004; Apaydın et al., 2017; Adıguzel and Kalender, 2020). Gallic acid is a common and important antioxidant reported to occur in various plants such as oak bark, tea leaves, green tea, apple peel, grapes (Zanwar et al., 2014). It has been stated that gallic acid reduces oxidative stress and increases antioxidant enzyme activities in rats (Fanaei et al., 2021, Adıguzel et al., 2021a). It was reported that gallic acid reduces the oxidative stress caused by FNT in rat blood as well (Adıgüzel et al., 2021b). In this study FNT caused to decrease enzyme activities. It is thought FNT inhibits enzyme activities or provokes deformations in structure of enzymes. Our findings suggest that a tested dose of gallic acid can some protective role, but not completely, against FNT-induced toxicity.

## 5.REFERENCES

Adıgüzel Ç, Kalender Y 2020. Bendiocarb Induced Nephrotoxicity in Rats and The Protective Role of Vitamins C and E. Environmental Science and Pollution Research, 27: 6449-6458.

Adıgüzel Ç, Apaydın FG 2021a. The Protective Role of Gallic Acid on Fenitrothion Induced Epididymal Toxicity in Rats. 4th International Eurasian Conference on Biological and Chemical Sciences (Eurasian BioChem 2021). Ankara, Türkiye, 24-26 November.

Adıgüzel Ç, Apaydın FG, Kalender Y 2021b. Fenitrothion'un Sıçan Kanında Meydana Getirdiği Oksidatif Stres Üzerine Gallik Asit'in Koruyucu Rolü, 3. Ulusal Başkent Disiplinler Arası Bilimsel Çalışmalar Kongresi, Ankara, Türkiye, 14-15 Mart.

Aebi H 1984. Catalase in vitro. Methods in Enzymology, 105: 121-126.

Afshar S, Farshid AA, Heidari, R, Ilkhanipour M 2008. Histopathological Changes in the Liver and Kidney Tissues of Wistar Albino Rat Exposed to Fenitrothion. Toxicology and Industrial Health, 24: 581–586.

Alam TR, Imam ST, Elmaaty AMA, Arisha HA 2019. Amelioration of Fenitrothion Induced Oxidative DNA Damage and Inactivation of Caspase-3 in the Brain and Spleen Tissues of Male Rats by N-Acetylcysteine. Life Sciences, 231: 116534.

Altuntas I., Delibas N, Doguc DK, Ozmen S, Gultekin F 2003. Role of reactive oxygen species in organophosphate insecticide phosalone toxicity in erythrocytes in vitro. Toxicology in vitro, 17: 153-157.

Apaydın FG, Baş H, Kalender S, Kalender Y 2017. Bendiocarb Induced Histopathological and Biochemical Alterations in Rat Liver and Preventive Role of Vitamins C and E. Environmental Toxicology and Pharmacology, 49: 148-155.

Banerjee DB, Seth V, Bhattacharya A, Pasha TS, Chakraborty KA 1999. Biochemical Effects of Some Pesticides on Lipid Peroxidation and Free Radical Scavengers. Toxicology Letters, 107: 33-47.

Cantelli-Forti G, Paolini M, Hrelia P 1993. Multiple end point procedure to evaluate risk from pesticides. Environmental Health Perspectives 101: 15-20.

Casida JE, Kathleen AD 2013. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects." Annual Review of Entomology 58: 99-117.

Del Rio D, Stewart AJ, Pellegrini N 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, metabolism and cardiovascular diseases, 15: 316-328.

El-Demerdash FM 2011. Lipid Peroxidation, Oxidative Stress and Acetylcholinesterase in Rat Brain Exposed to Organophosphate and Pyrethroid Insecticides. Food and Chemical Toxicology, 49: 1346–1352.

Elhalwagy MEA, Darwish NS, Zaher EM 2008. Prophylactic Effect of Green Tea Polyphenols Against Liver and Kidney Injury Induced by Fenitrothion Insecticide. Pesticide Biochemistry and Physiology, 91: 81–89.

Fanaei H, Mard SA, Sarkakil A, Goudarzi G, Khorsandi L 2021. Gallic acid Protects the Liver Against NAFLD Induced by Dust Exposure and High Fat Diet Through Inhibiting Oxidative Stress and Repressing the Inflammatory Signaling Pathways NF-kβ/TNF-α/IL-6 in Wistar Rats. Avicenna Journal of Phytomedicine, 11: 527-540.

Fridovich Irwin 1972. Superoxide radical and superoxide dismutase. Accounts of Chemical Research. 5: 321-326.

Galal AAA, Ramadan RA, Metwally MMM, El-Sheikh SMA 2019. Protective effect of N-acetylcysteine on fenitrothion-induced toxicity: The antioxidant status and metabolizing enzymes expression in rats. Ecotoxicology and Environmental Safety, 171: 502-510.

Goldfarb AH 1999. Nutritional antioxidants as therapeutic and preventive modalities in exercise-induced muscle damage. Canadian Journal of Applied Physiology, 24: 249-266.

Halliwell B 1994. Free radicals and antioxidants: a personal view., Nutrition Reviews, 52: 253-265.

Kalender S, Kalender Y, Ogutcu A, Uzunhisarcıklı M, Durak D, Açıkgöz F 2004. Endosulfan-induced cardiotoxicity and free radical metabolism in rats: the protective effect of vitamin E. Toxicology, 202: 227-235.

Kalender Y, Yel M, Kalender S 2005. Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats: the effects of vitamin E and catechin. Toxicology 209: 39-45.

Kovacic Peter 2006. Mechanism of organophosphates (nerve gases and pesticides) and antidotes: electron transfer and oxidative stress. Current Medicinal Chemistry, 10: 2705-2709.

Lukaszewicz-Hussain A. 2010. Role of oxidative stress in organophosphate insecticide toxicity–Short review. Pesticide Biochemistry and Physiology, 98: 145-150.

Marklund S, Marklund G 1974. Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. European Journal of Biochemistry, 47: 469-474.

Milatovic D, Gupta RC, Aschner M 2006. Anticholinesterase toxicity and oxidative stress. The Scientific World Journal 6: 295-310.

Ogutcu A, Uzunhisarcikli M, Kalender S, Durak D, Bayrakdar F, Kalender Y 2006. The effects of organophosphate insecticide diazinon on malondialdehyde levels and myocardial cells in rat heart tissue and protective role of vitamin E. Pesticide Biochemistry and Physiology, 86: 93-98.

Ogutcu, Ayse, Zekiye Suludere, Yusuf Kalender 2008. Dichlorvos-induced hepatotoxicity in rats and the protective effects of vitamins C and E. Environmental Toxicology and Pharmacology 26.3: 355-361.

Ohkawa H, Ohishi N. Yagi K 1979. Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. Analytical Biochemistry, 95: 351–358.

Paglia DE, Valentine NW 1967. Studies on the Quantative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase. Journal of Laboratory and Clinical Medicine, 70: 158–169.

Patlevič P, Vaskova J, Svorc P, Vasko L, Svorc P 2016. Reactive oxygen species and antioxidant defense in human gastrointestinal diseases. Integrative Medicine Research, 5: 250-258.

Poovala VS, Huang H, Salahudeen AK 1999. Role of reactive oxygen metabolites in organophosphate-bidrin-induced renal tubular cytotoxicity. Journal of the American Society of Nephrology, 10: 1746-1752.

Rael LT, Gregory T, Michael C, Raphael P, David B 2004. Lipid peroxidation and the thiobarbituric acid assay: standardization of the assay when using saturated and unsaturated fatty acids. BMB Reports, 37: 749-752.

Sarikaya R, Selvı M, Erkoç F 2004. Investigation of acute toxicity of fenitrothion on peppered corydoras (*Corydoras pale*atus) (Jenyns, 1842). Chemosphere 56: 697-700.

Uzun FG, Kalender S, Durak D, Demir F, Kalender Y 2009. Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E. Food and Chemical Toxicology 47: 1903-1908.

Uzunhisarcikli M, Kalender Y, Dirican K, Kalender S, Ogutcu A, Buyukkomurcu F 2007. Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. Pesticide Biochemistry and Physiology 87: 115-122.

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J 2007. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology 39: 44-84.

Verma RD, Mehta A, Srivastava N 2007. In vivo chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins. Pesticide Biochemistry and Physiology, 88: 191-196.

Zanwar AA, Badole LS, Shende SP, Hegde VM, Bodhankar LS 2014. Role of Gallic Acid in Cardiovascular Disorders. Polyphenols in Human Health and Disease, 2: 1045-1047.

## *ORAL PRESENTATION*

# Production of ethanol and lactic acid from agricultural wastes by fermentation by *Rhizopus oryzae*

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## Abstract

Natural resources have progressively started to decline as a result of the quickly growing population and the following expanding industrialization. This situation has led to the search for renewable alternative sources instead of fossil-based resources. For production from renewable resources in a sustainable future, the conversion of carbohydrate by-products to value-added products is essential. Biomass-derived products are the only practical options for the production of transportation fuels and platform chemicals. Biomass can be converted into fuels and platform chemicals using fermentation processes with the aid of microorganisms. *Rhizopus oryzae* is a fungus, uses sugars from biomass to make ethanol, L-(+)-lactic acid, and fumaric acid. Thanks to the amylase enzymes in its content, it is useful in breaking down starch by using plant-based wastes and consequently obtaining products such as ethanol, enzymes, organic acids like lactic acid, antibiotics, proteins, and vitamins. In this study, ethanol and lactic acid production from potato peel wastes, horse chestnut seeds, and peanut shells were studied by using *R. oryzae*. FTIR analysis was performed to identify the functional groups of the waste materials used in the study. Lactic acid, ethanol, and glucose concentrations were measured by HPLC. The highest ethanol conversion efficiency with 86% was obtained from potato peel, and the highest lactic acid conversion efficiency with 4% was obtained from horse chestnut.

**Keywords:** *Rhizopus oryzae*, Ethanol, Lactic acid, Potato peel, Horse chestnut, Peanut shell

## INTRODUCTION

The increase in the world population, industrialization and excessive use of fossil resources, and the environmental problems that have reached global dimensions over time have changed the view of states on renewable energy sources. Renewable energy sources have become a significant power for countries that rely on foreign energy (Sabancı et al., 2009).

Fungi are eukaryotic organisms. They cannot perform photosynthesis, therefore, as heterotrophs, they readily obtain their nutritional needs from other living things. They develop aerobically and obtain energy by breaking down organic materials (Akkara and Tosun, 2014).

Fungi can be used in many industrial fermentative processes such as the production of vitamins, pigments, lipids, glycolipids, polysaccharides, and polyhydric alcohols (Adrio and Demain, 2003). They have antimicrobial activity (Ranadive et al., 2013). They are used in biomineralization (Das et al., 2012; Gadd, 2021). They are also used as food (Derbyshire and Delange, 2021; Dupont et al., 2017; Ranadive et al., 2013), and biofertilizers (Odoh et al., 2020) due to their high protein content. Fungi are preferred more than yeasts and bacteria because they are rich in B vitamins and amino acids, they are more resistant to factors such as pH and temperature, and they are economical in terms of cost.

Filamentous fungi can degrade lignocellulosic biomass and have a natural ability to convert their degradation products, for example glucose, into various organic acids (Liaud et al., 2014; Ruijter et al., 2002). These fungi are mostly *Aspergillus* (citric, gluconic, malic and itaconic acids) and *Rhizopus* (lactic and fumaric acids). Some of these organic acids can be produced by large-scale bioprocesses (Magnuson and Lasure, 2004). Organic acids have multiple industrial applications as food additives, pharmaceutical, and cosmetic excipients (Sauer et al., 2008).

Although ethanol production is quite common among certain species, the ability of filamentous fungi to ferment ethanol is not well known. However, many fungi belonging to several different genera can produce ethanol at low to moderate concentrations under oxygen-limited conditions compared to *Saccharomyces cerevisiae*. Studies using filamentous fungi for ethanol production have shown that these fungi can also be

used in industrial biofuel production (Skory et al., 1997). The ethanol production from cellulosic biomass by fungal fermentation is becoming increasingly attractive as it helps prevent environmental pollution problems.

*R. oryzae* is a filamentous fungus in the Mucoromycota branch Mucorales order. The genus *Rhizopus* is known to produce fermentation products such as ethanol, L-(+)-lactic acid, fumaric , and to a lesser extent L-(+) malic acid. *R. oryzae* is ubiquitous and is found in decaying organic matter. They can grow on a wide variety of carbon sources such as glycerol, ethanol, lactic acid, glucose, mannose, fructose, sucrose, xylose, cellobiose, fatty acids , and oils (Meussen et al., 2012). All the sugars mentioned are to be a substrate for the production of L-(+) lactic or fumaric acid. *R. oryzae* was first described in 1936 as a lactic acid producer. In addition, *R. oryzae* has amylolytic (Amadioha, 1998), xylanolytic (Bakir et al., 2001), pectinolytic (Saito et al., 2003) , and cellulolytic (Karmakar and Ray, 2010) properties,which provide enabling the conversion of polymeric agricultural wastes. *R. oryzae* can grow well in a wide temperature range (up to 40 °C) and pH range (from 4 to 9) therefore it has potential use in industrial applications.

Lactic acid is a naturally occurring organic acid. Since it has both carboxylic and hydroxyl groups, it is used in versatile application areas in the food, pharmaceutical, textile, leather, and other industries. Lactic acid can be produced by chemical synthesis or by microbial fermentation. However, optically pure L-(+) or D-(-) lactic acid can only be obtained by a biotechnological fermentation process with suitable microorganisms. Compared to chemical synthesis, the biotechnological process offers several advantages for lactic acid production, such as low substrate costs, production temperature, and energy consumption.

There are two problems in the biotechnological production of pure lactic acid. The first problem is the cost of the substrate, because sugar needs to be added as a carbon source. This problem can be solved by using many economically cost-effective renewable raw materials (such as molasses, starch, lignocellulose , and agricultural wastes) , and agro-industrial residues as substrates for the fermentative production of lactic acid. However, most starchy and lignocellulosic materials need to be pretreated by physicochemical and enzymatic methods. Because these materials cannot be used directly by lactic acid fermenting microorganisms (Gao et al., 2011).

Fermentations made with *Rhizopus* strains compared to *Lactobacillus spp.* It is preferred more than bacterial systems since *Rhizopus* only synthesizes L-(+) lactic acid, while *Lactobacillus spp.* usually produces a mixture of isomers (Lin et al., 2007; Oda et al., 2003).

It is known that *R. oryzae* produces L-(+) lactic acid under minimum conditions by using different carbon sources, which reduces the fermentation process cost and facilitates the subsequent processes for product recovery (Açar et al., 2007; Bai et al., 2003; Efremenko et al., 2006; Londoño-Hernández et al., 2017; Maksimova, 2014).

Although many studies have been conducted on lactic acid and ethanol production from potato industry wastes with various microorganisms, lactic acid and ethanol production by *R. oryzae* using potato peel has not been studied before. Also, there is no study in the literature on the use of horse chestnut seeds for ethanol and/or lactic acid fermentation. This study will be the first in which horse chestnut is used as a substrate for ethanol and lactic acid production with *R. oryzae*. Microorganisms such as *Aspergillus niger, Bacillus coagulans, Geobacillus stearothermophilus* were used in a few studies where peanut shell was used as a substrate for ethanol production. No previous studies have been conducted on the production of ethanol and/or lactic acid from peanut shells by *R. oryzae*. This study aims to demonstrate the production potential of lactic acid and ethanol from potato peel, horse chestnut seed and peanut shell by *R. oryzae*.

## MATERIALS AND METHODS

### Microorganism, Media Preparation and Incubation Medium

*R. oryzae* ATCC 9363 strain was used in the study. *R. oryzae* inoculated on solid media containing "Potato Dextrose Agar" (PDA) was incubated at 30 °C for three days, then spores were collected and spore suspension was prepared. Spore counts were made using a hemocytometer. The composition of the liquid media, excluding the carbon source, is 2 g/L $(NH_4)_2SO_4$, 0.65 g/L $KH_2PO_4$, 0.25 g/L $MgSO_4.7H_2O$ and 0.05 g/L $ZnSO_4.7H_2O$. In the experiments, potato peel, horse chestnut, and peanut shell as agricultural waste were used and 2 g/L glucose as a carbon source as well as these wastes were added to the medium at a concentration of 40 g/L. The media were prepared in 250 mL flasks with a final volume of 100 mL. Then, the flasks containing these media were sterilized in an autoclave at 121 ºC for 15 minutes. The flasks, which were cooled to room temperature after autoclaving, were inoculated with a spore number of $10^5$ spores/ml and incubated at 150 rpm, 30 °C in a shaking incubator.

The potatoes and peanuts were bought from local markets, and the horse chestnuts were collected from a horse chestnut tree. The potatoes were washed and peeled. The potato peels were dried in an oven at 75 °C for three

days and ground with the help of a chopper. Afterward, the ground potato peels were sieved with a range of 1-2 mm with a sieve shaker.The horse chestnuts were ground to powder form after removing their shells and kept in an oven at 75 °C for three days. The outer shells of peanuts were ground (Figure 1).

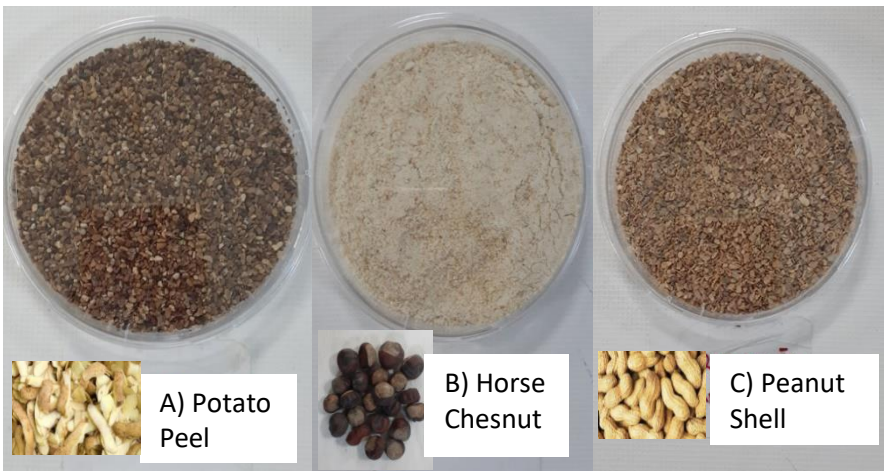


**Figure 1.** The ground agricultural wastes. A) Potato peel B) Horse chestnut C) Peanut shell

## Analysis Methods

In order to evaluate and define the starch content of potato peel, horse chestnut and peanut shells used in the study, Fourier Transform Infrared (FTIR, Perken-Elmer) analysis was performed with ATR equipment in the wavelength range of 4000-650 $cm^{-1}$ at Yıldız Technical University Science and Technology Application and Research Centre. FTIR spectra show absorption bands with characteristic frequency attributed to different functional groups.

In the experiments, the changes in the glucose, lactic acid and ethanol concentrations in the fermentation media were determined by HPLC. HPLC analyzes were performed using Phenoemenex Rezex Cal Organic acid column (300x7.8 mm), 0.005 N $H_2SO_4$ as mobile phase and refractive index detector as detector. The flow rate was set at 0.6 mL/min and the column temperature was set at 70 °C. The injection volume is 20 µL for standard solutions and samples.

## RESULTS and DISCUSSION

### FT-IR Analysis of Potato Peel, Horse Chestnut and Peanut Shell

FTIR is a powerful technique for identifying chemical bond types and functional polymer groups. FTIR analyzes were carried out to identify the functional groups of the waste materials used in the study and the FTIR spectra are given in Figure 2. According to the FTIR spectra, the plant wastes used in the study contain similar basic peaks. For the production of lactic acid and ethanol, complex carbohydrates in agricultural wastes must be converted into simple sugars by *R. oryzae*. Therefore, the presence of polysaccharide peaks in the FT-IR spectra attracts attention.




**Figure 2.** FT-IR spectra of agricultural wastes A) Potato peel B) Peanut shell C) Horse chestnut

**Lactic Acid and Ethanol Production with Potato peel**

It can be seen in Figure 3 that *R. oryzae* consumes the 0.2% (w/v) glucose that was added at the beginning within 21 hours, and after the 21st hour, it decomposed the starch in the potato peel with amylase activity and converted it into glucose. The glucose concentration in the medium increased up to 0.15% (w/v) due to simultaneous saccharification and fermentation, but remained in the range of 0.07-0.03% (w/v) until the fermentation was lasted.

Ethanol production started at the 21st hour and reached the highest concentration (0.878% (w/v)) at the 54th hour. It is thought that the decrease in ethanol concentration until the end of fermentation is due to evaporation. Theoretically, the highest ethanol yield obtained from 1 g glucose is 0.511 g ethanol. Considering that the starch ratio in the potato peels (dry weight) used in the study was 52% according to the literature, and considering that all of the glucose used in the medium was used as a result of the initial glucose and saccharification, The ethanol conversion efficiency was 86%.

Lactic acid production started at the 31st hour and reached the highest concentration (0.07% (w/v)) at the 46th hour. Although *R. oryzae* is known as a lactic acid producer, it is seen in this study that ethanol production is approximately 12 times higher than lactic acid production. Huang et al. (2005) obtained about 28 times this amount in their study with potato starch wastewater. Theoretically, the highest lactic acid yield obtained from 1 g glucose is 1 g lactic acid. The lactic acid conversion efficiency obtained in this study was 3%.

Especially at low glucose concentrations, pyruvate decarboxylase enzyme works faster than lactate dehydrogenase enzyme due to the low pyruvate concentration. Therefore, ethanol production is higher at low glucose concentrations (Büyükkileci, 2007). In addition, potato peels added to the medium increase the viscosity and adversely affect the oxygen distribution in the environment. During aerobic growth, *R. oryzae* produces L-lactic acid from lactate dehydrogenase-mediated reduction of pyruvate, while producing mainly ethanol under $O_2$-limiting conditions (Skory et al., 1998).

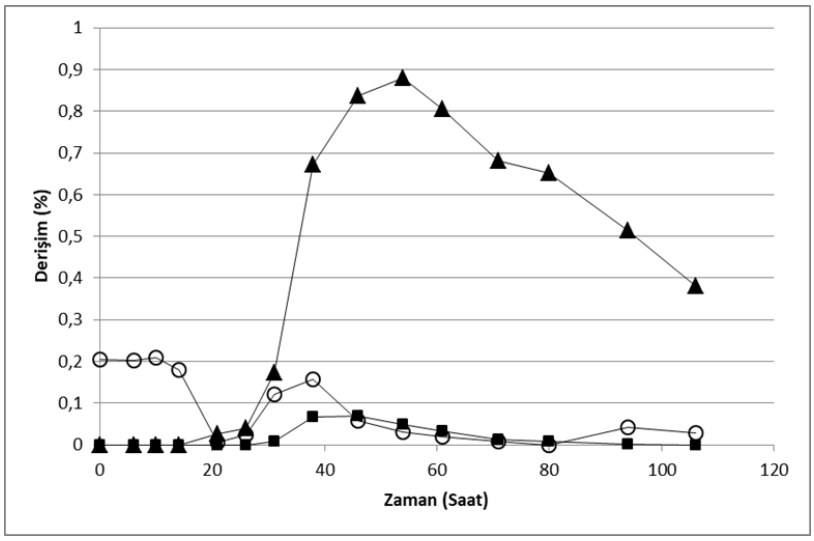


**Figure 3.** The time-dependent variations in glucose (○), lactic acid (■), and ethanol (▲) concentrations (%) in potato peel-containing fermentation media.

**Lactic Acid and Ethanol Production with Horse Chestnut**

In the study conducted using horse chestnut seeds as agricultural waste, it was determined that *R. oryzae* consumed 0.2% (w/v) glucose that was added at the beginning and the glucose (0.6% (w/v) in total) contained in the horse chestnut within 71 hours, in the 21st hour. After the glucose concentration in the medium decreased to 0.36% (w/v), it is seen in Figure 4 that the glucose amount increased to 0.75% (w/v) at the 26th hour as a result of the breakdown of the starch contained in the horse chestnut seed with the amylase activity of *R. oryzae*. .

Ethanol production started from the 14th hour and reached the highest concentration (0.863% (w/v)) at the 46th hour. It is thought that the decrease in ethanol concentration until the end of fermentation is due to evaporation. Considering that the starch and sugar ratio in the horse chestnut seeds (dry weight) used in the study was 70% according to the literature, and considering that all the glucose formed as a result of the initial glucose and saccharification was used, the ethanol conversion efficiency was 59% (Table 4.3).

Lactic acid production started at the 21st hour and reached the highest concentration (0.12% (w/v)) at the 31st hour. When horse chestnut seeds are used as a substrate, it is seen that ethanol production is approximately seven times higher than lactic acid production. The lactic acid conversion efficiency obtained with this substrate was 4%. Since there is no other study in the literature that produced ethanol and/or lactic acid using horse chestnut seeds, the results obtained were compared only with potato peel.

Compared to potato peel, the amount of lactic acid produced by horse chestnut was 1.7 times higher. According to these results, it can be said that the initial glucose concentration is an effective parameter on lactic acid production.

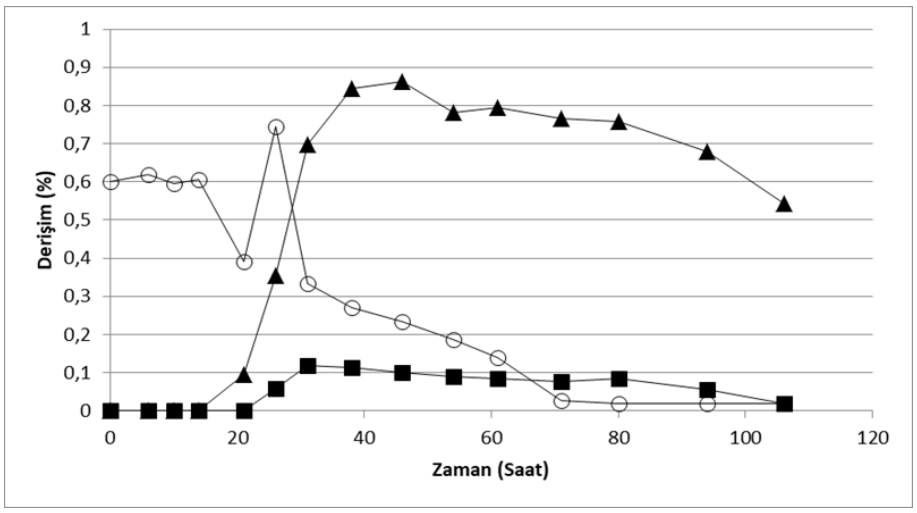


**Figure 4.** The time-dependent variations in glucose (○), lactic acid (■), and ethanol (▲) concentrations (%) in horse chestnut-containing fermentation media.

**Lactic Acid and Ethanol Production by Peanut Shell**

In the study using peanut shell as agricultural waste, it is seen in Figure 5 that *R. oryzae* consumed the 0.2% (w/v) glucose that was added at the beginning within 54 hours.

Ethanol production started from the 26th hour and reached the highest concentration (0.053% (w/v)) at the 38th hour. It is thought that the decrease in ethanol concentration until the end of fermentation is due to evaporation. The ethanol conversion efficiency was calculated as 45%, assuming that cellulose, hemicellulose and lignin in the peanut shells used in the study were not degraded by the cellulosic, hemicellulosic and lignocellulosic enzymes found in *R. oryzae*, and only the glucose added at the beginning was used for the production of ethanol and lactic acid.

Lactic acid production started at the 31st hour and reached the highest concentration (0.005% (w/v)) at the 38th hour. When peanut shell is used as a substrate, it is seen that ethanol production is about ten times higher than lactic acid production. The lactic acid conversion efficiency obtained with this substrate was 2%.

Again, *S. cerevisiae* was used for fermentation in a previous study made from peanut shells. In order to break down cellulose, hemicellulose and lignin, the enzyme Accellerase 1500 was added to the medium. In a short period of twelve hours, 0.22% ethanol was produced with a yield of 61.7% from the medium prepared with peanut shell. In this study, peanut shell medium was found to be suitable for bioethanol production (Huang et al., 2005). However, in the study with *R. oryzae*, since no pretreatment was done with enzymes that would increase the cost and *R. oryzae* could not produce cellulosic, hemicellulosic and lignocellulosic enzymes under fermentation conditions, their ethanol and lactic acid productions remained very low.

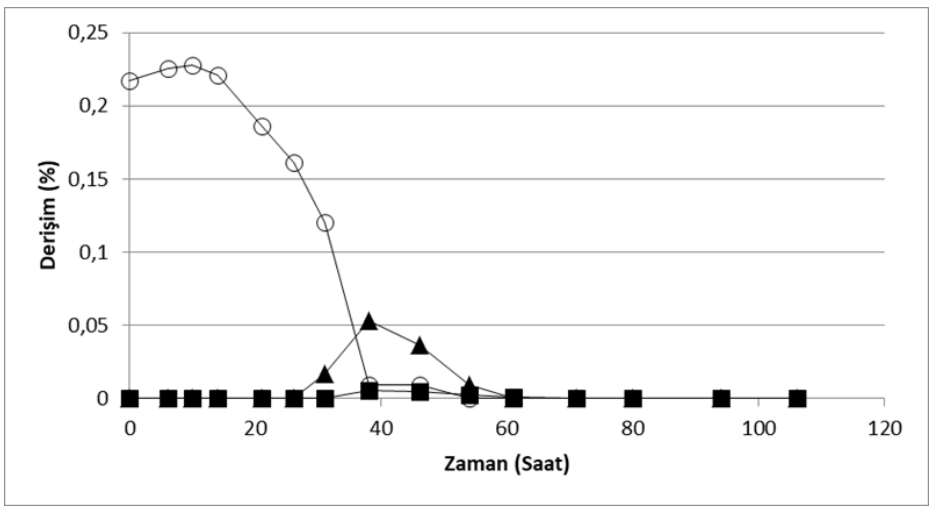


**Figure 5.** The time-dependent variations in glucose (○), lactic acid (■), and ethanol (▲) concentrations (%) in peanut shell-containing fermentation media.

## CONCLUSION

Potato peel, horse chestnut and peanut shells were used as agricultural waste in this study, which was carried out with the aim of reintroducing agricultural wastes that occupy a lot of space in the world.

It was determined that among the agricultural wastes used, the highest lactic acid production was obtained from horse chestnut, followed by potato peel in the second place, and the least amount of lactic acid production was obtained from peanut shell with a rate of 0.005%.

In addition, it was determined that for the ethanol production of *R. oryzae*, as a result of simultaneous saccharification and fermentation, the highest ethanol production was obtained from potato peel, followed by horse chestnut, and the least amount of ethanol production was obtained from peanut shell.

The initial glucose concentration in the medium has important effects on the physiology of *R. oryzae*. In this study, when the glucose concentration in the medium was 0.75%, ethanol production was higher than lactic acid. As a result of the study, it was determined that the ethanol production of *R. oryzae* was 0,879% the most, and the lactic acid production was 0,120% at the most. This study also showed that *R. oryzae* primarily uses lactic acid as a carbon source when glucose is depleted in the medium.

Bioethanol production from biomass can be achieved by using agricultural wastes as an alternative method to the increasing oil costs in recent years. In this case, the use of renewable resources instead of fossil resources has provided important results in terms of both preventing environmental pollution and providing cheap raw materials.

On the other hand, the production of lactic acid obtained from agricultural wastes provides cheap raw materials due to its use in many industries.

The products obtained by using agricultural wastes provide significant gains in terms of meeting the nutritional needs and reducing foreign dependency in today's world where the human population is increasing.

Another advantage is that the food used to obtain ethanol and lactic acid is used for nutritional needs and therefore it has an important value for the protection of forest areas that have been destroyed to open agricultural areas.

As a result, it has been proven that *R. oryzae* can play an active role in integrating agricultural wastes into production, as it can produce both ethanol and lactic acid by containing the starch-degrading amylase enzyme.

## ACKNOWLEDGEMENTS

This study was supported by Kocaeli University Scientific Research Projects Coordination Unit. Project Number: FYL-2020-2162.

## REFERENCES

Açar, Ş., Yücel, M., and Hamamci, H. (2007). Purification and characterisation of two isozymes of pyruvate decarboxylase from Rhizopus oryzae. *Enzyme and Microbial Technology*, *40*(4), 675–682. https://doi.org/10.1016/j.enzmictec.2006.05.028

Adrio, J. L., and Demain, A. L. (2003). Fungal biotechnology. *International Microbiology*, *6*(3), 191–199. https://doi.org/10.1007/s10123-003-0133-0

Akkara, M., and Tosun, H. (2014). Funguslardan Elde Edilen Endüstriyel Ürünler - Industrial Products Derived From Fungi Funguslardan Elde Edilen Endüstriyel Ürünler. *Electronic Journal of Food Technologies*, *9*(2), 46–53.

Amadioha, A. C. (1998). Effect of cultural conditions on the growth and amylolytic enzyme production by Rhizopus Oryzae. *Archives of Phytopathology and Plant Protection*, *32*(1), 41–48. https://doi.org/10.1080/03235409809383275

Bai, D. M., Jia, M. Z., Zhao, X. M., Ban, R., Shen, F., Li, X. G., and Xu, S. M. (2003). L(+)-lactic acid production by pellet-form Rhizopus oryzae R1021 in a stirred tank fermentor. *Chemical Engineering Science*, *58*(3–6), 785–791. https://doi.org/10.1016/S0009-2509(02)00608-5

Bakir, U., Yavascaoglu, S., Guvenc, F., and Ersayin, A. (2001). An endo-β-1,4-xylanase from Rhizopus oryzae: Production, partial purification and biochemical characterization. *Enzyme and Microbial Technology*, *29*(6–7), 328–334. https://doi.org/10.1016/S0141-0229(01)00379-9

Büyükkileci, A. O. (2007). *Investigation of sugar metabolism in* (Issue August). Middle East Technical University.

Das, S. K., Liang, J., Schmidt, M., Laffir, F., and Marsili, E. (2012). Biomineralization mechanism of gold by zygomycete fungi Rhizopous oryzae. *ACS Nano*, *6*(7), 6165–6173. https://doi.org/10.1021/nn301502s

Derbyshire, E. J., and Delange, J. (2021). Fungal Protein – What Is It and What Is the Health Evidence? A Systematic Review Focusing on Mycoprotein. *Frontiers in Sustainable Food Systems*, *5*(February). https://doi.org/10.3389/fsufs.2021.581682

Dupont, J., Dequin, S., Giraud, T., Le Tacon, F., Marsit, S., Ropars, J., Richard, F., and Selosse, M. A. (2017). Fungi as a source of food. *The Fungal Kingdom*, 1063–1085. https://doi.org/10.1128/9781555819583.ch53

Efremenko, E., Spiricheva, O., Varfolomeyev, S., and Lozinsky, V. (2006). Rhizopus oryzae fungus cells producing L(+)-lactic acid: Kinetic and metabolic parameters of free and PVA-cryogel-entrapped mycelium. *Applied Microbiology and Biotechnology*, *72*(3), 480–485. https://doi.org/10.1007/s00253-005-0297-y

Gadd, G. M. (2021). Fungal biomineralization. *Current Biology*, *31*(24), R1557–R1563.

Gao, C., Ma, C., and Xu, P. (2011). Biotechnological routes based on lactic acid production from biomass. *Biotechnology Advances*, *29*(6), 930–939. https://doi.org/10.1016/j.biotechadv.2011.07.022

Huang, L. P., Jin, B., Lant, P., and Zhou, J. (2005). Simultaneous saccharification and fermentation of potato starch wastewater to lactic acid by Rhizopus oryzae and Rhizopus arrhizus. *Biochemical Engineering Journal*, *23*(3), 265–276. https://doi.org/10.1016/j.bej.2005.01.009

Karmakar, M., and Ray, R. . (2010). Extra Cellular Endoglucanase Production by Rhizopus oryzae in Solid and Liquid State Fermentation of Agro Wastes. In *Asian Journal of Biotechnology* (Vol. 2, pp. 27–36).

Liaud, N., Giniés, C., Navarro, D., Fabre, N., Crapart, S., Herpoël- Gimbert, I., Levasseur, A., Raouche, S., and Sigoillo, J.-C. (2014). Exploring fungal biodiversity: organic acid production by 66 strains of filamentous fungi. *Fungal Biology and Biotechnology*, *1*(1), 1–10. http://linkinghub.elsevier.com/retrieve/pii/S1749461309000177

Lin, J., Zhou, M., Zhao, X., Luo, S., and Lu, Y. (2007). Extractive fermentation of l-lactic acid with immobilized Rhizopus oryzae in a three-phase fluidized bed. *Chemical Engineering and Processing: Process Intensification*, *46*(5), 369–374. https://doi.org/10.1016/j.cep.2006.06.015

Londoño-Hernández, L., Ramírez-Toro, C., Ruiz, H. A., Ascacio-Valdés, J. A., Aguilar-Gonzalez, M. A., Rodríguez-Herrera, R., and Aguilar, C. N. (2017). Rhizopus oryzae – Ancient microbial resource with importance in modern food industry. *International Journal of Food Microbiology*, *257*(June), 110–127. https://doi.org/10.1016/j.ijfoodmicro.2017.06.012

Magnuson, J. K., and Lasure, L. L. (2004). Organic Acid Production by Filamentous Fungi. *Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine*, 307–340. https://doi.org/10.1007/978-1-4419-8859-1_12

Maksimova, Y. G. (2014). Microbial biofilms in biotechnological processes. *Applied Biochemistry and Microbiology*, *50*(8), 750–760. https://doi.org/10.1134/S0003683814080043

Meussen, B. J., De Graaff, L. H., Sanders, J. P. M., and Weusthuis, R. A. (2012). Metabolic engineering of Rhizopus oryzae for the production of platform chemicals. *Applied Microbiology and Biotechnology*, *94*(4), 875–886. https://doi.org/10.1007/s00253-012-4033-0

Oda, Y., Yajima, Y., Kinoshita, M., and Ohnishi, M. (2003). Differences of Rhizopus oryzae strains in organic acid synthesis and fatty acid composition. *Food Microbiology*, *20*(3), 371–375.

Odoh, C. K., Eze, C. N., Obi, C. J., Anyah, F., Egbe, K., Unah, U. V., Akpi, U. K., and Adobu, U. S. (2020). Fungal biofertilizers for sustainable agricultural productivity. In A. Yadav, A., Mishra, S., Kour, D., Yadav, N., Kumar (Ed.), *Agriculturally Important Fungi for Sustainable Agriculture.* (1st ed., Vol. 1, pp. 199–225). https://doi.org/10.1007/978-3-030-45971-0_5

Ranadive, K. R., Belsare, M. H., Deokule, S. S., Jagtap, N. V, Jadhav, H. K., and Vaidya, J. G. (2013). Glimpses of antimicrobial activity of fungi from World. *Journal on New Biological Reports*, *2*(2), 142–162.

Ruijter, G. J. G., Kubicek, C. P., and Visser, J. (2002). Production of Organic Acids by Fungi. *Industrial Applications*, 213–230. https://doi.org/10.1007/978-3-662-10378-4_10

Sabancı, A., Ören, M. N., Yaşar, B., Öztürk, H. H., and Atal, M. (2009). Türkiye'de biyodizel ve biyoetanol

üretiminin tarım sektörü açısından değerlendirilmesi. *Ziraat Mühendisliği VII. Teknik Kongresi*, 1–19.

Saito, K., Kawamura, Y., and Oda, Y. (2003). Role of the pectinolytic enzyme in the lactic acid fermentation of potato pulp by Rhizopus oryzae. *Journal of Industrial Microbiology and Biotechnology*, *30*(7), 440–444. https://doi.org/10.1007/s10295-003-0071-z

Sauer, M., Porro, D., Mattanovich, D., and Branduardi, P. (2008). Microbial production of organic acids: expanding the markets. *Trends in Biotechnology*, *26*(2), 100–108.

Skory, C. D., Freer, S. N., and Bothast, R. J. (1997). Screening for ethanol-producing filamentous fungi. *Biotechnology Letters*, *19*(3), 203–206. https://doi.org/10.1023/A:1018337003433

Skory, C. D., Freer, S. N., and Bothast, R. J. (1998). Production of L-lactic acid by Rhizopus oryzae under oxygen limiting conditions. *Biotechnology Letters*, *20*(2), 191–194.

## *ORAL PRESENTATION*

# Mordenit/ZSM-5 Bizeolit Katalizör Üzerinde 1,2,4-Trimetilbenzen ile Pentanın Dealkilsiklizasyonu

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## Özet

Petrokimya sanayi için gerekli olan aromatik bileşiklerin büyük bir kısmı reforming ünitelerinde üretilmektedir. Reforming ünitesinden çıkan reformatlar ağır reformat ve hafif reformat olmak üzere iki kısıma ayrılmaktadır. Hafif reformat benzine direk eklenebililirken ağır reformatlar ise çevre kısıtlamalarından dolayı benzin içindeki aromatiklerin oranının %30'u geçmeyecek şekilde ilave edilmektedir. Durum böyle olunca dünya çapında ağır reformat arzında fazlalılık oluşmaktadır. Ağır reformat bileşiminin %39,0'ını 1,2,4-Trimetilbenzen (TMB) oluşturduğundan bu ürünün değerlendilmesi ayrı bir önem arz etmektedir. Bu ürünün özellikle ticari değeri yüksek ksilenlere özellikle p-ksilen ve o-ksilene dönüştürülmesi yoğun bir şekilde araştırılmaktadır. P-ksilenler poliester sanayinin girdileri olan saf tereftalik asit ve dimetil tereftalat üretimleridir. O-ksilenler Ftalik Anhidrid üretimi ve ilaç endüstrisinde kullanılmaktadır. Dünya genelinde ticari olarak aromatik bileşiklere önemli oranda talep olmasından dolayı aromatiklerin yüksek verimde ve ekonomik üretimi önem arz etmektedir. Petrol reforming ünitesinin ürünü olan ağır reformat içindeki aromatiklerin ticari değeri yüksek aromatik bileşlere dönüştürülmesi son zamanlarda üzerine yoğun çalışmalar yapılmaktadır. Bunlardan birisi de ağır reformat bileşiklerini hafif alkanlarla bir katalizör üzerinde reaksiyona sokarak kıymetli bileşiklerin eldesidir. Bu proseslerde katalizör olarak zeolit kullanımı ön plana çıkmaktadır. Zeolitler, katalitik reaksiyonların yürütülmesini sağlayan mikro gözenekler, kanal sistemi, yüksek yüzey alanı, iyon değiştirme özelliği ve güçlü asidik merkezleri olması ve seçici katalitik dönüşümleri sağlaması nedeniyle avantajlıdır. Bu kapsamda, Mordenit/ZSM-5 bizeolit katalizör üzerinde 1,2,4-TMB ile pentanın dealkilsiklizasyonu sabit yatak süreli bir sistemde deneysel olarak gerçekleştirilmiştir. Zeolit katalizörler Mordenit ve ZSM-5 1:2 oranında hazırlanmıştır. Parametreler, sıcaklık ve boşluk hızı (WHSV) olarak belirlenmiştir. Sırasıyla 400°C, 450 °C, 500°C ve 1,2,3 boşluk hızlarında deneyler yapılmış ve elde edilen sıvı ürünler GC-MS ile analiz edilmiştir. Elde edilen sonuçlara göre sıcaklık arttıkça dönüşüm ve ksilen verimlerinin arttığı görülmüştür. En yüksek m/p-ksilen verimi 500 °C de ve WHSV 2'de ve en yüksek o-ksilen verimi ise 500 °C de WHSV 1'de elde edilmiştir.

**Anahtar Kelimeler:** 1,2,4-Trimetilbenzen, Zeolit Katalizör, Mordenit, ZSM-5, p-Ksilen

# Dealkylcyclization of Pentane with 1,2,4-Trimethylbenzene on Mordenite/ZSM-5 Bizeolite Catalyst

## Abstract

Most of the aromatic compounds required for the petrochemical industry are produced in reforming units. Reformates coming out of the reforming unit are divided into two parts as heavy reformat and light reformat. Light reformate can be added directly to gasoline, while heavy reformates are added in such a way that the proportion of aromatics in gasoline does not exceed 30% due to environmental restrictions. When this is the case, there is an excess of heavy reformat supply around the world. Since 1,2,4-Trimethylbenzene (TMB) constitutes 39.0% of the heavy reformate composition, the evaluation of this product is of particular importance. Conversion of this product to xylenes with high commercial value, especially p-xylene and o-xylene, is being studied intensively. P-xylenes are the production of pure terephthalic acid and dimethyl terephthalate, which are the inputs of the polyester industry. O-xylenes are used in the production of Phthalic Anhydride and in the pharmaceutical industry. Since there is a significant demand for aromatic compounds commercially throughout the world, high efficiency and economic production of aromatics is important. The conversion of aromatics in heavy reformat, which is the product of the oil reforming unit, into aromatic compounds with high commercial value has recently been intensively studied. One of them is to obtain

valuable compounds by reacting heavy reformate compounds with light alkanes on a catalyst. The use of zeolite as a catalyst in these processes comes to the fore. Zeolites are advantageous because they have micropores, channel system, high surface area, ion exchange feature and strong acidic centers that enable catalytic reactions to be carried out, and provide selective catalytic conversions. In this context, dealkylationcyclization of pentane with 1,2,4-TMB on Mordenite/ZSM-5 bizolite catalyst was carried out experimentally in a fixed bed time system. Zeolite catalysts Mordenite and ZSM-5 were prepared in a 1:2 ratio. The parameters were determined as temperature and cavity velocity (WHSV). Experiments were carried out at 400 °C, 450 °C, 500 °C and 1,2,3 void speeds, respectively, and the liquid products obtained were analyzed by GC-MS. According to the results obtained, it was observed that the conversion and xylene yields increased as the temperature increased. The highest m/p-xylene yield was obtained at 500 °C and WHSV 2, and the highest o-xylene yield was obtained at 500 °C at WHSV 1.

**Keywords:** 1,2,4-Trimethylbenzene, Zeolite Catalyst, Mordenite, ZSM-5, p-Xylene

## GİRİŞ

Aromatik bileşiklerin petrokimya endüstrisinde çeşitli kullanım alanları mevcuttur. Benzenin yoğun talebinden dolayı dünyada üretimi en çok olan ikinci bileşendir (Karaduman vd., 2007). Bu aromatiklerin üretimi için mevcut süreçler bulunmaktadır ve bu süreçler pek çok araştırmacı tarafından çalışılmıştır. Bu çalışmalar son zamanlarda petrokimyada reforming prosesinden çıkan ağır raformatları değerlendirme şeklinde yönelmiştir. Bu ağır reformatların arz talebi olmaması için değerlendirip benzine katılması için çalışmalar mevcuttur. Üretim proseslerinde farklı tepkimeler ve katalizörler kullanımı mevcuttur. Bunlardan biri de hafif alkanlarla reaksiyonundan, bu ağır reformatları zeolit katalizörler üzerinde değerli aromatik bileşiklere dönüştürmektir. Ağır reformat bileşiminin %39,0'ını 1,2,4-Trimetilbenzen (TMB) oluşturmaktadır (Al-Khattaf vd., 2011). Bu ürünün değerlendilmesi ayrı bir önem arz etmektedir. Petrokimya sanayi için gerekli olan aromatik bileşiklerin büyük bir kısmı Reforming Proseslerinde üretilmektedir. Aromatik üretim için aromatik açıdan zengin reformata dönüştüren katalitik bir işlemdir.

Ksilen üretimi için transalkilasyon, hidrodealikasyon, disproporsiyon ve alkil siklizasyon gibi yöntemler mevcuttur (Al-Khattaf vd.2014). Kesilenlerle birlikte yoğun talebinden dolayı dünyada üretimi en çok olan ikinci bileşen olan benzen üretimi de oldukça önem arz etmektedir. Alkil grupları ve hafif ürünler ile ksilen ve benzen gibi değerli aromatik bileşikler elde edilebilir. Benzen üretimi için de alkanlar gibi hafif ürünlerin siklizasyonu kullanılmaktadır (Davis 1999).

Katalizör olarak zeolitlerin kullanımında rafine etme işleminde, zeolitlerin asitlik durumu oldukça önemli bulunmaktadır. Petrol ve endüstrisi sanayinde yer alan önemli zeolitler Mordenit ve ZSM-5'tir. ZSM-5 silika bakımından zengin, asidik ve etkin pencere yapısına sahip olan katalizörlerdir. Zeolitler önemli ölçüde iyi özelliklerinin yanında, reaksiyon ortamından güçlü bir şekilde özellikle koklaşma ve buhar veya sıcak sulu ortamla olumsuz etkileşimi gibi durumlardır. ZSM-5 zeolitlerin önemli özelliklerinden biri olan koklaşmaya karşı dirençli oluşu katalitik kraking için öne çıkarmıştır. Bu da petrol sanayide ve aromatik bileşik oluşumlarında önemli bir avantaj haline gelmektedir. Zeolit katalizörlerin yüksek adsorpsiyon kapasiteleri, yüksek yüzey alanları, katalitik reaksiyonların yürütülmesini sağlayan mikro gözenekler, kanal sistemi, iyon değiştirme özelliklerinden dolayı yaygın olarak kullanılmaktadırlar (Hajimirzaee vd., 2020).

## MATERYAL VE YÖNTEM

### Materyal

Mordenit katalizörü toz halde Zeolyst firmasından, ZSM-5 katalizörü granül halde ACS firmasından temin edilmiştir. Aldrıc ve Merck firmalarından sırasıyla 1,2,4-Trimetilbenzen ve Pentan kimyasalları temin edilmiştir.

## Katalizör Hazırlama

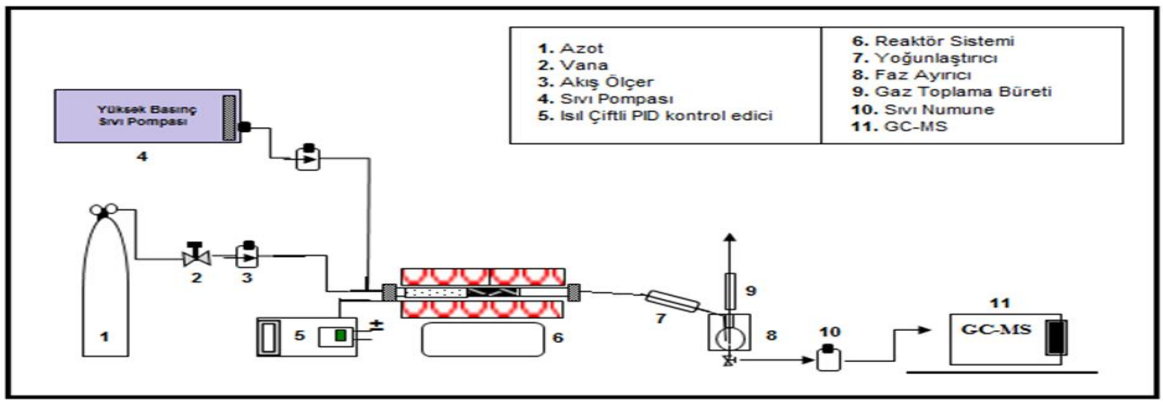
Mordenit ve ZSM-5 zeolitleri kullanılarak bizeolit katalizör sentezlenmiştir. Hazır alınan granül halde ZSM-5 ezilerek toz haline getirilmiş ve toz halde olan Mordenit ile Mordenit/ZSM-5 1:2 oranında, 7 g Mordenit ve 14 g ZMS-5 olacak şekilde tartılmış ve karıştırılmıştır. Daha sonra pellet haline getirmek için katalizöre 5:1 oranında olacak şekilde 4,2 g $\gamma$-$Al_2O_3$ eklenmiştir. Saf su ile hamur kıvamına getirilerek pelletler oluşturulmuştur. Hazırlanan pelletler bir gün oda koşullarında kurutulmuştur. Daha sonra 120°C'de 4 saat etüvde tutularak kurutma işlemi tamamlanmıştır. Kuru hale getirilmiş katalizörler kalsinasyon işlemine tabi tutularak D-formunda hazırlanmıştır.

## Katalizörün Karakterizasyonu

Hazırlanan Mordenit/ZSM-5 bizeolit katalizörünün dokusal özellikleri, Micromeritics 3 Flex gaz adsorpsiyon cihazı kullanılarak nitrojen sorpsiyon ölçümlerinden elde edildi. Numunelerin özgül yüzey alanı BET yöntemine (Barrett vd., 1951; Fager lund 1973) göre, gözenek hacmi ise BJH yöntemine (Barrett vd., 1951) göre hesaplandı. PANanytical Epsilon 4 cihazı ile bizeolit XRF (X-ışınları fluoresans spektroskopisi) analizi yapıldı.

## Katalitik Deneyler

Hazırlanan katalizin performansını belirlemek için besleme olarak bir 1,2,4-TMB + Pentan 1:1 oranında karışımı kullanılmıştır. Deneyler, atmosferik basınç altında kontrollü laboratuvar ölçekli bir deney sisteminde gerçekleştirilmiştir. Reaksiyon sabit yataklı gaz-katı katalitik reaktör (30 cm uzunluğunda ve 1 cm çapında paslanmaz çelik), soğutucu, gaz-sıvı ayırıcı ve yüksek basınçlı sıvı pompasından sisteminde gerçekleşmiştir. Reaktörün sıcaklık kontrolü oransal-integral-türev (PID) denetleyicisi kullanılarak gerçekleştirilmiştir. Reaktörün izotermal bölümünde 2 $cm^3$ katalizör sabitlendi, kalan boş hacim seramik boncuklarla doldurulduğu. Hidrojen, yüksek basınçlı ticari bir silindirden sağlandı. Sıvı besleme stoğu, reaktöre yüksek basınçlı sıvı pompası kullanılarak beslendi. Hidrojen gazı ve sıvı besleme, bunlar reaktöre eklenmeden önce karıştırıldı. Karışım seramik boncuklar üzerinden yatay reaktörden geçerken sıcaklığı reaksiyon sıcaklığına ulaşır ve böylece sıcaklıkları izotermal koşullara ulaşır. Kataliz, 6 saat boyunca atmosfer basıncı altında reaktörden 5 ml/dk azot gazı geçirilerek 400°C, 450 °C, 500°C ve 1,2,3 $h^{-1}$ WHSV parametrelerinde etkinleştirildi. Reaksiyon sonucu oluşan sıvı ürünler 60 m kapiler kolon bağlı Thermo-Finnigan Marka GC-MS cihazında analiz edilmiş ve oluşan ürünler tanımlanmıştır. Şekil 1'de deney düzeneği akış şeması verilmiştir.




**Şekil 1.** Deney sisteminin akış şeması

$W_{1,2,4\text{-TMB}}$ ve $W_{pentan}$ beslemedeki ve ürünlerdeki 1,2,4-Trimetilbenzen ve Pentanın kütlece oranlarıdır. Reaksiyon sonucunda dönüşüm ve verim (1) – (4) (Almulla vd., 2021) denklemleri arasında gösterildiği gibi hesaplandı. %Ksilen seçimliliği reaksiyon sonucunda oluşan ürünler içinden ksilenin diğer oluşan tüm ürünlere oranı şeklinde tanımlanmıştır. $N_{Ksilen}$ ürünlerdeki molce ksilendir ve denklem 5'teki gibi hesaplanmıştır.

$$\% \text{ 1,2,4-TMB Dönüşümü} = \frac{(W_{1,2,4-TMB})_{besleme} - (W_{1,2,4-TMB})_{ürün}}{(W_{1,2,4-TMB})_{besleme}} \times 100 \quad (1)$$

$$\% \text{ Pentan Dönüşümü} = \frac{(W_{Pentan})_{besleme} - (W_{Pentan})_{ürün}}{(W_{Pentan})_{besleme}} \times 100 \quad (2)$$

$$\% \text{ m/pKsilen Verimi} = \frac{W_{m/p-Ksilen}}{(W_{1,2,4-TMB})_{besleme}} \times 100 \quad (3)$$

$$\% \text{ o-Ksilen Verimi} = \frac{W_{o-Ksilen}}{(W_{1,2,4-TMB})_{besleme}} \times 100 \quad (4)$$

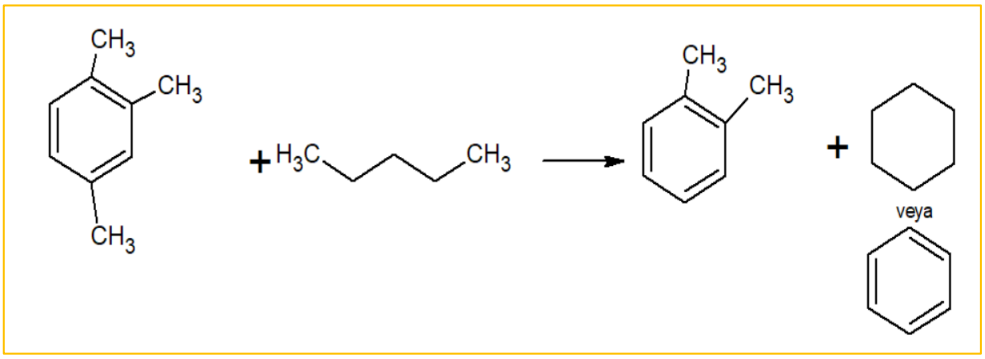
$$\% \text{ Ksilen Seçimliliği} = \frac{N_{Ksilen}}{N_{Diğer\ Ürünler}} \times 100 \quad (5)$$

Katalitik testlerin ardından kullanılan katalizörler 6 saat 200°C sıcaklıkta kurutulmuştur. 6 saat sonunda katalizör bir desikatöre alınmış ve yarım saat soğutulduktan sonra ikinci tartım yapılmıştır. Katalizör sabit tartıma gelinceye kadar 2 saat 200°C sıcaklıkta kurutma, soğutma ve tartım işlemlerine devam edilmiştir. Katalizör sabit tartıma geldikten sonraki kütle $m_1$'dir. Kurutulan katalizörler daha sonra kül fırınında 7 saat boyunca 25'ten önce 450°C daha sonra 625°C dereceye çıkarılıp yakma işlemi gerçekleştirilmiştir. Yakma işleminden sonra Yakılan katalizör numunesi sabit tartıma gelene kadar 2 saat 200°C kurutma devam etmiş ve sabit tartıma gelen katalizör kütlesi $m_2$ kabul edilmiştir. Denklem Çökelen kok miktarı denklem (6) (Rasouli vd., 2017) gibi hesaplanmıştır.

$$\% \text{ Kok Miktarı} = \frac{m_1 - m_2}{m_1} \quad (6)$$

## BULGULAR ve TARTIŞMA

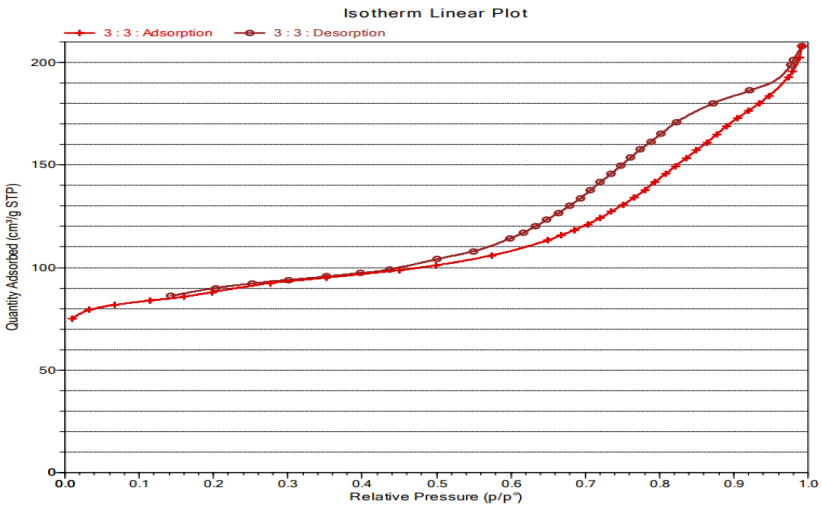
Ağır reformat olan 1,2,4-TMB'nin pentan ile verdiği ana reaksiyon denklemi Şekil 2'de verilmektedir. 1,2,4-TMB dealkilasyon gerçekleşerek ksilen oluşurken, pentanın siklizasyona uğraması ile benzen halkası meydana gelmiştir.




**Şekil 2.** 1,2,4-TMB ile Pentan'ın dealkilsiklizasyonu

### Azot Adsorpsiyon Analizi

The Brunauer, Emmett and Teller Model (BET) yüzey alanı, bizeolit katalizörün gözenek dağılımı Barrett-Joyner-Halenda (BJH) metoduna göre hesaplanmıştır. BET yüzey alanı 283,5800 $m^2/g$, BJH gözenek hacmi 0,317848 $cm^3/g$ ve BJH gözenek çapı 4,4834 nm olarak hesaplanmıştır.




**Şekil 3.** Mordenit/ZSM-5 bizeolit katalizörü için BET sorpsiyon grafiği

Şekil 3'te bizeolit katalizör için BET yöntemiyle çizilmiş adsorpsiyon-desorpsiyon izotermi verilmiştir. Grafik IUPAC sınıflandırılmasına göre tip IV izotermine uygun çıkmıştır (Betiha vd., 2015). Birinci tabakanın adsorpsiyon ısısı yoğunlaşma ısısından daha yüksek olan ve kılcal yoğunlaşmanın çok olduğu adsorpsiyon izotermleri bu eğriye benzemektedir. Şekil 3'te görülen adsorpsiyon ve desorpsiyon izotermleri farklı yollar izlemişlerdir buna adsorpsiyon histerezisi denir (Egeblad vd., 2007). Bu durum, dar ağızlardan dolan gözeneklerin geniş ağızlarından boşalması ile açıklanabilir. Genellikle mezo gözenek içeren katılardaki adsorpsiyon izotermleri bu tipe uymaktadır.

### XRF (X-ışınları fluoresans spektroskopisi) Analizi

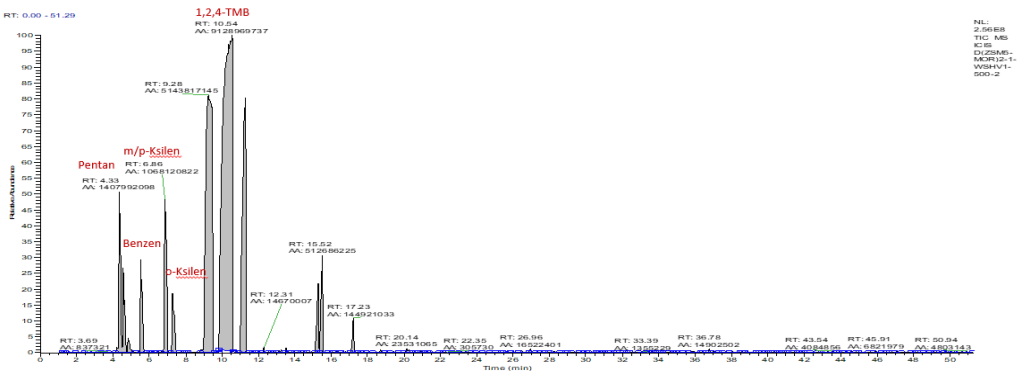
Katalizöre XRF analizi yapılmıştır ve sonuçları Tablo 1'de verilmektedir. Al ve Si elementlerine bakarak katalizörün başarıyla sentezlendiği söylenebilmektedir.

**Tablo 1.** Katalizörün XRF analiz sonucu

| Element | Al % | Si% | P% | Cl% | K% | Ca% | Ti % | Fe% |
|---|---|---|---|---|---|---|---|---|
| **ZSM-5/MOR** | 19,293 | 77,535 | 0,954 | 0,631 | 0,146 | 0,659 | 0,520 | 0,212 |

### Katalitik Aktivite Performans Değerlendirmesi

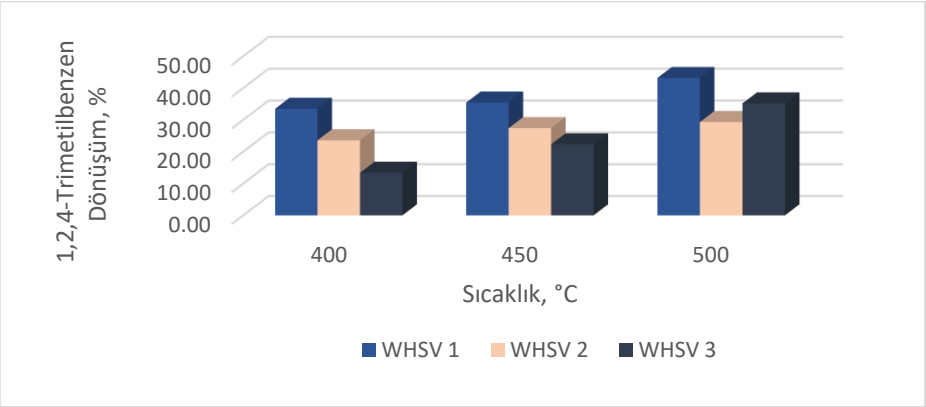
Modenit/ZSM-5 zeolit katalizörü üzerinde dealkilsiklizasyon testleri yapılmıştır. Yapılan çalışmada en yüksek pik veren sıcaklık ve WHSV'de yapılan test sonucunda oluşan GC-MS pikleri Şekil 4'te verilmiştir.




**Şekil 4.** 1,2,4-TMB-Pentan beslemesi GC-MS kromatogramı (500°C, WHSV 1)

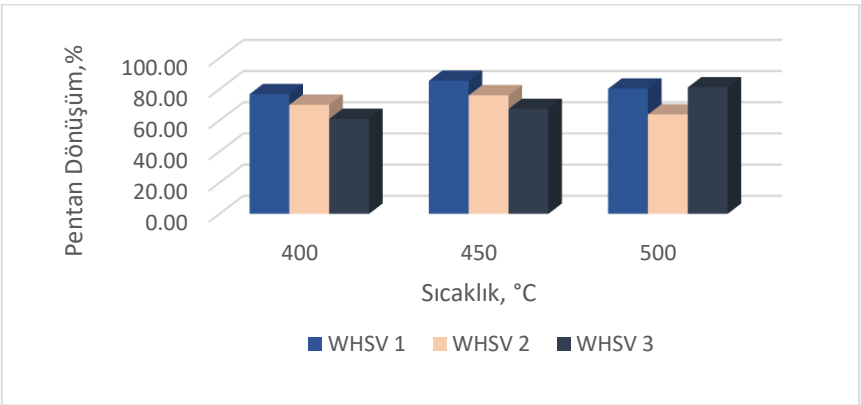
*Katalizörlerin dönüşüm performansı*

1:2 oranında hazırlanan Mordenit/ZSM-5 bizeolit katalizörü üzerinde 1,2,4-TMB dealkilsikzlizasyonu Şekil 5 incelendiğinde üç akış hızında da sıcaklık artımı ile dönüşümün arttığı gözlemlenmiştir. 400°C ve 450°C sıcaklıklarda, akış hızının arttırılmasıyla dönüşüm düşmüştür. 500°C sıcaklıkta ise WHSV 2'de en düşük dönüşüm gözlemlenmiştir. En yüksek dönüşüm yüksek sıcaklık 500°C ve düşük akış hızı WHSV 1'de %43,14 olarak bulunmuştur.




**Şekil 5.** Mordenit/ZSM-5 bizeolit katalizörü üzerinden farklı boşluk hızlarında 1,2,4-Trimetilbenzen dönüşümünün sıcaklıkla değişimi

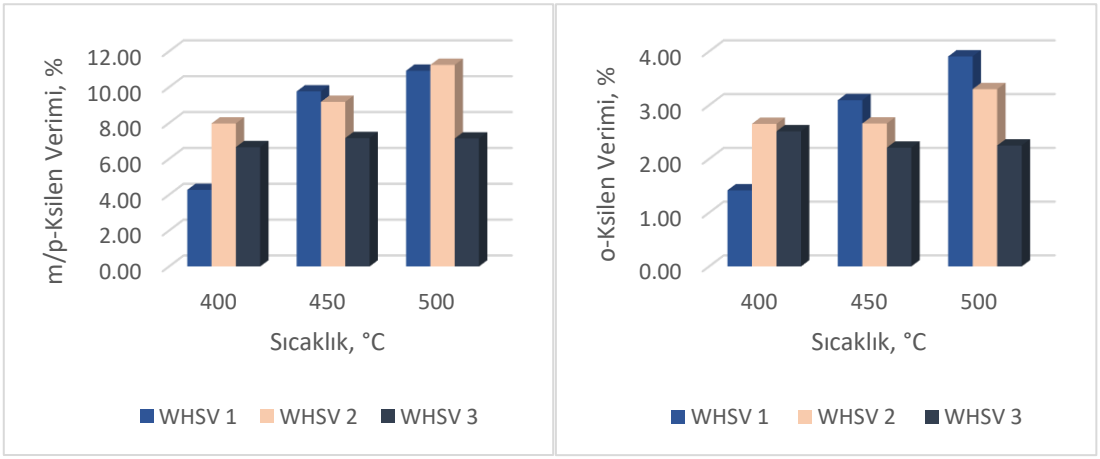
Şekil 6'da Mordenit/ZSM-5 bizeolit katalizörü üzerinden farklı boşluk hızlarında Pentan dönüşümünün sıcaklıkla değişimi verilmektedir.1:2 oranında hazırlanan Mordenit/ZSM-5 bizeolit katalizörü üzerinde pentanın dealkilsikzlizasyonu incelendiğinde en yüksek dönüşüm 450°C ve WHSV 1'de %85,33 olarak bulunmuştur. Aynı WHSV'de değişen sıcaklıklarda dönüşüm, 500°C %80,23 ile dönüşüm gösterirken 400°C'de %76,75'tir. Aynı sıcaklıkta boşluk hızının artmasıyla dönüşümde 400°C ve 450°C azalma görülürken, 500°C'de WHSV 2'de azalıp WHSV 3'te artış gözlemlenmiştir.




**Şekil 6.** Mordenit/ZSM-5 bizeolit katalizörü üzerinden farklı boşluk hızlarında Pentan dönüşümünün sıcaklıkla değişimi

*Ksilen Verimi*

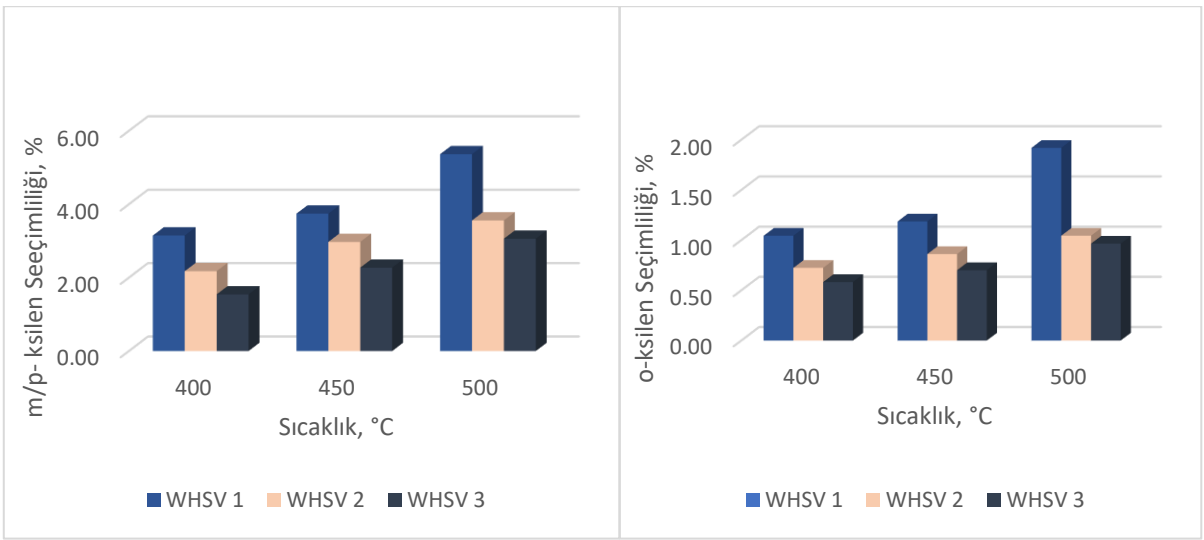
Şekil 7'de ksilen verim grafikleri ile sıcaklıkla ksilen verim artışı olduğu görülmektedir. 500°C'de WHSV 2 akış hızında %11,24 ile m/p ksilen için en yüksek verim değerine ulaşmıştır. Mordenit/ZSM-5 bizeolit katalizörü açısından m/p ksilen için 400°C ve 500°C derecede WHSV 2 akış hızı en verim verirken 450°C'de WHSV 1 akış hızında verim daha yüksektir. o-Ksilen için ise 450°C ve 500°C'lerde WHSV 1 akış hızında verim daha yüksekken, 400°C'de WHSV 2 akış hızında verimin yüksek olduğu gözlemlenmiştir.




**Şekil 7.** m/p-Ksilen ve o-Ksilen verimi

*Ksilen Seçimliliği*

Ksilen seçimliliği için ise yine verimde olduğu gibi sıcaklıkla seçimliliğin de arttığı söylenebilmektedir. En yüksek ksilen verimleri WHSV 1 ve 500°C de m/p ksilen için %5,36 ve o-ksilen için %1,92 olarak bulunmuştur. Şekil 8 incelendiğinde boşluk hızının artmasıyla tüm sıcaklıklarda doğrusal bir şekilde azalma gözlemlenmiştir. Mordenit/ZSM-5 bizeolit katalizörü üzerinde aynı boşluk hızında boşluk hızında sıcaklığın artması ile seçimlilik değerleri p-Ksilen için 400°C'de %3,15, 450°C'de ise %3,14 şeklindedir.




**Şekil 8.** m/p-Ksilen ve o-Ksilen seçimliliği

**Kok Tayini Sonuçları**

Reaksiyon sonrası kullanılan katalizöre yapılan kok miktarı testi sonucunda, tepkime sıcaklığının artması ile %kok oluşumu azalma gösterirken akış hızının azalması ile % kok artış göstermiştir. En yüksek kok oluşumu %0,55 ile 400°C WHSV 3 akış hızında bulunmuştur. En düşük kok oluşumu ise %0,052 ile 500°C ve WHSV1'dir.

**SONUÇ**

Çalışmada, Mordenit/ZSM-5 karışımından oluşturulmuş bizeolit destek üzerine petrokimya sanayinde ağır reformat ürünlerinden 1,2,4-trimetilbenzenin n-pentan varlığında dealkilsiklizasyonu sonucu oluşan ksilenlere etkisi araştırılmıştır. Bizeolit katalizörü ile farklı reaktör boşluk hızı (WHSV) ve sıcaklıklarındaki çalışmada, reaktör sıcaklığının artışı ile 1,2,4-TMB dönüşümünün, ksilenlerin veriminin ve seçimliliğinin arttığı belirlenmiştir. En yüksek p-ksilen verimi 500°C WHSV 2'de %11,24 olarak görülmüştür. WHSV arttıkça da 1,2,4-TMB dönüşümünün, ksilen seçimliliğin ve veriminin azaldığı gözlemlenmiştir. Düşük akış hızında beslemenin katalizör üzerinde daha fazla kalması ile etkileşimin artmasından kaynaklı olduğu söylenebilir. Kullanılan katalizörün üzerinde biriken koklar en çok yüksek sıcaklık ve düşük akış hızında görülmüştür. Bu

da yine beslemenin katalizör üzerinde geçirdiği sürenin uzunluğu ve yüksek sıcaklığın katalizörü koklaştırdığı şeklinde yorumlanmıştır.

## KAYNAKLAR

Ali, S. A., Aitani, A., M., Ercan, C., Wang, Y., Al-Khattaf, S. 2011. Conversion of Heavy Reformate into Xylenes Over Mordenite-Based Catalysts, Chem. Eng. Res. Des., 89(10): 2125–2135.

Almulla, Faisal M., et al. "On the influence of alumina as a binder on the performance of Pt-Beta catalys during the transalkylation of toluene and 1, 2, 4-Trimethylbenzene 2021." *Microporous and Mesoporous Materials* 320: 111095.

Al-Khattaf S., Syed A. A., Aitani A. M., Žilková N., Kubička D., Čejka J. 2014. Recent Advances in Reactions of Alkylbenzenes Over Novel Zeolites: The Effects of Zeolite Structure and Morphology. Catal. Rev., 56(4): 333-402.

Barrett EP, Joyner LG, Halenda PP. 1951. The Determination Of Pore Volume And Area Distributions in Porous Substances I. Computations From Nitrogen İsotherms. J Am Chem Soc. 73(1):373–80.

Betiha MA, Menoufy MF, Al-Sabagh AM, Hassan HM, Mahmoud SA. 2015. Acidic mesostructured aluminosilicates assembled from economic acidic template characterized by catalytic cracking reactions. Microporous Mesoporous Mater. 204:15–24.

Davis, B. H. 1999. Alkane Dehydrocyclization Mechanism. *Catalysis Today*, *53*(3), 443-516.

Egeblad, K., Kustova, M., Klitgaard, S. K., Zhu, K., & Christensen, C. H. 2007. Mesoporous zeolite and zeotype single crystals synthesized in fluoride media. Microporous and Mesoporous Materials, 101(1-2), 214-223.

Fagerlund G. 1973. Determination of specific surface by the BET method. Mate´riaux et Constr. 6(3):239–45.

Hajimirzaee, S., Soleimani Mehr, A., & Kianfar, E., 2020. Modified ZSM-5 Zeolite for conversion of LPG to aromatics. *Polycyclic Aromatic Compounds*, 1-14.

Karaduman A., Alp E., Yeniova H. & Alibeyli R.,2007, Poly-functional Catalyst Development for Benzene Production from Alkyl-aromatic Mixture via Hydrodealkylation, Petroleum Science and Technology, 25:479–490.

*ORAL PRESENTATION*

# Effects of smoke-water on physiological characteristics of wheat seedlings grown hydroponically

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## Abstract

Plant-derived smoke, which contains many stimulatory and inhibitory compounds, is known to affect various physiological processes in plants, including seed germination and somatic embryogenesis. In this research, different volumes of smoke-water were added to the hydroponic nutrient solution to determine smoke-induced physiological changes in wheat (*Triticum aestivum* L.) seedlings. The results showed that smoke-water supplementation at 1 and 2.5% significantly enhanced the length and root dry weight of wheat seedlings, respectively. In addition, these two treatments caused a decrease in the $H_2O_2$ and MDA contents of wheat roots compared to control conditions ($p < 0.05$). Furthermore, 2.5% smoke-water supplementation induced remarkable changes in DPPH scavenging activity, proline content, and total sugar concentration of wheat seedlings, especially in root tissues. Overall, the findings of the current study suggest that smoke-water can be utilized in hydroponic systems to promote plant growth and alleviate adverse environmental conditions.

**Keywords:** Smoke-water, wheat, seedling growth, oxidative metabolism, DPPH scavenging activity, hydroponics

## INTRODUCTION

Plant-derived smoke is recognized as an important germination cue for plants grown in fire-prone environments (Çatav et al., 2018a). Smoke has also been shown to stimulate the germination of many plant species from non-fire-prone areas (Nelson et al., 2012). The promotive effect of smoke on seed germination is associated with chemicals present in it, including karrikinolide ($KAR_1$), glyceronitrile, and syringaldehyde (Flematti et al., 2015; Cao et al., 2021). Studies on the mode of action of smoke compounds showed that $KAR_1$ enhanced the germination of *Avena fatua* by increasing the activities of α-amylase, β-amylase, and dehydrogenase and decreasing ABA content (Kępczyński et al., 2013; Cembrowska-Lech et al., 2015). In addition, it was found that glyceronitrile-induced seed germination was mediated by cyanide (Flematti et al., 2011). On the other hand, smoke is known to contain a number of compounds, such as 3,4,5-trimethylfuran-2(5*H*)-one and 5,5-dimethylfuran-2(5*H*)-one, which can cause germination inhibition at higher concentrations (Çatav and Akbaş, 2021).

The intriguing findings on smoke-stimulated germination have raised the question as to whether smoke and smoke-derived chemicals can improve plant growth. In this context, it has been demonstrated that smoke, $KAR_1$, and catechol enhance the root and shoot growth of many plant species (e.g., *Arabidopsis thaliana*, *Nicotiana attenuata*, maize, and okra) depending upon concentration and type of treatment (van Staden et al., 2006; Baldrianová et al., 2015; Wang et al., 2017; Çatav et al., 2018b). For instance, Baldrianová et al. (2015) revealed that in *Arabidopsis*, $KAR_1$ increased cotyledon surface area and root length and affected several physiological and metabolic processes, including carbohydrate metabolism, transcription control, and photosynthesis. Furthermore, recent studies have reported that smoke and $KAR_1$ can mitigate the adverse impacts of abiotic stress conditions, like boron toxicity and salinity, on plant growth and development (Jamil et al., 2014; Küçükakyüz and Çatav, 2021).

Water scarcity, heavy metal contamination, nutrient depletion, and erosion are the major challenges for crop production in cultivated soils worldwide (Magwaza et al., 2020). Hydroponic technology is an alternative approach to overcoming these obstacles in agriculture. In addition, hydroponic systems have several advantages over conventional agricultural systems, such as less space requirement, water conservation, and prevention of soil-borne diseases (Pandey et al., 2009). Supplementation of smoke-water to hydroponic nutrient solution has received increasing attention in recent years (Çatav et al., 2021; Dogrusoz, 2022). However, little is known concerning smoke-induced physiological responses in hydroponic systems. In this study, different volumes of smoke-water were added to the hydroponic solution in order to investigate the smoke-related changes in wheat roots and shoots. I especially focused on the effects of smoke-water on growth, photosynthetic pigment concentrations, oxidative and antioxidative status, and proline and total sugar levels. I

hypothesized that smoke-water had a concentration-dependent effect on tested parameters and that there would be significant differences in physiological responses to smoke between root and shoot tissues.

## MATERIALS AND METHODS

### Preparation of smoke-water

For the preparation of smoke-water, 20 g of filter paper and 20 g of dry twigs of kermes oak (*Quercus coccifera* L.) were burned in a bee smoker. The produced smoke was bubbled into 250 mL of distilled water for 12.5 min (Çatav et al., 2018a). The resultant solution was filtered through a Whatman filter paper (Grade No. 42) and considered a 100% concentration of smoke-water (pH: 3.57).

### Plant material, growth conditions, and experimental design

Wheat seeds (*Triticum aestivum* L. cv. Bayraktar-2000) were sterilized with 10% (v/v) commercial bleach for 20 min and rinsed with distilled water. Surface-sterilized seeds were germinated on filter papers moistened with distilled water in the dark at 20 °C. Seven-day-old seedlings were then transferred into quarter-strength Hoagland's nutrient solution (pH: 5.6) and grown at 22 °C under a 16-h photoperiod (5000 lux) for 3 days. After that, smoke treatments were started by adding different volumes of smoke-water (100%) into the nutrient solutions. The final concentration of smoke-water for each treatment was selected as 0, 0.1, 0.2, 0.5, 1, and 2.5% based on previous studies (Küçükakyüz and Çatav, 2021; Çatav et al., 2021). The pH of the nutrient solutions was adjusted to 5.6 with 1 N HCl or 1 N NaOH. Four replicates of 12 seedlings were used for each treatment, and the experiment was repeated 3 times. Wheat seedlings were harvested after 5 days of exposure to different concentrations of smoke-water under the same growth conditions, and one part of the fresh root and shoot samples were immediately stored at − 30 °C for biochemical analyses.

### Growth parameters

In order to determine the root and shoot lengths, four groups of 10 seedlings for each treatment were photographed with a ruler. Images were then imported into ImageJ software (version 1.8.0_172) and analyzed using "set scale" and "measure" functions. Dry weight measurements were carried out following the drying of plant samples at 70 °C for 48 h.

### Biochemical parameters

Photosynthetic pigment concentrations in wheat leaves were estimated according to the method described by Sumanta et al. (2014).

Malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) levels were quantified using the procedures outlined in Küçükakyüz and Çatav (2021), respectively. In brief, wheat roots and shoots were homogenized in 0.1% TCA solution in a ratio of 1:10 (w/v). The homogenates were centrifuged at 13.000 rpm for 10 min at 4 °C, and the resultant supernatants were used for both assays.

The total phenolic content of wheat roots and shoots was calculated by using Folin–Ciocalteu method (Maksimović and Živanović, 2012). The results were expressed as µg caffeic acid equivalent (CAE) per g of fresh weight (FW).

The antioxidant capacity of wheat roots and shoots was determined by the DPPH method. Briefly, 0.4 mL of methanolic plant extract and 1.6 mL of 0.2 mM DPPH solution were mixed and incubated at room temperature for 30 min. The absorbance of mixtures was then read at 517 nm (Çatav et al., 2021).

The phenol-sulfuric acid reaction was used for the determination of the total sugar content of root and shoot samples (Dubois et al., 1956). Concisely, 400 µL of ethanolic supernatant was mixed with 400 µL of 5% (w/v) phenol solution and 2 mL of concentrated sulfuric acid. The mixture was incubated at room temperature for 30 min, and the absorbance was read at 490 nm. D-glucose was used as a standard.

Proline content was quantified spectrophotometrically at 508 nm by the acid-ninhydrin method described by Shabnam et al. (2016).

### Data analysis

Prior to each analysis, Bartlett's and Anderson-Darling's tests were used to determine whether or not data met parametric assumptions, and if necessary, data transformations were applied. Data were then analyzed by one- or two-way ANOVA, and in case of a significant difference between groups, Tukey's HSD test was performed for post hoc comparisons. The significance level was set at $p < 0.05$ for all analyses.

## RESULTS and DISCUSSION

The main goal of this research was to determine physiological and biochemical changes in wheat seedlings grown in a hydroponic system supplemented with different concentrations of smoke-water. The results showed that 1% smoke-water treatment significantly increased the seedling length of wheat (Table 1). Moreover, it

was found that 2.5% smoke-water supplementation enhanced the root dry weight when compared to control conditions ($p < 0.05$). On the other hand, lower concentrations of smoke-water did not markedly affect any growth parameters of wheat seedlings. The current results are consistent with previous findings that smoke stimulates root and seedling growth of wild and agricultural plants, like okra, tomato, *Brassica napus*, *Lavandula latifolia*, and *Tragopogon longirostis* (Kulkarni et al., 2007; Moreira et al., 2010; Çatav et al., 2018a; Moyo et al., 2022).

**Table 1.** Effect of different concentrations of smoke-water on growth parameters of wheat seedlings

| Parameter | The concentration of smoke-water (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.1 | 0.2 | 0.5 | 1 | 2.5 |
| Root length (mm) | $124 \pm 6^{a}$ | $126 \pm 4^{a}$ | $131 \pm 5^{a}$ | $138 \pm 5^{a}$ | $140 \pm 5^{a}$ | $132 \pm 4^{a}$ |
| Shoot length (mm) | $196 \pm 6^{ab}$ | $193 \pm 7^{b}$ | $201 \pm 8^{ab}$ | $202 \pm 6^{ab}$ | $219 \pm 4^{a}$ | $193 \pm 5^{ab}$ |
| Seedling length (mm) | $320 \pm 6^{b}$ | $319 \pm 9^{b}$ | $332 \pm 11^{ab}$ | $340 \pm 10^{ab}$ | $359 \pm 7^{a}$ | $326 \pm 7^{ab}$ |
| Root dry weight (mg) | $5.6 \pm 0.2^{bc}$ | $5.4 \pm 0.2^{c}$ | $5.7 \pm 0.2^{abc}$ | $6.0 \pm 0.2^{abc}$ | $6.3 \pm 0.2^{ab}$ | $6.5 \pm 0.2^{a}$ |
| Shoot dry weight (mg) | $16.9 \pm 0.6^{ab}$ | $16.0 \pm 0.6^{b}$ | $17.2 \pm 0.5^{ab}$ | $17.3 \pm 0.5^{ab}$ | $18.6 \pm 0.4^{a}$ | $16.9 \pm 0.5^{ab}$ |
| Seedling dry weight (mg) | $22.5 \pm 0.8^{ab}$ | $21.4 \pm 0.8^{b}$ | $22.9 \pm 0.6^{ab}$ | $23.3 \pm 0.6^{ab}$ | $24.9 \pm 0.6^{a}$ | $23.4 \pm 0.7^{ab}$ |

Results are presented as mean + SE (n = 4 groups of 10 seedlings). Values with different superscripts on the same line are significantly different from one another ($p < 0.05$).

Photosynthetic pigments (e.g., chlorophyll a, chlorophyll b, and carotenoids) are an integral part of the photosynthetic apparatus and play an essential role in absorbing and transferring light energy. Furthermore, carotenoids have antioxidant properties that decrease photoinhibition and photodamage (Qiu et al., 2019; Simkin et al., 2022). Effects of smoke and $KAR_1$ on photosynthetic pigment concentrations have been examined in a number of studies (Abdelgadir et al., 2013; Papenfus et al., 2015; Kulkarni et al., 2019; Çatav et al., 2021). Some of these works indicated that smoke and $KAR_1$ solutions notably increased chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents in studied plants (Abdelgadir et al., 2013; Kulkarni et al., 2019). However, other research did not find any significant differences between control and smoke treatments with regard to photosynthetic pigment contents (Papenfus et al., 2015; Çatav et al., 2021). In addition, Ghebrehiwot et al. (2013) observed a considerable drop in the carotenoid content of *Eragrostis tef* plants treated with smoke-water and $KAR_1$. In this study, 2.5% smoke-water treatment led to a 35% rise in chlorophyll b concentration of wheat leaves as compared with the control group (Table 2). On the other hand, other smoke treatments had no impact on pigment concentrations ($p > 0.05$). Taken together, the influence of smoke and its components on photosynthetic pigment levels seem to depend on several factors, like solution concentration and studied plant species.

**Table 2.** Effect of different concentrations of smoke-water on photosynthetic pigment contents (µg/mL) of wheat seedlings

| Photosynthetic pigment | The concentration of smoke-water (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.1 | 0.2 | 0.5 | 1 | 2.5 |
| Chlorophyll a | $7.0 \pm 0.5^{ab}$ | $6.5 \pm 0.6^{ab}$ | $7.4 \pm 0.1^{ab}$ | $6.6 \pm 0.4^{ab}$ | $6.3 \pm 0.4^{b}$ | $8.3 \pm 0.3^{a}$ |
| Chlorophyll b | $2.0 \pm 0.1^{b}$ | $1.8 \pm 0.1^{b}$ | $1.9 \pm 0.1^{b}$ | $1.8 \pm 0.1^{b}$ | $1.8 \pm 0.0^{b}$ | $2.6 \pm 0.2^{a}$ |
| Total chlorophyll | $9.0 \pm 0.6^{ab}$ | $8.3 \pm 0.6^{b}$ | $9.3 \pm 0.2^{ab}$ | $8.4 \pm 0.5^{b}$ | $8.1 \pm 0.4^{b}$ | $11.0 \pm 0.4^{a}$ |
| Carotenoids | $1.6 \pm 0.1^{abc}$ | $1.7 \pm 0.1^{ab}$ | $1.6 \pm 0.0^{abc}$ | $1.4 \pm 0.1^{c}$ | $1.4 \pm 0.0^{bc}$ | $1.9 \pm 0.1^{a}$ |

Results are presented as mean + SE (n = 3). Values with different superscripts on the same line are significantly different from one another ($p < 0.05$).

Reactive oxygen species (ROS), such as $H_2O_2$ and superoxide anion radical are important molecules that modulate many biological processes in plants, including cellular proliferation and differentiation within the range between cytostatic and cytotoxic levels (Mittler, 2017). Nonetheless, cytotoxic ROS levels have been shown to induce lipid peroxidation, protein oxidation, and DNA damage in plant cells (Dumanović et al., 2021). In the current study, smoke supplementation at 0.5, 1, and 2.5% caused a fall in the $H_2O_2$ level of wheat roots ($p < 0.05$) whereas no appreciable effect of smoke was seen in the $H_2O_2$ content of wheat shoots (Figure 1A). In addition, a notable reduction in MDA content (an indicator of lipid peroxidation) was recorded in wheat seedlings treated with 1 and 2.5% smoke-water (Figure 1B). In a recent study, Çatav et al. (2021) revealed that smoke-water at 0.1% did not alter $H_2O_2$ and MDA levels of wheat plants grown in a hydroponic system. Moreover, Sunmonu et al. (2016) noted that smoke exerted mixed effects on the MDA content of different parts of maize and bean seedlings during the early growth stage. For example, MDA concentrations

of cotyledons and roots were increased and decreased, respectively, in response to smoke treatment. The data collectively suggest the interplay of smoke-water concentration, tissue type, and growth stage has a prominent effect on $H_2O_2$ and MDA levels in plants subjected to smoke.

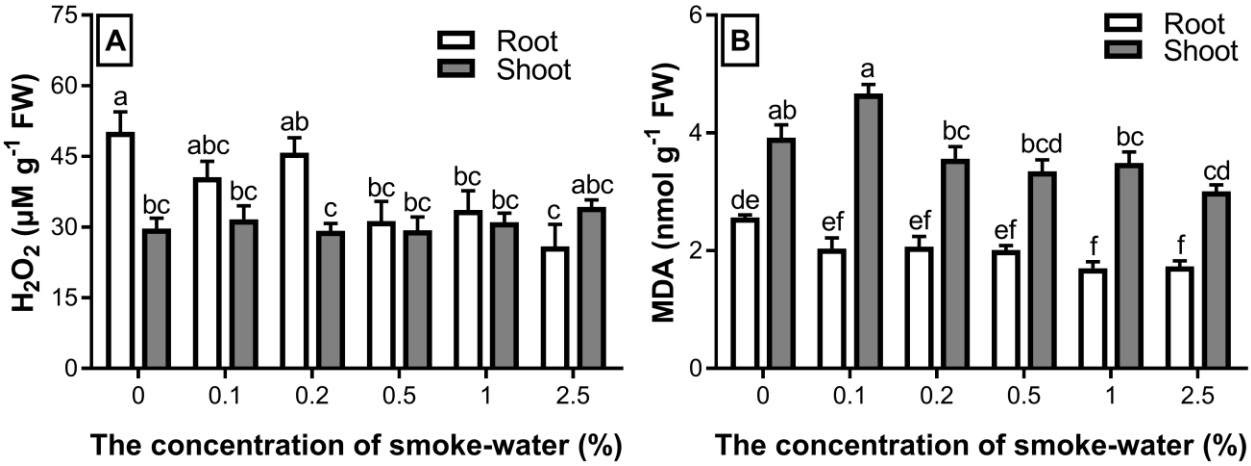


**Figure 1.** Effect of different concentrations of smoke-water on A) $H_2O_2$ and B) MDA levels of wheat seedlings. Results are presented as mean + SE (n = 3). Different letters on bars indicate that values are significantly different from one another ($p < 0.05$).

The antioxidant defense system, comprised of enzymatic and non-enzymatic antioxidants (e.g., catalase, superoxide dismutase, ascorbate, and phenols), has a crucial function in the regulation of ROS levels under both normal and stress conditions (Sancdev et al., 2021). The impacts of smoke and $KAR_1$ on antioxidant enzyme activities and total antioxidant capacity in plants have been investigated in several studies (Akhtar et al., 2017; Khan et al., 2017; Aremu et al., 2019; Çatav et al., 2021; İbrahim et al., 2022). The findings of these works showed that smoke and $KAR_1$ had varying effects on the antioxidant status of tested plant species. For instance, Aremu et al. (2019) found a marked increase in DPPH and beta-carotene antioxidant activities of *in vitro* plantlets and *ex vitro* leaves of *Eucomis autumnalis* treated with smoke and $KAR_1$. On the other hand, Akhtar et al. (2017) and İbrahim et al. (2022) demonstrated a considerable decrease in APX, POD, and SOD activities of rice and wheat seedlings exposed to smoke. In addition to these, some authors did not observe any significant change in the antioxidant enzyme activities of smoke-treated plants (Khan et al., 2017; Çatav et al., 2021). In this research, 2.5% smoke-water treatment gave rise to an increment ($p < 0.05$) in the DPPH radical scavenging activity of wheat roots compared to the control (Figure 2A). Phenolic compounds also constitute an important part of the antioxidant defense system. It has been shown that the total phenolic content of plants either remains unchanged or increases slightly in response to smoke (Aremu et al., 2012; Singh et al., 2014; Kulkarni et al., 2019). The present data, indicating that smoke had little or no effect on the phenolic content of wheat roots and shoots, is in agreement with these observations (Figure 2B).

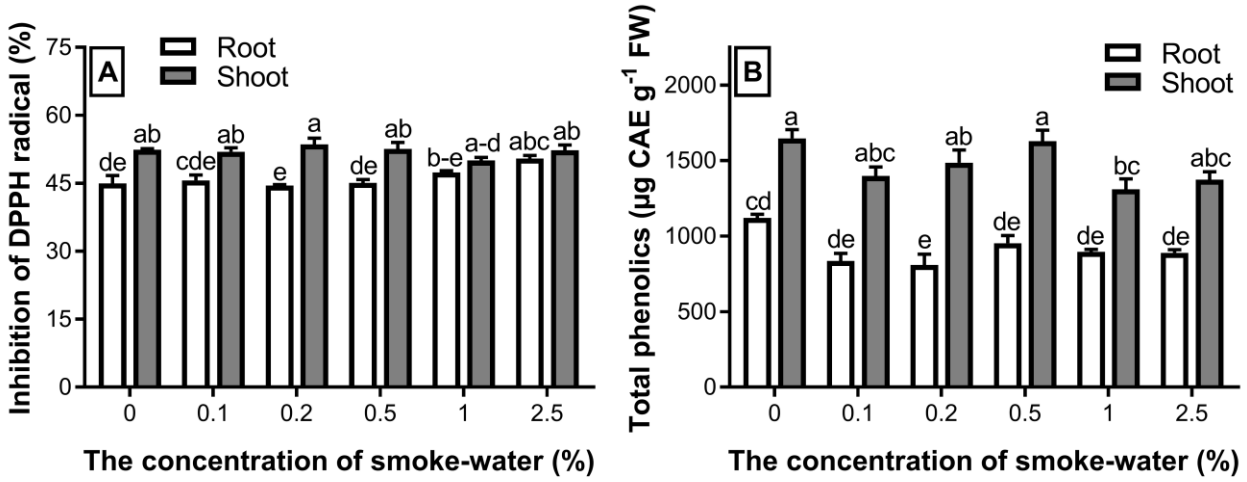


**Figure 2.** Effect of different concentrations of smoke-water on A) inhibition of DPPH radical (%) and B) total phenolic content of wheat seedlings. Results are presented as mean + SE (n = 3). Different letters on bars indicate that values are significantly different from one another ($p < 0.05$).

It is well-recognized that the accumulation of compatible solutes, such as amino acids, betaines, sugars, and polyamines in plant tissues increases tolerance to abiotic stress conditions (Kumar et al., 2018). These compounds have been reported to play a substantial role in many physiological processes, including osmoregulation, ROS scavenging, and stabilization of cellular membranes (Ahmed et al., 2021). In an NMR-based metabolomics study, Çatav et al. (2018b) unveiled that higher concentrations of smoke-water increased the fructose, glucose, isoleucine, leucine, and valine levels. However, they did not find any remarkable differences in levels of putrescine, an important polyamine, between control and smoke treatments. Moreover, some contradictory results were obtained regarding the effect of smoke on proline and total sugar concentrations in plants, like bean, rice, spinach, and wheat (Singh et al., 2014; Akhtar et al. 2017; Khan et al., 2017, Kulkarni et al., 2019; Çatav et al., 2021). The findings of this investigation pointed out that 2.5% smoke-water supplementation led to a rise ($p < 0.05$) in proline and total sugar levels of wheat roots (Figure 3A and 3B). A significant enhancement in the total sugar content was also detected in wheat shoots exposed to 2.5% smoke-water. Taken as a whole, because of the fact that smoke contains many inhibitory and promotive compounds, smoke-induced changes in compatible solutes seem to be associated with smoke-water concentration. In this regard, it can be thought of as some inhibitory compounds slightly induce stress-related responses in studied plant species depending on their levels in smoke-water.

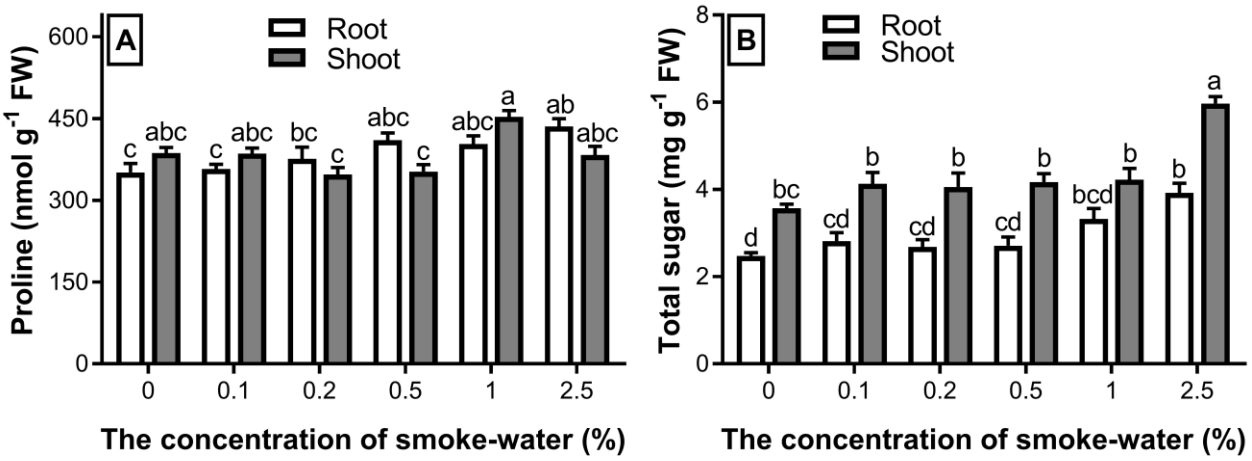


**Figure 3.** Effect of different concentrations of smoke-water on A) proline and B) total sugar levels of wheat seedlings. Results are presented as mean + SE (n = 3). Different letters on bars indicate that values are significantly different from one another ($p < 0.05$).

## CONCLUSION

This study provides evidence that smoke-water supplementation at a certain concentration range can significantly promote the seedling growth of bread wheat (*T. aestivum* L.). Additionally, smoke-water was shown to increase DPPH scavenging activity and decrease $H_2O_2$ and MDA contents of wheat roots compared to the control group. Furthermore, it was found that smoke caused alterations in the proline and total sugar contents of wheat seedlings. The findings of this work suggest that smoke-water can be utilized in hydroponic systems to enhance plant growth and mitigate stress conditions.

## ACKNOWLEDGEMENTS

I would like to thank Burcu Şahin for her support during the experiments. This study was supported by ÖYP coordinatorship of Mugla Sitki Kocman University.

## REFERENCES

Abdelgadir HA, Kulkarni MG, Aremu, AO, Van Staden J 2013. Smoke-water and karrikinolide ($KAR_1$) foliar applications promote seedling growth and photosynthetic pigments of the biofuel seed crop *Jatropha curcas* L. Journal of Plant Nutrition and Soil Science, 176: 743-747.

Ahmed S, Heo T-Y, Choudhury AR, Walitang DI, Choi J, Sa T 2021. Accumulation of compatible solutes in rice (*Oryza sativa* L.) cultivars by inoculation of endophytic plant growth promoting bacteria to alleviate salt stress. Applied Biological Chemistry, 64: 68.

Akhtar N, Khan S, Malook I, Rehman SU, Jamil M 2017. Pb-induced changes in roots of two cultivated rice cultivars grown in lead-contaminated soil mediated by smoke. Environmental Science and Pollution Research, 24: 21298-21310.

Aremu AO, Bairu MW, Finnie JF, Van Staden J 2012. Stimulatory role of smoke–water and karrikinolide on the photosynthetic pigment and phenolic contents of micropropagated 'Williams' bananas. Plant Growth Regulation, 67: 271-279.

Aremu AO, Masondo NA, Gruz J, Doležal K, Van Staden, J 2019. Potential of smoke-water and one of its active compounds (karrikinolide, $KAR_1$) on the phytochemical and antioxidant activity of *Eucomis autumnalis*. Antioxidants, 8: 611.

Baldrianová J, Černỳ M, Novák J, Jedelskỳ PL, Divíšková E, Brzobohatỳ B, 2015. Arabidopsis proteome responses to the smoke-derived growth regulator karrikin. Journal of Proteomics, 120: 7-20.

Cao D, Schöttner M, Halitschke R, Li D, Baldwin G, Rocha C, Baldwin IT 2021. Syringaldehyde is a novel smoke-derived germination cue for the native fire-chasing tobacco, *Nicotiana attenuata*. Seed Science Research, 31: 292-299.

Çatav ŞS, Akbaş K 2021. Yedi Akdeniz Lamiaceae Türünün Duman ve Duman Kökenli Bileşiklere Olan Çimlenme Tepkisi. Journal of Advanced Research in Natural and Applied Sciences, 7: 478-485.

Çatav ŞS, Küçükakyüz K, Tavşanoğlu Ç, Pausas JG 2018a. Effect of fire-derived chemicals on germination and seedling growth in Mediterranean plant species. Basic and Applied Ecology, 30: 65-75.

Çatav ŞS, Elgin ES, Dağ Ç, Stark JL, Küçükakyüz K 2018b. NMR-based metabolomics reveals that plant-derived smoke stimulates root growth via affecting carbohydrate and energy metabolism in maize. Metabolomics, 14: 143.

Çatav ŞS, Surgun-Acar Y, Zemheri-Navruz F 2021. Physiological, biochemical, and molecular responses of wheat seedlings to salinity and plant-derived smoke. South African Journal of Botany, 139: 148-157.

Cembrowska-Lech D, Koprowski M, Kępczyński J 2015. Germination induction of dormant *Avena fatua* caryopses by $KAR_1$ and $GA_3$ involving the control of reactive oxygen species ($H_2O_2$ and $O_2$-) and enzymatic antioxidants (superoxide dismutase and catalase) both in the embryo and the aleurone layers. Journal of Plant Physiology, 176: 169-179.

Dogrusoz MC 2022. Can plant derived smoke solutions support the plant growth and forage quality in the hydroponic system? International Journal of Environmental Science and Technology, 19: 299-306.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28: 350-356.

Dumanović J, Nepovimova E, Natić M, Kuča K, Jaćević V 2021. The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. Frontiers in Plant Science, 11: 552969.

Flematti GR, Dixon KW, Smith SM 2015. What are karrikins and how were they 'discovered' by plants? BMC Biology, 13: 108.

Flematti GR, Merritt DJ, Piggott MJ, Trengove RD, Smith SM, Dixon KW, Ghisalberti EL 2011. Burning vegetation produces cyanohydrins that liberate cyanide and stimulate seed germination. Nature Communications, 2: 360.

Ghebrehiwot H, Kulkarni M, Bairu M, Van Staden J 2013. Plant-derived aerosol-smoke and smoke solutions influence agronomic performance of a traditional cereal crop, tef. Experimental Agriculture 49: 244-255.

Ibrahim M, Nawaz S, Iqbal K, Rehman S, Ullah R, Nawaz G, Almeer R, Sayed AA, Peluso I 2022. Plant-derived smoke solution alleviates cellular oxidative stress caused by arsenic and mercury by modulating the cellular antioxidative defense system in wheat. Plants, 11: 1379.

Jamil M, Kanwal M, Aslam MM, Khan SU, Malook I, Tu J, Rehman S 2014. Effect of plant-derived smoke priming on physiological and biochemical characteristics of rice under salt stress condition. Australian Journal of Crop Science, 8: 159-170.

Kępczyński J, Cembrowska-Lech D, Van Staden J 2013. Necessity of gibberellin for stimulatory effect of $KAR_1$ on germination of dormant *Avena fatua* L. caryopses. Acta Physiologiae Plantarum, 35: 379-387.

Khan MHU, Khattak JZK, Jamil M, Malook I, Khan SU, Jan M, Din I, Saud S, Kamran M, Alharby H, Fahad S 2017. *Bacillus safensis* with plant-derived smoke stimulates rice growth under saline conditions. Environmental Science and Pollution Research, 24: 23850-23863.

Küçükakyüz K, Çatav ŞS 2021. Physiological effects of smoke-water and karrikinolide on wheat seedlings grown under boron stress. Russian Journal of Plant Physiology, 68: 552-558.

Kulkarni MG, Ascough GD, Van Staden J 2007. Effects of foliar applications of smoke-water and a smoke-isolated butenolide on seedling growth of okra and tomato. HortScience, 42: 179-182.

Kulkarni MG, Rengasamy KR, Pendota SC, Gruz J, Plačková L, Novák O, Doležal K, Van Staden J 2019. Bioactive molecules derived from smoke and seaweed *Ecklonia maxima* showing phytohormone-like activity in Spinacia oleracea L. New Biotechnology, 48: 83-89.

Kumar V, Khare T, Shaikh S, Wani SH 2018. Compatible solutes and abiotic stress tolerance in plants, in: Metabolic Adaptations in Plants during Abiotic Stress. CRC Press, pp. 213-220.

Magwaza ST, Magwaza LS, Odindo AO, Mditshwa A 2020. Hydroponic technology as decentralised system for domestic wastewater treatment and vegetable production in urban agriculture: A review. Science of the Total Environment, 698: 134154.

Maksimović JJD, Živanović BD 2012. Quantification of the antioxidant activity in salt-stressed tissues, in: Plant Salt Tolerance. Springer, New York, pp. 237-250.

Mittler R 2017. ROS are good. Trends in Plant Science, 22: 11-19.

Moreira B, Tormo J, Estrelles E, Pausas JG 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. Annals of Botany, 105: 627-635.

Moyo M, Amoo SO, Van Staden J, 2022. Seed priming with smoke water and karrikin improves germination and seedling vigor of *Brassica napus* under varying environmental conditions. Plant Growth Regulation, 97: 315-326.

Nelson DC, Flematti GR, Ghisalberti EL, Dixon KW, Smith SM 2012. Regulation of seed germination and seedling growth by chemical signals from burning vegetation. Annual Review of Plant Biology, 63: 107-130.

Pandey R, Jain V, Singh KP 2009. Hydroponics Agriculture: Its status, scope and limitations. pp. 20-29.

Papenfus HB, Kulkarni MG, Stirk WA, Rengasamy KR, Salomon MV, Piccoli P, Bottini R, van Staden J 2015. Interactions between a plant growth-promoting rhizobacterium and smoke-derived compounds and their effect on okra growth. Journal of Plant Nutrition and Soil Science, 178: 741-747.

Qiu NW, Jiang DC, Wang XS, Wang BS, Zhou F 2019. Advances in the members and biosynthesis of chlorophyll family. Photosynthetica, 57: 974-984.

Sachdev S, Ansari SA, Ansari MI, Fujita M, Hasanuzzaman M 2021. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. Antioxidants, 10: 277.

Shabnam N, Tripathi I, Sharmila P, Pardha-Saradhi P 2016. A rapid, ideal, and eco-friendlier protocol for quantifying proline. Protoplasma, 253: 1577-1582.

Simkin AJ, Kapoor L, Doss C, Hofmann TA, Lawson T, Ramamoorthy S 2022. The role of photosynthesis related pigments in light harvesting, photoprotection and enhancement of photosynthetic yield in planta. Photosynthesis Research, 152: 23-42.

Singh S, Kulkarni MG, Van Staden J 2014. Biochemical changes associated with gibberellic acid-like activity of smoke-water, karrikinolide and vermicompost leachate during seedling development of *Phaseolus vulgaris* L. Seed science research, 24: 63-70.

Sumanta N, Haque CI, Nishika J, Suprakash R 2014. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Research Journal of Chemical Sciences, 4: 63-69.

Sunmonu TO, Kulkarni MG, Van Staden, J 2016. Smoke-water, karrikinolide and gibberellic acid stimulate growth in bean and maize seedlings by efficient starch mobilization and suppression of oxidative stress. South African Journal of Botany, 102: 4–11.

van Staden, J., Sparg, S.G., Kulkarni, M.G., Light, M.E., 2006. Post-germination effects of the smoke-derived compound 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, and its potential as a preconditioning agent. Field Crops Research, 98: 98-105.

Wang M, Schoettner M, Xu S, Paetz C, Wilde J, Baldwin IT, Groten K 2017. Catechol, a major component of smoke, influences primary root growth and root hair elongation through reactive oxygen species-mediated redox signaling. New Phytologist, 213: 1755-1770.

## *ORAL PRESENTATION*

# Tip 3 Diyabet

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## Özet

"Beyinde Diyabet" olarak adlandırılan Tip 3 Diyabet, Tip 2 Diyabet ve Alzheimer/Daemans ile birlikte görülür. Uzun süreli kontrolsüz yüksek kan şekeri damarlarda hasara neden olabilir. Beyin damarları da bu durumdan etkilenir. Diyabet ayrıca beyindeki belirli kimyasalların dengesini bozarak yüksek kan şekeri ile birlikte beyinde iltihaplanmaya neden olabilir ve beyin hücrelerine zarar verebilir. Beyindeki insülin direncinin yanı sıra kandaki düşük insülin seviyeleri ile tanımlanan Alzheimer hastalığı ile tip 3 diyabet arasında kalıtsal bir bağlantı olduğu belirtiliyor. Tip 3 diyabet; Pankreas hasarı, bazı hastalıklar ve travma nedeniyle oluşur. Yapılan bir çalışmada, tip 2 diyabetli kişilerin Alzheimer riskinin %60 daha yüksek olduğu belirtilmiştir. Tip 3 Diyabet için 3 ana risk faktörü: yüksek tansiyon, aşırı kilolu veya obez olmak, depresyon ve polikistik over sendromudur (pkos). Bilim adamları, Alzheimer hastalarının beynin belirli bölgelerindeki glikozu işlemede sorun yaşadıklarını bulmuşlardır. Bu nedenle insülin direnci teşhisi konan kişiler bir yol ayrımına geldiklerini kabul etmelidirler. Küresel diyabet topluluğu, Alzheimer hastalığının beyin dokusunda insülin direncinin gelişmesi sonucu geliştiğini belirtmektedir. Diyabet hastalarının pankreasında görülen amiloid adı verilen bir maddeye tip 3 diyabet hastalarının beyin hücrelerinde de rastlanmıştır. Ayrıca günümüz beslenme sistemi ve hareketsiz yaşam tarzının vücuttaki serbest radikaller dediğimiz hücre öldürücü maddeleri artırdığını da yapılan çalışmalarla bildirilmiştir. Bu sayede Alzheimer riski artmış olur. Alzheimer hastalığında gösterilen insülin eksikliği ve direncinin düzeltilmesi yeni tedavi yaklaşımlarının geliştirilmesine yardımcı olacaktır. İnsülin ve onun alt yolları, gelecekte hafıza işlevlerinin geri kazanılması ve korunmasında mevcut önemini korumaya devam edecektir. Diyabetin tipinin belirlenmesi tedavi süreci için son derece önemlidir. Örneğin, tip-2 tedavisi için Glikazid kullanılır, ancak tip 3c için o kadar etkili olmayabilir. Bu gibi nedenlerle tanının doğru konması hem hastanın sağlığı açısından hem de tedavi süresince ekonomik açıdan faydalı olacaktır. Bu sayede yanlış teşhisten kaynaklanan zaman ve para kaybı ortadan kalkacaktır. Bu nedenlerle hem sağlık çalışanları hem de toplum tip 3c hakkında yeterince bilgilendirilmeli ve diyabet gibi önemli bir hastalık asla göz ardı edilmemelidir.

**Anahtar Kelimeler**: Alzheimer, İnsülin direnci, Pankreas hasarı

# Type 3 Diabetes

## Abstract

Type 3 Diabetes, called "Diabetes in the Brain", is seen together with Type 2 Diabetes and Alzheimer's/Dementia. Long-term uncontrolled high blood sugar can cause damage to the vessels. Brain vessels are also affected by this situation. Diabetes can also disrupt the balance of certain chemicals in the brain, causing inflammation in the brain along with high blood sugar, and can damage brain cells. It is stated that there is a hereditary link between Alzheimer's disease, which is defined by low insulin levels in the blood, as well as insulin resistance in the brain, and type 3 diabetes. type 3 diabetes; It occurs due to pancreatic damage, some diseases and trauma. In one study, it was stated that people with type 2 diabetes had a 60% higher risk of Alzheimer's. The 3 main risk factors for Type 3 Diabetes are: high blood pressure, being overweight or obese, depression and polycystic ovary syndrome (pkos). Scientists have found that people with Alzheimer's have problems processing glucose in certain areas of the brain. Therefore, people diagnosed with insulin resistance should accept that they have come to a crossroads. The global diabetes community states that Alzheimer's disease develops as a result of the development of insulin resistance in the brain tissue. A substance called amyloid, which is seen in the pancreas of diabetic patients, has also been found in the brain cells of type 3 diabetes patients. It has also been reported by studies that today's nutrition system and sedentary lifestyle increase cell-killing substances in the body, which we call free radicals. In this way, the risk of Alzheimer's increases. Correction of insulin deficiency and resistance shown in Alzheimer's disease will help develop new treatment approaches. Insulin and its sub-pathways will continue to maintain their current importance in restoring and preserving memory functions in the future. Determining the type of diabetes is extremely important for the treatment process. For example, Glycazide is used for type-2 treatment, but may

not be as effective for type 3c. For such reasons, making the diagnosis correctly will be beneficial both for the health of the patient and economically during the treatment. In this way, the waste of time and money due to misdiagnosis will be eliminated. For these reasons, both healthcare professionals and the public should be adequately informed about type 3c, and an important disease such as diabetes should never be ignored.

**Keywords**: Alzheimer, İnsulin resistance, Pancreatic damage

## GİRİŞ

Tip 1 diyabet otoimmün bir hastalık olup çoğunlukla daha genç kişilerde ortaya çıkmasına rağmen nadir olarak yaşlılarda da görülmektedir. Çoğunlukla bu hastalığın sorumlusu, pankreastaki insülin üreten hücrelere saldıran bir bağışıklık sistemi reaksiyonudur. Bu yüzden bağışıklık sistemi pankreasta normalde insülin üreten beta hücrelerini (Langerhans-adacıkları) tahrip eder. Bunun sonucu olarak mutlak bir insülin eksikliği ortaya çıkar. İnsülin eksikliği nedeniyle hücreler son derece yetersiz miktarda glikoz emilimi gerçekleştirir, bu yüzden kanda çok fazla glikoz birikir. Hastalığın seyri sırasında aşırı susama, sık idrara çıkma, kilo kaybı, yorgunluk ve halsizlik gibi şikayetler görülür. Tip 1 diyabet hastalığında hastaya ömür boyu insülin iğnesinin uygulanması gerekmektedir (Donnelly et al., 2000). Tip 2 diyabet, diyabet hastalıkları arasında en yaygın görülen türdür. Diyabet vakalarının %85'e yakınını Tip 2 diyabet oluşturmaktadır. Önceleri, Tip 2 diyabette metabolizma bozukluğu çoğunlukla daha ileri yaşlarda ortaya çıktığı için bu hastalık "erişkin tipi diyabet" olarak da adlandırılmıştır. Ancak gün geçtikçe insülin hormonu düzgün çalışmayan daha fazla genç yaştaki kişilerede Tip 2 diyabet tanısı konmuştur (Brownlee, 2001). Tip 2 diyabet türünde pankreas yeterli insülin hormonu üretebildiği halde bu hormon vücutta etkin olarak kullanılamaz. Sonuç olarak vücutta insülin üretimi yavaşlar ve vücut insüline daha az duyarlı hale gelir. Bu duruma insülin direnci denir (Baysal, 2000). Tip 1 ve tip 2 diabetes mellituslu (DM) bazı hastalarda bilişsel işlev bozuklukları mevcuttur. Tip 1 DM, bilgi işleme hızında, psikomotor etkinlikte, dikkatte, zihinsel esneklikte ve görsel algıda azalma ile ilişkilendirilmiştir. Tip 1 DM'nin uzunlamasına epidemiyolojik çalışmaları, tekrarlayan şiddetli hipoglisemiden ziyade kronik hiperglisemi ve mikrovasküler hastalığın DM ile ilişkili bilişsel işlev bozukluğunun patogenezi ile ilişkili olduğunu göstermiştir. Bununla birlikte, şiddetli hipoglisemik ataklar, DM'li yüksek riskli hastalarda bilişsel işlev bozukluğuna katkıda bulunabilir. Tip 2 DM, hafıza açıkları, psikomotor hızın azalması ve ön lob/yürütme işlevinde azalma ile ilişkilendirilmiştir. Tip 2 DM'de, kronik hiperglisemi, DM'nin uzun sürmesi, vasküler risk faktörlerinin (örn., hipertansiyon ve obezite) varlığı ve mikrovasküler ve makrovasküler komplikasyonlar, artan bilişsel işlev bozukluğu geliştirme riski ile ilişkilidir. DM'li bireylerde bilişsel işlev bozukluğunun patofizyolojisi şunları içerir: (1) hipergliseminin rolü (Barbagallo and Dominguez, 2014), (2) vasküler hastalığın rolü (Szeman et al., 2012) , (3) hipogliseminin rolü (Futamura et al., 2015) ve (4) insülin direnci ve amiloidin rolü (Jayaraj et al., 2020; Barbgallo and Dominguez., 2014). Son zamanlarda, bazı araştırmacılar tip 3 DM'nin sporadik Alzheimer hastalığı ile ilişkili olduğunu öne sürmüşlerdir. DM2, günümüzde sıklıkla yaşlanma ile ilişkilendirilen en hızlı büyüyen salgınlardan biridir. DM2'nin karakteristik özellikleri arasında insülin hareketlerinde ve sinyalleşmede bozulmalar yer alır. Periferik dokularda insülin direnci hiperglisemi ve hiperinsülinemi ile sonuçlanır. AD en yaygın nörodejeneratif bozukluktur ve insidansı yaşla birlikte artan bir hastalıktır (Futamura et al., 2015). AD, nöron kaybı, hücre dışı amiloid beta birikintilerinden oluşan yaşlılık plaklarının oluşumu, beyinde kümelenmiş hiperfosforile tau proteinlerinden oluşan hücre içi nörofibriler yumaklar, astrositlerin çoğalması ve mikroglia aktivasyonu dahil olmak üzere çeşitli patolojik işaretlerin varlığı ile karakterize edilir. Bu özelliklere mitokondriyal disfonksiyon ve nöronal sinapslardaki değişiklikler eşlik eder (Futamura et al., 2015). AD'nin altında yatan moleküler ve patofizyolojik mekanizmalar hala birçok karanlık tarafa sahiptir. AD'nin patolojik değişikliklerini tetikleyen etiyoloji ve kesin mekanizma hala net olmasa da, çoğu çalışma, amiloid-beta öncü proteininin (amiloid kaskad hipotezi) anormal bir şekilde işlenmesinin neden olduğu toksik amiloid-beta peptidinin birikmesinin, AD patogenezini başlattığı ve katkıda bulunabildiği söylenmektedir (Kukull et al., 2002; Mattson, 2004; Barbagallo and Dominguez, 2014).

## TARTIŞMA

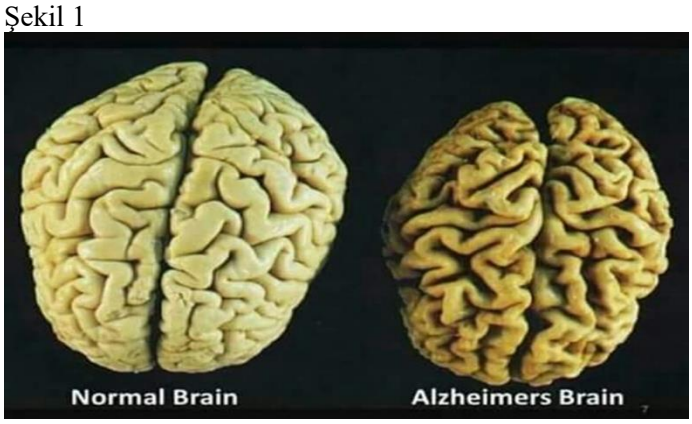
Artan epidemiyolojik ve biyolojik kanıtlar, yaşlanmayla ilgili bu iki hastalık arasında bir bağlantıyı desteklemektedir. Her şeyden önce, diabetes mellitus bilişteki değişiklikler ve bilişsel işlev bozukluğu ile ilişkilidir. Diyabetli kişilerde bilişsel gerileme ve AD insidansının daha yüksek olduğu bildirilmiştir (Ott et al., 1999; Barbagallo and Dominguez, 2014). 9 yıla kadar süren bir kohort çalışmasında, diyabetli kişilerde Alzheimer hastalığı geliştirme riski diyabetik olmayan kontrollere göre %65 daha yüksek olduğu bulunmuştur (Arvanitakis et al., 2004; Janson et al., 2004). Toplum temelli kontrollü bir çalışmada (What is Type 3c diabetes?, Diabetes UK, 2022), AD'li hastalarda diyabet ve glukoz intoleransı prevalansı ve AD'si olmayan kontrol katılımcıları incelemiştir. Çalışma, AD'li hastaların %80'ine kadarında açık diyabet (%35) veya glukoz intoleransının (%46) mevcut olabileceğini öne sürmüştür. De la Monte, AD'nin, diyabet ile örtüşen moleküler ve biyokimyasal özelliklerle beyni seçici olarak içeren bir diyabet formunu temsil edebileceğini belirterek,

AD'nin "tip 3 diyabet" olarak adlandırılabileceğini bile öne sürmüştür (De la Monte and Wand, 2008; Barbagallo and Dominguez, 2014). Beyin yaşlanmasında insülinin rolünün önemi uzun zamandır bilinmektedir. İnsülinin beyinde önemli nörotrofik özellikleri vardır. Hormon, insülin reseptörlerinin aracılık ettiği bir taşıma mekanizması ile kan-beyin bariyeri yoluyla hızla merkezi sinir sistemi seviyesine taşınmaktadır. Bu reseptörlerin esas olarak hipokampus, entorinal korteks ve hafıza ve öğrenme gibi işlevlerde yer aldığı bilinen frontal alanlarda lokalize olduğunu belirtmektedir. İnsülin ayrıca asetilkolin ve norepinefrin gibi önemli nörotransmitterlerin üretiminde de rol oynar (Barbagallo and Dominguez, 2014). Dolaşımdaki insülin seviyelerinde akut bir artışın, tokluk sonrası dönemde meydana geldiği gibi, beyindeki hormon konsantrasyonlarında fizyolojik paralel bir artışı belirlediği bilinmektedir. İnsülin direnci koşullarında ve DM2'de meydana geldiği gibi bir kronik hiperinsülinemi durumu, kan-beyin bariyerindeki insülin reseptörlerinin aşağı regülasyonunu belirleyebilir, böylece beyinde insülin taşınmasını azaltabilir. DM2'de AD'nin patogenezi olarak öne sürülen diyabetli kişilerde meydana gelebilen, beyinde amiloid öncüllerinin birikmesi ve metabolizmadaki bir değişikliği ilişkilendiren kanıtlar vardır. Amiloid öncü proteini, 770 amino asitten oluşan bir transmembran proteinidir; AD etyopatogenezinde rol oynayan amiloid betanın öncüsü olduğu bilinmektedir. Amiloid beta ve izoformlarının rolü henüz aydınlatılamamış olsa da, birçok fizyolojik süreçte yer aldığı görülmektedir. İnsülin önemli bir nörotrofik faktör olsa bile klinik hiperinsülinemi AD için nasıl bir risk faktörü olabilir? Bu iki belirgin paradoksal bulgu, insülin direnci kavramıyla uzlaştırılabilir. İnsülin orta konsantrasyonlarda nörotrofik bir faktör iken, beyinde yüksek konsantrasyonlarda insülin bulunan hiperinsülinemi, ortak ve ana indirgeme mekanizmaları olan "İnsülin-Degrading Enzim" (IDE) için rekabet nedeniyle azalmış amiloid-beta klirensi ile ilişkili olabilir. İnsülin, hücre içi birikimi azaltan amiloid öncü proteinin metabolizmasını modüle eder. İnsülin, amiloid betanın metabolizmasında ve bozulmasında da yer alan IDE tarafından parçalanır. Bu çok işlevli enzim, AD beynindeki amiloid-beta peptidi ile birlikte DM2 patolojisi ile ilgili peptidler olan insülin ve amilin'i bozar (Qiu and Folstein, 2006; Barbagallo and Dominguez, 2014). Bozulmuş açlık glukozu ve bozulmuş glukoz toleransı ile kognitif bozukluk arasındaki ilişki hakkında hala sınırlı bilgi olmasına rağmen, bu prediyabetik durumların yaşlı hastalarda AD riskini artırabileceğine dair artan kanıtlar vardır. Son 5 yıldaki ortalama glukoz konsantrasyonlarına göre diyabetik ve diyabetik olmayan kişilerde demans riski artmıştır. Diyabet tedavisinin ve glisemik kontrolün demans riski üzerindeki etkisi daha az nettir. DM2'li hastalarda bilişsel performansın korunmasında glisemik kontrolün rolü olabileceği öne sürülmüştür (Barbagallo and Dominguez, 2014). HbA1c ayrıca daha yüksek beyin atrofisi oranı için ek bir risk faktörü olarak tanımlanmıştır. Enzinger ve arkadaşlarının (2005). Avusturyada İnmeyi Önleme Çalışmasında, 201 katılımcıda MRG ile 6 yıl boyunca yıllık beyin hacmi değişikliklerini ölçmüşler ve HbA1c düzeylerinin dörtte birlik bölümlerine göre beyin atrofi oranlarında önemli farklılıklar bulmuşlardır (Enzinger et al., 2005). Yüksek HbA1c'li kişilerde metabolik sendrom olarak adlandırılan faktörlerle ilişkili faktörlerin kümelenmesi, insülin direnci ve hiperinsülinemi ile ilişkili bu sendrom ile ileri yaştaki beyin dokusu kaybı arasında bir bağlantı olduğunu düşündürmektedir (Barbagallo and Dominguez, 2014). Tip 2 diabetes mellitus (T2DM), nüfus arasında hızla büyüyen bir etiyolojidir. Tedavi stratejilerindeki gelişmeler ve diyabetlilerin kapsamlı bakımı ile hayatta kalma oranı önemli ölçüde artmıştır. Bu nedenle, nüfusun büyük bir kısmı diyabetle daha uzun yaşamakta ve komplikasyonlarıyla uğraşmaktadır (Li et al., 2015; Mittal and Katre, 2016). Son zamanlardaki çalışmalardan bazıları, çeşitli biyolojik süreçlerin ve sinyal yollarının bozulmasında insülin direncinin etkilerini büyük ölçüde olduğunu açıklamışlardır. Biyokimyasal, histopatolojik ve moleküler düzeyde anormalliklere yol açan bir demans türü olan Alzheimer hastalığı (AH), T2DM'nin ortaya çıkan komplikasyonlarından biridir (Talbot et al., 2012; Mittal and Katre, 2016). Deneysel kanıtlar, bozulmuş glikoz metabolizmasının bilişsel yeteneklerde erken anormalliklerle sonuçlandığını göstermiştir. Beyne glikoz alımı doyurulabilir bir süreçtir, ancak beyinde glikoz alımı arttığında, insülin reseptörlerinin yukarı regülasyonunu gerektirecektir. Ancak bu reseptörlerin aktivitesi tehlikeye girdiğinde, glikoz metabolizmasını azaltan fonksiyonel hipoglisemiye yol açabilir. Öte yandan, glikoz beyne normal olarak giriyorsa ancak insülin miktarı yeterli değilse, glikolize son ürünlerin oluşumuna yol açacaktır. İnsülin bilişsel işlevlerde çok önemli bir rol oynar. İnsülin akut olarak verilirse hafıza ve biliş fonksiyonlarını iyileştirecek, ancak kronik olarak verildiğinde biliş yeteneklerini etkileyerek insülin direncine, inflamasyona ve hiperglisemiye yol açacaktır. Ayrıca insülinin kan beyin bariyerindeki duyarlılığını azaltır ve dolayısıyla beyindeki glikoz metabolizmasını etkiler. Yani bu durumda enerji yakıtı vücudun içindeyken beyin hücreleri bunu kullanamadığında beyin hücrelerinde açlık oluşur (Craft et al., 1998; Convit, 2005; Monte et al., 2006; Mittal and Katre, 2016).

Çalışmalar, AD'nin Braak evresinin, kortikal nöronlarda ChAT'nin insülin veya IGF-1 reseptörü ile azalmış immünoreaktivitesiyle sonuçlanan kolin asetil transferaz (ChAT) ekspresyonunun düşük seviyesi ile ilişkili olduğunu göstermiştir. Deneysel veriler, tau proteini ve ChAT gen ekspresyonunun insülin ve IGF-1 reseptörü tarafından kontrol edildiğini göstermiştir (Monte et al., 2006; Mittal and Katre, 2016; River et al., 2005). Bu nedenle, insülin direnci veya eksikliği beyin plastisitesine, sinaptik işlev bozukluğuna ve nörodejenerasyona katkıda bulunur ve bilişsel yetenekleri bozarak Diabetes mellitus ile AD arasında biyokimyasal bir bağlantı kuran asetilkolin üretimini azaltır (Mittal and Katre, 2016). AD'de tip 3 DM fikrini destekleyen en güçlü kanıt,

intraserebroventriküler enjeksiyon yoluyla diyabet öncesi bir ilaç olan streptozotosin alan sıçanların deneysel bir çalışmasından elde edilmiştir. Streptozotosin ile tedavi edilen sıçanlar, uzaysal öğrenme ve hafıza eksikliği, beyin insülin direnci ve eksikliği ve AD benzeri nörodejenerasyon ile karakterize edilen bilişsel işlev bozuklukları geliştirmiştir (Lester et l., 2006; Weinstock and Shohm, 2004). Obezite, yağ metabolizmasında bozukluk, hareketsizlik, yüksek kolesterol değerleri ve yüksek tansiyon Tip 2 diyabetin gelişiminde öne çıkan risk faktörleridir. 2016'da yapılan bir araştırmaya göre, tip 2 diyabeti olan kişilerde Alzheimer hastalığı veya vasküler demans gibi başka bir demans türü geliştirme olasılığı yüzde 60'ın da üzerine kadar çıkabildiği gözlemlenmiştir (What is Type 3c diabetes?, Diabetes UK, 2022 ). Diyabet, beyninizde Alzheimer'ı tetikleyebilecek kimyasal dengesizliklere de neden olabilir. Ayrıca, yüksek kan şekeri seviyeleri, beyin hücrelerine zarar verebilecek iltihaplanmaya yol açar. Bu nedenlerle diyabet, vasküler demans adı verilen bir durum için bir risk faktörü olarak kabul edilir. Vasküler demans, kendi semptomları olan tek başına bir tanıdır veya Alzheimer hastalığının tetikleyicisidir. Zamanla, tedavi edilmeyen diyabet, beyninizdeki damarlar da dahil olmak üzere kan damarlarınıza zarar verebilir. Tip 2 diyabetli birçok kişi, teşhis ve uygun tedavi önlemlerini geciktirebilecek duruma sahip olduklarını bilmiyor. Bu nedenle, tip 2 diyabetlilerde, özellikle teşhis edilmemiş diyabetlilerde bu tür hasar riski daha yüksektir (Relationship Between Type 3c Diabetes and Pancreatic Cancer, The ASCO Post, 2020).

Şekil 1




(Relationship Between Type 3c Diabetes and Pancreatic Cancer, The ASCO Post, 2020).

Tip 3c diyabetin batı ülkelerinde yaklaşık %5-10 oranında olduğu düşünülmektedir. Genellikle tip 2 diyabet olarak yanlış teşhis edilebildiğinden, belki de oranı sanıldığından daha yüksektir. Bununla birlikte tip 3c diyabet tanısının doğru konması, hem uygun tedavinin verilmesi, hem de pankreas kanserinin erken teşhisine yardımcı olabilmesi açısından çok önemlidir. İspanyol Ulusal Kanser Araştırma Merkezi (CNIO) yaptığı bir araştırmanın sonuçlarını paylaşmıştır. Çalışmada pankreas kanserli her 4 hastadan 1'inde (%26) tip 3c diyabet geliştiği gözlenmiştir. Tip 3c diyabet nasıl gelişir? (Molina-Montes et al., 2021). Pankreasla ilgili gelişen hastalıklar tip 3c diyabete neden olmaktadır. Bu hastalıklar arasında pankreastit (pankreas iltihaplı hastalığı), pankreas kanseri, kistik fibrozis, hemokromatoz (demir birikmesi / bronz diyabet) sayılabilir. Pankreas ameliyatları sonrasında da Tip 3 c diyabet gelişebilmektedir (Molina-Montes et al., 2021).

Alzheimer hastalığı (AH), ilerleyici nöronal doku kaybına yol açan kronik nörodejeneratif bir hastalıktır. AD'ye benzer şekilde, tip 2 diabetes mellitus (T2DM) de benzer bir görünüme sahiptir. İki hastalığın küresel yükü hızlı bir eğimde olduğundan, T2DM'nin AD'nin ilerlemesi veya kötüleşmesi üzerindeki etkisinin araştırılması gerekli görülmektedir. Geç başlangıçlı bunamanın en yaygın nedeni olan AD, sinir sisteminin ilerleyici bir dejeneratif bozukluğudur. Amerika Birleşik Devletleri'nde yaklaşık 5.5 milyon Amerikalıyı (Silzer et a1., 2019) etkileyen ve dünya çapında yaklaşık 34 milyon insanı etkileyen altıncı önde gelen ölüm nedeni olduğu bilinmektedir (Barnes et al., 2011). Hastalık çok faktörlü olduğundan, hem genetik hem de çevresel yönlerin bir kombinasyonu, bireyin bu rahatsızlığı yaşamda geliştirme olasılığını artırır. Hastalığın göze çarpan bir özelliği, tau ve Amiloid beta (A-beta) protein birikimine bağlı yoğun nöron kaybıdır (Baram et al., 2016). Bununla birlikte, genetik bileşenin nadir formlardan biri olan erken başlangıçlı ailesel AD'ye neden olduğu bilinmektedir. A-beta polimerinin metabolizmasını içeren genler kendiliğinden mutasyona uğrayarak erken hastalık belirtilerine yol açabilir. Bunlar arasında en yaygın olanları amiloid öncü protein (APP), presenilin1 (PSEN1) ve presenilin2 (PSEN2)'dir. Bu, beynin hafıza bölgelerinde, özellikle hipokampus ve kortekste A-beta oligomerlerinin birikmesine yol açar (Baram et al., 2016, Ruiz et al., 2016; Nisar et al., 2020).

Tip 3 diyabet semptomları, erken Alzheimer hastalığında görülenler gibi demans semptomları olarak tanımlanır: Günlük yaşamı ve sosyal etkileşimleri etkileyen hafıza kaybı, tanıdık görevleri tamamlamada zorluk, objeleri, eşyaları sık sık yanlış yerleştirmek, bilgiye dayalı yargıda bulunma yeteneğinin azalması, kişilik veya tavırda ani değişiklikler olarak tanımlanabilir. Hem tip 2 diyabetiniz hem de Alzheimer hastalığınız

varsa, tip 2 diyabetinizin tedavisi demansın ilerlemesini yavaşlatmaya yardımcı olmak için önemlidir. Metformin ve insülin, 2014 yılında yapılan bir araştırmaya göre, diyabetin neden olduğu beyin hasarı geliştirme riskini de azaltabilen bir anti-diyabet ilaçlarıdır (Kirpichnikoc et al., 2002; Hundal, 2003). İnsan beyninin otopsi sonuçlarında, diyabetik hastalarda artmış seviyelerde nörofibriler yumaklar ve amiloid beta plakları görüldü. Amiloid beta plakları, APP'nin beta-sekretaz tarafından uygun olmayan şekilde bölünmesinin sonuçlarıdır. Bu anormal amiloid beta, sinyal yollarıyla etkileşime girer ve nörofibriler yumaklar oluşturmak üzere bir araya gelen tau proteinini hiperfosforile eder. Glikojen sentaz kinaz-3 (GSK-3) her iki patolojide de tau proteininin fosforilasyonunda önemli bir rol oynamıştır. Dolayısıyla, GSK-3'ün inhibisyonu, T3D tedavisi için potansiyel bir hedef olabilir (Bhat et al., 2004).

## SONUÇ

Alzheimer hastalığı kanda düşük insülin seviyesi ve beyinde insülin direnciyle tanımlanır. Serebrovasküler sistemin bozulması, yani beyindeki kan damarlarının hasarı, diyabet ve demansta etkili olan ve bu hastalıklarla ilişkilendirilen bir başka faktördür. Bu nedenle iyi bir diyabet tedavisi her zaman önemli bir demans önleme yöntemi olarak da görülmektedir. Diyetinizde değişiklikler yapmak ve günlük rutininize egzersizi dahil etmek gibi yaşam tarzı değişiklikleri tedavinizin büyük bir parçası olabilir. Dengeli beslenmenin yanı sıra yaşam tarzının değiştirilmesi, fazla kiloların verilmesi ve fiziksel aktivitenin artırılmasıyla iyileşme sağlanabilir. Yağ oranı düşük ve meyve ve sebzeler açısından zengin bir diyet semptomları iyileştirmeye yardımcı olabilir. Bu derleme, hem tanının doğru konması hem de hastanın sağlığı için faydalı olabilir. Bu sayede yanlış teşhisten kaynaklanan zaman ve para kaybı da önlenmiş olacaktır. Bu nedenlerle hem sağlık çalışanları hem de toplum tip 3c hakkında yeterince bilgilendirilmeli ve diyabet gibi önemli bir hastalık asla göz ardı edilmemelidir.

## KAYNAKLAR

Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA 2004. Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. Arch Neurol, 61: 661-666.

Baram M, Atsmon-Raz Y, Ma B, Nussinov R, Miller Y 2016. Amylin-Aβ oligomers at atomic resolution using molecular dynamics simulations: a link between type 2 diabetes and Alzheimer's disease. Phys Chem Chem Phys, 18: 2330-2338.

Barbagallo M, Dominguez LJ 2014. Type 2 diabetes mellitus and Alzheimer’s disease. World J Diabetes, 5(6): 889-893

Baysal A 2006. Beslenme. Hatiboğlu Yayınları:93, Ankara, Şahin Matbaası.

Barnes DE, Yaffe K 2011. The projected effect of risk factor reduction on Alzheimer’s disease prevalence. Lancet Neurol, 10: 819-828.

Brownlee M 2001. Biochemistry and molecular cell biology of diabetic complications. Nature, 414: 813-820

Bhat V, Haeberlein B, Avila J 2004. Glycogen synthase kinase 3: a drug target for CNS therapies J Neurochem, 89 (6): 1313-1317

Convit A 2005. Links between cognitive impairment in insulin resistance: an explanatory model. Neurobiol Aging, 26 (1): 31-35

Craft S, Peskind E, Schwartz W, Schellenberg D, Raskind M, Porte D 1998. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease Relationship to severity of dementia and apolipoprotein E genotype. Neurology, 50 (1): 164-168.

De la Monte SM, Wands JR. 2008. Alzheimer’s disease is type 3 diabetes-evidence reviewed. J Diabetes Sci Technol, 2: 1101-1113.

Donnelly R, Emslie-Smith AM, Gardner ID, Morris AD 2000. ABC of arterial and venous disease: vascular complications of diabetes. BMJ, 320: 1062-1066

Enzinger C, Fazekas F, Matthews PM, Ropele S, Schmidt H, Smith S, Schmidt R. 2005. Risk factors for progression of brain atrophy in aging: six-year follow-up of normal subjects. Neurology, 64: 1704-1711

Futamura A, Mori Y, Kawamura M. 2015. Diabetes and Dementia. Brain Nerve, 67(6): 725-32.

Hundal RS, Inzucchi SE 2003. Metformin: new understandings, new uses. Drugs, 63: 1879-94.

Janson J, Laedtke T, Parisi JE, O’Brien P, Petersen RC, Butler PC 2004. Increased risk of type 2 diabetes in Alzheimer disease. Diabetes, 53: 474-481.

Jayaraj RL, Azimullah S, Beiram R 2020. Diabetes as a risk factor for Alzheimer's disease in the Middle East and its shared pathological mediators. Saudi J Biol Sci, 27(2): 736-750.

Kukull WA, Higdon R, Bowen JD, McCormick WC, Teri L, Schellenberg GD, van Belle G, Jolley L, Larson EB 2002. Dementia and Alzheimer disease incidence: a prospective cohort study. Arch Neurol, 59: 1737-1746.

Lester-Coll N, Rivera EJ, Soscia SJ, Doiron K, Wands JR, de la Monte SM 2006. Intracerebral streptozotocin model of type 3 diabetes: relevance to sporadic Alzheimer's disease. J Alzheimers Dis, 9: 13-33.

Li X, Song D, Leng SX 2015. Link between type 2 diabetes and Alzheimer's disease: from epidemiology to mechanism and treatment. Clin Interv Aging, 10,10: 549-60.

Mattson MP 2004. Pathways towards and away from Alzheimer's disease. Nature, 430: 631-639.

Molina-Montes E, Coscia C, Gómez-Rubio P 2021. On behalf of the PanGenEU Study Investigators, et alDeciphering the complex interplay between pancreatic cancer, diabetes mellitus subtypes and obesity/BMI through causal inference and mediation analyses. Gut, 70: 319-329.

Monte D, Tong M, Lester N, Plater M, Wands Rs 2006. Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: relevance to Alzheimer's disease. J Alzheimer's Dis, 10 (1): 89-109

Mittal K, Katare DP 2016. Shared links between type 2 diabetes mellitus and Alzheimer's disease: A review. Diabetes Metab Syndr, 10(2-1): 144-9.

Nisar O, Pervez H, Mandalia B, Waqas M, Sra HK 2020. Type 3 Diabetes Mellitus: A Link Between Alzheimer's Disease and Type 2 Diabetes Mellitus. Cureus, 12(11): 11703

Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, Breteler MM 1999. Diabetes mellitus and the risk of dementia: The Rotterdam Study. Neurology, 53:1937-1942

Relationship Between Type 3c Diabetes and Pancreatic Cancer, The ASCO Post. https://ascopost.com/issues/november-25-2016/recent-progress-and-concepts-in-pancreatic-cancer/

Rivera J, Goldin A, Fulmer N, Tavares R, Wands R, Monte D, et al. (2005. Insulin and insulin-like growth factor expression and function deteriorate with progression of AD: link to brain reductions in acetylcholine. J Alzheimer's Dis, 8 (3): 247-268

Ruiz HH, Chi T, Shin AC, et al. 2016 .Increased susceptibility to metabolic dysregulation in a mouse model of Alzheimer's disease is associated with impaired hypothalamic insulin signaling and elevated BCAA levels. Alzheimers Dement, 12: 851-861.

Silzer T, Barber R, Sun J, Pathak G, Johnson L, O'Bryant S, Phillips N 2019. Circulating mitochondrial DNA: new indices of type 2 diabetes-related cognitive impairment in Mexican Americans. PLoS One.14: 213527

Szémán B, Nagy G, Varga T, Veres-Székely A, Sasvári M, Fitala D, Szollosi A, Katonai R, Kotyuk E, Somogyi A, Orv Hetil 2012. Changes in cognitive function in patients with diabetes mellitus, 4,153(9): 323-9.

Talbot K, Wang Y, Kazi H, Han Y, Bakshi Stucky A, Arnold E 2012. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. J Clin Invest, 122 (4):1316-1338,

Qiu WQ, Folstein MF 2006. Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis. Neurobiol Aging, 27:190-198.

Weinstock M, Shoham S 2004. Rat models of dementia based on reductions in regional glucose metabolism, cerebral blood flow and cytochrome oxidase activity. J Neural Transm (Vienna), 111:347-66.

What is Type 3c diabetes?, Diabetes UK. https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447,2022

## *ORAL PRESENTATION*

# Recent 3D Bioprinting Applications in Food and Agriculture

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## Abstract

Due to the rapid developments in the food and agriculture sector, all professionals in the industry follow the latest trends, best practices and effective tools to use in the field of production. One of these innovations is three-dimensional (3D) printing technology, an innovative technology adopted to develop customized products. It is a new computer-aided technology that offers the opportunity to produce products with complex geometric structures that cannot be created with classical production methods. 3D printing technology is based on the creation of the product in layers using computer design programs. With this technology, it is a prominent issue as it adapts to a personalized diet, prevents waste, minimizes personnel mistakes and increases production efficiency, and creates advantages in terms of producing quality products with less waste. In addition, process costs can be minimized by using less raw materials and energy. In this way, it plays an important role in reducing the ecological carbon footprint. However, it can also be used in the production of functional or specialized foods that contribute to a healthy diet and have the potential to improve health and reduce the risk of disease. 3D printing technologies have made great advances in medicine, pharmacy and cosmetics, and they are one of the current topics studied in the field of food and agriculture in recent years. In this review, information about the general principles of 3D printing technology, 3D printing stages, 3D printers, current developments and methods of the 3D printing process in the food and agriculture sector are given. It is thought that 3D printing technologies will take place more in the future within the concept of Society 5.0, which has emerged in order to increase the quality of life of people and to accelerate sustainable development by using technology to ensure cooperation between societies.

**Keywords:** 3D Bioprinting, biotechnology, food, agriculture, food design, plant science, sustainability, innovation

## Introduction

With the Fourth Industrial Revolution (Industry 4.0), technology is developing rapidly and developments have been experienced in many areas. Fourth Industry 4.0 process (4IR) has many sub-branches and it can be shaped depending on the conditions of the age (Chaka, 2020). Big data, cloud systems, cyber security, robotic applications are some of the areas that can be investigated under 4IR practices (Oztemel and Gursev, 2020). Production is one of the most important areas where these developments are experienced. Thanks to 3D printing technology, which is one of the components of Industry 4.0, positive developments have been achieved in production. Although 3D printing technology is seen as a new technology, it actually appeared for the first time in 1984. Various improvements have been made on this technology over time. Additive manufacturing is carried out thanks to devices called 3D printers. It is more advantageous in terms of cost and production speed compared to the production made with the traditional method. 3D printing technology can also be considered as an area with a high potential, since it has a wide application area in a wide range of industrial processes. It is seen that the technique used in 3D printers is different from the processing or shaping techniques used in traditional production (Shahrubudin et al. 2019).

3D printing or additive manufacturing technology involves processing the material by layering it until a certain shape is obtained to produce a 3D object. Today, 3D printers are used in aerospace, health, education, automotive, food, jewelry, defense industry, textile, construction, etc. has taken its place in many areas (Manstan & McSweeney, 2020; Burke-Shyne et al., 2020). Many products that you can imagine can be produced in hours or even minutes by creating models with computer-aided software. Unlike traditional manufacturing methods, 3D printing technology attracts attention due to its personalized geometry, reduced production cost, shortened production cycle and potential for use even in visually complex products (He et al., 2020). Unlike the traditional chip removal technique, which is based on the removal of materials as a result of processes such as drilling or cutting, it has been understood that production with 3D printers is a technology for adding materials. In order to produce a product with traditional production technology, a computer-prepared drawing of the product, supporting production equipment (mold, tool, fixture, etc.), various manufacturing machines and raw materials are needed. In the 3D printing technology, which is very practical and efficient, there is no need for any other hardware other than computer drawing, a 3D printer with material agglomeration and raw materials. At the same time, many different materials are used. In traditional printing, the preparation of the prototype before printing takes a long time and this can cause a waste of time, effort and energy by keeping the printing time longer than it should be (Attaran, 2017). With the use of 3D printers, the time spent to produce these prototypes has been reduced by many times. In addition, it is known that by using 3D printing technology, the raw material requirement and the amount of waste are considerably less than the other classical methods used. Thus, flexible and cost-effective production can be achieved with 3D modeling (Huang and Lin, 2017).

3D printing technologies; It is used in the production of artificial tissues, organs and personalized prostheses in the field of health. It is used in the production of spare parts for vehicles in the automotive industry, defense industry and aerospace fields. It is used in the construction industry in the construction of houses that are faster and less costly. In the field of textiles, the modeling of clothes, the creation of patterns and the production of clothes are carried out. It is used in the design of personalized and customized foods in the food industry, in the production of cakes and chocolates, and in the production of functional foods. In the agricultural sector, it is used in irrigation systems, sustainable closed gardens, customized production vehicles, and the production of spare parts (Zhang et al., 2022). 3D printers, which bring revolutionary changes and innovations to production technology, force the imagination of human beings. Creative ideas and designs can quickly turn into real models, end products and parts. It is thought that this new generation technology, which has produced very special and interesting products as well as all kinds of products imaginable since the first day of its use, will shape the future in an incredible way. With the use of 3D printing technology, such as airplanes, automobiles, spare parts, shoes, bicycles, toys, jewelry, accessories, flower pots, hand tools, chairs, seats, glasses, forks, spoons, chemicals, medical equipment, drugs, dentures, medical prosthesis all kinds of products and most importantly artificial organs designed to be transplanted into the human body will be produced. For this reason, 3D technology finds more and more application opportunities in different sectors (Horne and Hausman, 2017).

3D bioprinting technology is another side of 3D printing technology. In bioprinting, cells are used as bioinks. Human tissue is created from modified printer cartridges and patient biopsies (Agarwala, 2016). Cells are collected and filled into the cartridge to make bioink (Zhuang et al. 2021). The resulting bioink is put into the bioprinter together with the hydrogel. After this process, a layer of hydrogel and then a layer of bioink cells are printed. And the layers are combined. Hydrogels are 3D hydrophilic cross-linked polymers. Three-dimensional cross-linked swelling polymer structures Swell in the presence of water due to its hydrophilic structure Insoluble due to chemical cross-links It has a porous structure. 3D bioprinting technology is used in contact lenses, membrane artificial heart and artificial skin materials for biosensors, molecular separation systems, gel-based activators, valves, controlled release systems of drugs and other substances, artificial muscle for robotic instruments, reversible adsorbents, coatings (Dani et al. 2021).

The aim of this review is to explain the stages of printing in relation to 3D printing technology, to introduce the materials used, to give information about different printing technologies, to summarize the applications and benefits of 3D printing technology in the field of food and agriculture.

**Stages of 3D Bioprinting Technology**

3D printing technology is a controlled process in which food is created in layers by utilizing a product's 3D computer design program. It consists of a four-stage process: the selection of the material, the creation of the 3D model of the product, the printing of the product and the execution of the post-processing applications (**Figure 1**). At the stage of creating the 3D model of the product, the model is designed with a computer program and after the model is created, the design information is sent to the printer and the model is divided into layers. Then, these layers are combined in the determined 3D cross-section model (Michaeli et al., 2017). Creating a 3D model is a process that includes designing the model with a computer aided drawing program (CAD) and converting the designed model to an STL (stereolithography) file. In order to make the designed model understandable for the 3D printer, the STL format file needs to be converted to the G-code file format, which is a kind of numerical control (NC) programming language. Slicing software programs are used for this transformation (Godoi et al., 2016; Michaeli et al., 2017). The slicing software is an intermediate driver for the process route planning between the 3D model and the 3D printer and for calculating the motion steps of these process segments. By transferring the information generated as a result of the slicing software to the printer, the model is sliced in layers to obtain the outline of each section. Three-dimensional objects are created by depositing the sliced layers within the scope of the designed 3D model (Guo et al., 2019). After the 3D food is produced, the processes used to ensure the stability of the created shape are drying and rapid cooling. In order to ensure the shape stability of the obtained 3D food during cooking, careful selection of the material and amount used in the formulation and sometimes the addition of additives may be required (He et al., 2020). Finally, the printed objects can be subjected to trimming, baking or baking processes depending on the characteristics of the product to be designed (Yang et al., 2017).

The key to determining which process to use in food 3D printing technology is the nature and type of materials being printed. There are many different food materials made up of monomers and polymers, each with its own unique combination of structure, workability, and adhesion. Writable materials such as hydrogel, soft cheese, hummus and chocolate can be extruded smoothly with the help of an injector and can maintain their shape stably without the need for any extra finishing after the process. On the other hand, foods such as rice, meat, fruit and vegetables cannot be 3D printed due to their nature. Some foods in solid and semi-solid form such as these can be modified with various pretreatments (enzyme addition, use of hydrocolloids, etc.) to make them printable (Değerli and El, 2017; Kim et al., 2017; Chuanxing et al., 2018; Dankar et al., 2018b).

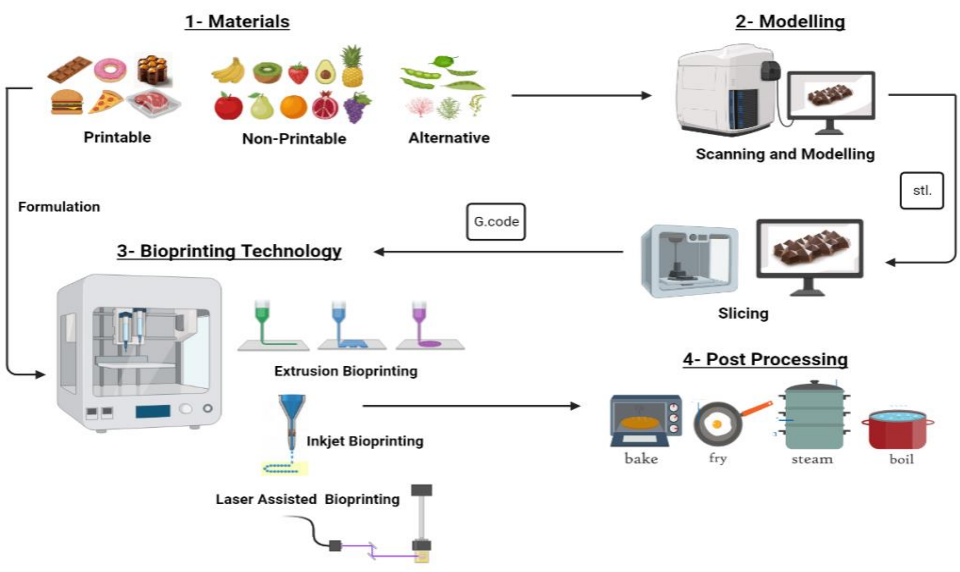


**Figure 1.** Stages of 3D Bioprinting Processes (created in BioRender.com)

**3D Bioprinting Technologies**

It is classified as liquid, powder and cell culture according to the materials used in three-dimensional food printers. Liquid foods are carried out by precipitation, extrusion and inkjet processes. Powdered foods are produced in the printer by applying a heat source (laser or hot air) or particle binder. Cell culture is used to produce meat-like structures on the printer (Wegrzyn et al., 2012). This production technology; selective laser sintering, layered stack modelling, printing with binder fluids, etc., which differ significantly in terms of theoretical principles and practical applications. consists of various techniques (Derossi et al., 2020). Depending on the processing process and materials used, these techniques have both strengths and weaknesses. The most important factors to consider when choosing an appropriate production technology for a particular purpose are precision, time and manufacturing cost (Shirazi et al., 2015).

**Extrusion Based Printing**

In extrusion-based printing systems, also known as fused deposition modeling (FDM), polymers are used as raw materials. The polymers are usually heated to a molten state and then extruded from the head of the machine (3D printer) (Mwema & Akinlabi, 2020). In the FDM method, which has been adapted to the food field since recent years, printing heads with mobility in -x, -y and -z axes are used. The food material, which is extruded in these heads and transferred to the platform just above the melting point, is provided to quickly transform into solid form. Then, the head moving in the z-axis starts to print the second layer on the food material, which it has transferred to the platform and is now in solid form. In this way, 3D production in layers is completed (Hao et al., 2010). One of the important points in this system is considered to be the relationship between the temperature of the outside environment and the temperature of the printed material. The printing temperature must be just above the melting point of the material, and the temperature of the outside environment must be lower than the melting point. Thus, the printed food material can quickly go into solid form (Kading and Straub, 2015).

**Inkjet Based Printing**

This method is based on the principle of conventional printers used to print paper in daily life. The material to be printed is sprayed onto the printing platform in the form of very small droplets via a nozzle. In this method, which can be dosed at intervals or continuously according to the geometric shape desired to be printed, very fast and error-free solutions are offered in the completion of pre-prepared products (for example: adding tomato sauce on the pizza base, adding chocolate sauce on the cookies being baked on the moving belt) (Godoi et al., 2016).

**Binder Jetting**

Printing with binder liquids is based on the creation of the desired layer shape with the binder, in which the powdered material is spread over a layer and is typically used in the form of a polymeric liquid (Mostafaei et al., 2020). In this method, the printing platform design and material form are similar to the SLS method. However, laser is not used as a power source. Shapes are created by applying liquid along the -x and -y axes via systems with multiple printer heads on the printing platform, which consists of powdered food raw materials such as sugar and starch. Then, the second layer is passed and as the layers are created, the printing platform moves downwards in the -z axis (Southerland et al., 2011).

**Selective Laser Sintering**

The principle of this technique is the use of sintering welding to fuse the powder particles and form a solid layer (Le-Bail et al., 2020). In this technique, the raw material in powder form is heated and sintered with a laser beam. Thus, a 3D layer is formed. While the printing surface moves as a platform, after each movement, a second head passes over the surface to prepare the new powder layer to be sintered, and then the raw material on the surface is shaped by heating -according to the model in the STL file-. As each layer is heated, the platform descends in the -z axis and maintains the 3D shape. While the print head moves in the -x and -y axes, the printing platform also moves in the -z axis (Godoi et al., 2016).

**The Benefits of 3D Bioprinting for Food and Agriculture**

One of the technological areas in which progress has been made recently is 3D bioprinting technology. In fact, the term bioprinting is a field that includes cell studies. However, its difference from traditional tissue engineering studies is that it works with biomaterials by using different and many layers by using the latest technological opportunities, and it also provides the opportunity to scale in studies and is practical. In this way, the synthesis of complex structures is also possible. As different studies in the field indicate, the variety of bioprinters exceeds 70 and more than half of them utilize extrusion technology. However, not only with this technique, but also with each printing type used in bioprinters, there are different application advantages specific to the product to be printed (Mallakpour et al. 2021).

Considering the reflections of 3D bioprinting in food and agriculture, it is noteworthy that the field is developing, although not as fast as in 3D printing technology. Especially in the last decade, commercial interactions have increased for reasons such as low cost, prone to customization and allowing sensitive work. This situation has enabled the 3D bioprinting technique to be scrutinized in terms of food and agriculture (Whenish et al. 2021). The technique has many advantages in studies in the field of food and agriculture. Since it is known that 95% of the source of food raw materials comes from soil-based materials, it does not seem possible to consider this area independently of the agricultural area. For this reason, the fields of food and agriculture industry are often mentioned together. Some of the areas where 3D bioprinting technology benefits in the food and agriculture industry concern printing materials, while some focus on the technique and technology of the work; In some subjects, the printing process is examined (Sanyé-Mengual et al. 2015).

There are many applications made possible by 3D bioprinters in the food and agricultural industries. When the advantages of these applications are evaluated due to the subject, the disadvantages should also be considered in the background and the bioethical aspect of the process should also be examined. However, when it is mentioned in a general context, one of the biggest advantages seen in the mentioned areas is the practicality and speed achieved in material printing compared to conventional methods (Kirillova et al. 2020). The system, which enables personalized printing, makes it possible to produce products with different geometric shapes from the same type of food. While increasing the nutritional value of food products, the principle of preserving the balance of nature, which is closely related to both this field and agricultural and environmental activities, is also supported. Because 3D bioprinting technology uses the power of the available printing materials very well, while protecting the environment (Whenish et al. 2022). At the same time, it is thought that bioprinting technology will provide a solution as an alternative technique to solve the nutritional problem in plant and animal production in the periods when there is a shortage of raw materials due to animal production in a limited area in the field of agriculture (Wu et al. 2014).

**3D Printing Applications in Food Production**

Producing food means both meeting the nutritional needs of people and using science and technological opportunities to catch up with the changing characteristics of the age while providing it. Knowing that the safety of food products should also be ensured, it is also an advantage to have developed techniques to ensure this (Pinstrup-Andersen and Watson II, 2011). Personalization is a triggering factor in developments in many different fields, including food production (Ordovas et al. 2018). 3D printing is also a way to achieve this. In addition to the 3D printing technique, 3D bioprinting technique accompanies the studies in the food field. In this way, textural examination of the food material designed for printing is also performed (Zheng et al. 2021). Here, the innovative aspect of the 3D bioprinting technique is due to the fact that it includes techniques that consider tissue diversity and cellular arrangements in the product to be printed, as well as providing a personalized product. Some of the areas where 3D printing technology is applied in the field of food are explained below, both by giving technical information and enriching with examples.

**Printing Technology and Materials**

The food processing process usually consists of rapid and repetitive processing steps (Zhu et al. 2021). Therefore, performing a food production process with automation systems can increase the efficiency of the process and the quality of the resulting food. Although both robotic-based food production and 3D food

printing technology can automate the food processing process and reduce human workload, both production technologies actually create a completely different user experience (Sun et al. 2015). While the former aims to reduce human involvement and workload by automating various manual processes, 3D printing technology puts users' creativity and control at the center of the process, enabling users to produce a variety of foods as desired. 3D printing or additive manufacturing technology involves processing the material by layering it until a certain shape is obtained to produce a three-dimensional object. Unlike traditional production methods, 3D printing technology attracts attention due to its customized geometry, reduced production cost, shortened production cycle and potential for use even in visually complex products. This production technology; selective laser sintering, layered stack modelling, printing with binder liquids, etc., which differ significantly in terms of theoretical principles and practical applications, consists of various techniques. Selective laser sintering can be classified into two types according to the type of sintering used: selective laser sintering or selective hot air sintering. The principle of this technique is the use of sintering welding to fuse the powder particles and form a solid layer. In the layered bulk modeling technique, polymers are used as raw materials. The polymers are usually heated to a molten state and then extruded through the head of the machine (3D printer). Printing with binder liquids is based on forming the desired layer shape with the binder, in which the powdered material is spread over a layer and is typically used in the form of a polymeric liquid. Depending on the processing process and materials used, these techniques have both strengths and weaknesses. The most important factors to consider when choosing a suitable production technology for a particular purpose are precision, time and cost of manufacture (Kruth, 2005).

Components used for 3D food printing are divided into three categories and can be classified as a) naturally printable materials, b) non-printable traditional foodstuffs, and c) alternative materials. Naturally printable foodstuffs are defined as inks with suitable viscoelastic properties that allow direct extrusion without the addition of ingredients such as gum or binders. Substances in this category can be easily extruded through a cap, and chocolate, dairy products and hydrogels are considered within this scope. These materials are stable enough to retain their shape after printing and do not require further processing (Baiano, 2022). Food products made from naturally printable materials are fully customizable in terms of taste, nutritional value and texture. Traditional food items that cannot be printed include meat, rice, vegetables and fruits. Hydrocolloids are added to them to make them suitable for extrusion used in 3D printing. The most widely used of these hydrocolloids are xanthan gum and gelatin (Sun et al. 2018). It can be used as a source of protein and fiber in alternative materials; components extracted from algae, fungi, seaweed and insects. Because these components contain high levels of bioactive components. Additionally, the alternative protein components in the insect powder were found to be slightly higher than the protein found in conventional meat products. For this reason, steak has been printed from nutrients obtained from algae or insects, and it is thought that nutrient absorption in the body can be increased by reducing the particle sizes of these components by using modern techniques. Thus, it is estimated that the use of alternative components in food printing can help develop healthier products, and the use of these components in 3D food printing gains importance in terms of emphasizing the sustainability aspect of the 3D method (Van Huis, 2016).

As a result, whatever source is used, the fluidity of the material is achieved by plasticizing and melting. In a multi-component system, differences in protein, carbohydrate and fat fraction affect the melting behavior, glass transition temperature and plasticization of food materials during liquid-based and powder-based 3D food printing processes. After printing, the food must retain its structure, which can be achieved by changing the processing temperature and/or gelling as a result of the addition of an additive.

**People with dysphagia**

Dysphagia is the medical term for difficulty swallowing. It is usually caused by nerve or muscle problems. This condition, which can be painful, is more common in the elderly and infants. Patients may have difficulty swallowing liquid or solid foods. In the type of dysphagia called paradoxical dysphagia, the patient; swallows solids more easily than liquids. In some people, difficulty in swallowing may be accompanied by pain. In some cases where swallowing difficulties are severe, swallowing may become impossible. Individuals with swallowing difficulties have difficulty sending the food and beverages they take into their mouths to their

stomachs, they cannot swallow, and they may escape into their lungs (Kelly et al. 2010). In this way, diseases called aspiration pneumonia can occur in the lungs. Therefore, they cannot consume very liquid or very solid foods or inhomogeneous foods. Because such patients are usually neurological or inpatients, they need meat-based foods with a high protein content. In order to achieve success in the treatment of dysphagia, it is important to research the root cause. In cases where dysphagia cannot be resolved in the treatment process that different methods are tried, eating and swallowing techniques can help to cope with this situation. Thus, the situation becomes less of a burden on the daily life of individuals. Treatments may include pulling the chin in, turning the head a certain way to help food move down the esophagus more efficiently, or changing the consistency of food eaten to aid swallowing. Rehabilitative approaches to the treatment of dysphagia aim to improve swallowing skills and provide permanent improvement. Treatments may include exercises that increase facial and esophageal muscle strength, or learning a swallowing technique that blocks the airway and prevents food from going from the windpipe to the lungs. Health professionals may also recommend that individuals change the way they eat. These may include taking small bites, chewing better, eating softer foods, or pureeing the food. A very good example that can be used at this point is the use of 3D printing technology in food in the treatment of dysphagia (Sura et al. 2012). Thanks to 3D printing technology, the production of many things has become easier. In addition to the production of physical equipment, various tissues and organs for patients can be created with 3D printers. In the food industry, innovative foods can be prepared using a 3D printer (Yan, et al. 2018). In a study, functional food was produced using 3D food printers for individuals with dysphagia. In the production made within the scope of the study, chicken meat, beef, capia pepper, celery powder, red beet powder were used. Thanks to the use of completely natural foods, a natural and healthy food has emerged. The criteria set by the institutions operating in the field of dysphagia and dysphagia were complied with and it was ensured that people with swallowing difficulties could easily consume these foods. Thanks to 3D printing technology, the production of many things has become easier. In addition to the production of physical equipment, various tissues and organs for patients can be created with 3D printers. In the food industry, innovative foods can be prepared using a 3D printer. In some studies, functional food was produced using 3D food printers for individuals with dysphagia. In the production made within the scope of the studies, chicken meat, beef, capia pepper, celery powder, red beet powder were used. Thanks to the use of completely natural foods, a natural and healthy food has emerged and the criteria set by the institutions operating in the field of dysphagia have been complied with and it has been ensured that people with swallowing difficulties can easily consume these foods (Lorenz et al. 2022; Tan et al. 2018; Prakash et al. 2018).

**Personalized Nutrition**

Today, 3D printing is no longer an idea, but a reality that is revolutionizing food innovation and production through creativity, customizability and sustainability. In the food industry, 3D printing enables the edible production of shapes, textures, flavors and forms that are difficult to create by hand. The main idea behind 3D printed food is the possibility of a desktop 3D printer that can print affordable and delicious meals without the need for cooking knowledge. Because 3D food printers follow digital instructions, the idea of making personalized food with the right percentage of nutrients for a given age or gender doesn't seem far off. Food printers can help easily quantify vitamins, carbohydrates, and fats. Thus, 3D printed foods are suitable for athletes or pregnant individuals etc. fully adaptable for individuals (Zhao et al. 2021). Personalization is made possible by technology's cellulose nano-crystals (CNC), a calorie-free, edible fiber type. The CNC's features include self-assembly to enable the binding of different food ingredients such as protein, carbohydrates and fats. This allows the CNC to create hard materials at low water content and gel-like materials at high water content. CNC stabilizes the oil droplets in the water and interacts with the proteins, thus creating a base for different textural elements. The base can then be given the desired amount of nutrients to create a delicious meal with special textures (Sandhya et al. 2016). Additionally, users are given the option to bake, bake, fry or grill while printing the food. As technology enables users to customize their own food using a low-calorie base, the resulting product is a quick, healthy meal and can be eaten right away. For example, when a 3D printer is developed that can print pasta, which is a food product shaped in different ways, it will be possible for consumers to quickly and easily print their own computer-aided design files in 3D with different pasta designs.

**Functional Foods**

In recent years, functional foods have gained popularity in health and wellness circles. Functional foods, also known as nutraceuticals, are highly nutritious and associated with a number of powerful health benefits. For example, they may protect against disease, prevent nutrient deficiencies, and support growth and development. Some examples include foods fortified with vitamins, minerals, probiotics or fiber. Nutrient-rich ingredients such as fruits, vegetables, nuts, seeds, and grains are also generally considered functional foods (Siró et al. 2008). Today, personalized foods have begun to be produced commercially by using different food components with functional functions such as increasing body resistance, helping hair and skin repair, strengthening the immune system, and supporting mental development. Within the scope of functional foods, the quality of life of individuals can be improved by producing products with reduced fat, salt and sugar content with 3D printing technology. In addition to customizing foods, it is thought that portion control can be achieved in foods to be created for individuals by making the relevant settings of the printer. It is predicted that the use of 3D printing technique in the production of functional foods will attract more attention from the food industry in the coming years, due to its advantages such as reducing raw material losses and inclusion of wastes in the production of functional foods, reducing carbon footprint, saving time and cost for people or businesses while producing prototypes (Tomašević et al. 2021).

**3D Printing Applications in Agriculture**

Adding efficiency, customizability and accessibility to manufacturing, 3D printing is changing the dynamics of the way that people do things. Today, innovative 3D-printed products and solutions can offer solutions ranging from the production of a variety of materials that were previously impossible to achieving formerly impractical goals for designers and engineers (Crisostomo and Dizon, 2021). Detailed information about the use of 3D printing technology in agriculture is given below.

**Printing Technology and Materials**

The boundaries of the agricultural industry are very wide. Based on the rapid increase in the world population, agriculture is at the top of the sectors that will provide resources to meet the increasing food need. Similar to the rapidly increasing population, 3D printing technique will become one of the methods that can be applied to meet the needs in the near future, based on the rapid development of technology. The use of technology covers not only product production and processing, but also product supply chain and marketing steps. The use of 3D printing technology in the agricultural sector facilitates the operations applied by the farmers in the field and makes the operation of agricultural activities more practical. It also allows for personalized designs for farmers to increase functionality in their fields, thus enabling on-site production (Crisostomo and Dizon, 2021). In addition, it has an infrastructure that can easily cooperate with smart agriculture technologies in more economical conditions by saving time and energy. All materials used in agriculture, which is the basis for food production, have critical importance in raw material and product processing. If the maintenance of the machines used and the quality of the materials that make up the machines are good, the quality of the processed product and therefore the food product produced is also good. 3D printing technology is also used in the production of agricultural equipment, thus providing solutions to increase the quality of the material. For this reason, possible risks are reduced, occupational safety is increased and production wastage is reduced. 3D printing technology is used in many scales in the production of tools used in agricultural production. Different types of thermoplastics have a large place in the production of equipment such as sprinklers and hose splitters used in irrigation. Using 3D printing technology, farmers have also been able to produce equipment made from biodegradable and recycled materials (Ishengoma and Mtaho, 2014).

The 3D printing technology used in agriculture is based on the principle of melting the printing materials used and creating layers during printing, based on the data transmitted by the designed structure. The manufacturing process changes almost all of the way parts are manufactured. At the same time, it makes use of bionic designs to produce geometrically shaped materials that cannot be produced with the use of previous technologies. Some of these materials are much lighter than those produced using traditional methods (Van Wijk and van Wijk, 2015). With the production of agricultural equipment using 3D printers, modern drawing and design programs created synergy with simulative applications and the positive aspects of individual effects in

production were reflected. In this way, the production of materials that are resistant to harsh weather conditions or unexpected work conditions in the classical production line has been ensured. An example of these conditions is the harsh terrain environment. The parts used in the off-road vehicle design have distinguished themselves from traditional methods with their differences from the main equipment to the driver's cabin.

**Irrigation and Water Management**

After planting on the agricultural land, it is necessary to irrigate the plant at the appropriate time and technique according to the type. Managing this is an important feature of agricultural production. Until today, they have developed different techniques to prepare a practical routine for irrigation with farmers. However, these methods sometimes cause waste of time, sometimes financial damage, and sometimes waste of labor. This is the part about irrigation techniques mentioned here, and there is an important agricultural parameter such as effective water management (Manoharan et al. 2020). More than half of the water used in agricultural production is lost due to leakage or evaporation. In addition, the pipe design used in irrigation systems is sometimes far from functionality due to its material properties and its location in the field. When the remarkable disadvantages in this regard were synthesized, special polymer pipes for irrigation systems were designed and printed in 3D printing technology. In this way, it is possible to transfer the moisture in the soil to the plant, considering the soil properties. In summary, 3D printing technology has been a promising area in order to turn the opportunities available in irrigation techniques and water management into advantage, and to match the crop and land characteristics at the optimum level (Kalossaka et al. 2021).

**Urban Farming**

With urban agriculture, it has been possible for consumers and producers to grow different foods in residences or in restaurants and cafes. With the hardware power of 3D printing technology, it is possible to produce on a large scale to achieve this. Although the designed system is of an infrastructural nature, the fact that the harvest obtained in order to test the product obtained with the 3D printer is in a wide product range has been an important data. Both system design and harvesting activities point to many difficulties and yield losses with traditional methods. Harvesting even a small area could be difficult, as if it were on an equivalent scale to harvesting the field or garden (Ng and Mahkeswaran, 2021).

Thanks to the use of 3D printers, there are many advantages such as on-site production of some system elements, production from functional materials, and more aesthetic design of the system among the innovations recorded in urban agriculture. Thus, a different understanding that can address the product, the consumer and the place of production, instead of the stereotypical production outputs of traditional production, has met the sector. 3D printing technology offers products that facilitate the application not only in the cultivation area, but also in harvesting tools and equipment in urban agriculture. The materials used in the equipment support units produced are biodegradable and recyclable. Thus, when both the system and the support equipment are considered holistically, it is once again understood that 3D printing technology provides advantages to support sustainability by not using or eliminating unnecessary materials (Crisostomo and Dizon, 2021).

**Scale Models**

If it is desired to make progress in an agricultural production, there is a need to invest in the facility. This investment may require a high cost consisting of factors such as additional units and additional equipment. When it comes to yield at harvest, producers need to match the available opportunities very well with the activities that need to be implemented. There is a need for 3D printing technology to be used in this field in order to achieve efficiency optimization with less effort and effective operation (Arrieta-Escobar et al. 2020). For this reason, farmers can realize what they want to do with the help of 3D models instead of trying to build and demolish them repeatedly during construction. In addition, by using the same modeling, they can integrate smart agriculture technologies into the system and conduct land surveys. This scanning option provides farmers with opportunities such as understanding the land structure and predicting possible risks. The parameters tested using scale modeling give an idea about the structural properties such as flood resistance and soil strength that may occur in the field in the future (Morris et al. 2008).

**Replacement Parts**

While the equipment used in agricultural production is sometimes used in standard sowing, mowing and processing operations, sometimes it is qualified and costly to be used in special operations. When there is any malfunction or malfunction during the process, it may take time to repair these equipments and it can be very costly to buy new equipment. While maintenance-repair operations are high, especially for parts whose production dates back to previous years, spare part options can be very few and expensive. Thanks to the use of 3D printers, manufacturers operating in agricultural areas can produce by spending much less than the time spent in classical methods (Mikula et al. 2021). If there is an existing design for the system that will produce spare parts, they can develop it or they can produce the parts they want to produce from scratch with their own designs. With this method, they both produce in more economical conditions and can reduce the loss of efficiency in the process with their production in a short time (Pearce, 2013).

**Indoor Gardening**

Over time, access to open spaces has become more difficult due to the physical decrease in green open space. In addition, due to the intense work schedule and time constraints, people are trapped in urban life and practically every individual cannot find the opportunity to go to open green spaces. Due to these and many other reasons, indoor gardening application has become an increasing trend day by day. With this current, people can grow different fruits and vegetables indoors (Wang et al. 2019). It is a good output that the harvested products have agricultural practice in a different place. At the same time, the activity makes a great contribution to the concept of a sustainable world and raises awareness about the subject. In the design of indoor gardening area, it is possible to create site-specific design options and test them in online programs with 3D printing technology. In this way, designs that can give maximum efficiency in small areas are obtained. In addition to the area settlement efficiency, it is possible to make different design models that can place agricultural products or different plants using 3D printing technology. The application provides the opportunity for non-professionals in plant care in terms of collecting the product that will spread over a wide area in a compact field and serves life cycle assessment analysis by reducing the carbon footprint (Dixit, 2019; Liu et al. 2016).

**Future Approaches of 3D Printing Technology**

When evaluated in general; 3D printing technology is being adopted in most industries, thanks to its advantages over other production techniques, allowing geometrically complex shapes and offering individual customization of products. It allows the material to be processed easily with the layer-by-layer processing process. In addition, from an economic point of view, it is cheaper to create complex shapes with 3D printing methods than to produce simple and filled shapes using traditional methods. Because in 3D printing technology, less waste is given and less material is processed in the production process, and the machine is operated in a much shorter time (Lipton, 2017).

In the future, the traditional food supply chain can be simplified by using 3D printing technologies. In this process, under an e-commerce platform, consumers can configure food designs and produce physical products using a nearby manufacturing facility. To reduce the process from design to market, many innovative food design websites and mobile applications can assist users in designing and ordering customized food products (Sun et al., 2015). Thus, by enabling the manufacturing activities to be moved closer to the customers gradually, advantages will be provided that deliver the products to the consumers in a shorter time and reduce the transportation volume. In addition, this system will reduce costs such as packaging and distribution (Liu et al., 2017). Especially in recent years, personalized nutrition that corresponds to the individual's health status and body type needs has started to gain importance. When 3D food printing is integrated with a nutrition model, users can precisely calculate the calorie and ingredient components of the manufactured product and create an exact nutritional distribution. Soft, nutritious and desired textured foods can be produced for the elderly and children. Customized foods enriched with healthy ingredients such as vitamins, fibers and phytochemicals can also be printed for athletes and expectant mothers. Therefore, 3D food printing technology will provide a convenient way to digitize people's nutrition and energy intake and offer better control over individual nutrition (Sun et al., 2015). Another of the most important breakthroughs of 3D printing technology

for the future is that it can reduce food waste by reusing the materials to be discarded. With this technology, it will be able to use food by-products and transform them into visually and psychologically more appealing food products, making it more likely for individuals to consume them (Burke-Shyne et al., 2020). At the same time, since it is possible to prepare edible food packages with this technology, it will be possible to reduce packaging waste and thus reduce the damage of waste to the environment (Lupton & Turner, 2016). 3D food printing is also a highly efficient process that can combine multiple processes in one step during processing. When evaluated in terms of the service sector; no food will be produced unless ordered, and as a result, both food waste will not occur and the need for water and energy spent for food that is not consumed will be eliminated (Dankar et al., 2018a).

Technology plays a huge role in agriculture by simplifying many processes, from food production to shipping and marketing. The use of 3D printing technologies in the agricultural industry can significantly increase the efficiency of the daily activities of farmers in the fields. In the field of crop cultivation, 3D-printed tools can be used to adapt to certain activities. At the same time, 3D printing technologies allow users such as farmers to produce or print on-site so that they can create quality tools and manage planting processes more quickly (Manoharan et al., 2020; Crisostomo and Dizon, 2021). Accordingly, 3D printing technology can be used in the design of model devices upon requests that save energy and money in remote locations. All kinds of machinery used in food processing and agriculture, from tractors and combines to seeders and fertilizer spreaders, are also very important. It is important that these machines not only run well, but also that good tires are fitted to the machines. In this way, with the increase in the traction power of the machines used by the farmers, the fuel consumption will decrease and therefore the productivity will increase. It will also increase safety by preventing slips and slides that could endanger the operator or destroy fields (Nayana et al., 2022). Agricultural equipment used in the agricultural industry is both very large and very expensive. Therefore, replacing a part can also be expensive. In addition, it can be difficult to find the old parts of the machine. By creating spare parts of the machines with 3D printer technologies, the continuity of the operation can be ensured by saving time and money without interrupting the production process (Herrera et al., 2022). Although many applications made using 3D printing in the agricultural sector are already successful, it is thought that they will continue to develop in the coming years and these applications will become more integrated into the agricultural industry. This technology has many benefits to the agricultural sector, from tools to more elaborate devices. The most important thing is that these practices bring together recycling. For example, 3D printing technology can be a great approach to recycling plastic and metal materials found around a farm. These waste materials can be used to reconstruct entirely new agricultural tools or device that could have another function.

**Conclusion**

The Fourth Industrial Revolution (E4.0) emerged when machines started to manage themselves and their production processes without the need for manpower, thanks to the hybrid technology that emerged with the blending of computer, internet and communication technologies. It is possible to produce almost anything with 3D printer technology, which allows the realization of additive production, which is one of the basic components of Industry 4.0. The prototyping process in traditional methods is both costly and time consuming. By using 3D printers, the desired prototypes can be produced within hours. Thanks to this technology, it is possible to save time and capital that would often be separated from traditional production methods that go through long and circuitous processes. It helps us stick to sustainable and efficient production as it continually finds useful applications in many different industries and fields.

However, in traditional production, decentralized methods are used in the production of any food, and the machines in the factory are integrated with each other and perform different operations on the raw material. In 3D food printing technology, a central structure is created using only printers and many operations on raw materials are carried out with a single equipment. Therefore, 3D food printing technology eliminates and simplifies many steps compared to the traditional process. Technological advances, especially in 3D printing, in recent years have drawn the attention of various food technologists and businesses to study and multiply the potential of this application for food. Because 3D printing technology can customize the needs of consumers according to their preferences. In addition, preparing a complex food design is also possible with this method,

because all processes are supported by 3D model software and are done fully automatically. However, to unlock the potential of 3D food printing technology, certain technical challenges must be overcome. Therefore, more research is needed on the printing materials to be used, their behavior in different printing methods (optimizing the printing technology), and their impact on the food market and customer acceptance.

This article presents the advantages and applications of 3D printing technologies in food processing and agriculture. As a result, 3D printer technology is becoming more and more widespread, although it takes its place in many sectors in our lives. With rapidly developing technologies and innovations brought by these technologies, our lives are facilitated and we keep up with these technologies.

**Acknowledgement**
The authors are thankful to 5th International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2022) Organization Committee for access to the facilities.

**References**

Agarwala, S. (2016). A perspective on 3D bioprinting technology: Present and future. *Am. J. Eng. Applied Sci*, *9*, 985-990.

Arrieta-Escobar, J. A., Derrien, D., Ouvrard, S., Asadollahi-Yazdi, E., Hassan, A., Boly, V., ... & Dignac, M. F. (2020). 3D printing: An emerging opportunity for soil science. *Geoderma*, *378*, 114588.

Attaran, M. (2017). The rise of 3-D printing: The advantages of additive manufacturing over traditional manufacturing. *Business horizons*, *60*(5), 677-688.

Baiano, A. (2022). 3D printed foods: A comprehensive review on technologies, nutritional value, safety, consumer attitude, regulatory framework, and economic and sustainability issues. *Food Reviews International*, *38*(5), 986-1016.

Burke-Shyne, S., Gallegos, D., Williams, T., (2020). 3D food printing: nutrition opportunities and challenges. *British Food Journal*.

Chaka, C. 2020. Skills, competencies and literacies attributed to 4IR/Industry 4.0: Scoping review. *IFLA journal*, *46*(4), 369-399.

Chuanxing, F., Qi, W., Hui, L., Quancheng, Z., Wang, M. (2018). Effects of pea protein on the properties of potato starch-based 3D printing materials. *Int. J. Food Eng*, *14*(3).

Crisostomo, J. L. B., & Dizon, J. R. C. (2021). 3D Printing Applications in Agriculture, Food Processing, and Environmental Protection and Monitoring. *Advance Sustainable Science, Engineering and Technology (ASSET)*, *3*(2), 0210201.

Dani, S., Ahlfeld, T., Albrecht, F., Duin, S., Kluger, P., Lode, A., & Gelinsky, M. (2021). Homogeneous and reproducible mixing of highly viscous biomaterial inks and cell suspensions to create bioinks. *Gels*, *7*(4), 227.

Dankar, I., Haddarah, A., Omar, F. E., Sepulcre, F., Pujolà, M. (2018a). 3D printing technology: The new era for food customization and elaboration. *Trends Food Sci. Technol.*, 75, 231-242.

Dankar, I., Haddarah, A., El Omar, F., Sepulcre, F., Pujolà, M. (2018b). Assessing the microstructural and rheological changes induced by food additives on potato puree. *Food Chem*. 240, 304-313.

Değerli, C., El, S. N. (2017). Üç Boyutlu (3D) Yazıcı Teknolojisi ile Gıda Üretimine Genel Bakış. *Turk. tarım gıda bilim teknol. derg.*, *5*(6), 593-599.

Derossi, A., Paolillo, M., Caporizzi, R., Severini, C., (2020). Extending the 3D food printing tests at high speed. Material deposition and effect of non-printing movements on the final quality of printed structures. *Journal of Food Engineering* 275, 109865.

Dixit, M. K. (2019, June). 3-D printing in building construction: a literature review of opportunities and challenges of reducing life cycle energy and carbon of buildings. In *IOP Conference Series: Earth and Environmental Science* (Vol. 290, No. 1, p. 012012). IOP Publishing.

Godoi, F. C., Prakash, S., Bhandari, B. R., (2016). 3D printing technologies applied for food design: Status and prospects. *Journal of Food Engineering*. 179, 44-54.

Guo, C., Zhang, M., Bhandari, B., (2019). Model building and slicing in food 3D printing processes: a review. *Comprehensive Reviews in Food Science and Food Safety.* 18, 1052-69.

Hao L, Mellor S, Seaman O, Henderson, J, Sewell N, Sloan, M. (2016). Material characterisation and process development for chocolate additive layer manufacturing. *Virtual and Physical Prototyping*. 5(2), 57-64.

He, C., Zhang, M., Fang, Z., (2020). 3D printing of food: Pretreatment and post-treatment of materials. *Critical reviews in food science and nutrition* 60, 2379-92.

Herrera, R.M., Blanco, M.A., Carabeo, J.C., Ramilo, F.D., Silva, J.R., Nuqui, N.O., Espino, M.T., Tuazon B.J., Dizon, J.R.C. (2022). 3D-Printed Ergonomic Tool Handles, *Advance Sustainable Science, Engineering and Technology (ASSET)*.4(2), 0220210-01 ~ 0220210-10.

Horne, R., & Hausman, K. K. (2017). *3D printing for dummies*. John Wiley & Sons.

Huang, T. C., & Lin, C. Y. (2017). From 3D modeling to 3D printing: Development of a differentiated spatial ability teaching model. *Telematics and Informatics*, *34*(2), 604-613.

Ishengoma, F. R., & Mtaho, A. B. (2014). 3D printing: developing countries perspectives. *arXiv preprint arXiv:1410.5349*.

Kalossaka, L. M., Sena, G., Barter, L. M., & Myant, C. (2021). 3D printing hydrogels for the fabrication of soilless cultivation substrates. *Applied Materials Today*, *24*, 101088.

Kelly, J., D'Cruz, G., & Wright, D. (2010). Patients with dysphagia: experiences of taking medication. *Journal of advanced nursing*, *66*(1), 82-91.

Kim, H. W., Bae, H., Park, H. J. (2017). Classification of the printability of selected food for 3D printing: Development of an assessment method using hydrocolloids as reference material. *J. Food Eng*, *215*, 23-32.

Kirillova, A., Bushev, S., Abubakirov, A., & Sukikh, G. (2020). Bioethical and legal issues in 3D bioprinting. *International Journal of Bioprinting*, *6*(3).

Kruth, J. P., Mercelis, P., Van Vaerenbergh, J., Froyen, L., & Rombouts, M. (2005). Binding mechanisms in selective laser sintering and selective laser melting. *Rapid prototyping journal*.

Le-Bail, A., Maniglia, B. C., Le-Bail, P., (2020). Recent advances and future perspective in additive manufacturing of foods based on 3D printing. *Current Opinion in Food Science.* 35, 54-64.

Lipton, J. I., (2017). Printable food: the technology and its application in human health. *Current opinion in biotechnology*. 44, 198-201.

Liu, Z., Jiang, Q., Zhang, Y., Li, T., & Zhang, H. C. (2016, June). Sustainability of 3D printing: A critical review and recommendations. In *International Manufacturing Science and Engineering Conference* (Vol. 49903, p. V002T05A004). American Society of Mechanical Engineers.

Liu, Z., Zhang, M., Bhandari, B., Wang, Y., (2017). 3D printing: Printing precision and application in food sector. *Trends in Food Science & Technology* 69, 83-94.

Lorenz, T., Iskandar, M. M., Baeghbali, V., Ngadi, M. O., & Kubow, S. (2022). 3D Food Printing Applications Related to Dysphagia: A Narrative Review. *Foods*, *11*(12), 1789.

Lupton, D., Turner, B., (2016). 'Both fascinating and disturbing': Consumer responses to 3D food printing and implications for food activism. *Digital Food Activism, edited by Tanja Schneider, Karin Eli, Catherine Dolan and Stanley Ulijaszek, by Routledge, London, Forthcoming*.

Mallakpour, S., Sirous, F., & Hussain, C. M. (2021). Current achievements in 3D bioprinting technology of chitosan and its hybrids. *New Journal of Chemistry*, *45*(24), 10565-10576.

Manoharan, H., Basha, A. R., Teekaraman, Y., & Manoharan, A. (2020). Smart home autonomous water management system for agricultural applications. *World Journal of Engineering*.

Manstan, T., McSweeney, M. B., (2020). Consumers' attitudes towards and acceptance of 3D printed foods in comparison with conventional food products. *International Journal of Food Science & Technology* 55, 323-31.

Michaeli, J. G., DeGroff, M. C., Roxas, R. C. (2017). Error aggregation in the reengineering process from 3D scanning to printing. *Scanning*.

Mikula, K., Skrzypczak, D., Izydorczyk, G., Warchoł, J., Moustakas, K., Chojnacka, K., & Witek-Krowiak, A. (2021). 3D printing filament as a second life of waste plastics—A review. *Environmental Science and Pollution Research*, *28*(10), 12321-12333.

Morris, M., Hanson, G., & Hassan, M. (2008). Improving the accuracy of breach modelling: why are we not progressing faster?. *Journal of Flood Risk Management*, *1*(3), 150-161.

Mostafaei, A., Elliott, A. M., Barnes, J. E., Li, F., Tan, W., Cramer, C. L., et al., (2020). Binder jet 3D printing–process parameters, materials, properties, and challenges. *Progress in Materials Science*. 100707.

Mwema, F. M.& Akinlabi, E. T. (2020). Basics of Fused Deposition Modelling (FDM). Fused Deposition Modeling: *Springer*. 1-15.

Nayana, N.P., Mallikarjuna, Ramachandra, C.T., Mahesh Kumar, G., Bhat S.N. & Manvi, D. (2022). Bioprinting: A Review on Technology and its Application in Food and Agriculture, *Mysore Journal of Agricutural Sciences*, 56(2), 26-38.

Ng, A. K., & Mahkeswaran, R. (2021, August). Emerging and disruptive technologies for urban farming: A review and assessment. In *Journal of physics: Conference series* (Vol. 2003, No. 1, p. 012008). IOP Publishing.

Ordovas, J. M., Ferguson, L. R., Tai, E. S., & Mathers, J. C. (2018). Personalised nutrition and health. *Bmj*, *361*.

Oztemel, E., & Gursev, S. 2020. Literature review of Industry 4.0 and related technologies. *Journal of Intelligent Manufacturing*, *31*(1), 127-182.

Pearce, J. (2013). Applications of open source 3-D printing on small farms. *Organic Farming*, *1*(1), 19-35.

Pinstrup-Andersen, P., & Watson II, D. D. (2011). Food policy for developing countries. In *Food Policy for Developing Countries*. Cornell University Press.

Prakash, S., Bhandari, B. R., Godoi, F. C., & Zhang, M. (2019). Future outlook of 3D food printing. In *Fundamentals of 3D food printing and applications* (pp. 373-381). Academic Press.

Sandhya, K. Y., Saritha, A., & Joseph, K. (2016). Liquid crystalline polymers from renewable resources: synthesis and properties. In *Liquid Crystalline Polymers* (pp. 273-306). Springer, Cham.

Sanyé-Mengual, E., Orsini, F., Oliver-Solà, J., Rieradevall, J., Montero, J. I., & Gianquinto, G. (2015). Techniques and crops for efficient rooftop gardens in Bologna, Italy. *Agronomy for sustainable development*, *35*(4), 1477-1488.

Shahrubudin, N., Lee, T. C., & Ramlan, R. (2019). An overview on 3D printing technology: Technological, materials, and applications. *Procedia Manufacturing*, *35*, 1286-1296.

Shirazi, S. F. S., Gharehkhani, S., Mehrali, M., Yarmand, H., Metselaar, H. S. C., Kadri, N. A., et al., (2015). A review on powder-based additive manufacturing for tissue engineering: selective laser sintering and inkjet 3D printing. *Science and technology of advanced materials*.

Siró, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—A review. *Appetite*, *51*(3), 456-467.

Southerland D, Walters P, Huson D. (2011). Edible 3D printing. In Proceeding of NIP & digital fabrication conference. *Society for Imaging Science and Technology*. 2, 819–822.

Sun, J., Zhou, W., Huang, D., Fuh, J. Y., & Hong, G. S. (2015). An overview of 3D printing technologies for food fabrication. *Food and bioprocess technology*, *8*(8), 1605-1615.

Sun, J., Zhou, W., Huang, D., & Yan, L. (2018). 3D food printing: Perspectives. *Polymers for food applications*, 725-755.

Sura, L., Madhavan, A., Carnaby, G., & Crary, M. A. (2012). Dysphagia in the elderly: management and nutritional considerations. *Clinical interventions in aging*, 287-298.

Tan, C., Toh, W. Y., Wong, G., & Li, L. (2018). Extrusion-based 3D food printing–Materials and machines. *International Journal of Bioprinting*, *4*(2).

Tomašević, I., Putnik, P., Valjak, F., Pavlić, B., Šojić, B., Markovinović, A. B., & Kovačević, D. B. (2021). 3D printing as novel tool for fruit-based functional food production. *Current Opinion in Food Science*, *41*, 138-145.

Wang, H., Dai, X., Wu, J., Wu, X., & Nie, X. (2019). Influence of urban green open space on residents' physical activity in China. *BMC public health*, *19*(1), 1-12.

Wegrzyn, T.F., Golding, M., & Archer, H.R., (2012). Food Layered Manufacture: A New Process For Constructing Solid Foods. *Trends in Food Science & Technology*, 27, 66-72.

Whenish, R., Ramakrishna, S., Jaiswal, A. K., & Manivasagam, G. (2022). A framework for the sustainability implications of 3D bioprinting through nature-inspired materials and structures. *Bio-Design and Manufacturing*, *5*(2), 412-423.

Wu, G., Fanzo, J., Miller, D. D., Pingali, P., Post, M., Steiner, J. L., & Thalacker-Mercer, A. E. (2014). Production and supply of high-quality food protein for human consumption: sustainability, challenges, and innovations. *Annals of the New York Academy of Sciences*, *1321*(1), 1-19.

Van Wijk, A. J. M., & van Wijk, I. (2015). *3D printing with biomaterials: Towards a sustainable and circular economy*. IOS press.

Van Huis, A. (2016). Edible insects are the future?. *Proceedings of the Nutrition Society*, *75*(3), 294-305.

Yang, F., Zhang, M., Bhandari, B., (2017). Recent development in 3D food printing. *Critical reviews in food science and nutrition* 57, 3145-53.

Yan, Q., Dong, H., Su, J., Han, J., Song, B., Wei, Q., & Shi, Y. (2018). A review of 3D printing technology for medical applications. *Engineering*, *4*(5), 729-742.

Zhao, L., Zhang, M., Chitrakar, B., & Adhikari, B. (2021). Recent advances in functional 3D printing of foods: A review of functions of ingredients and internal structures. *Critical reviews in food science and nutrition*, *61*(21), 3489-3503.

Zhang, J.Y., Pandya, J.K., McClements, D.J., Lu, J. & Kinchla, A.J. (2022). Advancements in 3D food printing: a comprehensive overview of properties and opportunities, *Critical Reviews in Food Science and Nutrition*, 62(17), 4752-4768.

Zheng, L., Liu, J., Liu, R., Xing, Y., & Jiang, H. (2021). 3D printing performance of gels from wheat starch, flour and whole meal. *Food Chemistry*, *356*, 129546.

Zhu, L., Spachos, P., Pensini, E., & Plataniotis, K. N. (2021). Deep learning and machine vision for food processing: A survey. *Current Research in Food Science*, *4*, 233-249.

Zhuang, P., Greenberg, Z., & He, M. (2021). Biologically Enhanced Starch Bio-Ink for Promoting 3D Cell Growth. *Advanced Materials Technologies*, *6*(12), 2100551.

## *ORAL PRESENTATION*

# Van ilinde yetişen vişne (*Prunus cerasus*) çekirdeği talaşi üzerinde kristal viyolenin adsorpsiyonunun kinetik hesaplamaları

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**Özet**

Atık su arıtımında adsorpsiyon, atık sulardaki belirli maddeleri uzaklaştırmak amacıyla bu maddeleri tutabilecek özellikler gösteren adsorbent maddelerin kullanılması işlemidir. Şehirlerde biriken atık sular çevreye zararlı olduğundan arıtılması gerekir. Yaptığımız bu orijinal çalışmada hazırladığımız kristal viyole çözeltisini vişne çekirdeği talaşı üzerine adsorbsiyon yaparak, çözeltiyi arıtmayı amaçladık.**3** kg vişne pazardan alınarak araştırma laboratuvarına getirildi. Çekirdekleri çıkarıldı. Saf su ile yıkandı. 48 saat açık havada kurutuldu. Havan da dövüldü ,250 mesh elekte elendi. Böylece adsorbent hazırlandı. Farklı derişimlerde kristal viyole çözeltileri hazırlanarak, 500 ml çözeltiye 1,5 gram vişne çekirdeği talaşı katılarak ,farklı zaman aralıklarında 5 er ml çözelti alınarak, santrifüjlenerek, UV cihazında 590 nm de adsorplanan boyar madde miktarı ppm olarak ölçüldü ve kayıt edildi.Yapılan deneysel çalışmada 20 ppm Kristal Viyole çözeltisinde 298 K'da %89,77 , 308 K' da %90,049 , 318 K'da %91,77 oranında Vişne çekirdeği talaşının boyar madde adsorpladığı tesbit edilmiştir. Birinci derece kinetik modelde 20 ppm Kristal Viyole derişimindeki regresyon sabiti değerleri 298 K, 308 Kve 318 K'de sırasıyla 0,769, 0,845 ve 0,726 olarak bulundu. İkinci derece kinetik modelde ise, 20 ppm Kristal viyole derişimindeki regresyon sabiti değerleri: 298 K, 308 K ve 318 K'de sırasıyla 1, 1 ve 1 olarak bulundu.Bu sonuçlar , Vişne çekirdeği talaşının atık suların arıtılmasında, filtrasyon amaçlı kullanılabileceğini göstermektedir.

**Anahtar Kelimeler:** Vişne Çekirdeği, Kristal Viyole, Adsorpsiyon, Kinetik, Çevre.

## Kinetic calculations of adsorption of crystal violet on cherry (prunus cerasus) seed saw

**Abstract**

Adsorption in wastewater treatment is the process of using adsorbent substances that can hold these substances in order to remove certain substances in wastewater. Waste water accumulated in cities should be treated as it is harmful to the environment. In this original study, we aimed to purify the solution by adsorption of the crystal violet solution we prepared on the cherry seed shavings. 3 kg of sour cherry was taken from the market and brought to the research laboratory. The nuclei were removed. It was washed with pure water. Air dried for 48 hours. The mortar was also beaten and sifted through a 250 mesh sieve. Thus, the adsorbent was prepared. Crystal violet solutions of different concentrations were prepared, 1.5 grams of sour cherry seed shavings were added to 500 ml of solution, 5 ml of solution was taken at different time intervals, centrifuged, and the amount of dyestuff adsorbed at 590 nm in the UV device was measured in ppm and recorded. In the experimental study, it was determined that sour cherry seed shavings adsorbed dyestuff in 20 ppm Crystal Violet solution at a rate of 89.77% at 298 K, 90.049% at 308 K, and 91.77% at 318 K. In the first-order kinetic model, the regression constant values at 20 ppm Crystal Violet concentration were found to be 0.769, 0.845 and 0.726 at 298 K, 308 K, and 318 K, respectively. In the second order kinetic model, the regression constant values at 20 ppm Crystal violet concentration were found to be 1, 1 and 1 at 298 K, 308 K and 318 K, respectively. These results show that sour cherry seed shavings can be used for filtration purposes in wastewater treatment.

**Keywords:** Cherry Seed, Crystal Violet, Adsorption, Kinetics, Environment.

# GİRİŞ

## Vişne

vişnenin muhtemel anavatanı olarak Hazar Denizi ile Kuzey Anadolu dağları arasında kalan bölge kabul edilmektedir. Vişnenin botanikteki latince adı olan *P. cerasus* bugünkü Giresun'un eski adı olan Kerasus'tan gelmektedir.. (Vişne, 2022).

## Kristal Viyole

Kristal viyole veya jansiyen moru; heksametil pararosanilin klorür veya metil viyole 10B olarak da bilinen histolojik bir boya ve Gram boyama yönteminde bakterileri sınıflandırmak için kullanılan bir triarilmetan boyadır.. (Kristal Viyole, 2022).

## Adsorpsiyon

Molekül ya da atomların herhangi bir katı yüzeyinde kohezyon çekim kuvvetleri ile bulunmasına **adsorpsiyon** denir.(Acemioğlu, Aksu, 2004) .

## Lagergren Modeli (Yalancı Birinci Derece Kinetik denklemi):

Lagergren'in (Lagergren, 1898) bulduğu kinetik model, birinci derecededir. Bu modelin hesabı aşağıda verilen eşitlik ile yapılmaktadır. (Ho and Mckay, 1998a), (Yavuz ve Altunkaynak, 2002).
Bu eşitlikte
k1 Biyokütlenin yaptığı adsorpsiyonun birinci derece hız sabitini gösterir
qe, çözeltide dengede adsorplanmış olan madde miktarını
qt ise t süre sonunda adsorplanan madde miktarını belirtmektedir.

$$ln(qm - qt) = lnqm - k_1 t \quad (1)$$

## Ho McKay Modeli (İkinci Derece Kinetik Model):

Pseudo second order modeli ise Ho ve McKay'ın geliştirdiği kinetik denklemdir. (Ho, Mckay, 1998b ). Adsorplama kapasitesinin zamana bağlı olarak nasıl değiştiğini gösteren ifadedir.
(Ali Rıza Kul ve ark, 2019)

$$t\ qt = 1\ K_2 qe\ 2 + t\ qe \quad (2)$$

# MATERYAL VE METOT

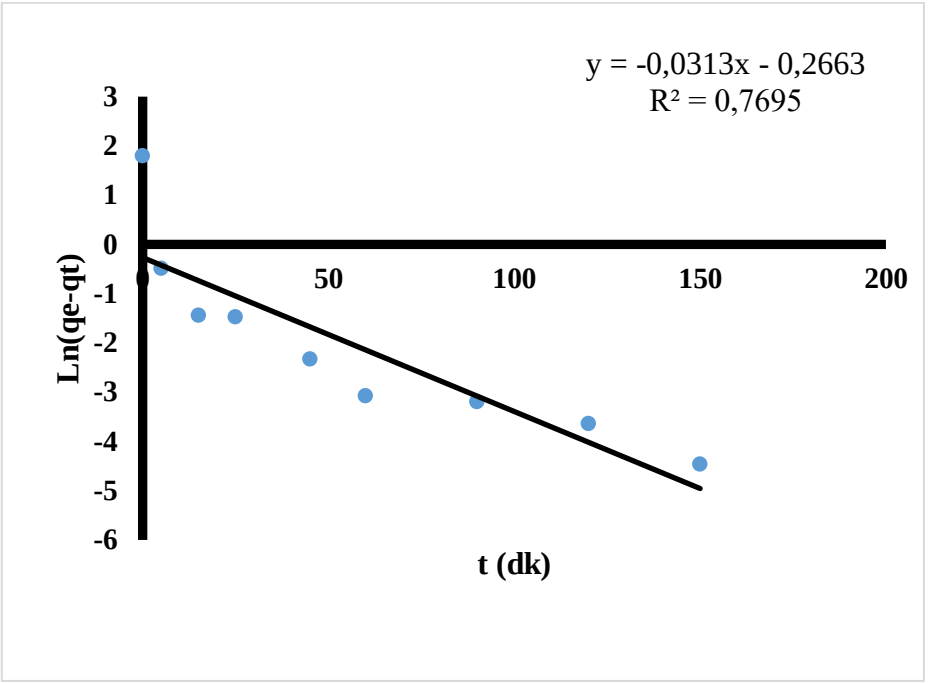
## Materyaller

a) Van ilinden toplanan Vişne
b) Kristal viyole
c) UltraViyole -VIS mini 1240 SHIMADZU marka Spektrometre cihazı
d) Laboratuvar Termometre

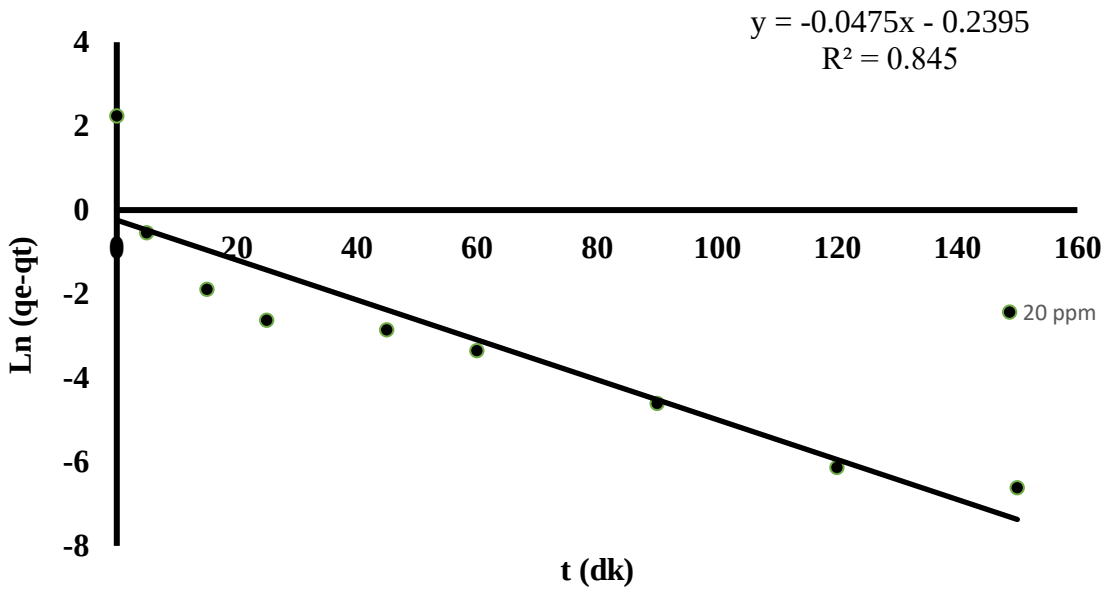
## Yöntem

Van ilinde yetişen vişnelerden 3 kilogram alındı. Kimya Araştırmaları laboratuvarına getirildi. Vişne çekirdekleri çıkarıldı, 48 saat açık havada kurutuldu. vişne çekirdekleri havanda dövülerek çok ince taneli hale getirildi. 250 mesh lik ince elekten geçirildi. Böylece vişne çekirdeği talaşı (V.Ç.T) biyokütle adsorbantı hazırlandı. Daha sonra farklı derişimlerde, 500'er mililitre cam beherde 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, 5 adet Malahit Yeşili çözeltileri hazırlandı. Hazırlanan çözeltileri sırası ile 298 K, 308 K, 318 K derece sıcaklıklarda ayrı ayrı her 500 ml çözeltiye 1,5'er gram vişne çekirdeği talaşı eklenerek magnetik karıştırıcı ile karıştırıldı. Çözelti PH' ları 5,5 olarak ayarlandı. Değişik zaman aralıklarında, 5-10-15-20-25-30-35-40-50-55-60-70-80-90-100-110-120-140-160-180. Dakikalarda çözeltilerden 5 ml örnekler alınarak ,UltraViyole -VIS mini 1240 SHIMADZU marka Spektrometre cihazında, 590 nanometre dalga boyunda, ne kadar adsorbsiyon yaptığı okundu ve kayıt edildi.

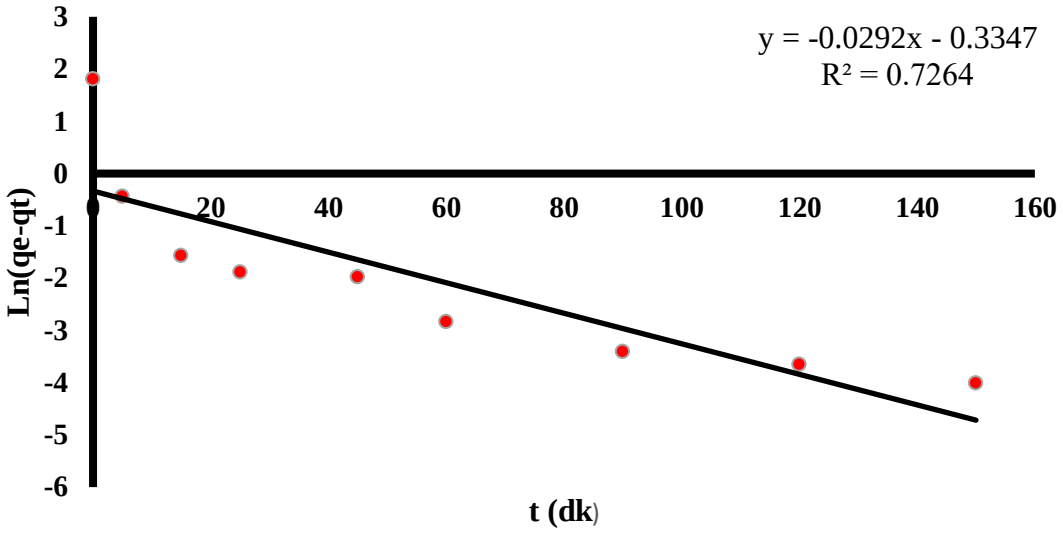
**BULGULAR**




Şekil 1. 20 ppm, 298 K da Kristal Viyolenin V.Ç.T Yüzeyinde Adsorpsiyonunun Yalancı Birinci Dereceden Kinetik Grafiği




Şekil 2. 20 ppm, 308 K da Kristal Viyolenin V.Ç.T Yüzeyinde Adsorpsiyonunun Yalancı Birinci Dereceden Kinetik Grafiği

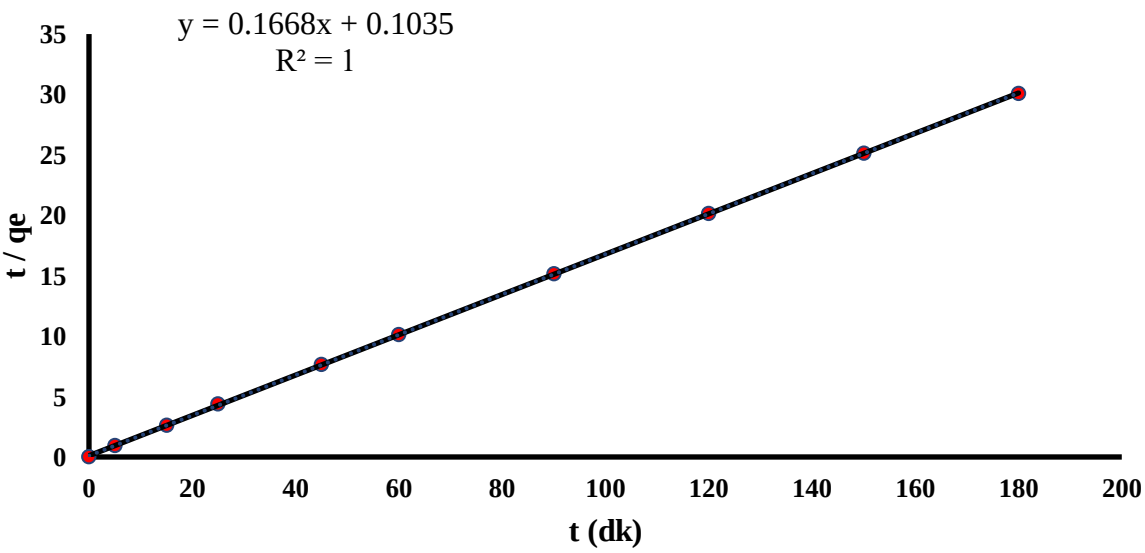



Şekil 3. 20 ppm, 318 K da Kristal Viyolenin V.Ç.T Yüzeyinde Adsorpsiyonunun Yalancı Birinci Dereceden Kinetik Grafiği

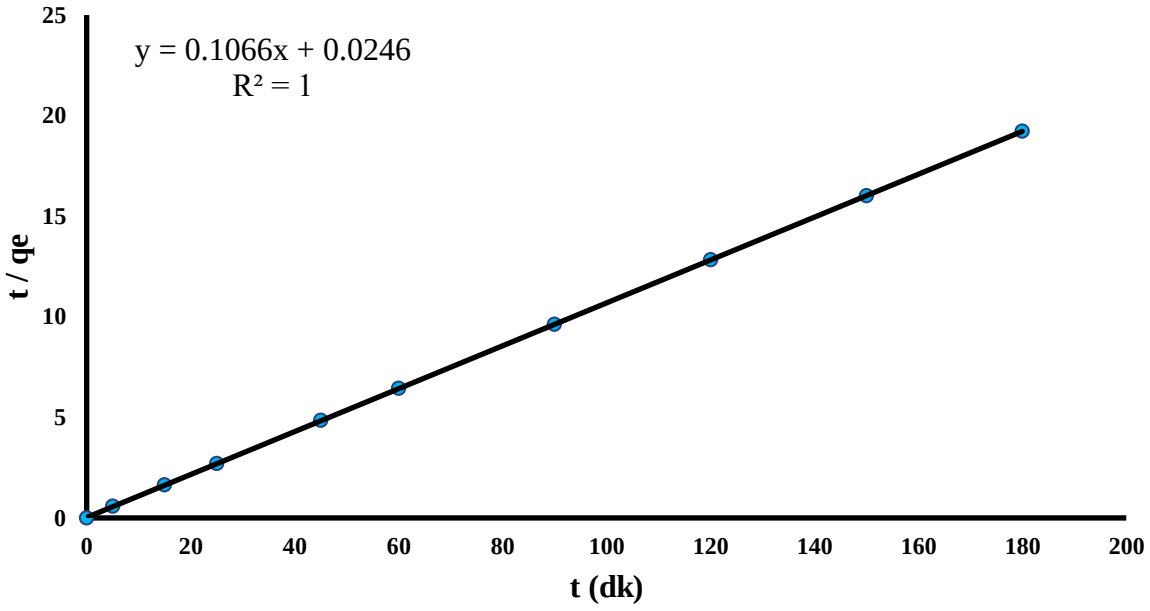
**Tablo 1.** 20 ppm, de Kristal Viyolenin V.Ç.T Yüzeyinde Adsorpsiyonunun Yalancı Birinci Dereceden Kinetik hesaplamaları

| T (K) | $k_1$ | Teorik $q_e$ (mg/g) | Deneysel $q_e$ (mg/g) | $R^2$ |
|---|---|---|---|---|
| 298 | 0,0313 | 0,7662 | 5,9847 | 0,7695 |
| 308 | 0,0475 | 0,787 | 9,366 | 0,845 |
| 318 | 0,0292 | 0,715 | 6,1186 | 0,7264 |

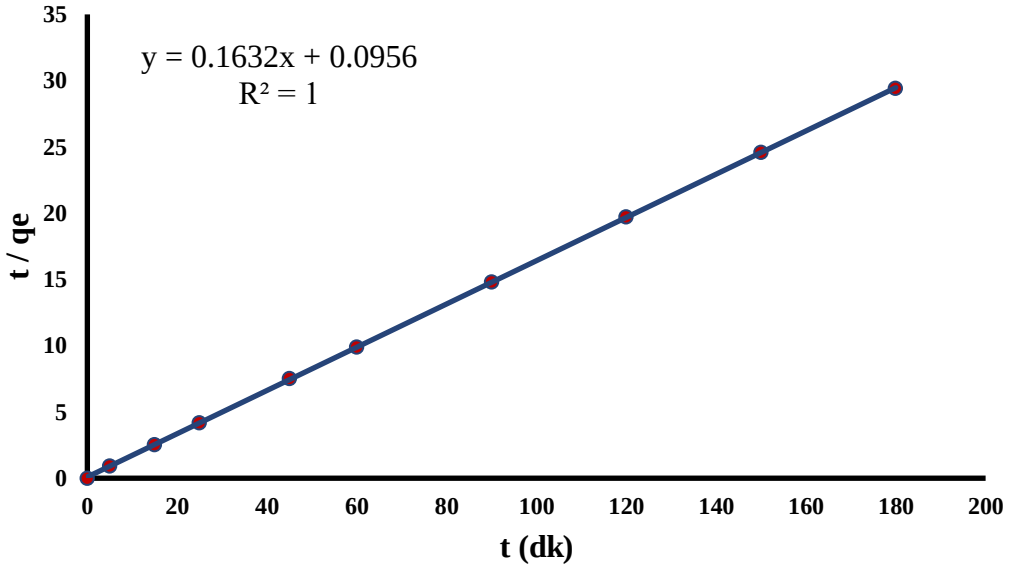
Tablo 1 de Kinetik Hesaplamalar da 3088 K'de $R^2$ değerinin 0,845 ile en yüksek çıkması ile adsorpsiyonun 3088 K' de daha yüksek olduğunu göstermektedir.



Şekil 4. 20 ppm, 298 K da Kristal Viyolenin V.Ç.T Yüzeyinde Adsorpsiyonunun Yalancı İkinci Dereceden Kinetik Grafiği




Şekil 5. 20 ppm, 308 K da Kristal Viyolenin V.Ç.T Yüzeyinde Adsorpsiyonunun Yalancı İkinci Dereceden Kinetik Grafiği




Şekil 6. 20 ppm, 318 K da Kristal Viyolenin V.Ç.T Yüzeyinde Adsorpsiyonunun Yalancı İkinci Dereceden Kinetik Grafiği

Tablo 2. Kristal Viyolenin V.Ç.T Yüzeyinde Yalancı İkinci Dereceden Kinetik Hesaplamaları (20 mg/L).

| **T (K)** | **$k_2$** | **Theoric $q_e$ (mg/g)** | **Experimental $q_e$ (mg/g)** | **$R^2$** |
|---|---|---|---|---|
| 298 | 0,2688 | 5,9952 | 5,9847 | 1 |
| 308 | 0,4619 | 9,3808 | 9366 | 1 |
| 318 | 0,045 | 6,1274 | 6,1186 | 1 |

Tablo 2 de $R^2$ değerlerinin her üç sıcaklık ta (298 K,308 K,318 K) 1 çıkması yalancı ikinci Dereceden Kinetik Hesaplamalarında adsorpsiyonun yüksek olduğunu göstermektedir.

## TARTIŞMA

Van ilinde yetişen vişne ağaçlarından alınan vişne çekirdekleri Talaşı kullanılarak atık sulardan Kristal viyolenin uzaklaştırılmasında, Ortam PH'sı 5,5 ta ayarlanarak çalışma yapılmış, sonucta alınan örnekler santrifüj edildikten sonra spektro fotometrede okunmuş ve değerler birinci derece yalancı ve ikinci derece yalancı kinetik denklemlerine hesaplanmıştır. Hesaplamalarda işlemin kolerasyon değerinin ikinci derece kinetik modelde, $R^2$= 1 (298 K) , $R^2$=1 (308 K) ,$R^2$=1 (318 K) birinci dereceden $R^2$=0,7695 (298 K) , $R^2$=0,845 (308 K) ,$R^2$=0,7264 (318 K) büyük olması nedeniyle, İkinci derece kinetik model, 20 ppm Kristal Viyole derişimindeki regresyon sabiti değeri 298 K,308 K, 318 K de
$R^2$= 1 olarak bulunmuş ve ikinci derece kinetik modeline uygun olduğu tesbit edilmiştir.

## SONUÇ

298 K, 308 K,318 K Farklı üç sıcaklıkların adsorpsiyon verimini değiştirmesi şöyle olmuştur: Sıcaklık arttıkça, 20 ppm Kristal Viyole konsantrasyonunun sırasıyla %89,77 , %90,049 , %91,77 , 298 K, 308 K ve 318 K olduğu bulunmuştur. Bu sonuç Van ilinde yetişen Vişne ağaçlarından alına Vişne Çekirdeği Talaşından hazırlanan biyokütlenin adsorban olarak atık su temizlenmesinde filtre olarak kullanılabileceğini ortaya çıkarmaktadır.

## KAYNAKLAR

Acemıoglu B, Adsorption of Congo red from aqueous solution onto calcium- rich fly ash. (2004). J. Colloid Interface Sci., 274: 371-379. 1 :21-52

Ali Rıza KUL,Hasan ELİK,Adnan ALDEMİR,Hasan ERGE,Cemile KUL ESER. Ispec 5th ınternatıonal conference on engıneerıng & natural scıences december 20-22, 2019 / van, turkey.Trabzon fındığından (corylus avellana) alınan kabuklar üzerinde metilen mavisi adsorpsiyonu çalışmasının kinetik hesaplamaları. Ankara: ıspec publications -; 2019. s. 204.

Ho Y S., Mckay G., (1998a). A Comparison Of Chemisorption Kinetic Models Applied To Pollutant Removal On Various Sorbents, Process Safety and Environmental Protection, 76,B, 332-340.

Ho Y S., Mckay G., (1998b). Kinetic Models For The Sorption Of Dye From Aqueous Solution By Wood To Pollutant Removal On Various Sorbents, Process Safety and Environmental Protection, 76, B, 183-191.

Kristal Viyole 2022, https://tr.wikipedia.org/wiki/Kristal_viyole Erişim Tarihi: 15.10.2022

Vişne ,2022, https://tr.wikipedia.org/wiki/Vi%C5%9Fne Erişim Tarihi: 15.10.2022

Yavuz Ö, Altunkaynak Y, Güzel F, 2002. Removal of copper, nickel, cobalt and manganese from aqueous solution by kaolinite. Water Research, 37, 4, 948-952.

## *ORAL PRESENTATION*

# Evaluations on the planning of honey forests in terms of forest-public relations

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## Abstract

Honey forests in Turkey is a significant instrument in terms of regulating forest-public relations and benefiting from forests. In recent years, honey forest facilities have get up to speed with the honey forest action plans carried out by the General Directorate of Forestry. Honey forests have an important potential for the on-site development of the poor forest villagers. Honey forests are also significant agroforestry zones where the state's forestry efforts directly translate into a source of income for the villagers who live in the forests. For this reason, it is necessary to determine and plan the facility areas of honey forests with a certain strategy. For this purpose, it is assumed that the use of some parameters in the planning and regulation of the distribution of honey forests at the provincial level will lead to more rational results. The current situation of the provinces for honey forest establishment has been revealed by using parameters such as Number of Businesses, Number of Colonies, Honey Production, Number of Honey Plants and Established Honey Forest Area. This study was conducted in order to provide practitioners with ideas for more rational and rural development-focused action plans for various forest products and services.

**Keywords:** Honey forest, multi-criteria approach, agro-forestry

## INTRODUCTION

Beekeeping in Turkey can be considered as one of the instruments that can be used in rural development, especially for forest villagers who have difficult living conditions and low incomes (Akyol ve Cazağ 2018; Gültekin 2019; Diktas-Bulut vd. 2022, OGM 2022). In different parts of Turkey, different types of honey are produced. In addition, beekeeping in Turkish forests: such as honey production by nomadic beekeepers in Pinus brutia forests (Korkmaz et al. 2018), honey production from Castanea sativa forests (Diktas-Bulut et al. 2022), and honey production from rhododendrons in Black Sea forests (Sıralı and Cınbırtoğlu, 2018).

The advantage of benefiting from forests in the process of carrying out beekeeping activities has been an significant opportunity for the forest organization to regulate forest-human relations (Gültekin 2019, OGM, 2022) and has caused beekeeping activities to be supported in various ways. Forest villagers have been supported for many years by state support, which is called ORKOY for many years. When the forestry statistics of the beekeeping supports realized by the forestry organization in Turkey are examined; From 1974 to 2020 (except for the years 2001-2002 and 2006-2007), it has been given as economic support under the name of "scientific beekeeping" (OGMSTAT-2021). In addition, to support beekeeping activities in many ways, special quality forests; "honey forests" began to be established. Honey forests is formed by either the rehabilitation of existing forests or the establishment of new forests in order to “produce honey and thus generate income for the local people and control soil protection and erosion in the process” (OGM, 2022). In the process of creating honey forests, to increase the positive effect of honey forest on beekeeping; first of all, the focus is on vegetation and in the studies carried out, issues such as vegetation diversity, ensuring that the bee can collect pollen-nectar as long as possible, appropriate topographic structure and the development of the local people are discussed (OGM, 2022). When the literature studies are examined;

In recent years; In the studies on the determination of the most ideal areas and potential in the honey forest facility; Güngör and Ayhan (2016) type of the trees; and Sönmez and Gencal (2019) carried out a study, considering tree species, wind direction and distances to residential areas. At the same time, Eker and Öner (2014) reported the general situation of honey forests in Çankırı; Sönmez and Gencal (2019) identified the potential honey forests in Bursa and Güngör and Ayhan (2016) in Bartın, Yıldız and Fakir (2019) the plant potential of the honey forest in Isparta; Öztürk, (2019) Determination of optimal honey forest areas in Artvin Borcka; Yildirim et al. (2020) Flora of honey forest in Yığılca district of Düzce; and Gültekin (2019) Effects on rural development; Gedik et al (2021) studied the socioeconomic effects of honey forests in Sivas

Koyulhisar district. In addition; In the Eastern Black Sea region, Küçük et al. (2022) and Altunel and Ölmez (2019) in Kastamonu Region carried out studies on forest villagers engaged in beekeeping.

In this study, at the provincial level in Turkey; The distribution of honey forests as of 2021 has been revealed in terms of factors that may affect the planning of honey forests in the future, such as honey forests established and beekeeping statistics. The aim of this study is to raise awareness among practitioners in order to plan the distribution of honey forests within the borders of the country with a multi-criteria approach, which can be used as an important tool in rural development and can improve beekeeping activities.

## MATERIALS AND METHODS

In this study is focused on the establishment of the "honey forest", which supports the beekeeping activities in Turkey and will positively affect the forest-human relations of the forestry organization. In the study is thought that the areas where honey forests will be established should be evaluated from different perspectives. For this purpose, the study was carried out on the provinces of Turkey. Some parameters were used at the provincial level. While choosing these parameters, it was thought that the number of honey plants in the provinces would have a significant impact on beekeeping and honey forest facilities. For this reason, the number of honey plants in the list of important honey plants in each province included in the Honey Forest Action Plan (2018-2023) action plan of GDF was determined by counting one by one (GDF, 2022). However, data on Bilecik province could not be obtained. In addition, parameters showing the current beekeeping activities of the provinces were preferred. These parameters are T.C. Taken from Turkey Province-Based Beekeeping Statistics (2021), which is available on the official website of the Ministry of Agriculture and Forestry, Directorate of Apiculture Research Institute (ARI, 2022). These parameters are listed as Number of Businesses, Number of Colonies, Honey Production, Number of Honey Plants and Established Honey Forest Area. In addition, the number of enterprises engaged in beekeeping activities in the provinces was obtained from TUIK (TUİK, 2022). The resulting data was mapped using ArcGIS 10.3. It was thought that with mapping, an easier interpretation could be made on the geographical distribution of beekeeping parameters.

## RESULTS

In the study, various data sets were obtained by moving from the point that the facility areas of honey forests should be evaluated and planned at the provincial level. The data sets obtained were organized at the provincial level. The distribution of beekeeping businesses by provinces as of 2021 in Turkey is given in Figure 1.

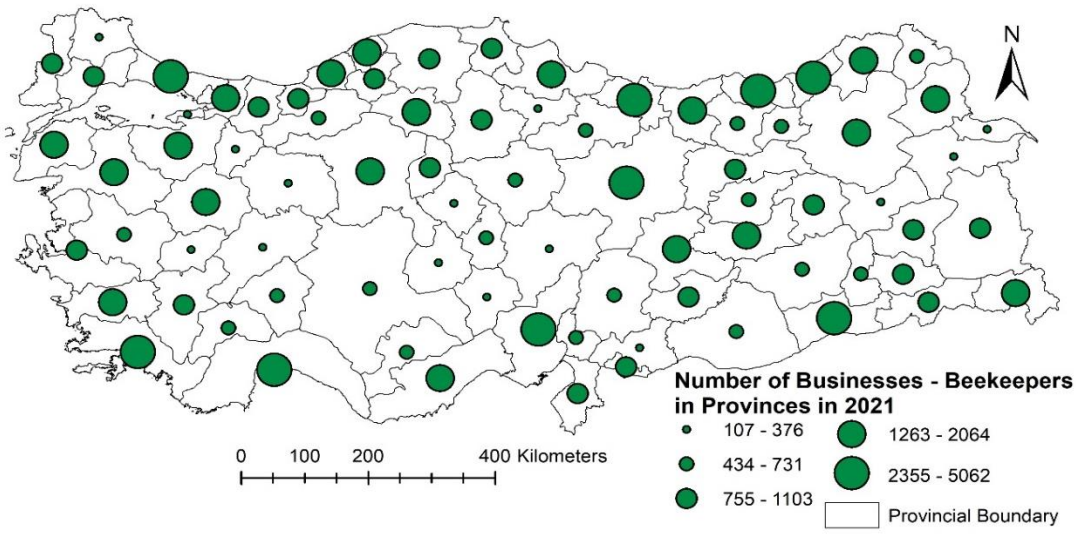


**Figure 1.** Number of Beekeeping Enterprises in the Provinces (2021)

When Figure 1 is examined; It is seen that the number of enterprises along the Black Sea, Mediterranean and Aegean coastlines and partially in the east-southeast regions is higher than in a few cities in Central Anatolia and the East. While the provinces of Muğla, Ordu, Rize and Sivas have the most enterprises, respectively, Kırıkkale, Kırşehir, Uşak and Kilis are the provinces with the least number of enterprises.

The number of colonies in the provinces is examined in Figure 2.

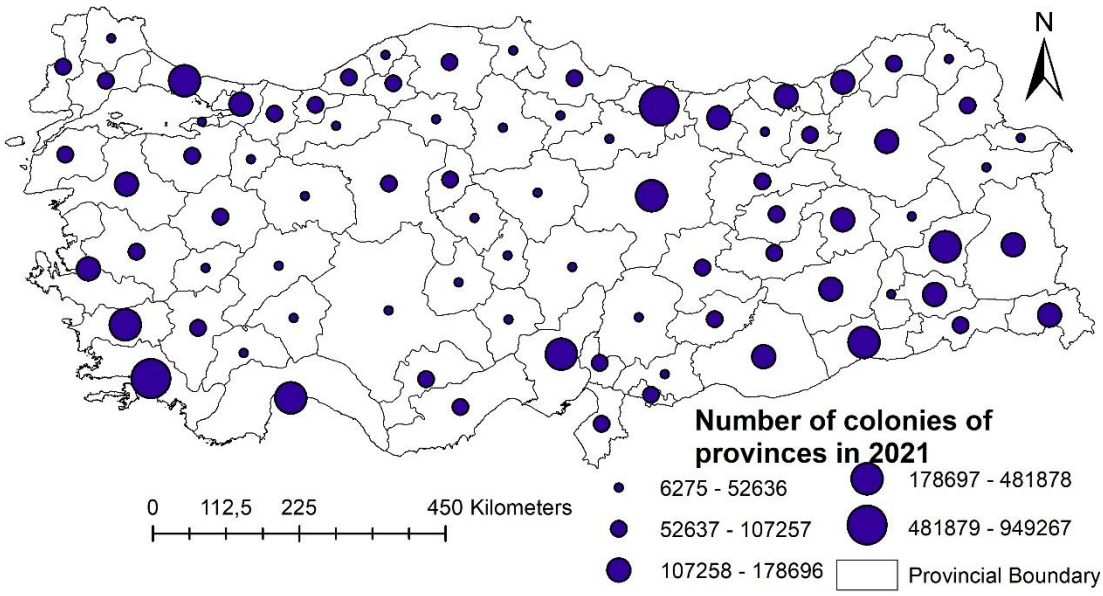


**Figure 2.** Number of Beekeeping Enterprises in Enterprise (2021)

When Figure 2 is examined; In parallel with the number of enterprises, it is seen that the lowest number of colonies in the provinces of Kırıkkale, Kırşehir, Kilis and Uşak and the highest number of colonies in the provinces of Ordu, Muğla and Adana. Especially in the Western Black Sea region and towards the inner parts, it is seen that the number of colonies decreases compared to the number of enterprises.

In the study, honey production at the provincial level is given in Figure 3.

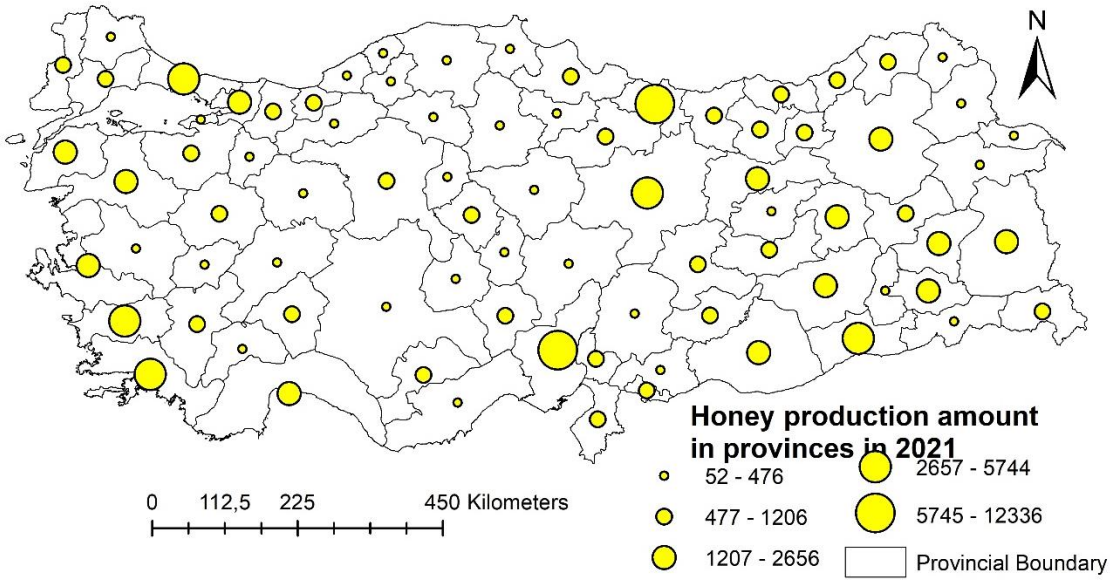


**Figure 3**. Honey Production Amount at Provincial Level (2021)

When the honey production amounts of the provinces are examined, it is expected to be compatible with the number of enterprises and the number of colonies; It is seen that the provinces with high honey production are distributed more intensively and individually in the Northern Marmara, Aegean coasts and the east of the country compared to the inner parts.

Some important honey plants of the provinces were included in the 2013-2017 and 2018-2023 honey forest action plans prepared by OGM. The distribution of these plants at the provincial level is given in Figure 4.

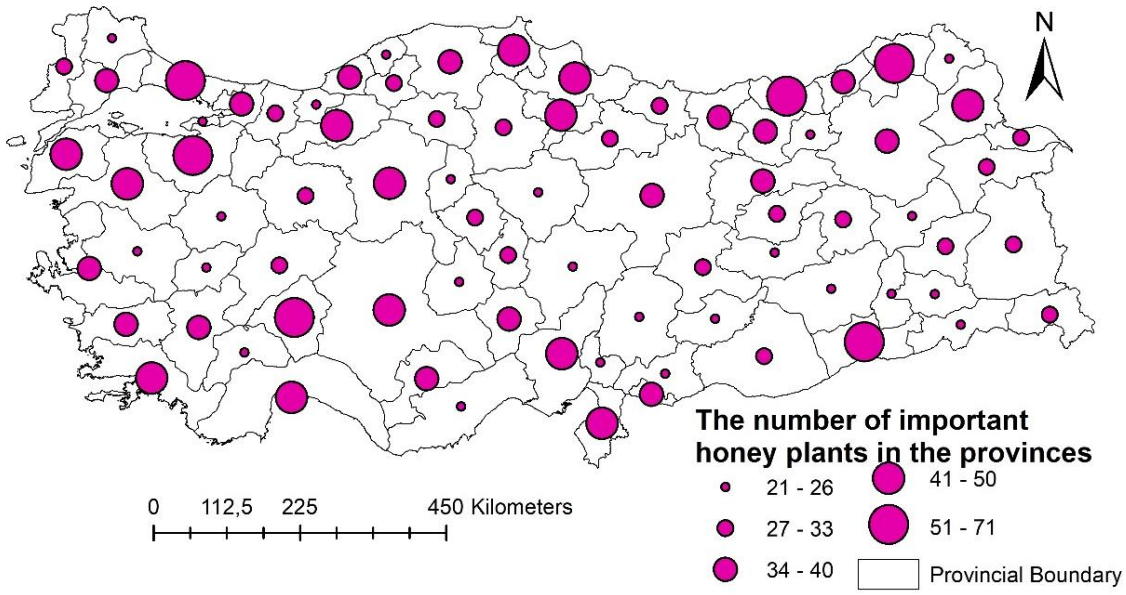


**Figure 4**. The Distribution of Important Honey Plants at the Provincial Level

When the distribution of some important honey plants included in the action plans is examined, it is seen that honey plants are densely located along the northern and southern lines of the country.

The spatial distribution of honey forests established between 2013-2021, which is aimed as an important support for the rural development of forest villagers, and which is also an agro-forestry study, according to the provinces is given in Figure 5.

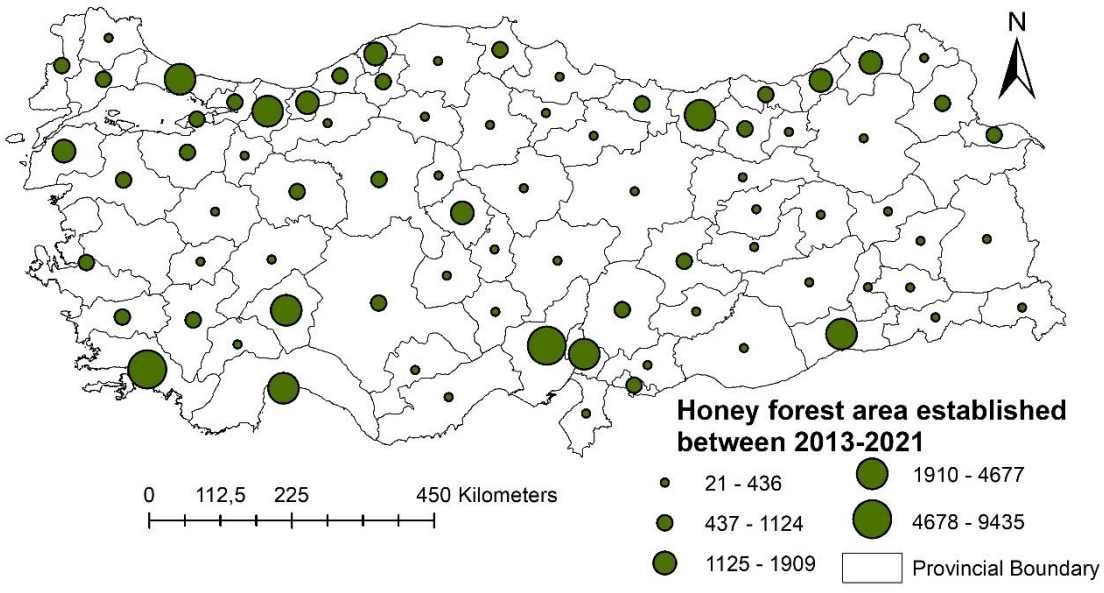


**Figure 5**. The Spatial Distribution of Honey Forests Established Between 2013-2021

When the distribution of honey forest areas established between 2013 and 2021 is examined; It is observed that honey forest facilities are concentrated in the provinces in small groups located in the northwest, northeast and south of the country.

## DISCUSSION and CONCLUSION

In order to determine the areas where honey forests will be established, which is considered as an significant instrument of Turkish forestry in terms of regulating forest-human relations, is very important. As in the studies of Güngör and Ayhan (2016) and Sönmez and Gencal (2019), it is important to determine the areas where honey forest can be established. In this context in this study, a macro-scale evaluation was made. In this study, the current situation of beekeeping activities and honey forests was evaluated by using various parameters. Beekeeping statistics are quite low in the inner parts of the country, and honey forest facilities are also less compared to other regions, is seen. In addition, considering that there is less forest in the interior, more importance should be given to the establishment of honey forests in the interior regions, both as new forest establishment works and to support rural development. In addition, there is a significant amount of enterprises and colonies in the east of the country. Honey forest facilities can be realized more intensively in order to support and sustain the existing businesses in these areas. In addition, the participation of local people in beekeeping activities should be ensured with the support of ORKOY in regions where honey forests are densely established. Trainings on beekeeping should be provided to the local people through joint studies with the Provincial Directorates of Agriculture and Forestry.

## REFERENCES

Akyol, A., Cazağ, S. N. 2018. Arıcılığın geliştirilmesine yönelik arı yetiştiricilerinin beklenti ve değerlendirmeleri. ISNOS-MED 2018.

Altunel, T., Olmez, B. 2019. Beekeeping as a rural development alternative in Turkish northwest. Appl. Ecol. Environ. Res, 17(3), 6017-6029.

ARI, 2022. Tarım ve Orman Bakanlığı, Arıcılık Araştırma Enstitüsü Müdürlüğü. Arıcılık İstatistikleri (tarimorman.gov.tr) Erişim(26/10/2022)

Diktas-Bulut, N., Daşdemir, İ., Bozlar, T. 2022. Economıc analysıs of chestnut honey productıon ın the natural chestnut forests of eastern black sea regıon, Turkey. Journal of Animal & Plant Sciences, 32(5), 1287-1298.

Eken, Ö., Öner, M. N. 2014. Yerel halkın sosyoekonomik durumunun iyileştirilmesi kapsamında bal ormancılığı: Çankırı Ahlât köyü bal ormanı örneği. II. Ulusal Akdeniz Orman ve Çevre Sempozyumu, 22–24 Ekim 2014, Isparta.

Gedik, S., Sönmez, B., Kayıkçı, S. 2021. Bal Ormanlarının Sosyo-Ekonomik Boyutunun İncelenmesi: Koyulhisar Örneği. Bartın Orman Fakültesi Dergisi, 23(3), 1013-1025.

GFD, 2022. Bal Ormanı Eylem Planı (2018-2023), Tarım ve Orman Bakanlığı Orman Genel Müdürlüğü. Pp: 149, Ankara https://www.ogm.gov.tr/tr/e-kutuphane-sitesi/Yayinlar/Bal% 20Orman%C4% B1%20Eylem%20Plan%C4% B1%20(2018-2023). pdf Erişim(26/10/2022)

Gültekin, Y. S., 2019. Yığılca Bal Arısının Kırsal Kalkınma ve Ekogirişimcilik Kapsamında Değerlendirilmesi. Düzce Üniversitesi Bilim ve Teknoloji Dergisi, 7(1), 911-921.

Güngör, E., Ayhan, A. 2016. Bartın Yöresi orman kaynaklarının bal üretim potansiyeli ve ekonomik değeri. Turkish Journal of Forestry, 17(1), 108-116.

Korkmaz, M., Avcı, M., Özçelik, R. 2018. Kızılçam Ormanlarında Çam Balı Üretimi Kapsamında Göçer Arıcılığın Sorunları. ISNOS-MED 2018. 1.st International Symposium on Silvopastoral Systems and Nomadic Societies in Mediterranean Countries.

Küçük, A., Saylam, A., Akın, A. L., Şahinoğlu, O. Y. 2022. Orman köylerinde arıcılık sorunları ve çözüm önerileri (Trabzon Orman Bölge Müdürlüğü örneği). Ormancılık Araştırma Dergisi, 9(2), 15-27.

Öztürk, H. 2019. Borçka yöresinde arıcılık faaliyetleri, bal ormanı potansiyelinin belirlenmesi ve orman kaynaklarının planlanmasına etkileri (Master's thesis, Artvin Çoruh Üniversitesi).

Sıralı, R., Cınbırtoğlu, Ş. 2018. Ormangülü (rhododendron) türlerinin bazı özellikleri ve arıcılık açısından önemi. Arıcılık Araştırma Dergisi, 10(2), 45-53.

Sönmez, T., Gencal, B. 2019. Bursa Orman Bölge Müdürlüğü sınırlarında bulunan bazı odunsu bitki türleri ile kurulabilecek potansiyel bal ormanı alanlarının belirlenmesi. Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi.

Taştan, F. 2021. Bal Arıları (Apis mellifera) İçin Yozgat Florasındaki Önemli Bitkiler. Türler ve Habitatlar, 2(2), 134-160.

TUİK, 2022. Türkiye İstatistik Kurumu Web Sitesi https://biruni.tuik.gov.tr/medas/?kn=101&locale=tr Erişim(26/10/2022)

Yıldırım, E. A., Özkan, N. G., Kılıç, N. K., 2020. Yığılca (Düzce) Balköy Bal Ormanı Florası. Düzce Üniversitesi Orman Fakültesi Ormancılık Dergisi, 16(2), 45-69.

Yıldız, S., Fakir, H., 2019. Bal Üretim Ormanları İçin Potansiyel Bitki Türleri: Isparta Keçiborlu Güneykent Bal Üretim Ormanı Örneği. Bilge International Journal of Science and Technology Research, 3(2), 213-222.

## *ORAL PRESENTATION*

# Cell Culture Systems As Therapeutic Modalities In Regenerative Medicine -A Review

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## Abstract

Introduction: Cell culture systems are the most important tools in molecular biology, tissue engineering, drug discovery, and regenerative medicine. One crucial requirement of cell culture system is their ability to resemble key aspects of their *in vivo* counterpart. This review identifies emerging the developments in the literature of regenerative medicine based on relevant articles and published reviews. Multipotent stem cells include adult stem cells that can self-renew or to differentiate into specialized cell types. Methods: Multiple datasets of publications relevant to regenerative medicine are constructed through topic search and citation expansion to ensure adequate coverage of the field. The collected data obtained from different references representing the literature of regenerative medicine that visualized based on the electronic database. Results and discussion: Stem cells occupy an active and growing area of basic science and clinical research due to their ability to self-renew and differentiate into mature cell types. New developments are identified in terms of newly emerged clusters and research areas. The research in induced pluripotent stem cells remains the most prominent area in the field of regenerative medicine. Conclusions: Stem cells have the unique ability to self-renew or differentiate into various cell types in response to appropriate signals. These properties provide stem cells with unique capabilities for tissue repair, replacement and regeneration, making them valuable research tools in regenerative medicine and stem cell therapies.

**Keywords:** Stem cells, Cell based therapy, Regenerative medicine, Cell culture automation.

## INTRODUCTION

Organ and tissue loss through disease and injury motivate the development of therapies that can regenerate tissues and decrease reliance on transplantations (Sebastian et al., 2022). The regenerative medicine, an interdisciplinary field that applies engineering and life science principles to promote regeneration, can potentially restore diseased and injured tissues and whole organs (Bacakova et al., 2018). Since the inception of the field several decades ago, a number of regenerative medicine therapies, including those designed for wound healing and orthopedics applications are now commercially available (Weissman and Shizuru, 2008). Cell culture is a principal technique used for the analysis of drug activity, metabolism, and toxicity in vivo. Different types of cells require different types of environments to grow in vitro (Mehrian, et al., 2020). Primary cells and stem cells grow anchorage-dependent and blood cells and secondary cell lines grow in suspension culture or anchorage-independent (Cao et al., 2021). Stem cells hold great potential in regenerative medicine as these cells are endorsed with indefinite self-renewal characteristics and can be differentiated into any cells of the body (Bissels et al., 2016). The cell-based therapies could revolutionize health care and regenerative medicine. Based on their differentiation potential, stem cells can be categorized as unipotent or pluripotent. Cell therapy applies the biochemical and biophysical properties of stem cells to generate healthy tissues and repair damaged organs, preventing them from further damage (Mahla, 2016). The bone marrow serves as a source of stem cells from where hematopoietic stem cells, neural stem cells, and mesenchymal stem cells can be derived for therapeutic purposes However, cell therapies share limitations in manufacturing that may compromise the administration of a successful therapy to patients and increase costs (Aijaz et al., 2018). The present review focuses on the cell culture systems as important tools in molecular biology and regenerative medicine.

## METHODS

This review inspect key specialized papers and issues belong to the bio-processing cell-based therapy from literature search and published research papers. Recent developments are encouraging adoption of quality approaches for bio-processing of cell-based therapies. The growth of the literature over the past few years is enormous. These novel cellular therapy bioprocesses are successful clinical applications. Although there are an increasing number of science programming systems and generic tools, few systems are readily accessible

and specifically designed to meet the needs for generating a systematic review of a fast-moving and complex field, especially with features to facilitate the detection and interpretation of emerging trends. Such review aims to identify new developments in the field of regenerative medicine. The structure and dynamics of the literature of cellular culture are analyzed in terms of progressively synthesized networks derived from citations made by citing articles that meet various selection criteria. The selected data and information were checked for their relation to the cell culture systems and regenerative medicine, specially to the following important topics: cellular regeneration, cell culture systems, regenerative medicine, cell based therapies**.**

## RESULTS AND DISCUSSION

This review was carried out to comprehensively assess to cell culture systems with a detailed description of the methods that are available on regenerative medicine, the promising alternatives to traditional tissue / organ transplantations and drug treatments, by using the so called cell based therapy. All the selected data and information were checked for their relation to the regenerative therapies, specially to the following important topics:

### 1. Cellular regeneration

Generally, regeneration means the re-growth of a damaged or missing organ part from the remaining tissue, our skin is constantly being renewed and repaired. Unfortunately many other human tissues don't regenerate, and a goal in regenerative medicine is to find ways to kick-start tissue regeneration in the body, or to engineer replacement tissues (Rodriguez-Hernandez, 2014). So, it is a natural process that allows any living organism to replace or restore damaged or missing cells, tissues, organs, and even entire body parts to full function. Scientists are studying regeneration for its potential uses in medicine, such as treating a variety of injuries and diseases (Emmerson E and Knox S, 2018). Perhaps one of the best-known types of cellular regeneration, stem cell therapy has been in the press a lot lately. That's because doctors have been using it to treat joint and tissue damage with success (Bissels U et al., 2016). Cellular regeneration is the science in which the procedure of recovery and development that makes genomes, cells, living beings and biological systems. Living organisms have some ability to regenerate as part of natural processes to maintain tissues and organs (Bacakova et al., 2018). These include the forming thick scars in tissues and skin to promote the healing of injured or amputated body parts, re-growing hair and skin, and healing a bone fracture by using new tissue to knit the bone pieces together (Venkata et al., 2021). Cellular regeneration involves healthy cells utilizing interactive feedback such as contact inhibition, preventing healthy cells from multiplying and stacking beyond their physiological role. Planar cell culture models with enhanced cell adhesion features significantly lack a vertical profile and may not reflect the ability of cells to simulate wound closure based on cell–cell interaction alone. The first *in vitro* mammalian cell culture technique was per-formed in the first decade of the twentieth century and the technique was named Harrison's hanging drop technique (Langer and Joensson, 2020). Mammalian cell culture is a principal technique used for the analysis of drug activity, metabolism, and toxicity *in vivo*. **(Fig. 1)**

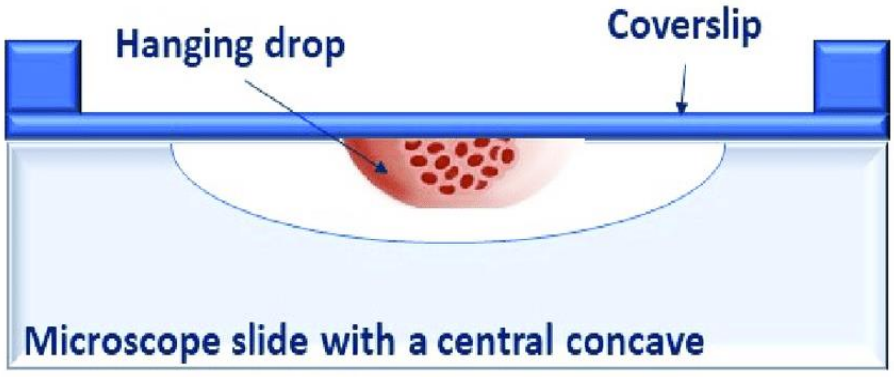


**Fig. 1: Harrison's hanging drop technique for mammalian cell culture.**

### 2. Regenerative medicine

The current therapy of transplantation of intact organs and tissues to treat organ and tissue failures and loss suffers from limited donor supply and often severe immune complications, but these obstacles may potentially be bypassed through the use of the regenerative medicine strategies (Hunsberger et al., 2018). Regenerative medicine is an interdisciplinary field that applies engineering and life science principles to promote regeneration, can potentially restore diseased and injured tissues and whole organs (He et al., 2017). Organ and tissue loss through disease and injury motivate the development of therapies that can regenerate tissues and decrease reliance on transplantations (Fričová et al., 2020). Stem cells hold great potential in regenerative

medicine as these cells are endorsed with indefinite self-renewal characteristics and can be differentiated into any cells of the body. A stem cell is a cell with the unique ability to develop into specialized cell types in the body. They may be used to replace cells and tissues that have been damaged or lost due to disease. Stem cells provide new cells for the body as it grows, and replace specialized cells that are damaged or lost (Sayali et al., 2022). They have two unique properties that enable them to do this:

1- They can divide over and over again to produce new cells (progenitor).
2- As they divide, they can change into the other types of cell that make up the body (differentiated). **(Fig. 2)**

The stem cells have several uses including: (a) research that helps to understand the basic biology of how living things work and what happens in different types of cell during disease, and (b) therapy used to replace the lost or damaged specialized cells in the harmed organs that can't replace naturally.

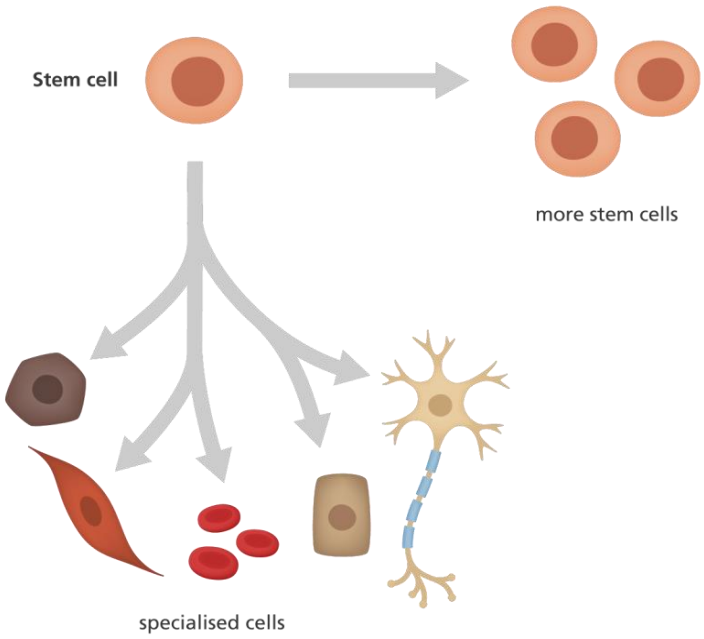


**Fig. 2: Stem cell giving rise to more stem cells or specialized cells.**

Regenerative medicine has the potential to heal or replace tissues and organs damaged by age, disease, or trauma, as well as to normalize congenital defects. Promising preclinical and clinical data to date support the possibility for treating both chronic diseases and acute insults, and for regenerative medicine to abet maladies occurring across a wide array of organ systems and contexts, including dermal wounds, cardiovascular diseases and traumas, treatments for certain types of cancer, and critical cases (Kumar R et al., 2010). The field of regenerative medicine encompasses numerous strategies, including the use of materials and de novo generated cells, as well as various combinations thereof, to take the place of missing tissue, effectively replacing it both structurally and functionally, or to contribute to tissue healing. The body's innate healing response may also be lever-aged to promote regeneration, although adult humans possess limited regenerative capacity in comparison with lower vertebrates (Kumar S et al., 2019).

### 3. Cell-based therapies

The cell-based therapy involves modifying a patient's own cells or cells from a donor to fight disease and alleviate medical conditions. The cell-based therapies could revolutionize health care and regenerative medicine. Based on their differentiation potential, stem cells can be categorized as unipotent or pluripotent (Cierpka-Kmiec et al., 2019). Cell therapy applies the biochemical and biophysical properties of stem cells to generate healthy tissues and repair damaged organs, preventing them from further damage. It offers the promise of treating and altering the course of diseases which cannot be addressed adequately by existing pharmaceuticals. Cell therapies are a diverse group across cell types and therapeutic indications and have been an active area of research for many years but are now strongly emerging through translation and towards successful commercial development and patient access (Liu et al., 2020). The cell-based therapies could revolutionize health care and regenerative medicine. Based on their differentiation potential, stem cells can be categorized as unipotent or pluripotent. Cell therapy applies the biochemical and biophysical properties of stem cells to generate healthy tissues and repair damaged organs, preventing them from further damage (Deinsberger et al., 2020). This type of therapy represents the most recent phase of the biotechnology

revolution in medicine. As with many remedies, cell therapies are based on ground-breaking scientific discoveries and technology advancements. **(Fig. 3).**

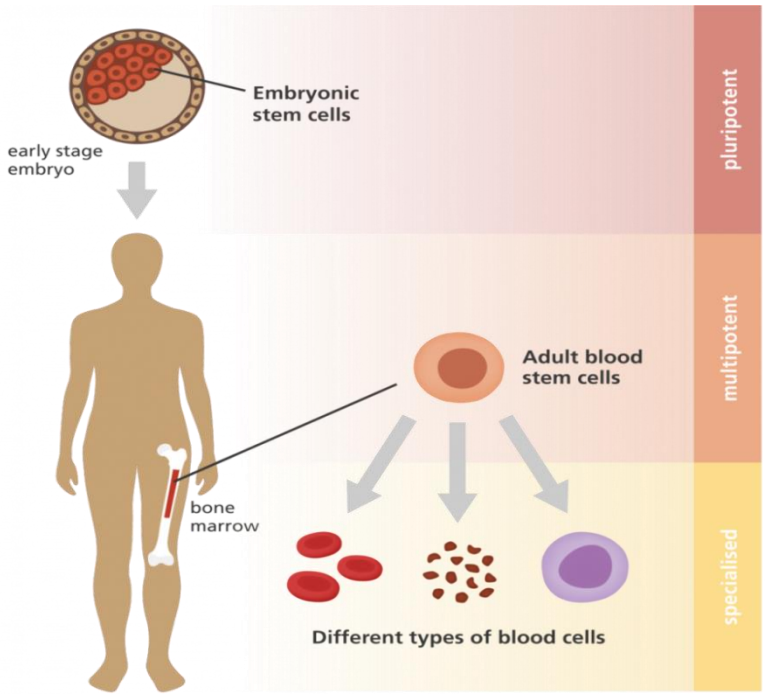


**Fig. 3: The pluripotent and multipotent stem cells.**

Most cell-based therapies are currently experimental, with a few exceptions such as haematopoietic stem cell (HSC) transplantation which is already a well-established treatment for blood related disorders (Cao et al., 2021). Cell therapies can be classified by the therapeutic indication they aim to address, e.g. neurological, cardiovascular, ophthalmological; by whether they comprise cells taken from and administered to the same individual (autologous) or derived from a donor (allogeneic). Most cell-based therapies are currently experimental, with a few exceptions such as haematopoietic stem cell (HSC) treatment which is already a well-established treatment for blood related disorders (Costariol et al., 2019).

## 4. Types of stem cell

Cells in the human body have specific purposes, but stem cells are cells that do not yet have a specific role and can become almost any cell that is required. Stem cells originate from two main sources: embryos tissues and adult body tissues. Scientists are also working on ways to develop stem cells from other cells, using genetic "reprogramming" techniques. A person's body contains stem cells throughout their life (Mehrian et al., 2020). The body can use these stem cells whenever it needs them. The cells are in a non-specific state, but they are more specialized than embryonic stem cells. They remain in this state until the body needs them for a specific purpose, say, as skin or muscle cells. The bone marrow serves as a source of stem cells from where hematopoietic stem cells (HSCs), neural stem cells (NSCs), and mesenchymal stem cells (MSCs) can be derived for therapeutic purposes. However, cell therapies, they share limitations in manufacturing (e.g., cell harvesting, expansion and purification; cell phenotype preservation; and development of a reproducible formulation) that may compromise the administration of a successful therapy to patients and increase costs. The current therapy of transplantation of intact organs and tissues to treat organ and tissue failures and loss suffers from limited donor supply and often severe immune complications, but these obstacles may potentially be bypassed through the use of the regenerative medicine strategies **(**Rothmiller et al., 2020).

A stem cell is a cell with the unique ability to develop into specialized cell types in the body. They may be used to replace cells and tissues that have been damaged or lost due to disease. Stem cells provide new cells for the body as it grows, and replace specialized cells that are damaged or lost. They have two unique properties that enable them to do this:

**1-** They can divide over and over again to produce new cells (progenitor).

**2-** As they divide, they can change into the other types of cell that make up the body.

Stem cells are present inside different types of tissue. Scientists have found stem cells in tissues, including: Brain, bone marrow, blood and blood vessels, skeletal muscles, skin, and the liver. However, stem cells can be difficult to find. They can stay non-dividing and non-specific for years until the body summons them to

repair or grow new tissue. Adult stem cells can divide or self-renew indefinitely. This means they can generate various cell types from the originating organ or even regenerate the original organ, entirely (Vitillo et al., 2020). In general, there are three main types of stem cells: Embryonic stem cells, adult stem cells, and induced pluripotent stem cells. Embryonic stem cells supply new cells for an embryo as it grows and develops into a baby. These stem cells are said to be pluripotent, which means they can change into any cell in the body. **(Fig. 4)**

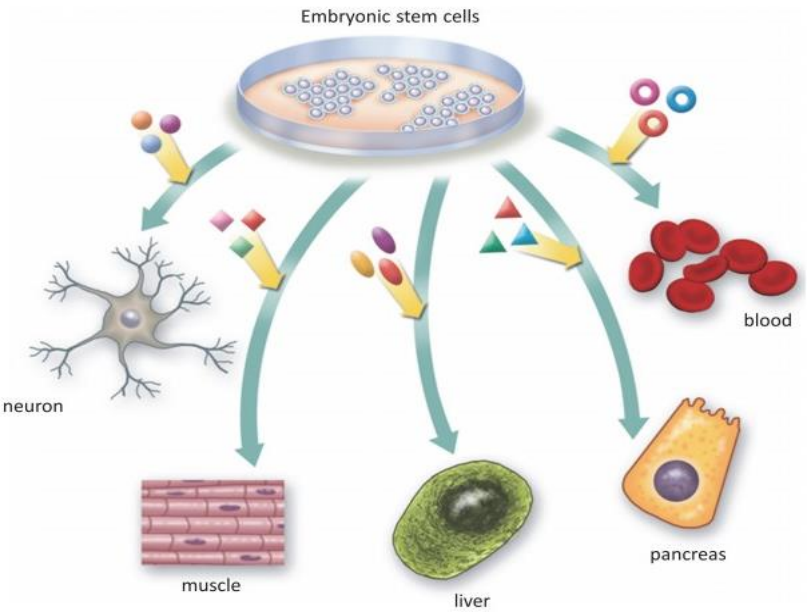


**Fig. 4: Embryonic stem cells that can change into any cell in the body.**

Adult stem cells supply new cells as an organism grows and to replace cells that get damaged. These stem cells are said to be multi-potent, which means they can only change into some cells in the body, not any cell, for example: Blood (or 'haematopoietic') stem cells can only replace the various types of cells in the blood, and skin (or 'epithelial') stem cells provide the different types of cells that make up our skin and hair. Induced pluri-potent stem cells (iPS cells) are stem cells that scientists make in the laboratory. The 'induced' means that they are made in the lab by taking normal adult cells, like skin or blood cells, and reprogramming them to become stem cells. Just like embryonic stem cells, they are pluripotent so they can develop into any cell type (Nishino et al., 2021).

## 5. New trends in 3D cell culture method

Recently, microfluidics and 3D cell culture technologies have merged, to increase the potential of *in vitro* modeling, in order to get even closer to Nature (Jensen and Teng 2020). The successful control of the cellular environment thanks to microfluidics in a 3D cell culture environment allows the study at the single-cell scale with mechanical control of cell shape, physicochemical properties, shear-stress, the rigidity of substrates, temperature and gas gradient. The perpetual increase of knowledge in histology, physiology and in technology, allow to drastically improve tools for repairing lesions or diseased organs of living organisms (Christian et al., 2021). Nowadays, 3D cell culture microfluidic devices are used in two main fields: as *in vitro* models for fundamental research, drug trial, toxicity assays / or in the cell therapy to manufacture tissue prior to implantation (Langer and Joensson, 2020). For instance regenerative medicine, which is still in the early stage of development, consists in repairing lesions or diseased organs by replacing the damaged parts. **(Fig. 5)**

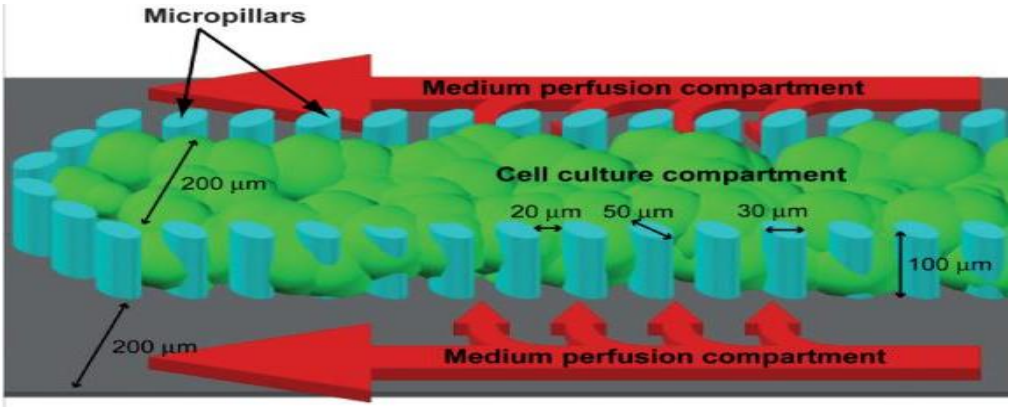


**Fig. 5: The 3D cell culture system using micropillars with perfusion medium.**

This approach follows the principle of cell therapy , and uses stem cells , undifferentiated or de-differentiated cells cultured *in vitro*, and transplant them *in vivo* (Mehrian, et al., 2020). The development of these technologies will have a huge impact on healthcare as a solution for many currently untreatable diseases : heart failure, kidney failure, genetic disorders, orphan diseases, and cancer. The 3D cell culture improve the *in vitro* model. *In vivo*, cells are surrounded by a well-designed extracellular matrix (ECM) structure. The ECM is composed of macromolecules secreted by the cells (*e.g.*, collagen, elastin, glycosaminoglycan, glycoprotein). Accordingly, There are four types of 3D cell culture method:

**1-** Hanging Drops, cells are floating in a medium droplet.
**2-** Hydrogels are made of polymerized hydrophilic polymers.
**3-** Paper-based scaffolds give cells a 3D cellulose architecture.
**4-** Fiber-based scaffolds are made of nano-fibers of different sizes. **(Fig. 6)**

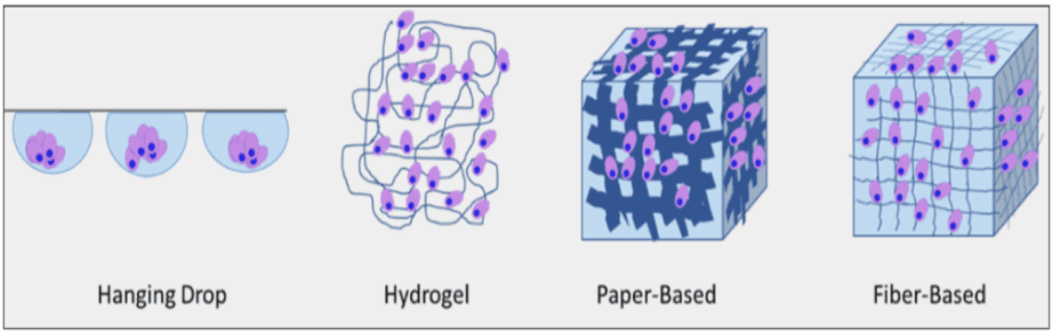


**Fig. 6: The four different types of 3D cell culture.**

Hydrogel solutions are hydrophilic networks of polymers and macromolecules of different composition, which under the right condition, can crosslink into a porous gel, with properties similar to one of the extra cellular matrix. These hydrogels are easy to produce and are often used as alternative to ECM to grow cells in a 3D configuration. Cells will grow and migrate inside the gel, and will be surrounded by small molecules increasing the surface exchanges. Hydrogels can be made of synthetic (Polyacrylamide and Polyethylene glycol) or natural polymers from the ECM (collagen, fibrin, hyaluronic acid), It's composition is adapted to the cell types studied, by tuning the substrate thickness and porosity. Paper-based scaffolds are a porous, biocompatible, stackable and foldable material onto which cells can adhere and grow. Paper sheets have been employed to generate complex 3D structures, and mimic the cell environments (Sebastian et al., 2022). The cellulose network provides a rigid architecture for cells to grow. These materials are very stable and can undergo several surface treatments: chemical treatment, plasma, or sterilization.

## CONCLUSION

It is widely recognized that cell-based therapies could revolutionize health care for a range of diseases. However, there are gaps in the overarching framework and technologies to generate clinical success. Recent developments are encouraging adoption of automation and quality engineering approaches for bio-processing of cell-based therapies. Regenerative medicine has the potential to heal or replace tissues and organs damaged by age, disease, or trauma, as well as to normalize congenital defects. Promising preclinical and clinical data to date support the possibility for treating both chronic diseases and critical cases, and for regenerative

medicine to abet maladies occurring across a wide array of organ systems and contexts, including dermal wounds, cardiovascular diseases and traumas, treatments for certain types of. The body's innate healing response may promotes regeneration process, although adult humans possess limited regenerative capacity.

## REFERENCES

1. Aijaz, A., Li, M., Smith, D., Khong, D., Leblon, C., Fenton, O 2018. Biomanufacturing for clinically advanced cell therapies. *Nat. Biomed. Eng.* 2, 362–376. doi: 10.1038/s41551-018-0246-6
2. Bacakova L, Zarubova J, Travnickova M, Musilkova J, Pajorova J, Slepicka P, Kasalkova NS, Svorcik V, Kolska Z, Motarjemi H, Molitor M 2018. Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review. Biotechnol Adv.; 36:1111–1126. [PubMed] [Google Scholar]
3. Bissels U, Diener Y, Eckardt D, Bosio A 2016. Characterization and classification of stem cells. In: Steinhoff G, editor. Regenerative Medicine – From Protocol to Patient. Cham, Switzerland: Springer International Publishing, 1-25. [Google Scholar]
4. Cao W, Guan L, Li X, Zhang R, Li L, Zhang S, Wang C, Xie X, Jiang Z, Wan D, Chi X 2021. Clinical analysis of bloodstream infections during agranulocytosis after allogeneic hematopoietic stem cell transplantation. Infect Drug Resist. 14:185–192. [PubMed] [Google Scholar]
5. Christian MG, Yujue W, Huiting X, Katarzyna K, Fredric E. Wand Hatem E. S 2021. Optimized 3D Culture of Hepatic Cells for Liver Organoid Metabolic Assays. *Cells*, 10 (12), 3280; https://doi.org/10.3390/cells10123280
6. Cierpka-Kmiec, K., Wronska, A., and Kmiec, Z 2019. In vitro generation of pancreatic β-cells for diabetes treatment. I. β-like cells derived from human pluripotent stem cells. *Folia Histochem. Cytobiol.* 57, 1–14. Google Scholar
7. Costariol, E., Rotondi, M., Amini, A., Hewitt, C., Nienow, A., Heathman, T., et al. 2019. Establishing the scalable manufacture of primary human T-cells in an automated stirred-tank bioreactor. *Biotechnol. Bioeng.* 116, 2488–2502. doi: 10.1002/bit.27088. PubMed Abstract | CrossRef Full Text | Google Scholar
8. Deinsberger J, Reisinger D, Weber B 2020. Global trends in clinical trials involving pluripotent stem cells: a systematic multi-database analysis. NPJ Regen Med;5:15. [PMC free article] [PubMed] [Google Scholar]
9. Emmerson E and Knox S 2018. Salivary gland stem cells: A review of development, regeneration and cancer. Genesis, 56 (5): e23211. [PMC free article] [PubMed] [Google Scholar]
10. Fričová D, Korchak JA, Zubair AC 2020. Challenges and translational considerations of mesenchymal stem/stromal cell therapy for Parkinson's disease. NPJ Regen Med. 5:20. [PMC free article] [PubMed] [Google Scholar]
11. He L, Zhong J, Gong Q, Cheng B, Kim SG, Ling J, et al. 2017. Regenerative endodontics by cell homing. Dent Clin North Am; 61:143–159. [PubMed] [Google Scholar]
12. Hunsberger J., Shupe, T., and Atala, A 2018. An industry-driven roadmap for manufacturing in regenerative medicine. *Stem Cells Transl. Med.* 7, 564–568. doi: 10.1002/sctm.18-0060
13. Jensen C, Teng Y 2020. Is it time to start transitioning from 2D to 3D cell culture? Front Mol Biosci 7:33. https://doi.org/10.3389/fmolb.2020.00033. CAS Article Google Scholar
14. Kumar R, Sharma A, Pattnaik AK, Varadwaj PK. 2010. Stem cells: An overview with respect to cardiovascular and renal disease. J Nat Sci Biol Med.;1:43–52. [Google Scholar]
15. Kumar S, Delgado, M., Mendez, V., and Joddar, B 2019. Applications of stem cells and bioprinting for potential treatment of diabetes. *World J. Stem Cells* 11, 13–32. Google Scholar
16. Langer K., and Joensson, H. N 2020. Rapid production and recovery of cell spheroids by automated droplet microfluidics. *SLAS Technol.* 25, 111–122. Google Scholar
17. Liu XY, Yang LP, Zhao L 2020. Stem cell therapy for Alzheimer's disease. World J *Stem Cells*.12:787–802. [PMC free article] [PubMed] [Google Scholar]
18. Mahla RS. 2016. Stem Cells Applications in Regenerative Medicine and Disease Therapeutics. Int J Cell Biol. 6940283. [PMC free article] [PubMed] [Google Scholar]
19. Mehrian, M., Lambrechts, T., Marechal, M., Luyten, F. P., Papantoniou, I., and Geris, L 2020. Predicting in vitro human mesenchymal stromal cell expansion based on individual donor characteristics using machine learning. *Cytotherapy* 22, 82–90. Google Scholar
20. Nishino K, Takasawa K, Okamura K, Arai Y, Sekiya A, Akutsu H, Umezawa A. 2021. Identification of an epigenetic signature in human induced pluripotent stem cells using a linear machine learning model. Hum Cell; 34: 99–110. [PMC free article] [PubMed] [Google Scholar]

21. Rodriguez-Hernandez 2014. Cell culture: history, development and prospects. Int J Curr Res Aca Rev 2(12):188–200. CAS Google Scholar
22. Rothmiller, S., Balszuweit, F., Menacher, G., Steinritz, D., Kehe, K., Thiermann, H., et al. 2020. Validation of automated pipetting systems for cell culture seeding, exposure and bio-analytical assays in sulfur mustard toxicology. *Toxicol. Lett.* 320, 80–86. Google Scholar
23. Sayali M, Garima Y, and Rajnish K 2022. Recent trends in stem cell-based therapies and applications of artificial intelligence in regenerative medicine. World J *Stem Cells.* 26; 13(6): 521–541. doi: 10.4252/wjsc.v13.i6.521.
24. Sebastian K, Roland S, Jürgen F, Joachim W, Joachim P, Wolf D, Cornelia K, and Dominik E 2022. Innovative Platform for the Advanced Online Monitoring of Three-Dimensional Cells and Tissue Cultures. *Cells*, 11 (3), 412; https://doi.org/10.3390/cells11030412
25. Venkata SV, Yoshio Y, Akira K, Masahiko I, Yusuke K, Masato N, Shigeto S, Keisuke H, and Masahiro S 2021. Clinical Applications of Cell-Scaffold Constructs for Bone Regeneration Therapy. *Cells*, 10 (10), 2687; https://doi.org/10.3390/cells10102687
26. Vitillo L, Durance C, Hewitt Z, Moore H, Smith A, Vallier L. 2020. GMP-grade neural progenitor derivation and differentiation from clinical-grade human embryonic stem cells. Stem Cell Res Ther;11:406. [PMC free article] [PubMed] [Google Scholar]
27. Weissman IL, Shizuru JA. 2008. The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. Blood 112, 3543–3553. ( 10.1182/blood-2008-08-078220) [PMC free article] [PubMed]

## *ORAL PRESENTATION*

# *Hypericum perforatum* L. (sarı kantaron) bitki ekstraktlarının *Fusarium oxysporum* üzerine antifungal etkisi

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## Özet

Bu çalışmada, önemli tıbbi ve aromatik bitki olan *Hypericum perforatum*' un *Fusarium oxysporum* üzerine antifungal aktivitesi incelenmiştir. *H. perforatum* çiçek, gövde ve yapraklarının bazı çözücülerdeki (metanol, etanol, aseton ve kloroform) ekstraktlarının %2'lik konsantrasyonu *F. oxysporum'*a karşı test edilmiştir. Ekstraktların antifungal aktiviteleri bu patojen ile mücadelede kullanılan ve sentetik fungusit olan Captan ile kıyaslanmıştır. Bitkinin gövde kısmının etanol ektraktı tüm örnekler içerisinde %52.3 ile en yüksek engelleme oranına sahip olmuş ve sentetik fungusitten (%19.0) daha yüksek aktivite sergilediği tespit edilmiştir. Genel olarak incelendiğinde, *F. oxysporum* patojeni üzerine her üç bitki kısmı için en etkili ekstraktın kloroformda çözünen ekstraktlarda olmasına rağmen, en düşük etki ise metanol ekstraktlarında kaydedilmiştir. Sonuç olarak, *H. perforatum* bitkisinin gövde kısımlarında *F. oxysporum* patojenine karşı etkili olan bileşik veya bileşiklerin olduğu, ayrıca metanol, aseton, ve kloroform çözücülerine kıyasla etanol çözücüsünde daha etkili formda çözündüğü tespit edilmiştir.

**Anahtar Kelimeler:** Antifungal, *Fusarium oxysporum*, *Hypericum perforatum*

## Antifungal effect of extracts of *Hypericum perforatum* L. (St. John's Wort) *on Fusarium oxysporum*

## Abstract

In this study, the antifungal activity of *Hypericum perforatum*, an important medicinal and aromatic plant, on *Fusarium oxysporum* was investigated. The 2% concentration of extracts in some solvents (methanol, ethanol, acetone and chloroform) from flowers, stems and leavesof *H. perforatum* was tested against *F. oxysporum.* The antifungal activities of these extracts were compared with Captan, a synthetic fungicide used to combat this pathogen. Among all samples, the ethanol extract of the stem part of the plant had the highest inhibition rate with 52.3% and showed higher activity than the synthetic fungicide (19.0%). Generally, for all plant parts on the *F. oxysporum* pathogen, although the strongest effect was in chloroform extracts, the lowest was recorded in methanol extracts. As a result, it was determined that there are compound or compounds that are effective against *F. oxysporum* pathogen in the stem parts of the *H. perforatum* plant, and also dissolve more effectively in ethanol solvent compared to methanol, acetone, and chloroform solvents.

**Keywords:** Antifungal, *Fusarium oxysporum*, *Hypericum perforatum*

## GİRİŞ

Bitkilerin iyileştirici etkileri çok eskilerden beri bilinmektedir (Walker ve ark., 2001). Son yıllarda bitkisel kaynaklı ürünler konusunda yapılan bilimsel çalışmalardan ortaya çıkan olumlu sonuçlar bu eğilimi desteklemektedir. *Hypericum perforatum* L. halk arasında sarı kantaron ve binbirdelik otu olarak bilinir. 30-100 cm boyunda, yapraklar 1-3.5 cm uzunlukta, oval elips şeklindedir. Yol kenarları, çayırlar ve tarla alanlarında 0-2500 m yükseklikte yetişir (Davis, 1967). Özellikle günümüzde ekstraktları Avrupa ve ABD'de depresyon tedavisinde bir ilaç olarak yaygın şekilde kullanılmaktadır (Duke, 1985). Ayrıca, bitki ekstraktının deri yaralanmalarının iyileşmesinde etkilidir (Baytop, 1999). Yapılan çalışmalarda sarı kantaron bitkisinin drogunda %0.1-0.3 oranında dianthron (hiperisin pseudohypericin ve hiperisine benzer maddeler), flavonoit, %3 hiperforin, %0.2-1 uçucu yağ ve tanenli maddelerin bulunduğu belirtilmektedir (Nahrstedt ve Butterweck, 1997).

Türkiye, dünyada yetiştiricilik yapılan ülkeler arasında önemli bir yere sahiptir. Yetiştiriciliği kısıtlayan ve önemli verim kayıplarına neden olan faktörler arasında ilk sıralarda yer alan, bitki patojeni fungal hastalık etmenleridir. Yetiştiricilik sırasında karşılaşılan toprak patojenlerinden *Fusarium oxysporum* bitkilerde kök ve kök boğazı çürüklüğü ve solgunluğa neden olmaktadırlar (Soylu ve ark., 2020).

*Fusarium oxysporum*, bir Ascomycota bölümü Nectriaceae familyasına ait bir fungal hastalıktır. *F. oxysporum* cinsi içindeki birçok tür, özellikle tarımsal alanlarda bitkiler için patojeniktir. Domates, patlıcan, muz, nohut, mercimek gibi birçok bitkide zarar oluşturmaktadır. *Fusarium oxysporum*, iletim demetlerinde solgunluğa yol açanfungal bir hastalıktır. İlk başta bitkideki yaprakların bir kısmı sararır ve solar. Sonuç olarak ise bitkinin tamamı ölür. İletim demetlerinde kahverengi renk bozulması olur ve gövde enine ya da boyuna kesildiğinde bu belirtiler görülebilir.

Çalışma kapsamında, tıbbi ve aromatik bitkilerden biri olan *Hypericum perforatum*' un *Fusarium oxysporum* üzerine antifungal aktivitesi incelenmiştir. Metanol, etanol, aseton ve kloroform çözücülerinde *H. perforatum* bitkisinin çiçek, gövde ve yaprak ekstraktlarının %2'lik konsantrasyonu *F. oxysporum'*a karşı test edilmiştir.

## MATERYAL VE METOT

### Bitki örnekleri

*H. perforatum* L. türü toprak üstü kısımları doğal ortamlarından %100 çiçeklenme döneminde, 30 birey olacak şekilde ( Çorum, Bayat 40º41'16N, 34º7'51E 1372 rakım) toplanmıştır. Bitkilerin çiçek, yaprak ve sap kısımlarını ayırdıktan sonra 20 ± 2 °C'de gölgede kurutulmuştur. Bitki materyalleri Prof. Dr. Osman Tugay (Selçuk Üniversitesi, Eczacılık Fakültesi, Farmasötik Botanik Bölümü) tarafından teşhis edilmiş ve KNYA herbaryumunda (Selçuk Üniversitesi, Fen Fakültesi, Konya, Türkiye) depolanmıştır (herbaryum numarası: 28283).

### Ekstraksiyon

Ekstraksiyon için *Hypericum* türünün toprak üstü kısımları (çiçek, yaprak ve gövde) kullanılmıştır. Bu kısımlar gölgede kurutulmuş ve bir karıştırıcı ile mekanik olarak öğütülmüştür. Her öğütülmüş bitki materyalinden 4 g örnek tartılmış,40 ml çözücü (% 100 metanol, etanol, aseton ve kloroform) içerisinde 40 °C'de 24 saat boyunca ayrı ayrı özümlenmiştir. Nihai çözeltiler, whatman kağıt ile süzülerek çözücüler 40 °C'de evaporator yardımıyla uzaklaştırılmıştır. Elde edilen ekstraktlardan methanollü ekstraktlar su, diğer çözücü ekstraktları ise aseton ile çözülerek stok solüsyonlar oluşturulmuştur.

### Hastalık etmeni

Çalışmada patojen olarak kullanılan *Fusarium oxysporum* Bozok Üniversitesi, Ziraat fakültesi, Bitki koruma bölümü laboratuvarından stok kültürlerden temin edilmiştir.

### Ekstrakların antifungal etkilerinin saptanması

*Hypericum*. türlerinin farklı kısımlarından farklı çözücülerle elde edilen ekstraktlarının %2'lik konsantrasyonu *F. oxysporum 'a* karşı kullanılmıştır. PDA besi ortamı otoklavda 121 °C'de 20 dakika sterilize edilmiştir. PDA besi ortamı her bir steril petriye (60 mm çap) yaklaşık 10 ml dökülmüştür. Öncedeki çalışmada yapılan metodoloji revize edilerek; önceden dozları ayarlanan ekstrakt solüsyonundan 100 µl alınarak PDA besin ortamına yayılmıştır. Önceden PDA besi yerlerinde geliştirilen patojen kültürlerinden birer disk (4 mm) alınarak ortamın tam ortasına bırakılmıştır. İnokulasyondan sonra petrilerin etrafı parafilm ile kapatılıp, 23±1°C'de inkübasyona bırakılmıştır. Negatif kontrol olarak ekstrakt çözücüleri olan su ve aseton çözücüleri ekstrakt yerine kullanılmıştır. Pozitif kontrol olarak Captan etkili maddeli ticari fungisit denemedeki dozda (%2) hazırlanarak aynı uygulama şekliyle patojenlere karşı denenmiştir.

### Deneme deseni ve istatistik analiz

Deneme 3 tekerrürlü, 2 tekrarlı olarak yürütülmüştür. Günlük olarak petriler kontrol edilerek kontrol petrilerinde koloni çapları petriyi kapladığı gün (5 gün) fungal koloni çapları ölçülerek kaydedilmiştir. Koloni çapının ölçümü fungus koloni çapının birbirine dik iki yönde ölçülmesi şeklinde yapılmıştır. Kontrole göre bitki ekstraktlarının % engelleme oranları aşağıdaki formül yarımıyla hesaplanmıştır.

E=K–M/Kx100

E= Engelleme (%)

K= Kontrolde koloni çapı (mm)

M= Muamelede koloni çapı (mm)

Denemelerde karakterler arasındaki farklılıkların önem dereceleri varyans analizi (ANOVA) ile belirlenmiş ve Duncan çoklu karşılaştırma testi ile ortalamalar kıyaslanmıştır.

## BULGULAR ve TARTIŞMA

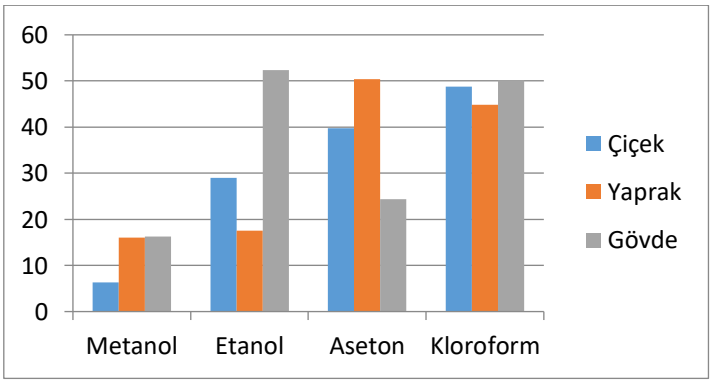
*F. oxysporum* patojeni üzerine en etkili ekstraktların (çiçek, gövde ve yaprak) kloroformda çözünen ekstraktlarda gözlenmiştir. En yüksek engelleme oranı, aseton yaprak ekstraktında %50, kloroform ve etanol gövde ekstraktlarında ise sırasıyla %50 ve %52 belirlenmiştir.

**Tablo 1**. Bitki patojeni *Fusarium oxysporum* türü üzerine *Hypericum perforatum* ekstraktlarının etkisi

| **Bitki Kısımları** | **Çözücüler (misel çapı (cm))** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Metanol | | | Etanol | | | Aseton | | | Kloroform | | |
| **Çiçek** | 5,63±0,22 | b | A | 4,26±0,19 | b | B | 2,98±0,15 | c | C | 3,08±0,22 | b | C |
| **Yaprak** | 5,03±0,15 | b | A | 4,95±0,30 | b | A | 4,04±0,46 | b | B | 3,31±0,24 | b | B |
| **Gövde** | 5,02±0,37 | ab | A | 2,86±0,32 | c | B | 3,40±0,36 | bc | B | 2,99±0,13 | b | B |
| **Kontrol** | 6,00±0,00 | b | | 6,00±0,00 | a | | 6,00±0,00 | a | | 6,00±0,00 | a | |
| **%2 Captan** | 4.3±0.07 | | | | | | | | | | | |

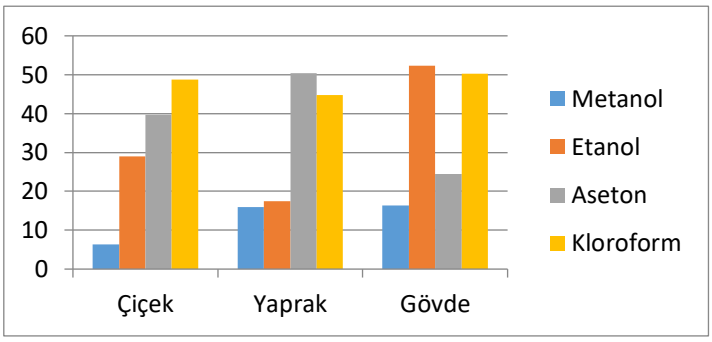
*Aynı sütunda farklı küçük harflerle ve aynı satırda büyük harfle gösterilen veriler arasında Duncan testi sonuçlarına göre 0.05 düzeyinde farklılık görülmüştür.

*Hypericum perforatum* bitkisinin *F. oxysporum* patojenine karşı etkisi incelendiğinde en yüksek misel çapı kontrole uygulaması ile istatistiki olarak aynı grupta olan 5.63±0.22 cm ile metanol çiçek ekstraktı olmuştur. En küçük misel çapına ise 2,86±0,32cm ile etanol gövde ekstraktı sahip olmuştur (Tablo 1).




**Şekil 1.** *Hypericum perforatum* bitki parçalarının çözücüler üzerine etkisi

Şekil 1 incelendiğinde *H. perforatum* bitkisinin *F. oxysporum* patojenine karşı metanol ekstraktları hariç tüm ekstraktların yüksek % engelleme aktivitesi sergilediği tespit edilmiştir. En yüksek % engelleme aktivitesi etanol ve kloroformlu gövde ekstraktları sahip (sırayla %52 ve %50) olmuştur.




**Şekil 2.** Çözücülerin *Hypericum perforatum* bitki parçaları üzerine etkisi

Şekil 1 incelendiğinde *H. perforatum* bitkisinin *F. oxysporum* patojenine karşı kloroform ekstraktları tüm bitki parçalarında yüksek % engelleme aktivitesi sergilediği tespit edilmiştir. Methanol ekstraktları ise en düşük çözücü etkisi sergilemiştir.

Çalışmada *H. perforatum* bitkisinin farklı kısımlarından metanol, etanol, aseton ve kloroform çözücülerinde elde edilen ekstraktlarının %2'lik konsantrasyonu *F. oxysporum'a* karşı kullanılmıştır. Genel olarak, *H. perforatum* türleri bu çalışmadaki fungal patojen olan *F. oxysporum'a* karşı en iyi çözücü kloroform ve en etkili bitki kısmı ise sırasıyla; gövde, yaprak ve çiçek olarak gözlenmiştir.

Daha önce farklı araştırıcıların yapmış olduğu çalışmalarda *Hypericum* spp. türlerinin ekstraktlarının bazı bitki fungal hastalıklarına karşı etkili olduğu görülmüştür. Kırbağ ve ark. (2005) *Hypericum scabrum* ekstraktını ve Bahraminejad (2012) ise hem *Hypericum scabrum* hem de *Hypericum perforatum* ekstraktlarını birçok fungal etmene karşı denemişler ve başarılı sonuçlar elde etmişleridir. *Alternaria alternata, Colletotrichum graminicola, Phytophthora sp., Rhizoctonia solani ve Sclerotinia rolfsii, Fusarium moniliforme, Rhizoctonia solani, Sclerotinia sclerotiorum ve Phytophthora capsici, Fusarium oxysporum* gibi bir çok fungal patojene karşı bitki ekstraktlarını kullanmışlar ve önemli derecede antifungal etkisi olduğunu belirtmektedirler (Çakır ve Yeğen 1991, Türküsay ve Onoğur 1998, Yanar ve ark. 2001, Bonventure, 2009, Basım ve Basım 2013, Erdoğan ve ark. 2014).

## SONUÇ

*Hypericum perforatum* bitkisinin gövde kısımlarında *F. oxysporum* patojenine karşı etkili olan bileşik veya bileşiklerin olduğu ve aseton, etanol, kloroform çözücülerin tarafından daha etkili formda çözündüğü tespit edilmiştir. İleriki çalışmalarda, *H. perforatum* bitki kısımlarındaki fitokimyasallarının ayrıntılı olarak incelenmesi ve bu patojene karşı etkili olabilecek bileşik veya bileşik gruplarının belirlenmesi konunun aydınlatılmasında daha etkili olacaktır.

## TEŞEKKÜR

Çalışma Yozgat Bozok Üniversitesi Bilimsel Araştırma Projeleri (BAP) tarafından 6602C-ZF/17-137 proje kodu ile desteklenmiştir. Yozgat Bozok Üniversitesi Bilimsel Araştırma Projeleri birimine katkılarından dolayı teşekkür ederiz.

## KAYNAKLAR

Bahraminejad S 2012. In vitro and In vivo antifungal activities of Iranian plant species against Pythium aphanidermatum, Annals of Biological Research, 3 (5): 2134-2143.

Basım E, Basım H 2013. Chemical composition, antibacterial and antifungal activities of turpentine oil of Pinus slyvestris L. against plant bacterial and fungal pathogens. Journal of Food, Agriculture & Environment, 11 (3-4): 2261-2264.

Baytop T 1999. Türkiye'de Bitkiler ile Tedavi (Geçmişte ve Bugün). İstanbul Üniversitesi Eczacılık Fakültesi, İstanbul, s.166-167.

Bonventure O, Wagai S and Odhiambo G 2009. Fungıtoxic Properties of Four Crude Plant Extracts on Fusarium oxysporum Schl. f.sp phaseoli, African Journal of Food Agricultural and Nutritional Development, 9(8): 1652-1666.

Çakır C, ve Yeğen O 1991. Antalya ve Çevresindeki Bazı Bitkilerin ve Uçucu Yağlarının Fungitoksik Potansiyellerinin Araştırılması. VI. Türkiye Fitopatoloji Kongresi, 213-218.

Davis PH 1967. Flora of Turkey and the East Aegean Islands. Edinburgh University Press. 2:355-401.

Duke JA 1985. Handbookof Medicinal Herbs. CRC, Boca Raton, Florida, p. 242.

Erdoğan O, Çelik A, Yıldız Ş, Kökten K 2014. Pamukta Fide Kök Çürüklüğü Etmenlerine Karşı Bazı Bitki Ekstrakt ve Uçucu Yağlarının Antifungal Etkisi. Türk Tarım ve Doğa Bilimleri Dergisi,1(3), 398-404.

Kırbağ S, Kurşat M, ve Kırbağ Zengin F 2005. Elazığ'da Tıbbi Amaçlar İçin Kullanılan Bazı Bitki Ekstraktlarının Antimikrobiyal Aktiviteleri. Doğu Anadolu Bölgesi Araştırmaları.

Nahrstedt A ve Butterweck V 1997. Biologicially Active and Other Chemical Constituents of the Herb from Hypericum perforatum L. Pharmacopsychiatry, 30:p.129-134.

Soylu EM, Soylu S, Kara M, Kurt Ş 2020. Sebzelerde Sorun Olan Önemli Bitki Fungal Hastalık Etmenlerine Karşı Vermikomposttan İzole Edilen Mikrobiyomların in vitro Antagonistik Etkilerinin Belirlenmesi. KSÜ Tarım ve Doğa Derg 23(1): 7-18, 2020.

Türküsay H, ve Onoğur E 1998. Bazı Bitki Ekstraktlarının In Vitro Antifungal Etkileri Üzerine Arastırmalar, Tr. J. of Agriculture and Forestry, 22: 267-271.

Walker L, Sirvent T, Gibson D and Vance N 2001. Regional Differences in Hypericin and Pseudohypericin Concentrations and Five Morphological Traits Among Hypericum perforatum Plants in the Northwestern United States. Canadian Journal of Botany, 79 (10): p.1248-1.

Yanar Y, Kadıoğlu İ, Kutluk ND, Çeşmeli İ, Hangün A 2001. Bazı bitki ekstraktlarının farklı bitki patojeni funguslara karşı antifungal etkilerinin belirlenmesi, Türkiye Herboloji Dergisi, 4(1): 58-63.

## *ORAL PRESENTATION*

# Antidiabetic activities of *Rubus tereticaulis* P. J. Müll. and its main compounds against α-amylase and α-glucosidase

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## Abstract

In the current study, our aim is to evaluate the inhibitory effects of various fractions (hexane, chloroform, ethyl acetate, and aqueous ethanol) obtained from *Rubus tereticaulis* leaves ethanol extract and the major compounds, kaempferol-3-*O*-β-D-glucuronide (MC1) and quercetin-3-*O*-β-D-glucuronide (MC2), previously isolated from its active fraction (ethyl acetate) against α-glucosidase and α-amylase enzymes based on the traditional uses of *Rubus* species for antidiabetic purposes. Also, the major compounds were compared with each other in terms of Structure Activity Relationship (SAR). While *R. tereticaulis* ethyl acetate fraction (RTEA) with $IC_{50}$ values of 28,32 and 52,53 µg/mL showed the best inhibitory activity against α-amylase and α-glucosidase enzymes, *R. tereticaulis* hexane fraction (RTH) exhibited the lowest activity with $IC_{50}$ values of 39,31 and 1031,00 µg/mL against these enzymes, respectively. The major compounds of RTEA, MC1 and MC2 with $IC_{50}$ values of 12,35 and 11,68 µg/mL were found to have a strong inhibitory effect against α-amylase enzyme compared to standard acarbose with an $IC_{50}$ values of 23,91 µg/mL. It has been suggested that the hydroxyl group at C3′ and C4′ on the B ring may potentiate the inhibitory activity of flavonoids against alpha amylase enzyme. This may explain why MC2 showed slightly better activity than MC1 in the present study. Also, MC1 and MC2 exhibited moderate inhibitory activity against α-glucosidase enzyme with $IC_{50}$ values of 106,70 and 132,60 µg/mL as compared to standard acarbose with an $IC_{50}$ values of 61,01 µg/mL. This study is first report on α-glucosidase (except MC1) and α-amylase inhibitory activity of *Rubus tereticaulis* leaves, MC1 and MC2. After all, these results suggest that major compounds may potentially be responsible for the antidiabetic effect of the RTEA against α-amylase enzyme. In addition, this study confirms the ethnobotanical use of the plant for the treatment of diabetes.

**Keywords:** *Rubus tereticaulis*, ethyl acetate fraction, major flavonoids, antidiabetic activity

## INTRODUCTION

Diabetes mellitus, a chronic disease, is characterized by high blood glucose level (hyperglycemia) as a result of irregularities in insulin level (Lawag et al., 2012).

According to the International Diabetes Federation (IDF), the worldwide prevalence of type II diabetes is expected to increase from 8.8% in 2015 to 10.4% in 2040. At the same time, IDF reported that the prevalence of type II diabetes in Turkey was 12.8% in 2015, and with this value, it was seen that Turkey has the third leading prevalence in the European region (Bulucu-Büyüksoy et al., 2020**).**

Deficiency in insulin secretion causes blood glucose levels to rise, resulting in serious damage to body systems such as blood vessels and nerves. Reducing postprandial hyperglycemia by delaying glucose absorption through inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase is one of the therapeutic approaches (Wang et al., 2010) Although antihyperglycemic drugs such as acarbose and miglitol, which are currently in use, can inhibit the activities of α-amylase and α-glucosidase enzymes, they have been reported to have some side effects such as diarrhea, flatulence, and liver disease (Wang et al., 2022). Therefore, extraction or isolation of α-glucosidase and α-amylase inhibitors from medicinal plants used in traditional folk medicine for the treatment of diabetes is important for the discovery and development of new drugs with fewer adverse effects against glucose enzymes.

The genus *Rubus*, a member of the Rosaceae family, is represented by naturally grown 16 taxa in Turkey (Bizim Bitkiler, 2022). Different parts of *Rubus* species (fruit, leaves and roots) are used in the treatment of many different diseases in traditional medicine, as well as in the treatment of diabetes (Tuzlacı, 2016; Kültür, 2007; Küpeli Akkol et al., 2011; Akalın, 1993). Phytochemical analyses of *Rubus* species have indicated the

presence of flavonoids (quercetin, kaempferol, etc.), phenolic acids (caffeic acid, chlorogenic acid, etc.), anthocyanins, terpenoids and steroids (Gudej and Tomczyk, 2004; Rocabado e al., 2008).

The present study aims to identify the potential inhibitory effects of various fractions (hexane, chloroform, ethyl acetate, and aqueous ethanol) obtained from *Rubus tereticaulis* leaves ethanol extract and the major compounds, kaempferol-3-*O*-β-D-glucuronide (MC1) and quercetin-3-*O*-β-D-glucuronide (MC2), previously isolated from its active fraction (ethyl acetate) against α-glucosidase and α-amylase enzymes.

## MATERIALS AND METHODS

### Plant material

Leaves of *Rubus tereticaulis* were collected in the flowering period from Sile district of Istanbul province of Turkey on June 15, 2016 and identified by Dr. Ahmet Dogan. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE No: 18573).

### Extraction and Isolation

Dried and ground leaves of *Rubus tereticaulis* (10 g) for *in vitro* activities were extracted with 96% EtOH (3×100 mL) at room temperature, using maceration methods. After filtration and evaporation, the obtained ethanol extract was dissolved in 50 mL of 50% aqueous ethanol and subjected to solvent–solvent partition between *n*-hexane (3×50 mL), chloroform (3×50 mL) and ethyl acetate (3×50 mL). The obtained *n*-hexane, chloroform, ethyl acetate and aqueous ethanol fractions were coded as RTH, RTC, RTEA and RTAE, respectively. Also, detailed information on the isolation of major compounds (kaempferol-3-O-β-D-glucuronide [MC1] and quercetin-3-O-β-D-glucuronide [MC2]) from *Rubus tereticaulis* leaves ethanol extract was reported in our previous study (Figure 1) (Şen et al., 2022).

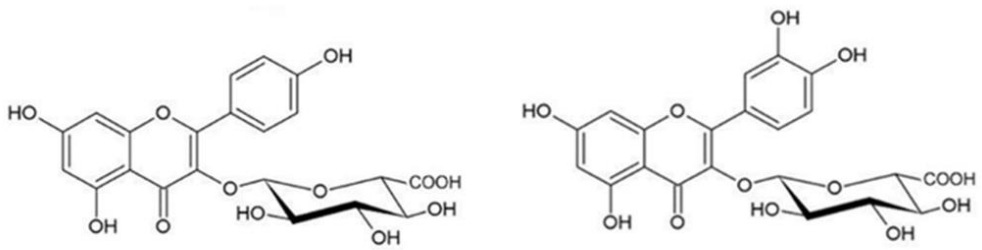


**Figure 1.** Chemical structures of major compounds isolated from *R. tereticaulis*

### Antidiabetic activity

#### *α-amylase inhibitory activity*

The α-amylase inhibitor activity was evaluated with slightly modified method of Ramakrishna et al. (2017). The method was adapted to a 96-well microplate format. 10 µl of sample, 15 µl of 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M NaCl) and 25 µl of porcine-amylase (0.5 mg/ml-15 units) prepared in buffer were mixed. The mixture was incubated at 25 ° C for 10 minutes. Then, 25 µl of a 1% starch solution prepared in buffer was added to each well. The mixtures were again incubated at 25°C for 10 minutes. The reaction was stopped with 50 µL of dinitrosalicylic acid (DNSA) and incubated in boiling water bath for 10 minutes. The solutions were cooled to room temperature and then were read absorbance at 540 nm diluting with 225 µL of ultra pure water. Acarbose was used as standard. The percentage inhibitory activity of the samples and standard against α-amylase enzyme were calculated according to the following:

$$\alpha\text{-amylase inhibitor activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where $A_0$ is the absorbance of the control (containing all reagents except the test compounds), and $A_1$ is the absorbance of the sample/standard. Sample or standard concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotting inhibition percentage against sample or standard concentration. Tests were carried out in triplicate.

### *α-glucosidase inhibitory activity*

10 µl of sample was mixed with 40 µl of 0.1 M sodium phosphate buffer (pH 6.9), and 100 µl of α-glucosidase (obtained from *Saccharomyces cerevisiae*) prepared in buffer. The mixtures were incubated at 25 ° C for 10 minutes. Thereafter, 50 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG) prepared in buffer to the solutions was added. The mixtures were re-incubated at 25°C for 5 minutes, and their absorbance was recorded by reading in the microplate reader before and after incubation at 405 nm. Acarbose was used as standard.
The percent inhibitory activity of samples and standard against α-glucosidase enzyme were calculated according to the following:

α-glucosidase inhibitor activity (%) = $[(A_0–A_1)/A_0]\times100$

where $A_0$ is the absorbance of the control (containing all reagents except the test compounds), and $A_1$ is the absorbance of the oil/standard. Sample or standard concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotting inhibition percentage against sample or standard concentration. Tests were carried out in triplicate (Ramakrishna et al., 2017).

### Statistical analysis

The data were given as means ± standard deviations and statistically analysed by analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using the GraphPad Prism 5. Differences between means at $p<0.05$ levels were considered significant.

## RESULTS and DISCUSSION

This is first study on *in vitro* antidiabetic activity of various fractions obtained from *Rubus tereticaulis* leaves ethanol extract. In the current study, the antidiabetic activities of the samples were investigated by two methods, α-amylase and α-glucosidase inhibition methods.

Among the fractions tested, RTEA had the best α-amylase inhibitory activity followed by RTAE, RTC and RTH ($p < 0.05$, Figure 1, Table 1). Although the activity of the fractions was lower than the standard acarbose, the activity of the major compounds (MC1 and MC2) obtained from RTEA was higher than the standard ($p < 0.05$, Figure 1, Table 1). In the α-glucosidase inhibition test, RTEA showed the strongest activity among all tested fractions, followed by RTAE, RTC and RTH, respectively ($p < 0.05$, Figure 2, Table 1). In addition, it was revealed that the RTEA had a better inhibitory activity when compared with standard acarbose. It is also seen that the samples have inhibitory activity on amylase rather than α-glucosidase.

**Table 1.** Antidiabetic activity of fractions and major compounds of *R. tereticaulis* ethanol extract

| Samples/standard | Antidiabetic activity ($IC_{50}$, µg/mL) | |
|---|---|---|
| | α-amylase inhibitory activity | *α*-glucosidase inhibitory activity |
| RTH | $39,31 \pm 1,05^{e}$ | $1031,00 \pm 21,92^{g}$ |
| RTC | $36,73 \pm 0,21^{de}$ | $254,80 \pm 2,76^{f}$ |
| RTEA | $28,32 \pm 1,92^{c}$ | $52,53 \pm 1,23^{a}$ |
| RTAE | $35,72 \pm 2,66^{d}$ | $98,47 \pm 1,44^{c}$ |
| MC1 | $12,35 \pm 0,02^{a}$ | $106,70 \pm 7,68^{d}$ |
| MC2 | $11,68 \pm 0,01^{a}$ | $132,60 \pm 9,86^{e}$ |
| Acarbose | $23,91 \pm 0,09^{b}$ | $61,01 \pm 0,93^{b}$ |

* The n-hexane, chloroform, ethyl acetate and aqueous ethanol fractions obtained by liquid–liquid extraction of *R. tereticaulis* ethanol extract were coded as RTH, RTC, RTEA and RTAE, respectively. Also, major compounds of *R. tereticaulis*, kaempferol-3-O-β-D-glucuronide and quercetin-3-O-β-D-glucuronide were coded as MC1 and MC2, respectively.
** Each value in the table is represented as mean ± SD (n = 3). Different letter superscripts in the same column indicate significant differences ($p < 0.05$).

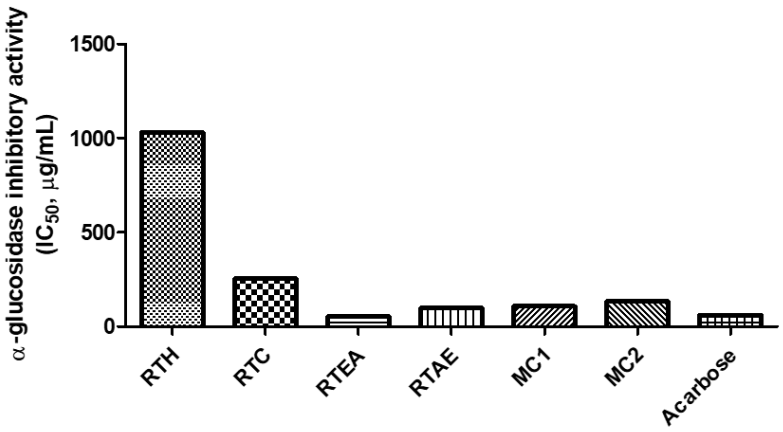


**Figure 1.** α-glucosidase inhibitory activity of major compounds, fractions and etanol extract of *R. tereticaulis*

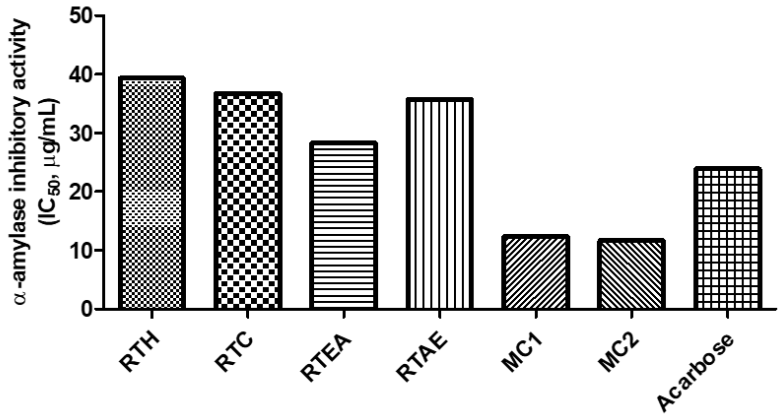


**Figure 2.** α-amylase inhibitory activity of major compounds, fractions and etanol extract of *R. tereticaulis*

Although there are no studies on the α-amylase and α-glucosidase inhibitory activity of *Rubus tereticaulis* extracts, previous studies have been reported on extracts from different *Rubus* species. In one of these reports, Grochowski et al. (2016) observed that diethyl ether and ethyl acetate fractions of *Rubus caesius* leaves exhibited inhibitory activity against α-amylase and α-glucosidase enzymes (Grochowski,et al., 2019). In another study, Zengin et al. (2019) investigated antidiabetic activity of *Rubus sanctus* and *Rubus ibericus* leaves and reported that extracts of *R. ibericus* (ethyl acetate) and *Rubus sanctus* (water and ethyl acetate) against α-amylase and α-glucosidase enzymes showed significant anti-diabetic activity (Zengin et al., 2019). In these studies, it is seen that the active fraction of *Rubus* species is generally the ethyl acetate fraction, which supports the results of our current study.

Also, it has been suggested that the hydroxyl group at C3′ and C4′ on the B ring may potentiate the inhibitory activity of flavonoids against alpha amylase enzyme. This may explain why MC2 showed slightly better activity than MC1 in the present study (Zhao et al., 2021). It has been reported that –OH groups at the 5 position of the A ring and the 3'- and/or 4'-positions of the B ring in flavonoids contribute significantly to the alpha glucosidase inhibitory activity (Proença et al., 2017; Alagesan et al., 2012). The activities of MC-1 and MC2 may also be due to these OH groups.

## CONCLUSION

These results showed that RTEA had significant antidiabetic activity against α-amylase and α-glucosidase enzymes. However, further studies, such as *in vivo* experiments are needed to fully evaluate the efficacy of the extract. Also, these results suggest that major compounds may potentially be responsible for the antidiabetic effect of the RTEA against α-amylase enzyme.

## ACKNOWLEDGEMENTS

The author would like to thank Assist. Prof. Ahmet Doğan for his help in collection and identification of the plant material.

## REFERENCES

Akalın E 1993. Tekirdağ İlinde İlaç ve Gıda Olarak kullanılan yabani bitkiler (Wild plants used as medicines and foods in Tekirdağ province), Master thesis, Health Sciences Institute, İstanbul University, İstanbul, 1993 (In Turkish).

Alagesan K, Thennarasu P, Kumar V, Sankarnarayanan S, Balsamy T 2012. Identification of α-glucosidase inhibitors from *Psidium guajava* leaves and *Syzygium cumini* Linn. seeds. International Journal of Pharma Sciences and Research, 3(2): 316-322.

Bizim bitkiler 2022. https://www.bizimbitkiler.org.tr/v2/hiyerarsi.php?c=Rubus (Erişim Tarihi: 14.11.2022).

Bulucu-Büyüksoy GD, Karataş N 2020. The effect of care provided at home by public health nurse on control of type II diabetes mellitus. Florence Nightingale Journal of Nursing, 28(3): 287-298.

Grochowski DM, Uysal S, Zengin G, Tomczyk M 2019. *In vitro* antioxidant and enzyme inhibitory properties of *Rubus caesius* L. International Journal of Environmental Health Research, 29(3): 237-245.

Gudej J, Tomczyk M 2004. Determination of flavonoids, tannins and ellagic acid in leaves from *Rubus* L. species. Archives of Pharmacal Research, 27: 1114-1119.

Kültür S, 2007. Medicinal plants used in Kirklareli province (Turkey). Journal of Ethnopharmacology, 111: 341-364.

Lawag IL, Aguinaldo AM, Naheed S, Mosihuzzaman M 2012. α-glucosidase inhibitory activity of selected Philippine plants. Journal of Ethnopharmacology, 144(1): 217-219.

Proença C, Freitas M, Ribeiro D, Oliveira EFT, Sousa JLC, Tomé SM, Ramos MJ, Silva AMS, Fernandes PA, Fernandes E 2017. α-glucosidase inhibition by flavonoids: an in vitro and in silico structure-activity relationship study. Journal of Enzyme Inhibition and Medicinal Chemistry, 32(1): 1216-1228.

Ramakrishna R, Sarkar D, Schwarz P, Shetty K 2017. Phenolic linked anti-hyperglycemic bioactives of barley (*Hordeum vulgare* L.) cultivars as nutraceuticals targeting type 2 diabetes. Industrial Crops and Products, 107: 509-517.

Rocabado GO, Bedoya LM, Abad, MJ, Bermejo P 2008. *Rubus*-A review of its phytochemical and pharmacological profile. Natural Product Communications, 3(3): 423-436.

Süntar I, Koca U, Keleş H, Küpeli Akkol E 2011. Wound healing activity of *Rubus sanctus* Schreber (Rosaceae): Preclinical study in animal models. Evidence-Based Complementary and Alternative Medicine, 2011, 816156.

Şen A, Özbeyli D, Teralı K, Göger F, Yıldırım A, Ertaş B, Doğan A, Bitiş L, Şener G 2022. Protective effects of *Rubus tereticaulis* leaves ethanol extract on rats with ulcerative colitis and bio-guided isolation of its active compounds: A combined in silico, in vitro and in vivo study. Chemico-Biological Interactions, 110263.

Tuzlacı E 2016. Türkiye bitkileri geleneksel ilaç rehberi (Turkey's plants traditional medicine guide), İstanbul: İstanbul Tıp Kitabevleri (In Turkish).

Wang H, Du YJ, Song HC 2010. α-glucosidase and α-amylase inhibitory activities of Guava leaves. Food Chemistry, 123(1): 6-13.

Wang S, Li Y, Huang D, Chen S, Xia Y, Zhu S 2022. The inhibitory mechanism of chlorogenic acid and its acylated derivatives on α-amylase and α-glucosidase. Food Chemistry, 372, 131334.

Zengin G, Ferrante C, Senkardes I, Gevrenova R, Zheleva-Dimitrova D, Menghini L, Orlando G, Recinella L, Chiavaroli A, Leone S, Brunetti L, Picot-Allain CMN, Rengasamy KRR., Mahomoodally MF 2019. Multidirectional biological investigation and phytochemical profile of *Rubus sanctus* and *Rubus ibericus*. Food and Chemical Toxicology, 127: 237-250.

Zhao Y, Wang M, Huang G 2021. Structure-activity relationship and interaction mechanism of nine structurally similar flavonoids and α-amylase. Journal of Functional Foods, 86: 104739.

## *ORAL PRESENTATION*

# Determination of in vitro immunostimulatory effects of vaccine formulations developed against visceral leishmaniasis

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## Abstract

Leishmaniasis is one of the most important public health problems in the world and in our country. According to the latest assessments of the World Health Organization, the disease is endemic in approximately 98 countries, including Turkey. While 1.5 million people worldwide suffer from cutaneous leishmaniasis, one of the clinical forms of the disease, approximately 500 thousand people suffer from visceral leishmaniasis, which is the lethal form of the disease. The fact that an effective and safe vaccine formulation suitable for use on humans has not been developed, causes the disease threat to continue. In this study, it was aimed to formulate an effective vaccine candidate for visceral leishmaniasis.
Soluble leishmania antigens (SLA) obtained from *L. infantum* parasites, which is a visceral leishmaniasis agent, by freeze-thaw and sonicator digestion methods were prepared at different rates and formulated with alum and saponin adjuvants. The immunostimulatory activity of the prepared vaccine formulations was determined in vitro on the murine J774 macrophage cell line. Nitric oxide (NO) and cell viability analyzes were performed in the study. According to the data obtained from the study, it was determined that the vaccine formulations developed with alum did not cause toxic effects for the host cell, the immunostimulatory activity increased with increasing concentration, and the highest value was obtained at 200 µg/ml concentration. In the alum formulation of SLA obtained by freeze-thaw method, cell viability rates varied between 99% and 96%, and the nitric oxide (NO) value was 6.22 nmol/mL with a 27% increase at 200 µg/ml concentration compared to the control group. ml was determined. It was determined that the NO value at 200 µg/ml concentration was 7.171 nmol/ml with an increase of 43% compared to the control group, and the cytotoxicity was 99% in the combinations of SLA and alum obtained by sonicating. In formulations where saponin is used as an adjuvant, it is clearly seen that the value of cell viability at 200 µg/ml concentration is 23% and 22%, thus negatively affecting the cell.
As a result, it can be said that vaccine formulations developed with SLA and alum obtained by sonicator degradation method can be used as potential vaccine candidates for visceral leishmaniasis. It is recommended to conduct experimental studies on experimental animals as soon as possible to examine the in vivo antileishmanial performance of the developed formulations.

**Keywords:** Visceral leishmaniasis, *L. infantum*, Adjuvant, Leishmaniasis vaccine

## INTRODUCTION

Leishmaniasis is a parasitic disease that is endemic in approximately 98 countries including Turkey (Brag et al.,2014). It has different clinical forms, such as Cutaneous Leishmaniasis (CL), which causes deep lesions on the skin, and Visceral Leishmaniasis (VL), which causes death if left untreated. VL, one of the notifiable communicable diseases, poses a serious threat to public health worldwide. It is believed that in the fight against leishmaniasis, vaccines can be a much more economical and safer option than treatment methods that usually use chemotherapeutic drugs. It is known that there is no effective and safe antileishmanial vaccine developed against human VL to date (Abdellahi et al.,2022). Although many vaccines against Leishmaniasis have been formulated in the last few decades, none of the strategies developed have provided full protection. It is desired that the vaccines to be developed for leishmaniasis, like other infections, should be reliable, stable, long-term protection, low cost, and easy to manufacture (Allahverdiyev et al.,2010). Development of an effective vaccine depends not only on the selection of suitable vaccine candidates, but also on the selection of the right adjuvant

or administration tool (Iborra et al.,2018; Reis et al.,2010) When the literature is examined, it is seen that adjuvants such as Freund's, alum, saponin, monophosphoryl lipid A, nanoparticles have been investigated in vaccine formulations developed against VL (Askarizadeh et al.,2020; Abamor et al.,2017; Badiee et al.,2013).

For this purpose, in our study, as a vaccine candidate against VL, the immunostimulatory activities of soluble Leishmania antigens prepared by us with different methods were evaluated under in vitro conditions by combining them with different adjuvants.

## MATERIALS AND METHODS

### *L. infantum* culture

The VL agent *L. infantum*, which was present in the laboratory cryobank, was incubated in RPMI 1640 medium in a $27^0$C cooled oven. 7 ml of medium was added to 25 $cm^2$ flasks and parasites were inoculated from the culture at a rate of $3x10^5$ parasites/ml. The growth of promastigotes was monitored daily using an inverted microscope. When the number of parasites/ml reached $10^7$ in a 25 $cm^2$ flask, *L. infantum* promastigotes were transferred to a 75 cm2 flask and incubated for 48 hours by adding 30 ml of RPMI 1640 medium with 5% FBS. After incubation, it was centrifuged at 4000 rpm at + 4°C for 25 minutes, and the supernatant was discarded and the pellet was washed with 1 ml of PBS. The supernatant was removed by centrifugation again at +4°C at 4000 rpm for 15 minutes. The resulting pellet was brought to -20°C for later use.

### Soluble leishmania antigen by freeze-thawing

Freeze-thaw method was used for the preparation of soluble Leishmania antigens (CLA). In the freeze-thaw method, parasites removed from -20 °C were dissolved in a water bath at 37 °C by adding 1 ml of PBS in a 15 ml falcon. The thawed cells were then placed in a liquid nitrogen container to be covered with liquid nitrogen, and the parasites were allowed to freeze for 15 minutes. Frozen parasites were kept in a 37 °C water bath for 15 minutes and thawed. The parasites suspended in PBS in 15mL falcons were frozen with liquid nitrogen and thawed in a water bath 5 times, then centrifuged at 10,000 rpm for 3 minutes and the supernatant was taken. Quantification of protein in the lysate was performed using the Warburg-Christian method in UV spectrometry at 280 and 260 nm wavelengths.

### Soluble leishmania antigen by sonication

Parasite cells in a 15 ml falcon, which was raised to -20 ˚C, were vortexed. It was centrifuged at 1000 rpm for 5 min. After centrifugation, the supernatant was discarded. Cells were suspended with 1 ml of PBS. A 15 ml falcon was connected to the sonicator probe. Falkon was placed in a flask filled with icy water and surrounded by ice. The sonicator was set for 40 seconds and 3 cycles. The power potentiometer of the sonicator generator was set to 40%. Sonication was repeated 5 times. After sonication, the falcon was centrifuged at 10,000 rpm for 3 minutes. After centrifugation, the supernatant in the falcon was taken and the amount of protein in the antigen was determined and the measurements were taken at 260 and 280 nm in the spectrophotometer. The measured lysate was stored at -20 ˚C.

### J774 macrophage cell culture

The J774 mouse macrophage cell line was used to detect immunostimulatory activity. Cells were cultured in 75$cm^2$ (Polystyrene surface, NEST) flasks using appropriate media. Stock media were prepared by adding 1% penicillin-streptomycin and 1% L-glutamine. Growth and proliferation of cells were carried out in RPMI-1640 medium containing 10% FBS. The passage numbers of the cell lines used in the study are between 10 and 15. During the experimental period, the cells were kept at 37°C, 95% humidity and 5% $CO_2$ incubation conditions required for culture. Cell growth was monitored daily with an inverted microscope. Cells were seeded at $1x10^5$ cells/mL per well in 96-well plates and incubated for 24 hours.

### Measuring nitric oxide productions

The amount of nitric oxide (NO) produced by the cells was determined by the Griess method to determine the immunostimulatory activity of different CLA concentrations alone or in combination with adjuvants in the J774 macrophage cell culture system. Antigen formulations prepared at six different concentrations (25 µg/ml, 40 µg/ml, 80 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml) after incubation of macrophage cells in 37 ºC oven containing 5% $CO_2$ for 24 hours and adjuvant combinations (alum and saponin) were added by adjusting with the medium. After 48 hours of incubation, the supernatants were collected and reacted with Griess reagent. Griess reagent was prepared by adding 2.5 ml of phosphoric acid, 0.1 g of N-(1-Naphthyl) Ethylenediamine and 1 g of Sulfanilamide to 100 ml of distilled water. 50 µl of the culture medium to be measured for NO measurement was added to 96-well plates. 50 µl of Griess reagent was added to the samples and incubated for

10 minutes at room temperature. After incubation, absorbance values were measured at 540 nm using ELISA Reader. All studies were performed in 3 replicates.

**Cell viability assay**

For cell viability analysis, MTT test was performed on cells incubated for 48 hours under 37°C temperature, 95% humidity and 5% $CO_2$ incubation conditions. Cell viability rates in cell lines were evaluated with MTT containing 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltatrazilium bromide. 10 µl of MTT solution was added to each well on the well plate and the cells were incubated in the dark at 37°C for 3 hours. After incubation, liquids containing MTT solution were aspirated and removed from the environment. Then, after adding 100 µl of dimethylsulfoxide (DMSO) to each well at room temperature, the well plates were kept in the dark for 30 minutes. After the formazan crystals were completely dissolved, the plates were placed in the ELISA reader. Cell viability analysis was performed by measuring the optical density in the well plates at a wavelength of 570 nm. Each experimental group was repeated three times and the repetitions were averaged. Cell viability analysis data were obtained using the equation and data plots were generated. Cell viability was calculated according to equation 1.

$$\text{Cell Viability}(\%)=(\text{sample absorbance}/\text{control absorbance})*100 \quad (1)$$

**Statistical Analysis**

The data of the experiment were analyzed by using IBM SPSS 25.0 (IBM Corporation, Armonk, NY, ABD) program.

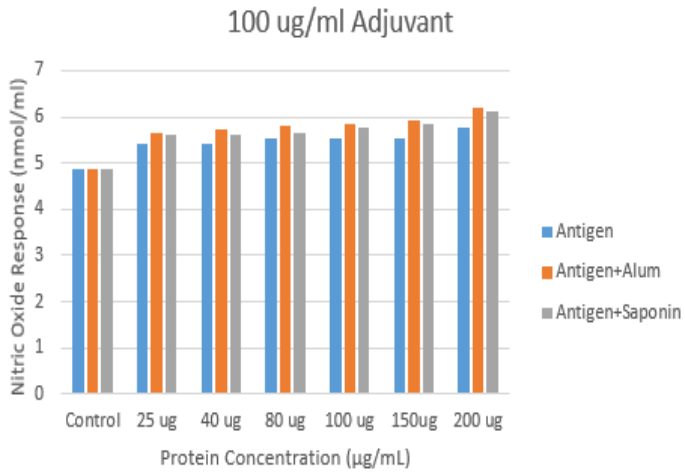
**RESULTS and DISCUSSION**

In this research experiment, soluble leishmania antigens were obtained from *L. infantum* parasites using the freeze-thaw method and ultrasonic lysis method. To design a vaccine formulation against VL, their cytotoxic and immunostimulatory activities were determined by applying their combination with antigens and adjuvants to the J774 macrophage cell line.

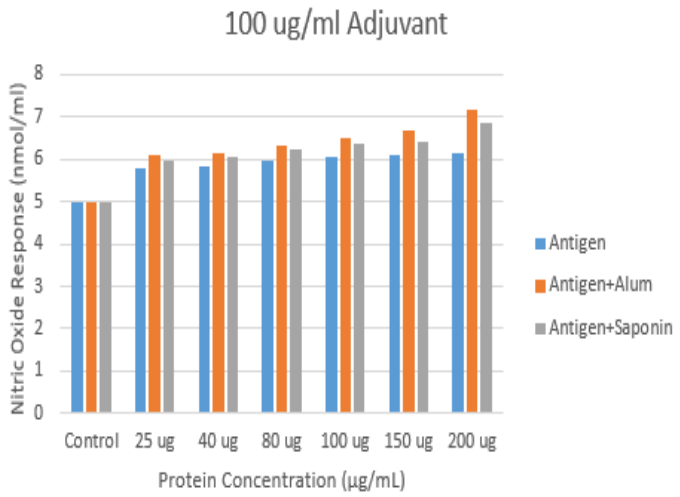
The cell viability of the positive control group was 100% and the cell viability of the other groups was determined according to that which was indicated as the rate of the alive cells. Cell viability is analyzed by using the MTT assay at the end of 48th hours of incubation for every group. The evaluations were done with the % cell viability results.

In the study, it was seen that the immunostimulatory activity increased with increasing concentration and the highest value was obtained at 200 µg/ml concentration. When nitric oxide (NO) values were examined, it was determined that the vaccine formulation obtained by combining the antigen obtained by freeze-thaw method with alum was 6.22 nmol/ml with a 27% increase in 200 µg/ml concentration compared to the control group. In the combinations of soluble leishmania antigen and alum obtained by fragmentation in a sonicator, the NO value at 200 µg/ml concentration was 7.171 nmol/ml with an increase of 43% compared to the control group. Graphics of NO data are presented in Table 1.

It is observed that cell viability rates vary between 99% and 96% in the alum formulation of SLA obtained by freeze-thaw method. It is observed that cell viability rates are 99% in alum formulations with SLA obtained by sonicator. In formulations where saponin is used as an adjuvant, it is clearly seen that the value of cell viability at 200 µg/ml concentration is 23% and 22%, thus negatively affecting the cell. Graphics of MTT data are presented in Table 1.

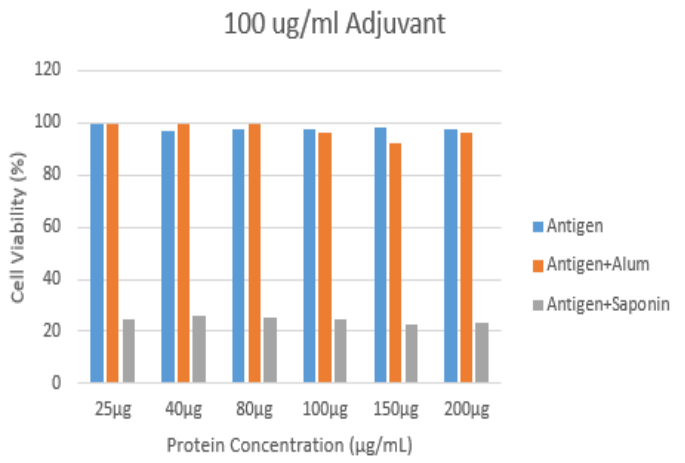



NO data of antigen obtained by freeze-thaw method

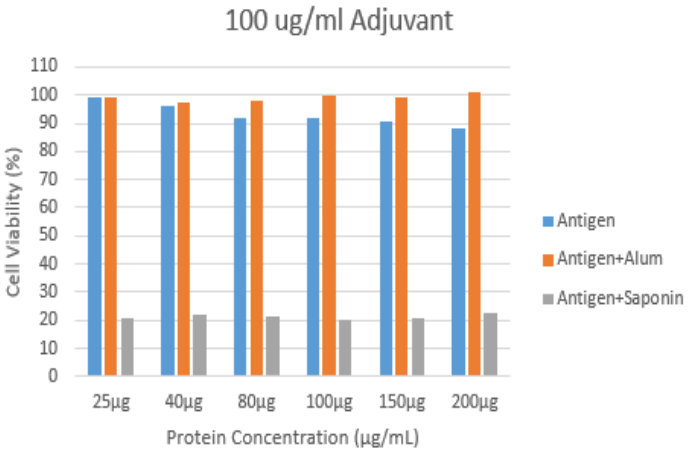



NO data using sonicator-acquired antigen

**Figure 1.** NO analysis data




MTT data of antigen obtained by freeze-thaw method




MTT data using sonicator-acquired antigen

**Figure 2.** MTT analysis data

## CONCLUSION

It is known that chemotherapy drugs are generally used in the treatment of visceral leishmaniasis, which is one of the notifiable diseases (Ouellette et al.,2004). However, although these treatment methods are not effective enough on their own, they exhibit toxic effects in living things (Bekhit et al.,2018). Due to the limited and disadvantageous treatment methods available, it is important for individuals to gain immunity before contracting the disease. For this purpose, the use of vaccines is appropriate. An effective and reliable human vaccine has not yet been produced for the prevention of leishmaniasis. In this study, soluble leishmania antigens prepared from *L. infantum* parasites by different methods were produced to prepare a new vaccine formulation. It is also known that an appropriate adjuvant must be added to the vaccine formulation to increase the efficacy and immunogenicity of the vaccine. The immunostimulatory activity was determined by applying the prepared vaccine formulation to J774 macrophage cells. In addition, nitric oxide and cell viability analyzes were performed by in vitro experiments.

As a result, it can be said that vaccine formulations developed with SLA and alum obtained by sonicator degradation method can be used as potential vaccine candidates for visceral leishmaniasis. These vaccine combinations are known to be highly efficient with low cost and their ability to induce immunity. It is recommended to conduct experimental studies on experimental animals as soon as possible to examine the *in vivo* antileishmanial performance of the developed formulations.

**REFERENCES**

Abamor, E. S. (2017). Antileishmanial activities of caffeic acid phenethyl ester loaded PLGA nanoparticles against Leishmania infantum promastigotes and amastigotes in vitro. Asian Pacific journal of tropical medicine, 10(1), 25-34.

Abdellahi, L., Iraji, F., Mahmoudabadi, A., & Hejazi, S. H. (2022). Vaccination in Leishmaniasis: A Review Article. Iranian Biomedical Journal, 26(1), 1.

Allahverdiyev, A., Bağirova, M., Oztel, O. N., Elçıçek, S., Ateş, S. C., & Karaca, T. D. (2010). Approaches and problems in vaccine development against leishmaniasis. Turkiye Parazitolojii Dergisi, 34(2), 122-130.

Askarizadeh, A., Badiee, A., & Khamesipour, A. (2020). Development of nano-carriers for Leishmania vaccine delivery. Expert Opinion on Drug Delivery, 17(2), 167-187.

Badiee, A., Shargh, V. H., Khamesipour, A., & Jaafari, M. R. (2013). Micro/nanoparticle adjuvants for antileishmanial vaccines: present and future trends. Vaccine, 31(5), 735-749.

Bekhit, A. A., El-Agroudy, E., Helmy, A., Ibrahim, T. M., Shavandi, A., & Bekhit, A. E. D. A. (2018). Leishmania treatment and prevention: Natural and synthesized drugs. European journal of medicinal chemistry, 160, 229-244.

Braga, M. S., Neves, L. X., Campos, J. M., Roatt, B. M., Soares, R. D. D. O. A., Braga, S. L., ... & Castro-Borges, W. (2014). Shotgun proteomics to unravel the complexity of the Leishmania infantum exoproteome and the relative abundance of its constituents. Molecular and biochemical parasitology, 195(1), 43-53.

Iborra, S., Solana, J. C., Requena, J. M., & Soto, M. (2018). Vaccine candidates against leishmania under current research. Expert review of vaccines, 17(4), 323-334.

Ouellette, M., Drummelsmith, J., & Papadopoulou, B. (2004). Leishmaniasis: drugs in the clinic, resistance and new developments. Drug Resistance Updates, 7(4-5), 257-266.

Reis, A. B., Giunchetti, R. C., Carrillo, E., Martins-Filho, O. A., & Moreno, J. (2010). Immunity to Leishmania and the rational search for vaccines against canine leishmaniasis. Trends in parasitology, 26(7), 341-349.

*ORAL PRESENTATION*

**Pyometralı kedi ve köpeklerde medikal tedavi seçenekleri**

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**Özet**

Pyometra, progesteronun uterus üzerindeki fizyolojik etkilerinin önemli rol oynadığı bir üreme bozukluğudur. Kedi ve köpeklerde her yaşta görülebildiği gibi, özellikle 8 yaş üstü dişilerde daha sık rastlanır. Pyometra için geleneksel tedavi ovariohisterektomidir. Ovariohisterektominin medikal tedaviye göre avantajı, pyometra nüksü için hem iyileştirici hem de önleyici olmasıdır. Bununla birlikte, operatif müdahale anestezi riskini de beraberinde getirir. Son 10 yılda hem açık hem de kapalı serviks pyometrayı tedavi etmek için çok sayıda medikal tedavi önerilmiştir. Küçük yan etkileri olan en etkili tıbbi tedavi, düşük dozlarda prostaglandinlerle ek tedavi ile veya ek tedavi olmaksızın aglepristonun tekrar tekrar uygulanmasıdır.

**Anahtar Kelimeler:** Pyometra, Aglepriston, Prostaglandin, Kabergolin

**Medical treatment options in cats and dogs with pyometra**

**Abstract**

Pyometra is a reproductive disorder in which the physiological effects of progesterone on the uterus play an important role. As it can be seen in cats and dogs at any age, it is more common especially in females over 8 years old. The traditional treatment for pyometra is ovariohysterectomy. The advantage of ovariohysterectomy over medical treatment is that it is both curative and preventive for pyometra recurrence. However, operative intervention carries the risk of anesthesia. Numerous medical treatments have been proposed in the last 10 years to treat both open and closed cervical pyometra. The most effective medical treatment with minor side effects is repeated administration of aglepristone with or without additional treatment with low doses of prostaglandins.

**Keywords:** Pyometra, Aglepristone, Prostaglandin, Cabergoline

## GİRİŞ

Pyometra, orta yaşlı ve yaşlı dişi evcil hayvanlarda potansiyel olarak yaşamı tehdit eden bir hastalıktır. Her yaştaki kedi ve köpeklerde görülebilmesine rağmen daha çok hiç doğum yapmayan yaşlı hayvanlarda sık rastlanır. Pyometra; bir lüteal dönem hastalığıdır ve genellikle östrustan sonraki 4 ay içinde teşhis edilir (Nak ve Kaşıkçı, 2013). Hastalık gelişiminde hormonlar ve bakteriler rol oynar. Kontrasepsiyon amacıyla progestinler gibi steroid hormonlar ve progesterona yanıtı artıran östrojen bileşikleri ile yapılan eksojen tedaviler, hastalık riskinin artmana sebep olur. (Nelson ve Feldman, 1986). Kistik endometriyal hiperplazi (KEH) gibi endometriyum bozuklukları, predispoze faktörler olarak kabul edilir, ancak pyometra ve KEH bağımsız olarak da gelişebilir. Pyometra oluşumunda yaşa ve ırka bağlı önemli farklılıklar vardır ve genetik faktörler, yüksek riskli ırklarda artan hassasiyete katkıda bulunabilir (Johnston ve ark., 2001). Tanı; vaka öyküsüne, fiziksel muayeneye, hematoloji ve biyokimya laboratuvar testlerindeki klinik belirti ve bulgulara ve rahim içi sıvıyı tanımlayan tanısal görüntülemeye dayanır (Günay Uçmak ve ark., 2021). Peritonit, endotoksemi ve sistemik inflamatuar yanıt sendromu, pyometranın sık görülen komplikasyonlarıdır ve daha ciddi hastalıklarla ilişkilidir. Tanı, prognoz ve tedavi değerlendirmesi için değerli olabilecek çeşitli biyobelirteçler ve inflamatuar değişkenler tanımlanmıştır (Enginler ve ark., 2014). Pyometranın en güvenli ve en etkili tedavisi, enfeksiyon kaynağını doğrudan ortadan kaldıran ve nüksü önleyen cerrahi ovariohisterektomidir. Tamamen tıbbi (farmakolojik) tedavi, açık serviks pyometralı ve diğer rahim veya yumurtalık patolojileri olmayan daha genç ve sağlıklı damızlık hayvanlarda bir alternatif olabilir (Hagman 2022). Bu makalede, kedi ve köpeklerde pyometranın tedavisinde kullanılan medikal tedavi protokolleri, bunların iyileşme ve fertilite üzerine verilerinin derlenmesi amaçlanmaktadır.

## MATERYAL VE METOT

Medikal tedavi tüm pyometra olguları için uygun bir seçenek değildir. Medikal tedavinin bir seçenek olabilmesi için belirli şartların olması gerekir. Gelecekte yavru alınması düşünülen, 6 yaşlı ve daha genç, normal kan üre nitrojen değerleri ile birlikte sistemik olarak hastalık bulgusu göstermeyen, hipotermi gibi endotokseminin klinik bulguları olmayan ve serviksin açık olduğu durumlarda medikal tedavi protokolleri uygulanabilir (Mateus ve Eilts, 2010). Pyometranın medikal tedavisi için farklı protokoller geliştirilmiş olsa da hepsinin ortak bir amacı vardır. Medikal tedavideki amaç; lüteolizisi uyarmak veya progesteron reseptörlerini bağlamak suretiyle progesteronun etkisine engel olmak, serviksin açılmasını sağlamak, uterus kontraksiyonlarını uyarmak ve uterusun boşalmasını sağlamak, geniş spektrumlu veya spesifik antibiyotiklerin kullanımı ile bakteriyel büyümenin ve gelişmenin engellenmesi, uterus dejenerasyonu şekillenen hayvanlarda uterusun rejenerasyonunu sağlamaktır (Verstegen ve ark., 2008). Kedi ve köpeklerde pyometranın medikal tedavisinde kullanılan ilaçlar; doğal prostaglandinler, prostaglandin analogları, progesteron reseptör antagonistleri, östrojenler, dopamin agonistleri ve bunların kombinasyonlarıdır (Nak ve Kaşıkçı, 2013).

## BULGULAR ve TARTIŞMA

Medikal tedaviye uygun adaylar, açık serviksli ve yumurtalık kisti olmayan genç ve sağlıklı damızlık dişi kedi ve köpeklerdir. Kullanılan bazı ilaçların terapötik etkisinin görülmesi 48 saate kadar sürebileceğinden, hastaların stabil olması ve kritik hasta olmaması önemlidir (Fieni, 2006). Kontrendikasyonlar arasında sistemik hastalık, ateş veya hipotermi, intrauterin fetal kalıntılar, organ işlev bozuklukları ve peritonit veya sepsis gibi komplikasyonlar bulunur. Olumsuz ilaç etkileri meydana gelebilir ve endotoksemi ve sepsis, klinik olarak stabil bir pyometrayı hızla acil duruma dönüştürebilir. Bu nedenle, yakın izleme, destekleyici tedaviler ve hızlı müdahaleye izin vermek için hastaneye yatış önerilir. Vajinal akıntının klinik belirtileri, azalması ve temizlenmesi; rahim boyutu; ve laboratuvar anormallikleri 1 ila 3 hafta içinde kademeli olarak normale döner (England ve ark., 2007). Komplikasyonlar ortaya çıkarsa veya genel sağlık durumu bozulursa ve dirençli vakalarda gecikmeden ovariohisterektomi gerekli olabilir. Pyometra tedavisi için tek başına antimikrobiyaller hastalığı azaltabilir ve ilerlemesini önleyebilir ancak uterusun iyileşmesi mümkün olmaz (Hagman, 2022).

Medikal tedavi stratejileri, progesteronun üretimini ve/veya etkisini engelleyerek etkilerini en aza indirmek, uterus enfeksiyonunu ortadan kaldırmak, serviksin gevşemesini ve intralüminal irin atılmasını teşvik etmek ve uterus iyileşmesini kolaylaştırmaktır. Yaygın olarak kullanılan ilaçlar, doğal prostaglandin F2a (PGF2a) veya sentetik analogu kloprostenol, dopamin agonistleri (kabergolin ve bromokriptin) veya progesteron reseptör blokerleridir (aglepriston) (Verstegen ve ark., 2008). Antimikrobiyal ilaç ve uygulama protokolü, optimal etkiyi elde etmek için bakteri kültürü, duyarlılık testleri ve farmakokinetik/farmakodinamiklere dayanmalıdır. Fizik muayene ve laboratuvar test sonuçlarına bağlı olarak damar içi sıvılar ve elektrolit desteği dahil olmak üzere ek destekleyici tedavi sağlanır (Lopate, 2017).

Prostaglandinler, lüteolizisin uyarılması, miyometriyal kontraksiyonların arttırılması ve serviksin açılması gibi fizyolojik etkilere sahiptirler. Plazma progesteron konsantrasyonunun azalması neticesinde glandular sekresyon azalır. Bu etkilerin hepsi uterustaki içeriğin dışarı atılmasını sağlar (Verstegen ve ark., 2008). Prostaglandin $F_{2\alpha}$ ile tedavi açık servik pyometra olgularında endikedir. Kapalı serviks olgularında tek başına kullanımı, uterus rupturu, frajil uterus duvarı ve oviduktlardan uterus içeriğinin abdomene boşalmasıyla peritonit şekillenme riski nedeniyle tavsiye edilmemektedir. Tedavide prostaglandin analoglarına (kloprostenol) nazaran doğal prostaglandinler (dinoprost trometamin) tercih edilmelidir. Çünkü doğal prostaglandinler sadece luteolizisi uyarmakla kalmayıp, uterus kontraksiyonlarına da sebep olurlar. Prostaglandin analogları ise daha az yan etkiye sahip olmalarına rağmen, uterusun yavaş boşalmasını sağlayan zayıf kontraksiyonlara neden olurlar (Mateus ve Eilts, 2010). Prostaglandin $E_2$ ise serviksin açılmasına neden olur ve östrojen ile sinerjik etkilidir. Hem açık hem de kapalı serviks pyometranın tedavisinde aglepriston ve misopristolun kombine kullanımı mevcuttur (Romagnoli ve ark., 2006). Prostaglandin E2'nin intravajinal olarak mı yoksa oral yolla mı verildiğinin tıbbi tedavi protokollerinde faydalı olan bir servikal gevşeme sağlayıp sağlamadığı henüz araştırılmamıştır (Lopate, 2017). Köpeklerde en önemli lüteotropik hormon prolaktindir. Prolaktin inhibitörleri olan dopamin agonistlerinin tekrarlayan uygulamaları plazma progesteron seviyesinde hızlı ve kalıcı bir azalmaya neden olur. Son yıllarda, prostaglandinlerin daha düşük dozlarda kullanımını sağlamak ve yan etkilerini azalmak için kabergolin veya bromokriptin gibi dopamin agonistleri doğal ve sentetik prostaglandin analogları ile kombine olarak kullanılmışlardır (Corrado ve ark., 2006). Kedilerde pyometra tedavisi için kabergolin ve bromokriptin üzerinde hiçbir klinik çalışma yayınlanmamıştır. Mifepriston ve aglepriston gibi progesteron reseptör antagonistleri, doğal progesteron ile yarışa girerek, progesteron reseptörlerine etkili ve rekabetçi bir şekilde ve progesteron hormonunun herhangi bir etkisini uyarmadan bağlanır. Böyle progesteronun immun sistem ve uterus üzerindeki etkileri ortadan kaldırılmış olur. Yan etkiler genellikle nadirdir veya şiddetli değildir. Uygulama yerinde tüylerde dökülme ve uygulama bölgesindeki yağ dokuya penetre olarak hedef reseptörlere bağlanamama gibi yan etkileri vardır. Bu sebeple,

aglepristonun uygulanma yeri, tüylerden ve yağ dokudan fakir bir bölge olan arka bacağın iç kısmında kalan deri altıdır. Enjeksiyon sonrası 48 saat içinde servikal gevşeme indüklenir (Fieni, 2006). Herhangi bir tedavi protokolüne geniş spektrumlu antibiyotikler ilave edilmelidir. İdeal olan, vajinanın kranialinden alınan irinli akıntıdan etken izolasyonu ve kültür antibiyogram testi sonucuna göre uygun antibiyotiğin seçilmesidir. İnvitro antibiyotik duyarlılık sonuçlarına göre, başlangıç için, amoksisilin, amoksisilin+klavulonik asit, sefalosporinler ve sülfanomidler iyi birer antibiyotik seçeneğidir (Nak ve ark., 2003). Septik şok kriterlerine sahip hayvanlarda öncelikle metabolik asidoz kompanze edilmeli ve hızla plazma hacmi arttırılmalıdır. Bu amaçla %5 dekstroz ve sodyum bikarbonat verilmesini takiben dengeli elektrolit solüsyonları kullanılmalıdır (Nak ve Kaşıkçı, 2013). Pyometranın lokal tedavi yöntemlerinin de etkili olduğu gösterilmiştir, ancak köpeklerde klinik uygulamada henüz yaygın olarak kullanılmamaktadır ve kedilerde bildirilmemiştir. Prostaglandinlerin ve antimikrobiyallerin intravajinal infüzyonu, tedavi edilen 17 dişinin 15'inde, 12 ay sonra yan etki veya nüks olmaksızın başarılı sonuçlar elde edildiği bildirilmiştir (Gabor ve ark., 1999). Aglepristonun, intrauterin antimikrobiyallerle kombinasyonu halinde, 11 dişinin 9'unda başarılı olmuştur (Gürbulak ve ark., 2005). Transservikal kateterler yoluyla intrauterin drenajın, dirençli vakalarda iyileşmeyi kolaylaştırabileceği rapor edilmiştir (Lagerstedt ve ark., 1987). Kedilerde KEH-pyometra olgularında başlangıçta östrojen enjeksiyonu, bunu takiben oksitosin ve antibiyotik enjeksiyonu birkaç gün süreyle uygulanarak uterusun boşaltılması sağlanabilir fakat kapalı serviks olgularında etkisi sınırlıdır. Östrojen uterusun kas tonusunu ve kontraktilitesini, bakteriyel enfeksiyonlara karşı direncini arttırır ve serviksin gevşemesini sağlar. Fakat birçok yan etkisinden dolayı östrojenlerin kullanımı önerilmemektedir (Agudelo, 2005). Bunun yerine kedilerde pyometra olgularında, mifepristone, aglepriston ve onapriston gibi antiprogestinler tercih edilir. Aglepriston, uterus reseptörlerine bağlanmak için doğal progesteron ile yarışırken köpeklerde 3, kedilerde 9 kat daha üstün bağlanma oranına sahiptir (Tek ve Beceriklisoy, 2013). Kedi ve köpeklerde pyometranın medikal tedavisinde kullanım alanı bulmuş olan ve yukarıda etki mekanizmaları bahsedilen ilaçlara ait tedavi protokolleri ve tedavi başarısına ait veriler mevcuttur. Köpeklerde açık serviks pyometra medikal tedavi protokolleri Tablo 1.'de, kedilerde açık serviks pyometrada medikal tedavi protokolleri ise Tablo 2.'de sunulmuştur.

**Tablo 1.** Köpeklerde açık serviks pyometranın sağaltımında kullanılan medikal tedavi protokolleri

| İlaç | Protokol ve Dozu | Sonuç ve Yan Etkileri | Kaynak |
|---|---|---|---|
| **Doğal Prostaglandinler** | 20µg/kg günde 3 kez, 8 gün süreyle | İyileşme oranı: %75<br>Nüks riski: %0 (10 ay boyunca)<br>Fertilite oranı: %86 | Hubler ve ark., 1991 |
| | 250µg/kg, günde 1 kez, 5 gün süreyle | İyileşme oranı:%75<br>Fertilite oranı:%100 | Renton ve ark., 1993 |
| | 100-500µg/kg günde 1 kez, 5 gün süreyle, eğer gerekirse 1-2 hafta sonra doz tekrarlanabilir. | İyileşme oranı: %82<br>Nüks oranı: %12<br>Fertilite oranı: %82 | Nelson ve ark., 1982 |
| | 250-500µg/kg günde 1 kez, 3 gün süreyle | İyileşme oranı: %100<br>Nüks oranı: %40 (1 yıl)<br>Fertilite oranı: %40 | Meyers-Wallen ve ark., 1986 |
| | 500µg/kg günde 1 kez, 5 gün süreyle | İyileşme oranı: %60<br>Nüks oranı: %10 (24 ay boyunca) | Nak ve ark., 2005 |
| | Kabergolin 5µg/kg oral + Kloprostenol 1µg/kg 7 gün süreyle günde 1 kez, tedaviye cevap vermeyenlere Kloprostenol aynı dozda 14.güne kadar devam edilmiş. | İyileşme oranı: %80<br>Nüks oranı: %21<br>Fertilite oranı: %50<br>Hafif yan etkiler kaydedildi. | Corrado ve ark., 2006 |

| | | | |
|---|---|---|---|
| **Kabergolin + Prostaglandin Analogları** | Kabergolin 5µg/kg oral günde 1 kez, 10 gün süreyle + Kloprostenol 5µg/kg, günde 1 kez, her 3 günde bir 10 gün süreyle | İyileşme oranı: %95<br>Nüks oranı: %19<br>Fertilite oranı: %63<br>Yan etkiler: Öğürme, kusma, hafif karın kasılması, ishal ve uygulamadan sonra 60 dakikaya kadar nefes nefese kalma | England ve ark., 2007 |
| **Aglepriston + Prostaglandin Analogları** | Aglepriston 10 mg/kg 1, 3, 8 ve ihtiyaç olduğunda 15.günde + Kloprostenol 1µg/kg 3.günde ve 8.günde | İyileşme oranı: %100<br>Nüks oranı: %20<br>Fertilite oranı: %100<br>Yan etki bildirilmedi. | Gobello ve ark., 2003 |
| **Aglepriston** | Aglepriston 10 mg/kg 1, 2, 7.günde ve ihtiyaç olduğunda 14.günde | İyileşme oranı: %70<br>Nüks oranı: %4<br>Fertilite oranı: %90 | Hoffmann ve ark.,2000 |
| | Aglepriston 10 mg/kg 1, 2, 7.günde | İyileşme oranı: %92<br>Nüks oranı: %9 (3 ay boyunca), %18 (1 yıl boyunca)<br>Fertilite oranı: %83 | Trasch ve ark., 2003 |
| | Aglepriston 10 mg/kg 1, 2, 8.günde ve ihtiyaç olduğunda 15.günde | İyileşme oranı: %60 | Fieni, 2006 |
| | Aglepriston 10 mg/kg 2, 7 ve 14.günde | %100 iyileşme; 54 aya kadar tekrarlama %12; Çiftleşen 17 dişinin %12'sinde doğurganlık. | Jurka ve ark., 2010 |
| | Aglepriston 10 mg/kg 1, 2, 7, 15 ve 23.günlerde ve tedavi edilmezse 29.günde | %75 iyileşme (klinik belirtilerin giderilmesi); nüks: 6 yıla kadar %48; 13 çiftleşmiş dişi köpeğin %69'unda doğurganlık. | Ros ve ark., 2014 |
| | Aglepriston 10 mg/kg 1, 2, 7, ve 14.günlerde | İyileşme oranı : %46 | Gürbulak ve ark., 2005 |

**Tablo 2.** Kedilerde açık serviks pyometranın sağaltımında kullanılan medikal tedavi protokolleri

| İlaç | Protokol ve Dozu | Sonuç ve Yan Etkileri | Kaynak |
|---|---|---|---|
| **Doğal $PGF_{2\alpha}$** | 0,1 mg/kg SC 12–24 saat arayla, 3–5 gün süreyle (6 kedide);<br>0,25 mg/kg dozunda ise 15 kedide uygulandı fakat daha etkili değildi. | Pyometra belirtilerinin ortadan kalkma ve siklusun başlama oranı: %95.<br>Doğurganlık oranı: %81.<br>İki farklı doz arasında fark saptanmadı (önerilen; daha düşük doz).<br>Hastaların %76'sında gözlenen geçici yan etkiler: Ses çıkarma, nefes nefese kalma, huzursuzluk, tımarlama, tenesmus, salivasyon, ishal, yuvarlanma, midriyazis, kusma, idrara çıkma ve 60 dk kadar süren lordoz.<br>Pyometra nüks oranı: %14 | Davidson ve ark., 1992 |
| **Sentetik $PGF_{2\alpha}$ analoğu (kloprostenol)** | 5mg/kg SC, 24 saat arayla ardışık 3 gün | Pyometra belirtileri %100 olarak çözülür; 1 yıl sonra tekrarlama yok; %40 doğurganlık.<br>Geçici yan etkiler: İshal, kusma, ses çıkarma. | Garcia Mitacek ve ark., 2014 |
| **Progesteron reseptör blokörleri (Aglepriston)** | 10 mg/kg SC, günde 1 kez, 1., 2. ve 7. günlerde ve tedavi edilmediyse 14. günde. | Pyometra belirtileri %90 çözülür; 2 yıllık takipten sonra nüks yok; hiçbir yan etki gözlenmedi. | Nak ve ark., 2009 |

## SONUÇ

Sonuç olarak, pyometra olgularında medikal tedaviye uygun adaylar, açık serviksli ve yumurtalık kisti olmayan genç ve sağlıklı damızlık dişi kedi ve köpeklerdir. Bu şartları sağlayan hastalar için seçilecek olan uygun tedavi yöntemi; terapötik etkinliği yüksek, yan etkisi az ve nüks ihtimali düşük protokolleri içermelidir. Kedi ve köpeklerde pyometranın medikal tedavisinde bu kriterleri sağlayan en etkili ilacın aglepriston olabileceği sonucuna varıldı.

## KAYNAKLAR

Agudelo CF 2005. Cystic endometrial hyperplasia-pyometra complex in cats. Veterinary Quarterly, 27: 173-182.

Corrado Y, Arias R, Rodriguez R, Tortora M, Gobello C 2006. Combination dopamin agonist and prostaglandin agonist treatment of cystic endometrial hyperplasia-pyometra complex in the bitch. Theriogenology, 66: 1557-1559.

Davidson AP, Feldman EC, Nelson RW 1992. Treatment of pyometra in cats, using prostaglandin F2 alpha: 21 cases (1982-1990). The Journal of the American Veterinary Medical Association, 200:825–828.

Enginler SO, Ateş A, Diren Sığırcı B, Sontaş BH, Sönmez K, Karaçam E, Ekici H, Evkuran Dal G, Gürel A 2014. Measurement of C-reactive protein and prostaglandin F2α metabolite concentrations in differentiation of canine pyometra and cystic endometrial hyperplasia/mucometra. Reproduction in domestic animals = Zuchthygiene, 49(4): 641–647.

England GC, Freeman SL, Russo M 2007. Treatment of spontaneous pyometra in 22 bitches with a combination of cabergoline and cloprostenol. Veterinary Record, 160: 293–296.

Fieni F 2006. Clinical evaluation of the use of aglepristone, with or without cloprostenol, to treat cystic endometrial hyperplasia-pyometra complex in bitches. Theriogenology, 66: 1550–1556.

Gabor G, Siver L, Szenci O 1999. Intravaginal prostaglandin F2 alpha for the treatment of metritis and pyometra in the bitch. Acta Veterinaria Hungarica, 47: 103–108.

García Mitacek MC, Stornelli MC, Tittarelli CM, Nuñez Favre R, de la Sota RL, Stornelli MA 2014. Cloprostenol treatment of feline open-cervix pyometra. Journal of Feline Medicine and Surgery, 16: 177–179.

Gobello C, Catex G, Klima L, Rodriguez R, Corrada Y 2003. A study of two protocols combining aglepristone and cloprostenol to treat open cervix pyometra in the bitch. Theriogenology, 60: 901-908.

Günay Uçmak Z, Kurban İ, Uçmak M 2021. Assessment of Hematological Parameters and Uterine Hemodynamic Indices in Bitches with Pyometra. Acta Scientiae Veterinariae, 49.

Gürbulak K, Pancarci M, Ekici H, Konuk C, Kirşan I, Uçmak M, Toydemir S 2005. Use of aglepristone and aglepristone intrauterine antibiotic for the treatment of pyometra in bitches. Acta Veterinaria Hungarica 2005;53:249–55.

Hagman R 2022. Pyometra in Small Animals 2.0. Veterinary Clinics: Small Animal Practice, 52(3): 631-657.

Hoffmann B, Lemmer W, Bostedt H, Failing K 2000. Application of the antiprogestin aglepristone fort he concervative treatment of pyometra in dog. Tierärztliche Praxis, 28: 323-329.

Hubler M, Arnold S, Casal M, Fluckiger M, Hauser B, Corboz L, Rusch P 1991. Use of prostaglandin $F_2$ alpha dose in the bitch. Schweizer Archiv fur Tierheilkunde, 133: 323-328.

Johnston SD, Root Kustritz MV, Olson PNS 2001. Canine and feline theriogenology. 1st edition. Philadelphia: Saunders.

Jurka P, Max A, Hawrynska K, Snochowski M 2010. Age related pregnancy results and further examination of bitches after aglepristone treatment of pyometra. Reproduction in Domestic Animal, 45: 525-529.

Lagerstedt A-S, Obel N, Stavenborn M 1987. Uterine drainage in the bitch for treatment of pyometra refractory to prostaglandin F2a. Journal of Small Animal Practice, 28: 215–222.

Lopate C 2017. Pyometra, cystic endometrial hyperplasia (hydrometra, mucometra, hematometra). In: Greco DS, Davidson AP, editors. Blackwell's five-minute veterinaryconsult clinical companion, small animal endocrinology and reproduction. Hoboken (NJ): Wiley-Blackwell, pp. 53–62.

Mateus L, Eilts BE 2010. Cystic endometiral hyperplasia and pyometra. In: Ettinger SJ, Feldman EC.(Editors). Textbook of veterinary internal medicine disease of the dog and the cat. 7th edition. Canada: Saunders, 1913-1921.

Meyers-Wallen VN, Goldschmidt MH, Flickinger GL 1986. Prostaglandin $F_2$ α treatment of canine pyometra. JAVMA, 189: 1557-1561.

Nak D, Çetin C, Nak Y, Aşyemez A, Keskin A 2003. Pyometralı köpek ve kedilerde izole edilen bakterilerin identifikasyonu ve uygun antibiyotik seçeneğinin belirlenmesi. Veterinarium, 14: 28-31.

Nak D, Nak Y, Keskin A 2005. Köpeklerde açık pyometranın tedavisinde Prostaglandin $F_2$ α ve sulfadoksin/trimetoprim kombinasyonunun kullanımı üzerine çalışmalar. Veteriner Bilimleri Dergisi, 21: 19-24.

Nak D, Nak Y, Tuna B 2009. Follow-up examinations after medical treatment of pyometra in cats with the progesterone-antagonist aglepristone. Journal of Feline Medicine and Surgery, 11: 499–502.

Nak D, Kaşıkçı G 2013. İnfetilite. In: Kedi ve Köpeklerde Doğum ve Jinekoloji. Kaymaz M., Fındık M., Rişvanlı A., Köker A. (Editörler). 1.basım, Anakra: Medisan, pp. 173-210.

Nelson RW, Feldman EC, Stabenfeldt GH 1982. Treatment of canine pyometra and endometritis with prostaglandin $F_2$α. JAVMA, 181: 899-903.

Nelson RW, Feldman EC 1986. Pyometra in the bitch. In: Morrow DA. (Editor). Current theraphy in theriogenology 2. Philedelphia: Saunders Company WB, pp.484-489.

Renton JP, Boyd JS, Harvey MJA 1993. Observations on the treatment and diagnosis of open pyometra in the bitch (*canis familiaris*). Journal of Reproduction and Fertility Supply, 47: 465-469.

Romagnoli S, Fieni F, Prats A, Gardey L, Vannozzi I, Rota A 2006. Treatment of canine open-cervix and closed-cervix pyometra with combined administration of aglepristone and misoprostol. Proceedings of congresseuropian veterinary society for small animal reproduction, Budapest, Hungary.

Ros L, Holst BS, Hagman R 2014. A retrospective study of bitches with pyometra, medically treated with aglepristone. Theriogenology, 82: 1281–1286.

Tek Ç, Beceriklisoy BH 2013. İnfetilite. In: Kedi ve köpeklerde doğum ve jinekoloji. Kaymaz M., Fındık M., Rişvanlı A., Köker A. (Editörler). 1.basım, Ankara: Medisan, pp. 333-341.

Trasch K, Wehrend A, Bosdtedt H 2003. Follow-up examinations of bitches after conservative treatment of pyometra with the antiprogestagen aglepristone. Journal of veterinary medicine. A, Physiology, pathology, clinical medicine, 50: 375-379.

Verstegen J, Dhaliwal G. Verstegen-Onclin K 2008. Mucometra, cystic endometrial hyperplasia, and pyometra in the bitch: advances in treatment and assessment of future reproductive success. Theriogenology, 70: 364–374.

*ORAL PRESENTATION*

**Türkiye'den *Lavandula stoechas* çiçeklerinin uçucu yağ bileşenleri**

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**Özet**

*Lavandula stoechas*, Lamiaceae familyasına ait ve birçok ülkenin geleneksel tıp uygulamalarında aktif olarak kullanılan bir tıbbi-aromatik bitkidir. Bu çalışmada Tokat ilinde doğal yayılış gösteren *Lavandula stoechas* türünün uçucu yağ bileşenleri incelenmiştir. Bitkide materyal olarak çiçek salkımları kullanılmıştır. Clevenger cihazında elde edilen uçucu yağlar gaz kromatografi-alev iyonlaştırma detektörü-kütle spektrometresinde (GC(FID)/MS) analiz edilmiştir. Uçucu yağ bileşenlerinin toplamda 52 bileşik ile %91.83 tanımlanmış, linalool ve linalyl acetate ana bileşen olarak kaydedilmiştir. Sonuç olarak, elde edilen veriler önceki analizlerle kıyaslanmış ve bu lokasyonun farkı ortaya çıkartılmıştır.

**Anahtar Kelimeler:** Lavanta, *Lavandula stoechas,* uçucu yağ, GC/MS, fitokimyasal

**Essential oil components of *Lavandula stoechas* flowers from Turkey**

**Abstract**

*Lavandula stoechas* is a medicinal-aromatic plant belonging to the Lamiaceae family and actively used in traditional medicine practices of many countries. In this study, essential oil components of *Lavandula stoechas* species, which is naturally distributed in Tokat province, were investigated. Inflorescences of the plant were used as material. The essential oils obtained by the clevenger apparatus were analyzed by gas chromatography-flame ionization detector-mass spectrometry (GC(FID)/MS). The essential oil components were identified 91.83%, with a total of 52 compounds, linalool and also linalyl acetate were recorded as the main components. As a result, the findings were compared with data of analyzes and the difference of this location was revealed.

**Keywords:** Lavander, *Lavandula stoechas,* essential oil, GC/MS, phytochemical

## GİRİŞ

Lavandula L. (Lavanta) cinsi, Lamiaceae familyasına ait, Kuzey Atlantik'ten Orta Doğu'ya kadar Akdeniz havzasında doğal olarak yayılış gösteren, Akdeniz'e özgü küçük çalı formunda 20-60 cm boyunda büyüyen, odunsu bir gövdeye sahip, çok yıllık otsu bitki bitkilerdir (Héral ve ark., 2021). Bu cins bitkileri genellikle asidik toprakları tercih ederler ve karşılıklı, kesik yapraklı, dörtgen gövdeli bitkiler ile karakterize edilir. Çiçekleri mor, mavi, menekşe, pembe veya leylak renginde salkım kümeleri halinde olup, Haziran ortasından Temmuz ortasına kadar tam çiçeklenme gösterirler (Ez zoubi ve ark., 2020; Héral ve ark., 2021). *Lavandula* türleri, dünya çapında süs bitkisi ve tıbbi bitki olarak yetiştirilmekte ve onların uçucu yağları parfümeride, kozmetikte, gıda işlemede ve aromaterapide ciddi pazar değerine sahiptir (Lis-Balchin, 2002). Yaprakları çay, krem, kapsül ve uçucu yağının masaj şeklinde uygulamaları antidepresan etkili olarak kullanılmaktadır (Firoozeei ve ark., 2021). Ayrıca, geleneksel olarak da astım, bronşit, kolik, soğuk algınlığı ve yorgunluk tedavisinin yanı sıra iştah açıcı amacıyla kullanılmaktadır (Baytop, 1984).

*Lavandula* cinsinin taksonomisi Upson ve Andrews tarafından, dünyada 39'dan fazla tür ve 79 tür içi takson ve melezden oluştuğu rapor edilmiştir (Upson ve Andrews, 2004). Bunlar içerisinde en iyi bilinen ve ekonomik olarak değerli olan tarımı yapılan türler arasında *L. angustifolia, L. stoechas, L. latifolia, L. lanata* ve L. x intermedia (*L. angustifolia* ve *L. latifolia'nın* bir melez) hibrit'dir. Ayrıca bu cinsin birçok hibrit ve yüzlerce

çeşiti bulunmaktadır (Moja ve ark., 2016; Passalacqua ve ark., 2017; Adaszyńska-Skwirzyńska ve Dzięcioł, 2017).

Ekonomik olarak değerli olan türler arasındaki yer alan *L. stoechas*, Türkiye doğal dağılış gösteren ve halk arasında Karabaş otu olarak bilinen bir türdür (Güner, 2012). Akdeniz bitkisi olup, Afrika, Avrupa ve Asya kıtalarında dağılış göstermektedir (Ez zoubi ve ark., 2020). Bu tür 30–100 cm'e kadar uzayabilen çok yıllık yarı çalımsı bir bitkidir. Yaprakları uzun, grimsi ipeksi tüylü 1–4 cm'dir. Çiçekler pembe-mordur, 10–30 cm uzunlukta sivri uçlu ince yapraksız saplar üzerindeki 2–3 cm uzunluğunda çiçekler görülür. Her bir çiçekte 4–8 mm uzunluğunda bir taç yaprak bulunur. Sivri ucun üstündeki çiçekler daha büyük, uzun ve parlak lavanta mavisi renklerdedir (nadiren beyaz) (Anonim, 2022).

*L. stoechas,* Akdeniz bölgesinde geleneksel yemeklerin ve bitki çaylarının hazırlanmasında ve kozmetik amaçlı kullanılmaktadır (Ristocelli ve ark., 1999; Zuzarte ve ark., 2013). Etnobotanik çalışmalara göre, birçok ülkede geleneksel olarak, baş ağrıları, depresyon, diyabet, yaralara, idrar yolu enfeksiyonlarına, egzamaya karşı olumlu etkileri, analjezik, sedatif, antiseptik, romatizmal hastalıkları ve nefrotik sendromları tedavi etmek, spazm önleyici, ağrı ve inflamatuar sorunları azaltmak için kullanılmaktadır (El-Hilaly ve ark., 2003; Sebai ve ark., 2013; Ez zoubi ve ark., 2016). Bu amaç için bitkinin hava kısımları, yaprakları, çiçekleri ve saplı çiçekleri tedavi amaçlı kullanılmıştır (Ez zoubi ve ark., 2020). Ayrıca, *L. stoechas* uçucu yağlarının ve ekstraktlarının antibakteriyel (Ez zoubi ve ark., 2017), antifungal, böcek öldürücü, anti-leishmanial (Bouyahya ve ark., 2017), antioksidan ve anti-inflamatuar (Ez zoubi ve ark., 2014) gibi biyolojik aktivitelere sahip olduğu rapor edilmiştir. Yapılan çalışmalar, tıbbi özelliğini uçucu yağına atfetmişlerdir. Bir yabani formdaki uçucu yağın kompozisyonu habitatına bağlı olarak varyasyon gösterebilmektedir (Kırmızıbekmez ve ark., 2009; Ez zoubi ve ark., 2017). Bu nedenle, bu çalışmada, *L. stoechas* çiçeklerinin uçucu yağ içeriğinin incelenmesi amaçlanmıştır.

## MATERYAL VE METOT

### Bitki Materyali

*Lavandula stoechas* L. Haziran 2017 yılında bitkinin çiçekli döneminde, Türkiye Tokat'dan (Turhal) toplanmıştır. Bir numune Mustafa Kemal Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü Herbaryumu'na MKUH-1329 herbaryum numarası ile bırakılmıştır.

### İzolasyon Prosedürü

Doğadan toplanan bitkilerin çiçek kısımları ayrıldı ve oda koşullarında gölgede kurutuldu. Kuru materyaller öğütülerek 100 g tartıldı ve 1000 litre su içerisinde, Clevenger tipi bir aparat kullanılarak 3 saat boyunca damıtma yöntemi ile uçucu yağlar elde edildi. Yağlar susuz $Na_2SO_4$ üzerinde kurutuldu ve kullanılıncaya kadar 4°C'de saklandı.

### Gaz Kromatografisi (GC(FID)/MS)

Uçucu yağ analizleri, alev iyonizasyon dedektörü (FID) ve kütle spektrometresi ile bölünmüş modda (50:1) bir GC/MS (gaz kromatografi/kütle spektrometresi) PerkinElmer, Clarus 500 Serisi (Shelton, ABD) ile kapiler kolon (30 m × 0.25 mm, 0.25 um i.d., SGE Analytical Science- Trajan Scientific and Medicinal) kullanılarak gerçekleştirilmiştir. Enjeksiyon sıcaklığı sabit ve FID 250°C'de ayarlanmıştır. Taşıyıcı gaz olarak 1.0 ml/dk (Split 1/20) oranında helyum kullanılmıştır. Numunenin enjeksiyon hacmi 5uL olarak alınmıştır. Fırın sıcaklığı 50°C'de başlatıldı ve ardından 8°C/dakikalık bir hızla 220°C'ye yükseltilmiştir. İyonizasyon enerjisi 70 eV, ve transfer hattının sıcaklığı kütle spektrometresinin 250°C olarak ayarlanmıştır.

Uçucu yağ bileşiklerinin tanımlanması, n-alkanların (C8-C20) homolog serileri referans alınarak hesaplanmış (RI) ve veri tabanındaki ve kütüphanelerdeki örneklerle karşılaştırılan kütle spektrumlarındaki parçalanma modeli ile yapılmıştır. Adams ve NIST veri tabanının yanı sıra standartların birlikte enjeksiyonu kullanılmıştır. Miktar tayini, temsili bileşiklerin GC/FID analizinden elde edilen kalibrasyon eğrisi kullanılarak harici standartlar yöntemiyle hesaplanmıştır.

## BULGULAR ve TARTIŞMA

*Lavandula stoechas* türünün çiçek kısımlarının uçucu yağ bileşen analizleri sonucu yağda toplamın %91.83'ünü temsil eden 52 bileşen tespit edilmiştir (Tablo 1). Yağının ana bileşenleri linalool ve linalyl acetate olarak kaydedilmiştir.

Önceki çalışmalarda, farklı Akdeniz ülkelerinden elde edilen *L. stoechas* türünün çiçek, yaprak ve toprak üstü kısımlarının uçucu yağ bileşenleri incelenmiş ve ana bileşenleri arasında önemli bir varyasyon olduğu gözlenmiştir (Ez zoubi ve ark., 2020). En yaygın tanımlanan ana bileşenler Fenchone, camphor, ve 1,8-cineole

olarak kaydedilmiştir (Melpomeni ve ark., 1999; Zrira ve ark., 2003; Sultan Giray ve ark., 2008; Messaoud ve ark., 2012; Fabíola ve ark., 2013; Zuzarte ve ark., 2013; Alejandrove ark., 2015).

**Tablo 1.** *Lavandula stoechas* türünün çiçek kısmına ait uçucu yağ bileşenleri

| Bileşen sayısı | Peak | RT | RI | Bileşenler | % |
|---|---|---|---|---|---|
| 1 | 5 | 9,11 | 929 | α-Pinene | 0,51 |
| 2 | 6 | 9,62 | 952 | Camphene | 0,51 |
| 3 | 7 | 10,459 | 974 | Sabinene | 0,16 |
| 4 | 8 | 10,586 | 979 | β-Pinene | 0,46 |
| 5 | 9 | 10,87 | 986 | 3-Octanone | 1,13 |
| 6 | 10 | 11,039 | 991 | β-Myrcene | 1,21 |
| 7 | 11 | 11,538 | 1004 | Pseudolimonen | 0,11 |
| 8 | 13 | 12,282 | 1023 | β-Cymene | 0,08 |
| 9 | 14 | 12,562 | 1032 | Eucalyptol | 7,36 |
| 10 | 15 | 12,741 | 1037 | β-Ocimene | 2,48 |
| 11 | 16 | 13,132 | 1049 | trans-β-Ocimene | 2,97 |
| 12 | 17 | 13,524 | 1060 | γ-Terpinene | 0,05 |
| 13 | 18 | 13,913 | 1070 | cis-Sabinene hydrate | 0,16 |
| 14 | 19 | 14,047 | 1074 | cis-Linalool oxide | 0,43 |
| 15 | 20 | 14,618 | 1086 | Isoterpinolene | 0,63 |
| 16 | 21 | 15,372 | 1099 | Linalool | 30,33 |
| 17 | 26 | 16,299 | 1135 | cis-Limonene oxide | 0,02 |
| 18 | 27 | 16,488 | 1138 | trans-Limonene oxide | 0,01 |
| 19 | 28 | 16,568 | 1142 | cis-Verbenol | 0,02 |
| 20 | 29 | 16,78 | 1143 | Camphor | 4,12 |
| 21 | 30 | 16,883 | 1144 | β-Terpineol | 0,04 |
| 22 | 31 | 17,194 | 1157 | Isoborneol | 0,02 |
| 23 | 32 | 17,342 | 1161 | trans-β-Terpineol | 0,03 |
| 24 | 33 | 17,57 | 1167 | endo-Borneol | 4,74 |
| 25 | 34 | 17,671 | | endo-Borneol-isomer | 0,12 |
| 26 | 35 | 17,753 | 1174 | cis-Linalool 3,7-oxide | 0,07 |
| 27 | 38 | 18,208 | 1184 | Cryptone | 0,25 |
| 28 | 46 | 20,223 | 1246 | Carvone | 0,02 |
| 29 | 47 | 20,308 | 1250 | 3-exo-Hydroxy-1,8-cineole | 0,04 |
| 30 | 48 | 20,8 | 1257 | Linalyl acetate | 26,76 |
| 31 | 51 | 21,297 | 1285 | Bornyl acetate | 0,05 |
| 32 | 52 | 21,727 | 1283 | Neryl formate | 1,94 |
| 33 | 63 | 24,912 | 1384 | β-Bourbonene | 0,05 |
| 34 | 65 | 25,096 | 1391 | β-Elemene | 0,01 |
| 35 | 69 | 25,682 | 1409 | α-Gurjunene | 0,05 |
| 36 | 70 | 26,041 | 1419 | β-Caryophyllene | 1,77 |
| 37 | 71 | 26,298 | 1432 | β-Copaene | 0,02 |
| 38 | 72 | 26,429 | 1435 | α-Bergamotene | 0,07 |
| 39 | 73 | 26,648 | 1444 | cis-β-Farnesene | 0,05 |
| 40 | 74 | 26,77 | 1448 | Isogermacrene D | 0,01 |
| 41 | 75 | 27,019 | 1457 | β-trans-Farnesene | 1,21 |
| 42 | 76 | 27,224 | 1460 | Lavandulyl butyrate | 0,04 |
| 43 | 77 | 27,352 | 1461 | Alloaromadendrene | 0,01 |
| 44 | 78 | 27,534 | 1463 | cis-Muurola-4(15),5-diene | 0,07 |

| | | | | | |
|---|---|---|---|---|---|
| 45 | 79 | 27,74 | 1466 | epi-β-Caryophyllene | 0,02 |
| 46 | 80 | 27,908 | 1473 | γ-Gurjunene | 0,50 |
| 47 | 81 | 28,445 | 1503 | Isodaucene | 0,05 |
| 48 | 82 | 28,574 | 1508 | Lavandulyl isovalerate | 0,37 |
| 49 | 83 | 28,866 | 1513 | γ-Cadinene | 0,04 |
| 50 | 84 | 29,09 | 1524 | β-Sesquiphellandrene | 0,05 |
| 51 | 96 | 32,715 | 1645 | Lavandulyl caproate | 0,01 |
| 52 | 98 | 33,625 | 1684 | α-Bisabolol | 0,60 |
| Toplam | | | | | 91,83 |

İlginç bir şekilde, Türkiye'nin farklı lokasyonlarından toplanan *L. stoechas* türünün uçucu yağ bileşenlerinin ana bileşenleri arasında da ciddi bir varyasyon saptanmıştır (Gören ve ark., 2002; Dadalioglu ve Evrendilek, 2004; Kırmızıbekmez ve ark., 2009). Gören ve ark. (2002) bitki örneklerinin ülkenin batısından toplamış ve yaprak örneklerindeki uçucu yağ bileşenleri arasından pulegone (%40.4), menthol (%18.1) ve menthone (%12.6) ana bileşen olarak rapor edilmiştir. Dadalioglu ve Evrendilek (2004) ülkenin güney bölgelerinden topladığı toprak üstü örneklerinin uçucu yağ ana bileşenlerini fenchone (%55.6), camphor (%18.2) ve 1,8-cineole (%8.0) olarak keydetmişlerdir. Kırmızıbekmez ve ark. (2009) ülkenin batısından toplanan bitkilerin yaprak ve çiçek kısımlarının uçucu yağ ana bileşenlerini yaprak için α-fenchone (%41.9), 1,8-cineole (%15.6), camphor (%12.1), çiçek için α-fenchone (%39.2), myrtenyl acetate (%9.5), α-pinene (%6.1), camphor (%5.9) olarak bildirilmiştir. Arabaci ve Bayram (2006) camphor (%47.0), 1,8-cineol (%41.7) ve α-pinene'ni (%8.3) *L. stoechas*'ın çiçekleri için ana bileşenler olarak kaydetmiştir. Giray ve ark. (2008) güney bölgesindeki kültür bitkilerinin çiçek kısımlarında fenchon (%32.0), camphor (%14.7), myrtenyl acetate (%11.7) ve 1,8-cineol (%7.7) bileşenleri ana bileşen kaydetmiştir. Bizim çalışmamıza benzer olarak, Özcan ve ark. (2018) bitkinin çiçek kısmına ait uçucu yağın ana bileşenlerini linalool (%49.9) ve linalyl acetate (%14.4) olduğunu bildirmiştir.

## SONUÇ

Kimyasal profildeki bu farklılıklar, önemli derece farklı kemotiplerin varlığı ile ifade edilebileceği gibi farklı çevresel koşullar, coğrafi kökenler, ekstrakte edilen bitkinin bölümleri ve ekstraksiyon yöntemleri ile açıklanabilir. *L. stoechas* türündeki kemotip farklılıklarının aydınlatılması ıslah çalışmalarında büyük öneme sahip olacaktır.

## KAYNAKLAR

Adaszyńska-Skwirzyńska M, Dzięcioł M 2017. Comparison of phenolic acids and flavonoids contents in various cultivars and parts of common lavender (*Lavandula angustifolia*) derived from Poland. Natural product research, 31(21): 2575-2580.

Alejandro C, Vanessa OR, Ramiro MG, Virginia T, Jose T 2015. *Lavandula stoechas* essential oil from Spain: aromatic profile determined by gas chromatography–mass spectrometry, antioxidant and lipoxygenase inhibitory bioactivities. Indus Crop Prod. 73: 16–27.

Anonim 2022. https://tr.wikipedia.org/wiki/Lavandula_stoechas. Erişim, 04.11.2022.

Arabaci O, Bayram E 2006. Evaluation of chemical composition, essential oil and morphological traits in wild populations of *Lavandula stoechas* L. in the Mediterranean environment. Asian Journal of Chemistry, 18: 371-380.

Baytop T 1984. Treatment with plants in Turkey. İstanbul University, 3255, İstanbul, Turkey.

Bouyahya A, Et-Touys A, Abrini J, Talbaoui A, Fellah H, Bakri Y, Dakka N 2017. *Lavandula stoechas* essential oil from Morocco as novel source of antileishmanial, antibacterial and antioxidant activities. Biocatal Agric Biotechnol.,12:179–84.

Dadalioglu I, Evrendilek GA. 2004. Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas* L.) and fennel (*Foeniculum vulgare*) on common foodborne pathogens. Journal of Agricultural and Food Chemistry, 52: 8255-8260.

El-Hilaly J, Hmammouchi M, Lyoussi B 2003. Ethnobotanical studies and economic evaluation of medicinal plants in Taounate Province (northern Morocco). J Ethnopharmacol., 86:149–58.

Ez zoubi Y, Bousta D, El Mansouri L, Boukhira S, Siham L, Achour S, Farah A 2016. Phytochemical screening, anti-inflammatory activity and acute toxicity of hydro-ethanolic, flavonoid, Tannin and

Mucilage Extracts of *Lavandula stoechas* L from Morocco. Int J Pharmacognosy Phytochem Res., 8(1):31–7.

Ez zoubi Y, El Ouali Lalami A, Bousta D, Polissiou M, Daferera D, Lachkar M, El Khanchoufi A, Farah A 2017. Chemical composition, antioxidant and antimicrobial activities of the essential oil and its fractions of *Lavandula stoechas* L. From Morocco. Int J Curr Pharm Rev Res., 8(1):60–7.

Ez zoubi Y, Bousta D, Farah A 2020. A Phytopharmacological review of a Mediterranean plant: Lavandula stoechas L. Clinical Phytoscience, 6(1): 1-9.

Fabíola M, Maria GM, João D, Florência V, Cristina M, Ana ID, Cristina AF, José GB, Luís GP 2013. Antioxidant capacity of the essential oils from Lavandula luisieri, L. stoechas subsp. lusitanica, *L. stoechas* subsp. *lusitanica x L. luisieri* and *L.viridis* grown in Algarve (Portugal). J Essent Oil Res., 21:327–36.

Firoozeei TS, Feizi A, Rezaeizadeh H, Zargaran A, Roohafza HR, Karimi M 2021. The antidepressant effects of lavender (*Lavandula angustifolia* Mill.): A systematic review and meta-analysis of randomized controlled clinical trials. Complementary therapies in medicine, 59: 102679.

Giray ES, Kirici S, Alpaslan Kaya D, Türk M, Sönmez O, Inan M 2008. Comparing the effect of sub-critical water extraction with conventional extraction methods on the chemical composition of *Lavandula stoechas*. Talanta, 74: 930-935.

Goren CA, Topcu G, Bilsel G, Bilsel M, Aydogmus Z. Pezzuto M 2002. The chemical constituents and biological activity of essential oil of *Lavandula stoechas* ssp. *stoechas*. Zeitschrift für Naturforschung, 57c: 797-800.

Güner A, Aslan S, Ekim T, Vural M, Babaç MT 2012. Türkiye bitkileri listesi (Damarlı bitkiler). Nezehat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, sayfa 578, İstanbul.

Héral B, Stierlin É, Fernandez,X, Michel T 2021. Phytochemicals from the genus Lavandula: A review. Phytochemistry Reviews, 20(4): 751-771.

Kırmızıbekmez H, Demirci B, Yeşilada E, Başer KHC, Demirci F 2009. Chemical composition and antimicrobial activity of the essential oils of Lavandula stoechas L. ssp. stoechas growing wild in Turkey. Natural Product Communications, 4(7): 1934578X0900400727.

Lis-Balchin M (ed) 2002. Lavender: the genus *Lavandula*. Taylor & Francis, Milton Park

Melpomeni S, Chedly A, Eugene K 1996. Essential oil variation of Lavandula stoechas L. ssp. Stoechas growing wild in Crete (Greece). Biochem Syst Ecol., 24(3):255–60.

Messaoud C, Chograni H, Boussaid M 2012. Chemical composition and antioxidant activities of essential oils and methanol extracts of three wild *Lavandula* L. species. Nat Prod Res., 26(21):1976–84.

Moja S, Guitton Y, Nicolè F et al 2016. Genome size and plastid trnK–matK markers give new insights into the evolutionary history of the genus *Lavandula* L. Plant Biosyst Int J Dealing Asp Plant Biol., 150:1216–1224.

Özcan MM, Starovic M, Aleksic G, Figueredo G, Juhaimi FA, Chalchat JC 2018. Chemical composition and antifungal activity of lavender (*Lavandula stoechas*) oil. Natural Product Communications, 13(7): 1934578X1801300728.

Passalacqua NG, Tundis R, Upson TM 2017. A new species of *Lavandula* sect. *Lavandula* (Lamiaceae) and review of species boundaries in *Lavandula angustifolia*. Phytotaxa, 292:161.

Ristocelli D, Tomi F, Casanova J 1999. 13C-NMR as tool for identification and enantiomeric differentiation of major terpenes exemplified by the essential oil of *Lavandula stoechas* L. ssp. *stoechas*. Flavour Fragrance J., 13:154–8.

Sebai H, Selmi S, Rtibi K, Souli A, Gharbi N, Sakly M 2013. Lavender (*Lavandula stoechas* L.) essential oils attenuate hyperglycemia and protect against oxidative stress in alloxan-induced diabetic rats. Lipids Health Dis., 12(1):189.

Sultan Giray E, Saliha K, Alpaslan Kaya D, Murat T, Özgür S, Memet I 2008. Comparing the effect of sub-critical water extraction with conventional extraction methods on the chemical composition of Lavandula stoechas. Talanta., 74:930–5.

Upson T, Andrews S 2004. The genus *Lavandula*. Royal Botanic Gardens, Kew.

Zrira S, Benjilali B 2003. The constituents of the oils of Lavandula stoechas L. ssp. atlantica Br.-Bl. and L. stoechas ssp. stoechas from Morocco. J Essent Oil Res., 15:68–9.

Zuzarte M, Goncalves MJ, Cavaleiro C, Cruz MT, Benzarti A, Marongiu B, Maxia A, Piras A, Salgueiro L 2013. Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils. Ind Crop Prod., 44:97–103.

## *ORAL PRESENTATION*

### Kanatlı mezbaha atıksularının arıtımı için yenilikçi hibrit oksidasyon yöntemleri uygulamaları

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**Özet**

Kanatlı sektörü tüketiciye ucuz protein ve istihdam sağlamasına karşın kanatlı et üretimindeki artışa bağlı olarak her bir işlem için kullanılacak su miktarı ve kesimhane atığı üretimi artmaktadır. Kanatlı kesimhaneleri, kan, yağ, gres ve protein gibi organik maddeleri ihtiva ettiğinden dolayı biyokimyasal oksijen ihtiyacı (BOİ) ve kimyasal oksijen talebi (KOİ), azot ve fosfor gibi yüksek seviyelerde organik maddeler içeren kanatlı atık sularını meydana getirdiğinden çevresel kirlilik oluşturmaktadır. Kanatlı kesimhane atık suyunun deşarjından önce arıtılmasıyla ciddi çevresel ve sağlığı tehdit edici tehditlerin önlenmesine olanak tanır. Kanatlı atık suyu arıtımında geleneksel yöntemler olarak kullanılan pıhtılaşma, flokülasyon, havalandırma, yüzdürme ve biyolojik arıtım gibi sistemlerde, yüksek çamur üretimi, bazı biyolojik bileşiklerin ve rengin daha az verimli şekilde uzaklaştırılması, yüksek enerji talebi (havalandırma için), uzun hidrolik alıkonma süreleri gibi bazı dezavantajlar vardır. Kanatlı atık suyu gibi özellikle endüstriyel çıkış suları yüksek seviyelerde KOİ ve yağ-gres içerdiğinden dolayı bu atık suların tekil proseslerle arıtılması zordur. Kanatlı endüstrisi gibi organik kirlilik yükü yüksek atık suların sadece fiziksel ve/veya kimyasal arıtıma maruz kalarak kentsel atık su arıtma tesislerine deşarj edilmesiyle tesislerdeki kirlilik yükü ve arıtım maliyeti artacaktır. Klasik arıtım teknolojilerine alternatif ileri oksidasyon prosesleri (İOPs) tamamlayıcı bir arıtma seçeneği olup kanatlı mezbaha atık suları için bugünkü kullanılan biyolojik proseslere ön arıtım veya ileri arıtım olarak kullanılabilmektedirler. İOP'lar, hidroksil radikalleri (·OH) gibi reaktif oksijen türlerinin (ROS) oluşumu tarafından dayanıklı organik kirleticilerin veya hedef kirleticilerin parçalanması ve mineralizasyonu için yüksek potansiyel gösterdiklerinden umut vaadedici bir yöntem olarak düşünülmektedir. Hibrit reaktör konfigüsayonları, kütle transferi ve yüzey reaksiyonlarını arttırarak yüksek miktarda biyolojik olarak yavaş parçalanabilir organik bileşikler içeren kanatlı sektörü atık sularının arıtımı ve bu organik kirleticilerin tamamlanmış oksidasyonu için yenilikçi bir yöntem olarak düşünülmektedir. Bu çalışmadaki amaç, hibrit İOP'larla şimdiye kadarki gerçek zamanlı kanatlı sektörü atık sularının arıtılması alanında yapılan sayılı çalışmaların arıtım performansı ve ekonomik yönden avantaj ve dezavantajlarının tartışılmasıdır.

**Anahtar Kelimeler:** Kanatlı mezbaha atıksuyu, hibrit proses, ileri oksidasyon prosesleri, atıksu arıtımı

### Applications of innovative hybrid oxidation methods for the treatment of poultry slaughterhouse wastewater

**Abstract**

Although the poultry sector provides cheap protein and employment to the consumer, the amount of water to be used for each process and the production of slaughterhouse waste increase due to the increase in poultry meat production. Poultry slaughterhouses create environmental pollution because they contain organic materials such as blood, oil, grease and protein, and because they produce poultry wastewater containing high levels of organic substances such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD), nitrogen and phosphorus. It allows to prevent serious environmental and health threats by treating poultry slaughterhouse wastewater before discharge. It allows to prevent serious environmental and health threats by treating poultry slaughterhouse wastewater before discharge. There are some disadvantages in systems such as coagulation, flocculation, aeration, flotation and biological treatment, which are used as traditional methods in poultry wastewater treatment, such as high sludge production, less efficient removal of some biological compounds and color, high energy demand (for aeration), long hydraulic retention times. Especially industrial effluents, such as poultry wastewater, contain high levels of COD and oil-grease, so it is difficult to treat these wastewaters by single processes. By exposing wastewater with high organic pollution load, such as the poultry industry, to municipal wastewater treatment plants only by being exposed to physical and/or chemical treatment, the pollution load and treatment cost in the facilities will increase. Advanced oxidation processes

(AOPs), alternative to conventional treatment technologies, are a complementary treatment option and can be used as pre-treatment or advanced treatment to the biological processes used today for poultry slaughterhouse wastewater. AOPs are considered a promising method as they show high potential for degradation and mineralization of resistant organic pollutants or target pollutants by the formation of reactive oxygen species (ROS) such as hydroxyl radicals (·OH). Hybrid reactor configurations are considered as an innovative method for the treatment of poultry industry wastewater containing high amounts of slowly biodegradable organic compounds by increasing mass transfer and surface reactions and for the complete oxidation of these organic pollutants. The aim of this study is to discuss the treatment performance and economic advantages and disadvantages of the few studies carried out in the field of real-time poultry sector wastewater treatment with hybrid AOPs.

**Keywords:** Poultry slaughterhouse wastewater, hybrid process, advanced oxidation processes, wastewater treatment

## 1. GİRİŞ

Günümüzde, Sürdürülebilir Kalkınma için 2030 gündeminde su sıkıntısı çeken bölgelerde su kıtlığı sorununun üstesinden gelmek için, ekonomik ve yenilikçi atık su arıtma teknolojilerini uygulayarak su kalitesinin iyileştirilmesini, geri dönüşümün artırılmasını ve suyun güvenli bir şekilde yeniden kullanılmasını içeren suyun sürdürülebilir yönetimi hedeflenmektedir. Birleşmiş Milletler (BM) tarafından ana hatları yayınlanan 17 Sürdürülebilir Kalkınma Hedefleri (SKH)'nden "2" ve "6" sayıları "Sıfır Açlık" ve "Temiz Su ve Sanitasyon" dur (UN General Assembly, 2015). Bu iki hedef, büyümenin ve ticarileştirmenin bir sonucu olarak et endüstrisi ile doğrudan ilgilidir. İklim değişikliği gibi küresel bir çevre krizinin yanı sıra, 628 milyondan fazla teyit edilmiş vaka (WHO, 2022) ile tüm kıtaları etkileyen COVID-19 pandemisi, gıda üretimi açısından kendi kendine yeterli bir ülke olmanın önemini göstermiştir. Kanatlı eti sektörü, ekonomik ve besleyici bir protein kaynağı olması nedeniyle dünyanın birçok yerinde en büyük ve hızla büyüyen tarıma dayalı endüstrilerden biri olarak kabul edilmektedir (Ngobeni ve ark., 2022; Meiramkulova ve ark., 2021). Yüksek ekonomik kazanımlara rağmen, kesim, tüy yolma, iç organlarının çıkarılması ve yıkama gibi kanatlı süreçleri, kanatlı başına ortalama 26.5 L tüketilen aşırı yoğun suyun kullanıldığı süreçlerdir (Fatima ve ark., 2021). Kanatlı kesimhaneleri, kan, yağ, gres ve protein gibi organik maddeleri ihtiva ettiğinden dolayı biyokimyasal oksijen ihtiyacı (BOİ) ve kimyasal oksijen talebi (KOİ), azot ve fosfor gibi yüksek seviyelerde organik maddeler içeren kanatlı atık sularını meydana getirdiğinden çevresel kirlilik oluşturmaktadır (Matsumura ve Mierzwa, 2008). Tüm bu nedenle kanatlı sektörü atık suları, olası birçok çevre felaketine yol açmamak için alıcı ortama deşarj edilmeden mutlaka verimli bir şekilde arıtılmalıdır (Debik ve Coskun, 2009). Son zamanlarda, maksimum verim elde etmek için farklı su arıtım metotlarının kullanılmasına doğru ilgi artmıştır. Kanatlı endüstrisi gibi organik kirlilik yükü yüksek atık suların sadece fiziksel ve/veya kimyasal arıtıma maruz kalarak kentsel atık su arıtma tesislerine deşarj edilmesiyle tesislerdeki kirlilik yükü ve arıtım maliyeti artacaktır. Buna karşılık, ileri oksidasyon prosesleri (İOP) biyolojik olarak parçalanamayan organik bileşikleri içeren atık suların arıtılması için geniş kapsamlı olarak kullanılmaktadır. Son yıllarda tekil İOP'lar hibrit olarak bir araya getirilerek, kütle transferi ve yüzey reaksiyonları arttırılmakta ve böylece yüksek miktarda biyolojik olarak yavaş parçalanabilir organik bileşikler içeren kanatlı sektörü atık suları gibi endütriyel atıksuların arıtımı gerçekleştirilmektedir. Hibrit İOP'lar, atıksulardaki bu organik kirleticilerin tamamlanmış oksidasyonu için yenilikçi bir yöntem olarak düşünülmektedir. Bu çalışmada, tekil ve hibrit İOP'larla kanatlı sektörü atıksularının arıtım verimleri kıyaslanarak hibrit proseslerin tekil proseslere göre avantajlarının ortaya koyulması amaçlanmıştır.

## 2. KANATLI MEZBAHA ATIKSUYU ÖZELLİKLERİ

Kanatlı mezbaha atıksuları, kan, yağ-gres, protein, ağır metaller ve temizlik için deterjanlar gibi farklı karmaşık organik ve inorganik maddelerin yanı sıra yüksek konsantrasyonlarda biyokimyasal oksijen ihtiyacı (BOİ), kimyasal oksijen ihtiyacı (KOİ), azot (N) ve fosfor (P) gibi besin maddeleri, askıda ve çözünmüş katılar ve patojenler içermektedir (Bustillo-Lecompte ve ark., 2016). Tablo 1'de, literatürde çeşitli çalışmalarda elde edilen kanatlı atıksuyu karakterizasyonu ve Türkiye'de 31/12/2004 tarihli ve 25687 sayılı Resmi Gazete'de yayımlanarak yürürlüğe giren Su Kirliliği Kontrol Yönetmeliği (SKKY) "Büyükbaş, Küçükbaş Hayvan Besiciliği ve Tavukhaneler" için yasal deşarj limitleri verilmiş olup BOİ, KOİ ve mikrobiyal diğer kirlilik parametrelerine bakıldığında kanatlı mezbahanın yüksek derecede kirli olarak nitelendirilen atık su ürettiği gözlenmektedir. Yeraltı ve yüzey sularında tespit edilen kanatlı mezbaha atıksuları (Cao ve ark., 2021), aşırı azot ve fosfor nedeniyle ötrofikasyona ve kontamine sularla enfekte olan insanlar için sağlık sorunlarına yol açarken, oksijen konsantrasyonunun tükenmesi su ekosistemini tahrip etmektedir (Yaakob ve ark., 2018). Ayrıca, Türkiye gibi gelişmekte olan ülkelerde, düşük kâr marjları, teknik bilgi, arıtma ekipman ve

teknolojileri için yetersiz maliyet ve finansal yetersizlik nedeniyle kanatlı hayvan işleme tesislerinin bir çoğu, kanunlarla belirlenen deşarj limitlerine uygun değildir (Bullo ve ark., 2022).

**Tablo 1.** Kanatlı mezbaha atıksularının karakteristiği

| Parametreler | Dogdu ve Sen (2022) | Ngobeni ve ark. (2022) | Yaakob ve ark. (2018) | Bustillo-Lecompte ve ark. (2016) | Bayar ve ark. (2011) | SKKY Deşarj Limit Değeri |
|---|---|---|---|---|---|---|
| **pH** | 7.37±0.03 | 6.19-7.24 | 8.02±0.042 | 4.90-8.10 | 6.73 | 6.0-9.0 |
| **Sıcaklık, ºC** | 23.03±0.06 | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | - |
| **Redoks Potensiyeli** | 25.73±0.38 mV | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | - |
| **Elektriksel İletkenlik** | 1356±2.65 µS/cm | 950-2470 µS/cm | Belirtilmemiş | Belirtilmemiş | 2858 µS/cm | - |
| **Bulanıklık** | 722±2.52 NTU | 316-1000 NTU | Belirtilmemiş | Belirtilmemiş | 176.6 NTU | - |
| **Yağ-gres** | 5647 ± 19.3 mg/L | 280-1668 mg/L | Belirtilmemiş | Belirtilmemiş | 143.1 mg/L | - |
| **Renk** | 6127±46.2 Pt/Co | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | - |
| **AKM** | 493±2.52 mg/L | 405-8319 mg/L | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | 150 mg/L |
| **TAKM** | Belirtilmemiş | Belirtilmemiş | 3438±2696.1 mg/L | 300-2800 mg/L | Belirtilmemiş | - |
| **KOİ** | 3280±39.1 mg/L | 3750-14681 mg/L | 5422±2282.69 mg/L | 1250-15900 mg/L | 2171 mg/L | 400 mg/L |
| **BOİ** | Belirtilmemiş | Belirtilmemiş | 1602±242.7 mg/L | 610-4635 mg/L | 1123 mg/L | - |
| **$NH_4$-N** | 95±5 mg/L | 53-312 mg/L | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | 15 mg/L |
| **$NO_2$-N** | 6.67±0.15 mg/L | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | - |
| **$NO_3$-N** | 30±1 mg/L | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | - |
| **$NO_3$** | Belirtilmemiş | Belirtilmemiş | 2.241±0.58 mg/L | Belirtilmemiş | Belirtilmemiş | - |
| **$PO_4$-P** | 105±2 mg/L | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | 9.65 mg/L | 2 mg/L |
| **$PO_4^{-3}$** | Belirtilmemiş | 30.8-56 mg/L | 12.256±4.25 mg/L | Belirtilmemiş | Belirtilmemiş | - |
| **Görünüş** | Kırmızımsı kahverengi | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | - |
| **T-N** | 305±5 mg/L | Belirtilmemiş | 361.25±215 mg/L | 50-841 mg/L | Belirtilmemiş | - |
| **T-P** | 47.67±1.53 mg/L | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | Belirtilmemiş | - |
| **Toplam Organik Karbon** | Belirtilmemiş | Belirtilmemiş | 419.3±222.66 mg/L | 100-1200 mg/L | Belirtilmemiş | - |

## 3. KANATLI MEZBAHA ATIKSULARININ FARKLI İOP'LARLA ARITILMASI

Literatürde kanatlı mezbaha atıksularının arıtılması için uygulanan geleneksel fiziksel, kimyasal ve biyolojik yöntemler mevcuttur. Bunlar; aerobik/anaerobik bozunma, filtrasyon, çökeltme, pıhtılaşma, flokülasyon, çözünmüş hava flotasyonudur (Hilares ve ark., 2021). Ancak bu prosesler sadece düşük mukavemetli atık sular için geçerli olup askıda katı maddelerin ve yağların birikmesi ve aşındırıcı pıhtılaştırıcı maddelerin depolanmasındaki zorluk nedeniyle bu her bir arıtma yönteminin daha yüksek çamur oluşumu, uzun hidrolik bekletme süreleri, yüksek enerji ve malzeme girişi, yüksek bakım ve sermaye yatırımı, ikincil kirletici üretimi, düşük arıtma verimliliği gibi çeşitli dezavantajları vardır (Ngobeni ve ark., 2022; Meiramkulova ve ark., 2021). Bu nedenle, çevreye duyarlı, yenilikçi ve verimli arıtma teknolojileri sağlamak, bu inatçı, karmaşık ve yüksek organik dayanımlı kanatlı atıksularının belirli deşarj kriterlerine ve sürdürülebilir atık kalite hedeflerine ulaşması için vazgeçilmezdir.

Özellikle hidroksil radikalleri (·HO) gibi oldukça reaktif radikal türlerin üretimine dayanan ileri oksidasyon prosesleri (İOP'lar), atık sudaki organik kirleticilerin bozunması ve mineralizasyonunda oldukça etkilidir (Stando ve ark., 2021). Klasik arıtım teknolojilerine alternatif ileri oksidasyon prosesleri (İOPs) tamamlayıcı bir arıtma seçeneği olup kanatlı mezbaha atık suları için bugünkü kullanılan biyolojik proseslere ön arıtım veya ileri arıtım olarak kullanılabilmektedirler (Bustillo-Lecompte ve ark., 2016). Son yıllarda artan atıksu arıtımı gereksinimleri yanında ileriki yıllarda yasal düzenlemeler ile birlikte istenilen koşulların sağlanabilmesi adına daha ileri bir arıtım teknolojilerini içeriğinde bulunduran, etkin maliyetli bir arıtma sistemine ihtiyaç duyulmaya başlanacağı öngörülmektedir. Hibrit ileri oksidasyon proseslerinin avantajları olarak, güçlü oksitleme kabiliyetine sahip daha fazla serbest ·OH radikal üretimi meydana gelirken, birden fazla İOP konfigüre edilerek kütle transferi ve katalizör yüzey reaksiyonları arttırılır (Yang ve ark., 2021). Hibrit İOP'lar, yüksek miktarda biyolojik olarak yavaş parçalanabilir organik bileşikler, kararlı ve biyolojik olarak parçalanamayan kirleticiler içeren kanatlı sektörü atık sularının arıtımı ve bu organik kirleticilerin tamamlanmış oksidasyonu için yenilikçi bir yöntem olarak düşünülmektedir. Ayrıca hibrit proseslerin su/atık su sistemlerindeki hedef kirleticilerin mühendislik açısından bozunma verimliliğini tekil proseslere kıyasla arttırdığı ve bunların kombinasyonlarının işletme maliyetini, reaktif miktarını, işletim süresini ve mineralizasyon için enerji gereksinimlerini azaltması beklenmektedir (Al-Bsoul ve ark., 2020). İOP'lar biyolojik arıtım gibi diğer hibrit teknolojilerle arıtım öncesi veya arıtım sonrası olarak görev alırlar. İOP'lar sadece bozunma yerine daha çok mineralizasyon kabiliyetlerinden dolayı oldukça etkilidirler. İOP teknikleri arasında, heterojen fotokataliz (HFK), organik kirletici bozunma reaksiyonlarını katalize eden elektron deliği çiftlerinin ($e^-/h^+$) üretilmesine dayanan umut verici süreçlerden biridir (Silva ve ark., 2022). $TiO_2$, ZnO, CuO, $SnO_2$, CdS en çok kullanılan yarı iletkenler olup doğal veya yapay ışıma ortamlarına maruz kaldıklarında

valans bant boşlukları ($h_{VB}^+$), iletim bant elektronları ($e_{CB}^-$), hidroksil radikalleri (·OH) ve süperoksit radikalleri ($O_2^{\cdot-}$) oluştururlar (Denklem 1-7) (Antonopoulou ve ark., 2021):

Katalizör + hv → $TiO_2$ ($e_{CB}^-$ + $h_{VB}^+$) (1)
Katalizör ($h_{VB}^+$) + $H_2O$ → Katalizör + $H^+$ + $HO^\bullet$ (2)
Katalizör ($h_{VB}^+$) + $HO^-$ → Katalizör + $HO^\bullet$ (3)
Katalizör ($e_{CB}^-$) + $O_2$ → Katalizör + $O_2^{\bullet-}$ (4)
$O_2^{\bullet-} + H^+ \rightarrow HO_2^\bullet$ (5)
$2HO_2^\bullet \rightarrow O_2 + H_2O_2$ (6)
$H_2O_2 + O_2^{\bullet-} \rightarrow HO^\bullet + OH^- + O_2$ (7)

Samsudin ve ark. (2019) sentezledikleri Pt@$BiVO_4$/g-$C_3N_4$ fotokatalizörünü kullanarak gerçek endüstriyel kanatlı atıksuyunun solar ışık ışıması altında arıtımını gerçekleştirmişlerdir. Yapılan bu çalışma, % 1.5 Platin ile hazırlanan Pt@$BiVO_4$/g-$C_3N_4$ fotokatalizörünün pH 8'de üst düzey bir performans sergileyerek 3 saat içerisinde %93.5 KOİ arıtımı gerçekleştirdiğini ortaya koymuştur. Kanafin ve ark. (2022) yaptıkları çalışmada, hidrojen peroksit, potasyum persülfat, titanyum dioksit ve demir tuzlarının gerçek kanatlı mezbaha atıksuyu arıtımını kesikli fotoreaktörde 150 dakikada UV-C ışıması (254 nm) altında incelemişlerdir. UV fotolizi veya Fe katkılı $TiO_2$ fotokatalizi pratik olarak verimsizken, katkısız $TiO_2$ uygulaması 60 dakikalık UV ışımasından sonra %44 toplam organik karbon (TOK) giderimi ile sonuçlanmıştır. UV ışığı altında 98 mM $H_2O_2$ kullanımı, 150 dakika sonra %74 TOK giderimine yol açmıştır. Bu sisteme Fe(II) iyonlarının eklenmesi, ilk pH ayarının 3'e getirilmesiyle birleştiğinde, 150 dakika sonra TOK giderimini %82.5'e yükseltmiştir. Son olarak, UV'nin 15 mM $K_2S_2O_8$ ile kombinasyonu, %85 TOK giderimiyle sonuçlanmıştır. Literatürde kanatlı sektörü atık suları immobilize $TiO_2$ katalizörü kullanılarak fotokataliz yöntemiyle arıtılmaya çalışılmıştır. Asha ve Kumar (2015), 56 W granüler aktif karbon (GAK)–$TiO_2$ sisteminde maksimum BOİ giderim hızı sabiti 0.987/dk ve maksimum BOİ giderim hızı 320 mg/L/dk olarak elde etmiştir. Literatürde son yıllarda endüstriyel atıksuların arıtımında en çok ilgi gören elektrokimyasal İOP'lardan biri olan elektro-Fenton (EF) prosesi Denklem 8'deki reaksiyondan üretilen $H_2O_2$'nin katalizör olarak $Fe^{2+}$ veya $Fe^{3+}$ içeren kontamine asit çözeltisine sürekli tedarikine dayanan ileri elektrokimyasal oksidasyon prosesidir. ·OH daha sonra ortamda Fenton'un demir iyonu ve hidrojen peroksit arasındaki reaksiyonu ile üretilir (Denklem 10).

$O_2 + 2H^+ \rightarrow H_2O_2$ (8)
$Fe(OH)^{2+} + e^- \rightarrow Fe^{2+} + OH^-$ (9)
$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + H_2O + \cdot OH$ (10)

Bu katalitik reaksiyon, esas olarak katotta $Fe^{3+}$'ün indirgenmesiyle gerçekleşen $Fe^{2+}$ rejenerasyonundan yayılır (Denklem 9). Davarnejad ve Nasiri (2017) çalışmalarında kanatlı mezbaha atıksuyunu elektro-Fenton prosesiyle arıtmışlardır. Çalışma sonucunda, pH 3.39'da, reaksiyon süresi 49.22 dakikada, 3.62 $H_2O_2/Fe^{2+}$ molar oranında, 67.90 mA/$cm^2$ akım yoğunluğda, 1.44 ml/l $H_2O_2$/atıksu hacim oranında %88.06 renk giderimi elde edilmiştir. Thirugnanasambandham ve ark. (2015), kanatlı işleme endüstrisi atıksularını EF prosesiyle arıtımını gerçekleştirmiş olup pH 3'de, 10 mA/$cm^2$ akım yoğunluğuda, 20 ml/l $H_2O_2$ dozunda ve 30 dk elektroliz süresinde maksimum %97 KOİ ve %93 bulanıklık giderimi elde ederken 0.009 kW sa/l elektrik enerjisi tüketmişlerdir. Cui ve ark. (2021) yaptıkları çalışmada, yenilikçi hibrit Fenton oksidasyonu ve alg yetiştiriciliği sistemiyle kanatlı atıksuyu arıtımını incelemiş olup alg büyümesini engelleyebilecek aşırı amonyak azotunu azaltmak için Fenton oksidasyonu (FO) benimsenmiştir. Tekil FO ön arıtımı sırasıyla %70.5, %96.7, %86.1 ve % 96.2 TN, Toplam amonyum azotu (TAN), TP ve KOİ giderimi gerçekleştirmiştir. En yüksek biyokütle (235.8 mg/L/gün) ve lipid (77.3 mg/L/gün) verimlilikleri, optimize edilmiş hibrit FO ve alg sisteminde 7 günlük kesikli ekimden sonra elde edilmiştir. Nutrient giderimi neredeyse %100'e ulaşmıştır. Yetilmezsoy ve Sakar (2008), kanatlı atıksuyunun 8.0 günlük hidrolik bekleme süresinde, pilot ölçekli yukarı akışlı anaerobik çamur yataklı anaerobik reaktörde (UASB), 63. günde %90.7'lik bir arıtma verimliliği ile toplam KOİ giderimi üzerinde dikkate değer bir performans elde etmiştir. Bununla birlikte, çalışma ham kanatlı atık suyunun KOİ'sinin yaklaşık %99.3'ünün bir UASB işlemi ve ardından bir son arıtma ünitesi olarak kullanılan Fenton oksidasyon teknolojisi ile etkin bir şekilde giderilebileceğini göstermiştir.

Kanatlı atıksuyu arıtımında literatürde en çok kullanılan bir diğer elektrokimyasal İOP da elektrokoagülasyon metodudur. Bu proseste, metal iyonları anotta oluşturulur ve katotta hidroksit iyonlarıyla reaksiyona girer. Atıksudaki askıda katı ve/veya kolloid katılarla reaksiyona girerek metal hidroksitler üretilir ve bir çökelek meydana getirilir. Böylece, toksik maddelerin giderimi gerçekleştirilir (Denklem 11-13) (Sridhar ve ark., 2012).

Anotta; $M \rightarrow M^{n+} + ne^-$ (11)
Katotta; $nH_2O + ne^- \rightarrow \frac{n}{2}H_2 + nOH^-$ (12)

Burada M anot materyaline karşılık gelirken n ise oksidasyon/redüksiyon reaksiyonunda yer alan elektron sayısına karşılık gelmektedir.

$M^{n+} + nOH \rightarrow M(OH)_n$ (13)

Thirugnanasambandham ve ark. (2014), kanatlı işleme endüstrisi atıksularını elektrokoagülasyon prosesiyle arıtımını gerçekleştirmiş olup %30 seyreltilmiş atıksuyun pH 6'da, 14 mA/cm$^2$ akım yoğunluğuda, 1075 mg/l elektrolit dozunda maksimum %95 KOİ giderimi elde ederken 3.48 KW sa/l elektrik enerjisi tüketmişlerdir. Gomes ve ark. (2018), kanatlı işleme endüstrisi atıksularını elektrokoagülasyon prosesiyle arıtımını gerçekleştirmiş olup yatay olarak yerleştirilmiş demir elektrotlar için elde edilen en yüksek KOİ giderme verimliliği, 12 dakikadan sonra 4 mA/cm$^2$ akım yoğunluğunda %89'dir. Dikey olarak yerleştirilmiş demir elektrotlar için ise, elde edilen en yüksek KOİ giderme verimliliği, 16 dakikadan sonra 4 mA/cm$^2$ akım yoğunluğunda %96'dir.

Son yıllarda organik maddelerin oksidasyonunu sağlayan ultrasonik ışınlama (US) veya sonoliz uygulamasına su ve atık su arıtımı için ilgi artmaktadır. Bu proses, dairesel çekirdeklenme, yayılma ve kavitasyon adı verilen mikro baloncukların başarılı bir şekilde parçalara ayrılmasına dayanır (Denklem 14-15) (Joseph ve ark., 2009). Ultrases teknolojisinin geleneksel İOP'lara kıyasla avantajları arasında reaktiflere ihtiyaç duymaması, daha kısa çalışma süresi, daha yüksek oksidasyon oranı bulunur (Kıdak ve Doğan, 2017). Buna karşın, sonoliz prosesi uygulamalarının en büyük zorluğu ise, termal durumda enerji tüketimi ve işletme maliyetinin yüksek olmasıdır.

$H_2O +))) \rightarrow \cdot OH + OH^-$ (14)

$H_2O +))) \rightarrow ½\ H_2 + ½\ H_2O_2$ (15)

Abdelhay ve ark. (2021), ultrasonik ışıma yoluyla gerçek kanatlı mezbaha atıksuyundan KOİ giderimin incelemişlerdir. Uygulanan frekans etkisi, güç yoğunluğu, ışınlama süresi, pH ve $H_2O_2$ ilavesinin KOİ giderimi üzerine etkisi çalışmanın ana hatlarını oluşturmuştur. KOİ giderimi, 180 dakikalık ışınlama süresinden sonra nihai seviyelere ulaşmıştır. 1142 ve 578 kHz çalışma frekanslarında, güç yoğunluğu 160'tan 1200 W/L'ye arttırıldığında sırasıyla KOİ giderim verimi %2'den %43'e ve %2'den %49'a yükselmiştir. Atıksu pH'ı 7'den 9'a çıkarıldığında, KOİ giderimim %51'den %13'e azalmıştır. Düşük güç yoğunluklarında, yüksek frekans (1142 kHz) düşük frekansa göre (578 kHz) KOİ gideriminde daha verimli olurken hibrit sonoliz ve $H_2O_2$ KOİ gideriminde tekil sonoliz sisteminden daha yüksek performans (yaklaşık %50 KOİ giderimi) sergilemiştir.

Sonikasyonun, kirleticilerin giderim verimini (%) arttırarak heterojenik sonokatalitik prosesin geliştirilmesi için nanokatalizörle birleştirilmesi gerçekleştirilmesi son yıllardaki en ilgi çeken yöntemlerden biri haline gelmiştir (Porhemmat ve ark., 2017). Böylece, alternatif prosesler arasında, toksik olmaması, yüksek kimyasal stabilite göstermesi, elektriksel ve optik özellikleri nedeniyle organik maddenin oksidasyonunda ultrases veya ultraviyole (UV) radyasyonlu, nano boyutlu $TiO_2$ olarak fotokatalizör kullanılabilmektedir (Sponza ve Oztekin, 2013). Sonofotokimyasal proseste, fotokatalizörler (genellikle $TiO_2$ veya ZnO gibi bir yarı iletken) ultrasonik ses dalgalarının varlığında ultraviyole radyasyon ile ışınlanır.

Fotokataliz (UV+$TiO_2$) prosesinin ultrases yöntemiyle birleştirilmesindeki asıl amaç ise, askıda $TiO_2$ partikülleri boyunca yayılmasından dolayı ultrasesin fiziksel etkisinin genişlemesi ve böylece proses boyunca kümelenmenin oluşumunun önlenmesidir. Böylece, bu etki katalizörün yüzey alanının artması sonucu oluşan ·OH miktarının oluşumunun artışını geliştirecektir (Al-Bsoul ve ark., 2020). Dogdu ve Sen (2022) çalışmasında, hibrit sonofotokataliz reaktörünü kullanarak gerçek kanatlı mezbaha atıksuyundan KOİ, yağ ve gres giderimini incelemişlerdir. Yapılan bu çalışmanın sonucunda, 2.5 g/L ZnO fotokatalizörüyle, pH 2'de ve $UVC_{254}$ 180 dakika sonunda maksimum KOİ giderimi gerçekleştirilirken 1.5 g/L ZnO fotokatalizörüyle, pH 6'da, 5 mmol/L $H_2O_2$ konsantrasyonu ve $UVC_{254}$ 30 dakika maksimum yağ ve gres giderimi gerçekleştirilmiştir. Maksimum KOİ ve yağ&gres giderimi sırasıyla %54 ve %99 olarak elde edilmiştir. Buna karşın, tekil sonoliz (US), UVC ışıma, ZnO adsorpsiyonu ve UVC+ZnO prosesleriyle sırasıyla %5, %7, %9 ve %28 KOİ giderim verimi elde edilmiştir. Bu durum hibrit İOP teknolojisinin kanatlı sektörü atıksularının giderilmesinde daha etkili olduğunu ortaya koymuştur.

## SONUÇ

Çevre ve halk sağlığının korunması için önemli miktarda kararlı, biyolojik olarak bozunabilir ve bozunamayan kirleticiler ve yoğun yağ ve gres içeren kanatlı mezbaha atksularının yenilikçi ileri oksidasyon prosesleriyle arıtılması ve su kalitesinin iyileştirilmesine yönelik önemli araştırmalar yapılmış ve literatür bulguları tartışılmıştır. Kanatlı endüstrisi ve kanatlı kesim prosesleri endüstriyel işlemlerin arıtımında, çevresel tahribat ve halk sağlığının sorunlarının dinamik çözümlerinde oldukça etkinlik gösterir. Yüksek dirençli karakterdeki kararlı kirleticileri içeren atıksuların arıtılmasında hibrit proseslerin tekil proseslere göre daha verimli performans sergiledikleri görülmüştür. Özellikle

İOP'ların aerobik/anaerobik biyolojik proseslere ön arıtım olarak kullanılmasının da oldukça başarılı bir performans gösterdiği de ortaya konulmuştur. Gelecekteki araştırmalar değişen çevresel koşullar altında hibrit İOP süreçlerinin optimizasyonu ve ardından ölçeklenebilirliği üzerine odaklanmalıdır.

## TEŞEKKÜR

"Hibrit ultrases ve ileri oksidayon prosesleri kullanılarak kanatlı endüstrisi atıksularının arıtılması" isimli "2021.09.02.1498" numaralı proje Bolu Abant İzzet Baysal Üniversitesi BAP Koordinasyon Birimi tarafından desteklenmiştir.

## KAYNAKLAR

Abdelhay A, Othman AA, Albsoul A 2021. Treatment of slaughterhouse wastewater using high-frequency ultrasound: optimization of operating conditions by RSM. Environmental Technology, 42(26): 4170–4178.

Al-Bsoul A, Al-Shannag M, Tawalbeh M, Al-Taani AA, Lafi WK, Al-Othman A, Alsheyab M 2020. Optimal conditions for olive mill wastewater treatment using ultrasound and advanced oxidation processes. Science of the Total Environment, 700:134576.

Antonopoulou M, Kosma C, Albanis T, Konstantinou T 2021. An overview of homogeneous and heterogeneous photocatalysis applications for the removal of pharmaceutical compounds from real or synthetic hospital wastewaters under lab or pilot scale. Science of The Total Environment, 765:144163.

Asha RC, Kumar M 2015. Photocatalytic degradation of poultry wastewater using activated carbon-supported titanium dioxide. Desalination and Water Treatment, 54(12): 3279-3290.

Bayar S, Yıldız YŞ, Yılmaz AE, İrdemez Ş 2011. The effect of stirring speed and current density on removal efficiency of poultry slaughterhouse wastewater by electrocoagulation method. Desalination, 280:103-107.

Bullo TA, Bayisa YM, Bultum MS 2022. Optimization and biosynthesis of calcined chicken eggshell doped titanium dioxide photocatalyst based nanoparticles for wastewater treatment. SN Applied Sciences, 4(17).

Bustillo-Lecompte CF, Ghafoori S, Mehrvar M 2016. Photochemical degradation of an actual slaughterhouse wastewater by continuous UV/$H_2O_2$ photoreactor with recycle. Journal of Environmental Chemical Engineering, 4: 719–732.

Cao ST, Tran HP, Le HTT, Bui HPK, Nguyen GTH, Nguyen LT, Nguyen BT, Luong AD 2021. Impacts of effluent from different livestock farm types (pig, cow, and poultry) on surrounding water quality: a comprehensive assessment using individual parameter evaluation method and water quality indices. Environmental Science and Pollution Research, 28:50302–50315.

Cui H, Yu J, Zhu X, Cui Y, Ji C, Zhang C, Xue J, Jia X, Qin S, Li R 2021. Advanced treatment of chicken farm flushing wastewater by integrating Fenton oxidation and algal cultivation process for algal growth and nutrients removal. Journal of Environmental Management, 298:113543.

Davarnejad R, Nasiri S 2017. Slaughterhouse Wastewater Treatment Using an Advanced Oxidation Process: Optimization Study. Environmental Pollution, 223:1–10.

Debik E, Coskun T 2009. Use of the Static Granular Bed Reactor (SGBR) with anaerobic sludge to treat poultry slaughterhouse wastewater and kinetic modeling. Bioresource Technology, 100: 2777–2782.

Dogdu G, Sen NE 2022. An innovative solution for the treatment of poultry industry wastewater with advanced hybrid technology sono-photocatalysis. Global NEST Journal, 24(XX):1-11.

Fatima F, Du H, Kommalapati RR 2021. Treatment of Poultry Slaughterhouse Wastewater with Membrane Technologies: A Review. Water, 13:1905.

Gomes AJG, Atambo DO, Das KK, Cocke DL, Das KP 2018. Electrochemical remediation of chicken processing plant wastewater. Journal of Environmental Chemical Engineering 6:6028–6036.

Hilares RT, Atoche-Garay DF, Pagaza DAP, Ahmed MA, Andrade GJC, Santos JC 2021. Promising physicochemical technologies for poultry slaughterhouse wastewater treatment: A critical review. Journal of Environmental Chemical Engineering, 9:105174.

Joseph CG, Puma GL, Bono A, Krishnaiah D 2009. Sonophotocatalysis in advanced oxidation process: A short review. Ultrasonics Sonochemistry, 16:583-589.

Kanafin YN, Makhatova A, Meiramkulova K, Poulopoulos SG 2022. Treatment of a poultr slaughterhouse wastewater using advanced oxidation processes. Journal of Water Process Engineering, 47:102694.

Kıdak R, Doğan Ş 2018. Medium-high Frequency Ultrasound and Ozone Based Advanced Oxidation for Amoxicillin Removal in Water. Ultrasonics Sonochemistry, 40(Pt B):131-139.

Matsumura EM, Mierzwa JC 2008. Water conservation and reuse in poultry processing plant — a case study. Resources, Conservation & Recycling, 52 (6):835–842.

Meiramkulova K, Mkilima T, Kydyrbekova A, Nikitin Y, Zandybay A, Khussainov M, Uryumtseva T 2021. Contribution of Electrolysis within an Integrated System for a Poultry Slaughterhouse Wastewater Treatment. Sustainability, 13(22). 12430.

Ngobeni PV, Basitere M, Thole A 2022. Treatment of poultry slaughterhouse wastewater using electrocoagulation: a review. Water Practice & Technology, 17(1): 38-59.

Porhemmat S, Rezvani A, Ghaedi M, Asfaram A, Goudarzi A 2017. Ultrasonic treatment of wastewater contaminated with variousdyes using tin oxide hydroxide nanoparticles loaded on activated carbon: Synthesis, performance, mechanism andstatistical optimization. Applied Organometallic Chemistry, 31(12).

Samsudin MFR, Jayabalan PJ, Ong W-J, Ng, YH, Sufian S 2019. Photocatalytic degradation of real industrial poultry wastewater via platinum decorated BiVO4/g-C3N4 photocatalyst under solar light irradiation. Journal of Photochemistry & Photobiology A: Chemistry, 378:46–56.

Silva AMT, Nouli E, Carmo-Apolinário ÂC, Xekoukoulotakis NP, Mantzavinos D 2007. Sonophotocatalytic/H2O2 degradation of phenolic compounds in agro-industrial effluent. Catalysis Today, 124:232–239.

Sponza DT, Oztekib R 2013. Treatment of the Olive Mill Industry Wastewater with Ultrasound and Some Nano-Sized Metal Oxides. Journal of Chemical Engineering & Process Technology, 4(2):1000147.

Sridhar R, Sivakumar V, Prince Immanuel V, Prakash Maran J 2012. Development of model for treatment of pulp and paper industry bleaching effluent using response surface methodology. Environ Progress& Sustainable Energy, 31: 558–65.

Stando K, Kasprzyk P, Felis E, Bajkacz S 2021. Heterogeneous Photocatalysis of Metronidazole in Aquatic Samples. *molecules*, 26:7612.

Thirugnanasambandham K, Kandasamy, S, Sivakumar V, Kumar RK, Mohanavelu R 2015. Modeling of by-product recovery and performance evaluation of Electro-Fenton treatment technique to treat poultry wastewater. Journal of the Taiwan Institute of Chemical Engineers, 46:89–97.

Thirugnanasambandham K, Sivakumar V, Prakash Maran J 2014. Efficiency of electrocoagulation method to treat chicken processing industry wastewater—modeling and optimization. Journal of the Taiwan Institute of Chemical Engineers 45: 2427–2435.

UN General Assembly 2015. Transforming our world: the 2030 Agenda for Sustainable Development, A/RES/70/1. Available at: http://www.refworld.org/docid/57b6e3e44.html [21.10.2015].

WHO 2022. WHO Coronavirus (COVID-19) Dashboard. Available at: http://covid19.who.int [11.11.2022].

Yaakob MA, Mohamed RMSR, Al-Gheethi AAS, Kassim AHM 2018. Characteristics of chicken slaughterhouse wastewater. Chemical Engineering Transactions, 63:637-642.

Yang Y, Wu ZS, Yang RP, Li YF, Liu XC, Zhang LH, Yu B 2021. Insights into the mechanism of enhanced photocatalytic dye degradation and antibacterial activity over ternary ZnO/ZnSe/$MoSe_2$ photocatalysts under visible light irradiation. Applied Surface Science, 539:148220.

Yetilmezsoy K, Sakar S 2008. Improvement of COD and color removal from UASB treated poultry manure wastewater using Fenton's oxidation. Journal of Hazardous Materials 151:547–558.

# Preparation and characterization of controlled release polyurethane-based fragrance capsules including medicinal lavender oil for aromatheraphy

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## Abstract

Aromatherapy is a supplementary therapy that uses essential oils (EOs) as the primary therapeutic agents to treat a variety of ailments. Various methods are used to extract EOs from the plant's flowers, barks, leaves, roots, stems, fruits, etc. Microencapsulation technology is an efficient method for controlling the release properties of EOs or perfumes, thereby extending the functional life of cosmetic textiles. In this paper, we investigate polyurethane microcapsules based on polyethylene glycol ($PEG_{1000}$) and polypropylene glycol ($PPG_{1200}$) for the release of medicinal lavender oil smells for textiles. The interfacial interaction of 2,4-toluene diisocyanate (TDI) with $PEG_{1000}$-$PPG_{1200}$ at the shell enclosed the original lavender oil in a polyurethane shell. Fourier transform infrared spectroscopy (FT-IR), particle size distribution (PSD), polarized optical microscopy (POM), and scanning electron microscopy (SEM) were used to investigate the fragrances capsules. The fragrance capsules' release behavior was determined by periodic weight checks over 30 days, and an encapsulation ratio was derived based on the release data. The FT-IR spectrum analysis revealed the particular peaks of -NH, -C=O, and -CN of the PU, with the average particle size ranging from 15 to 45µm. POM demonstrated that particles might be suspended in water and viewed. SEM analysis revealed a spherical shape size distribution. The microcapsules produced with toluene diisocyanate encapsulated 39.55 and%, 35.53 % of lavender oil, respectively, according to the release analysis.

**Keywords:** Essential oils, Fragrance capsules, Polyurethane polymers, Controlled release

## INTRODUCTION

Aromatherapy is an alternative and complementary therapy that uses EOs as the primary therapeutic agents to treat a variety of ailments (Ali et al., 2015). R.M. Gattefosse, a French cosmetic chemist, invented the term aromatherapy in the late 1920s after noticing the remarkable antibacterial capabilities and skin permeability of EOs (Buchbauer and Jirovetz, 1994). EOs are the complex combinations of various organic molecules extracted from different parts of plants by different methods and their chemical composition influences their biological activity and aroma. These natural chemicals have a wide range of qualities that make them useful in a variety of applications (Sousa et al., 2022). Scientific advancements have enhanced the production of novel goods containing EOs in textile fabrics. Microencapsulation is one of the most adaptable processes because small microcapsules may be efficiently made and applied to fabrics, increasing their dynamics, comfort, or utility. Microcapsules are tiny particles that have a wide range of commercial applications. Some well-known examples are controlled release of pharmaceuticals, textiles, cosmetics, adhesives, and inhibitors (Song et al., 2021).

Microencapsulation technology seeks to add additional functionality through the use of various approaches in manufacturing and applications. They concentrated on increasing textile thermal comfort qualities by incorporating phase-changing polymers (Mondal, 2008; Sarier and Onder, 2012; Xiao et al., 2015). The popularity of odor capsule-containing textile goods is growing by the day. Because of its commercial relevance, the manufacture of these capsules is yet unknown. Because of their volatile qualities, fragrances cannot be applied directly to textiles. As a result, they are used in textile materials to encase smells in a shell and preserve them from external effects (Lu et al., 2020; Rodrigues et al., 2008). The majority of commercially available fragrance microcapsules are amine-formaldehyde-based, however their use is restricted due to their environmental impact (Leclercq et al., 2009). A sophisticated conversion procedure can be used to create microcapsules containing volatiles from gelatin and Arabic gum in a variety of sizes. Because the microcapsules are often low molecular weight polymers, and the mechanical strength of the microcapsule to external forces is low and breaks quickly, the microcapsules can cling to each other in this approach, and the particle size can create coarser particles at different intervals. The interfacial polymerization process is one of the most effective approaches for making polyurethane odor capsules (Specos et al., 2010). The mechanical properties of the microcapsules and the release time are directly affected by the molecular weight of the diol

utilized in manufacture. The mechanical strength and release time increase with increasing molecule weight (Pušla, 2015). Various EOs (such as orange, lemon, jasmine, and lavender) with varying particle sizes have been produced and applied to textile fabrics, with the release, washing, and friction qualities examined (Hong and Park, 1999; Rodrigues et al., 2009). According to the literature review, the polymerization technique, mixing rate, fragrance type, wall material components, capsule size, and mechanical properties of the produced capsules all have a substantial impact on the odor release qualities of the microcapsules. It has also been demonstrated that the washing resistance of textiles varies depending on the application methods and crosslinkers utilized, with the length of application and the amount of microcapsules sticking to the fabric being directly affected (Azizi et al., 2014; Biswas et al., 2015; Feczkó et al., 2010; Tekin et al., 2013).

Lavender oil is one of the most researched perfumes, as EOs have been extensively researched as a signal molecule (Sharifi and Ryu, 2018). Lavender oil's main EOs components are linalool, linalyl acetate, and cineole. The most essential of these is linalyl acetate, which defines the quality of lavender (Ünal, 2018). EOs can be consumed orally, by inhalation, or through massage. When we look at the research on the benefits of lavender oil, we see that linalool and linalyl acetate in lavender oil have narcotic and sedative properties (Song et al., 2021). EOs can be consumed orally, by inhalation, or through massage. When we look at the research on the benefits of lavender oil, we see that linalool and linalyl acetate in lavender oil have narcotic and sedative properties. Gednay et al. placed a cotton muslin cloth with 5 drops of lavender oil next to the patients. They discovered that breathing increased sleep quality in a short period of time (Gedney et al., 2004). Furthermore, Peng et al. discovered the soothing effect of acetylcholine released by lavender oil on the sleep transition (Song et al., 2021). Several writers have given overviews of the potential applications of encapsulated EOs in food and medicine (Bhatt et al., 2022; Delshadi et al., 2020; Lammari et al., 2020; Maes et al., 2019).

However, due to EOs volatile nature, the effect of lavender oil was very limited. Capsules that can release more effective lavender oil for a longer period of time can be produced using microencapsulation technology, and ease of use can be provided by applying them to textile materials. In this study, medicinal lavender oil was selected to produce polyurethane fragrance capsules by reacting a diol with diisocyanates. It aims to comparatively demonstrate the possible PU shell materials for the controlled release of medicinal lavender oil. The aim is not only to optimize the shell structure, but also to clarify the chemical structure-property relationships in the shell. These capsules were found to be candidates for controlled release materials.

## MATERIALS AND METHODS

### Materials

The study employed TDI, $PEG_{1000}$, $PPG_{1200}$, Polyvinyl alcohol (Moviol 3-96), Sigma Aldrich, Dibutyltindilaurat (DBDTL), Merck, and lavender EO produced from naturally cultivated lavender flowers in the province of Tokat (Kocatürk pharmacy).

### Methods

Polyurethane fragrance capsules were created using the interfacial polymerization approach as shown Figure 1. (stirring at 200 rpm for 2 hours at 70 °C in a jacketed glass reactor). The fragrance capsules were then cleaned with 30% ethanol and allowed to dry at room temperature.




**Figure 1.** Experimental set-up for the preparation of lavender microcapsules.

Table 1. lists the recipes used in polymerization. The oil phase was made by combining 20 mL of lavender oil with 7.5 mL of isocyanate, while the first water phase was made with 120 mL of water and 2 g of Moviol 3-96 (protective colloid). The oil-water phase was then combined in the homogenizer for 5 minutes while being stirred at 4000 rpm. The emulsion was pumped into the glass batch reactor. The second water phase was made and combined with the emulsion in the reactor, containing 60 mL water, 0.5 mL DBDTL, and another mass of $PEG_{1000}$ and $PPG_{1200}$.

**Table 1.** The composition and chemical system of produced lavender microcapsules

| Microcapsules | Phase | Lavender Oil (mL) | Water (mL) | Isocyanate (mL) | PVA (g) | PEG-PPG (mL) | DBTDL (mL) |
|---|---|---|---|---|---|---|---|
| Mic-1 | O.P | 20 | | 7.50 TDI | | | |
| | $W.P_1$ | | 120 | | 2 | | |
| | $W.P_2$ | | 50 | | | 52.53 $PEG_{1000}$ | 0.50 |
| Mic-2 | O.P | 20 | | 7.50 TDI | | | |
| | $W.P_1$ | | 120 | | 2 | | |
| | $W.P_2$ | | 50 | | | 12.60 $PPG_{1200}$ | 0.50 |

Microcapsule encapsulation rates were calculated using the following formula for the 30-day controlled release results. The calculated encapsulation rates were 39.55% and 35.53% for Mic-1 and Mic-2, respectively.

$$\%ER = \frac{FDW - LDW}{FDW} x100$$

E.R: Encapsulation rate, L.D.W: Weight on last day and F.D.W: Weight on first day.

## RESULTS and DISCUSSION

### PSD Analysis Results

PSD and size graphs of the prepared capsules were determined using Malvern MS2000 E model PSD. Prior to particle size measurement, 0.5 g of microcapsules, 2-3 drops of surfactant (Triton-X 100) and 50 ml of deionized water were mixed with a homogenizer at a speed of 10000 rpm for 15 minutes to obtain a homogeneous microcapsule dispersion.

The particle size measurements were conducted three times, and the results are shown in Figure 2. The PSD demonstrated that the particle size was primarily dispersed in the range of 6-68 μm for usage in aramorephatic textile materials, with the average particle size of microcapsules being around 35 μm. The fact that the peak size distribution is quite narrow and simple implies that the microcapsule system is quite stable and homogenous.

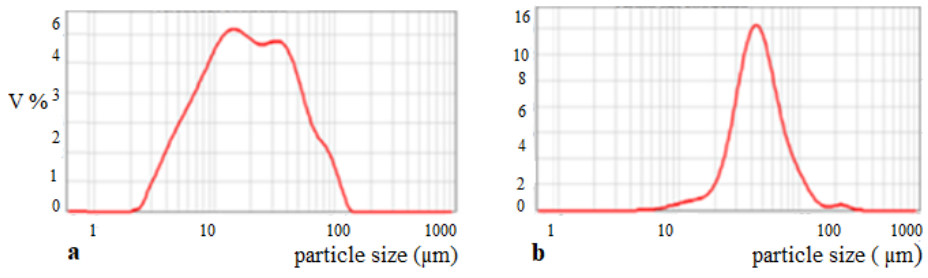


**Figure 2.** PSD graphs of a; Mic-1 and b; Mic-2 fragrance capsules with medicinal lavender oil

The weight percentage of the microcapsules (d (0.1)) was shown in Table 2: d (0.1%): the size of particles in the first 10%, d (0.5): the size of particles in the first 50%, and d (0.9): 90% of the original surface area, the surface area weighted average (d [3,2]), and the volume weighted average of particle diameters (d [4,3]).

**Table 2.** PSD parameters of medicinal lavender Mic-1 and Mic-2.

| Microcapsules | d (0.1) (μm) | d(0.5) (μm) | d(0.9) (μm) | Specific Surface Area ($m^2/g$) | d(3,2) (μm) | d(4,3) (μm) |
|---|---|---|---|---|---|---|
| **Mic-1** | 5.75 | 18.58 | 58.24 | 0.46 | 12.97 | 26.47 |
| **Mic-2** | 29.16 | 44.43 | 67.35 | 0.15 | 40.26 | 47.25 |

**FT-IR Spectroscopy results**

As the core ingredient, medicinal lavender oil was encapsulated with PU polymers produced via interactions of TDI-$PEG_{1000}$ and $PPG_{1200}$. Figure 3. shows the FT-IR spectra of empty PU capsules (made with TDI-$PEG_{1000}$ interactions under the same conditions), Mic-1and Mic-2.

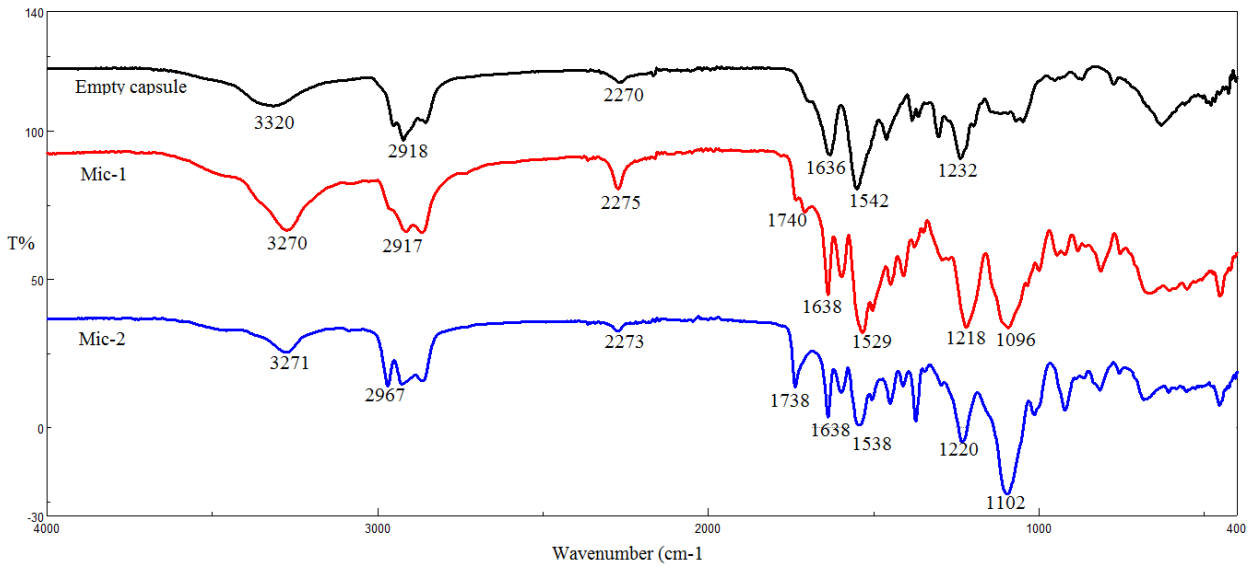


**Figure 3.** FT-IR spectra of PU empty capsule, Mic-1 and Mic-2

The N-H stretching mode is allocated about at 3300 $cm^{-1}$, the urea carbonyl at 1638 $cm^{-1}$, and the amide II and amide III vibrational modes are assigned at 1538 and 1220 $cm^{-1}$, respectively. The vibration at 1100 $cm^{-1}$ corresponds to the ether group's C-O-C stretching mode. These unique peaks were discovered in the structure of PU and were observed in the fragrances capsules that were manufactured. Because of the residual isocyanate, which was known to be essential in PU manufacturing, the peak was not well diffused and at roughly 2270 $cm^{-1}$. According to the FT-IR spectra, the wavenumbers and peak kinds of the PU fragrances capsules were successfully manufactured according to the FT-IR spectra peaks and wave numbers were listed in Table 3.

**Table 3.** Spectral peak assignments and corresponding wavenumbers of Mic-1 and Mic-2

| Peak assignment | Wave numbers ($cm^{-1}$) | |
|---|---|---|
| | **Mic-1** | **Mic-2** |
| -NH (stretching) | 3270 | 3271 |
| -CH (stretching) | 2918 | 2967 |
| -CH (asymmetric stretching) | 2860 | 2860 |
| -C=O (stretching) | 1738 | 1738 |
| -NH, (bending), -CN (stretching) | 1638 | 1638 |
| -NH, (bending), -CN (stretching) | 1529 | 1538 |
| -C-O-C (stretching) | 1096 | 1102 |

**POM and SEM investigations**

The lavender oil was successfully prepered in a spherical microencapsulated form with polyurethane shells, which matched the shape of the microcapsules investigated by POM (bright field at 1200x magnification) and SEM. Figure 4. depicts POM images, while Figure 5. depicts the embrittlement visible in SEM photos.

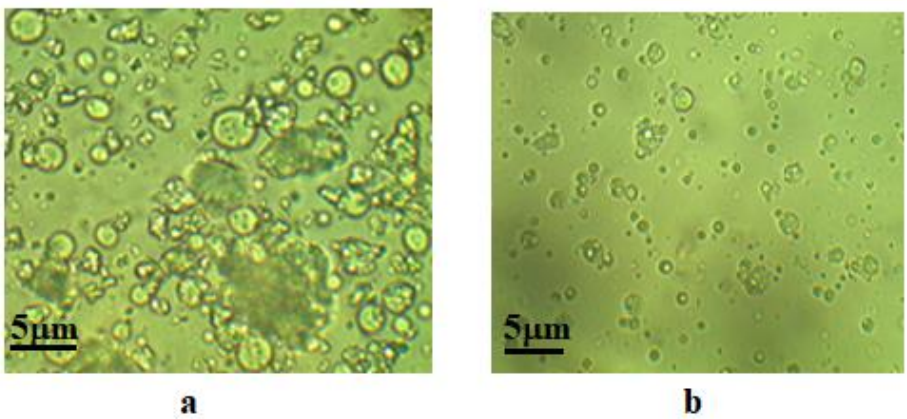


**Figure 4.** POM images of a; Mic-1 and b; Mic-2

The POM figures revealed that the microcapsules are spherical in shape and have smooth surfaces. POM depicted the particles in their true sizes and shapes, despite the fact that figures were obscured due to the depth of the suspension system. Because particles were disseminated to water using a surfactant and there was no coagulation or collapsed particles.

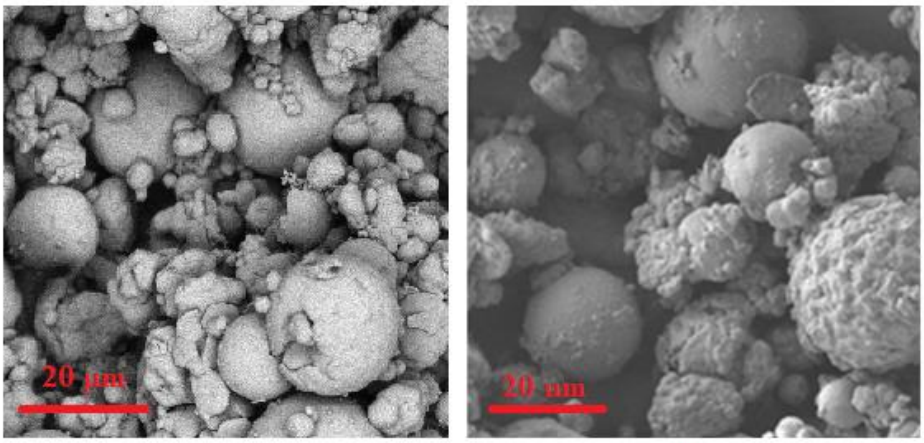


**Figure 5.** SEM images of a; Mic-1 and b; Mic-2

SEM micrographs of medicinal lavender-loaded microcapsules were shown in Figures 5. These observations clearly show that the capsules had a spherical form and a wide range of sizes. SEM images showed the microcapsules 3D morphology better than POM because that they were not distributed into water by means of a surfactant.

**Controlled release analysis of Mic-1 and Mic-2**

The manufactured therapeutic lavender oil capsules were placed in a petri dish and weighed daily for 30 days. The fragrance's weight percentage losses were tracked for 30 days, and it was obvious that they shed 40-45 weight percent on average in each try. Mic-1 and Mic-2 capsules made have the same controlled release behavior for 1 month as shown in Figure 6. At the same time, the release of lavender oil from the microcapsules was identified in the controlled release graphs, corresponding to the calculated encapsulation rate; the reason for the lesser release here is that a tiny amount of lavender oil stays in the capsules.

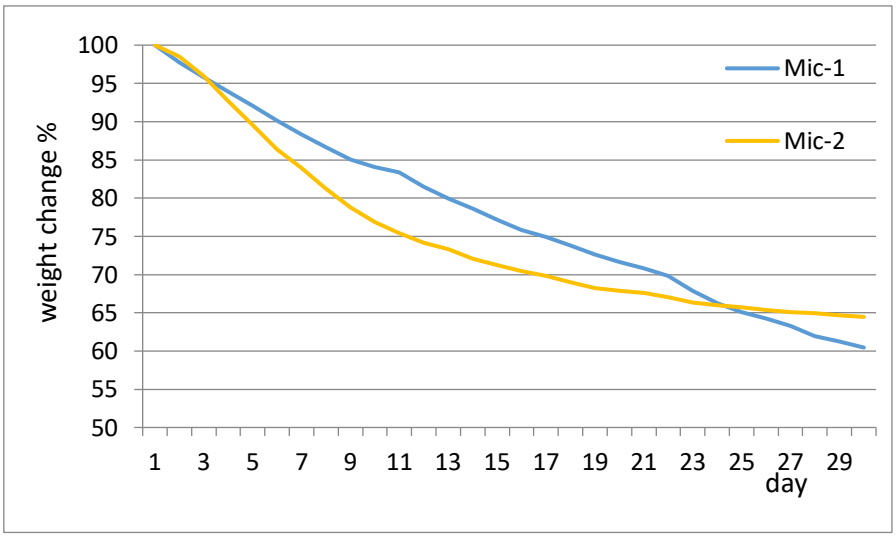


**Figure 6.** Weight percentage of Mic-1 and Mic-2 for 30 days.

## CONCLUSION

Medicinal lavender oil-filled polyurethane fragrance capsules were produced via interfacial polymerization. The microcapsules' 6-70 µm diameter makes them suitable for use aromatrephy in textile applications. By using FT-IR analysis, functional groups of polyurethane were discovered, proving the wall material. The spherical and compact structure of microcapsules was disclosed by the POM/SEM results, and it was found that the fragrances capsules could release lavender oil. At the conclusion of the release test, the fragrance capsules had lost 40–45% of their original weight, and the fragrances had been dispersed into the surrounding air.

Innovative textile materials can be made effectively with the help of microcapsules for aromatrephy for a long time. The smart fabrics could be utilized as both reusable and disposable textiles. In the suggested material system, medical lavender could be used to treat patients for extended periods of time, particularly palliative patients or those with sleep issues.

## ACKNOWLEDGEMENTS

This work was created from Ali Özsevinç's PhD thesis, which he finished from Prof. Cemil Alkan's guidance. The authors would like to thank Cihat Demiryürek, who grew therapeutic lavender plants and made lavender oil in Turkey's Tokat city.

## REFERENCES

Ali, B., Al-Wabel, N.A., Shams, S., Ahamad, A., Khan, S.A., Anwar, F., 2015. Essential oils used in aromatherapy: A systemic review. Asian Pacific Journal of Tropical Biomedicine 5, 601–611. https://doi.org/10.1016/j.apjtb.2015.05.007

Azizi, N., Chevalier, Y., Majdoub, M., 2014. Isosorbide-based microcapsules for cosmeto-textiles. Industrial Crops and Products 52, 150–157. https://doi.org/10.1016/j.indcrop.2013.10.027

Bhatt, L., Kholiya, R., Tewari, S., 2022. Containers for Encapsulation of Fragrances/Aroma/Odour for Textile Applications. pp. 155–178. https://doi.org/10.1007/978-981-16-8146-2_7

Biswas, D., Chakrabarti, S.K., Saha, S.G., Chatterjee, S., 2015. Durable fragrance finishing on jute blended home-textiles by microencapsulated aroma oil. Fibers and Polymers 16, 1882–1889. https://doi.org/10.1007/s12221-015-4829-5

Buchbauer, G., Jirovetz, L., 1994. Aromatherapy—use of fragrances and essential oils as medicaments. Flavour and Fragrance Journal 9, 217–222. https://doi.org/10.1002/ffj.2730090503

Delshadi, R., Bahrami, A., Tafti, A.G., Barba, F.J., Williams, L.L., 2020. Micro and nano-encapsulation of vegetable and essential oils to develop functional food products with improved nutritional profiles. Trends in Food Science & Technology 104, 72–83. https://doi.org/10.1016/j.tifs.2020.07.004

Feczkó, T., Kokol, V., Voncina, B., 2010. Preparation and characterization of ethylcellulose-based microcapsules for sustaining release of a model fragrance. Macromolecular Research 18, 636–640. https://doi.org/10.1007/s13233-010-0701-z

Gedney, J.J., Glover, T.L., Fillingim, R.B., 2004. Sensory and Affective Pain Discrimination After Inhalation of Essential Oils. Psychosomatic Medicine 66, 599–606. https://doi.org/10.1097/01.psy.0000132875.01986.47

Hong, K., Park, S., 1999. Preparation of polyurethane microcapsules with different soft segments and their characteristics. Reactive and Functional Polymers 42, 193–200. https://doi.org/10.1016/S1381-5148(98)00068-6

Lammari, N., Louaer, O., Meniai, A.H., Elaissari, A., 2020. Encapsulation of Essential Oils via Nanoprecipitation Process: Overview, Progress, Challenges and Prospects. Pharmaceutics 12, 431. https://doi.org/10.3390/pharmaceutics12050431

Leclercq, S., Milo, C., Reineccius, G.A., 2009. Effects of Cross-Linking, Capsule Wall Thickness, and Compound Hydrophobicity on Aroma Release from Complex Coacervate Microcapsules. Journal of Agricultural and Food Chemistry 57, 1426–1432. https://doi.org/10.1021/jf802472q

Lu, Y.F., Wang, Y.M., Chen, X.D., Miao, M.H., Zhang, D.H., 2020. Synthesis and shape memory behavior of hyperbranched polyimides from thiol-ene click reaction. Express Polymer Letters 14, 192–204. https://doi.org/10.3144/expresspolymlett.2020.16

Maes, Bouquillon, Fauconnier, 2019. Encapsulation of Essential Oils for the Development of Biosourced Pesticides with Controlled Release: A Review. Molecules 24, 2539. https://doi.org/10.3390/molecules24142539

Mondal, S., 2008. Phase change materials for smart textiles – An overview. Applied Thermal Engineering 28, 1536–1550. https://doi.org/10.1016/j.applthermaleng.2007.08.009

Pušla, J., 2015. Using Chain Extenders to Modify Release Rates of Orange Oil from Poly(Urea-Urethane)

Microcapsules. Acta Chimica Slovenica 700–708. https://doi.org/10.17344/acsi.2015.1434

Rodrigues, S.N., Fernandes, I., Martins, I.M., Mata, V.G., Barreiro, F., Rodrigues, A.E., 2008. Microencapsulation of Limonene for Textile Application. Industrial & Engineering Chemistry Research 47, 4142–4147. https://doi.org/10.1021/ie800090c

Rodrigues, S.N., Martins, I.M., Fernandes, I.P., Gomes, P.B., Mata, V.G., Barreiro, M.F., Rodrigues, A.E., 2009. Scentfashion®: Microencapsulated perfumes for textile application. Chemical Engineering Journal 149, 463–472. https://doi.org/10.1016/j.cej.2009.02.021

Sarier, N., Onder, E., 2012. Organic phase change materials and their textile applications: An overview. Thermochimica Acta 540, 7–60. https://doi.org/10.1016/j.tca.2012.04.013

Sharifi, R., Ryu, C.-M., 2018. Revisiting bacterial volatile-mediated plant growth promotion: lessons from the past and objectives for the future. Annals of Botany 122, 349–358. https://doi.org/10.1093/aob/mcy108

Song, X., Peng, J., Jiang, W., Ye, M., Jiang, L., 2021. Effects of aromatherapy on sleep disorders. Medicine 100, e25727. https://doi.org/10.1097/MD.0000000000025727

Sousa, V.I., Parente, J.F., Marques, J.F., Forte, M.A., Tavares, C.J., 2022. Microencapsulation of Essential Oils: A Review. Polymers 14, 1730. https://doi.org/10.3390/polym14091730

Specos, M.M.M., García, J.J., Tornesello, J., Marino, P., Vecchia, M. Della, Tesoriero, M.V.D., Hermida, L.G., 2010. Microencapsulated citronella oil for mosquito repellent finishing of cotton textiles. Transactions of the Royal Society of Tropical Medicine and Hygiene 104, 653–658. https://doi.org/10.1016/j.trstmh.2010.06.004

Tekin, R., Bac, N., Erdogmus, H., 2013. Microencapsulation of Fragrance and Natural Volatile Oils for Application in Cosmetics, and Household Cleaning Products. Macromolecular Symposia 333, 35–40. https://doi.org/10.1002/masy.201300047

Ünal, K.S., 2018. Aromatherapy Application in Nursing Care: Systematic Analysis of Studies Conducted Over the Past Decade in Turkey. Journal of Traditional Medical Complementary Therapies 1, 61–69. https://doi.org/10.5336/jtracom.2018-62137

Xiao, Z., Li, W., Zhu, G., 2015. Effect of wall materials and core oil on the formation and properties of styralyl acetate microcapsules prepared by complex coacervation. Colloid and Polymer Science 293, 1339–1348. https://doi.org/10.1007/s00396-015-3515-x

## *ORAL PRESENTATION*

# Karışık ligantlı Co(II) ve Ni(II)-2,6-piridindikarboksilik asit komplekslerinin sentezi, karakterizasyonu ve biyolojik etkilerinin incelenmesi

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## Özet

Bu çalışmada $[Co(pdca)(phen)H_2O].H_2O$ (pdca: 2,6-piridindikarboksilik asit, phen: 1,10-fenantrolin), $[Co_2(pdca)_2(H_2O)_5].2H_2O$, $[Ni(pdca)(phen)H_2O]$ ve $[Ni(pdca)_2]$ kompleksleri sentezlendi. Komplekslerin yapıları X-ışınları tek kristal difraksiyon yöntemi, FT-IR, UV-Vis. ve elementel analiz yöntemleriyle belirlendi. Sentezlenen komplekslerin sitotoksik etkinliği A 549 (ATCC® CCL-185™) insan akciğer kanser hücre hatlarında XTT ((2,3-bis[2-metoksi- 4-nitro- 5-sulfofenil]- 2H-tetrazolyum- 5-karboksianilit iç tuzu) yöntemi ile *in vitro* olarak araştırıldı.

**Anahtar Kelimeler:** 2,6-piridindikarboksilik asit, kobalt(II), nikel(II), 1,10-fenantrolin

# Synthesis, characterization and investigation of biological effects of mixed liganded Co(II) and Ni(II)-2,6-pyridinedicarboxylic acid complexes

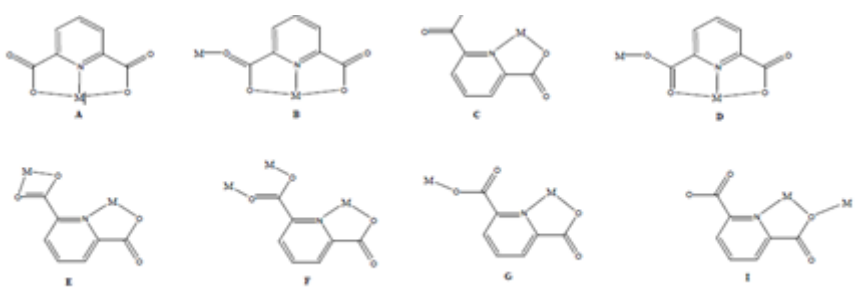
## Abstract

In this study $[Co(pdca)(phen)H_2O].H_2O$ (pdca: 2,6-pyridinedicarboxylic acid, phen: 1,10-phenanthroline), $[Co_2(pdca)_2(H_2O)_5].2H_2O$, $[Ni(pdca)(phen)H_2O]$ and $[Ni(pdca)_2]$ complexes were synthesized. Structures of complexes X-ray single crystal diffraction method, FT-IR, UV-Vis. and elemental analysis methods. Cytotoxic activity of the synthesized complexes in A 549 (ATCC® CCL-185™) human lung cancer cell lines XTT ((2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide) salt) method was investigated in vitro.

**Keywords:** 2,6-pyridinedicarboxylic acid, cobalt(II), nickel(II), 1,10-phenanthroline

## GİRİŞ

2,6-piridindikarboksilik asit geçiş metallerine ya iki dişli olarak, metal merkezleri arasında karboksilat köprüleri yoluyla polimerik veya dimerik kompleksler oluşturmak üzere ya da üç dişli olarak (O, N, O') bir metal iyonuna şelat oluşturmak üzere bağlanırlar (Aghabozorg ve ark., 2008). 2,6-piridindikarboksilik asitte (dipikolinik asit) merkez piridin halkası ve iki karboksilat grubu arasında 120$^o$ lik bir açı olması, metal kompleksleri için çok çeşitli koordinasyon türleri sağlamaktadır (Wang ve ark., 2004) (şekil 1). 2,6-piridindikarboksilik asit analitik kimyada (Kanai 1990), nükleer reaktörlerin arıtılması, inhibisyon uygulamaları ve biyolojik aktivite gibi alanlardaki uygulamaları ile tanınmaktadır (Bailey ve ark., 1965, Setlow ve ark., 1993).



**Şekil 1.** 2,6-Piridindikarboksilik asit için olası metal bağlanma yerleri

## MATERYAL VE METOT

### Materyaller

Komplekslerin sentezinde; $CoCl_2.6H_2O$ (Aldrich), $NiCl_2.6H_2O$ (Aldrich), 2,6-PDCA (Sigma), 1,10-fenantrolin monohidrat (Merck), Aspartik asit (Sigma), Glutamik asit (Sigma), NaOH (Sigma-Aldrich), Ultra saflıkta su, Etil alkol (Merck) ve 2-propanol (Merck) kullanıldı.

### Cihazlar

Sentezlenen komplekslerin tek kristal X-ışınları, grafit monokromatörlü Photon III C14 dedektörlü Bruker D8 Quest difraktometresi ile alındı. FT-IR spektrumları, 4000–400 $cm^{-1}$ frekans aralığında Shimadzu Prestige 21 FT-IR ATR spektrofotometresi ile alındı. UV-vis. soğurma spektrumları, Varian Cary 50 UV-vis. spektrofotometresi ile 200 ile 800 nm dalga boyu aralığında tarandı. Erime noktaları, Buchi 540 erime noktası tayin cihazı kullanılarak belirlendi. Komplekslerin elementel (C, H ve N) analizleri Costech ECS 4010 marka Elementel Analiz cihazı ile yapıldı.

### Komplekslerin Sentezi

$[C_{19}H_{13}CoN_3O_5 \cdot H_2O]H_2O$

$CoCl_2.6H_2O$ (1 mmol, 0,1189 g) (10 mL) sulu çözeltisine 2,6-PDCA (0,5 mmol, 0,0835 g) (10 mL) sulu çözeltisi eklendi. Oluşan turuncu renkli çözelti 60 °C'de iki saat karıştırıldı. Üzerine 1,10-fenantrolin (1 mmol, 0,1982 g) (10 mL) sulu çözeltisi eklendi. Oluşan çözeltinin rengi daha da koyulaştı. 60 °C'de iki saat karıştırılan çözelti kristallenmeye bırakıldı. Oda sıcaklığında 1-2 hafta sonra turuncu renkli tek kristal elde edildi.

$[C_{14}H_{20}N_2O_{15}Co_2]$

$CoCl_2.6H_2O$ (1 mmol, 0,1189 g) (10 mL) sulu çözeltisine 2,6-PDCA (0,5 mmol, 0,0835 g) (10 mL) sulu çözeltisi eklendi. Oluşan turuncu renkli çözelti 60 °C'de iki saat karıştırıldı. Üzerine aspartik asit (1 mmol, 0,1331 g) (10 mL) sulu çözeltisi eklendi. Oluşan kırmızı-kahverenkli çözelti 60 °C'de iki saat karıştırılıp kristallenmeye bırakıldı. Oda sıcaklığında 1-2 hafta sonra kırmızı-kahverenkli tek kristal elde edildi.

$[C_{19}H_{13}N_3NiO_5 \cdot H_2O]$

$NiCl_2.6H_2O$ (1 mmol, 0,2376 g) (10 mL) sulu çözeltisine 2,6-PDCA (0,5 mmol, 0,0835 g) (10 mL) sulu çözeltisi eklendi. Oluşan mavi renkli çözelti 60 °C'de iki saat karıştırıldı. Üzerine 1,10-fenantrolin (1 mmol, 0,1982 g) (10 mL) sulu çözeltisi eklendi. Oluşan çözeltinin rengi daha da koyulaştı. 60 °C'de iki saat karıştırılan çözelti kristallenmeye bırakıldı. Oda sıcaklığında 1-2 hafta sonra mavi renkli tek kristal elde edildi.

$[C_{14}H_{12}N_2NiO_{11}]$

$NiCl_2.6H_2O$ (1 mmol, 0,2376 g) (10 mL) sulu çözeltisine 2,6-PDCA (0,5 mmol, 0,0835 g) (10 mL) sulu çözeltisi eklendi. Oluşan turuncu renkli çözelti 60 °C'de iki saat karıştırıldı. Üzerine glutamik asit ( 1 mmol, 0,1471) (10 mL) sulu çözeltisi eklendi. Oluşan mavi renkli çözelti 60 °C'de iki saat karıştırılıp kristallenmeye bırakıldı. Oda sıcaklığında 1-2 hafta sonra mavi renkli tek kristal elde edildi.

### Biyolojik Activite

Numunelerin sitotoksik etkinliği A 549 (ATCC® CCL-185™) insan akciğer kanser hücre hatlarında XTT ((2,3-bis[2-metoksi- 4-nitro- 5-sulfofenil]- 2H-tetrazolyum- 5-karboksianilit iç tuzu) yöntemi ile *in vitro* olarak araştırılmıştır.

**Hücre Kültürü:** A549 hücreleri (ATCC® CCL-185™) 37 $^oC$ ve %5 $CO_2$ içeren ortama sahip inkübatörlerde havalandırmalı T75 flasklar içerisinde kültüre edilmiştir. Kültür ortamı olarak kullanılan besiyeri içeriği; F-12K medyum (ATCC, USA), %10 Fetal Calf serum (PAN Biotech, Aidenbach, Germany) 1mM SodyumPürivat (PAN Biotech, Aidenbach, Germany).1 mM L- Glutamin (Sigma Aldrich, Darmstadt, Germany), 100U/ml Penisilin- 100 µg/ml Streptomisin (Bioch-rom AG, Berlin, Germany) ile oluşturulmuştur.

## BULGULAR ve TARTIŞMA

Sentezlenen komplekslerin renk, erime (bozunma) sıcaklığı ve molekül ağırlığı gibi bazı fiziksel özellikleri çizelge 1'de verilmektedir.

**Çizelge 1.** Sentezlenen komplekslerin bazı fiziksel özellikleri

| Kompleksler | Renk | E.N.(°C) | M.K. (g/mol) |
|---|---|---|---|
| $[C_{19}H_{13}CoN_3O_5 \cdot H_2O]H_2O$ | Turuncu | > 310 | 458,28 |
| $[C_{14}H_{20}N_2O_{15}Co_2]$ | Kırmızı-Kahverengi | > 310 | 574,17 |
| $[C_{19}H_{13}N_3NiO_5 \cdot H_2O]$ | Mavi | > 310 | 440,05 |
| $[C_{14}H_{12}N_2NiO_{11}]$ | Mavi | > 310 | 442,97 |

Sentezlenen komplekslerin elementel analiz sonuçları ve yüzde verimleri Çizelge 2'de verilmektedir. Komplekslerin hepsi kristal halinde elde edildi ve yapıları X-ışını kırınımı yöntemiyle aydınlatıldı.

**Çizelge 2.** Sentezlenen komplekslerin verimi ve elementel analiz sonuçları[a]

| Kompleks | % Bileşim | | | % Verim |
|---|---|---|---|---|
| | C | H | N | |
| $[C_{19}H_{13}CoN_3O_5 \cdot H_2O]H_2O$ | 49,87 (49,79) | 3,85 (3,73) | 9,29 (9,16) | 78 |
| $[C_{14}H_{20}N_2O_{15}Co_2]$ | 29,38 (29,26) | 3,39 (3,48) | 4,91 (4,87) | 83 |
| $[C_{19}H_{13}N_3NiO_5 \cdot H_2O]$ | 51,73 (51,85) | 3,54 (3,43) | 9,68 (9,54) | 70 |
| $[C_{14}H_{12}N_2NiO_{11}]$ | 37,74 (37,96) | 2,87 (2,73) | 6,19 (6,32) | 71 |

[a]Hesaplanan değerler parantez içinde verilmiştir.

**X-Işını Kırınımı**

**1.** $[Co(pdca)(phen)H_2O].H_2O$

Triklinik sistemde kristallenen $[Co(pdca)(phen)H_2O].H_2O$ kompleksi birim hücrede iki (2) molekül içerir. Komplekse ait kristalografik veriler Çizelge 3'te verilmektedir.

Komplekste kobalt (II) iyonuna üç dişli olarak koordine olan 2,6-PDCA ligandı ve iki dişli olarak koordine olan phen ligandı, kobalt (II) iyonu çevresinde bozulmuş oktahedral geometri oluşturmaktadır (Şekil 2).

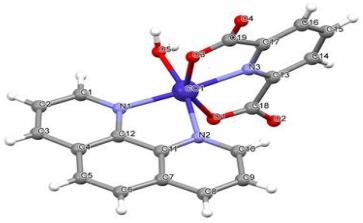
Koordinasyon küresinde kobalt (II) iyonuna şelat şeklinde koordine olan 2,6-PDCA ve phen ligantlarının şelat açılarının (N3–Co1–O5 91,24 (17)°, N1–Co1–N2 78,30 (16)°, N3–Co1–O3 75.86 (15)°, N1–Co1–O3 95.42 (15)°, N2–Co1–O3 88.78 (17)°, N2–Co1–O1 89,89 (15)° olması koordinasyon geometrisinde bozulmaya neden olmaktadır.

**2.** $[Co_2(pdca)_2(H_2O)_5].2H_2O$

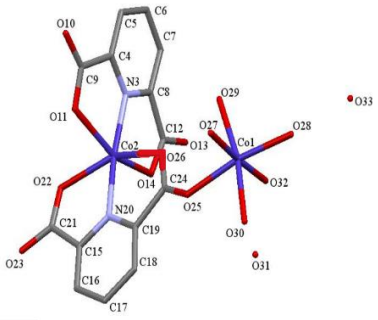
Monoklinik sistemde kristallenen $[Co_2(pdca)_2(H_2O)_5].2H_2O$ kompleksi birim hücrede dört (4) molekül içerir. Komplekse ait kristalografik veriler Çizelge 3'te verilmektedir.

Komplekste kobalt (II) iyonuna üç dişli olarak koordine olan 2,6-PDCA ligandı kobalt (II) iyonu çevresinde bozulmuş oktahedral geometri oluşturmaktadır (Şekil 3).

Koordinasyon küresinde kobalt (II) iyonuna şelat şeklinde koordine olan 2,6-PDCA ligandının şelat açılarının (O25–Co1–O30 79,9(2)°, O22–Co1–O11 85,9(2)°, O22–Co2–N20 75,6(2)°, O14–Co2–N3 75,8(2)° olması koordinasyon geometrisinde bozulmaya neden olmaktadır.



**Şekil 2.** [Co(pdca)(phen)$H_2O$].$H_2O$ kompleksin yapısı.



**Şekil 3.** [$Co_2$(pdca)$_2$($H_2O$)$_5$].2$H_2O$ kompleksin yapısı.

**Çizelge 3.** [Co(pdca)(phen)$H_2O$].$H_2O$ ve [$Co_2$(pdca)$_2$($H_2O$)$_5$].2$H_2O$ komplekslerine ait kristalografik veriler.

| | | |
|---|---|---|
| Kimyasal formül | $C_{19}H_{13}CoN_3O_5 \cdot 2(H_2O)$ | $C_{14}H_{20}N_2O_{15}Co_2$ |
| Formül kütlesi | 458,28 | 574,17 |
| Kristal sistemi | Triklinik | Monoklinik |
| Uzay grubu | P-1 | C21 |
| a (Å) | 7,826 (3) | 8,3807 (7) |
| b (Å) | 9,281 (3) | 27,3469 (17) |
| c (Å) | 14,069 (5) | 9,6147 (6) |
| ά (º) | 81,409 (12) | 90,095 (5) |
| β (º) | 81,264 (13) | 98,285 (6) |
| γ (º) | 73,109 (11) | 89,968 (6) |
| Birim hücre hacmi, V (Å$^3$) | 960,3 (6) | 2180,60 (3) |
| Birim hücredeki molekül sayısı, Z | 2 | 4 |
| Hesaplanan yoğunluk, ρ (g cm$^{-3}$) | 1,585 | 1,725 |
| Elektron sayısı, F (000) | 470 | 1148 |
| Çizgisel soğurma katsayısı, μ(mm$^{-1}$) | 0,94 | 1,592 |
| Kristal boyutları (mm) | 0,09 × 0,05 × 0,04 | 0,09 × 0,14 × 0,16 |
| Veri toplama sıcaklığı, T (K) | 296 | 293 (2) |
| Tmin; Tmax. | 0,945; 0,963 | 3,363 ; 28,923 |
| h, k, l aralığı (º) | 10, 12,18 | 11, 30,12 |
| Parametre sayısı | 289 | 309 |
| Toplanan yansıma sayısı | 4434 | 8883 |
| Bağımsız yansımalar ($R_{int}$) | 0,074 | 0,0858 |
| R[$F^2 > 2\sigma(F^2)$] | 0,067 | 0,0764 |
| wR ($F^2$) | 0,173 | 0,1933 |
| $F^2$ | 1,11 | 1,149 |
| $\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å$^{-3}$) | 0,65; – 0,48 | 0,193, – 0,076 |

**3.** [Ni(pdca)(phen)$H_2O$]

Kristal sistemi monoklinik olan [Ni(pdca)(phen)$H_2O$] kompleksi birim hücrede oniki (12) molekül içerir. Komplekse ait kristalografik veriler Çizelge 4'te verilmektedir.

Komplekste nikel (II) iyonuna üç dişli olarak koordine olan 2,6-PDCA ligandı ve iki dişli olarak koordine olan phen ligandı, nikel (II) iyonu çevresinde bozulmuş oktahedral geometri oluşturmaktadır (Şekil 4).

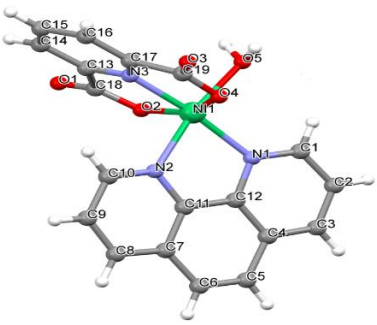
Koordinasyon küresinde nikel (II) iyonuna şelat şeklinde koordine olan 2,6-PDCA ve phen ligantlarının şelat açılarının (N1–Ni–N2 79,84(11)°, N3–Ni–N2 93,89(10)°, N2–Ni–O2 93,01(9)°, N2–Ni–O4 91,25(9)°, N3–Ni–O2 78,69(11)°) olması koordinasyon geometrisinde bozulmaya neden olmaktadır.

**4.** $[Ni(pdca)_2]$

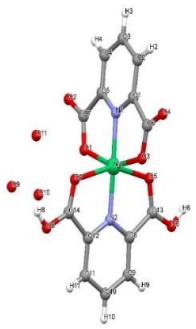
Triklinik sistemde kristallenen $[Ni(pdca)_2]$ kompleksi birim hücrede dört (4) molekül içerir. Komplekse ait kristalografik veriler Çizelge 4'te verilmektedir.

Komplekste nikel (II) iyonuna üç dişli olarak koordine olan 2,6-PDCA ligandı nikel (II) iyonu çevresinde bozulmuş oktahedral geometri oluşturmaktadır (Şekil 5).

Koordinasyon küresinde nikel (II) iyonuna şelat şeklinde koordine olan 2,6-PDCA ligandının şelat açılarının (N1–Ni1–O3 78.83 (8)°, N2–Ni1–O5 78.58 (9)°, N1–Ni1–O1 77.23 (8)º, N2–Ni1–O1 99.33 (8)º olması koordinasyon geometrisinde bozulmaya neden olmaktadır.




**Şekil 4.** $[Ni(pdca)(phen)H_2O]$ kompleksinin yapısı.



**Şekil 5.** $[Ni(pdca)_2]$ kompleksinin yapısı.

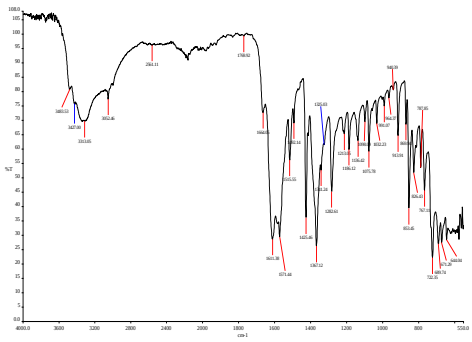
**Çizelge 4.** $[Ni(pdca)(phen)H_2O]$ ve $[Ni(pdca)_2]$ komplekslerine ait kristalografik veriler.

| | | |
|---|---|---|
| Kimyasal formül | $C_{19}H_{13}N_3NiO_5.H_2O$ | $C_{14}H_{12}N_2NiO_{11}$ |
| Formül kütlesi | 440,05 | 442,97 |
| Kristal sistemi | Monoklinik | Triklinik |
| Uzay grubu | P21/c | P2(1) |
| a (Å) | 10,6828 (15) | 13,685 (5) |
| b (Å) | 27,275 (4) | 10,048 (4) |
| c (Å) | 19.229 (3) | 13,792 (5) |
| ά (º) | 90 | 90 |
| β (º) | 104,835(4) | 115,205 (9) |
| γ (º) | 90 | 90 |
| Birim hücre hacmi, V (Å$^3$) | 5416,1 (14) | 1715,9 (12) |
| Birim hücredeki molekül sayısı, Z | 12 | 4 |
| Hesaplanan yoğunluk, ρ (g cm$^{-3}$) | 1,619 | 1,715 |
| Elektron sayısı, F (000) | 2712,0 | 904 |
| Çizgisel soğurma katsayısı, μ(mm$^{-1}$) | 1,119 | 1,197 |
| Kristal boyutları (mm) | 0,14 × 0,09 × 0,08 | 0,09 × 0,14 × 0,16 |
| Veri toplama sıcaklığı, T (K) | 296 | 296 |
| Tmin; Tmax. | 0,886; 0,914 | 2,611; 28,364 |
| h, k, l aralığı (º) | 14, 36, 25 | 18, 13,18 |
| Parametre sayısı | 820 | 273 |
| Toplanan yansıma sayısı | 13426 | 4269 |
| | | 0,081 |

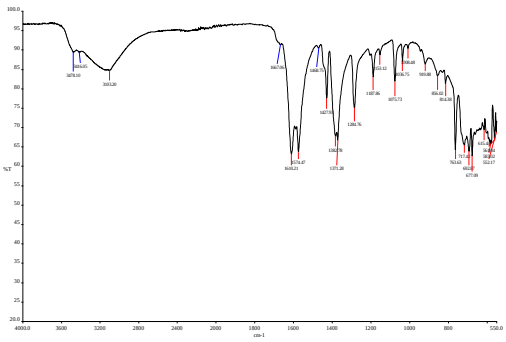
| | | |
|---|---|---|
| Bağımsız yansımalar ($R_{int}$) | 0,068 | 0,123 |
| $R[F^2 > 2\sigma(F^2)]$ | 0,0395 | 0,173 |
| wR ($F^2$) | 0,118 | 1,15 |
| $F^2$ | 1,15 | 0.56, −0.60 |
| $\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å$^{-3}$) | 0.56, −0.60 | |

**FTIR**

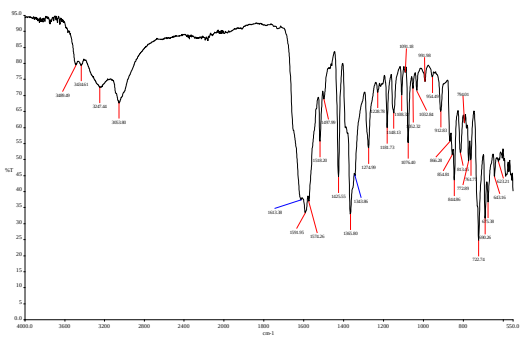
İki dişli ligant içeren nikel(II) ve kobalt(II) 2,6-piridindikarboksilik asit komplekslerine ait IR spektrumları (şekil 6-9) verilirken, spektrumlardaki bazı karakteristik soğurma bantları da çizelge 5'te listelenmiştir.



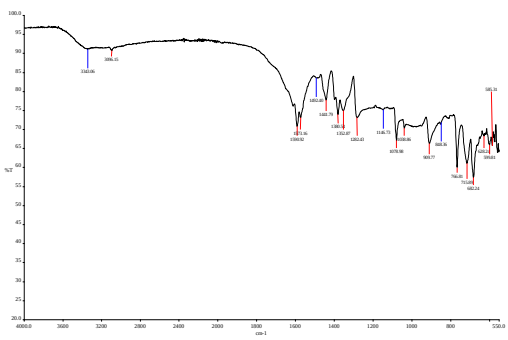
**Şekil 6.** [Co(pdca)(phen)$H_2O$].$H_2O$ kompleksinin IR spektrumu.



**Şekil 7.** [$Co_2$(pdca)$_2$($H_2O$)$_5$].2$H_2O$ kompleksinin IR spektrumu.



**Şekil 8.** [Ni(pdca)(phen)$H_2O$] kompleksinin IR spektrumu.



**Şekil 9.** [Ni(pdca)$_2$] kompleksinin IR spektrumu.

**Çizelge 5.** Nikel(II) ve kobalt(II) komplekslerin önemli IR titreşimleri ($cm^{-1}$)

| **Kompleks** | **ν(OH)** | **ν(N-H)** | **ν(C-H)** | **ν(C=O)** | **ν(C=N)** | **ν(C=C)** | **ν(C-O)** | **ν(C-N)** |
|---|---|---|---|---|---|---|---|---|
| $[Ni(pdca)(phen)H_2O]$ | 3489o<br>3434o | 3247o | 3053o | 1613şy<br>1591ş<br>1574ş | 1425çş | 1365çş | 1228o | 1148ş |
| $[Ni(pdca)_2]$ | - | 3343z | 3096z | 1590o<br>1573o | 1441o | 1352o | 1282o | 1146z |
| $[Co(pdca)(phen)H_2O].H_2O$ | 3483o<br>3427o | 3313y | 3052o | 1611ş<br>1571ş | 1425çş | 1367çş | 1282ş | 1136ş |
| $[Co_2(pdca)_2(H_2O)_5].2H_2O$ | 3478z<br>3416z | - | 3103y | 1610ş<br>1574ş | 1427ş | 1371ş | 1284ş | 1153o |

çş: çok şiddetli, ş: şiddetli, o: orta, y: yayvan, z: zayıf, dalga sayısı; $cm^{-1}$

**UV-vis.**

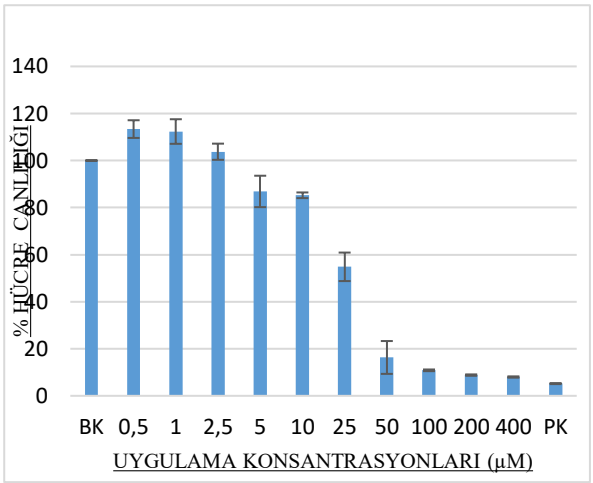
$[Co(pdca)(phen)H_2O].H_2O$, $[Co_2(pdca)_2(H_2O)_5].2H_2O$, $[Ni(pdca)(phen)H_2O]$ ve $[Ni(pdca)_2]$ komplekslerinin UV-vis. spektrumları $1x10^{-4}$ M sulu çözeltide alındı.
Çizelge 5'te komplekslerine ait UV−vis. spektroskopik verileri görülmektedir.

**Çizelge 6.** Nikel(II) ve kobalt(II) komplekslerinin UV−vis. spektroskopik verileri.

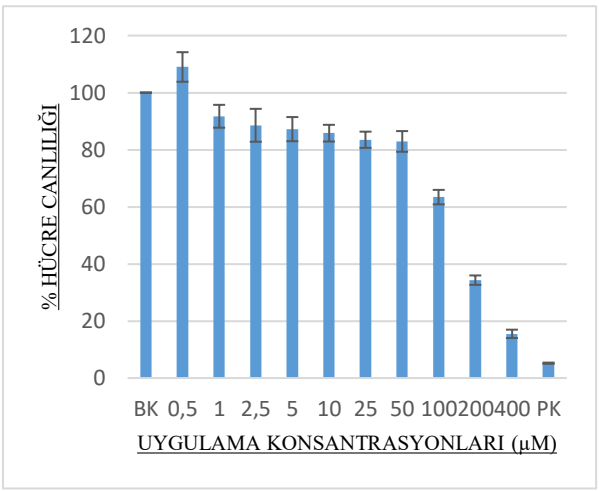
| Kompleks | λ (nm) | ε ($dm^3$/mol.cm) |
|---|---|---|
| $[Co(pdca)(phen)H_2O].H_2O$ | 285<br>294 | 7277<br>3618 |
| $[Co_2(pdca)_2(H_2O)_5].2H_2O$ | 310 | 11050 |
| $[Ni(pdca)(phen)H_2O]$ | 285<br>294 | 19384<br>5534 |
| $[Ni(pdca)_2]$ | 290 | 19470 |

**Biyolojik Aktivite**

$[Co(pdca)(phen)H_2O].H_2O$ ve $[Ni(pdca)(phen)H_2O]$ komplekslerinin sitotoksik etkinliği A 549 (ATCC® CCL-185™) insan akciğer kanser hücre hatlarında XTT (((2,3-bis[2-metoksi- 4-nitro- 5-sulfofenil]-2H-tetrazolyum- 5-karboksianilit iç tuzu) yöntemi ile *in vitro* olarak araştırılmıştır.
Hücre Kültürü: A549 hücreleri (ATCC® CCL-185™) (Şekil 15) 37 °C ve %5 $CO_2$ içeren ortama sahip inkübatörlerde havalandırmalı T75 flasklar içerisinde kültüre edilmiştir. Kültür ortamı olarak kullanılan besiyeri içeriği; F-12K medyum (ATCC, USA), %10 Fetal Calf serum (PAN Biotech, Aidenbach, Germany) 1mM SodyumPürivat (PAN Biotech, Aidenbach, Germany).1 mM L- Glutamin (Sigma Aldrich, Darmstadt, Germany), 100U/ml Penisilin- 100 µg/ml Streptomisin (Bioch-rom AG, Berlin, Germany) ile oluşturulmuştur (Roehm ve ark., 1991).
XTT yöntemi üç tekrar olarak uygulanmış ve % canlılık değerlerinin ortalamaları verilmiştir. Yöntemde pozitif kontrol olarak $H_2O_2$ (300 µM) kullanılmıştır. Tekrar deneylerinde elde edilen % canlılık değerleri içerisinden seçilen dört farklı konsantrasyon ile oluşturulan grafiklerin fonksiyon denkleminden IC 50 değerleri hesaplanmış ve ortalama olarak verilmiştir.
XTT testi sonrasında elde edilen % canlılık değerleri Şekil 10 ve Şekil 11'de verilmiştir.




**Şekil 10.** [Co(pdca)(phen)$H_2O$].$H_2O$ için XTT sonucunda elde edilen % hücre canlılık değerleri grafiği.
(BK; Büyüme Kontrol, PK; Pozitif Kontrol $H_2O_2$)




**Şekil 11.** [Ni(pdca)(phen)$H_2O$] için XTT sonucunda elde edilen % hücre canlılık değerleri grafiği.
(BK; Büyüme Kontrol, PK; Pozitif Kontrol $H_2O_2$)

## SONUÇ

[Co(pdca)(phen)$H_2O$].$H_2O$, [$Co_2$(pdca)$_2$($H_2O$)$_5$].2$H_2O$, [Ni(pdca)(phen)$H_2O$] ve [Ni(pdca)$_2$] kompleksleri sentezlendi. Komplekslerin yapıları X-ışınları tek kristal difraksiyon yöntemi, FT-IR, UV-Vis. ve elementel analiz yöntemleriyle karakterize edildi. Sentezlenen komplekslerin bozulmuş oktahedral geometri oluşturdukları gözlendi. XTT sonucunda elde edilen % hücre canlılık değerlerine bakıldığında [Co(pdca)(phen)$H_2O$].$H_2O$ ve [Ni(pdca)(phen)$H_2O$] kompleksleri için konsantrasyon arttıkça hücre canlılığının azaldığı görülmektedir.

Elde edilen sonuçlardan [Co(pdca)(phen)$H_2O$].$H_2O$ kompleksi için IC50 değeri 37,286 ± 17,318 ($R^2$= 0,829 ± 0,007) ve [Ni(pdca)(phen)$H_2O$] kompleksi için IC 50 değeri 195,786 ± 10,713 µM ($R^2$= 0,903 ± 0,012) belirlendi. IC 50 değerlerine bakıldığında [Co(pdca)(phen)$H_2O$].$H_2O$ kompleksinin daha aktif olduğu görülmektedir.

## TEŞEKKÜR

FHIZ-2022-727 projesine maddi desteğinden dolayı Bursa Uludağ Üniversitesi Bilimsel Araştırma Projeleri Birimine teşekkür ederiz.

## KAYNAKLAR

**1)** Aghabozorg, H., Manteghi, F., Sheshmani S 2008. *Journal of the Iranian Chemical*, 5(2): 184-227.
**2)** Bailey, G.F., Karp, S., Sacks, T.E 1965. Ultraviolet-absorption spectra of dry bacterial spores. *J. Bacteriol.*, 89: 984-987.
**3)** Kanai, Y 1990. Simultane ousdetermination of iron(II) and iron(III) oxides in geological materials by ion chromatography. *Analyst*, 115: 809-812.
**4)** Roehm NW, Rodgers GH, Hatfield SM, Glasebrook AL 1991. An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT. J Immunol Methods 13:142(2):257-65.
**5)** Setlow, B., and Setlow, P 1993. Dipicolinic Acid Greatly Enhances Production of Spore Photoproduct in Bacterial Sporesupon UV Irradiation. *Appl. Environ. Microbiol.*, 59(2): 640-643.
**6)** Wang, X.L., Qin, C., Wang, E., Changwen, H., Xu, L 2004. *J. Mol. Struct.*, 692: 187-193.

## *ORAL PRESENTATION*

# Synthesis and characterization of some novel 2-methoxy-5-[(1-acetyl-3-alkyl/aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates

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## Abstract

In the present study, a series of 2-methoxy-5-[(1-acetyl-3-alkyl/aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (2).were obtained from the reactions of 2-methoxy-5-[(3-alkyl/aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (1), which were synthesized by the reaction of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones with 3-(3-nitrobenzoxy)-4-methoxybenzaldehyde, with acetic anhydride. The newly synthesized compounds were fully characterized.

**Keywords:** 4,5-Dihydro-1*H*-1,2,4-triazol-5-one, Acetylation, Synthesis.

## INTRODUCTION

Triazoles are heterocyclic compounds with three nitrogen and two carbon atoms in their structure. A large number of triazoles are associate with diverse biological properties such as anti-inflammatory (Li et al. 2020, Hamoud et al. 2022), antioxidant (Beyzaei et al. 2020), antibacterial (Bitla et al., 2021; Sanina et al., 2022), antiviral (Simurova and Maiboroda 2021) , antitumor(Liu et al. 2022) and antiepileptic(Amanlou et al. 2022) properties. Schiff is formed by the reaction of primer amines with aldehydes or ketones, which have a C=N imine group in their structure (Beytur et al. 2018, Mahmood et al. 2022). In addition, Schiff bases are also known to have various properties like antihyperlipidemic (Kathiravan et al. 2016), anticancer (Cai et al. 2021), antiviral (Kumar et al. 2010), antioxidant (Yüksek et al. 2015), antimicrobial (Tamer et al. 2016), anti-inflammatory (Rakesh et al. 2015), antifungal (Santos Oliveira et al. 2022), analgesic (Chinnasamy et al. 2010) and antitumor (Hu et al. 2012). Therefore, syntheses of Schiff base containing 1,2,4 triazole have attentions of organic chemists in the past decades.

Due to the above-mentioned properties of 1,2,4 Triazole and Schiff bases, we were studied to synthesize of Schiff base containing 1,2,4 triazole. In the study, the the synthesis of 5 novel 2-methoxy-5-[(1-acetyl-3-alkyl/aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (Ilgar and Demirci 2014) (2).were obtained from the reactions of 2-methoxy-5-[(3-alkyl/aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (Aytemiz 2013) (1), which were synthesized by the reaction of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones with 3-(3-nitrobenzoxy)-4-methoxybenzaldehyde with acetic anhydride.

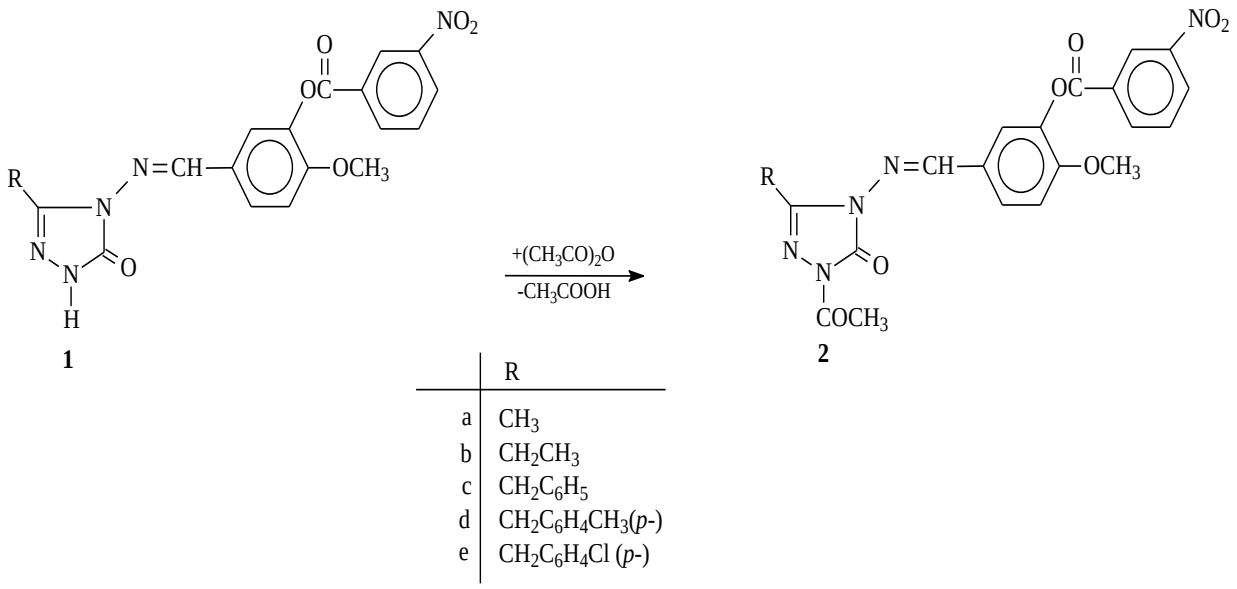
## MATERIALS AND METHODS

### Chemicals and Instruments

Chemical reagents and all solvents used in this study were purchased from Merck AG, Aldrich and Fluka. Melting points which were uncorrect were determined in open glass capillaries using an WRS-2A microprocessor digital melting point apparatus. The IR spectra were obtained on a ALPHA-P BRUKER FT-IR spectrometer. UV spectra were obtained a PG Instruments Ltd T80 UV/VIS Spectrometer device.

### General procedure for the synthesis of 2-methoxy-5-[(1-acetyl-3-alkyl/aryl)-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (2)

The corresponding compound **1** (0.01 mol) was boild with 20 mL acetic anhydride for 30 minutes. Then, 100 mL absolute ethanol was added to the solution and the reaction mixture was refluxed for 30 minutes. The solid was recrystallized from the same solvent and purified by drying in vacuo to obtain pure compounds 2 as colourless crystals (Scheme **1**).




| | R |
|---|---|
| a | $CH_3$ |
| b | $CH_2CH_3$ |
| c | $CH_2C_6H_5$ |
| d | $CH_2C_6H_4CH_3$(*p*-) |
| e | $CH_2C_6H_4Cl$ (*p*-) |

**Scheme 1.** Synthesis route of compounds **2**

**Table 1.** Melting point, yield, IR spectrum and UV spectrum data of 2-methoxy-5-[(1-acetyl-3- methyl 4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (2a)

| | |
|---|---|
| Melting Point | 220 ºC. |
| Yield | % 87.26 |
| IR ($cm^{-1}$) | 3061 (C=CH) $cm^{-1}$<br>1744, 1718 (C=O) $cm^{-1}$<br>1612 (C=N) $cm^{-1}$<br>1528 ve 1349 ($NO_2$) $cm^{-1}$<br>1251 (COO) $cm^{-1}$<br>808 ve 717 (1,3-disubstitue aromatic ring) $cm^{-1}$ |
| UV (Ethanol, %95) $\lambda_{max}$ (ε) | $\lambda_1$ = 306 (19622) nm<br>$\lambda_2$ = 260 (15856) nm<br>$\lambda_3$ = 230 (23140) nm |

**Table 2.** Melting point, yield, IR spectrum and UV spectrum data of 2-methoxy-5-[(1-acetyl-3-ethyl 4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (2b)

| | |
|---|---|
| Melting Point | 204 ºC |
| Yield | % 90.82 |
| IR ($cm^{-1}$) | 3065 (C=CH) $cm^{-1}$<br>1785, 1749, 1698 (C=O) $cm^{-1}$<br>1603 (C=N) $cm^{-1}$<br>1527 ve 1347 ($NO_2$) $cm^{-1}$<br>1274 (COO) $cm^{-1}$<br>822 ve 715 (1,3-disubstitue aromatic ring) $cm^{-1}$ |
| UV (Ethanol, %95) $\lambda_{max}$ (ε) | $\lambda_1$ = 302 (16078) nm<br>$\lambda_2$ = 296 (16548) nm<br>$\lambda_3$ = 254 (16662) nm<br>$\lambda_4$ = 236 (16608) nm |

**Table 3.** Melting point, yield, IR spectrum and UV spectrum data of 2-methoxy-5-[(1-acetyl-3- benzyl 4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (2c)

| | |
|---|---|
| Melting Point | 187 ºC |
| Yield | % 84.43 |
| IR ($cm^{-1}$) | 3065 (C=CH) $cm^{-1}$<br>1781, 1768, 1723 (C=O) $cm^{-1}$<br>1608 (C=N) $cm^{-1}$<br>1535 ve 1346 ($NO_2$) $cm^{-1}$<br>1252 (COO) $cm^{-1}$<br>762 ve 712 (monosubstitue aromatic ring) $cm^{-1}$.<br>809 (1,3-disubstitue aromatic ring) $cm^{-1}$. |
| UV (Ethanol, %95) $\lambda_{max}$ (ε) | $\lambda_1$ = 306 (18931) nm<br>$\lambda_2$ = 258 (16732) nm<br>$\lambda_3$ = 232 (20305) nm |

**Table 4.** Melting point, yield, IR spectrum and UV spectrum data of 2-methoxy-5-[(1-acetyl-3-*p*-methylbenzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (2d)

| | |
|---|---|
| Melting Point | 201 ºC |
| Yield | % 81.92 |
| IR ($cm^{-1}$) | 3062 (C=CH) $cm^{-1}$<br>1766, 1746, 1694 (C=O) $cm^{-1}$<br>1603(C=N) $cm^{-1}$<br>1533 ve 1344 ($NO_2$) $cm^{-1}$<br>1247 (COO) $cm^{-1}$<br>804 (1,4-disubstitue aromatic ring) $cm^{-1}$<br>778 ve 713 (1,3-disubstitue aromatic ring) $cm^{-1}$ |
| UV (Ethanol, %95) $\lambda_{max}$ (ε) | $\lambda_1$ = 306 (21851) nm<br>$\lambda_2$ = 260 (17749) nm<br>$\lambda_3$ = 230 (27926) nm |

**Table 5.** Melting point, yield, IR spectrum and UV spectrum data of 2-methoxy-5-[(1-acetyl-3-*p*-chlorobenzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)iminomethyl]phenyl 3-nitrobenzoates (2d)

| | |
|---|---|
| Melting Point | 213 ºC |
| Yield | % 88.36 |
| IR ($cm^{-1}$) | 3062 (C=CH) $cm^{-1}$<br>1766, 1746, 1694 (C=O) $cm^{-1}$<br>1603(C=N) $cm^{-1}$<br>1533 ve 1344 ($NO_2$) $cm^{-1}$<br>1247 (COO) $cm^{-1}$<br>804 (1,4-disubstitue aromatic ring) $cm^{-1}$<br>778 ve 713 (1,3-disubstitue aromatic ring) $cm^{-1}$ |
| UV (Ethanol, %95) $\lambda_{max}$ (ε) | $\lambda_1$ = 306 (21851) nm<br>$\lambda_2$ = 260 (17749) nm<br>$\lambda_3$ = 230 (27926) nm |

## RESULTS AND DISCUSSION

Five new compounds were synthesized using an acetylation reaction, which was a single-step reaction in which a secondary amine carbon group was condensed with acedic anhydrite using a reflux distillation method at a controlled temperature. The obtained compounds 2a-2e were dried using an evaporator. Weighing apparatus was used to calculate the percentage yield. Physical and chemical properties were investigated. WRS-2A microprocessor digital melting point apparatus was used to determine the melting point.

Spectroscopic techniques such as UV/VIS (Ultraviolet-Visible Region Spectroscopy) and FTIR (Fourier Transform Infrared spectroscopy) were used to confirm the synthesis of novel compounds 2a-2e (Table No. 1-5).

## CONCLUSION

In the present study, new Schiff derivatives (**2a-2e**) were designed and synthesized. The new synthesized compounds were identified using spectral data (IR and UV).

## REFERENCES

Amanlou, A., Nassireslami, E., Hosseini, F.S., Dehpour, A.R., Rashidian, A., Chamanara, M. 2022. Synthesis, docking and antiepileptic activity of new 2-((1,5-Diphenyl-1 *H* -1,2,4-Triazol-3-yl)Thio)- *N* -Phenylacetamide Derivatives.: Polycyclic Aromatic Compounds 42, 6429–6443.

Aytemiz, F. 2013. 3-Alkil(Aril)-4-[3-(3-nitrobenzoksi)-4-metoksibenzilidenamino]-4,5-dihidro-1H-1,2,4-triazol-5-on bileşiklerinin sentezi, yapılarının aydınlatılması ve bazı özelliklerinin incelenmesi. Fen Edebiyat Fakültesi, Kafkas Üniversitesi.

Beytur, M., Kardaş, F., Akyıldırım, O., Özkan, A., Bankoğlu, B., Yüksek, H., Yola, M.L., Atar, N. 2018. A highly selective and sensitive voltammetric sensor with molecularly imprinted polymer based silvergold nanoparticles/ionic liquid modified glassy carbon electrode for determination of ceftizoxime.: Journal of Molecular Liquids 251, 212–217.

Beyzaei, H., Ghanbari Kudeyani, M., Samareh Delarami, H., Aryan, R. 2020. Synthesis, antimicrobial and antioxidant evaluation, and molecular docking study of 4,5-disubstituted 1,2,4-triazole-3-thiones.: Journal of Molecular Structure 1215, 128273.

Bitla, S., Gayatri, A.A., Puchakayala, M.R., Kumar Bhukya, V., Vannada, J., Dhanavath, R., Kuthati, B., Kothula, D., Sagurthi, S.R., Atcha, K.R. 2021. Design and synthesis, biological evaluation of bis-(1,2,3- and 1,2,4)-triazole derivatives as potential antimicrobial and antifungal agents.: Bioorganic & Medicinal Chemistry Letters 41, 128004.

Cai, L., Zhu, P., Huan, F., Wang, J., Zhou, L., Jiang, H., Ji, M., Chen, J. 2021. Toxicity-attenuated mesoporous silica Schiff-base bonded anticancer drug complexes for chemotherapy of drug resistant cancer. Colloids and Surfaces B: Biointerfaces 205, 111839.

Chinnasamy, R.P., Sundararajan, R., Govindaraj, S. 2010. Synthesis, characterization, and analgesic activity of novel schiff base of isatin derivatives. Journal of Advanced Pharmaceutical Technology & Research 1, 342–347.

Hamoud, M.M.S., Osman, N.A., Rezq, S., A. A. Abd El-wahab, H., E. A. Hassan, A., Abdel-Fattah, H.A., Romero, D.G., Ghanim, A.M. 2022. Design and synthesis of novel 1,3,4-oxadiazole and 1,2,4-triazole derivatives ascCyclooxygenase-2 inhibitors with anti-inflammatory and antioxidant activity in LPS-stimulated RAW264.7 Macrophages. Bioorganic Chemistry 124, 105808.

Hu, G., Wang, G., Duan, N., Wen, X., Cao, T., Xie, S., Huang, W. 2012. Design, synthesis and antitumor activities of fluoroquinolone C-3 heterocycles (IV): s-triazole Schiff–Mannich bases derived from ofloxacin.: Acta Pharmaceutica Sinica B 2, 312–317.

Ilgar, E.G., Demirci, S. 2014. Bazı Yeni 1-Asetil-3-Alkil(Aril)-4-[3-(3-Nitrobenzoksi)-4-Metoksibenzilidenamino]-4,5-Dihidro-1H-1,2,4-Triazol-5-On Bileşiklerinin Sentezi. Fen Edebiyat Fakültesi, Kafkas Üniversitesi.

Kathiravan, M.K., Vidyasagar, N., Khiste, R., Chote, A., Jain, K. 2016. Synthesis and antihyperlipidemic activity of some novel 4-substituted-2-substitutedmethyltriazino[6,1-b]quinazolin-10-ones and 2,4-disubstituted-6,7-dimethoxy quinazoline. Arabian Journal of Chemistry 9, S395–S403.

Kumar, K.S., Ganguly, S., Veerasamy, R., De Clercq, E. 2010. Synthesis, antiviral activity and cytotoxicity evaluation of Schiff bases of some 2-phenyl quinazoline-4(3)H-ones. European Journal of Medicinal Chemistry 45, 5474–5479.

Li, S.-M., Tsai, S.-E., Chiang, C.-Y., Chung, C.-Y., Chuang, T.-J., Tseng, C.-C., Jiang, W.-P., Huang, G.-J., Lin, C.-Y., Yang, Y.-C., Fuh, M.-T., Wong, F.-F. 2020. New methyl 5-(halomethyl)-1-aryl-1H-1,2,4-triazole-3-carboxylates as selective COX-2 inhibitors and anti-inflammatory agents: Design, synthesis, biological evaluation, and docking study. Bioorganic Chemistry 104, 104333.

Liu, J., Liu, F., Li, Z., Li, C., Wu, S., Shen, J., Wang, H., Du, S., Wei, H., Hou, Y., Ding, S., Chen, Y. 2022. Novel 4-phenoxypyridine derivatives bearing imidazole-4-carboxamide and 1,2,4-triazole-3-carboxamide moieties: Design, synthesis and biological evaluation as potent antitumor agents. Bioorganic Chemistry 120, 105629.

Mahmood, W., Ahmad, I., Khan, M.A., Ali Shah, S.A., Ashraf, M., Shahzad, M.I., Pervaiz, I., Sajid-ur-Rehman, M., Khurshid, U. 2022. Synthesis, characterization, molecular docking and biological evaluation of Schiff Base derivatives of cefpodoxime. Heliyon 8, e11332.

Rakesh, K.P., Manukumar, H.M., Gowda, D.C. 2015. Schiff's bases of quinazolinone derivatives: Synthesis and SAR studies of a novel series of potential anti-inflammatory and antioxidants. Bioorganic & Medicinal Chemistry Letters 25, 1072–1077.

Sanina, N.A., Yakuschenko, I.K., Ya Gadomskii, S., Utenyshev, A.N., Dorovatovskii, P.V., Lazarenko, V.A., Emel'yanova, N.S., Zagainova, E.A., Ovanesyan, N.S., Mumyatova, V.A., Balakina, A.A., Terent'ev, A.A., Aldoshin, S.M. 2022. Synthesis, structure and antibacterial activity of dinitrosyl iron complexes (DNICs) dimers functionalized with 5-(nitrophenyl) -4-H-1,2,4-triazole-3-thiolyls. Polyhedron 220, 115822.

Santos Oliveira, I., Marrote Manzano, C., Hideki Nakahata, D., Brentini Santiago, M., Bernadelli Sousa Silva, N., Henrique Gomes Martins, C., Pimentel Respíndula, F., Henrique Pereira, D., Paulo Corbi, P. 2022. Antibacterial and antifungal activities in vitro of a novel silver(I) complex with sulfadoxine-salicylaldehyde Schiff base. Polyhedron 225, 116073.

Simurova, N.V., Maiboroda, O.I. 2021. Antiviral activity of 1,2,4-triazole derivatives (microreview).: Chemistry of Heterocyclic Compounds 57, 420–422.

Tamer, T.M., Hassan, M.A., Omer, A.M., Baset, W.M.A., Hassan, M.E., El-Shafeey, M.E.A., Eldin, M.S.M. 2016. Synthesis, characterization and antimicrobial evaluation of two aromatic chitosan Schiff base derivatives. Process Biochemistry 51, 1721–1730.

Yüksek, H., Koca, E., Gürsoy-Kol, Ö., Akyıldırım, O., Çelebier, M. 2015. Synthesis, in vitro antioxidant activity, and physicochemical properties of novel 4,5-dihydro-1H-1,2,4-triazol-5-one derivatives. Journal of Molecular Liquids 206, 359–366.

## *ORAL PRESENTATION*

### Atık EVOH ve PE ile oluşturulan EVOH/PE filmlerinin bariyer özelliklerinin incelenmesi

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**Özet**
Plastik malzemelerin önemi geçmişten günümüze kadar her yıl daha fazla artarak gelişmeye devam etmektedir. Hayatın neredeyse her alanında kullanılan plastikler ne kadar önemli ise bu malzemelerin çevresel döngü içerisinde tutulması da bir o kadar önem arz etmektedir. 1970'den günümüze kadar plastik kullanımı ve tüketimi neredeyse 10 kat artmıştır. Bu plastiklerin kullanımının büyük bir payı ise esnek ambalaj sektöründe oluşmaktadır. Ambalaj sektöründe yer alan esnek ambalajlar üzerinde kullanılan çok katmanlı filmlerde geleceğin en önemli konularından birisi hammadde krizleri diğeri ise geri dönüşüm ve sürdürülebilirliktir. Bu çalışmada; çok katmanlı şişirme ile kalıplama yöntemiyle üretilen PE/EVOH/PE yapılı esnek ambalaj filmi atığının, aynı PE/EVOH/PE yapıdaki esnek ambalaj filmi içerisinde değerlendirilmesi, malzemenin bariyer özellik değişimlerinin incelenmesi amaçlanmıştır. Sürdürülebilir çevre ve ekonomi, hammadde maliyetini azaltmak, yaklaşık olarak benzer bariyer özellikleri elde etmek çalışmanın amaçları arasındadır. PE/EVOH/PE filminden açığa çıkan atıklar tek vidalı ekstruderde granül haline getirilmiştir. Daha sonrasında bu atık EVOH granüllerinin miktarı toplam film içerisinde ağırlıkça %5, %10, %20 ve %25 olacak şekilde 7 katlı şişirme ile kalıplama film ekstruderinde saf EVOH filmi referans alınarak üretimleri gerçekleştirilmiştir. 50µm kalınlığında üretilen filmlerden standartlara uygun şekilde numuneler alınmıştır. Numunelere; oksijen bariyeri geçirgenliği (OTR), su buharı geçirgenliği (WVTR), mikroskop, parlaklık, pusluluk, testleri yapılmıştır. Yapılan testlerin sonuçlarına göre hurda EVOH kullanımı hakkında analizler sonuçlandırılmıştır.

**Anahtar Kelimeler:** EVOH, sürdürülebilirlik, esnek ambalaj, PE, geri dönüşüm, bariyer filmleri,

### Analysis of barrier properties of EVOH/PE films produced with waste EVOH and PE

**Abstract**
The importance of plastic materials continues to develop more and more every year from past to present. As important as the plastics used in almost every area of life are, it is equally important to keep these materials in the environmental cycle. Since 1970, the use and consumption of plastic has increased almost 10 times. A large share of the use of these plastics occurs in the flexible packaging sector. In the multi-layer films used on flexible packaging in the packaging industry, one of the most important issues of the future is the crisis of raw materials, and the other is recycling and sustainability. In this study, it is aimed to evaluate the PE/EVOH/PE structured flexible packaging film waste produced by multilayer blow molding method in the same PE/EVOH/PE flexible packaging film and to examine the barrier properties of the material. Sustainable environment and economy, reducing the cost of raw materials, obtaining approximately similar barrier properties are among the objectives of the study. The wastes released from the PE/EVOH/PE film were granulated in a single screw extruder. Then, the amount of these waste EVOH granules was 5%, 10%, 20% and 25% by weight in the total film, and their production was carried out with reference to the pure EVOH film in a 7-layer blow molding film extruder. Samples were taken from the films produced in 50µm thickness in accordance with the standards. to samples; oxygen barrier permeability (OTR), water vapor permeability (WVTR), microscope, gloss, haze, tests were carried out. According to the results of the tests, analyzes on the use of scrap EVOH were concluded.
**Keywords:** EVOH, sustainability, flexible packaging, PE, recyclability, barrier films,

## 1. GİRİŞ

Plastik sektörünün en önemli kollarından birisi olan ambalaj sektörüne talep her gün artmaktadır. Ambalaj sektöründe hem sert hem de esnek ambalaj kategorilerinde sektörel olarak ihtiyaçları karşılayabilecek üstün özelliklerde çeşitli ürün grupları üretilmektedir. Gıda, gıda dışı, ilaç, hijyen gibi birçok alanın çeşitli ambalaj talepleri esnek ambalaj ile birlikte sağlanmaktadır. İstenilen özelliklere uygun film yapıları ise çok katmanlı film üretimleri ile birlikte sağlanabilmektedir. Çok katmanlı filmler kompozit malzemelere benzer şekilde,

istenilen mekaniksel, fiziksel, ışık geçirgenliği, bariyer gibi birçok üstün özelliğe göre uygun şekilde katmanlı olarak üretilebilir. Bu durum da esnek ambalaj sektörünün rağbetinin artmasında oldukça önemli bir rol oynamaktadır (Blue Weave, 2022). Sektörün hızla büyümesi, üretimsel süreçlerin hızlanması, teknolojinin gelişmesi ile birlikte çevresel ekonomi ve sürdürülebilirlik gün geçtikçe önemini oldukça arttırmıştır. Ambalaj atıklarının tekrar kullanılması, hammadde tüketimlerinin azaltılması, üretim yöntemlerine tekrar kazandırılması, tek tip malzeme ile film oluşturulması gibi birçok sürdürülebilir çözümler üretilmeye ve yasal olarak uygulanmaya başlanmıştır (TMMOB, 2021). Bu noktada polimerlerin genel yapılarından yola çıkarak malzemelerin yapılarının iyice araştırılması ve tekrar kullanımları ile ilgili çalışmalar da giderek artmaktadır. Geri dönüşüm yöntemi olarak en çok tercih edilenler ile mekanik ve kimyasal geri dönüşüm yöntemleridir (Horodytska O. ve ark., 2018).
Bu çalışmada ambalaj sektöründeki yaşanılan hammadde sorunları, çevre kirliliğini azaltma durumlarına katkı sağlaması için, toplam 50 mikron kalınlığında 7 katlı şişirme ile şekillendirme (blown ekstruder) ile üretilen PE/EVOH/PE filminin içerisine, ağırlıkça %5, %10, %20 ve %25 oranlarında atık (PE/EVOH/PE) ve uyumlaştırıcı olarak PE-g-MA belirli oranlarda beslenerek PE/EVOH/PE filmi içerisinde tekrar kullanıma alınması sonrasında malzemenin bariyer özellikleri detaylı olarak incelenmiştir.

## 2. MATERYAL VE METOT

### *2.1 Materyal*

Dow Chemical Company firmasından düşük yoğunluklu polietilen (LDPE 310 E), polietilen aşılanmış maleik anhidrit (BYNEL 4157) ve atık kullanımı için özel olarak üretilen polietilen aşılanmış maleik anhidrit (Retain 3000), Exxon Mobil firmasından düşük yoğunluklu polietilen (Enable 2010 Series Blown 2010MA), Chang Chun Petrochemicals firmasından etilen vinil alkol (EVASIN EV3251F) ve son olarak üretim sırasında ortaya çıkmış, granül haline getirilmiş atık PE/EVOH granülü kullanılmıştır.

### *2.2 Tek Vidalı Ekstruder ile Atık PE/EVOH Granüllerinin Hazırlanması*

50 mikron kalınlığındaki çok katmanlı PE/EVOH filmlerinin üretimi sırasındaki fireleri, atıkları, bobin üzerinden traşlanan bölümleri bir kenara ayrılmıştır. Sepetlerde muhafaza edilmiştir ve Erema tek vidalı ekstruderine, re-ekstrude edilmek üzere ayrıştırılmıştır. Bant sistemi üzerinde kırpılmış esnek ambalaj atıkları yerleştirilmiştir. Beslemeden sonra ilk olarak ekstrudere giriş öncesinde kırpılmış filmleri daha küçük boyutlara getirmek, kolay işlenmesini sağlamak için Erema makinesinin ön öğütücü kısmında 104°C sıcaklıkta ön öğütme işlemine tabi tutulmuş ve atık filmler kendi içinde karıştırılmış, daha küçük parçalara ayrılmıştır. Bu işlemden sonra toplam 7 ısıtma bölgesi bulunan Erema tek vidalı ekstruder içerisine kırpılan atık filmler beslenmiştir. 240 rpm (devir/dakika) hızında 120-200°C derece sıcaklık aralığında malzemenin eriyik hale geçmesi sağlanmıştır. Aşağıdaki Tablo 2.1'de ekstruder çalışma sıcaklıkları görülmektedir.

**Tablo 2.1.** Tek vidalı erkstrüzyon makinesi çalışma aralığı

| İşlem | 1. Isıtma | 2. Isıtma | 3. Isıtma | 4. Isıtma | 5. Isıtma | 6. Isıtma | 7. Isıtma | Kafa Sıcaklığı |
|---|---|---|---|---|---|---|---|---|
| Sıcaklık (°C) | 130 | 190 | 190 | 200 | 190 | 200 | 200 | 230 |

**basınç ortalama 160 bar ve vida dönme hızı 240rpm*

Daha sonrasında kafa bölgesindeki delikli bölgeden spagetti şeklinde çıkan eriyik, bıçak yardımı ile ufak parçalara ayrılmıştır ve yaklaşık oda sıcaklığında bulunan (23°C) soğutma suyunda soğutulmuştur. Soğutma işleminde soğutulan granüller titreşim ile birlikte elekten geçirilmiştir. Son işlem olarak da kurutuculara taşınan granül kurutulduktan sonra 25 kg lık çuvallara doldurulmuştur. Tek vidalı ekstruderden geçirilen atık filmler ve en son oluşan granüller Şekil 2.1 de görülmektedir.



**(a)** **(b)**

**Şekil 2.1** Tek vidalı ekstruderden üretilen granüller a) atık PE/EVOH esnek ambalaj filmleri b) atık PE/EVOH granülleri

### *2.3 Çok Vidalı Ekstruder ile Atık PE/EVOH Granüllerinin Kullanımı*

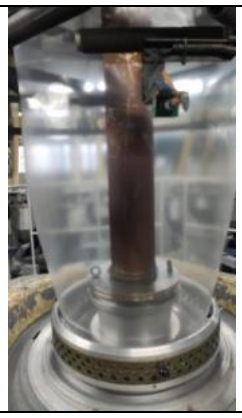
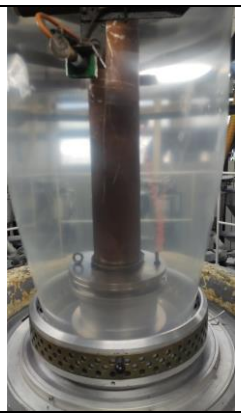
Elde edilen atık PE/EVOH granülleri artık çok katmanlı ekstruderde kullanıma uygun forma getirilmiştir. 7 katlı Kiefel (2006) Kirion model blown (şişirme) ekstruderiyle 50µ kalınlığında farklı atık PE/EVOH oranlarında filmler üretilmiştir. 7 katlı ekstruderde aşağıdaki görülen kat dağılımlarında filmin reçetesi oluşturulmuştur. Belirtilen A-B-C-D-E-F-G katları çok katmanlı ekstruder filmindeki her bir vida-kovanı ifade etmektedir. A-B ve F-G katları LDPE ve LLDPE polimerinin beslendiği katını ifade etmektedir. C ve E katları yapıştırıcı (tie) katını ifade etmektedir. D katı ise EVOH malzemesinin beslendiği, bariyer katıdır. Üretilen filmlerin içeriklerinde Tablo 2.3 de görüldüğü üzere filmin ağırlıkça %5, %10, %20 %25 i atık içeren ve saf halde olmak üzere toplam 5 farklı bariyerli ekstruder filmi üretilmiştir. Üretim sırasında öncelikle referans oluşturması için atıksız saf PE/EVOH filmi üretilmiştir. Tablo 2.4 de toplam filmdeki atık PE/EVOH oranı ağırlıkça 50µ filme oranı olarak hesaplanmıştır.

**Tablo 2.2.** 7 katlı ekstruder kat dağılımları toplam 50µ

| **A (Kat 1)** **13µ** | **B (Kat 2)** **7µ** | **C (Kat 3)** **4µ** | **D (Kat 4)** **2µ** | **E (Kat 5)** **4µ** | **F (Kat 6)** 7µ | **G (Kat 7)** **13µ** |
|---|---|---|---|---|---|---|
| LDPE LLDPE | LDPE LLDPE | g-Maleik Anhidrit PE | EVOH | g-Maleik Anhidrit PE | LDPE LLDPE | LDPE LLDPE |

Yapıştırıcı katı atık PE/EVOH oranı C ve E vidalarının ağırlıkça toplamının 50µ filme oranı ile hesaplanmıştır. Aynı şekilde PE katı atık PE/EVOH oranı A-B-F-G vidalarının ağırlıkça toplamının 50µ filme oranı ile hesaplanmıştır. Yapıştırıcı katı Retain 3000 oranı C ve E vidalarının, PE katı Retain 3000 oranı da A-B-F-G vidalarının ağırlıkça toplamlarının 50µ filme oranı ile hesaplanmıştır. Tablo 2.4 de ekstruder katlarındaki kullanılan atık PE/EVOH oranları görülmektedir. Ekstruderlerden çıkan filmlerin görüntüleri Şekil 2.2 de görülmektedir.

**Şekil 2.2**. Farklı atık PE/EVOH oranındaki filmlerin ekstruder kafasından çıkışı

|  |  |  |  |  |
|---|---|---|---|---|
| Saf PE/EVOH | %5 Atık PE/EVOH | %10 Atık PE/EVOH | %20 Atık PE/EVOH | %25 Atık PE/EVOH |

**Tablo 2.3.** Atık PE/EVOH ağırlıkça karışım oranları

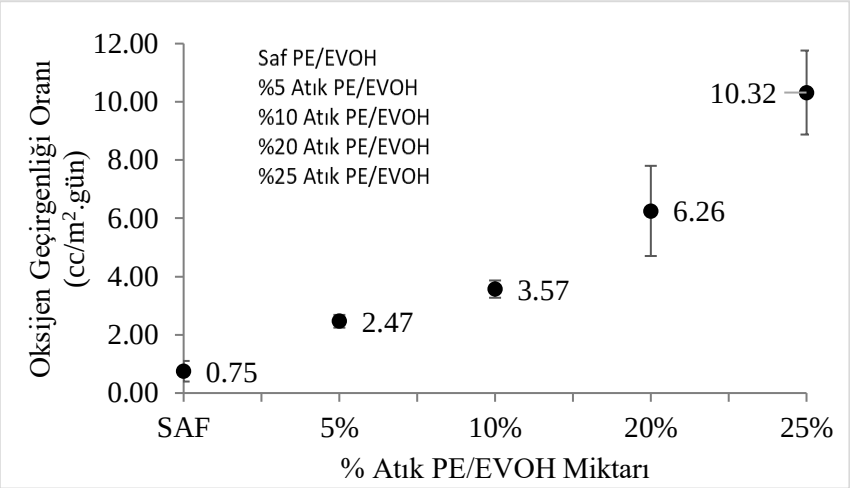
| 50 Mikron | Yapıştırıcı (C-E) Katı Atık PE/EVOH Oranı[1] (%) | Yapıştırıcı (C-E) Katı Retain 3000 Oranı (%) | PE (A-B-F-G) Katı Atık PE/EVOH Oranı[1] (%) | PE (A-B-F-G) Katı Retain 3000 Oranı (%) |
|---|---|---|---|---|
| Saf PE/EVOH | - | - | - | - |
| %5 Atık PE/EVOH | 0 | 0 | 17,25 | 0,5 |
| %10 Atık PE/EVOH | 25 | 0,5 | 27,5 | 1 |
| %20 Atık PE/EVOH | 50 | 1 | 41,5 | 1,5 |
| %25 Atık PE/EVOH | 50 | 1,5 | 55 | 2 |

*1: atık PE/EVOH %5 EVOH granülü içermektedir.*

## 3. BULGULAR VE TARTIŞMA

### *3.1 Oksijen Geçirgenlik Hızı (OTR)*

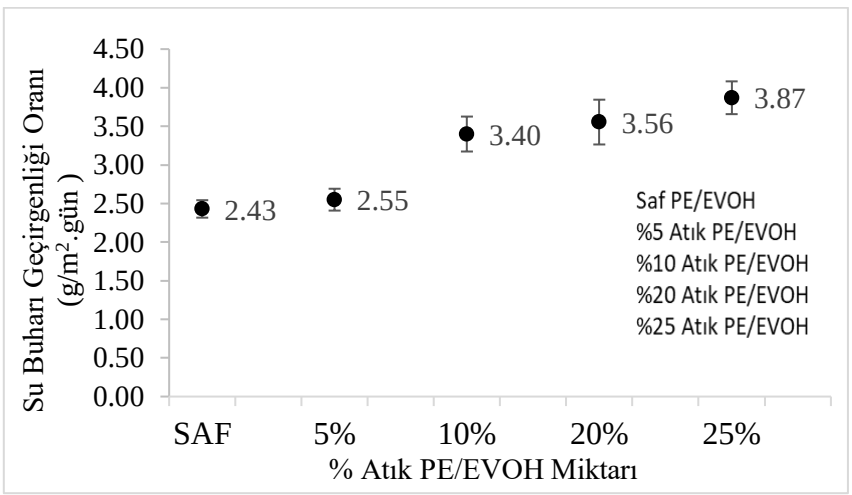
Oksijen insanlar için temel olarak gerekliyken, yağlar, vitaminler üzerinde de oldukça büyük bir önem arz etmektedir. Oksijen, koku, renk, tat ve besin maddelerinin bozulması için önemli bir etkendir. Gıda ürünlerinin oksijene duyarlılığı sebebiyle gıda ambalajlarının oksijene karşı bariyer özelliği kazandırılması, paketin içerisinde bulunan ürünün raf ömrünü uzatmak için oldukça önemlidir. Bu bariyer özelliğinin kazandırılabilme için de yüksek oksijen bariyeri içeren EVOH polimerinin çok katmanlı filmlerde kullanılması oldukça yaygın bir yöntem olmuştur (Wang L., 2019). Oksijen geçirgenliği hızı testi ise bir filmin diğer yüzeyine geçirdiği oksijen miktarının gün bazında karşılığının ölçülmesiyle oluşan bariyer özellikleri belirleme yöntemidir. OTR değerleri <1 ise çok yüksek, 1-5 arasında yüksek, 6-100 arasında orta >100 düşük olarak sıralandırılır (Martenen O., 2015). Yapılan deneyler sonucunda elde edilen OTR sonuçları Şekil 3.1'de verilmiştir. Deney sonuçlarına göre en az oksijen geçirgenliği (OTR) değeri 0,75 cc/m$^2$.gün ile saf PE/EVOH dan oluşan film örneğinde olduğu görülmüştür. Saf PE/EVOH filmine %5 oranında atık EVOH ve PE karışımı granülü eklendiğinde bu değerin 2,47 cc/m$^2$.gün değerine yükseldiği görülmektedir. İçindeki atık EVOH ve PE karışımı granülü %10 a çıkarıldığında değerin 3,57 cc/m$^2$.gün olarak ölçülmüştür. Yüksek bir oranda değişkenlik göstermediği görülmektedir. Oranın %20 seviyesine çıkartılması sonrasında değerin önemli ölçüde arttığı ve 6,26 cc/m$^2$.gün seviyesine çıktığı görülmüştür. %25 seviyesindeki değerde ise artık artış oranının daha da yükseldiği gözlemlenmiş ve 10,32 cc/m$^2$.gün değerinde olduğu görülmüştür.




**Şekil 3.1** Atık EVOH ve PE ile oluşturulan PE/EVOH filmlerinin OTR değerleri grafiksel gösterimi

### *3.2 Su Buharı Geçirgenlik Hızı (WVTR)*

Malzemelerde fiziksel ve kimyasal bozulmaları nem içeriğine bağlı olan paketlenmiş gıda ürününün su buharı bariyeri özellikleri, raf ömrünün korunması veya uzatılması için büyük önem taşımaktadır. Su buharı bariyeri, bir ambalaj malzemesinde birim alan ve zaman başına nüfuz eden su buharı miktarını gösteren su buharı geçirgenlik katsayıları (WVPC) ile ölçülür (kg·m/(m$^2$·s·Pa)). Taze gıdalar için su kaybını önlemek önemlidir, fırın veya şarküteri ürünleri için ise su sızmasını önlemek önemlidir. Su buharı iletim hızı (WVTR) cc/m$^2$·s (veya g·/m$^2$·gün) cinsinden ifade edilir (Siracusa V., 2012). Bu noktada WVTR ifadesinde, bir malzemenin su buharı bariyer özelliklerinin >100 düşük, 6-100 orta, 1-5 yüksek, <1 çok yüksek olarak sıralandırılır (Khalifa Y.I.M., 2016). Yapılan deneyler sonucunda elde edilen WVTR sonuçları Şekil 3.2'de verilmiştir.



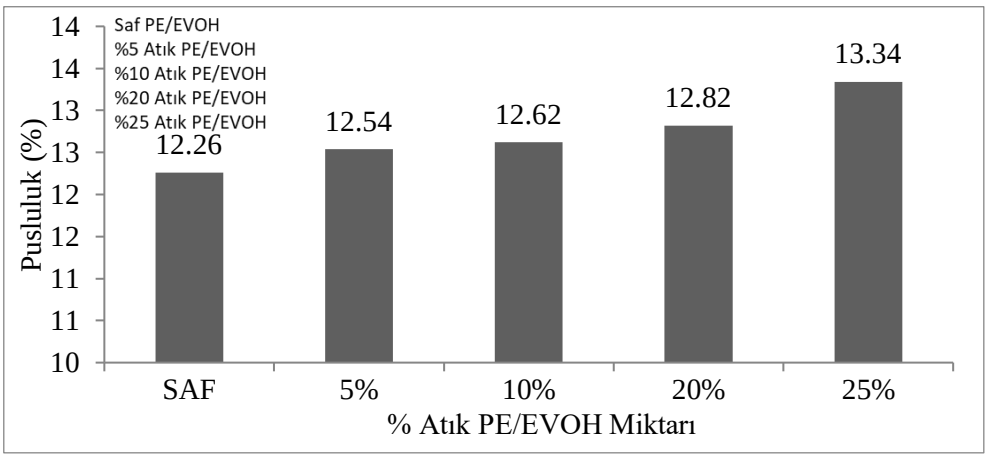

**Şekil 3.2** Atık EVOH ve PE ile oluşturulan PE/EVOH filmlerinin WVTR değerleri grafiksel gösterimi

Deney sonuçlarına göre en az su buharı geçirgenliği (WVTR) değeri 2,43 g/m$^2$.gün ile saf PE/EVOH dan oluşan film örneğinde olduğu görülmüştür. Saf PE/EVOH filmine %5 oranında atık EVOH ve PE karışımı granülü eklenen örnekte bu değerin 2,55 g/m$^2$.gün değerine yükseldiği ve önemli ölçüde bir değişiklik olmadığı görülmektedir. Atık EVOH ve PE karışımı granülü %10 içeren örnekte değerin 3,40 g/m$^2$.gün 'e yükseldiği görülmüştür. %20 oranındaki atık EVOH ve PE karışımı granülü içeren film örneğinde de 3,56 g/m$^2$.gün değerinde yaklaşık olarak yakın bir değer aralığında olduğu tespit edilmiştir. Atık EVOH ve PE karışımı granülü miktarı %25 olan filmin test sonuçlarına bakıldığında değerin 3,87 g/m$^2$.gün civarında olduğu görülmüştür.

### *3.3 Pusluluk Oranı*

Optik özellikleri esnek ambalaj filminde tayin edilmesi gereken önemli özelliklerden birisidir. Pusluluk oranı bir esnek ambalaj filminin içerisinde kullanılan polimerlerin kristalinite derecesine göre gözlemlenmektedir (Robertson G.L., 2005). Bu kristalinite değeri esnek ambalaj filminin içerisinde kullanılan katkılar, çok katmanlı filmlerdeki katların bileşen farklılıkları gibi noktalar ile değişmektedir. Esnek ambalaj filminde pusluluk, düzlemsel bir tabaka halinde bulunan film örneğinin belirli ışık iletme ve geniş açılı ışık saçılmasının

ilk ve son ölçümlerinin birbirine oranı ile hesaplanmaktadır. Bu değer % olarak ifade edilmektedir. Yapılan deneyler sonucunda elde edilen pusluluk oranı sonuçları Şekil 3.3'de verilmiştir.

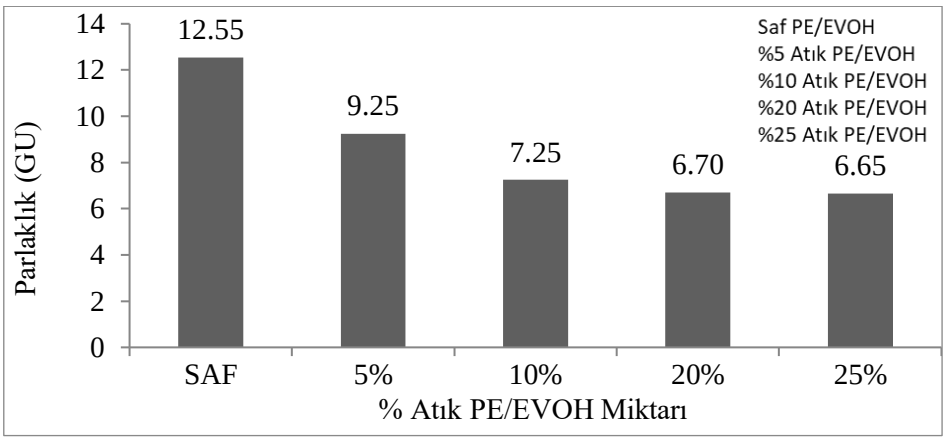



**Şekil 3.1** Atık EVOH ve PE ile oluşturulan PE/EVOH filmlerinin pusluluk oranı değerleri grafiksel gösterimi

Deney sonuçlarına göre pusluluk oranı en yüksek çıkan film %25 atık PE ve EVOH granülü içeren örnek olduğu belirlenmiştir. Şekil incelendiğinde saf PE/EVOH filminde pusluluk oranı %12,26 değerinde olduğu görülmüştür. %5 atık PE ve EVOH granülü içeren örnekte pusluluk değerinin %12,54 değerine çıktığı belirlenmiştir. Atık PE ve EVOH granülü %10' a çıkarıldığında pusluluk artışı devam ettiği ve değerin %12,62 olduğu belirlenmiştir. %20 atık PE ve EVOH granülü içeren filmde ise pusluluk artışı devam etmiştir ve çıkan değer %12,82 olmuştur. %25 atık PE ve EVOH granülü içeren filmde en yüksek pusluluk değeri gözlemlenmiştir ve bu değer %13,34 olarak ölçülmüştür.

### *3.4 Parlaklık Oranı*

Parlaklık oranı tayini, hem şeffaf hem de opak filmlere yapılan film yüzeyinin yansıttıkları ışımaların ölçümü olarak adlandırılmaktadır. Hem opak hem de şeffaf olan filmlerin yansıttıkları ışımaların ölçümüdür. Ölçümde çıkan sonuç GU olarak belirtilmiştir. Malzemenin parlaklık değeri arttığında bu durum ışımayı daha çok yansıtan, parlak yüzeye sahip bir film elde edildiği anlamına gelmektedir. Yapılan deneyler sonucunda elde edilen WVTR sonuçları Şekil 3.4'de verilmiştir. Deney sonuçlarına göre maksimum parlaklık değeri 12,55 GU değeri ile saf PE/EVOH filmine aittir. Grafik incelendiğinde saf PE/EVOH filmi içerisine %5 atık PE ve EVOH granülü eklendiğinde değer 9,58 GU değerinde çıkmış ve parlaklık azalmıştır. %10 atık PE ve EVOH granülü eklendiğinde ise değer 7,25 GU değerine gerilemiştir. %20 ve %25 atık PE ve EVOH granülü eklendiğinde ise değerlerde tekrar azalma görülmüştür. 6,70 ve 6,65GU değerinde sonuçlar ile birbirine yakın parlaklık oranı gözlemlenmiştir.

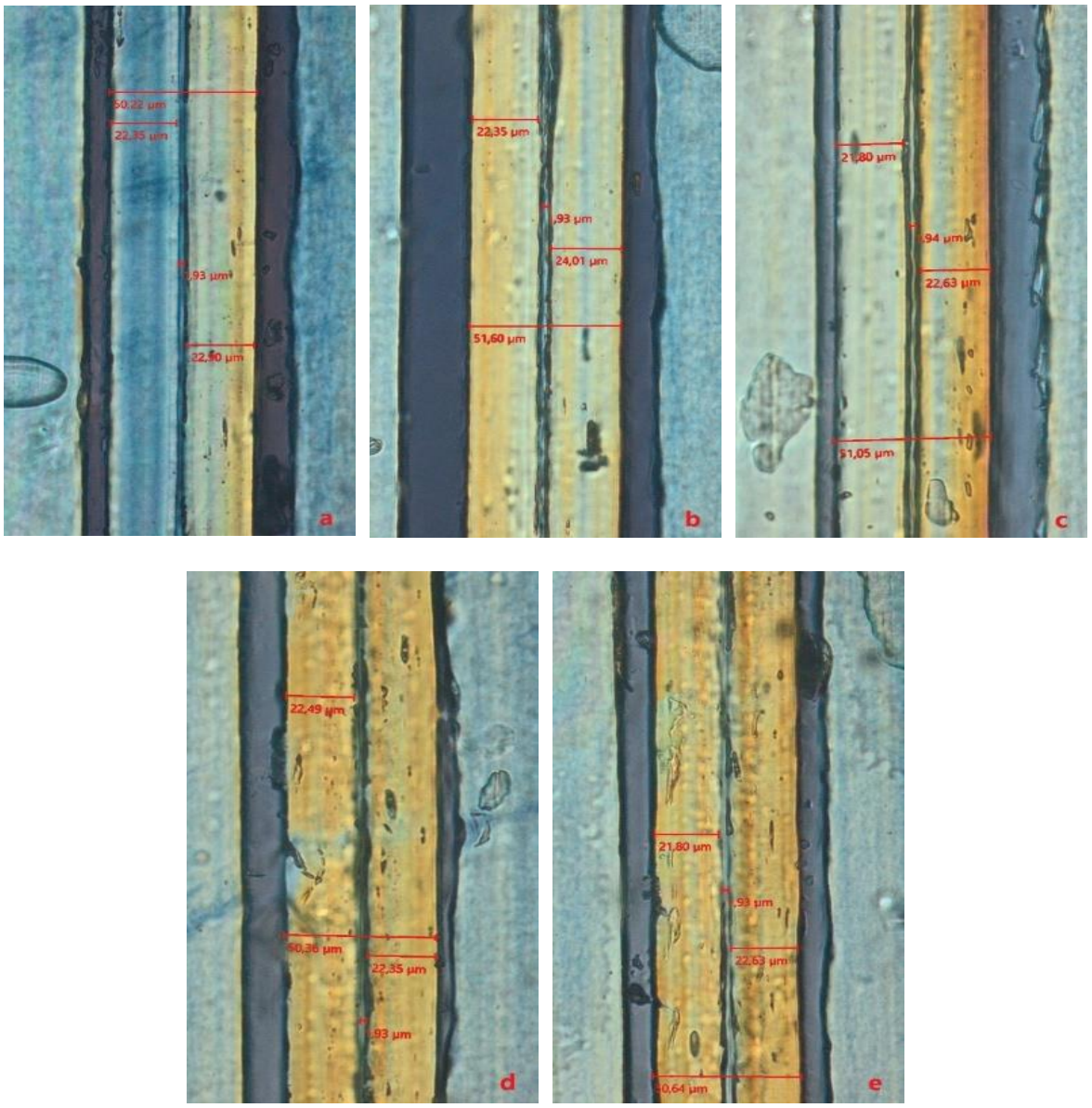



**Şekil 3.2** Atık EVOH ve PE ile oluşturulan PE/EVOH filmlerinin parlaklık oranı değerleri grafiksel gösterimi

### *3.5 Polarize Optik Mikroskop*

Şekil 3.5'de siyah kısımlar test numunesinin tutulduğu kısımlardır. Ölçeklendirmenin olduğu bölge ise numunenin bulunduğu kısımdır. İlk bölge PE ve g-maleik anhidrit katları, orta kısım EVOH katı ve tekrar simetrik şekilde PE ve g- maleik anhidrit katlarıdır. Mikroskop görüntülerinden yola çıkılarak atık miktarının artması ile kesit alanındaki katların temiz görüntüsü gittikçe bozulduğu gözlemlenmiştir. Saf PE/EVOH örneği

olan a şeklinde 50,22 µm olarak toplam film kalınlığı ölçülmüştür. PE ve yapıştırıcı katı birbirleri ile girişim yaparak tek bir katmana benzer şekilde örnek numunenin sol kısmında 22,35 µm, sağ kısmında ise 22,90 µm olarak ölçülmüştür. EVOH katmanında ise 1,93 µm olarak ölçüm alınmıştır. %5 atık PE/EVOH örneği olan b şeklinde 51,60 µm olarak toplam film kalınlığı ölçülmüştür. PE ve yapıştırıcı katları burada da birbirleri ile girişim yaparak tek bir katmana benzer şekilde örnek numunenin sol kısmında 22,35 µm, sağ kısmında 24,01 µm olarak ölçülmüştür EVOH katmanında ise 1,93 µm olarak benzer bir ölçüm gözlemlenmiştir. %10 atık PE/EVOH örneği olan c şeklinde 51,05 µm olarak toplam film kalınlığı ölçülmüştür. Burada da yine benzer bir şekilde PE ve yapıştırıcı katları birbirine girişim yaparak örnek numunenin sol kısmı 21,80 µm sağ kısmı ise 22,63 µm olarak ölçülmüştür. EVOH katmanı ise 1,94 µm olarak görülmektedir. Bu noktada PE, yapıştırıcı katmanlarında saf hale göre malzemenin birbiri arasında homojen görüntüsünün azalmaya başladığı görülmektedir. %20 atık PE/EVOH örneği olan d şeklinde 50,36 µm toplam film kalınlığı ölçülmüştür. Aynı şekilde burada da PE ve yapıştırıcı katları birbirine girişim yaparak örnek numunenin sol kısmı 22,49 µm sağ kısmı ise 22,35 µm olarak ölçülmüştür. EVOH katı ise 1,93 µm olarak ölçülse de incelip kalınlaşmalar artmaya başlamıştır. Buradaki görüntüde ise filmin artık homojenliği daha azaldığı ve girinti çıkıntının artış gösterdiği görülmektedir. %25 atık PE/EVOH örneği olan e şeklinde 50,64 µm toplam film kalınlığı ölçülmüştür. %20 atık PE/EVOH filmine benzer şekilde PE ve yapıştırıcı katının girişimi sonucunda sol kısım 21,80 µm ve sağ kısım 22,63 µm olarak ölçülmüştür. EVOH katı ise 1,93 µm olarak ölçülmüş ve %20 atık PE/EVOH filmine göre daha fazla girinti çıkıntı görülmektedir.




**Şekil 3.3** Atık EVOH ve PE ile oluşturulan PE/EVOH filmlerinin mikroskop görünümleri a) Saf PE/EVOH, b) %5 Atık PE/EVOH, c) %10 Atık PE/EVOH, d) %20 Atık PE/EVOH, e) %25 Atık PE/EVOH

## 4.SONUÇ

Bu çalışmada atık PE/EVOH filmleri granül haline getirildikten sonra tekrar PE/EVOH çok katmanlı filmlerin içerisinde başarılı bir şekilde kullanılmıştır. Genel olarak bakıldığında artan atık PE/EVOH granül miktarı ile birlikte esnek ambalaj filminin oksijen bariyeri özellikleri yüksek bariyerden, orta bariyere doğru gerilemiştir. Su buharı bariyerinde ise toplam yapının içerisindeki artan PE miktarı göz önünde bulundurularak filmde önemli ölçüde değişiklik gözlemlenmemiş, küçük bir miktar artış görüşmüştür. Başka bir çalışmada 3 katlı farklı g-maleik anhidrit içerikli ekstruder ve 5 katlı ekstruder filminin kıyaslamasında LLDPE' nin OTR değerinin EVOH' tan yüksek olduğu görülmüştür aynı zamanda WVTR değerinin de daha düşük olduğunu gözlemlemiştir (Villalpando O. ve ark., 1999). Aynı zamanda başka bir çalışmada ise EVOH PE karışımının içindeki etilen miktarının artması ile OTR değerinin arttığı belirtilmiştir (Caroline M. ve ark., 2018) ve regrind içeriğinin PP-EVOH levha özelliklerine etkisi ve oryantasyonun EVOH çok katmanlı filmlerin geçirgenliğine etkisi çalışması incelediğinde EVOH kopolimeri içerisindeki etilen miktarının artışına göre WVTR değerinin azaldığı bilgisini vermiştir (Wang L., 2019). Bu sonuçlar OTR ve WVTR değerleri için benzer sonuçların ortaya çıktığını göstermektedir. Bariyerli filmlerle ilgili bir diğer çalışmada ise yapıştırıcı katmanlarını kullanmadan ağırlıkça farklı oranlardaki EVOH ve LLDPE-g-maleik anhidrit kullanarak malzemenin OTR değerindeki değişkenliğini incelemiştir. Bu noktada ise LLDPE-g-maleik anhidrit malzemesinin LDPE katlarında kullanıldığında OTR değerinde önemli ölçüde bir değişiklik olmadığını sonucuna varmıştır (Changfeng Ge ve ark., 2016). Toplam film yapısının içerisindeki atık miktarı arttıkça malzemenin şeffaf ve saydam görüntüsü azalmaya başlamış ve pusluluk oranının da arttığı gözlemlenmiştir. Bir çalışmada ise EVOH/nano $SiO_2$ filmini hem eriyik harmanlamayı çift vidalı ekstruderde yapıp hem de şişirme ile kalıplama yöntemi ile test ettiklerinde karıştırma tiplerinin farklılığıyla ve karıştırma hızı farklılıklarıyla kristalinitelerinde değişiklik gözlemlemiş ve pusluluk oranlarında çift vidalı ekstruder kullanıldığında azalma olduğunu tespit etmiştir (Liu, Y. Ve ark, 2010). Çalışmamızda pusluluk aralığının çok yüksek değerlerde farklılık göstermemiştir olması gelecekte umut vaad edici bir sonuca varıldığını göstermektedir. Parlaklıkta ise pusluluk ile ters orantılı olarak malzemenin parlaklık değeri atık miktarı ile birlikte azalmıştır. Tüm örneklemelerin kesit alanlarına optik mikroskop altında bakıldığında ise atık miktarının artması ile EVOH katındaki kalınlık dengesizlikleri artmaya başlamış, PE ve yapıştırıcı katlarındaki homojen karışım da olumsuz yönde ilerleme sağlamıştır. Bu noktalara bakıldığında kullanılan Retain 3000 (MAH g-PE) malzemesinin çok katlı ekstruderlerde farklı oranlarda beslenmesi sonucunda %20 ye kadar atık PE/EVOH granülü içeren filme kadar başarılı bir görünüm verdiği ve geliştirilmesi için umut vaad ettiği söylenebilir.

## TEŞEKKÜR

Çalışmamıza tüm üretim desteğini sağlayan Korozo Ambalaj firmasına öncelikle Sayın İsa Atik, Özgür Akdemir, Mustafa Zorluca, Engin Yılmaz, Umutcan Taze, Özge Özcan'a ve Nilay Helvacıoğlu'na teşekkür ederiz.

## KAYNAKLAR

https://www.globenewswire.com/en/news-release/2022/07/05/2474204/0/en/Europe-Flexible-Packaging-Market-to-Projected-to-Cross-USD-71-3-Billion-by-2028-BlueWeave-Consulting.html, Erişim:2022

Horodytska, O., Valdés, F. J., & Fullana, A. (2018). Plastic flexible films waste management–A state of art review. Waste management, 77, sayfa 413-425

Ge, C., Fortuna, C., Lei, K., & Lu, L. X. (2016). Neat EVOH and EVOH/LDPE blend centered three-layer co-extruded blown film without tie layers. Food Packaging and Shelf Life, 8, sayfa 33-40.

Khalifa, Y. I. M. (2016). Effect of the printing remedies and lamination techniques on barrier properties "WVTR and OTR Value" for Polypropylene Film. EC Nutrition, 5(2), sayfa 1089-1099.

Liu, Y., Liu, Y., & Wei, S. (2010). Processing technologies of EVOH/nano-SiO2 high-barrier packaging composites. In Proc. 17th IAPRI World Conf. Packaging (pp. 269-74). Tianjin: Scientific Research Publishing.

Maes, C., Luyten, W., Herremans, G., Peeters, R., Carleer, R., & Buntinx, M. (2018). Recent updates on the barrier properties of ethylene vinyl alcohol copolymer (EVOH): A review. Polymer Reviews, 58(2), sayfa 209-246.

Mertanen, O. (2015). Innovation opportunity mapping – MFC film as a packaging barrier, sayfa 7

Robertson, G. L. (2005). Food packaging: principles and practice. CRC press, sayfa 45.

Siracusa, V. (2012). Food packaging permeability behaviour: A report. International Journal of Polymer Science, 2012.

TMMOB Kimya Mühendisleri Odası İstanbul Şubesi Plastik ve Kauçuk Komisyonu, (2021) Türkiye`de Plastik Geri Dönüşümü ve Atık İthalatı Raporu, sayfa 5

Villalpando-Olmos, J., Sánchez-Valdes, S., & Yáñez-Flores, I. G. (1999). Performance of polyethylene/ethylene-vinyl alcohol copolymer/polyethylene multilayer films using maleated polyethylene blends. Polymer Engineering & Science, 39(9), sayfa 1597-1603.

Wang, L. (2019). Effect of Regrind Content on PP-EVOH Sheets Properties and Effect of Orientation on Permeability of EVOH Multilayer Films (Doctoral dissertation, Ecole Polytechnique, Montreal (Canada)),

## *ORAL PRESENTATION*

# Human Resources Training For Socio-Economic Development Of Tuyen Quang Province And Northwest Area – In case study of Tan Trao University

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**Abstract:**

This paper aims to evaluate training, scientific research and technology transfer activities of Tan Trao University. The research data used in the article are aggregated from 2016 to 2021. By descriptive, statistics, comparative methods, the paper points out its impacts on the socio-economic development of the locality. The region, in which there are the University's contributions in the fields of: training high-quality human resources, scientific research, technology transfer, economic development within Tuyen Quang province and Northwest region as well as a bridge with domestic and foreign partners to serve the needs of socio-economic development of Tuyen Quang province and the Northwest region.

**Key words:** social - economic development, Tan Trao University, Tuyen Quang province, Vietnam

## 1. Introduction

For the locality and the region, the university is not only a place to provide quality human resources but also actively contribute to the socio-economic development of the locality and the region (Valero & Van Reenen, 2016).

Tan Trao University is a public university with multidisciplinary training in Vietnam. The Tan Trao University was established in 2013 and was assigned by the Ministry of Education and Training, Tuyen Quang province to train and associate training human resources with college, university and graduate degrees; scientific research and technology transfer; international cooperation in order to serve the needs of economic, cultural and social development of the local as well as the region and the whole country. In 2019, Tan Trao University is one of 128 universities in the country that has met the quality accreditation standard of higher education institutions (Tan Trao University, 2020). Currently, the University has been training pupils and students from Tuyen Quang province and from over 30 other provinces in the country. Over the years, Tan Trao University has made great contributions to improving people's intellectual standards, training human resources and fostering talents for education and training, contributing to economic development. - society of Tuyen Quang province, the Northwest region as well as the whole country.

## 2. Methods

The paper uses qualitative research methods (methods of analysis, comparison, synthesis, descriptive statistics...) and the collected data are secondary data selected from previous research results. as well as from reports from Tuyen Quang province, TanTrao Unversity on human resources training. These methods are used to clarify the research contents in order to point out the urgency of the article on human resources training of Tan Trao Unversity for socio-economic development in Tuyen Quang province and northwest area; at the same time, analyze and evaluate the current situation to highlight the results in human resource training achieved by Tan Trao University in recent times and point out the limitations and causes. From there, the paper analyzes, evaluates and predicts trends and solutions that need to be given to accelerate the process of training human resources for socio-economic developemt in Tuyen Quang province and northwest area.

## 3. Results

### *3.1 Contributions of Tan Trao University in the socio-economic development of the locality and the Northwest region in recent years*

*3.1.1 Knowledge training cradle*

The establishment of the university was intended to help develop the local economy. Valero and Reenen (2016) have demonstrated that if a locality has only one university, the contribution of this university will be 10% to the

total income of that locality. In addition, the benefits brought by the university also spread to the localities and neighboring areas; benefit more or less depends on geographical distance. From that, it can be seen that the economic benefits brought by the university are huge (Valero & Van Reenen, 2019).

Tan Trao University is currently training 24 undergraduate majors in most fields, basically matching the social needs as well as the strengths of Tuyen Quang province and the region such as: agriculture, forestry, economics & management business management, culture, tourism, pedagogy, especially in the field of medicine - pharmacy.

In the 2017-2021 period, Tan Trao University has trained, coordinated, and fostered over 34,075 people, of which 2,766 are full-time university students in various disciplines; training and granting certificates of all kinds to 18,936 people (Table 3.1).

**Table 3.1. The reality of training and fostering vocational skills for employees at Tan Trao University in the period 2017 - 2021**

| Type of training and retraining | *2017 (person)* | *2018 (person)* | *2019 (person)* | *2020 (person)* | *2021 (person)* | Rate (%) |
|---|---|---|---|---|---|---|
| 1. Certificate of IT | 1.170 | 2,500 | 1,500 yen | 1,000 yen | 700 | 87.9 |
| 2. Certificate of Foreign Language | 776 | 1,400 | 700 | 500 | 500 | 89.6 |
| 3. Certificate of Education Management | 88 | 100 | 100 | 100 | 100 | 103.2 |
| 4. Certificate of Culinary Arts | 190 | 100 | 110 | 121 | 133 | 91.5 |
| 5. Certificate of Education Skills | 48 | 0 | 300 | 300 | 300 | 158.1 |
| 6. Certificate of Vocational Education | 0 | 2,000 won | 1,500 yen | 1,000 yen | 700 | 108.8 |
| **Total** | **2,272** | **6,100** | **4.510** | **3,321** | **2.733** | **104.7** |

The source:(Tan Trao University, 2021)

Through the survey, in the period 2017-2021, over 97% of the officials, civil servants, public employees, teachers and students of Tuyen Quang province were trained and retrained to improve their qualifications at Tan Trao University.

The quality of training is also confirmed by the fact that on average, 70% of graduates have jobs (in 2019) and over 90% in 2020, some majors reach 100% such as accounting, land management, and materials. environmental management, culture and tourism (Tan Trao University, 2021).

*3.1.2 Scientific research and technology transfer*

Scientific and technological activities create a foundation of knowledge, understanding and skills of employees, both promoting and serving as a basis for production and social development. (Charles, D, 2011). The application of scientific and technological advances to production contributes to accelerating the fundamental and comprehensive transformation of production, improving productivity, quality and efficiency of production and business. The economic structure is shifted towards rapidly increasing production industries with high science and technology content and high added value, contributing to speeding up economic development.

Awareness of the important role of applying scientific and technological advances to production and life, over the years, Tan Trao University has paid great attention to developing scientific research activities. and technology transfer by both lecturers and students. That is:

- *Scientific research activities* : The results of scientific research activities of lecturers of Tan Trao University have increased significantly in quantity and quality in recent years (Table 2.2), especially the marked increase in the number of articles published in prestigious international scientific conferences and journals, specifically from 13 articles in 2017, the total number of these articles will increase to 107 articles by 2021. the growth rate reached 115.3%. Number of good quality articles published in international scientific journals with ISI index, Scopus achieved a growth rate of 151%. The Science Journal of CTU is ranked 71 out of 86 scientific journals with IF and H-index scores in more than 600 scientific journals of universities, research institutes and ministries and branches in the field of science and technology. the whole country (Tan Trao University, 2021).

**Table 3.2 Scientific research products of Tan Trao University in the period of 2017-2021**

| Type of research | 2017 | 2018 | 2019 | 2020 | 2021 | total | Ratio (%) | High school (%) |
|---|---|---|---|---|---|---|---|---|
| Provincial projects and above | 4 | 8 | 6 | 4 | 4 | 27 | 1.91 | 105.7 |
| University level projects | 23 | 53 | 38 | 2 7 | 43 | 184 | 13.00 | 116.9 |
| international proceedings | 13 | 4 | 37 | 30 | 23 | 107 | 7.56 | 115.3 |
| Article of ISI/ Scopus | 5 | ten | 21 | twelfth | 26 | 74 | 5.23 | 151.0 |
| domestic proceedings | 220 | 189 | 147 | 166 | 217 | 939 | 66.36 | 99.7 |
| Textbooks, references | 13 | 9 | 14 | 25 | 23 | 84 | 5.94 | 115.3 |

*Source : ( Department of Scientific Management and International Cooperation , Tan Trao University , 2020 )*

- *Technology transfer activities* : Every year, the University provides over 2.5 million agricultural and forestry seedlings such as bananas, sugarcane, acacia, eucalyptus... produced by tissue and cell culture methods, changing the structure of forestry tree varieties of Tuyen Quang province and some northern mountainous provinces. Training and transferring science and technology to over 200 individuals and households on economic development, sustainable agricultural development, and homestay tourism in districts such as Na Hang, Lam Binh, and Ham Yen.

*3.1.3 Attracting talents and high-quality workforce*

Attracting and retaining talent is one of the important strategies for the development of the university (Bradley, A. P, 2016). Improving working conditions and environment, developing appropriate policies and constantly improving rankings on university rankings are important factors to attract and retain qualified and skilled lecturersv(Huong, 2013). This is necessary not only for the development of the university but for the development of the locality itself.

**Table 3.3 Number of staff and lecturers at Tan Trao University in the period of 2017 - 2021**

| No | Level | 2017 | 2018 | 2019 | Year 2020 | Year 2021 | TDTU (%) | Ratio (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | Professor, Assoc Prof | first | first | 23 | 50 | 52 | 268.5 | 14.5 |
| 2 | Doctor | 21 | 25 | 36 | 65 | 98 | 147.0 | 27.3 |
| 3 | Master | 149 | 169 | 173 | 205 | 209 | 108.8 | 58.2 |
| 4 | BA | forty six | 34 | 38 | 20 | 0 | 0.0 | 0.0 |
| | **totTotal** | 217 | 229 | 270 | 340 | 359 | 113.4 | 100.0 |

The source:(Tan Trao University, 2021)

In 2017, Tan Trao University had 217 staff and lecturers, including 21 PhDs (accounting for 9.7% of direct teaching teachers), 01 Associate Professor (accounting for 0.5% of directteaching lecturers). By the end of 2021, the University has a total of 359 lecturers and researchers, 100% of the teaching staff have a master's degree or higher (the growth rate is 108.8%); the number of lecturers is PhD daccounts for is 27.3%; the number of lecturers is Assoc Prof, Prof reaches 14,5% of the total number of lecturers.

Thus, the number of staff and lecturers of Tan Trao University has increased in quantity and quality over the years. The university has been doing a good job of attracting and retaining talents. However, at present, Tan Trao University still has limitations that need to be overcome soon, that is, the number of doctorates is not many compared to the total number of lecturers and is uneven in professions; The training capacity of the University in some fields oexcept for pedagogy is not high; Tan Trao University is not really confident to go beyond the area of Tuyen Quang province to participate in the market of training and scientific research in Northwest aea as well as the whole country.

*3.1.4 Connecting and building cooperative relationships with domestic and foreign partners*

The university is the unit with the most favorable conditions in connecting and cooperating with domestic and foreign organizations and businesses, contributing to promoting investment attraction in the locality (Dung & Huong, 2017).

By 2022, Tan Trao University has established cooperative relationships with nearly 30 universities, research institutes and international organizations in ASEAN such as Thailand, Philippines, Singapore, Malaysia, Indonesia, Laos; countries in Asia such as: Korea, China; European countries such as: Russia, Belarus, Poland

(Tan Trao University, 2020). The content of cooperation focuses on the fields of training, scientific research, publishing scientific products, exchanging students and lecturers. From these cooperative relationships, the university has created opportunities for students to experience, access to a professional and modern learning environment from advanced countries around the world.

Every year, the universityl receives hundreds of students from other countries to practice at Tan Trao University and sends hundreds of students from Tan Trao University to do internships at universities abroad, from which students Tan Trao University has improved foreign language skills and working skills, gradually approaching the labor market of Southeast Asia. Besides, through cooperation activities, the university has contributed to introduce and promote the image and people of Tuyen Quang province to domestic and international friends.

***3.2 Some challenges for the development of Tan Trao University in the current context***

- Currently in Vietnam, the problem of competition between universities is increasing, especially the competition between newly established universities and those with "thickness" and "brand name". The trend of vocational training increased, higher education decreased. Many large enterprises attract workers without training.
- Conflict among the needs to improve the quality of training while the cost of training is low. There is a contradiction between the University's desire to expand, develop, grow and raise the level the university within ít conditions, to meet the university's needs of society, and the human and material capabilities of Tan Trao University, of the locality.
- The level of education and income of the people in the regions and localities of Tuyen Quang province in particular and the northern mountainous region in general are still low.
- Tan Trao University is located far away from big cities, cultural and social centers of the country.
- The university's autonomy is still low, most of it still relies on investment resources from the lprovincial budget.
- The University's human resources are highly qualified and fluent in foreign languages (accounting for 10% of the total number of lecturers in Tan Trao University). This affects and limits international integration and cooperation with foreign universities.

***3. 3. Proposing some solutions to promote the role of Tan Trao University in the socio-economic development of Tuyen Quang province and the Northern mountainous region in general***

*3.3.1 Strengthening the role of training high-quality human resources*

*Improve the quality of training the workforce to meet social needs.* In the long-term development strategy, Tan Trao University has determined that "training quality is the core factor for development". Therefore, in order to train high-quality human resources for socio-economic development, first of all, it is necessary to identify leading fields and industries that society needs; then the university needs to continuously innovate training programs, learn with practice, make practical contacts, diversify forms of training in the new situation; focus on training necessary skills for students so that after graduation, they can meet the recruitment needs of large enterprises and corporations. Orientation to train learners capable of working at home and abroad, timely adapting to the development of the industrial revolution 4.0.

*Focus on training high-quality human resources for the rapid development of priority areas of Tuyen Quang province and the northern mountainous regions.* The universityl needs to coordinate with other universities, localities and businesses in continuing to train high-level human resources (from university and above). It is necessary to focus resources on areas in which the university has strengths and at the same time suitable for the needs of human resources of the society. Specifically, a number of key industries can focus on the following training:

- Firstly, to train human resources to meet the educational development needs, especially primary and preschool education for the locality and neighboring provinces.
- Secondly, training human resources to meet the requirements of agricultural development to produce high-value-added goods, green development on the basis of application of modern technology and organized with advanced production forms such as: agricultural product value chain, agro-industrial complex, forestry-industrial complex, specialized production areas; production associated with branding and geographical indications, traceability; development of e-commerce and digital economy.
- Thirdly, training human resources to meet the requirements of sustainable development of processing, manufacturing and mineral processing industries in the direction of green development.
- Fourthly, training human resources to meet the development requirements of the service sector, in which trade and tourism are strongly developed to effectively promote the local cultural capital, production and consumption habits. of locals as well as of tourists from all over the world.
- Fifly, training human resources to meet the health care requirements of people as well as foreigners coming to work, live and visit in the locality.

*3.3.2 Promote its role as a center for scientific research and transfer of science and technology*

For many years, Tan Trao University has implemented and conducted effective research on many scientific research topics with high application and practicality for Tuyen Quang province and some localities in the region. In the

coming time, the university needs to continue to promote and improve the quality of scientific research and technology transfer activities for the development of the community and society.

First, the school needs to create motivation for lecturers to actively participate in scientific research, and at the same time improve the quality of facilities for scientific research activities such as: upgrading the school library, supplementing adding more database sources to be able to provide a full range of information sources and references to serve the scientific research process of the lecturers. There is a clear and specific policy mechanism in the "reward-punishment", especially the reward system for lecturers who have a high number of scientific research hours.

Tan Trao University needs to focus on researching scientific topics towards transforming growth models, attaching importance to improving productivity, quality, efficiency and competitiveness associated with green and sustainable development for the future. localities; Besides, research on topics in the field of value chains, global distribution chains and digital economy development should also be considered.

*3.3.3 Maintain and expand international cooperation relationships with domestic and foreign partners*

Tan Trao University needs to continue to maintain and develop the ongoing cooperation with domestic and foreign universities in scientific research, training, student exchange, and cultural exchange. … suitable for the new normal conditions during and after the current Covid-19 epidemic.

Actively organize and participate in online activities about training, cultural exchange, and exchange of study materials with other universities at home and abroad.

Strengthen the signing of training programs, cooperate with domestic and foreign enterprises, prestigious universities and enterprises abroad on the basis of investment in building facilities to meet international standards. national and international. Focus on high-tech field and seek output for scientific research products.

## 4. Conclusion

Tan Trao University has played an important role in the socio-economic development of Tuyen Quang province and the region. In which, the role of improving the quality of human resources, basically meeting the training needs of Tuyen Quang province and the region. The training professions are suitable with the province's socio-economic potential strengths and the needs of the society, the number of graduates who have jobs and the achievement of accreditation standards for higher education institutions have created a good change in the position of Tan Trao University in the region and the university system of the country.

In order to maximize the role of Tan Trao University in the socio-economic development of Tuyen Quang province and the northern mountainous region in general, some key solutions to focus on are: i) Strengthening the role of training high-quality human resources; (ii) Promote its role as a center for scientific research and transfer of science and technology; (iii) Maintain and expand international cooperation relationships with domestic and foreign partners.

**Acknowledgements:** This study is supported by Tan Trao University, Tuyen Quang and University of Transport Technology.

## References

Valero, A., & Van Reenen, J. (2016). How universities boost economic growth. *CEPR Policy Portal* .

Bradley, AP (2016). Talent management for universities. *Australian Universities' Review* , 58(1), 13-19.

Dung, NK, & Huong, PT (2017). The reality of university-business cooperation in Vietnam. *JOURNAL OF SCIENCE-EDUCATION SCIENCE* , Vol. 14, No. 4:29-41.

Huong, NT (2013). Develop teaching staff in universities - Situations and solutions. *VNU Journal of Science: Legal Studies* , 28(2), 110 - 116.

Valero, A., & Van Reenen, J. (2019). The economic impact of universities: Evidence from across the globe. *Economics of Education Review* , 68, 53-67.

Tan Trao University. (2021). *List of scientific research products of faculty and staff of Tan Trao University.* Office of Science Management & International Relations.

Charles, D. (2011). The role of universities in building knowledge cities in Australia. *Built Environment* , 37(3), 281-298.

Tan Trao University. (2020). *Report on the implementation of the strategy of science and technology development and international cooperation of Tan Trao University in the period 2014-2020.* Tan Trao University.

Tan Trao University. (2021). *Development strategy of TanTtrao U nversity to 2025, orientation to 2030.* Tan Trao University.

## *ORAL PRESENTATION*

### Astım tedavisinde kullanılan ilaç etken maddeleri üzerine yapılan analitik çalışmalar

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**Özet**

Astım, hava yollarının kronik olarak seyreden enflamasyonu olarak tanımlanabilecek bir solunum yolu hastalığıdır. Hava yollarının daralması ile ortaya çıkar ve ataklar (krizler) halinde devam eder. Astımın farmakolojik tedavisinde 3 grup ilaç kullanılmaktadır. Bunlar hastalığın kontrolünü sağlamak için kullanılan ilaçlar, semptomları gidermek için kullanılan rahatlatıcı ilaçlar ve hastalığı ağır geçirenler ile ilk iki gruptan sonuç alınamadığında kullanılan ilave ilaçlardır. Çalışmanın amacı; dünya üzerinde 300 milyonu aşkın insanı etkileyen astım hastalığının tedavisinde kullanılmakta olan beta 2 reseptör agonisti salbutamol ve lökotrien antagonisti montelukast etken maddeleri için son yıllarda yapılmış ve ICH tarafından onaylanmış parametrelerin kanıtlandığı analitik çalışmaların incelenmesi, yorumlanması ve sunulmasıdır. Salbutamol etken maddesi için LC-ESI-MS/MS, LC-MS, HPTLC, UPLC-MS/MS ve SFC-MS/MS, GC-MS, HPLC-DAD, RP-HPLC, HRMS; montelukast etken maddesi için HPLC-MS/MS çalışmaları incelenmiştir. Çalışmaların sonuçları incelendiğinde; araştırmacıların etken maddelerin miktar tayinleri için basit, kesin, doğru, hassas, hızlı ve ekonomik yöntemler geliştirdikleri söylenebilir.

**Anahtar Kelimeler:** Astım, salbutamol, montelukast, analitik çalışmalar

### Analytical studies on drug active substances used in the treatment of asthma

**Abstract**

Asthma is a respiratory disease that can be defined as chronic inflammation of the airways. It occurs with the narrowing of the airways and continues in attacks (crises). Three groups of drugs are used in the pharmacological treatment of asthma. These are drugs used to control the disease, reliever drugs used to relieve symptoms, and additional drugs used when the first two groups do not get results. Purpose of the study; it is the examination, interpretation and presentation of analytical studies conducted in recent years for the beta 2 receptor agonist salbutamol and the leukotriene antagonist montelukast, which are used in the treatment of asthma that affects more than 300 million people around the world, and which have proven the parameters approved by ICH. For the salbutamol active ingredient LC-ESI-MS/MS, LC-MS, HPTLC, UPLC-MS/MS with SFC-MS/MS, GC-MS, HPLC-DAD, RP-HPLC, HRMS; for the montelukast active ingredient HPLC-MS/MS studies were examined. When the results of the studies are examined; It can be said that researchers have developed simple, precise, accurate, sensitive, fast and economical methods for the quantification of active substances.

**Key Words:** Asthma, salbutamol, montelukast, analytical studies

## GİRİŞ

Astım, kronik reaktif hava yolları hastalığı olup, çeşitli uyaranlara karşı duyarlılık ve hava yollarının inflamasyonuyla karakterize bir hastalıktır. Çocukluk dönemi astımında önemli risk faktörlerinin başında erkek cinsiyet gelir. On dört yaş altında astım prevalansına bakıldığında erkeklerde kızların iki katına ulaştığı görülse de (Erdoğan ve ark., 2015; Selçuk ve ark., 1997) yaş büyüdükçe bu farklılık azalmakta ve yetişkin dönemde kadınlarda daha sık görülmektedir. Bunun yanında cinsiyet, hastalığın klinik remisyonunu ve kalıcılığını da etkileyebilmektedir (Şekerel ve ark., 2006). Atopi varlığında çocuk ve genç yetişkinlerde astım sıklığı artmaktadır. Son yıllarda yapılan çalışmalar alerjik duyarlanmanın yaş ilerledikçe astım görülme sıklığında artışa neden olduğunu göstermiştir. Hamam böceği, küf ve ev tozu akarı alerjenleri yetişkin astımı için önemli risk faktörleridir (Jaakkola ve ark., 2006; Busse ve ark., 2013). Bunun yanında polen duyarlılığı özellikle de Parietaria polen duyarlılığı yetişkin astımında risk faktörü olarak bulunmuştur (Di Lorenzo ve ark., 1997). Hava yoluyla bulaşan çevresel maruziyetler, tütün dumanı, kirleticiler ve ozon dahil astım riskini artırır. Atopik durumlar ve inhalasyon alerjenlerine karşı duyarlılık da astım gelişimi ile ilişkilidir. Mikrobiyomun etkileri, D vitamini, kimyasal maruziyet, diyet değişiklikleri, stres ve metabolitlerin etkileri astım gelişiminde

rol oynayan diğer faktörlerdir (Mims, 2015). Astımda genetik faktörler, hastalığın ortaya çıkışında etkili risk faktörlerinin başında yer alır (Ober, 2005). Çevresel faktörler ise genellikle astım alevlenmesine yol açarlar. Genler hem çevresel faktörlerle hem de birbirleriyle etkileşerek bireyin astıma eğilimini artırırlar (Holgate, 1999). Astımın patogenezinde birçok genin rolü bulunmaktadır (Holgate, 1999; Şahiner ve ark., 2014). Günümüzde astım ile ilişkili 600'ün üzerinde aday gen tanımlanmıştır (Toskala ve Kennedy, 2015).

Astım tedavisinde hedef, risklerin gözetilmesi, klinik kontrolün sağlanması ve devamlılığın korunmasıdır. Tedavide kullanılmakta olan ilaçlar üç gruptan oluşur. Bunlar kontrol ediciler, semptom gidericiler (rahatlatıcı/kurtarıcı), ağır astımlılarda kullanılmakta olan ve fenotipik tedavi adı verilen "ilave ilaçlar" dır.

Antiinflamatuar etkilerinden dolayı astımın kontrol altında tutulmasına yardımcı olan kontrol ediciler, genellikle her gün ve uzun süreli kullanılan ilaç grubudur. Hızlı etkileriyle bronkokonstriksüyonu geri döndürerek semptomların giderilmesini sağlayan semptom gidericiler ise gerektiğinde kullanılırlar. Üçüncü ve son gurup olan "ilave ilaçlar" ise tek başlarına kullanılmayan kontrol edici ilaçlarla birlikte kullanılan ilaçlardır (Global Initiative for Asthma (GINA), 2020; Ulusal Astım Tanı ve Tedavi Rehberi, 2016). Düzenli olarak düşük dozda inhaler kortikosteroid kullanımının ve gerektiğinde kısa etkili beta 2 reseptör agonisti takviyesinin astımın kontrolünde işe yaramadığı durumlarda, uzun etkili beta 2 reseptör agonistlerinin inhaler steroid ile kombine tedavisi önerilir (Ducharme ve ark., 2010). Uzun etkili beta 2 agonistlerin inhaler steroidlerle kombine tedavisi sonucunda astımın klinik bulgularında iyileşme ve astım ataklarda azalma görülür (Juniper ve ark., 1999; Ni Chroinin ve ark., 2009). Yetişkin hafif persistan astımlı hastalardan inhaler steroid kullanımı ile yan etki gelişenler veya kullanmak istemeyenler veya eşlik eden alerjik riniti olanlar başlangıç ve idame tedavinde anti-lökotrien ilaçlar kullanabilirler (Leff ve ark., 1998; Reiss ve ark., 1998). Lökotrien reseptör antagonisti olan montelukast anti-lökotrien ilaçlardan Türkiye'de kullanılan tek ilaçtır. Çalışmanın amacı astım tedavisinde kullanılan beta 2 reseptör agonisti salbutamol ve lökotrien antagonisti olan montelukast etken maddeleri hakkında bilgi vermek ve son yıllarda analitik yöntemler kullanılarak çalışılmış olan etken maddelerinin miktar tayinlerine ilişkin literatür çalışmalarının incelenmesi, bunların değerlendirilerek derleme oluşturulmasıdır.

## MATERYAL VE METOT

### Etken Madde Analiz Yöntemleri

*Elektrosprey iyonizasyon başlıklı - Sıvı Kromatografisi - Ardışık (Tandem) Kütle Spektrometresi (LC-ESI-MS/MS):* Sıvı kromatografi-kütle spektrometresi/kütle spektrometresi (LC-MS/MS), sıvı kromatografisinin (HPLC) kütle spektrometrisinin (MS) kütle analizi yetenekleri ile fiziksel ayırma yeteneklerini birleştiren bir analitik kimya tekniğidir. Eşleşmiş kromatografi - MS sistemleri kimyasal analizde popülerdir çünkü her tekniğin bireysel yetenekleri sinerjik olarak geliştirilmiştir. Sıvı kromatografisi, karışımları çoklu bileşenlerle ayırırken, kütle spektrometresi, yüksek moleküler özgüllük ve algılama duyarlılığı ile bireysel bileşenlerin yapısal kimliğini sağlar. LC ünitesinden ayrıştırılmış analitler iyonlaştırılarak MS/MS ünitesine gönderilirler. MS/MS ünitesinde iyonlar ilk quadropolden kütle/yük (m/z) oranlarında ayrıştırılarak collision cell olarak bilinen girişim hücresine yönlendirilirler. Burada yüksek saflıkta azot gazı ile çarpıştırılan moleküller parçalanarak ikinci quadropole gönderilirler. İkinci quadropol ünitesindeki bu ikinci iyonlar da kütle/yük (m/z) oranlarında ayrıştırılarak kütlesel olarak tayin edilirler. Aynı m/z oranına sahip pek çok molekül olabilecekken aynı parçalanma iyonlarına sahip moleküllerin doğada bulunma oranı 1/10000'dir. Bu tandem tekniği, çevre ve biyolojik kaynaklı karmaşık örneklerde yaygın olarak bulunan biyokimyasal, organik ve inorganik bileşikleri hassas bir şekilde analiz etmek için tercih edilir. Bu nedenle, LC-MS/MS, biyoteknoloji, çevre izleme, gıda işleme ve farmasötik, zirai ilaç ve kozmetik endüstrileri gibi çok çeşitli sektörlerde uygulanabilir. LC-MS/MS, analitlerin kalitatif ve kantitatif olarak analiz edilmesine imkan verir. Karmaşık ortamlarda yer alan tekli veya çoklu analitlerin analizinde, eser düzeydeki maddelerin analizinde, ilaç etken maddelerinin yanında safsızlık ve metabolitlerinin analizinde, gıdalardaki katkı maddeleri veya pestisitlerinin analizlerinde, kan, idrar, doku gibi biyolojik numunelerin analizlerinde yüksek hassaslık ve doğruluk ile sonuç almak mümkündür. LC-MS/MS, çoklu analit tayininin yanı sıra numunedeki eser miktardaki analitlerin tayini için tercih edilen en hassas metotlardan biridir. Örnekte bulunan bileşenlerin miktarı, yapısı ve molekül ağırlığı hakkında bilgi verir. Küçük farmasötik bileşiklerden büyük proteinlerin tayinine kadar, polar iyonik, termal kararsız ve uçucu olmayan bileşiklerin analizleri gibi çok geniş bir kullanım alanına sahiptir. Sistem ESI (electrospray ionization) ve APCI (atmospheric pressure chemical ionization) iyon kaynakları içermekte ve bu sayede daha geniş bir analiz skalası elde etmektedir.

*Sıvı Kromatografisi - Kütle Spektrometresi (LC-MS*): Sıvı kromatografinin (veya HPLC) fiziksel ayırma yeteneklerini kütle spektrometrisinin (MS) kütle analizi yetenekleriyle birleştiren bir analitik kimya tekniğidir.

*Yüksek Performanslı İnce Tabaka Kromatografisi (HPTLC- High Performance Thin Layer Chromatography):* İnce Tabaka Kromatografisinde (İTK) ayırımı etkileyen birçok parametrenin kontrol altına alındığı, standardize edildiği, son teknoloji İTK'dır. Farklı adımları otomatikleştirmek, elde edilen çözünürlüğü

artırmak ve daha doğru nicel ölçümlere izin vermek için ince tabaka kromatografisinin temel yönteminde bir dizi iyileştirme yapılabilir. Otomasyon, numune TLC plakasına elle uygulandığında damlacık boyutundaki ve konumundaki belirsizliğin üstesinden gelmek için yararlıdır. Otomasyona bir yaklaşım, numuneyi uygulamak için piezoelektrik cihazların ve mürekkep püskürtmeli yazıcıların kullanılması olmuştur. Spot kapasitesi (HPLC'deki pik kapasitesine benzer), iki boyutlu kromatografi kullanılarak plakanın iki farklı solvent ile geliştirilmesiyle arttırılabilir. Prosedür, ilk çözücü ile numune yüklü plakanın geliştirilmesiyle başlar. Çıkartıldıktan sonra plaka 90° döndürülür ve ikinci bir solvent ile geliştirilir.

*Ultra Performanslı Sıvı Kromatografisi - Ardışık (Tandem) Kütle Spektrometresi (UPLC-MS/MS) ve Süperkritik Akış Ekstraksiyon - Ardışık Kütle Spektrometresi (SFC-MS/MS):* Ultra Performanslı Sıvı Kromatografisi teknolojisi yüksek basınçta çalışan ve çok hassas femtomolar seviyede analizler yapan sistemdir. Genellikle, şekerlerin analizinde kullanılan, başka ufak moleküllerin ayrıştırılması ve karakterizasyonunda kullanılır.

Geleneksel LC/MS (likit kromatografi kütle spektrometre) ve GC/MS (gaz kromatografi kütle spektrometre) tekniklerinde, kullanıcılar zahmetli ve uzun süren numune hazırlığı, hassas bileşiklerin numune hazırlığı esnasında degradasyonu ve gürültünün içinde görünmeyen pikler gibi zorluklarla karşı karşıya kalmaktadır. SFC Kromatografi tüm bu zorluklara üst düzey çözümler sunar. Bunlar; Tam otomatik çevrimiçi numune hazırlama ve analizi, Kararsız bileşiklerin bile bozulma olmadan analizi, Birleştirilmiş analiz hızı, hassasiyet ve çözünürlüktür. SFC için mobil faz olarak kullanılan süper kritik karbondioksit düşük polarite ve viskozite, diğer organik çözücülerle karışabilirlik, buharlaşma ve çözücü maliyet düşüklüğü gibi benzersiz avantajlar sunar.

*Gaz Kromatografisi - Kütle Spektrometresi (GC-MS):* GC (Gaz Kromatografi) ve MS (Kütle Spektrometresi) ünitelerinin beraber çalıştığı yapı analizi ve miktar tayininde kullanılan cihazdır.

*Ters Fazlı Sıvı Kromatografisi (RP- HPLC):* Yüksek basınçlı veya yüksek performanslı sıvı kromatografisi (HPLC), bir karışımda yer alan her bir bileşeni ayırmak, tanımlamak ve miktarını belirlemek için kullanılan bir ayırma tekniğidir.

*Yüksek çözünürlüklü kütle spektrometresi (HRMS):* Bir bileşiğin kütlesinin belirlenmesinde kesinliğin iyi ve doğruluğun yüksek olması için bazı faktörlerin optimize edilmesi önemlidir. Bu faktörler, pik şekli, iyon bolluğu, çözünürlük ve kalibrasyondur. Pik şeklini etkileyen faktörlerden bir tanesi iyon bolluğudur. Sinyalin çok yoğun olması dedektör ölçümünün baskılanmasına; yeterli seviyede olmaması ise pik şeklinin kötü olmasına neden olmaktadır. HRMS ile yapılan ölçümlerde daha dar pik şekilleri elde edilebilmekte ve bu da belirsizliği azaltmaktadır. HRMS'nin, çok küçük kütle farklılıklarını algılayarak yüksek ayırma kapasitesine sahip olması, kromatografik ayrım ihtiyacını düşürmesi ve metabolitlerin tekli kütle analizörü ile geniş kapsamda miktarını belirlemesi bu cihazın kullanımının yaygınlaşmasını sağlamıştır.

## BULGULAR ve TARTIŞMA

Literatürde son yıllarda yapılan salbutamol ve montelukast etken maddelerinin tayin yöntemleri üzerine yapılan çalışmalar incelenmiş ve çalışmaları yapan araştırmacılar ile bu araştırmacıların buldukları sonuçlar derlenmiştir.

**İdrar örneklerinde salbutamol tespiti için kiral sıvı kromatografisi-kütle spektrometrisi (LC-ESI-MS/MS) yöntemi geliştirme** adlı makalede domuz idrarındaki salbutamol enantiyomerlerinin tespiti ve miktarının belirlenmesinde likit kromatografi-elektrosprey iyonizasyon-tandem kütle spektrometrisi (LC-ESI-MS/MS) kullanılarak sıralı bir katı faz ekstraksiyon (SPE) yöntemi geliştirilmiş ve doğrulanmıştır. Domuz idrar örnekleri, Helix pomatia'dan elde edilen beta-glukuronidaz/arilsülfataz ile hidrolize edilmiş ve ardından önce Abs-Elut Nexus SPE ve ardından Bond Elut Fenilboronik Asit (PBA) SPE kullanılarak bir çift katı faz ekstraksiyonuna (SPE) tabi tutulmuştur. Salbutamol enantiyomerleri, 15°C'de tutulan Astec CHIROBIOTIC™ T HPLC kolonu (3,0x100 mm; 5 µm) kullanılarak 0,4 mL/dk'lık bir akış hızında 15 dakikalık izokratik bir çalışma ile ayrılmıştır. Mobil faz, metanol içinde 5 mM amonyum formattan oluşturulmuştur. Salbutamol ve salbutamol tert-butil-d9 (iç standart) çoklu reaksiyon izleme modu ile izlenmiş ve miktarı belirlenmiştir. Yöntem, 0,3 ng/mL'de kantifikasyon limiti ile 0,1-10 ng/mL aralığı için doğrusallık göstermiştir. QC numunelerinin analizi, günler içi ve arası kesinliklerin %5,04'ten az olduğunu ve geri kazanımın %83,82 ile %102,33 arasında değiştiğini göstermiştir. Sıralı SPE yönteminin, gün içi ve günler arası doğrulama ile zenginleştirilen domuz idrarındaki salbutamol enantiyomerlerinin belirlenmesi için seçicilik/özgüllük, doğruluk, kesinlik, doğrusallık, geri kazanım ve tekrarlanabilirlik sağladığı sonucuna varılabildiği görülmüştür. Hem gün içi hem günler arası doğrulama çalışması, %10'dan daha az hassasiyetle tüm QC numuneleri için yöntem doğruluğu ve %15'ten daha az geri kazanım bildirmiş ve salbutamol enantiyomerleri nicelemesinde kullanıma uygun olduğu görülmüştür. Yöntemin LOQ'su (konsantrasyon tayin limiti) 0,30 ng/mL salbutamol'de (her enantiyomer için 0,15 ng/mL) belirlenmiştir (Chan ve ark., 2016).

**Sıvı kromatografisi ve tandem kütle spektrometrisi ile domuz kıllarında raktopamin ve salbutamolün tayini** adlı makalede domuz kılı numunelerini ön işleme tabi tutmak için konsantre sodyum hidroksit kullanılmış ve ardından katı faz ekstraksiyonu (SPE) prosedürü ile saflaştırılmıştır. Ekstrakte edilen solüsyon buharlaştırılmış ve pozitif çoklu reaksiyon izleme (MRM) modunda çalışan elektrosprey iyonizasyon (ESI) ile alet içinde enjeksiyon için yeniden oluşturulmuştur. Raktopamin ve salbutamol ayrımı, gradyan modda C18 analitik kolonunda gerçekleştirilmiştir. İç standart kalibrasyon eğrisi, 0,5 ile 100 ng/mL konsantrasyon aralığında doğrusal olduğu görülmüştür. Domuz kılı örneklerinde 100, 250 ve 500 ng/mL'lik üç eklenmiş konsantrasyonda analiz edilen bu yöntemin geri kazanımları, raktopamin için %79-82 ve salbutamol için %77-96 olduğu görülmüştür. Göreceli standart sapma (% RSD) olarak ifade edilen karşılık gelen günler arası ve gün içi kesinlik sırasıyla %3,8-6,4 ve %3,8-8,6 elde edilmiştir. Bir numune için analitik süre 8 dakika olarak belirlenmiştir. Bu yöntemin saptama sınırı, raktopamin ve salbutamol için sırasıyla 0,6 ve 8,3 ng/mL olarak belirlenmiştir. Geliştirilen bu yöntem, domuz yeminde bulunabilen beta agonistleri salbutamol ve raktopamin kullanımının domuz kılında izlenmesi için uygulanabileceği görülmüştür. Veterinerlerin kullandıkları ilaçların kalıntıları, olası olumsuz etkiler nedeniyle kamuoyunu ilgilendiren bir konu haline gelmiştir, bu da yasaklanmış ilaç kullanımının sürekli izlenmesinin önemli olduğu anlamına gelmektedir. Domuz kıllarında raktopamin ve salbutamol tayini için LC-MS kullanan basit, hızlı ve güvenilir bir yöntem geliştirilmiştir. Bu yöntem, şirketler ve devlet kurumları tarafından, domuzun büyüme döneminden kesilmesine kadar raktopamin ve salbutamol kullanımını izlemek için kullanılabileceği görülmüştür (Chang ve ark., 2018).

**İnhalasyon ürünlerinden salbutamol sülfat ve beklometazon dipropionatın eşzamanlı tayini için hassas yüksek performanslı ince tabaka kromatografi yöntemi** adlı makalede inhalasyon ürününden beklometazon dipropionat ve salbutamol sülfatın eş zamanlı tayini için hassas bir yüksek performanslı ince tabaka kromatografi (HPTLC) yöntemi geliştirilmiştir. Kromatografik ayırma, sabit faz olarak silika jel G60 $F_{254}$ ve hareketli faz olarak metanol:etil asetat:toluen:amonyak (3:1:3:0,15) ile önceden kaplanmış alüminyum plakalar üzerinde gerçekleştirilmiştir. Dansitometrik değerlendirme 232 nm'de yapılmıştır. Önerilen yöntemin sağlamlığı deneysel tasarım yaklaşımı kullanılarak değerlendirilmiş ve sonuçlar grafiksel olarak analiz edilmiştir. Önerilen HPTLC yöntemi ve rapor edilen HPLC yöntemi ile numune analizi sonuçları istatistiksel olarak kararlaştırılmıştır. Salbutamol sülfat ve beklometazon dipropionatın $R_f$ değeri sırasıyla 0,38±0,02 ve 0,72±0,02 olarak bulunmuştur. Beklometazon dipropiyonat (BD) için tespit limiti ve miktar tayin limiti sırasıyla 27 ng/spot ve 84 ng/spot ve salbutamol sülfat (SAL) için sırasıyla 40 ng/spot ve 112 ng/spot olarak bulunmuştur. Önerilen HPTLC yöntemi, inhalasyon ürününde beklometazon dipropiyonat ve salbutamol sülfatın aynı anda belirlenmesi için kesin, doğru ve tekrarlanabilir nicel analiz sağladığı görülmüştür. Yöntem, ICH yönergelerine göre doğrulanmıştır. Yöntemin beklometazon dipropiyonat için 100-500 ng/spot ve salbutamol sülfat için 200-1000 ng/spot aralığında lineer olduğu bulunmuştur. Önerilen yöntemlerin sağlamlığını ölçmek için DoE (deney tasarımı) kullanılmış ve deneysel koşullarda yapılan kasıtlı değişikliklere karşı sağlam olduğu bulunmuştur (Patel ve Parmar, 2018).

**Besleme maddelerinde beta-agonistlerin taranması için kütle spektrometrisine bağlı sıvı kromatografisi ve süperkritik akışkan kromatografisi arasında karşılaştırma** adlı makalede beta-agonistik ilaçların, 1980'lerin sonlarından beri Avrupa'da hayvanların yetiştirilmesinde büyüme destekleyicileri olarak kullanımı yasaklanmıştır. UHPLC-MS/MS'ye dayalı spesifik ve hassas analitik yöntemler, bu maddelerin büyük bir setinin izlenmesine izin vermektedir. Geleneksel HPLC'de ters faz kromatografisi kullanılırken genellikle boşluk hacminde ayrıştırılan en polardan en apolar olanlara kadar β-agonistlerin büyük elüsyon penceresini kapsayacak şekilde bir SFC-MS/MS (SFC; süperkritik akışkan kromatografisi) yaklaşımı geliştirilmiştir. Amaç, yem maddelerinde, Avrupa Birliği tarafından önerilen seviyeye yani 50 µg/kg'a ulaşmaktır. LC/MS ve SFC/MS performansları, 6 β-agonistik ilaç olan bromobuterol, klenbuterol, izoksuprin, raktopamin, salbutamol ve zilpaterol için analitik doğrulama verileri (doğrusallık, seçicilik, geri kazanım oranları, tekrarlanabilirlik, bileşikler tanımlama, doğruluk, karar limiti ve tespit kabiliyeti) açısından kapsamlı bir şekilde karşılaştırılmıştır. Her iki analitik yaklaşımda önerilenden daha düşük bir konsantrasyonda (50 µg/kg) hedeflenen β-agonistlerin saptanmasını sağlamıştır. Her iki kromatografik yaklaşım, yani SFC ve LC, 6 β-agonist (bromobuterol, klenbuterol, izoksuprin, raktopamine, salbutamol ve zilpaterol) için aynı özütler ve aynı kütle spektrometresi kullanılarak kapsamlı bir şekilde karşılaştırılmıştır. Bu çalışma ayrıca 4 ilave ve zorlu β-agonist (metaproterenol, izoproterenol, izoetarin ve salmeterol) için bu kromatografik yaklaşımların tamamlayıcılığını da göstermiştir. Besleme matrislerinde β agonist analizi için ana avantaj olarak, bir Torus 2-PIC kolonu kullanan SFC, RP-LC yaklaşımına kıyasla metaproterenol ve izoproterenol gibi hem polar bileşikler hem de salmeterol gibi apolar bileşikler için gelişmiş bir tutma sunmuştur. Ayrıca, seçicilik geliştirilmiş ve SFC kullanılarak bazı kodlama sorunlarının üstesinden gelmişlerdir. Doğrulama sürecinden sonra sonuçlar, hem UHPLC-MS/MS hem de SFC-MS/MS analitik yöntemlerinin doğrulanmış 6 β-agonist için 2002/657/EC sayılı karara uygun olduğunu göstermiştir. Ayrıca, doğrulama verileri, UPLC ayrımına dayanan yöntem için daha iyi bir doğrusallık ve doğruluk gösterirken, SFC performanslarının tanımlama, tekrarlanabilirlik ve karar sınırı için daha iyi olduğu sonucuna varılmıştır. Ayrıca, SFC için daha iyi bir

duyarlılık gözlenmiştir. Sonunda hem SFC hem de LC, yemde önerilen 50 µg/kg konsantrasyonunun çok altında saptama sınırlarına izin verdiği görülmüştür. Bu iki yaklaşımın uygulama alanı genişletilmiş ve bu analitik yöntemler, beslemede verimli bir kontrolü garanti etmek için artık 28 β-agonisti aynı anda izleyebilecek hale gelmiştir. Ancak, 28 β-agonistin tam listesi, verimli bir rutin kontrol sağlamak için gizli tutulmuştur (Herpin ve ark., 2018).

**Mikro-hacimli kan örneklemesi kullanılarak salbutamol miktar tayini–pediatrik astım alevlenmelerine yönelik uygulamalar** adlı makalede kan mikro hacimlerinde (10 µL) salbutamol miktarını belirlemek için yeni bir gaz kromatografi-kütle spektrometrisi (GC-MS) yöntemi geliştirilmiştir. Şiddetli akut astımın pediatrik terapötik doz izleminde (TDM) potansiyel bir uygulamadır. Çocuklara çoklu dozlarda salbutamol (inhale, nebülize ve bazen intravenöz) verilir, ancak yetersiz konsantrasyonlar nedeniyle tedaviye yanıt vermeyen çocukları toksisitesi olanlardan ayırt etmek zordur, semptomlar benzerdir. Geleneksel kurutulmuş kan lekeleri (DBS) ile yeni geliştirilen hacimsel soğurucu mikro-örnekleme (VAMS) tekniği arasında bir karşılaştırma yapılmış ve kuruma süresinin analit geri kazanımı üzerindeki etkisine ilişkin özel bir araştırma yapılmıştır. Her iki örnekleme tekniği için de son analiz hem normal terapötik hem de toksik aralık dahil olmak üzere test edilen konsantrasyon aralığında (3-100 ng/mL) iyi bir kesinlik ve doğruluk göstermiştir. Test, inhalasyon yoluyla 1 mg salbutamol verilen üç sağlıklı gönüllüde kan örneklerinde salbutamol konsantrasyonlarının nicelleştirilmesine başarıyla uygulanmıştır. Böylece, akut durumda önemli ölçüde daha yüksek salbutamol dozlarının uygulanacağı pediatrik TDM çalışmalarında kullanım potansiyelini göstermiştir. Çalışma ile salbutamol için bir TDM yönteminin VAMS kullanılarak gerçekleştirildiği ve DBS tarafından sağlanan tüm avantajlarının yanında geleneksel DBS koleksiyonunun doğal örnekleme hacmi yanlışlıklarının ortadan kaldırdığı ilk durum olmuştur. Çalışma ile salbutamol dozlanmış gönüllülerin mikro hacimli kan örneklerinden alınan kan salbutamol düzeylerinin tayini için VAMS'ın onaylanmış bir GC-MS yöntemiyle kullanılması bir ilk olmuştur. Yöntemin duyarlılığı, gönüllü bir çalışmada 10 µL mikro örnekleme DBS/VAMS kullanılarak 1 mg salbutamol uygulamasından 2 saat sonrasına kadar kan salbutamol düzeylerinin ölçümüne uygulanmasına izin vermek için yeterli olduğu görülmüştür. Çalışmada uygulanan doz, çocuklar için normal astım giderici dozun üzerinde olmasına rağmen, akut astımlı alevlenme sırasında uygulanan yüksek doz β 2 agonistleri düşünüldüğünde düşük bulunmuştur. Bu analizin ön gönüllü deneylere uygulanması, geliştirilen yöntemin, düşük hacimli, azaltılmış invazivlik (invaziv, zarar verici olan doku oluşumlarını nitelemek adına kullanılan bir kelimedir) örneklemesinin oldukça tercih edildiği pediatrik hastalarda kan salbutamol konsantrasyonlarının izlenmesi için geçerli olacağını göstermiştir (Cordell ve ark., 2018).

**İdrar numunelerinde salbutamol tayini için katı faz ekstraksiyonu ve HPLC-DAD** adlı makalede idrar numunelerinde salbutamol tayini için bir katı faz ekstraksiyonunun ve yüksek performanslı bir sıvı kromatografi analizi ve validasyonu anlatılmıştır. Numunelerin ön işlem prosedüründen 0,5 ve 24 saat sonra inhaler uygulamayı takiben idrar numunelerinde salbutamol tayini için HPLC-DAD yöntemi kullanılmıştır. Agilent ODS (5 µm, 4,6x250 mm) 220 nm'de algılama ile 1 mL/dk akış hızında asetonitril ve su karışımından (ortofosforik asit kullanılarak pH = 3'e ayarlanmış) (90:10, v/v) oluşan mobil faz ve C18 sabit faz ile yöntemin geliştirilmesi için iç standart olarak Bambuterol HCl kullanılmıştır. Katı faz ekstraksiyonu, hem metabolize edilmemiş salbutamol hem de metabolize salbutamol için Oasis MCX kartuşu kullanılarak sırasıyla ortalama %92,24 ve %91,52 geri kazanım yüzdeleri ile yapılmıştır. Tarif edilen yöntem, 0,15 µg/mL kadar düşük konsantrasyonda insan idrarında salbutamolün saptanmasına izin vermiştir. Önerilen yöntem, sağlıklı gönüllüye 0,5 ve 24 saat sonra Ventolin®'in ölçülü doz inhalerinin (MDI) solunmasından sonra idrar örneklerinde salbutamol tayini için başarıyla uygulanmıştır. Önerilen yöntem, salbutamolün farmakokinetik çalışması ve akciğerde göreceli birikiminin belirlenmesi için kullanılabileceği görülmüştür. İdrar matrisinde salbutamol tespiti için birkaç RP-HPLC yöntemi bildirilmiştir. Yayınlanmış yöntemlerden bazıları hem metabolize edilmemiş hem de metabolize olmuş salbutamolün belirlenmesi için uygun olmayan ve karmaşık saflaştırma adımlarına ihtiyaç duyan gelişmiş teknikler gerektirdiğinden bu çalışma ile bu zorlukların üstesinden gelinmesi düşünülmüştür. Dolayısıyla bu, HPLC-DAD yöntemiyle idrar numunelerinde salbutamolün saptanması için oldukça kısa bir çalışma süresi ve basit SPE izolasyon prosedürü ile bir ekstraksiyon yöntemine ilişkin ilk makale olmuştur. Önerilen yöntem, iç standart olarak Bambuterol HCl kullanılarak geliştirilmiş, optimize edilmiş ve valide edilmiştir ve sonraki diğer klinik çalışmalarda kullanılması ve oral uygulamadan sonra salbutamolün belirlenmesi için uygulanabilirliği görülmüştür (Abdelrahman, 2018).

**İyonik sıvı destekli sulu mobil faz kullanılarak terbutalin ve salbutamolün tayini için alternatif kromatografik yöntem** adlı makalede bazik bileşiklerin silika bazlı sabit faz üzerinde ters faz kromatografisi kullanılarak ayrılması, bazik bileşiklerin katyonik bölgeleri ile sabit fazın anyonik silanolleri arasındaki etkileşimden dolayı büyük bir zorluk meydana gelmektedir. Bu çalışma, mobil faz katkı maddesi olarak oda sıcaklığında iyonik sıvının (Room temperature ionic liquits (RTIL)) kullanıldığı terbutalin ve salbutamol tayini için basit, güvenilir ve organik solventsiz sıvı kromatografik bir yöntem sunmuştur. RTIL'lerin türleri ve

konsantrasyonu gibi iki bileşiğin tutulmasını etkileyen çeşitli mobil faz parametreleri ve mobil fazın pH'ı araştırılmıştır. Geliştirilen yöntem, Uluslararası Uyum Konferansı (ICH) yönergelerine göre valide edilmiş ve farmasötik preparatlarda salbutamol sülfatın belirlenmesinde etkin bir şekilde uygulanmıştır. Farmasötiklerin %70'inden fazlasını oluşturan bazik bileşiklerin analizi, RP ayrımları için uzun süredir devam eden bir zorluğu temsil etmektedir. Bu çalışmada, iki temel bileşiğin, terbutalin ve salbutamolün aynı anda belirlenmesi için RTIL'ler ile modifiye edilmiş bir sulu mobil faz kullanan etkili bir RP-HPLC yöntemi tanımlanmıştır. 1-etil-3-metilimidazolyum bromür [EMIM][Br], incelenen RTIL'ler arasında en uygun mobil faz katkı maddesi olarak belirlenmiştir. [EMIM][Br] konsantrasyonunun, mobil faz pH'ının ve kolon sıcaklığının analitlerin kromatografik davranışı üzerindeki etkisi detaylı olarak araştırılmıştır. Yöntemin duyarlılığı, doğruluğu ve kesinliği tatmin edici bulunmuştur. Önerilen yöntem, farmasötik müstahzarlarda salbutamol sülfat miktarını belirlemek için başarıyla uygulanmıştır. Diğer resmi yöntemlerle karşılaştırıldığında, bu yöntemde hiçbir organik çözücü kullanılmadığından daha az tehlikeli aynı zamanda seçicilik ve pik şekilleri açısından da üstün olduğu ortaya çıkmıştır (Mai ve ark., 2020).

**Salbutamol sülfat, terbutalin sülfat, budesonid ve asetilsistein ile karıştırılan Atrovent®'in (ipratropyum bromür) uyumlu stabilite ve aerosol özellikleri** adlı makalede aynı anda soluma için farklı soluma solüsyonlarını karıştırmak solunum yolu hastalıklarının tedavisinde yaygın bir uygulamadır. Günümüzde farklı inhalasyon ilaçlarının fizikokimyasal uyumluluğunu ve aerosol özelliklerini değerlendiren az sayıda çalışma yayınlanmıştır. Ancak hiçbiri Atrovent®'i incelememiştir. Çalışmada, Atrovent® ile ilgili çalışmaların eksikliğinin giderilmesi amaçlanmıştır. Karışım oluşturulduktan sonra belirli zaman aralıklarında her bir aktif bileşenin pH tayini, ozmolarite ölçümü ve yüksek performanslı sıvı kromatografisi (HPLC) testi ile fizikokimyasal uyumluluğa bakıldı. Aerosol davranışlarını karakterize etmek için geometrik ve aerosol partikül boyutu dağılımı, aktif ilaç verme oranı ve verilen toplam aktif ilaç ölçülmüştür. Test süresi boyunca pH değerinde, ozmotik basınçta veya katkı maddelerinin aktif bileşenlerinde önemli bir değişiklik bulunmamıştır. Karıştırma sonrası nebulizasyon hacmindeki artışla birlikte, ince partikül dozu ve verilen toplam aktif ilaç istatistiksel olarak anlamlı iyileşmeler gösterirken aktif ilaç verme oranı tek ilaçlı preparasyonlara kıyasla azalmıştır. Bu sonuçlar, Atrovent®'in diğer inhalasyon ilaçları ile karıştırıldığında 1 saat boyunca fizikokimyasal uyumluluğunu desteklemiştir. Aerosol özellikleri göz önüne alındığında, eşzamanlı inhalasyonun daha verimli olduğu görülmüştür (Chen ve ark., 2020).

**Çeşitli hayvan bazlı gıda matrikslerinde eser düzeylerde β-agonistlerin yüksek çözünürlüklü kütle spektrometrisi tabanlı tespiti ve miktar tayini** adlı makalede β-agonistler, hayvancılıkta büyümeyi teşvik edici amaçlar için yasa dışı olarak kullanılmıştır ve hayvan bazlı gıda tüketicileri arasında akut toksik reaksiyonlara neden olabilecek kalıntı konsantrasyonlarına yol açmıştır. Yalnızca düşük konsantrasyonlarda β-agonist kalıntılarının saptanmasına değil, aynı zamanda izlenecek bileşiklerin sayısının arttırılmasına da ihtiyaç duyulmuştur. Bu çalışmada çeşitli matrikslerde (kas, karaciğer, plazma, süt ve idrar) çok çeşitli farklı β-agonistleri (bazı metabolitler dahil 20 analit) tespit edebilen birleşik bir yöntemin geliştirilmesi hedeflenmiştir. Geliştirilen prosedür, sınırlı emek girdisi ve sarf malzemesi tüketimi ile numunelerin hızlı işlenmesine izin vermiştir. Yöntem algılama, yüksek çözünürlüklü kütle spektrometrisine bağlı ultra yüksek performanslı kromatografiye dayandırılmıştır. Doğrulama iki farklı cihazda (Orbitrap ve uçuş süresi) gerçekleştirilmiştir. Elde edilen miktar belirleme sınırı (0,05 ile 0,5 µg/kg ve en karmaşık matriste (karaciğer) %78'lik ortalama geri kazanım) mevcut düzenleyici gereksinimleri karşılamıştır. Bu çalışmada önerilen tamamen doğrulanmış yöntem, ilgili farklı matrikslerde gerekli konsantrasyon seviyelerinde ilgili tüm β-agonistlerin saptanmasına izin vermiştir. Analitik yöntemin hızlı olduğu, birçok numunenin paralel işlenmesine izin verdiği ve maliyetli sarf malzemelerinin kullanılmasını gerektirmediği görülmüştür. Uygulanan kimyasal hidroliz adımının kısa ve nispeten yumuşak olduğu görülmüştür. Bu, yüksek bir örnek çıktısına izin vermiştir. Konjuge hedefli bileşiklerin izlenmesi, ana bileşiklerin türevlerinin saptanmasına izin vermelidir. Bu, idrar gibi bazı matriksler için geçerlidir. Bu tür bileşiklerin bulunması, hidroliz koşullarının (zaman ve/veya sıcaklık) optimizasyonuna izin verecektir. Kantitatife yakın bir hidrolize ulaşıldığı ancak hiçbir ana ilaç bozulmasının kaydedilmediği koşullar, muhtemelen tüm analitler ve hatta analitler ve matriks kombinasyonları için aynı olmayacaktır. Analitlerin düşük maksimum kalıntı seviyeleri, yüksek bir saptama seçiciliğine sahip olmasını gerektirir. Bu, HRMS (hem TOF hem de Orbitrap) ile başarılabilir. Çalışmada numune matriksine ve kullanılan enstrümantasyona bağlı olarak, kitle ekstraksiyon pencerelerinin dikkatli seçimi veya hedeflenen edinme modlarının kullanımı, analitleri en düşük konsantrasyon seviyelerinde izlemek için gerekli olduğu sonucuna varılmıştır (Kaufmann ve ark., 2021).

**Hücre bazlı sistemlerde montelukast miktarının tayini için basit ve hassas bir HPLC-MS/MS analizi in vitro pulmoner ilaç geçirgenliği çalışması** adlı makalede montelukast, sisteinil lökotrien reseptörü 1 alt tipinin (CysLT1) güçlü ve seçici bir antagonistidir ve astım profilaksisi ve tedavisi için oral tabletler ve granüller şeklinde yaygın olarak kullanılır. Son zamanlarda, pulmoner inhaler uygulamanın sistemik dolaşımdaki montelukast dağılımını sınırlayabilmesi, ilk geçiş metabolizmasını önleyebilmesi ve solunum yolu hastalıkları tedavisinde daha iyi terapötik etkilere sahip olabilmesi nedeniyle, montelukastın inhalasyon

yoluyla verilmesi gibi alternatif uygulama yollarını araştırmak, montelukast için yeni bir araştırma trendi oluşturmuştur. Mevcut çalışmanın amacı, bir in vitro hücre bazlı pulmoner farmakokinetik sistem modelinde montelukast tayini için basit, doğru, oldukça hassas ve seçici bir sıvı kromatografi-tandem kütle spektrometrisi yöntemi (LC-MS/MS) geliştirilmesi ve doğrulanması olmuştur. İnhaler montelukastın akciğerlerdeki akıbetinin daha iyi anlaşılması için kullanılabilecek olan bu çalışmada montelukast, etiketli montelukast içeren asetonitril ile protein çökeltme yoluyla ekstrakte edilmiştir. Kromatografi, 35 °C'de çalışan bir Agilent Eclipse plus C8 kolonu (4,6x100 mm, 3,5 µm) üzerinde gerçekleştirilmiştir. Mobil faz asetonitrilden oluşturulmuştur: 20 mM amonyum format tamponu (80:20, v/v), 0,5 mL/dk'lık bir akış hızında verilmiştir. Montelukast ve iç standardın her ikisi de 4,2 dakikada ayrıştırılmıştır. 0,5 ile 600 ng/mL arasında bir analitik aralıkta kalibrasyonu gerçekleştirmek için doğrusal ($1/x^2$) bir ilişki kullanılmıştır. LLOQ aralığı dahil olmak üzere dört QC numunesi için CV olarak ifade edilen gün içi ve günler arası kesinlik %1,14-%6,25 arasında olduğu görülmüştür. Dört montelukast konsantrasyonu için gün içi ve günler arası doğruluğun %95,19-%104,1 aralığında olduğu görülmüştür. Yöntem, ICH'nin tüm biyoanalitik yöntem doğrulama gereksinimlerini karşılamış ve in vitro hücre bazlı pulmoner farmakokinetik sistem modelinde montelukast testi için uygun olduğu görülmüştür. Montelukast'ın hücre bazlı sistemlerde kantifikasyonu için basit, doğru, yüksek derecede duyarlı ve seçici bir LC-MS/MS yöntemi in vitro pulmoner ilaç geçirgenliği çalışması geliştirilmiştir. Araştırmacılar, ticari olarak mevcut bir iç standart, kolay ve hızlı bir numune hazırlama ve basit bir LC-MS/MS sistemi kullanmıştır. Yöntem, özgüllük, seçicilik, duyarlılık, doğruluk, kesinlik, doğrusallık, matris etkisi, özütleme geri kazanımı aktarımı ve kararlılığının tüm gereksinimlerini karşılamıştır. Testin, hücre bazlı sistemler pulmoner taşıma deneylerinin belirlenmesi için uygun olduğu görülmüştür (Wang ve ark., 2021).

**İnsan plazmasında levosetirizin dihidroklorür ve montelukast sodyum'un LC–MS/MS ile eşzamanlı belirlenmesi: bir insan farmakokinetik çalışmasına geliştirme, doğrulama ve uygulama** adlı makalede iç standart olarak feksofenadin hidroklorür kullanılarak insan plazmasında levosetirizin dihidroklorür (LCZ) ve montelukast sodyumun (MLK) eş zamanlı tayini için basit, hızlı, seçici ve hassas bir yüksek performanslı sıvı kromatografi-tandem kütle spektrometrisi (LC-MS/MS) yöntemi geliştirilmiştir. Numune hazırlama ve analiz için hem ilaçların hem de iç standardın plazmadan etil asetata sıvı-sıvı ekstraksiyonu kullanılmıştır. Hem ilaçların hem de iç standardın ayrılması, izokratik bir asetonitril mobil fazı ve 0,7 mL/dk akış hızında (73:27 v/v) bileşiminde 50 µL amonyum hidroksit ile pH 8'e ayarlanmış 10 mM amonyum format kullanılarak bir İnertsil ODS-3 (4,6 mm × 50 cm, dp 5 µm, partikül boyutu) kolonunda sağlanmıştır. LC–MS/MS, bir elektrosprey iyonizasyon tekniği kullanılarak çoklu reaksiyon izleme modu (MRM) altında çalıştırılmıştır. Kütle parametreleri, levosetirizin dihidroklorür için m/z $[M + H]^+$ 389,0 → 200,8, montelukast sodyum için m/z $[M + H]^+$ 586,2 → 422,2 ve feksofenadin hidroklorür için m/z $[M + H]^+$ 502,2 → 466,0 geçişleri izlemek üzere optimize edilmiştir. Yöntemin her iki ilaç için de 1-500 ng/mL aralığında lineer olduğu bulunmuştur. Gün içi ve günler arası kesinliğin sırasıyla %0,96– %1,92 ve %1,03–1,55 aralığında olduğu görülmüştür. Matriks etkisinin %RSD <15 ile kabul edilebilir olduğu görülmüştür. Önerilen yöntem doğrulanmış ve farmasötik preparatlarının oral yoldan verilmesinden sonra insan plazmasında her iki ilacın farmakokinetik çalışması için başarıyla uygulanmıştır. İnsan plazmasında LCZ ve MLK'nın eşzamanlı tespiti için basit, hassas, doğru ve tekrarlanabilir bir LC–MS/MS yöntemi geliştirilmiş ve doğrulanmıştır. Bu LC-MS/MS yöntemi, oral uygulama ve 10 mg MLK'dan sonra 6 sağlıklı aç erkek gönüllü üzerinde farmakokinetik çalışmalara başarıyla uygulanmıştır. Geliştirilen yöntemin avantajları arasında ticari olarak temin edilebilen bir iç standart ile basit numune hazırlama prosedürü, kısa analiz süresi (numune başına 2 dakika) ve yüksek hassasiyet (LLOQ = 1 ng/mL) olarak görülmüştür (Rashed ve Nasr, 2019).

## SONUÇ

Astım dünya üzerinde 300 milyonu aşan sayıda insanı etkileyen yaşam standartlarını bozan hava yollanın kronik enflamatuvar bir hastalığıdır. Hastalık yaşam kalitesinde önemli bozukluklara yol açmasının yanı sıra önemli iş gücü ve okul günü kayıplarına yol açmakta ciddi tedavi maaliyetleri doğurmaktadır. Sık tekrarlayan hırıltılı solunum atakları, geceleri uykudan uyandıran nefes darlığı ve/veya öksürük atakları, egzersiz esnasında nefes darlığı hırıltı gibi yakınmaları olan hastalarda astımdan şüphelenilmelidir. Astım tanısı anamnez ve solunum fonksiyonları ölçümü ile konur. Astım tedavisinin en temel öğesi eğitim olup, ilaç tedavisinde kronik inflamasyonu baskılamak amacıyla en sık kullanılan ilaç grubu inhaler steroidlerdir.

Bu çalışmada; astımın tanımı, tarihçesi, epidemiyolojisi, patofizyolojisi, risk faktörleri, tanı kriterleri, tedavisi anlatılmış ve astım hastalığının tedavisinde kullanılan salbutamol ve montelukast etken maddelerinin miktar tayinleri için araştırmacıların modern analitik yöntemler ile geliştirdikleri çalışmaları literatür taraması yaparak incelenmiştir. Son yıllarda yapılan miktar tayini çalışmalarını incelendiğinde salbutamol etken maddesi için; LC-ESI-MS/MS, LC-MS, HPTLC, UPLC-MS/MS ve SFC-MS/MS, GC-MS, HPLC-DAD, RP- HPLC ile HRMS, montelukast etken maddesi için; HPLC-MS/MS yöntemlerinin kullanıldığını ve araştırmacıların

buldukları sonuçlarının doğruluğu, kesinliği, hassaslığını belirli parametreleri göz önüne aldıklarında güvenli ve uygun olduğu gösterilmiştir.

Yapılan çalışmalara bakıldığında, daha ucuz materyaller kullanılarak, daha az çalışma gücü ve alanı ve daha az çalışma süresi ile etken maddelere uygulanan farklı kimyasal analiz yöntemleri ile alınan sonuçların daha basit, duyarlı, seçici, verimli ve uygun maliyetli olduğu gösterilmiştir.

## KAYNAKLAR

Abdelrahman MM 2018. Solid-phase extraction and HPLC-DAD for determination of salbutamol in urine samples. Analytical Chemistry Letters, 8(1): 35-45.

Busse PJ, Cohn RD, Salo PM, Zeldin DC 2013. Characteristics of allergic sensitization among adult asthmatics >55 years: Results from the National Health and Nutrition Examination Survey 2005–2006. Ann Allergy Asthma Immunol, 110(4): 247-252.

Chan SH, Lee W, Asmawi MZ, Tan SC 2016. Chiral liquid chromatography–mass spectrometry (LC–MS/MS) method development for the detection of salbutamol in urine samples. Journal of Chromatography B, 1025: 83-91.

Chang KC, Chang YT, Tsai CE 2018. Determination of ractopamine and salbutamol in pig hair by liquid chromatography tandem mass spectrometry. Journal of food and drug analysis, 26(2): 725-730.

Chen Y, Du S, Zhang Z, He W, Lu E, Wang R, Ma Y 2020. Compatible stability and aerosol characteristics of atrovent® (Ipratropium bromide) mixed with salbutamol sulfate, terbutaline sulfate, budesonide, and acetylcysteine. Pharmaceutics, 12(8): 776.

Cordell RL, Valkenburg TS, Pandya HC, Hawcutt DB, Semple MG, Monks PS 2018. Quantitation of salbutamol using micro-volume blood sampling–applications to exacerbations of pediatric asthma. Journal of Asthma, 55(11): 1205-1213.

Di Lorenzo G, Mansueto P, Melluso M, Morici G, Norrito F, Pellitteri ME, Caruso C 1997. Non-specific airway hyperresponsiveness in mono-sensitive Sicilian patients with allergic rhinitis. Its relationship to total serum IgE levels and blood eosinophils during and out of the pollen season. Clinical and Experimental Allergy, 27(9):1052-1059.

Ducharme FM, Ni Chroinin M, Greenstone I, Lasserson TJ 2010. Addition of long-acting beta2-agonists to inhaled corticosteroids versus same dose inhaled corticosteroids for chronic asthma in adults and children. Cochrane Database Syst Rev, CD005533.

Erdoğan T, Karakaya G, Kalyoncu AF 2015. Comorbid diseases in aspirin-exacerbated respiratory disease, and asthma. Allergol Immunopathol (Madr), 43(5): 442-448.

Global Initiative for Asthma (GINA) 2020. Global strategy for asthma management and prevention. Global strategy for asthma management and prevention, Avaible at: https://ginasthma.org/wp-content/uploads/2020/06/GINA-2020-report_20_06_04-1-wms.pdf [20.06.04]

Herpin L, Bichon E, Rambaud L, Monteau F, Le Bizec B 2018. Comparison between liquid chromatography and supercritical fluid chromatography coupled to mass spectrometry for beta-agonists screening in feeding stuff. Journal of Chromatography B, 1086: 130-137.

Holgate ST 1999. Genetic and environmental interaction in allergy and asthma. J Allergy Clin Immunol, 104: 1139-1146.

Jaakkola MS, Ieromnimon A, Jaakkola JJK 2006. Are atopy and specific IgE to mites and mo lds important for adult asthma? J Allergy Clin Immunol, 117: 642-648.

Juniper EF, Svensson K, O'Byrne PM, Barnes PJ, Bauer CA, Lofdahl CG, Ullman A 1999. Asthma quality of life during 1 year of treatment with budesonide with or without formoterol. European Respiratory Journal, 14(5):1038-1043.

Kaufmann A, Widmer M, Maden K, Butcher P, Walker S 2021. High resolution mass spectrometry-based detection and quantification of β-agonists at relevant trace levels in a variety of animal-based food matrices. Food Additives & Contaminants: Part A, 38(8): 1350-1363.

Leff JA, Busse WW, Pearlman D, Bronsky EA, Kemp J, Hendeles L, Reiss TF 1998. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exerciseinduced bronchoconstriction. New England Journal of Medicine, 339(3):147-152.

Mai XL, Choi Y, Truong QK, Nguyen TNV, Han SB, Kim KH 2020. Alternative chromatographic method for the assay test of terbutaline and salbutamol using ionic liquid assisted aqueous mobile phase. Analytical Science and Technology, 33(4): 169-176.

Mims JW 2015. Asthma: definitions and pathophysiology. International Forum of Allergy & Rhinology, 5(S1): p. 2-6.

Ni Chroinin M, Greenstone I, Lasserson TJ, Ducharme FM 2009. Addition of inhaled long-acting beta2-agonists to inhaled steroids as first line therapy for persistent asthma in steroid-naive adults and children. The Cochrane database of systematic reviews, 4:CD005307.

Ober C 2005. Perspectives on the past decade of asthma genetics. Journal of Allergy and Clinical Immunology, 116: 274-278.

Patel N, Parmar VK 2018. A sensitive high-performance thin layer chromatography method for simultaneous determination of salbutamol sulphate and beclomethasone dipropionate from inhalation product. Pharmaceutical Sciences, 24(2): 131-140.

Rashed NS, Nasr ZA 2019. Simultaneous determination of levocetirizine dihydrochloride and montelukast sodium in human plasma by LC–MS/MS: development, validation, and application to a human pharmacokinetic study. Acta Chromatographica, 31(3): 194-200.

Reiss TF, Chervinsky P, Dockhorn RJ, Shingo S, Seidenberg B, Edwards TB 1998. Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma: a multicenter, randomized, double-blind trial. Montelukast Clinical Research Study Group. Arch Int Med, 158(11):1213-1220.

Selçuk ZT, Çağlar T, Enünlü T, Topal T 1997. The prevalence of allergic diseases in primary school children in Edirne, Turkey. Clin Exp Allergy, 27: 262-269.

Şahiner ÜM, Semic-Jusufagic A, Curtin JA, Birben E, Belgrave D, Sackesen C, Simpson A, Yavuz TS, Akdis CA, Custovic A, Kalaycı O 2014. Polymorphisms of endotoxin pathway and endotoxin exposure: in vitro IgE synthesis and replication in a birth cohort. Allergy, 69(12): 1648-1658.

Şekerel BE, Civelek E, Karabulut E, Yıldırım S, Tuncer A, Adalıoğlu G 2006. Are risk factors of childhood asthma predicting disease persistence in early adulthood different in the developing world? Allergy, 61(7): 869-877.

Toskala E, Kennedy DW 2015. Asthma risk factors. Int Forum Allergy Rhinol, 5(1): 11-16.

Ulusal Astım Tanı ve Tedavi Rehberi. Türk Toraks Derneği 2016. ASTIM. Official Journal of the Turkish Thoracic Society, 17.

Wang S, Ni XJ, Wen YG, Xie HS, Chen JR, Luo YL, Li PL 2021. A simple and sensitive HPLC-MS/MS assay for the quantitation of montelukast in cell-based systems in vitro pulmonary drug permeability study. Journal of Pharmaceutical and Biomedical Analysis, 192: 113657.

## *ORAL PRESENTATION*

# Osteoporoz tedavisinde kullanılan ilaç etken maddeleri üzerine yapılan analitik çalışmalar

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**Özet**
Osteoporoz, kemik yapısının bozulması ile karakterize metabolik bir kemik hastalığıdır. Hastalık ilerledikçe kemik kırıkları oluşabilir. Osteoporoz, hastanın yaşam kalitesini büyük ölçüde azaltan, önlenebilir ve tedavi edilebilir bir hastalıktır. Bu nedenle zamanında önlem almak ve tedaviyi doğru adımlarla uygulamak çok önemlidir. Çalışma osteoporozun önlenmesi, farmakolojik ve farmakolojik olmayan tedavileri hakkında bilgiler içermektedir. Çalışmanın amacı; osteoporoz tedavisinde kullanılan alendronat, risedronat, denosumab, raloksifen ve teriparatidin etken maddeleri için son yıllarda yapılan analitik çalışmaları ICH tarafından onaylanan parametrelerle incelenmesi, yorumlanması ve sunulmasıdır. Alendronat etken maddesi tayini için HILIC, HPLC-MS/MS, HPLC-DAD ile HPLC-FD, MSPE ile LC-MS/MS, HPLC, HPLC-MS, asit baz potansiyometrik titrasyonu yöntemleri ile, risedronat etken maddesi tayini için HPLC-UV, SPE ile LC–MS/MS, HPLC yöntemleri ile, denosumab etken maddesi tayini için SE-HPLC ile RP-HPLC yöntemleri ile, raloksifen etken maddesi tayini için HPLC yöntemi ile, teriparatid etken maddesi tayini için LC-MS/MS, UHPLC-MS/MS yöntemleri ile ilgili çalışmalar incelenmiştir. İnceleme sonucunda çalışmaların; çevre dostu, ekonomik, hassas, basit, hızlı, doğru ve kesin olduğu görülmüştür.

**Anahtar Kelimeler:** Osteoporoz, alendronat, risedronat, analitik çalışmalar

## Analytical studies on drug active substances used in the treatment of osteoporosis

**Abstract**
Osteoporosis is a metabolic bone disease characterized by deterioration of bone structure. Bone fractures may occur as the disease progresses. Osteoporosis is a preventable and treatable disease, which greatly reduces the patient's quality of life. For this reason, it is very important to take timely measures and to apply the treatment with the right steps. The study includes information about the prevention of osteoporosis, its pharmacological and non-pharmacological treatments. The aim of the study; to interpret, analyze and present analytical studies conducted in recent years for the active ingredients of alendronate, risedronate, denosumab, raloxifene and teriparatide, which are used in the treatment of osteoporosis, with parameters approved by ICH. For the alendronate active ingredient HILIC, HPLC-MS/MS, HPLC-DAD with HPLC-FD, MSPE with LC-MS/MS, HPLC, HPLC-MS, acid-base potentiometric titration, for the risedronate active ingredient HPLC-UV, SPE with LC–MS/MS, HPLC, for the denosumab active ingredient SE-HPLC with RP-HPLC, for the raloxifene active ingredient HPLC, for the teriparatide active ingredient LC-MS/MS, UHPLC-MS/MS studies were examined. As a result of the examination, it has been seen that the studies are environmentally friendly, economical, sensitive, simple, fast, accurate and precise.

**Key Words:** Osteoporosis, alendronate, risedronate, analytical studies

## GİRİŞ

Osteoporoz, düşük kemik kütlesi ve mikro yapıdaki kemik dokusunun bozulması ile karakterize, patolojik kırıkların meydana gelebildiği, ilerleyici bir metabolik hastalık olarak tanımlanmaktadır. Daha çok postmenopozal dönemdeki kadınlarda gözlenen osteoporoz, kişinin hayat kalitesini oldukça düşürmektedir. 21. yüzyılın önemli sağlık sorunlarından biri olan hastalığın, ortalama yaşam süresinin artmasıyla birlikte görülme olasılığının da artacağı düşünülmektedir. Bu yüzden zamanında tedbir alınması, doğru adımlarla tedavinin uygulanması oldukça önem arz etmektedir (Hernlund ve ark., 2013; Chakuleska ve ark., 2018). Osteoporoz iki temel sınıfa ayrılmaktadır: primer ve sekonder osteoporoz. Primer osteoporoz denilen sınıf, menopoz sonrası osteoporotik kadınları ifade eder (Riggs ve Melton, 1995). Sekonder osteoporoz ise yetişkin nüfusta görülen osteoporozu ifade eder. Osteoporozun önlenmesinde öncelikle mevcut yaşam tarzının kemik

sağlığı açsından uygunluğuna bakılır. Duruma göre yaşam tarzında iyileştirilmelere gidilmektedir. Tedavide hem farmakolojik hem de nonfarmakolojik yaklaşımlar mevcuttur. Hastalığın evrelerine göre seçilen ilaç grupları ile tedavi uygulaması yapılır. Kalsiyum ve D vitamini kombine tedavisi, osteoporotik kırılganlığın önlenmesinde önemli bir role sahiptir (Kışlak ve Genç, 2019). Osteoporoz, antirezorptif ilaçların farmakolojik etkisinden yararlanılarak tedavi edilebilmektedir. Alendronik asit, osteoporoz ve Paget kemik hastalığını tedavi etmek için kullanılan bir bisfosfonat ilaçtır. Kullanım genellikle D vitamini, kalsiyum takviyesi ve yaşam tarzı değişiklikleri ile birlikte önerilir. Genellikle sodyum tuzu risedronat sodyum olarak kullanılan risedronik asit, bir bisfosfonattır. Kemiği parçalayan hücreleri yavaşlatır. Osteoporozu tedavi etmek veya önlemek ve Paget kemik hastalığını tedavi etmek için kullanılır. Denosumab osteoporoz, tedaviye bağlı kemik kaybı, kemiğe metastaz ve kemiğin dev hücreli tümörünün tedavisi için bir insan monoklonal antikorudur. Denosumab, kemiği parçalayan hücreler olan osteoklastların gelişimini önleyerek çalışan bir RANKL (nükleer faktör kappa -B ligandının reseptör aktivatörü) inhibitörüdür. Raloksifen, postmenopozal kadınlarda ve glukokortikoid kullananlarda osteoporozu önlemek ve tedavi etmek için kullanılan bir ilaçtır. Osteoporoz için bifosfonatlara göre daha az tercih edilmektedir. Yüksek risk altındaki kişilerde meme kanseri riskini azaltmak için de kullanılmaktadır. Teriparatid, hormonun biyoaktif kısmı olan ilk 34 amino asitten oluşan bir paratiroid hormonu (PTH) şeklidir. Bazı osteoporoz türlerinin tedavisinde kullanılan etkili bir anabolik (kemik oluşumunu teşvik eden) ajandır. Ayrıca bazen kırık iyileşmesini hızlandırmak için kullanılır. Teriparatid, insan paratiroid hormonunun bir kısmı ile aynıdır ve aralıklı kullanım, osteoblastları osteoklastlardan daha fazla aktive eder., bu da kemikte genel bir artışa yol açar.

Çalışmanın amacı, osteoporoz tedavisinde kullanılan alendronat, risedronat, denosumab, raloksifen ve teriparatid etken maddeleri hakkında bilgi verilmesi ve son yıllarda analitik yöntemler kullanılarak yapılmış alendronat, risedronat, denosumab, raloksifen ve teriparatid etken maddelerinin miktar tayinlerine ilişkin literatür çalışmalarının incelenmesi, bunların değerlendirilerek bir derleme oluşturulmasıdır.

## MATERYAL VE METOT

### Etken Madde Analiz Yöntemleri

*Hidrofilik etkileşim sıvı kromatografisi (HILIC):* İyon kromatografisi ve ters faz sıvı kromatografisi gibi diğer kromatografik uygulamalarla kısmen örtüşen normal fazlı sıvı kromatografisinin bir çeşididir. HILIC de, ters faz tipi eluentler ile hidrofilik sabit fazlar kullanır. HILIC ayırma modu, bazı biyomoleküllerin, organik ve bazı inorganik moleküllerin polarite farklılıkları ile ayrılması için yaygın olarak kullanılır. Metabolik süreç genellikle hücresel dokudan eliminasyonu arttırmak için polar grupların eklenmesiyle sonuçlandığından, metabolitler için analiz edilirken biyolojik numuneler için basitleştirilmiş numune hazırlama nedeniyle faydası artmıştır.

*Diyot dizisi dedektörlü-yüksek performanslı sıvı kromatografisi (HPLC-DAD) ile floresans dedektörlü-yüksek performanslı sıvı kromatografisi (HPLC-FD):* Yüksek performanslı sıvı kromatografisi (HPLC), bir karışımda yer alan her bir bileşeni ayırmak, tanımlamak ve miktarını belirlemek için kullanılan bir ayırma tekniğidir. Diyot dizisi dedektörler, UV ve görünür bölgede ışık yayarlar. Böylelikle 190 nm ile 950 nm aralığında ölçüm yapabilirler. Floresans dedektörler, belli bir dalga boyunda ışığı absorpladıktan sonra başka bir dalga boyunda ışın yayan yani florasans özellik gösteren maddelerin yaydığı ışık şiddetini ölçerler.

*Manyetik katı faz ekstraksiyonu (MSPE) ile Sıvı Kromatografisi - Ardışık (Tandem) Kütle Spektrometresi (LC-MS/MS):* Sıvı kromatografi-kütle spektrometresi/kütle spektrometresi (LC-MS/MS), sıvı kromatografisinin (HPLC) kütle spektrometrisinin (MS) kütle analizi yetenekleri ile fiziksel ayırma yeteneklerini birleştiren bir analitik kimya tekniğidir. Eşleşmiş kromatografi - MS sistemleri kimyasal analizde popülerdir çünkü her tekniğin bireysel yetenekleri sinerjik olarak geliştirilmiştir. Sıvı kromatografisi, karışımları çoklu bileşenlerle ayırırken, kütle spektrometresi, yüksek moleküler özgüllük ve algılama duyarlılığı ile bireysel bileşenlerin yapısal kimliğini sağlar. LC ünitesinden ayrıştırılmış analitler iyonlaştırılarak MS/MS ünitesine gönderilirler. MS/MS ünitesinde iyonlar ilk quadropolden kütle/yük (m/z) oranlarında ayrıştırılarak collision cell olarak bilinen girişim hücresine yönlendirilirler. Burada yüksek saflıkta azot gazı ile çarpıştırılan moleküller parçalanarak ikinci quadropole gönderilirler. İkinci quadropol ünitesindeki bu ikinci iyonlar da kütle/yük (m/z) oranlarında ayrıştırılarak kütlesel olarak tayin edilirler. Aynı m/z oranına sahip pek çok molekül olabilecekken aynı parçalanma iyonlarına sahip moleküllerin doğada bulunma oranı 1/10000'dir. Bu tandem tekniği, çevre ve biyolojik kaynaklı karmaşık örneklerde yaygın olarak bulunan biyokimyasal, organik ve inorganik bileşikleri hassas bir şekilde analiz etmek için tercih edilir. Bu nedenle, LC-MS/MS, biyoteknoloji, çevre izleme, gıda işleme ve farmasötik, zirai ilaç ve kozmetik endüstrileri gibi çok çeşitli sektörlerde uygulanabilir. LC-MS/MS, analitlerin kalitatif ve kantitatif olarak analiz edilmesine imkan verir. Karmaşık ortamlarda yer alan tekli veya çoklu analitlerin analizinde, eser düzeydeki maddelerin analizinde, ilaç etken maddelerinin yanında safsızlık ve metabolitlerinin analizinde, gıdalardaki katkı maddeleri veya pestisitlerinin analizlerinde, kan, idrar, doku gibi biyolojik numunelerin analizlerinde yüksek hassaslık ve doğruluk ile sonuç almak

mümkündür. LC-MS/MS, çoklu analit tayininin yanı sıra numunedeki eser miktardaki analitlerin tayini için tercih edilen en hassas metotlardan biridir. Örnekte bulunan bileşenlerin miktarı, yapısı ve molekül ağırlığı hakkında bilgi verir. Küçük farmasötik bileşiklerden büyük proteinlerin tayinine kadar, polar iyonik, termal kararsız ve uçucu olmayan bileşiklerin analizleri gibi çok geniş bir kullanım alanına sahiptir. Manyetik katı faz ekstraksiyonu ise temel olarak analitlerin manyetik sorbentler kullanılarak büyük numune hacimlerinden ayrılması ve izolasyonuna dayanan bir tekniktir. Manyetik katı faz ekstraksiyonunda sorbentlerin paramanyetik özellikleri, analitlerin harici bir manyetik alan kullanarak çözeltilerden kolay ve hızlı ayrılması sayesinde mevcut numune hazırlama proseslerinde yaygın olarak kullanılır.
*Katı faz ekstraksiyonu (SPE) ile Sıvı Kromatografisi - Ardışık (Tandem) Kütle Spektrometresi (LC-MS/MS):* Katı faz ekstraksiyon metodu, klasik sıvı-sıvı ekstraksiyon ile karşılaştırıldığında daha hızlı, az çözücüye ihtiyaç duyan, emülsiyon oluşumun şekillenmediği, çok daha ucuz bir tekniktir.
*Boyut dışlamalı yüksek performanslı sıvı kromatografisi (SE-HPLC) ile Ters fazlı yüksek performanslı sıvı kromatografisi (RP- HPLC):* Yüksek basınçlı veya yüksek performanslı sıvı kromatografisi (HPLC), bir karışımda yer alan her bir bileşeni ayırmak, tanımlamak ve miktarını belirlemek için kullanılan bir ayırma tekniğidir. Ters fazlı (RP-Reverse Phase) yüksek performanslı sıvı kromatografi (HPLC) en yaygın kullanılan tekniktir. Polar olmayan bileşiklerin, zayıf polar bileşiklerin ve iyonik bileşiklerin analizine uygulanabilmesi tekniğidir. Ters fazlı kromatografide; sabit faz apolar (Silikajel-Polimer ve üzerine bağlanmış C18, oktil veya fenil grupları, metil, etil ve $–NH_2$ gruplu dolgu maddeleri) iken, mobil faz polar (Metanol, Asetonitril, Tetrahidrofuran gibi güçlü organik çözücülerin zayıf çözücü olan sulu veya tamponlanmış, pH ayarı yapılmış karışımları) olur. Yüksek polariteye sahip analit kolondan ilk çıkar. Benzer özelliklere sahip maddelerin birbiri içinde dağılma özelliği olduğu için yüksek polariteye sahip analit mobil fazda çok iyi çözünür ve kolondan ilk önce çıkar. Ayrıca yine aynı özellik sebebiyle polar analit apolar sabit fazla az etkileştiğinden dolayı kolonda kısa süre tutunabilir. Alıkonma zamanını kısaltmak için mobil fazın polaritesi azaltılır. Alıkonma zamanını arttırmak için mobil fazın polaritesi artırılır. Ters Faz Kromatografide yüksek polariteye sahip mobil fazda alıkonma zamanları uzun, polaritesi azaltılmış mobil fazda alıkonma zamanları kısadır.
*Ultra Performanslı Sıvı Kromatografisi - Ardışık (Tandem) Kütle Spektrometresi (UPLC-MS/MS):* Ultra performanslı sıvı kromatografisi teknolojisi yüksek basınçta çalışan ve çok hassas femtomolar seviyede analizler yapan sistemdir. Genellikle, şekerlerin analizinde kullanılan, başka ufak moleküllerin ayrıştırılması ve karakterizasyonunda kullanılır.

## BULGULAR ve TARTIŞMA

Literatürde son yıllarda yapılan alendronat, risedronat, denosumab, raloksifen ve teriparatid etken maddelerinin tayin yöntemleri üzerine yapılan çalışmalar incelenmiş ve çalışmaları yapan araştırmacılar ile bu araştırmacıların buldukları sonuçlar derlenmiştir.
**Zwitteriyonik hidrofilik etkileşim kromatografisi kullanan alendronat sodyum için doğrulanmış stabilite gösterge yöntemi ve yüklü aerosol tespiti (Raju ve ark., 2015)** Alendronat sodyum, osteoporoz, malign hipokalsemi ve Paget hastalığının tedavisinde yaygın olarak kullanılan bir bifosfonat türevi ilaçtır. Alendronat sodyumun tayini için HPLC yönteminin geliştirilmesi oldukça güçtür. Çünkü HPLC için önem arz eden kromoforik grup alendronat sodyumda bulunmaz ve ayrıca yüksek polariteye sahip olması istenmeyen bir durumdur. Bu zorluklardan yola çıkarak araştırmacılar mevcut çalışmalarında, hidrofilik etkileşim sıvı kromatografisi (HILIC) kullanmışlardır. HILIC sistemi, özellikle polar anilitler için geliştirilmiş retansiyon ve böylece daha iyi çözünürlük avantajı sağlar. Çalışmada, yüklü bir aerosol dedektörü ile birleştirilmiş kısa ve basit bir izokratik yöntem geliştirilmiştir. Araştırmacıların geliştirdikleri yöntem, bu etken maddeyi içeren ve pazarlanan bir formülasyonun analizi için de başarıyla uygulanmıştır. Korona yüklü aerosol dedektörü (Corona CAD), kimyasal yapısından bağımsız olarak, uçucu özellik göstermeyen herhangi bir analiti ölçmek için yaygın olarak kullanılan çok yönlü, kütleye duyarlı bir dedektördür. Bu çalışma sonucunda araştırmacılar, alendronat sodyum gibi polar bileşiklerin analizi için HILIC-CAD'in uygulanabilirliğini göstermişlerdir.
**Metal içermeyen HPLC-MS/MS kullanılarak insan plazmasında ve diyalizatta alendronatın son derece hassas tayini (Yamada ve ark., 2018)** Araştırmacılar trimetilsilildiazometan ile metilasyonun ardından MonoSpin™ SAX® ekstraksiyonu ve metal içermeyen yüksek basınçlı sıvı kromatografisi (HPLC)-tandem kütle spektrometrisi (MS/MS) kullanılarak insan plazmasından ve diyalizatta alendronatın analizi için oldukça hassas bir yöntem geliştirmişlerdir. Alendronat ve alendronat-d6 için türevlerinin kromatografik ayrımını, 10mM amonyum asetattan (pH 6,80) ve asetonitrilden oluşan bir lineer gradyan elüsyon sistemi ile bir L-column2 ODS metal içermeyen kolonda (50x2 mm i.d., partikül büyüklüğü 3 µm) 0,3 mL/dk'lık bir akış hızında gerçekleştirmişlerdir. Yapılan miktar tayini, pozitif iyon elektrosprey iyonizasyonu (ESI) ile çoklu reaksiyon izleme modunda (MRM) ile yapılmış, 1 dakika içinde her kanalda alendronat ve iç standart için belirgin pikler gözlenmiştir. Bilindiği kadarıyla bu çalışma, insan plazmasında ve diyalizatta alendronatın

belirlenmesi için metal içermeyen bir HPLC-MS/MS sistemini bildiren ilk çalışmadır. Bulunan bu yöntem, klinik analizlerde alendronatın basit ve hassas tayini için faydalı olacaktır.

**Tabletlerde ve idrarda alendronat tayini için spektroskopik ve HPLC yöntemleri (Al Deeb ve ark., 2004)** Araştırmacılar tablet dozaj formunda alendronat sodyumun analizi için iki yöntem (spektrofotometrik ve HPLC) geliştirmiş ve doğrulamışlardır. Her iki yöntem için de alendronat sodyumun o-ftalaldehit (OPA) ile bazik pH'ta reaksiyona girerek bir ışık soğurucu türev üretme yeteneğine bağlı olduğu bildirilmiştir. Ne türevlendirme ajanının ne de analitin herhangi bir absorbsiyona sahip olmadığı 330 nm'de türevin maksimum absorbsiyona sahip olduğu bulunmuştur. Bu nedenle spektroskopik yöntem, 333 nm'de alendronat sodyumun türevlendirme ile indüklenen absorbsiyonuna ve HPLC yöntemi 333 nm'de algılama ile tabletlerdeki diğer bileşenlerden oluşan türevin ayrılmasına dayanmakta olduğu bildirilmiştir. Ayrıca, idrarda alendronat sodyum miktarının belirlenmesi için floresans dedektörlü bir HPLC-FD yöntemi geliştirmişlerdir. Yöntem alendronatın OPA ile türevlendirmesine dayanmakta fakat yöntemde floresans dedektör kullanılmaktadır. Araştırmacıların önerdikleri HPLC-DAD yöntemi, tabletlerde alendronatın belirlenmesi için hassas ve güvenilir bir yöntem olmuştur. İdrarda alendronatın tayini için, floresans tespitine (HPLC-FD) dayalı daha hassas ve seçici bir analiz tekniği bulunmuştur. HPLC-FD'nin oldukça hassas olduğu ve genellikle biyolojik numunelerde bulunan düşük alendronat konsantrasyonlarını (0,6 ng/mL) tespit etmek için kullanılabileceği bildirilmiştir.

**Plazmada alendronat tayini için çekirdek-kabuk yapılı titanyum dioksit kaplı demir oksit mikroküreler ile yerinde türevlendirmenin ardından manyetik katı faz ekstraksiyonu (She ve ark., 2021)** Bu çalışmada, plazmada alendronatı belirlemek için ilk kez manyetik katı faz ekstraksiyonu (MSPE) *in-situ* türevlendirme ile birleştirilmiştir. Çekirdek-kabuk yapılı $TiO_2$ kaplı $Fe_3O_4$ mikroküreler ($Fe_3O_4$@$TiO_2$) polidopamin kaplama, titanyum iyonları immobilize etme ve kalsinasyon aşamaları ile sentezlenmiştir. Hazırlandığı gibi mikroküreler, alendronatı seçici olarak özütleyebilir ve plazmadan hızla izole edebilirler. İlaca adsorbe edilen $Fe_3O_4$@$TiO_2$ mikroküreler daha sonra, türevlendirilmiş alendronatın $TiO_2$'ye afinitesini kaybettiği ve kendiliğinden daha fazla LC-MS/MS saptaması ile ayrıştırıldığı ve bu nedenle *in-situ* türevlendirme ve elüsyon prosedürünü gerçekleştirmek için türevlendirme reaktifi çözeltisinde doğrudan inkübe edilmiştir. *İn-situ* türevlendirme ile dispers manyetik katı faz ekstraksiyonunu birleştirmeye yönelik yapılan çalışmada tatmin edici sonuçlar elde edilmiştir. Araştırmacıların geliştirdikleri yöntem valide edilmiş ve iyi doğrusallık (0,05-500 ng/mL), düşük saptama limiti (20 pg/mL), yüksek doğruluk (%100,6 ile %105,3) ve kesinlik (RSDs < %5,27) gösterdiği bulunmuştur. Manuel çalıştırma ve analiz süresi, makalede bildirilen diğer yöntemlere kıyasla büyük ölçüde azaltılabilir bulunmuştur. Araştırmacılar yöntemi, av köpeklerinde yapılan bir farmakokinetik çalışmaya başarıyla uygulamışlardır.

**Alendronat sodyum ve bunun farmakopeyle ilgili safsızlığının eş zamanlı tespiti için Hantzsch kolon öncesi türevlendirme: floreskamin kullanılarak senkron florometri ile karşılaştırmalı çalışma (El-Yazbi ve ark., 2019)** Alendronat sodyum (ALN) ve bununla ilgili ana safsızlık, 4-aminobutanoik asidin (ABA) eş zamanlı belirlenmesi için, farmakope sınırları içerisinde, Hantzsch kondenzasyon reaksiyonuna dayalı bir kolon öncesi türevlendirme ile HPLC yöntemi uygulanmıştır. ALN ve ABA'nın renkli yoğuşma ürünlerinin ayrılması Agilent Zobrax Eclipse SB-C18 analitik kolonunda (250x4,6 mm, 5 µm) üzerinden asetonitril-0,1 M asetat tamponu, pH 5,0'de (15:85, v/v) içeren bir mobil faz kullanılarak gerçekleştirilmiştir. Akış hızı 1 mL/dk olarak belirlenmiştir. Bu analiz, foto diyot dizi dedektörü kullanılarak 340 nm'de gerçekleştirilmiştir. ALN ve ABA için sırasıyla 10-500 ve 0,2-40 µg/mL aralığında lineer regresyon bulunmuştur. Araştırmacıların önerdikleri bu yöntem doğrusallık, kesinlik, doğruluk, özgüllük ve sağlamlık için doğrulanmıştır. Bu yöntem, farmakope sınırında (%0,5) ABA (ilgili safsızlık) varlığında ALN'nin saflığını kontrol etmek için kullanılmıştır. Karşılaştırma amacıyla, ALN'nin floreskamin ile reaksiyonundan sonra senkron floresans ölçümünü içeren başka bir yöntem önerilmiştir. Bu yöntemde, üçüncü türev senkron spektrumları, çok yüksek hassasiyet gösteren, sırasıyla 24 ve 73 ng/mL LOD ve LOQ ile ALN tayini için 339 ile 370 nm arasında analiz edilmiştir. Dökme ve farmasötik dozaj formunda ALN tayini için tabletlerde veya oral solüsyonda katkı maddelerinin girişimi olmadan her iki yöntem de uygulanmıştır. Bu çalışma, ALN'nin hammaddede ve farmasötik dozaj formunda analizi için iki yöntemin geliştirildiğini bildirmektedir. İlk yöntem, hızlı, basit, seçici bir ve çevre dostu kolon öncesi türevlendirme HPLC-DAD yöntemini tanımlar ve bu yöntemin ALN'nin farmakope sınırında (%0,5) ana safsızlığı olan ABA'nın varlığında, hassas olarak belirlenmesine imkan sağlar. Öte yandan, önerilen ikinci yöntem, çeşitli eksipiyanlardan girişim olmaksızın farklı farmasötik dozaj formlarında ALN'nin hassas analizine izin veren basit, oldukça hassas ve seçici bir üçüncü türev senkron florometrik yöntemini açıklamaktadır.

**İnsan idrarında alendronatı belirlemek için yüksek performanslı sıvı kromatografi-kütle spektrometrisi yönteminin geliştirilmesi ve uygulanması (Tarcomnicu ve ark., 2007)** Bifosfonatlar gibi oldukça polar bileşikler üzerinde elektrosprey iyonizasyonun sunduğu yüksek potansiyele rağmen, birkaç uygulama geliştirilmiştir. Bu tür moleküller için uygun olan HPLC ayırma yöntemleri, yüksek uçucu olmayan tuz içeriği nedeniyle kütle spektrometrisi (MS) ile birlikte kullanılamaz; aynı zamanda biyolojik sıvılarda numune hazırlamada da bu durum zorlu bir problemdir. Geçmişte iyon çifti kromatografisi esas olarak bifosfanatların

HPLC-MS durumunda kullanılmakta iken kantitatif farmakokinetik (PK) çalışmalarda herhangi bir uygulama sunulmamıştır. Bu çalışmada, zayıf bir duyarlılık gösteren iyon çifti kromatografisi ile yapılan ön testlerden sonra, amino grubu ve bifosfonatın kombine bir türevlendirmesi geliştirilmiş ve bir PK çalışmasında test edilmiştir. Bu analitik yaklaşımı kullanarak idrarda 6,667-4860,0 ng/mL aralığında alendronat miktarı tam olarak doğrulanabilmiştir (numune hacmi 2,0 mL), her analitik çalışma 5,0 dakika uzunluğunda olmuştur. Elde edilen duyarlılık, tüm idrar toplama periyodu boyunca alendronat idrar atılımının doğru bir şekilde değerlendirilmesine izin vermiştir. Numune hazırlama, karmaşıklığına rağmen, bir iş gününde 200 numuneye kadar işlem ve analiz etme imkanı sağlamakta olduğu bildirmiştir.

**Alendronat sodyumun sulu çözeltideki pKa değerlerinin asit-baz potansiyometrik titrasyona dayalı parçalı lineer regresyon ile belirlenmesi (Ke ve ark., 2016)** Alendronik asidin bir mono-sodyum tuzu formu olarak alendronat sodyum, dört hidroksil grubunun ayrışması için çok iyi bir iyonizasyon sunar. Bu çalışmada, asit-baz potansiyometrik titrasyon reaksiyonuna dayalı olarak titrant hacmi ile pH değeri arasındaki parçalı doğrusal ilişki incelenerek alendronat sodyumun ayrışma sabitleri belirlenmiştir. Belirlenen pKa değerlerine göre alendronat sodyum dağılım eğrileri çizilmiştir. Alendronat sodyumun 4 dissosiasyon sabiti (sırasıyla $pKa_1$=2,43, $pKa_2$=7,55, $pKa_3$=10,80, $pKa_4$=11,99) ve farklı pH ortamlarında bulunan 4'ü göz ardı edilebilecek 12 mevcut formu bulunmuştur. Parçalı lineer regresyon ile birleştirilmiş potansiyometrik titrasyon yöntemlerinin son derece hassas, doğru ve kullanımı kolay olması gibi birçok avantajı vardır. Kaçınılmaz rastgele hatalar nedeniyle geleneksel grafik yönteminde veri noktaları daha dağınıktır bu nedenle hata daha büyüktür. Parçalı lineer regresyon ise, grafik yönteminin zayıflığının üstesinden gelir ve sonuçlar doğru ve güvenilirdir. Ayrıca, programlama ile karşılaştırıldığında yöntem, parçalı lineer regresyon ile daha hızlı ve basittir. Bu çalışmada araştırmacılar, alendronat sodyumun ayrışma sabitlerini belirlemek için bu yöntemi kullanmış ve güvenilir bir sonuç aldıklarını bildirmişlerdir. Bu arada dağılım farklı pH'lar altında alendronat sodyumun 12 formunun eğrileri, 4 form göz ardı edilebilecek koşullarda elde edilmiştir. Bu çalışmada, alendronat sodyumun ilaç özelliklerini keşfetmek için önemli bir temel sağlayacağı sonucuna ulaşılmıştır.

**İçerik tekdüzeliği, çözünme ve stabilite testine uygulama ile dökme ve tablet dozaj formunda risedronat tayini için çevre dostu bir HPLC-UV yöntemi (Moustapha ve ark., 2020)** Risedronat, menopoz sonrasında osteoporozun tedavisi ve önlenmesi için kullanılan nitrojen içerikli bir bifosfonattır. Bu çalışmada araştırmacılar, risedronat sodyumun dökme (ambalajlanmamış toz) ve tablet formüllerinde hızlı analizi için yeni çevre dostu bir HPLC-UV yöntemi geliştirmeyi amaçlamışlardır. Analiz edilen numuneler, 1:2 oranında pH 2,9 değerine sahip potasyum fosfat tamponu ve potasyum edetat tamponundan (pH 9,5) oluşan çevre dostu bir mobil faz kullanılarak Waters Atlantis dC18 kolonunda ayrılmıştır. Son pH değeri, fosforik asit ile 6,8 olarak ayarlanmış mobil faz 1,0 mL/dk akış hızında pompalanmıştır. Kolon sıcaklığı 30 °C'ye ayarlı olarak çalışılan numuneler 263 nm dalga boyunda tespit edilmiş ve kromatografik çalışma süresi 3,0 dk olarak belirlenmiştir. Yöntemin, 14-140 µg/mL konsantrasyon aralığında 0,9994 katsayı değeri ($R^2$) ile doğrusal olduğu bulunmuştur. Önerilen yöntemin faydalı olduğu, ticari Actonel® tabletlerde analiz, içerik tekdüzeliği ve çözünme testi yapılarak doğrulanmıştır. Uygulanan yöntem, risedronat analizi için daha önce bildirilen yöntemlerle karşılaştırıldığında, tüm analiz prosedürlerinin sulu ortamda yürütüldüğü ve dolayısıyla çevrede tehlikeye yol açmadan güvenli bir şekilde uygulanabildiği için, çevre dostu bir analiz yaklaşımıdır. Bu sonuçlar ile, önerilen yöntemin risedronatın dökme ve ticari formülasyonlarında rutin bir analiz için başarıyla kullanılabileceğini araştırmacılar bildirmişlerdir.

**İnsan plazmasındaki risedronat konsantrasyonlarının belirlenmesi için yüksek performanslı sıvı kromatografisi/tandem kütle spektrometrisi (LC–MS/MS) kullanan hassas bir katı faz ekstraksiyon (SPE) yönteminin geliştirilmesi ve doğrulanması (Ghassabian ve ark., 2012)** Risedronat, kemik bozukluklarının tedavisi için yaygın olarak reçete edilen bir bifosfonattır. Yüksek polaritesi ve düşük oral biyoyararlanımı nedeniyle, insan plazmasında düşük risedronat konsantrasyonları beklenir ve bu nedenle farmakokinetik çalışmalarda hizmet vermek için hassas bir analiz gereklidir. Bu çalışmada araştırmacılar, insan plazmasındaki risedronat konsantrasyonlarının ölçümü için bir LC-MS/MS analizinin geliştirilmesini ve doğrulanmasını açıklamışlardır. Risedronat ve iç standart, risedronat-d4, bir anyon değişimli katı faz ekstraksiyon kartuşunda türevlendirilmiştir. Termal olarak kararlı ve nispeten toksik olmayan türevlendirme maddesi olan trimetilsilildiazometan, risedronat fosfonik asit gruplarını metillemek ve analit polaritesini azaltmak için kullanılmıştır. Ekstraksiyonun ardından, analitler bir Phenomenex Gemini C18 kolonu (150x2,0 mm, 5 µm) üzerinde amonyum asetat 10 mM ve 300 µL/dk akış hızında asetonitril kullanılarak ayrılmıştır. Test kalibrasyon aralığı 0,2-25 ng/mL olmuştur. Altı ayrı plazma numunesine eklenen risedronat standartlarının kalibrasyon eğrisi doğrusal olarak bulunmuştur. Ortalama geri kazanım, risedronat için %54 ve iç standart için %51 olmuştur. Risedronat, insan plazma örneklerinde oda sıcaklığından en az 5 saat, -80 °C'de dondurularak 101 gün, 10 °C'de bir otomatik numune alma cihazında 72 saat ve üç dondurma/çözme döngüsü için stabil olarak bulunmuştur. Doğrulanmış analiz yöntemi, tek bir 35 mg risedronat tableti uygulanan, bilgilendirilmiş rızası alınmış sağlıklı gönüllülerden alınan plazma numunelerindeki risedronat konsantrasyonlarını başarılı bir şekilde ölçmüştür.

**UV dedektörlü iyon çifti yüksek performanslı sıvı kromatografisi ile sıçan plazma örneklerinde risedronat tayini (Jia ve ark., 2006)** Bu çalışmada sıçan plazmasındaki rutin analiz için bifosonatların bir üyesi olan risedronat tayini için analitik bir yöntem anlatılmaktadır. Numune ön işlemi, protein çökeltmesini, alkali pH'ta kalsiyum ile birlikte çökeltmeyi, olası pirofosfat türevlerinin hidrolizini ve yeniden çökeltmeyi içermektedir. Ters fazlı bir kolon (Hypersil ODS-2 C18, 4,6x250 mm, 5 µm) kullanılarak iyi bir ayırma elde edilmiştir. Mobil faz olarak sulu bir tampon çözeltisi (iyon çifti reaktifi olarak 1,5 mM EDTA-2Na, 1mM sodyum etidronat, 11mM sodyum fosfat ve iyon çifti reaktifi olarak 5 mM tetrabutilamonyum bromür) – metanol (88:12, v/v) 1 M NaOH kullanılarak pH 6,75'e ayarlanmıştır. Akış hızı 1 mL/dk yapılmıştır. 10-500 ng/mL konsantrasyon aralığındaki risedronatı ölçmek için UV dedektör (λ = 262 nm) kullanılmıştır. Risedronat için tespit sınırı ve miktar tayini sırasıyla 7 ve 10 ng/mL olarak ölçülmüştür. Yöntem, risedronat mini haplarının oral yoldan verildiği Wistar sıçanlarından alınan plazma örneklerine başarıyla uygulanmıştır. Hassasiyet, ekstraksiyon geri kazanımları ve ayrıca doğruluk sonuçları tatmin edici olmuş ve risedronat retansiyon zamanında hiçbir değişiklik bulunmamıştır. Bu nedenle araştırmacılar yöntem için, sıçan plazmasında risedronatı izlemek için uygundur demişlerdir.

**Monoklonal antikor denosumab'ın biyoanaliz ve doğrulanmış LC yöntemleri ile miktar tayini (Perobelli ve ark., 2018)** Monoklonal antikor Denosumab (DmAb) klinik olarak osteoporoz ve kemik kaybını tedavi etmek için kullanılır. Araştırmacılar DmAb'nin, nükleer faktör-κB ligandının (RANKL) insan reseptör aktivatörünün RAW 264,7 hücrelerinden türetilen osteoklastların oluşumunu uyarma etkisini engelleme yeteneğine dayanan bir biyoanaliz geliştirmişlerdir. Biyoanaliz, boyut dışlamalı yüksek performanslı sıvı kromatografisi (SE-HPLC) ile diyot dizisi algılamalı ters fazlı yüksek performanslı sıvı kromatografisi (RP-HPLC) yöntemleri (DAD), bu biyoteknolojiden türetilen ilacın miktar tayini için geliştirmişlerdir. *In vitro* osteoklastlar antiproliferatif biyoanaliz ve valide edilmiş SE-HPLC (DAD) ve RP-HPLC (DAD) yöntemlerinin biyoteknoloji süreci sırasında DmAb'yi ölçmek, kararlılığını izlemek, kalite kontrolünü iyileştirmek ve bu dökme ve ticari formunda biyoteknolojiden türetilen ilacın seriden seriye tutarlılığını sağlamak için uygulanabilecek büyük bir gelişmeyi temsil ettiğini bildirmişlerdir.

**Raloksifen hidroklorürün HPLC analizi ve ilaç kalite kontrol çalışmalarına uygulanması (Trontelj ve ark., 2005)** Raloksifen hidroklorür, seçici bir östrojen reseptör modülatörüdür ve şu anda menopoz sonrası kadınlarda osteoporozun önlenmesi için kullanılmaktadır. Bu makalede, raloksifen hidroklorürün tespiti için HPLC yöntemi ultraviyole (UV) ve kulometrik dedektörler kullanılarak geliştirilmiş ve doğrulanmıştır. Kantifikasyon limiti (LOQ), kulometrik ve ultraviyole dedektörler için sırasıyla 0,336 ve 0,610 mg/L olmuştur. Yöntem, 0,5-10 mg/L arlığında her iki dedektör için de çok iyi doğruluk, kesinlik ve doğrusallık göstermiştir. Beklendiği gibi kulometrik dedektör, ultraviyole dedektöre kıyasla daha düşük bir miktar belirleme sınırı göstermiştir. Kullanılan dedektörlerden bağımsız olarak, araştırmacılar önerdikleri yöntemin tablet dozaj formundaki raloksifen hidroklorür içeriğinin kalitatif ve kantitatif tespiti ve in vitro çözünme çalışmalarında elde edilen numuneler için uygun olduğunu kanıtlamışlardır. HPLC'nin çok yönlülüğünü dikkate alınarak yöntemin çeşitli ilaç kontrol çalışmalarında başarıyla kullanılabilir olduğu araştırmacılar tarafından bildirilmiştir.

**Bozulmamış teriparatid molekülünün doğrudan tespiti yoluyla insan serumunda teriparatidin (paratiroid hormon fragmanı 1-34) belirlenmesi için son derece seçici ve hassas LC-MS/MS yönteminin geliştirme ve doğrulanması (Kusuma ve ark., 2021)** Teriparatid, osteoporoz tedavisi için önerilen insan paratiroidinin ilk 1-34 amino asidinin yeni bir rekombinant peptit fragmanıdır. Terapötik proteinler ve peptitler rutin olarak ligand bağlama deney formatları kullanılarak analiz edilir, ancak küçük moleküllerin biyoanalizinde rutin olarak kullanılan LC-MS/MS tekniği, LBA'lara (ligand bağlama analizleri) göre sunabileceği geliştirilmiş doğruluk, seçicilik ve antikorsuz yöntem geliştirme avantajları nedeniyle artık büyük molekül biyoanalizinde önem kazanmıştır. Bu makale, tandem kütle spektrometrik tespiti ile hizalanmış ultra performanslı sıvı kromatografisi kullanılarak insan serumunda teriparatidin belirlenmesi için hassas bir biyoanalitik yöntem sunmaktadır. Teriparatid, katı faz ekstraksiyonu kullanılarak insan serumundan izole edilmiştir. Peptitlerin/proteinlerin, şişelere/tüp duvarlarına yapışmasının neden olduğu spesifik olmayan bağlanma etkisi, bloke edici ajan olarak BSA (Bovine Serum Albumin) solüsyonu kullanılarak önemli ölçüde azaltılmıştır. Araştırmacılar buldukları sonuçlara göre bu yöntemin insan serumunda teriparatidin kantitatif tayini için duyarlı, spesifik ve tekrarlanabilir olduğunu ve farmakokinetik çalışmalardan teriparatid içeren serum numunelerini analiz etmek için kullanılabileceğini göstermektedir demişlerdir.

**İnsan plazmasında insan paratiroidi 1-34'ün (teriparatid) doğrudan ölçümü için yüksek hassasiyetli LC–MS/MS yöntemi (Chambers ve ark., 2013)** İnsan paratiroid hormonunun 1-34 fragmanı olan teriparatid, yeni kemik oluşumunu uyararak yüksek kırık riski olan osteoporoz hastalarını tedavi etmek için kullanılır. Rutin olarak teriparatid, radyoimmumoanaliz kullanılarak ölçülür ancak bu çalışmada anlatılan LC-MS/MS yöntemi ile daha yüksek doğruluk ve kesinlik elde etme potansiyeli sahip ve rutin biyoanalitik laboratuvarlarda kolayca uygulanılabilir bir yöntem geliştirildiği söylenmiştir. Bu nedenle, teriparatidi yakından ilişkili endojen peptidlerden seçici olarak ayırmak etkileşimleri azaltmak için uygun LC ayırma ve seçilmiş reaksiyon izleme

(SRM) MS saptama ile etkili numune hazırlamayı birleştiren eksiksiz bir yöntem geliştirilmiştir. 2025 yılına kadar %50 oranında artması beklenen osteoporoza bağlı kırıkların küresel insidansı ile ayrıca tedavi ve izleme seçeneklerinin geliştirilmesine ve dolayısıyla teriparatidin tanımlanması ve miktarının belirlenmesine olan ilginin bilim camiası için giderek daha önemli olması beklenmektedir. Sadece teriparatide olan ilginin kesin olarak artması değil, aynı zamanda peptit terapötiklerinin gelişiminin artması ve ilginç bir şekilde peptit boyutunun artması yönündeki eğilimdir. Terapötikler, büyük peptitlerin biyoanaliziyle ilişkili spesifik zorlukların anlaşılmasını ve ele alınmasını özellikle önemli hale getirir. LC-M/MS ile teriparatidin nicelleştirilmesinin birçok belirgin avantajı vardır ve bu analiz, spesifik olmayan bağlanma, zayıf kromatografik pik şekli, taşıma, zayıf doğrusallık, zayıf tekrarlanabilirlik, protein bağlanması ve zayıf MS duyarlılığı dahil olmak üzere birçok analitik engelin üstesinden gelmiştir. Bu makale, µElution SPE ve LC-M/MS kullanılarak teriparatidin farklılaştırılması ve nicelleştirilmesi için yeni bir yöntem sunar ve rutin biyoanalitik laboratuvarında LC-MS ile büyük molekül analizi ile ilgili zorlukları anlamak, tanımlamak ve çözümlemek için araç ve teknikleri vurgular.

**Teriparatidin subkutan enjeksiyonundan sonra UHPLC-MS/MS ile plazmada PTH 1-34'ün sub-pikomolar ölçümü ve PTH 1-33'ün, onun bozunma ürününün tanımlanması (Eugster ve ark., 2019)**

Teriparatid (PTH 1-34 Forsteo), yüksek kırık riski olan hastalarda osteoporoz tedavisi için önerilen doğal endojen paratiroid hormonunun (PTH 1-84) biyoaktif bir N-terminal fragmanıdır. PTH 1-34 proteolize uğrayabileceğinden, bozulmamış PTH 1-34'ü ELİSA ile aynı hassasiyetle subpikomolar seviyede (LLOQ 0,4 pM'de) açık bir şekilde ölçmek için ultra yüksek performanslı sıvı kromatografi tandem kütle spektrometrisi (UHPLC-MS/MS) yöntemini araştırmacılar çalışmışlardır. Kromatografik çalışma 16,5 dakikada bitmiştir. Araştırmacılar çalışmaları için, UHPLC-MS/MS enstrümantasyonunun immuno ekstraksiyona dayalı bir numune hazırlama ile birlikte kullanımının, özgüllük endişeleri olmaksızın immunojenik testlere benzer bir duyarlılık sağladığını göstermektedir demişlerdir. Sonuç olarak, PTH ve diğer peptitler için daha iyi analizler arayan araştırmacıların ve klinisyenlerin ihtiyaçlarını karşılayabilir bir yöntem geliştirdiklerini belirtmişlerdir.

## SONUÇ

Osteoporoz veya kemik erimesi, kemik metabolizmasındaki bir bozukluk sonucunda kemikteki protein örgüsünün seyrelmesiyle iskelette ortaya çıkan ve kemiklerin çok kolay kırılabilmesine sebep olan bir hastalıktır. Osteoporoz, ülkemizde ve dünyada giderek artış gösteren 21. yüzyılın önemli bir sağlık problemine dönüşmüştür. Kemiğin birim hacimdeki mineral yoğunluğunun azalması sonucu kemikler daha kolay kırılır hale gelir. En çok omurlarda, kalça ve bilek kemiklerinde görülse de vücuttaki bütün kemikler bu durumdan etkilenir. Her iki cinste de görülebilmekle beraber hastaların %80'i kadındır. Osteoporoz hakkında farkındalığın kazandırılması, hastalığın tedavisi ve önlenmesinde oldukça önemlidir. Bu çalışmada osteoporozun tanımı, önemi, sınıflandırılması, tanı kriterleri, tedavisi anlatılmış ve ayrıca osteoporoz tedavisinde kullanılan ilaç etken maddeleri üzerine yapılan analitik çalışmalar derlenmiştir. Bu amaçla literatürde son yıllarda yapılan alendronat, risedronat, denosumab, raloksifen ve teriparatid etken maddelerinin tayin yöntemlerini inceledim. Çalışmanın neticesinde alendronat etken maddesi tayini için HILIC, HPLC-MS/MS, HPLC-DAD ile HPLC-FD, MSPE ile LC-MS/MS, HPLC, HPLC-MS, asit baz potansiyometrik titrasyonu; risedronat etken maddesi tayini için HPLC-UV, SPE ile LC–MS/MS, HPLC; denosumab etken maddesi tayini için SE-HPLC ile RP-HPLC; raloksifen etken maddesi tayini için HPLC; teriparatid etken maddesi tayini için LC-MS/MS, UHPLC-MS/MS yöntemlerinin kullanıldığı görülmüştür. Çalışmaların sonuçlarının doğruluğu, kesinliği, hassaslığı belirli parametreler göz önüne alınarak güvenli ve uygun bulunduğu makaleler incelendiğinde görülmüştür.

## KAYNAKLAR

Al Deeb SK, Hamdan II, Al Najjar SM 2004. Spectroscopic and HPLC methods for the determination of alendronate in tablets and urine. Talanta, 64(3): 695–702.

Chakuleska L, Simeonova R, Danchev N 2018. Review on the prarmacology of osteoporosis. Pharmacia, 65(3): 49-56.

Chambers EE, Lame ME, Bardsley J, Hannam S, Legido-Quigley C, Smith N, Fountain KJ, Collins E, Thomas E 2013. High sensitivity LC–MS/MS method for direct quantification of human parathyroid 1–34 (teriparatide) in human plasma. Journal of Chromatography B, 938: 96–104.

El-Yazbi AF, El-Kimary EI, Youssef RM 2019. Hantzsch pre-column derivatization for simultaneous determination of alendronate sodium and its pharmacopoeial related impurity: Comparative study with synchronous fluorometry using fluorescamine. Journal of Food and Drug Analysis, 27(1): 208-220.

Eugster PJ, Chtioui H, Herren A, Dunand M, Cappelle D, Bourquin J, Buclin T, Grouzmann E 2019. Sub-picomolar quantification of PTH 1-34 in plasma by UHPLC-MS/MS after subcutaneous injection of teriparatide and identification of PTH 1-33, its degradation product. Journal of Pharmaceutical and Biomedical Analysis, 166: 205–212.

Ghassabian S, Wright LA, deJager AD, Smith MT 2012. Development and validation of a sensitive solid-phase-extraction (SPE) method using high-performance liquid chromatography/tandem mass spectrometry (LC–MS/MS) for determination of risedronate concentrations in human plasma. Journal of Chromatography B, 881–882: 34–41.

Hernlund E, Svedbom A, Ivergard M, Compston J, Cooper C, Stenmark J, McCloskey EV, Jonnson B, Kanis JA 2013. Osteoporosis in the European Union: Medical menagment, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporosis, 8 (1-2): 136.

Jia HJ, Li W, Zhao K 2006. Determination of risedronate in rat plasma samples by ion-pair high-performance liquid chromatography with UV detector. Analytica Chimica Acta, 562(2): 171–175.

Ke J, Dou H, Zhang X, Uhagaze DS, Ding X, Dong Y 2016. Determination of pKa values of alendronate sodium in aqueous solution by piecewise linear regression based on acid-base potentiometric titration. Journal of Pharmaceutical Analysis, 6(6): 404-409.

Kışlak P, Genç F 2019. Osteoporoz ve Tedavisi. Lectio Scientific Journal of Health and Natural Sciences, 3(1): 1-18.

Kusuma MB, Kashibhatta R, Gavande A, Kiran R, Jagtap S, Vithala P, Moorkoth S, Bhat K 2021. Bioanalytical method development and validation of highly selective and sensitive LC MS/MS method for determination of teriparatide (parathyroid hormone fragment 1–34) in human serum through direct detection of intact teriparatide molecule. Journal of Chromatography B, 1187-123046.

Moustapha ME, Kamal M, Elgamal RM 2020. An eco-friendly HPLC-UV method for the determination of risedronate in its bulk and tablet dosage form with application to content uniformity, dissolution and stability testing. Saudi Pharmaceutical Journal, 28(11): 1301–1308.

Perobelli RF, Xavier B, Silveira AR, Remuzzi GL, Motta LGJ, Dalmora SL 2018. Quantitation of the monoclonal antibody Denosumab by bioassay and validated LC methods. International Journal of Biological Macromolecules, 119: 96–104.

Raju SPK, Narayanam M, Kumar BK, Tejaswee S, Singh S 2015. Validated Stability-Indicating Method for Alendronate Sodium Employing Zwitterionic Hydrophilic Interaction Chromatography Coupled with Charged Aerosol Detection. Chromatographia, 78: 1245–1250.

Riggs LB, Melton LJ 1995. Osteoporosis: Etiology, Diagnosis and Management. Lippincott-Raven Publishers, Philadelphia, New York, 335-350.

She X, Li J, Zhu J, Huang T, Li Y 2021. Magnetic solid phase extraction followed by in-situ derivatization with core-shell structured titanium dioxide coated ferriferrous oxide microspheres for determination of alendronate in plasma. Journal of Chromatography A, 1637-461809.

Tarcomnicu I, Silvestro L, Savu SR, Gherase A, Dulea C 2007. Development and application of a high-performance liquid chromatography–mass spectrometry method to determine alendronate in human urine. Journal of Chromatography A, 1160(1-2): 21–33.

Trontelj J, Vovk T, Bogataj M, Mrhar A 2005. HPLC analysis of raloxifene hydrochloride and its application to drug quality control studies. Pharmacological Research, 52(4): 334–339.

Yamada M, Lee X, Fujishiro M, Iseri K, Watanabe M, Sakamaki H, Uchida N, Matsuyama T, Kumazawa T, Takahashi H, Ishii A, Sato K 2018. Highly sensitive determination of alendronate in human plasma and dialysate using metal-free HPLC-MS/MS. Legal Medicine, 30: 14-20.

## *ORAL PRESENTATION*

# In vitro determination of immunostimulant and cytotoxic activities of vaccine formulations developed against gastric cancer on cells of the innate immune system

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**Abstract**

Cancer is the disease that results from the uncontrolled proliferation of cells and has the highest mortality rate in the world after cardiovascular diseases. Although stomach cancer is one of the most common cancer types in the world, GLOBOCAN 2020 caused approximately 800,000 deaths and approximately 1.1 million new gastric cancer cases were detected. Although methods such as surgery, chemotherapy and drug therapy are frequently used in the treatment of stomach cancer, they do not give effective results. For this reason, before stomach cancer occurs, immunity should be created to prevent the disease before it starts, or a rapid response should be obtained from the treatments applied thanks to the immunity obtained. Since vaccination activates the immune system against cancer, studies in the literature reveal promising results in cancer vaccines. In this study, antigens obtained from AGS gastric cancer cell line by freeze-thaw method were combined with Hydroxyapatite and Calcium phosphate adjuvants against gastric cancer, and the immunostimulant and cytotoxic effects of the developed vaccine formulations were determined in J774 murine macrophage and L929 fibroblast cells. According to the results of the study, it was determined that the generated antigens showed the highest immunostimulatory activity at a concentration of 40 µg/ml, the immunostimulatory activities of the vaccine formulations developed with the antigen-adjuvant combination were 1.38 times higher than the control groups and their toxic effects were low. It can be said that the results obtained can be used in the development of vaccine formulations for gastric cancer and that these formulations should be examined in vivo.

**Keywords:** Gastric cancer, vaccine, antigen, immunostimulant activity, cytotoxicity

## INTRODUCTION

Cancer is one of the most common diseases worldwide with a high mortality rate. There are many cancer types However, breast, lung, colorectal, prostate, and gastric cancer have higher mortality rates than the others(Krieghoff-Henning et al. 2017).

Gastric cancer comes in fourth among the deaths related to cancer. According to GLOBOCAN 2020 statistics, 7,7% of cancer-related deaths are caused by gastric cancer (Siegel et al. 2020). Helicobacter Pylori (H. Pylori) is known to be the crucial factor that causes gastric cancer along with mutations in tumor suppressive genes, and changes in the expressions of oncogenes (Khatoon et al. 2016).

Like any other cancer type, early diagnosis is crucial for gastric cancer treatment. However, even if it can be diagnosed early, the desired outcome may not be sufficient enough in terms of treatment since the drugs used for cancer may cause resistance in the body. Therefore, patients need to have immunity before they are diagnosed with gastric cancer. So that the response to treatment can be faster and more efficient (Sexton et al. 2020).

Many proteins can be used as vaccine antigens for cancer vaccines. In addition, cancer cell antigens can be used as a source of vaccine. Freeze-thaw, autoclave, or sonication methods can be used to obtain antigens from cell sources. For further applications, these antigens can be combined with adjuvants to increase their activity and achieve a greater immune response (Sangdee and Thummabenjapone 2012).

In this research, antigens were obtained from the human gastric cell line AGS by using the freeze-thaw method. Vaccine formulations were prepared with just antigen and its combination with different adjuvants (Hydroxyapatite and Calcium Phosphate). Cytotoxic effects and immunostimulant activities of the vaccine formulation were investigated against J774 murine macrophage and L929 fibroblast cell lines.

## MATERIAL AND METHOD

### Preparation of Cell Culture

In this research, L929 fibroblast and L774 macrophage cell lines with 10-15 passages were used. 75cm$^2$ flasks were used for culture. Stock mediums were prepared with 1% L-glutamine and 1% penicillin-streptomycin solutions. For cell proliferation, DMEM medium containing 10% FBS was used for the L929 cell line while RPMI-1640 with 10% FBS was used for the J774 cell line. prepared cell cultures were left for incubation under optimum conditions with 37$^o$C, 95% humidity, and 5% $CO_2$. J774 cell line was collected by physical method and centrifuged for 5 minutes at 1000 rpm under 25$^o$C while the L929 cell line was collected by enzymatic method. After that, the cells were seeded to 96 well plates, each well had 1x10$^5$ cells. The prepared plates were left for incubation (Kitala et al. 2018).

### Preparation of Antigens

For the cell proliferation of AGS gastric cancer cells, RPMI-1640 medium which contains 10% FBS was used. After the cell proliferation, AGS cultures were washed with PBS and stored at -20°C. To prepare the AGS antigens, the freeze-thaw method was used. For this method, the cells were taken out from -20°C, and they were thawed by using a water bath at 37°C. Once the cells were completely thawed, the cells were put inside liquid nitrogen for 15 minutes and they started to freeze. To thaw the frozen cells, the water bath was used again for another 15 minutes. This process was repeated 5 times. After that, the cells were centrifuged for 3 minutes at 1000 rpm and the supernatant was obtained. To determine the protein content inside the lysate, the Warburg-Christian method was used with a UV-vis spectrometer under 280 and 260 nm wavelengths (Weber et al. 2021).

### Nitrite Oxide Analyses

The Griess method was used to determine the NO (Nitrite oxide) content to investigate the immunostimulant activity against macrophage and fibroblast cell lines. Antigens were prepared by using the freeze-thaw method. The different concentrations of antigens were applied to the macrophage and fibroblast cell lines as well as their combinations with adjuvants. After the incubation for 24 hours, different concentrations of antigen (25 µg/ml, 40 µg/ml, 80 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml) and 100 µg/ml adjuvant (Hydroxyapatite and Calcium phosphate) combınations were applied to the wells. After the incubation for 48 hours, supernatants undergo a reaction with Griess reactive. Griess reactive was prepared by adding 2,5 ml Phosphoric acid, 0,1 g N-(1- Naphthyl) Ethylenediamine, and 1 g Sulfanilamide to distilled water. 50 µl cell culture and 50 µl of Griess reactive were added to the 96 well plates. The plate was left for incubation for 10 minutes at room temperature. After that, the absorbance was measured using ELISA Reader with 540 nm wavelength (Guevara et al. 1998).

### MTT Assay

The MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was conducted to analyze cell viability. 10 µl of MTT solution was added to each well in the plate. The plates were left for incubation in the dark at 37°C for 3 hours. MTT solutions were aspirated after incubation and 100 µl of DMSO (Dimethyl sulfoxide) was added to each well. The plates were left to incubate in the dark for 30 minutes. Cell viability was analyzed using a UV-vis spectrometer under 570 nm wavelength (Kumar et al. 2018). Each experiment group was tested 3 times and the mean of the data was calculated. Cell viability is measured by using the equation given below and graphics were created according to the data obtained from the experiment.

$$\text{Cell Viability}(\%) = (\text{sample absorbance}/\text{control absorbance}) * 100 \quad (1)$$

### Statistical Analyzes

The data of the experiment were analyzed by using IBM SPSS 25.0 (IBM Corporation, Armonk, NY, ABD) program.

## RESULTS AND DISCUSSION

In this research experiment, antigens were obtained from AGS gastric cancer cell lines using the freeze-thaw method. To design a vaccine formulation against gastric cancer, the antigens and their combinations with adjuvants were applied to J774 macrophage and L929 fibroblast cell lines, and their cytotoxic and immunostimulant activity was determined.

The cell viability of the positive control group was 100% and the cell viability of the other groups was determined according to that which was indicated as the rate of the alive cells. Cell viability is analyzed by using the MTT assay at the end of 48th hours of incubation for every group. The evaluations were done with the % cell viability results.

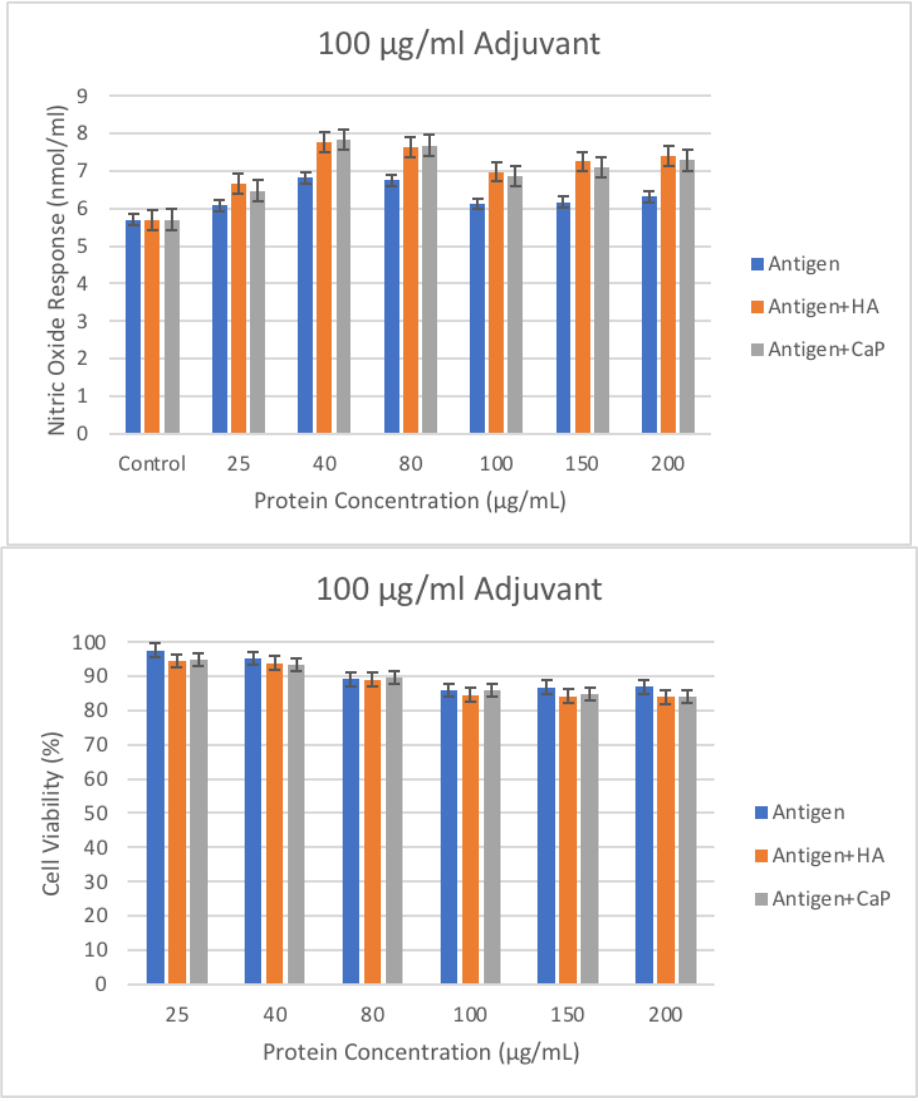


**Figure 1.** The immunostimulant and cytotoxic activity of 100 ug/ml antigen-adjuvant combinations against L929 fibroblast cell line

The immunostimulant and cytotoxic activity of 100 ug/ml antigen-adjuvant combinations against the L929 fibroblast cell line is given in figure 1. The antigens were obtained from the AGS gastric cell line by the freeze-thaw method. In this research, the highest immunostimulant activity is observed with 40 ug/ml concentration of the antigen. For the 40 ug/ml concentrations of antigen, the highest immunostimulant activity against the fibroblast cell line was observed as 6,822 mol/ml.

For the cytotoxicity analysis, the cell viability is observed as %95,23. In this study, the combinations of 100 ug/ml Hydroxyapatite and Calcium phosphate adjuvants with antigens are applied to the fibroblast cell culture to determine its immunostimulant activity. The highest immunostimulant activity was observed as 7,752 mol/ml when 40 µg/ml antigen-100 ug/ml Hydroxyapatite adjuvant combination was applied. Cell viability rates are given as 93,79% when 40 µg/ml antigen-100 ug/ml Calcium phosphate adjuvant combination is applied to fibroblast cells, the highest immunostimulant activity is observed as 7,826 mol/ml. while cytotoxicity assay is determined as 93,21% cell viability.

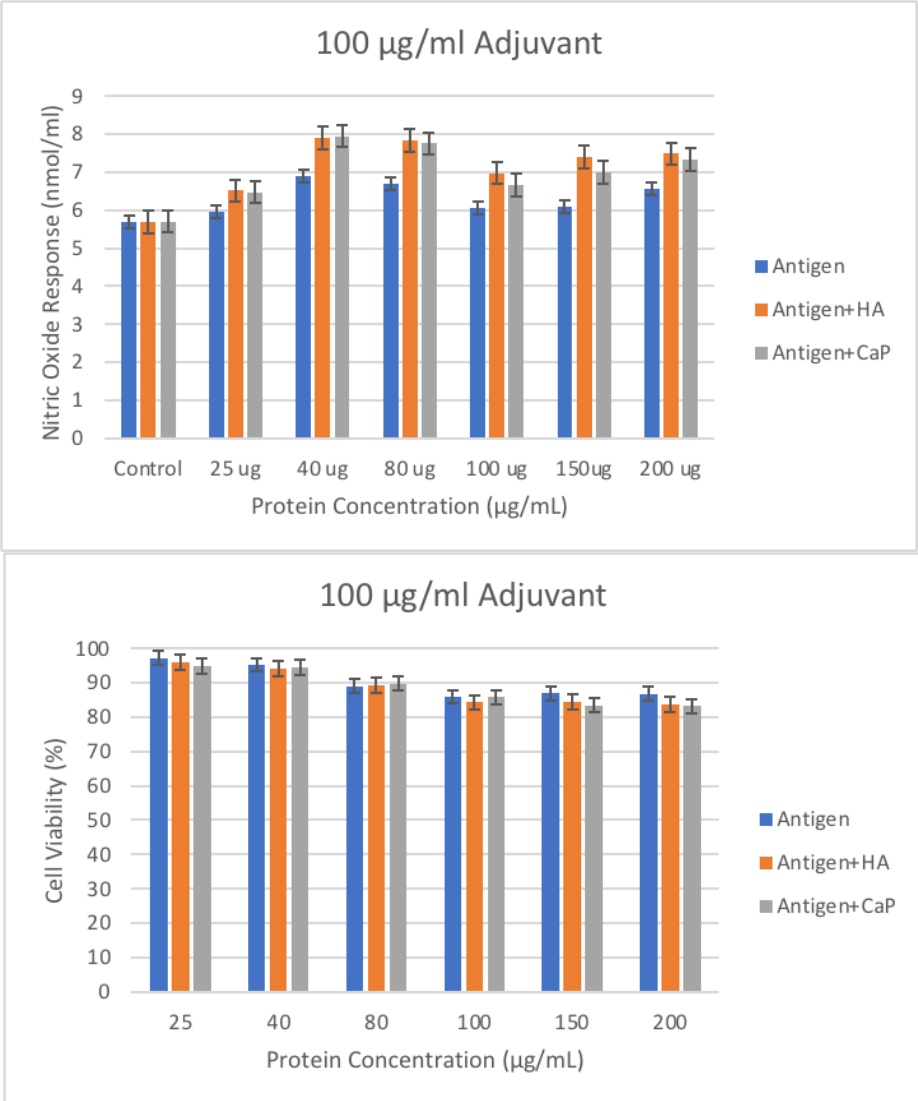


**Figure 2.** The immunostimulant and cytotoxic activity of 100 ug/ml antigen-adjuvant combinations against the J774 macrophage cell line

The immunostimulant and cytotoxic activity of 100 ug/ml antigen-adjuvant combinations against the J774 macrophage cell line is given in figure 1. The antigens were obtained from the AGS gastric cell line by the freeze-thaw method. In this research, the highest immunostimulant activity is observed with a 40 ug/ml concentration of the antigen. for the 40 ug/ml concentrations of antigen, the highest immunostimulant activity against the macrophage cell line was observed as 6,899 mol/ml. For the cytotoxicity analysis, the cell viability is observed as %95,16.

In this study, the combinations of 100 ug/ml Hydroxyapatite and Calcium phosphate adjuvants with antigens are applied to the macrophage cell culture to determine its immunostimulant activity. The highest immunostimulant activity was observed as 7,907 mol/ml when 40 µg/ml antigen-100 ug/ml Hydroxyapatite adjuvant combination is applied. while cell viability rates are given as 94,16%. When 40 µg/ml antigen-100 ug/ml Calcium phosphate adjuvant combination is applied to macrophage cells, the highest immunostimulant activity is observed as 7,946 mol/ml. while cytotoxicity assay is determined as 94,58% cell viability.

Gastric cancer is known to be the most common cancer type around the globe. to treat gastric cancer, chemotherapy, radiotherapy, gene therapy, immunotherapy, targeted therapy, etc. can be used. However, these therapies that are currently used are not efficient enough on their own. Therefore, it is crucial to have immunity before the patient gets cancer and vaccines can be used for this goal. In this research, AGS gastric cancer

antigens obtained from AGS gastric cancer cell lines were chosen to prepare a new vaccine formulation. It is also known that to increase the efficiency and the immunogenicity of the vaccine, a suitable adjuvant must be added to the vaccine formulation as well. Antigens that were obtained from gastric cancer cells with the freeze-thaw method were combined with Hydroxyapatite and Calcium phosphate adjuvants to achieve a stronger immune response against gastric cancer. The immunostimulant activity was determined by applying the prepared vaccine formulation to J774 macrophage and L929 fibroblast cells. In addition, nitric oxide and cell viability analysis were conducted with in vitro experiments. According to the data obtained from experiments, freeze-thaw antigens increased the immunostimulant activity by 1,2 times compared to control groups while Antigen-adjuvant combinations increased this activity by 1,38 times. Therefore it was determined that the immunostimulant activity of Calcium phosphate adjuvant is higher than Hydroxyapatite.

## CONCLUSION

In this research, it was observed that 40 µg/ml antigen-100 ug/ml Calcium Phosphate adjuvant combination has the highest potential that can be used in vaccine formulation. These vaccine combinations are known to be highly efficient with low cost and their ability to induce immunity. Therefore further in vivo experiments should be conducted in the future with this vaccine formulation to design a new treatment method against gastric cancer.

## REFERENCES

Guevara I, Iwanejko J, Dembińska-Kieć A, Pankiewicz J, Wanat A, Anna P, Szczudlik A 1998. *Determination of nitrite/nitrate in human biological material by the simple Griess reaction.* Clin Chim Acta, *274*(2), 177-188.

Khatoon J, Rai RP, Prasad KN 2016. *Role of Helicobacter pylori in gastric cancer: Updates*. World J Gastrointest Oncol, *8*(2), 147-158.

Kitala D, Klama-Baryła A, Kraut M, Łabuś W, Kawecki M 2018. *Isolation, culturing and preparation for transplantation of amniotic mesenchymal stem cells Repetitive and reproducible laboratory, technical protocol.* Ann Biol Clin, *76*(5), 562-567.

Krieghoff-Henning E, Folkerts J, Penzkofer A, Weg-Remers S 2017. *Cancer – an overview*. Med Monatsschr Pharm, *40*(2), 48-54.

Kumar P, Nagarajan A, Uchil PD 2018. *Analysis of Cell Viability by the MTT Assay*. Cold Spring Harb Protoc, *2018*(6).

Sangdee K, Thummabenjapone P 2012. *Evaluation of Antigen Preparation Methods for Polyclonal Antibody Production against Streptomyces spp*. British Microbiology Research Journal, *2*, 137-145.

Sexton RE, Al Hallak MN, Diab M, Azmi AS 2020. *Gastric cancer: a comprehensive review of current and future treatment strategies*. Cancer Metastasis Rev, *39*(4), 1179-1203.

Siegel RL, Miller KD, Jemal A 2020. *Cancer statistics, 2020.* CA Cancer J Clin, *70*(1), 7-30.

Weber D, Sittig C, Hubbuch J 2021. *Impact of freeze-thaw processes on monoclonal antibody platform process development.* Biotechnol Bioeng, *118*(10), 3914-3925.

*ORAL PRESENTATION*

# Vietnam's Digital Transformation In Agriculture – Opportunity And Challenge

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**Abstract**
The digital transformation, application of modern technology in agriculture are gradually becoming an inevitable trend, making an important contribution to changing production methods, liberating labor, reducing costs, and improving productivity, product quality. However, the digital transformation in agriculture still has many "bottlenecks", which need to be cleared. Therefore, the objective of this paper is to evaluate the reality status of digital transformation in agriculture in Vietnam; thereby point out Vietnam's opportunities and challenges in the process of implementing this digital transformation including difficulties in capital, human resources, digital infrastructure and production models and methods; On that basis, the paper proposes some solutions to accelerate Vietnam's digital transformation in the near future.
**Key words:** agriculture , digital transformation, digital transformation in agriculture, Vietnam, opportunity, challenge

## INTRODUCTION

Agriculture is an economic sector with a important position in the Vietnamese economy. Although according to the upward development of Vietnam in particular and countries in the world in general, the proportion of the agricultural sector contributing to GDP is decreasing compared to other economic sectors such as industry and services, but agriculture still plays a strategic role in the long term, is an important foundation for food security, social security and people's security of the country. This role is even more evident when the country is facing major problems, especially during the recent Covid-19 pandemic and the ongoing war between Russia and Ukraine, which showed that industry and services were stagnated, disrupted, caused. This makes many countries at risk of falling into a food shortage crisis, people's lives are seriously affected. It is worth mentioning that Vietnam is a country with a long tradition of agricultural production with thousands of years, but in the context of the 4th industrial revolution, international integration, climate change, epidemics and many other problems, which make Vietnam's agricultural industry not catch up with the agricultural development level of many countries in the world, such as the methods of smart agricultural production, clean agriculture, and high-tech agriculture. In other words, it is Vietnam's agriculture that needs a big shift from traditional production methods that are fragmented, small, and manual, using a lot of pesticides and inorganic fertilizers and being heavily dependent on agriculture from nature to a modern and advanced agriculture, strongly applying scientific and technological achievements to agricultural production. That is the process of digital transformation in agriculture in Vietnam that needs to aim at it. This is an inevitable trend, a "golden key" for the sustainable development of Vietnam's agricultural industry in the future. Digital transformation in agriculture also helps farmers and businesses reduce production costs, improve product quality, and increase competitiveness in not only domestic but also international markets, and profit maximization. This goal is being promoted by the Government, all sectors at all levels, with the expectation of creating a breakthrough in

productivity, quality, profit and competitiveness for the country's agricultural products. However, besides those expectations, the digital transformation in agriculture in Vietnam is also facing many opportunities and challenges that need to be overcome; thereby the digital transformation will be successful. This is the goal of the article that the authors wants to mention.

## MATERIALS AND METHODS

The article uses qualitative research methods (methods of analysis, comparison, synthesis, descriptive statistics...) and the collected data are secondary data selected from previous research results as well as from Vietnam's reports on digital transformation in agriculture. These methods are used to clarify the research contents in order to point out the urgency of the article on digital transformation in agriculture in Vietnam; at the same time, analyze and evaluate the current situation to highlight the results achieved by Vietnam in recent times and point out the opportunities and challenges that Vietnam is facing. From there, the article propose the solutions to accelerate the process of digital transformation in in agriculture in Vietnam.

## Results and Discussion

### *The current situation of Vietnam's digital transformation in agriculture*

*The reality of digital transformation in cultivation and processing of Vietnamese agricultural products*

Vietnam's agriculture is strongly shifting to commodity agriculture associated with quality standards, brands, added value and sustainable development. Initially, Vietnam has built a foundation for the digital transformation of agriculture and rural areas (such as a C/O tracing system for agricultural products, an information system and database for the management of animal feed and livestock farming facilities, planting area code management system, etc.), put into operation, catching up with the changes of the Industrial Revolution 4.0. Today, the whole country has 4.8 million hectares of planting area (including fruit trees, rice production and industrial plants), of which there are about 4,000 growing areas and 2,000 packing units that have been granted the number code (Phu, 2022).

In 2021, the growth rate of crop production value will reach 2.7%, the proportion of cultivation accounting for 44.6% of the total production value of agriculture, forestry and fishery. The export value of agricultural products from cultivation will reach over USD 21 billion in 2021, an increase of 13.5% compared to 2020. In July 2022 alone, out of a total of nearly USD 32.3 billion in agricultural, forestry and aquatic product exports, agricultural products, the main group of agricultural products for export reached US$13.3 billion, up 8.4% over the same period last year. Commodities, such as: coffee reached over $2.6 billion (up 46.2%); rice over USD 2 billion (up 9.0%); pepper over USD 661 million (up 11.7%); vegetables and fruits reached over USD 1.9 billion…. (Hieu, 2022).

These are important steps in the process of developing commodity agriculture in association with sustainable agricultural development, improving quality, added value, efficiency and competitiveness, focusing on ecological environment protection, contributing to increase income for farmers.

Digital agriculture can solve the problem of Vietnam's agriculture industry, with a large number of farmers, but small farming scale, low investment capital, old and outdated equipment, leading to fragmented and small production, depending on intermediary traders, instead, through electronic trading floors, eliminating intermediary stages, connecting directly between sellers and buyers, regardless of geographical distance, allowing collect data on market prices in various locales and countries, compare prices automatically, update daily, weakly or monthly information and even predict future prices in order to propose a competitive and appropriate selling price of agricultural products, farmers in mountainous and rural areas do not have to go to big cities to look for markets but can do it in their own localities (Dinh & Dung, 2021).

Through digital transformation, a digital agricultural data platform will be created for farmers to know and best exploit their advantages, not just based on qualitative experience, less on quantitative data and there is almost no information about its own cultivated land such as weather, light, rainfall or minerals. It is government agencies that can generate useful data for farmers by analyzing soil conditions, satellite images, weather data

or other factors. The Soil Health Cards initiative with 158 million land health cards for each region in India has improved farmers' productivity by up to 40% over the past 5 years in this way (Fitzpatrick, Millner, & Ginn, 2022), or Kenya provides a weather insurance service that pays for farmers if bad weather conditions occur, using 30 weather stations that automatically collect weather data, thereby determine the circumstances in which compensation is received (Dordley, 2022).

Up to now, many agricultural products of Vietnam have been granted certificates, trademarks as well as geographical indications or C/O: until August 1st , 2022, the whole country has had 1,682 collective certificates granted; there are many protected local collective brands granted such as: Quang Nam, Hai Phong, Bac Giang, An Giang...; there are many protected geographical indications, typically: Yen Bai, Ha Giang, Thanh Hoa 6, Ben Tre; In particular, there are a number of agricultural products certified with geographical indications or collective brands that meet international quality standards and are being exported to high-ranking markets around the world, such as Thai Nguyen tea, Phin Ho Shan Tuyet organic tea , Gia Lai pepper, Buon Ma Thuot coffee, Luc Ngan lychee, Phu Quoc fish sauce. (Hieu, 2022).

***The reality of digital transformation in the consumption of Vietnamese agricultural products***

Digital transformation plays the role of connecting farmers with processing and trading businesses associated with consumers in the country and around the world. Thereby transforming the small and inefficient production model into modern agricultural production; at the same time, applying 4.0 technology to production brings high economic efficiency to farmers. Digital transformation can help farmers easily access credit just with the smartphone they have. Through transaction and credit histories, farmers have access to loans with suitable interest rates.

There are more than 2 million agricultural farming households nationwide who have been trained in digital transformation skills and nearly 50,000 agricultural products have been posted on e-commerce platforms. In the past time, some localities have applied e-commerce in agricultural product consumption and obtained positive results. Bac Giang is one of the leading localities in supporting the consumption of lychee as well as other agricultural products on e-commerce exchanges such as Postmart, Alibaba, Sendo, 24h,.... As a result, in 2021, over 215,000 tons of lychee will be consumed, the highest consumption rate, in addition, the consumption market will be expanded and there is great room for development, including 8,000 tons of agricultural products consumed on e-commerce platforms (Son, 2022).

The fact that farmers in the provinces and cities using facebook, zalo ... to interact and sell agricultural products is also effective and achieved unexpected success. Farmer Ly Van Bon - Can Tho Excellent Farmer in 2021 who raises more than 10 types of rare fish on the Mekong River and combines it with community tourism, is chosen by many tourists as a destination when coming to Can Tho. Farmer Hoang Quang Dong - Excellent Vietnamese farmer 2021 Hung Yen, specializing in planting, processing and exporting turmeric to Japan and Europe with a total revenue of VND 15 billion (Mr. L., 2021) and so on.

Agriculture is theindustry that accounts for 14% of Vietnam's GDP, accounts for nearly 40% of the labor force. Agriculture has developed better, becoming the foundation for the economy (Vietnam Newws, 2021). The goal of Vietnam is a farmer who produces quality agricultural products at the lowest cost but sells them at the highest price. Therefore, digital transformation in agriculture or digital agriculture will be one of the keys to successfully accomplish this goal.

***Opportunities and challenges in Vietnam's digital transformation in agriculture***

*Opportunities in Vietnam's digital transformation in agriculture*

*Firstly, enhancing connectivity for farmers*

The biggest limitation of farmers is the lack of information about production and life. Therefore, digital transformation will give them the opportunity to connect information, gradually narrow the geographical distance, update new knowledge, open up a new way of thinking, a new way of doing things, willing to change,

catch up with development trend of the country. Farmers can easily access information and technology like a business to build their brand.

According to a survey by the Asian Crop Life Association, nearly 50% of Vietnamese rice, fruit and vegetable planting farmers surveyed said they want to apply digitalization in agriculture (Binh, 2022). This shows that Vietnamese farmers are very interested in digitization, which is also a premise for Vietnam to deploy training programs for farmers to understand and apply digital technology effectively, safely and efficiently. sustainably, with the goal of ensuring access and increasing the rate of technology application in farming.

*Secondly, develop digital economic activities through the Digital Agriculture Database*

When farmers have access to more information, they will have a firmer basis for making production decisions, thereby reducing production costs, increasing labor productivity and production efficiency. In addition, the access to information on the Digital Agriculture Database such as: input services of clear origin (fertilizers, plant protection drugs...), weather forecasting services, buyers' demand, price information, market situation ..., along with the application of advanced technologies, automation, help optimize the production process, ensure food safety and environmental issues .

Thanks to digital transformation in cantaloupe production such as: production management system with smartphone, automatic irrigation system, QR code traceability stamp..., Kien Tho agricultural service cooperative (Thanh Hoa) has reduced labor costs by up to 90%, productivity is 15% to 20% higher than traditional production models in the area. (Dang, 2022)

*Thirdly, access quality sales, logistics, and transportation channels at a reasonable cost*

Farmers have access to advanced logistics chains at the post-harvest stage through a value chain management system, traceability to ensure food safety, transparent information and sales through e-commerce channels.with digital platforms provided by businesses. These platforms also take care of logistics and transportation with advanced digital technologies such as Blockchain, IoT, AI... developed by Vietnamese enterprises. All of this is encapsulated on a digital technology platform, so farmers will have access to a suitable cost instead of searching for sales, logistics and transportation channels through middlemen.

*Challenges in Vietnam's agricultural digital transformation in agriculture*

*Firstly, the small, fragmented and scattered model of agricultural production*

In recent years, Vietnam's agriculture has achieved remarkable results, with the achievement of exporting many agricultural products ranked in the "Top" in the world, reaching a turnover of many billions of USD per year. However, Vietnam's agricultural industry still has a state of fragmented production, which makes it difficult to make a breakthrough, the lives of farmers are precarious, and incomes are unstable.

The per capita agricultural land area in Vietnam is 0.25 ha, while that in the world is 0.52 ha and in the region is 0.36 ha with 70% of the population engaged in agriculture. Vietnam's agriculture is developed mainly based on about 10 million individual farmer households with over 76 million plots and scattered small plots. (Vietnam News, 2019)

The average cultivated area ranges from 0.2 to 2 ha/household and only 0.34 ha/labor, small land size, little change, number of households not directly using land increased from 18.23 % to 21.38%, the number of households using 0.2 ha to less than 0.5 ha (i.e. decreasing from 32.29% to 27.11%), the number of households having agricultural land area under 0 ,2 ha only increased from 21.17% to 22.49%, the number of households with agricultural land size from 0.5 ha to less than 1 ha (i.e. an increase from 16.44% to 16.75%) only. about 5% of farming households have a land plot of more than 3 hectares (Vien, 2020).

Besides, Vietnam's agriculture has also made efforts to participate in the global agricultural value chain with many products with high export value such as coffee, pepper, seafood, fruits... However, because of the lack of chain linkages, most Vietnamese agricultural products that want to go to the world market have to go through intermediaries and are sold at low prices.

The global ranking of selling prices (USD/ton) of some key agricultural export products of Vietnam shows that the growth of Vietnam's agricultural exports is mainly based on price competition (cheap prices), low quality segment, no labels, not yet based on competitive advantage in quality (Vien, 2020).

Most of the farmers lack the ability to access the market, produce according to experience and rely on subjective calculations about the market. Vietnam's agricultural products can only participate in the stages of cultivation, collection, preliminary processing and export of raw products, which are the stages with the lowest added value in the value chain of agricultural products. In stages with high added value such as: research and development (R&D), processing, distribution (retail) and marketing... we have not been able to participate or the level of participation is still very low. The stages of collection, processing and consumption of agricultural products are incomplete and unsustainable, with many intermediate levels. Farmers mainly sell agricultural products to traders and purchasing agents, while these stages have not been carried out according to the technical process, so the quality of agricultural products is not high and the price is low.

*Secondly, the low quality and qualifications of agricultural human resources*

Today, because the lack of skilled and highly specialized labor, agricultural production in Vietnam still depends mainly on human strength and experience. In addition, most agribusiness and production units employ untrained laborers to work seasonally, are middle-aged or older, so they are only capable of doing jobs with. simple technical operation. If the enterprise invests in modern equipment and technology, it does not have enough engineers to do it, so the enterprise must both retrain seasonal workers and recruit quality human resources.

The labor force in rural areas has nearly 36.7 million people (accounting for 67% of the national labor force), but the proportion of trained workers with primary degrees or certificates or higher in the rural areas only 16% in rural areas, much lower than urban areas (39.3%). In 2020, human resources in the agricultural sector lack about 3.2 million trained workers. In addition, agricultural workers now lack skills in management, administration, connection in production and consumption, and lack of industrial style. (Feng, 2022)

*Thirdly, the shortage of investment capital for digital transformation in agriculture*

Over the past time, the National Assembly and the Government have issued many policies and incentives to invest from the state budget as well as to attract social investment in the agricultural sector, especially investment in digital transformation in agriculture towards sustainable agricultural development.

At the end of April 2021, credit for agricultural and rural development reached VND 2,363,955 billion, up 3.8% compared to 2020 and accounting for 24.69% of the total outstanding loans of the whole economy. However, the investment capital of the state budget for agriculture is still limited and not effective. In the past time, investment resources for agriculture and rural areas only meet 55% to 60% of demand, in addition, because of the lack of investment capital, material and technical facilities for agricultural production are still backward. To achieve modern and sustainable agriculture, agriculture must be based on digital agriculture (Thiet, 2021).

For foreign direct investment (FDI) in Vietnam, FDI inflows into the agricultural sector are still modest, the number of registered projects in agriculture is still small and accounts for a very low proportion of the total yearly registered capital according. Until the end of 2020, FDI in agriculture accounted for about 0.97% of total FDI into Vietnam, while the global average was 3% of total FDI (Lam, 2020).

In addition, access to bank credit for small businesses and households still faces many difficulties. The people who are eligible for agricultural concessional loans are still limited because the criteria for preferential loans are those directly engaged in high-tech agricultural production, excluding those who involved in the production and supply chain along the value chain. Ignoring the remaining stages in the supply chain also makes it difficult for the development of high-tech agriculture. On the other hand, the number of linkage models is still small and ineffective, so it is difficult to access preferential credit.

***Some basic solutions to promote Vietnam's digital transformation in agriculture***

*Firstly, continue to prioritize the development of cooperative models and closed chains for agricultural products*

To solve these difficulties in the context of small and fragmented production is not possible, many experts emphasized. They believe that, in order to expand digital transformation in agriculture, it is necessary to strengthen and develop cooperative groups and cooperatives, especially the cooperation model with the participation of enterprises, in which enterprises are the leading people, farmers are central. On that basis, we actively builds closed linkage models from research, deployment to farming, processing and consumption to ensure inputs and outputs for farmers and businesses, avoid falling into a strong situation that everyone does.

The construction and development of chain of links and cooperatives has been proposed by the Vietnam Cooperative Union in the Project on building and replicating models of cooperation, linkages, production and business along the value chain, considering this a breakthrough in production development and consolidating and improving operational efficiency for cooperatives.

To accomplish this goal, we need to support credit for agricultural and rural development, land reclamation, lease of land use rights to accumulate land, and encourage businesses to invest in agriculture and agriculture, supporting the development of agricultural production of commodities and OCOP[1] products to consolidate and build production and product consumption links.

Focus on supporting those who in building business linkage projects; building trademarks, geographical indications; promote and develop value chain brands; applying science and technology to production, improving productivity, quality and competitiveness of products; complete the product to meet the standards of the importing country.

*Secondly, strengthen the training of rural human resources to serve digital transformation in agriculture*

A huge challenge and an urgent requirement in training and developing human resources to promptly supply human resources for the digital transformation of the agricultural sector is that localities and units need to build training models on the basis of linking training and scientific research with production activities.

On the other hand, encourage localities and businesses to establish vocational training institutions in agricultural technology and implement training cooperation programs in agricultural production areas.

Strengthen the opening of training courses at production units for rural workers, so that they can quickly access and promptly apply high technology to actual production, in parallel with the application of information technology in agricultural services activities.

*Thirdly, diversify investment sources for digital transformation in agriculture*

In recent years, investment resources for agriculture and rural areas are limited, only meeting 55%-60% of demand. Therefore, the system of policies and laws on public investment in agriculture and rural areas needs to continue to be adjusted and perfected in the coming time, such as increasing investment spending in the agricultural and rural areas; implement state budget allocation to ensure harmony between the interests of localities with favourable conditions for industrial development with those of purely agricultural provinces. In addition, to attract private investment in the agricultural sector, especially through the form of PPP[2], it is necessary to have a breakthrough solution to take advantage of capital, qualifications, management capacity and strength about the supply chains of the world's leading corporations, as well as Vietnam's in the agricultural sector (Mr. T., 2021).

[1] OCOP: One Commune, One Product
[2] Public-Private Partnerships

## Conclusions

Thus, digital transformation in agriculture is an inevitable trend in the context of the industrial revolution 4.0 and is an objective requirement to improve the competitiveness and value of agricultural goods compared to the traditional farming model. However, to implement an effective digital transformation in agriculture, Vietnam needs to implement many synchronous solutions because Vietnam is a country with nearly 70% of its population engaged in agriculture, but so far, although agriculture has the long history of development, many new farmers only have knowledge and skills of production through experience and traditional farming techniques, so labor productivity is not high along with limitations, difficulties in capital and qualifications of farmers, who have not followed the new, advanced and modern methods. If these solutions are implemented, the process of digital transformation in agriculture will be as effective as expected, successfully contributing to a shift in economic structure in general and in agriculture in particular towards a modern and advanced direction./.

## Acknowledgments

This research is supported by University of Transport Technology, Hanoi and by Tan Trao University in Tuyen Quang, Vietnam

## References

Anh, L. (2021). Economy. Retrieved from Online newspaper of the Communist Party of Vietnam: https://dangcongsan.vn/tinh-te/co-hoi-cho-nong-dan-but-pha-voi-chuyen-doi-so-trong-nong-nghiep - 598689.html

Binh, D. (2022). Business. Retrieved from Vietnamplus: https://www.vietnamplus.vn/chuyen-doi-so-nong-dan-viet-nam-tren-hanh-trinh-cong-nghe-so/771287.vnp

Dang, H. (2022). Digital transformation . Retrieved from Online newspaper Thanh Hoa: https://baothanhhoa.vn/chuyen-doi-so/chuyen-doi-so-trong-san-xuat-king-doanh-nong-san/163612.htm

Dinh, T., & Dung. (2021). News. Retrieved from Vietnam Agriculture: https://vietnamagriculture.nongnghiep.vn/solve-the-5-agricultural-problems-by-digital-transformation-d294386.html

Dordley, L. (2022). Innovation. Retrieved from FoodForAfrika: https://foodforafrika.com/2022/06/20/kenyan-farmers-embrace-new-weather-insurance-product/

England, T. (2021). Economy. Retrieved from Nhan Dan online newspaper: https://nhandan.vn/day-nhanh-chuyen-doi-so-nganh-nong-nghiep-post680033.html

England, V. (2020). Farmer initiatives. Retrieved from Vietnam Farmer's Union: http://vietnamfarmerunion.vn/sitepages/news/1087/91436/lam-dong-effective-high-tech-agricultural-production-model

Fitzpatrick, I. C., Millner, N., & Ginn, F. (2022). Governing the soil: natural farming and bionationalism in India. Agriculture and Human Values , 1-16.

Hieu, P. (2022). Agri projects. Retrieved from Vietnam Agriculture Newspaper: https://vietnamagriculture.nongnghiep.vn/giving-identities-to-vietnamese-agro-products-d330386.html

Lam, H. (2020). Politics. Retrieved from Online newspaper Bien Phong: https://www.bienphong.com.vn/hut-fdi-vao-nong-nghiep-post435819.html#:~:text=C%E1%BB%A5%20th%E1%BB%83%2C%20n%C4%83m%202020%2C%20v%E1%BB%91n,USD%20%C4%91%E1%BB%95%20v%C3%A0o%20n%C3%B4ng% 20ghi%E1%BB%87p.

Lammers, T., Tomidei, L., & Regattieri, A. (2018). What causes companies to transform digitally? An overview of drivers for Australian key industries. USA: 2018 Portland International Conference on Management of Engineering and Technology.

Meinke, H. (2019). The role of modeling and systems thinking in contemporary agriculture- Sustainable Food Supply Chains. Italy: Academic Press.

Phong, T. (2022). Agriculture in the country. Retrieved from Vietnam Academy of Agricultural Sciences : https://vaas.vn/en/nong-nghiep-trong-nuoc/nganh-nong-nghiep-thieu-nguon-nhan-luc-chat-luong

Phu, G. (2022). Investment. Retrieved from Vietnamese Agriculture Newspaper: https://vietnamagriculture.nongnghiep.vn/planting-area-code-is-an-international-passport-for-vietnamese-agricultural-products-d329160.html#:~:text=According%20to%20the%20Department%20of,of%20%E2%80%8B%E2%80%8B300%2C000%20hectares.

Son, N. V. (2022). digital transformation in agricultural production consumption linkage. State management Review, 84-87.

Thiet, T. T. (2021). Research - Exchange. Retrieved from Financail and monetary market Review: https://thitruongtaichinhtiente.vn/hoan-thien-chinh-sach-ve-von-cho-chuyen-doi-nong-nghiep-so-phat-trien-nganh-nong-nghiep- ben-vung-36911.html

Vien, T. D. (2020). Scientific research. Retrieved from VietNam national University of Agricuture: https://www.vnua.edu.vn/tin-tuc-su-kien/nghien-cuu-khoa-hoc/nong-nghiep-viet-nam-nhung-van-de-ton-tai-51105

Vietnam News. (2019). Society. Retrieved from Vietnam News: https://vietnamnews.vn/society/537428/land-policies-restrain-viet-nam-agricultural-development.html

Vietnam News. (2021). Economy. Retrieved from Vietnam News: https://vietnamnews.vn/economy/1081786/digital-transformation-to-create-new-growth-engine-for-viet-nam-s-agriculture.html

## *ORAL PRESENTATION*

### Biyokütle artıklarından derin ötektik çözücüler ile pektin elde edilmesi ve karakterizasyonu

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**Özet**

Gıda, kozmetik, ilaç endüstrileri gibi birçok endüstriyel alanda pektin yaygın bir şekilde kullanılmaktadır. Bir biyopolimer olan pektin asidik heteropolisakkaritlerden oluşmaktadır ve meyveler pektinin ana kaynağıdır. Pektinlerin %90' ından fazlası meyve yan ürünlerinden elde edilmektedir. Günümüzde ticari amaçla kullanılan pektinler turunçgil meyvelerinin (limon, portakal, greyfurt, mandalina) kabuğundan, elma posasından, şeker pancarı posasından üretilmektedir. Pektin geleneksel olarak asidik, alkali ya da enzimatik olarak elde edilmektedir. Günümüzde, geleneksel kimyasal yöntemlerin yerine çevre üzerinde daha az olumsuz etki üreten alternatif kimyasal yöntemler ve yeşil çözücülerin kullanımı araştırılmaktadır. Bu amaçla pektin eldesinde derin ötektik çözücülerin (DES) kullanılabileceğine dair çalışmalar yapılmaktadır. DES'ler son yıllarda klasik çözücülere kıyasla çevreci ve ekonomik olması sebebi ile tercih edilmektedirler. Bu amaçla bu çalışmada pektin kaynağı olarak karpuz kabuklarının kullanılması ve pektin ekstraksiyonunun DES varlığında gerçekleştirilmesi amaçlanmıştır. Ekstraksiyon ortamı DES varlığında, 90$^{o}$C sıcaklıkta, 450 rpm karıştırma hızında ve 45 dakika süreyle hazırlanmıştır. Etilen Glikol:Sitrik Asit (EG:SA) ile hazırlanan DES bileşiminin farklı molar oranlarının (1:1, 1:2, 2:1) ekstraksiyon verimine etkisi incelenmiştir. Yapılan ekstraksiyon sonucu pektin verimi sırasıyla %25, %13,2 ve %32,6 olarak hesaplanmıştır. Elde edilen pektinlerin esterleşme derecesi belirlenmiştir. Esterleşme dereceleri titrimetrik yöntem kullanılarak hesaplanmış ve sırasıyla %52, %48 ve %56 olarak bulunmuştur. Bu sonuçlar karpuz kabuğu pektininin yüksek metoksilli pektin (HMP) özelliği gösterdiğini kanıtlar nitelikte olup literatür ile uyum göstermektedir.

**Anahtar Kelimeler:** Pektin, Derin Ötektik Çözücüler (DES), Ekstraksiyon, Karpuz Kabuğu

### Extraction and characterization of pectin from biomass residues by deep eutectic solvents

**Abstract**

Pectin is widely used in many industrial areas such as food, cosmetics and pharmaceutical industries. Pectin is a biopolymer which consists of acidic heteropolysaccharides and fruits are the main source of pectin. More than 90% of pectins are derived from fruit by-products. Pectins, which are used for commercial purposes today, are produced from the peel of citrus fruits (lemon, orange, grapefruit, tangerine), apple pulp, sugar beet pulp. Pectin is traditionally obtained acidic, alkaline or enzymatic. Today, alternative chemical methods that produce less negative effects on the environment and the use of green solvents are being investigated instead of traditional chemical methods. For this purpose, studies are carried out on the use of deep eutectic solvents (DES) in the production of pectin. DESs have been preferred in recent years due to being environmentally friendly and economical compared to conventional solvents. For this purpose, in this study, it was aimed to use watermelon peels as a source of pectin and to perform pectin extraction in the presence of DES. The extraction medium was prepared in the presence of DES, at 90$^{o}$C, at a stirring speed of 450 rpm for 45 minutes. The effect of different molar ratios (1:1, 1:2, 2:1) of DES compound prepared with Ethylene Glycol:Citric Acid (EG:CA) on extraction efficiency was investigated. As a result of the extraction, the pectin yield was calculated as 25%, 13.2% and 32.6%, respectively. The degree of esterification of the obtained pectins was determined. Esterification degrees were calculated using the titrimetric method and were found to be 52%, 48% and 56%, respectively. These results prove that watermelon peel pectin has high methoxyl pectin (HMP) properties and are in agreement with the literature.

**Keywords:** Pectin, Deep Eutectic Solvents (DES), Extraction, Watermelon Peel

## GİRİŞ

Meyve ve sebze atıklarında yüksek oranlarda bulunan pektinler, gıda sanayinde E-440 kodu ile tanınan bir gıda katkı maddesidir. Gıda katkı maddesi olarak endüstride kıvam verici, jelleştirici, stabilize edici ve emülsifiye edici şeklinde kullanılmaktadır (May, 1990). Bir biyopolimer olan pektin asidik heteropolisakkaritlerden oluşmaktadır. Meyveler pektinin ana kaynağıdır ve ticari pektinlerin %90' ından fazlası meyve yan ürünlerinden elde edilmektedir (Cui ve ark., 2019). Pektin kompleks yapılıdır ve içeriğindeki metil esterden dolayı uygun koşullar altında şeker ve asitle birlikte jelimsi bir madde oluşturur. Bu madde pektinin ana bileşeni D-Galakturonik asittir. Pektin geleneksel olarak asidik, alkali ya da enzimatik olarak elde edilmektedir. Yapılan çalışmalar pektin eldesinde derin ötektik çözücülerin (DES) kullanılabileceğini göstermektedir. Derin ötektik çözücüler iki veya daha fazla maddeden oluşan ve erime noktası kendisini meydana getiren maddelerin erime noktasından düşük olan karışımlar olarak da tanımlanmaktadır. DES'ler düşük buhar basıncına, ayarlanabilir fiziksel ve kimyasal özelliklere, düşük toksisiteye ve biyolojik olarak parçalanabilir özelliğe sahiptirler (Zhao ve ark., 2013). Pektinin en önemli özellikleri esterleşme derecesi, galakturonik asit içeriği, nötr şeker içeriği ve moleküler ağırlık dağılımıdır. Esterleşme derecesi (DE), esterlenmiş galakturonik asit gruplarının toplam galakturonik asit gruplarına oranıdır. Metanol ile esterleşme derecesine göre pektin, yüksek metoksil pektin (DE>%50) ve düşük metoksil pektin (DE<%50) olarak sınıflandırılır.

Günümüzde, ticari pektin en fazla turunçgil kabuğu (%85.5), ikinci olarak elma posası (%14) ve şeker pancarı posasından (%0.5) üretilmektedir (Chan ve ark., 2017). Son yıllarda portakal, limon ve elma gibi hammaddeler dışında pomelo kabuğu, karpuz kabuğu, nar kabuğu, muz kabuğu ve ayçiçeği tablası gibi diğer kaynaklarla da çalışmalar yapılmaktadır (Şen ve ark, 2020). Alternatif pektin kaynakları hakkında yapılan araştırmada karpuz kabuğu da potansiyel pektin kaynakları arasına girmiştir (Hartati ve ark., 2014). Literatürde karpuz kabuğundan pektin eldesine ilişkin az sayıda çalışmaya rastlanmıştır. Bu konuda yapılan çalışmaların artması ve karpuz kabuğu pektininin karakterize edilmesi, yeni uygulamalarda kullanılması için fayda sağlayacaktır. Literatürde yapılan bir diğer çalışmada karpuz kabuğundan geleneksel yöntem ile elde edilen pektinlerin esterleşme derecesi %60 bulunarak yüksek metoksilli pektin özellikleri gösterdiği saptanmıştır (Petkowicz ve ark., 2017). Daha önce yapılan çalışmalarda karpuz kabuğundan DES varlığında pektin ekstraksiyonu için bir çalışmaya rastlanmamıştır.

Bu çalışmada; bir gıda endüstrisi atığı olan karpuz kabuklarının DES varlığında pektin eldesinde değerlendirilmesi amaçlanmıştır. Elde edilen pektinlerin fiziksel ve kimyasal özellikleri belirlenecek ve ticari pektinin özellikleri ile karşılaştırılacaktır. Bu amaçla Etilen Glikol: Sitrik Asit (EG:SA) DES sistemi seçilmiş ve farklı mol oranlarında (1:1, 1:2, 2:1) hazırlanmışlardır. Bu şekilde hazırlanan DES ortamlarında ekstraksiyon işleminden sonra elde edilen ekstraktların ekstraksiyon verimi ve esterleşme dereceleri belirlenmiştir.

## MATERYAL VE METOT

Bu çalışmada pektin kaynağı olarak karpuz kabukları kullanılmıştır ve pektin ekstraksiyonu DES varlığında gerçekleştirilmiştir. Ekstraksiyon işleminde DES bileşimi olarak Etilen Glikol: Sitrik Asit (EG:SA) seçilmiş ve 1:1, 1:2, 2:1 olmak üzere 3 farklı molar oranda ekstraksiyon ortamı hazırlanmıştır. Tüm DES bileşimleri için çözelti asitlik derecesi ölçülmüş olup pH<1 değerindedir.

Kurutup öğütülen karpuz kabuklarının DES ile pektin ekstraksiyonu 45 dakika süre ile, 450 rpm karıştırma hızında, 90$^{o}$C sıcaklıkta manyetik karıştırıcıda gerçekleştirilmiştir. Ekstrat süzülerek ayrılmış ve pektin içeren süzüntü kısmı hacminin 2 katı kadar etanol ile çöktürülmeye bırakılmıştır. Çökmenin tamamlanması amacıyla 24 saat buzdolabında bekletilmiş ve vakumlu distilasyon ile süzme işlemi yapılmıştır. Elde edilen pektin 96 % etanol ile yıkanmış ve etüvde 40$^{o}$C sıcaklıkta sabit tartıma gelene dek kurutulmuştur. İzlenilen deneysel yöntemin aşamalarına dair görsel Şekil 1' de verilmiştir.
Ekstrakte edilen pektine karakterizasyon çalışması gerçekleştirmek üzere titrimetrik yöntem ile esterleşme derecesi tayini yapılmış ve pektin verimleri hesaplanmıştır.



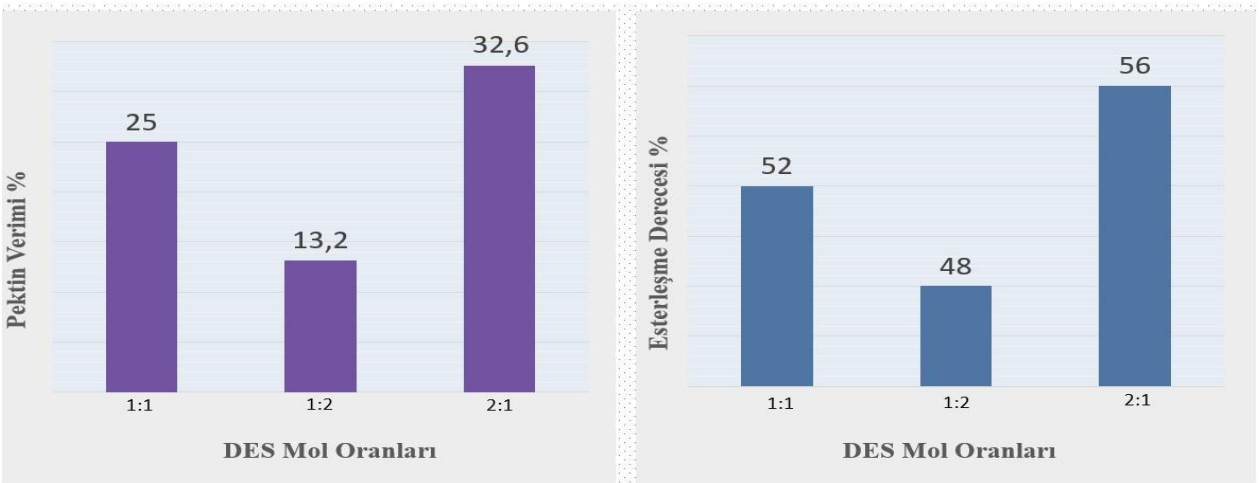

**Şekil 1.** Deneysel yöntemin aşamaları

Karpuz kabuklarından elde edilen pektin verimleri aşağıdaki denklem kullanılarak belirlenmiştir. Bu denklemde kuru pektin ağırlığı ekstraksiyon sonucu elde edilen toz pektini, numune miktarı ise başlangıçta ekstraksiyona tabi tutulan kuru karpuz kabuğu miktarını temsil etmektedir.

$$\% \text{ Verim} = \frac{Kuru\ pektin\ ağırlığı\ (g)}{Numune\ miktarı\ (g)} \times 100 \quad (1)$$

## BULGULAR ve TARTIŞMA

Etilen Glikol: Sitrik Asit DES varlığında ekstraksiyonu gerçekleştirilen karpuz kabuklarının pektin verimi 1:1, 1:2 ve 2:1 molar oranları için sırasıyla %25, %13,2 ve % 32,6 olarak hesaplanmıştır. Elde edilen pektinlere titrimetrik yöntemle esterleşme derecesi tayini yapılmış ve sırasıyla %52, %48 ve %56 olarak hesaplanmıştır. Farklı mol oranlarındaki DES sistemleri ile elde edilen ekstraksiyon verimi ve esterleşme derecesi ilişkisi Şekil 2' de verilmiştir. Literatürde konuyla ilgili çalışmalar incelendiğinde metil esterifikasyon derecelerinin, karpuz kabuğu pektinleri için %29.56-85.76 arasında değer gösterdiği görülmektedir (Mendez ve ark., 2021).




**Şekil 2.** Pektin verimi ve esterleşme derecesi tayinleri

## SONUÇ

Karpuz kabuklarından DES ekstraksiyonu yöntemiyle yüksek verimde ve ticari pektin özelliklerine yakın özelliklere sahip pektin elde etmek için farklı deney koşullarında pektin ekstraksiyonları yapılmıştır. Ekstrakte edilen pektinlerin özelliklerinin belirlenmesi için yapılan karakterizasyon çalışmalarında pektinlerin özelliklerinin ticari pektinin özelliklerine yakın olduğu bulunmuştur. Esterifikasyon dereceleri elde edilen pektinlerin yüksek metoksilli pektinler olduğunu göstermektedir. Optimum koşulda elde edilen pektin DES ekstraksiyonunda (90ºC, 45 dakika, 450 rpm, pH<1); karpuz kabuğu için %32,6 verimde ve %56 esterleşme derecesine sahip olarak bulunmuştur. DES ile ekstraksiyon sonuçları; etilen glikol/sitrik asit DES sistemlerinin pektin ekstraksiyonunda kullanılabileceğini göstermiştir.

## KAYNAKLAR

Chan, S.Y., Choo W.S., "Pectin as a rheology modifier: Origin, structure, commercial production and rheology". *Carbohydrate Polymers*, 161, 118-139, 2017.

Cui, J., Lian, Y., Zhao, C., Du, H., Han, Y., Gao, W., et al. (2019). Dietary fibers from fruits and vegetables and their health benefits via modulation of gut microbiota. Comprehensive Reviews in Food Science and Food Safety, 18(5), 1514–1532

Hartati, I., Riwayati, I. and Subekti, E., 2014, Microwave Assisted Extraction of Watermelon Rind Pectin With Different Kind of Acid Solution, ICETIA, 27-30pp.

May, C.D., 1990, Industrial pectins: Sources, production and applications, Carbohydr Polym, 12: 79–99 p.

Mendez, D.A., Fabra, M.J., Martínez-Abad, A., Martínez-Sanz, M., Gorria, M. and López-Rubio, A., 2021, Understanding The Different Emulsification Mechanisms of Pectin: Comparison Between Watermelon Rind and Two Commercial Pectin Sources, Food Hydrocolloids, 106957.

Petkowicz, C.L.O., Vriesmann, L.C. and Williams, P.A., 2017, Pectins from food waste: extraction, characterization and properties of watermelon rind pectin, Food Hydrocoll, 65: 57–67 p.

Şen, E., 2020, Meyve kabuğu atıklarından pektin ekstraksiyonu ve karakterizasyonu, Kimya Mühendisliği Bölümü, Mühendislik Fakültesi, Pamukkale Üniversitesi, Denizli, Türkiye.

Zhao, H. and Baker, G.A., (2013). Ionic liquids and deep eutectic solvents for biodiesel synthesis: a review. *Journal of Chemical Technology and Biotechnology*, 88(1): 3-12.

## *ORAL PRESENTATION*

# Sürdürülebilir Su Kaynakları Yönetiminde Hibrit Modeller: Hidrolojik Çalışmalarda Tahminleme İçin Yapay Sinir Ağ Modellerinin Rolü

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**Özet**

Değişmekte olan Dünyada, nüfus artışına paralel olarak artan temiz su ihtiyacı ve insan kaynaklı küresel iklim değişikliği gibi çevresel faktörler sonucunda içilebilir su kaynakları tehlike altına girmektedir. Sürdürülebilir su kaynaklarının yönetimi içinde bulunduğumuz çağda sosyo-ekonomik büyüme, ekosistem sürdürülebilirliği ve insan sağlığı açısından hayati önem teşkil eder. Bu amaçla bilim adamları tarafından farklı yöntemler kullanılmaktadır ve bu yöntemlerden günümüzde en popüler olanı Yapay Sinir Ağı (YSA) modelleridir. Yapay sinir ağları su kaynaklarının değişkenliklerini tahmin eden ve önceden haber veren son derecede faydalı araçlardır. YSA modeli, tarihsel verilerdeki gizli ilişkileri ortaya çıkarabilir, böylece su kaynakları bileşenlerinin (kalite, miktar, talep vb.) tahmin ve öngörüsünü kolaylaştırır.

Bu çalışmada, sürdürülebilir su kaynaklarının kalite, talep ve akış tahmini için kullanılan yapay sinir ağlarının çalışma şekilleri ve yapılan farklı hidrolojik tahmin çalışmaları incelenmiştir. Sonuç olarak geliştirilen hibrid modellerin diğer geleneksel modellere göre üstünlüğü yapılan çalışmalarda kanıtlanmıştır. Ancak, mevcut modellerin değişen küresel şartlara uyarlanması gerektiği de dikkat çekmiştir.

**Anahtar Kelimeler:** Yapay Sinir Ağları, Sürdürülebilirlik, Su Kalitesi, Su Kaynakları Yönetimi

# Hybrid Models in Sustainable Water Resources Management: The Role of Artificial Neural Network Models for Forecasting in Hydrological Studies

**Abstract**

In the changing world, drinking water resources are endangered as a result of environmental factors such as the increasing need for clean water in parallel with population growth and global climate change. The management of sustainable water resources is of vital importance in terms of socioeconomic growth, ecosystem sustainability, and human health. For this purpose, different methods are used by scientists, and today, the most popular of these methods are Artificial Neural Network (ANN) models. The ANN model can reveal hidden relationships in historical data, thus facilitating the prediction and forecasting of water resource components (quality, amount, demand etc.).

In this study, the working methods of ANNs used for quality, demand, and flow prediction of sustainable water resources and different hydrological forecasting studies were examined. As a result; It has been reported in studies that the developed hybrid models are more efficient than other conventional models. However, it has been also noted that existing models need to be adapted to changing global conditions.

**Key Words**: Artifial Neural Networks, Sustainable, Water Quality, Water Resources Management

## GİRİŞ

Su, hayat için gerekli olan en önemli doğal kaynak olup canlıların yaşamlarının devamı için kilit taşıdır. Canlı organizmalar hayatlarının sürekliliği için gerekli ölçüde kaliteli suya ihtiyaç duymaktadırlar. Türlerinin tolere edebilecek oldukları belli bir su kirlilik değeri bulunmaktadır. Bu değerlere ulaşılması sonucunda, canlıların hayatları olumsuz etkilenmekte ve ileri aşamalarda yaşam şanslarını ortadan kaldırmaktadır **(Aldhyani ve ark., 2020).** Dünyamızın ¾'ü suyla örtülü olmasına karşın, bunun yalnızca %1 kadarı tatlı sudur **(Al Aani ve ark., 2019**). 2050 yılına gelindiğinde her dört insandan en az birinin kronik veya tekrarlayan içme suyu sıkıntısı bulunan bir ülkede yaşama olasılığı yüksektir. Dünya genelinde temel temizlik ihtiyacını karşılamadan yaşamakta olan 2,3 milyar insan bulunmaktadır. Mevcut duruma baktığımızda oluşan bütün doğal afetlerin %90'ı su ile ilgili olup tatlı suya ihtiyaç devamlı olarak artış göstermektedir. Bu artışın nedenleri; artan nüfus (yaklaşık 7 milyar olup, 2050'de ise 9 milyar olacağı düşünülüyor), yaşam standarttı artmış olan ülkeler ve tüketimin artmasıdır (**Czajkowski ve ark., 2021).**

Su tüketiminin devam ettiği süreçte yapılmış olan doğru politikalar etkisiz kalması sonucunda, 2030 yılı için ihtiyaç duyulacak olan suyun yalnızca %60'ı dünyaya verilmiş olacaktır (**Connor, 2015).** 2030 Sürdürülebilir Kalkınma Hedefleri (SKH'ler) olarak; Temiz su kalitesi, niceliksel mevcudiyet ve güvenli ve uygun fiyatlı içme suyunun sürdürülebilir yönetiminin sağlanması bu sürecin uygulanmasında çok önemli faktörlerdir. **(Manzar ve ark., 2022).** Birleşmiş Milletler'in yayınlamış olduğu Dünya Su Geliştirme Raporu'nun 2018 baskısında, dünya da 2050 yılına gelindiğinde su talebinin artacağı ve yaklaşık 6 milyar insanın temiz su kıtlığı sorunu yaşayacağı belirtildi. Su talebindeki artışın kaynağı, su kirliliğinin artış göstermesi ve su kaynaklarının yok olmasının bir sonucudur. Su kıtlığının yanı sıra nüfus ve ekonomide düzensiz büyüme ve erişilebilirlik faktörlerinin sonuçları da dikkate alınmadığında temiz su sorununun 2050 yılına kadar katlanarak daha vahim bir hal alacağı düşünülmektedir **(Boretti ve Rosa, 2019).** Sürdürülebilir su kaynakları yönetiminde amaç mevcut toplum ve gelecekle ilgili varsayımların ekolojik, ekonomik ve hidrolojik içeriğinin devamını korumak için planlanır ve uygulanılır (Ali ve ark., 2019). Su kaynakları, sürdürülebilir kalkınmanın başlıca öğesidir. Son yıllarda, insanlar su kaynaklarının ve hizmetlerinin, sosyoekonomik kalkınma ve eko-çevresel korumaya duyulan ihtiyacı daha önemli olduğunu anlamaya başladılar (**Bakker, 2012**; **Gleeson ve ark., 2012; UNEP, 2012**). Kavram olarak sürdürülebilirlik, varmak istenen bir nokta değil, daha yakın bir bağlantı oluşturmak ve insan ile doğal ihtiyaçların yanı sıra her ikisi arasındaki etkileşimleri daha iyi algılamak düşüncesiyle sürekli bir süreçtir **(Wallace ve ark., 2003).** Su kaynaklarının sürdürülebilirliği suyun kalite kavramıyla da eşdeğer görülmelidir. Bir su kaynağının kalitesi; su kirliliğinin derecesini ortaya çıkarmak için kullanılmaktadır **(Abdel-Satar ve ark., 2017).** Bir diğer ifadeyle, suyun kalitesi, suyun belirli bir amaç doğrultusunda kullanımıyla ilgili olarak uygun olduğunu yansıtan bir ölçektir ve kullanıcıların ihtiyaçlarına ne kadar faydalı olacağını gösterir (**Ayers ve Westcot, 1989**). Dolayısıyla, su kaynaklarının sürdürülebilir kullanımında su kalitesinin bilinmesi, ileriye yönelik planlamalarda değişmez bir unsur olarak görülmektedir.

Sürdürülebilir su kaynaklarının geliştirilmesinin sağlanmasında mükemmel su kalitesi (WQ), mecburi bir elemandır. Birleşmiş Milletler'in Sürdürülebilir Kalkınma Hedeflerinin 3. (sağlık ve esenlik), 6. (temiz su ve sanitasyon) ve 14. (su altındaki yaşam) öğeleriyle yüksek oranda birliktelik vardır. Bu nedenle, politika yapıcılar her zaman WQ'yu verimli ve etkin bir şekilde yönetmek için stratejiler aramışlardır (**Wai ve ark., 2022).** Su kalitesinin artan bozulması, dünya çapında tarım, sanayi ve haneler için endişe verici bir durum haline gelmiştir. Bu nedenle, su kirliliğinin neden olduğu WQ azalmasını önemli ölçüde etkileyen iklimsel, hidrolojik ve jeolojik faktörler hem su kalitesini hem de miktarının belirlenmesi için büyük önem taşımaktadır **(Abba ve ark., 2022).** Düzenleyici standartlara ve WQ parametrelerine dayalı olarak dünya çapında çok sayıda WQ indeksi formüle edilmiştir **(Abba ve ark., 2019).** Özellikle, Kanada Çevre Bakanlar Konseyi WQI (CCMEWQI) (**Sharma, 2002; Khan ve ark., 2004**), Oregon WQI (OWQI) (**Boyacioglu, (2007**), ABD Ulusal Sanitasyon Vakfı WQI (NSFWQI) **(Horton, 1965; Katyal, 2011**) ve Ağırlıklı Aritmetik Su Kalitesi İndeksi (WAWQI) (**Zotou ve ark., 2020)** hesaplama yöntemleri kullanılmaktadır. Yapay sinir ağ modelleri su ile ilgili birçok hidrolojik alanda (su kalitesi (WQ) su talep tahmini, su-yağış-akış su talep tahmini ve su talep tahmini gibi) uygulanarak başarılı tahmin sonuçları çalışmalar ile sergilenmiştir. Yapay sinir ağları su kaynaklarının değişkenliklerini tahmin eden ve önceden haber veren son derecede faydalı araçlardır. Bu yüzden sinir ağ modellerinin geliştirilmesi ve uygulanması su kaynaklarının hakkında bilgi sağlanması sürdürülebilirlik açısından çok büyük önem taşımaktadır.

**Su kalitesi (WQ) Tahmini**

Su kalitesi endeksi, çeşitli su kalitesi parametreleri baz alınarak elde edilen genel su kalitesi hakkında fikir veren eden tek bir sayıdır. WQI'nin amacı, karmaşık bilimsel parametreleri anlamaktan farklı olarak, meslekten olmayan bir kişinin su kalitesinin durumunu hesaplayarak fikir sahibi olmasına yardımcı olmaktır. Halka ve araştırmacılara bölgenin potansiyel su sorunları hakkında kabaca bir fikir sağlamaktadır (**Prathibha ve ark., 2022).**

Su kalitesi indeksi (WQI), su kaynakları yönetimi ve planlaması için önemli bir araçtır. Bununla birlikte, önemli dezavantajları vardır: kimyasal atık üretimi, maliyetli ve zaman alıcıdır (Alves ve ark., 2018). WQ istatistikleri, büyük miktarda veriyi yönetmekte zorluk yaşamaktadır. Birçok çalışmada nehir kirliliğini WQ ile tahmin etmeye çalışmıştır. Ancak, WQ numune toplama, test etme ve veri işleme, kimyasal, ekipman ve insan gücü açısından zaman alıcı ve maliyetlidir. Geleneksel yaklaşımlar, suyla ilgili projelerde zorluklara yol açmakta ve ekonomik olarak yük oluşturmaktadır. Bu durum araştırma gruplarında programlarının karar vermesini ve verimliliğini etkilemektedir (**Chapman, 1992; Ongley, 2000; Tung ve Yaseen, 2020).**

WQI, su kalitesinin genel durumunu kapsamlı bir şekilde ortaya çıkaran, evrensel bir gösterge olarak kabul görmüştür. Fakat yaygın geleneksel su kalitesi endeksleri (WQI'ler), 'gölgede kalma' ve 'belirsizlik' gibi bazı olumsuz yanları mevcuttur. Yapay sinir ağları (YSA) gibi yapay zeka teknikleri, geleneksel WQI'lerin bu eksik yanlarını gidermek için büyük önem kazanmıştır (**Nayak ve ark., 2021).** Dünyada bazı ülkelerdeki laboratuvarlardaki cihaz ve kimyasal sarf malzemelerinin bulunmaması nedeniyle su kimyasal analizinin gerekli ölçülerde yapılamamaktadır. Bunun sonucu olarak ortaya çıkan düşük doğrulukta WQ'ya sebep olmaktadır. Bu nedenle, bu belirsizliği ortadan kaldıracak net ve hızlı WQ değerlendirmesi için kullanılabilecek olan uygun maliyetli ve gelecek vaat eden araçları keşfetmek gerekli hale gelmiştir. Yapay zeka (AI) teknikleri kullanılarak oluşturulan modelleme teknikleri, WQI başarılı bir şekilde tahmin etmek için modelleri oluşturmak ve mevcut deneysel parametre verilerine dayanarak WQI'yi tahmininde kullanılabilecek seçenekler sağlamaktadır. Bu yaklaşımın en önemli avantajı, analiz için pahalı analitik sarf malzemelerin veya reaktörlerin ihtiyaç duyulmayış olmasıdır (**Manzar ve ark., 2022).** Su kalitesi modellemesi, kirlilik kaynakları ile belirli bir su kütlesinin akarsu içi su kalitesi süreci arasındaki bağlantıdır. Özetle, bir modelin bir su kütlesinde meydana gelen fiziksel, kimyasal ve biyolojik su kalitesi süreçleri ve mekanizmalarının bir temsilinden daha fazlası olarak algılayamayız (**Othman ve ark., 2020).** Bu nedenle, su kalitesinin değerlendirilmesi için iş yükünü azaltmak ve altta yatan veri sorununun üstesinden gelmek için gelişmiş ve güvenilir değerlendirme tekniklerine ihtiyaç vardır **(Bozorg-Haddad ve ark., 2017; Aryafar ve ark., 2019; Shah ve ark., 2021).**

Temiz su, insanların ve diğer canlıların bağlı olduğu vazgeçilmez bir temel kaynaktır. Bu nedenle, gelecekteki su kalitesi koşullarını tahmin etmek için bir su kalitesi tahmin modelinin oluşturulması önemli bir sosyal ve ekonomik değere sahiptir **(Wu ve Wang, 2022).** Küresel bir sorun haline gelen erişilebilir temiz suyun çözümü için, içilebilir su kalitesinin iyileştirilmesi yoluyla insanların temiz suya ulaşmasının sağlanması gereklidir. Bu sorun dünya genelinde nüfusun sürekli artması ve yüksek kirliliğe sahip içme suyu ile daha da hayati bir konu olmuştur **(Ghazinoory, 2005; Dawood ve ark., 2021).**

Yapay sinir ağları (YSA) çok yönlüdür ve birçok su parametresinin tahmininde kullanılmıştır. Bunlar; su tuzluluğunu (**Maier ve Dandy, 1996),** toplam çözünmüş katıları, elektriksel iletkenlik, bulanıklık (**Najah ve ark., 2009),** kimyasal oksijen ihtiyacını, biyokimyasal oksijen ihtiyacını (**Rene ve Saidutta, 2008)** tahmin etmek için uygulanmıştır. Ayrıca ötrofikasyon (**Kuo ve ark., 2007),** yağış **(Wu ve ark., 2010**) ve asılı tortu yükü tahmini (**Chen ve Chau 2016; Olyaie ve ark., 2015**), klorofil-a seviyesi modellemesi (**Hacisolomou ve ark., 2021**), su kalitesi parametrelerinin tahmini **(Alizadeh ve diğerleri, 2018)** ve potansiyel zararlı alg aşırı çoğalması olayı (**Deng ve ark., 2021)** gibi diğer değişkenlerin tahmini içinde kullanılmıştır.

**Su-Yağış-Akış Tahmini**

Su, yalnızca doğal yaşam için gerekli bir kaynak değil, aynı zamanda sürdürülebilir kalkınmanın özü olan doğal bir kaynaktır. Su, sağlıklı ekosistemlerin, sosyal-ekonomik gelişmenin ve insanın hayatta kalmasının kökenidir. Uygun su yönetim, mevcut su kaynaklarının yetersiz oluşu sebebiyle su talebi ve kullanılabilirliği arasındaki boşluğu tamamlamak için en etkili önlemdir (**Verma, 2022).** Aynı zamanda son yıllarda dahada etkisini hissettirmeye başlayan iklim değişikliği insanları, ekosistemleri ve ekonomileri etkileyen tayfun, sel ve tsunami gibi beklenmedik olayların sıklıkla ortaya çıkmasıyla etkilemeye başlamıştır. Sonuç olarak, bu

durum gelecekteki iklimi tahmin etmenin önemini canlılar için daha da artmıştır (**Sulaiman ve ark., 2022).** Su kaynakları yönetimi dikkate alındığında, erişilebilir su kaynaklarının doğru tahmini ve temel faktörler arasındaki etkileşimler konusunda bilginin önemi büyüktür. Bu konu ile ilgili olarak, gelecekte erişilebilir su kaynaklarının tahmininde çok önemli bir rol oynayan akış verisi popüleritesi artan bir konu olmuştur. Mevcut su kaynaklarının miktarı ve kalitesi ile bunların yönetimi için hidrolik yapıların tasarım kapasitesi ve sel gibi doğal afetler ve ilgili çevre sorunları ile ilgili akışları tahmin etmek temel bir durum olmuştur (**Mohammadi ve ark., 2022).**

Yağış-akış ilişkisi en doğal komplekslerden biridir. Böyle bir ilişkinin bilincine varmak coğrafi olarak değişkendir ve çok zordur. İklim değişikliğine bağlı belirsizlikler altında yüzey akışının simülasyonu, özellikle taşkın yoğunluğunun tahmininde ve su kaynakları idaresi konusunda çok kritiktir (**Kalhori ve ark., 2022).** İklim değişikliği etkisinin hidrolojik tahmin uygulamalarına dahil edilmesi gerekli olan bir konudur. Bölgesel hidrolojiyi etkileyen farklı faktörlerin dahil edilmesi, hidrolojik tahminleri daha zor hale getirmekte, bu nedenle veri madenciliği yöntemleri gibi daha karmaşık modellerin kullanılması bir mecburiyet doğurmaktadır **(Yoosefdoost ve ark., 2022).**

**Su Talep Tahmini**

Su talebi tahmini, su kaynaklarının optimal tahsisinde temel aşamadır ve artan talep ile sınırlı su kaynakları arzı arasındaki çatışmayı hafifletmek için karar vericilerin uygun yönetim şemaları tasarlamalarına yardımcı olabilecek gelecekteki talebin simüle edilmiş görünümünü sağlamayı amaçlamaktadır. Bu amaçla, birçok araştırmacı su talebini modellemek ve tahmin etmek için farklı yöntemler önermiştir (Li ve ark., 2022). Su talebi tahmini ile ilgili mevcut literatür, çoğunlukla su talebinin doğru nokta tahminlerini vermeye odaklanmaktadır. Ancak, verilerin belirsizlik düzeyi arttığında su talep noktası tahmini, bilgi vermeyen ve güvenilmez sorunlarla karşılaşacaktır (Du ve ark., 2022). Maalesef şu an çoğu pratik problemde elde edilen dağılım fonksiyonu, özellikle acil durumlarda ve geçmiş verilerinin eksikliği söz konusu olduğunda, gerçek frekansa yeterince yakın değildir. Ayrıca su talebi verileri, uzmanlar tarafından verilmesi gereken ölçümlerdeki yanlışlıkların neden olduğu belirsiz özelliklere de sahiptir. Bu nedenle, belirli su talebi verilerindeki belirsizliklerle başa çıkmada yeni bir matematiksel araç uygulamak için çalışmalar yürütülmüştür **(Li ve ark., 2022).**

İklim değişkenleri kentsel su talebini etkilediğinden, iklim değişikliğinin de özellikle kurak ve yarı kurak bölgelerde sıcaklıklar arttıkça gelecekteki kentsel su talebinde önemli dalgalanmalara neden olması beklenmektedir. (**Waha ve ark., 2017; Perea ve ark., 2019).** Dahası, iklim değişikliği dünyadaki birçok şehirde kritik sorunlar çıkarmıştır. Bu sorunlar arasında kötüleşen altyapı, sürdürülebilirlik ve azalan sosyal sağlık sıkıntıları gibi durumlar yer almaktadır **(Keath and Brown, 2009**; **Stone ve ark., 2010**; **Harlan ve Ruddell, 2011**; **Leichenko, 2011; Salimi ve Al-Ghamdi ,2020**). İklim değişikliği aynı zamanda kentsel su kalitesi ve miktar yönetimini de önemli ölçüde etkilemektedir (**Stakhiv, 1998**; **Narsimlu ve ark., 2013**; **Özerol ve ark., 2020**; **Sharafati ve ark., 2021).**

Kentsel yaşam alanlarında su talebini tahmin etmek, yerel yetkililer ve su şebekesi planlamacıları için su altyapısına yatırım tahsis etmede de etkili bir yol göstericidir. Gelecekteki iklim değişikliği, kentleşme, nüfus artışı, sosyoekonomik kalkınma, su fiyatlandırması ve su yönetimi programlarına ilişkin tahminler, gelecekteki su talebini tahmin etmede temel veriler olarak kabul edilir. Bununla birlikte, kentsel dokuda sürekli bir değişim ve arazi kullanımında dinamik geçişler içermektedir. Ayrıca arazi kullanımı/arazi örtüsü evrimi ve kentsel yığılmaların ve nüfus dağılımının değişmesi su talebini de etkileyen faktörlerdir **(Al-Shaar ve ark., 2022).**

Artan nüfus ve beraberinde getirmiş olduğu yiyecek kıtlığı ve su kaynaklarına olan yüksek talep göz önüne alındığında, su kaynakları yönetiminin sürdürülebilirliği ve bu kaynakların rasyonel kullanımını sağlamak için acil bir önlem politikasının oluşturulması acil bir konudur **(El Mezouari ve ark., 2022).** Güvenilir su mevcudiyetinin sağlanmasına yardımcı olmak ve yüksek su kullanımını en aza indirmek için, güvenilir kentsel su talebi tahmin modelleri geliştirilmelidir. Bu tür tahmin modelleri, su hizmetlerinin operasyonel ve stratejik kararlar almasına yön verir ayrıca su güvenliğinin ve su tüketimin sürdürülebilirliğinin sağlanmasına da katkıda bulunur **(Beal ve Stewart, 2011).**

Son yıllarda yapılan birçok çalışma, çeşitli mühendislik alanlarında modelleme doğruluğunu artırmak ve ilerletmek için geleneksel yapay zekâ (AI) modellerini evrimsel algoritmalar (EA) ile birleştiren hibrit

tekniklerin uygulanmasını önermiştir. Genel olarak hibrit modeller hidrolojik tahmin problemlerinin çoğunda daha iyi sonuçlar çıkarmıştır (**Sun ve ark., 2014**; **Roshni ve ark., 2019; Abba ve ark., 2022).**

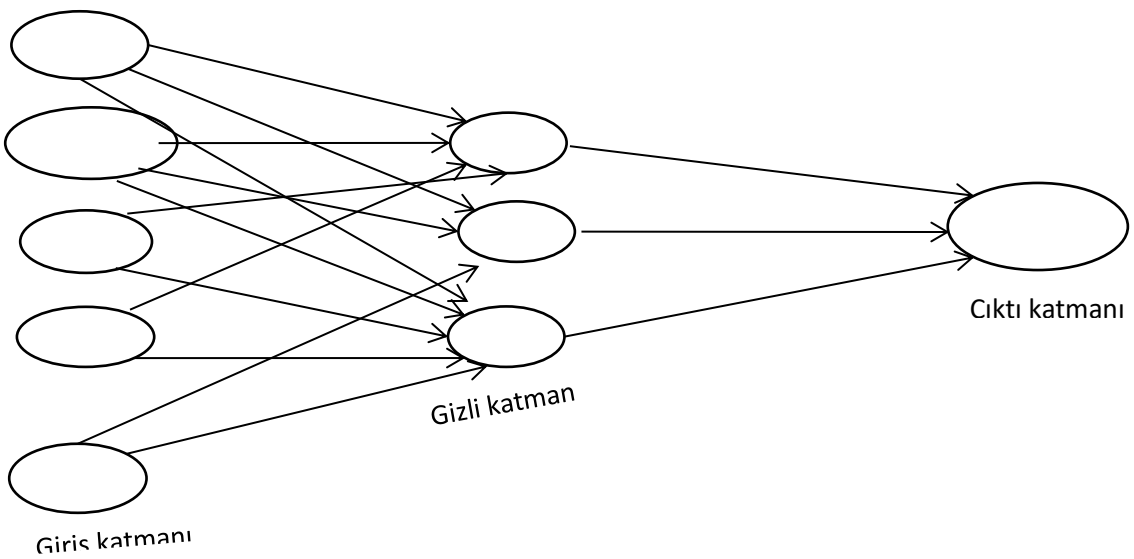
## YAPAY SİNİR AĞI (YSA) MODELLERİ

### YSA mimarisi

YSA modeli bir insan beyni sistemi gibi çalışır. İnsan beyni, gerçek yaşam durumlarının deneyimlerini farklı perspektiflerden alır ve beynin önceki deneyimlere dayanarak uygun bir karar almasını sağlar. Genel olarak deneyimleri çok olan bir kişinin daha iyi bir karar vereceğine inanılmaktadır. Bu, insan beyninin çok sayıda deneyime maruz kalması üzerine eğitilmesi şeklinde gelişen bir olayın sonucudur. Aynı şekilde çok sayıda veri seti sağlanarak YSA modelinin eğitimi gerçekleştirilerek deneyim kazanması sağlanır. Her veri seti için ayrıca hedef adı verilen beklenen sonuç da verilmiş olunur. Hedef değer, kendisine verilen girdilere dayalı olarak yaygın bir şekilde kullanılmakta olan bir yöntemle elde edilmiştir. İnsan beyninde biyolojik nöronlar birbiriyle ilişkili olup sürekli olarak birbirlerine sinyal iletirler. YSA modelinde yapay nöronlar mevcuttur bunlar birbirine iyi bağlanmış ve birbirlerine bilgi aktarma işini görmektedirler **(Nayak ve ark., 2021).**

YSA'ların üç veya daha fazla katmanı vardır: girdi katmanı, gizli katmanlar ve çıktı katmanları. Düğümlerin girdi katmanının tek rolü, girdi bilgilerini birincil gizli katmanın nöronlarına iletmektir **(Elzwayie ve ark., 2017**). Çıktı katmanının rolü, belirtilen girdi için bir çıktı üretmektir **(Liu ve Chen, 2012).** Aradaki gizli katmanlar (yalnızca tek bir katman olabilir) bir dizi özellik dedektörü olarak işlev görür. Uygun bir ağ çerçevesinin tanımlanması, sistemin modellenmesindeki en önemli ve karmaşık görevlerden biridir **(Sudheer ve ark., 2002; Karimi ve ark., 2013; Ghorbani ve ark., 2018).** Şekil.1 bu 3 katmanlı sistemin genel bir YSA modelinin şematik temsilini gösterir.

YSA'nın performansı büyük ölçüde hem öğrenme algoritmasına hem de başlangıç ağırlıklarına bağlıdır. Geri yayılım (BP) algoritması tarafından eğitilmiş çok katmanlı ileri beslemeli ağa sahip YSA, hızlı yanıt ve tatmin edici modelleme doğruluğu nedeniyle en olgun ve yaygın olarak kullanılan paradigmalardan biridir (**Deng ve ark., 2021**; **Ghritlahre ve Prasad, 2018; Wu ve ark., 2019)**. Gradyan iniş algoritmalı BP ağı, öngörülen ve verilen çıktılar arasındaki farkı hesaplayarak hata oranını en aza indirir ve daha sonra rastgele başlatılan ağırlıkları, önceden belirlenmiş bir doğruluk elde edilene kadar hata oranını azaltma yönünde rastgele başlatılan ağırlıkları yinelemeli olarak ayarlar (**Hadjisolomou ve ark., 2021).** Eğitim tamamlandıktan sonra ağırlık matrisi sabitlenir ve model eğitimsiz verileri test etmek için kullanılabilir **(Deng ve ark., 2021).** Özgün bir YSA, Şekil 4'te gösterildiği gibi verilebilir. Burada giriş katmanının girdileri aldığı, gizli katmanların bilgiyi işlediği ve çıktı katmanının sonucu oluşturduğu bir birbirine bağlı nöron sistemi ile temsil edilebilir **(Chia ve diğerleri, 2020).**




Şekil 1. YSA mimarisi.

## WQI Tahmini İçin Kullanılan Modeller

### Geleneksel modeller

Nehir suyu kalitesini modellemek için genel olarak en geleneksel ve sık kullanılan yöntemler regresyon tekniklerini baz almaktadır. Çoklu doğrusal regresyon (MLR), çoklu doğrusal olmayan regresyon (MNLR) ve oto-regresif entegre hareketli ortalama (ARIMA) modelleri bu amaçla yaygın olarak tercih edilmektedir. Bununla birlikte, verilerde doğrusallık ve durağanlık varsayarlar ve hidro-çevresel verilerde yer alan durağan olmayanları ve doğrusal olmayanları yakalama konusunda sınırlı bir yeteneğe sahiptirler (**Nourani ve Komasi, 2013; Rajaee ve ark., 2020).** MLR, bağımsız (açıklayıcı) değişkenler ile bağımlı (yanıt) değişkenler arasındaki doğrusal ilişkiyi modellemek için kullanılan istatistiksel tekniklerden biridir **(Meerasri ve Sothornvit, 2022).** Buna ek olarak, MLR, yanıtın değerini tahmin etmek için bir dizi farklı tahmin edici kullandığından, basit doğrusal regresyonun bir uzantısıdır (**Azrour ve ark.,2022).** Çoklu doğrusal olmayan regresyon (MNLR**)** modelinin temeli, girdi ve çıktı değişkenleri arasında doğrusal olmayan bir ilişki kurmaktır. MNLR modeli, MLR modelinden farklı olarak, bağımlı ve bağımsız değişkenler arasında doğrusal olmayan bir ilişki kurarak tahmin edebilir **(Adamowski ve ark., 2012).**

Otoregresif Entegre Hareketli Ortalama (ARIMA) modeli, araştırma uzmanları arasında çok popüler bir zaman serisi tahmin modelidir. 1970 yılında Box ve Jenkins tarafından önerilen bir zaman serisi veri tahmin yöntemidir (**Box ve ark., 2015)**. ARIMA modeli basit bir kurulum ve doğrusal ilişkileri yakalama konusundaki avantajı ile tercih edilmektedir. Geleneksel zaman serisi tahmin yöntemlerini kullanarak dalgacık ayrıştırmasından sonra trend terimlerini modelleyerek daha doğru tahmin sonuçları elde edilebilme yeteneğine sahiptir (**Zhou ve ark., 2022).** ARIMA modeli durağan olmayan verileri doğrudan tahmin edemez, bu nedenle önceden durağan verilere dönüştürülmesi gerekir. Bu modelin su kalitesini kesin olarak tahmin edememesinin bir başka sebebi de zaman serisi tahmini dışında başka faktörleri çok fazla hesaba katamamasıdır (**Mei ve ark., 2022).**

Tekrarlayan sinir ağları (RNN), geri yayılım algoritmasını benimsediğinde kaybolan bir gradyan sorununa sahiptir. Bu sorunu çözmek için Sepp Hochreite ve Jurgen Schmidhuber, RNN tabanlı yeni bir derin öğrenme sinir ağı olan uzun kısa süreli bellek (LSTM) modelini önermiştir **(Shu ve ark., 2021).** Hochreiter ve Schmidhuber (1997) tarafından önerilen LSTM ağı öğrenmeyi ek alan bilgisi olmadan model eğitimi ile birleştiren bir RNN çeşididir. Geliştirilmiş LSTM yapısı, tipik RNN'de gradyan kaybolması probleminden kurtulmak için yardımcı olur. Bu, LSTM'nin uzun vadeli bağımlılığı yakalamada ve doğrusal olmayan dinamikleri modellemede avantajlara sahip olduğu ve uzun dizi verileriyle başa çıkmak için kullanılabileceği anlamına gelmektedir (**Ye ve ark., 2019).**

Kapılı tekrarlayan birim (GRU), LSTM'ye benzer yapıdadır. Fakat GRU ek olarak bellek hücrelerinde kapı birimlerine sahiptir, ayrı bir bellek kapısına sahip değildir **(Cho ve ark., 2014).** GRU ağının yapısı LSTM' den daha basit yapıdadır. Bu yapı sayesinde ayarlamak için daha az parametre gerektirir ve eğitim hızı daha hızlıdır, LSTM ile benzer tahmin performansları gösterirler (**Yang ve ark., 2022).**

Özetle, bilim adamları su kalitesi tahmini alanında çok sayıda araştırma yöntemi uygulamıştır. Fakat geldiğimiz noktada geleneksel istatistiksel modellerin tahmin sonuçları, büyük dalgalanmalar ve uzun vadeli eğilimler içeren zaman serileri için tatmin edici olmadığı sonucu çıkmaktadır. Örnek olarak, regresyon analizi modeli nispeten basittir, ama istatistiksel veriler için gereksinimleri yüksektir ayrıca büyük bir örneklem ve iyi bir dağılım modeline sahip veriler gereklidir. Zaman serisi modeli nispeten sağlam bir teorik temele sahiptir lakin tahmin doğruluğu zayıftır. Gri tahmin modeli, küçük ve süreksiz geçmiş veriler için uygundur, ancak model, büyük bir tahmin hatasıyla sonuçlanan kararsız verilerin etkisine karşı hassastır. Destek vektör makinesi küçük örnek verileri için uygundur, fakat parametre ve çekirdek fonksiyonlarının seçimine daha duyarlıdır. Ek olarak, geri yayılım sinir ağı (BPNN) ve RBFNN gibi geleneksel tekli derin öğrenme modelleri, geçmiş bilgiler için bellek yeteneğine sahip değildirler. Ayrıca, eksik veri doldurma yöntemlerinin çoğu zaman, veri kümesindeki zaman serisi bilgilerini etkin bir şekilde işleyemez ve bu da eksik değerlerin tahmininde büyük hatalara neden olmaktadır (**Wu ve Wang, 2022).**

Son yıllarda, su kaynakları üzerinde yapılan çalışmalarda YSA daha fazla ilgi görmektedir. Bunun sebebi olarak özellikle değişkenler arasındaki ilişkinin açık biçiminin bilinmediği durumlarda, karmaşık doğrusal olmayan ilişkileri simüle etmek için kullanılan sağlam bir hesaplama algoritması oluşudur. Örneğin, son yıllarda bulanık mantık (FL), su kaynaklarında hidrolojik bileşenlerin analizinde ve karar vermede güçlü bir teknik olarak ortaya çıkmıştır. Hidroloji ile ilgili problemler genellikle bulanık mantık tabanlı modellerle çok iyi bir şekilde ele alınabilen hata payı ve belirsizlik ile ilgilidir (**Kambalimath ve Deka, 2020).** Bulanık mantık (FL) modelleri, bulanık kümeler teorisini kullanarak bir dizi girdi verisi ile bir dizi çıktı verisi arasındaki ilişkileri geliştirmek için bir strateji sağlamaktadır **(Zadeh, 1978; Mazhar ve ark., 2019**). Bulanık mantık çıkarım sistemlerinde kullanılan dilsel hesaplama, WQI değerlendirmesi için cebirsel bir ifadeden daha iyi bir ifade verir**.** Diğer yöntemlerde nadir görülen bireysel mevcut bilgi ve deneyimi bilgi olarak kullanır. Bu yöntemin benzersizliği, sonucu etkileyen çok sayıda karmaşık çevresel sorunlarla (su kalitesi göstergelerinin su kalitesi üzerindeki etkisi gibi) karşılaşabilmesidir **(Chau, 2006; Lermontov ve ark., 2009; Ellina ve ark., 2020; Oladipo ve ark., 2021).** Literatür, bulanık mantığın yüzey suyu hidrolojisinde, yeraltı suyu hidrolojisinde, sulama teknolojisinde vb. geniş bir uygulanabilirliğini göstermektedir (**Kambalimath ve Deka, 2020).**

***Genetik algoritmalar (GA)***, evrimsel genetik ve biyolojik seçilim ilkelerine bağlı olarak karmaşık çözümleri bulmaya ve optimize etmeye yönelik metasezgisel ve stokastik bir yaklaşımdır (**Goldberg, 2013; Aldrees ve ark., 2022).** Genetik algoritmalar (GA'lar), çeşitli uygulama alanlarında, özellikle buluşsal yöntemlerin yeterli sonuç vermediği problemler için arama, uyarlama ve öğrenme için çok yönlü bir problem çözme mekanizması sağlayabilir. Bunlar, evrim ve doğal genetik ilkeleri tarafından yönlendirilen rastgele arama ve optimizasyon tekniklerdir. Verimli, uyarlanabilir ve sağlam arama süreçleridir, optimuma yakın çözümler üretirler ve büyük miktarda örtük paralelliğe sahiptirler. Bu nedenle, hesaplama gereksinimlerinin optimizasyonu ve sağlam, hızlı ve yakın yaklaşık bir çözüm gerektiren rezervuar su kalitesi yönetimi için GA'ların benimsenmesinin uygun ve başarılı olacağı belirtilmektedir **(Kuo ve ark., 2006).**

***Genetik programlama (GP)***, genetik değerlendirme sürecini benimsemiş bir makine öğrenmesi tekniğidir. GP, genel olarak sinirsel ve regresyon teknikleri ilkesine göre çalışmaktadır. Deneysel formülleştirme özelliklerinden ötürü, sinir ağı tabanlı formülasyonlar genellikle geliştirilemeyecek kadar karmaşıktır **(Gholampour ve ark., 2017).** GP, su kaynakları mühendisliği alanındaki birçok problemin karmaşık ve oldukça doğrusal olmayan davranışını anlamaya yardımcı olan fiziksel olarak anlamlı algoritmalar oluşturmak için sıklıkla kullanılmaktadır. Örneğin: yeraltı suyu kalitesi tahmini **(Aryafar vd., 2019),** gözenekli ortamlarda yüksek düzeyde çözünen madde taşınması **(Hill vd., 2007)** ve kentsel drenaj (yağış-akış) modellemesi **(Savic vd., 1999; Siriwardene ve Perera, 2006)** gibi çalışmalarda başarıyla uygulanmıştır.

***Destek vektör makinesi (SVM),*** hem sınıflandırma hem de regresyon problemleri için kullanılabilecek hataları en aza indirmek için bir hiper-düzlem oluşturmaya dayanan ayırt edici bir yaklaşımdır **(Aldhyani ve ark., 2020).** SVM, yeterli düzeyde veri olmadığında bile iyi sonuçlar çıkarmaktadır. Fakat büyük veri kümeleriyle çalışırken eğitim uzun zaman alıcı olmaktadır. Ayrıca, kişisel koşulların ve farklı ağırlıkların yarattığı sorunlar nedeniyle algılamak ve kavramak gibi birtakım sorunları olabilir **(Nasir ve ark., 2022).**

***İleri Beslemeli Sinir ağı (FFNN),*** kendisine insan beyninin biyolojik yapısını rol edinmiş ve çok karmaşık sistemlerin davranışını kavramak ve çözümlemek için kullanılan YSA tabanlı bir modeldir. FFNN modeli, deneyimden öğrenir ve bilgi edinir. Bu teknik, tahmin ve tanıma gibi dinamik ve doğrusal olmayan sorunların çözümünde özellikle etkilidir. FFNN, su kaynakları verilerinin sınıflandırılması ve regresyonu için en sık olarak kullanılan YSA tasarımlarından biridir (**Ahmad ve ark., 2022).**

***Oto-regresif entegre hareketli ortalama (ARIMA) modeli***, zaman serilerinin zaman alanı analizi yöntemlerinden biridir. Temel olarak AR modeli, MA modeli, ARIMA modeli, otoregresif koşullu değişen varyans modeli (ARCH) ve türev modellerini içerir (**Song ve Yao, 2022).** ARIMA, hidrolojik değişkenleri tahmin etmek için kullanılan doğrusal istatistiksel modellerden biridir **(Choubin ve Malekian, 2017).** Avantaj olarak basit bir kurulumun ve doğrusal ilişkileri yakalama konusunda daha iyi yeteneğe sahiptir. Ayrıca geleneksel zaman serisi tahmin yöntemlerini kullanarak dalgacık ayrıştırmasından sonra trend terimlerini modelleyerek daha iyi doğruluk elde edilebilir (**Zhou ve ark., 2022**). Fakat ARIMA modellerinin kısa vadeli tahminler yapmada regresyon modellerinden daha kötü performans gösterdiğini bildirmiştir **(Yan ve Ma, 2016; Al-Musaylh ve ark., 2018).**

**Hibrit Modeller**

Sunulan su kalitesi modellemesi dışındaki kategorilerden başka geliştirilmiş modeller de kullanılmıştır. Bu kategori, doğadan ilham alan algoritmalar (NI'ler), dalgacık modelleri (WL'ler) ve diğer birleştirilmiş veya hibrit algoritmalar gibi modeller bulunmaktadır**.**

***Doğadan ilham alan algoritmalar*** (NI'ler), evrimsel algoritmaları (EA), sürü zekâsı tabanlı (SI) algoritmaları ve fizik tabanlı (PB) algoritmaları kapsar **(Jaddi ve ark., 2017).** Doğadan ilham alan algoritmalar, zorlu ortam yanıtında sağlamlık ve performans açısından avantajlara sahiptir **(Ishibuchi ve ark., 2003).** Tartışmasız fayda ve avantajlara rağmen, tüm optimizasyon problemini çözecek tek bir algoritma yoktur **(Wolpert ve Macready, 1997).**

Bir WL modeli, bir sinyalin zaman ve frekans alanlarında bilgi sağlayarak, verinin fiziksel yapısı hakkında değerli bilgiler sağlar **(Rajaee, 2010).** Örneğin, dalgacık dönüşümü (WT) yerel analiz ve durağan olmayan sinyal analizi yapabilme yeteneğine sahip olup sinyal analizi için Fourier dönüşümüne dayalı geliştirilmiş bir matematiksel yöntemdir. Ek olarak bir dizi ölçek boyunca zaman-frekans alanındaki hidro-iklimsel sinyallerin birçok doğal özelliğini ortaya çıkarmak için güçlü bir araçtır. **(Grinsted ve ark., 2004; Penalba ve Vargas 2004; Rhif ve ark., 2019; Hao ve Lu 2021).** Ayrıca dalgacık dönüşümü (WT), su kalitesi parametreleri ve su kaynakları yönetimi ve hidrolojisi ile ilgili çalışmaların tahmininde yaygın olarak kullanılmaktadır **(Parmar ve ark. 2019; Bayatvarkeshi ve ark. 2020; Jamei ve ark. 2020).**

***Uyarlanabilir ağ tabanlı bulanık çıkarım sistemi*** (ANFIS), ileri beslemeli bir YSA kullanan gelişmiş bir bulanık çıkarım sistemidir. Bu hibrit teknik, hem AI (öğrenme algoritması) tekniklerinden hem de bulanık sistemden yararlanma özelliği göstermektedir. Bundan dolayı gelişmiş tahmin doğruluğu ve daha hızlı yakınsama faydaları ile daha etkili olarak kabul edilir **(Esfahanipour ve Mardani, 2011; Khadr ve Elshemy, 2017, Tiwari ve ark., 2018, Adıgün ve Oyedele, 2019; Abd El-Mageed ve ark., 2022).** Uyarlanabilir nöro-bulanık çıkarım sistemi (ANFIS) ve hibrit ANN modelleri gibi makine öğrenimi (ML) modelleri, sağlam bir WQI tahmin modeli geliştirmek için her parametrenin duyarlılığını ve öznel davranışını dikkate alarak doğrusal olmayan su kalitesi modellerini yakalayabilme yeteneğine sahiptir **(Tabbussum ve Dar, 2021).**

Biyolojik evrime bağlı olan Genetik algoritma (GA), yaygın olarak kullanılan bir optimizasyon tekniğidir **(Holland, 1992; Haldurai ve ark., 2016).** GA, kombinatoryal optimizasyon, sezgisel arama veya süreç planlama problemleri için optimum çözümler sağlayan "Darwinian" doğal seçilim ve genetik teorisine dayanan bir arama yinelemeli yaklaşımlar sınıfına aittir (**Holland, 1992; Shahabi ve ark., 2022).** GA, YSA'yı tasarlamak için çeşitli şekillerde uygulanabilir. Son zamanlarda, hidrolojide yağış, su kalitesi ve su akışı tahmini için hibrit GA-ANN tabanlı modeller üzerinde çalışmalar yapılmaktadır **(Nasseri ve ark., 2008; Gowda ve Mayya 2014; Ding ve ark., 2014).**

Birçok çalışma, ***doğrusal olmayan otoregresif sinir ağı*** (NAR), doğrusal olmayan otoregresif sinir ağı (NARX) **(Lee ve ark., 2017),** NAR-MA **(Telab ve ark., 2017),** uzun kısa süreli bellek sinir ağı (LSTM) gibi sinir ağı modelleri **(Liu ve ark., 2019),** diğer geleneksel yöntemlerden daha iyi tahmin sonuçları verir. Fakat, ARIMA ve NAR-MA sinir ağlarının doğrusal olmama veya zaman serisi değişkenliği için etkisiz olduğunu ortaya koymuştur **(Tealab ve ark., 2017).** Ayrıca araştırmacılar, güvenilirlikleri ve doğrulukları sebebiyle makine öğrenmesi (ML) modelleri gibi yumuşak bilgi işlem araçlarını kullanarak yüzey ve yeraltı WQ modelleme fikrini geliştirmişlerdir **(Chen ve ark., 2020; Rajaee ve ark., 2020).** Bununla birlikte, ML modelleri, modelleme parametreleri arasındaki karmaşık ve oldukça doğrusal olmayan ilişkiyi ele almak için genellemenin yetersiz olduğunu göstermiştir (**Barzegar ve ark., 2016).** Modelleme yaklaşımı içerisinde son yıllarda popüleritesi artan hibrit YSA modelleri esnek ve verimli modeller oluşturmak için kullanılmaktadır. Diğer geleneksel ve daha gelişmiş modelleme teknikleriyle entegre olma yeteneklerin dolayı modellemede büyük rol oynayan hibrit modellerini kullanma eğilimi her geçen gün artmaktadır (**Yan ve ark., 2019; Chen ve ark., 2020).**

## Literatür

Yapay zekâ teknikleri küresel olarak birçok araştırmacı tarafından hem yüzey hem de yeraltı suyu kaynakları için WQI'nin tahmini ve değerlendirilmesi için kullanılmıştır. Örneğin, **Than ve ark., (2021)** Dong Nai Nehir suyu kalitesini farklı zaman serilerine göre sınıflandırmak için hibrit LSTM-MA (uzun kısa süreli bellek-hareketli ortalama sinir ağı) modeli başarıyla geliştirilmiştir. Hibrit LSTM-MA modelinin ARIMA (otoregresif entegre hareketli ortalama), NAR (doğrusal olmayan otoregresif sinir ağı), NAR-MA (doğrusal olmayan otoregresif- hareketli ortalama sinir ağı) ve LSTM (uzun kısa süreli bellek sinir ağı) modellerinden daha hızlı eğitim süresine ve daha kararlı ve güvenilir öngörülebilirliğe sahip olduğu tespit edilmiştir. Ayrıca LSTM-MA hibrit modelin, su kalitesini geleneksel yöntemlerden bağımsız olarak sınıflandırabileceği bildirilmiştir.

Hibrit AI modeli, GA-EANN (genetik algoritma-duygusal yapay sinir ağı) Malezya Kinta Nehri'ndeki WQI'yi tahmin etmek için kullanılmıştır. Önerilen hibrit GA-EANN, daha az girdi değişkeni ile WQI'nin tahmin doğruluğunu arttırdığı ve bu nedenle, düşük kullanılabilir WQ verisine sahip havzalar için faydalı bir araç olarak hizmet edebileceği raporlanmıştır **(Abba ve ark., 2022).**

Yamuna Nehri'nin WQ'yu tahmin etmek için MLR, yapay zekâ tabanlı modeller (YSA) ve Uyarlanabilir Nöro-Bulanık Çıkarım Sistemi (ANFIS) kullanılmış ve modellerin performans doğrulukları karşılaştırılmıştır. Hem YSA hem de ANFIS modelleri, çalışma alanındaki WQI tahmininde MLR (çoklu doğrusal regresyon) modeline göre daha üstünlük göstermiştir (**Gaya ve ark., 2020).**

**Othman ve ark., (2020)** WQI tahmininde parametrelerden biri olmadığında, parametrelerin indekslerini kullanmak yerine girdi parametrelerinden WQI'yi hesaplamak için YSA'ları (Yapay Sinir Ağları) kullanarak bir girdi yaklaşımı oluşturmuşlardır. YSA modeli kullanılarak hesaplanan WQI değerlerinin, belirtilen WQI ile oldukça iyi bir uyum içinde olduğunu göstermiştir. Bu sonuca göre YSA modelinin faydalı ve uygun olduğu neticesine varılmıştır.

**Al-Sulttani ve ark., (2021)** Irak Fırat Nehri'nin aylık biyokimyasal oksijen ihtiyacı (BOİ) değerlerini tahmin etmek için Quantile Regresyon Ormanı (QRF), Random Forest (RF), Radyal Destek Vektör Makinesi (SVM), Stokastik Gradient Boost (GBM) ve Gradient Boosting Machines (GBM_H2O) olmak üzere beş farklı topluluk makine öğrenmesi (ML) modeli geliştirilmiştir. Araştırmanın sonucu olarak, Temel Bileşenler Analizi-Quantile regresyon ormanı (PCA-QRF) modelinin, diğer yerleşik modellere kıyasla BOİ tahmininde güvenilir bir performans sağlamış olduğu tespit edilmiştir.

**Banadkooki ve ark., (2020**), başka bir çalışmada toplam çözünmüş katıları (TDS) tahmin etmek için yeni hibrit ML algoritmaları kullanmıştır.

**Liu ve ark., (2016)** bir hibrit model, yani ampirik mod ayrıştırma (EMD)-BPNN önerilmiştir. Sunulan EMD-BPNN yöntemi, doğrusal olmayan, durağan olmayan ve güçlü karmaşıklık su kalitesi verileriyle büyük ölçekli yoğun su ürünleri yetiştiriciliğinde su kalitesi tahmini için hem oldukça uygun hem de verimli olduğu bildirilmiştir.

**Ye ve ark., (2019)** LSTM-RNN modeline dayalı nehir suyu kalitesi parametrelerinin bir tahmin yöntemi sunulmuştur. Önerilen LSTM-RNN modelinin tahmin doğruluğunun, diğer tekrarlayan sinir ağları (RNN) ve geleneksel tahmin modellerinden daha yüksek olduğu sonucuna varılmıştır.

**Lu ve Ma, (2020a)** kısa vadede su kalitesini tahmin etmek için iki hibrit karar tabanlı makine öğrenme modeli önermiştir. İki hibrit modelin temel modelleri, aşırı gradyan artırma ve rastgele ormandır.

**Zhou ve ark., (2022**) W-ARIMA-GRU modeli olarak kısaltılan önerilen entegre Dalgacık Ayrıştırma, Otoregresif Entegre Hareketli Ortalama (ARIMA) ve Kapılı Tekrarlayan Birim (GRU) modeli, üç geleneksel su kalitesi göstergesi ile karşılaştırılmıştır. Önerilmiş olan W-ARIMA-GRU su kalitesi göstergesi tahmin modelinin iyi bir evrenselliğe ve yüksek verimliliğe sahip olduğu ve diğer nehirler, göller ve rezervuarlar için su kalitesi koşullarında bozulma eğilimi olduğunda olası su kalitesi kirliliği riskini değerlendirmek için kullanılabileceği söylenmiştir.

**Wan ve ark., (2022)** fiziksel sürece dayalı simülasyon-gözlem farkı (SOD) modüler, uzamsal özellikleri yansıtan görsel geometri (VGG) modülerine sahip SOD-VGG-LSTM adlı yeni bir birleştirilmiş hibrit derin öğrenme modeli geliştirilmiştir. Kurulan model, sadece aşırı değer tahmini sorununun üstesinden gelmekle

kalmamış, ayrıca farklı zaman veya bölgelerdeki mekansal özelliklerin su kalitesi üzerindeki etkisini de ortaya çıkartmıştır.

**Song ve Yao, (2022)** su kalitesi tahmini için hibrit senkro-sıkıştırılmış dalgacık dönüşümü ve serçe arama algoritması ile optimize edilmiş derin aşırı öğrenme makinesini entegre eden yeni bir su kalitesi tahmin modeli (SWT-SSA-DELM) geliştirmiştir. SWT-SSA-DELM'ye dayalı su kalitesi tahmin modelinin emsal modellerden daha iyi performans gösterdiği tespit edilmiştir.

**Mei ve ark., (2022)** geleneksel hidrolik modelin karmaşık çalışması ve zayıf hassasiyeti sorununu çözmek için bir evrişimsel sinir ağı (CNN)- kapı tekrarlayan birim (GRU)- Dikkat (A) birleştirilmiş olan hibtit modeli sunmuşlardır. Önerilen (CGA) hibrit model, erken uyarı ve su yönetimi için etkili ve basit bir araç olarak kullanılabileceği tavsiye edilmiştir.

**Kadkhodazadeh ve Farzin, (2022a)** ilk kez, WQI değişkenlerinden olan elektriksel iletkenliğin (EC) ve toplam çözünmüş katıların (TDS) tahmini için uyarlanabilir nöral bulanık çıkarım sistemi-ayrık dalgacık dönüşümü-gradyan tabanlı optimizasyon (ANFIS-DWT-GBO) gibi yeni bir hibrit çerçeve uygulanmıştır. Önerilmiş olan modelin gücünü göstermek için ANFIS, ANFIS-DWT ve ANFIS-GBO dahil olmak üzere üç karşılaştırma algoritması kullanılmıştır. ANFIS-DWT-GBO'nun kıyaslama veri kümeleri modellemesinde ve su kalitesi parametreleri (WQP'ler) tahmininde diğer algoritmalara kıyasla çok başarılı sonuca ulaşmışlardır.

**Paul ve ark., (2022)** hibrit Uzun Kısa Vadeli Bellek modeli ile Serçe Arama Optimizasyonu (WQP-SSHLSTM) kullanılarak su kalitesi (WQ)'nin tahmini yapılmıştır. WQP-SSHLSTM modelinin WQ için mükemmel bir sınıflandırma yöntemi olduğu belirtilmiştir.

**Xia ve Zeng, (2022)** araştırmada optimizasyon destek vektör makinesi (SVM) modeline dayalı olarak Chl-a konsantrasyonunun tahminine yardımcı olmak için çevre faktörlerini (su kalitesi ve meteorolojik veriler) kullanmayı amaçlamıştırlar. SVM'yi optimize etmek için geleneksel parçacık sürü optimizasyonu (PSO) ve gri kurt optimize edici (GWO) en son optimizasyon algoritması ile yüksek doğruluklu Chl-a konsantrasyonu tahmin sonuçları karşılaştırılmıştır. Sonuçlar GWO-SVM modeli hem eğitim hem de doğrulama süreçlerinde diğer modellere göre daha yüksek doğruluğa sahip olduğu tespit edilmiştir.

**Kadkhodazadeh ve Farzin, (b2022)** ilk kez, en küçük kareler destek vektörü makine-aritmetik optimizasyon algoritması (LSSVM-AOA) adlı yeni bir hibrit makine öğrenme modeli önerilmiştir. Bu algoritmanın sonuçları beş farklı klasik ve hibrit algoritma ile karşılaştırılmıştır. LSSVM-AOA'nın klasik ve hibrit algoritmalara (yapay sinir ağı) kıyasla su kalitesi parametrelerinin (WQP'ler) tahmin edilmesinde başarılı sonuçlara sahip olduğunu belirtilmiştir.

**Wu ve Wang, (2022)** Jinjiang Nehri'nin su kalitesini tahmin etmek için yapay sinir ağına (YSA), ayrık dalgacık dönüşümüne (DWT) ve uzun kısa süreli belleğe (LSTM) dayalı bir model oluşturulmuştur. Çalışmada önerilen ANN-WT-LSTM modelinin birçok değerlendirme indeksinde önceki modellerden daha iyi performans göstermiş olduğu raporlanmıştır.

**Abba ve ark., (2022)** Malezya Kinta Nehri'ndeki WQI'yi tahmin etmek için bir hibrit GA-EANN (genetik algoritma-duygusal yapay sinir ağı) model önerilmiştir. Önerilen hibrit GA-EANN, daha az girdi değişkeni ile WQI'nin tahmin doğruluğunun artmış olduğu bulunmuştur. Bu nedenle, düşük kullanılabilir WQ verisine sahip havzalar için faydalı bir araç olarak hizmet edebileceğini raporlanmıştır

Yağış-akış modelleme çalışmaları iklim değişikliği etkilerini anlamak, sel ve kuraklık gibi aşırı olaylara karşı önceden tedbir alınması için daha önemli hale gelmiştir. Bu açıdan son yıllarda farklı modelleme yaklaşımlarının ortaya çıkarılmasının önemi dahada artmıştır (**Sezen ve Partal, 2022).** Örneğin**,**

**Li ve ark., (2022)** iklim değişikliği bağlamında tasarım sel tahmini ve ilgili risk analizi için ortak bir olasılıksal yağış-akış modeli (JPRR) önerilmiştir. JPRR, Çin'de farklı iklim bölgelerini ve manzaraları temsil eden dört bozulmamış havzaya uygulanmış. Sonuçlar, JPRR'nin yüksekten aşırıya akış simülasyonlarında iyi bilinen üç ML modelinden (yani, rastgele orman, yapay sinir ağları ve uzun kısa süreli bellek) önemli ölçüde daha iyi performans gösterdiğini ortaya çıkarmıştır.

**Sezen ve Partal, (2022)** çalışmalarında, genellikle kurak ve değişken iklim koşullarına sahip olan Türkiye Konya Kapalı Havzası'nın üç alt havzasında Génie Rural à 6 parametreli Journalier (GR6J)-dalgacık tabanlı

genetik algoritma-yapay sinir ağı 1 (GR6J-WGANN 1) ve GR6J-dalgacık tabanlı genetik algoritma-yapay sinir ağı 2 (GR6J-WGANN 2) modelleri, yağış-akış modelleme performansını geliştirmek için önerilmiştir. Hibrit modellerin, özellikle aşırı akış tahminleri ile ilgili olarak, kavramsal ve veriye dayalı modellerden daha iyi performans gösterdiği tespit edilmiştir.

**Sulaiman ve ark., (2022)** Malezya'nın doğu kıyısı Yarımadası'nda önerilen hibrit Destek Vektör Sınıflandırmasını (SVC) ve İlgili Vektör Makineleri (RVM'ler) kullanılarak yağışlı günlerin sıklığına dayalı olarak yağış miktarını tahmin etmişlerdir. SVC-RVM tarafından yağış değerlerinin tahmini, bu hibrit modelin gerçek yağış değeri modelini takip edebildiğini ve aşırı yağış değerlerini yakalayabildiğini de gösterdiği bildirilmiştir.

**Ahmedi ve ark., (2019)** başka bir çalışmada hibrit algoritmanın özellikle yağışlı mevsimde akış tahmini konusunda daha iyi sonuçlar elde ettiğini bulmuştur**.**

**Gomes ve ark., (2022)** yağış tahminlerini elde etmek için bir yapay sinir ağı (YSA) ve uyarlanabilir nöro-bulanık çıkarım sistemi (ANFIS) ile maksimum örtüşen ayrık dalgacık (MODWT) yöntemine dayalı bir hibrit model geliştirilmiştir.

**Tiwari ve ark., (2022)** Kolar Nehir Havzası'nda yapay sinir ağı ile dalgacığın hibrit yaklaşımını kullanarak akış modellemesi yapılmıştır. Radyal temel fonksiyon sinir ağı modelleri (RBF) sinir ağına dalgacık girişi ile birlikte ve dalgacık girişi olmadan yağış-akış modellemesinin oluşturulması için incelenmiştir. Bu modelin daha fazla sayıda giriş kombinasyonu ve farklı nehir havzalarından gelen verilerle daha da geliştirilme potansiyeline sahip olduğu söylenmiştir.

**Jahandideh-Tehrani ve ark., (2021)** Güneydoğu Queensland, Avustralya'daki Nerang Nehri Havzası'ndaki gerçek zamanlı akış akışını tahmin etmek amacıyla yapay sinir ağı (YSA) parametrelerini eğitmek için parçacık sürüsü optimizasyonu (PSO) ve genetik algoritma (GA) olmak üzere iki evrimsel algoritmanın uygulanması yapılmıştır. Sonuçlar, hibrit ANN-PSO modelinin yakınsama hızı, doğruluk ve uygunluk fonksiyonu değerlendirmesi açısından ANN-GA modelinden önemli ölçüde daha iyi performans gösterdiği raporlanmıştır.

Literatür taramasından, kentsel kısa vadeli su talebi için mevcut tahmin modelinin, tahmin doğruluğunu daha etkili bir şekilde artırabilecek hibrit modellerin yönüne doğru ilerlediğini gördük. Son yıllarda, çeşitli ülkelerden bilim adamları, genetik algoritma, karınca kolonisi optimizasyon algoritması, yapay balık sürüsü algoritması, yapay bağışıklık algoritması, Parçacık Sürüsü Optimizasyonu (PSO) ve Meyve Sineği Optimizasyon Algoritması (FFOA) gibi hayvan davranışlarından birçok optimizasyon algoritması (sürü zekâsı algoritması) çıkarmışlardır **(Lu ve ark., 2020b). Örneğin,**

**Liu ve ark., (2022)** Şangay'da gerçekleştirdikleri su talebi çalışması için 744 veri noktasına sahip olan saatlik bir su temini akış serisi kullanılmıştır. Saatlik su talebinin tahmini için üç tek model ARIMA, Yapay Arı Kolonisi (BP) ve SVM ile üç hibrit model (EEMD-ARIMA, EEMD-BP ve EEMD-SVM) için topluluk ampirik mod ayrıştırması (EEDM) uygulanarak karşılaştırılmış. Sonuçların EEMD-SVM, altı tahmin modeli içinde en iyi performansı gösteren algoritma olmuştur.

**Chen ve ark., (2022)** su talebi tahmininde veri temizliğinde Mevsimsel Hibrit Aşırı Öğrenci Sapması (S-H-ESD) yöntemini uygulamıştır. Conv1D-GRU (Tek Boyutlu Evrişim Kapılı Tekrarlayan Birim) adı verilen bir model önerilmiştir. Önerilmiş olan bu modelin yeteneğini göstermek için, Kapılı Tekrarlayan Birim Ağı (GRUN) ve YSA modellerinden elde edilen sonuçlarla kıyaslanmıştır.

**Li ve ark., (2022)** su talebini tahmini için ARIMA yöntemiyle önerilen model ile karşılaştırılmıştır. Pekin kentinin su talebini tahmin etmek için Belirsiz Otoregresif (UAR) modeli kullanılmıştır. Sonuçlar, önerilen modelin su talebi tahminleri için belirtilen geleneksel modele göre çok daha iyi doğruluk değeri verdiği belirlenmiş.

**Pan ve ark., (2022)** 2014'ten 2019'a kadar olan veriler kullanılarak, Çin'deki endüstriyel farklılaşmaların tespiti ve farklı önde gelen endüstrilerin su tüketiminin belirlenmesi için Gri Niş Modeli (W-CGM) önerilmiştir. Sonuç olarak gelecekte kullanılacak olan suyun miktarına ilişkin W-CGM modeli daha başarılı olduğu bildirilmiştir.

**Du ve ark., (2022**) kentsel su talebinin aralığını tahmin etmek için yeni hibrit bir model (KDE-PSO-LSTM) önerilmiştir. Çin'in Suzhou kentindeki bir su şebekesinden alınan gerçek veriler, çalışmada önerilen modelin doğruluğun kanıtlanması için kullanılmıştır. Önerilen, KDE-PSO-LSTM' nin su talep aralığı tahmininin kalitesini etkili bir şekilde artırabileceğini gösteren daha kapsamlı bir değerlendirme performans sonucu sunulmuştur.

**Ristow ve ark., (2021**) aylık kentsel su talebi tahmini içinMevsimsel Otoregresif Entegre Hareketli Ortalama (SARIMA) yönteminin karşılaştırılan beş kategoriden dördünde su tüketimini tahmin etmek için daha yeterli olduğunu belirlenmiştir. Sunulan yöntemin, yeterli veri olduğu sürece herhangi bir yerdeki aylık kentsel su tüketimi tahminlerini kolayca gerçekleştirebileceği söylenmiştir.

**Lu ve ark., (2020)** Austin, Teksas'taki aylık su talebini tahmin etmek için simüle edilmiş bir Tavlama Algoritması (SA), Genetik Çapraz Faktör (GCF), Meyve Sineği Optimizasyon Algoritması (FFOA) ve Destek Vektör Makinesini (SVM) ile birleştirilerek hibrit model önerilmiştir. FFOA-GCFSA-SVM'nin PSO-SVM ve Geri Yayılım Sinir Ağları (BPNN) gibi diğer akıllı modellerle karşılaştırılmıştır. Yeni hibrit algoritmanın küresel optimizasyon performansının daha iyi çıktığı ve dolayısıyla tahmin doğruluğunun daha yüksek olduğu tespit edilmiştir.

**Oyebode ve Ighravwe, (2019)** su tüketimini tahminine yönelik gerçekleştirilen çalışmada, feed forward yapay sinir ağları ve bir destek vektör makinesi (SVM) seçilerek uyugulanmıştır. Çalışmada yumuşak hesaplama tekniklerinin (ANN-CG, ANN-DE ve SVM) performansının karşılaştırılması, su tüketimi için öngörücü modeller olarak ES ve MLR ile yapılmıştır. Elde edilen veriler doğrultusunda DE ile eğitilen YSA modelinin, CG tarafından eğitilmiş YSA ve diğer tahmine dayalı modellerden (SVM, ES ve MLR) daha iyi performans göstermiş olduğu bulunmuştur.

**Xu ve ark., (2018)** Çin'in Zhuzhou kentindeki günlük kentsel su talebini tahmin etmek için yeni bir Çift Ölçekli Derin İnanç Ağı (DSDBN) yöntemi geliştirilmiştir. Önerilen bu yaklaşım modelinin tahmin performansını doğrulamak ve değerlendirmek için, EEMD-ARIMA, EEMD-FFNN, EEMD-SVR ve tekli DBN modelleri ile karşılaştırılmıştır. Çalışma sonuçlarına göre DSDBN yaklaşımının, Çin Zhuzhou'daki kentsel su işlerinin günlük su talebi tahmininde tüm karşılaştırma modelleri arasında en yüksek tahmin doğruluğu sonucuna varılmıştır.

**Altunkaynak ve Nigussie, (2017)** İstanbul'un aylık su tüketimini tahmin etmek için MSA– MLP geliştirilmiştir. Kombine MSA- MLP'nin tahmin teslim süreleri boyunca en iyi performansı vermiş olduğu bulunmuştur. Bu da Çarpımsal Mevsim Algoritması (MSA)'nın orijinal verilerden durağanlığı kaldırmada, MLP modelinin doğruluğunu artırmada ve tahmin teslim süresini uzatmada Ayrık Dalgacık Dönüşümü (DWT)'den daha basit ancak daha iyi bir ön işleme tekniği olduğu sonucuna varılmıştır.

## SONUÇ

Su kalitesi değerlendirmesi; sürdürülebilir su kaynakları yönetiminde kirliliğe yönelik mücadelede önemli bir çıkarım sunmaktadır. Yapılacak olan çalışmalarda hidrolojik durumlarla ilgili tahminlerin yapılması, su yönetimi için çok değerli olan bilgiye ulaşmayı sağlar. Bu bilgiler ışığında yapılacak olan uyarılar sel, su miktarı ve hatta su kalitesi gibi hidrolojik alanlarda kirli bilginin önlenmesi için anahtar görevi görür. İlaveten, su kalitesinin bozulması durumunda sadece su yaşamının değil, aynı zamanda doğrudan/dolaylı yolla etkileşimde bulunan tüm canlı cansız ekosistem unsurlarının da de olumsuz sonuçlar görebileceği ihtimali unutulmamalıdır. Dolayısıyla, herhangi bir alandaki sucul sistemlerin yakın geleceğinin planlanmasının önemi göz ardı edilememektedir.

Su kaynaklarının geleceğinin planlanmasını da içeren çalışmalarda önerilen modeller, akıllı su koruma geliştirme aşamasında noktasal olmayan kaynak kirliliğini çözmek, su talebi, sel, akış ve yağış için anahtar bağlantı olabilecek akıllı bir havza su kalitesi tahmin modeli geliştirme çabasında olmuştur. Önerilen modeller gelecekte, havza taşkın tahmininin kontrolü ve risk yönetimi için etkin karar desteği sağlayabilirler. Çünkü kurulan modeller, hidrolojik sürecin zamanında ve verimli bir şekilde erken uyarısını sağlayabilme yeteneğine sahip olacaktır. Su kalitesi tahmini ile ilgili yapılan çalışmalarda çoğu araştırmacı yalnızca su kalitesi değişikliklerine entegre olmuş bir sistem üzerinden ilerlemiştir. Ancak gerçekte, temel su parametrelerin yanı sıra arıtma mekanizması gibi kullanım amacına yönelik birçok etkenin de tahmini su kalitesi üzerine olan etkisinin dikkate alınması gerekmektedir. Bu nedenle, gelecekteki çalışmalar için gerçek su kalitesini

belirlerken bu faktörleri dikkate alan kapsamlı bir tahmin modelinin geliştirilmesine ihtiyaç olduğu çıkarımlanmıştır. Diğer bir çıkarım ise, tahmin sonuçlarını pratik amaçlar için daha uygun hale getirmek amacıyla algılama ve çözümleme kapasitesinin arttırılmalısıdır **(Mei ve ark., 2022).** Örneğin, sinir ağının daha iyi performans göstermesi için, daha iyi eğitilmesi için yüksek doğrulukta verilere ihtiyaç duyulmaktadır. Evapotranspirasyon, yeraltı suyu şarjı, havzanın morfometrisi, güneş radyasyonu, rüzgâr hızı vb. gibi diğer verilerin de akış analizi için dikkate alınması gerekmektedir **(Tiwari ve ark., 2022).** Ayrıca, su kaynaklarının kalitesi ile talebi dikkate alınarak; sürdürülebilir su kaynaklarının gelecekteki durumunun öngörülmesi, su kaynakları yöneticilerinin başarılı ve kritik kararlar almasına yardımcı olacaktır.

Bu kapsamda popüleritesi her geçen gün artan hibrit modeller, araştırmacıların hidrolojik alanlardaki tahminlerinin doğruluğunu artırmak için kullanılması gereken en etkili araç olacaktır. Kapsamlı bir hibrit model hem ön işleme hem de sezgisel algoritma tekniklerini içerdiğinden en doğru sonuca ulaştırması mümkündür. Sonuç olarak, son yıllarda geliştirilen hibrit modellerin literatürde en çok kullanılan yöntemler arasında olması şaşırtıcı olmayacaktır. Artık, birçok çalışma YSA modellerinin tahminleme için mükemmel modeller olduğunu ve değişen dünya şartlarına uyum sağlayarak sürekli gelişmeye ihtiyaç duyduklarını göstermiştir.

## TEŞEKKÜRLER

Hakan IŞIK (YÖK 100/2000) proje bursiyeridir.

## KAYNAKÇALAR

Abba SI, Abdulkadir RA, Sammen SS, Pham QB, Lawan AA, Esmaili P, Al-Ansari N 2022. Integrating feature extraction approaches with hybrid emotional neural networks for water quality index modeling. Applied Soft Computing, 114, 108036.

Abba SI, Gaya MS, Yakubu ML, Zango MU, Abdulkadir RA, Saleh MA, Wahab NA 2019. Modelling of uncertain system: a comparison study of linear and non-linear approaches. In 2019 IEEE International Conference on Automatic Control and Intelligent Systems (I2CACIS) (pp. 1-6). IEEE.

Abd El-Mageed AM, Enany TA, Goher ME, Hassouna ME 2022. Forecasting water quality parameters in Wadi El Rayan Upper Lake, Fayoum, Egypt using adaptive neuro-fuzzy inference system. The Egyptian Journal of Aquatic Research, 48(1), 13-19.

Abdel-Satar AM, Ali MH, Goher ME 2017. Indices of water quality and metal pollution of Nile River, Egypt. The Egyptian Journal of Aquatic Research, 43(1), 21-29.

Adamowski J, Fung Chan H, Prasher SO, Ozga-Zielinski B, Sliusarieva A 2012. Comparison of multiple linear and nonlinear regression, autoregressive integrated moving average, artificial neural network, and wavelet artificial neural network methods for urban water demand forecasting in Montreal, Canada. Water Resources Research, 48(1).

Adıgun OH, Oyedele OJ 2019. Coastal Water Level Prediction Model Using Adaptive Neuro-fuzzy Inference System. Journal of Advances in Computer Engineering and Technology, 5(1), 11-18.

Ahmad M, Al Mehedi MA, Yazdan MMS, Kumar R 2022. Development of Machine Learning Flood Model Using Artificial Neural Network (ANN) at Var River. Liquids, 2(3), 147-160.

Ahmadi M, Motamedvaziri B, Ahmadi H, Moeini A, Zehtabiyan GR 2019. Assessment of climate change impact on surface runoff, statistical downscaling and hydrological modeling. Physics and Chemistry of the Earth, Parts A/B/C, 114, 102800.

Al Aani S, Bonny T, Hasan SW, Hilal N 2019. Can machine language and artificial intelligence revolutionize process automation for water treatment and desalination?. Desalination, 458, 84-96.

Aldhyani TH, Al-Yaari M, Alkahtani H, Maashi M 2020. Water quality prediction using artificial intelligence algorithms. Applied Bionics and Biomechanics, 2020.

Aldrees A, Khan MA, Tariq MAUR, Mustafa Mohamed A, Ng AWM, Bakheit Taha A T 2022. Multi-Expression Programming (MEP): Water Quality Assessment Using Water Quality Indices. Water, 14(6), 947.

Alizadeh MJ, Kavianpour MR, Danesh M, Adolf J Shamshirband, S, & Chau, K W 2018. Effect of river flow on the quality of estuarine and coastal waters using machine learning models. Engineering Applications of Computational Fluid Mechanics, 12(1), 810-823.

Al-Musaylh MS, Deo RC, Adamowski JF, Li Y 2018. Short-term electricity demand forecasting with MARS, SVR and ARIMA models using aggregated demand data in Queensland, Australia. Advanced Engineering Informatics, 35, 1-16.

Al-Shaar W, Nehme N, Haidar H, Lakiss H 2022. Forecasted water demand using Extended Cellular Automata Markov Chain Model: case of Saida and Jezzine regions in Lebanon. Sustainable Water Resources Management, 8(3), 1-11.

Al-Sulttani A, O Al-Mukhtar M, Roomi AB, Farooque AA, Khedher KM, Yaseen ZM 2021. Proposition of new ensemble data-intelligence models for surface water quality prediction. IEEE Access, 9, 108527-108541.

Altunkaynak A, Nigussie TA 2017. Monthly water consumption prediction using season algorithm and wavelet transform–based models. Journal of Water Resources Planning and Management, 143(6), 04017011.

Alves EM Rodrigues RJ, dos Santos Corrêa C, Fidemann T, Rocha JC, Buzzo JLL, Núñez EGF 2018. Use of ultraviolet–visible spectrophotometry associated with artificial neural networks as an alternative for determining the water quality index. Environmental monitoring and assessment, 190(6), 1-15.

Aryafar A, Khosravi V, Zarepourfard H, Rooki R 2019. Evolving genetic programming and other AI-based models for estimating groundwater quality parameters of the Khezri plain, Eastern Iran. Environmental earth sciences, 78(3), 1-13.

Ayers RS, Westcot DW 1989. Salinity problems. Water quality for agriculture. FAO, Rome, Italy, 1-32.

Azrour M, Mabrouki J, Fattah G, Guezzaz A, Aziz F 2022. Machine learning algorithms for efficient water quality prediction. Modeling Earth Systems and Environment, 8(2), 2793-2801.

Bakker K 2012. Water security: research challenges and opportunities. Science, 337(6097), 914-915.

Banadkooki FB, Ehteram M, Panahi F, Sammen SS, Othman FB, Ahmed ES 2020. Estimation of total dissolved solids (TDS) using new hybrid machine learning models. Journal of Hydrology, 587, 124989.

Barzegar R, Adamowski J, Moghaddam AA 2016. Application of wavelet-artificial intelligence hybrid models for water quality prediction: a case study in Aji-Chay River, Iran. Stochastic environmental research and risk assessment, 30(7), 1797-1819

Bayatvarkeshi M, Mohammadi K, Kisi O, Fasihi R 2020. A new wavelet conjunction approach for estimation of relative humidity: wavelet principal component analysis combined with ANN. Neural Computing and Applications, 32(9), 4989-5000.

Beal C, Stewart R, Huang T, Rey E 2011. South East Queensland residential end use study. Brisbane, Australia: Urban Water Security Research Alliance.

Boretti A, Rosa L 2019. Reassessing the projections of the world water development report. NPJ Clean Water, 2(1), 1-6.

Box GE, Jenkins GM, Reinsel GC, Ljung GM 2015. Time series analysis: forecasting and control. John Wiley & Sons.

Boyacioglu H 2007. Development of a water quality index based on a European classification scheme. Water Sa, 33(1).

Bozorg-Haddad O, Soleimani S, Loáiciga HA 2017. Modeling water-quality parameters using genetic algorithm–least squares support vector regression and genetic programming. Journal of Environmental Engineering, 143(7), 04017021.

Chapman D 1992. Water Quality Assessments, Published on behalf of UNESCO/WHO/UNEP.

Chau KW 2006. A review on integration of artificial intelligence into water quality modelling. Marine pollution bulletin, 52(7), 726-733.

Chen L, Yan H, Yan J, Wang J, Tao T, Xin K, Qiu J 2022. Short-term water demand forecast based on automatic feature extraction by one-dimensional convolution. Journal of Hydrology, 606, 127440.

Chen XY, Chau KW 2016. A hybrid double feedforward neural network for suspended sediment load estimation. Water Resources Management, 30(7), 2179-2194.

Chen Y, Song L, Liu Y, Yang L, Li D 2020. A review of the artificial neural network models for water quality prediction. Applied Sciences, 10(17), 5776.

Chia MY, Huang YF, Koo CH, Fung KF 2020. Recent advances in evapotranspiration estimation using artificial intelligence approaches with a focus on hybridization techniques—a review. Agronomy, 10(1), 101.

Cho K, Van Merriënboer B, Bahdanau D, Bengio Y 2014. On the properties of neural machine translation: Encoder-decoder approaches. arXiv preprint arXiv:1409.1259.

Choubin B, Malekian A 2017. Combined gamma and M-test-based ANN and ARIMA models for groundwater fluctuation forecasting in semiarid regions. Environmental Earth Sciences, 76(15), 1-10.

Connor R 2015. The United Nations world water development report 2015: water for a sustainable world (Vol. 1). UNESCO publishing.

Czajkowski A, Remiorz L, Pawlak S, Remiorz E, Szyguła J, Marek D, Paszkuta M, Drabik G, Baronu G, Paduch J, Antemijczuk O 2021. Global Water Crisis: Concept of a New Interactive Shower Panel Based on IoT and Cloud Computing for Rational Water Consumption. Applied Sciences, 11(9), 4081.

Dawood T, Elwakil E, Novoa HM, Delgado JFG 2021. Toward urban sustainability and clean potable water: Prediction of water quality via artificial neural networks. Journal of Cleaner Production, 291, 125266.

Deng T, Chau KW, Duan HF 2021. Machine learning based marine water quality prediction for coastal hydro-environment management. Journal of Environmental Management, 284, 112051.

Ding YR, Cai YJ, Sun PD, Chen B 2014. The use of combined neural networks and genetic algorithms for prediction of river water quality. Journal of applied research and technology, 12(3), 493-499.

Du B, Huang S, Guo J, Tang H, Wang L, Zhou S 2022. Interval forecasting for urban water demand using PSO optimized KDE distribution and LSTM neural networks. Applied Soft Computing, 122, 108875.

El Mezouari A, El Fazziki A, Sadgal M 2022. A hybrid artificial neural network: An optimization-based framework for smart groundwater governance. Water Supply, 22(5), 5237-5252.

Ellina G, Papaschinopoulos G, Papadopoulos BK 2020. Research of fuzzy implications via fuzzy linear regression in data analysis for a fuzzy model. Journal of Computational Methods in Sciences and Engineering, 20(3), 879-888.

Elzwayie A, El-Shafie A, Yaseen ZM, Afan HA, Allawi MF 2017. RBFNN-based model for heavy metal prediction for different climatic and pollution conditions. Neural Computing and Applications, 28(8), 1991-2003.

Gaya MS, Abba SI, Aliyu MA, Tukur AI, Saleh MA, Esmaili P, Wahab NA 2020. Estimation of water quality index using artificial intelligence approaches and multi-linear regression. IAES International Journal of Artificial Intelligence, 9(1), 126.

Gholampour A, Gandomi AH, Ozbakkaloglu T 2017. New formulations for mechanical properties of recycled aggregate concrete using gene expression programming. Construction and Building Materials, 130, 122-145.

Ghorbani MA, Khatibi R, Karimi V, Yaseen ZM, Zounemat-Kermani M 2018. Learning from multiple models using artificial intelligence to improve model prediction accuracies: application to river flows. Water resources management, 32(13), 4201-4215.

Ghritlahre HK, Prasad RK 2018. Application of ANN technique to predict the performance of solar collector systems-A review. Renewable and Sustainable Energy Reviews, 84, 75-88.

Gleeson T, Wada Y, Bierkens MF, Van Beek LP 2012. Water balance of global aquifers revealed by groundwater footprint. Nature, 488(7410), 197-200.

Goldberg DE 2013. Genetic algorithms. pearson education India.

Gomes EP, Blanco CJC, da Silva Holanda P, de Oliveira Júnior JF 2022. MODWT-ANN hybrid models for daily precipitation estimates with time-delayed entries in Amazon region. Environmental Monitoring and Assessment, 194(4), 1-13.

Gowda CC, Mayya SG 2014. Comparison of back propagation neural network and genetic algorithm neural network for stream flow prediction. Journal of Computational Environmental Sciences, 2014.

Grinsted A, Moore JC, Jevrejeva S 2004. Application of the cross wavelet transform and wavelet coherence to geophysical time series. Nonlinear processes in geophysics, 11(5/6), 561-566.

Hadjisolomou E, Stefanidis K, Herodotou H, Michaelides M, Papatheodorou G, Papastergiadou E 2021. Modelling freshwater eutrophication with limited limnological data using artificial neural networks. Water, 13(11), 1590.

Haldurai L, Madhubala T, Rajalakshmi R 2016. A study on genetic algorithm and its applications. International Journal of Computer Sciences and Engineering, 4(10), 139.

Hao Y, Lu J 2021. Teleconnection between climate oscillations and riverine nutrient dynamics in Southeast China based on wavelet analysis. Environmental Science and Pollution Research, 28(31), 41807-41820.

Harlan SL, Ruddell DM 2011. Climate change and health in cities: impacts of heat and air pollution and potential co-benefits from mitigation and adaptation. Current opinion in environmental sustainability, 3(3), 126-134.

Hill DJ, Minsker BS, Valocchi AJ, Babovic V, Keijzer M 2007. Upscaling models of solute transport in porous media through genetic programming. Journal of Hydroinformatics, 9(4), 251-266.

Hochreiter S, Schmidhuber J 1997. Long short-term memory. Neural computation, 9(8), 1735-1780.

Holland JH 1992a. Adaptation in natural and artificial systems: an introductory analysis with applications to biology, control, and artificial intelligence. MIT press.

Holland JH 1992b. Genetic algorithms. Scientific american, 267(1), 66-73.

Horton RK 1965. An index number system for rating water quality. J Water Pollut Control Fed, 37(3), 300-306.

Jaddi NS, Alvankarian J, Abdullah S 2017. Kidney-inspired algorithm for optimization problems. Communications in Nonlinear Science and Numerical Simulation, 42, 358-369.

Jahandideh-Tehrani M, Jenkins G, Helfer F 2021. A comparison of particle swarm optimization and genetic algorithm for daily rainfall-runoff modelling: a case study for Southeast Queensland, Australia. Optimization and Engineering, 22(1), 29-50.

Jamei M, Ahmadianfar I, Chu X, Yaseen ZM 2020. Prediction of surface water total dissolved solids using hybridized wavelet-multigene genetic programming: New approach. Journal of Hydrology, 589, 125335.

Kadkhodazadeh M, Farzin S 2022a. A novel hybrid framework based on the ANFIS, discrete wavelet transform, and optimization algorithm for the estimation of water quality parameters. Journal of Water and Climate Change, 13(8), 2940-2961.

Kadkhodazadeh M, Farzin S 2022b. Introducing a novel hybrid machine learning model and developing its performance in estimating water quality parameters. Water Resources Management, 36(10), 3901-3927.

Kalhori M, Ashofteh PS, Moghadam SH, Singh VP 2022. Investigating the effect of uncertainty of AOGCM-TAR and AOGCM-AR5 climate change models on river runoff. Arabian Journal of Geosciences, 15(13), 1-16.

Kambalimath S, Deka PC 2020. A basic review of fuzzy logic applications in hydrology and water resources. Applied Water Science, 10(8), 1-14.

Karimi S, Kisi O, Shiri J, Makarynskyy O 2013. Neuro-fuzzy and neural network techniques for forecasting sea level in Darwin Harbor, Australia. Computers & Geosciences, 52, 50-59.

Katyal D 2011. Water quality indices used for surface water vulnerability assessment. International journal of environmental sciences, 2(1).

Keath NA, Brown RR 2009. Extreme events: being prepared for the pitfalls with progressing sustainable urban water management. Water Science and Technology, 59(7), 1271-1280

Khadr M, Elshemy M 2017. Data-driven modeling for water quality prediction case study: The drains system associated with Manzala Lake, Egypt. Ain Shams Engineering Journal, 8(4), 549-557.

Khan AA, Paterson R, Khan H 2004. Modification and application of the Canadian Council of Ministers of the Environment Water Quality Index (CCME WQI) for the communication of drinking water quality data in Newfoundland and Labrador. Water Quality Research Journal, 39(3), 285-293.

Kuo JT, Hsieh M, H Lung WS, She N 2007. Using artificial neural network for reservoir eutrophication prediction. Ecological modelling, 200(1-2), 171-177.

Kuo JT, Wang YY, Lung WS 2006. A hybrid neural–genetic algorithm for reservoir water quality management. Water research, 40(7), 1367-1376.

Lee CC, Sheridan SC, Barnes BB, Hu C, Pirhalla DE, Ransibrahmanakul V, Shein K 2017. The development of a non-linear autoregressive model with exogenous input (NARX) to model climate-water clarity relationships:

reconstructing a historical water clarity index for the coastal waters of the southeastern USA. Theoretical and Applied Climatology, 130(1), 557-569.
Leichenko R 2011. Climate change and urban resilience. Current opinion in environmental sustainability, 3(3), 164-168.
Lermontov A, Yokoyama L, Lermontov M, Machado MAS 2009. River quality analysis using fuzzy water quality index: Ribeira do Iguape river watershed, Brazil. Ecological Indicators, 9(6), 1188-1197.
Li H, Wang X, Guo H 2022. Uncertain time series forecasting method for the water demand prediction in Beijing. Water Supply, 22(3), 3254-3270.
Li K, Huang G, Wang S, Razavi S, Zhang X 2022. Development of a Joint Probabilistic Rainfall-Runoff Model for High-to-Extreme Flow Projections Under Changing Climatic Conditions. Water Resources Research, 58(6), e2021WR031557.
Li L, Jiang P, Xu H, Lin G, Guo D, Wu H 2019. Water quality prediction based on recurrent neural network and improved evidence theory: a case study of Qiantang River, China. Environmental Science and Pollution Research, 26(19), 19879-19896.
Liu X, Zhang Y, Zhang Q 2022. Comparison of EEMD-ARIMA, EEMD-BP and EEMD-SVM algorithms for predicting the hourly urban water consumption. Journal of Hydroinformatics, 24(3), 535-558.
Liu P, Wang J, Sangaiah AK, Xie Y, Yin X 2019. Analysis and prediction of water quality using LSTM deep neural networks in IoT environment. Sustainability, 11(7), 2058.
Liu S, Xu L, Li D 2016. Multi-scale prediction of water temperature using empirical mode decomposition with back-propagation neural networks. Computers & Electrical Engineering, 49, 1-8.
Liu WC, Chen WB 2012. Prediction of water temperature in a subtropical subalpine lake using an artificial neural network and three-dimensional circulation models. Computers & Geosciences, 45, 13-25.
Lu H, Matthews J, Han S 2020. A hybrid model for monthly water demand prediction: a case study of Austin, Texas. AWWA Water Science, 2(2), e1175.
Lu H, Ma X 2020a. Hybrid decision tree-based machine learning models for short-term water quality prediction. Chemosphere, 249, 126169.
Lu H, Matthews J, Han S 2020b. A hybrid model for monthly water demand prediction: a case study of Austin, Texas. AWWA Water Science, 2(2), e1175.
Maier HR, Dandy GC 1996. The use of artificial neural networks for the prediction of water quality parameters. Water resources research, 32(4), 1013-1022.
Manzar MS, Benaafi M, Costache R, Alagha O, Mu'azu ND, Zubair M, ... & Abba SI 2022. New generation neurocomputing learning coupled with a hybrid neuro-fuzzy model for quantifying water quality index variable: A case study from Saudi Arabia. Ecological Informatics, 101696.
Mazhar S, Ditta A, Bulgariu L, Ahmad I, Ahmed M, Nadiri AA 2019. Sequential treatment of paper and pulp industrial wastewater: Prediction of water quality parameters by Mamdani Fuzzy Logic model and phytotoxicity assessment. Chemosphere, 227, 256-268.
Meerasri J, Sothornvit R 2022. Artificial neural networks (ANNs) and multiple linear regression (MLR) for prediction of moisture content for coated pineapple cubes. Case Studies in Thermal Engineering, 33, 101942.
Mei P, Li M, Zhang Q, Li G 2022. Prediction model of drinking water source quality with potential industrial-agricultural pollution based on CNN-GRU-Attention. Journal of Hydrology, 127934.
Mohammadi B, Safari MJS, Vazifehkhah S 2022. IHACRES, GR4J and MISD-based multi conceptual-machine learning approach for rainfall-runoff modeling. Scientific Reports, 12(1), 1-21.
Najah A, Elshafie A, Karim OA, Jaffar O 2009. Prediction of Johor River water quality parameters using artificial neural networks. European Journal of scientific research, 28(3), 422-435.
Narsimlu B, Gosain AK, Chahar BR 2013. Assessment of future climate change impacts on water resources of Upper Sind River Basin, India using SWAT model. Water resources management, 27(10), 3647-3662.
Nasir N, Kansal A, Alshaltone O, Barneih F, Sameer M, Shanableh A, Al-Shamma'a A 2022. Water quality classification using machine learning algorithms. Journal of Water Process Engineering, 48, 102920.
Nasseri M, Asghari K, Abedini MJ 2008. Optimized scenario for rainfall forecasting using genetic algorithm coupled with artificial neural network. Expert systems with applications, 35(3), 1415-1421.
Nayak JG, Patil LG, Patki VK 2021. Artificial neural network based water quality index (WQI) for river Godavari (India). Materials Today: Proceedings.
Nourani V, Komasi M 2013. A geomorphology-based ANFIS model for multi-station modeling of rainfall–runoff process. Journal of Hydrology, 490, 41-55.
Oladipo JO, Akinwumiju AS, Aboyeji OS, Adelodun AA 2021. Comparison between fuzzy logic and water quality index methods: A case of water quality assessment in Ikare community, Southwestern Nigeria. Environmental Challenges, 3, 100038.
Olyaie E, Banejad H, Chau KW, Melesse AM 2015. A comparison of various artificial intelligence approaches performance for estimating suspended sediment load of river systems: a case study in United States. Environmental monitoring and assessment, 187(4), 1-22.
Ongley ED 2000. Water quality management: design, financing and sustainability considerations-II. In Invited presentation at the World Bank's Water Week Conference: Towards a strategy for managing water quality management.
Othman F, Alaaeldin ME, Seyam M, Ahmed AN, Teo FY, Ming Fai C, ... El-Shafie A 2020. Efficient river water quality index prediction considering minimal number of inputs variables. Engineering Applications of Computational Fluid Mechanics, 14(1), 751-763.

Oyebode O, Ighravwe DE 2019. Urban water demand forecasting: a comparative evaluation of conventional and soft computing techniques. Resources, 8(3), 156.

Özerol G, Dolman N, Bormann H, Bressers H, Lulofs K, Böge M 2020. Urban water management and climate change adaptation: A self-assessment study by seven midsize cities in the North Sea Region. Sustainable Cities and Society, 55, 102066.

Pan X, Cai K, Wu L 2022. Using a Grey Niche Model to Predict the Water Consumption in 31 Regions of China. Water, 14(12), 1883.

Parmar KS, Makkhan SJS, Kaushal S 2019. Neuro-fuzzy-wavelet hybrid approach to estimate the future trends of river water quality. Neural Computing and Applications, 31(12), 8463-8473.

Paul V, Ramesh R, Sreeja P, Jarin T, Kumar PS, Ansar S, ... Said Z 2022. Hybridization of long short-term memory with Sparrow Search Optimization model for water quality index prediction. Chemosphere, 307, 135762.

Penalba OC, Vargas WM 2004. Interdecadal and interannual variations of annual and extreme precipitation over central-northeastern Argentina. International Journal of Climatology: A Journal of the Royal Meteorological Society, 24(12), 1565-1580.

Perea RG, Poyato EC, Montesinos P, Díaz JAR 2019. Optimisation of water demand forecasting by artificial intelligence with short data sets. Biosystems engineering, 177, 59-66.

Prathibha KS, Kumar RK, Joseph RS, Subramani S 2022. Predicting the parameters of water quality and calculating the Water Quality Index of Ulsoor Lake, Bangalore, India using Deep Learning Techniques. In 2022 International Conference on Advances in Computing, Communication and Applied Informatics (ACCAI) (pp. 1-11). IEEE.

Rajaee T 2010. Wavelet and Neuro-fuzzy Conjunction Approach for Suspended Sediment Prediction. CLEAN–Soil, Air, Water, 38(3), 275-286.

Rajaee T, Khani S, Ravansalar M 2020. Artificial intelligence-based single and hybrid models for prediction of water quality in rivers: A review. Chemometrics and Intelligent Laboratory Systems, 200, 103978.

Rene ER, Saidutta MB 2008. Prediction of water quality indices by regression analysis and artificial neural networks.

Rhif M, Ben Abbes A, Farah IR, Martínez B, Sang Y 2019. Wavelet transform application for/in non-stationary time-series analysis: a review. Applied Sciences, 9(7), 1345.

Ristow D, Henning E, Kalbusch A, Petersen CE 2021. Models for forecasting water demand using time series analysis: a case study in Southern Brazil. Journal of Water, Sanitation and Hygiene for Development, 11(2), 231-240.

Roshni T, Jha MK, Deo RC, Vandana A 2019. Development and evaluation of hybrid artificial neural network architectures for modeling spatio-temporal groundwater fluctuations in a complex aquifer system. Water Resources Management, 33(7), 2381-2397.

Savic DA, Walters GA, Davidson JW 1999. A genetic programming approach to rainfall-runoff modelling. Water resources management, 13(3), 219-231.

Sezen C, Partal T 2022. New hybrid GR6J-wavelet-based genetic algorithm-artificial neural network (GR6J-WGANN) conceptual-data-driven model approaches for daily rainfall–runoff modelling. Neural Computing and Applications, 1-25

Shah MI, Javed MF, Abunama T 2021. Proposed formulation of surface water quality and modelling using gene expression, machine learning, and regression techniques. Environmental Science and Pollution Research, 28(11), 13202-13220.

Shahabi M, Ghorbani MA, Naganna SR, Kim S, Hadi SJ, Inyurt S, ... Yaseen ZM 2022. Integration of Multiple Models with Hybrid Artificial Neural Network-Genetic Algorithm for Soil Cation-Exchange Capacity Prediction. Complexity, 2022.

Sharafati A, Asadollah SBHS, Shahbazi A 2021. Assessing the impact of climate change on urban water demand and related uncertainties: a case study of Neyshabur, Iran. Theoretical and Applied Climatology, 145(1), 473-487.

Sharma TC 2002. Canadian water quality index determination for four sites in the Mackenzie River Basin. Ecological Monitoring and Assessment Network, Burlington, ON, Canada, 58.

Shu W, Li J, Lan Z, Xu G, Nie J, Liu S 2021. Missing Value Imputation and Prediction of River Water Quality Based on GRU–Autoencoder with Input-decay. In 2021 40th Chinese Control Conference (CCC) (pp. 8169-8174). IEEE.

Siriwardene NR, Erera BJC, 2006. Selection of genetic algorithm operators for urban drainage model parameter optimisation. Mathematical and Computer Modelling, 44(5-6), 415-429.

Song C, Yao L 2022. Application of artificial intelligence based on synchrosqueezed wavelet transform and improved deep extreme learning machine in water quality prediction. Environmental Science and Pollution Research, 29(25), 38066-38082.

Stakhiv EZ 1998. Policy implications of climate change impacts on water resources management. Water Policy, 1(2), 159-175.

Stone B, Hess JJ, Frumkin H 2010. Urban form and extreme heat events: are sprawling cities more vulnerable to climate change than compact cities? Environmental health perspectives, 118(10), 1425-1428.

Sulaiman NAF, Shaharudin SM, Ismail S, Zainuddin NH, Tan ML, Abd Jalil Y 2022. Predictive Modelling of Statistical Downscaling Based on Hybrid Machine Learning Model for Daily Rainfall in East-Coast Peninsular Malaysia. Symmetry, 14(5), 927.

Sun AY, Wang D, Xu X 2014. Monthly streamflow forecasting using Gaussian process regression. Journal of Hydrology, 511, 72-81.

Tabbussum R, Dar AQ 2021. Performance evaluation of artificial intelligence paradigms—artificial neural networks, fuzzy logic, and adaptive neuro-fuzzy inference system for flood prediction. Environmental Science and Pollution Research, 28(20), 25265-25282.

Tealab A, Hefny H, Badr A 2017. Forecasting of nonlinear time series using ANN. Future Computing and Informatics Journal, 2(1), 39-47.

Than NH, Ly CD, Van Tat P 2021. The performance of classification and forecasting Dong Nai River water quality for sustainable water resources management using neural network techniques. Journal of Hydrology, 596, 126099.

Tiwari DK, Tiwari HL, Nateriya R 2022. Runoff modeling in Kolar river basin using hybrid approach of wavelet with artificial neural network. Journal of Water and Climate Change, 13(2), 963-974.

Tiwari S, Babbar R, Kaur G 2018. Performance evaluation of two ANFIS models for predicting water quality index of River Satluj (India). Advances in Civil Engineering, 2018.

Tung TM, Yaseen ZM 2020. A survey on river water quality modelling using artificial intelligence models: 2000–2020. Journal of Hydrology, 585, 124670.

UNEP 2012. Entegre Yaklaşımların Su Kaynakları Yönetimine Uygulanmasına İlişkin Durum Raporu

Verma R 2022. ANN-based Rainfall-Runoff Model and Its Performance Evaluation of Sabarmati River Basin, Gujarat, India. Water Conservation Science and Engineering, 1-8.

Waha K, Krummenauer L, Adams S, Aich V, Baarsch F, Coumou D, ... Schleussner CF 2017. Climate change impacts in the Middle East and Northern Africa (MENA) region and their implications for vulnerable population groups. Regional Environmental Change, 17(6), 1623-1638.

Wai KP, Chia MY, Koo CH, Huang YF, Chong WC 2022. Applications of Deep Learning in Water Quality Management: A State-of-the-Art Review. Journal of Hydrology, 128332.

Wallace JS, Acreman MC, Sullivan CA 2003. The sharing of water between society and ecosystems: from conflict to catchment–based co–management. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 358(1440), 2011-2026.

Wan H, Xu R, Zhang M, Cai Y, Li J, Shen X 2022. A novel model for water quality prediction caused by non-point sources pollution based on deep learning and feature extraction methods. Journal of Hydrology, 612, 128081.

Wolpert DH, Macready WG 1997. No free lunch theorems for optimization. IEEE transactions on evolutionary computation, 1(1), 67-82.

Wu B, Zhang Y, Hong H, Hu M, Liu H, Chen X, Liang Y 2019. Hydrophobic organic compounds in drinking water reservoirs: Toxic effects of chlorination and protective effects of dietary antioxidants against disinfection by-products. Water Research, 166, 115041.

Wu CL, Chau KW, Fan C 2010. Prediction of rainfall time series using modular artificial neural networks coupled with data-preprocessing techniques. Journal of Hydrology, 389(1-2), 146-167.

Wu J, Wang Z 2022. A hybrid model for water quality prediction based on an artificial neural network, wavelet transform, and long short-term memory. Water, 14(4), 610.

Xia J, Zeng J 2022. Early warning of algal blooms based on the optimization support vector machine regression in a typical tributary bay of the Three Gorges Reservoir, China. Environmental Geochemistry and Health, 1-15.

Xu Y, Zhang J, Long Z, Chen Y 2018. A novel dual-scale deep belief network method for daily urban water demand forecasting. Energies, 11(5), 1068.

Yan J, Xu Z, Yu Y, Xu H, Gao K 2019. Application of a hybrid optimized BP network model to estimate water quality parameters of Beihai Lake in Beijing. Applied Sciences, 9(9), 1863.

Yan Q, Ma C 2016. Application of integrated ARIMA and RBF network for groundwater level forecasting. Environmental Earth Sciences, 75(5), 1-13.

Yang H, Liu S 2022. Water quality prediction in sea cucumber farming based on a GRU neural network optimized by an improved whale optimization algorithm. PeerJ Computer Science, 8, e1000.

Ye Q, Yang X, Chen C, Wang J 2019. River water quality parameters prediction method based on LSTM-RNN model. In 2019 Chinese Control And Decision Conference (CCDC) (pp. 3024-3028). IEEE.

Yoosefdoost I, Khashei-Siuki A, Tabari H, Mohammadrezapour O 2022. Runoff simulation under future climate change conditions: Performance comparison of data-mining algorithms and conceptual models. Water Resources Management, 36(4), 1191-1215.

Zadeh LA 1978. Fuzzy sets as a basis for a theory of possibility. Fuzzy sets and systems, 1(1), 3-28.

Zhou S, Song C, Zhang J, Chang W, Hou W, Yang L 2022. A Hybrid Prediction Framework for Water Quality with Integrated W-ARIMA-GRU and LightGBM Methods. Water, 14(9), 1322.

Zotou I, Tsihrintzis VA, Gikas GD 2020. Water quality evaluation of a lacustrine water body in the Mediterranean based on different water quality index (WQI) methodologies. Journal of Environmental Science and Health, Part A, 55(5), 537-548.

## *ORAL PRESENTATION*

# Synthesis and characterization of some novel 1-(4-piperidinecarboxyamide-1-yl-methyl)-3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones

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**Abstract**

In this study, some 3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones) (2) were obtained from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (1) with 4-hydroxybenzaldehyde. The synthesized 2 type Schiff bases were reacted with formaldehyde and 4-piperidinecarboxyamide to afford 1-(4-piperidinecarboxyamide-1-yl-methyl)-3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (3). The structures of new compounds were characterized using IR, $^{1}$H NMR and $^{13}$C NMR spectroscopic methods

**Keywords:** 4,5-Dihydro-1*H*-1,2,4-triazol-5-one, Schiff base, Mannich base, Synthesis.

## INTRODUCTION

The triazole compounds are one of the very important structures used not only in organic chemistry but in anorganic chemistry, polymer chemistry, pharmaceutical chemistry, metal chemistry. These heteroaromatic compounds are composed of three nitrogen and two carbon atoms (Sahu et al., 2013). Since triazole nucleus has acidic hydrogen and three nitrogen atoms, ıt reacts easly and ıts new derivatives can be synthesized easly. Metal complexes can be synthesized as a result of reaction of triazoles with variety metal as Vo,Cu, Ru, Fe, Ni, Cd, Se, Pt and these derivatives have used in many field such as industry, pharmacology (El-Metwaly et al.,2020; Al-Radadi et al.,2020; Sumrra et al., 2020; Haasnoot et al.,2000). The Schiff Baze derivatives of triazole are found in many drugs such as flucanozole, itraconazole and are used new drug studies (Shcherbyna et al.,2018; Beyzaei et al.2019; Jia et al.,2019). The Mannich derivatives of triazole are also used in industry, polymer chemistry, agriculture, metal science (Almajan et al.,2009; Roman et al.,2015; Bala et.al.2013)
In this study, 2 type Schiff Base derivatives and 3 type Mannich Base derivatives of 1,2,4-triazoles were synthesized. Firstly, some of 3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones) (2) were obtained from the reactions of 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (1) with 4-hydroxybenzaldehyde. And then 2 type Schiff Bases were reacted with formaldehyde and 4-piperidine-carboxyamide to afford nine new 1-(4-piperidinecarboxyamide-1-yl-methyl)-3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones. The structure properties of new compounds were illuminated using a range of spectroscopic techniques such as IR, $^{1}$H NMR and $^{13}$C NMR.

## METERIALS AND METHODS

The chemical substances and solvents used in this experimental study were purchased from Merck AG, Aldrich and Fluka. The starting compounds 1,2,4-triazoles were synthesized according to method which developed from iminoester hydrochlorides synthesized by the pinner method. 3-Alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones were derivatived from 1 type 1,2,4- triazole compounds. 1 type 1,2,4-Triazole compounds were reacted with 4-hydroxybenzaldehyd in acetic acid medium for to obtain 2 type Schiff bases (Alkan, 2001; Boy, 2018). The 3-Alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones were reacted with formaldehyde and 4-piperidinecarboxyamide to get 3 type 1-(4-piperidinecarboxyamide-1-yl-methyl)-3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones *N*-Mannich bases through Mannich reaction. The IR spectra data of compounds were obtained on a Perkin-Elmer Instruments Spectrum One FT-IR spectrometer. $^{1}$H and $^{13}$C NMR spectra data also were recorded in deuterated dimethyl sulfoxide with TMS as internal standard using a Varian Mercury spectrometer at 200 MHz and 50 MHz, respectively.

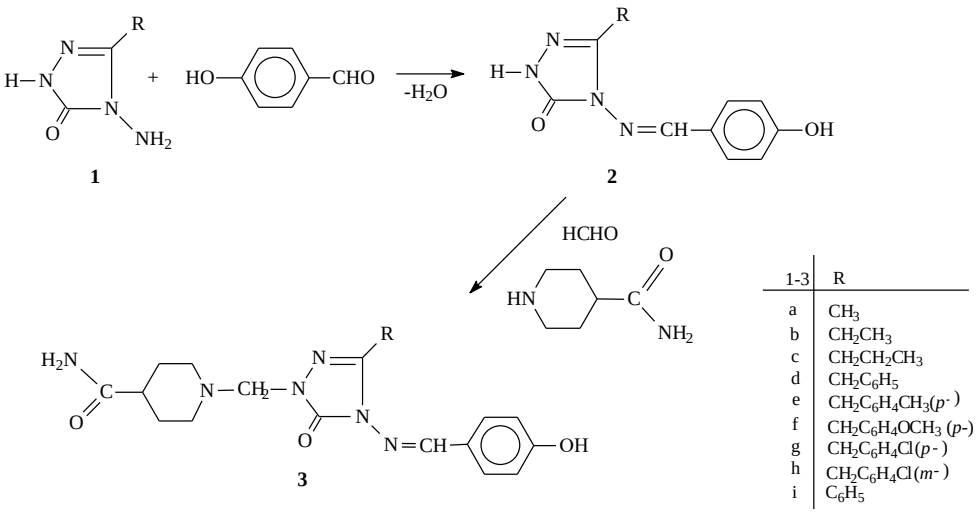


Figure 1. Synthesized scheme of type 3 compounds

**General procedure for the synthesis of compound 3.** 2 type 3-alkyl(aryl)-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones ( 0,01 mol) were dissolved in 100 ml ethyl alchol. 4-piperidine-carboxyamide (0,01 mol) and (0,02mol), (% 37) formaldehyde solution were added to this solution and boiled under reflux for 3 hours. The obtained triazole derivative compounds were cooled at room temperature and kept at -18 overnigt. The compounds were filtered and washed with cold alchol, dried and again crystallized several times in ethyl alchol. 3 Type triazole compounds were obtained after the dried in vacuum.

## RESULTS

**1-(4-Piperidinecarboxyamide-1-yl-methyl)-3-methyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3a):** Yield 82 %. M.P. 221 °C. IR (ATR) ($cm^{-1}$): 3410 (NH), 3156 (OH), 1686, 1577 (CO), 1595,1577 (C=N), 840 (1,4-disubstituted benzenoid ring). $^{1}$HNMR (DMSO-*d*6): δ 2,25 (s, $CH_3$), [1.46-1.49 (m, 2H, $CH_2$), 1.63-1.65 (m, 2H, $CH_2$), 1.90-1.93 (m, 1H, CH), 2.21-2.24 (m, 2H, $CH_2$), 2.86-2.89 (m, 2H, $CH_2$)] (Piperidine ring H), 4.49 (s, $NCH_2N$), 6.70 (s, $NH_2$), 6.86 (d, 2H, ArH; *J* = 8.00 Hz), 7.66 (d, 2H, ArH;, *J* =8.00 Hz), 9.49 (s, N=CH), 10.15(s, OH). $^{13}$CNMR (DMSO-*d*6): δ 10.10 ($CH_3$), [28.86 ($2CH_2$), 41.54 (CH), 50.17 ($2CH_2$)] (Piperidine ring C), 66.50 ($NCH_2N$), [116.33 (2CH), 124.66 (C), 130.23 (2CH), 161.20 (C)] (ArC), 143.26 (Triazole C3), 150.82 (Triazole C5), 155.47 (N=CH), 176.88 ($CONH_2$)

**1-(4-Piperidinecarboxyamide-1-yl-methyl)-3-ethyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3b):** Yield 84 %. M.P. 203 °C. IR (ATR) ($cm^{-1}$): 3366 (NH), 3193 (OH), 1680,1644 (CO), 1617, 1598 (C=N), 835 (1,4-disubstituted benzenoid ring). $^{1}$HNMR (DMSO-*d*6): δ 1,23 (t, $CH_3$; *J* = 7.20 Hz), 2.70 (q, $CH_2$; *J* = 7.20 Hz), [1.50-1.51 (m, 2H, $CH_2$), 1.67-1.70 (m, 2H, $CH_2$), 1.95-1.98 (m, 1H, CH), 2.25-2.30 (m, 2H, $CH_2$), 2.91-2.94 (m, 2H, $CH_2$), 2.92 (d, 2H, $CH_2$; *J*=11.60 Hz)] (Piperidine ring H), 4.56 (s, $NCH_2N$), 6.72, 7.17 (s, $NH_2$), 6.90 (d, 2H, ArH; *J* = 8.80 Hz), 7.70 (d, 2H, ArH; *J* =8.80 Hz), 9.53 (s, N=CH), 10.18 (s, OH). $^{13}$CNMR (DMSO-*d*6): δ 10.12 ($CH_2\underline{C}H_3$), 18.45 ($\underline{C}H_2CH_3$), [(28.41 ($2CH_2$), 41.11 (CH), 49.75 ($2CH_2$)], (Piperidine ring C), 66.31 ($NCH_2N$), [115.90 (2CH), 124.26 (C), 129.73 (2CH), 160.76 (C)] (ArC), 146.57 (Triazole C3), 150.51 (Triazole C5), 155.07 (N=CH), 176.39 ($CONH_2$)

**1-(4-Piperidinecarboxyamide-1-yl-methyl)-3-propyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3c):** Yield 75 %. M.P. 187 °C IR (ATR) ($cm^{-1}$): 3354 (NH), 3185 (OH), 1680, 1649 (CO), 1597 (C=N), 835 (1,4-disubstituted benzenoid ring). $^{1}$HNMR (DMSO-*d*6): δ 0.97 (t, $CH_3$), 1.66, 1.74 (sext), 2.67 (t, $CH_2$; *J* = 7.20 Hz), [1.50-1.57 (m, 2H, $CH_2$), 1.66-1.70 (m, 2H, $CH_2$), 1.94-1.97 (m, 1H, CH), 2.25-2.30 (m, 2H, $CH_2$), 2.91-2.94 (m, 2H, $CH_2$), 2.92 (d, 2H, $CH_2$; *J*=11.60 Hz)] (Piperidine ring H), 4.55 (s, $NCH_2N$), 6.72, 7.17 (s, $NH_2$), 6.90 (d, 2H, ArH; *J* = 8.40 Hz), 7.69 (d, 2H, ArH; *J* =8.80 Hz), 9.53 (s, N=CH), 10.18(s, OH). $^{13}$CNMR (DMSO-*d*6): δ 13.39 ($CH_2\underline{C}H_3$), 18.93, 26.58 ($\underline{C}H_2CH_3$), [28.43 ($2CH_2$), 41.12 (CH), 49.75 ($2CH_2$)] (Piperidine ring C) 66.26 ($NCH_2N$), [115.91 (2CH), 124.26 (C), 129.72 (2CH), 160.76 (C)] (ArC), 145.36 (Triazole C3), 150.45 (Triazole C5), 155.07 (N=CH), 176.37 ($CONH_2$)

**1-(4-Piperidinecarboxyamide-1-yl-methyl)-3-benzyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3d):** Yield 83%. M.P. 189 °C IR (ATR) ($cm^{-1}$): 3359 (NH), 3185 (OH), 1692,1648 (CO), 1601, 1581 (C=N), 832 (1,4-disubstituted benzenoid ring), 769 and 705 (monosubstituted benzenoid ring). $^{1}$HNMR (DMSO-*d*6): 4.08 (s, $CH_2$), [1.49-1.58 (m, 2H, $CH_2$), 1.68-1.70 (m, 2H, $CH_2$), 1.95-2.01 (m, 1H,

CH), 2.26-2.31 (m, 2H, $CH_2$), 2.92-2.95 (m, 2H, $CH_2$), 2.93 (d, 2H, $CH_2$; *J*=11.60 Hz) (Piperidine ring H), 4.57 (s, $NCH_2N$), 6.75,7.20 (s, $NH_2$), 6.89 (d, 2H, ArH; *J* = 8.40 Hz), 7.22-7.26 (m, 4H, ArH), 7.33-7.36 (m, 4H, ArH), 7.66 (d, 2H, ArH; *J* =8.80 Hz), 9.50 (s, N=CH), 10.18(s, OH). $^{13}$CNMR (DMSO-*d*6): δ 30.91 ($CH_2$), [28.41 ($2CH_2$), 41.11 (CH), 49.75 ($2CH_2$)] (Piperidine ring C), 66.39 ($NCH_2N$), [115.68 (2CH), 124.20 (C), 129.76 (2CH), 160.77 (C)] (ArC), [126.72 (CH), 128.47 (2CH), 128.67 (2CH), 135.76 (C)] (ArC linked to C3), 144.67 (Triazole C3), 150.36 (Triazole C5), 154.68 (N=CH), 176.38 ($CONH_2$)

**1-(4-Piperidinecarboxyamide-1-yl-methyl)-3-p-methylbenzyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3e):** Yield 81 %. M.P. 167 °C IR (ATR) ($cm^{-1}$): 3369 (NH), 3187 (OH), 1685,1647 (CO), 1608,1596 (C=N), 835 and 811 (1,4-disubstituted benzenoid ring). $^1$HNMR (DMSO-*d*6): 2.26 (s, $CH_3$), 4.01 (s, $CH_2$), [1.51-1.57 (m, 2H, $CH_2$), 1.67-1.70 (m, 2H, $CH_2$), 1.95-1.99 (m, 1H, CH), 2.28-2.31 (m, 2H, $CH_2$), 2.92-2.95 (m, 2H, $CH_2$), 2.93 (d, 2H, $CH_2$; *J*=11.20 Hz)] (Piperidine ring H), 4.56 (s, $NCH_2N$), 6.73, 7.19 (s, $NH_2$), 6.89 (d, 2H, ArH; *J* = 8.80 Hz), 7.13 (d, 2H, ArH; *J* =7.60), 7.21 (d, 2H, ArH; *J* =8.00), 7.66 (d, 2H, ArH, *J* =8.40 Hz), 9.49 (s, N=CH), 10.20 (s, OH). $^{13}$CNMR (DMSO-*d*6): δ 20.57 ($CH_2\underline{C}H_3$), (30.52 ($CH_2$), [28.41 ($2CH_2$), 41.12 (CH), 49.75 ($2CH_2$)] (Piperidine ring C), 66.37 ($NCH_2N$), [115.89 (2CH), 124.22 (C), 129.76 (2CH), 160.76 (C))] (ArC), [128.55 (2CH), 129.04 (2CH), 132.62 (C), 135.79 (C)] (ArC linked to C3), 144.81 (Triazole C3), 150.38 (Triazole C5), 154.68 (N=CH), 176.38 ($CONH_2$)

**1-(4-Piperidinecarboxyamide-1-yl-methyl)-3-*p*-methoxybenzyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3f):** Yield 79 %. M.P. 161 °C IR (ATR) ($cm^{-1}$): 3364 (NH), 3187 (OH), 1685, 1648 (CO), 1608, 1582 (C=N), 837 and 810 (1,4-disubstituted benzenoid ring). $^1$HNMR (DMSO-*d*6): 3.72 (s, $CH_3$), [1.48-1.57 (m, 2H, $CH_2$), 1.67-1.70 (m, 2H, $CH_2$), 1.95-2.01 (m, 1H, CH), 2.25-2.31 (m, 2H, $CH_2$), 2.92-2.95 (m, 2H, $CH_2$), 2.93 (d, 2H, $CH_2$; *J*=11.60 Hz)] (Piperidine ring H), 4.56 (s, $NCH_2N$), 6.73, 7.18 (s, $NH_2$), 6.89 (d, 2H, ArH; *J* = 8.00 Hz), 7.25 (d, 2H, ArH; *J* =8.80 Hz), 7.67 (d, 2H, ArH; *J* =8.80 Hz), 9.50 (s, N=CH), 10.20 (s, OH). $^{13}$CNMR (DMSO-*d*6): δ 55.02 ($CH_2\underline{C}H_3$), 30.06 ($CH_2$), [28.41 ($2CH_2$), 41.11 (CH), 49.75 ($2CH_2$)] (Piperidine ring C), 66.37 ($NCH_2N$), [115.90 (2CH), 124.24 (C), 129.76 (2CH), 160.76 (C)] (ArC), [113.92 (2CH), 127.51 (C), 129.74 (2CH), 158.10 (C)] (ArC linked to C3), 144.97 (Triazole C3), 150.39 (Triazole C5), 154.69 (N=CH), 176.38 ($CONH_2$)

**1-(4-Piperidinecarboxyamide-1-yl-methyl)-3-*p*-chlorobenzyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3g):** Yield 73 %. M.P. 187 °C IR (ATR) ($cm^{-1}$): 3338 (NH), 3232,3192 (OH), 1700,1637 (CO), 1596 (C=N), 842 and 805 (1,4-disubstituted benzenoid ring). $^1$HNMR (DMSO-*d*6): 4.09 (s, $CH_2$), [1.50-1.54 (m, 2H, $CH_2$), 1.67-1.70 (m, 2H, $CH_2$), 1.96-2.00 (m, 1H, CH), 2.25-2.31 (m, 2H, $CH_2$), 2.91-2.94 (m, 2H, $CH_2$), 2.92 (d, 2H, $CH_2$; *J*=11.60 Hz)](Piperidine ring H), 4.56(s, $NCH_2N$), 6.73,7.18 (s, $NH_2$), 6.88 (d, 2H, ArH; *J* = 8.80 Hz), 7.36-7.40 (m, 2H, ArH), 7.65 (d, 2H, ArH; *J* =8.80 Hz), 9.50 (s, N=CH), 10.19 (s, OH). $^{13}$CNMR (DMSO-*d*6): δ 30.06 ($CH_2$), [28.40 ($2CH_2$), 41.07 (CH), 49.72 ($2CH_2$)] (Piperidine ring C), 66.41 ($NCH_2N$), [115.90 (2CH), 124.15 (C), 129.79 (2CH), 160.81 (C)] (ArC), [128.42 (2CH), 130.61 (2CH), 131.45 (C), 134.75 (C) (ArC linked to C3),144.34 (Triazole C3), 150.37 (Triazole C5), 154.80 (N=CH), 176.36 ($CONH_2$)

**1-(4-Piperidinecarboxyamide-1-yl-methyl)-3-*m*-chlorobenzyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3h):** Yield 76 %. M.P. 180 °C IR (ATR) ($cm^{-1}$): 3450 (NH), 3346, 3184 (OH), 1697, 1645 (CO), 1612, 1597 (C=N), 838 (1,4-disubstituted benzenoid ring), 795 and 638 (1,3-disubstituted benzenoid ring). $^1$HNMR (DMSO-*d*6): 4.11 (s, $CH_2$), [1.50-1.54 (m, 2H, $CH_2$), 1.67-1.70 (m, 2H, $CH_2$), 1.68 (d, 2H, $CH_2$; *J*=10.40 Hz), 1.96-2.00 (m, 1H, CH), 2.25-2.31 (m, 2H, $CH_2$), 2.92 ,2.95 (m, 2H, $CH_2$), 2.93 (d, 2H, $CH_2$; *J*=11.60 Hz)] (Piperidine ring H), 4.57 (s, $NCH_2N$), 6.74, 7.18 (s, $NH_2$), 6.88 (d, 2H, ArH; *J* = 8.80 Hz), 7.28-7.33 (m, 3H, ArH), 7.38 (d, 1H, ArH; *J* =8.00 Hz), 7.66 (d, 2H, ArH; *J* =8.40 Hz), 9.50 (s, N=CH), 10.19 (s, OH). ). $^{13}$CNMR (DMSO-*d*6): δ 30.53 ($CH_2$), [28.41 ($2CH_2$), 41.12 (CH), 49.76 ($2CH_2$)] (Piperidine ring C), 66.42 ($NCH_2N$), [115.87 (2CH), 124.17 (C), 129.79 (2CH), 160.80 (C)] (ArC), [126.77 (CH), 127.43 (CH), 128.88 (CH), 130.30 (C), 132.97 (CH), 138.17(C)] (ArC linked to C3), 144.16 (Triazole C3), 150.35 (Triazole C5), 154.71 (N=CH), 176.36 ($CONH_2$)

**1-(4-Piperidinecarboxyamide-1-yl-methyl)-3-phenyl-4-(4-hydroxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (3i):** Yield 86 %. M.P. 212 °C. IR (ATR) ($cm^{-1}$): 3300 (NH), 3189 (OH), 1682, 1640 (CO), 1597, 1579 (C=N), 837 (1,4-disubstituted benzenoid ring), 768 and 692 (monosubstituted benzenoid ring). $^1$HNMR (DMSO-*d*6): [1.53-1.59 (m, 2H, $CH_2$), 1.70-1.72 (m, 2H, $CH_2$), 1.71 (d, 2H, $CH_2$; *J*=10.40 Hz), 1.68-2.02 (m, 1H, CH), 2.34-2.39 (m, 2H, $CH_2$), 2.98-3.01 (m, 2H, $CH_2$), 3.00 (d, 2H, $CH_2$; *J*=11.60 Hz)] **(**Piperidine ring H), 4.69 (s, $NCH_2N$), 6.74, 7.17 (s, $NH_2$), 6.91 (d, 2H, ArH; *J* = 8.80 Hz), 7.53-7.56 (m, 3H, ArH), 7.91-7.94 (m, 2H, ArH), 7.69 (d, 2H, ArH; *J* =8.80 Hz), 9.44 (s, N=CH), 10.28 (s, OH). $^{13}$CNMR (DMSO-*d*6): δ [28.45 ($2CH_2$), 41.50 (CH), 49.75 ($2CH_2$)] (Piperidine ring C), 66.82 ($NCH_2N$), [115.99 (2CH), 124.19 (C),

129.93 (2CH), 161.03 (C)] (ArC), [126.85 (C), 127.92 (2CH), 128.54 (2CH), 130.16 (CH] (ArC linked to C3), 142.92 (Triazole C3), 150.61 (Triazole C5), 158.12 (N=CH), 176.42 ($CONH_2$)

## CONCLUSION

In this research study, nine new Mannich Base derivatives (3a-i) that contain 1,2,4-triazole rings were synthesised. The characterization of novel compounds was analysed through infrared spectroscopy (IR), $^{1}H/^{13}C$ nuclear magnetic resonance (NMR).

## REFERENCES

Alkan, M., "Bazı 3-alkil(aril)-4-amino-4,5-dihidro-1*H*-1,2,4-triazol-5-on bileşiklerinin sentezi ve reaksiyonlarının incelenmesi", Doktora Tezi, KTÜ Fen Bilimleri Enstitüsü, Trabzon, (2001).

Almajan, G. L., Barbuceanu, S. F., Almajan, E. R., Draghici, C., & Saramet, G., Synthesis, characterization and antibacterial activity of some triazole Mannich bases carrying diphenylsulfone moieties. *European journal of medicinal chemistry*, *44*(7), 3083-3089, (2009).

Al-Radadi, N. S., Zayed, E. M., Mohamed, G. G., & Abd El Salam, H. A. (2020). Synthesis, spectroscopic characterization, molecular docking, and evaluation of antibacterial potential of transition metal complexes obtained using triazole chelating ligand. *Journal of Chemistry*, *2020*.

Bala, S., Sharma, N., Kajal, A., Kamboj, S., & Saini, V. (2014). Mannich bases: an important pharmacophore in present scenario. *International journal of medicinal chemistry*, *2014*.

Beyzaei, H., Khosravi, Z., Aryan, R., & Ghasemi, B., A green one-pot synthesis of 3 (5)-substituted 1, 2, 4-triazol-5 (3)-amines as potential antimicrobial agents. *Journal of the Iranian Chemical Society*, *16*(12), 2565-2573. (2019).

Boy, S., "Bazı 4, 5-dihidro-1H-1, 2, 4-triazol-5-on türevlerinden potansiyel biyolojik aktif yeni heterosiklik mannich bazlarının sentezi ve bazı özelliklerinin incelenmesi", Doktora Tezi, Kafkas üniversitesi, Fen Bilimleri Enstitüsü, Kars, (2018).

El-Metwaly, N., Farghaly, T. A., & Elghalban, M. G. (2020). Synthesis, analytical and spectral characterization for new VO (II)-triazole complexes; conformational study beside MOE docking simulation features. *Applied Organometallic Chemistry*, *34*(4), e5505.

Haasnoot, J. G. (2000). Mononuclear, oligonuclear and polynuclear metal coordination compounds with 1, 2, 4-triazole derivatives as ligands. *Coordination Chemistry Reviews*, *200*, 131-185

Jia, M., Wang, Y., Wang, D., Teng, M., Yan, J., Yan, S., ... & Zhu, W., The effects of hexaconazole and epoxiconazole enantiomers on metabolic profile following exposure to zebrafish (Danio rerio) as well as the histopathological changes. *Chemosphere*, *226*, 520-533, (2019).

Roman, G., Mannich bases in medicinal chemistry and drug design. *European Journal of Medicinal Chemistry*, *89*, 743-816, (2015).

Sahu, J. K., Ganguly, S., & Kaushik, A., Triazoles: A valuable insight into recent developments and biological activities. *Chinese journal of natural medicines*, *11*(5), 456-465, (2013).

Shcherbyna, R., Parchenko, V., Martynyshyn, V., & Hunchak, V., Evaluation of acute and subacute toxicity of oil liniment based on 4-((5-(decylthio)-4-methyl-4H-1, 2, 4-triazol-3-yl) methyl) morpholine. *Journal of Faculty of Pharmacy of Ankara University*, *42*(1), 43-52, (2018).

Sumrra, S. H., Habiba, U., Zafar, W., Imran, M., & Chohan, Z. H. (2020). A review on the efficacy and medicinal applications of metal-based triazole derivatives. *Journal of Coordination Chemistry*, *73*(20-22), 2838-2877.

## *ORAL PRESENTATION*

# Synthesis and characterization of some new Mannich bases

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**Abstract**

In the present study, a series of 1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4-(3-benzenesulfonyloxy-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (II) were synthesized from the reactions of 3-alkyl(aryl)-4-(3-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (I) with formaldehyde and morpholine. The newly synthesized compounds were fully characterized.

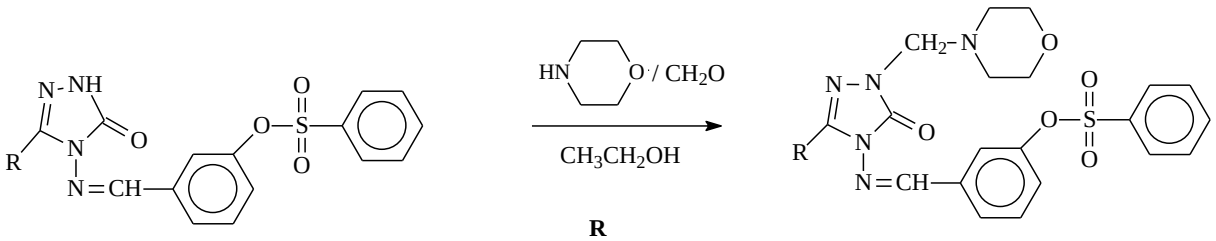
**Keywords:** Characterization, Synthesis, Mannich base

## INTRODUCTION

1,2,4-Triazole derivatives are recorded to own a wide variety of pharmacological activities like antimicrobial (Al-Abdullah et al., 2014), antioxidant (Gürsoy-Kol et al., 2014), anti-inflammatory (Uzgören-Baran et al., 2012), antiviral (Henen et al., 2012), antitumor (Demirbas et al., 2002), anti-HIV (Li et al., 2013), antihypertensive and diuretic (Ali et al., 2011) properties.

The Mannich reaction is an important reaction for the direct construction of new C-C and C-N bonds. Its endproducts, Mannich bases have withdrawed more and more attention of chemists owing to their specific biological activities, such as antiviral, acetylcholinesterase inhibitory, antioxidant, antiproliferative. What's more, Mannich bases are also key precursors to access amino alcohols, peptides, lactams and optically active amino acids (Xie et al., 2018).

Taking into account, about the development of new hetero moieties by combining potential biological active scaffolds, an venture was made here to obtain 1,2,4-triazoles bearing morpholine ring. In this respect, the synthesis of 5 novel 1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4-(3-benzenesulfonyloxy-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**IIa-e**) were synthesized by the reactions of 3-alkyl(aryl)-4-(3-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**Ia-e**) with formaldehyde and 4-piperidinecarboxamide (Scheme **1**).




**Scheme 1.** Synthesis route of compounds **II**

## EXPERİMENTAL

Chemical reagents and all solvents used in this study were purchased from Merck AG and Aldrich. Melting points which were uncorrect were determined in open glass capillaries using an Stuart SMP30 digital melting point apparatus. The IR spectra were obtained on a ALPHA-P BRUKER FT-IR spectrometer. $^{1}$H and $^{13}$C NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as internal standard using a Bruker Ultrashield Plus Biospin spectrometer at 400 MHz and 100 MHz, respectively.

### General procedure for the synthesis of 1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4-(3-benzenesulfonyloxy-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (II)

The corresponding compound **I** (5 mmol) (Harmankaya et al., 2018) was dissolved in 100 mL of ethanol followed by addition of morpholine (0.06 mol) and formaldehyde (37%, 10 mmol). The reaction mixture was refluxed for 3 hours. Then the reaction mixture were cooled and filtrated. Several recrystallizations of the residue from an appropriate solvent gave pure compounds **IIa-e** as colourless crystals..

*1-(morpholine-4-yl-methyl)-3-methyl-4-(3-benzenesulfonyloxy-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (IIa)*

White solid. Yield: 71 %; mp; 131°C. IR (cm$^{-1}$): 1702 (C=O), 1600-1599 (C=N), 1375-1190 ($SO_2$), 793 and 715 (1,3-disubstituted benzenoid ring), 775 and 683 (monosubstituted benzenoid ring). $^{1}$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: [2.35 (s, 3H, $CH_3$), 2.71 (t, 4H, $CH_2NCH_2$; $J$=4,80 Hz)], 3.71 (t, 4H, $CH_2OCH_2$; $J$=4,80 Hz)], 4.61 (s, 2H, $NCH_2N$), 7.09-7.12 (m,1H, ArH), 7.38 (t, 1H, ArH; $J$=8.00 Hz), 7.42 (t, 1H, ArH; $J$=2.00 Hz), 7.53-7.57 (m, 2H, ArH), 7.62 (d, 1H, ArH; $J$=8.00 Hz), 7.67-7.69 (m, 1H, ArH), 7.85-7.88 (m, 2H, ArH), 9.77 (s, 1H, N=CH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 11.34 ($CH_3$), 50.46 ($CH_2NCH_2$), 66.62 ($NCH_2N$), 66.81 ($CH_2OCH_2$), [120.84-125.08-127.03-128.52(2C)], 129.24(2C), [130.13-134.37-135.41-135.78-150,98] (ArC), 143.84 (Triazole $C_3$), 150.06 (Triazole $C_5$), [154.56- 152.07] (N=CH).

*1-(morpholine-4-yl-methyl)-3-ethyl-4-(3-benzenesulfonyloxy-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (IIb)*

White solid. Yield: 79 %; mp; -. IR (cm$^{-1}$): 1698 (C=O), 1587 (C=N), 1382-1190 ($SO_2$), 807 and 732 (1,3-disubstituted benzenoid ring), 755 and 687 (monosubstituted benzenoid ring); $^{1}$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.37 (t, 3H, $CH_2CH_3$; $J$=7.20 Hz), 2.69 (q, 2H, $CH_2CH_3$; $J$=7.20 Hz), 2.72 (t, 4H, $CH_2NCH_2$; $J$=4,40 Hz), 3.71 (t, 4H, $CH_2OCH_2$; $J$=4,40 Hz), 4.63 (s, 2H, $NCH_2N$), 7.12-7.13 (m, 1H, ArH), 7.38-7.40 (m, 2H, ArH), 7.53-7.56 (m, 2H, ArH), 7.62 (d, 1H, ArH; $J$=8.00 Hz), 7.67-7.70 (m, 1H, ArH), 7.85-7.88 (m, 2H, ArH), 9.79 (s, 1H, N=CH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 10.43 ($CH_2\underline{C}H_3$), 19.04 ($\underline{C}H_2CH_3$), 50.43 ($CH_2NCH_2$), 66.57 ($NCH_2N$), 66,70 ($CH_2OCH_2$), 120.74-125.06-127.02-128.50(2C), 129.42(2C), [130.14-134.30-135.36-135.83-151.13] (ArC), 147.75 (Triazole$_C$3), 150.06 (Triazole $C_5$), 151.97 (N=CH).

*1-(morpholine-4-yl-methyl)-3-benzyl-4-(3-benzenesulfonyloxy-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (IIc)*

White solid. Yield: 76 %; mp; 102°C. IR (cm$^{-1}$): 1690 (C=O), 1607 (C=N), 1371 and 1184 ($SO_2$), 796 and 736 (1,3-disubstituted benzenoid ring), 755 and 688 (monosubstituted benzenoid ring); $^{1}$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.72 (t, 4H, $CH_2NCH_2$; $J$=4,80 Hz), 3.72 (t, 4H, $CH_2OCH_2$; $J$=4,80 Hz), 4.05 (s, 2H, $CH_2Ph$), 4,65 (s, 2H, $NCH_2N$), 7.04-.07 (m, 1H, ArH), 7.25-7.27 (m, 1H, ArH), 7.32-7.37 (m, 5H, ArH), 7.44 (t, 1H, ArH; $J$=2.00 Hz), 7.51-7.55 (m, 3H, ArH), 7.65 (d, 1H, ArH; $J$=7.60 Hz), 7.85-7.87 (m, 2H, ArH), 9.71 (s, 1H, ArH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 31.82 ($CH_2Ph$), 50.46 ($CH_2NCH_2$), 66.72 ($NCH_2N$), 66,85 ($CH_2OCH_2$), 120.78-124.98-127.09-128.47(2C), 31.82 ($CH_2Ph$), 50.46 ($CH_2NCH_2$), 66.72 ($NCH_2N$), 66,85 ($CH_2OCH_2$), 120.78-124.98-127.09-128.47(2C), 129.25(2C)-130.09-134.38-135.08-135.81-151.01 (ArC), 127.17, 128.75(2C), 128.87(2C), 134.38 ($C_3$-ArC), 145.62 (Triazole $C_3$), 150.05 (Triazole $C_5$), 151.97 (N=CH).

*1-(morpholine-4-yl-methyl)-3-(p-methylbenzyl)-4-(3-benzenesulfonyloxy-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (IId)*

White solid. Yield: 80 %; mp; 98°C. IR ($cm^{-1}$): 1697 (C=O), 1607 (C=N), 1385 and 1192 ($SO_2$), 830 (1,4-disubstituted benzenoid ring), 801 and 704 (1,3-disubstituted benzenoid ring), 759 and 683 (monosubstituted benzenoid ring).$^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.32 (s, 3H, $PhCH_3$), 2.72 (t, 4H, $CH_2NCH_2$; $J$=4,80 Hz), 3.71 (t, 4H, $CH_2OCH_2$; $J$=4,80 Hz), 4.01 (s, 2H, $CH_2Ph$), 4,64 (s, 2H, $NCH_2N$), 7.05-7.07 (m, 1H, ArH), 7.13 (d, 2H, ArH; $J$=7.60 Hz), 7.21 (d, 2H, ArH; $J$=8.00 Hz), 7.35 (t, 1H, ArH; $J$=8.00 Hz), 7.45 (t, 1H, ArH; $J$=2.00 Hz), 7.51-7.56 (m, 3H, ArH), 7.65 (d, 1H, ArH; $J$=7.20 Hz), 7.85-7.88 (m, 2H, ArH), 9.70 (s, 1H, ArH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 21.05 ($PhCH_3$), 31.38 ($CH_2Ph$), 50.46 ($CH_2NCH_2$), 66.69 ($NC_{H2}N$), 66,85 ($CH_2OCH_2$), 120.79-124.92-127.08-128.48(2C), 129.25(2C), 130.09-134.38-135.43-135.85-151.02 (ArC), 128.76(2C), 129.44(2C), 131.97-136.82 ($C_3$-ArC), 145.82 (Triazole $C_3$), 150.05 (Triazole $C_5$), 151.92 (N=CH).

*1-(morpholine-4-yl-methyl)-3-(p-chlorobenzyl)-4-(3-benzenesulfonyloxy-benzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones (IIe)*

White solid. Yield: 80 %; mp; 122°C. IR ($cm^{-1}$): 1696 (C=O), 1572 (C=N), 1382 and 1193 ($SO_2$), 827 (1,4-disubstituted benzenoid ring), 800 and 705 (1,3-disubstituted benzenoid ring), 760 and 684 (monosubstituted benzenoid ring). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.71 (t, 4H, $CH_2NCH_2$; $J$=4,80 Hz), 3.72 (t, 4H, $CH_2OCH_2$; $J$=4,80 Hz), 4.03 (s, 2H, $CH_2Ph$), 4.65 (s, 2H, $NCH_2N$), 7.03-7.05 (m, 1H, ArH), 7.26-7.37 (m, 5H, ArH), 7.50-7.56 (m, 4H, ArH), 7.66 (d, 1H, ArH; $J$=7.60 Hz), 7.85-7.87 (m, 2H, ArH), 9.72 (s, 1H, ArH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 31.22 ($CH_2Ph$), 50.45 ($CH_2NCH_2$), 66.75 ($NCH_2N$), 66,83 ($CH_2OCH_2$), 120.66, 124.98-127.20-128.47(2C), 129.27(2C), 130.15, 31.22 ($CH_2Ph$), 50.45 ($CH_2NCH_2$), 66.75 ($NCH_2N$), 66,83 ($CH_2OCH_2$), 120.66-124.98-127.20-128.47(2C), 129.27(2C), 130.15-134.40-135.38-135.68-150.98 (ArC), 128.93(2C), 130.27(2C), 133.15-135.38 ($C_3$-ArC), 145.18 (Triazole $C_3$), 150.06 (Triazole $C_5$), 152.16 (N=CH).

## RESULTS and DISCUSSION

This study, the structures of five new 1-(morpholine-4-yl-methyl)-3-alkyl(aryl)-4-(3-benzenesulfonyloxy-benzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**IIa-e**), which were synthesized by the reactions of 3-alkyl(aryl)-4-(3-benzenesulfonyloxybenzylidenamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**Ia-e**) with formaldehyde and morpholine, were identified using IR, $^1$H- and $^{13}$C-NMR the observed spectral values were seen to be compatible with literature values (Yuksek et al., 2013; Aktas-Yokus et al., 2015].

## REFERENCES

Aktas-Yokus, O., Yuksek, H., Gursoy-Kol, O. & Alpay-Karaoglu, S. (2015). Synthesis and biological evaluation of new 1,2,4-triazole derivatives with their potentiometric titrations. *Medicinal Chemistry Research*, 24, 2813‑2824.

Al-Abdullah, E. S., Al-Tuwaijri, H. M., Hassan, H. M., Haiba, M. E., Haiba, E. E. & El-Amam, A. A. (2014). Antimicrobial and hypoglycemic activities of novel N-Mannich bases derived from 5-(1-adamantyl)-4-substituted-1,2,4-triazoline-3-thiones. *International Journal Molecular Sciences*, 15 (12), 22995‑23010.

Ali, K. A., Ragab, E. A., Farghaly, T. A., & Abdalla, M. M. (2011). Synthesis of new functionalized 3-substituted[1,2,4]triazolo[4,3-a]pyrimidine derivatives: potential antihypertensi ve agents. *Acta Poloniae Pharmaceutica*, *68*(2), 237–47.

Demirbaş, N., Ugurluoglu, R., & Demirbas, A. (2002). Synthesis of 3-alkyl(aryl)-4-alkylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones and 3-alkyl-4-alkylamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones as antitumor agents. *Bioorganic and Medicinal Chemistry*, *10*(12), 3717–3723. Doi:10.1016/S0968-0896(02)00420-0.

Gürsoy-Kol, Ö., & Ayazoglu, E. (2014). Antioxidant activities and acidic properties of some novel 4-[3,4-di-(4-nitrobenzoxy)-benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives. *Arabian Journal of Chemistry*, 1‑9. doi:10.1016/j.arabjc.2013.11.015.

Harmankaya, A., Yılmaz, Y., Beytur, M., Manap, S., Yüksek, H. 2018. Synthesis and Antioxidant Activities of Some Novel 3-(3-Alkyl-4,5-dihydro-1*H*-triazol-5-on-4-yl-azomethine)-phenyl Benzenesulfonates, *4th International Turkic Conference on Chemical Sciences and Technologies*, Sakarya University, Kiev, Ukraine, Book of Processing, S: 1-4, 7-10 September.

Henen, M. A., El Bialy, S. A. A., Goda, F. E., Nasr, M. N. A., & Eisa, H. M. (2012). [1,2,4]Triazolo[4,3-a]quinoxaline: Synthesis, antiviral, and antimicrobial activities. *Medicinal Chemistry Research*, *21*(9), 2368–2378. Doi:10.1007/s00044-011-9753-7.

Li, Z., Cao, Y., Zhan, P., Pannecouque, C., Balzarini, J., & Clercq, E. De. (2013). Synthesis and anti-HIV evaluation of novel 1,2,4-triazole derivatives as potential non nucleoside HIV-1 reverse transcriptase inhibitors. *Letters in Drug Design & Discovery*, *10*(1), 27–34. Doi:10.2174/1570180811309010027.

Uzgören-Baran, A., Tel, B. C., Sargöl, D., Öztürk, E. I., Kazkayas, I., Okay, G., Tozkoparan, B. (2012). Thiazolo[3,2-b]-1,2,4-triazole-5(6H)-one substituted with ibuprofen: Novel non-steroidal antiinflammatory agents with favorable gastrointestinal tolerance. *European Journal of Medicinal Chemistry*, *57*, 398–406. Doi:10.1016/j.ejmech.2012.07.009.

Xie, L., Cheng, J., Luo, Z., & Lu, G. (2018). Mannich reaction of indole with cyclic imines in water. Tetrahedron Letters, 59 pp. 457-461.

Yuksek, H., Akyıldırım, O., Yola, M. L., Gürsoy-Kol, Ö., Çelebier, M. & Kart, D. (2013). Synthesis, In vitro antimicrobial and antioxidant activities of some new 4,5-dihydro-1*H*-1,2,4-triazol-5-one Derivatives. *Archiv der Pharmazie*, 346, 470‑480.

## *ORAL PRESENTATION*

# Molecular, Electronic and Thermodynamic Properties of 3-Ethyl-4-(*p*-methylthiobenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one with Density Functional Theory

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## Abstract

3-Ethyl-4-(*p*-methylthiobenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one compund was obtained by the reaction of 3-ethyl-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one with 4-(methylthio)benzaldehyde in good yields. The 3-ethyl-4-(*p*-methylthiobenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one was optimized using B3LYP/6-311G(d,p) basis set. The molecular properties such as bond angles, bond length and dihedral angles of the studied compound were calculated using the B3LYP/6311G(d,p) basis set. The calculated IR data of compound were calculated in gas phase by using of the same basis sets of same methods. In the identification of calculated IR data was used the veda4f program. In Addition, $^{1}$H-NMR and $^{13}$C-NMR spectral data values were calculated according to the method of GIAO. The using the calculated the highest occupied molecular orbital energies ($E_{HOMO}$) and the lowest unoccupied molecular orbital energies ($E_{LUMO}$), electronic properties of the studied molecules such as energy gap ($\Delta E=E_{LUMO}-E_{HOMO}$), chemical potential (μ), electrophilic index (ω), ionization potential (IP), electron affinity (EA), electronegativity (χ), molecular softness (S), molecular hardness (η) were obtained. Finally, The thermodynamic parameters and Mulliken atomic charges of titled compound has been investigated the same basis set. All computational studies have been performed with the Gaussian 09W program.

**Keywords:** Triazol, B3LYP, Molecular, Electronic, Spectroscopic, Thermodynamic.

## INTRODUCTION

In the past years, by increasing development of computational chemistry, theoretically properties of Schiff bases were investigated. Quantum chemical calculation methods have widely been used to theoretically predict the structural, spectroscopic, thermodynamic and electronic properties of molecular systems. The quantum chemical calculation methods provide support for experimental structural and spectroscopic studies (Uğurlu et al., 2007; Beytur et al., 2019; Irak and Beytur, 2019; Uğurlu and Beytur, 2020; Beytur and Avinca, 2021). In the present study, 3-Ethyl-4-(*p*-methylthiobenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one (Yüksek and Kardaş, 2022) were optimized using DFT method and B3LYP/6-311G(d,p) basis set (Figure 1).

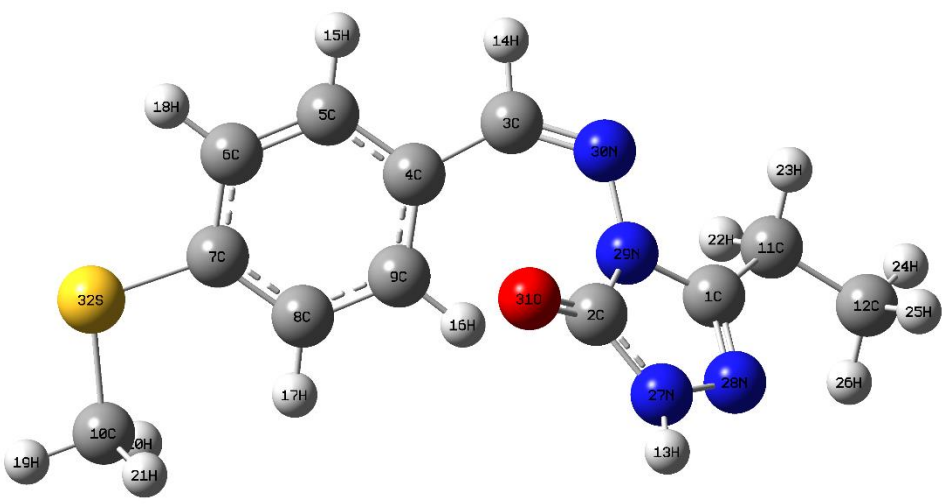


**Figure 1.** The optimized molecular structure (Gaussview Appearance) of titled molecule with B3LYP/6-311G (d,p) basis set.

## MATERIALS AND METHODS

Bond angles, bond lengths, dihedral angles of the molecule were calculated with B3LYP/6-311G(d,p) basis set and DFT method. The calculated IR data of titled compound were calculated in gas phase. The assignments of fundamental vibrational modes of the title molecule were performed on the basis of total energy distribution (TED) analysis by using VEDA 4f program (Jamroz, 2004). $^{1}$H-NMR and $^{13}$C-NMR spectral data values were calculated according to the method of GIAO. The chemical shift values obtained theoretically were compared to the experimental results and found to be compatible. The using the calculated the highest occupied molecular orbital energies and the lowest unoccupied molecular orbital energies, electronic properties of the studied molecules such as energy, chemical potential, electrophilic index, ionization potential, electron affinity, electronegativity, molecular softness, molecular hardness were obtained. Finally, The thermodynamic parameters and Mulliken atomic charges of titled compound has been investigated the same basis set. All quantum chemical calculations were carried out by using Gaussian 09W (Frisch et al., 2009; Wolinski, Hilton & Pulay, 1990) program package and the GaussView molecular visualization program (Frisch, Nielson & Holder, 2003).

## RESULTS and DISCUSSION

### Molecular Structures

In the study, in order to see the compatibility of the bond lengths of 3-ethyl-4-(*p*-methylthiobenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one with experimental data, the C-C bond lengths of the benzene ring and the C-H bond lengths compared with the experimental data recorded in the literature (Table 1). In the literature, all C-H bonds in the benzene ring are of equal length and measured as 1.084 $A^0$ (Gökçe et al., 2014). The C-H bond lengths measured as 1.082 $A^0$ according to the DFT calculated for the benzene ring found in the studied molecule. These results revealed that the calculated bond lengths were in full agreement with the experimental values (Yüksek and Kardaş, 2022). Another interesting result obtained from the examination of the theoretical bond lengths. When the bond lengths calculated according to the B3LYP method of the investigated compound examined, C2-N-H bond length found to be 1.372 $A^0$ according to B3LYP. It has been revealed that the values given in the literature are between C-C single bond length and C=C double bond length (Table 1). This showed that the electron pair remaining on the N atom and the carbonyl group delocalized because of resonance. The fact that the average length of the C2-N-H bond is shorter than that of B3LYP can be explained by the following resonance structures (Figure 2). The molecular geometrical parameters such as bond angles and dihedral angles of the the compound were calculated (Table 2 and Table 3).

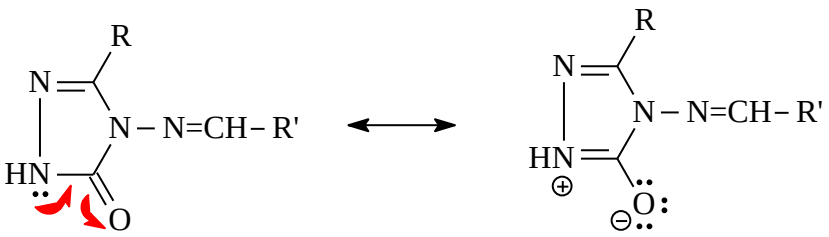


**Figure 2.** Resonance structures of 4,5-dihydro-1H-1,2,4-triazol-5-one

**Table 1.** The bond lengths of the titled compound calculated according to the B3LYP method

| Bond Lengths | | B3LYP ($A^0$) | Bond Lengths | | B3LYP ($A^0$) |
|---|---|---|---|---|---|
| **1** | C1-C11 | 1.4924 | **18** | C8-C9 | 1.3883 |
| **2** | C1-N28 | 1.2975 | **19** | C8-H17 | 1.0815 |
| **3** | C1-N29 | 1.3907 | **20** | C9-H16 | 1.0805 |
| **4** | C2-N27 | 1.3721 | **21** | C10-H19 | 1.0902 |
| **5** | C2-N29 | 1.4188 | **22** | C10-H20 | 1.0904 |
| **6** | C2-O31 | 1.2135 | **23** | C10-H21 | 1.0904 |
| **7** | C3-C4 | 1.4644 | **24** | C10-S32 | 1.8209 |
| **8** | C3-H14 | 1.0901 | **25** | C11-C12 | 1.5294 |
| **9** | C3-N30 | 1.2874 | **26** | C11-H22 | 1.0964 |
| **10** | C4-C5 | 1.4071 | **27** | C11-H23 | 1.0953 |
| **11** | C4-C9 | 1.403 | **28** | C12-H24 | 1.0923 |
| **12** | C5-C6 | 1.3837 | **29** | C12-H25 | 1.0917 |

| | | | | | |
|---|---|---|---|---|---|
| **13** | C5-H15 | 1.085 | **30** | C12-H26 | 1.0916 |
| **14** | C6-C7 | 1.4035 | **31** | H13-N27 | 1.0062 |
| **15** | C6-H18 | 1.0842 | **32** | N27-N28 | 1.3814 |
| **16** | C7-C8 | 1.401 | **33** | N29-N30 | 1.3926 |
| **17** | C7-S32 | 1.7736 | | | |

**Tablo 2.** The bond angles of the titled compound calculated according to the B3LYP method ($^0$)

| | **Bond Angles** | **B3LYP ($^0$)** | | **Bond Angles** | **B3LYP ($^0$)** |
|---|---|---|---|---|---|
| **1** | C11-C1-N28 | 125.9356 | **28** | H19-C10-H20 | 108.8733 |
| **2** | C11-C1-N29 | 122.696 | **29** | H19-C10-H21 | 108.9314 |
| **3** | N28-C1-N29 | 111.3493 | **30** | H19-C10-S32 | 105.1974 |
| **4** | N27-C2-N29 | 101.1969 | **31** | H20-C10-H21 | 110.6596 |
| **5** | N27-C2-O31 | 130.3305 | **32** | H20-C10-S32 | 111.5934 |
| **6** | N29-C2-O31 | 128.4722 | **33** | H21-C10-S32 | 111.384 |
| **7** | C4-C3-H14 | 113.5804 | **34** | C1-C11-C12 | 113.4032 |
| **8** | C4-C3-N30 | 135.5027 | **35** | C1-C11-H22 | 108.5045 |
| **9** | H14-C3-N30 | 110.8408 | **36** | C1-C11-H23 | 107.705 |
| **10** | C3-C4-C5 | 116.6712 | **37** | C12-C11-H22 | 110.4809 |
| **11** | C3-C4-C9 | 125.5606 | **38** | C12-C11-H23 | 111.0312 |
| **12** | C5-C4-C9 | 117.6182 | **39** | H22-C11-H23 | 105.3505 |
| **13** | C4-C5-C6 | 121.6384 | **40** | C11-C12-H24 | 109.9054 |
| **14** | C4-C5-H15 | 119.262 | **41** | C11-C12-H25 | 111.1781 |
| **15** | C6-C5-H15 | 119.0967 | **42** | C11-C12-H26 | 111.3268 |
| **16** | C5-C6-C7 | 120.1767 | **43** | H24-C12-H25 | 108.3441 |
| **17** | C5-C6-H18 | 119.8825 | **44** | H24-C12-H26 | 108.4068 |
| **18** | C7-C6-H18 | 119.9398 | **45** | H25-C12-H26 | 107.5732 |
| **19** | C6-C7-C8 | 118.7772 | **46** | C2-N27-H13 | 124.7185 |
| **20** | C6-C7-S32 | 116.6556 | **47** | C2-N27-N28 | 114.1321 |
| **21** | C8-C7-S32 | 124.5661 | **48** | H13-N27-N28 | 120.0741 |
| **22** | C7-C8-C9 | 120.6284 | **49** | C1-N28-N27 | 104.7625 |
| **23** | C7-C8-H17 | 120.7188 | **50** | C1-N29-C2 | 108.0532 |
| **24** | C9-C8-H17 | 118.6506 | **51** | C1-N29-N30 | 121.3989 |
| **25** | C4-C9-C8 | 121.1335 | **52** | C2-N29-N30 | 125.6749 |
| **26** | C4-C9-H16 | 120.4399 | **53** | C3-N30-N29 | 119.2735 |
| **27** | C8-C9-H16 | 118.4166 | **54** | C7-S32-C10 | 103.8931 |

**Table 3.** The dihedral angles of the titled compound calculated according to the B3LYP method ($^0$)

| | **Dihedral Angles** | **B3LYP ($^0$)** | | **Dihedral Angles** | **B3LYP** ($^0$) |
|---|---|---|---|---|---|
| **1** | N28-C1-C11-H22 | 118.6485 | **14** | N30-C3-C4-C5 | 168.0901 |
| **2** | N28-C1-C11-H23 | -127.781 | **15** | N30-C3-C4-C9 | -16.4882 |
| **3** | N29-C1-C11-H12 | 177.2211 | **16** | H20-C10-S32-C7 | -63.0269 |
| **4** | N29-C1-C11-H22 | -59.6244 | **17** | H21-C10-S32-C7 | 61.2007 |
| **5** | N29-C1-C11-H23 | 53.9462 | **18** | C1-C11-C12-H25 | -60.068 |
| **6** | C11-C1-N28-N27 | 179.4018 | **19** | C1-C11-C12-H26 | 59.8565 |
| **7** | C11-C1-N29-N30 | -18.8692 | **20** | H22-C11-C12-H24 | 57.9204 |
| **8** | N28-C1-N29-N30 | 162.6322 | **21** | H22-C11-C12-H26 | -62.2044 |
| **9** | N27-C2-N29-N30 | -162.281 | **22** | H23-C11-C12-H24 | -58.5901 |
| **10** | O31-C2-N29-C1 | 173.233 | **23** | H23-C11-C12-H25 | 61.3606 |
| **11** | O31-C2-N29-N30 | 17.9262 | **24** | H13-N27-N28-C1 | -171.426 |
| **12** | H14-C3-C4-C5 | -15.4703 | **25** | C1-N29-N30-C3 | 148.9969 |
| **13** | H14-C3-C4-C9 | 159.9514 | **26** | C2-N29-N30-C3 | -58.7351 |

**Vibrational frequencies**

The harmonic vibrational frequencies were calculated by using B3LYP method with the 6-311G(d,p) basis set. Theoretically found excess values can be adjusted by applying the scaling factors B3LYP (0.9608) (Foresman, 1996; Avcı, & Atalay, 2008). The titled compound has 32 atoms and the number of the normal vibrations are

90. The observed and calculated vibrational frequencies, the calculated IR intensities and assignments of selected vibrational frequencies for title compound are summarized in Table 4 and simulated IR spectra were given in Figure 3. It has been seen that the theoretical values and the experimental values are compatible (Yüksek and Kardaş, 2022).

**Table 4.** The calculated frequencies values of the molecule.

| Selected Vibrational Types | Experimental | scaled DFT |
|---|---|---|
| C-C, C-H | 809 | 826 |
| N=C | 1593 | 1584 |
| O=C | 1695 | 1711 |
| Ar C-H | 3052 | 3042 |
| Ar C-H | 3086 | 3063 |
| N-H | 3170 | 3497 |

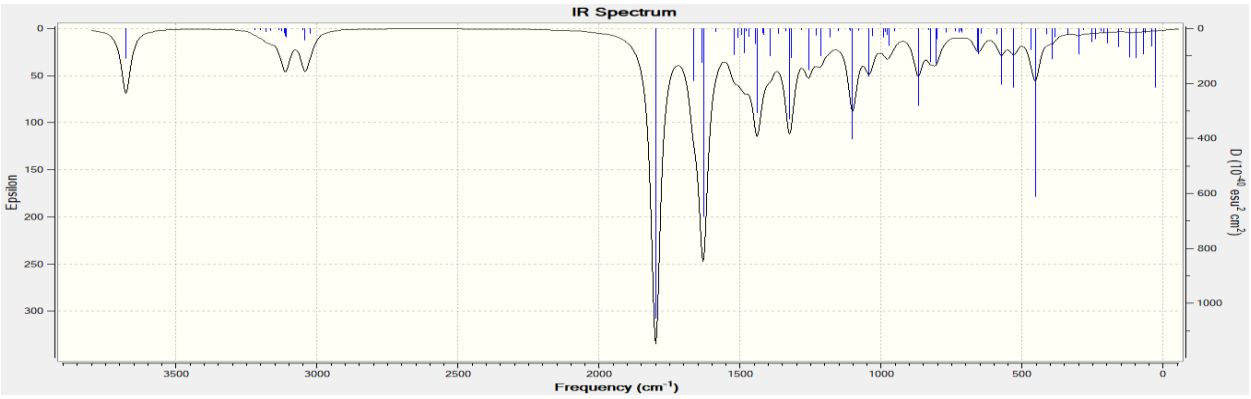


**Figure 3.** IR spectra simulated with DFT/B3LYP/6-311G(d,p) level of the titled compound.

**NMR Spectral Analysis**

In the present study, the optimized the 3-ethyl-4-(*p*-methylthiobenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one was obtained in DMSO solvent by using B3LYP method with 6-311G(d,p) basis level. The $^{1}$H and $^{13}$C NMR chemical shift values were calculated at the same level by using Gauge-Independent Atomic Orbital method (Table 5 and Figure 4) (Wolinski, Hinton, & Pulay, 1990; Wade, 2006). In Table 5, the $^{13}$C chemical shift value of the molecule are observed at 155,08, 149,77 and 153.22 ppm for the triazole C1, C2 and N=CH carbon atoms, respectively (Anderson et al., 2004). DMSO solvent the calculated ppm values (DFT/B3LYP). The $^{1}$H-NMR spectrum of the titled compound was observed belong to H13 proton peak at 11.80/7.54 (Experimental/B3LYP) ppm because acidic show feature (Yüksek, 1992; Yüksek et al., 2005; Yüksek et al., 2006). Theoretically, DMSO solvent these values for the mentioned proton atoms were found as 9.66/8.61 ppm (Experimental/B3LYP). It has been seen that the theoretical values and the experimental values are compatible (Yüksek and Kardaş, 2022).

**Table 5.** Theoretically and experimentally $^{13}$C and $^{1}$H-NMR (B3LYP/(DMSO)) chemical shift values of the titled molecule according to the TMS standard (δ/ppm)

| No | Experimen. | DFT/DMSO | Diff./DMSO | No | Experim. | DFT/DMSO | Diff./DMSO |
|---|---|---|---|---|---|---|---|
| **1C** | 155.08 | 158.02 | -2.94 | **13H** | 11.80 | 7.51 | 4.29 |
| **2C** | 149.77 | 153.59 | -3.82 | **14H** | 9.66 | 8.17 | 1.49 |
| **3C** | 153.22 | 165.28 | -12.06 | **15H** | 7.74 | 7.54 | 0.20 |
| **4C** | 131.65 | 132.84 | -1.19 | **16H** | 7.74 | 7.62 | 0.12 |
| **5C** | 129.75 | 138.57 | -8.82 | **17H** | 7.35 | 7.21 | 0.14 |
| **6C** | 127.33 | 129.67 | -2.34 | **18H** | 7.35 | 7.84 | -0.49 |
| **7C** | 144.65 | 158.47 | -13.82 | **19H** | 2.52 | 2.23 | 0.29 |
| **8C** | 127.33 | 125.43 | 1.90 | **20H** | 2.52 | 2.47 | 0.05 |
| **9C** | 129.75 | 134.10 | -4.35 | **21H** | 2.52 | 2.49 | 0.03 |
| **10C** | 15.94 | 17.52 | -1.58 | **22H** | 2.67 | 2.65 | 0.02 |
| **11C** | 20.33 | 23.11 | -2.78 | **23H** | 2.67 | 2.88 | -0.21 |
| **12C** | 11.83 | 7.74 | 4.09 | **24H** | 1.21 | 1.22 | -0.01 |
| | | | | **25H** | 1.21 | 1.22 | -0.01 |
| | | | | **26H** | 1.21 | 1.38 | -0.17 |

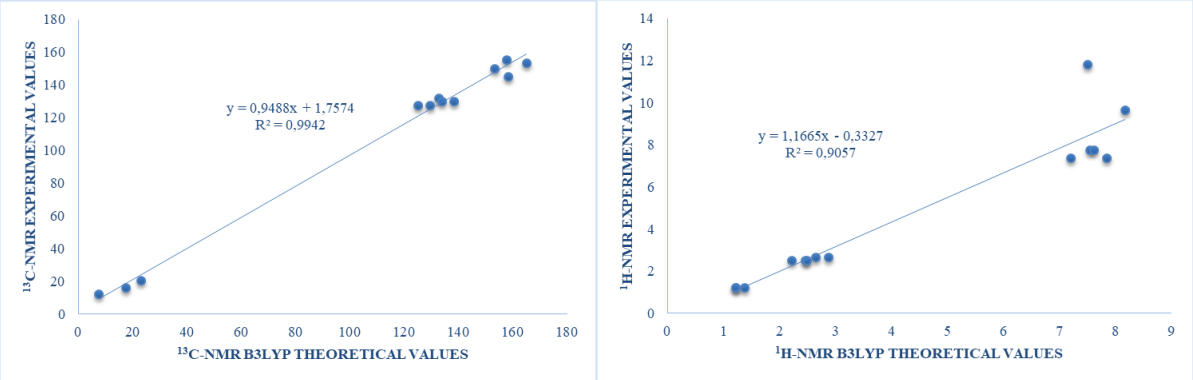


**Figure 4.** Regression analysis of the theoretical values calculated with experimental data of $^{13}$C-NMR and $^{1}$H-NMR chemical shift values by B3LYP method and 6-311G(d.p) basis set

## Electronic Properties

In these chemical reactions, HOMO energy is defined as electron donor tendency (π-donor), and LUMO energy is defined as electron acceptor tendency (π-acceptor) (Figure 5) (Fukui, 1982). The electronic properties were calculated by applying the 6-311G(d,p) diffused and polarized basis set and the B3LYP method to the molecule. From the calculated HOMO-LUMO energies, electronic parameters such as I; Ionization potential, A; electron affinity, η; molecular hardness, S; molecular softness and χ; electronegativity, total energies, dipole moment values were determined (Table 6).

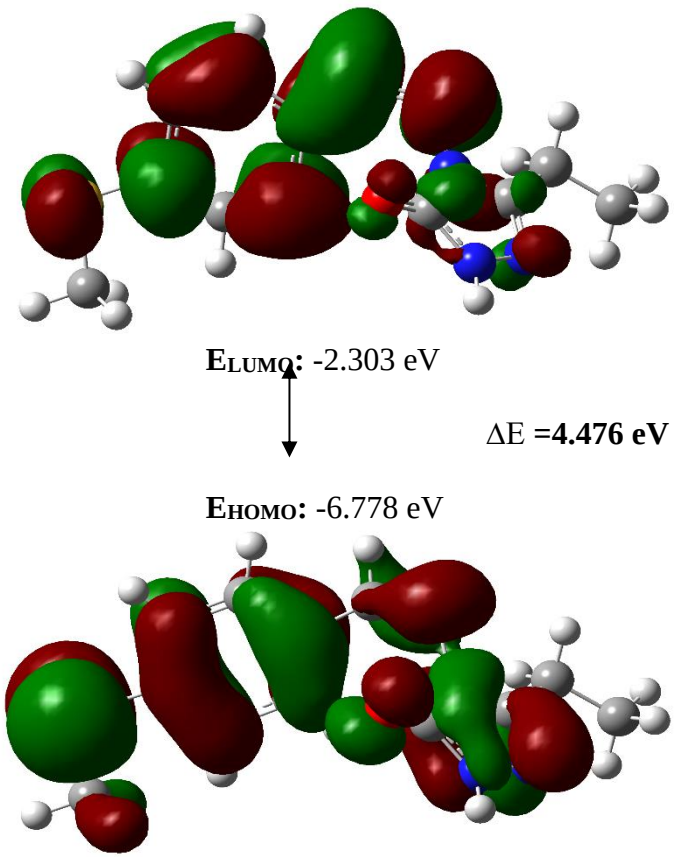


**Figure 5.** Calculated HOMO-LUMO shapes of the titled compound by B3LYP method 6-311G(d.p) basis set

**Table 6.** Electronic properties of the titled compound calculated according to B3LYP method and 6-311G(d.p) basis set

| Electronic Features | B3LYP (eV) | B3LYP (Kcal/mol) | B3LYP (KJ/mol) |
|---|---|---|---|
| A; Electron Affinity | 1,930 | 44,496 | 186,174 |
| I; Ionization Potential | 6,052 | 139,575 | 583,990 |
| ΔE; Energy Gap | 4,123 | 95,079 | 397,816 |
| χ; Electronegativity | 3,991 | 92,036 | 385,082 |
| μ; chemical potential | -3,991 | -92,036 | -385,082 |
| ω: electrophilic index | 3,863 | 89,090 | 372,756 |
| ε: Nucleophilic index | -0,302 | -6,973 | -29,174 |
| S; Molecular Softness | 1,031 | 23,770 | 99,454 |
| η; Molecular Hardness | 2,061 | 47,540 | 198,908 |

**Thermodynamic Properties**

Several thermodynamic parameters were calculated using the B3LYP method with the 6-311G(d,p) basis set of the compound whose structure was investigated, and these calculated parameters are given in Table 7. An accurate estimate of the zero-point vibrational energy and entropy is multiplied by the data. The change in total energies and total entropy of the related compound with the same theoretical method at room temperature is also presented.

**Table 7.** thermodynamic properties of titled compound

| | |
|---|---|
| Zero-point vibrational energy (Kcal/Mol) | 157.17884 |
| Thermal correction to Energy | 0.267955 |
| Thermal correction to Enthalpy | 0.268900 |
| Thermal correction to Gibbs Free Energy | 0.203011 |
| Sum of electronic and zero-point Energies | -1158.071 |
| Sum of electronic and thermal Energies | -1158.054 |
| Sum of electronic and thermal Enthalpies | -1158.053 |
| Sum of electronic and thermal Free Energies | -1158.119 |
| Thermal Energies E (Kcal/mol) | |
| Translational | 0.889 |
| Rotational | 0.889 |
| Vibrational | 166.367 |
| Total | 168.145 |
| Thermal Capacity CV (Cal/Mol-Kelvin) | |
| Translational | 2.981 |
| Rotational | 2.981 |
| Vibrational | 58.728 |
| Total | 65.687 |
| Entropy S (Cal/Mol-Kelvin) | |
| Translational | 42.590 |
| Rotational | 33.909 |
| Vibrational | 62.175 |
| Total | 138.673 |

**Mulliken Charge Analysis**

It is clear that in the molecule Mulliken populations give one of the simplest pictures of the charge distribution, and Mulliken charges form net atomic populations. The charge distributions of the studied molecule were calculated by Mulliken method at B3LYP/6-311G(d) basis levels. The results are given in Table 8.

**Table 8.** Mulliken atomic charge data of the molecule by B3LYP method 6-311 G(d.p) basis set.

| No | B3LYP | No | B3LYP | No | B3LYP |
|---|---|---|---|---|---|
| **1C** | -0.147935 | **12C** | -0.733380 | **23H** | 0.200120 |
| **2C** | 0.124850 | **13C** | 0.368985 | **24H** | 0.142018 |
| **3C** | -0.671233 | **14H** | 0.195701 | **25H** | 0.172736 |
| **4C** | 1.630997 | **15H** | 0.159652 | **26H** | 0.164919 |
| **5C** | -0.693259 | **16H** | 0.140691 | **27N** | -0.133910 |
| **6C** | -0.358540 | **17H** | 0.166701 | **28N** | -0.080164 |
| **7C** | 0.971681 | **18H** | 0.184828 | **29N** | 0.170494 |
| **8C** | -0.463164 | **19H** | 0.192865 | **30N** | -0.196852 |
| **9C** | -0.505496 | **20H** | 0.190424 | **31O** | -0.320813 |
| **10C** | -0.474973 | **21H** | 0.195941 | **32S** | -0.748534 |
| **11C** | -0.006511 | **22H** | 0.161161 | | |

## CONCLUSION

In the paper, 3-ethyl-4-(*p*-methylthiobenzylideneamino)-4,5-dihydro-1*H*-1,2,4-triazol-5-one was optimized by using Gaussian G09W software. The bond lengths of the compound named C-C bond lengths of the benzene ring and the d C-H bond lengths registered in the literature were compared. The molecular geometrical parameters such as bond angles and dihedral angles of the the compound were calculated. The harmonic vibrational frequencies were calculated by using B3LYP method with the 6-311G(d,p) basis set. It has been seen that the theoretical values and the experimental values are compatible. , the optimized titled compound was obtained in DMSO solvent by using B3LYP method with 6-311G(d,p) basis level. The $^{1}H$ and $^{13}C$ NMR chemical shift values were calculated at the same level by using Gauge-Independent Atomic Orbital method. It has been seen that the theoretical values and the experimental values are compatible. The electronic properties were calculated by applying the 6-311G(d,p) polarized basis set and the B3LYP method to the molecule from the calculated HOMO-LUMO energies. An accurate estimate of the zero-point vibrational energy and entropy is multiplied by the data. The change in total energies and total entropy of the related compound with the same theoretical method at room temperature is also presented. Finally, It is clear that in the molecule Mulliken populations give one of the simplest pictures of the charge distribution, and Mulliken charges form net atomic populations.

## REFERENCES

Anderson RJ, Bendell DJ, Groundwater PW 2004. Organic Spectroscopic Analysis, The Royal Society of Chemistry: Sanderland, UK.

Avcı D, Atalay Y 2008. Theoretical analysis of vibrational spectra and scaling-factor of 2-aryl-1,3,4-oxadiazole derivatives, International Journal of Quantum Chemistry, 109: 328-341.

Beytur M, Avinca I 2021. Molecular, Electronic, Nonlinear Optical and Spectroscopic Analysis of Heterocyclic 3-Substituted-4-(3-methyl-2-thienylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones: Experiment and DFT Calculations. Heterocyclic Communications, 27: 1-16.

Frisch A, Nielson AB, Holder AJ 2003.Gaussview User Manual, Gaussian, Inc., Wallingford, CT.

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery JA, Vreven T, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin N, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin LR, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ 2009. Gaussian Inc., Wallingford, CT.

Foresman JBE, Frisch (Ed.) 1996. Exploring Chemistry with Electronic Structure Methods: A Guide to Using Gaussian, Gaussian Inc., Pittsburg, PA.

Fukui K 1982. Role of frontier orbitals in chemical reactions, Science, 747-754

Gökçe H, Akyıldırım O, Bahçeli S, Yüksek H, Gürsoy-Kol Ö 2014. The 1-acetyl-3-methyl-4-[3-methoxy-4-(4-methylbenzoxy)benzylidenamino]-4,5-dihydro-1H-1,2,4-triazol-5-one molecule investigated by a joint spectroscopic and quantum chemical calculations. Journal of Molecular Structure, 1056-1057: 273-284.

Jamróz MH 2004. Vibrational Energy Distribution Analysis: VEDA 4 program, Warsaw.

Turhan Irak Z, Beytur M 2019. 4-Benzilidenamino-4,5-dihidro-1H-1, 2, 4-triazol-5-on Türevlerinin Antioksidan Aktivitelerinin Teorik Olarak İncelenmesi. Journal of the Institute of Science and Technology, 9 (1): 512-521.

Uğurlu G, Beytur M 2020. Theoretical Studies on The Structural, Vibrational, Conformational Analysis and Nonlinear Optic (NLO) Property of 4-(Methoxycarbonyl) Phenylboronic Acid. Indian Journal of Chemistry-Section A, 59 (10): 1504-1512.

Uğurlu G, Kasap E, Kantarci Z, Bahat . 2007. A theoretical study of the linear, nonlinear optical properties and conformational analysis of 3-phenylthiophene and its fluoro derivatives with torsional dependence. Journal of Molecular Structure, 834-836: 508–515.

Wade Jr LG 2006. Organic Chemistry, 6nd ed.; Pearson Prentice Hall: New Jersey.

Wolinski K, Hinton JF, Pulay P 1990. Efficient implementation of the gauge-independent atomic orbital method for NMR chemical shift calculations. Journal of the American Chemical Society, 112(23): 8251-8260.

Yüksek H 1992. 3-Alkil(aril)-4-amino-4,5-dihidro-1,2,4-triazol-5-on'ların bazı reaksiyonlarının incelenmesi. Doktora Tezi, KTÜ Fen Bilimleri Enstitüsü, Trabzon.

Yüksek H, Üçüncü O, Alkan M, Ocak Z, Bahçeci Ş, Özdemir M 2005. Synthesis and Determination of pKa Values of Some New 3,4-Disubstituted-4,5-Dihydro-1H-1,2,4-triazol-5-one Derivatives in Non-aqueous Solvents, Molecules 10: 961-970.

Yüksek H, Küçük M, Alkan M, Bahçeci S, Kolaylı S, Ocak Z, Ocak U, Şahinbas E, Ocak M 2006. Synthesis and antioxidant activities of some new 4-(4-hydroxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives with their acidic properties, Asian Journal of Chemistry, 18 (1): 539-550.

Yüksek H, Kardaş F 2022. Synthesis and Characterization of Novel Potential Biologically Active 3-Alkyl(Aryl)-4-(4-methylthiobenzylideneamino)-4,5-dihidro-1H-1,2,4-triazol-5-ones, The Pharmaceutical and Chemical Journal, 7(3): 19-22.

## ORAL PRESENTATION

# Antioxidant and Antimicrobial Properties of Silver Nanoparticles Synthesized from Sebin Walnut Oil

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**Abstract**

The origin of walnut samples in this study is from Giresun Province, Şebinkarahisar district, Çileder neighborhood, Çakır Village. The weight of the shell fruit at the origin is 9.40 g, the internal weight of the fruit is 6.60 g, the yield is 63% and the total oil content is 69.40%. It is a productive variety and bears fruit early. The number of fruits in the cluster is 2-4. Having a total tocopherol content of 36.3% plays a major role in the enhanced antioxidant properties of Sebin walnut. Considering the fatty acid content, it has been reported that Sebin walnut contains 55.03% linoleic acid, 14.17% linolenic acid, 19.06% oleic acid and 7.81% palmitic acid. The inner walnut is easily separated from the shell and comes out as a whole. When the tocopherol content of 100 g oil obtained from Sebin walnut was examined, the total amount of tocopherol was 36.9 mg, of which 1.4 mg α-Tocopherol, 33.1 mg γ + β-Tocopherol, 2.4 mg δ-Tocopherol were determined. In our study, we extracted the walnut oil from Sebin *Juglans Regia* fruits by using hexane extraction. The obtained walnut oil was used to reduction of the $AgNO_3$ to silver nanoparticles. Synthesized silver nanoparticles (AgNPs) showed promising antioxidant properties by using DPPH radical scavenging technique and Folin-Ciocalteu method. The antimicrobial effects of produced AgNPs were observed against to *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) bacteria cultures. According to Folin-Ciocalteu method results, the excellent correlation coefficient ($R^2$) value was found to be as 0.9608 for AgNPs obtained from Sebin Walnut Oil. The antibacterial activity of *E. Coli* and *S. Aureus* are observed and the area of death bacteria cells are expanded by 1.4 and 1.5 folds, respectively. The liquid bacteria cultures, *E. coli* and *S. Aureus* were exposed to synthesized AgNPs that were prepared as two different concentrations; 1.5 g/l and 2.5 g/l. As the AgNPs concentration increases, the bacteria cells concentration that belong to *E. Coli* and *S. Aureus* were decreased as 1 and 1.5 folds, respectively.

**Keywords:** Sebin Walnut, silver nanoparticles, antioxidant, antimicrobial

## 1.Introduction

Nanoparticles can be created using a variety of techniques, including physical, chemical, photochemical, and biological ones. Each technique has benefits and drawbacks. Cost, stability, particle size, and particle size dispersion are often encountered issues in nanoparticle production [1]. The use of natural resources to create environmentally friendly nanoparticles and nanostructures under mild reaction conditions is sparked by green and sustainable chemistry.

Noble metals have so far helped humanity. Due to their enhanced chemical, physical, and optical capabilities, the conversion of metals to their nanoscale is becoming increasingly important in the current environment [2,3]. The use of metal and metal oxide nanoparticles, such as $TiO_2$, $Ag_2O$, ZnO, and others, in the field of medicine has shown significant therapeutic value. Metallic NPs have also been used in applications for biosensors, diagnostic imaging, and medication administration [4,5].

Silver nanoparticles (AgNPs) are very poisonous to a variety of bacteria. These nanoparticles are frequently employed in biological research and environmental sanitation because of their antibacterial qualities. Due to their high surface to volume ratio and large active surface area, AgNPs exhibit exceptional features. However, due to this aspect of the active surface, the nanoparticles clump together [6].

In comparison to other biological processes, the generation of NPs by plants is more advantageous because the cell culture is preserved and maintained without disruption. In contrast to microbes, plant-mediated synthesis is a simple, one-step process with no mutation hazards. For the large-scale synthesis of NPs, extraction and separation may also be simply scaled up [7]. Plant components must have a variety of reducing agents with electron-shuttling chemicals, secondary metabolites, enzymes, and proteins in order to synthesize metal NPs. The location of NPs during the bioaccumulation process depends on the presence of particular enzymes or proteins. It takes time and money to collect these NPs from plant tissues, and enzymes are needed to break down the cellulose components that surround them [8]. Therefore, downstream processing and scaling up of NPs are simple when generating metal NPs utilizing plant extracts. There are three primary phases in the creation of metal nanoparticles from plant extracts: [i] initial phase; [ii] activation phase, [iii] termination phase. Metal ion reduction and the nucleation of reduced metal atoms take place in the first stage. Nanotubes, nanoprisms, nanohexahedrons, and other irregularly shaped NPs can all be formed more easily as a result of the

aggregation. As the metal NPs are stabilized by the plant extract, they eventually adopt the most energetically advantageous shape [9-11].

*Juglans regia* is said to have its genetic roots in Türkiye. Every region of the nation is home to wild walnut trees, which vary greatly in terms of fruit characteristics and vegetative growth. Particularly its natural hybrids and geographical variants, which have significant potential for use as cultivars, Türkiye is rich in genetic resources for walnut seedlings. The expansion of walnut agriculture in this nation will rely heavily on the use of this variability in the selection of exceptional seedlings with desirable features. These seedling selections are suitable for the soil and local climate, and they will also provide nuts of a high standard. From this population, the Persian walnut cultivar "Sebin" was chosen.

A standard in many Turkish regions that produce walnuts, "Sebin" is a Persian walnut cultivar that began as a seedling in Sebinkarahisar. The high kernel ratio, outstanding yield, high nut quality, and precocity of "Sebin" are all factors in its appeal. The mean nut weight ranged from 9.95 to 12.40 g, the mean kernel weight ranged from 6.25 to 7.85 g [12]. Sebin walnut (*Juglans regia*) has a medium growth rate and 50% of its lateral buds are fruitful. It begins to leaf out at the beginning of April and has nuts that weigh an average of 11 g and have 67% kernel. At the end of September, fruit begins to ripen [13,14]. Its shell thickness is 0.65 mm, its kernel weight is 7 g, its oil content is 65%, protein content is 18%, ash content is 1.7% [15].

In this study, we developed a synthesis method for the reputable Sebin walnut based silver nanoparticles (S-AgNPs) and then we examined the antioxidant and antibacterial effects of S-AgNPs. Firstly, we applied Folin-Ciocalteu and DPPH techniques in order to prove the antioxidant effects of S-AgNPs. Secondly, we tested the antibacterial effect of synthesized S-AgNPs on liquid and solid cultures of both Escherichia Coli (*E.coli*) and Staphylococcus aureus (*S. aureus*) bacteria types.

## 2. Materials and method

### 2.1 Materials

Walnut samples (Juglans regia L.) were collected during the September of 2022 harvest from Giresun Province, Şebinkarahisar district, Çileder neighborhood, Çakır Village that is located in the Black Sea region of northeastern Türkiye. Air-dried and unshelled samples were ground into small pieces by using a mortar. n-Hexane (98%), Silver nitrate ($AgNO_3$) and Sodium Hydroxide (99%) were purchased from ZAG Chemicals, Türkiye.

### 2.2 Experimental method

#### 2.2.1 Soxhlet extraction

Unshelled and dried samples (91,79 g) of walnut, were exhaustively extracted with n-hexane as a solvent in a 500-mL Soxhlet apparatus (Figure 1) for 6 h. Thereafter, the extracts were separated from the solvents by rotary evaporation. Total oil amounts obtained as 272,36 ml oil/kg dry sample were determined gravimetrically.



**Figure 1.** Soxhlet extraction apparatus for hexane extraction of Sebin Walnut

### 2.2.2 Synthesis of silver nanoparticles

A 100 ml volume of 1.25 mmol $AgNO_3$ solution was prepared. 100 ml of 1 mmol NaOH was prepared. The pH of the $AgNO_3$ solution was adjusted to 11 by dropping NaOH little by little into the $AgNO_3$ solution. 0.6 ml of Sebin walnut oil was added to AgNO3 solution adjusted to pH 11 and this solution was stirred in a magnetic stirrer for 6 hours.

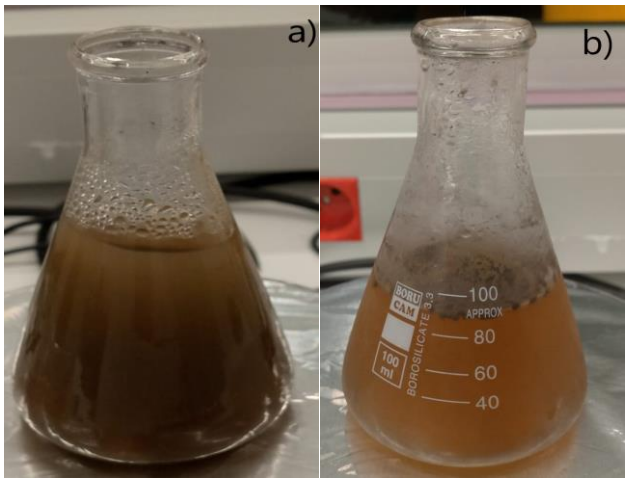


**Figure 2.** a) Before the reduction of $AgNO_3$ solution with the oil b) After the reduction of $AgNO_3$ to silver nanoparticles

After the reduction of the $AgNO_3$ to silver nanoparticles, the colour of the solution turned to dark greenish colour to orange-red-brown colour as seen in Figure 2.

### 2.3 Characterization studies

The biosynthesized nanoparticles were characterized using various analytical techniques. UV visible (UV-vis) spectra of S-AgNPs reaction mixture was recorded between 200 - 600 nm wavelength by using Shimadzu UV-1900i spectrophotometer, Japan. The functional groups were analysed by using Jasco FT/IR-4700 spectrometer of Japan over the wavelength range from 500 to 4000 $cm^{-1}$. Morphology studies of the synthesized nanoparticles were carried out by using scanning electron microscopy of Zeiss EVO M10 SEM, Jena, Germany. In order to prepare the samples for SEM characterization, the S-AgNPs solution was dripped and dried onto the microscope slides.

### 2.4 Antioxidant assay

In order to measure the antioxidant properties of silver nanoparticles synthesized by using Sebin Walnut oil (S-AgNPs), the Folin- Ciocalteu procedure was applied. First 6 test tubes were taken and labelled as blank and s1-s5 (sample 1 - sample 5). Secondly, 0.5 mL of Gallic acid sample solutions were added to the related labeled tubes (s1-s5). Thirdly, 0.5 mL of distilled water was added to the blank tube. After that, 2 mL of diluted Folin-Ciocalteu's phenol reagent was added to each tube and mixed well. In next step, 1 mL of $Na_2CO_3$ solution was added to each tube and mixed well. The samples were incubated at room temperature for 30 min in dark. Finally, their absorbances were measured at 765 nm against the blank. Also, for comparison, Gallic acid was used as the standard in the concentration range of 20-100 mg/L. The results were determined from the standard curve and were expressed as Gallic acid equivalent (mg GAE/mL of extract).

### 2.5 Antimicrobial assay

The *E. coli* (gram negative bacteria) and *S. aureus* (gram positive bacteria) were provided by University of Health Sciences, Haydarpasa Numune Training and Research Hospital, Microbiology Laboratuary, Türkiye. 100 ml distilled water was added to 4 g Luria Bertani (LB) broth-agar and then this LB both solution was autoclaved for 2 hours. After that, the LB broth-agar solution was pour into the petri dishes. After these broth was held for 2 hours and then *E. coli* and *S. aureus* bacteria cultures were cultivated in these petri dishes by using cell spreader. These bacteria cultivated sample bottles was held in drying oven at 37 ◦C for bacteria growth for 24 hours.

For the liquid bacteria cultures, 100 ml distilled water was added to 2.5 g Luria Bertani (LB) broth and then this LB broth solution was autoclaved for 2 hours. After that, the LB broth solution was pour into two different sample bottles for *E. coli* and *S. aureus*. Then, 1ml of each of the bacteria strains was put into separate sample bottles for *E. coli* and *S. aureus*. These bacteria cultivated sample bottles was held in incubator (Microtest) at 37 ◦C by applying the agitation at 150 rpm for bacteria growth for 24 hours.

## 3. Results and Discussion

The results of characterization studies, tests that were done for representing the antioxidant and antibacterial effects of S-AgNPs were presented and interpreted in this section.

### 3.1 UV-vis analysis results

The UV-vis analysis was performed to synthesized S-AgNPs. The UV-vis spectra of S-AgNPs is given in Figure 3.

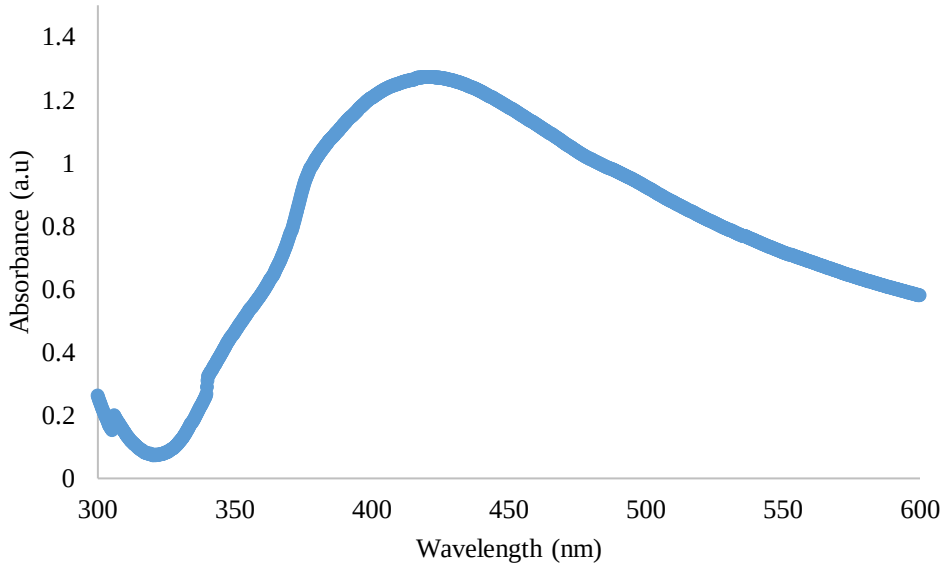


**Figure 3.** UV-vis analysis results of synthesized S-AgNPs.

The reaction mixture of silver nitrate with oil extract was turned into brown colour within 6 hours' incubation indicates starting of synthesis of silver nanoparticles. The SPR band was formed at 420 - 435 nm for silver nanoparticles synthesized from oil extract. Oil extract mediated synthesized silver nanoparticles shows SPR band at initial time of incubation at 420 nm with broadened indicates reduction process was started. The absorbance intensity was increased while increasing the time of reaction. After 24 hours, the maximum synthesis of nanoparticles was attained and the high peak at 420 nm was observed. This result is compatible with the previous studies [16,17].

### 3.2 FT-IR analysis results

In order to pinpoint the several chemical groups in biomolecules that appear to be in charge of the bio-reduction of $Ag^{+}$, capping, and stabilizing the S-AgNPs, FT-IR study was carried out. The obtained FT-IR spectra which belongs to S-AgNPs is presented in Figure 4.

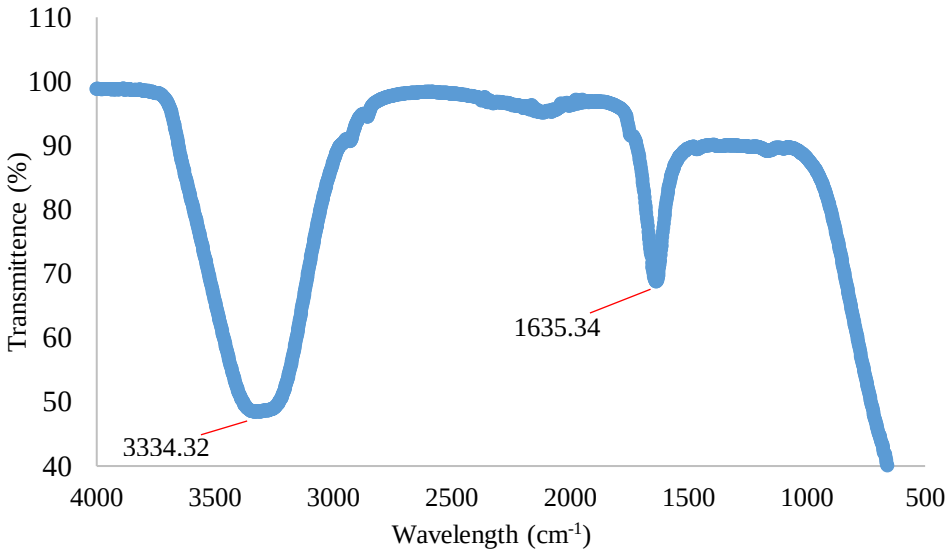


**Figure 4.** FT-IR spectra of silver nanoparticles synthesized using Sebin walnut oil

FT-IR spectrum shows the water soluble organic compounds from the oil extract may involve in the reduction of silver ions. A strong peak was observed at 3334.32 $cm^{-1}$ indicates presence of O-H stretching (phenolics) and N-H stretching of carboxylic acid and amides linkages groups in the organic molecules. O-H stretching vibration is brought on by the presence of phenols and alcohols [18]. A medium peak at 1635.34 $cm^{-1}$ shows C-H stretching of aromatics groups and represents the N–H (amine) band [19]. Also, in the spectra, this band is consistent with C-N and C-C stretching and denotes the presence of proteins. These carboxylic groups and amines from proteins of oil extract may cap the silver nanoparticles and form a layer on the surface of nanoparticles could acts reducing and stabilizing the nanoparticles.

### 3.3 SEM-EDX characterization results

The morphological characterization of silver nanoparticles synthesized from oil extract of Sebin Walnut (*Juglans Regia*) fruit was done by SEM (Figure 5a-b). SEM images represent the size, shape and distribution of nanoparticles. It illustrates that individual silver nanoparticles as well as number of aggregates and it illustrates the particles are predominantly spherical in shape with uniformly distribution. Figure 5c that including the EDX pattern demonstrates the crystalline and elemental composition of S-AgNPs.

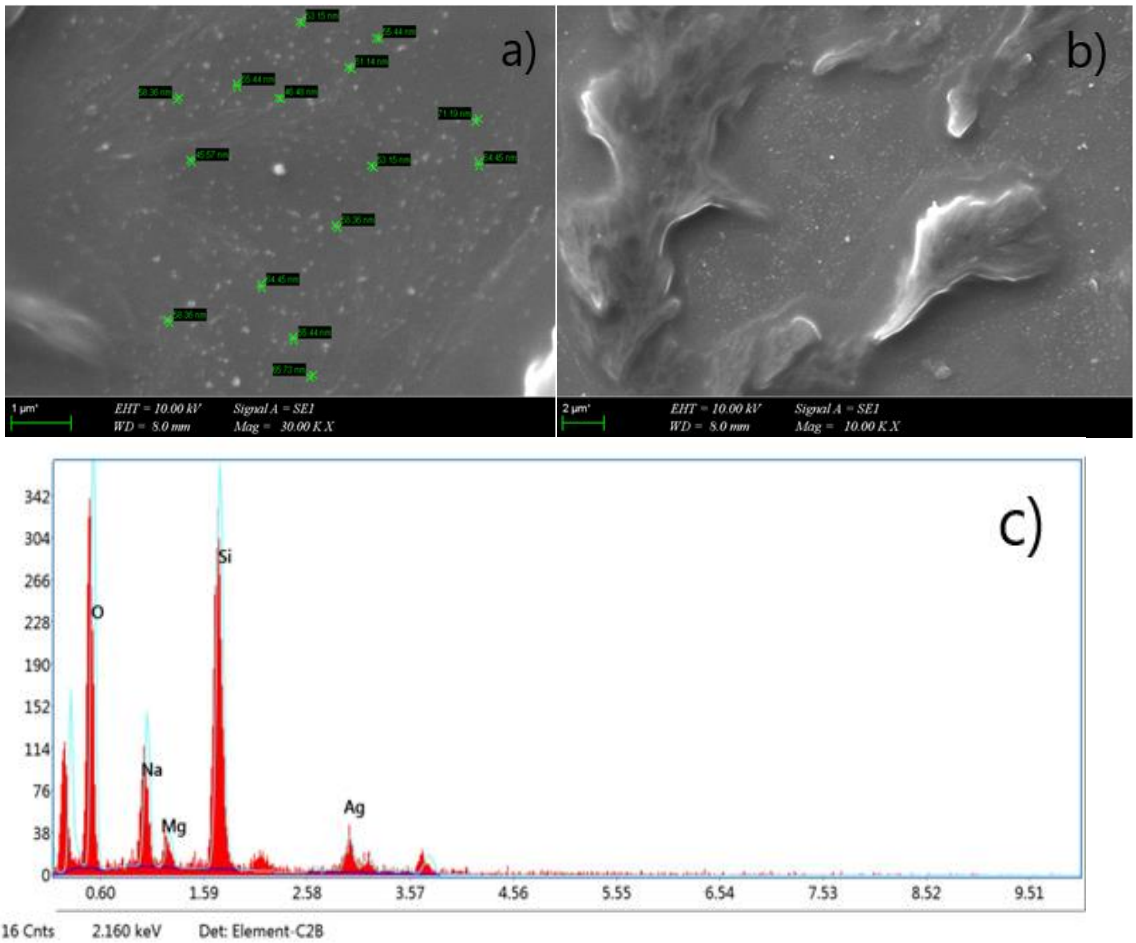


**Figure 5.** SEM-EDX results of S-AgNPs. (a) SEM image shows spherical shape of nanoparticles at a) 1µm and (b) 2 µm scale bar c) EDX report of AgNPs observed at 10 keV binding energy for Ag.

The average particle size of the synthesized nanoparticles was estimated as 57.781 nm according to SEM images. The SEM-EDX pattern representing the elemental composition of S-AgNPs was given in Table 1. According to EDX results, the weight (%) of Ag is the third highest element with 16.4 % after silica.

**Table 1.** EDX pattern representing the elemental composition of S-AgNPs

| Element | Weight (%) | Atomic (%) | Error (%) |
|---|---|---|---|
| O | 33,85 | 50,84 | 9,72 |
| Na | 13,55 | 14,16 | 9,67 |
| Mg | 2,77 | 2,74 | 16,99 |
| Si | 33,43 | 28,6 | 5,78 |
| Ag | 16,4 | 3,65 | 20,05 |

The significant signal in the silver region was observed at 10 keV for silver nanoparticles due to the surface plasmon resonance. Also another signal from 'O' is recorded it may due to the presence of organic moieties from the enzymes or proteins in the walnut oil. High silica content that is shown in Table 1 can be observed due to the microscope slide that we used during the sample preparation.

### 3.4 Antioxidant assay results

In order to determine the antioxidant capacity of S-AgNPs, Folin-Ciocalteu method was applied.

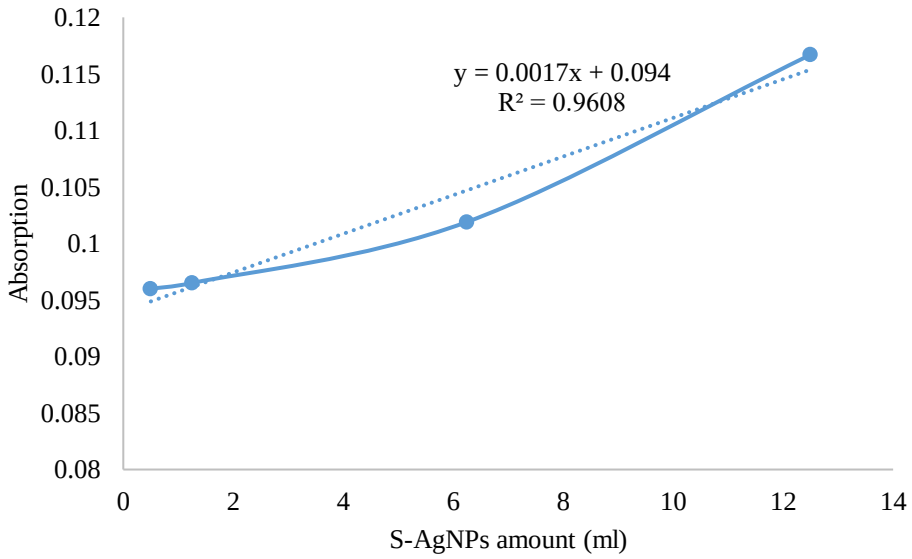


**Figure 5.** Folin-Ciocalteu method results of S-AgNPs

According to Folin-Ciocalteu results, the excellent correlation coefficient ($R^2$) value was found to be 0.9608 for AgNPs obtained from Sebin Walnut Oil as presented in Figure 5.

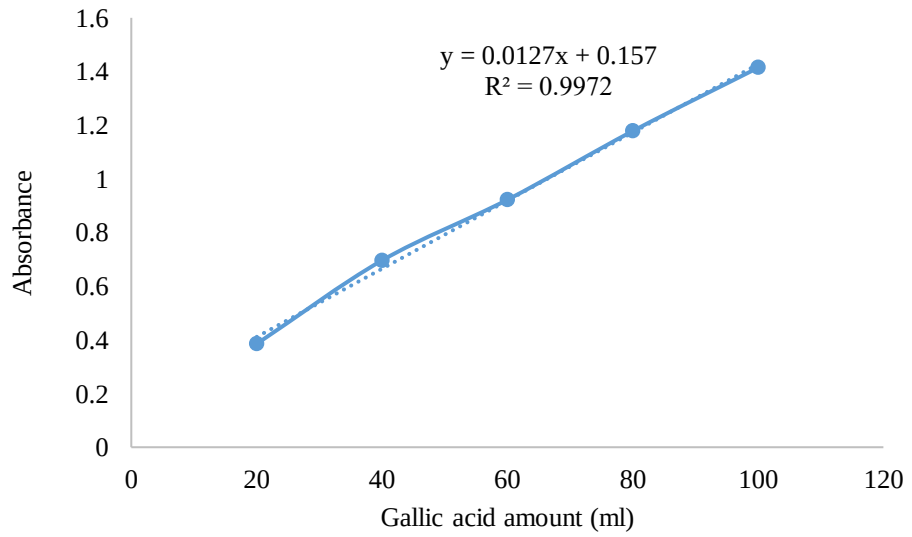


**Figure 6.** Folin-Ciocalteu method results of Gallic acid

According to Folin-Ciocalteu results, the excellent correlation coefficient ($R^2$) value was found to be 0.9972 for Gallic acid as presented in Figure 6.

### 3.5 Antibacterial assay results

#### 3.5.1 Antibacterial test results that conducting at solid form bacteria cultures

The antibacterial activity of *E. coli* and *S. aureus* are observed in Figure 7.

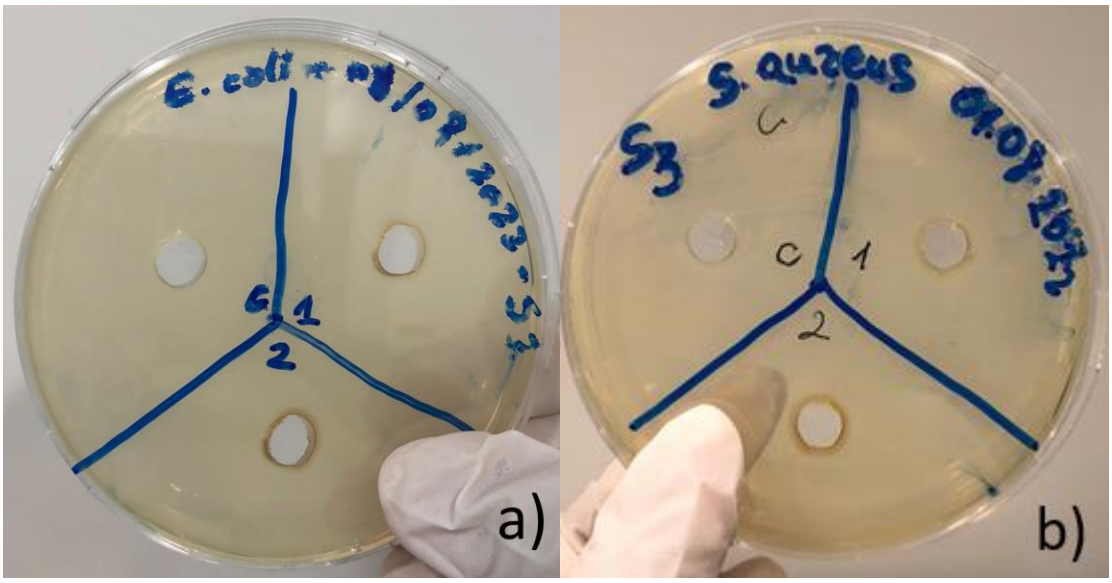


**Figure 7.** The images of the bacteria cultures (a)*E. Coli* and b) *S. Aureus*) after exposing to the S-AgNPs

After the expose of S-AgNPs, the areas of death bacteria cells are expanded by 1.4 and 1.5 folds for *E. coli* and *S. aureus*, respectively. The expansions of the perimeter of the area of bacteria cell death were given in Table 2.

**Table 2.** The expansion of the death area which different bacteria cell types on agar plate

| **Time (hr)** / **Bacteria Culture** | 1 | 2 | 3 | 36 |
|---|---|---|---|---|
| *E.coli* | 0.8 cm | 1 cm | 1 cm | 1.1 cm |
| *S. aureus* | 1 cm | 1 cm | 1.1 cm | 1.5 cm |

#### 3.5.2 Antibacterial test results that conducting at liquid form bacteria cultures

The liquid bacteria cultures, *E. coli* and *S. aureus* were exposed to synthesized S-AgNPs that were prepared as two different concentrations; 1.5 g/*l* and 2.5 g/*l*. The results were presented in Table 3.

**Table 3.** The decrease of the concentration of *E. coli* and *S. Aureus* bacteria cultures in liquid form in glass tube

| | ***E. coli*** | | | ***S. aureus*** | | |
|---|---|---|---|---|---|---|
| **$C_{AgNP}$** / **Time** | Control | 1.5 g/*l* | 2.5 g/*l* | Control | 1.5 g/*l* | 2.5 g/*l* |
| $t_0$ | 1.556 | 1.552 | 1.849 | 1.426 | 1.229 | 1.768 |
| $t_1$ | 1.151 | 1.689 | 1.846 | 1.484 | 1.668 | 1.936 |
| $t_2$ | 1.615 | 1.712 | 1.758 | 1.571 | 1.411 | 1.599 |
| $t_3$ | 1.741 | 1.782 | 1.913 | 1.594 | 1.532 | 1.897 |
| $t_{24}$ | 1.923 | 1.673 | 1.798 | 2.109 | 2.076 | 1.284 |

The decrease that have been observed on *E. coli* and *S. Aureus* were presented in Table 3. As S-AgNPs concentration increased, the bacteria cells concentration that belong to *E. Coli* and *S. Aureus* were decreased as 1 and 1.5 folds, respectively.

## 4. Conclusion

In this study, we developed a simple, fast, facile and green method for the synthesis of silver nanoparticles by using the oil extract from Sebin Walnut (*Juglans Regia*) fruit. After 24 hours from the synthesis, the maximum yield of nanoparticles was attained and the high peak at 420 nm was observed in UV-vis spectra. According to FT-IR results, a strong peak was observed at 3334.32 $cm^{-1}$ indicates presence of O-H stretching (phenolics) and N-H stretching of carboxylic acid and amides linkages groups in the organic molecules. Also, a medium peak at 1635.34 $cm^{-1}$ showing C-H stretching of aromatics groups and represents the N–H (amine) band was obstained in FT-IR spectra of S-AgNPs. The average particle size of the synthesized nanoparticles was estimated as 57.781 nm according to SEM images. According to EDX results, the weight (%) of Ag is the third highest element with 16.4 % after silica. High silica content can be observed due to the microscope slide that was used during the sample preparation. Also, another signal from 'O' is recorded it may due to the presence of organic moieties from the enzymes or proteins in the walnut oil. According to Folin-Ciocalteu results, the excellent correlation coefficient ($R^2$) value was found to be 0.9608 for S-AgNPs. According to the antibacterial test results that were conducted by using the solid form bacteria cultures, after the expose of S-AgNPs, the areas of death bacteria cells are expanded by 1.4 and 1.5 folds for *E. coli* and *S. aureus*, respectively. Also, according to the antibacterial test results that were conducted by using the liquid form bacteria cultures, as S-AgNPs concentration increased, the bacteria cells concentrations that belong to *E. Coli* and *S. Aureus* were decreased as 1 and 1.5 folds, respectively.

## Acknowledgement

This study was conducted at Bioengineering Basic and Application Laboratory of Üsküdar University. The authors thank to the head of Microbiology laboratory of Haydarpasa Numune Training and Research Hospital, Professor Sebahat Aksaray for providing *E. coli* and *S. aureus* bacteria cultures.

## Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## References

1. Rahimzadeh F, Ghadam P, Kasra-Kermanshahi R, Zarrabi M. In-situ production of silver nanobiocomposite using surface layer protein of Lactobacillus helveticus and aqueous extract of dried Juglans regia green husk and investigation of antibacterial activity. Polymer Bulletin. 2022;79(10):8353-67.
2. Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC. Nanoparticles in Medicine: Therapeutic Applications and Developments. Clinical Pharmacology & Therapeutics. 2008;83(5):761-9.
3. Yaqoob AA, Ahmad H, Parveen T, Ahmad A, Oves M, Ismail IMI, Qari HA, Umar K and Mohamad Ibrahim MN. Recent Advances in Metal Decorated Nanomaterials and Their Various Biological Applications: A Review. Front. Chem. 2020;8:341.
4. Yaqoob AA, Ahmad H, Parveen T, Ahmad A, Oves M, Ismail IMI, Qari HA, Umar K and Mohamad Ibrahim MN. Recent Advances in Metal Decorated Nanomaterials and Their Various Biological Applications: A Review. Front. Chem. 2020;8:341.
5. Katas H, Moden NZ, Lim CS, Celesistinus T, Chan JY, Ganasan P, Suleman Ismail Abdalla S. Biosynthesis and Potential Applications of Silver and Gold Nanoparticles and Their Chitosan-Based Nanocomposites in Nanomedicine. Journal of Nanotechnology, 2018;2018:1-13.
6. Salayova A, Bedlovicova Z, Daneu N, Balaz M, Lukacova Bujnakova Z, Balazova L, Tkacikova L. Green synthesis of silver nanoparticles with antibacterial activity using various medicinal plant extracts: morphology and antibacterial efficacy. Nanomaterials, 2021;11:1–20.
7. Veerasamy R, Xin TZ, Gunasagaran S, Xiang TFW, Yang EFC, Jeyakumar N, et al. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. Journal of Saudi Chemical Society. 2011;15(2):113-20.
8. Marshall AT, Haverkamp RG, Davies CE, Parsons JG, Gardea-Torresdey JL, van Agterveld D. Accumulation of Gold Nanoparticles in Brassic Juncea. International Journal of Phytoremediation. 2007;9(3):197-206.
9. Sigel H. (2000). Metal Ions in Biological Systems: Volume 37: Manganese and Its Role in Biological Processes (1st ed.). CRC Press.
10. Si S, Mandal TK. Tryptophan-based peptides to synthesize gold and silver nanoparticles: A mechanistic and kinetic study. Chemistry - A European Journal. 2007;13(11):3160-8.
11. Kim J, Rheem Y, Yoo B, Chong Y, Bozhilov KN, Kim D, Sadowsky MJ, Hur HG, Myung NV. Peptide-mediated shape-and size-tunable synthesis of gold nanostructures. Acta Biomaterialia. 2010;6(7):2681-9.
12. Akça Y. 'Şebin' Persian Walnut. Acta Hortic. 2014;1050,139-142.
13. Akca Y, Ozongun S. Selection of late leafing, late flowering, laterally fruitful walnut (*Juglans regia*) types in Turkey. New Zealand Journal of Crop and Horticultural Science, 2004;32(4):337-342.
14. Beyhan, O., GözIekçi, S., Gündoğdu, M., & Ercişli, S. (2016). Physico-Chemical and Antioxidant Characteristics in Fruits of Walnut (Juglans regia L.) Genotypes from Inner Anatolia. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44(2), 586–592.

**15.** Ertürk U, Şisman T, Yerlikaya C, Ertürk O, Karadeniz T. Chemical Composition and Nutritive Value of Selected Walnuts (*Juglans Regia L.*) from Turkey. Acta Horticulturae, 2014;1050:231-234.
**16.** Vilas V, Philip D, Mathew J. Catalytically and biologically active silver nanoparticles synthesized using essential oil. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;132:743-50.
**17.** Kumar R, Sharma P, Bamal A, Negi S, Chaudhary S. A safe, efficient and environment friendly biosynthesis of silver nanoparticles using Leucaena leucocephala seed extract and its antioxidant, antimicrobial, antifungal activities and potential in sensing. 2017;6(5):449-59.
**18.** Melkamu WW, Bitew LT. Green synthesis of silver nanoparticles using Hagenia abyssinica (Bruce) J.F. Gmel plant leaf extract and their antibacterial and anti-oxidant activities. Heliyon. 2021 Nov 24;7(11):e08459.
**19.** Huq MA. Green Synthesis of Silver Nanoparticles Using Pseudoduganella eburnea MAHUQ-39 and Their Antimicrobial Mechanisms Investigation against Drug Resistant Human Pathogens. Int J Mol Sci. 2020 Feb 22;21(4):1510.

## ORAL PRESENTATION

# Green synthesis of silver nanoparticles from Tarragon (*Artemisia dracunculus*) leaves and their antioxidant and antimicrobial activities

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**Abstract**

The most secure, environmentally friendly, economical, and risk-free method of producing nanoparticles (NPs) is known as "green synthesis." The aqueous leaf extract of Tarragon (*Artemisia dracunculus*) was used in the current work as a reducing, capping, and stabilizing agent during the green production of silver nanoparticles (AgNPs). In this study, we developed a fast and economical way for synthesis of Tarragon based AgNPs (T-AgNPs). Then, we observed the synthesized T-AgNPs by UV-vis spectroscopy results. According to UV-vis spectroscopy results, our synthesized T-AgNPs gave a peak at 420 nm. Then, we tested the antioxidant activities of the produced T-AgNPs by DPPH radical scavenging and Folin-Ciocalteu methods. According to Folin-Ciocalteu results, the excellent correlation coefficient ($R^2$) value was found to be 0.984 for T-AgNPs obtained from Tarragon (*Artemisia dracunculus*). For the antibacterial capabilites of the synthesized nanoparticles, we observed both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. Increasing the T-AgNPs amount 2 times raised up the antioxidant capability by 37%. The liquid bacteria cultures, *E.coli* and *S. aureus* were exposed to synthesized T-AgNPs that were prepared as different amounts between 0.6 - 1.8 ml by increasing 0.3 ml steps. *E.coli* growth was reduced from 64.67% to 6.41-9.79% with the addition of T-AgNP compared to the control group. Also, the decrease of the concentration of *S. aureus* culture in liquid form in glass tube was presented in Table 2. While *S. aureus* growth was 7.72% in the control group, this growth increased to 12.52% for 0.6 and 1.8 ml values with the addition of T-AgNP. The best results were obtained with the addition of 0.9 ml of T-AgNP, reducing the bacterial population by 8.3%. In the following results, bacterial growth was determined as 6.76% and 4.73% for 1.2 ml T-AgNP and 1.5 ml T-AgNP values, respectively. As a result, a decrease of 1-15% was achieved compared to the control group.

**Keywords:** Tarragon, *Artemisia dracunculus*, silver nanoparticles, antioxidant, antimicrobial

## 1.Introduction

An interdisciplinary field of study known as nanotechnology looks at the synthesis of nanomaterials (between 1 and 100 nm), the physical, chemical, and biological characteristics of nanostructures that exist at scales ranging from atoms or molecules to submicron sizes, as well as the integration of nanomaterials into larger systems [1]. $Ag^+$ ions are converted to $Ag^0$ by plant extracts and allow for nanoscale production [2]. Terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids are the primary phytochemicals involved. Water-soluble phytochemicals such flavones, organic acids, and quinones are in charge of the rapid ion reduction [3].

The plant *Artemisia dracunculus*, also known as "tarragon," belongs to the class of medicinal aromatic herbs. The tarragon plant is well-known for its usage in the treatment of intestinal worms, dyspepsia, hiccups, anxiety, and muscle dysfunction (pinworm) [4]. It is described as an expectorant, antipyretic, respiratory aid, and sleep regulator in the writings of illustrious medical researchers including Ibn-i Sina, Abu Mansur, and Ibn Baytar [5]. The primary factors influencing tarragon's broad use in the food business are its pleasant perfume and essential oil, as well as its antibacterial and antifungal activities [6]. In addition to its therapeutic applications, tarragon is also used to sweeten meals in many nations [7].

There are several phytochemicals in tarragon's aerial portions, including monoterpenoids, sesquiterpenoids, phenolics, polyacetylenes, and alkaloids [8]. Coumarins, flavonoids, and phenolic acids have been identified as the main classes of metabolic constituents. Quercetin, luteolin, narigenin, camphorol, chlorogenic acid, caffeic acid, caffeyolquinic acid, and chicoric acid are a few more flavonoids and phenolic acids that have been identified in a number of investigations [4,9-11].
In this study, we developed a synthesis method for the tarragon based silver nanoparticles and then we examined the antioxidant and antibacterial effects of the tarragon based silver nanoparticles. Firstly, we applied Folin-Ciocalteu and DPPH techniques in order to prove the antioxidant effects of T-AgNPs. Secondly, we tested the antibacterial effect of synthesized T-AgNPs on liquid and solid cultures of both Escherichia Coli (*E.coli*) and Staphylococcus aureus (*S. aureus*) bacteria types.

## 2. Materials and method

### 2.1 Materials

Tarragon (*Artemisia dracunculus*) plants were collected from Antalya Province, Türkiye and were purchased from Nebba Agricultural Products Distribution Marketing Industry and Trade Limited Company. Silver nitrate ($AgNO_3$) were purchased from ZAG Chemicals, Türkiye. Folin-Ciocalteu's phenol reagent was purchased from Merck, Germany. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Adrich, MilliporeSigma, Germany.

### 2.2 Tarragon leaves extraction

5 g of fresh tarragon leaves were weighed. It was centrifuged for 24 hours at a mixing speed of 4100 rpm by adding 50 ml of distilled water. The centrifuged samples were filtered by Macherey-Nagel™ MN 616 Grade Folded Filter Papers. After removing the filtrate over the filter paper, the permeate under the filter paper were used for the synthesis of silver nanoparticles as shown in Figure 1.



**Figure 1.** The tarragon (*Artemisia dracunculus*) extract

### 2.3 Synthesis of Silver Nanoparticles

A 100 ml volume of 1 mmol $AgNO_3$ solution was prepared. Then 4 ml of tarragon extract was added into it. It was mixed on a hot plate with magnetic stirrer by applying a temperature of 100 - 150 ˚C. Color change from yellow to brown was observed after 30 min as presented in Figure 2.




**Figure 2.** a) Before the reduction of $AgNO_3$ solution with the Tarragon extract b) After the reduction of $AgNO_3$ to silver nanoparticles by Tarragon extract

### 2.4 Characterization studies

The biosynthesized nanoparticles were characterized using various analytical techniques. UV Visible (UV-vis) spectra of AgNP reaction mixture was recorded between 200 - 600 nm wavelength by using Shimadzu UV-1900i spectrophotometer, Japan. The functional groups were analyzed by using Jasco FT/IR-4700 spectrometer of Japan over the wavelength range from 500 to 4000 $cm^{-1}$.

### 2.5 Antioxidant assay

In order to measure the antioxidant properties of silver nanoparticles synthesized by using tarragon extract, the Folin-Ciocalteu procedure and DPPH scavenging method were applied.

As a scope of Folin-Ciocalteu procedure, first 6 test tubes were taken and labelled as blank and s1-s5 (sample 1 - sample 5). Secondly, 0.5 mL of gallic acid sample solutions were added to the related labeled tubes (s1-s5). Thirdly, 0.5 mL of distilled water was added to the blank tube. After that, 2 mL of diluted Folin-Ciocalteu's phenol reagent was added to each tube and mixed well. In next step, 1 mL of $Na_2CO_3$ solution was added to each tube and mixed well. The samples were incubated at room temperature for 30 min in dark. Finally, their absorbances were measured at 765 nm against the blank. Also for comparison, gallic acid was used as the standard in the concentration range of 20-100 mg/L. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg GAE/mL of extract).

As a scope of DPPH technique, 1 liter of 304 µmol solution of DPPH was prepared using methanol as solvent. 0.5 ml of the 304 µmol DPPH solution prepared was placed in each of the 4 sample tubes. No.1 test tube labeled as control. Only 1.5 ml of methanol and 0.5 ml of DPPH were put into the test tube labeled as control. Silver nanoparticles in the range of 0.2-0.4 ml were placed in each test tube from test tube 2 to test tube 4, in 0.1 ml increments. Methanol was added to the test tubes from the 2nd test tube to the 4th test tube, respectively, in the range of 1.3 – 1.1 ml, by decreasing 0.1 ml. This test was carried out in the dark. The tubes were centrifuged at 1500 rpm for 15 min at room temperature in the centrifuge. After centrifugation was completed, photometry analyzes were performed in UV spectrometry at 517 nm using the control sample as reference.

### 2.6 Antimicrobial assay

The *E. Coli* (gram negative bacteria) and *S. aureus* (gram positive bacteria) were provided by University of Health Sciences, Haydarpasa Numune Training and Research Hospital, Microbiology Laboratuary, Türkiye. 100 ml distilled water was added to 4 g Luria Bertani (LB) broth-agar and then this LB both solution was autoclaved for 2 hours. After that, the LB broth-agar solution was pour into the petri dishes. After these broth was held for 2 hours and then *E.coli* and *S.Aureus* bacteria cultures were cultivated in these petri dishes by using cell spreader. These bacteria cultivated sample bottles was held in drying oven at 37 ˚C for bacteria growth for 24 hours.

For the liquid bacteria cultures, 100 ml distilled water was added to 2.5 g Luria Bertani (LB) broth and then this LB broth solution was autoclaved for 2 hours. After that, the LB broth solution was pour into two different sample bottles for *E. coli* and *S. aureus*. Then, 1ml of each of the bacteria strains was put into separate sample bottles for *E. coli* and *S. aureus*. These bacteria cultivated sample bottles was held in incubator (Microtest) at 37 ˚C by applying the agitation at 150 rpm for bacteria growth for 24 hours.

## 3. Results and Discussion

### 3.1 UV-vis analysis results

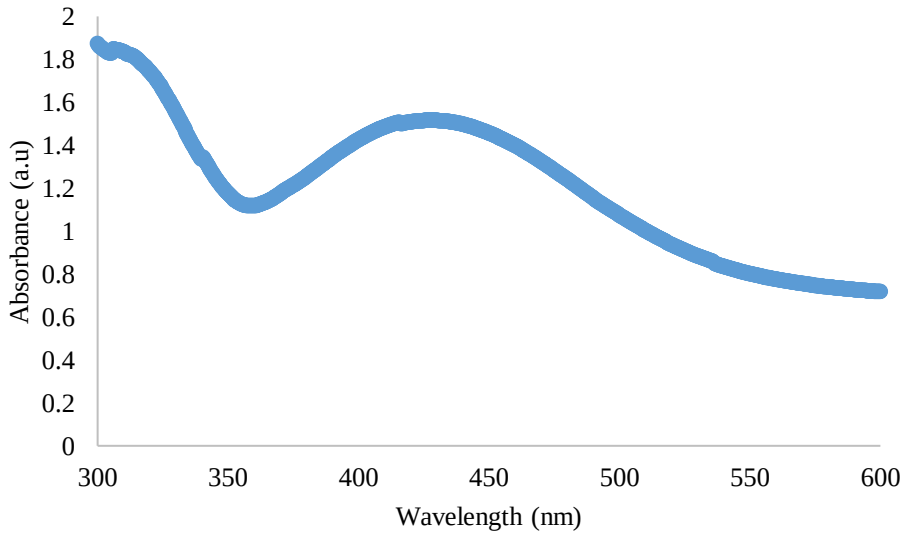


**Figure 3.** UV-vis spectra of AgNPs synthesized from Tarragon (*Artemisia dracunculus*)

The reaction mixture of silver nitrate with tarragon extract was turned into brown colour within 6 hours' incubation indicates starting of synthesis of silver nanoparticles. The SPR band was formed at 420 - 435 nm for silver nanoparticles synthesized from tarragon extract. Tarragon extract mediated synthesized silver nanoparticles shows surface plasmon resonance (SPR) band at initial time of incubation at 420 nm with broadened indicates reduction process was started. The

absorbance intensity was increased while increasing the time of reaction. After 24 hours, the maximum synthesis of nanoparticles was attained and the high peak at 420 nm was observed.

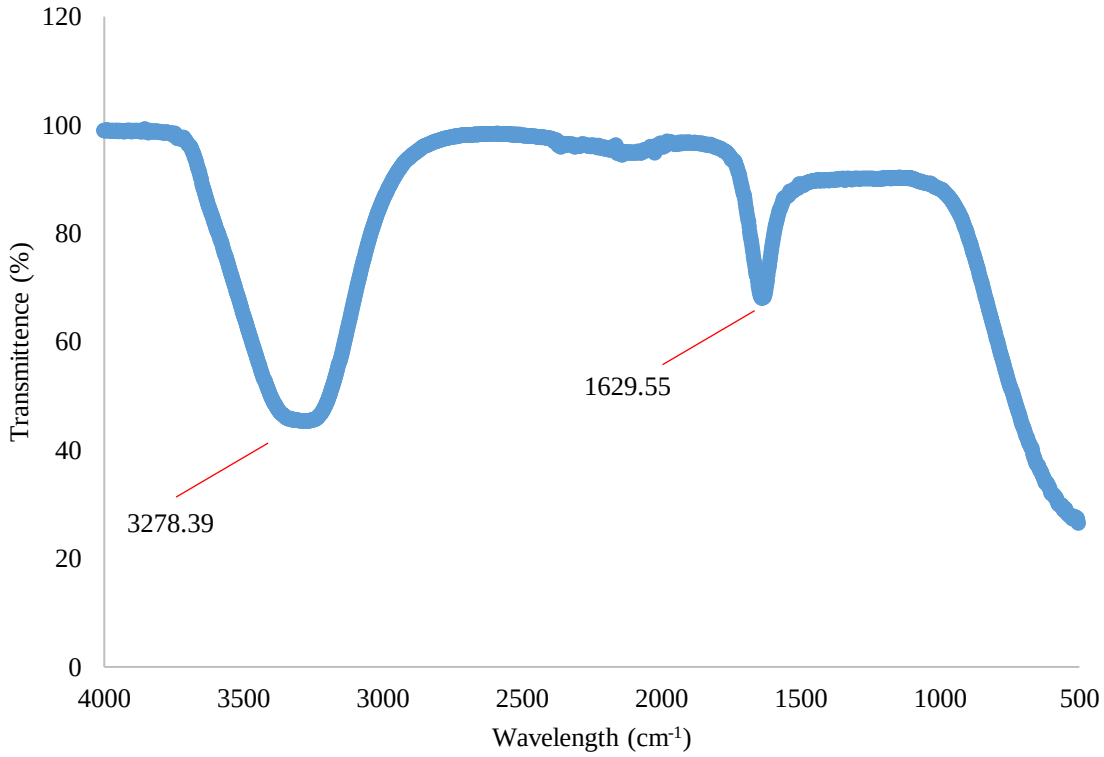


**Figure 4.** FT-IR spectrum of silver nanoparticles synthesized using Tarragon (*Artemisia dracunculus*)

FT-IR spectrum shows the water soluble organic compounds from the tarragon extract may involve in the reduction of silver ions. A strong peak was observed at 3278.39 $cm^{-1}$ indicates presence of O-H stretching and N-H stretching of carboxylic acid and amides linkages groups in the organic molecules. A medium peak at 1629.55 $cm^{-1}$ shows C-H stretching of aromatics groups (Figure 4). These carboxylic groups and amines from proteins of tarragon extract may cap the silver nanoparticles and form a layer on the surface of nanoparticles could acts reducing and stabilizing the nanoparticles.

### 3.3 Antioxidant assay results

#### 3.3.1 Folin-Ciocalteu method results

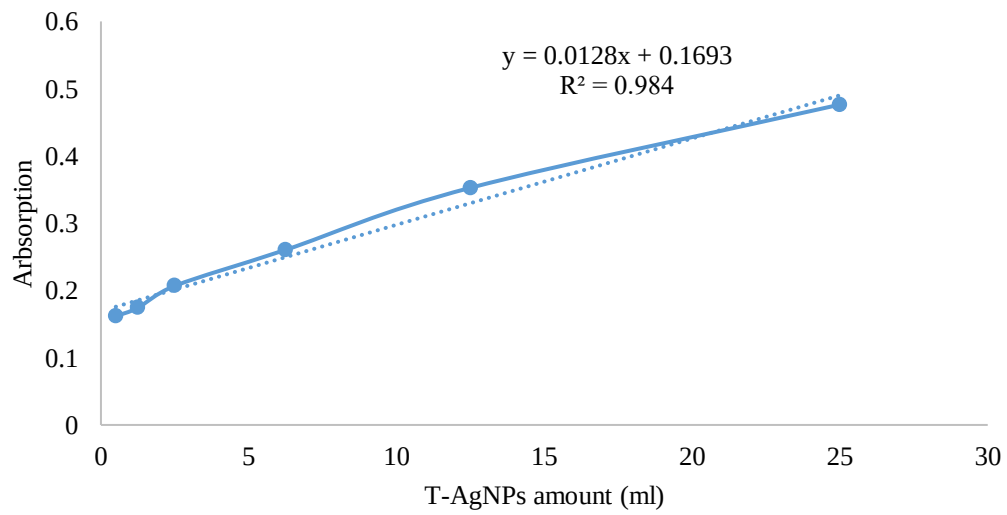


**Figure 5.** Folin Ciocalteu assay results of T-AgNPs synthesized from Tarragon (*Artemisia dracunculus*) leaves

According to Folin-Ciocalteu results, the excellent correlation coefficient ($R^2$) value was found to be 0.984 for AgNPs obtained from Tarragon (*Artemisia dracunculus*) leaves extract as presented in Figure 5.

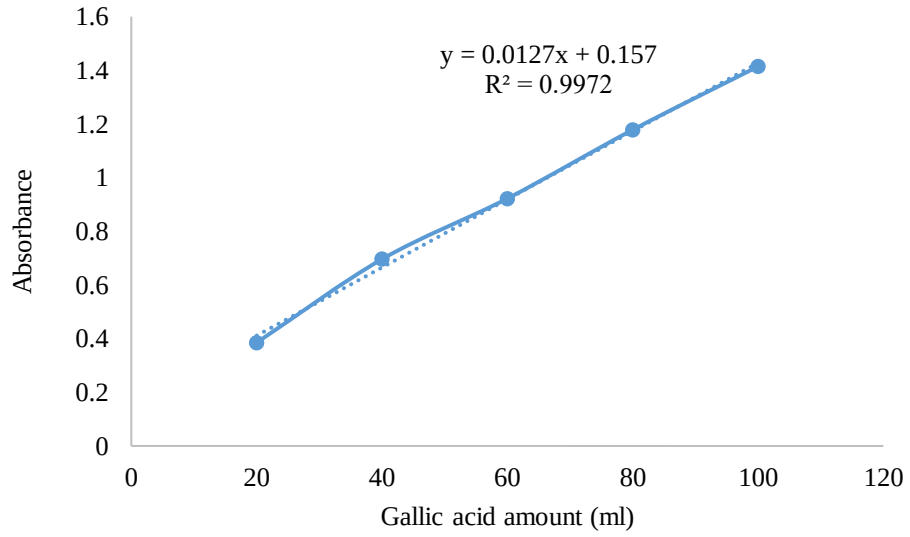


**Figure 6.** Folin-Ciocalteu method results of Gallic acid

According to Folin-Ciocalteu results, the excellent correlation coefficient ($R^2$) value was found to be 0.9972 for Gallic acid as presented in Figure 6.

### 3.3.2 DPPH scavenging method results

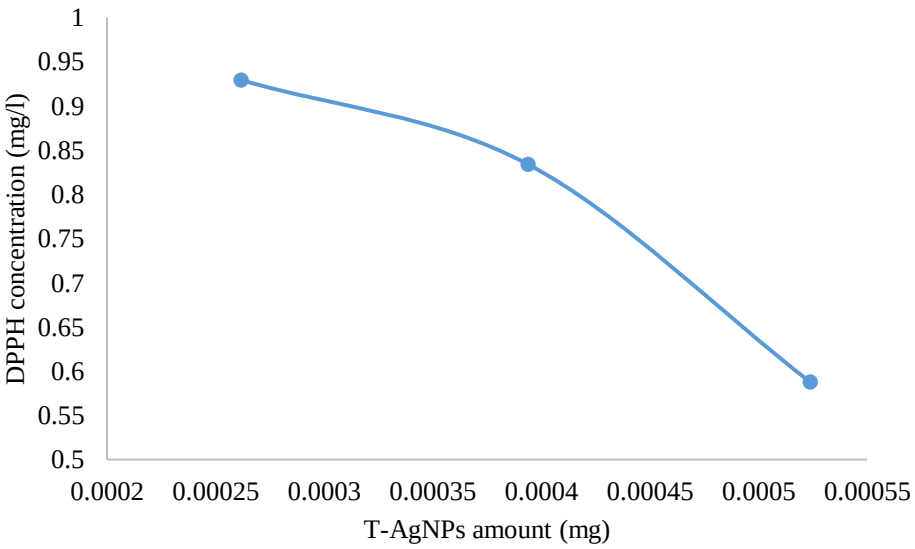


**Figure 7.** The relation between the T-AgNPs' amount and the DPPH concentration

As the amount of T-AgNPs increased, the the concentration of DPPH was decreased. Thus, it can be concluded that T-AgNPs created an antioxidant effect on DPPH.

### 3.4 Antibacterial assay results

### 3.4.1 Antibacterial test results that conducting at liquid form bacteria cultures

**Table 1.** The decrease of the concentration of *E.coli* cultures in liquid form in glass tube

| ***E. coli*** **(time-hr)** **(T-AgNP ml)** | **$t_0$** | **$t_1$** | **$t_2$** | **$t_3$** | **$t_{24}$** |
|---|---|---|---|---|---|
| Control | 1,6676 | 1,7536 | 1,7637 | 1,7039 | 2,2866 |
| 1 | 1,8267 | 1,7951 | 1,8406 | 1,8879 | 2,1298 |
| 2 | 1,5996 | 2,0181 | 2,0897 | 2,0986 | 1,9792 |
| 3 | 1,9285 | 1,9190 | 2,0575 | 2,1364 | 2,0909 |
| 4 | 1,9289 | 1,8191 | 1,9963 | 2,1254 | 2,0654 |
| 5 | 1,9414 | 1,8422 | 2,0361 | 2,0757 | 2,0321 |

We tested the antibacterial effect of the silver nanoparticles synthesized by using Tarragon (*Artemisia dracunculus*) on *E. coli* bacteria culture. The control sample was grown by 27%. The growth yield of *E. coli* was decreased to 14.22%, 19.18%, 7.77%, 6.61%, and 0.45% for Sample No.1, Sample No.2, Sample No.3, Sample No.4, and Sample No.5, respectively.

**Table 2.** The decrease of the concentration of *S. Aureus* culture in liquid form in glass tube

| ***S. aureus*** **(time-hr)** **(T-AgNP ml)** | **$t_0$** | **$t_1$** | **$t_2$** | **$t_3$** | **$t_{24}$** |
|---|---|---|---|---|---|
| Control | 1,9022 | 1,9956 | 1,9452 | 1,8279 | 2,1093 |
| 1 | 2,1559 | 2,1896 | 1,6404 | 2,0906 | 1,9634 |
| 2 | 1,8746 | 1,9794 | 0,6868 | 2,0376 | 2,2364 |
| 3 | 2,1935 | 2,1478 | 1,8056 | 2,1458 | 1,9341 |
| 4 | 2,1045 | 2,1317 | 2,1908 | 2,3318 | 2,2110 |
| 5 | 2,0817 | 2,1442 | 2,2598 | 2,3096 | 2,3681 |

We tested the antibacterial effect of the silver nanoparticles synthesized by using Tarragon (*Artemisia dracunculus*) on *S. Aureus* bacteria culture. The control sample was grown by 9.82%. The bacteria cultivated area of *S. Aureus* was decreased by 8,93% and 11.83 for Sample No.1 and Sample No.3, respectively. The bacteria cultivated area of *S. Aureus* was increased by 16.35%, 4.82%, 5.11% for Sample No.2, Sample No.4, Sample No.5, respectively.

**3.4.2 Antibacterial test results that conducting at solid form bacteria cultures**

The antibacterial activity of *E. Coli* and *S. aureus* are observed in solid bacteria cultures in petri dishes. The death area expansion of *E. coli* and *S. aureus* bacteria cultures were presented in Figure 8.

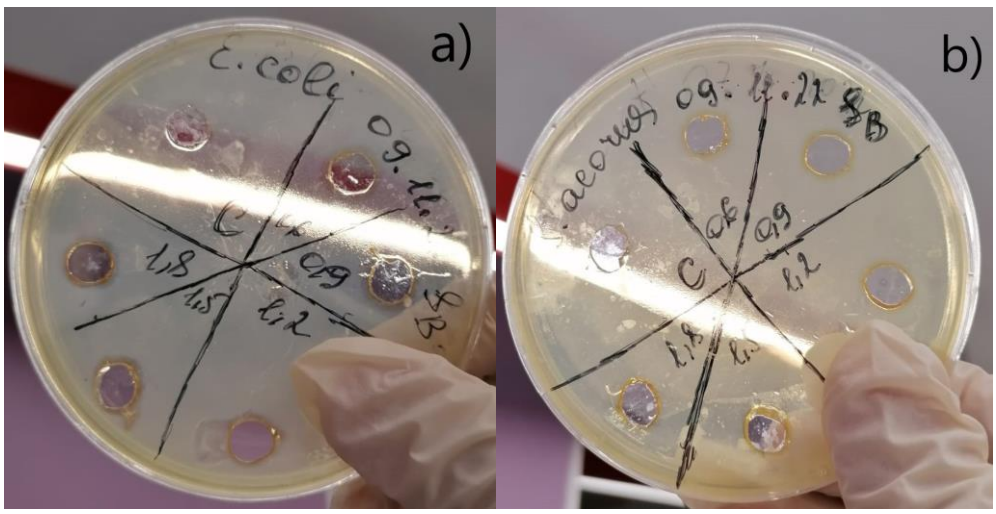


**Figure 8.** The death area growth of *E. coli* and *S. aureus* bacteria cultures

The results that belong to the antimicrobial test that we conducted in petri dishes are presented as in Table 3 and 4. The death area of *E. coli* was expanded as indicated in Table 3.

**Table 3.** The death area expansion of *E. coli*

| ***E. coli*** **time (hour)** **AgNPs (ml)** | **$t_1$** | **$t_2$** | **$t_{24}$** |
|---|---|---|---|
| Control | 0 cm | 0 cm | 0 cm |
| 0,6 | 1,1 cm | 1,1 cm | 1,2 cm |
| 0,9 | 1,2 cm | 1,1 cm | 1,2 cm |
| 1,2 | 0,9 cm | 1 cm | 1,5 cm |
| 1,5 | 1,1 cm | 1,1 cm | 1,1 cm |
| 1,8 | 0,9 cm | 0,9 cm | 0,9 cm |

The death area of *S. aureus* was expanded as indicated in Table 4.

**Table 4.** The death area expansion of *S. aureus*

| ***S. aureus*** **time (hour)** / **AgNPs (ml)** | **$t_1$** | **$t_2$** | **$t_{24}$** |
|---|---|---|---|
| Control | 0 cm | 0 cm | 0 cm |
| 0,6 | 0,9 cm | 1,1 cm | 1,1 cm |
| 0,9 | 1 cm | 0,9 cm | 1,3 cm |
| 1,2 | 0,9 cm | 0,9 cm | 1,3 cm |
| 1,5 | 1 cm | 0,9 cm | 1,5 cm |
| 1,8 | 0,9 cm | 0,9 cm | 1,3 cm |

As the concentration of T-AgNPs increases, the death area of *E. coli* and S. *aureus* increases. For example, the maximum death area was obtained as 5.055 $cm^2$ for 1.2 ml T-AgNPs solution. As the concentration of T-AgNPs increases the death area of S. *aureus* increases. For example, the maximum death area was obtained as 5.055 $cm^2$ for 1.5 ml T-AgNPs solution. Thus, it can be concluded that as the amount of T-AgNPs increased, the area of the death bacteria cells increased, too.

## 4. Conclusion

In this study, we developed a facile, green method for the synthesis of silver nanoparticles by using Tarragon (*Artemisia dracunculus*) extract. We proved the formation of silver nanoparticles by UV-vis and FT-IR spectroscopy techniques. Tarragon extract mediated synthesized silver nanoparticles (T-AgNPs) show surface plasmon resonance (SPR) band at initial time of incubation at 420 nm in UV-vis spectra. A strong peak was observed at 3278.39 $cm^{-1}$ indicates presence of O-H stretching and N-H stretching of carboxylic acid and amides linkages groups in the organic molecules. A medium peak at 1629.55 $cm^{-1}$ shows C-H stretching of aromatics groups according to FT-IR spectra of T-AgNPs. Also, we proved the antioxidant properties of synthesized T-AgNPs by both Folin-Ciocalteu and DPPH scavenging methods. According to Folin-Ciocalteu method's results, the excellent correlation coefficient ($R^2$) value was found to be 0.984 for T-AgNPs. Also, as the amount of T-AgNPs increased, the concentration of DPPH was decreased. Thus, it can be concluded that T-AgNPs created an antioxidant effect on DPPH.

Also, we tested the antibacterial effect of synthesized T-AgNPs on both *E. coli* and *S. aureus* liquid bacteria cultures. For liquid *E. coli* cultures, the control sample was grown by 27%. The growth yield of *E. coli* was decreased to 14.22%, 19.18%, 7.77%, 6.61%, and 0.45% for Sample No.1 (1 ml T-AgNPs), Sample No.2 (2 ml T-AgNPs), Sample No.3 (3 ml T-AgNPs), Sample No.4 (4 ml T-AgNPs), and Sample No.5 (5 ml T-AgNPs), respectively. For liquid *S. aureus* cultures, the control sample was grown by 9.82%. The bacteria cultivated area of *S. aureus* was decreased by 8,93% and 11.83 for Sample No.1 (1 ml T-AgNPs) and Sample No.3 (3 ml T-AgNPs), respectively. The growth area of *S. aureus* was increased by 16.35%, 4.82%, 5.11% for Sample No.2 (2 ml T-AgNPs), Sample No.4 (4 ml T-AgNPs), Sample No.5 (4 ml T-AgNPs), respectively. Also, we confirmed the antibacterial effect test results that obtained by liquid bacteria cultures with the solid bacteria cultures. As the concentration of T-AgNPs increased, the death area of *E. coli* and S. *aureus* had increased. As, the maximum death area of *E. coli* was obtained as 5.055 $cm^2$ for 1.2 ml T-AgNPs solution. For example, the maximum death area of S. *aureus* was obtained as 5.055 $cm^2$ for 1.5 ml T-AgNPs solution.

## Acknowledgement

This study was conducted at Bioengineering Basic and Application Laboratory of Üsküdar University. The authors thank to the head of Microbiology laboratory of Haydarpasa Numune Training and Research Hospital, Professor Sebahat Aksaray for providing *E. coli* and *S. aureus* bacteria cultures.

## Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## References

1. Nasrollahzadeh M, Sajadi SM, Sajjadi M, Issaabadi Z. Chapter 1 - An Introduction to Nanotechnology. In: Nasrollahzadeh M, Sajadi SM, Sajjadi M, Issaabadi Z, Atarod M, editors. Interface Science and Technology. 28: Elsevier; 2019. p. 1-27.
2. Rahman A, Kumar S, Bafana A, Dahoumane SA, Jeffryes C. Biosynthetic Conversion of $Ag^+$ to highly Stable $Ag^0$ Nanoparticles by Wild Type and Cell Wall Deficient Strains of Chlamydomonas reinhardtii. Molecules. 2018 Dec 28;24(1):98.
3. Shaikh WA, Chakraborty S, Owens G, Islam RU. A review of the phytochemical mediated synthesis of AgNP (silver nanoparticle): the wonder particle of the past decade. Appl Nanosci. 2021;11(11):2625-2660.

**4.** Aglarova AM, Zilfikarov IN, Severtseva OV. Biological characteristics and useful properties of tarragon (Artemisia dracunculus L.) (review). Pharmaceutical Chemistry Journal. 2008;42(2):81-6.
**5.** Sharafati Chaleshtori R, Rokni N, Razavilar V, Rafieian Kopaei M. The Evaluation of the Antibacterial and Antioxidant Activity of Tarragon (*Artemisia dracunculus L.*) Essential Oil and Its Chemical Composition. Jundishapur J Microbiol. 2013;6(9):e7877.
**6.** Hassanzadeh MK, Tayarani Najaran Z, Nasery M, Emami SA. Chapter 92 - Tarragon (Artemisia dracunculus L.) Oils. In: Preedy VR, editor. Essential Oils in Food Preservation, Flavor and Safety. San Diego: Academic Press; 2016. p. 813-7.
**7.** Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. J Agric Food Chem. 2005 Nov 30;53(24):9452-8.
**8.** Mumivand H, Babalar M, Tabrizi L, Craker LE, Shokrpour M, Hadian J. Antioxidant properties and principal phenolic phytochemicals of Iranian tarragon (*Artemisia dracunculus L.*) accessions. Horticulture, Environment, and Biotechnology, 2017;58, 414-422.
**9.** Onuchak LA, Kurkin VA, Minakhmetov RA. HPLC analysis of Artemisia dracunculus extracts. Chem Nat Compd. 2000;36:144-147.
**10.** Obolskiy D, Pischel I, Feistel B, Glotov N, Heinrich M. *Artemisia dracunculus L.* (Tarragon): A critical review of its traditional use, chemical composition, pharmacology and safety. J Agric Food Chem 2011;59:11367-11384.
**11.** Weinoehrl S, Feistel B, Pischel I, Kopp B, Butterweck V. Comparative evaluation of two different Artemisia dracunculus L. cultivars for blood sugar lowering effects in rats. Phytother Res 2012;26:625-629.

## *ORAL PRESENTATION*

# Evaluation of the *in vitro* cytotoxic and genotoxic profile of Allura red in different lymphoblastoid cell lines in terms of metabolic activity

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**Abstract**
Allura red (AR), E129 is a water-soluble and dark red, synthetic anionic monoazo dye. It is widely used in many beverages, juices, baked goods, meat and dessert products. Some studies have held AR responsible for some adverse effects on human health, including cancer, due to the reaction of aromatic azo compounds (R R' = aromatic). Its undesirable effects were determined on living organisms, where it was able to induce genotoxicity in mice tested when administered in high amounts. In this study, it was aimed to determine of in vitro cytotoxic and genotoxic potency of AR by using different cell lines which are TK6 and AHH1. For this purpose, the relative population doubling test (RPD%) and the single-cell alkaline comet assay were used for the determination of the cytotoxic and genotoxic potency of AR, respectively. Experimental applications were carried out with cell lines with different passage numbers, with 3-replicate analyzes independently of each other. After cell strains were treated with the different 5 dose concentrations of AR (0.12, 2.4, 3.6, 4.8 and 6.0 mM), they were incubated at 24 hours under cell culture conditions which are %5 CO2, 100% humidity at 37°C in a 6-well plate. RPMI 1640 medium supported with horse serum 10% and L-glutamine 1% was used for each cell line. The cytotoxic effect of Allura red was found to be higher in TK6 cells than in AHH1 cells at the same dosing and incubation conditions. In addition, the comet frequency was found to be significantly higher in TK6 cells than in AHH1. The fact that cytotoxic and genotoxic damage observed in the AHH1 cell, which is known to have some metabolism enzymes responsible for oxidation (CYP450), is less than in TK6 cells that do not have these enzymes, suggesting that metabolic activities mediated by CYP450s reduce the formation of toxic metabolites.

**Keywords:** Allura red, RPD%, comet assay, TK6, AHH1

## INTRODUCTION

A food coloring or additive is any dye, pigment, or other substance that imparts color added to a food, beverage, or any non-food application, including pharmaceuticals (Newsome et al., 2014). On the other hand, a color additive is also any chemical that reacts with another substance and causes color formation (de Boer, 2014). One of the extraordinary abilities of the eye is its ability to recognize colors, which is why dyes are used to increase the attractiveness of objects (Feng et al., 2012). Food dyes are used for some reasons such as compensating for color loss caused by exposure to light, air, temperature and storage conditions, increasing natural colors to make food more attractive and appetizing, and providing color to colorless foodstuffs (Kobylewski & Jacobson, 2012). Although food dyes can be classified according to various criteria such as opacity (transparent and opaque) and solubility (soluble and insoluble), they are mainly evaluated according to their origin (organic and inorganic; natural or synthetic). Nowadays, there are thousands of various types of dyes used widely in pharmaceuticals, textiles, cosmetics, plastics and foods (Feng et al., 2012).

In order to allow their use in the food industry, colorants are subjected to a wide variety of toxicity tests such as acute, subchronic and chronic toxicity, carcinogenicity, mutagenicity, teratogenicity, reproductive toxicity, accumulation in the body, bioenergy effects and detection of immune effects (Amchova et al., 2015). *In vivo* toxicity tests are performed in six species, at least three of which are mammals. Small rodents (rat, mouse, guinea pig) and special rabbit, dog, cat or pig breeds that are particularly close to human body physiology are used in most of the tests (Kumar & Madan, 2014). Today, an approach to avoiding animal testing under the 3R (reduce, replace, improve) rule is common. For this reason, genotoxicity tests have been more commonly performed *in vitro* for the last 30 years (Dural et al., 2020).

Azo dye group a large class of synthetic organic dyes that contain nitrogen as the azo group —N=N— as part of their molecular structures. All azo dyes contain one or more azo bonds. Although azo dyes can theoretically provide a full rainbow of colours, most commercial azo dyes are used for yellow, orange and red colours (Feng et al., 2012). More than half the commercial dyes belonging to this group. This group of colorants typically consists of substances that give bright colors. Approximately higher than 0.7 million tons of azo dyes are

synthesized each year (Feng et al., 2012). Recently, increasing attention has been paid to the toxicity of azo-dyes used in the ingredients of food and pharmaceutical products. However, the potential carcinogenicity threat posed by their reduction to carcinogenic metabolites by the gut microbiota is the main concern limiting their use (Feng et al., 2012).

Human intestinal bacteria are also involved in the reduction of various xenobiotics to metabolites, some of which are involved in genotoxicity and carcinogenesis. Human exposure to xenobiotics, including azo dyes, occurs through ingestion, skin contact, or inhalation. Azo dyes can be bioconverted to colorless aromatic amines, some of which may become less toxic than the original dye, but others such as arylamines and free radicals are potentially carcinogenic (Collier et al., 1993).

Allura red (AR) is a water-soluble anionic monoazo-dye with a red color. Toxicological risks have been reported in some regulatory bodies regarding the related azo dye. In vivo studies in mice have shown that different doses of Allura Red cause genotoxic damage to several organs. However, the observations carried out showed that the effect was not associated with cytotoxicity (Tsuda et al., 2001). The Scientific Opinion of the EFSA Panel reported that Allura Red may cause allergic reactions such as urticaria, asthma, especially when applied as a mixture with other synthetic color additives (Fallico et al., 2011). Although some studies classify Allura Red as non-genotoxic and allow its use as a food colouring, some studies have identified genotoxic properties (Bastaki et al., 2017). There is conflicting information in the literature regarding the toxicity potentials of Allura red.

Since Allura red is an azo dye, it has been reported that azo reduction occurring during its metabolism causes the formation of toxic metabolites. Carcinogenic activities of these metabolites have been reported in some studies. However, there is not enough information in the literature about how CYP450 activities affect its toxicity. The aim of this study was to investigate the toxicity potential of Allura red, an azo dye, in AHH1 cells with CYP450 activity and TK6 cells without CYP450 activity by cytotoxicity and genotoxicity tests after 24 hours of acute dosing. For this purpose, relative population doubling test (RPD%) and single-cell alkaline comet assay methods were applied to cells exposed to AR.

## MATERIALS AND METHOD

### Chemicals

RPMI 1640 (without L glutamine), horse serum and glutamine were obtained from Gibco (Thermo Fisher Scientific, U.K.). Dimethyl sulfoxide (DMSO), triton X-100, sodium chloride and ICP multi-element standard were obtained from Merck (Darmstadt, Germany). Allura red, low melting point agarose (LMPA), tris base, histopaque-1077, ethylenediaminetetraacetic acid-Na2 (2Na-EDTA), and sodium lauroyl sarcosinate were supplied from Sigma Aldrich (USA). Normal melting point agarose (NMPA) was purchased from Biomax. Phosphate-buffered saline (PBS) was taken from Wisent (Quebec, Canada). Ethidium bromide was had from MP Biomedical (France). All chemical solutions used in trace element and comet analyzes were prepared with ultrapure water with a conductivity of ≤0.055 µS/cm or an electrical resistance of ≤ 18.2 mΩ.cm at 25 °C, respectively.

### Instruments

Cell counting in the study was carried out with a inverted microscope (Euromex-OX.3120/25-Netherlands). An inverted microscope (Euromex, Holland) and the image processing system, consists a fluorescent microscope (Olympus AS52), a fluorescent light source, a camera attachment and an image processing software (BAB2000), were used in the COMET evaluation.

### Cell culture

The human lymphoblastoid cell lines TK6 and AHH1 were cultured in RPMI 1640 supplemented with 10% horse serum and 1% L-glutamine in an 80-cm2 flask at 37°C, 5% CO2 with 100% humidity. The cells were maintained at a concentration of 1–2 x 105/ml. Cell culture was initiated by thawing a frozen cell stock in 50 mL medium in TS-75 culture flasks. Cells were then cultured for experimental use. Cell density was determined manually by an heamocytometer cell counter and the cells were appropriately diluted to prevent overgrowth (<1 x 106 cells/min). Cell suspensions were prepared at 1 x 105 cells/mL and treated with serial dilution with one of the chemicals described above for 24 h. Cell lines TK6 and AHH1 were donated by Gareth Jenkins (Swansea University-UK).

Cells were exposed to 1.2, 2.4, 3.6, 4.8 and 6.0 mM concentrations of allura red for 24 hours and cytotoxicity and genotoxicity tests were performed to determine the acute effects. The %RPD test was used to determine cytotoxicity, and single-cell alkaline comet analysis was performed to determine genotoxicity.

RPD% measurement

Initially, cells were seed in the 6-well plate 1 x 105 cell/mL. After 24 hours, they were counted by a neaubeaur slide and then were dosed with the appropriate concentration of chemical. For 24 hours after. the cells were treated with these chemicals for 1 day. At end of the 24 hour cells were counted by the same way and it was calculated according to the RPD% equation. This equation was given in below.

RPD% = (Number of population doublings in treated cultures/Number of population doublings in control cultures) x 100

Population doubling = [log (Post − treatment cell number ÷ Initial cell number)] ÷ log2

DNA damage assay

In all studies, TK6 and AHH1cell were processed within 2 h. Cells centrifuged for 5 min at 1500 rpm and 25°C. The interface bands containing lymphocyte were washed with PBS and then collected by the 5 min centrifugation at 1500 rpm. The resulting pellets were resuspended in PBS to obtain approximately 20000 cells in 100 µL.

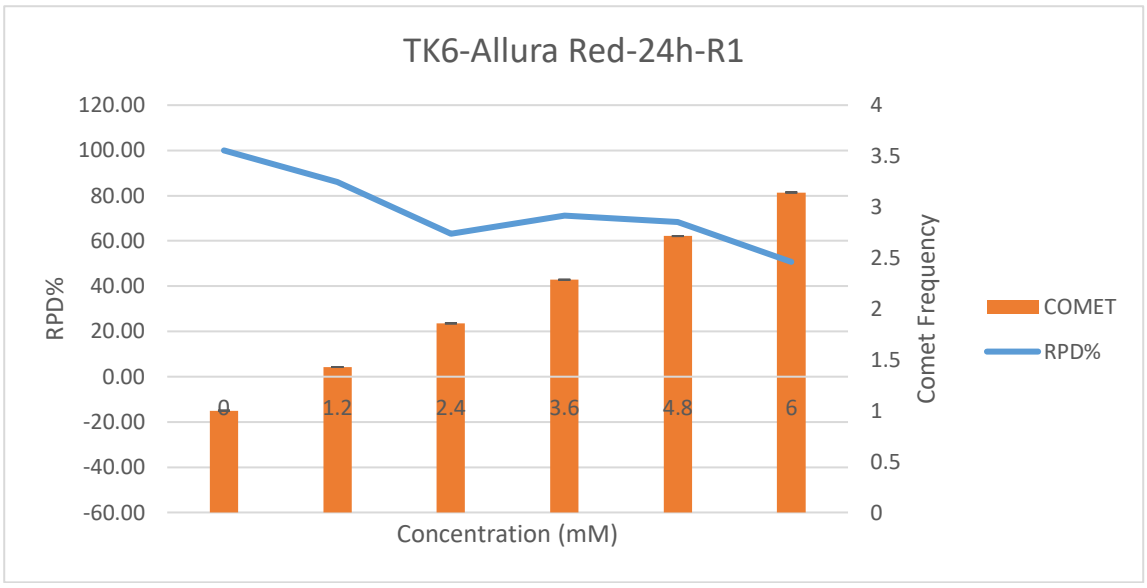
The comet assay was performed according to Singh et al. with the following modifications (Singh et al., 1988). Thus, 50 µL of fresh lymphocytes suspension were mixed with 100 µL of 0.75% LMA in PBS at 37°C. Subsequently, 150 µL mixture was layered onto the slide pre-coated with thin layers of 1% NMA and immediately covered with a coverslip. Slides were left for 5 min at 4°C to allow the agarose solidify. After removing cover slips, the slides were submersed in the lysing solution (2.5 M NaCl, 100 mM EDTA-2Na, 10 mM Tris-HCl, pH 10-10.5, 1% triton-X-100 and 10% DMSO added just before use) freshly prepared and at 4°C for 90 min. Slides were than immersed in freshly prepared alkaline electrophoresis buffer (0.3 mol/L NaOH and 1 mmol Na2EDTA, pH>13) at 4°C for unwinding (20 min) and then electrophoresed (25 V/300 mA, 20 min). All steps were carried out under minimal illumination. Then, slides were held in 5 mins water, 15 mins in neutralisation solution and 5 mins water again, respectively. Then they were leaved to the dryness overnight. Subsequently, the slides were stained with ethidium bromide (2 µg/mL in distilled water; 70 µL/slide), covered with a coverslip and analysed using a fluorescence microscope (Olympus AS52). Images of 100 randomly elected cells (50 cells from each of two replicates slides) were analysed visually from each subject. Each image was classified according to the intensity of the fluorescence in the comet tail and was given a value of either 0 (undamaged), 1 (low damaged), 2 (middle damaged), 3 (damaged) or 4 (high damaged), so that the total score of slide could be between 0 and 400 arbitrary units (AU).

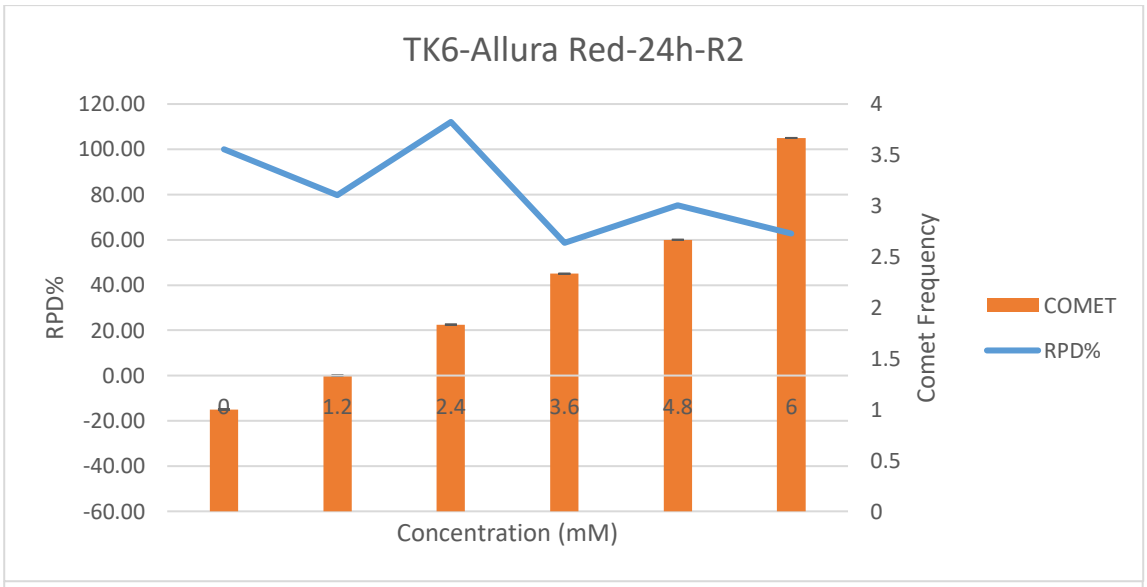
Arbitrary Units (AU) calculation equation was given at below:

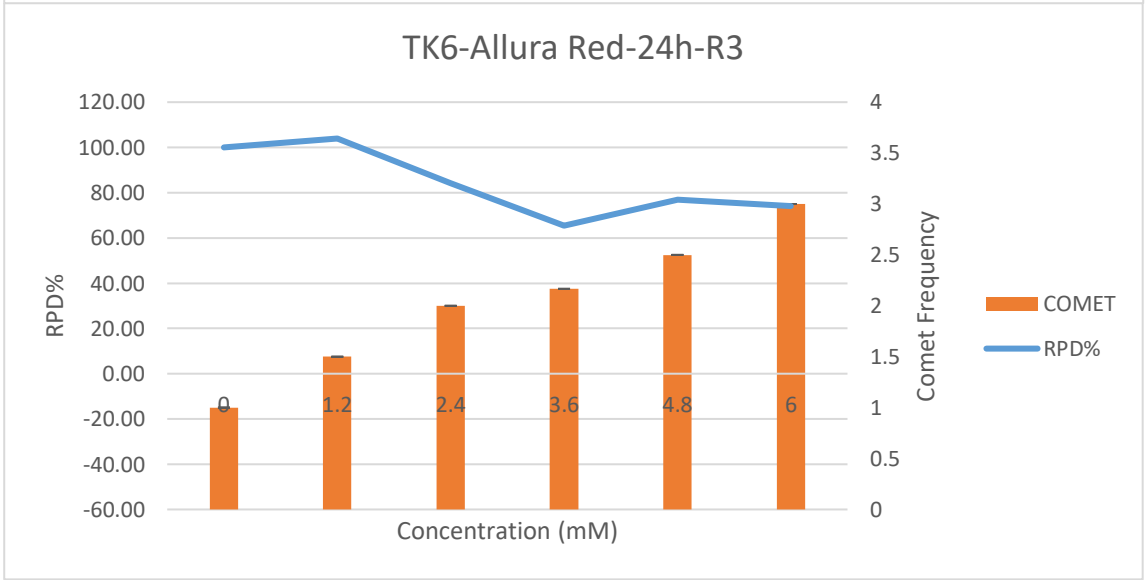
$$AU = (0\ x\ undamaged\ DNA\ number) + (1\ x\ low\ damaged\ DNA) + (2\ x\ middle\ damaged\ DNA) + (3\ x\ damaged\ DNA) + (4\ x\ high\ damaged\ DNA)$$

## RESULTS and DISCUSSION

It is known that the AHH1 cell line differs from the TK6 cell line in that it has CYP450 oxidation ability. The RPD% test results performed in two different cell lines showed that TK6 cells (Figure 1) were more affected by the cytotoxic effect of allura red. Similarly, in the analyzes performed with Comet assay, it was observed that TK6 cells had more DNA breaks than AHH1 cells (Figure 2) . The lack of CYP450 activity of TK6 cells (Figure 1) caused them to be more affected by the toxic effects of Allura red.


TK6-Allura Red-24h-R1
RPD%
Comet Frequency
Concentration (mM)
0
1.2
2.4
3.6
4.8
6
COMET
RPD%


TK6-Allura Red-24h-R2
RPD%
Comet Frequency
Concentration (mM)
0
1.2
2.4
3.6
4.8
6
COMET
RPD%


TK6-Allura Red-24h-R3
RPD%
Comet Frequency
Concentration (mM)
0
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2.4
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4.8
6
COMET
RPD%

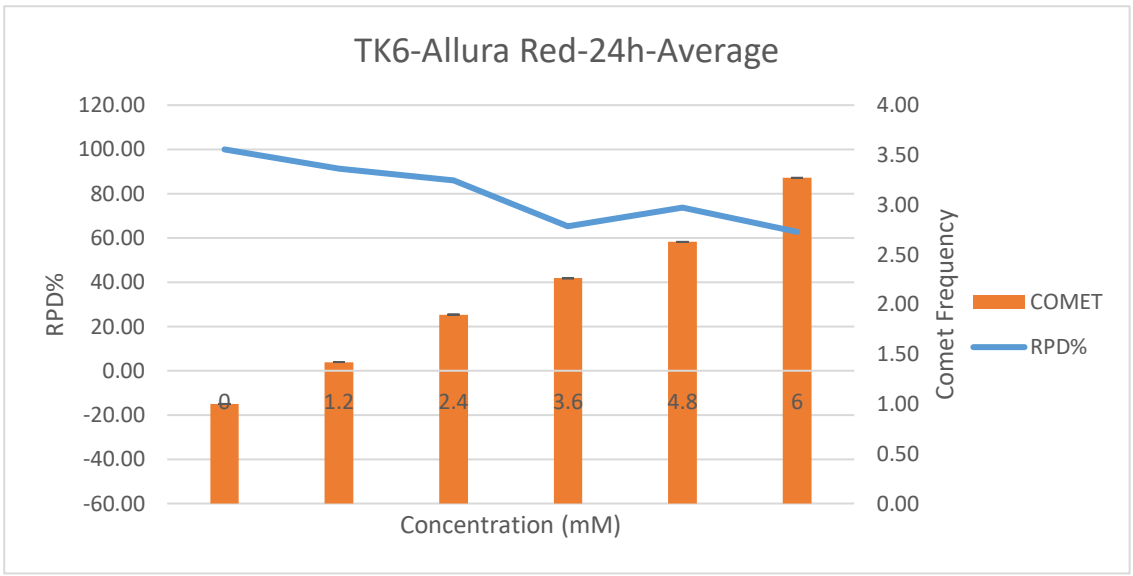
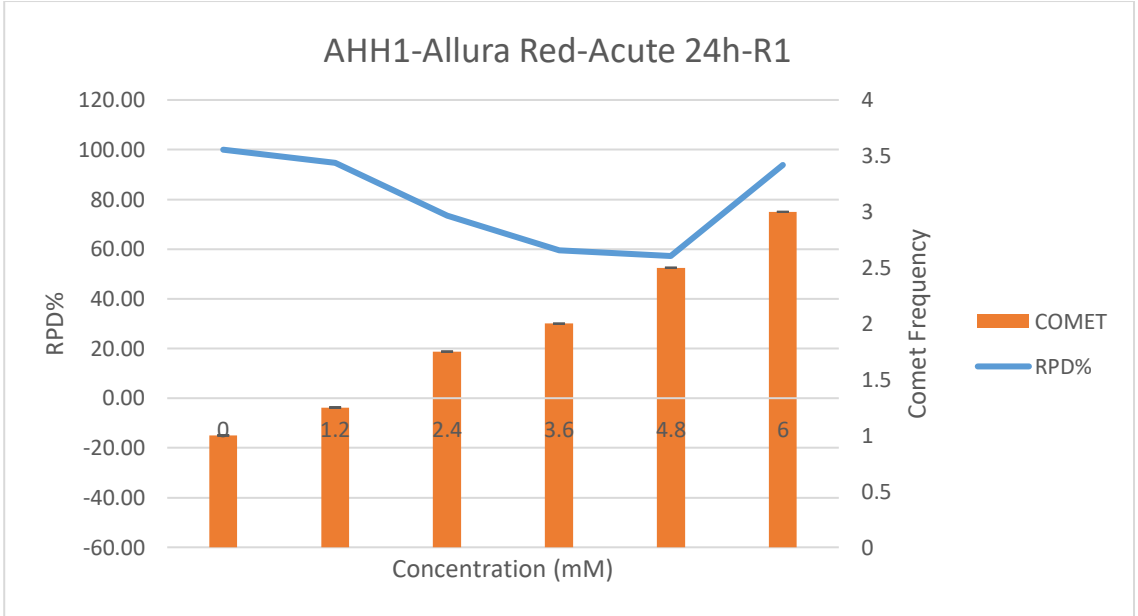



Figure 1. Cytotoxicity and genotoxicity results of Allura red in TK6 cell line (R1, R2, R3 and average)



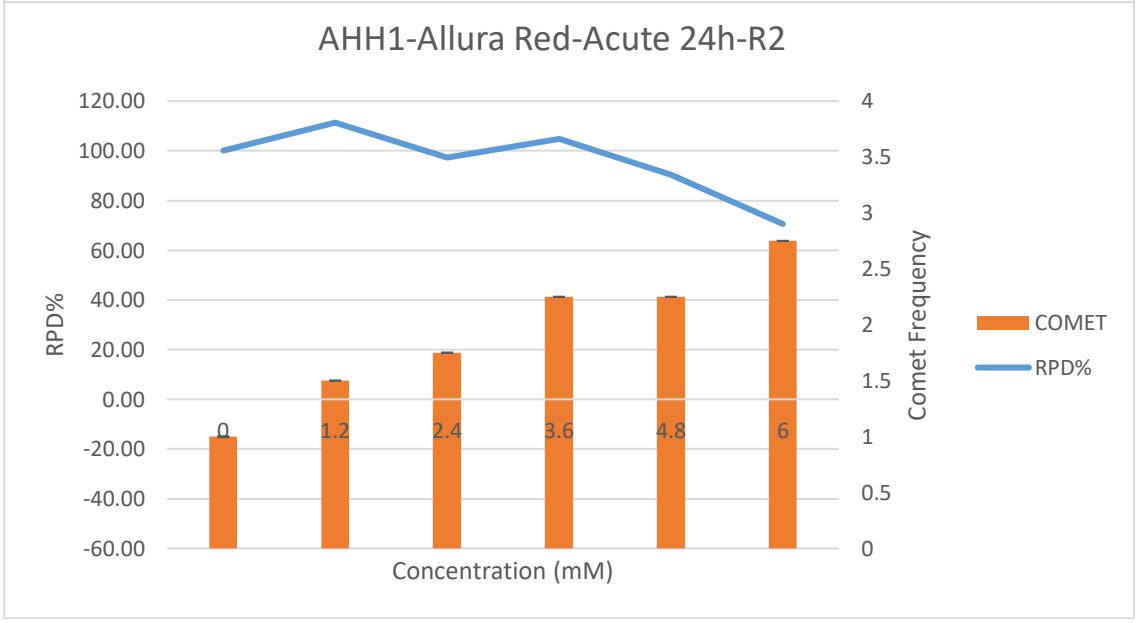






Figure 2. Cytotoxicity and genotoxicity results of Allura red in AHH1 cell line (R1, R2, R3 and average)

## CONCLUSION

The applied RPD% test and Comet assay were successfully applied to both cell lines. Studies in both TK6 and AHH1 cells show that AR causes both cytotoxic and genotoxic damage in a dose-dependent manner. Statistically significant DNA damages were observed in both TK6 and AHH1 cells from 3.6 mM AR concentration (at 4.8 and 6.0 mM concentrations) compared to control. When DNA damage frequencies caused by AR in TK6 and AHH1 cell lines were compared, it was seen that 6 mM AR concentration caused statistically significantly more DNA damage in TK6 cell lines. The observed results suggest that CYP450-based oxidation reactions carried out in the AHH1 cell result in a reduction of both the cytotoxicity and genotoxicity caused by AR.

## ACKNOWLEDGEMENTS

The author would like to thank Professor Gareth Jenkins (Swansea University-UK) who donated the cell lines used in this research, and Cumhuriyet University School of Medicine Research Laboratory (CUTFAM) laboratory authorities and staff, whose laboratory facilities we used during the research. This study was supported by Cumhuriyet University Scientific Research Projects Support Office within the scope of project numbered ECZ078.

## REFERENCES

Amchova, P., Kotolova, H., & Ruda-Kucerova, J. (2015). Health safety issues of synthetic food colorants. Regulatory Toxicology and Pharmacology, 73(3), 914–922.

Bastaki, M., Farrell, T., Bhusari, S., Pant, K., & Kulkarni, R. (2017). Lack of genotoxicity in vivo for food color additive Allura Red AC. Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association, 105, 308–314.

Collier, S. W., Storm, J. E., & Bronaugh, R. L. (1993). Reduction of Azo Dyes During in Vitro Percutaneous Absorption. Toxicology and Applied Pharmacology, 118(1), 73–79.

de Boer, L. (2014). Biotechnological production of colorants. Advances in Biochemical Engineering/Biotechnology, 143, 51–89.

Dural, E., Shah, U. K., Pritchard, D., Chapman, K. E., Doak, S. H., & Jenkins, G. J. S. (2020). The effect of chronic dosing and p53 status on the genotoxicity of pro-oxidant chemicals in vitro. Mutagenesis, 35(6), 479–489.

Fallico, B., Chiappara, E., Arena, E., & Ballistreri, G. (2011). Assessment of the exposure to Allura Red colour from the consumption of red juice-based and red soft drinks in Italy. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 28(11), 1501–1515.

Feng, J., Cerniglia, C. E., & Chen, H. (2012). Toxicological significance of azo dye metabolism by human intestinal microbiota. Frontiers in Bioscience - Elite, 4 E(2), 568–586. https://doi.org/10.2741/e400

Kobylewski, S., & Jacobson, M. F. (2012). Toxicology of food dyes. International Journal of Occupational and Environmental Health, 18(3), 220–246.

Kumar, A., & Madan, A. K. (2014). Color additives: legislative perspective in the United States, Europe, Australia, and India. International Journal of Pharmaceutical Compounding, 18(4), 293–300.

Newsome, A. G., Culver, C. A., & van Breemen, R. B. (2014). Nature's palette: the search for natural blue colorants. Journal of Agricultural and Food Chemistry, 62(28), 6498–6511. https://doi.org/10.1021/jf501419q

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research, 175(1), 184–191.

Tsuda, S., Murakami, M., Matsusaka, N., Kano, K., Taniguchi, K., & Sasaki, Y. F. (2001). DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicological Sciences : An Official Journal of the Society of Toxicology, 61(1), 92–99.

## *ORAL PRESENTATION*

# Effects of valproic acid on intestinal tissue due to spinal cord injury in rats

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## Abstract

Spinal cord injury (SCI**)** is a condition in which organs are damaged due to the activation of many pathways due to oxidative stress. Studies on finding suitable treatment drugs for this type of damage are quite scarce. Therefore, in our study, we wanted to investigate the effect of secondary damage that develops after spinal cord injury on intestinal tissue and whether valproic acid (VPA) has a therapeutic aspect in this damage. Sixteen Wistar albino rats were divided into two equal groups. In group 1, rats created SCI after laminectomy, but no medication was given. In group 2 rats, SCI was established after laminectomy, and VPA (single dose 300 mg/kg intraperitoneally) treatment was started. After the slaughter of rats, lipid peroxidation (such as malondialdehyde: MDA) and glutathione (GSH) levels, and superoxide dismutase (SOD) activities were examined in the collected tissues as markers of oxidative stress. To understand the role of VPA in intestinal tissue damage caused by SCI, lipid peroxidation, and enzyme levels were examined biochemically and histologically.VPA administration increased **GSH** and **SOD** levels and decreased **MDA** levels, which showed that it improved increased oxidative stress and impaired antioxidant status in SCI-induced damaged intestinal tissue homogenate. VPA has a statistically significant therapeutic effect, especially on the damaged intestinal tissue cell, and we observed that VPA reduces secondary damage after SCI by regulating the oxidant and antioxidant balance.

**Keywords:** Intestinal, Valproic acid, SOD, MDA, GSH

## Introduction

Spinal cord injury (SCI) leads to damage to many organs (such as the intestines), especially due to events such as damage to the autonomic nervous system and reduced blood flow. SCI consists of two phases and covers various cellular and biochemical events . Primary damage occurs at the beginning of the event and constitutes the first phase of the event. The secondary phase, on the other hand, is more common than the primary phase and constitutes the main mechanism of damage. In this phase, many tissues are damaged due to the occurrence of many physiopathological events such as oxidative stress, and apoptosis [1]. In addition, when treated with many therapeutic agents in this phase, the spread of damage is prevented because it responds to treatment [2]. For this purpose, when we gave VPA, an anti-epileptic drug, to rats in which we created experimental SCI, it was shown that the prevalence of secondary damage was reduced by reducing the level of MDA, which is a marker of oxidative damage. In addition, the increased activity of SOD, an enzymatic antioxidant that forms the first line of the body's defenses, as well as the increase in the level of GSH, confirm the purpose of this experiment. We think that the use of VPA with a neuroprotective effect will be beneficial, as it will contribute to currently limited treatments after SCI damage.

## Material and methods

In our study, two groups of eight rats were formed from Wistar albino rats with an approximate weight of 230 grams each. SCI (control) group: Only experimental SCI was created, but VPA was not applied. SCI+VPA group: After the experimental SCI was established, a single dose of intraperitoneal 300 mg/kg VPA was administered. At the end of three days, the experiment was terminated by cutting off the heads of the rats under anesthesia. After the spleen tissue hemojanation of the rats, the preparation of supernatants was started, and the MDA, SOD, and GSH levels were examined biochemically with the promise of commercial kits. The statistical analysis of the experimental results was performed using the SPSS 20 Windows package program. Kruskal-Wallis and Mann-Whitney tests were applied as statistical methods among the existing groups, and it was considered statistically significant if the 'p-value was below 0.05.

## Results

**Figures 1, 2, and 3:** The levels of MDA, SOD, and GSH in the intestinal tissue taken are shown. It was observed that MDA levels decreased statistically in the SCI + VPA group compared to the SCI (control) group, while SOD and GSH levels increased. Thus, the shift of negative findings to positive ones with the neuroprotective effect of VPA prevented secondary damage due to SCI **(p. *, 0.05).**

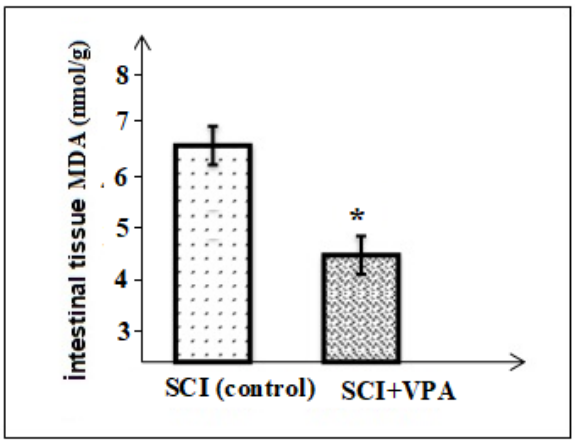


Figure 1: Graphic showing the mean values of MDA as nmol/g protein.

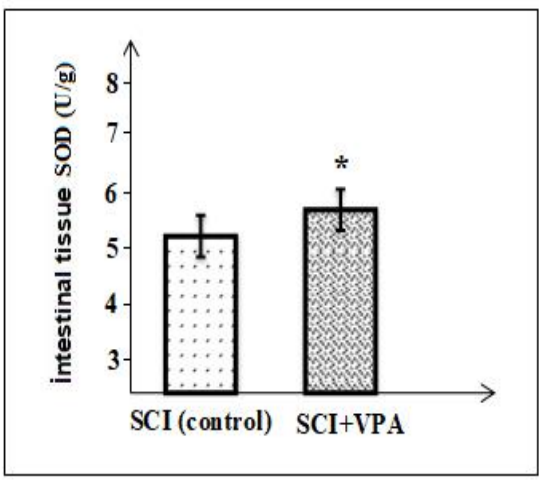


Figure 2: Graphic showing the mean values of SOD as (U/g) protein.

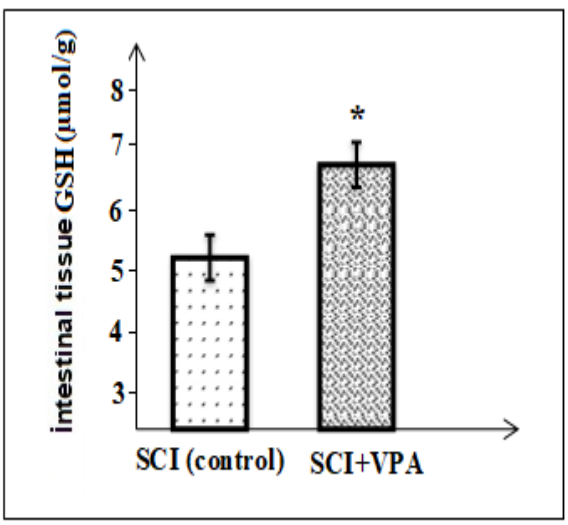


Figure 3: Graphic showing the mean values of GSH as µmol/g protein.

## Discussion

Causes such as autonomic nerve damage due to spinal cord injury and decreased blood flow lead to successive pathophysiological events. The most important of these phenomena is oxidative stress, which leads to the spread of damage to many organs. Damage that begins with a primary injury gradually leads to the emergence of secondary phenomena involving many organs. If the events that develop in secondary injury are not brought under control, they can progress to loss of function, as well as organ damage [1]. Oxidative stress is prevented with the help of antioxidant enzymes (such as SOD), which are the first line of defense. However, when this is not enough, it is tried to prevent such events with agents such as antiepileptic drugs. In our study, the decrease in MDA level and the increase in SOD and GSH levels in the SCI+VPA group compared to the SCI group confirm the information given. VPA, one of the antiepileptic agents, can prevent the spread of secondary damage by inhibiting neuronal damage with its neuroprotective property. In one study, the administration of VPA to rats with ischemia-reperfusion prevented the brain tissue from being affected by damage by preventing the oxidative stress that develops after ischemia [3]. In our study, the administration of VPA to rats with SCI shared a common feature with the mentioned study in terms of preventing damage that develops after SCI. As a result, the use of agents such as VPA in terms of preventing nerve damage events such as SCI can contribute to the fact that many organs are not affected by damage.

## References

[1] Anwar MA, Al Shehabi TS, Eid AH. Inflammogenesis of Secondary Spinal Cord Injury. Front Cell Neurosci. 2016, 13;10:98.

[2] Varma AK, Das A, Wallace G 4th, Barry J, Vertegel AA, Ray SK, Banik NL. Spinal cord injury: a review of current therapy, future treatments, and basic science frontiers. Neurochem Res. 2013;38(5):895-905.

[3] Costalonga EC, Silva FM, Noronha IL. Valproic Acid Prevents Renal Dysfunction and Inflammation in the Ischemia-Reperfusion Injury Model. Biomed Res Int. 2016;2016:5985903.

## *ORAL PRESENTATION*

# $2^3$ Full factorial design approach to the optimization of copper removal from aqueous solutions with bio-nano material

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## Abstract

It is known that the high amounts of copper in water are harmful to the living system and the environment. The removal or recovery of Cu(II) in water is important in many respects, such as being a precious metal. In this study, a bio-nanocomposite material was produced from bio-waste material and silver nanoparticles and used as a bio-adsorbent for the removal of Cu(II) from model aqueous solutions. Since the optimization study of many factors affecting the removal process with bio-adsorption will cause long time and financial loss with classical methods, the effect of factors on bio-adsorption and the interaction between factors have been carried out with the Experimental design method. With $2^3$ full factorial design models, the effects of 3 factors (time, amount of bio-adsorbent, initial Cu(II) concentration) on the bio-adsorption process were examined and their optimum values were found. From the results obtained, the percentage of Cu(II) removal of the bio-nanomaterial from aqueous solutions and the capacity of the bio-adsorbent were determined

**Keywords:** copper, full factorial design, bio-nano material

## INTRODUCTION

As a result of increasing industrial studies in recent years, aqueous environments are polluted with heavy metals. It is not surprising that copper, one of the most used heavy metals in daily life and industry, is found in aqueous environments. A certain level of copper is an essential element for living organisms. However, this level depends on what the living organisms are varied (Bozbaş K. and Boz 2016). The presence of copper in low concentration levels in the human body is necessary for many conditions such as enzyme synthesis, tissue, and bone development; however, high copper concentration has toxic and carcinogenic effects. Various reports in the literature indicate acute copper poisoning/toxicity causing stomach and intestinal disorders such as nausea, vomiting, diarrhea, and stomach cramps (Taylor et. al. 2020). WHO sets the permissible copper level in drinking water as 0.02 mg/L (Chain V. 2011).

Many methods and studies are carried out for the recovery and removal of copper(II) from aqueous environments. Methods such as chemical precipitation and filtration, electrochemical methods, ion exchange, reverse osmosis, evaporation and adsorption have been used for the removal and recovery of copper from aqueous media. Among these methods, the most preferred method is the adsorption method, which has many advantages such as easy applicability, use for all metals, and cheapness.

There are many materials used as adsorbent in the adsorption method. Some of these are newly synthesized materials, some are waste materials, some are bio-materials found in nature. The material to be used as an adsorbent is expected to be easy to obtain, cheap and reusable (Karayunlu Bozbas 2013, Zanin 2017)

The bio-nanomaterial used in this study consists of the powder of seashells collected from the seaside and silver nanoparticles. Ball milling method was chosen for the bio-nanomaterial we obtained. This material was used to remove the copper (II) ion from model aqueous solutions. The percent removal success and optimization conditions in the copper(II) removal of the material we obtained were realized with the $2^3$ full factorial design method.

## MATERIALS AND METHODS

### Materials

Copper was prepared from standard 1000 mg L-1 Cu(II) ion solutions nitrate salts (Merck, Germany). The Ag ion was obtained from $AgNO_3$ (Merck). Different types of seashells we collected from the seaside were first brushed and washed in tap water to purify them from external pollution. The washed peels were kept overnight in deionized water. It was then dried at 100 °C for 24 hours. Clean and dry shells were crushed into small pieces with hard materials such as hammers, then ground and sieved down to 45 µm. These powders are mixed with an $AgNO_3$ solution. The mixture crushed with ball-milling for 2 hours. After ball milling, the mixture was washed with distilled water, centrifuged for 5 minutes and then washed with ethanol. It was dried in vacuo overnight. The bio-nanomaterial was stored in airtight acid-washed polyethylene containers at room condition.

A Perkin Elmer Model AAnalyst 800 flame atomic absorption spectrophotometer (FAAS) was used for determine the Cu(II) concentration after bio-adsorption process.

All the chemicals used in the work were of analytical reagent grade.

### Methods

Factors such as metal concentration, pH, amount of bio-adsorbent, time and temperature affect adsorption. With the Full Factorial design, the total number of experiments required in traditional methods is reduced and the best optimization values are found. It is seen in the literature that experimental design methods are used for many studies with advantages such as improving quality, reducing costs and increasing the reliability of the results. A two-level full factorial design with three experimental factors was modeled to determine the optimum levels of effective factors in determining the maximum percentage of removal in the bio-adsorption process.

Using 20 mL of copper(II) solution at pH 5.5 at room temperature and at a stirring speed of 300 rpm; Factors such as time, copper (II) concentration and amount of bio-nanomaterial and their interaction effects were investigated.

All experiments were performed in 50 mL dark glass bottles containing 25 mL of Cu(II) solution. The bio-adsorption process was started by adjusting the amount of bio-nanomaterial, copper (II) concentration and time values specified in the design for each experiment. After reaching equilibrium, metal concentrations in the supernatant solutions were measured in FAAS (diluted if necessary). The percent removal of adsorbent was calculated from the following equation:

$$\%R = \left(\left(C_0 - C_e\right)/C_0\right)x100 \quad (1)$$

where %R is the percentage removal of Cu(II), $C_0$ and $C_e$ are initial and equilibrium concentrations of Cu(II) in the solution (mg/L), respectively.

For the $2^3$ experimental design, the high and low levels of the three independent factors and the results are listed in Table 1. Appropriate choices of high- and low-level values for the factors and other parameters were determined during previous experiments.

**Table 1.** Appropriate choices of high- and low-level values for the factors and other parameters were determined during previous experiments

| Run | Bio-nanomaterial amount (g) | Cu(II) concentration (mg/L) | Time (min) | R (%) | R (%) |
|---|---|---|---|---|---|
| 1 | 0.05 | 50 | 10 | 49.060 | 48.825 |
| 2 | 0.30 | 50 | 10 | 60.980 | 61.100 |
| 3 | 0.05 | 200 | 10 | 65.095 | 66.010 |
| 4 | 0.30 | 200 | 10 | 65.390 | 64.670 |
| 5 | 0.05 | 50 | 150 | 44.360 | 44.030 |
| 6 | 0.30 | 50 | 150 | 87.886 | 88.920 |
| 7 | 0.05 | 200 | 150 | 65.365 | 64.780 |
| 8 | 0.30 | 200 | 150 | 66.825 | 67.230 |

Using MINITAB 15 statistical software, $2^3$ full factorial design tables were created and the results were analyzed. The results were created by averaging the %R results of two parallel experiments. The order in which the experiments were carried out was chosen randomly to avoid systematic errors.

## RESULTS and DISCUSSION

Effects of optimum conditions and factors on the percentage of Cu(II) removal from model aqueous solutions by bio-nanomaterial; Eight parallel experiments were conducted and main effects were evaluated by Pareto plot, interaction plot, surface plot and analysis of variance of experiments.

Statistical parameters with analysis of variance (ANOVA), the quality of interactions between the independent factors and the model was performed based on a 95% confidence level P-value (Hines and Montgomery 1990). In Table 2, the singular effects of the factors and the two- and three-way interaction effects, the coefficients of the model (Coef.), the standard error (SE Coef) coefficients, Student test values (T) and probability (P) results are given.

**Table 2.**Estimated Effects and Coefficients for R (%) (coded units)

| Term | Effect | Coef | SE Coef | T | P |
|---|---|---|---|---|---|
| Constant | | 63.158 | 0.1102 | 573.06 | 0.000 |
| Bio-nanomaterial amount (g) (A) | 14.434 | 7.217 | 0.1102 | 65.49 | 0.000 |
| Cu(II) concentration (mg/L) (B) | 5.026 | 2.513 | 0.1102 | 22.80 | 0.000 |
| Time (min) (C) | 6.033 | 3.017 | 0.1102 | 27.37 | 0.000 |
| Bio-nanomaterial amount (g)* Time (min) (A*C) | 13.718 | -6.859 | 0.1102 | -62.24 | 0.000 |
| Bio-nanomaterial amount (g)* Cu(II) concentration (mg/L) (A*B) | 8.647 | 4.324 | 0.1102 | 39.23 | 0.000 |
| Cu(II) concentration (mg/L)* Time (min) (B*C) | -5.274 | -2.637 | 0.1102 | -23.93 | 0.000 |
| Bio-nanomaterial amount (g)* Cu(II) concentration (mg/L)* Time (min) (A*B*C) | -7.408 | -3.704 | 0.1102 | -33.61 | 0.000 |

S = 0.44 PRESS = 6.2191, R-Sq = 99.94% R-Sq(pred) = 99.75% R-Sq(adj) = 99.88%

In the Table 2, Effects were found significant at a 5% probability level ($P < 0.05$) in case all factors and factors interactions. In addition, the corrected regression coefficient R-Sq(adj) value of 99.88% was obtained, which shows the compatibility of the model with the experimental data (Tizi et al. 2013). Model equation for Cu(II) extraction by the new bio-nanomaterial:

%R= 63.158 + 7.217A+ 2.513 B +3.017 C -6.859 A*C + 4.324 A*B -2.637 B*C -3.704 A*B*C

The three independent factors examined according to the recovery equation formed from the table and all interactions between the factors are effective on the model. The negative and positive coefficients in the above equation indicate high or low factor settings (Mathialagan and Viraraghavan 2005).
The interaction plots, shown in Figure 1, show the significant interactions of the factors studied for the percent removal of Cu(II) from aqueous solutions. These graphs are used to understand the importance of different interactions on the bio-nanomaterial, in a sense to determine operating conditions.

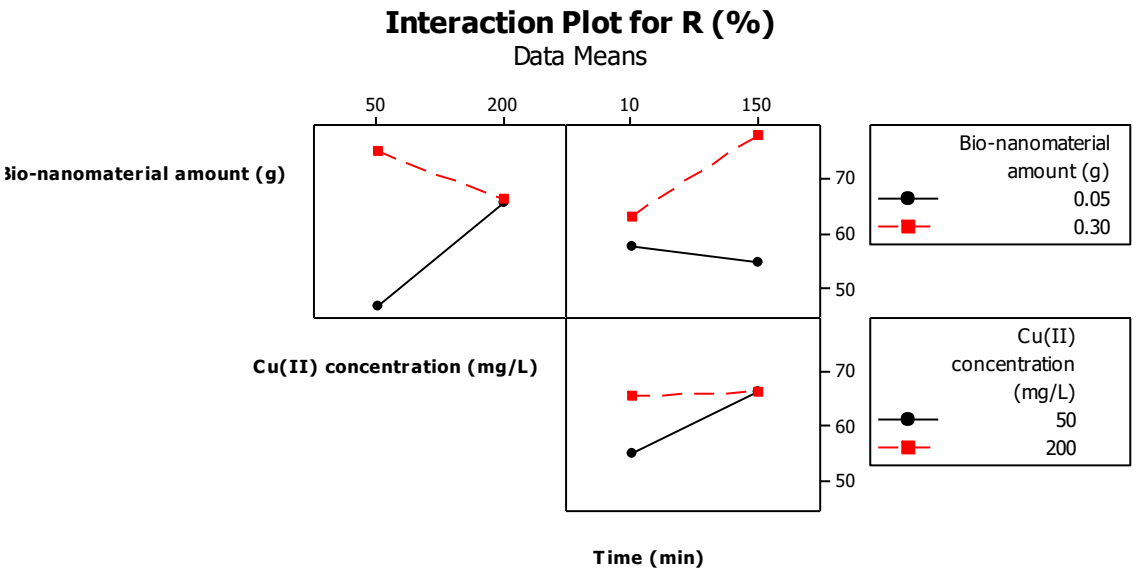


**Figure 1.** Interactions plot of factors

The MINITAB program plots the response for each combination of factors from the generated design and connects the points for the low and high level of the factor plotted on the x-axis. The variation of a factor's response from low to high, depending on the level of a second factor, tells us whether the factors are effective or not. If the lines are not parallel, it is concluded that the investigated factors are effective on bio-adsorption. It is seen that when the Cu(II) concentration is low, the highest %R value is obtained when high time is applied at low Cu(II) concentration and high bio-nanomaterial amount, again at high bio-nanomaterial amount. Cu(II) concentration and amount of bio-nanomaterial, Cu(II) concentration and time, time and amount of bio-nanomaterial; It was determined that the bilateral interactions were effective on the recovery percentage in this case.

The main effects of the three selected factors for the removal of Cu(II) from model aqueous solutions by the bio-adsorption process on the bio-nanomaterial on the recovery percentage with a change in the main factor level are shown in Figure 2. The sign of the selected factors defines whether the effect is positive or negative on the outcome (Srinivasan and Viraraghavan 2010).

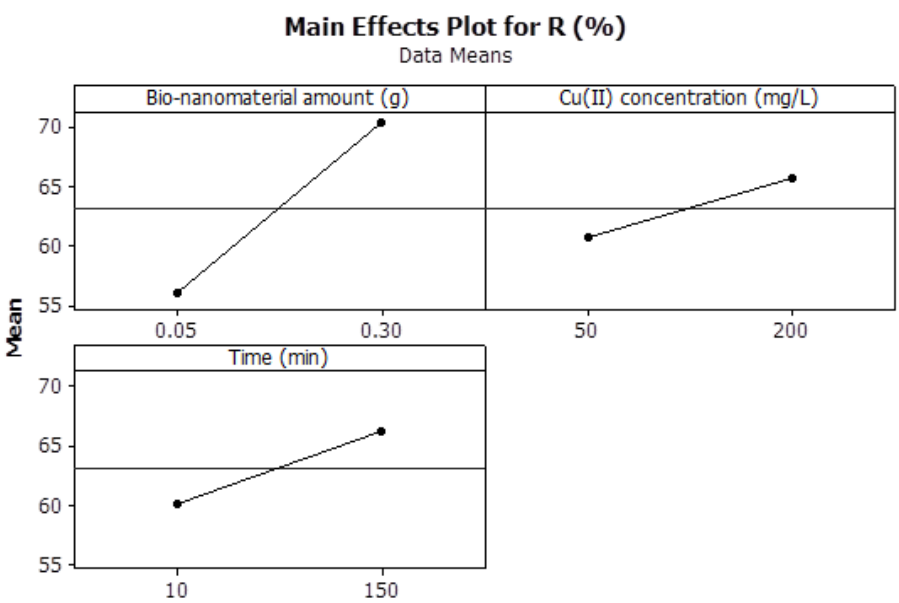


**Figure 2.**Main Effects of Each Factor

As seen in the figure above, while the effects of time and Cu(II) concentration on the bioadsorption process were similar, it was determined that the effect of the amount of bio-nanomaterial was more effective.

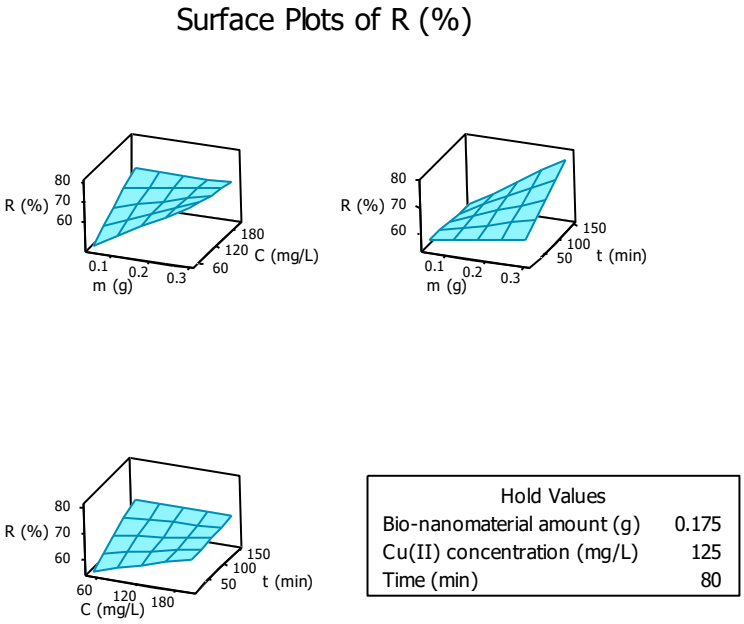


**Figure 3.** Surface plots for median of three factors

Response surface method was used to determine the optimum parameter values for the removal of Cu(II) from model aqueous solutions. The surface graphics of the design are as in Figure 3.
As seen in the graphs drawn based on the median values of the two interactions of the three factors, the highest R % value was determined to be below 90.
According to the optimization values given by the model, it was determined that the highest percent recovery value was 88.40% in the amount of 0.30 g bio-nanomaterial in 50 ppm Cu(II) concentration in 150 minutes. When the experiment was repeated with these optimization values, our test result was determined as 87.56%.

## CONCLUSION

As a result, the use of composite nanomaterial consisting of seashell powder and silver nano-particles as an adsorbent has been tried. The optimization values of the bio-adsorption process of the bio-nanomaterial were carried out by the experimental design method. The highest recovery percentage of the bio-nanomaterial in the removal of Cu(II) from model aqueous solutions was determined as 88.40%. This value was obtained at a concentration of 50 ppm Cu(II) and 0.30 g of bio-nanomaterial in 150 minutes.

## REFERENCES

Bingöl, D., Karayünlü Bozbaş, S. 2012. Removal of Lead (II) from Aqueous Solution on Multiwalled carbon nanotube by using response surface methodology. Spectrosc. Lett. 45: 324-329.

Bozbaş, SK., & Boz, Y. 2016. Low-cost biosorbent: Anadara inaequivalvis shells for removal of Pb (II) and Cu (II) from aqueous solution. Process Safety and Environmental Protection, 103, 144-152.

Chain V 2011 Guidelines for drinking-water quality, Forth. World Health Organization, Gutenberg

Hines, WW. Montgomery, D.C., 1990. Probability and Statistics in Engineering and Management Science, John Wiley and Sons.

Karayunlu Bozbas S., Ay U. & Kayan A. 2013. Novel inorganic–organic hybrid polymers to remove heavy metals from aqueous solution, Desalination and Water Treatment, 51:37-39, 7208-7215.

Mathialagan, T., Viraraghavan, T. 2005. Biosorption of pentachlorophenol by fungal biomass from aqueous solutions: A factorial design analysis. Environ. Technol. 26: 571-579.

Srinivasan, A., Viraraghavan, T. 2010. Oil removal from water by fungal biomass: A factorial design analysis, J. Hazard. Mater. 175: 695-702

Tizi, H., Berrama, T., Kaouah, F., Bendjama, Z. 2013. Study of the conditions of activated carbon preparation from an agriculture by-product for 4BA elimination in aqueous solution using full factorial design, Desalin. Water Treat. 51:7286-7295

Taylor AA, Tsuji JS, Garry MR et al 2020 Critical review of exposure and effects: implications for setting regulatory health criteria for ingested copper. Environ Manage 65:131–159

Ünügül, T., Nigiz, F.U., & Karayünlü Bozbaş, S. 2022. Application of Response Surface Methodology for Optimization of Copper Removal Using a Novel Polymeric Adsorbent. Journal of Polymers and the Environment, 1-15.

Zanin E, Scapinello J, de Oliveira M et al. 2017 Adsorption of heavy metals from wastewater graphic industry using clinoptilolite zeolite as adsorbent. Process Saf Environ Prot 105:194–200

## *ORAL PRESENTATION*

# Effect of vacuum packaging on shelf life of fresh crabs

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## Abstract

In this study, it was aimed to determine the shelf life of blue crab meat, which was caught and cleaned in the processing plant by the fishermen of the DALKO Fisheries Cooperative in the Köyceğiz Dalyan region. Blue crabs were taken from DALKO Fisheries Cooperative and brought to the Muğla Sıtkı Koçman University Fisheries Faculty Quality Control Laboratory under cold chain conditions. After the initial analyses (sensory, chemical, microbiological and nutrient content) were made for the fresh blue crabs, the remaining samples were shrink packed. Packaged samples were kept under refrigerator (+/- 4ºC) conditions, and shelf life analyses were carried out at certain periods during storage.

According to the results, the initial nutritional values (protein, lipid, moisture and ash) of freshly packaged crabs were determined as 16.22%, 1.06%, 81.17%, 1.45%, respectively. At the end of storage, these values were determined as 15.88%, 1.51%, 80.18%, 1.67%, respectively. According to the results of sensory, chemical and microbiological analysis, the consumable limit values were exceeded on the 4$^{th}$, 8$^{th}$ and 4$^{th}$ days, respectively. When sensory and microbiological analyses were considered, it was determined that the fresh crabs had a shelf life of 4 days in vacuum packaged refrigerator conditions.

**Keywords:** Blue crab, shelf life, vacuum packaging

## INTRODUCTION

DALKO Dalyan Fisheries Cooperative, is established by the people of the region in 1971 within the borders of Köyceğiz Lagoon, Dalyan town of Köyceğiz district in Muğla/Turkey. The cooperative was established in order to protect the small fishermen in the region, to provide job opportunities, to market the fishery products, to continue the old lagoon fishery, and to protect the environment and nature. The cooperative constitutes a source of livelihood for many families. Köyceğiz Dalyan is located in a world-famous town where tourism activities are intense. Caught fish and blue crabs are offered to local and tourists at fish restaurants and sales points. Undesirable situations at production and sales, because of inadequate and unconscious hygiene conditions, negatively affect our country's tourism, as well as create problems for the livelihood of hundreds of families in Dalyan.

DALKO cooperative was stated that they have difficulties in packaging of the blue crabs, which are offered fresh or cooked to the consumers. Tearing the vacuum bags during the packaging of shellfish products reduces the effect of packaging on the shelf life of the product. Blue crabs in the cooperative is sold to the consumer either in aluminum foils or wrapped in paper packages. The consumer has to consume the product that bought either immediately or in a short time under refrigerator conditions. It is thought that these punctures can be prevented when shrink packaging is applied to these samples taken into the bowl.

In addition, it is thought that the quality of supply in the market will be increased by having a knowledge about the nutritional compositions of aquatic products produced by DALKO Fisheries Cooperative and marketed both to the local and foreign markets. It is aimed to increase the demand of consumers for these products, which are healthy and rich in nutrients, and to gain consumption habits. Especially since the content and label information are not fully specified in the foreign market, our local products, which are kept in imperfect competition with the products produced by other countries, find buyers far below their value.

In line with these shortcomings, in this study, it was aimed to determine the nutritional content and shelf life of shrink packaged blue crab meat, which was caught by the fishermen of the DALKO Fisheries Cooperative in Köyceğiz Dalyan region and cleaned in the processing facility. The aim of our research is to contribute to our country's economy by providing added value to the product in the foreign market by promoting the consumption of our local products and to extend the shelf life by packaging.

## MATERIALS AND METHODS

### Material

In this study, blue crab caught from Köyceğiz Dalyan was used. Blue crabs were brought to Muğla Sıtkı Koçman University, Faculty of Fisheries, Quality Control Analysis Laboratory from DALKO Fisheries Cooperative within 1 hour under cold chain conditions after the upper shell part was removed. 200 mature crabs were used.

### Method

#### Vacuum Packaging of the samples

The crabs, which were brought to the laboratory from DALKO Fisheries Cooperative, were packed in plastic containers in 2 pieces, then packed with a vacuum shrink machine and stored in the refrigerator. Analyses were carried out during 8 days.

#### Nutritional Composition Analysis

In the fresh blue crab meat, nutritional compositions analyses; % protein; according to AOAC (2006a, 984.13) by Kjeldahl method, % lipid; according to Bligh and Dyer (1959), % moisture; according to AOAC (2006b, 934.01) and % ash analyses; according to AOAC (1990, 950.46) were carried out at the beginning and at the end of storage.

#### Sensory Analysis

In sensory analysis, hedonic scale test applied for fresh seafood products was used. Crab meat was evaluated over 5 points in terms of aroma, taste, color, texture, smell, appearance and general appreciation criteria.

#### Chemical Analysis

Over the 8-day period, from chemical analyses; according to Manthey et al. (1988) pH, according to Antonocoupoulos (1973) Total Volatile Basic Nitrogen (TVB-N), according to Tarladgis et al. (1960) Thiobarbituric Acid (TBA) analyses were carried out.

#### Microbiological Analysis

According to FDA/BAM (2001) microbiological analyses; total viable bacterial count (TVC) and psychrotrophic bacteria count were performed.

## RESULTS and DISCUSSION

### Nutritional Composition Analysis Results

At the beginning and at the end of storage nutritional composition analyses; protein, lipid, moisture and ash analyses were made in the fresh crab brought to the laboratory. At the beginning (Day 0) protein was determined as 16.22%, lipid 1.06%, moisture 81.17% and ash 1.45% (Table 1). At the end of storage, these values were determined as 15.88%, 1.51%, 80.18%, 1.67%, respectively. Protein and moisture content decreased slightly during storage. Zotti et al. (2016) find the moisture, protein and ash values of blue crabs (*Callinectes sapidus*), 80.12%, 15.13%, 1.63%, respectively, in their study in Acquatina Lagoon (SE Italy). The results are quite similar with the results in our study. Umer et al. (2021) determined the amount of lipid in commercial crab species; *P. pelagicus*, *P. sanguinolentus*, *S. serrata* and *C. feriatus* in the range of 0.25-1.86 g/100 g. The crabs have high protein values and low lipid values, as in our study.

**Table 1.** Nutritional composition analysis results

| | Nutritional Composition (%) | | | |
|---|---|---|---|---|
| | **Protein** | **Lipid** | **Moisture** | **Ash** |
| **Initial (Day 0)** | 16.22±0.28 | 1.06±0.04 | 81.17±0.26 | 1.45±0.04 |
| **End of the storage** | 15.88±0.55 | 1.51±0.12 | 80.18±0.29 | 1.67±0.18 |

(Mean ± SD, n:4)

### Sensory Analysis Results

According to the sensory analysis results, the consumable limit values were exceeded on the 6th day. The odor and texture deteriorated after the 4th day and these characteristics were evaluated unfavorable by the panelists. Color properties were also deteriorated.

### Chemical Analysis Results

TVB-N value was determined as 12.42 mg/100 g on day 0, 19.79 mg/100 g on day 2, 30.76 mg/100 g on day 4, 34.32 mg/100 g on day 6, 40.78 mg/100 g on day 8 (Figure 1). The limit value of 35 mg/100 g for seafood meat was exceeded on the 8th day.

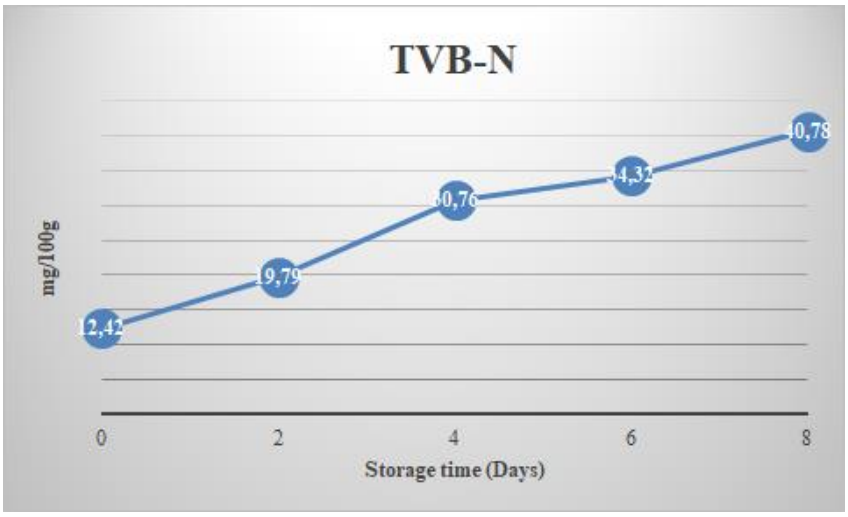


**Figure 1.** TVB-N analysis results

Lorentzen et al. (2016) in the study of shelf life of snow crab stored at 0 and 4ºC, in raw meat, the level of TVB-N was 20 mg/100 g from day 0 and it did not change during the storage period of 7 days. Sun et al. (2017) in their study about the effects of superchilling with modified atmosphere packaging on the shelf life of swimming crab, the TVB-N of air packaged samples (without MAP) increased rapidly, and the value reached 30.64 mg N/100 g on the 5th day of storage at 4ºC. Similar results were obtained in our study.

The pH value was determined as 6.31 on day 0, 6.61 on day 2, 6.96 on day 4, 6.96 on day 6, and 7.35 on day 8 (Figure 2). The limit value was exceeded on the 8th day.

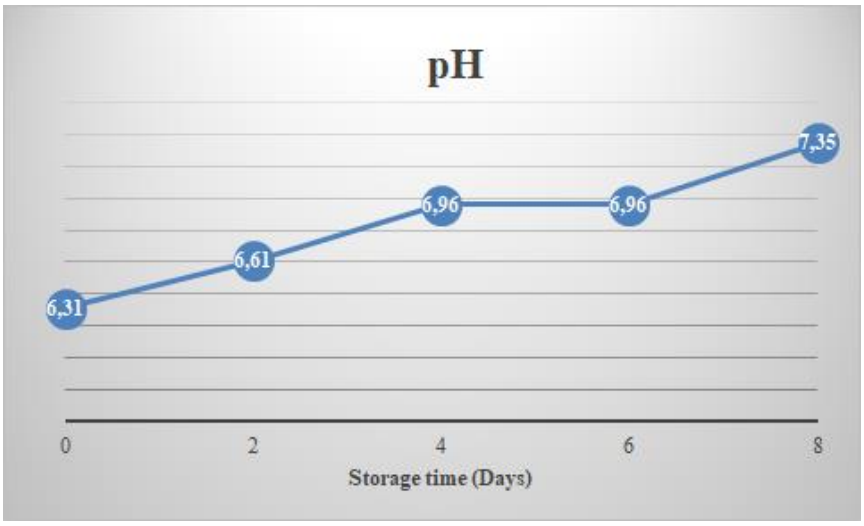


**Figure 2.** pH analysis results

Lorentzen et al. (2014) examined the shelf life of cooked red king crab at 4ºC, and the pH value was found between 7.3-7.9 during the 14-day study. Lorentzen et al. (2016) in the other study about shelf life of snow crab stored at 0 and 4ºC, The pH of raw leg crab meat was 6.5 at day 2 and the final pH was 7.0 at day 7. Similar results were obtained in our study.

TBA value was determined as 0.37 mg malonaldehyde/kg on day 0, 0.54 mg malonaldehyde/kg on day 2, 0.63 mg malonaldehyde/kg on day 4, 0.70 mg malonaldehyde/kg on day 6, 0.79 mg malonaldehyde/kg on day 8 (Figure 3). During the storage, values were obtained quite below the limit value of 8 mg malonaldehyde/kg. This value was not exceeded during storage.

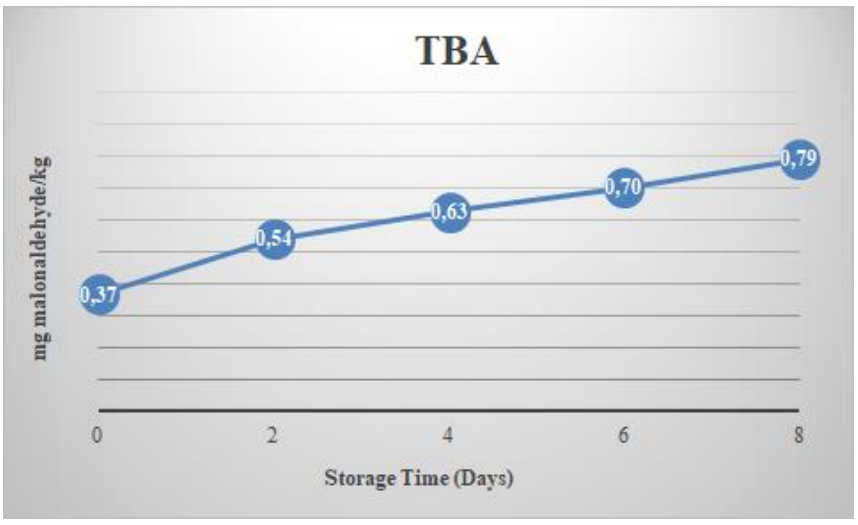


**Figure 3.** TBA analysis results

Sun et al. (2017) in the study that examined the effects of superchilling with modified atmosphere packaging on the shelf life of swimming crab, the TBARS content reached 1.5 mg MDA/kg of muscle at day 4 in for air packed (without MAP) samples. Higher results were obtained than our study.

**Microbiological Analysis Results**

**Total Viable Count Results (TVC)**

Total viable count was determined as log 4.82 CFU/g on day 0, log 5.18 CFU/g on day 2, log 5.67 CFU/g on day 4, log 7.44 CFU/g on day 6, log 7.22 CFU/g day 8 (Figure 4). An increase occurred during the storage. The recommended limit of $\log 10^6$ CFU/g for refrigerated and frozen crab meat according to ICMSF (1986) was exceeded on the 6$^{th}$ day.

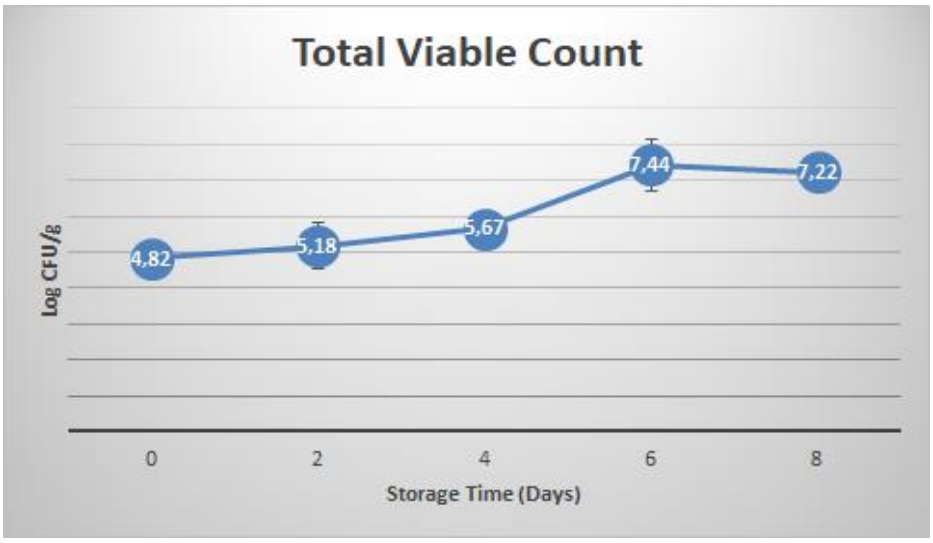


**Figure 4.** Total viable count results

Psychrotrophic bacteria count was determined as log 3.35 CFU/g on day 0, log 4.16 CFU/g on day 2, log 6.43 CFU/g on day 4, log 5.22 CFU/g on day 6, log 7.64 CFU/g on day 8 (Figure 5). An increase occurred during storage.

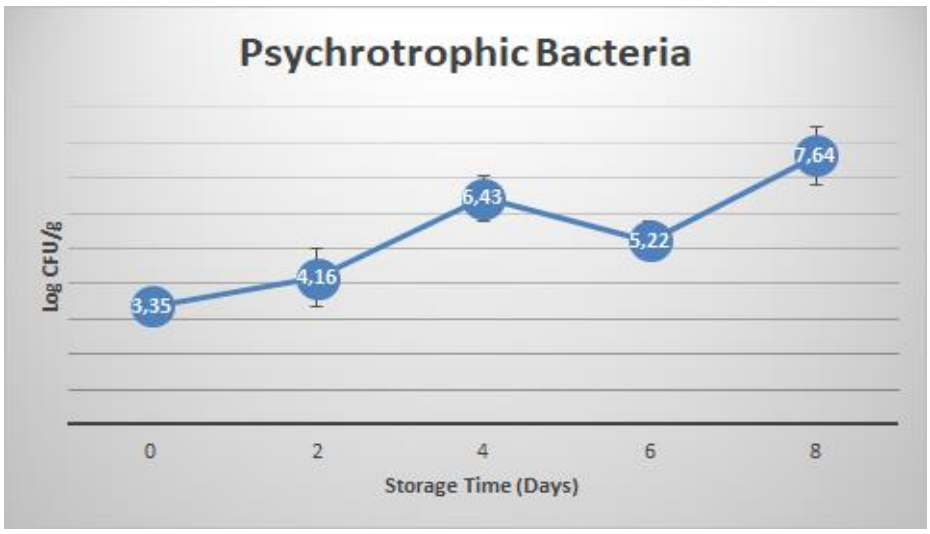


**Figure 5.** Psychrotrophic bacteria results

Lorentzen et al. (2014) in the study examined the shelf life of cooked red king crab at 4ºC, the TVC value was found below the viable count up to 5 days, and it was log 4.4 CFU/g on the 5$^{th}$ day. Due to the cooked sample, a lower result was obtained than the values of our study. Lorentzen et al. (2016), in the study of shelf life of snow crab stored at 0 and 4ºC, TVC increased to maximum level of log 5.5 CFU/g in cooked crab at 4ºC. The initial level of TVC of raw snow crab was approximately log 2.5 CFU/g and increased about one log unit over the next 7 days of storage at 0ºC. Lower values were obtained than our study due to storage at lower temperatures. Sun et al. (2017) in the study investigated the effects of superchilling with modified atmosphere packaging on the shelf life of swimming crab, for air packed (without MAP) samples, the initial total aerobic plate count (TPC) was 3.96 log CFU/g for fresh crab and TPC reached log10$^5$ CFU/g at day 4.

## CONCLUSION

In this study, shrink packaging was applied to fresh crabs and shelf life analyses were carried out during 8 days of cold storage. As a result of the analyses, it was concluded that the shelf life of the packaged crabs was 4 days according to the results of sensory and microbiological analysis. According to the results of the chemical analysis of the packaged crab samples, it was determined that they preserved their quality properties during storage up to 6 days. The high initial microbial loads of the samples prevented further extension of the shelf life. It is thought that pre-treatments such as cooking or disinfection before packaging to extend the shelf life will reduce the microbiological load as well as improve the product in terms of sensory and chemical properties. In addition, it is suggested that compliance with the personnel and plant hygiene rules during the

capture, processing and storage of the product is important for the future studies in terms of the quality of the product.

## ACKNOWLEDGEMENTS

This project was supported with the application number '1919B012103483' within the scope of TUBITAK 2209-A University Student Research Projects Program.

## REFERENCES

Antonocopoulus N 1973. Bestimmung des Fl_chtigen Basenstickstoofs, In: Ludorf W and Meyer V, Fische und Fischerzeugnisse. Aulage Verlag Paul Parey, Berlin, pp: 224-225.

AOAC 1990. Official Methods of Analysis (13th Ed.) Association of Official Analytical Chemists, Official method 950.46, Washington, D.C., USA.

AOAC 2006a. Crude protein in meat. In Official methods of analysis (17th ed.). 984.13. Gaithersburg, Maryland: Association of Official Analytical Chemists.

AOAC 2006b. Moisture content. Official method 934.01. Official Methods of Analysis (17th ed.). Gaithersburg: Maryland (USA): Association of Official Analytical Chemists.

Bligh EG, Dyer WJ 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37: 911-917.

FDA/BAM 2001. Food and Drug Analyses/Bacteriological analytical manual, Aerobic plate count, Edition 8, Chapter 3, January.

ICMSF (International Commission on Microbiological Specifications for Foods) 1986. Microorganisms in foods 2: sampling for microbiological analysis: principles and specific applications, 2nd edn. University of Toronto Press, Canada, pp: 1–293.

Lorentzen G, Skuland AV, Sone I, Johansen JO 2014. Determination of the shelf life of cluster of the red king crab (*Paralithodes camtschaticus*) during chilled storage. Food Control, 42: 207-213.

Lorentzen G, Rotabakk BT, Olsen SH, Skuland AV 2016. Shelf life of snow crab clusters (*Chionoecetes opilio*) stored at 0 and 4ºC. Food Control, 59: 454-460.

Manthey M, Karnop G, Rehbein H 1988. Quality changes of European catfish from warm-water aquaculture during storage ice. International Journal of Food Science and Technology, 23: 1-9.

Sun B, Zhao, Y, Ling J, Yu J, Shang H, Liu Z 2017. The effects of superchilling with modified atmosphere packaging on the physicochemical properties and shelf life of swimming crab. Journal of Food Science and Technology, 54(7): 1809–1817.

Tarladgis BG, Watts, BM, Younathan MT, Dugan TL 1960. A distillation method for quantitative determination of malonaldehyde in rancid foods. Journal of the American Oil Chemists' Society, 37: 44-48.

Umer, A, Syed MN, Tarar OM, Mushtaq S, Jalbani N, Saleem N, Haider MS, Ahmadi N 2021. Biochemical evaluation with reference to nutritional aspects of edible species of crabs collected from the coastal waters of Pakistan. Journal of Food Composition and Analysis, 100: 103877.

Zotti M, Coco LD, Pascali SAD, Migoni D, Vizzini S, Mancinelli G, Fanizzi FP 2016. Comparative analysis of the proximate and elemental composition of the blue crab *Callinectes sapidus*, the warty crab *Eriphia verrucosa*, and the edible crab *Cancer pagurus*. Heliyon, 2(2): e00075.

## *ORAL PRESENTATION*

# Balık parazitlerinin ülkeler arası yolculuğu

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## Özet

Su ürünleri yetiştiriciliğinin sürdürülebilirliğinde tehdit oluşturan ve sektörün gelişimini olumsuz yönde etkileyebilecek en önemli riskler arasında balık hastalıkları yer alır. Balık patojenlerinin uluslararası ticaret yoluyla farklı bölgelere taşınması ve taşındıkları yerlerde yayılma potansiyelinde oldukları bilinmektedir. Dünyada önemli bir sektör olan akvaryum balığı ticareti infeksiyöz hastalıkların sınır ötesine yayılımını kolaylaştırmaktadır. Balıklarda hastalık oluşturan etkenlerden olan parazitler, akvaryum balığı ticaretinde de dikkat edilmesi gereken problemler arasında yer alır. İthalatçı ülkeler ile özellikle Uzakdoğu menşeili ihracatçı ülkeler arasındaki ticaret sonucunda *Centrocestus formosanus, Camallanus cotti, Bothriocephalus acheilognathi* gibi kompleks yaşam siklusuna sahip parazitler, *Dactylogyrus anchoratus, D. vastator* gibi monogenean parazitler ile *Chilodonella sp., Trichodina sp., Ichthyophthirius multifiliis* gibi Ciliatalar yeni bölgelere, farklı coğrafyalara taşınmaktadırlar. Bu çalışmada, farklı ülkelerden akvaryum balığı ticareti ile taşınan parazit türleri, dünya üzerindeki parazit rotaları ve doğal fauna için olası riskler açıklanmaktadır.

**Anahtar Kelimeler:** Akvaryum balıkları, parazit transferi.

# Transboundary spread of fish parasites

## Abstract

Fish diseases are one of the most important risks threatening the sustainability of aquaculture, adversely affecting the development of the sector. It is known that fish pathogens have the potential to be transported and spread to different regions through international trade. The ornamental fish trade, which is an important sector in the world, facilitates the spread of infectious fish diseases across borders. Fish parasites are known as a common problem in the aquarium fish trade. As a result of trade between importing and exporting countries, especially by imports from the Far East, parasites with complex life cycles such as *Centrocestus formosanus, Camallanus cotti, Bothriocephalus acheilognathi*, monogenean parasites such as *Dactylogyrus anchoratus, D. vastator*, and ciliates like *Chilodonella* sp., *Trichodina* sp., *Ichthyophthirius multifiliis* move to different and various geographical regions. In this study, parasite species transported by ornamental fish trade from different countries, parasite routes around the world and possible risks for natural fauna are explained.

**Keywords**: Aquarium fish, parasite transfer.

## GİRİŞ

Süs balığı bakmak yüzlerce yıllık geçmiş olan popüler bir hobidir ve bu ilgiyle beraber 125'ten fazla ülkede artan süs balığı ticareti gerçekleştirilmektedir. Uluslararası süs balığı ticareti gelişmekte olan ülkelerde binlerce kişiye iş olanakları sağlamaktadır. Balık yetiştiriciliği, ulaşım ve akvaryum teknolojisindeki gelişmeler ile her yıl daha çok balık türü bu ticarete konu olmaktadır (Dey 2016).

2020 yılı verilerine göre süs balığı ithalatçısı ülkelerin başında Amerika ve Çin gelmektedir. 2020 yılında en büyük ithalatçı olarak Amerika 63.7 milyon dolarlık (%19.3 ) ithalat yapmıştır. Amerika'yı Çin 30.1 milyon dolarlık ithalat ile takip etmiştir. Diğer büyük ithalatçılardan bazıları Almanya (23.5 milyon dolar), İngiltere (22.2 milyon dolar), Fransa (19.4 milyon dolar), Japonya (14.8 milyon dolar), Hong Kong (12.6 milyon dolar), Hollanda (12.5 milyon dolar) ve Singapur'dur (9.09 milyon dolar). İhracatta ise Japonya 2020 yılında 43.1

milyon dolarlık ihracat ile birinci konuma sahip olmuştur. Diğer büyük ihracatçı ülkelerdan bazıları ise Endonezya (38.5 milyon dolar), Singapur (33.8 milyon dolar), Tayland (21.9 milyon dolar), Hollanda (21.9 milyon dolar), Çek Cumhuriyeti (18.7 milyon dolar) ve Malezya (16.1 milyon dolar) dır (FAO 2022).

Su ürünleri yetiştiriciliğinde sürdürülebilirliği risk altında tutan etkenlerden biri balık hastalıklarıdır. Balık hastalıkları balık türü, yetiştirme sistemi, çevresel etkenler ve patojen karakteristikleri gibi faktörlerin etkileşimi sonucu ortaya çıkmaktadır (Yavuzcan ve ark. 2010). Akvaryum balığı ticareti balık hastalıklarının ülkelerarası yayılımını kolaylaştırmaktadır. Birçok patojenik metazoan, protozoan, bakteri ve virüsün sucul organizmaların transferi ile hastalık salgınlarına yol açtığı bilinmektedir (Whittington ve Chong 2007). Son yıllarda yapılan çalışmalar uluslararası ticaret vasıtasıyla yoğun patojen hareketinin olduğunu ve ekolojik riskler kapsamında egzotik organizmaların ticareti ile patojen transferinin önemli hale geldiğini göstermektedir (Evans ve Lester 2001, Kim ve ark. 2002, Thilakaratne ve ark. 2003, Yildiz 2005, Şahin ve Yavuzcan 2005, Tripathi 2014a, Adel ve ark. 2015).

Paraziter balık hastalıkları balık yetiştiriciliğinin en önemli problemlerindendir ve balık parazitleri su ekosistemlerinin ayrılmaz bir parçasıdır (Jimenez- Garcı'a ve ark. 2001, Lintermans 2004, Copp ve ark. 2005, Gertzen ve ark. 2008, Trujillo-Gonzales ve ark. 2018). Parazitlerin çoğu enfekte balıkların ticareti esnasında taşınmaları ile farklı ülkelere ve bölgelere girmektedir (Velez-Hernández ve ark. 1998, Evans ve Lester 2001) ve bu ülkelerin faunası için tehlike yaratmaktadır. Bu çalışmada, farklı ülkelerden akvaryum balığı ticareti ile taşınan parazit türleri, dünya üzerindeki parazit rotaları ve doğal fauna için olası riskler açıklanmaktadır.

## AKVARYUM BALIĞI TİCARETİ İLE ÜLKELER ARASI PARAZİT TRANSFERİ

Patojenlerin artışına zemin sağlayan aşırı stoklama, taşıma, ilaç tedavisi, yetersiz beslenme, sıcaklık değişimleri ve su kirliliği gibi faktörler balıkların homeostatik dengesi üzerinde baskı yaratmakta ve akvaryum balıklarını parazitlere karşı hassaslaştırarak paraziter hastalıkların yayılmasına sebep olmakta ve önemli balık kayıplarıyla sonuçlanmaktadır.

Yetiştiricilik koşullarında hastalık nedeniyle balık kayıplarının % 10-20 olduğu ve paraziter hastalıklar neticesi ölümlerin bu kayıplar içersinde 25%'lik paya sahip olduğu bildirilmektedir (Möller 1987, Sinderman 1987, Yeler 1998, Özer ve Erdem 1999, Scholz 1999, Thilakaratne ve ark. 2003).

Akvaryum balığı ticareti, 60%'ından fazlası tatlı su balıklarından oluşan 5000'den fazla balık türünün dahil olduğu, 2021 yılı verilerine göre yıllık yaklaşık 5 milyar dolarlık küresel hacme sahip önemli bir sektördür.

Yılda 1 milyardan fazla balığın 100'den fazla ülkeye ihraç edildiği global akvaryum balığı ticareti enfeksiyöz paraziter balık hastalıklarının sınır ötesi yayılımını kolaylaştırmaktadır.

## İTHALATÇI ÜLKELERE PARAZİT GİRİŞİ – AVUSTRALYA ÖRNEĞİ

İthalatçı ülkelerden Avustralya'ya ithal edilen çeşitli balık türlerinde tespit edilen parazitler ve ilgili çalışmalar Tablo 1'de listelenmiştir.

Tablo 1. İthalatçı Ülkelere Parazit Girişi - Avustralya Örneği

| Balık | Parazit/Hastalık | İhraç Eden Ülke | Referans |
|---|---|---|---|
| Pembe diken, gurami, lepistes, | *Dactylogyrus* spp., *D. Vastator*, *Gyrodactylus* spp., *Carassius auratus*, *Dactylogyrus ostraviensis*, *Pethia conchonius*, *Trianchoratus* spp., *Trichopodus trichopterus*, *Trichopodus leerii*, *Urocleidoides reticulatus*, *Poecilia reticulata*, | Singapur, Malezya, Tayland, Sri Lanka | Trujillo-González ve ark. (2018) |
| *Poecilia reticulata*, *Xiphophorus maculatus*, *Paracheirodon innesi*, *Paracheirodon axelrodi*, *Gyrinocheilus aymonieri* | *Camallanus cotti*, *Centrocestus formosanus*, *Bothriocephalus acheilognathi*, *Urocleidoides reticulatus*, *Tetrahymena corlissi*, *Chilodonella piscicola*, *Hexamita* sp., *Cryptobia* sp., *Chloromyxum* sp. | Singapore, Indonesia, Hong Kong, Germany | Evans ve Lester (2001) |

## İTHALATÇI ÜLKELERE PARAZİT GİRİŞİ – BREZİLYA ÖRNEĞİ

İthalatçı ülkelerden Brezilya'ya ithal edilen çeşitli balık türlerinde tespit edilen parazitler ve ilgili çalışmalar Tablo 2'de listelenmiştir.

Tablo 2. İthalatçı Ülkelere Parazit Girişi - Brezilya Örneği

| Balık | Parazit/Hastalık | Referans |
|---|---|---|
| *Xiphophorus maculatus*, *Danio rerio*, *Paracheirodon axelrodi*, *Corydoras ephippifer* | *Ichthyophthirius multifiliis*, Digenea metacercariae, *Acanthostomum* sp. metacercariae, *Camallanus* spp., *Bothriocephalus acheilognathi*, *Echinorhynchus* sp. | Hoshino ve ark. (2018) |

## İTHALATÇI ÜLKELERE PARAZİT GİRİŞİ – PAKİSTAN ÖRNEĞİ

İthalatçı ülkelerden Pakistan'a ithal edilen çeşitli balık türlerinde tespit edilen parazitler ve ilgili çalışmalar Tablo 3'te listelenmiştir.

Tablo 3. İthalatçı Ülkelere Parazit Girişi - Pakistan Örneği

| Balık | Parazit/Hastalık | Referans |
|---|---|---|
| *Carassius auratus* | *Ichthyophthirius multifiliis,*<br>*Tetrahymena* sp.,<br>*Dactylogyrus* sp.,<br>*Gyrodactylus* sp.,<br>*Argulus foliaceus* | Iqbal ve Noreen (2014) |
| *Carassius auratus* | *Dactylogyru extensus,*<br>*Gyrodactylus* sp.,<br>*Ichthyophthirius multifiliis,*<br>*Trichodina* sp.,<br>*Argulus foliaceus* | Iqbal ve Rehaman (2014) |
| *Carassius auratus,*<br>*Poecilia reticulata,*<br>*Poecilia sphenops,*<br>*Xiphophorus maculatus,*<br>*Xiphophorus helleri* | *Gyrodactylus turnbulli,*<br>*Gyrodactylus* sp.,<br>*Dactylogyru extensus,*<br>*Trichodina* sp.,<br>*Chidonella* sp.,<br>*Ichthyophthirius multifiliis,*<br>*Piscinoodiniuö pillulare* | Iqbal ve Haroon (2014) |

## İHRACATÇI ÜLKELERDE PARAZİTİK BULGULAR – ENDONEZYA ÖRNEĞİ

İhracatçı ülkelerden Endonezya'dan ihraç edilen çeşitli balık türlerinde tespit edilen parazitler ve ilgili çalışmalar Tablo 4'te listelenmiştir.

Tablo 4. İhracatçı Ülkelerde Parazitik Bulgular - Endonezya Örneği

| Balık | Parazit/Hastalık | Referans |
|---|---|---|
| *Poecilia reticulate,*<br>*Carrasius auratus* | *Dactylogyrus* sp.,<br>*Gyrodactylus* sp.,<br>P*iscinodinium* sp.,<br>*Capillaria* sp. | Dewi ve Fadhilla (2018) |

## İHRACATÇI ÜLKELERDE PARAZİTİK BULGULAR – GÜNEYDOĞU ASYA ÖRNEĞİ (SİNGAPUR, MALEZYA, TAYLAND, SRİ LANKA)

İhracatçı ülkelerden Singapur, Malezya, Tayland ve Sri Lanka'dan ihraç edilen çeşitli balık türlerinde tespit edilen parazitler ve ilgili çalışmalar Tablo 5'te listelenmiştir.

Tablo 5. İhracatçı Ülkelerde Parazitik Bulgular - Güneydoğu Asya Örneği (Singapur, Malezya, Tayland, Sri Lanka)

| Balık | Parazit/Hastalık | Referans |
|---|---|---|
| *Carassius auratus,*<br>*Pethia conchonius* | *D. anchoratus ,*<br>*D. formosus ,*<br>*D. intermedius,*<br>*D. vastator,*<br>*D. ostraviensis* | Trujillo-González ve ark. (2018) |

## İHRACATÇI ÜLKELERDE PARAZİTİK BULGULAR – SRİ LANKA ÖRNEĞİ

İhracatçı ülkelerden Sri Lanka'dan ihraç edilen çeşitli balık türlerinde tespit edilen parazitler ve ilgili çalışmalar Tablo 6'da listelenmiştir.

Tablo 6. İhracatçı Ülkelerde Parazitik Bulgular - Sri Lanka örneği

| Balık | Parazit/Hastalık | Referans |
|---|---|---|
| *Poecilia reticulata*, *Carassius auratus*, *Xiphophorus maculatus*, *Poecilia sphenops*, *Pterophyllum scalare*, *Xiphophorus helleri*, *Hyphessobrycon* sp., *Capeota*, *Puntius* spp., *Colisa* sp., *Cyprinus carpio*, *Betta spelendens* | *Dactylogyrus extensus*, *Dactylogyrus vastator*, *Dactylogyrus* spp., *Gyrodactylus turnbulli*, *Gyrodactylus katherineri*, *Gyrodactylus* spp., *Trichodina nigra*, *Trichodina* spp., *Tetrahymena corlissi*, *Tetrahymena pyriformis*, *Ichthyophthirius multifiliis*, *Ichthyobodo necator*, *Piscinoodinium* spp., *Argulus foliaceus*, *Lernaea cyprinacea*, *Ergasilus ceylonensis* | Thilakaratne ve ark. (2003) |

## TÜRKİYE'YE İTHAL EDİLMİŞ AKVARYUM BALIKLARI VE PARAZİTLER

2020 yılı verilerine göre Türkiye'nin 2020 yılı süs balığı ithalat değeri 295211 dolar'dır. Aynı yıl Türkiye'den yapılan akvaryum balığı ihracat değeri ise 44066 dolar olmuştur (The Observatory of Economic Complexity (OEC) 2020).

Özer (2021) tarafında yapılan çalışmada Türkiye'de 2020 yılı sonuna kadar araştırılmış balık parazitleri ve bunların görüldüğü tatlı su, deniz ve akvaryum balıkları belirtilmiştir.

Ülkemizde akvaryum balıkları parazitleri üzerine yapılan bazı çalışmalar Tablo 7'de listelenmiştir.

Tablo 7. Türkiye'ye İthal Edilmiş Akvaryum Balıkları ve Taşıdıkları Parazitler

| Balık | Parazit/Hastalık | Referans |
|---|---|---|
| *Xiphophorus maculatus,*<br>*Poecilia reticulata,*<br>*Symphsodon aequifasciatus,*<br>*Xiphophorus helleri,*<br>*Pangasianodon hypopthalmus,*<br>*C. auratus auratus* | *Chilodonella sp.,*<br>*Dactylogyrus* sp.,<br>*Centrocestus metaserker,*<br>*Thaparocleidus caecus,*<br>*Thaparocleidus siamensis,*<br>*Ichthyophthirius multifiliis* | Şahin (2019) |
| *C. auratus auratus* | *Ichthyophthirius multifiliis,*<br>*Chilodonella sp.,*<br>*Trichodina sp.,*<br>*Dactylogyrus sp.,*<br>*Gyrodactylus sp.,*<br>*Argulus sp.,*<br>*Centrocestus metaserkerleri* | Şahin ve Yavuzcan (2005) |
| *Poecilia reticulata,*<br>*Chichlasoma nigrofasciatum,*<br>*Labidochromis caeruleus* | *Tetrahymena* spp.,<br>*Picinoodinium pillulare* | Kayış ve ark. (2013) |
| *Calico,*<br>*C. auratus auratus,*<br>*Xiphophorus helleri,*<br>*Xiphophorus maculatus,*<br>*Poecilia sphenops,*<br>*Ancistrus* sp. | *Gyrodactylus* sp.,<br>*Trichodina* sp.,<br>*Ichthyophthirius multifiliis,*<br>*Transversotrema* sp. | Mefüt ve ark. (2017) |

## ULUSLARARASI AKVARYUM BALIĞI TİCARETİ İLE YAYILAN BALIK PARAZİTLERİNİN YARATTIĞI SORUNLAR VE ALINABİLECEK ÖNLEMLER

Akvaryum balıklarının ülkeler arası ticareti esnasında beraberlerinde parazitlerin de taşınması sonucunda; ithal edilen ülkeye parazit girişi, parazitin ülkede doğal sularda bulunan balıklara yayılması, balık hastalıkları oluşturması, toplu balık ölümleri, ekonomik kayıplar, çevre ve insan sağlığı üzerine olumsuz etkiler gözlenmesi riskleri oluşur.

Bu riskleri minimize etmek için alınabilecek bazı önlemler şunlardır;

- ülkeye giriş öncesi sınır kontrol birimleri tarafından yapılacak kontroller
  - balık sağlık sertikası kontrolleri
  - balıkların görsel kontrolleri
  - mecburi karantina süresi uygulamaları
  - ölü balıkların doğru şekilde imhası
- ülkeye ithal edilebilecek balık türlerinden parazit taşıma riski yüksek türlerin sınırlandırılması
- balık ithal edilecen ülkelerden parazit taşıma yüksek olan ülkelerden balık ithalatının sınırlandırılması
- güvenilir ve hızlı sonuç veren diagnostiler
- dezenfeksiyon protokolleri
- atık akvaryum sularının dezenfeksiyonu
- merkezi olmayan bölgesel sağlık kontrol merkezleri

(Yavuzcan ve ark. (2010), Şahin (2019), Whittington ve Chong (2007))

## SONUÇ

Uluslarası akvaryum balığı ticareti dünya ekonomisinde taşıdığı yer ve ülke ekonomileri için taşıdığı önem yanında balık parazitlerinin ülkeler arası taşınması ve beraberinde getirdiği çevresel, sağlık ve ekonomik riskleri de taşımaktadır. Alınacak balık türü ve ithal edilen ülke bazlı sınırlamalar, sınır kontrolleri, görsel kontrollere ek hassas ve minimum zaman alan diyagnostik test metodları, etkin biyogüvenlik protokolleri bu riskleri minimize etmek için önemlidir.

## KAYNAKLAR

Adel, M., Ghasempour, F., Azizi, H.R., Shateri, M.H. and Safian, A.R., 2015. Survey of parasitic fauna of different ornamental freshwater fish species in Iran. In Veterinary Research Forum (Vol. 6, No. 1, p. 75). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Copp, G.H., Wesley, K.J. and Vilizzi, L., 2005. Pathways of ornamental and aquarium fish introductions into urban ponds of Epping Forest (London, England): the human vector. Journal of Applied Ichthyology, 21(4), pp.263-274.

Dewi, R.R., Fadhilla, A., 2018, February. The prevalence of parasites in ornamental fish from fish market in Medan. In IOP Conference Series: Earth and Environmental Science (Vol. 122, No. 1, p. 012110). IOP Publishing.

Dey VK, 2016. Infofish International, 4/2016, pp.52-55

Evans, B.B., Lester, R.J., 2001. Parasites of ornamental fish imported into Australia. Bulletin-European Association of Fish Pathologists, 21(2), pp.51-55.

FAO. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

Gertzen, E., Familiar, O. and Leung, B., 2008. Quantifying invasion pathways: fish introductions from the aquarium trade. Canadian Journal of Fisheries and Aquatic Sciences, 65(7), pp.1265-1273.

Hoshino, É.D.M., Hoshino, M.D.F.G., Tavares-Dias, M., 2018. Parasites of ornamental fish commercialized in Macapá, Amapá State (Brazil). Revista Brasileira de Parasitologia Veterinária, 27, pp.74-79.

Iqbal, Z., Haroon, F., 2014. Parasitic infections of some freshwater ornamental fishes imported in Pakistan. Pakistan Journal of Zoology, 46(3).

Iqbal, Z., Noreen, H., 2014. Parasitic Infection in an Imported Fish Fantail a Variety of Goldfish Carassius auratus L. in Pakistan. Pakistan Journal of Zoology, 46(6).

Iqbal, Z., Rehaman, B.G., 2014. Parasitic Infestation of an ornamental fish comet, *Carassius auratus* L. Imported to Pakistan. Biologia (Pakistan), 60(1), pp.103-107.

Jiménez-García, M.I., Vidal-Martínez, V.M. and López-Jiménez, S., 2001. Monogeneans in introduced and native cichlids in Mexico: evidence for transfer. Journal of Parasitology, 87(4), pp.907-909.

Kayış, Ş., Balta, F., Serezli, R. and Er, A., 2013. Parasites on different ornamental fish species in Turkey. J FisheriesSciences. com, 7, pp.114-120.

Kim, J. H., Hayward, C. J., Joh, S. J., Heo, G. J. (2002). Parasitic infections in live freshwater tropical fishes imported to Korea. Diseases of aquatic Organisms, 52(2), 169-173.

Lintermans, M., 2004. Human-assisted dispersal of alien freshwater fish in Australia. New Zealand Journal of Marine and Freshwater Research, 38(3), pp.481-501.

Mefut, A., Aksoy, Emre, N. ve Dal, İ. 2017. 19.Su Ürünleri Sempozyumu. İthal edilen tatlı su akvaryum balıklarında paraziter hastalık etkenlerinin tespiti. 12-15 Eylül 2017, Bildiri Özet Kitabı, 9, Sinot/Türkiye

Möller, H., 1987. Pollution and parasitism in the aquatic environment. International Journal for Parasitology, 17(2), pp.353-361.

Özer, A., 1999. The relationship between occurrence of ectoparasites, temperature and culture conditions: a comparison of farmed and wild common carp (Cyprinus carpio L., 1758) in the Sinop region of northern Turkey. Journal of Natural History, 33(4), pp.483-491.

Özer, A., 2021. Checklist of marine, freshwater and aquarium fish parasites in Turkey

Scholz, T., 1999. Parasites in cultured and feral fish. Veterinary parasitology, 84(3-4), pp.317-335.

Sindermann, C.J., 1987. Effects of parasites on fish populations: practical considerations. International Journal for Parasitology, 17(2), pp.371-382.

Şahin, G. 2019. Türkiye'ye ithal edilen bazı tatlısu akvaryum balıklarının parazitleri: hastalık profilleri ve risk analizi (Doktora Tezi. Ankara Üniversitesi Fen Bilimleri Enstitüsü, Su Ürünleri Anabilim Dalı, Ankara).

Şahin, G. ve Yavuzcan, H. Türkiye'ye ithal edilen altın balıkların (*Carassius auratus*) ektoparazitolojik olarak incelenmesi. 13. Su Ürünleri Sempozyumu. 3-5 Eylül 2005; Çanakkale/Türkiye

The Observatory of Economic Complexity (OEC), 2020), https://oec.world/en/profile/hs/ornamental-fish-live

Thilakaratne, I.D.S.I.P., Rajapaksha, G., Hewakopara, A., Rajapakse, R.P.V.J., Faizal, A.C.M., 2003. Parasitic infections in freshwater ornamental fish in Sri Lanka. Diseases of Aquatic Organisms, 54(2), pp.157-162.

Tripathi, A., 2014a. The invasive potential of parasitic monogenoids (platyhelminthes) via the aquarium fish trade: an appraisal with special reference to India. Reviews in Aquaculture, 6(3), pp.147-161.

Trujillo-González, A., Becker, J.A., Hutson, K.S., 2018. Parasite dispersal from the ornamental goldfish trade. Advances in parasitology, 100, pp.239-281.

Trujillo-González, A., Becker, J.A., Vaughan, D.B., Hutson, K.S., 2018. Monogenean parasites infect ornamental fish imported to Australia. Parasitology research, 117(4), pp.995-1011.

Vélez-Hernández, E.M., Constantino-Casas, F., García-Márquez, L.J. and Osorio-Sarabia, D., 1998. Short communication Gill lesions in common carp, *Cyprinus carpio* L. Mexico due to the metacercariae of *Centrocestus formosanus*. Journal of Fish Diseases, 21(3), pp.229-232.

Whittington, R.J., Chong, R., 2007. Global trade in ornamental fish from an Australian perspective: the case for revised import risk analysis and management strategies. Preventive veterinary medicine, 81(1-3), pp.92-116.

Yavuzcan, H., Pulatsü, S., Demir, N., Kırkağaç, M., Bekcan, S., Topçu, A., Doğankaya, L., Başçınar, N., 2010. Türkiye'de sürdürülebilir su ürünleri yetiştiriciliği. TMMOB Ziraat Mühendisliği VII. Teknik Kongresi, Bildiriler Kitabı-2, 767, p.789.

Yeler, S., 1998. Bodrum'da yetiştiricilik ortamındaki levrek balıkları (*Dicentrarchus labrax L.*) Parazitlerinin İncelenmesi (Doctoral dissertation, Yüksek Lisans Tezi. Ankara Üniversitesi Fen Bilimleri Enstitüsü, Su Ürünleri Anabilim Dalı, 45, Ankara).

Yıldız, H.Y., 2005. Infection with metacercariae of *Centrocestus formosanus* (Trematoda: Heterophyidae) in ornamental fish imported into Turkey. Bulletin of the European Association of Fish Pathologists, 25(5), pp.244-246.

## *ORAL PRESENTATION*

# Fish disease problems in recirculating aquaculture systems (RAS)

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**Abstract**

Recirculating aquaculture systems (RAS) involve the latest technologies used in aquaculture. In RAS, water in the system can be repeatedly reused, resulting in water quality changes. Since the water in RAS is reused continuously, the water environment can be suitable for the proliferation of harmful microorganisms and the emergence of bacterial, parasitic, fungal, and viral fish diseases. Furthermore, conditions causing stress in RAS such as low water quality or high fish density can suppress fish immune systems and fish may be more susceptible to the pathogens. High levels of ammonia, nitrite, carbon dioxide and suspended solid matter have been evidenced concerning disease outbreaks in RAS. Although RAS may provide many advantages, such as reducing total water use, they are not a perfect solution to prevent fish diseases. This study reviewed major diseases reported from the RAS and the factors that cause the diseases.

**Keywords:** Recirculating aquaculture systems (RAS), disease problems

## INTRODUCTION

Aquaculture production is among the fastest growing food production sectors in world. Global aquaculture production reached record of 122.6 million tonnes in 2020, 54.4 million tonnes of which were from inland waters and the share of production of aquatic animals reached record of 49.2% (FAO 2022). To meet the continuously increasing demand, not only production is increased, but also sustainable methods to increase productivity are sought for. Recirculating Aquaculture Systems (RAS) are among these methods with the promise of increased production with reduced resources.

RAS enable fish growing out at high densities within a controlled environment using minimum amount of water. They are considered to be an economical and sustainable alternative to open pond and flow-through systems where low densities of fish are reared which are prone to pollutants, stress, suboptimal growing conditions, predators and diseases. However, high fish densities and fluctuations in water quality parameters in RAS may suppress fish immune systems and these fish may become more vulnerable to diseases. Furthermore, many areas within RAS may serve as pathogen reservoirs and slower turnover of water promote proliferation of many pathogens. Some of the pathogenic diseases in recirculating aquaculture systems are reviewed in this study as well as some of the remedies for minimising these problems.

## RECIRCULATING AQUACULTURE SYSTEMS

Recirculating Aquaculture Systems (RAS) are usually indoor systems where high-density fish farming is carried out. High rate of water reuse and recycling in these systems is achieved by mechanical and biological filtration through which suspended matter and metabolites are removed (Fig.1). Most common examples of RAS are encountered in fish farms and public and private aquaria (Yanong 2004).

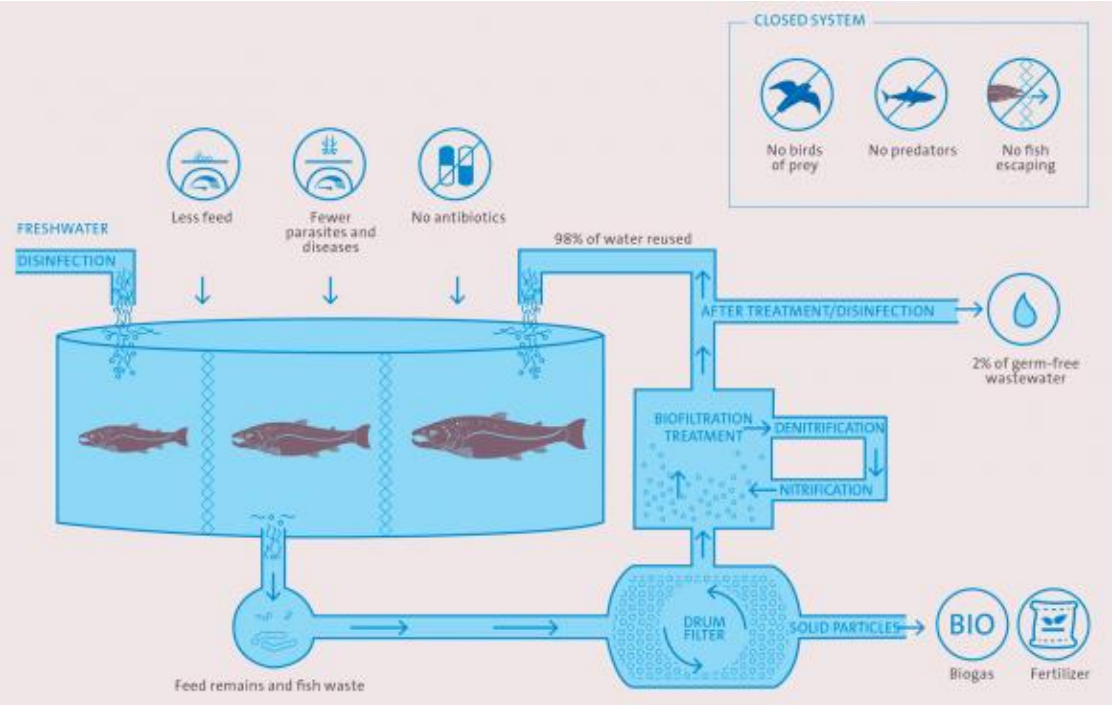


Figure 1. Recirculating aquaculture system with positive aquaculture characteristics (Hofmann 2019)

In raceway systems all the water is eliminated after making one pass through the fish tank which is why they are referred to as "open" or "flow through" systems. In RAS, reconditioned water circulates through the system and new water is supplied to the tanks just to compensate for the water lost through evaporation and splash out and for water lost while flushing out waste materials (Helfrich and Libey, 1991). RAS with water replacement ratios of less than 10% per day are called as 'intensive' or 'fully-recirculating' systems and the systems with water replacement ratios higher than 10% per day are referred to as 'partial replacement' systems (Murray et al., 2014).

In RAS, fish are kept in indoor or outdoor tanks which are controlled environments designed with the aim of providing the best possible conditions (like clean water supply with most suitable oxygen content and temperature) for fish to remain healthy and grow.

Recirculating systems utilize components for fine and dissolved solids removal, carbon dioxide removal, disinfection, biological filtration, waste solids removal and aeration (Fig.2). These systems should be designed and operated so that water quality parameters are kept within a range avoiding mortality, stress, or reduction in growth rate of fish (Noble and Summerfelt, 1996).

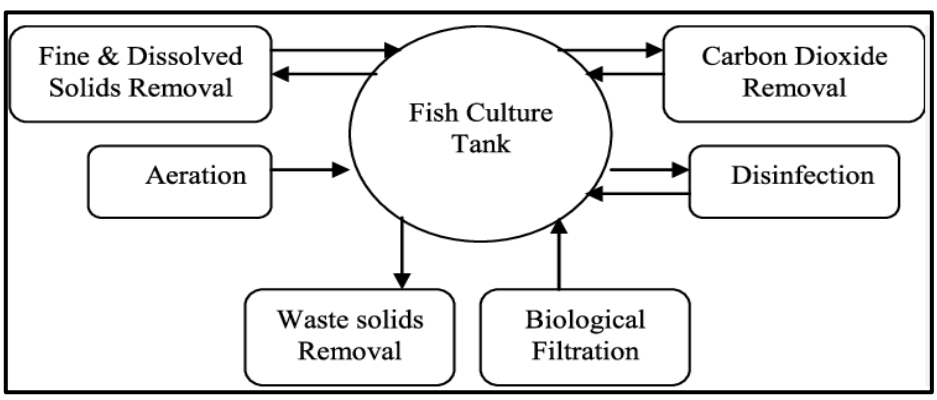


Figure 2. Recirculating aquaculture system components (Farghally et al., 2014)

Critical water quality parameters for fish health in recirculating systems include (Yanong 2004);

- ammonia and nitrite
- dissolved oxygen
- alkalinity
- carbon dioxide
- pH
- hardness
- salinity
- organics
- conductivity
- water temperature

Most of the species in aquaculture can be grown in RAS while some of the fresh water fish species suitable for recirculating aquaculture systems are channel catfish, hybrid striped bass, tilapia, rainbow trout, barramundi, yellow perch, sturgeon, and eel. Red drum, sea bass, sea bream, cobia, turbot, cod, and tuna are examples of sea fish reared in RAS (Murray et al., 2014, Noble and Sumerfelt, 1996, Rupp et al., 2019, Yanong 2003, Yanong 2004).

Recirculating systems have the advantages of using limited amount of water, improving water quality and providing better feed control in comparison to flow-through and pond systems. However, recirculating systems have some disadvantages as well. Main advantages and disadvantages of RAS compared to open pond and flow-through systems are listed in Table 1.

To be cost competitive, recirculating systems utilize continuous production and high fish densities compared to flow-through systems (Noble and Summerfelt, 1996). Low rate of replenishment of water, formation of biofilms, sediment and consequent development of pathogens in tanks, sumps, mechanical and biological filters may cause development of many pathogens and result in disease outbreaks (Yanong 2003). Changes in water quality parameters in RAS, like increases in ammonia or nitrite due to high fish densities, can cause suppressed immune systems in fish and result in greater susceptibility to pathogens like bacteria, parasites, fungi, and viruses.

Table 1: Main advantages and disadvantages of recirculating aquaculture systems

| Advantages | Disadvantages |
|---|---|
| Reduce overall water usage compared to flow-through systems | High capital costs (pumps, tanks, biologic/mechanical filters, UV lamps, etc) |
| Improved feed control and water quality control | High operational costs |
| Utilize minimum land area which is particularly advantageous where land is expensive or not readily available | Need for high technology equipment |
| Flexibility of location of facilities near large markets reducing transportation costs | Require careful supervision with skilled personnel |
| Production of various species without limitation due to their varying temperature requirements | Require uninterrupted power supply is required. Backup power supply with generators is needed as well. |
| Compatible with many contemporary goals for sustainable aquaculture | Risk of equipment malfunction and consequent loss of fish |
| Maximization of production with limited supply of land and water | Require skilled technical assistance to operate relatively complex systems and to conduct water quality analysis |

## DISEASE PROBLEMS IN RECIRCULATING AQUACULTURE SYSTEMS

Bacteria found in recirculating systems include *Aeromonas* spp., *Vibrio* spp., *Mycobacterium* spp., *Streptococcus* spp., and *Flavobacterium columnare*. Parasites which appear in RAS include *Trichodina* spp., *Ichthyophthirius multifiliis*, *Cryptocaryon irritans*, *Ichthyobodo* spp., and monogeneans. RAS can also favor

occurrence of fungi (i.e., *Saprolegnia*) and viruses (Infectious pancreatic necrosis) (Murray et al. 2014, Noble and Sumerfelt, 1996, Rupp et al., 2019, Yanong 2003, Yanong 2004).

Noble and Summerfelt (1996) studied diseases in rainbow trout in RAS. The diseases found in RAS farms in Europe at rainbow trout, tilapia and perch are mentioned in Murray et al. (2014)'s study. The diseases mentioned in respective studies are listed in Table 2. Rupp et al. (2019) evaluated diagnostic data from European perch from a warm water RAS in Switzerland in 2017 and 2018. Bacterial diseases were found to be among the most important diseases in perch and *Aeromonas* sp. was one of the most common bacteria identified.

Changes in water temperature and changes in levels of ammonia and nitrite, dissolved oxygen, carbon dioxide and pH may either stress fish and lead to infectious diseases or their levels may reach toxic levels and cause mortalities in fish through non-infectious problems (Noble and Sumerfelt, 1996). Elevated level of ammonia and nitrite in RAS is usually caused by lack of proper functioning of the biofilter. This malfunction of biofilter may take place at start up periods of RAS during which bacteria population in biofilters may not be enough to run the nitrification process which turns ammonia and nitrite into nitrate. This problem can also occur due to high fish density and/or high level of feeding of fish both of which elevate ammonia concentration in water and biofilter may not catch up with this excessive ammonia level. Water change, reduction of fish density, reduction of feeding, examination of biofilter are some of the first remedies to do in these situations to avoid ammonia and/or nitrite concentrations to reach toxic levels to fish.

Dissolved oxygen is reduced during periods of feeding due to increased level of consumption by fish. In case of excessive feeding or excessive loading of the system with fish, dissolved oxygen level may decrease to toxic levels for fish. To avoid this, dissolved oxygen level in water must be regulary controlled by RAS operators and in case of risk, aeration of water must be increased, number of fish and feeding in the tanks must be reduced and water flow must be increased.

Fish excrete carbon dioxide during respiration and carbon dioxide levels in water may reach toxic levels when fish density is high. Aeration of water must be increased to decrease carbon dioxide level in water and water must be adequately aerated during operation to prevent occurrence of this problem.

Alkalinity and pH levels in water are critically important for fish health. Carbon dioxide excretion by fish and decomposition of feed wastes make water more acidic with less alkalinity by time. Alkalinity is critical for biofilter bacteria as well and drop in alkalinity may avoid proper functioning of biofilter. To avoid alkalinity and pH to decrease to toxic levels for fish, water pH and alkalinity must be continuously monitored, water must be adequately aerated and changed, fish densities and feeding must be at controlled levels and sodium bicarbonate must be added as needed.

Table 2. Diseases in rainbow trout, tilapia, and perch in RAS

| Fish | Agent/Disease | Reference |
|---|---|---|
| | **Bacterial** | |
| Rainbow trout | Bacterial gill disease<br>Furunculosis<br>Bacterial kidney disease | Noble and Summerfelt (1996) |
| Rainbow trout, tilapia, and perch | *Aeromonas* spp., *Vibrio* spp., *Mycobacterium* spp., *Streptococcus* spp., *Flavobacterium* spp., *Fransicella asiatica* | Murray et al. (2014) |
| | **Parasites** | |
| Rainbow trout | *Gyrodactylus, Chilodonella, Trichodina, Ichthyopthirius, Ichtyobodo* | Noble and Summerfelt (1996) |
| Rainbow trout, tilapia, and perch | *Trichodina* spp., *Apiosoma* sp., *Ambiphrya* sp., *Epistylis* sp., *Chilodonella piscicola, Icthyobodo necator, Spironucleus salmonis, Gyrodactylus derjavinoides, Displostomum spathaceum* | Murray et al. (2014) |
| | **Fungus** | |
| Rainbow trout | *Saprolegnia* | Noble and Summerfelt (1996) |
| | **Viral** | |
| Rainbow trout | Infectious pancreatic necrosis virus, hemorhagic septicemia, infectious hematopoietic necrosis | Noble and Summerfelt (1996) |

## THE APPLICATIONS FOR PREVENTING THE DISEASE PROBLEMS IN RECIRCULATING AQUACULTURE SYSTEMS

Preventing disease outbreaks by complying with the basic principles of biosecurity is the most economical method for RAS. These principles include knowledge and sensitivity to species biology, nutrition, and water quality. Being knowledgable about species biology and corresponding suitable nutrition and water quality parameters helps RAS operators improve fish immune systems. In addition, complying with quarantine, sanitation and disinfection procedures helps prevent existence or propagation of pathogens (Yanong, 2004).

Biosecurity is a proactive management strategy in RAS to prevent entry of pathogens into the system and when they do, to minimize the number of pathogens in the system and to avoid propagation of the pathogens between systems. Isolation and quarantine of fish is the basic strategy to prevent entry of pathogens into RAS with new fish. Quarantine is not only an effective method to prevent entry of pathogens but also serves as an adjustment period for newly transferred fish during which the depressed immune system of fish, due to stress caused during transportation, is improved.

Through rigorus sanitation protocols the number of pathogens in the system may be reduced by keeping areas that may serve as pathogen reservoirs under close control. These pathogen reservoirs may be dead fish, wasted feed, plant floors, sediment or surfaces of system equipment like tanks and mechanical filters. System disinfection methods like ultraviolet (UV) sterilization may be an effective strategy to avoid spreading of pathogens. In good fish health management practices in recirculating aquaculture systems some details about the disinfection and clean environment are extremely important to remove pathogens. Some applications about good practices are presented in Table 3.

Table 3. Example of good management practices in RAS to inhibit disease problems

| Application example | The method |
|---|---|
| Clean water | Remove excessive nitrite, increase dissolved oxygen levels |
| Pathogen free water | Using UV for the water hygiene |
| Correct nutrition | Species- specific feed, high quality feed, non-toxic feed (i.e. no aflatoxin in feed) |
| Recognizing the diseases | Observation of any abnormal behavior, alert for the erratic swimming, lethargic or gulping air |
| Prevent stress | Avoid excessive handling |
| Provide good welfare | Avoid poor welfare conditions |
| Clean system | Using NaOH for the system disinfection |
| Clean equipment | Spraying iodine solutions to the surfaces |
| Staff | Change footwear and clothes in entrance to the system unit, washing hands |
| Visitors | Disinfect the footwear and “do not touch” application |

## CONCLUSION

Even though recirculating systems have many advantages compared to open pond and flow-through systems, they have their own set of challenges and disease susceptibility is among the most important of these challenges. Reduction of the risk of infection and ability to manage diseases in RAS is critical for a successful RAS operation. Better design of RAS, compliance with good management and biosecurity principles, understanding of common diseases and how treatments affect the overall system are part of an effective strategy to deal with disease problems in recirculating aquaculture systems.

## REFERENCES

FAO. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

Farghally, H.M., Atia, D.M., El-Madany, H.T. and Fahmy, F.H., 2014. Control methodologies based on geothermal recirculating aquaculture system. Energy, 78, pp.826-833.

Helfrich, L.A. and Libey, G.S., 1991. Fish farming in recirculating aquaculture systems (RAS). Virginia Cooperative Extension.

Hofmann, T., 2019. Recirculating aquaculture system-based salmon farming. Field Actions Science Reports. The journal of field actions, (Special Issue 20), pp.85-87.

Murray, F., Bostock, J. and Fletcher, D., 2014. Review of recirculation aquaculture system technologies and their commercial application.

Noble, A.C. and Summerfelt, S.T., 1996. Diseases encountered in rainbow trout cultured in recirculating systems. Annual Review of Fish Diseases, 6, pp.65-92.

Rupp, M., Knüsel, R., Sindilariu, P.D. and Schmidt-Posthaus, H., 2019. Identification of important pathogens in European perch (*Perca fluviatilis*) culture in recirculating aquaculture systems. Aquaculture International, 27(4), pp.1045-1053.

Yanong, R., 2004. Fish Health Management Considerations in Recirculating Aquaculture Systems-Part 1: Introduction and General Principles: Cir 120/FA099, 12/2003. EDIS, 2004(1).

Yanong, R.P., 2003. Fish Health Management Considerations in Recirculating Aquaculture Systems–Part 2: Pathogens. Circular, 121, pp.1-8.

Yanong, R., 2004. Fish Health Management Considerations in Recirculating Aquaculture systems-Part 3: General Recommendations and Problem-Solving Approaches: Cir 122/FA101, 12/2003. EDIS, 2004(1).

## *ORAL PRESENTATION*

# Synthesis and characterization of new some 1-acetyl-3-alkyl(aryl)-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones

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**Abstract**

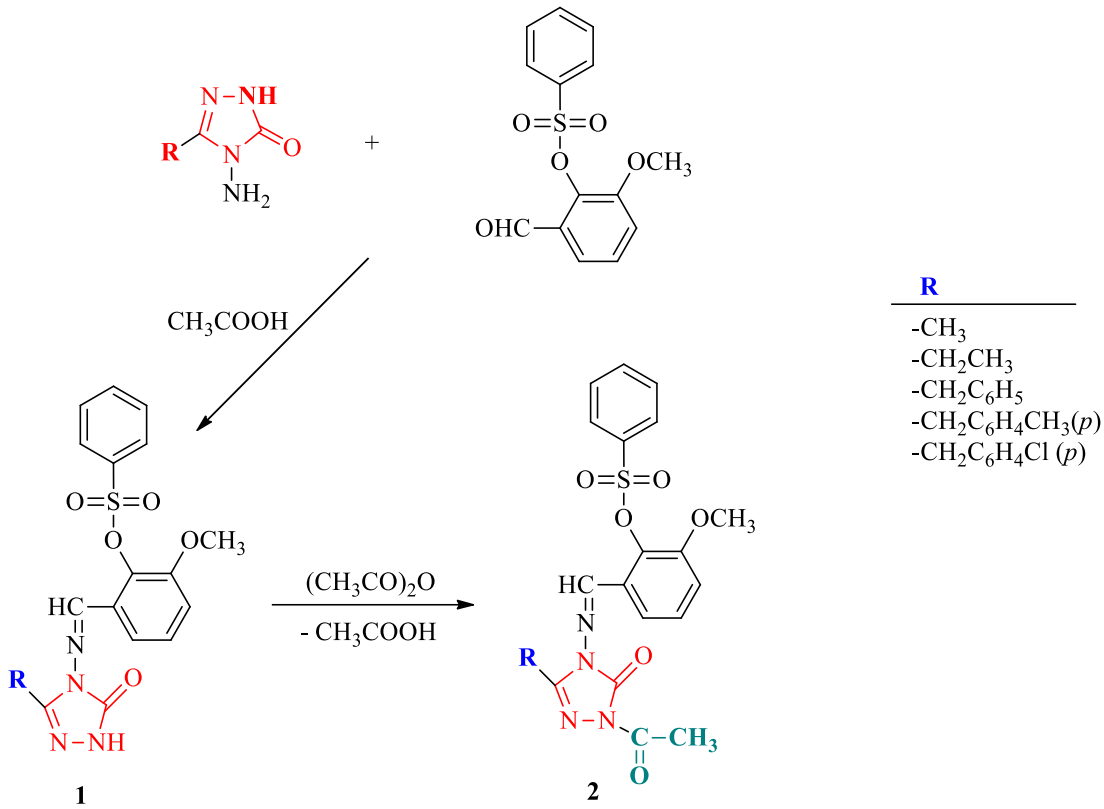
1,2,4-Triazoles are considerable heterocyclic rings that are extensively found in molecular structures with medicinal and pharmaceutical features. According to the importance of these rings, many studies were issued over the last few decades. In the present study, five novel 1-acetyl-3-alkyl(aryl)-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2**) were synthesized by the reactions of 3-alkyl(aryl)-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**1**) with acetic anhydride. The newly synthesized compounds were characterized using UV spectral data, IR, $^{1}$H-NMR and $^{13}$C-NMR spectroscopy.

**Keywords:** 1,2,4-Triazole, acetyl, synthesis.

## INTRODUCTION

Triazole is a five-membered diunsaturated ring structure composed of three nitrogen atoms and two carbon atoms at non-adjacent positions. Triazole, also known as pyrrodiazole, is one of the classes of organic heterocyclic compounds (Kharb et al., 2011). 1,2,4-Triazoles are biologically active heterocyclic organic compound. Many articles have presented studies of 1,2,4-triazoles with potent antibacterial properties (Ben Salah et al., 2015; Yang et al., 2021). As a result of these studies, it is accepted that many heterocyclic compounds with 1,2,4-triazole rings have antimicrobial, antioxidant, antiviral, anti-inflammatory, antitumor, analgesic, anticonvulsant, enzyme inhibitor, antihypertensive and anti-HIV properties (Maddila et al., 2013; Kharb et al., 2011).

In the light of this information, we aimed to synthesize new molecules carrying triazole rings, since heterocyclic compounds carrying 1,2,4-triazole units have important properties. For this reason, "3-alkyl(aryl)-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones" **1**, which were synthesized according to the literatüre (Yüksek et al., 2018), were treated with acetic anhydride and five new 1-acetyl-3-alkyl(aryl)-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2**) were synthesized (Scheme 1). Spectroscopic methods such as $^{1}$H-NMR, $^{13}$C-NMR and IR were used to determine the recent synthesized compounds.




**Scheme. 1.** Reaction equation of compounds **2**

## MATERIALS and METHODS

### General procedure for the synthesis of 1-acetyl-3-alkyl-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (2)

The corresponding compound **1** (0.01 mol) was refluxed with acetic anhydride (15 mL) for 0.5 h. After addition of absolute ethanol (50 mL), the mixture was refluxed for 1 h. Evaporation of the resulting solution at 40-45 °C *in vacuo* and several recrystallizations of the residue from an appropriate solvent gave pure compounds **2**.

### 1-Acetyl-3-methyl-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2a)

White solid; yield: 98.0%; m.p. 227°C; IR ($cm^{-1}$) $\upsilon_{max}$: 1761, 1697 (C=O), 1614 (C=N), 1369 and 1152 ($SO_2$), 1294 (COO), 749 and 688 (monosubstituted benzenoid ring). $^1$H-NMR (400 MHz, DMSO-$d_6$) (ppm) δ H: 2.35 (s, 3H, $CH_3$), 2.52 (s, 3H, $COCH_3$), 3.52 (s, 3H, $OCH_3$), 7.29 (d, 1H, ArH, *J* = 8.4 Hz), 7.44 (t, 1H, ArH, *J* = 8.4 Hz), 7.59 (d, 1H, ArH, *J* = 8.0 Hz), 7.68 (t, 2H, ArH, *J* = 7.6 Hz), 7.80 (d, 1H, ArH, *J* = 7.6 Hz), 7.88-7.90 (m, 2H, ArH), 9.72 (s, 1H, N=CH); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) (ppm) δ C: 11.69 ($CH_3$), 24.01 ($CO\underline{C}H_3$), 56.33 ($OCH_3$), [116.70, 118.00, 128.77 (2C), 128.90, 129.02, 130.03 (2C), 135.33, 135.62, 138.07, 149.30] (ArC), 147.14 (triazole-$C_3$), 148.20 (N=CH), 152.69 (triazole-$C_5$), 165.90 ($\underline{C}OCH_3$); UV [Ethanol, $\lambda_{max}$, nm (ε, $L.mol^{-1}.cm^{-1}$)]: 290 (12.390), 244 (16.255), 220 (31.195), 198 (29.000).

### 1-Acetyl-3-ethyl-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (2b)

White solid; yield: 75.7%; m.p. 209°C; IR ($cm^{-1}$) $\upsilon_{max}$: 1724 (C=O), 1608 (C=N), 1372 and 1156 ($SO_2$), 1294 (COO), 750 and 696 (monosubstituted benzenoid ring). $^1$H-NMR (400 MHz, DMSO-$d_6$) (ppm) δ H: 1.27 (t, 3H, $CH_2\underline{C}H_3$, *J* = 7.6 Hz), 2.52 (s, 3H, $COCH_3$), 2.76 (q, 2H, $CH_2\underline{C}H_3$, *J* = 7.6 Hz), 3.52 (s, 3H, $OCH_3$), 7.30 (d, 1H, ArH, *J* = 8.4 Hz), 7.45 (t, 1H, ArH, *J* = 8.0 Hz), 7.58 (d, 1H, ArH, *J* = 8.0 Hz), 7.68 (t, 2H, ArH, *J* = 7.6 Hz), 7.80 (t, 1H, ArH, *J* = 7.8 Hz), 7.88-7.90 (m, 2H, ArH), 9.79 (s, 1H, N=CH); $^{13}$C-NMR (100 MHz, DMSO-$d_6$) (ppm) δ C: 9.52 ($CH_2\underline{C}H_3$), 18.55 ($\underline{C}H_2CH_3$), 23.54 ($CO\underline{C}H_3$), 55.86 ($OCH_3$), [116.21, 117.40,

128.28 (2C), 128.43, 128.56, 129.54 (2C), 134.84, 135.10, 137.59, 150.09] (ArC), 147.89 (triazole-$C_3$), 148.83 (N=CH), 152.22 (triazole-$C_5$), 165.88 ($\underline{C}OCH_3$); UV [Ethanol, $\lambda_{max}$, nm (ε, L.mol$^{-1}$.cm$^{-1}$)]: 290 (11.690), 222 (30.580), 198 (30.190).

**1-Acetyl-3-benyl-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4c)**

White solid; yield: 74.5%; m.p. 153°C; IR (cm$^{-1}$) $\upsilon_{max}$: 1734 (C=O), 1608 (C=N), 1368 and 1154 ($SO_2$), 1291 (COO), 756 and 690 (monosubstituted benzenoid ring). $^1$H-NMR (400 MHz, DMSO-d$_6$) (ppm) δ H: 2.53 (s, 3H, $COCH_3$), 3.52 (s, 3H, $OCH_3$), 4.15 (s, 2H, $CH_2Ph$), 7.27-7.32 (m, 2H, ArH), 7.35-7.40 (m, 4H, ArH), 7.43 (t, 1H, ArH, *J* = 8.0 Hz), 7.50 (dd, 1H, ArH, *J* = 8.0, 1.6 Hz), 7.58-7.61 (m, 2H, ArH), 7.73-7.77 (m, 1H, ArH), 7.82-7.84 (m, 2H, ArH), 9.65 (s, 1H, N=CH); $^{13}$C-NMR (100 MHz, DMSO-d$_6$) (ppm) δ C: 23.59 ($CO\underline{C}H_3$), 30.94 ($CH_2Ph$), 55.84 ($OCH_3$), [116.20, 117.33, 128.21 (2C), 128.53, 129.01, 129.49 (2C), 134.78, 135.05, 137.65, 152.26] (ArC), [127.02, 128.35 (2C), 128.84 (2C), 134.62] (ArC linked C-3), 147.78 (triazole-$C_3$), 148.17 (N=CH), 152.26 (triazole-$C_5$), 165.85 ($\underline{C}OCH_3$); UV [Ethanol, $\lambda_{max}$, nm (ε, L.mol$^{-1}$.cm$^{-1}$)]: 292 (14.095), 244 (19.960), 222 (36.985), 198 (34.185).

**1-Acetyl-3-(*p*-methylbenzyl)-4-(2-benzenesulfonyloxy-3-methoxy)-benzylidene-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4d)**

White solid; yield: 78.4%; m.p. 174°C; IR (cm$^{-1}$) $\upsilon_{max}$: 1729 (C=O), 1613 (C=N), 1375 and 1154 ($SO_2$), 1295 (COO), 801 (1,4-disubstituted benzenoid ring), 759 and 691 (monosubstituted benzenoid ring). $^1$H-NMR (400 MHz, DMSO-d$_6$) (ppm) δ H: 2.28 (s, 3H, $PhCH_3$), 2.53 (s, 3H, $COCH_3$), 3.52 (s, 3H, $OCH_3$), 4.09 (s, 2H, $CH_2Ph$), 7.17 (d, 2H, ArH, *J* = 7.6 Hz), 7.27 (d, 2H, ArH, *J* = 8.0 Hz), 7.28 (d, 1H, ArH, *J* = 8.0 Hz), 7.43 (t, 1H, ArH, *J* = 8.0 Hz), 7.52 (dd, 1H, ArH, *J* = 8.0, 1.2 Hz), 7.58-7.62 (m, 2H, ArH), 7.73-7.77 (m, 1H, ArH), 7.82-7.85 (m, 2H, ArH), 9.73 (s, 1H, N=CH); $^{13}$C-NMR (100 MHz, DMSO-d$_6$) (ppm) δ C: 20.62 ($PhCH_3$), 23.58 ($CO\underline{C}H_3$), 30.54 ($CH_2Ph$), 55.83 ($OCH_3$), [116.17, 117.33, 128.21 (2C), 128.51, 128.89, 129.48 (2C), 134.78, 135.06, 137.64, 152.20] (ArC), [128.70 (2C), 129.08 (2C), 131.46, 136.15] (ArC linked C-3), 147.77 (triazole-$C_3$), 148.31 (N=CH), 152.20 (triazole-$C_5$), 165.85 ($\underline{C}OCH_3$); UV [Ethanol, $\lambda_{max}$, nm (ε, L.mol$^{-1}$.cm$^{-1}$)]: 292 (12.890), 222 (36.720), 198 (35.115).

**1-Acetyl-3-(*p*-chlorobenzyl)-4-(2-benzenesulfonyloxy-3-methoxy)-benzylidene-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-one (4e)**

White solid; yield: 71.2%; m.p. 162°C; IR (cm$^{-1}$) $\upsilon_{max}$: 1727 (C=O), 1615 (C=N), 1375 and 1153 ($SO_2$), 1290 (COO), 800 (1,4-disubstituted benzenoid ring), 754 and 692 (monosubstituted benzenoid ring). $^1$H-NMR (400 MHz, DMSO-d$_6$) (ppm) δ H: 2.52 (s, 3H, $COCH_3$), 3.50 (s, 3H, $OCH_3$), 4.17 (s, 2H, $CH_2Ph$), 7.27 (d, 1H, ArH, *J* = 8.0 Hz), 7.40-7.45 (m, 5H, ArH), 7.49 (dd, 1H, ArH, *J* = 8.0, 1.6 Hz), 7.61-7.65 (m, 2H, ArH), 7.75-7.79 (m, 1H, ArH), 7.84-7.86 (m, 2H, ArH), 9.70 (s, 1H, N=CH); $^{13}$C-NMR (100 MHz, DMSO-d$_6$) (ppm) δ C: 23.57 ($CO\underline{C}H_3$), 30.27 ($CH_2Ph$), 55.81 ($OCH_3$), [116.19, 117.33, 128.24 (2C), 128.50, 128.84, 129.48 (2C), 134.71, 135.07, 137.63, 152.16] (ArC), [128.37 (2C), 130.77 (2C), 131.74, 134.64] (ArC linked C-3), 147.22 (triazole-$C_3$), 148.52 (N=CH), 152.25 (triazole-$C_5$), 165.88 ($\underline{C}OCH_3$); UV [Ethanol, $\lambda_{max}$, nm (ε, L.mol$^{-1}$.cm$^{-1}$)]: 292 (14.955), 224 (38.740), 198 (36.700).

## RESULTS and DISCUSSION

In this present research work, synthesis of 1-acetyl-3-alkyl(aryl)-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones was conducted to provide pure compounds in good yields. The structures of all new compounds were elucidated using UV spectral data, $^1$H-$^{13}$C NMR and infrared. It is planned to study the synthesized compounds in terms of antioxidant and antibacterial activities in the next step.

## REFERENCES

Ben Salah, B., Chaari, N., Rekik, A., Ben Hsouna, A., Trigui, M., & Kossentini, M. 2015. Synthesis, Crystal Structure, and Antibacterial Activity of 1,2,4-Triazoles and 1,2,4-Triazol-3-one. *Journal of Heterocyclic Chemistry*, *52*(6), 1769-1775. https://doi.org/10.1002/jhet.2264.

Maddila, S., Pagadala, R., Jonnalagadda, BS. 2013. 1,2,4-Triazoles: A Review of Synthetic Approaches and the Biological Activity, Letters in Organic Chemistry; 10(10) .https://dx.doi.org/10.2174/15701786101010131126115448.

Kharb, R., Chander, SP., Shahar Yar, M. 2011. Pharmacological significance of triazole scaffold, Journal of Enzyme Inhibition and Medicinal Chemistry, 26:1, 1-21, DOI: 10.3109/14756360903524304.

Yang, P., Luo, JB., Wang, ZZ., Zhang, LL., Feng, J., Xie, XB., Shi, QS., Zhang, XG. 2021. Synthesis, molecular docking, and evaluation of antibacterial activity of 1,2,4-triazole-norfloxacin hybrids. *Bioorganic Chemistry*, *115*, 105270. https://doi.org/10.1016/j.bioorg.2021.105270.

Yüksek, H., Yıldız, Ç., Manap, S., Gürsoy-Kol, Ö., Beytur, M., Alkan, M., Aytemiz, F. 2018. Synthesis, *in-vitro* Antioxidant and Antimicrobial Activities of Some 3-Alkyl(Aryl)-4-(2-benzenesulfonyloxy-3-methoxy)-benzylideneamino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones and Newly Mannich Base Derivatives, *4th International Turkic Conference on Chemical Sciences and Technologies*, Sakarya University, Kiev, Ukraine, Book of Abstracts, S: 108, 7-10 September.

## *ORAL PRESENTATION*

### Türkiye'nin geleneksel değeri: salep

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**Özet**

Türkiye'nin çeşitli bölgelerinde doğal olarak yetişen orkide yumrularından üretilen salep, geleneksel Türk içeceğidir. Çiçekli bitkilerin bir familyası olan *Orchidaceae* familyası üyelerinden olan *Orchis*, *Serapias*, *Platanthera*, *Ophyris* ve *Dactylorhiza* yumruları salep orkidelerindendir. Çiçeklerinin görüntüsü, kokusu ve sağlığa etkileri ile büyüleyen orkidelerden elde edilen salep, İslamın kabulüyle birlikte Osmanlı Döneminde tüketilmeye başlamıştır. Bileşimi ve kalitesi toplandığı bölgenin coğrafik yapısına, elde edildiği türe ve hasat edildiği döneme göre değişmekte olup, yaklaşık olarak %16-55 oranlarında glikomannan, %2.7 nişasta, %2.4 mineral ve %12 nem içermektedir. Salebin kimyasal bileşiminde büyük yeri olan glikomannan, kıvam arttırıcı ve şekil koruyucu özelliklere sahiptir. Geçmişten beri mide rahatsızlıkları, felç, egzama, hepatit, romatizma gibi hastalıkların tedavisinde kullanılan salep orkideleri soğuk algınlığı ve bağırsak sorunlarında da kullanılmaktadır.

**Anahtar Kelimeler:** Geleneksel, Orkide, Salep, Türk içeceği

### Turkey's traditional value: salep

**Abstract**

Produced from orchid tubers that grow naturally in various regions of Turkey, salep is a traditional Turkish drink. *Orchis*, *Serapias*, *Platanthera*, *Ophyris* and *Dactylorhiza* tubers, which are members of the *Orchidaceae* family, a family of flowering plants, are among salep orchids. Salep, obtained from orchids, which fascinates with the appearance, smell and health effects of its flowers, started to be consumed in the Ottoman Period with the acceptance of Islam. Its composition and quality vary according to the geographical structure of the region where it is collected, the type from which it is obtained and the period it is harvested, and it contains approximately 16-55% glucomannan, 2.7% starch, 2.4% mineral and 12% moisture. Glucomannan, which has a great place in the chemical composition of salep, has thickening and shape-preserving properties. Salep orchids, which have been used in the treatment of diseases such as stomach ailments, paralysis, eczema, hepatitis and rheumatism since the past, are also used for colds and intestinal problems.

**Keywords:** Traditional, Orchid, Salep, Turkish beverage

## GİRİŞ

Salep, bazı *Orchidaceae* türlerinin kökleri veya yumruları olarak bilinmektedir (Kurt ve Kahyaoglu, 2017). Çiçekli bitkilerin ikinci büyük familyası olan Orkidegiller, familya ve tür bakımından oldukça zengindir. Genellikle tropik iklime sahip bölgelerde görülürler (Gülşen, 2017). Yumrulu otsu bitkiler olan orkideler, kırmızı, mor, pembe ve beyaz çiçekli, düz ve uzun yapraklı bitkilerdir (Ece Tamer ve ark., 2006). Dünyada yaklaşık 880 cins ve yaklaşık 22 bin tür; Türkiye'de ise 24 cins ve 60'dan fazla tür bulunmaktadır (Gülşen, 2017). Dilçıkık, çam çiçeği, çayır otu veya dildamak olarak da bilinen yabani orkide türlerinin yaklaşık %13'ü Türkiye'ye özgüdür (Tekinşen, 2006). *Orchidaceae* familyası üyelerinden olan *Orchis*, *Platanthera*, *Serapias*, *Ophyris* ve *Dactylorhiza*'nın yumruları salep orkideleri olarak adlandırılmaktadır (Gülşen, 2017; Kurt ve Kahyaoglu, 2017). Ovoid (yumurta şeklinde) olan yumrular *Orchis*, *Serapias* ve *Ophrys* cinslerine ait iken, parçalı yumrular *Plantanthera* ve *Dactylorhiza* cinslerine aittir (Tekinşen, 2006). Türkiye'de yaygın olan salep orkideleri Güney, Kuzey, Doğu ve Kuzeydoğu bölgelerinde görülmektedir (Ece Tamer ve ark., 2006). Türkiye, orkide çeşitliliği açısından diğer ülkelere kıyasla en zengin ülkeler arasında yer almaktadır (Yılmaz, 2015). Orkidelerin dünyadaki en önemli tedarikçilerinden biri de Türkiye'dir (Gülşen, 2017). Ülkemizde, yılda 45 milyon ile 180 milyon arasında orkide yumrusu toplanmakta, salep eldesi için orkideler iki yumrusu ile

sökülmekte ve tekrar kullanılmamaktadır. Türkiye'nin neredeyse tamamında yaygın olan orkideler şehirleşme, aşırı otlama, tarımsal faaliyetler, sanayileşme, ihracat amacıyla toplanmakta ve tehdit altına girmektedir (Şen, 2017).

Orkideler, yaklaşık 3000 yıldan beri çiçeklerinin görüntüsü ve kokusuyla, sağlığa faydaları ile insanları etkisinde bırakmıştır. Tarih, tıp ve ticaret gibi birçok alanda dikkatleri üstüne çeken orkidelerden elde edilen salep, geleneksel içeceklerimizden biri olmayı başarmıştır (Yılmaz, 2015). Bu derleme çalışmasında, geleneksel Türk içeceği olan salebin tarihi, bileşimi, eldesi, üretimi ve sağlığa etkileri incelenmiştir.

**Salebin Tarihçesi**

M.Ö. 250 yılında, botaniğin babası olan Theophrastus, yeraltındaki yumrulu köklere orkide adını vermiştir. Yunan filozof, orkidelerin tıbbi değeri olduğunu da belirtmiştir (Şen, 2017; Çalışkan ve Kurt, 2019). Yumrulu köklere sahip fazla sayıda cins ve orkide türlerinden hazırlanan sıcak içecek salep olarak isimlendirilmiştir (Çalışkan ve Kurt, 2019). 8. yüzyılda İslamın kabulüyle birlikte kımız, şarap gibi alkollü içecekler yasaklanmıştır. Alkollü içeceklerin yerini ise şıra, boza ve salep almıştır (Arabacı ve ark., 2017). Ortadoğu'ya özel bir içecek olan salep, Osmanlı Döneminde, sarayda, padişahlar için pişirilen macunlarla birlikte deftere kaydedilmiştir (Yılmaz, 2015). İngiliz devlet adamı Oliver Cromwell döneminde Londra sokaklarında dağıtılan salep, 17. ve 18. Yüzyılda oldukça popüler bir içecek haline gelmiştir. Avrupa'da, kadınlarda düşük, verem, zührevi rahatsızlıklar, mide problemleri ve grip gibi hastalıklarda kullanılan salep, besleyici özelliğinden dolayı 18. ve 19. yüzyıllarda, İngiliz ordusundaki hasta askerler tarafından yiyecek olarak kullanılmıştır (Çalışkan ve Kurt, 2019). Aynı zamanda salep, Avrupa'da kralların ve soyluların içeceği olurken, Avrupa ile Uzakdoğu arasındaki baharat ticaretinin de en pahalı ürünü olmuştur (Şen, 2017).

Hindistan, Afganistan ve Doğu Avrupa gibi birçok bölgede yetişen salebin asıl üreticisi Türkiye'dir (Çalışkan ve Kurt, 2019). Türkiye'de Kuzey Anadolu (Kastamonu), Güneybatı Anadolu (Muğla, Antalya), Güney Anadolu (Silifke), Güneydoğu Anadolu (Maraş), Doğu Anadolu (Van, Muş, Siirt, Hakkari) bölgelerinden elde edilen salep, ek olarak Çorum, Yozgat ve Çankırı'dan da elde edilmektedir (Yılmaz, 2015; Bilgin, 2019). Anadolu'dan İngiltere, Orta Doğu ve Almanya'ya yayılan salebin popülerliği çay ve kahvenin yaygınlaşmasıyla azalmıştır (Yılmaz, 2015; Çalışkan ve Kurt, 2019).

**Salebin Bileşimi ve Besin İçeriği**

Salebin bileşimi ve kalitesi, toplandığı bölgeye, hasat edildiği döneme, elde edildiği türe ve genetik özelliklere göre değişiklik göstermektedir (Gülşen, 2017; Şen, 2017; Bilgin, 2019). Salep yaklaşık olarak %2.7 oranında nişasta, % 2.4 oranında mineral, %12 oranında nem ve %16-55 oranlarında glikomannan içermektedir (Süren ve Kızıleli, 2021). Bazı kaynaklarda ise, salebin bileşiminde %1 oranında şeker, %2.7 oranında nişasta, %5 azotlu madde, % 2 oranında kül ve %48 oranında müsilaj olduğu belirtilmektedir. Salebin yapısındaki külün çoğu potasyum ve kalsiyum fosfat klorürlerinden oluşmaktadır (Ece Tamer ve ark., 2006; Yılmaz, 2015). Ek olarak salep içeceği, demir, fosfor, çinko, sodyum, A vitamini, tiamin, riboflavin, niasin, C vitamini ve kolestrolü de bileşiminde barındırmaktadır (Şen, 2017).

Farklı etkenlere bağlı olarak değişiklik gösteren kimyasal bileşimde, salebin kalitesini etkileyen asıl faktör glikomannan düzeyidir (Yılmaz, 2015; Şen, 2017). Salebin yapısında bulunan glikomannan, 1 mol glikoz ve 3 mol mannozun β(1-4) bağı ile bağlanmasıyla oluşmaktadır. Glikomannanlar, süt ve su eklenmesiyle birlikte şişerek viskoz hale gelmektedirler (Yılmaz, 2015). Hacminin yaklaşık 400 katı kadar suyu tutabilen glikomannanlar, kıvam artırıcı, erime hızını düzenleyici ve şekil koruyucu özelliklere sahiptir (Gülşen, 2017). Maraş dondurmasında da kullanılan salep, glikomannan içeriği ile dondurmaya sertlik ve geç erime özellikleri sağlamaktadır (Şen, 2017). Salebin yapısında bulunan az miktardaki nişasta da şişme özelliğine sahiptir. Bu özellikleri ile glikomannanlara kıvam arttırıcı olarak yardımcı olmaktadırlar (Bilgin, 2019).

## Salebin Eldesi ve Üretimi

*Orchis, Ophrys, Himantoglossum, Dactylorhiza, Anacamptis, Serapias* ve *Barlia* gibi yumrulu orkide türleri salebin elde edilmesinde kullanılmaktadır (Tekinşen, 2006). Yumrulu orkideler genellikle, yeni yıla ve bir önceki yıla ait olmak üzere iki adet yumru taşımaktadır (Yılmaz, 2015). Yeni yıla ait yumru genellikle küçük ve dolgun iken, bir önceki yıla ait yumru büyük ve buruşuktur (Tekinşen, 2006). Yumru toplama işlemi bitki çiçekliyken yapılmakta ve yeni yıla ait yumru toplanmaktadır (Tekinşen, 2006; Yılmaz, 2015). Bir önceki yıla ait eski yumru, bitki tohumunun gelişmesi için tekrar toprağa dikilmektedir. Bu sayede tohum gelişimi tamamlanmakta ve bitkinin soyu devam ettirilmektedir (Şen, 2017). Toplanan yumrular, üzerlerindeki kir ve topraktan arındırılmak amacıyla soğuk su ile yıkanmaktadır (Yılmaz, 2015). Sepete doldurulan yumrular, süt, peynir altı suyu, ayran veya suda haşlanmaktadır (Yılmaz, 2015; Şen, 2017). Yumruların suda kaldığı süre yaklaşık olarak 10-15 dakikadır (Tekinşen, 2006; Yılmaz, 2015). Haşlama işleminin amacı, yumruların gelişimini durdurmak ve salebe özgü aromanın oluşmasını sağlamaktır. Haşlama işleminden sonra sepet çıkarılmakta ve soğuk suya daldırılmaktadır (Yılmaz, 2015). Genellikle ipe dizilerek veya yere serilerek güneşte 7-10 gün boyunca kurutulan yumrular aynı zamanda, yere serilerek gölgede, altından hava akımı olacak şekilde yaklaşık 6 gün boyunca kurutulabilmektedir (Sezik, 2002; Tekinşen, 2006; Şen, 2017). Kurutma işlemi ile yumrular ağırlıklarının yaklaşık %80'ini kaybetmektedir (Sezik, 2002). Yumrular diş kesmeyecek kıvama geldiğinde kurutma işlemi sona ermekte ve kurutulmuş numuneler değirmende veya makinelerde öğütülerek toz haline getirilmektedir (Tekinşen, 2006; Şen, 2017). Ortalama 1000 tane yumrudan toz halde yaklaşık olarak 1 kg salep elde edilmektedir (Tekinşen, 2006).

Süt veya su içerisine toz salep ve şeker ilave edilip kaynatılarak hazırlanan salep, günümüzde yüksek sıcaklık uygulaması (UHT) ile de üretilebilmektedir (Ece Tamer ve ark., 2006; Bilgin, 2019). Yüksek sıcaklık uygulamasında; süte su, salep tozu, nişasta, salep ve tarçın aroması eklenerek karıştırılmaktadır. Sırasıyla, ön ısıtma, homojenizasyon ve sterilizasyon işlemleri uygulanan sıvı formdaki salep, sıcaklığı 25°C'ye ulaştığında aseptik dolum yapılarak paketlenmektedir (Ece Tamer ve ark., 2006).

## Salebin Sağlığa Etkileri

Orkideler, eski zamanlardan beri mide rahatsızlıkları, artrit, sarılık, felç, kolera, egzama, basur, iltihap, göğüs ağrısı, frengi, hepatit, romatizma, kulak ağrısı, astım, cinsel yolla bulaşan hastalıklar, tümör ve adet düzensizliği gibi farklı rahatsızlıkların tedavisinde kullanılmıştır (Hossain, 2011). Aynı zamanda, soğuk algınlığına, bağırsak sorunlarına ve öksürüğe iyi gelen salep, zihni açmak ve tokluk hissi oluşturmak için de kullanılmaktadır (Ece Tamer ve ark., 2006; Tekinşen, 2006). Yapılan çalışmalarda, salebin bileşenlerinden olan glukomannan sayesinde, yemekten sonra kan şekeri düzeyinin daha düşük olduğu görülmüştür. Glukomannanla zenginleştirilmiş diyetlerle beslenen kişilerde diyabet kontrolü yapılmış ve kan şekeri düzeylerinin iyileştiği görülmüştür (Ece Tamer ve ark., 2006). Müsilajlı yapıya sahip olan salebin prebiyotik olduğu, *Bifidobacterium* türlerinin gelişimine olanak sunduğu, karaciğeri koruyucu etkisi olduğu ve bronşit tedavisinde kullanıldığı belirtilmiştir. Fareler üzerinde yapılan çalışmalarda salebin, tüberküloz hastalığının tedavisinde kullanılan ilacın olumsuz etkilerini inhibe ettiği belirtilmiştir (Bilgin, 2019).

## SONUÇ

*Orchidaceae* familyası, çiçekli bitkilerin en geniş familyalarından biridir. Salep ise bazı *Orchidaceae* türlerinin yumruları olarak bilinmektedir. Türkiye'nin özellikle Güney, Kuzey, Doğu Anadolu ve Güneydoğu bölgeleri endemik olan orkideler bakımından zengindir. Türkiye'de yetişen orkidelerin yumrularından yıllar boyu salep elde edilmiş, 8. yüzyıldan beri İslamiyetle birlikte yasaklanan alkollü içkilerin yerini salep gibi alkolsüz içkiler almıştır. Osmanlı Devleti'nde salep kültürümüzden etkilen birçok Balkan ve Ortadoğu ülkesinde de salep tüketilmeye başlanmıştır. Kış aylarında sıcak içecek arayışına yetişen, yaz aylarında ise dondurmalarda kullanılan salebin Türk mutfağındaki yeri ayrıdır. Besleyici bileşimi olan salebin faydaları eski zamanlardan beri bilinmektedir. Salebin, ishal, bronşit, mide-bağırsak rahatsızlıkları, öksürük, cilt hastalıkları ve romatizma gibi birçok alanda faydası bulunmaktadır. Faydaları ve kullanım alanlarının geniş olmasından dolayı orkideler büyük önem taşımaktadır. Ülkemizde bulunan orkidelerin çoğu endemik türdedir ve doğadan bilinçsizce fazla miktarda sökülmesi, geriye dikilmemesi ile soylarının tükenme riski artmaktadır. Türk mutfak kültüründe geleneksel bir ürün olan salebin sürekliliğini devam ettirebilmesi için orkidelerin koruma altına alınması ve çiftçilerin bilinçlendirilmesi gerekmektedir.

**KAYNAKLAR**

Arabacı O, Tan U, Yıldız Ö, Tutar M 2017. Effect of Different Harvest Times on Some Quality Characteristics of Cultivated Sahlep orchid Serapias vomeracea (Burm. fill.) Brig. International Journal of Secondary Metabolite, 4(3, Special Issue 2), 445-451.

Bilgin AK 2019. Salebin taklit ve tağşişinin belirlenmesinde kullanılabilecek hızlı analiz yöntemlerinin geliştirilmesi.

Çalışkan Ö, Kurt D 2019. Tarihi kayıtlar ile geçmişten günümüze salep orkideleri. Türkiye Tarımsal Araştırmalar Dergisi, 6(3), 349-355.

Ece Tamer C, Karaman B, Utku Copur O 2006. A traditional Turkish beverage: salep. Food Reviews International, 22(1), 43-50.

Gülşen O 2017. Akdağmeni Salebin Kültüre Alınması Projesi Sonuç Raporu. Orta Anadolu Kalkınma Ajansı. Erişim adresi: https://www.oran.org.tr/images/dosyalar/20170915085853_0.pdf

Hossain MM 2011. Therapeutic orchids: traditional uses and recent advances—an overview. Fitoterapia, 82(2), 102-140.

Kurt A, Kahyaoglu T 2017. The Physicochemical and Structural Characteristics of Cultivated Sahlep. International Journal of Secondary Metabolite, 4(3, Special Issue 2), 488-498.

Sezik E 2002. Turkish orchids and salep. Acta Pharmaceutica Turcica, 44, 151-157.

Süren T, Kızıleli M 2021. Geleneksel Türk İçecekleri. Ankara Hacı Bayram Veli Üniversitesi Turizm Fakültesi Dergisi, 24(1), 46-71.

Şen MA 2017. Osmanlı Mutfağından Günümüze Gastronomik Değerimiz "Salep". Avrasya Bilimler Akademisi. Sosyal Bilimler Dergisi. 260.

Tekinşen KK 2006. Maraş Salebi. Akademik Gıda, 4(5), 28-30.

Yılmaz A 2015. Farklı formülasyonlarla hazırlanan salep benzeri içeceklerin reolojik ve duyusal özelliklerinin belirlenmesi.

## *ORAL PRESENTATION*

# Serbest Dolaşımlı (Free-Range) Sistemde Yetiştirilen Kaz, Ördek ve Tavuk Yumurtalarında Mikrobiyal Yükün Karşılaştırılması

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## Özet
Bu araştırma, Adana Çukurova Bölgesi Serbest Dolaşımlı (Free-Range) Sistemde yetiştirilen kaz, ördek ve tavuklardan elde edilen sofralık yumurtaların kabuk yüzeyindeki mikrobiyal yükün belirlenmesi amacıyla yürütülmüştür. Araştırma materyali sofralık yumurtalar; yumurtalama dönemine yeni başlamış 200 adet Çin Kazı (KY), 250 adet Pekin Ördeği (ÖY) ve 390 adet Akbay Yerli Beyaz (TY) genç sürüden günlük toplanarak *Staphylococcus* Aureus, Coliform, Enterococcus, E.coli, küf-maya, *Campylobacter* spp., toplam mezofolik bakteri yükü (TMAB), E.*coli* (Mac.C.), ve Shigella düzeyleri saptanmıştır. *Staphylococcus* Aureus en yüksek tavuk yumurtasında 6.61, sırasıyla kaz ve ördek yumurtalarında 5.86 ve 5.71 (log kob/yumurta) tespit edilimiştir. Toplam mezofolik bakteri yükü (TMAB) Serbest Dolaşımlı (Free-Range) sistemde yetiştirilen ördek yumurtalarında en yüksek 11.32 sırasıyla kaz ve tavuk yumurtalarında 9.92 ve 8.49 (log kob/yumurta) tespit edilimiştir. Enterococcus sadece kaz yumurtalarında (9.21 log kob/yumurta), Shigella TY'de (6.90 log kob/yumurta) tespit edilirken *Campylobacter* spp. sofralık yumurtaların hiçbirinde tespit edilememiştir.
E. coli düzeyi kaz, ördek ve tavuk yumurtalarında sırasıyla 7.55, 7.47 ve 7.47 en düşük küf düzeyi 3.01 log kob/yumurta olarak kaz yumurtalarında tespit edilmiştir. Toplam mezofolik bakteri yükü (TMAB), *Staphylococcus* Aureus, Enterococcus, Shigella, küf ve maya düzeyleri bakımından türler arasında saptanan farklılıklar önemli bulunmuştur (P≤0.05).
**Anahtar Kelimeler:** Kuluçkalık yumurta, mikrobiyal yük, free-range, kaz, ördek

## Abstract
This research was carried out to determine the microbial load on the shell surface of table eggs obtained from geese, ducks and chickens grown in Adana Çukurova Region at Free-Range System. Research material table eggs; were collected daily from young flocks that have just started laying period from 200 Chinese Goose (KY), 250 Peking Duck (ÖY) and 390 Akbay Native White (TY) hen herds and tested for Staphylococcus Aureus, Coliform, Enterococcus, E. coli, mold-yeast, *Campylobacter* spp., total mesopholic bacterial load (TMAB), E. coli (Mac.C.), and Shigella levels were determined. Staphylococcus Aureus found highest in chicken eggs as 6.61 log CFU/egg and 5.86, 5.71 (log CFU/egg) in goose and duck eggs respectively. Total mesopholic bacterial load (TMAB) in Free-Range system was highest in duck eggs as 11.32, 9.92 and 8.49 (log CFU/egg) in goose and chicken eggs respectively. Enterococcus was detected only in goose eggs (9.21 log CFU/egg), Shigella in TY as 6.90 log cfu/egg, while *Campylobacter* spp. was not detected in any of the table eggs.
E.coli levels were found in goose, duck and chicken eggs, respectively 7.55, 7.47 and 7.47, and the lowest mold level was 3.01 log CFU/egg in goose eggs. Significant differences between species were found in terms of total mesopholic bacterial load (TMAB), Staphylococcus Aureus, Enterococcus, Shigella, mold and yeast levels (P≤0.05).

**Key words:** hatching eggs, microbiological load, free-range, goose, duck

## Giriş

Kanatlı hayvanlardan elde edilen en önemli ürünlerden biri olan yumurta, günümüzde konvansiyonel kafes sistemlerinde yumurtacı hibrit tavuklar kullanılarak yapılmaktadır. Artan insan nüfusunun beslenme ihtiyacını karşılamak için üretimden maksimum verim alacağımız yoğun yetiştirme sistemlerini bu nedenle tercih edilmektedir. Ancak son yıllarda insanların tükettikleri ürünler ile ilgili güvenilir ürün endişesi duyması, hayvan refahının başta Avrupa Birliği olmak üzere dünya da pek çok ülkede giderek yaygınlaşması hem etlik piliç üretiminde hem de yumurta üretiminde alternatif yetiştirme sistemlerinin ortaya çıkmasını sağlamıştır (Anonim, 2000; İpek ve Sözcü, 2015). Bu sistemlerden biri olan free range (serbest dolaşımlı) yetiştirme sistemlerinde hayvanlar türe özgü doğal davranışlarını sergileyebilmekte, güneş ışığından faydalanmakta, biyolojik mücadeleye katkı sağlamakta ve rahatça hareket etmektedirler (Sossidou ve ark., 2011; Sözcü ve İpek, 2016). Benzer nedenlerden dolayı sadece tavukçulukta değil alternatif kanatlı hayvan yetiştiriciliğinde de serbest dolaşımlı yetiştirme sistemleri tercih edilmektedir. Bugün yumurta ihtiyacımızın neredeyse tamamı yumurtacı hibrit tavuklardan karşılanıyor olsa da diğer kanatlı türlerinin yumurtaları da tüketilmektedir. Bunlardan bazıları bıldırcın, kaz, ördek ve hindi yumurtasıdır. Ancak bu hayvanlardan özellikle kazlar yumurta veriminin azlığı, döllü yumurta elde edilmesindeki güçlükler, kuluçkada yaşanılan sıkıntılar ve yılın sadece belirli aylarında yani sezonluk yumurta yumurtalaması gibi nedenler kaz yetiştiriciliğine olan talebi azalTMABktadır (Boz ve ark., 2014). Az olan yumurta veriminden dolayı kazların yumurtaları çoğunlukla kuluçkalık olarak değerlendirilir. Ördekler ise yumurta verimi yıl boyu sürmektedir ve bir üretim döneminde (yaklaşık 10 ay) ortalama 150-200 adet arasında yumurta vermektedirler (Narahari ve ark., 1991; Alaşahan ve ark., 2019). Serbest dolaşımlı sistemlerde hayvanların folluklara yumurtalaması için hayvan sayısına uygun olarak gezinti alanına folluklar yerleştirilir, ancak bu sistemlerde yere yumurtalama davranışı görülebilmektedir. Ayrıca bu yetiştirme sisteminde hayvanlar doğrudan gübre ile temas halinde oldukları için ürüne bulaşma söz konusudur. İster yemeklik ister kuluçkalık yumurta olsun free range sistemlerde yetiştirilen hayvanlardan elde edilen ürünlerde mikrobiyal yük önemli bir konudur.

Bu çalışama serbest dolaşımlı (free range) sistemde yetiştirilen farklı kanatlı türlerinin yumurtalarındaki mikrobiyal yükü belirlemek amacıyla yapılmıştır.

## Materyal ve Metod

### Yumurta Materyali

Çalışmanın yumurta materyalini özel bir işletmede serbest dolaşımlı (free range) sistemde yetiştirilen kaz, ördek ve tavuklardan elde edilen yumurtalar oluşturmaktadır. Her türden 10' ar adet yumurta kullanılmıştır.

Çizelge 1. Çalışmada Kullanılan Kanatlı Türleri ve İşletme Kapasiteleri.

| Kanatlı Türü | Materyal | Genotip | Üretim Sistemi | İşletme Kapasitesi |
|---|---|---|---|---|
| **Kaz** | Yumurta | Çin Kazı | Free-Range Yetiştirme Sistemi | 200 Adet |
| **Ördek** | Yumurta | Pekin Ördeği | Free-Range Yetiştirme Sistemi | 250 Adet |
| **Tavuk** | Yumurta | Akbay Yerli Yumurtacı Hibrit | Free-Range Yetiştirme Sistemi | 390 Adet |

Kaz, ördek ve tavuklardan elde edilen yumurtalar üzerinde çalışılan mikrobiyolojik analizler (mezofilik aerobik bakteri, toplam koliform bakteri (Campylobacter, Salmonella, Enterecoc, *E.coli*), küf ve maya tespitleri olarak Çukurova Üniversitesi Ziraat Fakültesi Zootekni Bölümü Hayvan Besleme Laboratuvarı'nda yapılmıştır.

Mikrobiyolojik analizde kullanılan besiyerleri, Plate Count Agar (PCA), Chloramphenicol Agar (DRBC), Toplam Koliform bakteri sayısı, toplam Campylobacter sayısı, Salmonella varlığı/yokluğu, toplam Enterococci sayısı, Escherichia coli, toplam Küf-Maya sayısı, sayısını belirlemek için kullanılmıştır.

Her bir uygulama grubunda yer alan yumurtaların bulunduğu steril poşet içerisine 10 ml Maximum Recovery Dilvent (CM 07333; Oxoid) ilave edilmiş yumurta kabuk yüzeyi iki dakikalık süreyle ovulmuş, beş dakika ara verildikten sonra ikinci defa iki dakika süreyle ovma işlemi yapılmış ve kabuk yüzeyindeki mikrobiyal yükün sıvı içerisine tamamen geçişi sağlanmıştır. Daha sonra bu yıkama suyundan 1ml'lik örnekler

alınarak koliform, toplam bakteri, fekal koliform için $10^{-3}$'e kadar, küf-maya belirlemesi için ise $10^{-4}$ 'e kadar dilüsyonlar hazırlanarak ekimler yapılmıştır. Toplam bakteri ve küf-maya belirlenmesinde 0.1ml yayma yapılmıştır. Yayma işlemleri sonrasında örneklerden toplam ve koliform bakteri yoğunluğunu belirlemek için 35°C sıcaklıkta ve 48±2 saat, küf-mayayı belirlemek için ise 25 °C sıcaklıkta ve 5 gün süreyle inkübe edilmiştir. Fekal koliform ve E.coli FDA (United States Food and Drug Administration), BAM (Bacteriological Analytical Manual, 2002) EMS (en muhtemel sayı) ve aerobik koloni sayımlarında FDA, BAM (2001) EMS yöntemi kullanılmıştır. Koliform bakteri için VRB (Violet Red Bile Agar) besi yeri, fekal koliform grubu bakteri ve E.coli belirlemesinde MLSTB (Merck Lauryl Sulfate Broth) besi yeri kullanılmıştır. Toplam aerob mezofilik bakteri için Plate Count Agar (PCA; Merck), Staphylococcus bakteri belirlemesinde Braid Parker Agar besi yeri, küf-maya için DRBC (Dichloran Rose-Bengal Chloramphenicol Agar) ve Salmonella'nın belirlenmesinde RVS (Rappaport Vassiliadis Soy) besi yeri kullanılmıştır. Salmonella analizleri için var-yok olarak değerlendirilmiştir.
Mikrobiyolojik analizler Çukurova Üniversitesi Ziraat Fakültesi Zootekni Bölümü Yemler ve Hayvan Besleme Laboratuvarındaki steril kabin ( ESCO Class II Bio Hazard Safety Cabinet) otoklav (HIRAYAMA HV-110L), normal standart inkübatör, Stomacher Cihazı (Inter science Bag mixer® 400 P) anaerob jar, stomacher bag (030020057 - Standard Bags for Stomacher 80) gibi spesifik malzemeler kullanılarak yapılmıştır.

Aşağıdaki formül kullanılarak petrilerdeki sayılan koloniler (kob/yumurta) hesaplanmıştır.

$$\text{Koloni oluşturan birim sayısı (kob/yumurta)} = \frac{\text{Koloni sayısı x Dilüsyon faktörü}}{\text{Aşılama miktarı}}$$

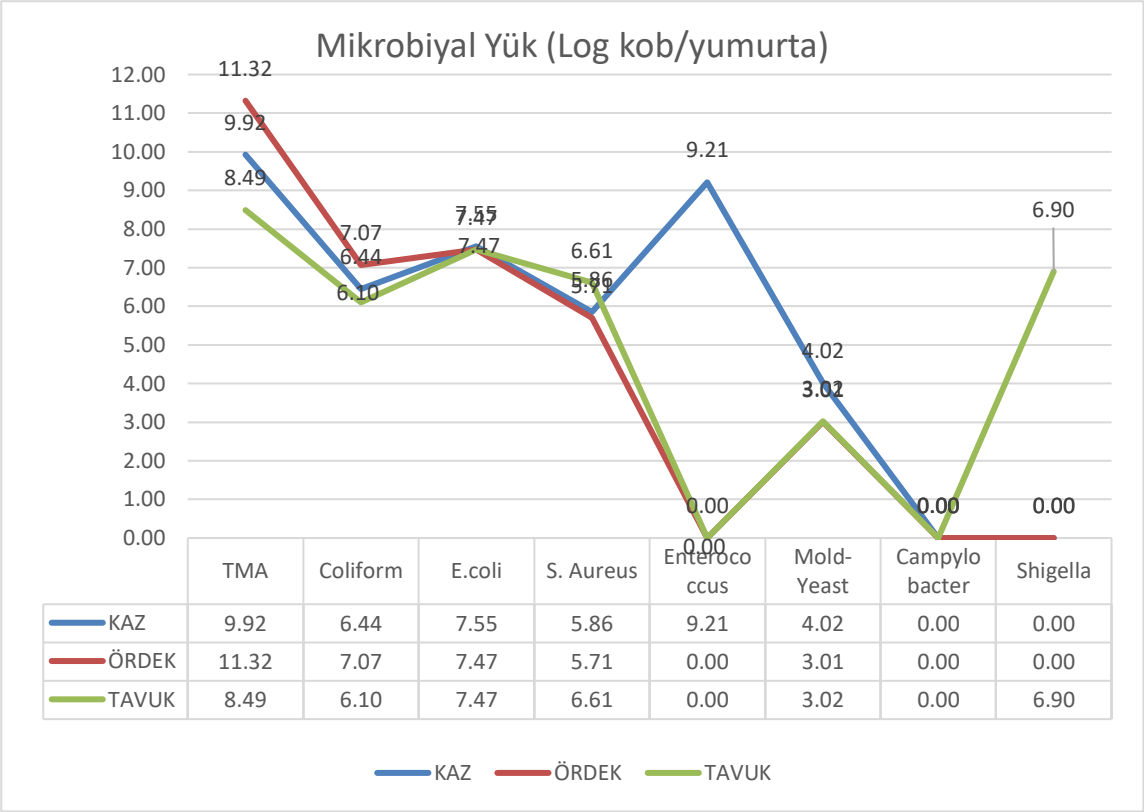
Saptanan bakteri kolonilerinin sayısı logaritmik ($\log_{10}$ kob/yumurta) değere çevrilmiştir.

**İstatiksel Analiz Yöntemi**

Yumurtalardaki mikroorganizma varlığını belirlemek amacıyla yapılan bu araştırmada üç farklı kanatlı türünün serbest dolaşımlı (free range) yetiştirme yöntemiyle ürettiği 890 adet yumurta örneği içinden 30 adet yumurta, beş ayrı tekerrür halinde dahil edilmiştir. Mikrobiyolojik analizler sonucu besiyerlerinde gelişme gösteren mikroorganizmalar ($\log_{10}$ kob/yumurta) olarak ifade edilmiştir. Belirlenen faktörlerin etkisini saptamak amacıyla bu veriler logaritmaya çevrilip SPSS 17.0 (Statistical Package for the Social Sciences) istatistiksel paket programından yararlanılarak varyans analizine tabi tutulmuştur. Çözümlemede tanımlayıcı istatistikler, Independent Samples T-Test, One-way Anova, Pearson Ki Kare analizi ve Tukey çoklu karşılaştırma testlerinden yararlanılmıştır. Analizlerde istatistiksel önemlilik düzeyi P değerinin 0,05'den küçük olmasıyla tanımlanmıştır.

**Araştırma Bulguları ve Tartışma**

Bu çalışmada serbest dolaşımlı sistemde yetiştirilen kaz, ördek ve tavuklardan elde edilen ve tüketici düzeyinde satışına izin verilen yumurtalar; toplam mezofilik bakteri (TMAB), koliform, E *coli,* Staphylococcus Aureus, Enterococcus, küf-maya, *Campylobacter* spp. ve Shigella sayısı yönünden analiz edilerek değerlendirilmiştir. Yumurta kabuğundan yıkanarak elde edilen örnek solusyonlardan yapılan ekimlerde Salmonella tespit edilmemiştir. Çalışma sonucunda kanatlı türüne göre tespit edilen mikroorganizma düzeyleri ile gıda güvenliği açısından veri tabanı oluşturulmaya çalışılmıştır (Veriler Çizelge 2'de $\text{Log}_{10}$ kob/yumurta olarak sunulmuştur).




| | TMA | Coliform | E.coli | S. Aureus | Enterococcus | Mold-Yeast | Campylobacter | Shigella |
|---|---|---|---|---|---|---|---|---|
| KAZ | 9.92 | 6.44 | 7.55 | 5.86 | 9.21 | 4.02 | 0.00 | 0.00 |
| ÖRDEK | 11.32 | 7.07 | 7.47 | 5.71 | 0.00 | 3.01 | 0.00 | 0.00 |
| TAVUK | 8.49 | 6.10 | 7.47 | 6.61 | 0.00 | 3.02 | 0.00 | 6.90 |

Çizelge 2 . Kaz, Ördek, Tavuk (AKBAY) Yumurtalarının Ortalama Mikrobiyolojik Analiz Sonuçları ($log_{10}$ kob/yumurta)

Yumurta kabuk yüzeyindeki mikrobiyal yük düzeyinin yüksek olması mikroorganizmaların, kabuktan yumurta sarısına geçme olasılığını artıran bir faktördür. Toplam mezofilik bakteri (TMAB) yükü sofralık yumurtaların en önemli hijyen göstergesidir. Yumurtacı tavuklarda yapılan çalışmalarda araştırmacılar yumurta kabuk yüzeyindeki TMAB seviyesinin $10^{3.8}$ ila $10^{7.0}$ log kob/yumurta arasında değiştiğini ve ortalama düzeyin $10^{4.5}$ log kob/yumurta olduğunu bildirmişlerdir (Jones ve ark., 2004; Musgrove ve ark., 2005; De Reu ve ark., 2008; 2009; Nordenskjöld, 2010; Englmaierova ve ark., 2014; Bulancak ve ark.., 2016; İncili ve ark., 2019, Yenilmez ve Bulancak, 2020). Yaptığımız çalışmada serbest dolaşımlı sistemde yetiştirilen kaz, ördek ve tavuk yumurtalarının kabuğunda TMAB düzeyi sırasıyle 9.92, 11.32 ve 8.49 log kob/yumurta olarak tespit edilirken türler arasında farkların istatistiksel açıdan önemli olduğu ($p \leq 0.05$) saptanmıştır (Çizelge 2). Jones ve Anderson (2013) kahverengi yumurtacılarda yetiştirme sistemine göre kabuk bakteri düzeylerini araştırmış, zeginleştrilmiş kafes, katlı kafes, kapalı yer sistemi ve serbest dolasımlı (free range) sistemde TMAB düzeyinin sırasıyla 3.31, 4.01, 4.49 ve 4.17 log kob/yumurta olarak tespit etmişlerdir. Jones ve ark. (2015) kapalı kafes sistemi ve zenginleştrilmiş kafes folluklarından aldıkları swap örneklerinde TMAB düzeylerini sırasıyla 5.5 ve 5.6 log kob/yumurta olarak saptamışlardır. Toy ve ark. (2018) yerde yetiştirilen tavuk, hindi ve bıldırcın yumurtalarında TMAB düzeyini sırasıyla 6.19, 5,36 ve 7.33 log kob/yumurta olarak tespit etmişler gruplar arası farklılıklar istatiksel olarak önemli bulmuşlardır. Daha önceki çalışmalarda elde edilen TMAB düzeylerine göre bu çalışmadaki sonuçların özellikle kaz ve ördek yumurtalarında yüksek olduğu saptanmıştır. *Staphylococcus* Aureus, *Enterococcus*, Shigella, küf ve maya düzeyleri bakımından türler arasında saptanan farklılıklar önemli bulunmuştur ($P \leq 0.05$). S. Aeureus bakteri düzeyleri kaz, ördek ve tavuk yumurtalarında sırasıyla 5.86, 5.71 ve 6.61 log kob/ yumurta olarak saptanmış. S. Aeureus en yüksek tavuk yumurtasında 6.61 log kob/ yumurta tespit edilirken (Çizelge 2), Toy ve ark. (2018) kapalı yer sisteminde yetiştirilen tavuklardan elde edilen sofralık yumurtalarda 5.84 log kob/yumurta olarak tespit etmişlerdi. Serbest dolaşımlı sistemde yetiştirilen tavuklardan elde edilen yumurtalarda yerde yetiştirilenlerden daha yüksek sonuçlar elde edilmiştir (yaklaşık 1 log kob/yumurta). Bulancak ve ark. (2017) S. Aeureus düzeyini, kapalı yer sisteminde yetiştirilen tavuklardan elde edilen kuluçkalık yumurtalarda 7.10 log kob/yumurta olarak tespit etmişlerdir.
Toy ve ark. (2018) tavuk, hindi ve bıldırcın yumurtalarının kabuğunda *Enterococcus* spp. düzeyini sırasıyla 4.48, 4.66 ve 6.03 log kob/yumurta olarak saptamışlardır. Bu çalışmada tavuk ve ördek yumurtalarında tespit edilmezken kaz yumurtasında oldukça yüksek düzeyde (9.21 log kob/yumurta) *Enterococcus* spp. saptanmıştır. Sadece tavuk yumurtasının kabuğunda Shigella bakterisi tespit edilmiştir ( 6.90 (log kob/yumurta).

Yaptığımız çalışmada; özellikle sofralık yumurtanın raf ömrünü doğrudan etkileyen küf maya düzeyleri; serbest dolaşımlı sistemde yetiştirilen kaz, ördek ve tavuk yumurtalarının kabuğunda sırasıyle 4.02, 3.01 ve 3.02 log kob/yumurta olarak tespit edilmiş ve türler arası farklılıklar önemli bulunmuştur (P≤0.05). Jones ve Anderson (2013) kahverengi yumurtacı Hy Line Brown, Silver Brown ve Playmouth Rock yumurtalarıyla yaptıkları çalışmada zeginleştrilmiş kafeste ırklara göre sırasıyle 0.99, 0.81 ve 0.86, katlı kafeste 1.31, 1.37 ve 0.64, kapalı yer sisteminde 1.73, 1.3 ve 0.00 ve serbest dolasımlı (free range) sistemde 1.99, 0.55 ve 0.84 log kob/yumurta olarak tespit etmişlerdir. Jones ve ark. (2015) kapalı kafes sistemi ve zenginleştrilmiş kafes sistemi yumurta folluklarından aldıkları swap örneklerinde sırasıyla 1.6 ve 2.7 log kob/yumurta olarak saptamışlardır.

Gıda maddelerinde hijyen göstergesi olarak da kullanılan koliformlar Enterobacteriaceae familyasında bulunan gram negatif spor oluşturmayan çubuk formunda kompleks bir bakteri grubudur. Yumurtalara Koliform bulaşması, dışkı veya dışkı ile temas etmiş yüzeyler aracılığyla gerçekleşir (EFSA; 2011). Esen(2019)'in farklı yetiştirme sistemlerinde üretilen yumurtalarda Salmonella ve bazı indikatör mikroorganizmaların varlığınının belirlenmesi çalışmasında yumurta kabuğunda koliform düzeyini serbest yetiştirme sisteminde ortalama 2.88 en yüksek 4.61 log kob/ml, yerde yetiştirme sisteminde ortalama 2.86 en yüksek 4.52 log kob/ml olarak tespit etmiştir. Toy ve ark. (2018) yerde yetiştirilen tavuk, hindi ve bıldırcın yumurtalarında koliform düzeyini sırasıyla 3.92, 3.98 ve 3.88 log kob/yumurta olarak tespit etmişler ve kanatlı türleri arasındaki istatistiki farkı çalışmamıza paralel olarak önemsiz bulmuşlardır. Karadal ve ark. (2018) market yumurtalarının % 16'sında ve köy yumurtalarının %17'sinde sırasıyle 3.69 ve 5.62 log kob/ml olarak tespit ederken Bulancak ve ark. (2016) kapalı yer sisteminde yetiştirilen damızlık tavukların kuluçkalık yumurtalarında koliform düzeyini 5.30 log kob/yumurta olarak tespit etmişlerdir. Jones ve ark. (2015) katlı kafes sisteminde yetiştirilen Lohman beyaz yumurtacıların yumurta folluğundan alınan swap örneklerinde koliform düzeyini 1.6, zenginleştirilmiş kafes sisteminde 2.7 log cfu/ml olarak tespit etmişlerdir. Bu çalışmada sofralık serbest dolaşımlı sistemde (free range) yetiştirilen kaz, ördek ve tavuk yumurtalarında koliform düzeyleri sırasıyla 6.44, 7.07, ve 6.10 log kob/yumurta olarak tespit edilmiş ve gruplar arası farklılıklar istatiksel olarak önemli bulunmamıştır (P>0.05). Escherichia coli (E. coli), Enterobacteriaceae familyasında yer alan fekal bulaşma göstergesi olarak kullanılan, patojen ve patojen olmayan suşları bulunan önemli bakteri türlerinden biridir. Yumurta kabuklarında bakteriyel patojenlerin varlığı ile ilgili yapılan bir çalışmada analiz edilen örneklerin %11,1'inde (Chousalkar ve ark., 2010), Ürdün'de yerel pazarlarda satışa sunulan yumurtaların mikrobiyal kalitesini değerlendirmek amacıyla gerçekleştirilen bir çalışmada analiz edilen yumurtaların %15'inde E. coli tespit edildiği bildirilmiştir(Al Momani ve ark., 2018). Adesiyun ve ark. (2007) inceledikleri yumurtaların %88,1'inin E. coli ile kontamine olduğunu bildirmiştir. Bulancak ve ark. (2016) damızlık yerde yetiştrilen tavukların kuluçkalık yumurtaların kabuğunda 3.66 log kob/yumurta düzeyinde Escherichia coli (E. coli) tespit ederken, Toy ve ark. (2018) kapalı yer sisteminde yetiştrilen tavukların sofralık yumurta kabuğunda 3.47 log kob/yumurta, Esen (2019) serbest gezen, kümeste kapalı yer sisteminde ve katlı kafes sisteminde yetiştirilen tavukların sofralık yumurta kabuğunda E. Coli düzeyini sırasıyla en fazla 3.74, 2.40 ve 1.36 ortalama 2.37, 1.78 ve 1.19 log kob/ml olarak tespit etmiştir. Esen (2019) Ecoli bakteri düzeyleri açısından yetiştirme sistemleri arasındaki farkı istatistiki olarak önemli bulmuştur. Yaptığımız çalışmada serbest dolasımlı sistemde yetiştrilen kaz, ördek, tavuk yumurtalarının kabuğunda Ecoli bakteri düzeyleri sırasıyla 7,55, 7,47 ve 7.47 log kob/yumurta olarak saptanmıştır. Türler arasındaki fark istatistiki olarak önemsiz bulunmuştur (p> 0.05).
Campylobacter spp. gıda kaynaklı zehirlenmeler açısından önem arz etmektedir. Campylobactere ilişkin bulaşmalar kanatlı yetiştiriciliğinde yatay olarak gerçekleşir. Özellikle altlıklar, hijyenik olmayan içme suyu, alet ekipman, diğer çiftlik hayvanları ve personel gibi faktörler bulaşmada önemli rol oynamaktadır. *Campylobacter* ile ilgili yapılan çalışmalar bu bakterinin yumurtalarda çok düşük oranlarda dahi olsa bulunduğunu göstermektedir. Yaptığımız çalışmada örneklenen yumurta kabuklarının yıkama solusyonlarında *Campylobacter* saptanmamıştır. Elde edilen bu sonuç Sulonen ve ark. (2007)'nın analiz ettikleri 360 yumurta kabuğunun sadece bir tanesinde tespit edilmiş olması ve Jones ve ark. (2012)'nın katlı kafes ve serbest gezen sistemlerde yetiştirilen yumurtaların kabuğunda *Campylobacter* spp. tespit edemedikleri sonuçlarıyla uyumlu bulunmuştur. Benzer şekilde yumurta içeriğinde yaptıkları *Campylobacter* spp. analizinde Dipineto ve ark. (2011) bakteri tespit etmediklerini bildirmişlerdir.

**Sonuç**

Ülkemizde organik sistem, serbest dolaşımlı sistem (free range), kapalı kümes yer sistemi, katlı kafes sistemi ve zenginleştirilmiş kafes sisteminde üretilen sofralık yumurtalar, üretildiği yetiştirilme sistemine göre kodlanarak tüketiciye sunulmaktadır. Bu çalışmada serbest dolaşımlı sistemde yetiştirilen kaz, ördek ve tavuklardan elde edilen ve tüketici düzeyinde satışına izin verilen yumurtalar; toplam mezofilik bakteri (TMAB), koliform, E *coli*, Staphylococcus Aureus, Enterococcus, küf-maya, *Campylobacter* spp. ve Shigella sayısı bakımından analiz edilmiştir. Analiz edilen parametreler açısından türler arasında farklılıkların olduğu

saptanmış ve daha önce yapılmış çalışmalarda yetiştirme sistemine göre elde edilen sonuçlarla karşılaştırılmıştır. Tavuklarda toplam mezofilik bakteri (TMAB) düzeyinin önceki çalışmalara göre serbest dolaşımlı yer sisteminde; yaklaşık 3 log kob/yumurta farkla daha yüksek çıktığı saptanmıştır. Koliform ve küf maya bakteri düzeyleri de önceki çalışmalara göre serbest dolaşımlı sistemde daha yüksek düzeyde tespit edilmiştir. Bu sonuçların yanında gıda güvenliği açısından serbest dolaşım sisteminde yetiştirilen kaz, ördek ve tavuk yumurtalarının kabuğunda *Salmonella* ve *Campylobacter* spp. saptanmamıştır. Yumurta üretiminin her basamağında uluslararası gıda ve kalite güvenlik sistemlerinin en yüksek düzeyde uygulanması, bakteriyel bulaşma risklerinin kontrol altına alınması ve yok edilmesi önem arzeden konulardır. Farklı kanatlı türlerinde ve yetiştirme sistemlerinde mikrobiyal düzeyin saptanması açısından ülkemiz genelinde daha çok çalışma yapılmasına ihtiyaç duyulmaktadır.

**Kaynaklar**

Adesiyun, A., Offiah, N., Seepersadsingh, N., Rodrigo, S., Lashley, V., Musai, L. 2007. Antimicrobial Resistance of Salmonella spp. and Escherichia Coli Isolated From Table Eggs. *Food Control*. 2007;18(4):306-11.

Al Momani, W., Janakat, S., Khatatbeh, M. 2018. Bacterial Contamination of Table Eggs Sold in Jordanian Markets. *Pakistan Journal of Nutrition*. 2018;17(1):15-20.

Alaşahan, S., Garip, M., Çağlayan, T., Ateş, C.T. 2019. Halk Elinde Yetiştirilen Kaz, Ördek ve Hindi Yumurtalarının Bazı Dış Kalite Özelliklerinin İncelenmesi. Harran Üniv Vet Fak Derg, 2019; 8 (1): 21-25.

Anonim. 2000. Report of The Scientific Committee on Animal Health and Welfare, The Welfare of Chickens Kept for Meat Production (Broilers). Europian Commission, Health and Consumer Protection Directorate General, March 2000.

BAM, 2002, Bacteriological Analytical Manual, 8th Ed.,Revision A.

Boz, m.A., Sarıca, M., Yamak, U.S. 2014. Yozgat İlinde Kaz Yetiştiriciliği. Tavukçuluk Araştırma Dergisi 11 (1): 16-20, 2014 Basılı ISSN:1302-3209 - Çevrimiçi ISSN:2147-9003.

Bulancak, A., Baylan, M., Ergınkaya, Z., Toy,N. (2016) Effects of propolis and thyme essential oil as a bio-fumigants on microbiological load of hatching eggs and incubation performance parameters.World's Poultry Science Journal, Volume 72, Supplement I, 5th *Mediterranean Poultry Summit*, Oral sessions, 20-25 October, Italy-Spain- France, page 41.

Chousalkar, K., Flynn, P., Sutherland, M., Roberts, J., Cheetham, B. 2010. Recovery of Salmonella and Escherichia Coli From Commercial Egg Shells and Effect of Translucency on Bacterial Penetration in Eggs. International Journal of Food Microbiology. 2010;142(1-2):207-13.

De Reu, K., Messens, W., Heyndrickx, M., Rodenburg, T.B., Uyttendaelle, M., Herman, L. 2008. Bacterial contamination of table eggs and the influence of housing systems. *World's Poult.Sci. J.,* 64: 5-19.

De Reu, K., Rodenburd, T.B., Grijspreerdt, K., Heyndrickx, M., Tuyttens, F.A.M., Sonck, B., Zoons, J., Herman, L. 2009. Bacteriological contamination, dirt, and crack of eggshells in furnished cages and noncage systems for laying hens: an international on-farm comparison. *Poultry Science,* 88: 2442-2448.

Dipineto, L., Gargiulo, A., Russo, T.P., De Luca Bossa, L.M., Borrelli, L., Menna, L.F., 2011. Campylobacter Jejuni, Campylobacter Coli, and Cytolethal Distending Toxin Genes in Laying Hens. Avian Diseases. 2011;55(1):103-5.

EFSA, 2011. Evaluation of the FoodEx, the Food Classification System Applied to the Development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal.9(3): p. 1970.

Englmaierova, M., Tůmová, E., Charvátová, V., Skřivan, M. 2014. Effects of laying hens housing system on laying performance, egg quality characteristics, and egg microbial contamination. *Czech J. Anim. Sci.*, 59 (8): 345-352.

Esen, Ş. 2019. Farklı Yetiştirme Sistemlerinde Üretilen Tavuk Yumurtalarında Salmonella ve Bazı İndikatör Mikroorganizmaların Varlığı, Dicle Üniversitesi, Sağlık Bilimleri Enstitüsü, Besin/Gıda Hijyeni ve Teknolojisi Anabilim Dalı, Tez Çalışması.

Karadal F. Niğde ve Kayseri'de Satışa Sunulan Köy ve Market Yumurtalarının Mikrobiyolojik Kalitesi. Erciyes Üniversitesi Veteriner Fakültesi Dergisi. 2018;15(1):51-7.

İncili, G.K., Durmuşoğlu, H., Güngören, A., İlhak, O.I. 2019. Investigation of Microbiological Quality of Conventional (cage type) and Village Eggs Sold in Elazig. *The Journal of Faculty of Veterinary Medicine,* 12 (2): 97-102.

İpek, A., Sözcü, A. 2015. Alternatif kanatlı yetiştirme sistemlerinde yetiştirme pratikleri ve refah standartları. Uludağ Üniversitesi Ziraat Fakültesi Dergisi 29(1):133-146.

Jones, D.R., Cox, N.A., Guard, J., Fedorka-Cray, P.J., Buhr, R.J., Gast, R.K., Abdo, Z., Rigsby, L.L., Plumblee, J.R., Karcher, D.M. and Robison, C.I., 2015. Microbiological impact of three commercial laying hen housing systems. *Poultry Science*, *94*(3), pp.544-551.

Jones, D.R. and Anderson, K.E. 2013. Housing System And Laying Hen Strain İmpacts On Egg Microbiology. *Poultry Science*. 2013; 92(8):2221-2225.

Jones, D. R., Anderson, K. E., Guard, J. Y. 2012. Prevalence of Coliforms, Salmonella, Listeria, and Campylobacter Associated with Eggs and the Environment of Conventional Cage and Free-Range Egg Production. *Poultry Science*. 2012; 91(5):1195-1202.

Jones, D.R., M.T. Musgrove and J.K. Northcutt, 2004. Variation in external and internal microbial populations in shell eggs during extended storage. *J. Food Prot*., 67: 2657-2660.

Musgrove, M.T., Jones, D.R., Northcutt, J.K., Cox, N.A., Harrison, M.A. 2005. Shell rinse and shell crush methods for the recovery of aerobic microorganisms and Enterobacteriaceae from shell eggs. *Journal of Food Protection*, 68: 2144-2148.

Nordenskjöld, J. 2010. Study of microflora on egg shells in egg production in Jordan. Independent project/degree project in food science Uppsala Biocenter University of agricultural sciences. EX0426 Independent project/degree project in Food Science. pp.1-30.

Sözcü, A. ve İpek, A. 2016. Kanatlı Yetiştiriciliğinde Serbest Dolaşımlı Sistemde (Free Range) Besleme Teknikleri. Hayvansal Üretim 57(2): 68-74.

Sossidou, E.N., Dal Bosco A., Elson, H.A., Fontes, C.M.G.A. 2011. Pasture-based systems for poultry production: implications and perspectives. *World's Poultry Science Journal* 67:47-58.

Sulonen, J., Kärenlampi. R., Holma, U., Hänninen, M.L. 2004. Campylobacter in Finnish Organic Laying Hens in Autumn 2003 and Spring 2004. *Poultry Science. 2007*;86(6):1223-8.

Toy, N., Kurşun, K., Açık, İ.P., Bulancak, A., Baylan, M. 2018. Yerde Yetiştirilen Kanatlılardan Elde Edilen Sofralık Yumurtaların Mikrobiyolojik Flora Açısından Karşılaştırılması. 3rd International Mediterranean Science and Engineering Congress (IMSEC 2018) Çukurova University, Congress Center, October 24-26, 2018, Adana / TURKEY Pages: 1-1, Paper ID:520.

Yenilmez, F., Bulancak, A. 2020. Microbiological Quality of Table Eggs Sold at Different Sales Location. Çukurova Tarım Gıda Bil. Der. *Çukurova J. Agric. Food Sci*. 35(2): 115-124.

## *ORAL PRESENTATION*

# Kara incimiz: Türk kahvesi

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## Özet

Rubiaceae familyasına ait olan kahve ağacı, tropikal iklime sahip bölgelerde gelişen odunsu bir bitkidir. *Coffea arabica* ve *Coffea robusta* olmak üzere iki önemli türü bulunan kahve, dünyada en çok tüketilen içecek olarak dikkatleri çekmektedir. Kahve ağacından hasat edilen meyve çekirdeğinden ayrılmakta ve kavurma, öğütme işlemlerinden sonra tüketime hazır hale gelmektedir. Kahvenin lezzetinde, kahve çekirdeği türünün, kavurma ve pişirme parametrelerinin etkisi oldukça fazladır. Bu işlemler esnasında çekirdekler bazı fiziksel ve kimyasal değişikliklere uğramaktadır. Uyarıcı etkisiyle keşfedilen kahve, insan sağlığına faydalı bileşenleri de yapısında barındırmaktadır. Polifenoller, kafein ve kahveol gibi bileşenler sayesinde antioksidan aktivite ve antibakteriyel etki göstermektedirler. Aynı zamanda kahve, ağrı kesici, kan basıncını düşürücü, bağırsak hareketlerini düzenleyici, tip II diyabet riskini azaltıcı ve bazı kanser türlerine karşı koruyucu etkilere de sahiptir.

**Anahtar Kelimeler:** *Coffea arabica, Coffea robusta*, kafein, kahve çekirdeği, Türk kahvesi

# Our black pearl: Turkish coffee

## Abstract

Belonging to the Rubiaceae family, the coffee tree is a woody plant that thrives in tropical climates. Coffee, which has two important types, *Coffea arabica* and *Coffea robusta*, draws attention as the most consumed beverage in the world. The fruit harvested from the coffee tree is separated from the seed and becomes ready for consumption after roasting and grinding processes. The effect of coffee bean type, roasting and cooking parameters is quite high on the flavor of coffee. During these processes, the nuclei undergo some physical and chemical changes. Discovered with its stimulating effect, coffee also contains components beneficial to human health. Thanks to components such as polyphenols, caffeine and coffeeol, they show antioxidant activity and antibacterial effect. At the same time, coffee has analgesic effects, lowering blood pressure, regulating bowel movements, reducing the risk of type II diabetes and protecting against some types of cancer.

**Keywords:** Coffea arabica, Coffea robusta, caffeine, coffee bean, Turkish coffee

## GİRİŞ

Yüzyıllar boyunca keyifle tüketilen kahve, dünyada en çok tüketilen içeceklerden biri olmuştur (Durmaz, 2015; Yıldız, 2019). Aynı zamanda yılda 8 milyon ton üretimi yapılan kahve, ekonomik açıdan da petrolden sonra gelen ticari açıdan değerli bir üründür (Ülger, 2015; Tatar, 2021). 70'den fazla ülkede yetiştirilen kahve bitkisinin üretiminde Kolombiya, Brezilya, Hindistan ve Etiyopya önde gelmektedir (Tatar, 2021). Kahve bitkisi, *Rubiaceae* (Kökboyasıgiller) familyasına aittir (Durmaz, 2015; Hasbahçe, 2020). Kahve ağacı, 18-24 °C sıcaklık ortalamasına sahip ılıman iklim bölgelerinde, yağmur yağışının çok olduğu, tropikal iklime sahip bölgelerde ve 25° Kuzey, 30° Güney paralelleri arasında bulunan bölgelerde yetiştirilen odunsu bitkidir (Yıldız, 2019; Hasbahçe, 2020). Kahve türleri, fiziksel görünüş, büyüme ortamları ve kimyasal bileşenleri ile birbirinden ayrılmaktadır (Yıldız, 2019). Çok sayıda tür ve cinse sahip olan kahve bitkisinin en önemli türleri *Coffea Canephora* (robusta) ve *Coffea Arabica*'dır (Durmaz, 2015). Antioksidan bileşikler açısından zengin olan *Coffea Canephora*, hava şartlarına karşı dayanıklı bir tür iken; *Coffea Arabica* hava şartları ve hastalıklara karşı dayanıklı değildir. Aynı zamanda, *Coffea Canephora*'dan elde edilen kahve, kafein açısından *Coffea Arabica*'dan elde edilen kahveye göre daha zengindir (Yıldız, 2019; Hasbahçe, 2020). Her iki tür de farklı aroma ve tada sahiptir. Ağaçtan hasat edilen meyvenin merkezindeki çekirdekler, yeşil çekirdek olarak

isimlendirilmekte ve sırayla kavurma, öğütme, demleme aşamalarından geçirilmektedir (Durmaz, 2015). Kahvenin duyusal özelliklerini ve kimyasal bileşimini etkileyen demleme yöntemi, kahve bileşenlerinin ekstrakte olup antioksidan aktivite göstermeleri açısından oldukça önem taşımaktadır (Ülger, 2015). Geçmişten günümüze kadar yaygın olan Türk kahvesi en eski demleme metodu olarak bilinmektedir (Durmaz, 2015; Ülger, 2015). Osmanlıdan, özgünlüğünü yitirmeden gelen Türk kahvesi geleneksel içeceklerimiz arasında önemli bir yere sahiptir (Durmaz, 2015). Bu derleme çalışmasında, Türk kahvesinin tarihi, bileşimi, eldesi, üretimi ve sağlığa etkileri incelenmiştir.

## KAHVENİN TARİHİ

Kahvenin kim tarafından keşfedildiği ve nereden geldiği hakkında birçok varsayım vardır (Yıldırım, 2022). Varsayımların çoğunda, kahvenin ilk keşfedildiği yer, Etiyopya (Habeşistan) olarak belirtilmiştir (Coşgun, 2022). Kahvenin keçiler tarafından bulunduğu, çoban Kaldi hikayesiyle sık sık karşılaşılmaktadır (Kaplan, 2011; Coşgun, 2022). Çoban Kaldi, sürüsündeki deve ve keçilerin, bir ağacın kırmızı meyvelerini yediğini ve yedikten sonra daha canlı ve hareketli olduklarını görmüştür (Kaplan, 2011). Aynı meyveleri kendisi tükettiğinde kendisini daha zinde hissetmiş ve daha uzun süre uykusuzluğa dayanabilmiştir (Ülger, 2015). Kaldi, bu durumu din alimlerine anlatmıştır. Din alimleri ise uzun süren ibadetlerinde kahve tanelerini suda kaynatarak tüketmeye başlamışlardır (Coşgun, 2022). Durumdan haberdar olan ünlü derviş Şazilî de aynı ağacın meyvelerini suda kaynatıp içtiğinde kendisini zinde hissetmiştir (Kaplan, 2011). 700'lü yıllarda Habeşistan'daki tekkesinden kovulan Şazilî, dağlara sürgün edilmiş ve kahve çekirdeklerinden ekmek yaparak hayatta kalmayı başarmıştır (Coşgun, 2022). Bu sayede kahvenin yüzyıllar süren serüveni başlamıştır. 'İslamın şarabı' ve 'Kara inci' olarak da adlandırılan kahve, Habeşistan'dan sonra sırayla Yemen'e, Mekke ve Medine'ye yayılmıştır. Daha sonra İran, Mısır ve Türkiye'ye islam gezginleri ile gelmiştir (Kaplan, 2011). Yemen Valisi Özdemir Paşa, 1517 yılında kahveyi İstanbul'a getirmiş ve Türkiye kahveyle tanışmıştır. Türkler tarafından keşfedilen yöntemle cezve veya güğümlerde pişirilen kahve 'Türk kahvesi' olarak adlandırılmıştır (Kıvançlı, 2011). Türk tarihçi Peçevi ise, 1554-1555 yıllarında İstanbul'daki ilk kahvehanenin açıldığını öne sürmüştür (Ülger, 2015).

Kanuni Sultan Süleyman döneminde, ulema sınıfı ve devlet yöneticileri tarafından zevkle tüketilmeye başlanan kahve, şehirde de halk tarafından tüketilmeye başlanmıştır. İstanbul halkı, kahveyi öğütmeyi, kavurmayı ve pişirmeyi öğrenmiş ve yemeklerden sonra kahve içmeyi adet haline getirmiştir (Bayramova, 2019). 1600'lü yıllarda Venedikli tüccarlar ile Avrupa'ya götürülen kahve, 1683 yılında, Viyana kuşatması ile birlikte Viyana, Fransa, İtalya, İngiltere gibi Avrupa ülkelerine de yayılmaya başlamıştır. Amerika'da ise, 19. yüzyılda deniz subayı Gabriel Mathieu de Clieu'nın kahve bitkisini bahçesine ekmesiyle yayılmaya başlamıştır (Coşgun, 2022).

## KAHVENİN BİLEŞİMİ

Kahve çekirdeği, protein, karbonhidrat, tanen, lipit, mineraller ve polifenollerden oluşmaktadır (Yıldız, 2019). Kahve çekirdeklerinin bileşimi, türe göre değişmektedir. Genel olarak, yaklaşık %12 yağ, %13 protein ve %50 polisakkaritten oluşmaktadırlar (Kıvançlı, 2011). Kahveol, kafein ve kafestoller gibi biyoaktif bileşikleri içeren kahve, merkezi sinir sisteminde büyük bir role sahiptir. Antioksidan aktiviteye sahip olan ve lipit metabolizmasını düzenlemeye yardımcı olan klorojenik asit gibi polifenolleri de içermektedir (Tatar, 2021). Acı bir tada sahip olan kafein ise kahvede doğal olarak bulunan alkaloiddir (Yıldız, 2019). Kahvedeki kafein miktarı, kahvenin kavrulma derecesine, türüne ve pişirme yöntemine göre değişebilmektedir (Tatar, 2021). Aynı zamanda, magnezyum, sodyum, potasyum, demir, kalsiyum, çinko, manganez, bakır, nikel, kurşun, fosfor, brom, kobalt, sülfat, baryum, kadmiyum ve daha birçok mineral ve alanin, sistein, arginin, glisin, histidin, izolösin, lisin, fenilalanin, glutamik asit, treonin, valin, prolin, lösin, tirozin, metiyonin gibi birçok amino asit kahvenin yapısında bulunmaktadır (Kıvançlı, 2011; Yıldız, 2019). Kahve çekirdeğinde, glukoz, galaktoz, sükroz, mannoz, arabinoz ve fruktoz gibi şekerler de bulunmaktadır (Yıldız, 2019). *Coffea arabica* ve *Coffea canephora* kahve çekirdeklerinin yeşil ve kavrulmuş haldeki bileşimi Tablol 1'de gösterilmiştir (Kıvançlı, 2011).

**Tablo 1:** *Coffea arabica* ve *Coffea canephora* kahve çekirdeklerinin yeşil ve kavrulmuş haldeki bileşimi (Kıvançlı, 2011).

| Bileşen | *Coffea canephora* (robusta) (% kuru madde) | | *Coffea arabica* (% kuru madde) | |
|---|---|---|---|---|
| | Yeşil kahve çekirdeği | Kavrulmuş kahve çekirdeği | Yeşil kahve çekirdeği | Kavrulmuş kahve çekirdeği |
| Proteinler | 11-13 | 13-15 | 11-13 | 13-15 |
| Yağlar | 9-13 | 11-16 | 12-18 | 14.5-20 |
| Mineraller | 4-4.5 | 4.6-5 | 3-4.2 | 3.5-4.5 |
| Oligosakkaritler | 5-7 | 0-3.5 | 6-8 | 0-3.5 |
| Toplam polisakkaritler | 37-47 | 0 | 50-55 | 24-39 |
| Kafein | 1.6-2.4 | 2 | 0.9-1.2 | 1 |
| Toplam klorojenik asitler | 7-10 | 3.9-4.6 | 5.5-8 | 1.2-2.3 |
| Alifatik asitler | 1.5-2 | 1-1.5 | 1.5-2 | 1-1.5 |
| Amino asitler | 2 | 0 | 2 | 0 |

## KAHVENİN ELDESİ ve İŞLENMESİ

Çoğu kişi tarafından sevilerek tüketilen kahve, tüketime hazır olana kadar hasat, kabuklarından ayırma, kavurma ve öğütme işlemlerinden geçmektedir (Durmaz, 2015; Kıvançlı, 2011). Bu işlemler, kahve kalitesini belirlemede büyük önem taşımaktadır (Durmaz, 2015).

### Hasat:

Kahve işlemenin ilk aşaması olan hasat işlemi kahvenin içim kalitesini etkileyen bir basamaktır (Coşgun, 2022). Kahve meyveleri ilk başta yeşil renkteyken, daha sonra sırasıyla sarı ve kırmızı renk almaktadır (Durmaz, 2015). Meyvelerin hasadı, yağış olmayan mevsimde, meyvenin olgunlaşıp kırmızı renge döndüğü zaman yapılmaktadır (Hasbahçe, 2020). Meyveler heterojen olgunlaşma sürelerine sahip olduğu için aynı ağaçta aşırı olgunlaşmış, olgunlaşmış ve olgunlaşmamış meyveler bulunmaktadır (Coşgun, 2022). Meyvelerin hasatında, elle toplama, makine ile toplama ve dalı sıyırma yöntemleri bulunmaktadır. El ile toplama metodu zahmetli ve maliyetli olduğundan genellikle dalı sıyırma veya makine ile toplama metotları tercih edilmektedir (Hasbahçe, 2020). Aynı zamanda, uygulanacak hasat metodunun seçiminde, arazi yapısı, işçilik maaliyeti, meyvelerin olgunluk dağılımı ve ağaç yüksekliği büyük önem taşımaktadır (Coşgun, 2022).

### Ayırma:

Kahve meyveleri hasat edildikten sonra çekirdeklerin meyveden zarar görmeden ayrılması gerekmektedir (Kıvançlı, 2011). Hasat sonrası yapılan ayırma, kuru, yaş ve yarı kuru olmak üzere üç çeşittir (Yıldırım, 2022). Çekirdeği meyveden ayırma işlemi, kahvenin duyusal kalitesini ve lezzetini etkileyebileceğinden uygun bir biçimde yapılmalıdır (Durmaz, 2015).

Özel ekipmanlara ve bol suya ihtiyaç duyulan yaş yöntem işlemi genellikle Arabica kahvelerinin işlenmesinde kullanılmaktadır (Kıvançlı, 2011). Meyveler su tanklarında yıkandıktan sonra kanallara aktarılmakta ve kanallarda akan su sayesinde çekirdeğin koruyucu tabakası sertliğini kaybetmektedir (Hasbahçe, 2020). Daha sonra palperlerde, kahve meyvesi, sabit ve hareketli iki yüzey arasında sıkıştırılmaktadır. Sıkıştırma işlemiyle meyve eti ve kabuğu ile parşömen kahve çekirdeği ayrılmaktadır (Kıvançlı, 2011). Kahve çekirdeğinin

etrafındaki zar ise fermantasyon işlemi ile uzaklaştırılmaktadır. Büyük tanklarda gerçekleştirilen fermantasyon işleminde, maya ve bakteriler kullanılarak alkoller ve şekerler yok edilmektedir (Durmaz, 2015; Yıldırım, 2022). Daha sonra kahve çekirdekleri, mekanik kurutuculardа veya güneşte kurutulmaktadır (Yıldırım, 2022). Mikrobiyal kontaminasyon riski bulunan kurutma işlemlerinde çekirdek etkilenebilmekte ve kahvenin duyusal özelliklerini bozabilmektedir (Durmaz, 2015).

Doğal ayırma yöntemi olarak da bilinen kuru yöntem, en kolay ve en eski yöntem olmasının yanı sıra makineye en az ihtiyaç duyulan yöntemdir (Kıvançlı, 2011). Meyvenin tamamının güneşte veya mekanik kurutuculardа kurutulması esasına dayanır (Kıvançlı, 2011; Yıldırım, 2022). Kurutma işlemi sırasında meyve çekirdekleri parşömenden ayrılmakta ve meyvenin içerdiği nem miktarı düşmektedir. Böylece kabuk kolaylıkla meyveden ayrılmaktadır (Yıldırım, 2022). Homojen kurutma işlemi yapılabilmesi için güneşte kurutulan meyveler alt-üst edilmektedir (Kıvançlı, 2011). Kurutma işlemi genelde açık havada 12-15 gün sürerken, bazen 4 haftada tamamlanabilmektedir (Durmaz, 2015). Kuru yöntem neredeyse tüm *C. robusta* çekirdeklerine uygulanmaktadır (Yıldırım, 2022).

Yarı kuru yöntem ise, yaş yöntemden fermantasyon işleminin çıkarılmış halidir. Meyvenin dışındaki etli kısım uzaklaştırılıp, meyve çekirdeği dış kısmındaki zar ile birlikte kurutulmaktadır (Yıldırım, 2022).

**Kavurma:**

Kahve çekirdeklerindeki kahveye özgü lezzet ve renk parametreleri kavurma işlemiyle birlikte ortaya çıkmaktadır. İstenilen renge ulaşılana kadar kahveye ısı uygulanmakta ve sürekli karıştırılarak homojen kavurma sağlanmaktadır (Kıvançlı, 2011). Kavurma işlemi, belirli bir ısı uygulaması ve kahve istenilen sıcaklığın üstüne çıkmaması için uygulanan soğutma işlemi olmak üzere iki aşamadan oluşmaktadır (Durmaz, 2015). Kahveye belirli bir ısı uygulanan ilk aşama, kurutma aşaması olarak da adlandırılmaktadır. Endotermik olan bu işlemde, sıcaklık 180 °C'ye kadar yükselmekte ve kahvede nem kaybı olmaktadır. Kavrulma esnasında kızarmış ekmek kokusunu andıran koku duyulmakta ve çekirdek renklerinde sarılaşma görülmektedir (Hasbahçe, 2020). Dönen bir ısıtılmış tamburda kavrulan kahve çekirdekleri soğutma tepsisinde sürekli olarak karıştırılmakta ve kahvelerin depolandığı kısma aktarılmaktadır (Kıvançlı, 2011). Kavurma işleminden sonra nem, %12'den %8 civarına düşmektedir (Durmaz, 2015). Kahvenin kavrulurken, çekirdekte koyu renk elde edildiğinde fiziksel ve kimyasal reaksiyonlar gerçekleşmektedir (Hasbahçe, 2020). Hidroliz, oksidasyon, polimerizasyon, indirgeme gibi kimyasal reaksiyonlarda, kütle, hacim, renk ve pH gibi parametrelerde değişiklikler meydana gelmektedir. Aynı zamanda uçucu bileşikler meydana çıkmaktadır (Durmaz, 2015; Hasbahçe, 2020).

Isıl işlem uygulanırken çekirdekte bulunan proteinler 50 °C ve üstünde denatüre olmaktadır. Çekirdekte şişmeye neden olan, organik bileşiklerin pirolizi 100 °C'nin üzerinde gerçekleşmekte ve esmerleşme meydana gelmektedir. Piroliz işleminin ardından kahve çekirdeklerinden duman çıkışı görülür ve çatlama sesi duyulur. 150 °C civarlarında ise karbonhidrat ve su çekirdekten uzaklaşır. Sıcaklık, 200 °C'ye ulaştığında ise kahve aroması ortaya çıkmaktadır (Yıldırım, 2022). Kavurma derecesi, çekirdeklerin aroması, lezzeti, rengi ve kimyasal değişimleri ile belirlenebilmektedir (Hasbahçe, 2020). Kavurma işleminde, Maillard ve Strecker gibi reaksiyonlar sonucunda karbondioksit açığa çıkmaktadır (Durmaz, 2015). Kahvenin kalitesini doğrudan etkileyen kavurma işleminde, uygulanan sıcaklık-süre ikilisi kontrol edilmeli ve kahveden yapılacak olan içeceğe göre belirlenmelidir (Kıvançlı, 2011). Örneğin, *C. arabica* çekirdeği, acılaşma olmaması için hafif derece kavrulmalı ve maksimum asitliğe sahip olmalıdır (Yıldırım, 2022). Kavurma işlemi genellikle 200 °C ile 230 °C ile 12-20 dakika aralığında yapılmaktadır (Hasbahçe, 2020).

**Öğütme:**

Kavrulan kahve çekirdeğinin tüketilebilmesi için öğütülmesi yani toz haline getirilmesi gerekmektedir (Yıldırım, 2022). Öğütme işlemi, kahvedeki çözünebilir tat ve aromaların su ile emülsiyon haline gelebilmesi için uygulanmaktadır (Hasbahçe, 2020; Yıldırım, 2022). Kavurma aşamasından yaklaşık 12-18 saat sonra öğütme işlemi uygulanabilmektedir (Coşgun, 2022). Kahveler, demleme metoduna göre kalın, orta, ince ve çok ince gibi farklı partikül boyutlarına göre endüstriyel kahve değirmenlerinde öğütülmektedir (Durmaz, 2015; Hasbahçe, 2020). Örneğin; Türk kahvesinde çok ince öğütülmüş, espresso makinelerinde ince öğütülmüş ve plunger tipi kahvelerde orta öğütülmüş kahve kullanılmaktadır (Kıvançlı, 2011).

Ortalama 3.4 $cm^2$ yüzey alanına sahip olan kahve çekirdeğinin öğütme sonrası, Türk kahvesinde 15000-35000 partiküle, filtre kahvede 500-800 partiküle, Espressoda 3500 partiküle, French press'te ise 100-300 partiküle

sahip olması gerekmektedir (Kıvançlı, 2011; Durmaz, 2015; Hasbahçe, 2020). Türk kahvesinde olması gereken partikül boyutu yakalanamaz ise, köpüksüz kahve elde edilir. Aynı zamanda, kahvenin lezzeti, aroması ve soğuma hızı istenilenden farklı olur (Durmaz, 2015). Tazeliği yaklaşık 2-3 gün süren kahveler genellikle vakumlanmış ambalajlarda saklanmaktadır (Kıvançlı, 2011; Hasbahçe, 2020).

## TÜRK KAHVESİ ÇEŞİTLERİ ve GELENEKSEL PİŞİRME YÖNTEMLERİ

Türklere özgü pişirme metodu bulunan Türk kahvesi için genellikle *C. arabica* kahve kullanılmaktadır. Orta kavrulmuş ve ince öğütülmüş kahve çekirdekleri, cezvede su ve isteğe bağlı şeker ilavesiyle kaynatıldığında Türk kahvesi hazırlanmaktadır (Kıvançlı, 2011; Yılmaz ve ark., 2017). Aynı zamanda Türk kahvesi, damla sakızı, karanfil, vanilya, vişne, tarçın, fındık gibi doğal aromalar ilave edilerek de yapılabilmektedir (Hasbahçe, 2020). Kahve, köpük ve telveden oluşan Türk kahvesinin kendine özgü kalıcı bir tadı, kadifemsi, yumuşak bir köpüğü ve aromatik, kıvamlı bir yapısı vardır (Yıldırım, 2022). İyi hazırlanmış Türk kahvesinin bazı püf noktaları bulunmaktadır (Kıvançlı, 2011). Örneğin, kahve demlenirken ilk önce cezvenin alt kısmındaki moleküller ısındığından moleküllerin yoğunluğu azalır ve yukarıya doğru hareket ederler. Fiziksel karışım olarak da adlandırılan moleküllerin bu hareketine ek olarak ateşteki cezve karıştırılırsa köpüksüz kahve elde edilir (Durmaz, 2015). Aynı zamanda soğuk su kullanılarak, kısık ateşte pişirilen Türk kahvesi, olması gereken sıcaklık değerlerine daha doğru zamanda ulaşır ve bol köpüklü kahve elde edilir (Kıvançlı, 2011; Yıldırım, 2022). Ateşe koyulan kahvenin ilk köpürmesi 80 °C'de başlar ve köpük taşmadan cezve alınır. Daha sonra köpük fincanlara eşit şekilde paylaştırılıp tekrar ateşe koyulur. 90 °C'de ikinci köpürme gerçekleşir ve birkaç saniye bekledikten sonra kahve fincanlara paylaştırılır. Türk kahvesinin demlenme süresi ise 2 ile 3 dakika civarında olmalıdır. Yanında su ile birlikte servis edilen Türk kahvesi, Dünya'da 'Turkish Mocca' olarak da bilinmektedir (Durmaz, 2015).

## KAHVENİN SAĞLIĞA ETKİLERİ

Uyarıcı etkisiyle bilinen kahve, insan sağlığına faydalı olan bileşenleri de yapısında barındırmaktadır (Kıvançlı, 2011; Durmaz, 2015). Bileşiminde bulunan polifenoller ve kafeik, kumarik, sinapik, klorojenik, protokateşik asitler sayesinde antioksidan aktiviteye sahip olan kahve aynı zamanda antibakteriyel etki de göstermektedir. Kahvenin yapısında bol miktarda bulunan metilksantinler, astımın etkilerini azaltmakta ve kardiyovasküler hastalıklarda olumlu sonuçlar vermektedir. (Coşgun, 2022). Ek olarak kahvedeki klorojenik asidin kan basıncını düşürdüğü, polifenollerin ise oksidatif stresi önlediği bilinmektedir (Yıldırım, 2022). Ağrı kesici, diüretik ve laksatif etkileri olduğu bilinen kahve, vücutta noradrenalin ve adrenalin hormonlarını arttırarak odaklanmayı da desteklemektedir. (Coşgun, 2022). Yapılan diğer çalışmalarda, kahve tüketiminin bağırsak hareketlerini düzenlediği; sirozu önlemeye destek olduğu; inme riskini azalttığı; Alzheimer ve Parkinson riskini azalttığı; mesane, pankreas, meme ve prostat gibi bazı kanser türlerine karşı koruyucu etkiye sahip olduğu ve tip II diyabet riskini azalttığı belirtilmiştir (Durmaz, 2015; Ülger, 2015; Coşgun, 2022; Altundağ, 2019). Günlük beslenmede yüksek miktarda alınan kahve, içeriğindeki akrilamid sebebiyle karsinojenik etki gösterebileceğinden uygun dozlarda tüketilmelidir (Altundağ, 2019). Yetişkinler için tüketilebilecek kahve miktarının bileşimindeki kafeine göre belirlendiği ve günde yaklaşık 300 mg kafein, yaklaşık 4 fincan kahve tüketilebileceği belirtilmiştir (Ilgaz, 2001).

## SONUÇ

*Rubiaceae* familyasından olan kahve ağacının, *C. robusta* ve *C. arabica* olmak üzere iki önemli türü bulunmaktadır. Uyarıcı etkisi ile keşfedilen kahve, gün geçtikçe günlük diyette önemli bir yere sahip olmuştur. Osmanlıdan günümüze kadar gelen, geleneksel içeceklerimizde biri olan Türk kahvesinin de tüketimi oldukça fazladır. Genellikle Türk kahvesi yapılırken, kavrulmuş ve ince öğütülmüş *C. arabica* kahve çekirdekleri kullanılmaktadır. Ülkemizde, su ve tercihe bağlı şeker ve aroma maddeleri eklenerek, farklı pişirme yöntemleri uygulanarak hazırlanan Türk kahvesi, kendine has bir kokuya ve tada sahiptir. Zengin polifenol içeriği sayesinde antioksidan aktivite gösteren kahvenin sağlık üzerine de birçok olumlu etkisi bulunmaktadır. Özellikle, kanser, Alzheimer, Parkinson, diyabet hastalığı riskini azaltmada yardımcı olduğu, kan basıncını düşürücü ve ağrı kesici etki gösterdiği yapılan çalışmalar sonucunda belirtilmiştir. Sağlık açısından olumlu etkileri bulunsa da Türk kahvesinin tüketim miktarı oldukça önemlidir. Ülkemizde sık sık tüketilen geleneksel içeceğimizin sağlık etkileri hakkında daha fazla çalışma yapılması gerekmektedir.

## KAYNAKLAR

Altundağ ÖÖ 2019. Türk Kahvesinin Sağlık Boyutu ve Etkileri. İzmir Democracy University Health Sciences Journal, 2(3), 183-193.

Bayramova G 2019. Türk Kahvesi Pişirme Yöntemleri ve Tarihsel Gelişiminin Ürün Tasarımı Bağlamında İncelenmesi. Yüksek Lisans Tezi. Endüstri Ürünleri Tasarımı Anasanat Dalı. Güzel Sanatlar Enstitüsü. T.C. Marmara Üniversitesi, İstanbul.

Coşgun G 2022. Depolama Koşullarının Türk Kahvesinin Fiziksel, Kimyasal ve Duyusal Özellikleri Üzerine Etkisi. Yüksek Lisans Tezi. Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı. T.C. Akdeniz Üniversitesi, Antalya.

Durmaz AA 2015. Farklı Kahve Çekirdekleri ve Pişirme Ekipmanlarıyla Hazırlanan Türk Kahvesinin Duyusal ve Kimyasal Analizlerle Optimizasyonu. Yüksek Lisans Tezi. Gastronomi Anabilim Dalı. T.C. Okan Üniversitesi Sosyal Bilimler Enstitüsü, İstanbul.

Hasbahçe G 2020. Türk Kahvesine Uygun İki Farklı Aroma Emülsiyonunun Oluşturulması Ve Türk Kahvesinde Kullanılabilirliğinin Araştırılması. Yüksek Lisans Tezi. Gıda Mühendisliği Anabilim Dalı. T.C. Ege Üniversitesi Fen Bilimleri Enstitüsü, İzmir.

Ilgaz Ş 2001. Kafein ve Sağlık Üzerine Etkileri. Sürekli Tıp Eğitimi Dergisi. Sağlık Bakanlığı, Ana Çocuk Sağlığı ve Aile Planlaması Genel Müdürlüğü, Ankara. Erişim adresi: https://www.ttb.org.tr/STED/sted0101/16.html#:~:text=Pek%20%C3%A7ok%20%C3%A7al%C4%B1%C5%9Fmada%2C%20yeti%C5%9Fkinler%20i%C3%A7in,b%C3%BCy%C3%BCk%20bardak%20%C3%A7ay)%20oldu%C4%9Fu%20belirtilmi%C5%9Ftir.

Kaplan M 2011. Bir Fincan Keyif: Kahvenin Öyküsü. Yurt ve Dünya Dergisi/The Journal of Homeland and The World, 2(2), 11-20.

Kıvançlı J 2011. Türk Kahvesinin Karakteristik Lezzetinin GC/MS ve Lezzet Profili Analizi Tekniği ile Belirlenmesi. Doktora Tezi. Gıda Mühendisliği Anabilim Dalı. Ege Üniversitesi Fen Bilimleri Enstitüsü, İzmir.

Tatar RÖ 2021. Türk Kahvesi Tüketiminin Tip 2 Diyabetli Hastalarda Hba1c Düzeyine Etkisi. Uzmanlık Tezi. T.C. İzmir Katip Çelebi Üniversitesi Tıp Fakültesi Aile Hekimliği Anabilim Dalı, İzmir.

Ülger N 2015. Türk Kahvesi ve Bazı Hazır Kahve Karışımlarının Total Fenol İçeriği Ve Antioksidan Aktivitelerinin Karşılaştırılması. Yüksek Lisans Tezi. Beslenme Bilimleri Programı. T.C. Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü, Ankara.

Yıldırım S 2022. Kahve Çekirdeği Kavurma Derecelerinin Türk Kahvesi, Filtre Kahve ve Espressodaki Antioksidan Kapasitesine Etkisinin Elektrokimyasal Yöntemle Belirlenmesi ve Gastronomik Açıdan Değerlendirilmesi. Yüksek Lisans Tezi. Gastronomi Anabilim Dalı. T.C İstanbul Okan Üniversitesi Lisansüstü Eğitim Enstitüsü, İstanbul.

Yıldız N 2019. Zayıflama Diyetine Eklenen Türk Kahvesinin Antropometrik Ölçümler ve Lipit Profilleri Üzerine Etkisi. Yüksek Lisans Tezi. Veterinerlik Biyokimyası Anabilim Dalı. Ondokuz Mayıs Üniversitesi Sağlık Bilimleri Enstitüsü, Samsun.

Yılmaz B, Acar-Tek N, Sözlü S 2017. Turkish cultural heritage: a cup of coffee. Journal of Ethnic Foods, 4(4), 213-220.

## *ORAL PRESENTATION*

# The Effect of *PDYN* rs910080 Polymorphism on The Risk of Alcohol Use Disorder in Turkish Men

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**Abstract**

Alcohol Use Disorder (AUD) is a complex brain disorder depending on environmental and biological factors. Prodynorphin (*PDYN*) which encodes the precursor molecule of dynorphins, a ligand of the kappa opioid receptor, is believed to be associated with negative moods. Epidemiological studies have shown high comorbidity between addiction and negative emotional states due to overlapping neurobiological abnormalities in neural circuits regulating emotion. In this study, the relationship of *PDYN* rs910080 gene polymorphism with AUD was investigated for the first time in Türkiye. For this purpose, 50 male patients diagnosed with AUD and 50 healthy males were included in the study. Genotyping was performed using the restriction fragment length polymorphism (RFLP) technique. The frequencies of the A and G alleles were 67.0% and 33.0% in healthy controls, and 69.0% and 31.0% in alcohol-dependent individuals, respectively. The allele frequencies were in Hardy-Weinberg equilibrium ($p>0.05$). No statistically significant difference was detected between patients and healthy individuals in terms of allele frequencies ($p=0.936$). This result indicated that the *PDYN* rs910080 polymorphism has no effect on the risk of AUD in Turkish men.

**Keywords:** Alcohol use disorder, prodynorphin, negative moods

## INTRODUCTION

Alcohol use disorder (AUD) is a relapsing and complex brain disorder (Edenberg and Foroud, 2013). It is characterised by physiological withdrawal and emerging several negative effects during this period (Kissler et al., 2014). AUD is associated with developing mental and behavioural disorders, non-contagious diseases and traffic accidents. In addition, it leads to social and economic problems both individually and countrywide (Arunachalam and Chandrasekaran, 2020). Even though alcohol intake is a common behaviour, some individuals are affected more adversely. One-time use of alcohol also causes discrete outcomes among individuals. This diversity is generally considered as a result of genetic differences (Wall et al., 2016). Studies on twins, adopted children and people with a family history of AUD have shown that AUD is associated with genetics (Kaya et al., 2017).

Molecular changes in endogenous opioid system induced by alcohol and other addictive substances can lead to neuroplastic adaptations that lead to addiction (Taqi et al., 2011). Human *PDYN* gene, located in chromosome 20, contains four exons and is 15.3 kb long. Dynorphins are obtained by proteolytic processing of prodynorphins encoded by the *PDYN* gene and bind to the kappa opioid receptors encoded by *OPRK1* gene (Faisal et al., 2014). As a result, dopamine release is blocked, and so dynorphins possess a protective or regulatory role in reducing extracellular dopamine after substance-induced dopamine increase, in substance addiction and tolerance. High dynorphin levels lead to dysphoria and anhedonia associated with negative craving and withdrawal (Flory et al., 2010).

It has been shown that dynorphins encoded by *PDYN* gene also cause negative moods. These negative mood states lead to negative craving and excessive alcohol consumption. However, there has been no study investigating the relationship between *PDYN* gene polymorphisms and AUD in Turkish population. Therefore,

it was aimed to investigate the effect of *PDYN* rs910080 polymorphism on alcohol use disorder in Turkish men.

## MATERIALS AND METHODS

A total of 50 individuals with AUD and 50 healthy controls were enrolled in this study. All AUD patients were entered in the Ankara University Faculty of Medicine, Department of Mental Health and Diseases. They were diagnosed with AUD according to International Classification of Diseases-10 (ICD-10) diagnostic criteria and fulfilled the DSM–5criteria for AUD. The exclusion criteria for patients with AUD were as follows: (i) substance use disorders other than alcohol and nicotine dependence, (ii) clinically significant comorbid psychiatric illness such as any psychotic disorders, schizophrenia, mental retardation, bipolar disorder and severe depression, (iii) subjects administered drugs for psychiatric illness such as depression. Controls were comprised of healthy individuals that satisfied the same exclusion criteria and had no past or current alcoholism (n=50). Controls were matched to AUD patients for gender and smoking habits.

Written informed consent was obtained from each subject who was eligible for the study. Samplings were performed in accordance with the principles of The Declaration of Helsinki and approval (approval number and year: I3-220-21 in 2021) for the use of human subjects was obtained from the institutional ethics committee. A small questionnaire was also used to gather socio-demographic information regarding age, marital, education and employment status, age onset of alcohol dependence.

Two ml of venous blood was taken from everyone into tubes with EDTA for DNA isolation. Genomic DNA was extracted from whole blood samples using the QIAamp DNA blood-kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. The *PDYN* rs910080 polymorphism genotyping was carried out using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP), as previously described by Hashemi et al. (2018). The sequences of the forward (F) and reverse (R) primers were 5'-CAATGCCCAGTGCGTATGT-3' and 5'-CTTTGGAGACGATGCTTTAGGT-3', respectively. PCR amplification was conducted on a Techne Tc 512 PCR System in a 25-µL reaction mixture containing 2mM of dNTPs, 10 pmol each of the forward (F) and reverse (R) primers, 1 U of Hot Star Taq DNA polymerase, 10×PCR buffer and 50 ng of genomic DNA. Then, the 497 bp- PCR products were digested with *SduI* at 37˚C for 1 hour. The undigested and digested PCR products were separated by gel electrophoresis on a 3% agarose gel, visualized by ethidium bromide staining under an UV illuminator, and then scanned and photographed using the Syngene Monitoring System (Figure 1).

The Statistical Package for Social Sciences (SPSS) version 21.0 software for Windows was used for the statistical analyses. All categorical data were shown as numbers, percentages and 95% confidence interval. For numerical data, the mean and standard deviation (SD) or median and the interquartile range (IQR) were given according to the normality of the data examined by the Kolmogorov-Smirnov test. The frequencies of the *PDYN* rs910080 alleles and genotypes were obtained by direct counting, and departure from the Hardy-Weinberg equilibrium was evaluated by the chi-square test. The relationship between the *PDYN* rs910080 and AUD was modelled by binary logistic regression analysis. $p<0.05$ was considered as statistically significant.

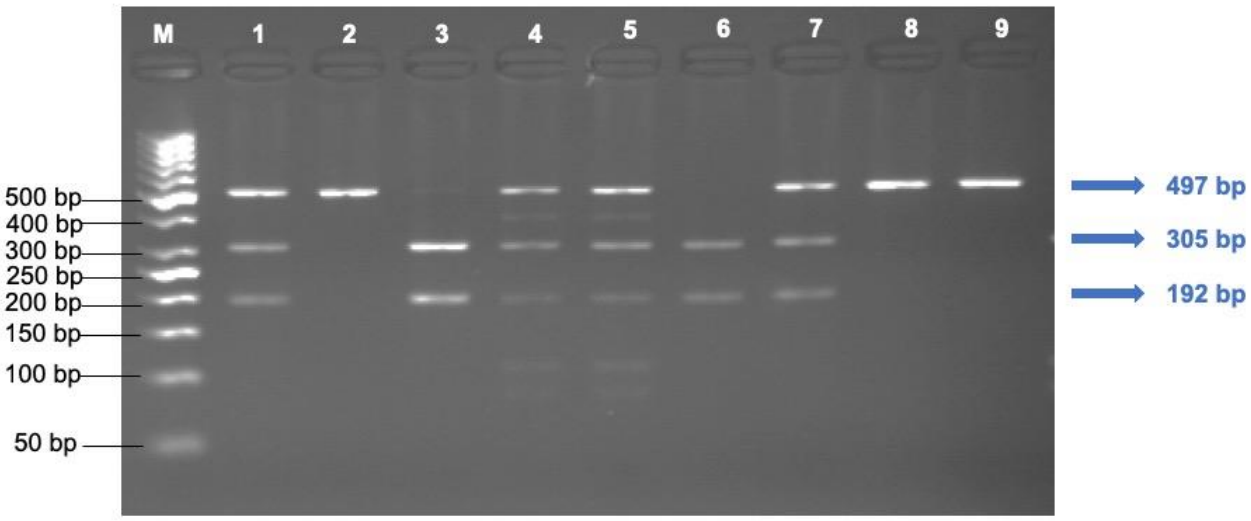


**Figure 1.** A representative agarose gel image of digested PCR products (497 bp) with *SduI* for the *PDYN* rs910080 polymorphism. M: 50 bp ladder; Lanes 2, 8 and 9: homozygous wild-type genotype (AA) (497 bp); Lanes 1, 4, 5 and 7: heterozygote genotype (AG) (497 bp, 305 bp and 192 bp); Lanes 3 and 6: homozygous mutant genotype (GG) (305 bp and 192 bp).

## RESULTS

The socio-demographic characteristics of 50 healthy controls (mean age: 40.6±8.9 years) and 50 individuals with AUD (mean age: 41.1±8.4 years) were presented on Table 1. All subjects in both the controls and AUD patients were smokers. Patients with AUD had a significantly higher duration of smoking (years) and pack years of smoking than healthy controls (24.5 vs. 15.0 years and 27.5 vs. 10.0 packet, respectively; $p<0.05$).

**Table 1**. Socio-demographics characteristics

| **Parameter** | **AUD (n=50)** | **Control (n=50)** | **p-value** |
|---|---|---|---|
| **Education** | **n (%)** | **n (%)** | |
| Primary/Secondary | 12 (24) | 9 (18) | $\chi2$=15.79 p=0.001 |
| High School | 20 (20) | 10 (20) | |
| Undergraduate | 18 (36) | 19 (38) | |
| Graduate | - | 12 (24) | |
| **Occupation** | | | |
| Working | 33 (66) | 47 (94) | $\chi2$=17.71 p=0.001 |
| Not working | 13 (26) | 1 (2) | |
| Retired | 4 (8) | 2 (4) | |
| **Marital status** | | | |
| Single | 15 (30) | 14 (28) | $\chi2$=18,03 p=0.001 |
| Married | 19 (38) | 35 (70) | |
| Widow/Divorced | 16 (32) | 1(2) | |

The allele and genotype frequencies in both AUD and control subjects were shown in Table 2 for the *PDYN* rs910080 polymorphism. The frequencies of the A and G alleles were 69.0% and 31.0% in healthy controls, and 67.0% and 33.0% in individuals with AUD. The genotype frequencies of the *PDYN* rs910080 polymorphism were consistent with Hardy–Weinberg equilibrium both in patients ($\chi2$=0.08; p=0.77) and controls ($\chi2$=0.28; p=0.59). The relationship between the *PDYN* rs910080 genotypes and AUD was examined by logistic regression analysis and the *PDYN* rs910080 polymorphism was not found to be associated with AUD (Table 2).

**Table 2.** Genotype frequencies of *PDYN* rs910080 polymorphism in AUD and healthy subjects**.**

| ***PDYN* rs910080 Genotypes** | **AUD (n=50)** | | **Healthy controls (n=50)** | | **p-value** | **Odds Ratio (95% CI)** |
|---|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | | |
| AA | 22 | 44 | 23 | 46 | p>0.05 | Reference |
| AG | 23 | 46 | 23 | 46 | | 1.307 (0.31-5.509) |
| GG | 5 | 10 | 4 | 8 | | 1.25 (0.297-5.256) |
| Variant allele freq. | 33% | | 31% | | | |
| HWE p-value | $\chi2$=0.08; p=0.77 | | $\chi2$=0.28; p=0.59 | | | |

## DISCUSSION

Dynorphin, which is expressed throughout limbic brain areas, is considered an integral part of the brain's stress response system, and release of dynorphin occurs during exposure to painful, noxious or stressful stimuli (Karkhanis et al., 2017; Knoll and Carlezon, 2010). Opioids are also profound activators of the brain stress system (Chavkin and Koob, 2016). Thus, the present study was undertaken with the aim of exploring whether *PDYN* rs910080 gene polymorphism have an effect on AUD due to the contribution of dynorphin to to the drug-seeking behaviour due to negative reinforcement. To the best of our knowledge, this is the first report examining the association of *PDYN* rs910080 gene polymorphism with AUD at the gene level in Turkish men. *PDYN* rs910080 is located in the 3′-UTR region of the *PDYN* gene and is in high linkage disequilibrium with *PDYN* rs2235749. It was demonstrated that rs2235749 polymorphism in the *PDYN* 3′-untranslated region (3′UTR) modifies striatal *PDYN* mRNA expression via impaired binding of miR-365, a microRNA that targets the 3′UTR of *PDYN* (Egervari et al., 2006). Although the nearly complete linkage disequilibrium of *PDYN* rs910080 with rs2235749, there was not a significant association between the *PDYN* rs910080 and AUD in the present study (p>0.05).

Although different results have been obtained from genetic studies, PDYN gene variations have been accepted as powerful candidates in terms of alcohol dependence (Winham et al., 2015). D'Addario and colleagues (2007) investigated the impact of *PDYN* gene polymorphism and promoter methylation on alcohol dependence in their study comparing alcohol dependents and healthy controls. They determined that DNA methylation increased in the peripheral blood samples of alcohol dependents. Moreover, a correlation was observed between polymorphism in the promoter region of *PDYN* gene and DNA methylation. Taqi and co-workers (2011), investigated the effect of polymorphism in the CpG islands of PDYN gene in the postmortem prefrontal cortex samples of alcohol dependents and healthy individuals and reported the relationship with alcohol dependence. Winham et al. (2015) evaluated the effect of *PDYN* rs2281285 gene polymorphism on alcohol dependence, negative craving and relapse in men and women. Post-treatment relapse was followed at 3, 6, 9 and 12 months over a 12-month period after treatment was completed. This polymorphism was found to be associated with negative craving and relapse in women (Winham et al., 2015).

In conclusion, this study indicated that the *PDYN* rs910080 polymorphism has no effect on the risk of AUD in Turkish men. A clear generalized statement about the role of the *PDYN* rs910080 polymorphism can be done with a larger sample including women alcohol users.

## ACKNOWLEDGEMENTS

This study was supported by the Ankara University Scientific Research Projects Coordination Unit (grant number: THD-2022-2409 awarded to Dilek Kaya-Akyüzlü).

## REFERENCES

Arunachalam U, Chandrasekaran A 2020. Genetic Polymorphism in Alcohol-dependent Genes: A Review. SBV Journal of Basic, Clinical and Applied Health Science, 3(1): 10–15. https://doi.org/10.5005/jp-journals-10082-02260.

Chavkin C, Koob GF 2016. Dynorphin, dysphoria, and dependence: the stress of addiction. Neuropsychopharmacology, 41(1): 373–374. https://doi.org/doi:10.1038/npp.2015.258.

D'Addario C, Shchetynsky K, Pucci M, Cifani C, Gunnar A, Vukojević V, Padyukov L, & Terenius L 2017. Genetic variation and epigenetic modification of the prodynorphin gene in peripheral blood cells in alcoholism. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 76: 195–203. https://doi.org/10.1016/j.pnpbp.2017.03.012.

Edenberg HJ, Foroud T 2013. Genetics and alcoholism. Nature Reviews Gastroenterology and Hepatology, 10(8): 487–494. https://doi.org/10.1038/nrgastro.2013.86.

Egervari G, Jutras-Aswad D, Landry J, Miller, ML, Anderson SA, Michaelides M, Jacobs MM, Peter C, Yiannoulos, G, Liu X, Hurd YL 2016. A Functional 3'UTR Polymorphism (rs2235749) of Prodynorphin Alters microRNA-365 Binding in Ventral Striatonigral Neurons to Influence Novelty Seeking and Positive Reward Traits. Neuropsychopharmacology, 41(10): 2512–2520. https://doi.org/10.1038/npp.2016.53.

Faisal M, Waseem D, Ismatullah H, Taqi MM 2014. A molecular prospective provides new insights into implication of PDYN and OPRK1 genes in alcohol dependence. Computers in Biology and Medicine, 53: 250–257. https://doi.org/10.1016/j.compbiomed.2014.07.021.

Flory JD, Pytte CL, Hurd Y, Ferrell RE, Manuck SB 2011. Alcohol dependence, disinhibited behavior and variation in the prodynorphin gene. Biological Psychology, 88(1): 51–56. https://doi.org/10.1016/j.biopsycho.2011.06.007.

Karkhanis A, Holleran KM, Jones SR 2017. Dynorphin/Kappa Opioid Receptor Signaling in Preclinical Models of Alcohol, Drug, and Food Addiction. In International Review of Neurobiology (Vol. 136, pp. 53–88). Academic Press Inc. https://doi.org/10.1016/bs.irn.2017.08.001.

Kaya H, Kaya Ö, Dilbaz N 2017. Genetics of Alcohol Use Disorder. Current Addiction Research, 1(1): 33–46. https://doi.org/10.5455/car.20170705081115.

Kissler JL, Sirohi S, Reis DJ, Jansen HT, Quock RM, Smith DG, Walker BM 2014. The one-two punch of alcoholism: Role of central amygdala dynorphins/kappa-opioid receptors. Biological Psychiatry, 75(10): 774–782. https://doi.org/10.1016/j.biopsych.2013.03.014.

Knoll AT, Carlezon WAN 2010. Dynorphin, stress, and depression. Brain Research, 1314: 56–73. https://doi.org/10.1016/j.brainres.2009.09.074.

Taqi MM, Bazov I, Watanabe H, Sheedy D 2011. Prodynorphin CpG-SNPs associated with alcohol dependence: Elevated methylation in the brain of human alcoholics. Addiction Biology, 16(3): 499–509. https://doi.org/10.1111/j.1369-1600.2011.00323.x.

Wall TL, Luczak SE, Hiller-Sturmhöfel S 2016. Biology, Genetics, and Environment: Underlying Factors Influencing Alcohol Metabolism. Alcohol Research: Current Reviews, 38(1): 59–68.

Winham SJ, Preuss UW, Geske JR, Zill P, Heit JA, Bakalkin G, Biernacka JM, Karpyak VM 2015. Associations of prodynorphin sequence variation with alcohol dependence and related traits are phenotype-specific and sex-dependent. Scientific Reports, 5: 1-9. https://doi.org/10.1038/srep15670.

## *ORAL PRESENTATION*

# Effect of zirconia reinforcement on the corrosion performance of poly(*N*-vinyl carbazole) films

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## Abstract

Zirconia reinforced poly(*N*-vinyl carbazole) (PNVC@$ZrO_2$) films were deposited electrochemically on 304-type stainless steel (304-SS) surface. Cyclic voltammetry was used for the electrodeposition of PNVC@$ZrO_2$ films from *N*-vinyl carbazole (NVC) monomer, tetrabutylammonium perchlorate (TBAP) supporting electrolyte and zirconia ($ZrO_2$) containing non-aqueous acetonitrile (ACN) solution. Three different amounts of zirconia (0.05%, 0.10% and 0.15%) were used during electrodeposition of poly(*N*-vinyl carbazole) (PNVC) and 304-SS surface was covered by PNVC@$ZrO_2$ films. Optimum zirconia amount was determined from corrosion tests by comparison of potentiodynamic polarization curves recorded for PNVC@$ZrO_2$ coated stainless steel electrodes (SS/PNVC@$ZrO_2$) in 1 M $H_2SO_4$ test solution. It has been found that 0.10% zirconia reinforced poly(*N*-vinyl carbazole) coatings [PNVC@$ZrO_2$(0.10%)] exhibited better corrosion performance than the other PNVC@$ZrO_2$ coatings. Characterization of PNVC@$ZrO_2$(0.10%) films was made by ATR-FTIR and SEM investigations. Corrosion protection performances of PNVC@$ZrO_2$(0.10%) films were evaluated by $E_{ocp}$-time curves, potentiodynamic polarization (PP) and electrochemical impedance spectroscopy (EIS) techniques in 1 M $H_2SO_4$ corrosive media and compared with the results of unreinforced poly(*N*-vinyl carbazole) coated stainless steel electrode (SS/PNVC). It has been found that the PNVC@$ZrO_2$(0.10%) films have much better (>90%) corrosion protection performance than the unreinforced PNVC films.

**Keywords:** Corrosion, Stainless steel, Poly(vinyl carbazole), Electropolymerization, Zirconia, Conducting polymer.

## INTRODUCTION

In the recent past, conducting polymers have been employed in the development of corrosion inhibiting coatings because of their good chemical stabilities and ease of synthesis (Carregher and Breslin, 2018). Polypyrrole, polyaniline, polythiophene, polycarbazole and their derivatives are the most commonly used conducting polymers for corrosion protection of active metals such as copper, aluminium, iron, as well as steels (Duran et al., 2013, 2017). It has been reported that inhibiting effect of a conducting polymer depends on synthesis conditions, nature of used dopant and degree of adhesion to the metal surface. In this respect, it is known that porosity could improve the adhesion of the polymer film (Garcia-Cabezon, et al., 2020)

Nanocomposites of conducting polymers with noble metal nanoparticles or metal oxides are highly promising materials because of incorporation of metal oxide particles into the polymer matrix increases the surface area, porosity, adhesion to the metal surface and mechanical properties as nano-sized fillers and minimizes the penetration of corrosive species so enhances the corrosion resistance (Mahmoudian et al., 2011, Demirel and Bozkurt, 2022). For this purpose, nanoparticles such as $TiO_2$, $MnO_2$, $SnO_2$, $ZrO_2$, ZnO etc. have been used as reinforcement material for different conducting polymers. Surveying of literature shows that relatively few study have been carried out for corrosion protection of steel by electrochemically synthesized polycarbazole-metal oxide nanocomposite films (Elangovan et al., 2018).

## MATERIAL AND METHODS

NVC, TBAP, $ZrO_2$, ACN, $H_2SO_4$ and other chemicals used in this study were all high purity (>98%) and purchased from Sigma-Aldrich, while cylindrical type 304-SS rods were obtained locally. Electropolymerization and corrosion experiments were carried out in five-necked and single-compartment glass cells with a volume of 10 mL and 100 mL, respectively. 304-SS rod was embedded into a Teflon holder by polyester and used as working electrode. Before deposition of polymer coating, the SS surface was mechanically ground with 1200 grit SiC abrasive paper by Forcipol 1V grinder/polisher; then degreased in 1:1

(v/v) acetone/ethanol mixture in an ultrasonic bath, finally rinsed with ACN. During electropolymerization, Pt sheet and Ag wire were employed as counter and pseudo reference electrode, respectively. Ag/AgCl electrode (3 M KCl, Gamry) and platinum wire (CHI115) was used as the reference and as the counter electrode in corrosion tests.

Computer controlled Gamry Reference 600 potentiostat/galvanostat was employed during electropolymerization and corrosion tests. Perkin Elmer/Spectrum 100 ATR-FTIR spectrometer was used for infrared spectroscopic analysis of the polymer coated SS samples while Hitachi Regulus 8230 model FE-SEM was used for surface analyses of polymer coated ITO materials.

0.02 M NVC, 0.1 M TBAP and 0.10% (w/v) $ZrO_2$ containing polymerization solution was prepared by using non-aqueous ACN as solvent. PNVC@$ZrO_2$ films were synthesized at 50 mV/s scan rate in the potential region of +0.3 V and +1.5 V by applying 10 cycles. At the end of electropolymerization, polymer coated SS electrode (SS/PNVC@$ZrO_2$) was removed from the polymerization solution, rinsed with ACN and dried in air, at room temperature.

Corrosion inhibition properties of PNVC@$ZrO_2$ films were investigated by open circuit potential-time ($E_{ocp}$-t) curves, PP and EIS techniques in 1 M $H_2SO_4$ solution. EIS and PP measurements were recorded after the working electrode was kept in test solution for 30 minutes to attain a stable $E_{ocp}$ value. EIS data were obtained by application of an AC current with 10 mV amplitude in the frequency range of 100 kHz to 10 mHz at around $E_{ocp}$. Afterwards, PP curves were recorded at a scan rate of 1 mV/s in the potential range of -0.4 V to +0.4 V according to the $E_{ocp}$. ZSimpWin 3.21 software was used for EIS analysis.

## RESULTS AND DISCUSSION

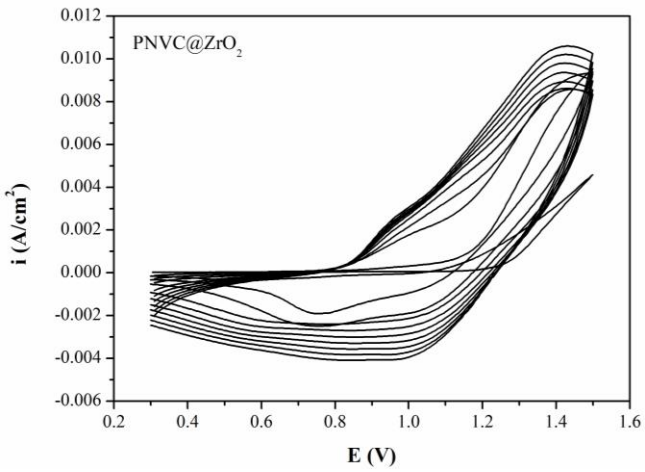


**Figure 1.** Cylic voltammetric synthesis of PNVC@$ZrO_2$ films on 304-SS from 0.1 M TBAP, 0.02 M NVC and 0.10% $ZrO_2$ containing ACN solution

PNVC@$ZrO_2$ films were synthesized by cyclic voltammetry in presence of 0.05%, 0.10% and 0.15% $ZrO_2$ containing polymerization solutions to find out best protective PNVC@$ZrO_2$ film. Figure 1 shows cyclic voltammograms recorded during PNVC@$ZrO_2$ synthesis in presence of 0.10% $ZrO_2$. Current increase starting around +1.15 V in the first forward scan leads to NVC oxidation and polymer film formation on the 304-SS surface starts with this oxidation process. The reduction of the freshly formed polymer can be observed from the first reverse scan at around +0.8 V and oxidation of polymer film can be seen from the second forward scan at around +1.0 V potential. In subsequent scans, regular increase of broad peak currents indicates conducting polymer growth on the steel surface (Tüken et al., 2006) and SS surface was covered with dark green and homogeneous PNVC@$ZrO_2$ film.

Zirconia reinforced PNVC films were synthesized in presence of three different amounts of $ZrO_2$ (0.05%, 0.10% and 0.15%) and corrosion performances of the PNVC@$ZrO_2$ coated SS electrodes (SS/PNVC@$ZrO_2$) were tested by PP method in 1 M $H_2SO_4$ solution to find out best protective polymer film; recorded PP curves were presented in Figure 2. As it is seen from Figure 2, when the SS surface covered with 0.10% $ZrO_2$ reinforced PNVC, PP curve shifts to the lower current density region. Application of Tafel extrapolation to these curves gives quantitative corrosion parameters such as corrosion potential ($E_{corr}$), corrosion current density ($i_{corr}$), anodic and cathodic Tafel constants ($\beta_a$ and $\beta_c$) and corrosion rate (CR); these parameters were listed in Table 1. Comparison of $i_{corr}$ values shows that the PNVC film synthesized in the presence of 0.10%

$ZrO_2$ has the lowest $i_{corr}$ value, so the PNVC@$ZrO_2$(0.10%) film has much protective property than the others. Hence, optimum $ZrO_2$ amount was determined as 0.10% for PNVC films.

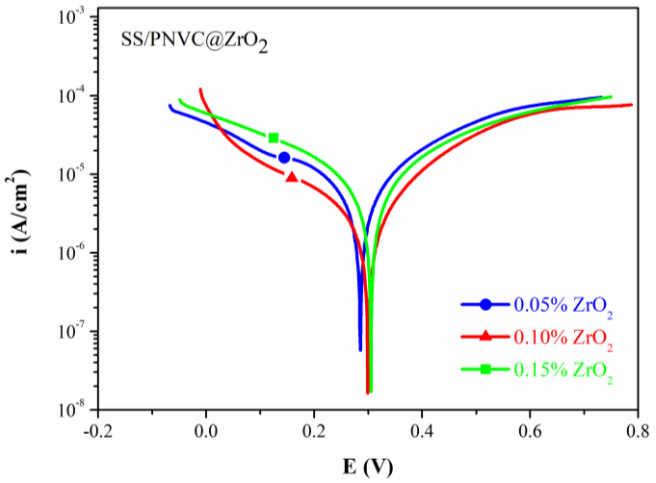


**Figure 2.** Potentiodynamic polarization curves of SS electrodes coated with PNVC films reinforced with $ZrO_2$ at 0.05%, 0.10% and 0.15% concentrations

**Table 1.** Corrosion parameters obtained from the PP curves of SS electrodes coated with the PNVC films reinforced with $ZrO_2$ at 0.05%, 0.10% and 0.15% concentrations

| Electrode | $E_{corr}$ (V) | $i_{corr}$ (A/cm$^2$) | $\beta_a$ (V/dec) | $\beta_c$ (V/dec) | CR (mpy) |
|---|---|---|---|---|---|
| SS/PNVC@$ZrO_2$ (0.05%) | 0.285 | $14.62\times10^{-6}$ | 0.378 | 0.530 | 6.681 |
| SS/PNVC@$ZrO_2$ (0.10%) | 0.299 | $4.76\times10^{-6}$ | 0.224 | 0.386 | 2.175 |
| SS/PNVC@$ZrO_2$ (0.15%) | 0.305 | $18.30\times10^{-6}$ | 0.475 | 0.581 | 8.351 |

Infrared spectra of PNVC@$ZrO_2$(0.010%) and PNVC coated SS samples were compared in Figure 3. Unreinforced and $ZrO_2$ reinforced PNVC films have the same chemical structure. The weak peaks at 3000 cm$^{-1}$ and 2800-2900 cm$^{-1}$ show aromatic and aliphatic C-H stretchings, respectively. The typical three bands appeared at 1400-1600 cm$^{-1}$ are assigned to aromatic C=C stretching vibrations of the benzene rings. Two medium peaks seen at 1250 and 1350 cm$^{-1}$ are belong to C-N stretching vibrations of *N*-vinyl pyrrole units of the carbazoles. The strong peak at 1100 cm$^{-1}$ indicates the Cl-O stretches of perchlorate ions incorporated into the polymer structure. A medium signal in the range of 700-750 cm$^{-1}$ denotes 1,2-disubstitution of the benzene rings. These observed characteristic peaks of PNVC confirm the polymerization of *N*-vinyl carbazole in the presence of zirconia (Frau et al., 2010).

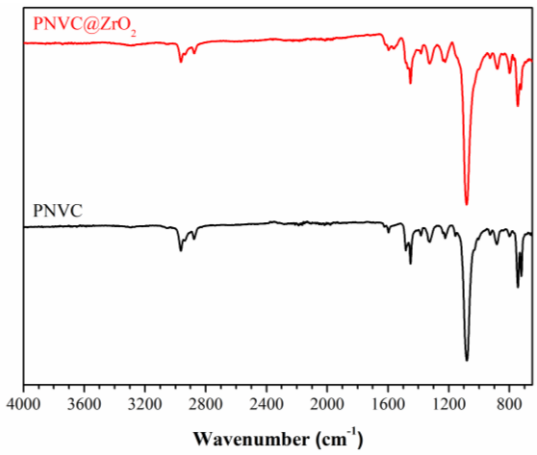


**Figure 3.** ATR-FTIR spectra of $ZrO_2$ reinforced and unreinforced PNVC coated SS samples

Figure 4 shows the SEM micrographs and EDS spectra of the PNVC@$ZrO_2$ and PNVC films. PNVC@$ZrO_2$ film consists of bigger polymeric grains and has more dense structure than the unreinforced PNVC film. EDS spectrum of PNVC@$ZrO_2$ coated sample verifies the incorporation of Zr into the polymer matrix.

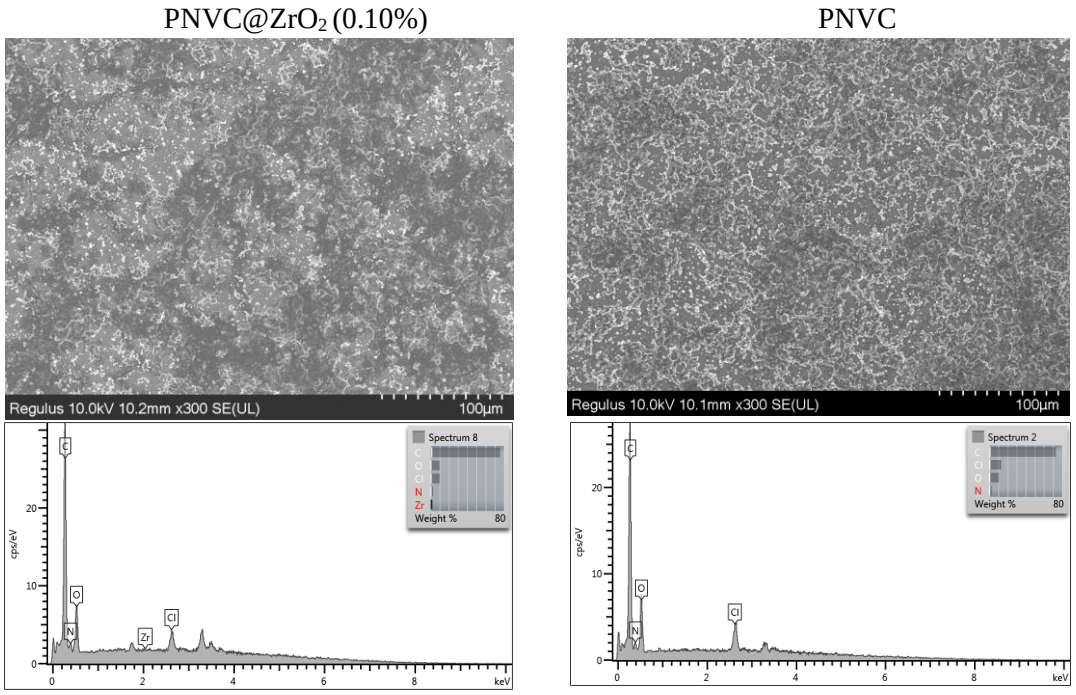


**Figure 4.** SEM micrographs and EDS spectra of $ZrO_2$ reinforced and unreinforced PNVC films

Corrosion protective properties of PNVC@$ZrO_2$ films have been explored in 1 M $H_2SO_4$ solution by $E_{ocp}$-time plots, PP and EIS methods and compared with the data of unreinforced PNVC film and uncoated SS. The open circuit potential values of SS/PNVC@$ZrO_2$, SS/PNVC and SS electrodes measured during 5 days (120 h) and plotted versus immersion time; this plot was presented in Figure 5. It can be seen that the $E_{ocp}$ values of the PNVC and PNVC@$ZrO_2$ coated SS electrodes are more positive than the uncoated SS electrode. This case is attributed to the anodic protection effect of conductive polymers in oxidized form (Duran et al., 2017). On the other hand, it can be said that incorporation of metal oxide particles into the polymer structure leads to a slight decrease in $E_{ocp}$ values, as it has been observed for poly(*N*-vinyl carbazole)-$TiO_2$ composite coated SS sample (Duran and Şimşek, 2022).

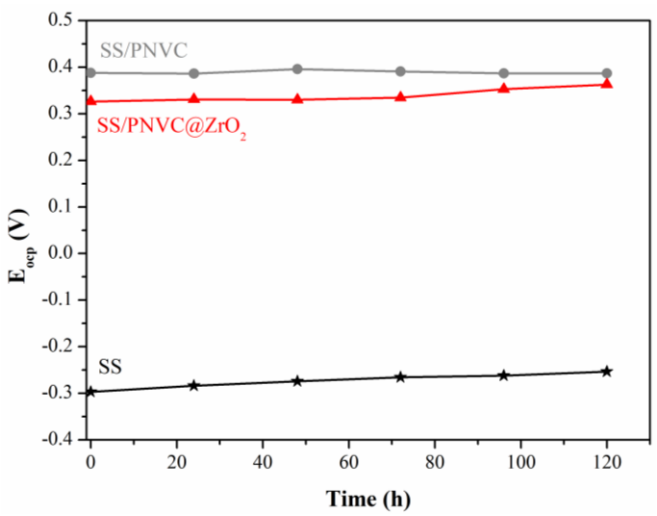


**Figure 5.** $E_{ocp}$-time curves of PNVC@$ZrO_2$ coated, PNVC coated and uncoated SS electrodes

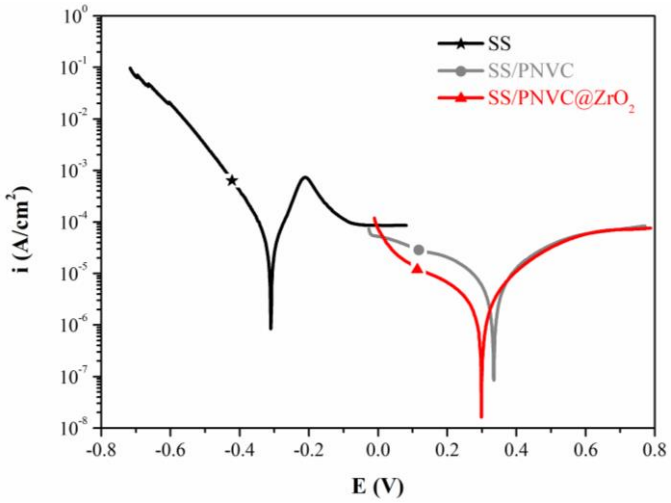


**Figure 6.** Potentiodynamic polarization curves of PNVC@$ZrO_2$ coated, PNVC coated and uncoated SS electrodes

**Table 2.** Corrosion parameters obtained from the PP curves of the SS, SS/PNVC and SS/PNVC@$ZrO_2$ electrodes

| Electrode | $E_{corr}$ (V) | $i_{corr}$ (A/cm$^2$) | $\beta_a$ (V/dec) | $\beta_c$ (V/dec) | CR (mpy) |
|---|---|---|---|---|---|
| SS | -0.310 | $66.0\times10^{-6}$ | 0.134 | 0.113 | 30.17 |
| SS/PNVC | 0.334 | $38.6\times10^{-6}$ | 0.760 | 1.459 | 17.63 |
| SS/PNVC@$ZrO_2$ (0.10%) | 0.299 | $4.76\times10^{-6}$ | 0.224 | 0.386 | 2.175 |

PP curves of the SS/PNVC@$ZrO_2$, SS/PNVC and SS electrodes were given in Figure 6 and corrosion parameters extracted from the Tafel extrapolation were listed in Table 2. Figure 6 and Table 2 demonstrate that the PNVC@$ZrO_2$ and PNVC coatings provide protection to the SS against corrosion due to the $i_{corr}$ and CR values of polymer film coated SS electrodes are lower than those of the bare SS electrode. Comparison of protective properties of PNVC@$ZrO_2$ and PNVC films shows that the PNVC@$ZrO_2$ film exhibits higher protection performance than the unreinforced PNVC coating. Positive shift in the $E_{corr}$ of the polymer film coated electrodes reveals that these coatings have anodic protection effect and provide corrosion protection by suppressing anodic dissolution of stainless steel.

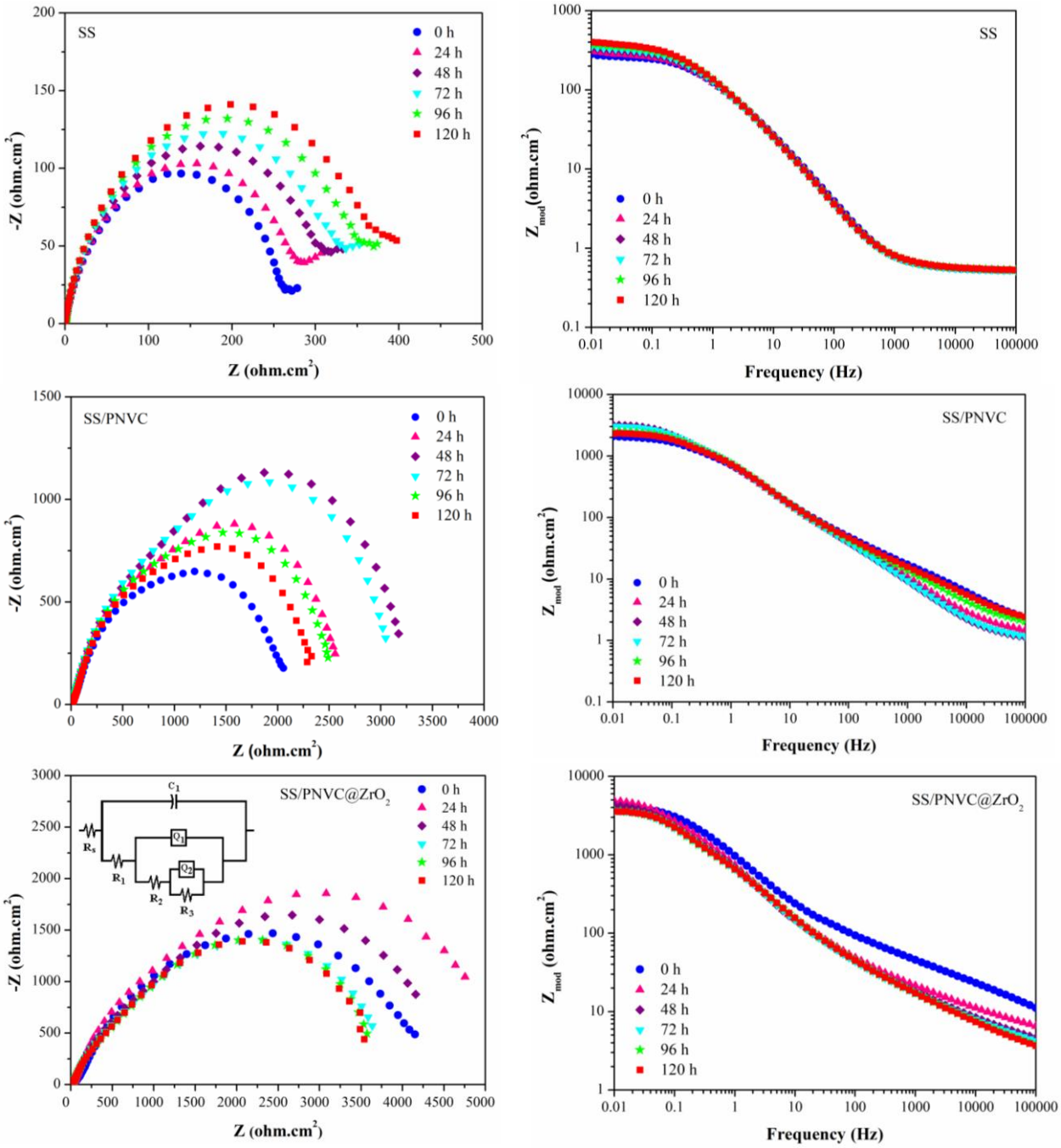


**Figure 7.** Nyquist and Bode plots of uncoated, PNVC coated and PNVC@$ZrO_2$ coated SS electrodes

Time dependant Nyquist and Bode impedance plots of SS, SS/PNVC and SS/PNVC@$ZrO_2$ electrodes were shown in Figure 7. The Nyquist plots consist of semicircular capacitive loops indicating electron transfer controlled corrosion process and the charge transfer resistance ($R_{ct}$) values can be obtained from the diameters of the semicircles (Bazzaoui et al., 2014). These $R_{ct}$ values can be also determined from the plateau region at low frequencies in Bode diagrams, while another plateau region at the high frequencies are related to solution resistance ($R_s$) (Tüken et al., 2006). Another way to reach detailed impedance data is equivalent circuit analysis. Impedance spectra of SS/PNVC@$ZrO_2$ electrode were analyzed using R(C(R(Q(R(QR))))) equivalent circuit model shown in the inset of Figure 7 and all of the impedance parameters were listed in Table 3. In this circuit model, $R_s$ shows electrolyte resistance, $R_1$ denotes to film resistance, $R_2$ corresponds to resistance of passive oxide film, $R_3$ reflects charge transfer resistance of metal. C shows capacitor of polymer film/solution interface, $Q_1$ represents to constant phase element of passive film/solution interface, $Q_2$ ascribes the constant phase element of metal/solution interface.

**Table 3.** Impedance parameters obtained from R(C(R(Q(R(QR))))) equivalent circuit analysis of the SS/PNVC@$ZrO_2$ electrode

| t (h) | $R_s$ (ohm.cm$^2$) | C (F/cm$^2$) | $R_1$ (ohm.cm$^2$) | $Q_1$ (SS$^{-n}$/cm$^2$) | n | $R_2$ (ohm.cm$^2$) | $Q_2$ (SS$^{-n}$/cm$^2$) | n | $R_3$ (ohm.cm$^2$) | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 6.65 | $2.38\times10^{-7}$ | 13.44 | $9.87\times10^{-5}$ | 0.64 | 103.4 | $1.59\times10^{-4}$ | 0.75 | 4528 | $1.60\times10^{-3}$ |
| 24 | 5.10 | $6.45\times10^{-7}$ | 4.98 | $1.67\times10^{-4}$ | 0.66 | 47.05 | $1.96\times10^{-4}$ | 0.74 | 5759 | $9.90\times10^{-4}$ |
| 48 | 3.45 | $7.70\times10^{-7}$ | 3.46 | $1.98\times10^{-4}$ | 0.65 | 38.81 | $2.22\times10^{-4}$ | 0.70 | 5227 | $1.03\times10^{-3}$ |
| 72 | 3.22 | $8.52\times10^{-7}$ | 3.49 | $1.71\times10^{-4}$ | 0.66 | 35.80 | $2.41\times10^{-4}$ | 0.69 | 4389 | $1.07\times10^{-3}$ |
| 96 | 2.77 | $9.04\times10^{-7}$ | 3.27 | $1.69\times10^{-4}$ | 0.66 | 34.96 | $2.50\times10^{-4}$ | 0.68 | 4403 | $1.56\times10^{-3}$ |
| 120 | 2.74 | $9.31\times10^{-7}$ | 3.34 | $1.62\times10^{-4}$ | 0.67 | 35.54 | $2.49\times10^{-4}$ | 0.68 | 4335 | $1.53\times10^{-3}$ |

$R_{ct}$ values of SS and SS/PNVC electrodes were determined from the Bode plots and given in Table 4. $R_{ct}$ values of polymer coated SS electrodes are higher than that of the uncoated SS for the same immersion time. This case is attributed to protective property of the polymer films. As it is seen from Figure 7 and Table 4, $R_{ct}$ values of the SS/PNVC@$ZrO_2$ electrode are also higher than that of the SS/PNVC electrode and this case indicates that the $ZrO_2$ reinforced PNVC coating provides better protection to the SS. $R_{ct}$ values of uncoated SS increase with time due to passivation of stainless steel in $H_2SO_4$ medium. Although this increase, $R_{ct}$ values are not as high as polymer coated SS electrodes. $R_{ct}$ values of SS/PNVC and SS/PNVC@$ZrO_2$ electrodes increase at the beginning of immersion time and then start to decrease due to deterioration of polymer film with time. At the end of 120 h, higher $R_{ct}$ value of SS/PNVC@$ZrO_2$ indicates much better corrosion protective property of zirconia reinforced poly(*N*-vinyl) carbazole film.

**Table 4.** Comparison of charge transfer resistance ($R_{ct}$) values of the SS, SS/PNVC and SS/PNVC@$ZrO_2$ electrodes

| Time (h) | $R_{ct}$ (ohm.cm$^2$) | | |
|---|---|---|---|
| | SS | SS/PNVC | SS/PNVC@$ZrO_2$ |
| 0 | 279 | 2077 | 4528 |
| 24 | 310 | 2582 | 5759 |
| 48 | 339 | 3209 | 5227 |
| 72 | 358 | 3067 | 4389 |
| 96 | 378 | 2490 | 4403 |
| 120 | 406 | 2358 | 4335 |

Protection efficiency ($\eta$) of the PNVC@$ZrO_2$ films can be calculated by using Equations (1-2) given below:

$$\eta_{PP}(\%) = [i^{o}_{corr} - i_{corr} / i^{o}_{corr}] \times 100 \quad (1)$$

$$\eta_{EIS}(\%) = [R_{ct} - R^{o}_{ct} / R_{ct}] \times 100 \quad (2)$$

In these equations $i^{o}_{corr}$ and $i_{corr}$ refer to corrosion current densities of the uncoated and polymer coated SS, while $R_{ct}^{o}$ and $R_{ct}$ denote charge transfer resistance of uncoated and polymer coated SS electrodes, respectively. When the protection efficiency of the PNVC@$ZrO_2$ coatings is calculated it can be found that the PNVC@$ZrO_2$ coating provides 93% protection to SS according to both of the PP and EIS results. These results showed that, there is an agreement between the protection efficiency values calculated from two different experimental methods.

## CONCLUSION

PNVC@$ZrO_2$ films were electrochemically synthesized on 304-SS surface by cyclic voltammetry. ATR-FTIR and SEM analyses confirmed the electrodeposition of PNVC@$ZrO_2$. $E_{ocp}$-time curves, PP and EIS methods revealed that the PNVC@$ZrO_2$ film exhibited better corrosion protection than the PNVC and 93% protection efficiency was observed against corrosion of SS in 1 M $H_2SO_4$ media and these films can be proposed as alternative coatings for the protection of 304 SS in 1 M $H_2SO_4$ corrosive environment.

## ACKNOWLEDGEMENTS

The authors thank to Eskişehir Osmangazi University for providing research facilities.

## REFERENCES

Bazzaoui M, Martins JI, Bazzaoui EA, Albourine A, Martins, L. 2014. Corrosion protection of stainless steel plates in fuel cells environment by conducting polymers. Materials and Corrosion, 65; 67-75.

Carragher U, Breslin CB. 2018. Polypyrrole doped with dodecylbenzene sulfonate as a protective coating for copper, Electrochimica Acta, 291; 362-372.

Demirel S, Bozkurt B. 2022. Coating of PANI/$SnO_2$, PANI/ZnO polymeric nanocomposite on 304 L stainless steel by galvanostatic method and investigation of corrosion behaviour. Protection of Metals and Physical Chemistry of Surfaces, 58; 53-64.

Duran B, Cakmakcı İ, Bereket G. 2013. Role of supporting electrolyte on the corrosion performance of poly (carbazole) films deposited on stainless steel. Corrosion Science, 77; 194-201.

Duran B, Ünver İÇ, Bereket G. 2017. Inhibition of steel corrosion by potentiodynamic deposition of poly(N-methyl carbazole). Journal of Adhesion Science and Technology, 31; 1467-1479.

Duran B, Şimşek E, Poly(N-vinyl carbazole)-$TiO_2$ composite coating for protection of steel, Journal of Adhesion Science and Technology, In press.

Elangovan N, Pugalmani S, Srinivasan A, Rajendiran N, Rajendran N. 2018. Synthesis and anticorrosive properties of novel PVK-$ZrO_2$ nano composite coatings on steel-substrate, E-Journal of Surface Science and Nanotechnology, 16; 5-13.

Frau AF, Pernites RB, Advincula RC. 2010. A conjugated polymer network approach to anticorrosion coatings: poly(vinylcarbazole) electrodeposition. Industrial & Engineering Chemistry Reserach, 49; 9789-9797.

Garcia-Cabezon C, Salvo-Comino C, Garcia-Hernandez C, Rodriguez-Mendez ML, Martin-Pedrosa F. 2020. Nanocomposites of conductive polymers and nanoparticles deposited on porous material as a strategy to improve its corrosion resistance, Surface and Coatings Technology, 403; 126395.

Mahmoudian MR, Basirun WJ, Alias Y. 2011. Synthesis and characterization of poly(N-methylpyrrole)/$TiO_2$ composites on steel. Applied Surface Science, 257; 3702-3708.

Tüken T, Yazıcı B, Erbil M. 2006. The use of polyindole for prevention of copper corrosion. Surface and Coatings Technology, 200; 4802-4809.

## *ORAL PRESENTATION*

# Estimation of Total Flavonoid, Antioxidant and Lung Protective Activity of *Plantago* ethanolic Extract on Albino Male Mice

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## Abstract

The impartial of this research was to determine whole flavonoid content (TFC) based on flavonoid-aluminum chloride ($AlCl_3$) and antioxidant action by spectrophotometric method and resolve of lung protective ability of *plantago* plant. One of these plant *plantago*. Results indicated that *plantago* possesses great flavonoid contents which was (281± 0.210 µg/ml) in addition to incredible antioxidant potential in a concentration dependent manner (ranged from 0.150 to 0.538 for 0.02 and 0.64 mg/ml respectively for reductive ability and possessed lung protective ability by counteract damage induced by $CCL_4$ treated albino male mice.

**Key words**: *plantago*, antioxidant, mice, flavonoid

## Introduction

Traditionally, consuming the medicinal plants for therapeutic of respiratory diseases for many years ago (1). Investigators have been informed that ginger plants could be active in treating asthma. Plantain distributed all completed the planet and is one of the most plentiful accessible medicinal herbs (2). Plantago lanceolata L. (Plantaginaceae) is a recurrent plant species with a worldwide distribution . It has many bioactive composites containing allantoin, aucubin, ursolic acid, flavonoids, and asperuloside.

Many technical studies also report that the plantain extract has a broad variety of biologic effects including "damage therapy action, anti-inflammatory, analgesic, antioxidant, weak antibiotic, immunomodulating, and anti-ulcerogenic activity" (3). Iridoid Glycosides are a class of monoterpene-developed combinations that have been documented in over 50 plant families (4). The major Iridoid Glycosides found in P. lanceolata are catalpol and its precursor aucubin (5).

The leaf was conventionally used to cure injuries, including respiratory infections combined with fever. This potted plant contains of phenol mixtures and has antioxidant estate (6). The valuable within cure of the soreness of the higher site of respiratory system because of containing Tanen and Mucilage. (7). Plantago major,) along with certain polysaccharides enhances damage. Study gets indicated that can be helpful in the medication chronic bronchitis (8). Numerous healing belongings involving beneficial impacts on gastrointestinal, bloodline and respiratory (asthma and dyspnea) ailments consume been described for the *Plantago lanceolata* in (9). So it is used within to overpower coughs related to bronchitis and upper respiratory inflammation, to decrease skin inflammation, medication of injuries and equally a laxative (10).

## Methods

### Plant collection, identification and extraction

Plants aerial parts (leaves) were supplied from the local markets of Baghdad during Sep. / 2021 and recognized by Dr. Ibrahim S. Al-Jubouri, College of Pharmacy, and Al-Mustansiriyah University, Iraq. Briefly, after plant parts cleaned and dried at shade for a week. Then, via electrical grinder the plant grinding and stored in air tight container and maintained at room temperature until used. The method of (11) were adopted in which about 50 grams of the plant leaf were extracted with 80% methanol (vol: m250 ml) at 65°C for 3 hours using the soxhlet apparatus then dryness in a rotary evaporator to yield dried crude extract.

**Dose of *plantago* in mice**

In albino male mice. A dose of 400 mg/kg was tested depending on LD50 of *Plantago lanceolata* to 2,940 mg/kg.

**Research laboratory Animals**

Biotechnology Research Centre (Al-Nahrain University) supplied the animals (Albino male mice).

**Experimental Design**

Experimental designs were divided into two groups, as explained below, (4 animals per group)

- **Group I:** Mice were managed with *Plantago* ethanolic extract at dose of (400mg/kg).
- **Group II:** Mice were administrated with the $CCL_4$ (7th days) at dose of (40mg/kg).

**Group III:** Mice were administrated with $CCL_4$ (1st and 2ed days) + *Plantago* ethanolic extract (from 3rd to 7th day).

The mice were injected intraperitoneally (IP) as a single dose (0.1ml) per a day for 7 days using plant extract and sacrificed in day 8.

**Determination of Total Flavonoids**

Total flavonoids substance was determined using spectrophotometer in methanolic extract of plantago as rutin (as standard) followed method of (12). In this assay methanolic extract at weight (3.2 mg) was dissolved in 5 ml of 50% methanol, then 1 ml of a 5% (w/v) sodium nitrite and 1 ml of a 10% (w/v) aluminum chloride solution was added after 5 and 6 minutes respectively. Finally 10 ml of a 10% (w/v) NaOH solution was added. The mixture was completed to 50 ml with distilled water. The absorbance was measured at 450 nm with a spectrometer after 15 min. same procedure followed to create equation of the standard curve.

**Assessment of Anti-oxidant Activity *in vitro* (reductive ability)**

The method described by (11) was adopted, briefly 1 ml of different plant concentrations (from 0.02 to 0.64 mg/ml) mixed with 1ml of 0.2M phosphate buffer (pH 6.6) and 1.5 ml of 1% potassium ferricyanide, and then after incubation for 20 min at 50°C. 1ml of 10% TCA was added to the mixture and centrifuged for 10 minutes and 2.5 ml of the supernatant was mixed with 2 ml of distilled water and 0.5 ml of freshly prepared 1% Ferric chloride. After that, the absorbance was measured at 700nm. Trolox used as control by following same method to estimate the reductive ability.

**Lung Tissue Preparation for Histology Preparation**

Lung of mouse are set for histopathologically learning by way of termed (10). Sections remained stable in (10%) formalin about 24h, afterward dehydration through measured series of alcoholic liquid ranged from (30-100%) for (5) min apiece. Formerly the tasters cleared in xylene and paraffin wax. Cross sections of (5)µm fatness are ready and stained with hematoxylin (Harison) and eosin rendering to typical technique. (Ibrahim *et al*., 2017).

**Results**

**Total flavonoid of plantago**

Total flavonoids content in methanolic extract of plant was (281± 0.210 µg/ml) flavonoids.

**Reductive ability**

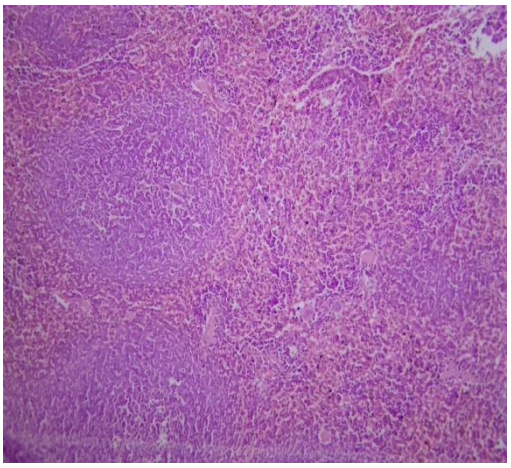
In all concentration tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml), plant possessed more reductive ability than trolox as sown in table 1 below (ranged from 0.150 to 0.538 for 0.02 and 0.64 mg/ml respectively).

**Table 1: Reductive ability of *plantago* ethanolic extract and trolox (vitamin E)**

| Concentration (mg/ml) | Reductive Ability Absorbance (Mean ± SD) | |
|---|---|---|
| | *H. triquetrifolium* Extract | Trolox (Vitamin E) |
| 0.02 | 0.150 ± 0.010 | 0.100 ± 0.001 |
| 0.04 | 0.292 ± 0.007 | 0.101 ± 0.001 |
| 0.08 | 0.351 ± 0.007 | 0.108 ± 0.001 |
| 0.16 | 0.460 ± 0.013 | 0.114 ± 0.004 |
| 0.32 | 0.488 ± 0.016 | 0.132 ± 0.007 |
| 0.64 | 0.538 ± 0.013 | 0.211 ± 0.015 |

**Lung histological section result**

In mice treated with **plant extract**, Section showing widening of white pulp and reduction of red pulp (H & E) (x40).figure 1



(Figure 1)

**The results** of mice treated with CCL4 indicated the ability to cause damage to organ tested. Histological examination of the CCL4 group showing interstitial lymphocytic inflammation and interstitial fibrosis and hyperplasia was also detected in the tissue (x200).figure 2

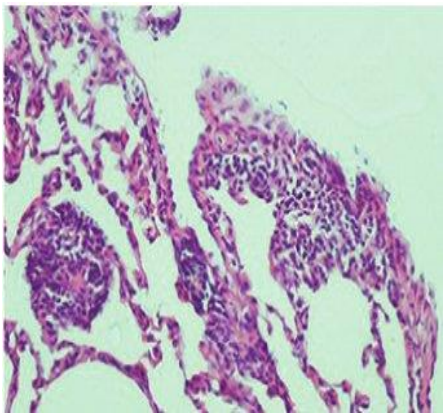


Figure 2

**The results** of interaction group Section of the lung showing thickening of alveolar septa with congestion, and mild inflammatory cells infiltration and destruction of alveolar septa forming the beginning of emphysema structure (H & E)(X40).figure 3

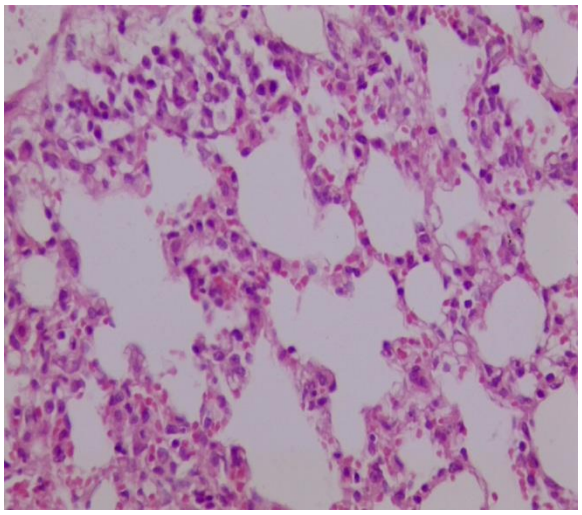


Figure 3

**Discussion**

Alcoholic extract of Plantago major is anti-phlogistic in carrageenan and pge1- stimulated in flammations in mice (13). Aceteoside, the most important phenyl ethanoid that prevents ara-chidonic acid stimulated mouse ear oedema (14). Therefor it can inhibit oedema in wound .Compounds such as the iiridoid glyciosides auccubin and catallpol, the aglycone aucubiigenin, and caffeiic acid products containing plant amajoside and acetonidethat have been remote from Plantago spp. and have established antimicrobial activity thus Plantago plant extracts can decline germ capability in wound site which is a suitable propensity to increase speed damage treatment.

P. major is one of the greatest plentiful and broadly distributed medicinal crops in the world. The dynamic chemical ingredients are aucubin (an anti-microbial agent), allantoin which stimulates cellular growth and tissue regeneration, and mucilage which reduces pain and discomfort (15). Composed, these components are supposed to provide plantain mild anti-inflammatory, antimicrobial, antihemorrhagic, and expectorant actions (16). In this research, in the group treated with the extract of plantago due to allantoin constituents, goblet cells in bronchioles were improved and regenerated. Additionally, due to mucilage compounds in plantago, the mice of lymphoid cells and nodules were small and decreased. Moreover, the thickness of epithelium was reduced and enhanced (17).

**References:**

Boskabady MH, Rakhshandah H, Afiat M, Aelami Z, Amiri S. 2006. Anti tussive effect of Plantago lanceolata in guinea pigs. Iran J Med Sci, 31: 143-146.
Pouraboli I, Hajizadeh S, Najafipour H, Khoshbaten A, Rasaei M. 2008. Does PGE2 mediate indomethacin and theophylline effects on joint diameter and vascular response to saphenous nerve stimulationin chronically inflamed rat knee joint. Physiol Pharmacol, 12: 68 – 75
Rahimian M. 2002. The study of the effect of inspiration of the extract of Darmaneh plant on the health and spirometric of asthema sickness. J med sci Yazd, 10: 20-25
Fatemi1 F, Allameh1 A, Khalafi H, Rezaei MB, Seyhoon M. 2010. The effect of essential oils and hydroalcoholic extract of caraway seed on oxidativestress parameters in rats suffering from acute lung inflammation before and after γ-irradiation. Iran J Med Aromatic Plant, 25: 44-49.
Fatemi1 F, Allameh1 A, Khalafi H, Rezaei MB, Seyhoon M. 2010. The effect of essential oils and hydroalcoholic extract of caraway seed on oxidativestress parameters in rats suffering from acute lung inflammation before and after γ-irradiation. Iran J Med Aromatic Plant, 25: 44-49.
Khosrou A, Sarvghadi F. 2006. The investigation of Hypogonadism and the level of the serum of free testostron in chemical injures who suffer from asthma which is the result of sulfur demostard. Military J Med Sci, 4: 975-979.
Sullivan P, Bekir S, Jaffar Z, Page C, Jeffery P, Costello J. 1994. Anti-inflammatory effects of low-dose oral theophylline in atopic asthma. Lancet.343:1006-1008.
Galvez M, Cordero MC, Cortes F, Ayus MY. 2003. Cytotixic effect of Plantago spp.on canceralllins. J Ethopharmacol, 88: 125-130.
Khosrou A, Sarvghadi F. 2006. The investigation of Hypogonadism and the level of the serum of free testostron in chemical injures who suffer from asthma which is the result of sulfur demostard. Military J Med Sci, 4: 975-979.
Sergejeva S, Ivanov S, Lotvall J, Lidn A. 2005. Interlukin-17 as a recruitment and survival factorfor airway macrophages in allergic airway inflammation. Am J Respir Cell Mol Biol, 33: 248-53.
Fua, W., Chena, J., Caia, Y., Leia, Y., Chenb, L., Peic, L., Zhoua, D., Lianga, X. and Ruana, J. (2010). Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch. et Sav.) Ching. J Ethnopharmacol., 130: 521-528.
Sakanaka, S., Tachibana, Y. and Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinohacha). Food Chem., 89: 569-575.
Bartlett NW, Walton RP, Edwards MR, Aniscenko J, Caramori G, Zhu J, Glanville N, Choy KJ, Jourdan P, Burnet J, et al. 2008. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. Nat Med, 14: 199-204.
Asthmatic airways and induces human bronchial fibroblasts to produce cytokines. J Allergy Clin Immunol, 108: 430-438.
Habibipour S, Oswald TM, Zhang F, Joshi P, Zhou XC, Dorsett-Martin W, Lineaweaver WC (2003). Effect of sodium diphenylhydantion on skin wound healing in rats. Plast. Reconstr. Surg. 112:1620-1627.
Herold A, Cremer L, Calugaru A (2003). Hydroalcoholic plant extracts with anti-inflammatory activity. Roum. Arch. Microbiol. Immunol. 62:117-129.
Mohamednoruldin Dh Hazim, Ruqaya M. Al-Ezzy and Ghufran M. Ealan.(2021). In vivo immunoloprotective potentials of Plantago lanceolata aqueous extract on methotrexate induced albino male mice . International Journal of Science and Research Archive, 03(02), 168–174.
Ibrahim NA, Ibrahim RM. Cytarabine induced reproductive histopathological changes in albino male mice. Jornal of Biotechnology Research Center. 2017;11(1).6-12.

## ORAL PRESENTATION

# Colorimetric $H_2O_2$ Detection with Magnetic Cobalt-Ferrite Nanoparticles

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## Abstract

In this study, the magnetic cobalt-ferrite nanoparticles ($CoFe_2O_4$ NPs) were synthesized via successive chemical reduction, hydrothermal, and calcination methods. The characteristic properties of the synthesized $CoFe_2O_4$ NPs were proven by TEM, FT-IR, XRD, and BET surface area analyzer. Accordingly, the synthesized particles have spherical structure in nano-scale, cobalt-ferrite crystal phase, and meso-pores with 56.51 $m^2/g$ surface area. The magnetic property of $CoFe_2O_4$ NPs was determined with an external magnet, which provides the easy separation of the material from the media. Then, the synthesized $CoFe_2O_4$ NPs were utilized as peroxidase-like catalyst for the colorimetric $H_2O_2$ detection. The experimental data were collected by UV-vis spectrophotometer as absorbance differences at 655 nm wavelength between in presence and absence of the catalyst of $CoFe_2O_4$ NPs. The effects of various environmental conditions such as pH, acetate buffer:TMB volume ratio, catalyst concentration, temperature, contact time, TMB concentration, and $H_2O_2$ concentration were investigated. The optimum conditions were observed as pH=3.0, catalyst concentration 1.333 g/L, acetate buffer:TMB (v:v) volume ratio 2:1, temperature 20 ˚C, contact time 75 min, and TMB concentration 8.0 mM. The calibration line was plotted to determine the minimum detection limit (LOD) of $CoFe_2O_4$ NPs for the colorimetric $H_2O_2$ detection, and the LOD value was calculated as 0.3407 µM from the linear plot in the $H_2O_2$ concentration range of 0.003-1.0 mM with the high regression coefficient. On the other hand, the UV-vis spectrum scanning was carried out for the mixtures of TMB+acetate buffer, TMB+acetate buffer+$CoFe_2O_4$ NPs, TMB+acetate buffer+$H_2O_2$, and TMB+acetate buffer+$H_2O_2$+$CoFe_2O_4$ NPs. Accordingly, the maximum peak intensities at 655 and 880 nm were obtained in presence of TMB, acetate buffer, $H_2O_2$, and $CoFe_2O_4$ NPs in the media, which indicated that $H_2O_2$ could be detected colorimetrically via catalyzing the oxidation of TMB with $CoFe_2O_4$ NPs as the peroxidase-like catalyst.

**Keywords:** Cobalt-ferrite nanoparticles, colorimetric $H_2O_2$ detection, minimum detection limit, peroxidase-like catalyst.

## INTRODUCTION

Hydrogen peroxide ($H_2O_2$), as the reactive oxygen species, has been widely used in various areas, containing chemical reactions, sensing applications, environmental monitoring, pharmaceutical synthesis, and food analysis (Zhao et al., 2022a). Furthermore, it is a common byproduct produced in the cellular metabolism process and its level is associated to various diseases and cell damages in human body. It has been reported that human blood $H_2O_2$ levels range from a possible low of 0.25 µM to a probable normal range of 1.0–5.0 µM, and a high range of 30–50 µM in certain disease states or during chronic inflammation (Forman et al., 2016). The excessive $H_2O_2$ in the human body can cause the fragility of blood vessels, osteoporosis, Alzheimer's disease, autoimmune diseases, cardiovascular diseases, and cancer by attacking proteins and DNA (Zhao et al., 2022a). Therefore, the precise quantitative detection of $H_2O_2$ in complex biological systems like human blood is crucial to carry out clinical research and diagnosis. Several conventional methods such as electrochemistry, liquid chromatography, chemiluminescence, fluorescence, and titrimetric or colorimetric assays are presently being used for the quantitative detection of $H_2O_2$. Among these methods, colorimetric methods, especially the peroxidase nanozymes-based detection systems, are attracted much attention due to their some advantages such as simplicity, cost-effectiveness, and quick readable results by visual determination via the naked eye or convenient portable instruments (Lu et al., 2020). Nanozymes have been preferred instead of natural enzymes in the colorimetric detection systems because natural enzymes have some disadvantages like high cost, poor stability, and easy inactivation in harsh conditions, and so on although they show high catalytic activity and selectivity in various chemical reactions (Bian et al., 2019). In the literature, a variety of nano-based materials have been developed and utilized as peroxidase-like catalysts, containing metal oxide

nanomaterials, noble metal nanomaterials, carbon-based nanomaterials, spinel ferrites, layered double hydroxides, and metal organic frameworks-based nanomaterials (Yang et al., 2020). Among them, spinel ferrites ($MFe_2O_4$, M = Zn, Co, Ni, Cd, Mn) have attracted growing attention due to their outstanding chemical and physical properties such as high catalytic activity, good structural stability, tunable shape, size, and morphology, and especially being magnetic which provides easier separation from the medium for further recycling. Their catalytic activity can be modified by doping with different metal ions. Various spinel ferrite nanomaterials given in Table 1 have been utilized as peroxidase-like catalysts in colorimetric detection systems. The literature survey showed that Co and Cu-based spinel ferrite nanomaterials have been rarely used as peroxidase-like catalyst. Therefore, the applicability of the magnetic cobalt-ferrite nanoparticles ($CoFe_2O_4$ NPs) as peroxidase-like catalysts for the colorimetric $H_2O_2$ detection was investigated in the present work.

**Table 1.** The reported spinel ferrite nanomaterials used as peroxidase-like catalysts

| Peroxidase-like catalyst | Detection system | Reference |
|---|---|---|
| $MoS_2$-decorated $MgFe_2O_4$ nanocomposite | Colorimetric detection of $H_2O_2$ and glucose | (Zhang et al., 2018) |
| Porphyrin functionalized $ZnFe_2O_4$ hollow nanospheres | Colorimetric detection of $H_2O_2$ and glucose | (Bian et al., 2018) |
| Nickel ferrite/nickel oxide nanoparticles co-loaded three-dimensional reduced graphene oxide (3DRGO-$NiFe_2O_4$/NiO NPs) | Colorimetric detection of organophosphorus pesticides (Dichlorvos) | (Wei et al., 2020) |
| Pt deposited on magnetic $CoFe_2O_4$ nanoparticles | Colorimetric detection of dopamine | (He et al., 2020) |
| Copper ferrite nanoparticles loaded on reduced graphene oxide nanozymes | Colorimetric detection of chromium ions | (Yi et al., 2022) |
| CTAB@$MgFe_2O_4$ nanocomposite | Colorimetric detection of $H_2O_2$ and glucose | (Singh et al., 2021) |
| Bismuth ferrite oxide ($Bi_2Fe_4O_9$) nanoparticles | Colorimetric detection of dopamine | (Razavi et al., 2022) |
| $NiFe_2O_4$ nanoparticles | Colorimetric detection of 2,4-dichlorophenoxyacetic acid herbicide | (Omar and Jabbar, 2022) |
| Meso-tetrakis(4-chlorophenyl)porphyrin functionalized $CuFe_2O_4$/$SiO_2$ nanocomposites | Colorimetric detection of glucose | (Xu et al., 2019) |

## MATERIALS AND METHODS

### Synthesis and Characterization of $CoFe_2O_4$ NPs

20 mL of 0.1 M $FeCl_3.6H_2O$ and 20 mL of 0.05 M $Co(NO_3).4H_2O$ were mixed and stirred magnetically at 750 rpm for 30 min. 50 mL of 0.1 M $NaBH_4$ was added dropwise to the iron-cobalt containing mixture and as-prepared mixture was stirred magnetically at 1000 rpm for 2 h. The homogeneous mixture was transferred to the stainless steel autoclave and heated at 137 °C for 10 h. After the autoclave was allowed to cool naturally, the mixture was centrifuged at 3460 RCF for 15 min and the precipitation was washed with ethanol for several times, and dried at 60 ºC for 24 h in the stove. Then, the obtained particles were ground well and it was calcined at 500°C for 4 h (Navadeepthy et al., 2020). The final product was denoted as $CoFe_2O_4$ NPs and it was again well-ground and used for further studies. The characterization studies of $CoFe_2O_4$ NPs were carried out Transmission Electron Microscope (TEM), X-ray powder diffraction (XRD), Fourier Transform Infrared (FT-IR), and Brunauer-Emmett-Teller (BET) surface area analyzer.

### Colorimetric $H_2O_2$ Detection with $CoFe_2O_4$ NPs

The colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs is based on the oxidation of TMB. Accordingly, the desired volume of acetate buffer solution at the known pH and the desired volume of $H_2O_2$ solution at the known concentration were added sequentially onto the desired volume of TMB solution at the known concentration in an UV-vis spectrophotometer cuvette. Then, the desired concentration of $CoFe_2O_4$ NPs as peroxidase-like catalyst was added to the cuvette. The absorbance value of the mixture at 60$^{th}$ min was recorded with a UV-vis spectrophotometer at 655 nm wavelength and this absorbance value was called $A_1$. For the control experiment carried out in the absence of $CoFe_2O_4$ NPs, the absorbance value at 655 nm wavelength was also recorded and this absorbance value was called $A_0$. The absorbance differences ($A_1$-$A_0$) were calculated as the experimental results. These experiments were repeated for the different environmental conditions such as pH (3.0-7.0), acetate buffer:TMB volume ratio (1:1-1:4), $CoFe_2O_4$ NPs concentration (0.133-2.67 g/L), temperature (20-70 ºC), TMB concentration (0.1-10 mM), and $H_2O_2$ concentration (0.001-500 mM).

The calibration line was plotted in the linear $H_2O_2$ concentration range of 0.003-1.0 mM in order to quantitatively detect of $H_2O_2$ with $CoFe_2O_4$ NPs. For this purpose, 1500 µL acetate buffer solution (pH=3.0), 1500 µL of $H_2O_2$ solution at different concentrations, and 0.267 g/L $CoFe_2O_4$ NPs were added sequentially onto 750 µL of 1.0 mM TMB solution in an UV-vis spectrophotometer cuvette. After 60 min, the absorbance values of the solutions containing the oxidation products of TMB were recorded with a UV-vis spectrophotometer at 655 nm wavelength. The limit of detection (LOD= 3σ/s) was calculated according to the signal which is equivalent to 3 times the standard deviation of the blanks (Liu et al., 2021).

## RESULTS AND DISCUSSIONS

### Characterization of $CoFe_2O_4$ NPs

$CoFe_2O_4$ NPs could be actuated quickly by an external magnet, and they dispersed rapidly with a slight shaking once the external magnet was separated. The fast response of $CoFe_2O_4$ NPs to an external magnetic force showed that they could be removed without any equipment from the medium, making it appropriate for practical applications.

TEM analysis was conducted to understand the morphology and size distribution of $CoFe_2O_4$ NPs. TEM image given in Figure 1 (a) indicated that $CoFe_2O_4$ NPs were spherical in shape and their mean size was calculated as 12.22±2.76 nm by Image-J program. It was also seen in Figure 1 that larger-size nanoparticles with few agglomerations formed due to the magnetic nature of $CoFe_2O_4$ NPs.

The structure and phase purity of $CoFe_2O_4$ NPs were confirmed by XRD pattern given in Figure 1 (b). As shown in Figure 1 (b), $CoFe_2O_4$ NPs contained the main peaks of monophasic cubic $CoFe_2O_4$ with a spinel structure at 35.46°, 57.28°, and 62.61° that can be assigned to (311), (511), and (440), respectively. In the literature, the reported $CoFe_2O_4$ NPs synthesized by various methods including one-pot hydrothermal (Paul et al., 2016), co-precipitation (Aslibeiki et al., 2022; Vishwas et al., 2022), modified solvothermal (Kalam et al., 2018), modified Pechini (Cheraghi et al., 2021), facile hydrothermal (Reddy et al., 2015) have the same planes associated to the standard pattern of cubic spinel $CoFe_2O_4$ structure.

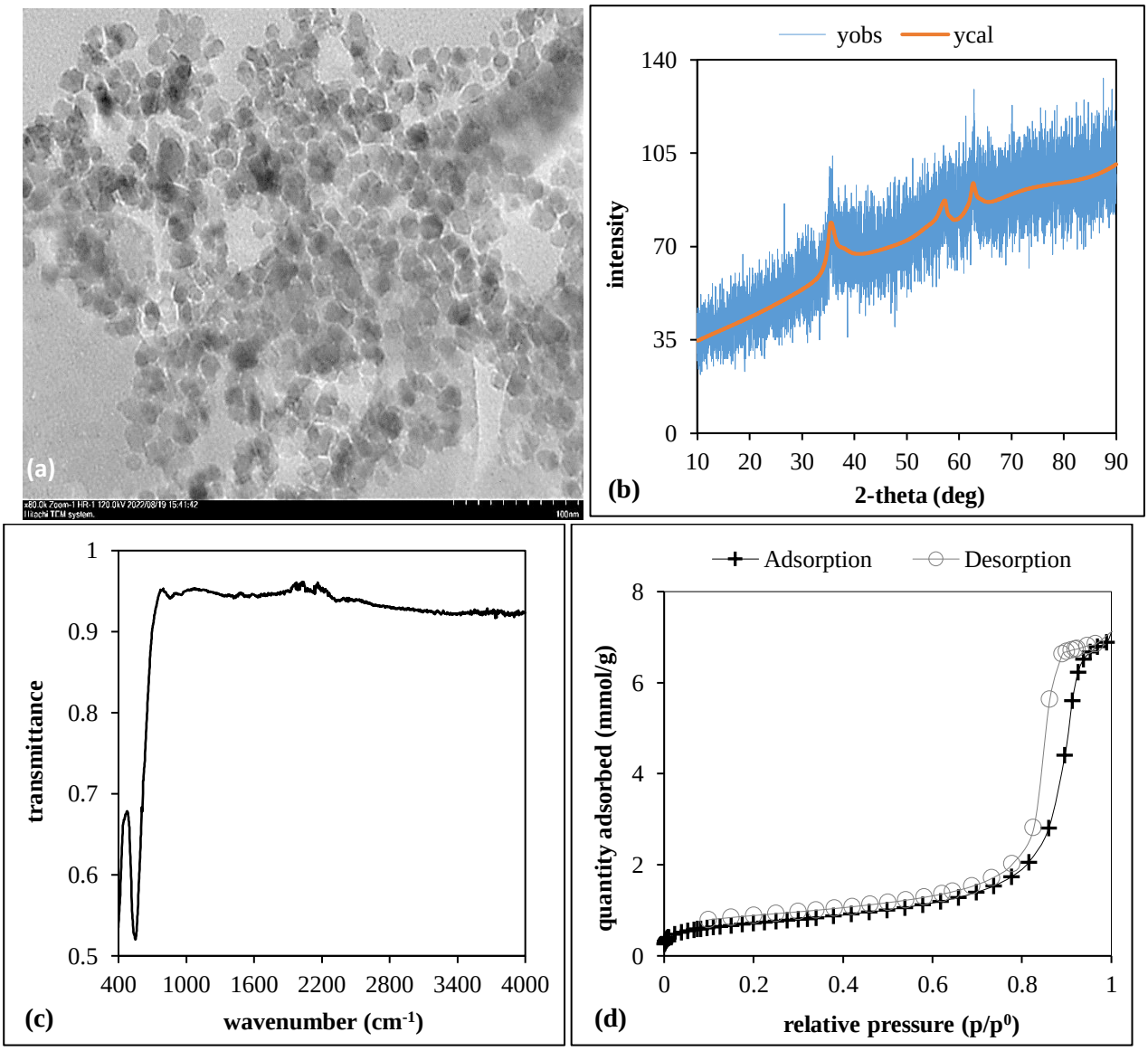


**Figure 1. (a)** TEM image of $CoFe_2O_4$ NPs, **(b)** XRD pattern of $CoFe_2O_4$ NPs, **(c)** FT-IR spectrum of $CoFe_2O_4$ NPs, **(d)** BET isotherm of $CoFe_2O_4$ NPs

FT-IR analysis was conducted in order to have knowledge of the identification of chemical functional groups present on $CoFe_2O_4$ NPs. From the FT-IR spectrum given in Figure 1 (c), it was observed that $CoFe_2O_4$ NPs contained characteristic peaks at 551 $cm^{-1}$ ($\upsilon_1$) related to the vibration of $Fe^{3+}$─$O^{2-}$ and 414 $cm^{-1}$ ($\upsilon_2$) related to the trivalent metal-oxygen vibration assigning to the tetrahedral coordination ($\upsilon_1$) and octahedral coordination ($\upsilon_2$) for the single-phase spinel structure (Makram Eyssa, 2015; Khizar et al., 2020; El-Masry and Ramadan, 2022).

The specific surface area ($S_{BET}$), pore width, and the nature of the porosity of $CoFe_2O_4$ NPs were determined via BET surface area analysis. ($S_{BET}$) of $CoFe_2O_4$ NPs was found to be 56.51 $m^2/g$. As depicted in Figure 1 (d), $N_2$ adsorption–desorption isotherm of $CoFe_2O_4$ NPs could be classified as a type IV isotherm with H1 hysteresis loop, which confirmed the formation of mesoporous (2-50 nm) (Vijayalakshmi et al., 2020). BJH adsorption average pore width of 16.34 nm with 0.2296 $cm^3/g$ pore volume confirmed the BET isotherm results. $S_{BET}$ of $CoFe_2O_4$ NPs was compared with that of cobalt ferrite nanoparticles used in various application areas in the literature and the results were summarized in Table 2. The $S_{BET}$ of $CoFe_2O_4$ NPs synthesized in this work was found to be lesser than that of the previously reported cobalt ferrite nanoparticles. The formation of aggregated structures may be the reason for the reduction of the specific surface area.

**Table 2.** The BET specific surface area of various $CoFe_2O_4$ nanoparticles used in various application areas in the literature

| BET specific surface area ($S_{BET}$, $m^2/g$) | Application area | Reference |
|---|---|---|
| **199.00** | Congo Red and Methyl Orange adsorption | (Simonescu et al., 2021) |
| **140.60** | Electrode material for supercapacitor | (Lv et al., 2013) |
| **113.4** | U and Pb removal | (Rahimi et al., 2018) |
| **90.11** | Acetone sensor | (Zhang et al., 2019) |
| **89.15** | Removal of lead | (Qi et al., 2019) |
| **85.40** | - | (Reddy et al., 2015) |
| **82.80** | Adsorption of Methyl Orange and Methyl Blue | (Wu et al., 2016) |
| **76.00** | Photocatalyst for degradation of Methylene Blue | (Kalam et al., 2018) |
| **75.30** | Supercapacitor | (Rani and Sahu, 2020) |
| **73.97** | - | (Qin et al., 2009) |
| **65.60** | Catalyst for epoxidation of styrene | (Liu et al., 2019) |
| **58.44** | Colorimetric $H_2O_2$ detection and catalytic oxidation of methylene blue | (Wu et al., 2018) |
| **56.51** | Colorimetric $H_2O_2$ detection | This work |
| **56.20** | Ethanol sensor | (Wei et al., 2022) |
| **50.40** | Removal of Zn (II) ions | (Asadi et al., 2020) |
| **49.77** | - | (Jiao et al., 2008) |
| **48.14** | Catalyst for Knoevenagel reaction | (Senapati et al., 2011) |
| **34.81** | Photocatalytic discoloration of aqueous methyl violet dye and energy storage applications | (Vijayalakshmi et al., 2020) |
| **16.42** | Photocatalyst for degradation of metronidazole, Methylene Blue, and Rhodamine B | (Cai-Yun et al., 2020) |
| **15.24** | Catalyst for oxidation of alcohols by periodic acid | (Paul et al., 2016) |

### Colorimetric $H_2O_2$ Detection with $CoFe_2O_4$ NPs

For the investigation of the mechanisms of the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs, the mixtures of TMB+acetate buffer, TMB+acetate buffer+$CoFe_2O_4$ NPs, TMB+acetate buffer+$H_2O_2$, and TMB+acetate buffer+$H_2O_2$+$CoFe_2O_4$ NPs were prepared and their spectrum scans between 500-1100 nm were recorded in the UV-vis spectrophotometer. According to the spectrum scans given in Figure 2, no significant peak could be obtained in the presence of only TMB and acetate buffer in the medium. In the case of adding $H_2O_2$ or $CoFe_2O_4$ NPs separately to the medium containing TMB and acetate buffer, it was observed that approximately the same intensity peaks were obtained at 655 and 880 nm wavelengths. The presence of TMB, acetate buffer, $H_2O_2$ as well as $CoFe_2O_4$ NPs in the medium caused a significant increase in the peak intensities at 655 and 880 nm. The peak at 655 nm belongs to the oxidized TMB (ox-TMB) product formed as a result of TMB oxidation. The increase in peak intensity at 655 nm as a result of the addition of $CoFe_2O_4$ NPs to the medium was realized by the catalysis of the oxidation of TMB with $CoFe_2O_4$ NPs.

The reaction steps predicted for the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs were presented in Figure 3. Accordingly, $H_2O_2$ decomposes with a Fenton-like reaction in the presence of $CoFe_2O_4$ NPs and forms OH• radicals, which cause the oxidation of the substrate of TMB. As a result of the reaction of OH• radicals with the substrate, TMB loses electrons to form a cation radical first, then the reaction ends with the formation of yellow diimine and blue colored oxidized TMB (oxTMB: charge transfer complex) (Wu et al., 2018). These predicted reaction steps are quite similar to the oxidation of TMB substrate as a result of the decomposition of $H_2O_2$ with horseradish peroxidase enzyme (HRP), a natural peroxidase enzyme, in the literature. Therefore, it was concluded that $CoFe_2O_4$ NPs could be evaluated as a peroxidase-like catalyst in the colorimetric $H_2O_2$ detection.

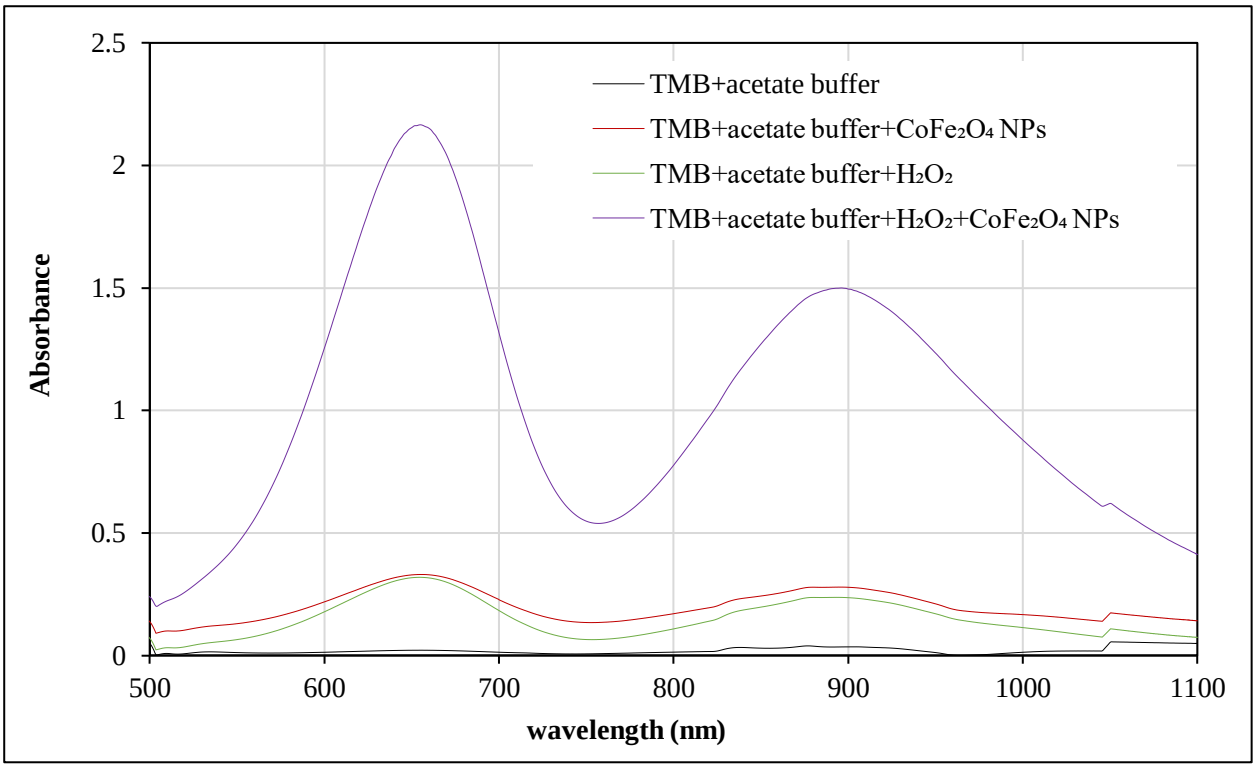


**Figure 2.** Spectrum scans of the prepared mixtures at optimum environmental conditions for the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs (Experimental conditions: 750 µL of 1.0 mM TMB, 1500 µL acetate buffer solution (pH=4.0), T=20 ºC, 1500 µL of 5.0 mM $H_2O_2$ solution, 0.267 g/L $CoFe_2O_4$ NPs

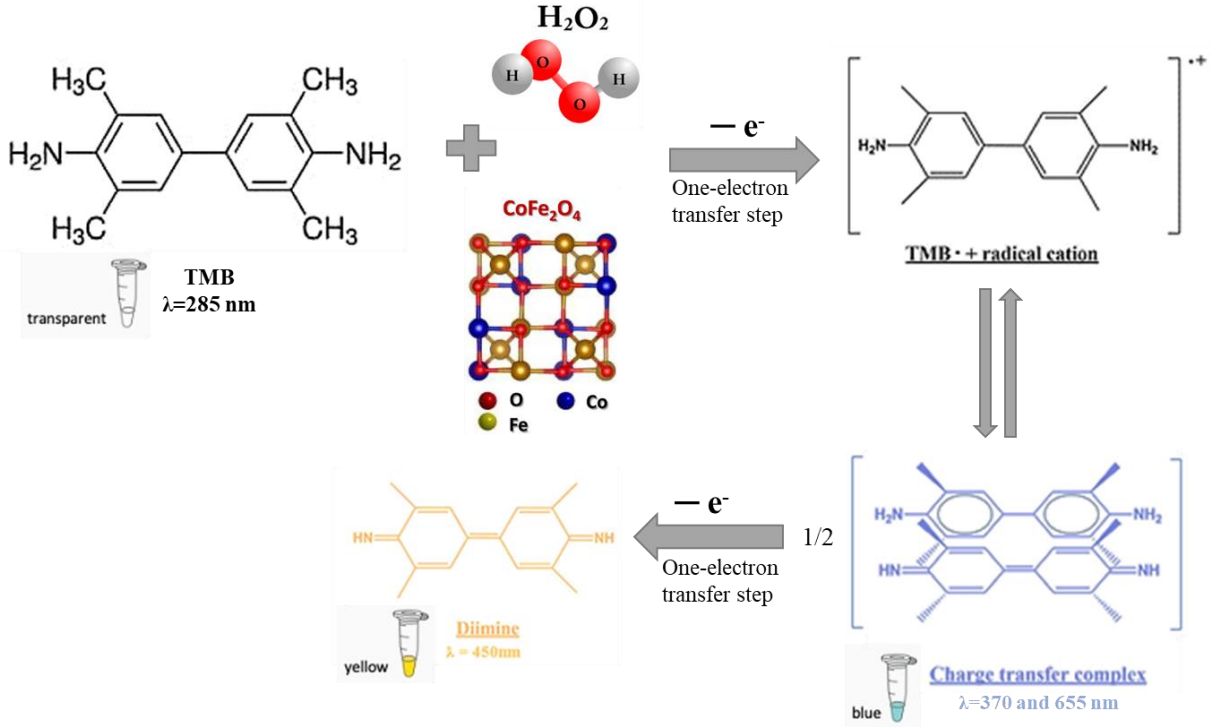


**Figure 3.** The predicted reaction steps for the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs

The effects of various environmental conditions such as pH (3.0-7.0), catalyst concentration (0.133-2.67 g/L), acetate buffer:TMB volume ratio (4:1-1:4), temperature (20-70 ºC), TMB concentration (0.1-10 mM), $H_2O_2$ concentration (0.0005-500 mM), and contact time (0-75 min) were investigated. The obtained data were plotted against various affecting parameters in Figure 4 (a)-(f).

For investigation of pH effect, 1500 µL acetate buffer solution at different pH values (pH=3.0-7.0), 1500 µL of 5.0 mM $H_2O_2$ solution, and 0.267 g/L of $CoFe_2O_4$ NPs were added sequentially into 750 µL of 1.0 mM TMB solution in an UV-vis spectrophotometer cuvette. The absorbance values of the solutions containing the oxidation products of TMB were recorded with an UV-vis spectrophotometer at 655 nm wavelength. Figure 4

(a) showed the effect of pH on the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs and it was seen that the absorbance values decreased with increasing pH. It has been reported in the literature that the colorimetric $H_2O_2$ detection with peroxidase-like catalysts is achieved by a Fenton-like reaction. It is known that in Fenton reactions, hydroxyl radicals (OH●) are formed more efficiently under strongly acidic conditions and the activity of the catalyst decreases due to the decomposition of $H_2O_2$ into less reactive hydroperoxyl radicals ($HO_2$●) as the pH increases. Depending on this situation, it was determined that the activity of $CoFe_2O_4$ NPs catalyst decreased as the pH increased. As a result, the optimum pH was determined as 3.0 for the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs.

The effect of the catalyst concentration on the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs was investigated in the range of 0.133-2.67 g/L of $CoFe_2O_4$ NPs. For this purpose, 1500 µL of acetate buffer solution (pH=3.0), 1500 µL of 5.0 mM $H_2O_2$ solution, and different concentration of $CoFe_2O_4$ NPs were added sequentially into 750 µL of 1.0 mM TMB solution in an UV-vis spectrophotometer cuvette. The absorbance values of the solutions containing the oxidation products of TMB were recorded with an UV-vis spectrophotometer at 655 nm wavelength. The obtained absorbance values changing with catalyst concentration was given in Figure 4 (b) and it was observed that the absorbance values increased up to the catalyst concentration of 1.333 g/L and then remained nearly constant. Accordingly, the concentration of the oxTMB product increased due to the increase in the formed OH• radicals as the concentration of $CoFe_2O_4$ NPs increased. Thus, the optimum catalyst concentration was found to be 1.333 g/L.
The effect of acetate buffer:TMB volume ratio was investigated in the range of 4:1-1:4 by adjusting the volume ratios of acetate buffer and TMB as the total volume of 2250 µL. For this purpose, the desired volume of acetate buffer solution (pH=3.0), 1500 µL of 5.0 mM $H_2O_2$ solution, and 0.267 g/L of $CoFe_2O_4$ NPs were added sequentially into the desired volume of 1.0 mM TMB solution in UV-vis spectrophotometer cuvette. The absorbance values of the solutions containing the oxidation products of TMB were recorded with an UV-vis spectrophotometer at 655 nm wavelength. According to Figure 4 (c), the absorbance values increased with changing acetate buffer:TMB volume ratio from 4:1 to 2:1, and then they decreased dramatically. When the acetate buffer:TMB (v:v) ratio was 4:1, a lower absorbance value was obtained than that of 2:1, since the required pH value for TMB oxidation could not be achieved. With the change of the acetate buffer:TMB (v:v) ratio from 2:1 to 3:2, although the concentration of TMB in the medium increased, the concentration of $H_2O_2$ and $CoFe_2O_4$ NPs was constant; thus, no more OH• radicals were formed, and in this case, no more TMB oxidation occurred. Accordingly, the absorbance values obtained at the acetate buffer:TMB (v:v) ratios of 3:2 and 2:1 were quite close to each other because the product concentration formed as a result of the oxidation of TMB remained approximately constant. When the acetate buffer:TMB (v:v) ratio was lower than 3:2, it was thought that the reason for the decrease in absorbance values despite the increase in the concentration of TMB in the medium was due to the fact that the excess concentration of TMB in the medium reduced the activity of $CoFe_2O_4$ NPs. As a result, the optimum acetate buffer:TMB (v:v) volume ratio was determined as 2:1 for the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs.

For investigation of temperature effect, 1500 µL of acetate buffer solution (pH=3.0), 1500 µL of 5.0 mM $H_2O_2$ solution were added sequentially into 750 µL of 2.0 mM TMB solution in UV-vis spectrophotometer cuvette. The prepared solutions were placed in a water bath and heated to the desired temperature. Then, 0.267 g/L of $CoFe_2O_4$ NPs was added to the solution at the desired temperature. The absorbance values of the solutions containing the oxidation products of TMB were recorded with an UV-vis spectrophotometer at 655 nm wavelength. As seen in Figure 4 (d), the activity of $CoFe_2O_4$ NPs decreased with increasing temperature; thus, the optimum temperature was obtained to be 20 ºC. In addition, although the absorbance values decreased as the temperature increased, it was determined that the oxidation reaction of TMB was completed in a shorter time. The reason for this situation can be explained as follows: as the temperature increases, the kinetic energies of the molecules in the medium increase; thus, the number of collisions per unit time increases and the oxidation reaction of TMB takes place in a shorter time.

The effect of TMB concentration was investigated in the range of 0.1-10 mM. For this purpose, 1500 µL of acetate buffer solution (pH=3.0), 1500 µL of 5.0 mM $H_2O_2$ solution, and 0.267 g/L of $CoFe_2O_4$ NPs were added sequentially into 750 µL of TMB solution at different concentrations in an UV-vis spectrophotometer cuvette. The absorbance values of the solutions containing the oxidation products of TMB were recorded with an UV-vis spectrophotometer at 655 nm wavelength. As shown in Figure 4 (e), it was determined that the absorbance values increased with the increase of TMB concentration up to 8.0 mM, and then remained approximately constant. If there is a sufficient concentration of $H_2O_2$ and $CoFe_2O_4$ NPs in the environment, TMB oxidation and thus absorbance values increase as the concentration of TMB increases. Accordingly, the optimum TMB concentration was determined as 8.0 mM in the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs.

The effect of $H_2O_2$ concentration was investigated in the range of 0.0005-500 mM. For this purpose, 1500 µL of acetate buffer solution (pH=3.0), 1500 µL of $H_2O_2$ solution at different concentrations, and 0.267 g/L of $CoFe_2O_4$ NPs were added sequentially into 750 µL of 2.0 mM TMB solution at different concentrations in an UV-vis spectrophotometer cuvette. The absorbance values of the solutions containing the oxidation products of TMB were recorded with an UV-vis spectrophotometer at 655 nm wavelength. It was seen in Figure 4 (f) that the obtained absorbance values increased with the increase of $H_2O_2$ concentration up to 400 mM, and then remained approximately constant. It can be explained as follows; in the case of a sufficient concentration of $CoFe_2O_4$ NPs in the environment, as the $H_2O_2$ concentration increased, more hydroxyl radicals (OH●) were formed in the environment and these radicals increased TMB oxidation and thus increased the absorbance values.

The calibration line for the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs was created by using the linear part of the curve in Figure 4 (f). Accordingly, a linear graph with high $R^2$ value was obtained in the range of 0.003-1.0 mM $H_2O_2$ concentration. The resulting line equation is A = 1.0641 x $C_{H_2O_2}$ (mM) + 0.2389. The intercept value of the line equation is due to the absorbance value of the control solution. For the control solution, 750 µL of 2.0 mM TMB, 1500 µL of acetate buffer solution (pH=3.0), 1500 µL of distilled water, and 0.267 g/L of $CoFe_2O_4$ NPs were mixed in an UV-vis spectrophotometer cuvette. 20 control solutions were prepared in the same composition and absorbance values were recorded at 655 nm. The standard deviation value (σ) of the control solution from the obtained absorbance values was calculated as 0.000121. The minimum detection limit (LOD=3σ/s) of $CoFe_2O_4$ NPs was calculated as 0.3407 µM using the slope of the calibration line and the standard deviation value of the control solution. A literature review was conducted to compare the linear detection range and the minimum detection limit of the colorimetric $H_2O_2$ detection process, and the results were summarized in Table 3. As can be seen in Table 3, the minimum colorimetric $H_2O_2$ detection limit of $CoFe_2O_4$ NPs was at a level that could compete with many peroxidase-like catalysts in the literature, and $CoFe_2O_4$ NPs had a relatively wide range of linear detection limit. Due to these properties, it was concluded that the $CoFe_2O_4$ NPs synthesized in this study could be utilized as an effective peroxidase-like catalyst in the colorimetric $H_2O_2$ detection in the presence of TMB.

**Table 3.** Linear ranges and minimum detection limits of various reported peroxidase-like catalysts for the colorimetric $H_2O_2$ detection process

| Peroxidase-like catalyst | LOD (µM) | Linear range | Reference |
|---|---|---|---|
| **$Fe_3O_4$@*Musa paradisiaca* L. nanoparticles** | 0.008 | 0.01 µM–0.9 mM | (Rasheed et al., 2022) |
| **Ferrous-metal organic framework** | 0.01804 | 40–5000 nM | (Huang et al., 2023) |
| **Cu-based MOFs** | 0.097 | 0.5–200 µM | (Chai et al., 2022) |
| **Hemin-$Fe_3O_4$@polypyrrole nanocomposite** | 0.18 | 0.2–100 µM | (Yang et al., 2021) |
| **$CoFe_2O_4$ NPs** | 0.3407 | 0.003-1.0 mM | This study |
| **$Co_xFe_{3-x}O_4$ nanocubes** | 0.36 | 1.0-60 µM | (Yang et al., 2014) |
| **CoFe layered double hydroxide nanoplates** | 0.40 | 1.0–20 mM | (Zhang et al., 2012) |
| **Fe/$FeS_2$@C nanoparticles** | 0.42 | 2–200 µM<br>200–1000 µM | (Zhao et al., 2022b) |
| **Reduced $Co_3O_4$ nanocomposite** | 0.43 | 1–30 µM | (Lu et al., 2020) |
| **Porphyrin modified $ZnFe_2O_4$/reduced graphene oxide** | 0.54 | 0.7–30 µM | (Bian et al., 2019) |
| **$Co_{0.5}Ni_{0.5}Fe_2O_4$-montmorillonite** | 0.5650 | 0.5-20 µM | (Nfuipe et al., 2022) |
| **FeS nanoparticles embedded in 2D carbon nanosheets** | 0.78 | 1.0–70 µM | (Song et al., 2022) |
| **Co/CuS-Montmorillonite nanocomposites** | 2.2 | 10–100 µM | (Wu et al., 2020) |
| **CoFe-LDH nanosheets/$CeO_2$** | 3.0 | 0.01–1.0 mM | (Yang et al., 2020) |
| **MOF(Co/2Fe)** | 5.0 | 10–100 µM | (Yang et al., 2017) |
| **Porous $CoFe_2O_4$** | 20 | 0.02-6.0 mM | (Wu et al., 2018) |

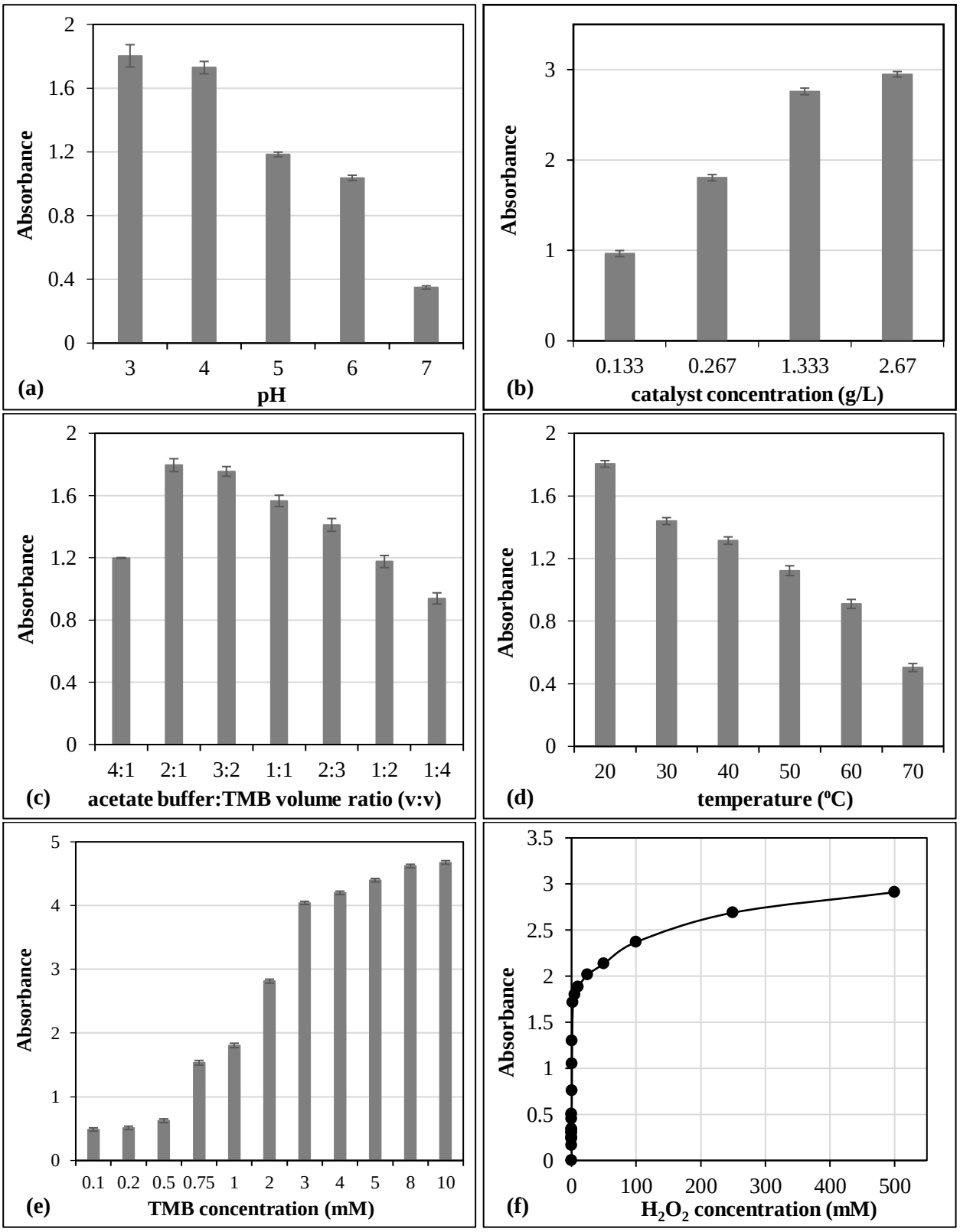


**Figure 4.** The effect of environmental conditions on the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs **(a)** pH, **(b)** catalyst concentration, **(c)** acetate buffer:TMB volume ratio, **(d)** temperature, **(e)** TMB concentration, **(f)** $H_2O_2$ concentration

The optimum environmental conditions of the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs was found to pH=3.0, the catalyst concentration of 1.333 g/L, the acetate buffer:TMB volume ratio of 2:1, the temperature of 20 ºC, and the TMB concentration of 8.0 mM.

An experiment was carried out to examine the effect of contact time under optimum environmental conditions and the absorbance values obtained over time were presented in Figure 5. As seen in Figure 5, it was determined that the absorbance values increased up to the 45$^{th}$ minute and then remained approximately

constant at the optimum conditions. It was concluded that the oxidation reaction of TMB completed in 45 minutes under the optimum environmental conditions for the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs.

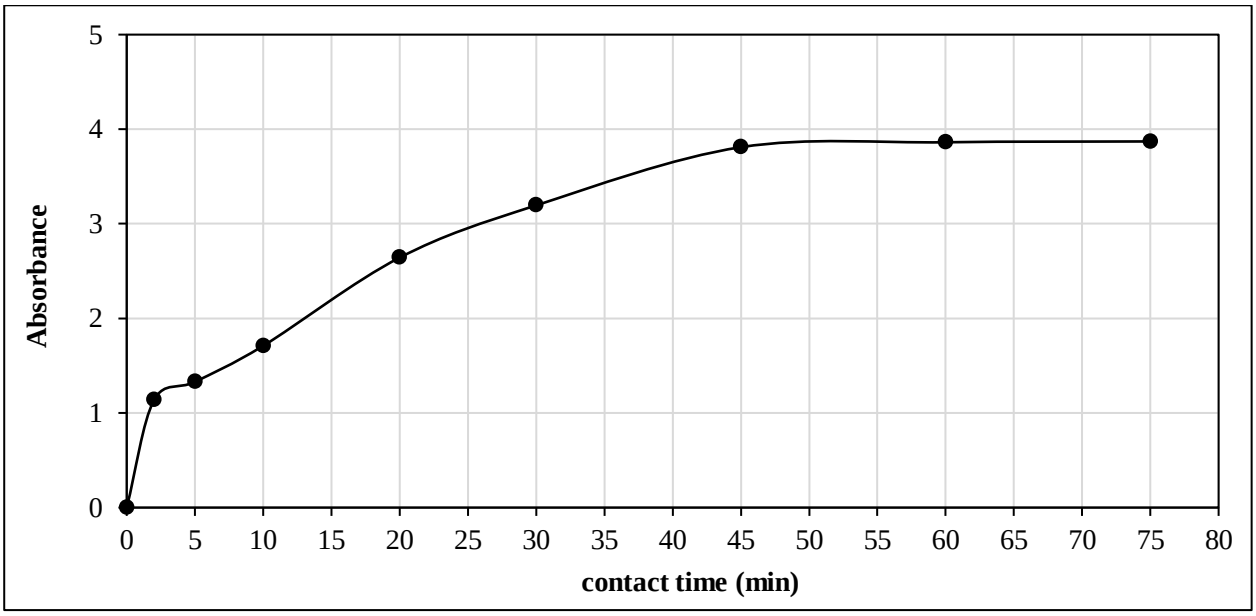


**Figure 5.** The effect of contact time on the colorimetric $H_2O_2$ detection with $CoFe_2O_4$ NPs (Experimental conditions: 750 µL of 8.0 mM TMB, 1500 µL acetate buffer solution (pH=3.0), T=20 ºC, 500 µL of 5.0 mM $H_2O_2$ solution, 1.333 g/L $CoFe_2O_4$ NPs)

## CONCLUSION

In this study, magnetic $CoFe_2O_4$ nanoparticles were synthesized by three-consecutive-step methods of co-reduction, hydrothermal, and calcination. The characterization studies confirmed that the synthesized material had spherical structure in nano-scale, cobalt-ferrite crystal phase, and meso-pores with 56.51 $m^2/g$ surface area. The study showed that as-prepared $CoFe_2O_4$ NPs exhibited good peroxidase-like activity for the colorimetric $H_2O_2$ detection. According to the predicted detection steps, $CoFe_2O_4$ NPs catalyzes the oxidation of TMB in the presence of $H_2O_2$ with a color change from colorless to blue. This peroxidase-like activity of $CoFe_2O_4$ NPs was resulted from the catalytic decomposition of $H_2O_2$ into hydroxyl radicals (OH●). The minimum detection limit of $CoFe_2O_4$ NPs was found to be 0.3407 µM, which was relatively lower than some reported peroxidase-like catalyst. Thus, it could be concluded that $CoFe_2O_4$ NPs can be utilized as a promising candidate of peroxidase mimics to be used in nanomaterials-based areas of colorimetric detection.

## REFERENCES

Asadi R, Abdollahi H, Gharabaghi M, Boroumand Z 2020. Effective removal of Zn (II) ions from aqueous solution by the magnetic MnFe2O4 and CoFe2O4 spinel ferrite nanoparticles with focuses on synthesis, characterization, adsorption, and desorption. Advanced Powder Technology, 31: 1480–1489.

Aslibeiki B, Eskandarzadeh N, Jalili H, Ghotbi Varzaneh A, Kameli P, Orue I, Chernenko V, Hajalilou A, Ferreira LP, Cruz MM 2022. Magnetic hyperthermia properties of CoFe2O4 nanoparticles: Effect of polymer coating and interparticle interactions. Ceramics International, 48: 27995–28005.

Bian B, Liu Q, Yu S 2018. Enhanced peroxidase-like activity of porphyrin functionalized ZnFe2O4 hollow nanospheres for rapid detection of H2O2 and glucose. New Journal of Chemistry, 42: 18189–18200.

Bian B, Liu Q, Yu S 2019. Peroxidase mimetic activity of porphyrin modified ZnFe2O4/reduced graphene oxide and its application for colorimetric detection of H2O2 and glutathione. Colloids and Surfaces B: Biointerfaces, 181: 567–575.

Cai-Yun L, Zheng-Feng Y, Paul B 2020. Facile Synthesis of Spinel CoFe2O4 Nanoparticle and Its Application as Magnetic Recoverable Photocatalyst for Degradation of Metronidazole and Some Selected Organic Dyes.pdf. Journal of nanoscience and nanotechnology, 20: 1209–1214.

Chai J, Yuan L, Wang S, Li T, Wu M, Huang Z, Yin H 2022. A series of novel Cu-based MOFs: syntheses, structural diversity, catalytic properties and mimic peroxidase activity for colorimetric detection of

H2O2. New Journal of Chemistry, 46: 12372–12380.

Cheraghi A, Davar F, Homayoonfal M, Hojjati-Najafabadi A 2021. Effect of lemon juice on microstructure, phase changes, and magnetic performance of CoFe2O4 nanoparticles and their use on release of anti-cancer drugs. Ceramics International, 47: 20210–20219.

El-Masry MM, Ramadan R 2022. The effect of CoFe2O4, CuFe2O4 and Cu/CoFe2O4 nanoparticles on the optical properties and piezoelectric response of the PVDF polymer. Applied Physics A: Materials Science and Processing, 128: 1–13.

Forman HJ, Bernardo A, Davies KJA 2016. What is the concentration of hydrogen peroxide in blood and plasma? Archives of Biochemistry and Biophysics, 603: 48–53.

He F, Li W, Zhao F, Zhu X, Liu Q, Liu Z, Zhang X, Zhang X 2020. Pt deposited on magnetic CoFe2O4 nanoparticles: Double enzyme-like activity, catalytic mechanism and fast colorimetric sensing of dopamine. Microchemical Journal, 158: 105264-105273.

Huang P, Chang Q, Jiang G, Wang X, Zhu H, Liu Q 2023. Rapidly and ultra-sensitive colorimetric detection of H2O2 and glucose based on ferrous-metal organic framework with enhanced peroxidase-mimicking activity. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 285: 121943-121952.

Jiao Z, Geng X, Wu M, Jiang Y, Zhao B 2008. Preparation of CoFe2O4 nanoparticles by spraying co-precipitation and structure characterization. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 313–314: 31–34.

Kalam A, Al-Sehemi AG, Assiri M, Du G, Ahmad T, Ahmad I, Pannipara M 2018. Modified solvothermal synthesis of cobalt ferrite (CoFe2O4) magnetic nanoparticles photocatalysts for degradation of methylene blue with H2O2/visible light. Results in Physics, 8: 1046–1053.

Khizar S, Ahmad NM, Ahmed N, Manzoor S, Hamayun MA, Naseer N, Tenório MKL, Lebaz N, Elaissari A 2020. Aminodextran coated CoFe2O4 nanoparticles for combined magnetic resonance imaging and hyperthermia. Nanomaterials, 10: 1–16.

Liu, C., Im, S. H. YT 2021. Synthesis of Au–Cu Alloy Nanoparticles as Peroxidase Mimetics for H2O2 and Glucose Colorimetric Detection. Catalysts, 11: 343–355.

Liu J, Meng R, Li J, Jian P, Wang L, Jian R 2019. Achieving high-performance for catalytic epoxidation of styrene with uniform magnetically separable CoFe2O4 nanoparticles. Applied Catalysis B: Environmental, 254: 214–222.

Lu J, Zhang H, Li S, Guo S, Shen L, Zhou T, Zhong H, Wu L, Meng Q, Zhang Y 2020. Oxygen-Vacancy-Enhanced Peroxidase-like Activity of Reduced Co3O4 Nanocomposites for the Colorimetric Detection of H2O2 and Glucose. Inorganic Chemistry, 59: 3152–3159.

Lv L, Xu Q, Ding R, Qi L, Wang H 2013. Chemical synthesis of mesoporous CoFe2O4 nanoparticles as promising bifunctional electrode materials for supercapacitors. Materials Letters, 111: 35–38.

Makram Eyssa N 2015. Phase Formation and Crystallinity-Dependent Magnetic Parameters of Co1-xFe2+xO4 Nanoparticals. American Journal of Physics and Applications, 3: 33-40.

Navadeepthy D, Thangapandian M, Viswanathan C, Ponpandian N 2020. A nanocomposite of NiFe2O4-PANI as a duo active electrocatalyst toward the sensitive colorimetric and electrochemical sensing of ascorbic acid. Nanoscale Advances, 2: 3481–3493.

Nfuipe F, Jut IBT, Bewboubhft PXO, Ejtbewboubhft BOE 2022. In situ decorating the surface and interlayer of montmorillonite with Co0. 5Ni0.5Fe2O4 nanoparticles: A sustainable, biocompatible colorimetric platform for H2O2 and acetylcholine. Nano Research, 1: 1–8.

Omar NA, Jabbar HS 2022. NiFe2O4 nanoparticles as nanozymes , a new colorimetric probe for 2 , 4-dichlorophenoxyacetic acid herbicide detection. Inorganic Chemistry Communications, 146: 110104-110110.

Paul B, Purkayastha DD, Dhar SS 2016. One-pot hydrothermal synthesis and characterization of CoFe2O4 nanoparticles and its application as magnetically recoverable catalyst in oxidation of alcohols by periodic acid. Materials Chemistry and Physics, 181: 99–105.

Qi G, Ren H, Fan H, Liu Y 2019. Preparation of CoFe2O4 nanoparticles based on high-gravity technology and application for the removal of lead. Chemical Engineering Research and Design, 147: 520–528.

Qin R, Li F, Jiang W, Liu L 2009. Salt-assisted low temperature solid state synthesis of high surface area CoFe2O4 nanoparticles. Journal of Materials Science and Technology, 25: 69–72.

Rahimi Z, Sarafraz H, Alahyarizadeh G, Shirani AS 2018. Hydrothermal synthesis of magnetic CoFe2O4 nanoparticles and CoFe2O4/MWCNTs nanocomposites for U and Pb removal from aqueous solutions. Journal of Radioanalytical and Nuclear Chemistry, 317: 431–442.

Rani B, Sahu NK 2020. Electrochemical properties of CoFe2O4 nanoparticles and its rGO composite for supercapacitor. Diamond and Related Materials, 108: 107978-107988.

Rasheed Q, Ajab H, Farooq M, Shahzad SA, Yaqub A 2022. Fabrication of colorimetric sensor using Fe3O4 @ Musa paradisiaca L. nanoparticles for detecting hydrogen peroxide: an application in environmental and biological samples. Applied Nanoscience (Switzerland), 12: 2841–2855.

Razavi M, Barras A, Ifires M, Swaidan A, Khoshkam M, Szunerits S, Kompany-Zareh M, Boukherroub R 2022. Colorimetric assay for the detection of dopamine using bismuth ferrite oxide (Bi2Fe4O9) nanoparticles as an efficient peroxidase-mimic nanozyme. Journal of Colloid and Interface Science, 613: 384–395.

Reddy MP, Mohamed AMA, Zhou XB, Du S, Huang Q 2015. A facile hydrothermal synthesis, characterization and magnetic properties of mesoporous CoFe2O4 nanospheres. Journal of Magnetism and Magnetic Materials, 388: 40–44.

Senapati KK, Borgohain C, Phukan P 2011. Synthesis of highly stable CoFe2O4 nanoparticles and their use as magnetically separable catalyst for Knoevenagel reaction in aqueous medium. Journal of Molecular Catalysis A: Chemical, 339: 24–31.

Simonescu CM, Tătăruş A, Culiţă DC, Stănică N, Ionescu IA, Butoi B, Banici AM 2021. Comparative study of cofe2o4 nanoparticles and cofe2o4-chitosan composite for congo red and methyl orange removal by adsorption. Nanomaterials, 11: 1–24.

Singh E, Kaur M, Sharma S 2021. Structural tuning of CTAB@MgFe2O4 nanocomposite as peroxidase mimic for H2O2 and glucose sensing. Materials Chemistry and Physics, 271: 124851-124865.

Song C, Liu H, Zhang L, Wang J, Zhao C, Xu Q, Yao C 2022. FeS nanoparticles embedded in 2D carbon nanosheets as novel nanozymes with peroxidase-like activity for colorimetric and fluorescence assay of H2O2 and antioxidant capacity. Sensors and Actuators B: Chemical, 353: 131131-131139.

Vijayalakshmi S, Elaiyappillai E, Johnson PM, Lydia IS 2020. Multifunctional magnetic CoFe2O4 nanoparticles for the photocatalytic discoloration of aqueous methyl violet dye and energy storage applications. Journal of Materials Science: Materials in Electronics, 31: 10738–10749.

Vishwas M, Venkatesha Babu KR, Arjuna Gowda K V., Babu Gandla S 2022. Synthesis, characterization and photo-catalytic activity of magnetic CoFe2O4 nanoparticles prepared by temperature controlled co-precipitation method. Materials Today: Proceedings, 68: 497–501.

Wei K, Huai H-X, Zhao B, Zheng J, Gao G-Q, Zheng X-Y, Wang C-C 2022. Facile synthesis of CoFe2O4 nanoparticles and their gas sensing properties. Sensors and Actuators B: Chemical, 369: 132279-132288.

Wei Z, Li H, Wu J, Dong Y, Zhang H, Chen H, Ren C 2020. 3DRGO-NiFe2O4/NiO nanoparticles for fast and simple detection of organophosphorus pesticides. Chinese Chemical Letters, 31: 177–180.

Wu K, Li W, Zhao S, Chen W, Zhu X, Cui G, Liu Z, Liu Q, Zhang X, Zhang X 2020. Cobalt tuned copper sulfide on montmorillonite: Peroxidase-like activity, catalytic mechanism and colorimetric sensing of hydrogen peroxide. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 602: 125063-125070.

Wu L, Wan G, Hu N, He Z, Shi S, Suo Y, Wang K, Xu X, Tang Y, Wang G 2018. Synthesis of porous CoFe2O4 and its application as a peroxidase mimetic for colorimetric detection of H2O2 and organic pollutant degradation. Nanomaterials, 275: 155-162.

Wu X, Wang W, Li F, Khaimanov S, Tsidaeva N, Lahoubi M 2016. PEG-assisted hydrothermal synthesis of CoFe 2 O 4 nanoparticles with enhanced selective adsorption properties for different dyes. Applied Surface Science, 389: 1003–1011.

Xu Z, Lyu X, Yang B, Cao W, Li R, Zhang X, Zhang X, Fan G, Kong X, Liu Q 2019. Meso-tetrakis(4-chlorophenyl)porphyrin functionalized CuFe 2 O 4 /SiO 2 nanocomposites with enhanced peroxidase-like activity conveniently using for visual biosensing at room temperature. Colloids and Surfaces A:

Physicochemical and Engineering Aspects, 569: 28–34.

Yang H, Yang R, Zhang P, Qin Y, Chen T, Ye F 2017. A bimetallic (Co/2Fe) metal-organic framework with oxidase and peroxidase mimicking activity for colorimetric detection of hydrogen peroxide. Microchimica Acta, 184: 4629–4635.

Yang W, Hao J, Zhang Z, Lu B, Zhang B, Tang J 2014. CoxFe3-xO4 hierarchical nanocubes as peroxidase mimetics and their applications in H2O2 and glucose detection. RSC Advances, 4: 35500–35504.

Yang W, Li J, Yang J, Liu Y, Xu Z, Sun X, Wang F, Ng DHL 2020. Biomass-derived hierarchically porous CoFe-LDH/CeO2hybrid with peroxidase-like activity for colorimetric sensing of H2O2 and glucose. Journal of Alloys and Compounds, 815: 152276-152288.

Yang W, Weng C, Li X, He H, Fei J, Xu W, Yan X, Zhu W, Zhang H, Zhou X 2021. A sensitive colorimetric sensor based on one-pot preparation of h-Fe3O4@ppy with high peroxidase-like activity for determination of glutathione and H2O2. Sensors and Actuators, B: Chemical, 338: 129844-129853.

Yi W, Zhang P, Wang Y, Li Z, Guo Y, Liu M, Dong C, Li C 2022. Copper ferrite nanoparticles loaded on reduced graphene oxide nanozymes for the ultrasensitive colorimetric assay of chromium ions. Analytical Methods, 14: 3434–3443.

Zhang HJ, Liu LZ, Zhang XR, Zhang S, Meng FN 2019. Microwave-assisted solvothermal synthesis of shape-controlled CoFe2O4 nanoparticles for acetone sensor. Journal of Alloys and Compounds, 788: 1103–1112.

Zhang Y, Tian J, Liu S, Wang L, Qin X, Lu W, Chang G, Luo Y, Asiri AM, Al-Youbi AO, Sun X 2012. Novel application of CoFe layered double hydroxide nanoplates for colorimetric detection of H2O2 and glucose. Analyst, 137: 1325–1328.

Zhang Y, Zhou Z, Wen F, Tan J, Peng T, Luo B, Wang H, Yin S 2018. A flower-like MoS2-decorated MgFe2O4 nanocomposite: Mimicking peroxidase and colorimetric detection of H2O2 and glucose. Sensors and Actuators, B: Chemical, 275: 155–162.

Zhao C, Shi G-M, Shi F-N, Wang X-L, Li S-T 2022a. The synthesis and excellent peroxidase-like activity for the colorimetric detection of H2O2 of core-shell Fe/FeS2@C nanoparticles. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 642: 128612.

Zhao C, Shi GM, Shi FN, Wang XL, Li ST 2022b. The synthesis and excellent peroxidase-like activity for the colorimetric detection of H2O2 of core-shell Fe/FeS2@C nanoparticles. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 642: 128612.

*ORAL PRESENTATION*

# Identification of EST-SSR Markers in Safflower (*Carthamus tinctorius* L.) using EST Databases

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## Abstract

Oil plants have a very important place in animal and human nutrition in terms of protein, carbohydrates, minerals and fat content. Vegetable oils are one of the main nutrients in the nutrition ring that people should take in order to lead a healthier life. In addition, the need for vegetable oils, which is the core material of biodiesel and evaluated especially in the energy sector, is increasing its importance day by day. In this case, it is inevitable to increase the production of oil crops in all sustainable agricultural lands. Throughout the world, oil crops are cultivated in two ways as annual and perennial. Oil crops grown for one year; soybean, rapeseed, sunflower, sesame, safflower, peanut, flax and camelina, perennial plants such as olive, coconut, jojoba, cocoa, palm and avocado. Safflower is an oilseed plant grown in winter and summer, belonging to the genus Carthamus of the Asteraceae (Compositeae) family, which shows foreign pollination and is grown for one year. It contains 20-50% oil in the seeds of the safflower plant and the fact that it is approximately 90% (oleic and linoleic) in terms of total unsaturated fatty acids is proof of how important the quality of edible oil is. EST-SSRs or genic SSRs have been used usually in analyses of functional diversity, genetic mapping and/or marker-assisted selection in plants. In present study 89,907 expressed sequence tags (ESTs) of *Carthamus tinctorius* L. were vused to search for simple sequence repeats (SSRs). A total of 11,677 EST-SSRs, which accounted for 12.99% of all the ESTs, were identified. The most abundant EST-SSR types were dimeric repeats (6,772, 58%) followed by trimeric (2,285, 19%), monomeric (1441, 13%), hexameric (597, 5%), pentameric (331, 3%) and tetrameric repeats (251, 2%). For SSR motifs, the most prevalent motifs AC/GT (1819, 30.9%), followed by G/C (246, 23.5%), AAA/CTT (377, 19%), AC/GG (449, 7.6%) and AT/TT (332, 5.6%). We suppose that the EST-SSR markers provided here can be species-specific markers for taxonomic identification of *C. tinctorius* L. from other Carthamus species. Thus, the set of EST-SSRs developed in the present study is a promising resource for the related studies of Carthamus species.

**Keywords:** *Carthamus tinctorius,* EST-SSR, microsatellite, safflower, sequence repeats

## INTRODUCTION

Oil plants have a very important place in animal and human nutrition in terms of protein, carbohydrate, minerals and fat content. Some of the fats required for nutrition are included in different foods, and some are provided by different consumption of oilseed plants (Şahin and Taşlıgil, 2016). One of the main nutrients in the nutrition ring that people should take in order to lead a healthier life is vegetable oils. In addition, the need for vegetable oils, which is the core material of biodiesel, which is evaluated especially in the energy sector, is increasing its importance day by day. In this case, it is inevitable to increase the production of oil crops in all sustainable agricultural lands (Akınerdem, 2011). Throughout the world, oil crops are cultivated in two ways as annual and perennial. Oil crops grown for one year; soybean, rapeseed, sunflower, sesame, safflower, peanut, flax and camelina, perennial plants such as olive, coconut, jojoba, cocoa, palm and avocado (Baydar and Erbaş, 2007).

The cultivation of safflower in the world has a very large climatic zone, from the tropical climate of India to the Mediterranean climate of Turkey, between 20° South and 40° North latitudes. It is accepted that the safflower plant was cultivated in Egypt about 3500 years ago and spread all over the world from there. In China, Japan, India and Iran, the petals of the safflower plant have been cultivated for food, fabric dyeing and medicinal use. In the following times, it was started to be cultivated for the amount of oil in its seed (Babaoğlu, 2007). Safflower is an oilseed plant grown in winter and summer, belonging to the genus Carthamus of the

Asteraceae (Compositeae) family, which shows foreign pollination and is grown for one year. 25 different safflower species have been identified worldwide (Singh and Nimbkar, 2006). Recently cultivated safflower (*Carthamus tinctorious* L.) was cultured from *Carthamus lanatus* L. (Saffron thistle) and *Carthamus oxyacantha* L. (Wild safflower) (Ahlawat, 2008). *Carthamus tinctorius* species is cultivated in more than 20 countries (Esendal, 2001; Singh and Nimbkar, 2006).

The seeds of the safflower plant contain 20-50% oil and the fact that they are approximately 90% (oleic and linoleic) in terms of total unsaturated fatty acids proves how important the quality of edible oil is (Öztürk et al., 2008). In addition, since safflower oil has antioxidant properties and contains high tocopherol-containing vitamin E, it is likely to be included in the diets of cardiovascular patients. The fact that it is very effective in the amount of cholesterol is an indication that this plant is very valuable (Arslan and Bayraktar, 2015). After extracting the oil from the safflower seed, the remaining pulp takes its place in animal nutrition (Uğur, 2010). For this reason, it is very important in the raw material source of the vegetable oil and compound feed sector. It can also be used in animal nutrition, grazing, silage and hay making, feed industry, pulp making (it has an important place in animal husbandry with its high protein rate of 25%), beekeeping and biodiesel production (İlkdoğan, 2012).

Microsatellites, or simple sequence repeats (SSRs), tandemly repeated nucleotide sequences (1-6 bases in lenght) and widely distributed across prokaryotic and eukaryotic genomes. SSRs have become the best choice among markers used in plant breeding programs, as they are practical, convenient, easy to use and inexpensive. Among all available molecular markers, SSRs are easy to score and have wide genomic distribution, codominant inheritance and a multiallelic nature (Chen et al., 2015; Han et al., 2016).

Expressed sequence tags (ESTs) are sequenced portions of complementary DNA copies of mRNA, and represent part of the transcribed region of the genome. They mainly correspond to relatively conserved sequences. Many applications, ESTderived markers (EST-SSR) can be used to cross-reference genes between species for enhancing the resolution in comparative genomics studies and identifying conserved genomic regions among species and genus (Poncet et al., 2006, Cheng et al., 2016). EST-SSRs offer the following advantages, for example: they should detect variation in the expressed portion of the genome, so that gene tagging should give ‘‘perfect’’ marker-trait associations, they can be developed at no cost from the EST databases [genomic SSRs (gSSRs) are expensive to develop] and once developed, these markers, unlike genomic SSRs, may be used across a number of related species (Gupta et al., 2003).

In current study, we have mined *Carthamus tinctorius* L. transcriptome and characterised genome-wide EST-SSR markers for this species and developed EST-SSRs serve as a potential marker to allow a clear differentiation of *Carthamus tinctorius* L. taxa from other morphologically similar Compositae and other family plants.

## MATERIALS AND METHODS

### Data Retrieval and Analysis

Illumina RNA-Seq data for *Carthamus tinctorius* L. was obtained from NCBI Short Read Achieve (SRA) database (https://www.ncbi.nlm.nih.gov/sra) under accession number SRP050023 with the BioProject accession number of PRJNA313459. The sequenced read length for forward and reverse sequences of paired-end data is 100 bp in length.

All readings were obtained in raw sequence data as “.sra” format and converted to “fastq” format for Illimuna by the NCBI (National Center for Biotechnology Information) SRA Toolkit. After cutting the low-quality readings (Phred quality (Q) score <20) and trimming adapters with FASTX toolkit, all clean readings were exposed to FastQC analysis to control reading qualities in terms of per-base sequence qualities.

All transcript sequences were subject to identify potential microsatellite or SSR motifs using a perl script called MIcroSAtellite tool (MISA, version 1.0). The parameters for identifying simple repeat motifs were as follows: (i) the SSR motifs are expected to contain mono-, di-, tri-, tetra-, penta-, and hexa-nucleotides with minimum repeat numbers of 10, 6, 5, 5, 4 and 3, respectively, (ii) a compound SSR motif was defined if the number of bases between two adjacent SSR motifs was ≤100.

## RESULTS and DISCUSSION

A total of 89,907 expressed sequence tags (ESTs) of *Carthamus tinctorius* L. were vused to search for simple sequence repeats (SSRs). A total of 11,677 EST-SSRs, which accounted for 12.99% of all the ESTs, were

identified. The most abundant EST-SSR types were dimeric repeats (6,772, 58%) followed by trimeric (2,285, 19%), monomeric (1441, 13%), hexameric (597, 5%), pentameric (331, 3%) and tetrameric repeats (251, 2%) (Figure 1). For SSR motifs, the most prevalent motifs AC/GT (1819, 30.9%), followed by G/C (246, 23.5%), AAA/CTT (377, 19%), AC/GG (449, 7.6%) and AT/TT (332, 5.6%). These top five motifs accounted nearly for 85.2% of total motifs whereas the remaining ten motifs accounted for 14.8% (Figure 2).

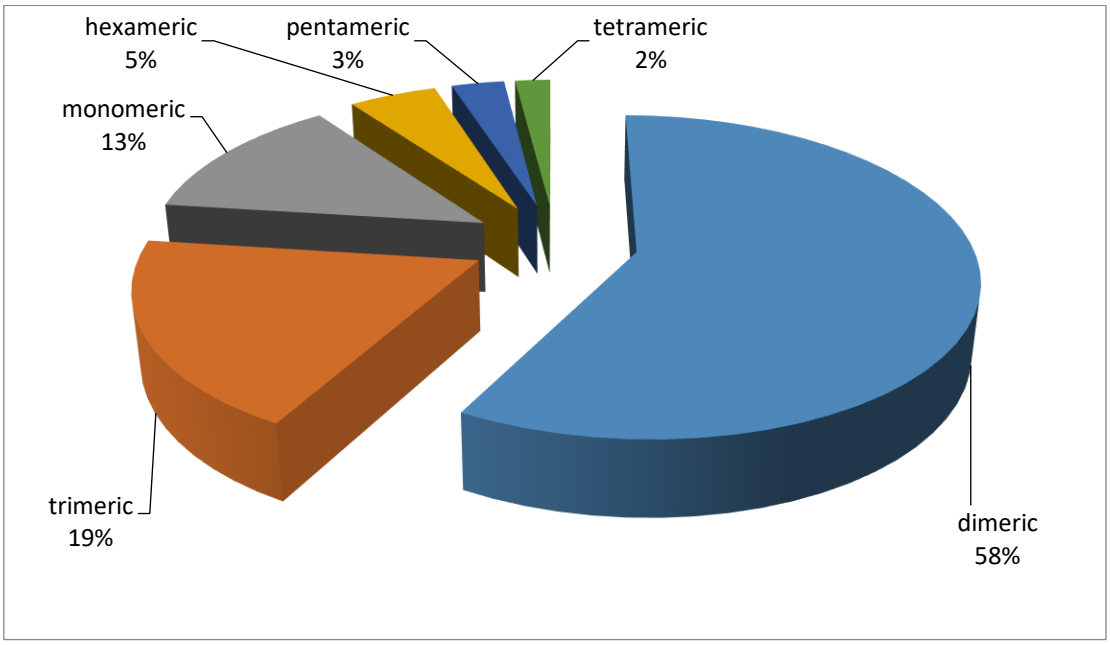


**Figure 1.** The frequency and distribution of EST-SSR types

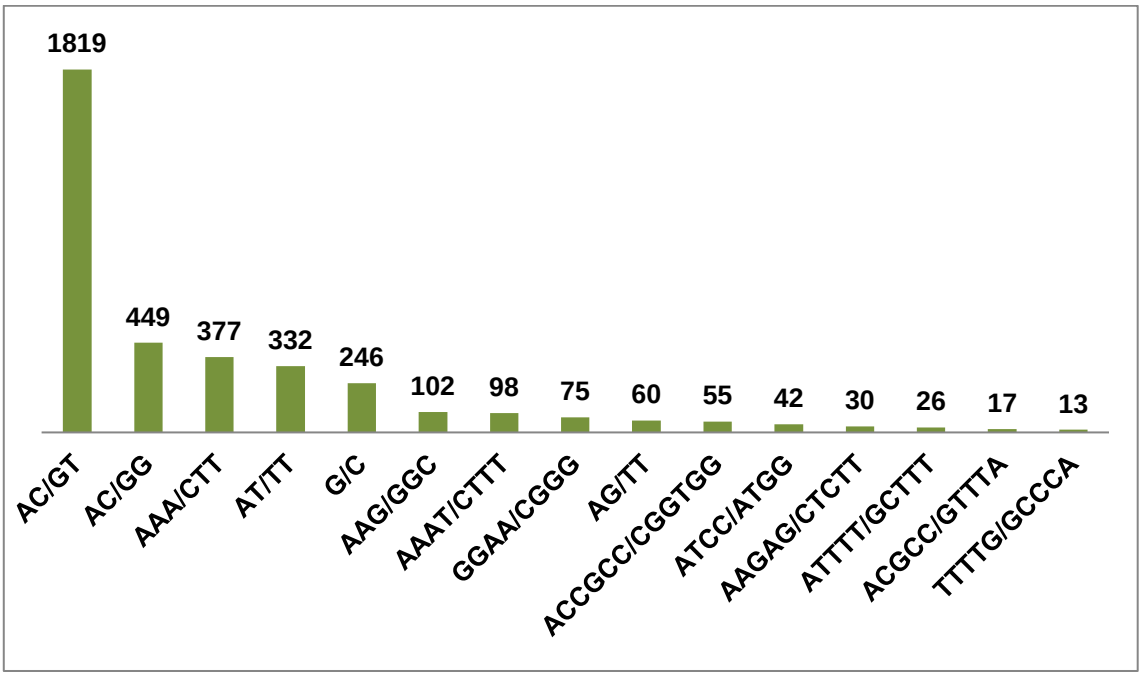


**Figure 2.** Basic characteristics and number of top 15 dominant EST-SSR motifs in *Carthamus tinctorius* L.

Microsatellites, known as simple sequence repeats (SSRs), are tandem repeat motifs of 1-6 nucleotides at high frequency scattered throughout the genome. For example (A)11, It is expressed as (GT)12, (ATT)9, (ATCG)8, (TAATC)6 and (TGTGCA)5. Most common SSR sequences dinucleotide (AC)n, (AG)n, (AT)n, trinucleotide (TCT)n, (TTG)n or tetranucleotide (TATG)n structure. The length of a microsatellite locus typically ranges from 5 to 40 repeats, although longer repeat sequences are possible. Dinucleotide, trinucleotide, and tetranucleotide repeats are the most common options used for molecular genetic studies (Wei et al., 2007).

Tri- and dinucleotide repeats are generally the most common motif found in both monocots and dicots (Eujayl et al. 2004; Varshney et al., 2005). However, they suggested that their result might have been due to the over-representation of untranslated regions (UTRs) compared with open reading frames (ORFs). Indeed, because of the absence of frameshift mutations when there are length variations in tri- and hexanucleotide repeats, these motifs are found in excess in both coding and noncoding sequences, but other repeat types are much less frequent in coding regions than in UTRs (Metzgar et al., 2000).

As most microsatellite libraries used for marker development are generally enriched in mono-, di-, tri- and tetra-nucleotide repeats, computational mining of EST databases mainly involves these types of motifs (Morgante et al., 2002; Eujayl et al., 2004). The AC/GT motif was the most abundant dinucleotide motif (30.9%) in *Carthamus tinctorius* L. ESTs. These motifs were also the most frequently observed SSRs in plants (Scott et al., 2000; Gao et al., 2003; Thiel et al., 2003; Saha et al., 2004). The most abundant trinucleotide repeat motif detected in the present study was AAA/CTT (19%), closely followed by mononucleotide repeat G/C (23.5%). These results are in agreement with other reports on safflower plants (Sehgal et al., 2005; Chapman et al., 2009; Mayerhofer et al., 2010; Hamdan et al., 2011).

Finally, EST databases provide a valuable resource for the development of SSR-markers, which are associated with transcribed genes. The EST-SSR identification, development and characterization in the *Carthamus tinctorius* L. very scarce, mainly due to the limited number of transcriptomic and genomic studies. We suppose that the EST-SSR markers provided here can be species-specific markers for taxonomic identification of *Carthamus tinctorius* from other Carthamus species. The EST-SSR data can also be used for this genus specific markers. However, EST-SSRs are the preferred method at genome-wide level because of their multi-allelic and co-dominant nature as well as quite high level of transferability across species/genus.

## CONCLUSION

EST-derived SSRs, which can easily be extracted from EST databases, can be used successfully and EST databases provide a valuable resource for the development of SSR-markers, which are associated with transcribed genes. In addition to mapping ESTs via microsatellite loci for locating the genes of putative functions, the EST-derived SSRs developed are a useful tool to study the genetic diversity within plants, although the potential limitations imposed by homoplasy and allele likeness require further attention. In the present study we comprehensively mined and characterised the publicly available transcriptome data from *Carthamus tinctorius* to identify EST-SSR markers at EST databases. Thus, the set of EST-SSRs developed in the present study is a promising resource for the related studies of Carthamus species.

## REFERENCES

Ahlawat IPS 2008. Agronomy-Rabi Crops Safflower. Division of Agronomy Indian Agricultural Research Institute, 10, New Delhi/India.

Akınerdem F 2011. Stratejik ve Ekonomik Değeri Yüksek Bir Tarım Alanı: Yağ Bitkileri ve Üretim Politikaları. Gübretaşla Verim Dergisi, 24: 22-24.

Arslan Y, Bayraktar N 2015. Farklı Azot ve Fosfor Seviyelerinin Kuru şartlarda Yetiştirilen Aspir (*Carthamus tinctorious* L.) Bitkisinin Bazı Verim Özellikleri Üzerine Etkisi. Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 1(2): 94-103.

Babaoğlu M 2007. Aspir ve Tarımı (*Carthamus tinctorius* L.). Trakya Tarımsal Araştırmalar Enstitüsü Yayınları, Edirne.

Baydar H, Erbaş S 2007. Türkiye'de Yemeklik Yağ ve Biyodizel Üretimine Uygun Aspir Islahı. 1.Ulusal Yağlı Tohumlu Bitkiler ve Biyodizel Sempozyumu, 378-386, 28-31 Mayıs, Samsun.

Chapman MA, Hvala J, Strever J, Matvienko M, Kozik A, Michelmore RW, Tang S, Knapp SJ, Burke JM 2009. Development, polymorphism, and cross-taxon utility of EST–SSR markers from safflower (Carthamus tinctorius L.). Theoretical and Applied Genetics, 120(1): 85-91.

Chen H, Wang L, Wang S, Liu C, Wohlgemuth Blair M, Cheng X 2015. Transcriptome Sequencing of Mung Bean (Vigna radiate L.) Genes and the Identification of EST-SSR Markers. PLoS ONE, 10(4): e0120273.

Cheng J, Zhao Z, Li B, Qin C, Wu Z, Trejo-Saavedra DL, Luo X, Cui J, Rivera-Bustamante RF, Li S, Hu K 2016. A comprehensive characterization of simple sequence repeats in pepper genomes provides valuable resources for marker development in Capsicum. *Scientific Reports*, 6: 18919.

Esendal E 2001. Safflower Production and Research in Turkey, Vth International Safflower Conference, Willinston, N.D., USA, July 23-27, pp 203-206.

Eujayl I, Sledge MK, Wang L, May GD, Chekhovskiy K, Zwonitzer JC, Mian MA 2004. Medicago truncatula EST-SSRs reveal cross-species genetic markers for *Medicago* spp. Theor Appl Genet 108(3): 414-422.

Gao LF, Tang JF, Li HW, Jia JZ 2003. Analysis of microsatellites in major crops assessed by computational and experimental approaches. Mol Breed, 12(3): 245-261.

Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS 2003. Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol Gen Genomics, 270: 315-323.

Hamdan YAS, García-Moreno MJ, Redondo-Nevado J, Velasco L, Pérez-Vich B 2011. Development and characterization of genomic microsatellite markers in safflower (Carthamus tinctorius L.). Plant Breeding, 130(2): 237-241.

Han B, Wang C, Tang Z, Ren Y, Li Y, Zhang D, Dong Y, Zhao X 2016. Genome-Wide Analysis of Microsatellite Markers Based on Sequenced Database in Chinese Spring Wheat (*Triticum aestivum* L.). PLoS ONE, 10(11): e0141540.

İlkdoğan U 2012. Türkiyede Aspir Üretimi Için Gerekli Koşullar ve Oluşturulacak Politikalar (Doktora Tezi). Ankara Üniversitesi Fen Bilimleri Enstitüsü Tarım Ekonomisi Anabilim Dalı. 108 s.

Mayerhofer R, Archibald C, Bowles V, Good AG 2010. Development of molecular markers and linkage maps for the Carthamus species C. tinctorius and C. oxyacanthus. *Genome*, *53*(4): 266-276.

Metzgar D, Bytof J, Wills C 2000. Selection against frameshift mutations limits microsatellite expansion in coding DNA. Genome Res, 10: 72-80.

Morgante M, Hanafey M, Powell W 2002. Microsatellites are preferentially associated with non-repetitive DNA in plant genomes. Nature Genet, 30: 194-200.

Öztürk E, Özer H, And Polat T 2008. Growth and Yield of Safflower Genotypes Grown Under Irrigated and Non-Irrigated Conditions in a Highland Environment. Plant Soil Environment, 54(10): 453–460.

Poncet V, Rondeau M, Tranchant C, Cayrel A, Hamon S, Kochko A, Hamon P 2006. SSR mining in coffee tree EST databases: potential use of EST–SSRs as markers for the *Coffea* genus. Mol Gen Genomics, 276: 436-449.

Saha MC, Mian MA, Eujayl I, Zwonitzer JC, Wang L, May GD 2004. Tall fescue EST-SSR markers with transferability across several grass species. Theor Appl Genet, 109(4): 783-791.

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ 2000. Analysis of SSRs derived from grape ESTs. Theor Appl Genet, 100(5):723-726.

Sehgal D, Raina SN 2005. Genotyping safflower (Carthamus tinctorius) cultivars by DNA fingerprints. Euphytica 146: 67-76.

Singh V, Nimbkar N 2006. Safflower (*Carthamus tinctorius* L.) Genetic Resources, Chromosome Engineering and Crop Improvement, Chapter 6, (Ed. Ram J. Singh), Genetic Resources, Chromosome Engineering, and Crop Improvement: Oilseed Crops, CRC Press, p. 320, Boca Raton.

Taşlıgil N, Şahin G 2016. Stratejik önemi artan bir endüstri bitkisi: Aspir (*Carthamus tinctorius* L.). Türk Coğrafya Dergisi, 66 (2016): 51-62.

Thiel T, Michalek W, Varshney RK, Graner A 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor Appl Genet, 106(3): 411-422.

Uğur E 2010. Bitkisel Yağ Sanayicileri Derneği Sunumu. Yağlı Tohumlu Bitkiler ve Bitkisel Yağlar Konferansı. 15 Eylül. İstanbul.

Varshney RK, Graner A, Sorrells ME 2005. Genic microsatellite markers in plants: features and applications. Trends Biotechnol, 23(1): 48-55.

Wei Z, Zhu SX, Van den Berg RG, Bakker FT, Schranz ME 2017. Phylogenetic relationships within Lactuca L.(Asteraceae), including African species, based on chloroplast DNA sequence comparisons. Genetic Resources and Crop Evolution, 64(1): 55-71.

## *ORAL PRESENTATION*

# Bibliometric analysis of research on *Papaver somniferum* L. based on Web of Science

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**Abstract**

*Papaver somniferum* is one of the most significant medicinal plants in the world because of its alkaloids. The major alkaloids like morphine and noscapine alkaloids shows anti-analgesic and anti-cancer effects. Turkey is among the countries where the traditional cultivation of poppy is done. The main purpose of this study is to examine the scope of research studies on *P. somniferum* during the period 2000-2022 years from the Web of Science (WoS) Core Collection. The documents that have in the title "*P. somniferum*" were recovered. The basic characteristics such as authors, publication years, document types, affiliations, institutions, of the published papers were studied. The 742 documents are detected from 2000 to 20th Jan 2022. The results show that research on *P. somniferum* reached the highest number in 2014. 200 authors from 68 different countries were determined. The top 3 authors were Facchini, PJ., Kutchan, TM. and Shukla, Sudhir Kumar. Among the countries, India, the United States and Canada were the dominant ones. The most enhanced thematic area corresponds to plant sciences, biochemsitry/molecular biology. The top 3 core journals were Acta Horticulturae, Phytochemistry and Industrial Crops and Products. It was detected that the Council of Scientific Industrial Research CSIR published the highest number of documents. The most used language is the English with the number of 701. Th highest citation was recorded in 2021. This study provides a comprehensive overview of *P. somniferum* using the specified specific bibliometric analysis factors. In addition, this article sets out the main research objectives and aspects of scientific literature production that can be addressed in future studies.

**Keywords:** Bibliometric analysis, *Papaver somniferum*, Web of Science

## INTRODUCTION

*Papaver sominferum* (opium poppy) is an annual plant which is a member of the Papaveraceae family. It is one of the most important medicinal plant for its benzylisoquinoline alkaloids (BIA) (Ziegler and Facchini, 2008). The meaning of the plant comes from ancient Greek that while Papaver corresponds to opium, somniferum corresponds to somnolent (*somni* to sleep + *ferre* to bring). It is not known exactly when the medicinal properties of poppy were first determined by humans (Brook et al., 2017). Opium poppy synthesises over 100 BIAs (Facchini and Park, 2003). Morphine and codeine, used for narcotic and analgesic properties, is the best known alkaloid among of all. Opium poppy is the only commercial source of them (Weid et al., 2004) . The isolation of morphine from opium by , the first isolation of a natural product, contributed greatly to the development of pharmacology as an independent discipline (Huxtable and Schwarz, 2001). Besides of its importance as an oil and food plant, opium poppy also synthesizes many, like antitussive codeine and noscapine, anticancer noscapine, antispasmodic papaverine (Alagöz et al, 2016). In addition, various components such as triterpenoids, phenylpropanoids, flavonoids, essential oils are also found in opium poppy (Da Cheng et al.,2015). The seed oil of opium poppy is rich in protein and comprises saturated palmitic, stearic, myristic and lauric acids and unsaturated fatty acids such as oleic, linoleic, alpha-linolenic acid (Untoro et al.,2006).

It is cultivated legally in Turkey, India, Australia, France, Spain, Hungary under the supervision of the United Nations. Besides, Turkey and India are reported by the United Nations as traditional poppy producer countries (Gümüşçü et al., 2008). The fact that it is not cultivated all around the world and is subjected to a special permission also limits the number of researcher working with this plant.

Numerous studies have been conducted on P. somniferum L. and its alkaloids. These studies have focused on the morphology and physiology of the plant, as well as the pathway and regulation of alkaloid production,

isolation and effects of alkaloids. Because of being the only source of morphine opium poppy is used as a model organism (Beaudoin and Facchini, 2014). The BIA pathway is a vey complex pathway and there are a lot of questions to be solved on it (Singh et al., 2019). New techniques in science leads new achievements on opium poppy studies.

Considered as an important quantitative analysis tool, bibliometric analysis can reveal the general trend of the development of a subject or field. Bibliometric analyses are frequently used to analyze scientific and technological production and development in different fields of science (Yuan and Sun, 2021a). These analysis have been conducted for some plants such as Morus alba (Peña-Borrego et al., 2019), cotton (Yuan and Sun, 2021b), endangered plants (Xu et al., 2022), potato (Yuan and Sun, 2022a) or plant related terms like agronomy (Cañas-Guerrero et al., 2013), ), critical dilution curve (Chen et al., 2021), rice and climate change (Yuan and Sun, 2022b) and biostimulants (Corsi et al., 2022).

The aim of this study was to determine publications on opium poppy from 2000 to 2022 by bibliometric science. Using these data we can answer the current research status, key authors, journals, research areas and institutions.

## MATERIALS and METHODS

The data were retrived from the Thomson Reuters Science Citation Index Web of Science (WoS) database within the period 2000–2022 (as of for 20 October 2022) that contain the phrase ‘’Papaver somniferum’’ in the titles (https://www.webofscience.com/wos/woscc/basic-search). WoS is an important platform covering scientific citation search and analytical information all over the world. It is one of the most comprehensive sources of academic information in the world due to its coverage of more than 12,000 academic journals (Yuan and sun, 2022b).

The data collected from the document types of articles, book chapters or review articles. The study is focused on the parameters, document types, number of publications, institutions, number of citations per year, number of authors per article, the document languages.

## RESULTS and DISCUSSION

### Type and number of published papers in WoS

The number of published academic articles is an important indicator for evaluating the development of scientific research. In this study we evaluated the articles, book chapters and reviews. In total 742 documents were detected during the period between 2000 and 2022 from the online version of WoS (Figure 1). The highest numbers of papers published per year were 59, 53 and 49 in 2014, 2021 and 2020, respectively. After 2010 the research on *P. somniferum* showed a significant growth. Among document types we evaluated, the ratio of articles was 98%, followed by reviews 9% and book chapters 2%. With the advance of biochemical and molecular biology tools especially next generation sequencing the studies have shifted towards this area.

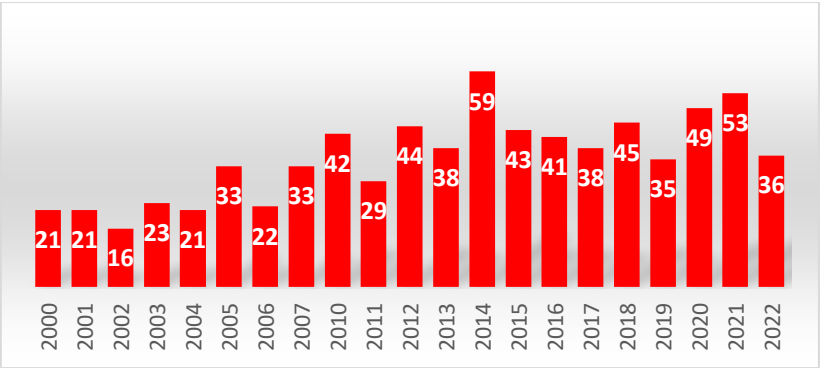


**Figure 1.** Number of published documents of *P. somniferum* research during 2000 to 2022

**Authors of the publications**

According to the analysis of the literature we found the authors who published the articles on *P. somniferum*. The results revealed that there were 200 authors who published the documents between 2000 and 2022. The top 3 authors were Facchini, PJ., Kutchan, TM and Shukla, SK. (Figure 2) Approximately 25% of the articles published by these authors. The authors are especially from Canada and India.

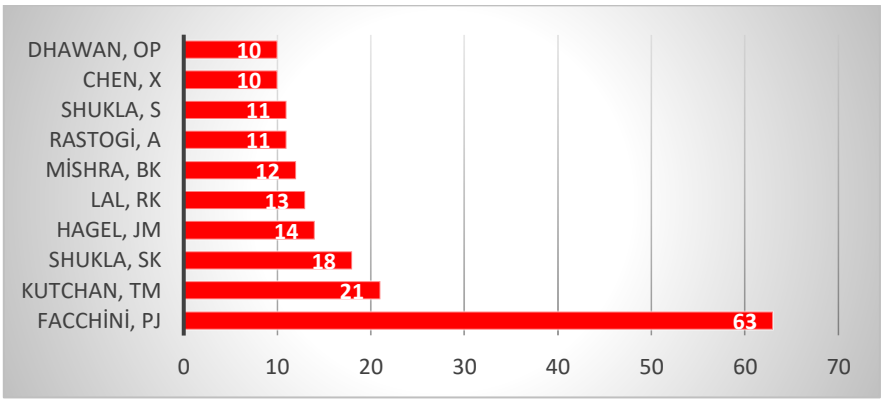


**Figure 2.** The number of publications of the top 10 most productive authors

**Most Productive Countries**

Here we detected 68 countries contributing the 895 papers. Figure 3 shows the countries having at least 10 documents. It is found that 85% of the documents published by these countries. Based on the number of publications, India was the predominant country with 117 documents, followed by USA, Canada, Germany and Turkey.

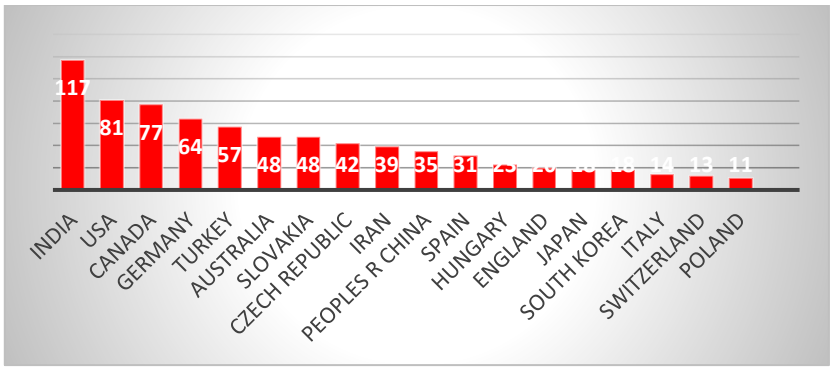


**Figure 3.** The name of the countries published more than 10 documents

**Top productive institutes published documents**

We found 200 institutes that published the documents including *P. somniferum* in the title. These institutes published 1002 publications. Studying this plant is a multidisciplinary field as it requires different fields such as biochemistry, molecular biology or analytical chemistry to work together, and different units are involved in this field. Council of Scientific Industrial Research Csir India, University of Calgary and Csir Central Institute of Medicinal Aromatic Plants CIMAP were the institutes which published the highest number of documents (Table 1)

**Table 1.** Names of institutes with more than 10 publications

| Names of Institutes | Number of Publications |
|---|---|
| Council of Scientific Industrial Research Csir India | 85 |
| University of Calgary | 64 |
| Csir Central Institute of Medicinal Aromatic Plants CIMAP | 48 |
| Csir National Botanical Research Institute NBRI | 32 |
| Comenius University Bratislava | 24 |
| Leibniz Institut fur Pflanzenbiochemie | 19 |
| Czech University of Life Sciences Prague | 18 |
| Martin Luther University Halle Wittenberg | 17 |
| Consejo Superior de Investigaciones Cientificas CSIC | 13 |
| Deakin University | 12 |
| Afyon Kocatepe University | 11 |
| Donald Danforth Plant Science Center | 11 |
| United States Department of Agriculture USDA | 11 |
| Universidad de Cordoba | 11 |
| University of Tasmania | 11 |

**Publishers of the documents**

According to the data that we obtained from WoS, 147 organizations produced 742 publications. The top three organizations are Elsevier, Springer Nature and Wiley. 45% of the documents were published by these organizations. Because being an medically and economically important plant, most of the documents published in high indexed publishers.

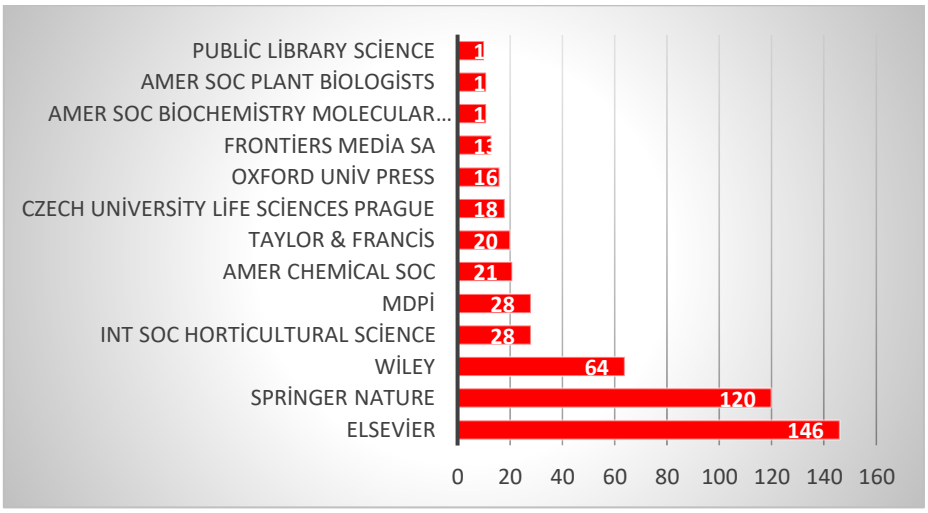


**Figure 4.** The number of publishers

**Funding agencies**

Here we found 199 funding agencies corresponding to 595 documents. Table 2 shows the agencies that support more than 10 projects. 38% of the documents were supported by these agencies. The data we obtained show direct proportion with the number of publications. Therefore, the more support is given to the studies, the more research and publications in the field increase. Although France is one of the countries that gave the most support, the country or the researchers do not come to the fore.

**Table 2.** Names of agencies and the number of publications

| Funding Agencies | Number of documents | Country |
|---|---|---|
| Natural Sciences And Engineering Research Council Of Canada Nserc | 38 | Canada |
| Cgiar | 28 | France |
| Council Of Scientific Industrial Research Csir India | 27 | India |
| Genome Canada | 15 | Canada |
| Turkiye Bilimsel Ve Teknolojik Arastirma Kurumu Tubitak | 15 | Turkiye |
| Genome Alberta | 14 | Canada |
| European Commission | 13 | European Union |
| Government Of Alberta | 13 | Canada |
| National Natural Science Foundation Of China Nsfc | 12 | China |
| Canada Foundation For Innovation | 11 | Canada |
| National Institutes Of Health NIH USA | 11 | USA |
| United States Department Of Health Human Services | 11 | USA |
| Australian Research Council | 10 | Australia |
| National Science Foundation | 10 | USA |

**Publication titles**

Based on the data that we collected from WoS, there are 189 journals and book series for *P. somnifrum* research. The journals that published over five articles on *P. somniferum* and their Quartile is given in Table 3. Acta Horticulturae comprised the 6% of the articles. Most of the articles are in Q1. It is important to publish an article in serious journals with high impact factors (White-Gibson et al., 2019).

Table 3: Top 10 journals publishing with the title of *P. somniferum*

| Journal | Publication | QC |
|---|---|---|
| Acta Horticulturae | 34 | 4 |
| Phytochemistry | 16 | 1 |
| Industrial Crops and Products | 14 | 1 |
| Journal of Biological Chemistry | 13 | 1 |
| Plant Journal | 13 | 1 |
| Plos One | 10 | 1 |
| Scientific Reports | 10 | 1 |
| Journal of Agricultural and Food Chemistry | 8 | 1 |
| Plant Physiology | 8 | 1 |
| Planta | 8 | 1 |

**Research areas**

*P. somniferum* research from 2000 to 2022 there are 71 research areas. Figure 6 displays the top 10. In research Areas, Plant Sciences (240, 21.97%), Agriculture (149, 13.64), Biochemistry Molecular Biology (106, 9.70), Chemistry (97,8.88%) and Biotechnology Applied Microbiology (56, 5.21%) comprises half of the documents. Besides the fact that there are journals with two or more research areas in WoS, documents can also cover different areas. The diversity of research areas indicates that a study area is multidisciplinary (Elango and Ho, 2017). In addition to being an oil plant, opium poppy is also an important medicinal plant have been used since ancient times. Since it is a model plant, it is suitable for multidisciplinary studies.

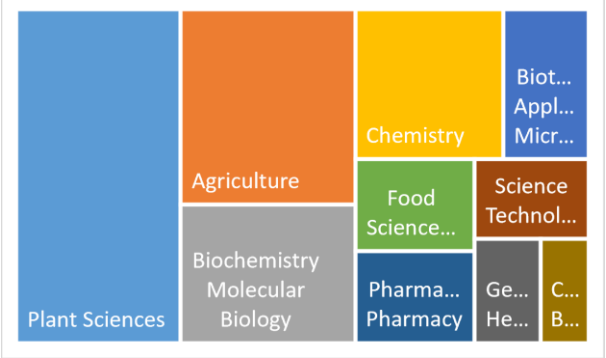


**Figure 5:** Publications on P. somniferum by top 10 research areas

**Language of publications**

The published document languages are shown in Figure 7. Among the documents, most of the documents were published in English (701,94.47%), Czech (21, 2.83%), German (5,0.67%), Chinese and Hungarian (3, 0.27%), Polish, Slovak and Spanish (2, 0.27%), Italian, Turkish and Ukranian (1, 0.13%). Many bibliometric analyses revealed that most of the documents were written in English (Yuan and Sun, 2021b).

**Number of citations**

The results showed that citations showed a steady increase and reached the highest level in 2021. In parallel with the increase in the number of publications, the number of citations also increases. In total the number of citations were 3349, without self-citations 2270 during the period of 2000-2022. The most cited paper was "Papaver somniferum 10-Gene Cluster for Synthesis of the Anticancer Alkaloid Noscapine" written by Winzer et al. in 2012 with the number of 215.

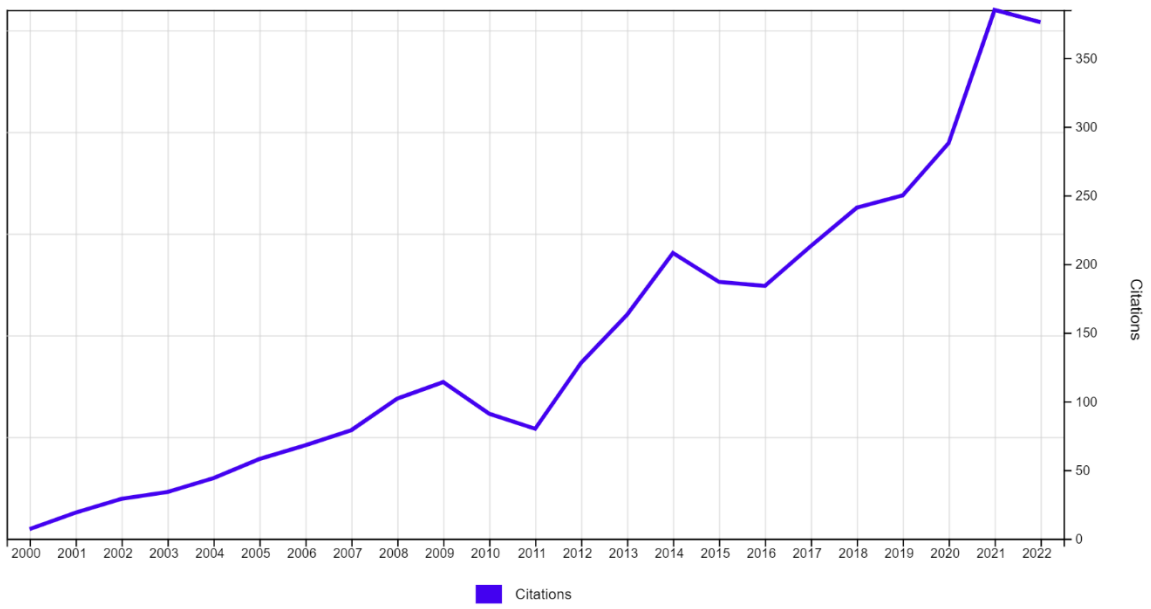


**Figure 7:** The trend of the number of citations regarding opium poppy from 2000 to 2022

## CONCLUSION

This study is the first to use bibliometric tools to understand the literature related to *P. somniferum* from 2000 to 2022. 742 documents were retrieved from WoS. It is found that the studies about opium poppy have gradually attracted the attention of scholars. It is detected that the annual output of the number of research documents is increased after 2010. Canadian and Indian national institutions and authors carried out more intensive research. Future research will focus on the genomic structure, producing morphine in other organisms and the regulation of BIA pathway.

## REFERENCES

Alagoz Y, Gurkok T, Zhang B, Unver T 2016. Manipulating the biosynthesis of bioactive compound alkaloids for next-generation metabolic engineering in opium poppy using CRISPR-Cas 9 genome editing technology. Scientific reports, 6(1):1-9.

Beaudoin GA, Facchini PJ 2014. Benzylisoquinoline alkaloid biosynthesis in opium poppy. Planta, 240(1):19-32.

Brook K, Bennett J, Desai SP 2017. The chemical history of morphine: an 8000-year journey, from resin to de-novo synthesis. Journal of Anesthesia History,. 1;3(2):50-5.

Cañas-Guerrero I, Mazarrón FR, Pou-Merina A, Calleja-Perucho C, Díaz-Rubio G 2013. Bibliometric analysis of research activity in the “Agronomy” category from the Web of Science, 1997–2011. European Journal of Agronomy, 50:19-28.

Chen R, Zhu Y, Cao W, Tang L 2021. A bibliometric analysis of research on plant critical dilution curve conducted between 1985 and 2019. European Journal of Agronomy, 123, 126199.

Corsi S, Ruggeri G, Zamboni A, Bhakti P, Espen,L, Ferrante A, Scarafoni, A 2022. A Bibliometric Analysis of the Scientific Literature on Biostimulants. Agronomy, 12(6):1257.

Da Cheng H, Xiao-Jie G, Pei GX. Phytochemical and biological research of Papaver pharmaceutical resources. Medicinal Plants: Chemistry Biology and Omics. 2015:217-51.

Elango B, Ho YS 2017. A bibliometric analysis of highly cited papers from India in science citation index expanded. Current Science, 112 (8):1653–58.

Facchini PJ, Park SU 2003. Developmental and inducible accumulation of gene transcripts involved in alkaloid biosynthesis in opium poppy. Phytochemistry, 1;64(1):177-86.

Gümüşçü A, Arslan N, Sarıhan EO 2008. Evaluation of selected poppy (Papaver somniferum L.) lines by their morphine and other alkaloids contents. Europan Food Research Technology, 226:1213–1220.

Huxtable RJ, Schwarz SK 2001. The isolation of morphine—first principles in science and ethics. Molecular interventions, 1;1(4):189.

Peña-Borrego MD, Fermoselle-Cumbá D, Peña-Rueda YF, Bécquer-Granados C 2019. Bibliometric analysis about the published studies on Morus alba L. Pastos y Forrajes, 42(1): 81-87.

Singh A, Menéndez-Perdomo IM, Facchini PJ 2019. Benzylisoquinoline alkaloid biosynthesis in opium poppy: an update. Phytochemistry Reviews, 18(6):1457-82.

Untoro J, Schultink W, West CE, Gross R, Hautvast JG 2006. Efficacy of oral iodized peanut oil is greater than that of iodized poppy seed oil among Indonesian schoolchildren. The American Journal of Clinical Nutrition, 84(5):1208-1214.

Weid M, Ziegler J, Kutchan TM 2004. The roles of latex and the vascular bundle in morphine biosynthesis in the opium poppy, Papaver somniferum. Proceedings of the National Academy of Sciences, 21;101(38):13957-62.

White-Gibson A, O'Neill B, Cooper D, Leonard M, O'Daly B 2019. Levels of evidence in pelvic trauma: A bibliometric analysis of the top 50 cited papers. Irish Journal of Medical Science, 188:155–59.

Xu J, Xiao P, Li T, Wang Z 2022. Research Progress on endangered plants: a bibliometric analysis. Biodiversity and Conservation, 1-23.

Yuan BZ, Sun J 2021a. Bibliometric analysis of Strawberry (Fragaria× ananassa Duch.) research publications from Horticulture category based on Web of Science. Journal of Berry Research, (Preprint), 1-18.

Yuan BZ, Sun J 2021b. Bibliometric analysis of cotton research from Plant Sciences category based on Web of Science. J Nat Fibers.

Yuan BZ, Sun J 2022a. Bibliometric analysis of potato research publications from Agronomy Category based on Web of Science from 2000 to 2021. Potato Research, 65(2): 233-253.

Yuan BZ, Sun J 2022b. Bibliometric analysis of rice and climate change publications based on Web of Science. Theoretical and Applied Climatology, 150(1): 347-362

Ziegler J, Facchini PJ 2008. Alkaloid biosynthesis: metabolism and trafficking. Annual Reviews of Plant Biology,59:735–69.

## *ORAL PRESENTATION*

# Effect of *PDYN* rs2281285 polymorphism on the intensity of anxiety symptoms and craving in patients with alcohol use disorder

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## Abstract

Alcohol use disorder (AUD) is associated with changes in reward and stress pathways and these changes may lead to an enhanced sensitivity to emotional stress and, thus, compulsive alcohol seeking. The co-occurrence of AUDs and anxiety disorders, which may act as a triggering factor for craving, is related with a complex clinical presentation. Dynorphins, encoded by the *PDYN* gene, are endogenous peptide ligands of the kappa-opioid receptors (KOR). The KOR/dynorphin interaction results in anhedonic/dysphoric (reluctance, lack of pleasure) effects. In this study, the relationship between *PDYN* rs2281285 gene polymorphism and the intensity of anxiety symptoms and craving in individuals with AUD was investigated. For this purpose, Turkish men diagnosed with AUD (n=45) were included in the study. *PDYN* rs2281285 gene polymorphism was analysed by Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) method. Craving and the intensity of anxiety symptoms were measured by Penn Alcohol Craving Scale (PACS) and Beck Anxiety Scale (BAS), respectively. The frequencies of *PDYN* rs2281285 A and G alleles were 78% and 22%, respectively. The genotype and allele frequencies of this polymorphism were consistent with Hardy–Weinberg equilibrium ($p>0.05$). The mean PACS score of individuals with AUD was 17.38±8.87 with a minimum of zero and a maximum of 30. The median BAS score of individuals with AUD was 15.0 (IQR:7.0-26.5). PACS score of the AA genotype (16.19±8.07) was lower than that of the AG+GG genotypes (19.0±9.84). On the other hand, the BAS score of the *PDYN* rs2281285 AA genotype was higher (15.5; IQR:7.75-28.0) than that of the AG+GG genotypes (13.0; IQR:5.0-25.0). These differences between *PDYN* rs2281285 genotypes were not statistically significant ($p>0.05$). In conclusion, this pilot study indicated that the *PDYN r*s2281285 polymorphism had no effect on anxiety symptoms and craving in Turkish men with AUD.

**Keywords:** Alcohol use disorder, craving, anxiety, *PDYN*, polymorphism

## INTRODUCTION

Alcohol use disorder (AUD) is a chronic psychiatric disorder leading to emotional, physical and social problems and resulting in harmful drinking behavior (Kaya et al., 2017). Individuals with such disorders have impaired control over alcohol consumption and exhibit a chronically heavy and often increased use of alcohol, despite the severely damaging costs to their health (Carvalho et al., 2019). Besides severe psychiatric and physical disorders, alcohol causes deteriorated interpersonal relations, increased family problems and also affects children adversely. Alcohol is considered as the main responsible for work and traffic accidents, injury, murder and suicide, loss of work and labour force (Özpoyraz et al., 1998). AUDs are common in all developed countries and observed more in men than women. Although the rates of such disorders are low in Mediterranean countries such as Greece, Italy and Israel and high in north and east Europe such as Russia and Scandinavians, they are responsible for the majority of the healthcare burden of almost all populations (Saxena, 1997; Schuckit et al., 2006). According to the data of the "Global Status Report of Health and Alcohol" published by World Health Organization (WHO) in 2014, 3.3 million deaths worldwide in 2012 were caused by alcohol consumption (IHME, 2015).

Dynorphins are synthesized by proteolytic processing of pyrodynorphin encoded by the *PDYN* gene (Horikawa et al., 1983). More than one active peptides namely as dynorphin A, dynorphin B and a/B neoendorphines are released while pyrodynophin is digested during processing (Horikawa et al., 1983; Mansour et al., 1995).

Dynorphins selectively binds to the kappa-opioid receptors (KOR) encoded by the *OPRK1* gene (Yuferov et al., 2004). It has been shown that dynorphin/KOR system plays a role in the motivational aspects of stress by mediating pro-depressive-like states with anhedonia/dysphoria and aversion elements in humans and laboratory animals (Knoll et al., 2007; Todtenkopf et al., 2004; Wadenberg, 2003).It has been found that *PDYN* mRNA and dynorphins were up-regulated in the dorsa-lateral prefrontal cortex in individuals with alcohol dependence (Bazov et al., 2011). This up-regulation which may be affected by the *PDYN* gene polymorphism and epigenetic mechanisms may contribute to the disruption of the cognitive control over alcohol drinking behaviour (Shippenberg et al., 2007; Taqi et al., 2011a,b).

Clinical studies indicate that negative mood status along with the alcohol withdrawal contributes to relapse (Zywiak et al., 2003; Zywiak et al., 2006). Research data show that negative craving is a more crucial marker of drinking compared to positive and obsessive craving (Victorio-Estrada and Mucha, 1996; Victorio-Estrada et al., 1997). It is known that co-occurrence of AUD and anxiety is common due to the contribution of anxiety to negative mood status. Furthermore, unsuccessful pharmacotherapy is supported by the co-occurrence of AUD and anxiety (Anker and Kushner, 2019).

The present study was undertaken with the aim of exploring whether *PDYN* rs2281285 polymorphism has an effect on the intensity of anxiety and negative craving in individuals with AUD due to the contribution of dynorphin to the negative emotional states. In the literature, the contribution of *PDYN* gene to the induction of negative craving was also investigated in AUD subjects (Karpyak et al., 2013; Preuss et al., 2013; Winham et al., 2015). However, to the best of our knowledge, this is the first report examining the association of variation in the *PDYN* gene with the intensity of anxiety symptoms at the gene level in patients with AUD.

## MATERIALS AND METHODS

A total of 45 individuals who had a current AUD by the International Classification of Diseases-10 (ICD-10) diagnostic criteria were enrolled in this study. They also fulfilled the DSM–5criteria for AUD. The inclusion criteria for patients with AUD were as follows: (i) they were at least 18 years of age, (ii) they had no acute health problems or clinically significant co-morbid psychiatric illness such as depression, (iii) they have not been administered drugs for psychiatric illness, (iv) they had no history of substance use disorders other than alcohol and nicotine. The study design was approved by the institutional ethics committee (Approval No: I3-220-21 in 2021). Samplings were performed in accordance with the principles of The Declaration of Helsinki. Written informed consent was obtained from each participant who was eligible for the study. Furthermore, all subjects completed a small questionnaire used to gather socio-demographic information regarding age, marital, education and employment status.

To investigate the effect of *PDYN* rs2281285 polymorphism on the intensity of anxiety and craving in AUD patients, the Beck Anxiety Scale (BAS) and Penn Alcohol Craving Scale (PACS) were assessed, respectively. The validities and reliabilities of Turkish versions of these scales were demonstrated (Evren et al., 2008; Ulusoy et al., 1998).

The venous blood sample was taken from each patients into tubes with EDTA and, then, genomic DNA was extracted using the QIAamp DNA blood-kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. The *PDYN* rs2281285 polymorphism was genotyped using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP), as previously described by Hashemi et al. (2018). PCR amplification was conducted on a Techne Tc 512 PCR System in a 25-µL reaction mixture containing 2mM of dNTPs, 10 pmol each of the forward (F) and reverse (R) primers, 1 U of Hot Star Taq DNA polymerase, 10×PCR buffer and 50 ng of genomic DNA. The sequences of the forward (F) and reverse (R) primers were 5'- GCTCAGATTTTCACTGTTCCGA -3' and 5'- AGCCAACATTCATGGGCTGA -3', respectively. After PCR amplification, the 423 bp- PCR products were digested with *HYP8I* at 37˚C for 1 hour. The undigested and digested PCR products were separated by gel electrophoresis on a 3% agarose gel, visualized by ethidium bromide staining under an UV illuminator, and then scanned and photographed using the Syngene Monitoring System (Figure 1).

The Statistical Package for Social Sciences (SPSS) version 21.0 software for Windows was used for the statistical analyses. The normality of numerical variables was examined using the Kolmogorow-Smirnow test. The median and the interquartile range (IQR) or mean and standard deviation (SD) were given according to the normality of the data. Numbers, percentages and 95% confidence interval were given for categorical data. The frequency of the *PDYN* rs2281285 alleles and genotypes were obtained by direct count, and the departure from the Hardy-Weinberg equilibrium ($p^2+2pq+q^2=1$) was evaluated by the chi-square test. Genotypes were

compared using Mann Whitney U test or t-test, as appropriate. The relationship between BAS and PACS scores was analysed using the Spearman correlation test. $p<0.05$ was considered as statistically significant.

## RESULTS

There was a total of 45 male patients in the cohort. The mean age of the subjects was 41.28±9.33 years with a minimum of 23 years and a maximum of 60 years. Seventy-six patients were employed, while 13 were unemployed. Fourteen patients were single, while 18 were married, and 13 were divorced. All subjects with AUD were smokers. The duration of smoking was 23.22±9.88 years. The pack years of smoking was 27 packets/year (IQR: 15.0-56.0 packets/year). The first age onset of alcohol was 16 years (IQR:14.0-18.0 years).

The allele and genotype frequencies, 95% confidence interval and Hardy–Weinberg equilibrium (HWE) of *PDYN* rs2281285 polymorphism in 45 Caucasian Turkish individuals with AUD were shown in Table 1. The frequencies of the A and G alleles were78.0% and 22.0%. The genotype frequency of *PDYN* rs2281285 polymorphism is consistent with Hardy–Weinberg equilibrium ($p>0.05$). There was only one homozygous GG individual in the cohort. Thus, subjects having GG genotype was merged with those having AG genotype and compared statistically to those having AA.

The mean PACS score of individuals with AUD was 17.38±8.87 with a minimum of zero and a maximum of 30. The median BAS score of individuals with AUD was 15.0 (IQR:7.0-26.5). PACS score of the AA genotype (16.19±8.07) was lower than that of the AG+GG genotypes (19.0±9.84). On the other hand, the BAS score of the *PDYN* rs2281285 AA genotype was higher (15.5; IQR:7.75-28.0) than that of the AG+GG genotypes (13.0; IQR:5.0-25.0). These differences between *PDYN* rs2281285 genotypes were not statistically significant ($p>0.05$) (Table 2). Moreover, a significant and positive correlation was found between craving and anxiety ($r^2=0.555$, $p=0.001$).

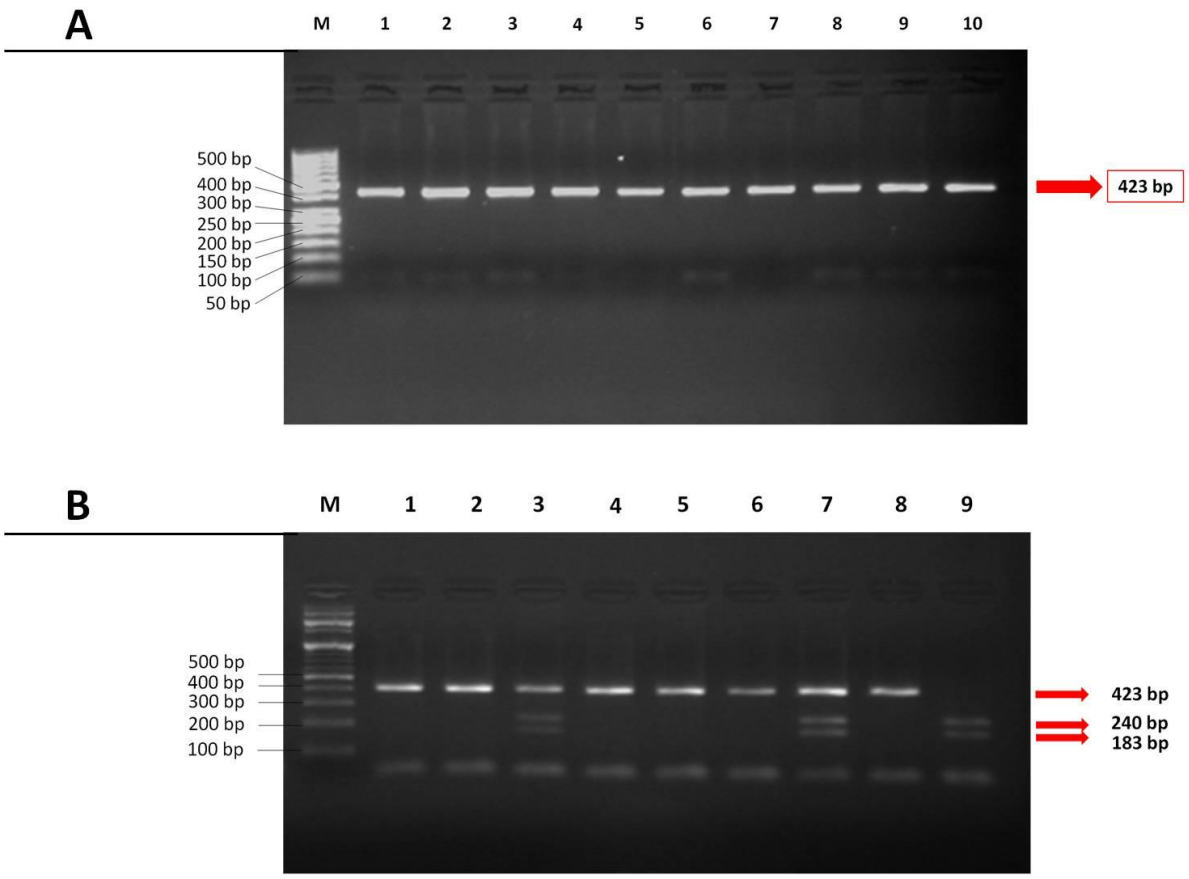


**Figure 1. A)** A representative agarose gel image of undigested PCR products (423 bp) for *PDYN* rs2281285 polymorphism (M: 50 bp ladder; 1-10: 423 bp PCR products). **B)** A representative agarose gel image of digested PCR products with *HPY8I* for the *PDYN* rs2281285 polymorphism. M: 50 bp ladder; Lanes 1,2,4,5,6 and 8: homozygous wild-type genotype (AA) (423 bp); Lanes 3 and 7: heterozygote genotype (AG) (423 bp, 240 bp and 183 bp); Lane 9: homozygous mutant genotype (GG) (240 bp and 183 bp).

**Table 1.** Allele and genotype frequencies of *PDYN* rs2281285 polymorphism observed in patients

| *PDYN* rs2281285 alleles | N (90) % Frequency | 95% CI |
|---|---|---|
| A | 70 (78%) | 78 (69.4-86.6) |
| G | 20 (22%) | 22 (13.4-30.6) |
| *PDYN* rs2281285 genotypes | N (45) % Frequency | 95% CI |
| AA | 26 (58%) | 58 (43.6-72.4) |
| AG | 18 (40%) | 40 (25,7-54.3) |
| GG | 1 (2%) | 2 (NA*) |
| HWE p-value | $\chi 2=1.11$; p=0.29 | |

N: number, CI: Confidence Interval, HWE: Hardy-Weinberg Equilibrium, *NA: non-available because calculation of CI requires n≥ 5.

**Table 2.** PACS and BAS total scores of AUD patients according to *PDYN* rs2281285 genotypes

| ***PDYN* rs2281285 Genotypes** | **PACS total score** | | **p-value** | **BAS total score** | | **p-value** |
|---|---|---|---|---|---|---|
| | Mean±S.D. | Min.-Max. | | Median | IOR | |
| | | | | | | |
| AA (n=26) | 16.19±8.07 | 0.0-30.0 | t=-1.05 p=0.299 | 15.5 | 7.75-28.0 | Z=-0.46 p=0.646 |
| AG+GG (n=19) | 19.0±9.84 | 3.0-30.0 | | 13.0 | 5.0-25.0 | |

IOR: Interquartile Range

## DISCUSSION

The *PDYN* rs2281285 polymorphism is in the second intron (-749 bp downstream of the exon 2/intron B border). It is thought that this area is in the regulation of transcription initiation, giving rise to one of two dominant transcripts: FL2-PDYN (in the hypothalamus and claustrum) and FL1-PDYN (in limbic-related structures) mRNAs (Nikoshkov et al., 2008). Allelic variability in this location may cause differential regulation of these 2 transcripts (Karpyak et al., 2013). The low risk rs2281285 A allele resides within the sequence that represents the DNA-binding element for several transcription factors of the POU (Pit-Oct-Unc) family. However, the high-risk rs2281285 minor G allele disables this sequence (Karpyak et al., 2013; Preuss et al., 2013). It was hypothesized that the members of the POU family of transcription factors are involved in the regulation of gene expression in the hypothalamus and pituitary gland, which have an important role in hormonal stress responses (Karpyak et al., 2013).

It is suggested that craving may occur due to the desire for the rewarding features of alcohol (positive craving) or the desire for drinking in the context of alleviating withdrawal symptoms (negative craving) Karpyak et al., 2013; Preuss et al., 2013). AUD subjects examined in the present study were characterized as possessing negative craving because of a positive significant correlation between craving and anxiety. In the current analysis, although it was not statistically significant (p>0.05), the *PDYN* rs2281285 AG+GG genotypes (19.0±9.84) had a higher PACS total score than AA genotypes (16.19±8.07), suggesting the association of negative craving with the *PDYN* rs2281285 variant. This is consistent with previous investigations on alcohol-dependent subjects, which demonstrated an association between the rs2281285 minor G allele and increased negative craving (Karpyak et al., 2013; Preuss et al., 2013; Winham et al., 2015).

Epidemiological and clinical studies have reported that the co-occurrence of anxiety and AUD is common and complex (Smith and Randall, 2012; Anker and Kushner, 2019). According to Kushner and colleagues (2012) all anxiety and mood disorders correlates with the risk for AUD by contributing to negative emotionality. Moreover, co-occurring anxiety disorders may cause a poor response to treatment for AUD. Investigations have focused on how treatment for one co-occuring disorder influence symptoms of the other disorder (Anker and Kushner, 2019). Thus, in the present study, we aimed to determine the effect of PDYN, a candidate gene

for both AUD and mood disorders, on the intensity of anxiety. Although there was not a statistically difference between rs2281285 genotype subgroups, the BAS score of the AA genotype was higher (15.5; IQR:7.75-28.0) than that of the AG+GG genotypes (13.0; IQR:5.0-25.0), which indicated that minor G allele could decrease the intensity of anxiety symptoms.

In conclusion, this pilot study indicated that the *PDYN r*s2281285 polymorphism had no effect on anxiety symptoms and craving in Turkish men with AUD. However, it was detected that there were differences between *PDYN* rs2281285 genotype subgroups in view of total scores of scales that evaluate the intensity of craving and anxiety symptoms. Thus, more research is necessary to determine the functional importance of the *PDYN* rs2281285 variant on anxiety in AUD subjects.

**ACKNOWLEDGEMENTS**
This study was supported by the Ankara University Scientific Research Projects Coordination Unit (grant number: THD-2022-2409 awarded to Dilek Kaya-Akyüzlü).

**REFERENCES**

Anker JJ, Kushner MG. 2019. Co-Occurring Alcohol Use Disorder and Anxiety: Bridging Psychiatric, Psychological, and Neurobiological perspectives. Alcohol Res 40:e1–e12.

Bazov I, Kononenko O, Watanabe H, Kuntiä V, Sarkisyan D, Taqi MM, Hussain MZ, Nyberg F, Yakovleva T, Bakalkin G. 2011. The endogenous opioid system in human alcoholics: Molecular adaptations in brain areas involved in cognitive control of addiction. Addiction Biology 18:161–169. doi:10.1111/j.1369-1600.2011.00366.x

Carvalho AF, Heilig M, Perez A, Probst C, Rehm J. 2019. Alcohol use disorder. Lancet 394:781–792.

Evren C, Flannery B, Çelik R, Durkaya M, Dalbudak E. 2008. Penn Alkol Aşerme Ölçeği (PAAÖ) Türkçe Şeklinin Yatarak Tedavi Gören Erkek Alkol Bağımlısı Hastalarda Geçerliği ve Güvenirliği. Journal of Dependence 9:128–134.

Horikawa Saburo, Takai Toshiyuki, Toyosato Mitsuyoshi, Takahashi Hideo, Masaharu Noda, Kakidani Hitoshi, Kubo Tai, Hirose Tadaaki, Inamaya Seiichi, Hayashida Hidenori, Miyata Takashi, Numa Shosaku. 1983. Isolation and structural organization of the human preproenkephalin B gene. Nature 306:611–613.

Institute for Health Metrics and Evaluation. Country Profiles. 2015; Available from: http://www.healthdata.org/results/country-profiles

Karpyak VM, Winham SJ, Preuss UW, Zill P, Cunningham JM, Walker DL, Lewis KA, Geske JR, Colby CL, Abulseoud OA, Hall-Flavin DK, Loukianova LL, Schneekloth TD, Frye MA, Bazov I, Heit JA, Bakalkin G, Mrazek DA, Biernacka JM. 2013. Association of the PDYN gene with alcohol dependence and the propensity to drink in negative emotional states. International Journal of Neuropsychopharmacology 16:975–985. doi:10.1017/S1461145712001137

Kaya H, Bolat Kaya Ö, Dilbaz N. 2017. Genetics of Alcohol Use Disorder. Current Addiction Research 1:33–46. doi:10.5455/car.20170705081115

Knoll AT, Carlezon WA. 2010. Dynorphin, stress, and depression. Brain Res 1314:56–73. doi:10.1016/j.brainres.2009.09.074

Kushner MG, Wall MM, Krueger RF, Sher KJ, Maurer E, Thuras P, Lee S. 2012. Alcohol Dependence is Related to Overall Internalizing Psychopathology Load Rather than to Particular Internalizing Disorders: Evidence from a National Sample. Alcohol Clin Exp Res 36:325–331. doi:10.1111/j.1530-0277.2011.01604.x

Mansour A, Hoversten MT, Taylor LP, Watson SJ, Akil H. 1995. The cloned m, d and K receptors and their endogenous ligands: Evidence for two opioid peptide recognition cores. Brain Res 700:89–98.

Nikoshkov A, Drakenberg K, Wang X, Cs Horvath M, Keller E, Hurd YL. 2008. Opioid neuropeptide genotypes in relation to heroin abuse: Dopamine tone contributes to reversed mesolimbic proenkephalin expression. PNAS 105:786–791.

Özpoyraz N, Taman L, Şentürk A. 1998. Alkol ve Madde Kullanım Bozuklukları [Alcohol and Drug Use Disorders]. Galenos 1:58–66.

Preuss UW, Winham SJ, Biernacka JM, Geske JR, Bakalkin G, Koller G, Zill P, Soyka M, Karpyak VM. 2013. PDYN rs2281285 variant association with drinking to avoid emotional or somatic discomfort. PLoS One 8:e78688. doi:10.1371/journal.pone.0078688

Saxena S. 1997. Alcohol, Europe and the developing countries. Addiction 92:43–48. doi:10.1111/j.1360-0443.1997.tb03394.x

Schuckit, M. A. (2006). Drug and Alcohol Abuse A clinical guide to diagnosis and treatment (Sixth)Springer Science and Business Media.

Shippenberg TS, Zapata A, Chefer VI. 2007. Dynorphin and the pathophysiology of drug addiction. Pharmacol Ther 116:306–321. doi:10.1016/j.pharmthera.2007.06.011

Smith JP, Randall CL. 2012. Anxiety and Alcohol Use Disorders Comorbidity and Treatment Considerations. Alcohol Res 414–431.

Taqi Malik Mumtaz, Bazov I, Watanabe H, Nyberg F, Yakovleva T, Bakalkin G. 2011. Prodynorphin promoter SNP associated with alcohol dependence forms noncanonical AP-1 binding site that may influence gene expression in human brain. Brain Res 1385:18–25. doi:10.1016/j.brainres.2011.02.042

Taqi Malik Mümtaz, Bazov I, Watanabe H, Sheedy D. 2011. Prodynorphin CpG-SNPs associated with alcohol dependence: Elevated methylation in the brain of human alcoholics. Addiction Biology 16:499–509. doi:10.1111/j.1369-1600.2011.00323.x

Todtenkopf MS, Marcus JF, Portoghese PS, Carlezon WA. 2004. Effects of κ-opioid receptor ligands on intracranial self-stimulation in rats. Psychopharmacology (Berl) 172:463–470. doi:10.1007/s00213-003-1680-y

Ulusoy M, Şahin NH, Erkmen H. 1998. Turkish Version of the beck anxiety inventory: psychometric properties. Journal of Cognitive Psychotherapy: An International Quarterly 12:163–172.

Victorio-Estrada A, Mucha RF. 1997. Inventory of drinking situations (IDS) in current drinkers with different degrees of alcohol problems. Addictive Behaviors 22:557–565.

Victorio-Estradaa A, Mucha RF, Stephand ER. 1996. Excessive drinking situations in German alcoholics: replication of a three-factor model used for North Americans. Drug Alcohol Depend 41:75–79.

Wadenberg M-LG. 2003. A Review of the Properties of Spiradoline: A Potent and Selective k-Opioid Receptor Agonist. CNS Drug Rev 9:187–198.

Winham SJ, Preuss UW, Geske JR, Zill P, Heit JA, Bakalkin G, Biernacka JM, Karpyak VM. 2015. Associations of prodynorphin sequence variation with alcohol dependence and related traits are phenotype-specific and sex-dependent. Sci Rep 5:1–9. doi:10.1038/srep15670

Yuferov V, Fussell D, Laforge KS, Nielsen DA, Gordon D, Ho A, Leal SM, Ott J, Kreek MJ. 2004. Redefinition of the human kappa opioid receptor gene (OPRK1) structure and association of haplotypes with opiate addiction. Pharmacogenetics 14:793–804.

Zywiak WH, Stout RL, Trefry WB, Glasser I, Connors GJ, Maisto SA, Westerberg VS. 2006. Alcohol relapse repetition, gender, and predictive validity. J Subst Abuse Treat 30:349–353. doi:10.1016/j.jsat.2006.03.004

Zywiak WH, Westerberg VS, Connors GJ, Maisto SA. 2003. Exploratory findings from the Reasons for Drinking Questionnaire. J Subst Abuse Treat 25:287–292. doi:10.1016/S0740-5472(03)00118-1

## *ORAL PRESENTATION*

# Increased keratinase production by *Yarrowia lipolytica* ISF7

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**Abstract**

Keratin is a structural fibrillar protein that exists in the skin of the birds, reptiles and mammals. Keratinase is an important enzyme and takes place in degradation of keratinous wastes, especially poultry and slaughterhouse wastes. The main purpose of this study was to investigate keratinase production capability of *Yarrowia lipolytica* ISF7 strain and increase the enzyme production using Taguchi design for optimization. Some of the significant environmental conditions were employed for optimization progress. Initial pH, incubation temperature and time were selected as the most effective environmental conditions for keratinase production. The results showed that maximum keratinase activity could be obtained approximately 2-fold-higher (32.63 U/mL) using 10 as initial pH, 34 ˚C for incubation temperature and 4 days for incubation time when compared with the unoptimized experimental run (15.21 U/mL). *Y. lipolytica* ISF7 has an excellent keratinase production capacity and these results may be used for further investigations.

**Keywords:** keratinase, statistical optimization, yeast

## INTRODUCTION

Keratin is the main fibrillar protein commonly exists in the keratinous tissues of animals like skin of mammals, reptiles and birds (Scott & Untereiner, 2004; Zhang et al., 2022). The structure of the keratin has excellent durability and resistance against the traditional proteolytic enzymes, like pepsin and trypsin, and common chemical reagents (Gupta et al., 2006). There are two types of keratins occur in nature. Type I has an acidic structure and Type II has a neutral structure (Scott & Untereiner, 2004). Keratinase (EC 3.4.4.25) is an important enzyme that catalyses the hydrolysation of the keratin into soluble peptides or amino acids (Sharma & Kango, 2021; Vidmar & Vodovnik, 2018). World's rising population causes environmental pollution depending on the consumption of animal-derived food. Keratinous materials require particular elimination methods. Their specific structure needs different degradation techniques other than the organic wastes such as fruit peels, vegetables, etc. Microbial keratinases have been considered as being stable, active at alkaline pH value (Zhang et al., 2022). The poultry waste materials, like chicken feather, have been used for animal feeding. The feather meal is commonly used for protein source, however; the degradation techniques including high pressured cooking and milling that cause reduction of many nutritional values of the feather meal. On the other hand, the microbial keratinolytic digestion of the feather meal is more environmentally and causes less nutritional loss (Riffel et al., 2003).

Keratinase enzyme has commonly been used in waste management, especially slaughterhouse and poultry waste like, chicken feather, cattle hair, nails and horns. These waste materials can be evaluated as feedstock (Cai et al., 2022; Etemadian et al., 2021; Murray-Tortarolo & Jaramillo, 2020). Keratinase-based degradation of keratinous wastes is more environmentally friendly and sustainable process (Zhang et al., 2022). Various application scopes for keratinase enzymes include industrial waste water treatments, organic fertilizer production, cosmetic industry and leather finishing process (Cai et al., 2022).

Statistical optimization tools have many advantages like random designed experimental runs, being a part of full factorial design, quick scanning, etc. The basic optimization method is one variable at a time method is the most preferred classical optimization method. However, the interactions of the factors cannot be estimated or random combinations could not be investigated. In this context, statistical optimization tools such as response surface methodology and Taguchi design, have been interested. These statistical optimization designs present opportunity for rapid screening and percentage contribution of each factor on the progress (Farid et al., 2013; Tasar, 2020; Tasar, 2022). The aim of this study was to investigate the keratinase production ability of *Y. lipolytica* ISF7 strain using powdered ram horn as the nitrogen source and to increase the enzyme production using Taguchi design for optimization.

## MATERIALS AND METHODS

### Microorganism and medium

The chemicals were purchased from Sigma (USA) and Merck (Germany). The keratinase producing yeast *Y. lipolytica* ISF was obtained from our laboratory culture collection. The ram horn was obtained from local slaughterhouse and then they were milled and powdered. To prepare the yeast starter culture, the microorganism was maintained on potato dextrose agar slants at 4 ˚C and was cultured bimonthly. One loop-full of 24h-old *Y. lipolytica* ISF7 was inoculated in a flask containing 100 mL of potato dextrose broth. The flask was taken into an orbital shaking incubator and incubated at 30 ˚C at 200 rpm for 24 h. For keratinase production the medium was designated as following (g/l): 10 glucose, 3 powdered ram horn, 1.5 $KH_2PO_4$, 0.05 $MgSO_4.7H_2O$, 0.05 NaCl. The pH was adjusted to the selected value using 1 N NaOH or 1 N HCl and the flasks were autoclaved at 121 ˚C for 20 min. After cooling to room temperature, one millilitre of the yeast starter culture was used as inoculum material ($OD_{600}$=1,5) for each flask containing 50 mL of medium. Each experimental run was done in duplicate and the results were taken as the means.

### Enzyme assay

The modified colorimetric method was used for the detection of the keratinase activity (Gonzalo et al., 2020; Letourneau et al., 1998; Suntornsuk & Suntornsuk, 2003). Keratin azure was used as the solution and was prepared as following: 0.4 g of keratin azure was taken into 100 mL of 0.01 M (pH 7.5) Tris-HCl buffer. The reaction mixture contains 1 mL of enzyme source (crude enzyme, culture filtrate) and 1 mL of keratin azure solution. The tubes were incubated at 50 ˚C at 200 rpm for one hour. In the next step, the tubes were taken to the water bath at 90 ˚C and was boiled for 5 min. The reaction mixture was centrifugated at 5000 x g for 20 min. The supernatant was measured at 595 nm for the release of the azo dye. One-unit keratinase activity was determined as the enzyme amount that obtained 0.1 absorbance increase between the sample and the control at 595 nm in one hour under experimental conditions.

### Taguchi design

Three effective factors with three levels were chosen to optimize keratinase production by *Y. lipolytica* ISF7 (Table 1). Taguchi L9 orthogonal array was used in the current study. Three different quality characteristics are employed in Taguchi design of experiment methodology. The larger-the better, the nominal-the better and the smaller-the better criterions affect the experiments. The aim of this study was to increase the keratinase production by *Y. lipolytica* ISF7 strain, hence, the larger-the better criterion was preferred that the equation shown below:

$$S/N = -10 \log_{10} \left(1/n \sum_{i=1}^{n} 1/Yi^{2}\right)$$

In the equation, the signal to noise (S/N) ratio determines the performance statistics, n determines the repetition numbers and the Yi determines the performance value of the $i^{th}$ experiment. The S/N value is strongly wanted to be higher by the investigators (Jean & Tzeng, 2003). Taguchi method uses S/N ratio to interpret the results instead of the means (Tan et al., 2005; Tasar, 2020).

**Table 1.** Optimization parameters and selected levels

| Serial No | Factors | Level 1 | Level 2 | Level 3 |
|---|---|---|---|---|
| 1 | Initial pH | 9 | 10 | 11 |
| 2 | Temperature (˚C) | 32 | 34 | 36 |
| 3 | Time (d) | 2 | 3 | 4 |

The analysis of variance (ANOVA) test was preferred to evaluate the effects of each factor and indicate the optimal levels. Both of Taguchi design and ANOVA analysis were carried out using Minitab® 21.2 Software Programme (USA).

## RESULTS and DISCUSSION

The production of keratinase enzyme is strongly affected by the environmental conditions. The results were illustrated in Table 2. Different combinations of the factors caused variation on keratinase production. The maximum keratinase production was carried out at 10 pH, 34 ˚C and 4 days in the current study. The minimum enzyme activity was obtained from the first experimental design. The results showed that longer incubation time generally resulted in higher enzyme production.

Optimal pH value of an enzyme varies depending on the microorganism and the media. In a previous paper, the production pH, incubation temperature and incubation time were reported to be 9.0, 37 ˚C and 5 days, respectively for *Bacillus* sp. FK46 (Suntornsuk & Suntornsuk, 2003). On the other hand, *Bacillus pumilis* KS1

was reported as 5 pH, 40 ˚C and 3 days for keratinase production (Gupta and Ramnani, 2006). *Y. lipolytica* ISF7 strain showed alkaline keratinase production on the contrast of many different microorganisms (Suh & Lee, 2001)

**Table 2.** Taguchi L9 orthogonal array and keratinase activity and S/N ratios

| Exp. No. | pH | Temperature | Time | Keratinase (U/mL) | S/N |
|---|---|---|---|---|---|
| 1 | 9 | 32 | 2 | 15.21 | 23.6426 |
| 2 | 9 | 34 | 3 | 27.86 | 28.8996 |
| 3 | 9 | 36 | 4 | 25.67 | 28.1885 |
| 4 | 10 | 32 | 3 | 26.33 | 28.4090 |
| 5 | 10 | 34 | 4 | 32.63 | 30.2723 |
| 6 | 10 | 36 | 2 | 18.25 | 25.2253 |
| 7 | 11 | 32 | 4 | 22.17 | 26.9153 |
| 8 | 11 | 34 | 2 | 20.15 | 26.0855 |
| 9 | 11 | 36 | 3 | 17.35 | 24.7860 |

Type of microorganism cause different environmental conditions. In a previous study, *Bacillus subtilis* FTC02PR1 strain was employed for chicken feather meal degradation. The keratinolytic protease activity was found as optimum at 60 ˚C and pH stability was determined between 6-11, while the maximum activity was found at 8 pH value (Ferrareze et al., 2016). Feather degradation is important depending on their evaluation in animal feeding industry. Microbially enzymatic digestion of the feather meal is more environmentally friendly (Daroit & Brandelli, 2014). Three psychrotolerant bacteria were isolated from penguin feathers and were investigated for their keratinase production capability. *Lysobacter* sp. A03 showed keratinase activity optimum at 40 ˚C and 9.5 pH, *Chrysobacterium* sp. A17U had maximum keratinase activity at 40 ˚C and 8.5 pH and *Arthrobacter* sp. A08 had maximum keratinase at 40 ˚C and 8.0 pH (Pereira et al., 2014). Table 3 illustrates the response data for S/N ratios. Delta value showed the rank value of each Delta. Incubation time had the first rank for Delta value, following by temperature and pH, respectively.

**Table 3.** Response table for means.

| **Level** | **pH** | **Temperature** | **Time** |
|---|---|---|---|
| 1 | 23.48 | 21.91 | 17.87 |
| 2 | 26.41 | 27.45 | 25.09 |
| 3 | 19.89 | 20.42 | 26.82 |
| Delta | 6.52 | 7.03 | 8.95 |
| Rank | 3 | 2 | 1 |

ANOVA analysis showed the rank values which were determined from the basis of the amplitude of S/N variation (Table 4). The analysis results showed that incubation time factor is more significant than the others ($P<0.05$). Taguchi DOE uses the ANOVA test to determine the optimum effects of the factors.

**Table 4.** Analysis of variance for means.

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| pH | 2 | 63.99 | 63.99 | 31.994 | 4.75 | 0.174 |
| Temperature | 2 | 82.28 | 82.28 | 41.138 | 6.11 | 0.141 |
| Time | 2 | 135.30 | 135.30 | 67.648 | 10.05 | 0.090 |
| Residual Error | 2 | 13.46 | 13.46 | 6.731 | | |
| Total | 8 | 295.02 | | | | |

DF: Degree of freedom; Seq SS: Sequential sum of square; Adj SS: Adjusted sum of square; Adj MS: Adjusted mean of squares; F: F value; P: P value.

Main effects plot for S/N ratios illustrated the optimal values for each factor (Figure 1). pH was found at 10.0, incubation temperature was found as 34 ˚C and the incubation time was found as 4 days as optimal values. The maximum keratinase activity was found as 32.63 U/mL from the 5$^{th}$ experimental run while the minimum activity was obtained from the first design as 15.21 U/mL

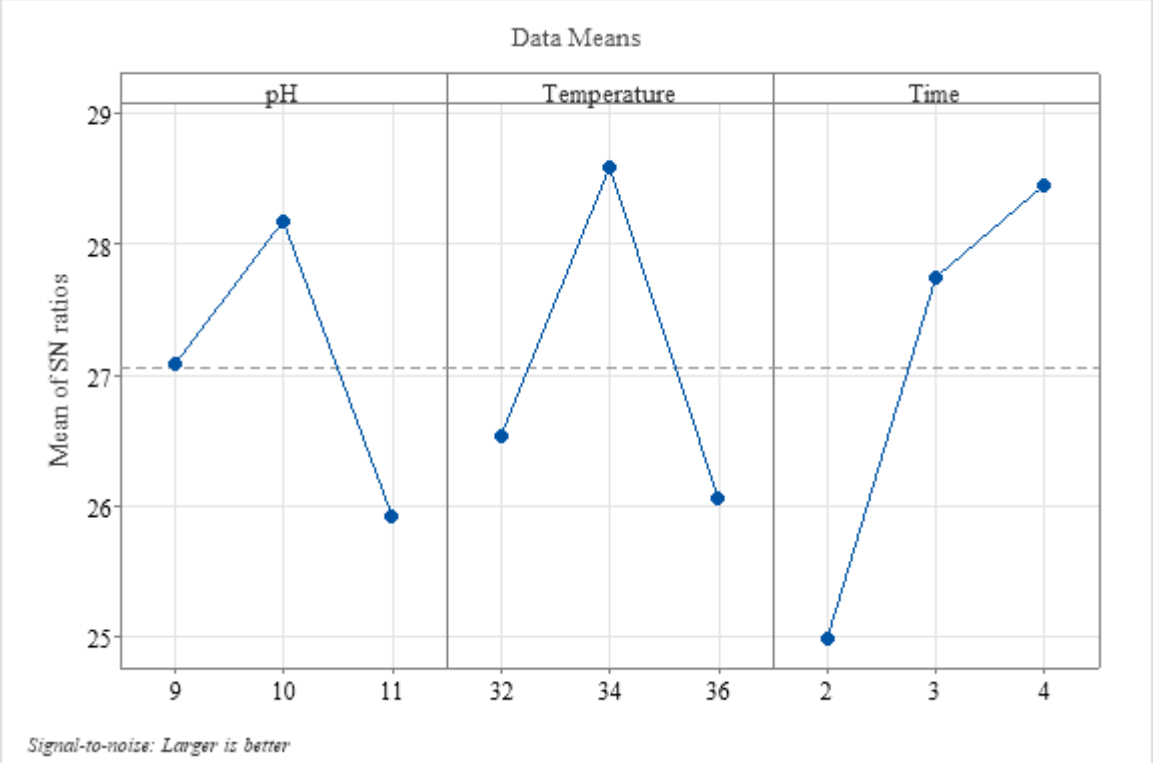


**Figure 1.** Main effects plot

## CONCLUSION

Keratinase has been used in elimination of keratinous waste materials. Enzymatic reactions have great advantages against heavy chemical applications. Keratinous waste materials occur all over the world and can be evaluated and recycled using keratinase enzyme to various useful materials such as animal feed. nitrogen source for microbial fermentation. In the current study keratinase production ability of an oleaginous yeast *Y. lipolytica* ISF7 strain was investigated. Furthermore. Taguchi design of experiment was preferred for optimization of environmental conditions. The maximum keratinase activity was obtained as 32.63 U/mL. *Y. lipolytica* ISF7 found as an excellent keratinase producer microorganism. Further studies may include the characterization of this enzyme and different studies can be carried out using other keratinous materials.

## REFERENCES

Cai G, Moffitt K, Navone L, Zhang Z, Robins K, & Speight R 2022. Valorisation of keratin waste: Controlled pretreatment enhances enzymatic production of antioxidant peptides. Journal of Environmental Management, 301(July 2021), 113945. Retrieved from https://doi.org/10.1016/j.jenvman.2021.113945

Daroit DJ, & Brandelli A 2014. A current assessment on the production of bacterial keratinases. Critical Reviews in Biotechnology, 34(4), 372–384. Retrieved from https://doi.org/10.3109/07388551.2013.794768

Etemadian Y, Ghaemi V, Shaviklo AR, Pourashouri P, Sadeghi Mahoonak AR, & Rafipour F 2021. Development of animal/ plant-based protein hydrolysate and its application in food, feed and nutraceutical industries: State of the art. Journal of Cleaner Production, 278. Retrieved from https://doi.org/10.1016/j.jclepro.2020.123219

Farid MA, Ghoneimy EA, El-Khawaga MA, Negm-Eldein A, & Awad GEA 2013. Statistical optimization of glucose oxidase production from *Aspergillus niger* NRC9 under submerged fermentation using response surface methodology. Annals of Microbiology, 63(2), 523–531. Retrieved from https://doi.org/10.1007/s13213-012-0497-5

Ferrareze PAG, Correa APF, & Brandelli A 2016. Purification and characterization of a keratinolytic protease produced by probiotic *Bacillus subtilis*. Biocatalysis and Agricultural Biotechnology, 7, 102–109. Retrieved from https://doi.org/10.1016/j.bcab.2016.05.009

Gonzalo M, Espersen R, Al-Soud WA, Cristiano Falco F, Hägglund P, Sørensen SJ, Jacquiod S 2020. Azo dying of α-keratin material improves microbial keratinase screening and standardization. Microbial Biotechnology, 13(4), 984–996. Retrieved from https://doi.org/10.1111/1751-7915.13541

Gupta R, Ramnani P 2006. Microbial keratinases and their prospective applications: an overview. Applied Microbiology and Biotechnology, 70, 21–33.

Jean MD, & Tzeng YF 2003. Use of Taguchi methods and multiple regression analysis for optimal process development of high energy electron beam case hardening of cast iron. Surface Engineering, 19(2), 150–156. Retrieved from https://doi.org/10.1179/026708403225002496

Letourneau F, Soussotte V, Bressollier P, Branland PV, & B 1998. Keratinolytic activity of *Streptomyces* sp. SK1-02: a new isolated strain. Letters in Applied Microbiology, (26), 77–80.

Murray-Tortarolo GN, & Jaramillo VJ 2020. Precipitation extremes in recent decades impact cattle populations at the global and national scales. Science of the Total Environment, 736, 139557. Retrieved from https://doi.org/10.1016/j.scitotenv.2020.139557

Pereira JQ, Lopes FC, Petry MV, Medina LF da C, & Brandelli A 2014. Isolation of three novel Antarctic psychrotolerant feather-degrading bacteria and partial purification of keratinolytic enzyme from *Lysobacter* sp. A03. International Biodeterioration and Biodegradation, 88, 1–7. Retrieved from https://doi.org/10.1016/j.ibiod.2013.11.012

Riffel A, Ortolan S, & Brandelli A 2003. De-hairing activity of extracellular proteases produced by keratinolytic bacteria. Journal of Chemical Technology and Biotechnology, 78(8), 855–859. Retrieved from https://doi.org/10.1002/jctb.828

Scott JA, & Untereiner WA 2004. Determination of keratin degradation by fungi using keratin azure. Medical Mycology, 42(3), 239–246. Retrieved from https://doi.org/10.1080/13693780310001644680

Sharma I, & Kango N 2021. Production and characterization of keratinase by *Ochrobactrum intermedium* for feather keratin utilization. International Journal of Biological Macromolecules, 166, 1046–1056. Retrieved from https://doi.org/10.1016/j.ijbiomac.2020.10.260

Suh H, & Lee HK 2001. Characterization of a keratinolytic serine protease from *Bacillus subtilis* KS-1. Journal of Protein Chemistry, 20, 165–169.

Suntornsuk W, & Suntornsuk L 2003. Feather degradation by *Bacillus* sp. FK 46 in submerged cultivation. Bioresource Technology, 86(3), 239–243. Retrieved from https://doi.org/10.1016/S0960-8524(02)00177-3

Tan O, Zaimoglu AS, Hinislioglu S, & Altun S 2005. Taguchi approach for optimization of the bleeding on cement-based grouts. Tunnelling and Underground Space Technology, 20(2), 167–173. Retrieved from https://doi.org/10.1016/j.tust.2004.08.004

Tasar ÖC 2020. Inulinase production capability of a promising medicinal plant: *Inula viscosa*. Commagene Journal of Biology, 4, 67–73. Retrieved from https://doi.org/10.31594/commagene.747618

Tasar OC 2022. Glucose oxidase production using a medicinal plant: *Inula viscosa* and optimization with Taguchi DOE. Journal of Food Processing and Preservation, 46(3), e16375. Retrieved from https://doi.org/10.1111/jfpp.16375

Vidmar B, & Vodovnik M 2018. Microbial keratinases: Enzymes with promising biotechnological applications. Food Technology and Biotechnology, 56(3), 312–328. Retrieved from https://doi.org/10.17113/ftb.56.03.18.5658

Zhang J, Su C, Kong X, Gong JS, Liu Y, Li H, Shi JS 2022. Directed evolution driving the generation of an efficient keratinase variant to facilitate the feather degradation. Bioresources and Bioprocessing, 9(1), 38. Retrieved from https://doi.org/10.1186/s40643-022-00524-4

Zhang RX, Wu ZW, Cui HY, Chai YN, Hua CW, Wang P, Yang TY 2022. Production of surfactant-stable keratinase from *Bacillus cereus* YQ15 and its application as detergent additive. BMC Biotechnology, 22(1), 26. Retrieved from https://doi.org/10.1186/s12896-022-00757-3

## ORAL PRESENTATION

# Gaziantep'te kurutmalık olarak tüketilen bazı sebzelerde (bamya, acı ve tatlı biber, kabak, patlıcan) total antioksidan/oksidan seviyenin araştırılması

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## Özet

Değişen dünya düzeni, küresel olarak artan insan nüfusu paralelinde iyi yaşam beklentilerini de beraberinde getirmiştir. Artan bu beklentiler kısıtlı olan kaynakların ve enerjilerin doğru kullanılmasını gerekli kılmaktadır. Bu anlamda mevsiminde taze olarak tüketilen sebzelerin kurutularak değerlendirilmesi ve böylece her mevsim tüketilebilir olması hem ekonomik hem duyusal bakımdan büyük önem taşımaktadır. Taze sebzeler yüksek miktarda içerdikleri antioksidan bileşikler ve polifenolikler sayesinde kardiyovasküler hastalıklar ve bulaşıcı olmayan hastalıklar başta olmak üzere birçok hastalığın önlenmesinde etkin rol oynamaktadır. Ozmotik, konvektif, akışkan yataklı, mikrodalga, vakum ve dondurarak kurutma işlemleri kurutma metodolojilerine birkaç örnektir. Gerçek kurutma aşamasından önce sebzelerin seçildiği, yıkandığı, ara sıra soyulduğu, çekirdeklerinin ayıklandığı ve küçültüldüğü bir ön işlem aşaması vardır. Sanayileşmiş kurutma işlemleri gıda kalitesinin korunmasını en üst düzeye çıkarmak için tasarlanmasına rağmen, ön işlem ve kurutmanın her ikisi de bazı besinlere, özellikle flavonoidler, karotenoidler ve vitaminler gibi ısıya dayanıklı bileşenlere zarar verme potansiyeline sahiptir. Kurutma esnasında taze sebzelerin bünyesindeki su miktarı %80-95'ten %10-20'lere kadar düşerken tat, renk ve besin değeri de bir miktar değişmektedir.
Bu çalışmanın amacı Gaziantep bölgesinde oldukça sık tüketilen kurutmalık sebzelerin (bamya, kabak, patlıcan, acı ve tatlı biber) total antioksidan/oksidan seviyenin araştırılmasıdır. Çalışmada kullanılan kurutmalık sebzeler, piyasada satılan tüketime hazır ürünlerden rastgele seçildi. Kurutmalık sebzelerin metanolde hazırlanan ekstraktlarında total antioksidan/oksidan seviyeleri ticari kitlerle spektrofotometrede analiz edildi. Bulgulara bakıldığında, kurutmalık bamyanın total antioksidan seviyesi en yüksek bulunurken (6.568±0.385) kurutmalık tatlı biberin total antioksidan seviyesi en düşük bulundu. Total oksidan seviyelerine bakıldığında ise en yüksek miktara sahip sebze kurutmalık patlıcanken (14.645±0.420) en düşük total oksidan seviyenin kurutmalık bamyada olduğu tespit edildi. (13.972±0.391). Son olarak, kurutmalık kabağın oksidatif stres indeksi (0.248±0.022) en yüksek bulunurken, kurutmalık bamyanın oksidatif stres indeksi (0.212±0.008) en düşük bulundu.
Sonuç olarak, halk tarafından oldukça sık ve sevilerek tüketilen kurutmalık sebzelerin total antioksidan/oksidan seviyeleri birbirinden farklı çıksa da kurutulan her sebzenin antioksidan ve oksidan seviyesini koruduğu kanaatini taşımaktayız.

**Anahtar Kelimeler:** Kurutmalık sebze, total antioksidan seviye, total oksidan seviye

# Investigation of total antioxidant/oxidant levels in some vegetables (okra, hot and sweet pepper, zucchini, eggplant,) consumed for drying in Gaziantep

## Abstract

The changing world order has brought good life expectations in parallel with the increasing human population globally. These increasing expectations necessitate the correct use of limited resources and energies. In this sense, it is of great importance both economically and emotionally that the vegetables that are consumed fresh in the season are used by drying and thus can be consumed in all seasons. Fresh vegetables play an active role in the prevention of many diseases, especially cardiovascular diseases and non-communicable diseases, thanks to the antioxidant compounds and polyphenolics they contain in high amounts. Osmotic, convective, fluidized bed, microwave, vacuum and freeze drying processes are a few examples of drying methodologies. Before the actual drying stage, there is a pre-processing stage in which the vegetables are selected, washed, occasionally peeled, seeded, and shrunk. Although industrialized drying processes are designed to maximize the

preservation of food quality, both pretreatment and drying have the potential to damage some nutrients, particularly heat-stable ingredients such as flavonoids, carotenoids and vitamins. During drying, the amount of water in the fresh vegetables decreases from 80-95% to 10-20%, while the taste, color and nutritional value change slightly.
The aim of this study is to investigate the total antioxidant/oxidant levels of dried vegetables (okra, zucchini, eggplant, hot and sweet pepper) that are consumed quite frequently in Gaziantep region. Dry vegetables used in the study were randomly selected from ready-to-eat products sold in the market. Total antioxidant/oxidant levels in the extracts of dried vegetables prepared in methanol were analyzed by spectrophotometer with commercial kits. According to the findings, the total antioxidant level of dried okra was found to be the highest (6.568±0.385), while the total antioxidant level of dried sweet pepper was found to be the lowest. When the total oxidant levels were examined, it was determined that the vegetable with the highest amount was dried eggplant (14.645±0.420), while the lowest total oxidant level was found in dried okra. (13,972±0.391). Finally, the oxidative stress index of dried zucchini was found the highest (0.248±0.022), while the oxidative stress index of dried okra (0.212±0.008) was found to be the lowest.

**Keywords:** Dry vegetables, total antioxidant level, total oxidant level

## GİRİŞ

Kurutma işlemi, çok eski çağlardan beri meyve ve sebzelerin muhafaza edilmesi için kullanılan bir muhafaza yöntemidir. Kurutmadaki temel amaç gıdaların tüm yıl boyunca mevcudiyetini sağlayarak, tüketime hazır hale getirmektir. Geleneksel kurutma işlemi genelde güneş enerjisi ile yapıldığı için oldukça doğal ve basittir fakat sanayi tipi kurutmada çok fazla ekipman kullanıldığından ve kurutma parametreleri sürecin her aşamasında dikkatlice incelenip optimize edildiğinden daha sofistike ve karmaşıktır. Kurutma sadece ürünü korumakla kalmaz, aynı zamanda baharatlarda, tıbbi bitkilerde, şifalı bitkilerde, biyoaktif enzimlerde ve kurutma sırasında katma değerli bileşikler üretilebilen kuruyemişlerde olumlu bir etki sağlar. Genel olarak tüketime hazır olan ürünler kuru meyvelerken, tüketilmeden önce haşlama, ısıtma, pişirme gibi rehidre edilerek tüketilebilen gıdalar ise kuru sebzelerdir (Rahman 2007; Szychowski ve ark., 2018).
Besinlerdeki istenmeyen mikroorganizmaların gelişmesine neden olan faktörlerin başında gelen su, besinlerin bünyesinde bağlı ve serbest olmak üzere iki formda bulunur ve besinler için önemli olan bağlı sudur. Bağlı su, besinin katı kısmının mikro yapısında tutulan sıvı ile karakterizedir. Bağlı suyun fazlalığı ile oluşan su ise serbest formdur. Bağlı su, katı bir matriste tutulan sıvı bir çözelti ile temsil edilir ve taze gıdaların termal kurutulması sırasında karmaşık işlemlerin bir arada olmasına neden olur. İlk olarak, sıcak kurutma ajanından taze ürüne enerji aktarılır. İkinci olarak, serbest su buharlaşır ve sonunda, hücresel yapı içinde sınırlanan, difüzyona ve dolayısıyla göçe maruz kalan su partikülleri, suyun nihai olarak buharlaştığı ürün yüzeyine aktarılır (Setty ve Murthy 2003).
Kurutmanın temel ve basit mantığı şu şekildedir: önce gıda maddesinin yapısından su buharlaştırılır, ardından bu buhar ürünün yüzeyinden uzaklaştırılır. Gıdanın yapısında bulunan mevcut suyun kaybı ile su aktivitesi mikroorganizmaların üreyebileceği değerin altına düştüğü için bakteri, küf ve mayaların neden olabileceği bozulmalar önlenir ve böylece gıdaların raf ömrü uzar. Ayrıca, besinlerin tat ve doku özellikleri değiştirilerek, daha sağlıklı alternatif atıştırmalıklar için yeni nesil ürünler elde edilir (Sehrawat ve ark., 2018).
Fiziksel ve kimyasal maddelerin DNA üzerindeki genotoksik etkilerini minimum seviyede tutabilmek için son yıllarda doğal bitkisel ürünlerin antigenotoksik etkilerinin araştırılması, doğal antioksidanların önemini arttırmıştır. İnsanlar maruz kaldıkları mutajenik maddelerin olumsuz etkilerine vücutlarında bulunan doğal antioksidanlar sayesinde direnirler (Uluman ve Kılıçle 2020). Besinlerde bulunan antioksidanlar, insan vücudunda oluşan serbest oksijen radikallerinin veya serbest nitrojen radikallerinin olumsuz etkilerini azaltabilen maddelerdir. Gıdaların antioksidan içerikleri ve antioksidanların biyoyararlanımları gıda maddesinin cinsine, hasat zamanına, hasat yöntemine, yetiştiği çevre şartlarına, depolamaya, hazırlanma koşullarına göre değişmektedir (Kasım ve Kasım, 2019). Sebze ve meyveler içerdikleri biyoaktif bileşikler sayesinde "süper antioksidanlar" olarak bilinmektedir (Deveci ve ark., 2016; Mattioli ve ark., 2018). Antioksidanların kan basıncını veya kan glikozunu dengeleme, antikanserojen, otoimmün destekleme, antibakteriyal, antifungal, antiviral, kolesterol düşürücü ve anti-trombotik etkileri vardır. Yapılan çalışmalarda, yeterli miktarda meyve ve sebze tüketiminin kardiyovasküler hastalıklar, kanser, diyabet, alzheimer ve parkinson gibi bulaşıcı olmayan hastalıkları ve yaşa bağlı vücut fonksiyonlarının azalması ile karakterize kronik hastalıkları önlemede önemli rol oynadığı kanıtlanmıştır. Sebze ve meyvelerin sağlık üzerindeki olumlu etkilerinin ortaya konulmasından sonra bu besinler hem yetiştikleri mevsimde taze olarak hem de mevsimlerinin dışında kurutma, dondurma gibi işlemlere maruz bırakılarak tüketilmektedir (Ülger ve ark.,2018).

Ülkemizde de meyve ve sebzeler mevsimlerinin dışında genellikle güneşte kurutularak veya derin dondurucularda dondurularak muhafaza edilmektedir. Özellikle ülkemizin güney ve doğu anadolu bölgesinde kurutmalıklar daha çok tüketilmektedir. Bu çalışmada, Gaziantep yöresinde doğal yöntemlerle kurutulmuş olan sebzelerin total antioksidan seviyesi ve total oksidan seviyesinin belirlenmesi amaçlanmıştır.

## MATERYAL VE METOT

### Kurutmalık sebzelerin eldesi

Çalışmada materyal olarak Gaziantep ilinde bulunan baharatçılardan 2021 yılının mahsulü kurutmalık sebzeler (bamya, acı ve tatlı biber, kabak, patlıcan) kullanıldı. Daha sonra bu kurutmalık sebze örnekleri öğütülerek toz haline getirildi. Toz haline getirilen kurutmalık sebze örnekleri ışık ve nemden korunacak şekilde koyu cam şişelerde saklandı.

### Kurutmalık Sebze Ekstraktlarının Hazırlanması

Toz halinde şişelerde saklanan kurutmalık sebze (bamya, acı ve tatlı biber, kabak, patlıcan) örneklerinden 10 g tartılıp, üzerine 500 ml metanol ($CH_3OH$) eklendi ve 60ºC'de yaklaşık 18 saat süreyle Soksalet cihazında ekstraksiyon sağlandı. Elde edilen ekstrakttan 48ºC'de vakumlu evaporatörde evaporasyon yöntemiyle metanol uzaklaştırıldı. Hazırlanan sebze ekstratları analiz edilinceye kadar -18ºC'de muhafaza edildi (Heleno ve ark., 2016).

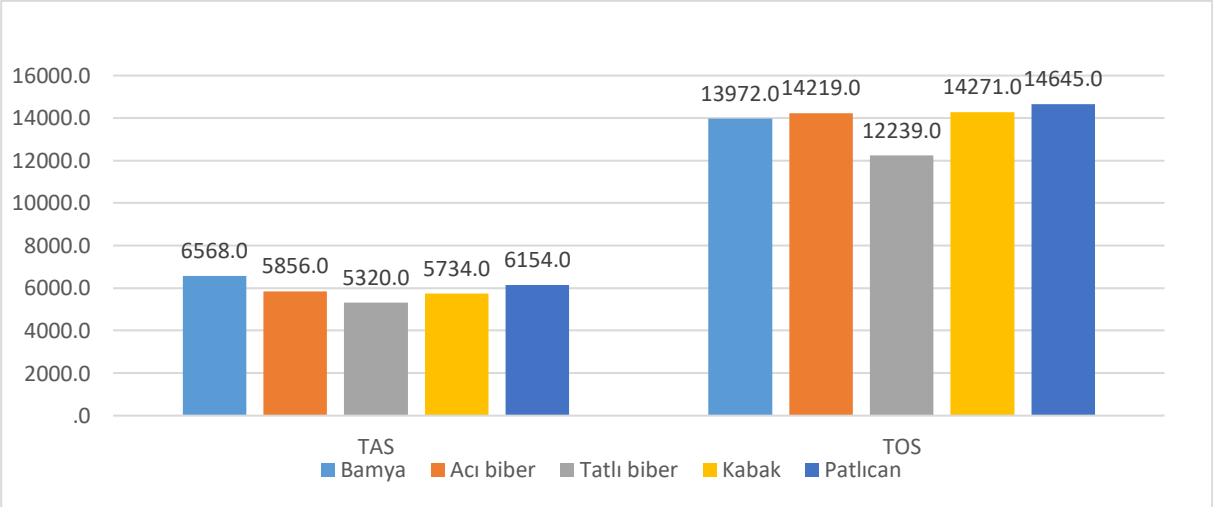
### Total Antioksidan/Oksidan Seviye Analizi

Çözücü olarak metanol kullanılarak hazırlanan sebze ekstratlarının total antioksidan seviyesi (TAS), Erel (2004) tarafından geliştirilen Total Antioxidant Status kiti (Rel Assay Kit Diagnostics, Türkiye) ile spektrofotometrik olarak analiz edildi. TAS değeri mmol Trolox eşdeğeri/L olarak hesaplandı. Sebze ekstratlarının total oksidan seviyesi (TOS) ise Erel (2005) tarafından geliştirilen Total Oxidant Status kiti (Rel Assay Kit Diagnostics, Türkiye) ile spektrofotometrik olarak analiz edildi. TOS değeri µmol $H_2O_2$ eşdeğeri/L olarak hesaplandı. TAS ve TOS analizleri üç tekrarlı yapılarak sonuçların ortalaması alındı. Elde edilen TOS değerinin TAS değerine oranının yüzde derecesi olarak ifade edilen oksidatif stres indeksi (OSİ) değeri ise TAS ve TOS sonuçlarına göre hesaplandı. OSİ değerleri hesaplanırken, TAS sonucu birimindeki mmol değeri TOS sonucundaki gibi µmol birimine çevrildi. OSİ değeri aşağıdaki formüle göre hesaplandı (Eren ve ark., 2015).

OSİ= [ (TOS/TAS) x 100 ]

## BULGULAR

Bu çalışmada Gaziantep ilinde satılan bazı kuru sebzelerin total antioksidan/oksidan seviyesi ölçülmüştür. Çalışma sonucunda elde edilen bulgular Şekil 1 ve Tablo 1'de açıkça gösterilmiştir.




**Şekil 1.** Kurutmalık sebzelerin TAS ve TOS değerleri

**Tablo 1.** Kurutmalık sebzelerin TAS ve TOS değerleri

| Parametreler<br>Kurutmalık Örnekleri | TAS<br>(mmol Trolox equiv./L) | TOS<br>(µmol $H_2O_2$ Equiv./L) | OSİ<br>(Arbitrary Unit) |
|---|---|---|---|
| **Kurutmalık bamya** | 6.568 ± 0.385 | 13.972 ± 0.391 | 0.212 ± 0.008 |
| **Kurutmalık acı biber** | 5.856 ± 0.420 | 14.219 ± 0.542 | 0.243 ± 0.020 |
| **Kurutmalık tatlı biber** | 5.320 ± 0.260 | 12.239 ± 0.418 | 0.230 ± 0.016 |
| **Kurutmalık kabak** | 5.734 ± 0.365 | 14.271 ± 0.395 | 0.248 ± 0.022 |
| **Kurutmalık patlıcan** | 6.154 ± 0.223 | 14.645 ± 0.420 | 0.238 ± 0.083 |

Şekil 1 ve Tablo 1'de belirtildiği gibi kurutmalık bamyanın total antioksidan seviyesi en yüksek bulunurken (6.568±0.385) kurutmalık tatlı biberin total antioksidan seviyesi en düşük bulundu. Total oksidan seviyelerine bakıldığında ise en yüksek miktara sahip sebze kurutmalık patlıcanken (14.645±0.420) en düşük total oksidan seviyenin kurutmalık bamyada olduğu tespit edildi. (13.972±0.391). Son olarak, kurutmalık kabağın oksidatif stres indeksi (0.248±0.022) en yüksek bulunurken, kurutmalık bamyanın oksidatif stres indeksi (0.212±0.008) en düşük bulundu.

## TARTIŞMA

Oksidatif reaksiyonlar, hücrelere zarar veren zincir reaksiyonlarını başlatarak serbest radikaller üreten tepkimelerdir. Antioksidanlar ise oluşan bu serbest radikalleri uzaklaştırarak zincir reaksiyonlarını sonlandıran böylece oksidasyonu yavaşlatan veya önleyen maddelerdir (Landete 2013). Meyve ve sebzeler hem yüksek antioksidan aktiviteye sahip olmaları hem de iyi bir antioksidan karışımı ve kombinasyonunu temsil etmeleri açısından önemli antioksidan kaynakları arasında sayılmaktadır (Koca ve Karadeniz 2003). Gıdalarda kullanılan yapay antioksidanların karsinojenik olabileceği yönündeki endişeler sağlık açısından daha güvenilir olan doğal antioksidanlara karşı ilgiyi artırmaktadır (Llorach ve ark., 2002). Kurutulmuş sebzelerde sıklıkla araştırılan bir kalite parametresi olan antioksidan aktivite, esas olarak vitamin ve polifenollerin varlığı ile ilgilidir. Kurutulmuş birçok sebze ile ilgili yapılmış çalışmalar mevcut iken bamya, biber, kabak ve patlıcan ile yapılmış çalışma sayısı oldukça azdır. Bu sebzelerin total antioksidan ve oksidan içeriğinin belirlenmesine yönelik kısıtlı sayıda çalışma olduğu için yapılan bu çalışmanın literatüre önemli katkı sağlayacağı düşünülmektedir.

Xu ve arkadaşlarının yaptığı bir çalışmada farklı yöntemlerle (dondurarak kurutma, güneşte kurutma, fırında kurutma ve mikrodalgada kurutma) kurutulan bamyaların antioksidan seviyeleri değerlendirilmiştir. Analiz edilen tüm bamya örneklerinde antioksidan seviyenin yüksek olduğu saptanmıştır (Xu ve ark., 2020). Bunun nedeni, bamyada bulunan fenolik bileşiklerin ısıl işlemlerle serbest bırakıldığında antioksidan görevi görmesinden kaynaklanmaktadır (Kim ve ark., 2006). Benzer şekilde bizim çalışmamızda da güneşte kurutulan bamyalarda total antioksidan seviyenin olduğu hatta güneşte kurutulan diğer örneklerle (kurutmalık acı biber, kurutmalık tatlı biber, kurutmalık kabak ve kurutmalık patlıcan) kıyaslandığında kurutmalık bamyanın total antioksidan seviyesinin diğer örneklerden yüksek olduğu belirlenmiştir. Total oksidan seviyesine bakıldığında ise bamyanınkinin kurutmalık acı biber, kurutmalık kabak ve kurutmalık patlıcandan düşük iken kurutmalık tatlı biberden fazla olduğu saptanmıştır.

Biberler, başta karotenoidler olmak üzere birçok biyoaktif fitokimyasal açısından zengin oldukları için mükemmel bir antioksidan kaynağı olarak kabul edilmektedir. Diyette düzenli olarak tüketilen bu maddelerin başta kanser ve kardiyovasküler hastalıklar olmak üzere birçok hastalığı önlediği bilinmektedir (Kaur ve Kapoor 2001). Karotenoidler, biberlere sarı, kırmızı ve yeşil rengini veren maddelerdir. Kırmızı biberdeki ana karotenoid kapsantindir. Genel olarak sarı-turuncu biberlerdeki karotenoidler α ve β karoten, zeaksantin, lutein ve β-kriptoksantindir (Pugliese ve ark., 2014). Turuncu renkli biberler zengin bir zeaksantin, violaksantin ve β karoten kaynağıdır; sarı biberlerde ise violaksantin, antheraksantin, lutein ve zeaksantin baskın karotenoidlerdir (Guzman ve ark., 2010). Yapılan bir çalışmada, farklı türdeki biberlerin antioksidan özellikleri değerlendirilmiştir. Çalışmadan elde edilen bulgulara bakıldığında, kırmızı ve chili (acı) biberlerin antioksidan özelliğinin yüksek olduğu saptanmıştır. Bu durumun nedeninin acı biberlerdeki karotenoid içeriğinin yüksek olmasından kaynaklandığı düşünülmektedir. Benzer şekilde bizim çalışmamızda da acı biberlerin total antioksidan seviyesi tatlı biberlerinkinden yüksek bulunmuştur. Fakat biberler arasındaki karotenoid içeriğindeki farklılıklar, genotip, olgunluk aşaması ve kurutma işlemlerinden etkilendiğinden dolayı bizim çalışmamızda bu farklılığın nedenini tam olarak saptayabilmek için daha ileri analizlere ihtiyaç vardır. Ayrıca çalışmamızda total antioksidan seviyeye paralel olarak acı biberlerin total oksidan seviyesinin tatlı biberlerin total oksidan seviyesinden yüksek olduğu saptanmıştır.

Sebzeler arasında makro ve mikro besin içeriği bakımından en zengin olan sebze kabaktır. Özellikle beta-karoten, fenolik bileşikler ve C vitamini gibi doğal antioksidan miktarı yeşil kabaklarda oldukça fazladır.

Ancak kabak, dilimlendikten sonra sertlik kaybı, esmerleşme ve çürüme nedeniyle hızla bozulan bir sebze olduğu için raf ömrü 1-2 gün ile sınırlıdır. Bu nedenle, kabak mevsiminde taze olarak tüketilecekse hemen tüketilmelidir. Mevsim dışı tüketilecekse muhafaza için çeşitli kurutma veya dondurma yöntemleri kullanılmalıdır (Eissa ve ark., 2013). Kabağın kurutulması için en sık başvurulan yöntem açık havada güneşte kurutmaktır. Her ne kadar bu yöntem en çok kullanılan olsa da hangi muhafaza yönteminin kabağın besin içerikleri üzerindeki etkisi için en ideal olduğu hakkında yeterli çalışma bulunmamaktadır. Martínez-Valdivieso ve arkadaşlarının taze kabaklarla yaptığı bir çalışmada, hem sarı hem yeşil kabakların antioksidan içeriğinin yüksek olduğu saptanmıştır. Kabakta bulunan lutein, β-karoten, zeaksantin ve dehidroaskorbik asit gibi aktif antioksidan bileşikler insan beslenmesini olumlu yönde etkilemektedir (Martínez-Valdivieso ve ark., 2017). Yapılan geniş literatür taramalarımız sonucu kurutulmuş kabakların antioksidan özelliğini değerlendiren herhangi bir çalışmaya rastlayamadık. Bu yönüyle çalışmamızın literature katkı sağlayacağını düşünmekteyiz.

Güneşte kurutma, patlıcanın uzun süre muhafaza edilmesi için kullanılan en geleneksel yöntemdir. Ayrıca sebzeler arasında en çok kurutularak tüketilen sebze de patlıcandır. Tüm bu özelliklerine rağmen kuru patlıcanın besinsel özellikleri ile ilgili yapılmış bir çalışma bulunmamaktadır. Literatürde daha önce yapılmış böyle bir çalışma bulunmadığından bu çalışmanın alana önemli katkılar sağlayacağı düşünülmektedir. Bu çalışmanın sonuçlarına bakıldığında ise patlıcan total antioksidan seviyesi sıralamasında kurutulmuş bamyadan sonra ikinci sırada gelmektedir. Total oksidan seviyesi bakımından ise ilk sırada yer almaktadır.

## SONUÇ

Sonuç olarak yapılan bu çalışmada, beş farklı kurutmalık sebzenin total antioksidan ve oksidan içeriği incelenmiştir. İncelenen sebzelerin tamamının antioksidan seviyesinin yüksek olduğu belirlenmiştir. Kurutmalık bamyanın total antioksidan içeriği en yüksek bulunurken kurutmalık tatlı biberin total antioksidan içeriği en düşük bulunmuştur. Halk tarafından oldukça sık ve sevilerek tüketilen bu kurutmalık sebzelerin total antioksidan/oksidan seviyeleri birbirinden farklı çıksa da kurutulan her sebzenin antioksidan ve oksidan seviyesini koruduğu kanaatini taşımaktayız.

## KAYNAKLAR

Alçay AÜ, Bostan K, Dinçel E, Yalçın S 2015. Dried foods from middle asia to Anatolia. Journal of Anadolu Bil Vocational School of Higher Education, 37: 83-93.

Deveci HA, Nur G, Kırpık MA, Harmankaya A, Yıldız Y 2016. Fenolik bileşik içeren bitkisel antioksidanlar. Kafkas Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 9(1): 26-32.

Eissa HA, GF Bareh, AA Ibrahim, RK Moawad, HS Ali 2013. The effect of different drying methods on the nutrients and non-nutrients composition of zucchini (green squash) rings. Journal of Applied Sciences Research, 9: 5380-89.

Guzman I, Hamby S, Romero J, Bosland PW 2010. Variability of carotenoid biosynthesis in orange colored *Capsicum spp.* Plant Science, 179(1-2): 49-59.

Heleno SA, Diz P, Prieto MA, Barros L, Rodrigues A, Barreiro MF, Ferreira IC 2016. Optimization of ultrasound-assisted extraction to obtain mycosterols from Agaricus bisporus L. by response surface methodology and comparison with conventional Soxhlet extraction. Food chemistry, 197: 1054-1063.

Erel O 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry, 37: 277-285.

Erel O 2005. A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry, 38: 1103-1111.

Eren Y, Dirik E, Neselioglu S, Erel O 2015. Oxidative Stress and Decreased Thiol Level in Patients With Migraine. Cross-Sectional Study. Acta Neurologica Belgica, 115(4): 643-649.

Kasım R, Kasım MU 2019. Renkli meyve ve sebzelerin bileşimi ve insan sağlığı açısından önemi. 3: 31.

Kaur C, Harish CK 2001. Antioxidants in fruits and vegetables–the Millennium's Health. International Journal of Food Science & Technology, 36(7): 703-25.

Kim SY, Jeong SM, Park WP, Nam KC, Ahn DU, Lee SC 2006. Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. Food Chemistry, 97(3): 472-79.

Koca N, Karadeniz F 2003. Serbest radikal oluşum mekanizmaları ve vücuttaki antioksidan savunma

sistemleri. Gıda Mühendisliği Dergisi, 16: 32-37.

Landete JM 2013. Dietary intake of natural antioxidants: vitamins and polyphenols. Critical Reviews in Food Science and Nutrition, 53(7): 706-21.

Llorach R, Juan CE, Tomas-Barberan FA, Ferreres F 2002. Artichoke *(Cynara Scolymus L.)* byproducts as a potential source of health-promoting antioxidant phenolics. Journal of Agricultural and Food Chemistry, 50(12): 3458-64.

Martínez-Valdivieso D, Font R, Fernandez-Bedmar Z, Merinas-Amo T, Gomez P, Alonso-Moraga A, Del Rio-Celestino M 2017. Role of zucchini and its distinctive components in the modulation of degenerative processes: genotoxicity, anti-genotoxicity, cytotoxicity and apoptotic effects. Nutrients, 9(7): 755.

Mattioli R, Mosca L, Sanchez-Lamar A, Tempera I, Hausmann R 2018. Natural bioactive compounds acting against oxidative stress in chronic, degenerative, and infectious diseases. Oxidative Medicine and Cellular Longevity.

Alessandro P, O'Callaghan Y, Tundis R, Galvin K, Menichini F, O'Brien N, Loizzo MR 2014. In vitro assessment of the bioaccessibility of carotenoids from sun-dried chilli peppers. Plant Foods for Human Nutrition, 69(1): 8-17.

Rahman MS 2007. Handbook of Food Preservation. CRC press.

Sehrawat R, Nema PK, Kaur BP 2018. Quality evaluation and drying characteristics of mango cubes dried using low-pressure superheated steam, vacuum and hot air drying methods. Lwt, 92: 548-55.

Setty YP, Murthy JV 2003. Development of a model for drying of solids in a continuous fluidized bed dryer. Indian Journal of Chemical Technology, 10(5): 477-482.

Szychowski PJ, Lech K, Sendra-Nadal E, Hernandez F, Figiel A, Wojdylo A, Carbonell-Barrachina AA 2018. Kinetics, biocompounds, antioxidant activity, and sensory attributes of quinces as affected by drying method. Food Chemistry. 255: 157-64.

Uluman E, Kilicle, PA 2020. The investigation of the possible antigenotoxic in vivo effects of pomegranate (Punica granatum L.) peel extract on mitomycin-C genotoxicity. Turkish Journal of Veterinary & Animal Sciences, 44(2): .

Ülger TG, Songur AN, Çırak O, Çakıroğlu FP 2018. Role of vegetables in human nutrition and disease prevention. Veg. Importance Qual. Veg. Hum. Health: 7-32.

Xu K, Martinez MM, Yang B, Guo M 2020. Fine structure, physicochemical and antioxidant properties of LM-pectins from okra pods dried under different techniques. Carbohydrate Polymers, 241: 116272.

## *ORAL PRESENTATION*

### CRISPR/Cas9 Sisteminin Tanımı, Kullanım Alanları, Kısıtlamaları ve Etik Problemleri

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**Özet**

Yaşam bilgisinin kaynağı olan DNA ile ilgili mekanizmaların anlaşılması ve moleküler teknolojik gelişmelerin şaşırtıcı hızı sonucunda, moleküler biyoloji alanında yüzyılın keşfi olan CRISPR/Cas[1] sitemi ile yeni bir DNA devrimi gerçekleştirilmiştir. Emmanuelle Charpentier ve Jennifer A. Doudna, bu sistemi kullanarak genom düzenlemeyi keşfettikleri için 2020 Nobel Kimya Ödülü'ne layık görülmüştür. "Programlanabilir Moleküler Makas" ve "Moleküler Cerrahi Bıçağı" gibi tanımlar CRISPR/Cas sistemi için literatüre girmiştir. Bakteri ve arkelerde organizmayı yabancı genetik elementlere karşı koruyan bu doğal sistem, moleküler teknolojik yöntemlerle ökaryotik hücrelere dahil edilerek genomda ucuz, kolay, hızlı ve nispeten güvenilir bir şekilde değişiklik yapma imkanı sağlar. Genom düzenleyici bu sistem, kalıtsal ve genetik hastalıklar ile kanserin tedavisi, bitki ve hayvan verimliliğini artırma, ilaç endüstrisi gibi pek çok alanda geniş bir kullanım olanağı sunmaktadır. Ancak teknolojinin uygulamalarının hedef dışı yan etkiler doğurabileceği ve aynı zamanda kötü amaçlı kullanımların biyoetik sorunların yaratılmasına yol açacağı endişeleri de gündemde olan önemli konulardır.
Bu çalışmada CRISPR/Cas9 sisteminin kullanımı ile bugüne kadar yapılan çalışmalardan örnekler, sistemin kısıtlayıcı sorunları, kısıtlamalara yönelik çözüm stratejileri ve gen düzenlemenin etik boyutu sunulmaktadır.

**Anahtar Kelimeler:** CRISPR/Cas9, gen düzenleme, genetik değişiklik, etik

### Definition, Application Areas, Restrictions and Ethtical Problems of the CRISPR/Cas9 System

**Abstract**

As a result of the understanding of the mechanisms related to DNA, which is the source of life information and the surprising speed of developments in molecular technology, a new DNA revolution has been realized with the CRISPR/Cas[2] system, which is the discovery of the century in the field of molecular biology. Emmanuelle Charpentier and Jennifer A. Doudna were awarded the 2020 Nobel Prize in Chemistry for their discovery of genome editing using this system. Definitions such as "Programmable Molecular Scissors" and "Molecular Surgical Knife" have entered the literature for the CRISPR/Cas system. This natural system, which protects the organism against foreign genetic elements in bacteria and archaea is included in eukaryotic cells by molecular technological methods, providing the opportunity to make changes in the genome in a cheap, easy, fast and relatively reliable way. This genome-editing system offers a wide range of uses in many fields such as hereditary, genetic and viral infectious diseases and cancer modeling and treatment and increasing plant and animal productivity. However, the concerns that these technological applications may cause off-target side effects and that malicious use will lead to the creation of bioethical problems are also important issues on the agenda.
In this study, examples from the studies carried out to date with the use of the CRISPR/Cas9 system, the restrictive problems of the system, the solution strategies for the restrictions and the ethical dimension of gene editing are presented.
**Keywords:** CRISPR/Cas9, gene editing, genetic modification, ethics

[1] Düzenli Aralıklarla Bölünmüş, Palindromik Tekrar Kümeleri / CRISPR ile ilişkili protein
[2] Clustered Regularly Interspaced Short Palindromic Repeats / CRISPR associated protein

## 1.GİRİŞ

Bu çalışmada son yıllarda keşfedilen ve 2020 Kimya Nobel ödülüne layık görülen genom düzenleme aracı olarak CRISPR/Cas9 sistemi bileşenlerinin tanımlanması, sistemin genetik mühendisliği alanındaki kullanım alanları, sisteme ilişkin kısıtlamalar ve çözümleri ile etik boyutu hakkında bilgiler verilmesi amaçlanmıştır.

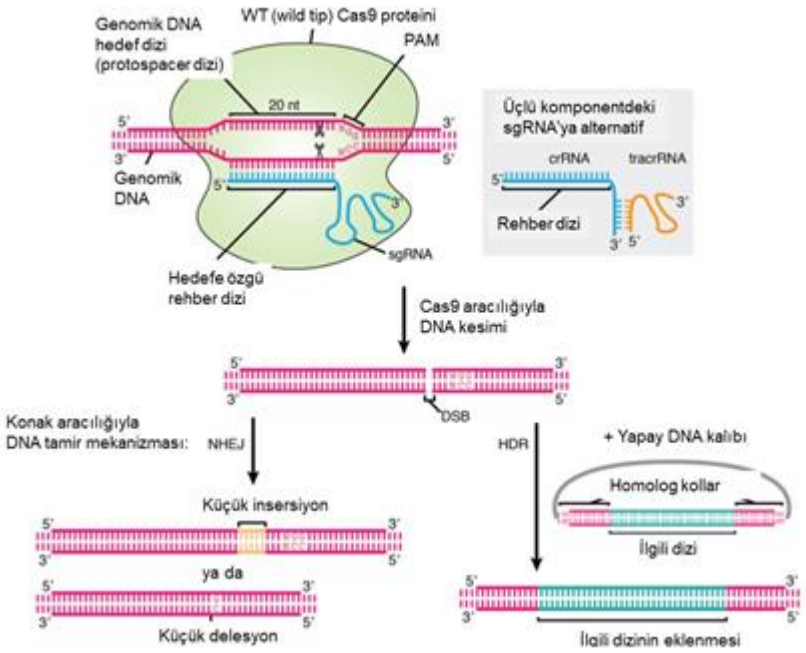
CRISPR doğada plazmidler, bakteriyel virüsler gibi yabancı genetik elementlere karşı bakterilere direnç sağlayan bağışıklık sistemi olarak mevcuttur. CRISPR yer aldığı canlının genomundaki, hücreye giren yabancı genetik elementlere karşı verilen savaşın bir sonucu olarak, organizmanın genomuna dâhil edilen palindromik tekrar dizileridir. Bu diziler bir sonraki aynı istilacı ile karşılaşıldığında "genetik hafıza" oluşturmakta ve bağışıklık sisteminin bir parçası olarak iş görmektedir. CRISPR ile ilişkili protein (Cas9) ise DNA'nın her iki ipliğini kesip, ekleyen bir CRISPR nükleazdır. CRISPR/Cas9 sistemi ise nükleaz temelli olarak hedeflenmiş gen düzenlemesi[3] imkânı sunan bir teknolojidir. Bu sistem, herhangi bir organizmanın genomundaki belirli bir gen bölgesini kesmek, etkisiz hale getirmek ya da tamamen değiştirmek için bir **"gen düzenleme aracı"** olarak büyük umut vaat etmektedir (Mittal, 2019).

CRISPR/Cas9 sistemi, Cas9 endonükleaz ve klavuz RNA (sgRNA/gRNA)[3]'dan oluşan iki anahtar bileşen içermektedir (Wang ve ark., 2017). Bakterilerden izole edilen Cas9 iki nukleaz bölgesi (RuvC ve HNH) olan ve hedef DNA dizisinde çift iplik kırığı (DSB)[4] oluşturmak için sgRNA ile birlikte iş gören bir proteindir. Tek bir klavuz RNA olan sgRNA ise crRNA (CRISPR RNA) ve tracrRNA[5] içeren ve hedef DNA sekansına tamamlayıcı kısa diziye sahip olmasından dolayı Cas9'u hedef DNA'ya yönlendiren bileşendir. Diğer önemli bileşen hedef DNA dizisinde bulunan ve Cas9'un kesim yerini belirlemesine klavuzluk yapan PAM[6] motifidir (Perez Rojo ve ark., 2018). Bu sistem hedef bölgede mutasyon, delesyon, gen baskılama ve aktive etme gibi uygulamaları yapmayı mümkün kılmıştır (Wang ve ark., 2017).

Genetik mühendisliği alanında bu yöntem ile genomda kesimin yapıldığı alanlarda DNA'nın yapısı hassas bir şekilde değiştirilebilmektedir. Hücrelerin hasarlı DNA'yı tanıma ve tamir edebilme yeteneğine sahip olmasından faydalanılarak genetik mühendisliği alanında bu teknoloji kullanılabilmektedir. CRISPR/Cas9 sistemi, genomun/genin istenilen bölgesinde oluşturulan kesme işlemi sonucunda DNA'daki DSB ile oluşan kesik uçların NHEJ[7] ya da HR[8] tamir mekanizmasıyla düzeltilmesi temeline dayanır. Sistemin bileşenleri düzenlenerek istenilen, tasarlanan genom/gen değişikliği gerçekleştirilebilmektedir. Tasarlanmış sgRNA dizisinin baz eşleşmesi yoluyla hedef DNA bölgesine bağlanması ile Cas9 nükleaz enzimi kesim bölgesini tanır ve nükleaz aktivitesi ile PAM motifinin (NGG) 3 nükleotid (nt) yakınından zincirin her iki ipliğini de keser ve endojen DNA tamiri başlatılmış olur. Bu aşamadan sonra eğer hedef genin çıkarılması amaçlanmışsa çift zincir kırığının hataya meyilli olan NHEJ yoluyla tamir edilmesi sağlanır. Bu sırada meydana gelen insersiyon/delesyon (InDel) mutasyonlar gen ürünlerinin kaybolmasına yol açar. Eğer yeni bir özelliğin genoma dahil edilmesi hedeflenmişse, Cas9/sgRNA kompleksi ile birlikte dışarıdan bir DNA dizisi verildiğinde, verici dizinin HDR yoluyla genoma dahil edilmesi (geni yenisiyle değiştirme) sağlanmış olur (Salsman ve ark., 2017). CRISPR/Cas9 sistemi kullanılarak genom/gen düzenlemesi Şekil 1'de gösterilmektedir (Agrotis ve Ketteler, 2015). CRISPR/Cas9 çeşitli hücre tipleri ve tüm organizmalarda uygulanabilmektedir. Cas9 temelli uygulamalarda gen düzenlemesi farklı şekillerde yapılabilmektedir (Lee ve ark., 2015) (Şekil 2).

Heyecan verici, çığır açan CRISPR/Cas9 buluşu, etkin, kolay uygulanabilir ve daha güvenli genom düzenleme aracı olarak, bitki ve hayvan ürünlerinin verimliliği, viral enfeksiyonlar ile kanserin de dahil olduğu kalıtsal ve sonradan kazanılmış insan hastalıklarının modellenmesi ve tedavisi, biyolojik kökenli ilaç geliştirilmesi gibi tıp, biyoloji ve biyoteknolojinin pek çok alanında uygulamalara imkân vermektedir (Saha ve ark., 2019).

[3] Gene editing
[3] sgRNA/gRNA: single guide RNA/guide RNA (tek klavuz RNA / klavuz RNA)
[4]DSB: Double Strand Break (Çift iplik kırığı)
[5] tracrRNA: trans-activating RNA (Trans aktive edici RNA)
[6] PAM: **P**rotospacer **A**djacent **M**otif (Protoaralık bitişik motif)
[7] **N**on-**H**omologous **E**nd-**J**oining (Homolog olmayan uç birleştirme)
[8] **H**omologous **R**ecombination ya da HDR: **H**omology-**D**irected **R**epair (Homolog rekombinasyon ya da HDR: Homoloji yönlendirmeli tamir)

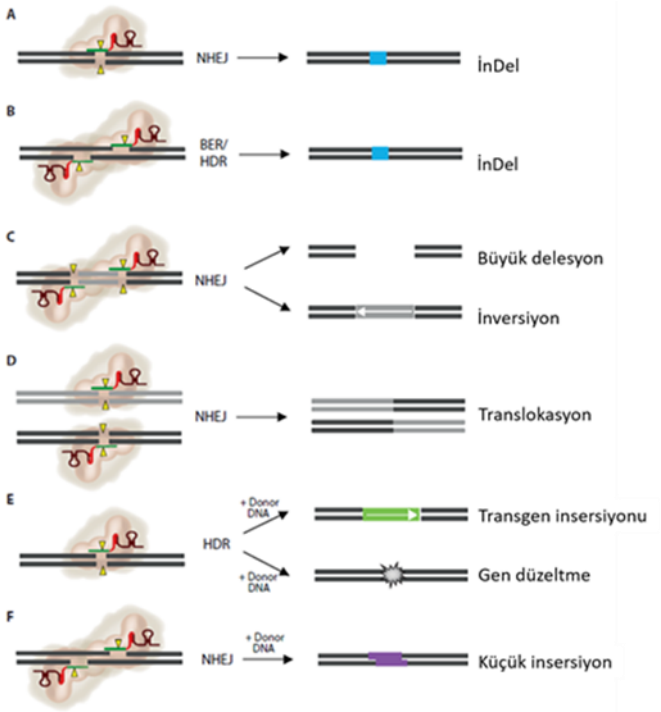



**Şekil 1.** CRISPR/Cas9 sistemi kullanılarak genom/gen düzenlemesi (Agrotis ve Ketteler, 2015).

Bir devrim niteliğindeki bu teknolojide baş edilmesi gereken sorunlar da gözardı edilemez. Bunlardan biri CRISPR/Cas9 temelli genom/gen düzenlemede hedef dışı etkilerin ortaya çıkmasıdır. Sistemin özgünlüğü ve hedef dışı etkilerin azaltılmasına yönelik olarak sgRNA tasarımı ve Cas9 modifikasyonları vb. konularda (Salsman ve ark., 2017) çözümler üretmek için çeşitli stratejiler geliştirilmektedir.

Nobel Fizik Ödülü sahibi Richard Feynman'ın "Bilim bize "cennetin" kapısını açan anahtarı verir, fakat aynı anahtar "cehennemin" de kapısını açar" sözü, bilim insanlarının CRISPR'ı kullanırken büyük bir sorumluluk altında olduğunu hatırlatmaktadır. Çünkü bu teknoloji yetişkin hücrelere uygulanabildiği gibi embriyonik gen düzenleme aracı olarak da kullanılabilmektedir. Tasarlanmış insan üretmeyi mümkün kılabilecek, bilim kurgudan gerçeğe insanı taşıyabilecek bu sistemin kullanımının güvence altına alınması gerekmektedir. Gözardı edilmemesi gereken etik sorular olması ve bunların tartışma platformlarında detaylı bir şekilde ele alınarak sistemin kullanımının güvence altına alınması için, embriyolarda yapılacak tüm klinik çalışmalara ara verilmiştir.

Bu çalışmada CRISPR/Cas9 sisteminin bileşenlerinin tanımlanması, genom düzenleme aracı olarak uygulama tipleri, sistemin uygulandığı alanlar ve bu alanda yapılmış çalışma örnekleri ile sisteme ilişkin kısıtlamalar ve çözüm yolları ve etik konuları literatür bilgileri ışığında değerlendirilmektedir.




**Şekil 2.** CRISPR/Cas9 kullanılarak genom düzenleme uygulamalarının tipleri (Lee ve ark., 2015).

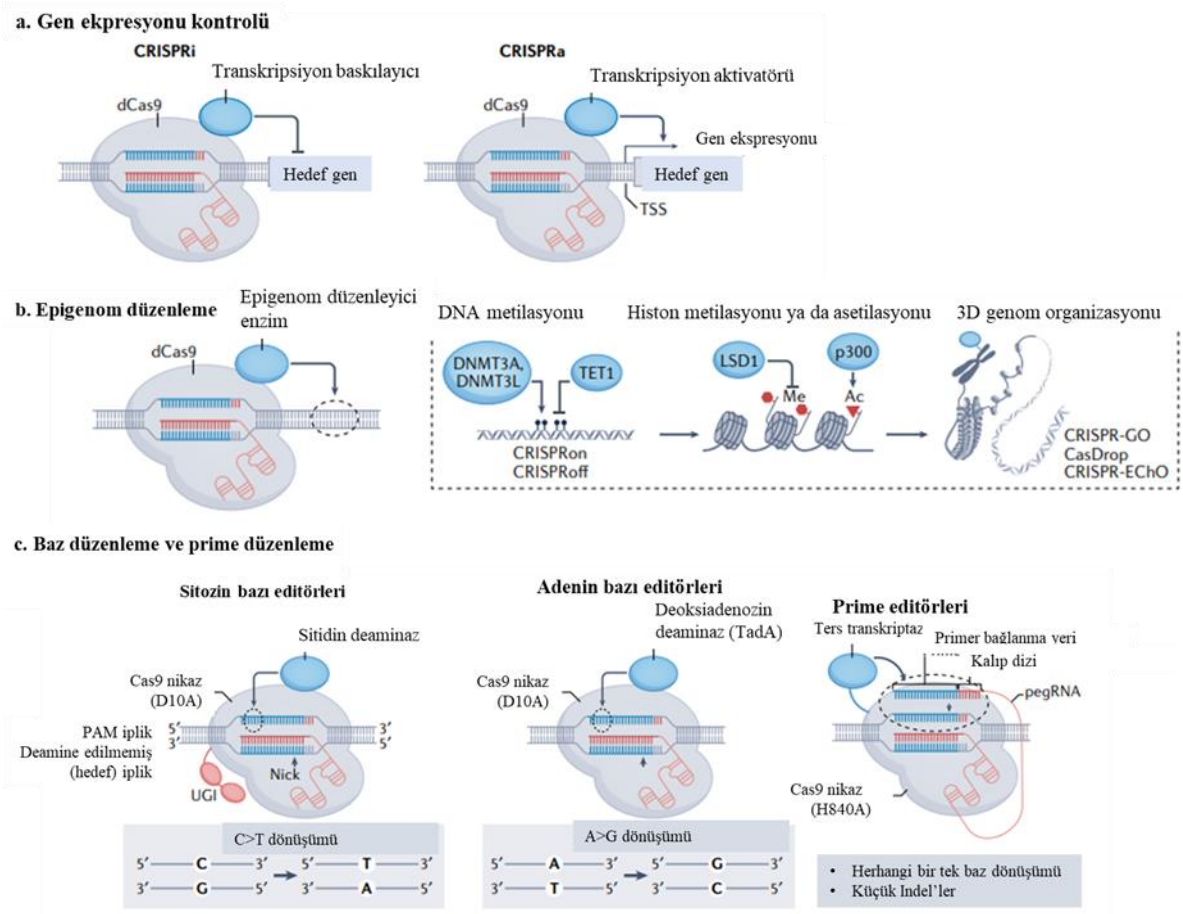
## 2.NÜKLEAZ EKSİKLİĞİ OLAN CAS9 İÇEREN CRISPR SİSTEMİ

Sistemin bileşenlerinden yabanıl tip (wild type) Cas9 üzerinde modifikasyonların yapılması farklı uygulamalara imkân tanımıştır. Cas9 enziminin alt ünitelerinden iki nukleaz bölgesi (RuvC ve HNH)'nin mutasyonlarla etkisizleştirilmesi hedef DNA'da çift iplik kırığı oluşturmadan, sgRNA ile yönlendirilerek araştırılan DNA'da belirli lokuslara bağlanmayı sağlamaktadır. Nükleaz eksikliği olan Cas9 ölü Cas9 (dCas9: dead Cas9) olarak adlandırılmaktadır. dCas9 içeren CRISPR sistemi, DNA'da baz değişikliği yapmadan, sisteme entegre edilen uygun effektör proteinler aracılığıyla genomdaki hedeflenen lokuslarda genom düzenlemenin ötesinde çalışmalara olanak tanımaktadır. Amaca uygun, çeşitli effektör protein içeren CRISPR/dCas9 aracılığıyla gen ekspresyonunun kontrolü (CRISPR interferens ve gen aktivasyonu), epigenomik düzenlemeler yapılabilmektedir (Şekil 3a) (Nishiga ve ark., 2022).

Yabanıl tip Cas9 proteininin iki nükleaz bölgesinden birinin etkisizleştirilmesi, diğerinin aktivitesini koruması ile hedef DNA'nın tek bir ipliğinde kırık oluşturabilmektedir. Bu tip Cas9'a Cas9 nikaz (Cas9 nickase) adı verilmektedir. Cas9 nikazlar baz düzenleme, prime düzenleme uygulamalarını yapmaya olanak tanımaktadır (Şekil 3b, 3c) (Nishiga ve ark., 2022).

## 3.CRISPR/Cas9 UYGULAMALARI

CRISPR/Cas9 ile genom/gen düzenleme tarım, hayvancılık, biyofarmositik (biyolojik kökenli tıbbi madde) ürün geliştirme, genomun yapısı, düzenlenmesi ve fonksiyonu ile ilgili çalışmalar, transgenik organizmalar ve hücre hatları oluşturma gibi biyoteknoloji, kalıtsal ve genetik hastalıkların modellenmesi ve tedavisi ile ilgili tıp gibi çeşitli pek çok alanlarda uygulama olanağı sunmaktadır (Saha ve ark., 2019).




**Şekil 3.** Çeşitli yeni CRISPR araçları. CRISPR/dCas9 aracılığıyla gen ekspresyonunun kontrolü (a), epigenomik düzenlemeler (b), CRISPR/Cas9 nikazlar ile baz düzenleme, prime düzenleme (c) (Nishiga ve ark., 2022).

Sistemi kullanarak gen nakavt, gen ekleme, baz değişiklikleri ile mutasyonlar oluşturma ve genetik hastalıklarda etkili olan gen mutasyonlarında baz değişikliği ile gen düzeltme (gene correction) işlemleri için farklı modellerle çalışılmaktadır. Hücre hatları (*in vitro*), organoidler (yetişkin ya da embriyonik kök hücrelerden köken alan üç boyutlu (3D) hücre kültürleri), bitki ve hayvan modelleri (*in vivo*), iPSC[9]'ler, hayvan embriyoları modellerinde teknoloji kullanılarak farklı alanlarda araştırmalar yapılmaktadır.

Moleküler biyolojik çalışmalarda hücre hatları ile gerçekleştirilen *in vitro* modeller kullanışlı, temin edilmesi, uygulanabilirliği kolay ve test tekrarlarına olanak tanıyan sistemlerdir. Herhangi bir hücresel işleyişi etkileyen olayların araştırılmasında öncü veriler elde edilmesinde oldukça önemli bir yere sahiptirler. CRISPR/Cas9 teknolojisi ile genlerde farklı herhangi bir yolla değişiklik oluşturulabileceğinin ilk kanıtları da *in vitro* çalışmalardan elde edilmiştir. *In vitro* Cas9 genetik mühendisliği çalışma prensibinin aşamaları genel hatları ile özetlenecek olursa; 1. İlgilenilen gen ve hedeflenecek bölgenin belirlenmesi, 2. Genomik hedefin seçilmesi, 3. sgRNA tasarlanması, 4. Cas9/sgRNA birleştirilmesi, 5. Kompleksin hücre hatlarına verilmesi, 6. Gen düzenleme olayları için yenilenen ve taranan transgenik hücrelerin doğrulanması, 7. CRISPR/Cas9 ile düzenlenmiş hücre hattında protein seviyelerinin analizi (Western Blot, vb teknikler), 8. Hücre hatlarında düzenlenmiş ilgilenilen hedef gen ürününün analizi (ELISA vb teknikler) (Giau ve ark., 2018).
Dokuyla sınırlı ASC[10] ya da ESC[11] ya da iPSC'den köken alan organoid kültürler, dokuların üç boyutlu (3D) organizasyonu ile çalışma olanağı sunan kültürlerdir. Organoid kültürler primer tek tabakalı hücre kültürleri ile *in vivo* çalışmalar arasındaki eksikliği tamamlayan uygun platformlardır. ASC kaynaklı organoidler sindirilmiş doku parçalarından ya da spesifik hücre popülasyonlarından oluşturulabilir. İstenilen hücreler izole edildikten sonra hücre tipine uygun içeriğe sahip kültür ortamında süspanse edilirler. PSC[12] kökeni olan organoidler ya ESC'ler ya da iPSC'lerden (örneğin deri fibroblastları kaynaklı) elde edilir. Farklı uygulama

[9] **i**nduced **P**luripotent **S**tem **C**ell (indüklenmiş pluripotent kök hücre)
[10] **A**dult **S**tem **C**ell (yetişkin kök hücre)
[11] **E**mbrionic **S**tem **C**ell (embriyonik kök hücre)
[12] **P**luripotent **S**tem **C**ell (pluripotent kök hücre)

protokollerine göre farklı organoid tiplerine *in vitro* olarak faklılaşabilirler. CRISPR teknolojisinde organoid çalışmaları tek gen hastalıkları ya da tümör oluşumunu *in vitro* modelleme, genom kapsamlı CRISPR taramaları ya da raportör organoidler oluşturmak amacıyla kullanılabilirler (Driehuis ve Clevers, 2017).

Embriyolarda gen düzenleme çalışmaları için çeşitli hayvan modelleri kullanılmaktadır. Embriyonik genomun düzenlenmesi CRISPR/Cas9 teknolojisi ile kolaylıkla başarılabilmektedir. Bu işlem üç aşamada gerçekleştirilir: 1) süper yumurtlamış dişilerden zigotların izolasyonu, 2) zigot içine sgRNA ve Cas9 mRNA'nın verilmesi, 3) bu işlemin ardından yaşayabilen jenerasyonu oluşturmak için yalancı-hamile hayvanlar içine embriyo transferi (Jin ve Li, 2016).

Somatik hücrelerden köken alan iPSC'ler insan hastalıklarının araştırılmasında, hastalardan elde edilerek üretilebilmesi ve embriyo yıkımına yol açmaması nedeniyle etik olarak da tercih edilen modellerdir. Genom düzenleme teknolojisi ile iPSC'ler, hastalık modellemede, kalıtsal bozuklukların da dahil olduğu çeşitli hastalıkların genetik, moleküler ve hücresel mekanizmalarının araştırılmasında, mutasyonların düzeltilmesinde kullanılmaktadır. CRISPR teknolojisinde hastalıkların modellenmesi ve araştırılmasında iPSC'ler iki farklı yaklaşım için kullanılmaktadır: a) hastalıklı iPSC'lerde mutasyona uğramış geni düzeltme (doğrulama); b) sağlıklı iPSC'lerde yabanıl tip (wild type) geni nakavt yaparak mutasyon taşıyan iPSC'ler oluşturmak. İçerdikleri mutasyonlar teknoloji aracılığıyla düzeltilmiş iPSC'ler potansiyel olarak sağlıklı farklılaşmış hücreler oluşturabilirler. Düzeltilmiş bu hücreler elde edildikleri hastalara aktarılarak rejeneratif tıp alanında kullanım potansiyeline de sahiptirler (Ben Jehuda ve ark., 2018).

### 3.1. Bitki ve Hayvanlarda

CRISPR/Cas9 sistemi ile hedef DNA'da sgRNA'lar aracılığıyla delesyonlar, insersiyonlar ve baz çiftlerinin modifikasyonlarını gerçekleştirerek çeşitli organizmalarda genom/gen düzenlemesi yapılabilmektedir. Fare, sıçan, domuz ve insanın da dahil olduğu memeliler ve pirinç, Arabidopsis, tütün, mısır ve soya gibi bitki türlerinde genom düzenleme ile ilgili çalışmalar raporlanmıştır (Wang ve ark., 2017).

İklim değişiklikleri ve çevre kirliliği dünya üzerinde yaşayan canlılar için büyük bir tehdit oluşturmaktadır. Ekosistemin besin zinciri gittikçe artan bir tehlike altındadır ve acil çözüme ihtiyaç vardır. Genomu CRISPR/Cas sistemi araçları ile düzenleyerek bitkisel ve hayvansal ürünlerin verimini artırmak çözüm yollarından biri olarak oldukça heyecan vericidir.

Bitki ve hayvanlarda genom/gen düzenlemesinde CRISPR/Cas9 uygulamalarının temel adımları; 1. Hedef gen ve PAM dizisinin belirlenmesi ve sgRNA sentezi, 2. sgRNA'nın uygun özellikte vektörde klonlanması, 3. Tek ya da çoklu hedef gen düzenlemenin sağlanması, 4. Uygun transformasyon yöntemi ile konak sisteme dağıtılması, 5. Transgeniklerin taranması ve doğrulanması, 6. Fenotipik değerlendirme.

#### 3.1.1. Tarım Alanında

Günümüzde sıcaklık artışı, kuraklık, artan toprak tuzluluğu ve sel gibi iklim değişikliklerinin yol açtığı abiyotik stresler ve virüs, bakteri, mantar gibi bitki patojenlerinin sebep olduğu biyolojik stres gıda üretim sistemini önemli ölçüde etkilemektedir. Ayrıca 2050 yılına kadar 9.7 milyar olacağı tahmin edilen dünya nüfusunun gıda ihtiyacını karşılamak için ürün verimliliğinin arttırılması ihtiyacı da gündemdedir. Tarım alanında CRISPR/Cas9 çalışmaları, ürün üretimini artırmak, bitki patojenlerine, herbisitlere karşı direnci artırmak, zararlı otlarla mücadele etmek, daha sağlıklı ve besleyici gıdalar üretmek konularında odaklanmıştır (Hamdan ve ark., 2022.).

Tarımsal ürünlerin verimliliğinin arttrılmasının temel yolu ürün verimliğini etkileyen tane büyüklüğü ve ağırlığı gibi özelliklerin kalitesinin iyileştirilmesi ile ilişkilidir. Bu özellikler bitki genomunun QTL[13] ile genetik olarak kontrol edilmektedir. QTL lokusundaki bazı özellikler moleküler biyolojik olarak analiz edilmiştir ve tanımlanmıştır (Zuo ve Li, 2014). CRISPR/Cas9 yöntemi ile tarım alanında ürün verimliliği ile ilgili çalışmaların öncülleri pirinç üzerine yapılanlardır. Pirinç bitkisinde QTL'deki tane büyüklüğünü negatif etkileyen GW2[14], GW5/qSW5[15], TGW6[16] genleri CRISPR/Cas9 sitemi kullanılarak gen nakavt yöntemi ile düzenlenmiştir (Xu ve ark., 201 6).

[13] kantitatif özellik lokusu (QTL: Quantitative trait loci)
[14] RING-type E3 ligase
[15] polyubiquitin-interacting protein
[16] IAA-glucose hydrolase

Bitkilerde çiçeklenme gün boyu mevsimsel değişikliklerle tetiklenmektedir. Ürünlerde gün boyu hassasiyet, coğrafi ekim alanlarını sınırlandırır ve bu nedenle fotoperiyot tepkisinin değiştirilmesi bitki ıslahında kritik önemdedir. Domates bitkisinde de gün boyu hassas çiçeklenme kaybı söz konusudur. Kayba neden olan baskılayıcı SP5G[17] geni CRISPR/Cas9 sistemi ile tasarlanan mutasyonlarla nakavt edilmiş, böylece tarla domateslerinin erken bir verime dönüşen hızlı çiçek üretimi sağlanmıştır (Soyk ve ark., 2017).

Tarımsal ürünlerin verimliliğini etkileyen bir diğer neden bitki patojenleri, herbisitlere dirençsizlik ve zararlı otlardan kaynaklanan kısıtlamalardır. Gen düzenleme ile bakteri, virüs ve funguslara karşı bitki patojen direnci ve herbisit direnci geliştirilmeye çalışılmaktadır.

CRISPR/Cas9 sistemi ile ya viral genom ya da konak genomu hedeflenerek bitkilerde viruslere karşı başarılı direnç geliştirme çalışmaları raporlanmıştır. CRISPR-Cas9 teknolojisi ile Arabidopsis'de TuMV[18]'e karşı, viral yaşam için gerekli olan konağa ait eIF(iso)4E geninde delesyon gerçekleştirilerek geliştirilen direnç çalışması bildirilmiştir (Pyott ve ark., 2016).

Pirinç tungro hastalığı tropikal Asya'da pirinç üretimini etkileyen çok önemli ciddi bir sorundur. Hastalığa RTSV[19] ve RTBV[20] arasındaki etkileşim sebep olmaktadır. Bu hastalığa karşı direnç konağın eIF4G[21] geni ile ilişkilidir. CRISPR/Cas9 düzenleme ile eIF4G geninde mutasyonlar oluşturularak RTSV'ye karşı direnç sağlayan yeni allel varyantına sahip bitkiler elde edilebilmiştir (Macovei ve ark., 2018).

Maksimum tarım ürünü gelişimi ve verimi, etkin bir zararlı ot mücadele yönetimi gerektirmektedir. Klasik yaklaşımda herbisitler tercih edilmektedir ancak, herbisitler bitkilerde yaşamsal proteinlerin üretilmesinde etkili olan sinyal yolaklarındaki proteinleri olumsuz etkilemektedir. Örneğin sıklıkla tercih edilen "glyphosate" herbisiti bitkilerde hayati öneme sahip aromatik amino asitlerin üretimini engellemektedir. Gen düzenlemesi ile bitkinin protein yapısında değişiklik yapılarak, herbisitlerin bu proteinlere bağlanmasının önlenmesi ile herbisit toleransı oluşturulabilmektedir. Ketengiller familyasından keten cinsinin en yaygın türü olan *Linum usitatissimum*'da EPSPS[22] geninde homolog rekombinasyon temelli CRISPR/Cas9 yöntemi ile iki tek nükleotid değişimi gerçekleştirilerek glyphosate'a karşı direnç sağlanmıştır (Sauer ve ark., 2016). Gen düzenleme ile tütün, soya, mısır ve pirinçte dallanmış zincirli amino asit sentezinde iş gören ALS[23] enzimini baskılayan herbisitlere karşı tolerans geliştirme çalışmaları da raporlanmıştır (Li ve ark., 2015; Svitashev ve ark., 2015; Endo ve ark., 2016).

Gıda tüketiminde daha sağlıklı ve besin değeri yüksek kalitede meyve ve sebzeler üretilmesi ilgi duyulan alanlardandır. Antioksidant etkinliği yüksek olan, pişirildiğinde kanserojen madde oluşumunun azaltıldığı sebze ve meyvelerin artırılması açısından genomun düzenlemesi çalışmaları hız kazanmaktadır. Örneğin, bazı bitkilerin kök, gövde, yaprak, çiçek ve meyve kısımlarında bulunan, güçlü bir antioksidant olan anthocyanin biyosentezi domates bitkisinde, CRISPR/Cas9 temelli olarak ANT1[24] geninin ifadesi artırılarak düzenlenmiştir (Cerma´k ve ark., 2015).

Tarım ve gıda alanında önemli bir yere sahip olan filamentoz fungiler pekçok seviyede insanlarla etkileşim halindedir. Yüksek protein sekrete etme yetenekleri, çeşitli enzimleri üretmeleri, ekonomik degeri olan farklı metabolitleri sentezlemeleri, antibiotikler, organik asitler, pigmentler, PUFA[25]'ların üretilmesinde kullanışlı olmaları gibi onları değerli yapan özelliklere sahiptirler. Bunun yanısıra gıda, tarım ürünleri ve hatta insanları kontamine etme risklerinin de bulunması ekonomik kayıplara yol açmaktadır. Bu nedenlerle onların hakkında genetik ve moleküler biyolojik yönden bilgi sahibi olmak ilgilenilen alanlarda kontrol altına almak bakımından önemlidir. CRISPR/Cas9 sistemi ile filamentoz fungilerle ilgili daha önce yapılmış genetik mühendisliği alanındaki araştırmaların kapısı biraz daha aralanmıştır. CRISPR/Cas9 ile filamentoz fungilerin farklı türlerinde gen nakavt ve gen ekleme çalışmaları, fonksiyonal genomik alanında bilgilerin ve yeni doğal ürünlerin elde edilmesinde katkılar sağlayacaktır (Shi ve ark., 2017).

[17] Self-Pruning 5G
[18] Turnip Mosaic Virus
[19] Rice Tungro Spherical Virus
[20] Rice Tungro Bacilliform Virus
[21] translation Initiation Factor 4 Gamma
[22] 5-Enolpyruvylshikimate-3-Phosphate Synthase
[23] Acetolactate Synthase
[24] Anthocyanin 1
[25] **P**oly**U**nsaturated **F**atty **A**cid (Polisatüre edilmemiş yağ asidi)

### 3.1.2. Hayvanlarda

#### *Çiftlik Hayvanlarında*

Yakın geçmişte genotipleme ve tüm genomu dizileme çalışmalarıyla çiftlik hayvanlarının genetik yapısını analiz etmek mümkün olmuştur. Sığır, koyun, keçi ve domuzların genomlarında ilgilenilen spesifik genlerde yapılan düzenleme ile gen ifadesinde değişiklikler yapılmış ve bu değişikliklerin zigotlarına ve organizmaya da aktarıldığı gösterilmiştir (Petersen ve Niemann, 2015).

CRISPR/Cas9 tekniği hayvancılık ürünlerinin üretimi, kalitesi ve hayvan verimliliğini artırmak, kısırlıkla mücadele ve hastalıklara karşı direnci geliştirmek için başarıyla kullanılmıştır (Jiang ve Shen, 2019).

Hayvan ürünlerinin kalitesini artırmak hedefli gen düzenleme sistemi aracılığıyla gen ekleme sonucu transgenik domuzlar elde edilebilmiştir. n-3PUFA[26]'lar bakımından zengin gıdalar insan sağlığını olumlu yönde etkileyen gıdalardır. *Caenorhabditis elegans*'ın fat-1[27] geni, CRISPR/Cas9 sistemi ile domuzlara dahil edilebilmiştir. Analiz sonuçlarında domuzlarda n-3PUFA seviyesinin önemli ölçüde arttığı bildirilmiştir (Li ve ark., 2018).

Hayvan verimliliğine yönelik CRISPR/Cas9 uygulaması çalışmasında keçi kılı gelişimini düzenleyen FGF5[28] geninde mutasyon oluşturularak kıl uzunluğunun artırıldığı raporlanmıştır. Bu çalışmada kaşmir keçi embriyolarına MSTN[29] ve FGF5 genini hedefleyen Cas9 mRNA ve sgRNA'lar mikroenjeksiyonla sokularak kaşmir ürün miktarı, kaşmir fiber çapı ve uzunluğu artırılmıştır (Wang ve ark., 2016).

Çiftlik hayvanlarında enfeksiyonlara karşı direnç kazandırılması üretimi olumlu yönde etkilemektedir. Domuzlarda PRRS[30] hastalığı domuz üretimini olumsuz etkileyen, üremeyi ve solunum sistemini bozan, başedilmesi gereken bir hastalıktır. PRRS'ye yol açan virüsün enfeksiyon geliştirmesinde makrofajların yüzeyinde bulunan CD163 reseptörü etkilidir. CRISPR/Cas9 ile domuz zigotlarında CD163'ü kodlayan SRCR5[31] geninin 7. ekzonunda delesyonlar oluşturulmuştur. Bu zigotlardan gelişen domuzlarda ters bir etkinin olmadığı, normal gelişebildikleri raporlanmıştır (Burkard ve ark., 2017).

#### *Kemirgenlerde*

Kemirgenlerde CRISPR/Cas9 sistemi ile genomun modifiye edilmesi ve gen ekspresyonun düzenlemesi ile ilgili oldukça başarılı çalışmalar bildirilmiştir (Wang ve ark., 2013; Yang ve ark., 2013; Yin ve ark.,2014).

CRISPR/Cas9 teknolojisi ile genetik olarak değiştirilen farelerin oluşturulmasındaki başarılı ilerlemeler ve haploid embriyonik kök hücre yaklaşımları gen fonksiyonu, insan hastalık modelleme ve gen terapisi için yeni platformlar sağlamaktadır (Ben Jehuda ve ark., 2018).

#### *İnsan Dışındaki Primatlarda*

Fare, sıçan gibi memeli hayvanlar insan genomu yapısı ve fonksiyonu ile hastalıkların oluşum mekanizmalarının anlaşılması konusunda model organizmalar olarak kullanılmaktadır. Ancak genetik ve fizyolojik özellikler bakımından insana büyük benzerlikler gösteren insan dışındaki primatlarda (NHPs: nonhuman primates) yapılacak çalışmalar insan biyolojisinin, hastalıklarının anlaşılması bakımından önemlidir. Geleneksel genom araştırma teknikleri primatlarda uygulamalar açısından oldukça zor ve kısıtlıydı. Her ne kadar NHP'lerle ilgili çalışmalar erken aşamada ise de CRISPR gen düzenleme aracı primat genom manipulasyonlarının kapısını açmıştır (Luo ve ark., 2016).

Çinli bilim insanları CRISPR/Cas9 sistemini ilk kez 2014 yılında kullanarak, cynomolgus maynunlarında gen düzenlemesini gerçekleştirmişlerdir. Tek hücreli evredeki embriyoya Cas9 mRNA'sı ve sgRNA'yı birlikte enjekte ederek, iki hedef gende (Ppar-g ve Rag1) insersiyon/delesyon mutasyonları oluşturmuşlar ve hedef dışı etkilerin de olmadığını bildirmişlerdir (Niu ve ark., 2014).

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[26] n-3 Polyunsaturated Fatty Acid
[27] Fatty Acid Desaturase 1: n-6PUFA'ları (n-6 Polyunsaturated Fatty Acids) N-3PUFA'ya çeviren enzim
[28] Fibroblast Growth Factor 5
[29] Myostatin: Kas büyüme ve gelişmesini negatif etkileyen protein
[30] Porcine Reproductive and Respiratory Syndrome
[31] Scavenger Receptor Cysteine-Rich Domain 5

Maymunlarda birçok gen nakavt (in/del yoluyla) çalışması bildirilmiştir, ancak maymunlardaki DNA onarım mekanizmalarının karmaşıklığından dolayı, CRISPR/Cas9 sistemi aracılığıyla gen ekleme ya da baz değişikliği mutasyonları oluşturmak ve gen nakavt etkinliğini artırmak pek de kolay işlemler değildir. Belirtilen durumların üstesinden gelmek, hedef dışı etkileri ve mozaik mutasyonları elimine etmek amacıyla çeşitli çözüm üretmeye yönelik araştırmalara odaklanılmıştır (Luo ve ark., 2016).

### 3.1.3. Tıp Alanında

Hastalık modelleme, hastalık teşhisi, genetik hastalıkların tedavisi ve gen terapisi gibi tıp alanında yapılan çalışmalar bu modern düzenleme teknolojisinin trend konularındandır. Moleküler cerrah bıçağı ile HIV[32], kanser, Parkinson, Alzheimer gibi pek çok genetik hastalığa çare bulmanın yolunun hız kazanacağı öngörülebilmektedir. Yeni jenerasyon terapötik ilaçların geliştirilmesinde de büyük umut vadetmektedir (Gupta ve Shukla, 2017).

#### 3.1.3.1. Hastalıkların Modellenmesi

CRISPR/Cas9 teknolojisi kanser, nörolojik, kardiyovasküler, infeksiyöz, immün bağışıklık gibi hastalıklarla ilgili *in vitro* ya da *in vivo* (hayvan) modeller oluşturarak gen fonksiyonunun ve gen düzenlenmesinin çalışılmasına imkân yaratmaktadır (Torres-Ruiz ve Rodriguez-Perales, 2017). Hastalıkların modellenmesi konusunda yapılan çalışmalardan örneklere Tablo 1'de yer verilmektedir.

**Tablo 1.** CRISPR/Cas9 uygulamaları ile yapılmış *in vitro* ve *in vivo* hastalık modelleme çalışmalarına bazı örnekler (Torres-Ruiz ve Rodriguez-Perales, 2017; Rahman ve ark., 2019).

| Hastalık | CRISPR uygulama |
|---|---|
| Kanser | MDA-MB-231 (meme kanseri hattı) hücre hattında KIBRA ve KIBRA gen nakavt |
| Kanser | *İn vitro* HEK293 (human embryonic kidney cells 293: insan embriyonik böbrek hücreleri 293) hücre hattı modelinde MET geni exon 14 delesyonu |
| Kanser | İnsan HEK293, mezenşimal ve hematopoetik hücrelerde t(11;22) and t(8;21) translokasyonlarının yeniden oluşturulması |
| Kanser | *İn vivo* karaciğer modelinde Pten and p53 genlerini nakavt etme ve beta-katenini aktive eden nokta mutasyonu oluşturma |
| Nörolojik | Fare modelinde Tenm1 (Teneurin Transmembrane Protein 1) nakavt |
| Nörolojik | iPSC'den köken almış nöronlarda GABAerjik alt tipinin floresans işaretlemesi ile SCN1A A5768G mutasyonunun epilepsi üzerindeki etkisini araştırma modeli |
| Nörolojik | Alzheimer hastalığı için ɣ-sekretaz-APP tanıma modelinde APP (β-amyloid precursor protein) geninde T48P, L52P ve K53N mutasyonlarının oluşturulması |
| Kardiyovasküler | Fare kardiyomyositlerinde AAV9 transformasyonu ile Myh6 delesyonu yapılarak kalbe özgü Cas9 transgenik fare oluşturma |
| Kardiyovasküler | *In vivo* fare karaciğer modelinde AAV transformasyonu ile Pcsk9 nakavt |
| İmmün bağışıklık | Fare B2m, Il2rg, Prf1, Prkdc ve Rag1 genleri hedeflenerek İmmün yetersizliği olan fare modeli oluşturma |

#### 3.1.3.2. Tedaviye Yönelik (Terapötik) Yaklaşımlar

Genetik hastalıkların tedavi edilebilmesi, gen düzenleme tekniklerinin kullanılması ile başarılabilir. Basitliği, yüksek özgünlüğü ve ucuz olması bakımından CRISPR/Cas9 sistemi gen tedavisinde büyük umut vaat eden bir teknolojidir (Li ve ark., 2018). Genom düzenleme terapötik uygulamaları ile ilgili çalışmalarda kalıtsal hastalıklar, hematolojik (kanla ilgili) hastalıklar, insan kanserleri, nörodejeneratif hastalıklar ve viral infeksiyöz hastalıklar (HIV, HBV[33]) üzerine odaklanılmıştır (Saha ve ark., 2019).

##### 3.1.3.2.1. Kalıtsal Hastalıklar

Duchenne musküler distrofi, dünyada yaygın görülen, ölümcül X'e bağlı resesif bir kas hastalığıdır. Distrofin genindeki okuma çerçevesini değiştiren mutasyonlar sonucunda kas membran bütünlüğünün azalmasına ve dejenerasyon duyarlılığının artmasına yol açan distrofin kaybıyla karekterize edilir. CRISPR / Cas9 teknolojisi,

[32] **H**uman **I**mmunodeficieny **V**irus (İnsan Bağışıklık Yetmezliği Virüsü)
[33] **H**epatitis **B** **V**irus (Hepatit B virüsü)

DMD[34] genindeki bozulmuş gen okuma çerçevesini eski haline getirebilen ve distrofin restorasyonuna yol açabilen kalıcı ekzon atlama potansiyeli nedeniyle DMD tedavisi için bir çözüm olarak ilgi görmektedir. DMD hastalığının tedavisinde CRISPR/Cas9'un etkinliği ve güvenilirliğine ilişkin yapılmış çeşitli *in vitro* and *in vivo* araştırmalar raporlanmıştır (Lim ve ark., 2018).

Kistik fibrozis CF[35] transmembran geçişi düzenleyici genindeki mutasyonların yol açtığı en yaygın öldürücü genetik hastalıklardan birisidir. CFTR[36] geni klorid ve bikarbonat salınımını sağlayan ekzokrin epiteliyal hücrelerinin apikal zarında yar alan klorid anyon kanalına bağlanan adenozin trifosfat kasetini transporte eden proteini kodlar. CF akciğer başta olmak üzere birkaç organı etkileyen bir hastalıktır. Ancak hastalıkla ilgili hala daha etkili bir çare bulunmuş değildir. CF'lu pediatrik hastalardan elde edilen kültüre edilmiş bağırsak kök hücrelerinin (organoidler) bir modelinde CRISPR / Cas9 tekniği kullanılarak tam CFTR protein işlevselliğinin restorasyonu gösterilmiştir. Aynı şekilde, CFTR mutasyonunun düzeltilmesi de CRISPR/Cas9 yaklaşımı ile iPSC modellerinde denenmiştir. CRISPR stratejisinin, yakın gelecekte CF tedavisi için önemli bir araç olabileceği belirtilmektedir (Marangi ve Pistritto, 2018).

***Fragile X Mental Retardasyon 1*** (FMR1) geninin 5' translasyona uğramayan bölgesi (UTR) içinde tekrar eden CGG trinükleotidinin genişlemesi, ***Fragile X sendromu'***nun baskın ve Fragile X ile ilişkili Tremor/Ataxia Sendromu ile Fragile X ile ilişkili primer yumurtalık yetersizliğin bilinen tek nedenidir. CRISPR/Cas9 sitemi ile insan iPSC, ESC ve nöronal progenitör hücrelerle yapılmış çalışmalar henüz başlangıç aşamasındadır. Bu konuda çalışılmaya ihtiyaç vardır (Yrigollen ve Davidson, 2019).

### 3.1.3.2.2. Hematolojik Hastalıklar

***Beta talasemiler,*** beta globin geni (HBB) içindeki genetik mutasyonların sebep olduğu, kalıtsal monogenetik hastalık grubuna dahil olan bir kan hastalığıdır. Hastalarda mutasyonun tipine göre ciddi kansızlık tablosu oluşur. CRISPR/Cas9 sistemi ile gen tedavisi amacıyla hastanın iPSC'leri kullanılarak HBB genindeki mutasyonların düzeltildiği çalışmalar raporlanmıştır (Jensen ve ark., 2019).

***Orak hücreli anemi***, HBB genindeki A (Adenin)'nin T (Timin)'e tek nükleotid değişimi mutasyonu sonucu β-globin proteinindeki Glu6Val yer değiştirmesi mutasyonuyla oluşan resesif otozomal hametopoetik bir hastalıktır. Bu hastalarda, kırmızı kan hücrelerinin deformasyonu sonucu hemolitik anemi, yanısıra iskemi, ilerleyici organ hasarı ve felç meydana gelir. CRISPR/Cas9 teknolojisi, HSPC[37]'lerde hastalıkla ilgili mutasyonun düzeltilmesinde kullanılarak gen tedavisi çalışmaları yapılmıştır (Jensen ve ark., 2019).

Kalıtsal hametolojik hastalıklardan ***Fanconi anemisi***'ne yol açan FA[38] gen mutasyonları (Osborn ve ark., 2015) ve X-SCID[39] hastalığında IL2Rɣ[40]'yı kodlayan IL2RG[41] geni (Pavel-Dinu ve ark., 2019) mutasyonlarının düzeltilmesinde CRISPR/Cas9 sistemi çalışmaları son yıllarda gerçekleştirilmiştir.

### 3.1.3.2.3. Nörodejeneratif Hastalıklar

Nörodejeneratif hastalıklar yaşlılardaki hastalıkların büyük bir kısmını oluşturur. Kompleks sporadik formlarda genetik faktörler önemli rol oynar. Ailesel fenotiplerde nörodejenerasyona yol açan birkaç gen tanımlanmıştır. Nörodejenerasyonun oluşum mekanizmasının belirlenmesinin tedavi stratejilerinin geliştirilmesinde katkısı büyüktür. Alzheimer, Parkinson, Amyotrofik Lateral Skleroz, Huntington hastalığı en yaygın görülen nörodejeneratif hastalıklar arasındadır (Kumar ve ark., 2018).
CRISPR/Cas sistemiyle gen düzenleme çalışmaları nörodejeneratif hastalıkların altında yatan gen mutasyonlarının oluşturularak hastalığın modellenmesi ve mutasyonlu genlerin düzeltilmesi üzerine olmuştur. Alzheimer, Parkinson ve Huntington gibi hastalıklarda, spesifik genlerdeki mutasyon sonucunda yanlış katlanmış ilişkili protein birikimi sonucu nöron hücresi hasarı meydana gelmekte ve nöron kaybına yol açmaktadır. Alzheimer ve Huntington gibi nörodejeneratif hastalıklarda CRISPR/Cas9 gen düzenleme ile mutasyon düzeltme çalışmaları yapılmaktadır (Rahman ve ark., 2019). Bu alandaki çalışmalardan birinde Alzheimer hastalığında, *in vitro* şartlarda bazal ön beyin kolinerjik nöronlarında, Presenilin 2 genindeki N141I

[34] **D**uchenne **M**uscular **D**ystrophy (Duchenne Musküler Distrofi)
[35] **C**ystic **F**ibrosis (Kistik fibrozis)
[36] CF Transmembrane Conductance Regulator (CF transmembran geçişli düzenleyici)
[37] **H**aematopoietic **S**tem and **P**rogenitor **C**ell (Hametopoetik kök ve öncül hücreler)
[38] Fanconi Anemisi
[39] X-linked Severe Combined Immunodeficiency (X'e bağlı kombine edilmiş ciddi immün defect)
[40] Interleukin-2 Receptor ɣ Chain: İnterlökin-2 reseptör ɣ
[41] Interleukin-2 Receptor Gamma

mutasyonunun sistem kullanılarak düzeltilmesinin ardından Aβ42/40 düzeylerinin normalize edilmesi başarılabilmiştir (Ortiz-Virumbrales ve ark., 2017).

### 3.1.3.2.4. Viral İnfeksiyöz Hastalıklar

HIV-1[42] ve HBV[43] enfeksiyonları, hastalardan viral DNA'yı yok etmede etkili tedavilerin eksikliği sebebiyle global sağlığı ilgilendiren önemli enfeksiyöz hastalıklardır. HIV-1 virüsü bulaştığı genoma genetik materyalini entegre ederek kronik enfeksiyona yol açar. HIV-GFP Jurkat hücre hattında HIV-1 DNA'nın sessizleştirilmesinde CRISPR/Cas9 sistemi ile çalışılmıştır. Etkili bir şekilde HIV-1 DNA'nın baskılanabileceği gösterilmiştir (Liao ve ark., 2015).

HBV dünyada 350 milyon kişiye bulaşmış, karaciğer sirozu ve karaciğer kanserine sebep olan bir hastalıktır (Liang ve ark., 2017). CRISPR-Cas9 ile HBV genomlarının gen düzenlemesi için *in vivo* ve *in vitro* HBV modelleri üzerinde çalışmalar yapılmaktadır, ancak tedaviye yönelik sisteme ilişkin kısıtlamalar hala daha aşılması gereken problemler arasındadır (Yang ve Yang, 2021).

### 3.1.3.2.5. Kanser

Kanser, tümör oluşumunun transformasyon, tümör gelişimi ve metastaz aşamalarında genetik ve epigenetik değişikliklerin rol oynadığı kompleks bir hastalıktır. Tümör baskılayıcı genlerin fonksiyon kaybına ya da onkogenlerin aşırı ifade edilmesine yol açan, DNA yeniden düzenlenmelerinin sonucu oluşan genetik değişiklikler doku kompartımanlarındaki hücreleri kanserleşmeye götürebilmektedir. Meydana gelen bu değişikliklerin karekterize edilmesi, ilgili kanser tipinde sebebin ortaya konmasını ve böylece tedavi stratejilerinin geliştirilmesine olanak tanıyabilir.

CRISPR ile ilgili çalışmalar hastalık modeli oluşturma, kanser teşhisi, kanser terapisi, ilaç validasyonu ve fonksiyonel gen çalışmaları üzerine odaklanmıştır (Tian ve ark., 2019). CRISPR/Cas9 kanser immünoterapi açısından da bir devrimdir. Kanser hastalarında tüm genom kütüphanelerinin geliştirilmesinde de önemli rol oynar. Teknoloji genetik değişikliklerin *in vitro*, *in vivo* ve *ex vivo* deneysel mutasyon modellemelerle (Tablo 1) ortaya konmasını sağlama potansiyeli taşımaktadır. Ayrıca CRISPR sisteminin tümörlere dağıtımı direkt ya da indirekt olarak kanser gelişimini baskılayabilir. Teknoloji klinik örneklerde düşük sayıda kanser hücresi veya nadir mutasyonları tespit etmek için de kullanılabilir (Yin ve ark., 2019).
CRISPR ile genomu düzenlenmiş, hücre kültürleri, organoidler kanserin başlaması ve ilerlemesine katılan genler arasındaki rolü ve etkileşimlerin anlaşılması veya ilaç tarama çalışmalarında kullanılmaktadır (Yin ve ark., 2019).

CRISPR, hayvan modelllerinde kanser oluşturmada da kullanılışlı bir yöntemdir. CRISPR'ın avantajı yetişkin hayvanlarda çabuk sonuca götüren kanser modelleri oluşturulabilmesindedir. Yanısıra, somatik hücrelere driver mutasyonların girişi ile yapılan genetik manipülasyon, kazanılmış bu tip mutasyonlara sahip az sayıda somatik hücrede onkogenezi başlatabilir. CRISPR/Cas9'un hücrelere verilmesi sistemi somatik hücrelerdeki genetik değişikliğin etkin girişi için anahtar rol oynar. Lentivirus, AAV (Adeno Associated Virüs), adenovirus ve plasmid; fare kanser modelleri kurulması için *in vivo* çalışmalarda kullanılabilir (Yin ve ark., 2019).

Özafagus, idrar kesesi, prostat, böbrek, küçük hücreli olmayan akciğer, lösemi, rahim ağzı, multiple myeloma, melanomun dahil olduğu çeşitli kanserlerde CRISPR/Cas9 ile tedaviye yönelik faz I ve II düzeyinde klinik çalışmalar da yapılmaktadır (Zhan ve ark., 2019).

## 3.1.4. Genom Kapsamlı Tarama (Genome-Wide Screening)

Fonksiyonel genomik, gen fonksiyonunu tanımlamak için, özellikle genom çapında bozulmalardan kaynaklanan hücresel fenotipleri değerlendirmede güçlü bir tekniktir. CRISPR/Cas9 teknolojisi uygulama alanındaki son çabalardan biri de genom kapsamlı tarama için sgRNA kütüphanelerinin havuzunun oluşturulmasıdır. Büyük içerikli genoma sahip insan, fare ve Drosophila'nın kodlayıcı ve düzenleyeci bölgelerinin çalışılması için sgRNA havuzları oluşturulmuştur. Bu da genomun fonksiyonel fenotipik taranmasını kolaylaştırmıştır (Agrotis ve Ketteler, 2015).

[42] Human Immmunodeficiency Virus-1 (İnsan bağışıklık yetmezliği virüsü-1)
[43] Hepatitis B Virüs (Hepatit B virüsü)

CRISPR taraması, pozitif veya negatif seçimle birleştirilen havuzlanmış kütüphane yaklaşımları veya alternatif olarak dizilenmiş kütüphaneler kullanılarak gerçekleştirilebilir. **Havuzlanmış kütüphane tarama**da, viral sgRNA kütüphanesi trandüksiyona uğramış hücreler ya da spesifik fenotiplerin seçimi yapılmadan önce düşük enfeksiyon düzeyindeki hücreler tek bir kap içine alınır. Tarama ile ilgili çıktı kontrol hücre popülasyonuna karşı seçilmiş hücre popülasyonundaki genomik DNA'nın derin dizilemesinden elde edilir, bu da seleksiyona cevapta her bir sgRNA'nın zenginleşmesi ya da eksilmesinin ölçümünü sağlamaktadır. **Dizilenmiş kütüphane tarama**da, farklı hedefleri olan sgRNA'lar array formatında gelişen hücrelere dağıtıldığı için daha fazla kütüphane tipi ve dağıtım metodu kullanılır. Fenotipler zorunlu olarak seçilme yerine her bir fenotipten sorumlu sgRNA'nın orijinal ayrıntılı kütüphanedeki lokalizasyonuna göre teşhis edilir. Bu metodda son çıktı, taramada hücreye giren her bir sgRNA'nın sonucu kazanılmış fenotipik ölçümüdür (Agrotis ve Ketteler, 2015).

## 4. SİSTEME İLİŞKİN KISITLAMALAR VE ETİK

### *Kısıtlamalar ve Çözümlerine İlişkin Stratejiler*

CRISPR/Cas9 sistemi uygulamalarında ve geliştirilmesinde hala baş edilmesi gereken konular bulunmaktadır. Hedef dışı etkiler ya da istenmeyen mutasyonlar, immün cevap gelişmesi, transfeksiyon ajanlarının toksisitesi, kalıtsal mutasyonlara yol açabilecek embriyolardaki genomik düzenleme teknolojinin uygulamalarında karşılaşılabilecek kısıtlamalardan bazılarıdır. Etik ve legal kaygıları güvence altına alacak atılımların da yapılmasına ihtiyaç vardır.

CRISPR/Cas9 teknolojisinin majör bileşenlerinin tümünü (Cas9, sgRNA ve plazmid) tek bir vektör içinde paketlemek oldukça güçtür. Uygun viral vektör geliştirmek de kolay değildir. Ayrıca Cas9 pozitif olarak yüklüdür. Geliştirilen vektörlerin de pozitif yüklü olması aralarında elektrostatik etkileşime yol açar. Bu durumların, transfer için viral olmayan uygun vektörler geliştirilmesi ile üstesinden gelinmesi mümkün olabilir (Wang ve ark., 2019). Sistemin mikroenjeksiyon, Lance Array nanoenjeksiyon, elektroporasyon, hidrodinamik enjeksiyon ile direkt hücreye transferi ve lipozomlar, nanopartiküller gibi viral olmayan vektörler ile indirekt hücreye salıverilmesi teknikleri gen düzenleme etkinliğini artırmak için geliştirilmektedir (He ve ark., 2017).

CRISPR/Cas9 hedef dışı etkilere yol açabilir. Çalışılan genin dışındaki bölgelerde modifikasyonlara sebep olabilir, spesifik olmayan genlerde mutasyonlar oluşturabilir. Bunun için sgRNA'nın optimize edilmesi, uygun Cas9 konsantrasyonunda çalışılması istenmeyen etkileri azaltabilir. Ayrıca Cas9/sgRNA ile hücrelerin uzun süre karşı karşıya kalması da hedef dışı etkilerin boyutunu genişletebilir. Kısa süreli etkileşim için Cas9/sgRNA'nın RNP (ribonükleoprotein) formunda hücrelere girişinin sağlanması hedef dışı etkiyle başetmeye yardımcı olabilir (Wang ve ark., 2019).

Fizyolojik koşullarda CRISPR/Cas9 siteminin kararlılığını korumak düzeltme işleminin etkinliği bakımından son derece önemlidir. Hücredeki proteazlar ve nükleazlar sistemin bileşenlerini yıkabilir. Bunu önlemek için uygun vektörlerin geliştirilmesine ihtiyaç vardır ve bu konuda çalışmalar yapılmaktadır (Wang ve ark., 2019).

Sistemin bileşenleri hedef hücrelerde immün cevabı tetikleyebilir. Cas9 geni Cas9 proteini ifadesi için hücrelere verildiğinde, konağın immün sistemi tarafından tanınarak immün cevap oluşmasına yol açabilir. Cas9 proteini ile hücreye verme sistemi, proteinin daha kısa süre hücrede kalması sebebiyle daha az immün yanıt oluşmasını sağlayabilir. Nonviral vektörler kullanılması, Cas9 sisteminin bileşenlerini vektör içinde tutacağı için immün cevap oluşma kısıtlama sorunuyla baş etmeye yardımcı olabilir. İmmün cevap oluşumunun üstesinden gelmenin anahtarı vektör tasarımında yatmaktadır (Wang ve ark., 2019).

Diğer bir baş edilmesi gereken konu, DNA tamir mekanizması etkinliğinin artırılmasıdır. Bunun için bölünmeyen hücrelerde ve postmitotik hücrelerde homoloji ile yönetilen rekombinasyon stratejilerinin ya da gen tedavisinde NHEJ yoluyla gen düzeltilmesinin geliştirilmesine ihtiyaç vardır (Hu ve ark., 2018).

### *Etik*

CRISPR / Cas9 ve diğer genom düzenleme teknolojileriyle, başarılı somatik ve germline genom düzenlemesi uygulanabilir hale gelmektedir. Ancak teknoloji ile germline ve embriyolarda yapılacak düzenlemelerin kalıtsal olması, insan genomunu düzenleme etiğiyle ilgili endişelerin artmasına neden olmuştur. Henüz cevabı olmayan bilimsel, etik ve politik sorulara yanıt arama çalışmaları çeşitli komitelerin platformlarında tartışılmaktadır.

ABD Ulusal Bilimler Akademisi ve Ulusal Tıp Akademisi, mevcut durumu gözden geçirmek ve önerilerde bulunmak için çoklu disipliner, uluslararası bir komite oluşturmuştur. Komite'nin Şubat 2017'de yayınlanan raporu, gen terapisi için mevcut etik ve düzenleyici standartların somatik (kalıtsal olmayan) bir insan genom düzenlemesine uygulanmasını önermektedir. Komite iki durumda deneysel germline genom düzenlemesine izin verilmesini tavsiye etmektedir: a) Ciddi bir hastalık ya da durumun bulaşmasının engellenmesiyle sınırlandırılması amacıyla yapılmasını, b) Hastalıkla ilişkili olmadığı bilinen bir ortak DNA sekansında değişiklik yapılmasını, c) Araştırmanın katı bir etik ve düzenleyici gereksinimler kümesi çerçevesinde yürütülmesini (Coller, 2019).

Amerikan İnsan Genetiği Topluluğu çalışma grubu ise, Mart 2017'de ASHG[44] Kurulu tarafından daha önce onaylanan konuyla ilgili raporu geliştirdi. Çalışma grubu, İngiltere Genetik Hemşireler ve Danışmanlar Derneği, Kanada Genetik Danışmanlar Derneği, Uluslararası Genetik Epidemiyoloji Derneği ve ABD Ulusal Genetik Danışmanlar Derneği'nden temsilcilerden oluşturulmuştu. Amerikan Üreme Tıbbı Derneği, Asya Pasifik İnsan Genetiği Topluluğu, İngiliz Tıbbi Genetik Derneği, Avustralya İnsan Genetiği Topluluğu, Asya Profesyonel Genetik Danışmanları Derneği ve Güney Afrika İnsan Genetiği Derneği gibi gruplar da son raporu destekledi. Raporda şu açıklamalara yer verildi: 1) Şu anda, cevaplanmamış bilimsel, etik ve politika ile ilgili soruların niteliği ve sayısı göz önüne alındığında, insanların hamileliğinde germline gen düzenlemesi yapılması uygun değildir. 2) Şu anda, gen düzenlemesinin gelecekteki klinik uygulamaları ile ilgili araştırmaları kolaylaştırmak için donörlerin uygun gözetimi ve rızasının olduğu insan embriyoları ve gametlerinde *in vitro* germline genom düzenlemesinin yasaklanması için bir neden yoktur. Bu araştırmayı desteklemek için kamu fonlarının kullanılabilir olmasında yasak olmamalıdır. 3) İnsan germline genom düzenlemesinin gelecekteki klinik uygulaması, a) zorlayıcı bir tıbbi gerekçe, b) klinik kullanımını destekleyen bir kanıt temeli, (c) etik bir gerekçe ve (d) kamu katılımı olmadıkça devam etmemelidir (llyse ve ark., 2019).

## 5. SONUÇ

CRISPR/Cas9 teknolojisi hastalıkların modellenmesi, nörodejeneratif, kalıtsal, genetik ve viral infeksiyöz hastalıklar ile kanserin tedavisi, bitki ve hayvan verimliliğini artırma gibi pek çok alanda geniş bir uygulama imkanı sunmaktadır. Teknolojinin ucuz, kolay, hızlı ve nispeten güvenli uygulanabilir olması gelecekte büyük umutlar vaat etmektedir.

CRISPR hala bebeklik döneminde olmasına rağmen, yetişkin dokusunda gen tedavisi konusunda muazzam bir potansiyele sahiptir. CRISPR bize, insan yaşamının genetik kodunu manipüle etme konusunda eşi görülmemiş bir güç veriyor. Bu, tüm dünyadaki insanlar için daha iyi bir yaşam sağlayabilir. Bununla birlikte, kötüye kullanılması durumunda, düşünülemez felaketlere yol açabilir. Bu nedenle, CRISPR'ın uygulamasını izlemek için sıkı bir düzenlemeye ihtiyaç vardır. Ek olarak, germline gen düzenleme, geniş toplumsal fikir birliği olana ve riskler iyice araştırılana kadar devam etmemelidir (Liang ve ark., 2017).

CRISPR teknolojisinin istenmeyen etkilerine rağmen, birkaç biyoteknoloji firması, Bill Gates ve Google gibi teknoloji devleri çeşitli aksaklıklarla mücadele etmek için çağın teknolojisi olarak CRISPR sisteminin geleceğini desteklemektedirler. Farmositik olmayan bu yatırımcılar ve şirketler CRISPR teknolojisinin geliştirilmesi ve uygulanması için milyonlarca dolarlık yatırımlar yapmaktadırlar. Bill Gates, "Dünya son birkaç on yıldaki olağanüstü ilerlemeye devam etmek istiyorsa, güvenlik ve etik kurallarına göre, CRISPR gibi gelecek vaat eden araçlardan yararlanmaya devam etmeye bilim insanları teşvik edilmelidir" yorumunu yapmıştır. Kısıtlamaları etkisizleştirmeye yönelik yaklaşımlardaki ilerleme ve etik ve yasal normlara bağlılık, CRISPR-Cas9 teknolojisinin iyi geliştirilmiş şekilde uygulanmasına yol açacaktır (Kaushik ve ark., 2019).

Nitelikli genetik, politika ve etik uzmanlarının germline ve embriyo araştırmalarının planlanması ve uygulanmasına dahil edilmemesi, konunun sorunlu yönlerinden yalnızca biridir. Klinik genetik uzmanları, genetik hastalığın yapısını ve gen düzenlemenin klinik ihtiyaç eşiğini nerede karşılayabileceğini daha yasal olarak tespit edebilir. Genetik ve biyoetik araştırmacıları, hastalar, aileler ve toplulukların da dahil edilmesi ile çok yönlü bakış açılarından perspektiflerin dahil edilmesini sağlayabilirler. Genetik ve insanla ilgili araştırmaların etik, yasal ve sosyal sonuçları konusunda uzmanlar, klinik fayda, bilimsel keşif ve hasta özerkliği hedeflerini ilerleten sağlam ve uyumlu protokollerin tasarlanmasına yardımcı olabilirler. Artan disiplinlerarası iş birliği, şeffaflık ve halkın katılımı ile insanlarda gen düzenlemesi için güvenli ve etik bir yol bulunması mümkün olacaktır (llyse ve ark., 2019).

[44] American Society of Human Genetics (Amerikan İnsan Genetiği Topluluğu)

## KAYNAKLAR

Agrotis A, Ketteler R 2015. A new age in functional genomics using CRISPR/Cas9 in arrayed library screening. Frontiers in Genetics, 6: 300.

Ben Jehuda R, Shemer Y, Binah O 2018. Genome editing in induced pluripotent stem cells using CRISPR/Cas9. Stem Cell Reviews and Reports, 14(3): 323-336.

Burkard C, Lillico SG, Reid E, Jackson B, Mileham AJ, Ait-Ali T, Whitelaw CB, Archibald AL 2017. Precision engineering for PRRSV resistance in pigs: Macrophagesfrom genome edited pigs lacking CD163 SRCR5 domain are fully resistant to bothPRRSV genotypes while maintaining biological function. PLOS Pathogens, 13(2): e1006206.

Cerma´k T, Baltes NJ, Cegan R, Zhang Y, Voytas DF 2015. High-frequency, precise modification of the tomato genome. Genome Biology, 16: 232.

Coller BS 2019. Ethics of Human Genome Editing. Annu Rev Med, 70: 289-305.

Driehuis E, Clevers H 2017. CRISPR/Cas 9 genome editing and its applications in organoids. American Journal of Physiology-Gastrointestinal and Liver Physiology, 312(3): G257-G265.

Endo M, Mikami M, Toki S 2016. Biallelic gene targeting in rice. Plant Physiol, 170(2): 667–677.

Giau VV, Lee H, Shim KH, Bagyinszky E, An SSA 2018. Genome-editing applications of CRISPR-Cas9 to promote in vitro studies of Alzheimer's disease. Clinical Interventions in Aging, 13: 221-233.

Gupta SK, Shukla P 2017. Gene editing for cell engineering: trends and applications. Critical Reviews in Biotechnology, 37(5): 672-684.

Hamdan MF, Karlson CKS, Teoh EY, Lau SE, Tan BC 2022. Genome editing for sustainable crop improvement and mitigation of biotic and abiotic stresses. Plants (Basel), 11(19): 2625.

He ZY, Men K, Qin Z, Yang Y, Xu T, Wei YQ 2017. Non-viral and viral delivery systems for CRISPR-Cas9 technology in the biomedical field. Science China Life Sciences, 60(5): 458-467.

Hu MS, Longaker MT, Wan DC 2018. Discussion: CRISPR Craft: DNA editing the reconstructive ladder. Plastic and Reconstructive Surgery, 142(5): 1365-1366.

Jensen TI, Axelgaard E, Bak RO 2019. Therapeutic gene editing in haematological disorders with CRISPR/Cas9. British Journal of Haematology, 185(5): 821-835.

Jiang S, Shen QW 2019. Principles of gene editing techniques and applications in animal husbandry. 3 Biotech, 9(1): 28.

Jin LF, Li JS 2016. Generation of genetically modified mice using CRISPR/Cas9 and haploid embryonic stem cell systems. Dongwuxue Yanjiu, 37(4): 205-213.

Kaushik I, Ramachandran S, Srivastava SK 2019. CRISPR-Cas9: A multifaceted therapeutic strategy for cancer treatment. Seminars in Cell and Developmental Biology, 4. pii: S1084-9521(18)30118-6.

Kumar S, Yadav N, Pandey S, Thelma BK 2018. Advances in the discovery of genetic risk factors for complex forms of neurodegenerative disorders: contemporary approaches, success, challenges and prospects. Journal of Genetics, 97(3): 625-648.

Lee JS, Grav LM, Lewis NE, Faustrup Kildegaard H 2015. CRISPR/Cas9-mediated genome engineering of CHO cell factories: Application and perspectives. Biotechnology Journal, 10(7): 979-94.

Li M, Ouyang H, Yuan H, Li J, Xie Z, Wang K, Yu T, Liu M, Chen X, Tang X, Jiao H, Pang D 2018. Site-specific fat-1 knock-in enables significant decrease of n-6PUFAs/n-3pufas ratio in pigs. G3 (Bethesda), 8(5): 1747-1754.

Li WY, Gao QP, Liu H 2018. CRISPR/Cas9 system and its advances in gene therapy of hematologic diseases. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 26(6): 1863-1867.

Li Z, Liu ZB, Xing A, Moon BP, Koellhoffer JP, Huang L, Ward RT, Clifton E, Falco SC, Cigan AM 2015. Cas9-guide RNA directed genome editing in soybean. Plant Physiology, 169: 960–970.

Liang P, Zhang X, Chen Y, Huang J 2017. Developmental history and application of CRISPR in human disease. Journal of Gene Medicine, 19(6-7). doi: 10.1002/jgm.2963.

Liao HK, Gu Y, Diaz A, Marlett J, Takahashi Y, Li M, Suzuki K, Xu R, Hishida T, Chang CJ, Esteban CR, Young J, Izpisua Belmonte JC 2015. Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. Nature Communications, 6:6413. doi:10.1038/ncomms7413.

Lim KRQ, Yoon C, Yokota T 2018. Applications of CRISPR/Cas9 for the treatment of Duchenne Muscular Dystrophy. Journal of Personalized Medicine, 8(4). pii: E38.

llyse M, Bombard Y, Isasi R, Michie M, Musunuru K, Ormond KE 2019. What do we do now?: Responding to claims of germline gene editing in humans. Genetics in Medicine, 27.

Luo X, Li M, Su B 2016. Application of the genome editing tool CRISPR/Cas9 in non-human primates. Dongwuxue Yanjiu, 37(4): 214-219.

Macovei A, Sevilla NR, Cantos C, Jonson GB, Slamet-Loedin I, Čermák T, Voytas DF, Choi IR, Chadha-Mohanty P 2018. Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus. Plant Biotechnology Journal, 16(11): 1918-1927.

Marangi M, Pistritto G 2018. Innovative therapeutic strategies for cystic fibrosis: moving forward to CRISPR technique. Frontiers in Pharmacology, 9: 396.

Mittal RD 2019. Gene editing in clinical practice: Where are we? Indian Journal of Clinical Biochemistry, 34(1): 19-25.

Nishiga M, Liu C, Qi LS, Wu JC 2022. The use of new CRISPR tools in cardiovascular research and medicine. Nature Reviews Cardiology. 19(8): 505-521.

Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, Kang Y, Zhao X, Si W, Li W, Xiang AP, Zhou J, Guo X, Bi Y, Si C, Hu B, Dong G, Wang H, Zhou Z, Li T, Tan T, Pu X, Wang F, Ji S, Zhou Q, Huang X, Ji W, Sha J 2014. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell, 156(4): 836-43.

Ortiz-Virumbrales M, Moreno CL, Kruglikov I, Marazuela P, Sproul A, Jacob S, Zimmer M, Paull D, Zhang B, Schadt EE, Ehrlich ME, Tanzi RE, Arancio O, Noggle S, Gandy S 2017. CRISPR/Cas9-correctable mutation-related molecular and physiological phenotypes in iPSC-derived Alzheimer's PSEN2 N141I neurons. Acta Neuropathologica Communications, 5(1): 1-20.

Osborn MJ, Gabriel R, Webber BR, DeFeo AP, McElroy AN, Jarjour J, Starker CG, Wagner JE, Joung JK, Voytas DF, von Kalle C, Schmidt M, Blazar BR, Tolar J 2015. Fanconi anemia gene editing by the CRISPR/Cas9 system. Human Gene Therapy, 26(2): 114-126.

Pavel-Dinu M, Wiebking V, Dejene BT, Srifa W, Mantri S, Nicolas CE, Lee C, Bao G, Kildebeck EJ, Punjya N, Sindhu C, Inlay MA, Saxena N, DeRavin SS, Malech H, Roncarolo MG, Weinberg KI, Porteus MH 2019. Gene correction for SCID-X1 in long-term hematopoietic stem cells. Nature Communications, 10(1): 1634.

Perez Rojo F, Nyman RKM, Johnson AAT, Navarro MP, Ryan MH, Erskine W, Kaur P 2018. CRISPR-Cas systems: ushering in the new genome editing era. Bioengineered, 9(1): 214-221.

Petersen B, Niemann H 2015. Molecular scissors and their application in genetically modified farm animals. Transgenic Research, 24: 381–396.

Pyott DE, Sheehan E, Molnar A 2016. Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free Arabidopsis plants. Molecular Plant Pathology, 17(8): 1276-1288.

Rahman S, Datta M, Kim J, Jan AT 2019. CRISPR/Cas: An intriguing genomic editing tool with prospects in treating neurodegenerative diseases. Seminars in Cell and Developmental Biology, pii: S1084-9521(18)30109-5.

Saha SK, Saikot FK, Rahman MS, Jamal MAHM, Rahman SMK, Islam SMR, Kim KH 2019. Programmable molecular scissors: applications of a new tool for genome editing in biotech. Molecular Therapy Nucleic Acids, 14: 212-238.

Salsman J, Masson JY, Orthwein A, Dellaire G 2017. CRISPR/Cas9 gene editing: From basic mechanisms to improved strategies for enhanced genome engineering *in vivo*. Current Gene Therapy, 17(4): 263-274.

Sauer NJ, Narváez-Vásquez J, Mozoruk J, Miller RB, Warburg ZJ, Woodward MJ, Mihiret YA, Lincoln TA, Segami RE, Sanders SL, Walker KA, Beetham PR, Schöpke CR, Gocal GF 2016. Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants. Plant Physiology, 170: 1917–1928.

Shi TQ, Liu GN, Ji RY, Shi K, Song P, Ren LJ, Huang H, Ji XJ 2017. CRISPR/Cas9-based genome editing of the filamentous fungi: the state of the art. Applied Microbiology and Biotechnology, 101(20): 7435-7443.

Soyk S, Müller NA, Park SJ, Schmalenbach I, Jiang K, Hayama R, Zhang L, Van Eck J, Jiménez-Gómez JM, Lippman ZB 2017. Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. Nature Genetics, 49(1): 162–168.

Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM 2015. Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiology, 169(2): 931–945.

Tian X, Gu T, Patel S, Bode AM, Lee MH, Dong Z 2019. CRISPR/Cas9- An evolving biological tool kit for cancer biology and oncology. Nature Partner Journals Precision Oncology, 3: 8.

Torres-Ruiz R, Rodriguez-Perales S 2017. CRISPR-Cas9 technology: applications and human disease modelling. Briefings in Functional Genomics, 16(1): 4-12.

Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, Jaenisch R 2013. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell, 153(4): 910-918.

Wang L, Zheng W, Liu S, Li B, Jiang X 2019. Delivery of CRISPR/Cas9 by novel strategies for gene therapy. ChemBioChem, 20(5): 634-643.

Wang X, Cai B, Zhou J, Zhu H, Niu Y, Ma B, Yu H, Lei A, Yan H, Shen Q, Shi L, Zhao X, Hua J, Huang X, Qu L, Chen Y 2016. Correction: disruption of FGF5 in cashmere goats using CRISPR/Cas9 results in more secondary hair follicles and longer fibers. PLoS One, 11(11): e0167322.

Wang Y, Geng L, Yuan M, Wei J, Jin C, Li M, Yu K, Zhang Y, Jin H, Wang E, Chai Z, Fu X, Li X 2017. Deletion of a target gene in Indica rice via CRISPR/Cas9. Plant Cell Reports, 36(8): 1333-1343.

Xu R, Yang Y, Qin R, Li H, Qiu C, Li L, Wei P, Yang J 2016. Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. Journal of Genetics and Genomics, 43(8): 529–532.

Yang H, Wang H, Shivalila CS, Cheng AW, Shi L, Jaenisch R 2013. One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. Cell, 154(6): 1370-1379.

Yang YC, Yang HC 2021. Recent progress and future prospective in HBV cure by CRISPR/Cas. Viruses. 14(1): 4.

Yin H, Xue W, Anderson DG 2019. CRISPR-Cas: a tool for cancer research and therapeutics. Nature Reviews Clinical Oncology, 16(5): 281-295.

Yin H, Xue W, Chen S, Bogorad RL, Benedetti E, Grompe M, Koteliansky V, Sharp PA, Jacks T, Anderson DG 2014. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nature Biotechnology, 32(6): 551-553.

Yrigollen CM, Davidson BL 2019. CRISPR to the rescue: Advances in gene editing for the FMR1 gene. Brain Sciences, 9(1). pii: E17.

Zhan T, Rindtorff N, Betge J, Ebert MP, Boutros M 2019. CRISPR/Cas9 for cancer research and therapy. Seminars in Cancer Biology, 55: 106-119.

Zuo J, Li J 2014. Molecular genetic dissection of quantitative trait loci regulating rice grain size. Annual Review of Genetics, 48: 99–118.

*ORAL PRESENTATION*

# Yonca bitkisinin bazı verim unsurlarına Rhizobium, azotobakter ve Rhizobium/azotobakter kombine aşılamanın etkisi

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## Özet

Verimi yükseltmek için kullanılan aşırı gübrelemenin, maliyeti artırdığı, toprağın biyolojik verimliliğini olumsuz olarak etkilediği, ayrıca bitkilerde depolanarak ve içme sularına karışarak insan ve hayvan sağlığı açısından önemli sorunlara neden olduğu belirlenmiştir. Bitkilerin azot gereksinimlerinin bir kısmını daha az masraflı olan biyolojik azot fiksasyonu ile karşılamanın önemli olduğu açık olarak belirlenmiştir. Araştırmada; kontrol, azotlu kontrol, rhizobium, azotobakter ve rhizobium/azotobakter'in kombine uygulamaları ile kontrollü şartlarda serada deneme kurulmuştur. Yonca bitkisinin tohumları %0.5'lik sodyum hipoklorit (NaClO) çözeltisi ile yüzey sterilizasyonu yapıldıktan sonra, bakteri (*Rhizobium meliloti*) aşılaması ($1x10^8$ CFU) yapılmıştır. Aşılanan tohumlar, steril kum+perlit içeren ortamlara ekilmiştir. Denemede kontrol ve azotlu kontrol gruplarına da yonca tohumlarının yüzey sterilizasyonu yapıldıktan sonra ekilmiştir. Bitkiler çiçeklenmenin %50'sini geçtiği dönemde hasat edilmiş ve bazı ölçümler (üst aksam ve kök uzunluğu, üst aksam yaş ve kuru ağırlık, kök yaş ve kuru ağırlık, nodül sayısı ve ağırlığı, üst aksam ve kökte azot) yapılmıştır. Elde edilen verilere göre; Bilensoy-80 yonca çeşidi bitkisinin tohumlarına rhizobium, azotobakter ve rhizobium/azotobakter kombine bakterilerinin aşılanmasının etkileri farklı olmuş ve bu farklılıklar istatistiksel olarak önemli ($p<0.05$) bulunmuştur. Denemede; bitki üst aksam uzunluğu, bitki üst aksam kuru ağırlık, nodül sayısı, nodül ağırlığı, bitki üst aksam azot ve kökte azot içeriğine rhizobium/azotobakter kombine aşılaması etkili olmuştur. Diğer taraftan kök uzunluğu, bitki üst aksam yaş ağırlık, kök yaş ve kuru ağırlık üzerine ise rhizobium aşılamasının etkili olduğu belirlenmiştir. Bazı verim unsurları ve nodülasyon verilerinin en düşük değerleri ise kontrol uygulamasında tespit edilmiştir.

**Anahtar kelimeler:** Azotobakter, Rhizobium, Kombine aşılama**,** Verim unsurları, Yonca

# The effect of rhizobium azotobacter and rhizobium/azotobacter combined inoculation on some yield components of alfalfa plant.

## Abstract

It has been determined that overfertilization used to raise productivity increases the cost, negatively affects the biological productivity of the soil, and causes fundamental problems in terms of human and animal health by being stored in plants and contaminated with drinking water. It has been ascertained that it is essential to meet some of the nitrogen requirements of plants with the less expensive biological nitrogen fixation. In the research, a trial was set up in the greenhouse under controlled conditions with the combined applications of control, nitrogen control, rhizobium, azotobacter, and rhizobium/azotobacter. Bacteria (*Rhizobium meliloti*) inoculation ($1x10^8$ CFU) was performed after surface sterilization of alfalfa seeds with 0.5% sodium hypochlorite (NaClO) solution. Seeds inoculated were sown in an environment containing sterile sand + perlite. In the trial, alfalfa seeds were planted in the control and nitrogen control groups after surface sterilization. Plants were harvested when they exceeded 50% bloom, and some measurements (upper part and root length, upper part fresh and dry weight, root fresh and dry weight, number and weight of nodule, upper part, and root nitrogen) were made. According to the data obtained, the effects of the inoculation of combined bacteria of rhizobium, azotobacter, and rhizobium/azotobacter on the seeds of Bilensoy-80 varieties of alfalfa were different, and these differences were found to be statistically significant ($p<0.05$). At trial, combined inoculation with Rhizobium/azotobacter was efficient on the plant's upper part length, upper part dry weight, number of nodules, nodule weight, plant's upper part nitrogen, and root nitrogen content. On the other hand, it was found that rhizobium inoculation was efficient on root length, upper part fresh weight, and root fresh and

dry weight. The lowest values of some yield components and nodulation data were determined in the control application.

**Keywords**: Azotobacter, Rhizobium, Combined inoculation Yield components, Alfalfa

## Giriş

Kimyasal gübrelerin sürekli kullanımı sadece çevreyi kirletmez, aynı zamanda mikrobiyal aktiviteyi ve topraktaki organik maddeyi azaltır (Pahalvi et al. 2021). Biyogübreler özellikle N ve P besin arzı ile ilgili olarak tarımsal üretime faydalı mikroorganizmalar içerir; bu yüzden, genel bitki büyümesi için faydalıdırlar ve bitki büyümesini teşvik eden rizobakteriler (PGPR) olarak bilinirler (Affy et al. 2019). PGPR ile aşılama yoluyla bitki büyümesinin desteklenmesi, temel metabolik süreçleri içeren azot fiksasyonu, fosfat çözünürlüğü ve bitki büyüme hormonlarının üretimi etkilerine aracılık eder (Sheteiwy et al. 2021a; Gao et al. 2020). Rhizobium ile aşılama azot fiksasyonu ve büyüme teşvikinde sadece konukçu baklagillere fayda sağlamaz aynı zamanda özellikle mantar hastalıklarına karşı bitkinin biyotik stres toleransını artırır (Das et al. 2017). Baklagil bitkilerinin kök nodüllerindeki mikroorganizmalar tarafından atmosferik azotun amonyağa dönüştürülmesi olan biyolojik azot fiksasyonu, küresel azot döngüsünde ve dünya tarımında önemli bir rol oynamaktadır (Santos et al. 2012). Herridge et al. (2008) bildirdiği gibi, rhizobium simbiyozu konukçu bitkinin köklerinde veya gövdelerinde nodüller oluşturur ve baklagil-rhizobium simbiyozu toplam biyolojik azot fiksasyonunun %60'ını oluşturur. Bu yüzden, etkili rhizobium ile baklagillerin aşılanması, toprak sağlığını korurken, baklagillerin verim ve verim unsurlarını artırdığı belirlenmiştir. Ayrıca, N fiksasyonunun iyileştirilmesi için kullanılan çevre dostu uygulamalar olması, artan sürgün büyümesi, bakla sayısı ve bakla tane verimi ile sonuçlanmıştır (Siczek ve Lipiec, 2016). Azotobakter sp. ve Azospirillum sp. farklı ürünlerde en yaygın kullanılan biyogübrelerdir (Vessey 2003). Rana et al. (2012), göre, bir biyogübre olarak azotobakterin kullanılması bitkilerin ve toprağın besin profilinin iyileştirilmesinde, ürünlerin artışında ve çevrenin korunmasında büyük öneme sahiptir. Ayrıca, azotobakter ile aşılama, aşılanmayan bitkilere göre mısırda dane verimini %35'lere kadar artırmıştır (Bandhu and Parbati 2013). Rodelas et al. 1999a; Z25 *Rhizobium leguminosarium* ve 5 farklı *Azotobakter chroococcum* veya *Azotobakter vinelandii* suşları ile bakla tohumlarının mix aşılanması, nodülasyon, bitki gelişimi ve çiçeklenme aşamasında nodüllü köklerin nitrogenaz aktivitesi üzerine etkileri önemli bulunmuştur. *A. chroococcum* H23 suşu ve *A. vinelandii* ATCC 12837 ve Dv42 suşları, nodülleri içeren tüm bitki parçalarında kuru madde birikimini ilaveten total N içeriğini önemli derecede artırırken bitki büyümesini teşvik etmiştir. Andjelkovic et al. 2014; yaptıkları bir çalışmada rhizobium (*Rhizobium meliloti*), azotobakter (*Azotobakter chroococcum*) ve aktinomiset (*Streptomyces spp*.) kültürlerinin bireysel ve kombine kültürleri ile ekim öncesi aşılamanın yonca bitkilerinin büyüme parametreleri (boy, gövde sayısı ve bitki ağırlığı) üzerine etkileri araştırılmıştır. Test edilen parametreler için çeşit ve aşılama arasındaki interaksiyon kontrolle karşılaştırıldığında pozitif bir etkiyle sonuçlanmıştır. Rodelas et al. 1999b, Rhizobium/Azospirillum ve Rhizobium/Azotobakter 4 farklı kombinasyonu ile aşılanan bakla bitkileri, sadece rhizobium ile aşılanan bitkilerle karşılaştırıldığında makro ve mikro elementlerin (K, P, Ca, Mg, Fe, B, Mn, Zn ve Cu) dağılımı, konsantrasyonu ve total içeriklerinde değişikliklere yol açtığı belirlenmiştir. Etkiler, kombine aşılama için seçilen Azotobakter ve Azospirillum suşları arasında büyük ölçüde farklılık göstermiştir. *Rhizobium leguminoarum* (Rh), *Azotobacter chroococcum* (AZ1) ve *Bacillus megaterium var phosphaticium* (BM3) ile inokülasyonun iki çeşit kuru fasulyede (Bronco and Paulista) NPK kimyasal gübrelerin tavsiye edilen dozunun %25 altında nodülasyon, N fiksasyonu, rizosfer mikroorganizmaların popülasyonu, NPK içeriği, verim ve kabuk kalitesi üzerine etkilerini çalışmak için 2007-2008 yıllarında Kahire Üniversitesi, Ziraat Fakültesinde bir araştırma yürütülmüştür. Sonuçlar, kombine biyogübrelerle aşılamanın, kuru fasulyenin büyüme parametreleri, nodülasyon ve $N_2$-fiksasyonu üzerine önemli bir etkisi olduğunu göstermiştir (Gharib et al. 2015). Rhizobium'un farklı suşları, kanatlı fasulye ve soya fasulyesinin kombine inokülasyonu için sıcak ve tropikal koşullar altında Azospirillumun birkaç suşları ile test edilmiştir. Bu bakterilerin bazı kombinasyonlarından elde edilen mix inokülasyon sebebiyle nodülasyon, $N_2$ fiksasyonu, sürgün kuru madde üretimi ve N kazancında önemli artışlar belirlenmiştir. Azospirillum'un hücre içermeyen ekstraktı, organizmanın kendisiyle aynı etkiyi üretirken, kültür süpernatantı da bazı durumlarda aynı şeyi göstermiştir (Iruthayathas et al. 1983). Doğal *Rhizobium legominosarum* F46 ve *Azotobacter chroococcom* AGO11 suşu ile aşılama ve azotlu gübrenin su stresi koşullarında bakla bitkisinin büyüme ve büyüme indekslerine etkileri çalışılmıştır. Sonuçlar, tek başına aşılamanın, rhizobium ve azotobakter'in birlikte aşılanmasının nodül sayısı, nodülasyon, total azot içeriği, bağıl su içeriği, kök kuru ağırlık, ortalama gün çimlenme ve gün çimlenme hızı gibi büyüme indekslerinin çoğunu artırdığını göstermiştir. Rhizobium ve Azotobakter'in birlikte aşılanması su stresi koşulları altında su ve besin alımını artırmış, dolayısıyla su kıtlığının etkisini hafifletmiştir. (Dashadi et al. 2011). Bitki gelişimini teşvik eden rizobakteriler (PGPR) ve rhizobium ile birlikte aşılamanın etkisi, fasulye (*Phaseolus vulgaris* L.) çeşitlerinin

verim ve verim unsurları üzerine tarla koşullarında iki yıl üst üste araştırmalar yapılmıştır. Rhizobium suşu ile aşılamanın tepkisi olarak bitki gelişiminde önemli bir varyasyon gözlemlenmiştir. PGPR ile aşılama bitki başına bakla sayısı, bakladaki tohum sayısı, 100 tane ağırlığı, bitki başına tohumların ağırlığı, tohum verimi ve protein içeriğinin yanı sıra R6'daki toplam kuru madde miktarını önemli derecede artırmıştır (Yadegari ve Rahmani, 2010). Bir araştırmada, bezelye bitkisinin büyüme, verim ve kalite özellikleri üzerine *Azotobacter chroococcum* ve *Rhizobium leguminosarum* ile birlikte aşılamanın etkisini değerlendirmek için ekim öncesi ve çıkış sonrası gerçekleştirilmiştir. *A. chroococcum* ve *R. leguminosarum*, yalnızca rhizobium ile aşılanmış ilgili kontrol bitkileri ile karşılaştırıldığında, bitki biyokütlesini, besin alımını ve yaprak fotosentetik pigmentlerini önemli ölçüde iyileştirmiştir. Ayrıca, kontrol bitkilerine kıyasla *A. chroococcum* ile aşılama nodülasyon verilerini (nodül sayısı, nodül çapı ve nodül kuru ağırlığı) ve nitrogenaz enziminin aktivitesini arttırmıştır. İlaveten, sonuçlarımız ekim öncesi veya ekim sonrası uygulamalar arasında önemli bir fark olmadığını ortaya koymuştur (Ibrahim et al. 2022). Bu çalışma; yonca bitkisinin bazı verim unsurları ve nodülasyonu üzerine azotlu kontrol uygulamalarına kıyasla rhizobium, azotobakter ve rhizobium/azotobakter kombine aşılamalarının etkisini belirlemek amaçlanmıştır.

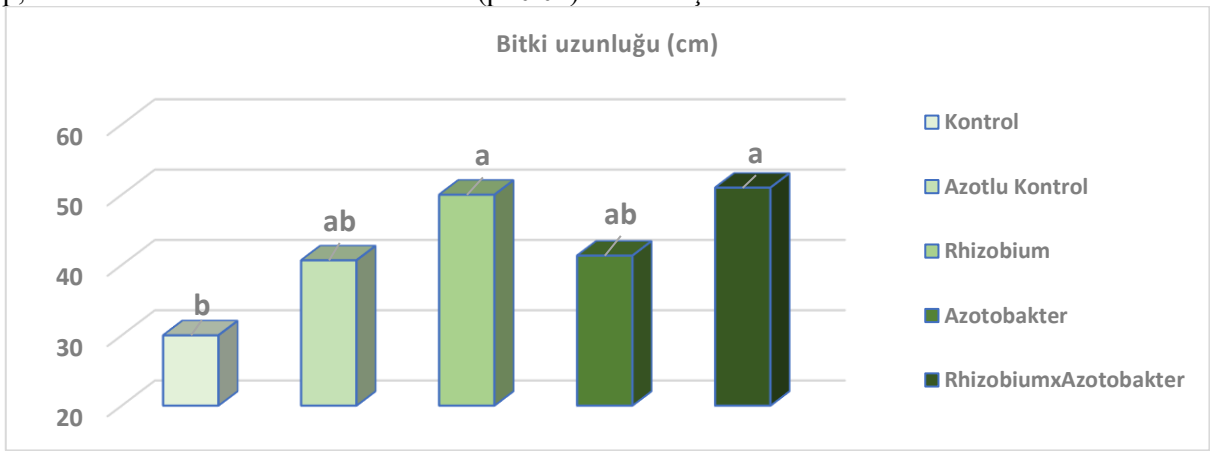
**Materyal ve Metot**

Araştırmada materyal olarak Bilensoy-80 Yonca çeşidi kullanılmıştır. Denemede aşılama materyali olarak Ankara Toprak, Gübre ve Su Kaynakları Merkez Araştırma Enstitüsü Müdürlüğünden temin edilen *Rhizobium meliloti* suşu kullanılmıştır. Deneme Selçuk Üniversitesi Ziraat Fakültesi Toprak Bilimi ve Bitki Besleme Bölümüne ait olan kontrollu serada yürütülmüştür. Yonca tohumlarının yüzey sterilizasyonu % 0.5'lik sodyum hipoklorit (NaClO) çözeltisi ile yapıldıktan sonra tohumlar bakteri kültürü ($1x10^8$ cfu) ile aşılanmıştır. Deneme süresince saksılar 1/5 oranında sulandırılmış azotsuz Jensen besin solüsyonu ile sulanmıştır. Sadece azotlu kontrol gruplarının besin solüsyonuna bitkinin ihtiyacı kadar azot verilmiştir. Bitkiler çiçeklenme döneminin %50'sini geçtikten sonra hasat edilmiştir. Yonca bitkilerinde; bitki üst aksam ve kök uzunluğu, bitki üst aksam yaş ve kuru ağırlığı, kök yaş ve kuru ağırlığı, nodül sayısı, nodül ağırlığı, bitki üst aksam ve kökte azot unsurları ölçülmüştür. Denemeden elde edilen veriler MINITAB ve MSTAT-C paket programlarından yararlanılarak varyans analizine tabi tutulmuştur. İstatistikî farklı grupların belirlenmesinde AÖF (LSD) testinden yararlanılmıştır (Yurtsever 1984).

**Araştırma Sonuçları ve Tartışma**

**Bitki üst aksam uzunluğu**

Araştırmada, kontrol, azotlu kontrol, rhizobium, azotobakter, rhizobium x azotokter kombine uygulamalarının Bilensoy-80 Yonca çeşidi bitkisinde bazı verim unsurları ve nodülasyon verileri ölçülmüştür. Araştırmadan elde edilen verilere göre; yapılan uygulamalar arasında bitkinin üst aksam uzunluklarında farklılıklar göstermiş olup, bu farklılıklar istatistik olarak önemli ($p<0.01$) bulunmuştur.



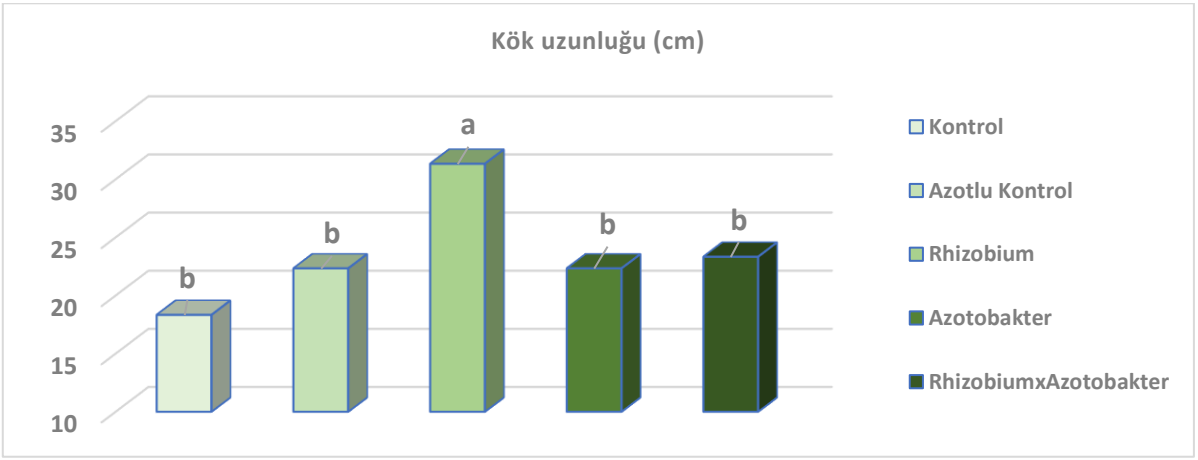

Şekil 1. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki üst aksam uzunluğu üzerine etkisi ($p<0.01$)

En yüksek bitki üst aksam uzunluğu 51 cm ile rhizobium x azotobakterin kombine uygulamasında belirlenmiştir. En düşük bitki üst aksam uzunluğu ise kontrol uygulamasında tespit edilmiştir (Şekil 1). Bitki üst aksam uzunlukları incelendiğinde bireysel ve kombine bakteri uygulamalarının kontrol ve azotlu kontrol uygulamalarına göre daha yüksek değerler olduğu tespit edilmiştir. Rhizobium ve rhizobium/azotobakter kombine aşılamalarının yonca bitkisinin üst aksam uzunluğuna azotlu kontrol uygulamasından daha yüksek değerler elde edildiği belirlenmiştir. Herridge et al. (2008) bildirdiği gibi, rhizobium simbiyozu konukçu bitkinin köklerinde veya gövdelerinde nodüller oluşturur ve baklagil-rhizobium simbiyozu toplam biyolojik azot fiksasyonunun %60'ını oluşturur. Bu yüzden, etkili rhizobium ile baklagillerin aşılanması, toprak sağlığını korurken, baklagillerin verim ve verim unsurlarını artırdığı tespit edilmiştir. Ayrıca, N fiksasyonunun

iyileştirilmesi için kullanılan çevre dostu uygulamalar olması, artan sürgün büyümesi, bakla sayısı ve bakla tane verimi ile sonuçlanmıştır (Siczek ve Lipiec, 2016).

**Bitki kök uzunluğu**

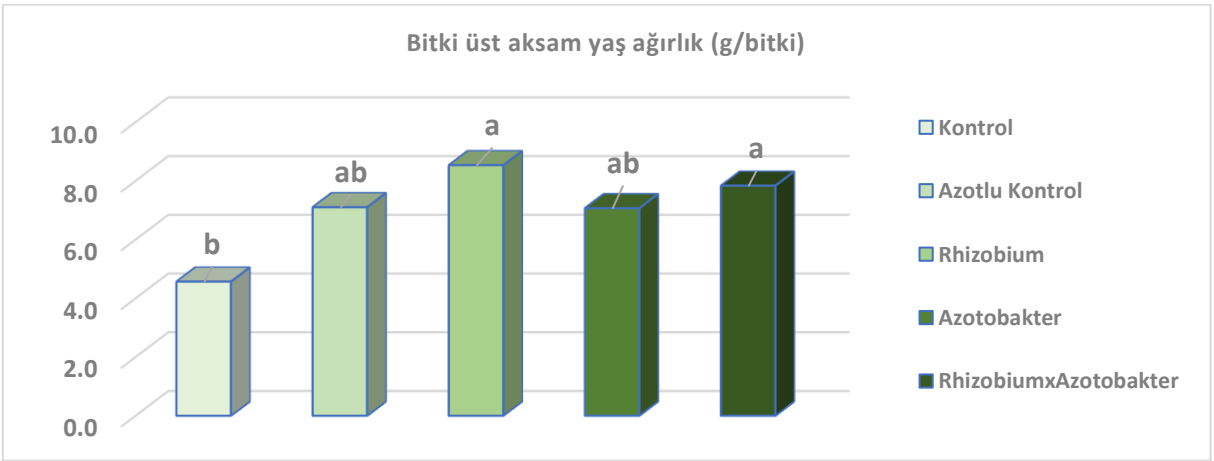
Araştırmadan elde edilen verilere göre; yapılan uygulamalar arasında bitkinin kök uzunluklarında farklılıklar göstermiş olup, bu farklılıklar istatistik olarak önemli ($p<0.05$) bulunmuştur. En yüksek bitki kök uzunluğu 31.3 cm ile rhizobium ile aşılanan tohumlardan elde edilen bitkilerde belirlenmiştir. En düşük bitki kök uzunluğu ise kontrol uygulamasında tespit edilmiştir (Şekil 2). Bilensoy-80 yonca bitkisinin kök uzunluklarının 18.3-31.3 cm arasında değiştiği belirlenmiştir. Rhizobium'un farklı suşları, kanatlı fasulye ve soya fasulyesinin kombine inokülasyonu için sıcak ve tropikal koşullar altında Azospirillumun birkaç suşları ile test edilmiştir. Bu bakterilerin bazı kombinasyonlarından elde edilen mix inokülasyon sebebiyle nodülasyon, $N_2$ fiksasyonu, sürgün kuru madde üretimi ve N kazancında önemli artışlar belirlenmiştir. Azospirillum'un hücre içermeyen ekstraktı, organizmanın kendisiyle aynı etkiyi üretirken, kültür süpernatantı da bazı durumlarda aynı şeyi göstermiştir (Iruthayathas et al. 1983)




Şekil 2. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki kök uzunluğu üzerine etkisi ($p<0.05$)

**Bitki üst aksam yaş ağırlık**

Rhizobium ve rhizobium/azotobakter kombine aşılamalarının kontrol uygulamaları ile kıyaslandığında bitki üst aksam yaş ağırlığı üzerine etkileri istatistiksel olarak önemli ($p<0.01$) bulunmuştur. Yonca bitkisinin üst aksam yaş ağırlıklarının 4.57-8.53 g arasında değiştiği belirlenmiştir.

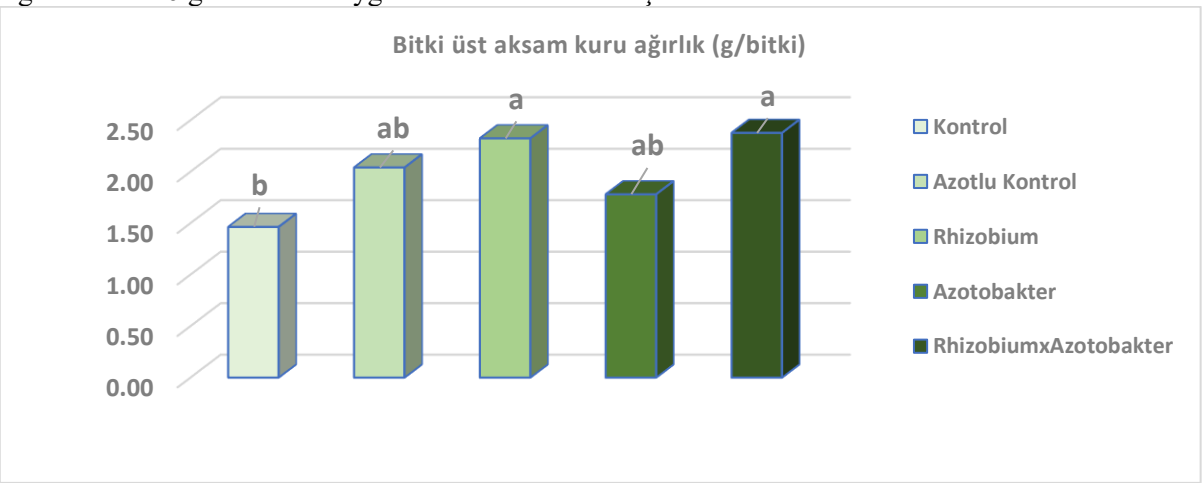



Şekil 3. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki üst aksam yaş ağırlığı (g/bitki) üzerine etkisi ($p<0.01$)

En yüksek bitki üst aksam yaş ağırlığı 8.53 g olarak rhizobium bakterisi ile aşılanan bitkilerden elde edilmiş olup en düşük yaş ağırlık ise 4.57 g ile kontrol uygulamasında belirlenmiştir. Rhizobium ve rhizobium/azotobakter kombine bakterilerle aşılanan tohumlardan elde edilen bitkilerin yaş ağırlıklarında azotlu kontrol grubundan daha yüksek değerler tespit edilmiştir. Bitki gelişimini teşvik eden rizobakteriler (PGPR) ve rhizobium ile birlikte aşılamanın etkisi, fasulye (*Phaseolus vulgaris* L.) çeşitlerinin verim ve verim unsurları üzerine tarla koşullarında iki yıl üst üste araştırmalar yapılmıştır. Rhizobium suşu ile aşılamanın tepkisi olarak bitki gelişiminde önemli bir varyasyon gözlemlenmiştir. PGPR ile aşılama bitki başına bakla sayısı, bakladaki tohum sayısı, 100 tane ağırlığı, bitki başına tohumların ağırlığı, tohum verimi ve protein içeriğinin yanı sıra R6'daki toplam kuru madde miktarını önemli derecede artırmıştır (Yadegari ve Rahmani, 2010).

**Bitki üst aksam kuru ağırlık**

Kontrol, azotlu kontrol, rhizobium, azotobakter ve rhizobium/azotobakter kombine uygulamalarının bitki üst aksam kuru ağırlığı üzerine etkileri istatistiksel olarak önemli ($p<0.05$) bulunmuştur. Yonca bitkisinin üst aksam kuru ağırlıklarının 1.46-2.38 g arasında değiştiği belirlenmiştir. En yüksek bitki üst aksam kuru ağırlığı 2.38 g olarak rhizobium/azotobakter kombine bakterileri ile aşılanan bitkilerden elde edilmiş olup en düşük yaş ağırlık ise 1.46 g ile kontrol uygulamasında belirlenmiştir.

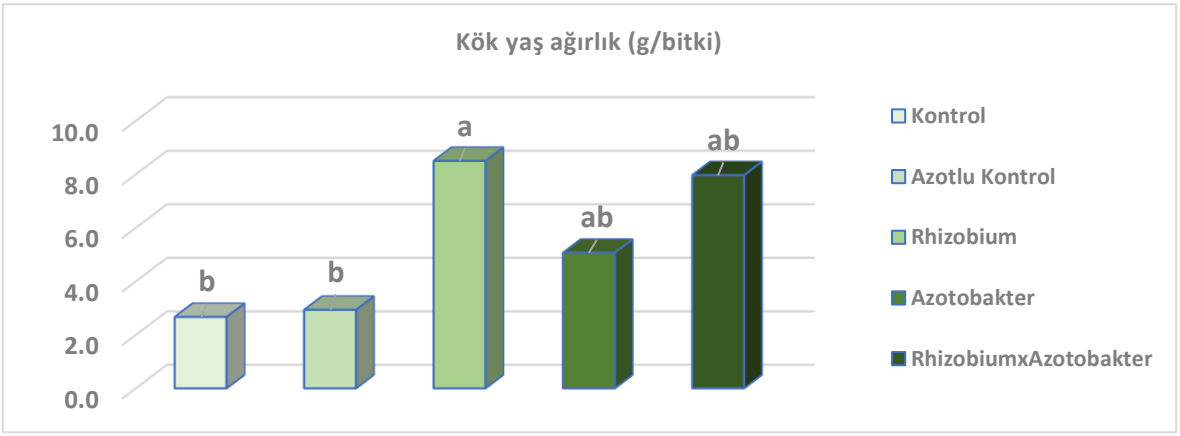



Şekil 4. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki üst aksam kuru ağırlık (g/bitki) üzerine etkisi ($p<0.05$)

Rhizobium ve rhizobium/azotobakter kombine bakterilerle aşılanan tohumlardan elde edilen bitkilerin kuru ağırlıklarında azotlu kontrol grubundan daha yüksek değerler tespit edilmiştir. Farklı seviyelerde azot ve biyo-gübrelerin kanola verimine etkisini araştırmak amacıyla İran, Kazvin'deki tesislerde bir araştırma yapılmıştır. Uygulamalar azotun 3 farklı dozu (0, 75 ve 150 $kgha^{-1}$) ve 4 farklı biyolojik gübreleri (kontrol, azotobakter, azospirillum ve azotobakter/azospirillum kombinasyonu) içermektedir. Sonuçlar, azot uygulamasının verim ve verim bileşenleri üzerinde önemli bir etkiye sahip olduğunu göstermiştir. Önemli artışlar biyo-gübre uygulanması ile tüm özelliklerde gözlemlenmiştir. Aynı zamanda biyogübrelerin kombine kullanımı diğer uygulamalardan istatistiksel olarak önemli bulunmuştur (Naderifar ve Daneshian, 2012).

**Kök yaş ağırlık (g/bitki)**

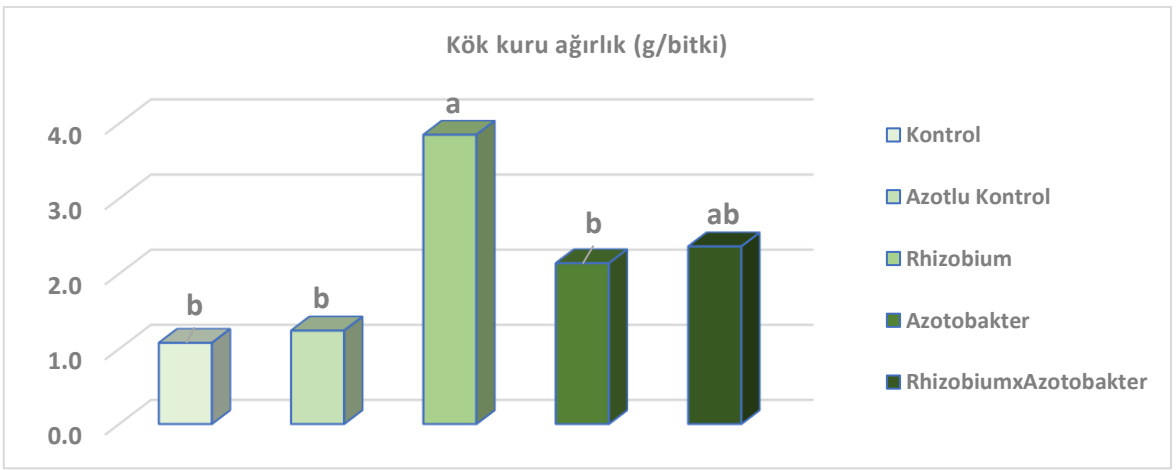
Araştırmadan elde edilen verilere göre; kontrol, azotlu kontrol, rhizobium, azotobakter ve rhizobium/azotobakter kombine aşılamalarının yonca bitkisinin kök yaş ağırlığı üzerine etkileri istatistiksel olarak önemli ($p<0.01$) olduğu belirlenmiştir. Bilensoy 80 yonca çeşidi bitkisinin kök yaş ağırlıklarının 2.67-8.50 g arasında değiştiği belirlenmiştir. Bitkinin en yüksek kök yaş ağırlığı 8.50 g olarak rhizobium bakterileri ile aşılanan bitkilerden elde edilmiş olup en düşük yaş ağırlık ise 2.67 g ile kontrol uygulamasında belirlenmiştir. Rhizobium ve rhizobium/azotobakter kombine bakterilerle aşılanan tohumlardan elde edilen bitkilerin kuru ağırlıklarında azotlu kontrol grubundan daha yüksek değerler tespit edilmiştir. Andjelkovic et al. 2014; yaptıkları bir çalışmada rhizobium (*Rhizobium meliloti*), azotobakter (*Azotobakter chroococcum*) ve aktinomiset (*Streptomyces spp*.) kültürlerinin bireysel ve kombine kültürleri ile ekim öncesi aşılamanın yonca bitkilerinin büyüme parametreleri (boy, gövde sayısı ve bitki ağırlığı) üzerine etkileri araştırılmıştır. Test edilen parametreler için çeşit ve aşılama arasındaki interaksiyon kontrolle karşılaştırıldığında pozitif bir etkiyle sonuçlanmıştır.




Şekil 5. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki kök yaş ağırlık (g/bitki) üzerine etkisi ($p<0.01$)

**Kök kuru ağırlık (g/bitki)**

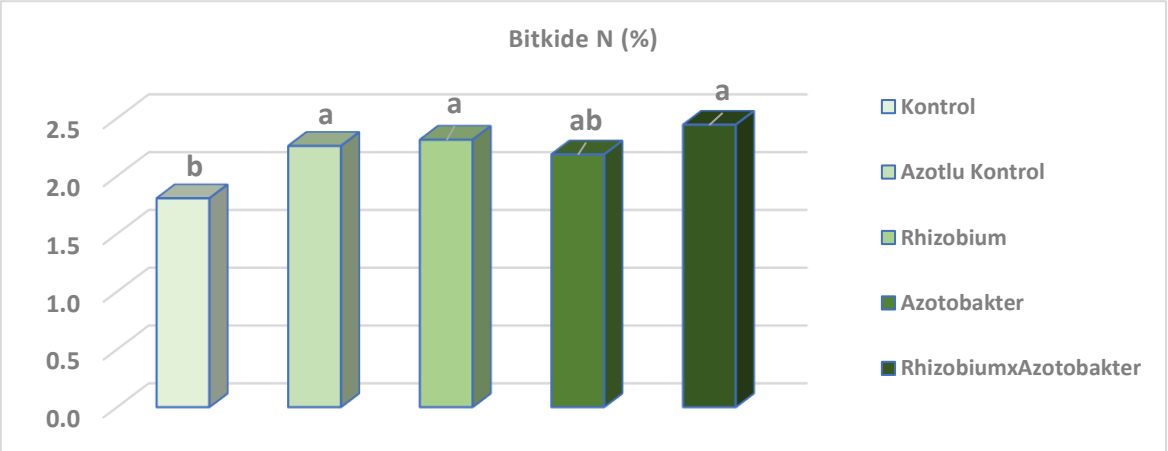
Yonca bitkisinin kök kuru ağırlığı üzerine; kontrol, azotlu kontrol, rhizobium, azotobakter ve rhizobium/azotobakter kombine uygulamalarının etkileri istatistiksel olarak önemli ($p<0.05$) bulunmuştur. Uygulamalar arasında Bilensoy 80 yonca çeşidi bitkisinin kök kuru ağırlıklarının 1.08-3.85 g arasında değiştiği belirlenmiştir. Yonca bitkisinin en yüksek kök kuru ağırlığı 3.85 g olarak rhizobium bakterileri ile aşılanan bitkilerden elde edilmiş olup en düşük kuru ağırlık ise 1.08 g ile kontrol uygulamasında tespit edilmiştir. En yüksek kök kuru ağırlığını (2.36 g) rhizobium/azotobakter kombine bakterilerle aşılanan tohumlardan elde edilen bitkilerin kuru ağırlıkları takip etmiştir. Doğal *Rhizobium legominosarum* F46 ve *Azotobacter chroococcom* AGO11 suşu ile aşılama ve azotlu gübrenin su stresi koşullarında bakla bitkisinin büyüme ve büyüme indekslerine etkileri çalışılmıştır. Sonuçlar, tek başına aşılamanın ve Rhizobium ve Azotobakter'in birlikte aşılanmasının nodül sayısı, nodülasyon, total azot içeriği, bağıl su içeriği, kök kuru ağırlık, ortalama gün çimlenme ve gün çimlenme hızı gibi büyüme indekslerinin çoğunu artırdığını göstermiştir. Rhizobium ve Azotobakter'in birlikte aşılanması su stresi koşulları altında su ve besin alımını artırmış, dolayısıyla su kıtlığının etkisini hafifletmiştir (Dashadi et al. 2011).




Şekil 6. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki kök kuru ağırlık (g/bitki) üzerine etkisi ($p<0.05$)

**Bitki üst aksam azot içeriği (%)**

Yonca bitkisinin üst aksam azot içeriğine, kontrol, azotlu kontrol, rhizobium, azotobakter ve rhizobium/azotobakter kombine uygulamalarının etkileri istatistiksel olarak önemli ($p<0.01$) olduğu tespit edilmiştir.

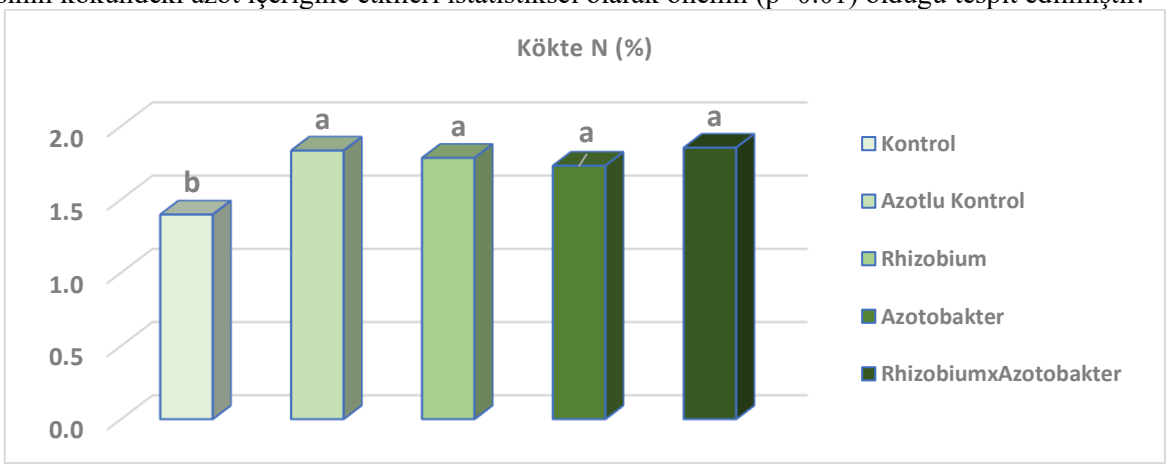



Şekil 7. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki üst aksam azot içeriğine etkisi ($p<0.01$)

Uygulamalar arasında Bilensoy 80 yonca çeşidi bitkisinin üst aksam azot içeriklerinin %1.81-2.44 arasında değiştiği belirlenmiştir. Yonca bitkisinin en yüksek üst aksam azot içeriği % 2.44 olarak kombine bakterileri ile aşılanan bitkilerden elde edilmiş olup en düşük üst aksam azot içeriği ise % 1.81 ile kontrol uygulamasında tespit edilmiştir. En yüksek üst aksam azot içeriğini % 2.31 ile rhizobium bakterileri ile aşılanan tohumlardan elde edilen bitkilerdeki azot içeriği takip etmiştir. Azotlu kontrol uygulamasının üst aksam azot içeriği % 2.26 olarak belirlenmiş olup azotobakterle aşılanan bitkilerin üst aksam azot içerikleri ise % 2.19 bulunmuştur. Rana et al. (2012), göre, bir biyogübre olarak Azotobakterin kullanılması bitkilerin ve toprağın besin profilinin iyileştirilmesinde, ürünlerin artışında ve çevrenin korunmasında büyük öneme sahiptir. Ayrıca, azotobakteri ile aşılama aşılanmayan bitkilere göre mısırda dane verimini %35 lere kadar artırmıştır (Bandhu ve Parbati 2013). Rodelas et al. 1999a; Z25 *Rhizobium leguminosarium* ve 5 farklı *Azotobakter chroococcum* veya *A. Vinelandii* suşları ile bakla tohumlarının mix aşılanması, nodülasyon, bitki gelişimi ve çiçeklenme aşamasında nodüllü köklerin nitrogenaz aktivitesi üzerine etkileri önemli bulunmuştur.

**Bitki kökü azot içeriği (%)**

Kontrol, azotlu kontrol, rhizobium, azotobakter ve rhizobium/azotobakter kombine aşılamalarının yonca bitkisinin kökündeki azot içeriğine etkileri istatistiksel olarak önemli ($p<0.01$) olduğu tespit edilmiştir.

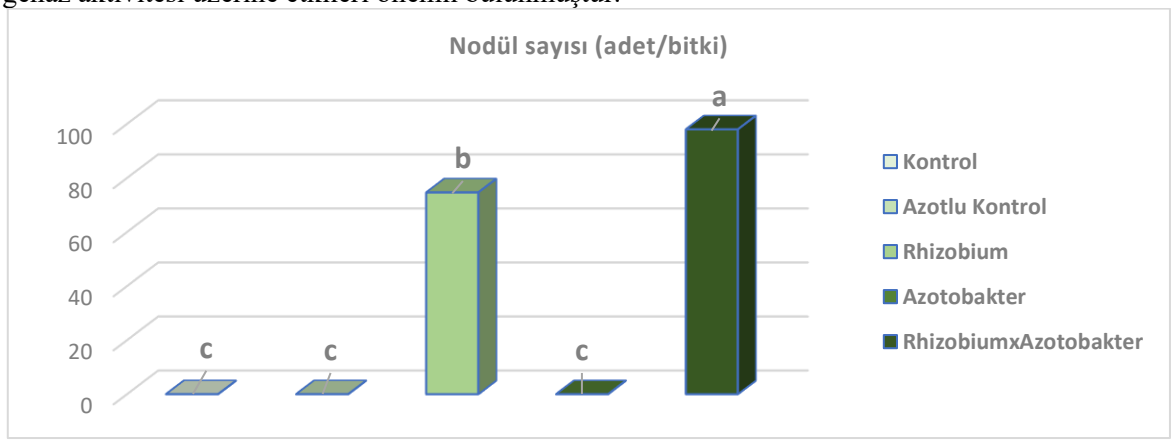



Şekil 8. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki kökündeki azot içeriğine etkisi ($p<0.01$)

Uygulamalar arasında Bilensoy 80 yonca çeşidi bitkisinin kökündeki azot içeriklerinin % 1.40-1.85 arasında değiştiği belirlenmiştir. Yonca bitkisinin kökündeki en yüksek azot içeriği % 1.85 olarak rhizobium/azotobakter kombine bakterileri ile aşılanan bitkilerden elde edilmiş olup en düşük azot içeriği ise % 1.40 ile kontrol uygulamasında belirlenmiştir. Bir araştırmada, bezelye bitkisinin büyüme, verim ve kalite özellikleri üzerine *Azotobacter chroococcum* ve *Rhizobium leguminosarum* ile birlikte aşılamanın etkisini değerlendirmek için ekim öncesi ve çıkış sonrası gerçekleştirilmiştir. *A. chroococcum* ve *R. leguminosarum*, yalnızca Rhizobium ile aşılanmış ilgili kontrol bitkileri ile karşılaştırıldığında, bitki biyokütlesini, besin alımını ve yaprak fotosentetik pigmentlerini önemli ölçüde iyileştirmiştir. Ayrıca, kontrol bitkilerine kıyasla *Azotobakter chroococcum* ile aşılama nodülasyon verilerini (nodül sayısı, nodül çapı ve nodül kuru ağırlığı) ve nitrogenaz enziminin aktivitesini arttırmıştır. İlaveten, sonuçlarımız ekim öncesi veya ekim sonrası uygulamalar arasında önemli bir fark olmadığını ortaya koymuştur (Ibrahim et al. 2022).

**Nodül sayısı (adet/bitki)**

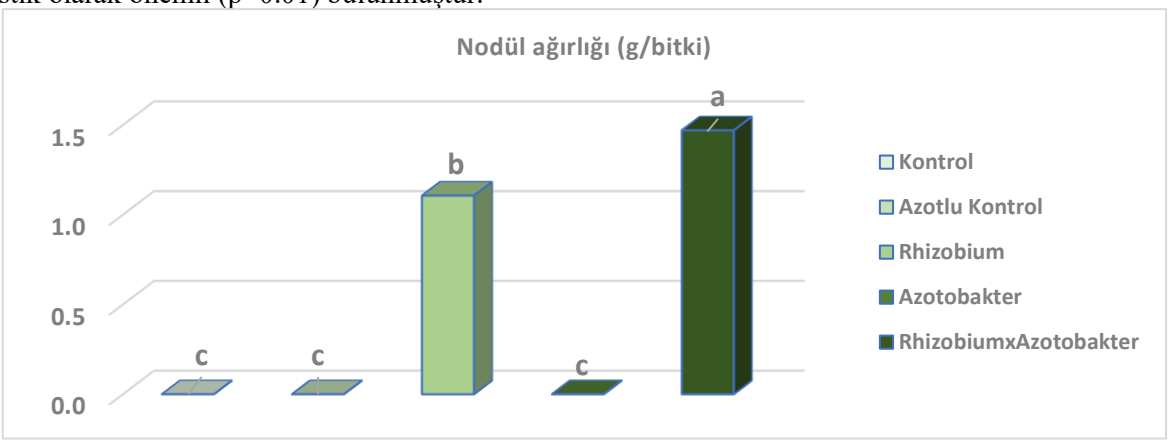
Farklı uygulamaların (kontrol, azotlu kontrol, rhizobium, azotobakter, rhizobium/azotobakter) sera koşullarında yetiştirilen yonca bitkisinin nodül sayısı üzerine etkileri farklılıklar göstermiş olup, bu farklılıklar istatistik olarak önemli ($p<0.01$) bulunmuştur. Elde edilen verilere göre uygulamalar arasındaki nodül sayılarının 0-98 adet arasında değiştiği belirlenmiş olup, en yüksek nodül sayısının 98 adet ile rhizobium/azotobakter kombine bakterilerle aşılanan bitkilerde belirlenmiştir. En düşük nodül sayısı ise 0 adet olarak kontrol, azotlu kontrol ve azotobakter uygulamalarında tespit edilmiştir. Rodelas et al. 1999a; Z25 *Rhizobium leguminosarium* ve 5 farklı *Azotobakter chroococcum* veya *A. vinelandii* suşları ile bakla tohumlarının mix aşılanması, nodülasyon, bitki gelişimi ve çiçeklenme aşamasında nodüllü köklerin nitrogenaz aktivitesi üzerine etkileri önemli bulunmuştur.




Şekil 9. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki kökündeki nodül sayısına (adet/bitki) etkisi ($p<0.01$)

**Nodül ağırlığı (g/bitki)**

Sera koşullarında yetiştirilen yonca bitkisinin nodül ağırlığı üzerine farklı uygulamaların (kontrol, azotlu kontrol, rhizobium, azotobakter, rhizobium/azotobakter) etkileri farklılıklar göstermiş olup, bu farklılıklar istatistik olarak önemli ($p<0.01$) bulunmuştur.




Şekil 10. Rhizobium, Azotobakter ve Rhizobium x Azotobakter kombine aşılamalarının bitki kökündeki nodül ağırlığına (g/bitki) etkisi ($p<0.01$)

Araştırma sonuçlarına göre uygulamalar arasındaki nodül ağırlıklarının 0-1.47 g arasında değiştiği belirlenmiş olup, en yüksek nodül ağırlığının 1.47 ile rhizobium/azotobakter kombine aşılama yapılan bitkilerin kökündeki nodüllerde belirlenmiştir. En düşük nodül ağırlığı ise 0 g olarak kontrol, azotlu kontrol ve azotobakter uygulamalarında tespit edilmiştir. *Rhizobium leguminoarum* (Rh), *Azotobacter chroococcum* (AZ1) ve *Bacillus megaterium var phosphaticium* (BM3) ile inokülasyonun iki çeşit kuru fasulyede (Bronco and Paulista) NPK kimyasal gübrelerin tavsiye edilen dozunun %25 altında nodülasyon, N fiksasyonu, rizosfer mikroorganizmaların popülasyonu, NPK içeriği, verim ve kabuk kalitesi üzerine etkilerini çalışmak için 2007-2008 yıllarında Kahire Üniversitesi, Ziraat Fakültesinde bir araştırma yürütülmüştür. Sonuçlar, kombine biyogübrelerle aşılamanın, kuru fasulyenin büyüme parametreleri, nodülasyon ve $N_2$-fiksasyonu üzerine önemli bir etkisi olduğunu göstermiştir (Gharib et al. 2015).

**Sonuç**

Kontrol, azotlu kontrol, rhizobium, azotobakter ve rhizobium/azotobakter kombine bakterilerle aşılamalarının Bilensoy-80 Yonca bitkisinin bazı verim unsurları ve nodülasyonuna etkisi istatistiksel olarak önemli ($p<0.01$; 0.05) bulunmuştur. Kontrol ve azotlu kontrol uygulamasına göre bakterilerin bireysel ve kombine aşılanması,

yonca bitkisinin verim unsurları ve nodülasyonunu artırmıştır. Sonuçlarımız yonca bitkisinin kök uzunluğu, üst aksam yaş ağırlık, kök yaş ağırlık ve kök kuru ağırlıkları üzerine bireysel olarak rhizobium bakterisi ile aşılamanın etkili olduğu belirlenmiştir. Diğer taraftan bitki uzunluğu, üst aksam kuru ağırlık, üst aksam ve kökte azot içeriği, nodül sayısı ve ağırlığı üzerine ise rhizobium/azotobakter bakterilerinin kombine aşılamalarının daha etkili olduğu tespit edilmiştir.

**Teşekkür**
Bu çalışma, Sadık İncekara tarafından yazılan Yüksek Lisans Tezinin bir bölümünün özetidir.

**KAYNAKLAR**

Affy AH, Hauka FIA, El-Sawah AM, Yanni YG, El-Saadany AY 2019. Inoculation with single, dual or consortia of *Rhizobium leguminosarum bv. trifollii*, *Pseudomonas stutzeri* and *Anabaena* sp. and their effect on yield components of rice plant. Journal of Agricultural Chemistry and Biotechnology, 10:189–193. https://doi.org/10.21608/jacb.2019. 60235.

Andjelković S, Vasić T, Lugić Z, Babić S, Milenković J, Jevtić G, Živković S 2014. The influence of individual and combined inoculants on development of alfalfa on acidic soil. Quantitative Traits Breeding for Multifunctional Grasslands and Turf, pp. 353–357.

Bandhu RB, Parbati A 2013. Effect of Azotobacter on growth and yield of maize. SAARC Journal of Agriculture, 11:141–147. https://doi.org/10. 3329/sja.v11i2.18409.

Das K, Prasanna R, Saxena A. 2017. Rhizobia: a potential biocontrol agent for soilborne fungal pathogens. Folia Microbiol (praha) 62:425–435. https://doi.org/10.1007/s12223-017-0513-z.

Dashadi M, Khosravi H, Moezzi A, Nadian H, Heidari M, Radjabi R 2011. Co-inoculation of Rhizobium and Azotobacter on growth indices of faba bean under water stress in the greenhouse condition. Advanced Studies in Biology, Vol. 3, no. 8, 373-385.

Gao C, El-Sawah AM, Ali DFI, Alhaj Hamoud Y, Shaghaleh H, Sheteiwy MS 2020. The integration of bio and organic fertilizers improve plant growth, grain yield, quality and metabolism of hybrid maize (*Zea mays* L.). Agronomy 10:319. https://doi.org/ 10.3390/agronomy10030319.

Gharib AA, Shahein MM, Ragab AA 2015. Influence of rhizobium inoculation combined with *Azotobacter chrococcum* and *Bacillus megaterium var phosphaticum* on growth, nodulation, yield and quality of two snap been (*Phaseolus vulgaris* l.) cultivars. Annals of Agricultural Science, Moshtohor., Vol. 53(2), 249–261.

Herridge DF, Peoples MB, Boddey RM 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil; 311:1–18.

Ibrahim HM, El-Sawah AM 2022. The mode of integration between Azotobacter and Rhizobium affect plant growth, yield, and physiological responses of Pea (*Pisum sativum* L.). Journal of Soil Science and Plant Nutrition. 22:1238–1251.

Iruthayathas EE, Gunasekaran S, Vlassak K 1983. Effect of combined inoculation of Azospirillum and Rhizobium on nodulation and $N_2$-fixation of winged bean and soybean. Scientia Horticulturae, 20: 231—240.

Naderifar M, Daneshian J 2012. Effect of seed inoculation with Azotobacter and Azospirillum and different nitrogen levels on yield and yield components of canola (*Brassica napus* L.). Iranian Journal of Plant Physiology, 3 (1), 619 – 626.

Pahalvi HN, Rafya L, Rashid S, Nisar B, Kamili AN 2021. Chemical fertilizers and their impact on soil health. In: Dar GH, Bhat RA, Mehmood MA, Hakeem KR (eds) Microbiota and biofertilizers, vol 2. Springer, Cham. https://doi.org/10.1007/ 978-3-030-61010-4_1

Rana A, Joshi M, Prasanna R, Shivay YS, Nain L 2012. Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. European Journal of Soil Biology, 50:118–126. https://doi.org/10.1016/j.ejsobi.2012.01.005.

Rodelas B, GonzaÂlez-LoÂpez J, Pozo C, SalmeroÂn VMV, MartõÂnez-Toledo MV 1999a. Response of Faba bean (*Vicia faba* L.) to combined inoculation with Azotobacter and *Rhizobium leguminosarum* bv. viceae. Applied Soil Ecology 12: 51-59.

Rodelas B, González-López J, Martínez-Toledo MV, Pozo C, Salmerón V 1999b. Influence of Rhizobium/Azotobacter and Rhizobium/Azospirillum combined inoculation on mineral composition of faba bean (*Vicia faba* L.). Biology and Fertility of Soils. 29:165–169.

Santos PC, Fang Z, Mason SW, Setubal JC, Dixon R 2012. Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. BMC Genomics; 13:162.

Sheteiwy MS, AbdElgawad H, Xiong YC, Macovei A, Brestic M, Skalicky M, Shaghaleh H, Hamoud YA, El-Sawah AM 2021a. Inoculation with *Bacillus amyloliquefaciens* and mycorrhiza confers tolerance to

drought stress and improve seed yield and quality of soybean plant. Physiologia Plantarum, 1–17. https://doi.org/10. 1111/ppl.13454.

Siczek A, Lipiec J 2016. Impact of faba bean-seed rhizobial inoculation on microbial activity in the rhizosphere soil during growing season. International Journal of Molecular Sciences. 17(5): 784.

Vessey JK 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586. https://doi.org/10.1023/A:10260 37216893.

Yadegari M, Asadi Rahmani H 2010. Evaluation of bean (*Phaseolus vulgaris*) seeds' inoculation with *Rhizobium phaseoli* and plant growth promoting Rhizobacteria (PGPR) on yield and yield components. African Journal of Agricultural Research Vol. 5(9), pp. 792-799.

Yurtsever N 1984. "Deneysel İstatistik Metotları" Tarım Orman ve Köy İşleri Bakanlığı. Köy Hizmetleri Genel Müdürlüğü Yayınları: 623. Ankara.

## *ORAL PRESENTATION*

# Fruit and Vegetable Based Probiotic Food Products

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## Abstract

Fruits and vegetables are widely known to be rich in nutrients, antioxidants, vitamins, dietary fiber, minerals, and a bioactive molecule. They are also an essential component of a balanced diet with multiple documented positive effects on human health. Probiotic cultures are beneficial microorganisms that can exert therapeutic effects on the organisms ingesting them. It is recommended to consume a minimum of $10^6$–$10^7$ CFU/mL daily to experience the health benefits of probiotics. Traditionally, probiotics are commonly recognized to be derived from yoghurt and other dairy. Dairy products are the major delivery vehicle for probiotics while non-dairy food offers some distinctive characteristics and benefits over dairy food products such as fulfilling the needs of the vegetarian population, absence of dairy-based allergens, providing low cholesterol content to consumers who suffer from cardiovascular or obesity problems, meeting the dietary habits of various ethnic groups, and an increase in the nutritional value. Therefore, there is a need to explore the potential of novel non-dairy food matrices to deliver these beneficial microorganisms to humans. In recent years, the demand for plant-based products is growing at a faster rate, with an increased demand for the incorporation of probiotics in fruit and vegetable juices. The increased awareness of the allergenicity and high levels of cholesterol and fats in milk has incited a shift in the preference of consumers to healthier alternatives, as even soybean products could also impose on the soy allergies of some consumers. Furthermore, lactose-intolerance and vegetarian, animal, and environmental activism have driven companies to expand their businesses into dairy-free and vegan-based drinks. This review describes the application of probiotic cultures in fruit and vegetable food products.

**Key words:** Probiotic foods, microorganisms, fruit, vegetable

## INTRODUCTION

Functional food was first used in Japan and the concept of food are designed to be medically beneficial to the consumer evolved during the 1980s (Junnarkar et al., 2019). Functional food can be determined as food or dietary components. Probiotic foods are the fastest growing field of functional food production in marketing (Aspri et al., 2020, Küçükgöz and Trzaskowska, 2022).

Several health benefits have been linked to the consumption of probiotic products (Natt and Katyal, 2021). These are *i)* treatment of diarrhea, *ii)* alleviation of symptoms of lactose intolerance, *iii)* reduction of blood cholesterol, *iv)* treatment of irritable bowel syndrome, *v)* inflammatory bowel disease, *vi)* anti-carcinogenic properties, *vii)* synthesis of vitamins, and *vii)* enhancing immunity (Vasudha and Mishra, 2013; Guergoletto et al., 2019). The health benefits of probiotic strains are mainly dependent on their ability to survive the passage through the upper GT, colonize, and multiply in the host intestine. In 2002, WHO/FAO has established the criterion that any food claimed to have a probiotic effect must contain at least $10^6$ to $10^7$ CFU/mL of viable probiotic bacterial (Aspri et al., 2020).

In food industry, many stages can affect the viability and survival of probiotic bacteria. The main stages are *i)* processing, *ii)* storage, *iii)* handling, *iv)* transport and *v)* shelf-life of the probiotic food. Moreover, following ingestion, probiotics must be able to survive the acidic conditions of the stomach as well as the bile salts in the small intestine, before they reach the lower gastrointestinal tract where they will provide beneficial effects (Aspri et al., 2020). This review describes the application of probiotic cultures in fruit and vegetable food products.

## FRUITS AND VEGETABLES BASED PROBIOTIC FOODS

Traditionally, probiotics were commonly recognized to be derived from dairy-based products such as yoghurt, cheese, and milk (Vasudha and Mishra, 2013). However, nowadays, non-dairy foods have been used for the isolation of potential probiotic isolates and to identify whether these cultures are suitable to produce novel nondairy probiotic products. Several non-dairy food products including meats, beverages, cereals, vegetables, and fruit have been utilized as delivery vehicles for probiotics. Probiotic cultures are also successfully applied in these food matrices (Min et al., 2018; Küçükgöz and Trzaskowska, 2022). The first non-dairy probiotic product came from a Swedish company Skane Dairy in 1994. Brand name is ProViva. The base substrate is oatmeal gruel being fermented by *Lactobacillus plantarum* 299v. The final product contains ~$5\times10^{10}$ CFU/L of probiotic culture. A similar product GoodBelly, prepared from oatmeal and *Lactobacillus plantarum* 299v was the first nondairy probiotic drink in US market in 2006 (Aspri et al., 2020). Fermented milk products have been conventionally considered as the most excellent carriers for probiotics; however, the use of milk-based products may be also limited by lactose-intolerance, allergies, dyslipidemia and vegetarianism (Patel, 2017).

Fruits and vegetables are considered healthy foods. These foods contain several beneficial nutrients such as various phytochemicals, antioxidants, minerals, vitamins, and dietary fibers. In contrast to dairy products, fruits and vegetables lack allergens, lactose, and cholesterol (Vasudha and Mishra, 2013). They are adversely affected certain groups of the population. Fruits are also healthy, refreshing, and have an appealing taste and flavor for all the population groups. Pineapple, cranberry, strawberry, sweet lime, mango, grapes, cashew apple, olive, carrot, beet root, and oranges are some examples of fruit juices used as food substrates for probiotic bacteria delivery (Natt and Katyal, 2021). Pectins and fruit fibers present in the tissue of fruits such as apples, guava, bananas, and melons are potential carriers of probiotic bacteria, and act as prebiotics to provide strong adhesion support for these bacterial. The use of fruits and vegetable juices in the fermentation process can increase the nutritional and functional properties of beverages, contributing beneficial effects on Health (Dahiya and Nigam, 2022). Many studies have demonstrated the feasibility of probiotic bacteria incorporation into fruit and vegetables. However, their survival and stability in such matrices have been found to be highly strain dependent. Several factors could limit probiotic viability and survival in juices. These are categorized as;

*i)* Intrinsic food parameters, such as titretable acidity, pH, molecular oxygen, water activity, presence of salt, sugar, artificial flavoring and coloring agents, and chemical or microbial preservatives like hydrogen peroxide and bacteriocins;

*ii)* Processing parameters- extent of heat treatment, incubation temperature, cooling rate, volume, packaging materials and storage techniques;

*iii)* Microbiological factors which mainly includes kind of probiotic strains, compatibility of different strains, inoculums proportion and rate (Patel, 2017).

A variety of types of probiotic fruit and vegetable products have been developed and commercialized (Table 1). Wide range of probiotic strains, mainly species of *Lactobacillus* spp. (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus paracasei, Lactobacillus rhamnosus GG, Lactobacillus plantarum, Lactobacillus fermentum*) and *Bifidobacterium* spp. (*Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium adolescentis, Bifidobacterium infantis, Bifidobacterium breve, Bifidobacterium lactis, Bifidobacterium laterosporus,*) have been widely used in the development of many fruit and vegetable probiotic products (Min et al., 2018).

**Table 1.** Fruit and vegetable based probiotic products sold commercially (adapted by Patel, 2017; Mojikon et al., 2022)

| Product Name | Manufacturer | Probiotic Strain (s) | Non-Dairy Substrate |
|---|---|---|---|
| Biomel | Biomel, UK | *B. bifidum, B. coagulans, Lb. plantarum* | Grape extract |
| GT's Organic Kombucha | GT, Los Angeles | *Lb. bacterium* (species not specified) and *Bacillus coagulans* GBI-30 6086 | Kiwi juice |
| KeVita Apple Cider Vinegar Tonics | KeVita, California | Water kefir (starter culture) and *Bacillus coagulans* GBI-30 6086 | Apple juice (to make apple cider), apple juice, and lemon extract |
| GoodBelly Juice Drink | Next Foods, Boulder, Colorado | *Lb. plantarum* LP299V | Mango: pear juice, mango puree, banana puree, Cranberry watermelon: grape juice, pear juice, cranberry juice, strawberry juice, watermelon juice, vegetable juice |
| Gut Shot® | Farmhouse Culture | Naturally occurring bacteria in the cabbage (Not specified) | Sauerkraut brine (fermented cabbage) and apple |
| Proviva | Skane Dairy, Sweden | *Lb. plantarum* LP299V | Orange, strawberry or blackcurrant juice |
| Rela | Biogaia, Sweden | *Lb. reuteri* MM53 | Apple juice |
| Biola | Valio Gefilus Ltd., Finland | *Lb. rhamnosus GG, Propionibacterium freudenreichii ssp. shermanii JS* | Seven varieties of juices |

## CONCLUSION

Fruit and vegetable based foods represent a suitable carrier for the delivery of probiotic cultures. However, there are several challenges to overcome, such as the survival of probiotics and their effects on the sensory attributes. There are some strategies (encapsulation, fortification with prebiotics, etc.) to overcome the issues, so preliminary studies are very promising and fascinating.

## REFERENCES

Aspri M, Papademas P, Tsaltas D 2020. Review on Non-Dairy Probiotics and Their Use in Non-Dairy Based Products. Fermentation, 6, 30. doi:10.3390/fermentation6010030.

Dahiya D, Nigam PS 2022. Nutrition and Health through the Use of Probiotic Strains in Fermentation to Produce Non-Dairy Functional Beverage Products Supporting Gut Microbiota. Foods, 11, 2760. Doi: 10.3390/foods11182760.

Guergoletto KB, Farinazzo FS, Mauro CSI, Fernandes MTC, Alves G, Prudencio SH, Garcia S 2019. Nondairy Probiotic and Prebiotic Beverages: Applications, Nutrients, Benefits, and Challenges. Editor(s): Alexandru Mihai Grumezescu, Alina Maria Holban. In Book: Nutrients in Beverages, Chapter 8, Academic Press, Pages 277-314, ISBN 9780128168424.

Junnarkar M, Pawar S, Gaikwad S, Mndal A, Jass J, Nawani N 2019. Probiotic potential of lactic acid bacteria from fresh vegetables: Application in food preservation. Indian Journal of Experimental Biology, 57: 825-838.

Küçükgöz K, Trzaskowska M 2022. Nondairy Probiotic Products: Functional Foods That Require More Attention. Nutrients, 14, 753. Doi: 10.3390/nu14040753

Min M, Bunt CR, Mason SL, Hussain MA 2018. Non-dairy probiotic food products: An emerging group of functional foods. Critical Reviews in Food Science and Nutrition, doi:10.1080/10408398.2018.1462760.

Mojikon FD, Kasimin ME, Molujin AM, Gansau, J.A.; Jawan, R. Probiotication of Nutritious Fruit and Vegetable Juices: An Alternative to Dairy-Based Probiotic Functional Products. Nutrients 2022, 14, 3457

Natt SK, Katyal P 2021. Current Trends in Non-Dairy Probiotics and Their Acceptance among Consumers: A Review. Agricultural Reviews. Doi: 10.18805/ag.R2172.

Patel AR 2017. Probiotic fruit and vegetable juices- recent advances and future perspective. International Food Research Journal 24(5): 1850-1857.

Vasudha S, Mishra HN 2013. Non-dairy probiotic beverages. International Food Research Journal, 20(1): 7-15.

## *ORAL PRESENTATION*

# DNA profiling by str method on a rootless dyed single hair strand

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## Abstract

A single strand of hair obtained from the crime scene is an important biological evidence in determining the course of the investigation and the case. Rooted hair is preferred in forensic genetic examinations. If the hair has fallen out with the root part, the identity of the perpetrator can usually be clarified by nuclear DNA tests. Since the small amount of DNA in hair is highly degraded and since the hair includes PCR inhibitors, working with this biological material in identification has serious difficulties. In DNA-based identification, small amounts of DNA must be successfully isolated, inhibitors should be removed or neutralized to increase the chance of correct typing of the hair. When hair is exposed to sunlight and chemical oxidation processes, these environmental effects make DNA analysis from hair even more difficult. Based on these limitations, in our study, hair samples were taken from 8 female and 8 male individuals aged between 20-65, using 4 different brands of hair dye selected randomly from the market. As the control group, 4 female and 4 male individuals with congenital black, brown, auburn and blond hair colors were included in the study. Silica-based DNA extraction was performed from the collected hair samples. DNA quality was checked by gel electrophoresis to determine whether DNA was obtained after isolation. In the measurements made to determine the amount of DNA, it was determined that 1.42 (±0.50) ng/mL DNA was obtained in dyed hair and 2.07 (±0.44) ng/mL DNA in the control group. PCR was performed with the STR kit containing primers specific to human genomic DNA. PCR products, which were analysed in the ABI 3130 genetic analyzer device, were genotyped using the Genemapper IDx software program. In antemortem samples, DNA is generally obtained as mtDNA from samples that are in the telogen phase (resting phase) or that do not contain hair follicles, while DNA was obtained with classical STR in only a few studies. In studies using STR in the literature, no interpretable profile could be obtained in an important ratio of the samples. In this study, our DNA isolation method which we modified and applied a purification protocol; is economical, fast and semi-manual compared to the literature methods, while successful DNA profiles were obtained in all dyed and undyed samples including rootless ones, with the STR kit, using a modified method. Since it is known that environmental conditions affect the amount and quality of DNA obtained, the amount of DNA obtained from dyed hair that we examined within the scope of our study is relatively lower than the amount of DNA obtained from undyed hair, However full DNA profiles could be obtained from dyed and undyed hair.

**Keywords:** Hair dyeing, DNA degradation, STR, Melanin oxidation, DNA Profiling and Identification

## INTRODUCTION

Hair and fiber have long been investigated as evidence in forensic cases (Gabel, 2014). Some of the defendants against whom this evidence was used turned out to be innocent in the past. In 1981, Donald Eugene Gates, 30, was arrested for the rape to and murder of Catherine Schilling a 21-year-old Georgetown University student (Yeshiva University, 2022). A police informant, Gerald Mack Smith, claimed that he and Gates were drinking in the park when Gates said he wanted to rob the victim, but when she resisted, he killed her. At Gates' trial in 1982, key testimony came from FBI forensic analyst Michael Malone who said that Gates' hairs were "microscopically indistinguishable" from hairs found on the victim's body. A paid police informant also testified that Gates had confessed the crime to him. On September 16, 1982, Gates was convicted. He was sentenced to 20 years to life in prison. In 1988, Gates sought DNA testing on the hair and tests were attempted, but DNA testing was in its infancy and the results were inconclusive. In 1997, an internal review of the FBI laboratory found that Malone and other analysts made false reports on cases across the country and performed inaccurate laboratory tests. In January, 2002, the U.S. Justice Department sent a letter to prosecutors in the

case, informing them that Malone's lab report was not supported by his notes but Gates' defense attorney was not notified. In 2007, Gates's former attorney, requested the case to be re-examined. In 2008, Gates sought DNA testing again, two years later, the request was granted and the tests, conducted on a sample of biological evidence found at the District of Columbia medical examiner's office, prooved his innocence. He was released on December 15, 2009. Ultimately, the real perpetrator who had worked as a janitor at the office building of the victim was identified through the DNA profile of the semen recovered post mortem.

DNA analysis on hairs serves justice even with a small strand, in solving even the most dark and hopeless files. A few strands of hair may fall to the crime scene without the criminal being aware of it, during a struggle or as a result of his rapid movement, or may be found between the fingers of the victims (Nickell & Fischer, 2014). Since our hair is also composed of cells, DNA can be obtained from our hair. Hair is a very special piece of evidence sought after in forensic cases to detect the culprit, as it is since it is personal. It is a priority choice because it is resistant to environmental conditions.

If a person has harmful habits, use drugs or is exposed to some poisons, drugs or other chemicals through the mouth, nose, injection, etc., a hair sample can tell us what they are and how long that person has been using them (Peacock et al., 2019). Because these substances accumulate in the hair. It is possible to have an idea about whether a person uses drugs, which opiate (poisonous substance) or not. Also, when the hair is routinely dyed, the chemical substances in the dyes are stored in the hair. Hair can also be useful in illuminating some historical events. Heavy metals can be dangerous for health, if exceed certain doses (Jaishankar et al., 2014). One of the ways of removing heavy metals from the body is hair. A human hair grows 1 cm per month, the last 3 cm of hair gives information for 3 months. Analysing hair for these substances and DNA, can solve the cases of crimes.

**The structure and the growth of the hair**

Hair is mainly composed of protein, keratin (65- 95%), lipids (1–9%), water (15–35%), and minerals (<1%). It has varying texture, color, and composition (Usman et al., 2019). Hair follicles arelocated 3–4-mm deep in the skin, in the epidermis of epithelium (Fig 1). The total hair length covers its bulb rooted in the follicle through the shaft and ends at its tip. Hair is composed of a root and shaft (K.-H. Kim et al., 2016). The shaft has three layers; a cuticle consisting of tightly packed colorless cells, a cortex containing natural color pigments that determine color, and a medulla as a hollow core.

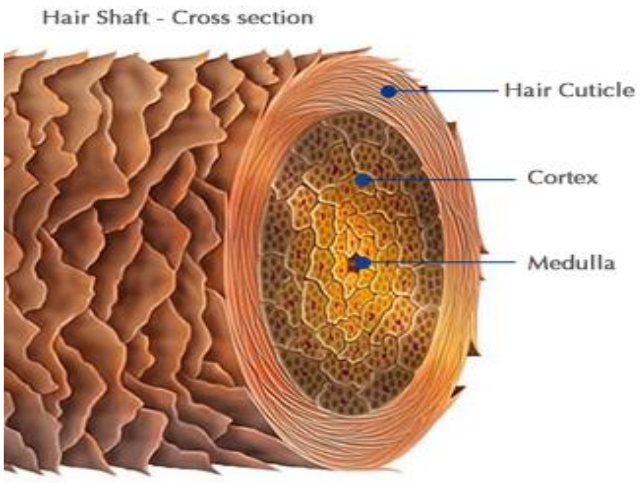


**Figure 1.** Hair follicle structure (modified from Robbins 2012)

According to region, age, gender, race and person to person; thickness (hair diameter) and color change (Fujimoto et al., 2008; Schneider et al., 2019; Tang et al., 2016). Different types of hairs grow at different rates, e.g., pubic hair 0.3 mm/day and axillary hair 0.4 mm/day (Usman et al., 2019). Beard hair grows with the speed of 0.27 mm/day, and is a good choice for drug/toxin analysis. It can also be collected daily for investigation of the rate of drug deposition. Hair has three phases of growth, called as anagen, catagen, and telogen phases (Fig 2). First years up to 6 is the anagen phase, while the root remains attached to the follicle for growth, giving the root bulb a flame-shape. During 2-3 weeks catagen phase, hair continues to grow more slowly and the roots gain an elongated shape as the root bulb shrinks and are pushed out of the hair follicle. Telogen phase begins after the soppage of the hair growth and the root acquires a club-shape. By the next 2 to 6 months, the hair is pushed out of the follicle, causing the hair to shed off naturally.




**Figure 2.** The growth phases of the hair

Hair follicle is wrapped around its outer and inner root sheaths. Glands exist located in and around the follicle (McLafferty et al., 2012). Sebaceous glands produce sebum to protect the skin. It also has a lubricating effect on the hair. Medulla can be absent or highly variable if present (Koch et al., 2020).

Each root of the hair is formed from a small group of stem cells in the hair follicle, providing a limited pool of mtDNA molecules, i.e. somatic bottleneck (Barrett et al., 2020). Growing hair follicles form hair root and shaft through mitosis. A stochastic segregation of DNA occurs in each part.

Hair can be found at crime scenes. Criminological research on this evidence has led to the development of various scientific methods over time. In forensic investigations, vertex posterior (back of the head) is the best area for sample collection (Kintz, 2018), because here, most of the hairs are in the same growth phase, the growth rate of most of the hairs is also the same and it is influences less from age and sex. The hair next to the scalp is cut and the location is noted (Usman et al., 2019). The hairs are stored in an envelope, aluminum foil, or a plastic zip lock bag and stored at ambient temperature. The amount sample depends on the drug to be tested and the methods of extraction and analysis. 3 cm hair shaft is taken, when segmental analysis of hair is to be performed especially in toxicological analyses. Length of 1 cm corresponds to 1-month growth, but this usually depends on the season and the hormonal balance of the body. Research shows that it grows faster during summer and daylight hours.

When biological material is transferred between a perpetrator and a victim in violent crimes such as murder or rape, the DNA recovered from the crime scene is used to identify the perpetrator (Mahajan et al., 2019). The best identification including a high power of discrimination and rapid analysis speed has been achieved with STR (short tandem repeat) DNA markers. STR DNA profiling technology has emerged as the most accurate and trusted scientific method to prove the crime and to get justice for the victims. When the hair will be used as a forensic evidence, first it should be discriminated morphologically, finding if it is a human or animal hair. Then DNA analysis is used for confirming this discrimination.If it is determined that it does not belong to a human, in the second stage, it is examined which animal the hair belongs to. Forensic genetics laboratories should first and always need genetic maps and DNA analysis of domestic animals such as cats or dogs. If it is a human hair, the below traditional workflow for generating DNA profiles is performed.

Identification of DNA from body samples found on different substrates:

1. Quantification of extracted DNA
2. Amplification of DNA using polymerase chain reaction (PCR) based STR reactions
3. Denaturation of amplified DNA
4. Genotyping using short tandem repeat (STR) technique
5. Analysis and comparison of generated DNA profiles.

STR analysis prediction result should be 99.99% in identification with DNA tests (Marjanović et al., 2009).

**Deformations formed by commercially available hair dyes on DNA identification of hairs**

It is inevitable to experience hair breakage, wear and some allergic reactions due to the use of dyes. The larger molecules of temporary hair dyes are unable to penetrate into the cortex; on the other hand, the smaller molecules of semi-permanent hair dyes easily penetrate into the cortex and diffuse out easily during washes

(K.-H. Kim et al., 2016). In the case of permanent hair dye, the smaller dye precursors penetrate into the cortex and undergo oxidation to form large colored molecules that tend to remain within the cortex. Permanent dyes wear out the hair fiber and damage the hair proteins and DNA.

**Can DNA be identified with a high probability ratio with current methods in literature?**

Since DNA isolation from rootless hairs is problematic, the literature studies on rootless hairs are focused on mtDNA analysis, instead of nuclear DNA; because of its relatively high copy number in hair shafts, and circular nature, and having smaller diameter highly variability allowing for an enhanced identification of individuals being less prone to degradation than nuclear DNA (Barrett et al., 2020). Although the high copy number of mitochondrial DNA relative to nuclear DNA routinely permits the recovery of mitochondrial DNA (mtDNA) data in these cases, mtDNA profiles do not offer the discriminatory power of nuclear DNA profiles (Brandhagen et al., 2018). However with mtDNA, a lower probability ratio in identification is obtained and fewer loci are studied, when compared with DNA analysis through a specially developed sensitive isolation method STR method (Barrett et al., 2020). Also mtDNA analysis is costlier and more time-consuming than STR analysis (G. H. Kim et al., 2020). If heterogenesis is low, mtDNA also won't help in identification of the culprit (Barrett et al., 2020). It is a lineage marker that cannot differentiate between maternally related genotypes (Grisedale et al., 2018).

In a study performed in 2021, results showed external decontamination procedures decreased drop-in alleles but also greatly reduced profile recovery (Gutierrez et al., 2021). In the literature, mini STRs are used for increasing success in the profiling however, in our study, a wide scale was used as 50-450 bps in PCR amplification. In the study of Martins et al., STRs with 60-120 bps are amplified with InnoTyper® 21 and Globalfiler™ kits. In spite of hair shafts exhibiting both low copy number and degradation, over 40% of the samples (25 hair shaft DNA extracts) produced interpretable profiles using the InnoTyper 21 kit. The method is fast however full profiles cannot be obtained in 60% of the samples (Martins et al., 2019). In nearly 23% of the samples (n = 9), the mobile elements-based kit allowed to obtain GPs with enough quality to be reported ($60 \leq GP \leq 99\%$ amplified alleles and complete GP), 10% (n = 4) of which presented a complete GP.

The ability of InnoTyper 21 to provide significant discrimination power, when compared with mtDNA results, is indicated to provide a significant benefit when genotyping highly degraded and trace nuDNA samples (Grisedale et al., 2018). It is known that some nuclear DNA is known to persist in telogen hairs, albeit at low quantities and highly variable qualities Brand hagen Grisedale et al couls obtain sufficient DNA to enable successful typing of nuclear DNA using the InnoTyper 21 amplification strategy in a number of hair shaft samples, and to a lesser extent, using the GlobalFiler kit. Overall, 42% of 60 hair samples tested produced genotype recoveries greater than 40% with InnoTyper 21. More than 70% of the hair shaft nuclear DNA extracts failed to amplify entirely using the GlobalFiler kit. Obtaining an interpretable nuDNA profile from a single hair shaft was observed to be largely dependent upon the hair donor, the DNA quantity recovered, and the Degradation Index.

Since the small amount of DNA in hair is highly degraded, working with this biological material in identification has serious difficulties. Another problem is PCR inhibitors in the hair. In DNA-based identification, small amounts of DNA must be successfully isolated, inhibitors should be removed or neutralized to increase the chance of correct typing of the hair. In particular, melanins, which are hair pigments, are known to inhibit PCR. When hair is exposed to sunlight and chemical oxidation processes, these environmental effects make DNA analysis from hair even more difficult. It is stated in the literature that; in order for DNA isolation to be successful, the hair must be in the active growth phase, that is, the anagen phase, or in the transition phase from the anagen phase to the catagen phase (regression period). In antemortem samples, while DNA is generally obtained as mtDNA from samples that are in the telogen phase (resting phase) or that do not contain hair follicles, while DNA was obtained with classical STR in only a few studies. The difficulty of successful STR typing of shed hairs is attributed primarily to the keratinization process, which degrades cellular organelles and nucleic acids (Brandhagen et al., 2018). In studies using STR in the literature, no interpretable profile can be obtained in an important ratio of the samples. So in this study, successful DNA profiles were obtained in all dyed and undyed samples from 16 individuals aged between 20-65, including rootless ones, with the STR kit, using a DNA isolation method that was modified, along with an original purification protocol which is economical, fast and semi-manual compared to the literature methods.

## MATERIAL AND METHOD

### Dyed rootless hair samples

In this study, it is aimed to examine the dyed hair samples, 4 men and 4 women, from 4 different age groups, which we used, in DNA analysis of chemical changes and whether there is a difference.A hair sample was placed in a 2 ml eppendorf tube by cutting it at 1 cm intervals and vortexed with clean sterile bi-distilled water for 1 minute and the water was poured. The Qiagen QIAamp® DNA Micro Commercial Kit Procedure has been modified and used. The prepared extract was divided into small sterile Eppendorf tubes and stored at -20 °C to reduce the risk of contamination. DNA quality was checked by gel electrophoresis to determine whether DNA was obtained after DNA isolation using the silica-based method.

Since we work with trace amounts and gradient samples at the cellular level, the purification protocol we developed was applied to the DNA isolates of the hair samples before PCR in order to increase the amount and quality of DNA.

AmpFlSTR® Identifiler® PCR Amplification kit (Applied Biosystems) was used as a DNA profiling system amplificating 15 otosomal (TPOX, D3S1358, D5S818, FGA, CSF1P0, D8S1179, D7S820, TH01, VWA, D13S317, D16S539, D18S51, D21S11, D2S1338, D19S433) and 1 gene identifying (amelogenin) STR loci. The primers included in the kit were developed using 5 different fluorescent dyes. Each of these dyes (PET, LIZ, 6-FAM, VIC, NED) fluoresces at different wavelengths up to 660 nm. In order to obtain optimum results in the PCR reaction, it is recommended by the manufacturer to analyze with a DNA amount in the range of 0.5-1.25 ng/µl. The allelic ladder included in the AmpFlSTR® Identifiers™ kit contains the most common alleles for each locus.

Also, GlobalFiler™ kit was used as an alternative method for confirmation. This kit is used in the amplification of 24 STR loci including D7S820, D5S818, CSF1PO, D1S1656, D13S317, D2S441, D12S391, D10S1248, D18S51, FGA, D21S11, D8S1179, VWA, D16S539, TH01, D3S1358, AMEL, D2S1338, D19S433, DYS391, TPOX, D22S1045, SE33,Y Indel repeat regions.In order to increase the success rate in gradient samples, the kit contains miniSTR loci, at least 10 of which are amplicon size less than 220 base pairs. The kit contains 2 STR loci, DYS391 and Y-Indel, in order to prevent false sex determination as a result of allele drop in Amelogenin or deletion in Y chromosome in mixed samples. The primers in the kit are marked with 5 different fluorescent labels. DNA size is labeled with a sixth fluorescent label different from the standard primers, allowing the length of PCR products to be determined.

### Electrophoresis of PCR products with ABI 3130

Electrophoresis of the samples was performed in 36 cm capillary and using polymer POP4 (Life Technologies) on ABI PRISM®3130 Genetic Analyzer instrument.

### Analysis of Data and Evaluation of Results

Visualization of raw data obtained from post-electrophoresis samples in ABI 3130, genotyping of electrophoreograms and evaluation of results were performed using GeneMapper® ID-X software v1.4 (Life Technologies) analysis program. GeneScan™-LIZ 500™/GeneScan™-LIZ 600™ DNA standards werer used so that the raw data could be properly evaluated in the GeneMapper program. In the study, scales including Allelic Ladder included in three separate PCR kits, including all common alleles of the studied loci, were used with the samples. Since allele types are known in these tables, the obtained peaks were evaluated according to them. RFU (Relative Fluorescent Unit) treshold was accepted as 50.

## RESULTS AND DISCUSSION

The presence of DNA strips was observed with 2% agarose gel electrophoresis in order to determine whether the isolation was successful and to observe the quality of the DNA obtained from the rootless hair samples (Fig 3).

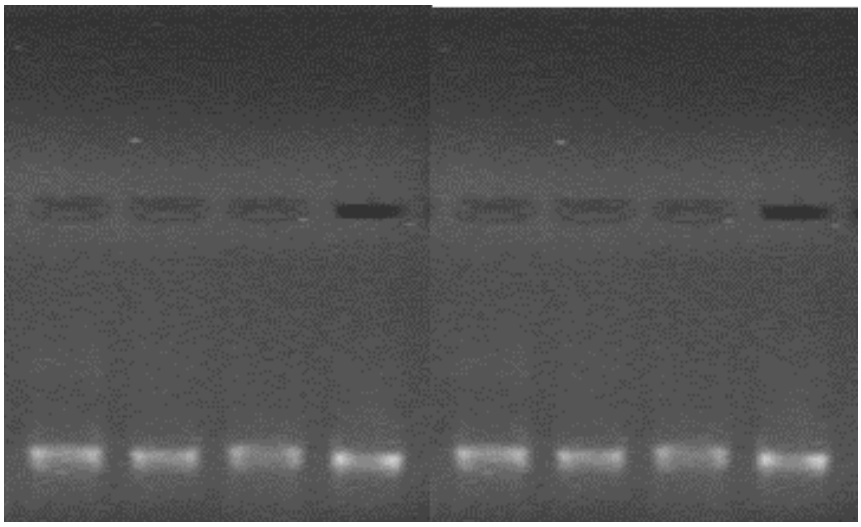

**Figure 3.** Gel electrophoresis results showing DNA quality in Biometra Gel Imaging System

In the measurements made to determine the amount of DNA, it was determined that 1.42 (±0.50) ng/mL DNA was obtained in dyed hair and 2.07 (±0.44) ng/mL DNA was obtained in the control group. A full profile was obtained by detecting 100% of the relevant alleles in each sample, that is, at all loci, for which DNA analysis was performed.

While hairs are excellent reservoirs for intact mitochondrial DNA (mtDNA), the increased time for mtDNA analysis and lack of discriminatory power are not ideal for common forensic casework (Gutierrez et al., 2021). In the studies performed with rootless hairs, thorough decontamination is performed before extractions to avoid potential contamination from exogenous DNA sources. The ability to remove any potential contamination and produce a single source genetic profile is an important factor to consider when extracting hair samples. This decontamination is highly successful at lowering the incidence of external contamination, but external surfaces of the hair can contain potentially probative DNA content from the hair donor. However in our study, we have performed a light decontamination technique in order not to lose DNA. Recent literature has also proven that most DNA content in rootless hair shafts is nuclear in origin, rather than mitochondrial (Brandhagen et al., 2018). Therefore, we can obtain nuclear DNA from rootless hair strands in our study.

## CONCLUSION

We cannot give a certain result morphologically or chemically in electron microscopy or normal microscopic studies, but we can directly find the perpetrator or the deceased with DNA. There is deterioration in the impulse structures of the cuticle layers of the hair exposed to chemicals such as dyes, etc. These may damage some of the DNA. The general problem in the analysis of DNA from hair is the lose of DNA while cleaning. Also the isolation techniques should be specialized for hair.

In rootless samples, DNA was obtained with classical STR in only a few studies. In studies using STR in the literature, no interpretable profile can be obtained in an important ratio of the samples. In this study, our DNA isolation method which we modified and applied a purification protocol; is economical, fast and semi-manual compared to the literature methods, while successful DNA profiles were obtained in all dyed and undyed samples including rootless ones, with the STR kit, using a modified method. Since it is known that environmental conditions affect the amount and quality of DNA obtained, the amount of DNA obtained from dyed hair that we examined within the scope of our study is relatively lower than the amount of DNA obtained from undyed hair, However full DNA profiles could be obtained from dyed and undyed hair.

**REFERENCES**

Barrett, A., Arbeithuber, B., Zaidi, A., Wilton, P., Paul, I. M., Nielsen, R. & Makova, K. D. (2020). Pronounced somatic bottleneck in mitochondrial DNA of human hair. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1790), 20190175. https://doi.org/10.1098/rstb.2019.0175

Brandhagen, M. D., Loreille, O. & Irwin, J. A. (2018). Fragmented Nuclear DNA is the Predominant Genetic Material in Human Hair Shafts. *Genes*, *9*(12), 640. https://doi.org/10.3390/genes9120640

Fujimoto, A., Kimura, R., Ohashi, J., Omi, K., Yuliwulandari, R., Batubara, L., Mustofa, M. S., Samakkarn, U., Settheetham-Ishida, W., Ishida, T., Morishita, Y., Furusawa, T., Nakazawa, M., Ohtsuka, R. & Tokunaga, K. (2008). A scan for genetic determinants of human hair morphology: EDAR is associated with Asian hair thickness. *Human Molecular Genetics*, *17*(6), 835–843. https://doi.org/10.1093/hmg/ddm355

Gabel, J. D. (2014). Realizing reliability in forensic science from the ground up. *The Journal Of Criminal Law & Criminology*, *104*, 284–351.

Grisedale, K. S., Murphy, G. M., Brown, H., Wilson, M. R. & Sinha, S. K. (2018). Successful nuclear DNA profiling of rootless hair shafts: a novel approach. *International Journal of Legal Medicine*, *132*(1), 107–115. https://doi.org/10.1007/s00414-017-1698-z

Gutierrez, R., LaRue, B. & Houston, R. (2021). Novel extraction chemistry and alternative amplification strategies for use with rootless hair shafts. *Journal of Forensic Sciences*, *66*(5), 1929–1936. https://doi.org/10.1111/1556-4029.14763

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B. & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, *7*(2), 60–72. https://doi.org/10.2478/intox-2014-0009

Kim, G. H., Kwon, S. Y., Lee, S.-Y., Seong, K. M., Kim, J. & Lim, S.-K. (2020). Evaluation of the InnoTyper21® system for the applications into trace and degraded DNA in the Korean population. *International Journal of Legal Medicine*, *134*(6), 2053–2059. https://doi.org/10.1007/s00414-020-02429-3

Kim, K.-H., Kabir, E. & Jahan, S. A. (2016). The use of personal hair dye and its implications for human health. *Environment International*, *89–90*, 222–227. https://doi.org/10.1016/j.envint.2016.01.018

Kintz, P. (2018). Hair Analysis in Forensic Toxicology: An Updated Review with a Special Focus on Pitfalls. *Current Pharmaceutical Design*, *23*(36). https://doi.org/10.2174/1381612823666170929155628

Koch, S. L., Tridico, S. R., Bernard, B. A., Shriver, M. D. & Jablonski, N. G. (2020). The biology of human hair: A multidisciplinary review. *American Journal of Human Biology*, *32*(2). https://doi.org/10.1002/ajhb.23316

Mahajan, V. B., Padale, V., Kudekar, D. Y., More, B. P. & Kulkarni, K. V. (2019). Hair-A Good Source of DNA to Solve the Crime. *Archives of Clinical and Biomedical Research*, *3*(4), 287–295.

Marjanović, D., Durmić-Pašić, A., Kovačević, L., Avdić, J., Džehverović, M., Haverić, S., Ramić, J., Kalamujić, B., Bilela, L. L., Škaro, V., Projić, P., Bajrović, K., Drobnič, K., Davoren, J. & Primorac, D. (2009). Identification of Skeletal Remains of Communist Armed Forces Victims During and After World War II: Combined Y-chromosome Short Tandem Repeat (STR) and MiniSTR Approach. *Croatian Medical Journal*, *50*(3), 296–304. https://doi.org/10.3325/cmj.2009.50.296

Martins, C., Ferreira, P. M., Carvalho, R., Costa, S. C., Farinha, C., Azevedo, L., Amorim, A. & Oliveira, M. (2019). Evaluation of InnoQuant® HY and InnoTyper® 21 kits in the DNA analysis of rootless hair samples. *Forensic Science International: Genetics*, *39*, 61–65. https://doi.org/10.1016/j.fsigen.2018.12.005

McLafferty, E., Hendry, C. & Farley, A. (2012). The integumentary system: anatomy, physiology and function of skin. *Nursing Standard*, *27*(9), 35.

Nickell, J. & Fischer, J. F. (2014). *Crime science: methods of forensic detection.* University Press of Kentucky.

Peacock, A., Bruno, R., Gisev, N., Degenhardt, L., Hall, W., Sedefov, R., White, J., Thomas, K. V, Farrell, M.

& Griffiths, P. (2019). New psychoactive substances: challenges for drug surveillance, control, and public health responses. *The Lancet*, *394*(10209), 1668–1684. https://doi.org/10.1016/S0140-6736(19)32231-7

Schneider, P. M., Prainsack, B. & Kayser, M. (2019). The Use of Forensic DNA Phenotyping in Predicting Appearance and Biogeographic Ancestry. *Deutsches Arzteblatt International*, *51–52*(51–52), 873–880. https://doi.org/10.3238/arztebl.2019.0873

Tang, W., Zhang, S. G., Zhang, J. K., Chen, S., Zhu, H. & Ge, S. R. (2016). Ageing effects on the diameter, nanomechanical properties and tactile perception of human hair. *International Journal of Cosmetic Science*, *38*(2), 155–163. https://doi.org/10.1111/ics.12269

Usman, M., Naseer, A., Baig, Y., Jamshaid, T., Shahwar, M. & Khurshuid, S. (2019). Forensic toxicological analysis of hair: a review. *Egyptian Journal of Forensic Sciences*, *9*(1), 17. https://doi.org/10.1186/s41935-019-0119-5

Yeshiva University. (2022). *The Innocence Project is affiliated with Benjamin N. Cardozo School of Law*. https://innocenceproject.org/cases/donald-eugene-gates

## *ORAL PRESENTATION*

# An approach to evaluate plasmid and insert DNA ligation efficiency and desired colony selectability by PCR

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## Abstract

Ligation is a process in which the desired DNA fragments are covalently attached to form recombinant DNA by DNA ligase. It is widely used to obtain desired and intact recombinant plasmid DNA. This is a critical step in gene manipulation or recombinant DNA technology. The success of this step is dependent on the well-defined reaction component and well-prepared DNA samples. Success in this process affects significantly transformation efficiency and selection of the desired colony in plasmid-based gene manipulations. Therefore, it may affect time, labor, and cost of study, and it should be checked before transformation to competent cells. In the current study, the PCR approach was applied to interpret ligation success. For this purpose, two primer pairs, which were sequencing primers, were used. These primers bind to downstream and upstream DNA parts of multiple cloning sites and enable the amplification of inserted DNA. After the ligation step, a tiny volume of ligation mixture, ~0.1 µl, was taken and added to the PCR mixture. After running of PCR at appropriate condition, PCR products were resolved by agarose gel electrophoresis. As a result, two different lengths of PCR products were obtained. The desired PCR product length was about insert length, which indicated recombinant plasmid in the mixture, whereas the undesired PCR product length was the same length obtained from the empty vector, which indicated wild-type plasmid occurrence in the ligation mix. The band intensity ratio of desired PCR product to the undesired PCR product may indicate the success of the ligation step and the possibility to find the desired colony. This approach can be used to determine ligation efficiency and desired colony selectability for cloning studies.

**Keywords:** Cloning, Ligation, Efficiency, Evaluation, PCR

## INTRODUCTION

Recombinant DNA technology is a molecular application that the genetic material of an organism is altered to obtain desired products from a living organism or improve the characteristics of living organisms (Khan et al., 2016, Nambisan, 2017). A dozen of studies have been carried out since its introduction by Cohen et al. (1973). Since linear DNA molecules from different cell sources are not suitable in host cells, DNA carriers called vectors are utilized for the replication, occurrence, and functioning of foreign DNA in the desired host cells. The major step in the approach is the recombination of insert DNA and vector DNA to obtain desired hybrid DNA. For this purpose, well-prepared DNA samples are required. PCR-directed construction of a gene or DNA fragment is used generally to prepare DNA samples for this technology. The obtained PCR products and vector DNA were then treated with unique restriction endonucleases to obtain cohesive ends for recombination. Restriction enzymes cut DNA at a specific sequence of bases, which has greatly stimulated the application of recombinant DNA technology (Bhagwat, 1995). Restriction enzyme enables proper alignment of duplex DNA strands due to cohesive ends. The gap between properly aligned DNA duplexes is filled by DNA ligase enzymes. DNA ligases catalyze the formation of phosphodiester bonds between the 3'-hydroxyl and 5'-phosphoryl end groups in oriented DNA ends, which makes the technique to be an indispensable tool for in vitro DNA manipulation in a wide range of applications in cloning (Engler and Richardson, 1982, Shore et al., 1981).

Even though studies in the field have improved the application of the method by eliminating the usage of restriction endonucleases and/or DNA ligases, restriction enzymes and DNA ligase treatment are still highly used protocols in this technology (Aslanidis and de Jong, 1990, Chaudhary et al., 2014, Chen et al., 2021, El Qaidi and Hardwidge, 2019, Li et al., 2011, Scholz et al., 2013, Wang et al., 2015, Zhou and Gomez-Sanchez, 2000). In the classical applications, well-performed restriction and ligation of insert DNA and vector DNA affect the success of an application. Therefore, a well-defined reaction component and well-prepared DNA samples are required. On the other hand, a controlled study can save time, labor, and cost of a study. Therefore,

controlling ligation reaction status can be useful to perform the following steps. For this purpose, here, a PCR-based approach was carried out to evaluate the ligation efficiency and selectability of the desired colony.

## MATERIALS AND METHODS

### Competent cell preparation

Competent cells were prepared by using the $CaCl_2$ method. For this purpose, 50 µl frozen stock culture of *Escherichia coli* DH5α strain was inoculated into 10 ml LB broth (5.0 g/L yeast extract, 10.0 g/L NaCl, 10.0 g/L tryptone) and incubated overnight at 37 ˚C and 200 rpm shaking. 200 µl of the overnight culture was transformed into 50 ml LB broth in 250 ml flask and incubated at 37 ˚C and 200 rpm shaking for ~3 hours. When the culture's optical density reached 0.4, the culture was transported on ice. Then, 50 ml culture was centrifuged at 6.000 rpm for 10 min at 4˚C**.** Ice-cold 100 mM $CaCl_2$ was added to the pellet and cells were resuspended. Resuspended cells were incubated on ice for ~4 hours. $CaCl_2$ treated cells were harvested at 6.000 rpm for 10 min at 4˚C and the supernatant was poured. Then, the ice-cold storage solution (85 mM $CaCl_2$, 15% glycerol) was added to the cell pellet and the pellet was resuspended. 100 µl of the resuspended cells were aliquoted in microcentrifuge tubes**.** The tubes were frozen in a liquid nitrogen tank, immediately.

### Insert and Plasmid DNA

In the study, previously digested (*EcoR*I and *BamH*I) and purified insert and plasmid DNA (pUC19) were used. DNA concentration (ng/µl) of the insert and plasmid DNA was determined by a spectrophotometer to determine amounts to be used in the ligation reaction.

### Ligation

Digested and purified plasmid and insert DNA were ligated by using a DNA Ligation Kit (Merck, Novagen). Ligation reaction contained 1 µl of 10X Ligase Buffer, 0.5 µl of 100 mM DTT, 1 µl of 10 mM ATP, 0.5 µl (50 ng) digested pUC19, 1 µl of T4 DNA Ligase (1:10 diluted), 1 µl (50 ng) digested insert (1011 bp), 5 µl of PCR-grade water. The ligation mixture was incubated at 16 ˚C for 16 hours. Then, inactivation was carried out at 70 ˚C for 20 min and tubes were stored at +4 ˚C for transformation.

### Ligation check PCR

To control ligation, a PCR approach was followed in the study. PCR ingredients of the assay was given in Table 1. Also, the used primer information was given in Table 2. Thermal cycling was set up as followed: an initial denaturation phase of 3 min at 94 °C, then 30 cycles of 20 s at 94 °C, 25 s at 55 °C, 75 s at 72 °C, and a final extension at 72 °C for 5 minutes.

**Table 1.** PCR composition for ligation check PCR.

| Volume (µl) | Component |
|---|---|
| **2.5** | 10X Taq Buffer |
| **0.2** | dNTP Mix, 20 mM each |
| **0.2** | Taq DNA Polymerase (5 U/µl) |
| **0.5** | Forward primer (10 µM) |
| **0.5** | Reverse primer (10 µM) |
| **2.5** | 25 mM MgCl2 |
| **17.5** | PCR-grade water |
| **0.1** | Ligation Mix |
| **25** | Total volume |

**Table 2.** The primers used in the study.

| Primer | Sequence (5'->3') | Length | Tm | GC% |
|---|---|---|---|---|
| **Forward** | GGAAACAGCTATGACCATG | 19 | 53.43 | 47.37 |
| **Reverse** | GTAAAACGACGGCCAGTG | 18 | 56.47 | 55.56 |

Agarose gel electrophoresis was carried out to determine PCR product lengths. Gel preparation (2%) and application were carried out as described previously (Arslan et al., 2021). After electrophoresis, the gel was visualized under ultraviolet (UV) light and the results were recorded.

### Transformation

For transformation, an aliquot (100 µl) of previously prepared competent cells was taken from a liquid nitrogen tank. Cells were thawed on ice. After thawing, 10 µl ligation mixture was added to 100 µl competent cells and incubated on ice for 1 hour. Tubes were shaken gently in every 15 minutes. Then, heat shock application was carried out at 42 ˚C for 2 min. Tubes were incubated on ice for 5 minutes, and LB broth was added and

incubated at 37 °C and 200 rpm shaking for 1 hour. 100 ul of the culture was spread onto LB ampicillin plate (50 µg/ml ampicillin). The plate was incubated at 37°C for 24 hours.

**Colony PCR**

For screening desired recombinant plasmid, colony PCR was carried out (Bergkessel and Guthrie, 2013). After transformation and incubation, colonies were selected randomly for colony PCR. Using sterile pipette tips, a part of the colonies was added to 20 µl NaOH solution (20 mM). The tubes were heated at 99 °C for 10 min in a thermal cycler. Tubes were stored at 4 °C until the PCR step. 1 µl from the tubes was used in the PCR reaction as template. In colony PCR analysis, the composition of PCR was very similar to the PCR described above (Table 1). The only difference was the used template. After PCR running, PCR products were resolved in 2 % agarose gel as described above.

**RESULTS**

After ligation, PCR analysis was carried out to check ligation success. As shown in Figure 1, the primer pairs used in the study enabled for amplification of 101 bp DNA region on wild-type plasmid (pUC19), while in the ligation mix, both 101 bp and 1085 bp DNA were amplified. According to the results, the ligation mix contained both wild-type pUC19 and recombinant plasmid. Also, the band intensity of 1085 bp product was higher than the 101 bp product, indicating that the recombinant plasmid ratio is higher than the wild-type plasmid in the ligation mix, and the selection of the desired colony can be easy.

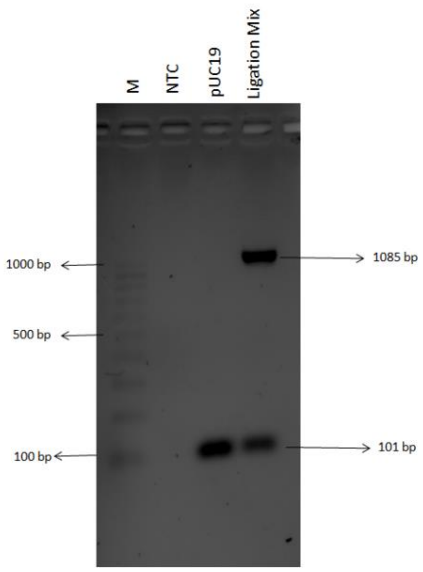


**Figure 1.** PCR results of ligation check PCR. pUC19 and ligation mix was used as template, also no template control (NTC) was used in the assay.

After the transformation of the ligation mix to competent cells, growing colonies were shown on the LB plate containing ampicillin (Figure 2). To determine the recombinant plasmid-containing colony, five colonies were selected randomly for colony PCR (blue circles indicated the selected colonies) (Figure 2).

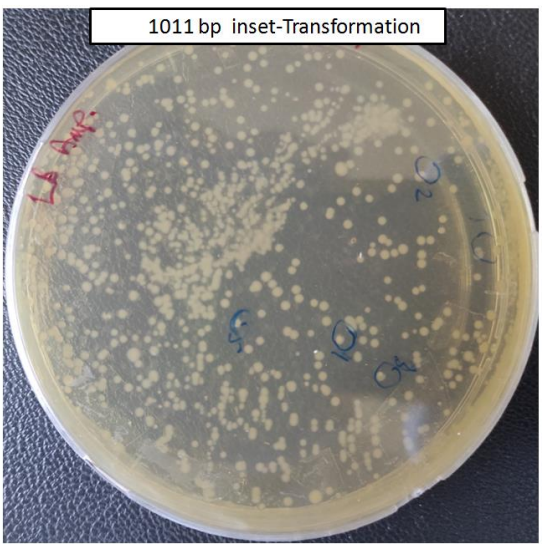


**Figure 2.** Growing colonies on the LB ampicillin plate after transformation. Blue circles indicate colonies selected for colony PCR.

After colony PCR, agarose gel electrophoresis was carried out to determine PCR product length (bp) (Figure 3). According to the results, 1085 bp DNA region, which contained insert DNA in the plasmid, was amplified in colony 1 and 2, while 101 bp DNA fragment was amplified in colony 2, 4, and 5. This result indicated that colony 1 and 3 contained recombinant plasmid, whereas colony 2, 4 and 5 contained wild-type plasmid (pUC19).

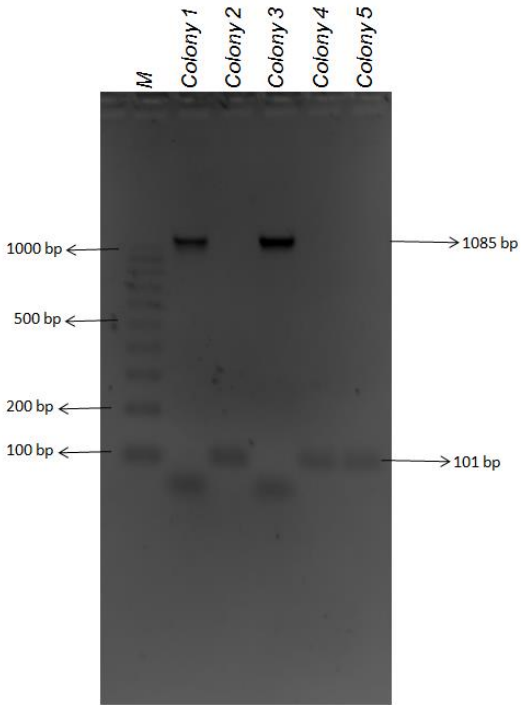


**Figure 3**. Agarose gel electrophoresis results of Colony PCR assay.

## DISCUSSION

Ligation is a fundamental step of recombinant DNA technology, where two or more DNA fragments are joined covalently by a ligase-catalyzed reaction. It can create new functional units from different DNA fragments (Takita et al., 2013). In the study, a PCR approach was described to check ligation and to interpret the selectability of the desired colony.

In this assay, the used primer pairs should bind upstream and downstream parts of the cloning region of the plasmid. An insertion into the cloning site increases plasmid DNA length, therefore, PCR product length increases. In this study, since *EcoR*I and *BamH*I digestion causes deletion of 15 bp DNA length, plasmid DNA was shortened. When 1011 bp DNA fragment is inserted into the region, the expected PCR product length should be 1085 bp. As shown in Figure1, the expected DNA fragment was amplified successfully. This indicates ligation occurred in the desired manner. Also, 101 bp PCR product was amplified (Figure 1). This indicates that there was some amount of wild-type plasmids in the ligation mixture. In the study, double-digested and purified plasmid DNA was used, which indicates that 15 bp DNA region of plasmid would be deleted during these processes and recircularization would not be occurred. However, the results indicate that plasmid DNA could not cut in properly. Therefore, it can be concluded that ligation control PCR can also show restriction problems. Furthermore, in some cloning studies, only one restriction enzyme is used and alkaline phosphates treatment is not carried (Dale and Greenaway, 1985). In these studies, recircularization of plasmid can occur and decreases cloning success. Ligation check PCR can also show recircularization and recombinant plasmid levels.

In the assay, band intensity difference between 1085 and 101 bp PCR products can indicate wild-type and recombinant plasmid ratio in the ligation mixture (Figure 1). This finding may indicate the selectability of the desired colony, however, long DNA fragments can give a more band intensity than shorter ones. In the colony PCR screening step, five colonies were selected and PCR evaluation indicated that two of them carried recombinant plasmid (Figure 3). Furthermore, some plasmids have advantages to allowing the screening of recombinant clones visually due to color phenotypes like `blue/white' color. pUC19 plasmid has a β-galactosidase (*lacZ*) gene on the cloning site, which can be used for blue/white screening (Langley et al., 1975, Maas, 1999). However, it requires the addition of X-gal, 5-bromo-4-chloro-3-indolyl-*β*-D-galactopyranoside, into agar medium. Since this increases the cost of study, the technique cannot be used. Therefore, other screening techniques such as colony PCR are utilized. Here, a similar protocol was followed. In this study, the ratio of selectability of the right colony was 40%.

In the defined ligation protocol or standard protocols, this approach can be ignored. However, undefined ligation and restriction enzyme mixtures or protocol optimizations, this simple step can be performed before the transformation. In this approach, PCR and agarose gel electrophoresis take ~2 hours. However, this can save time, labor, and cost of study.

## CONCLUSION

In the study, a PCR approach is described to check the ligation status. The approach could be used for new protocol optimizations before transformation, which could save time, labor, and cost and also may direct the researcher or applicant to find the problem. This protocol can also enable interpretation of how many colonies should be screened to find desired recombinant clone in the selection process.

## REFERENCES

Arslan M, Tezcan E, Camcı H, Avcı MK 2021. Effect of DNA concentration on band intensity and resolution in agarose gel electrophoresis. Van Sağlık Bilimleri Dergisi, 14:326-333.

Aslanidis C, de Jong PJ 1990. Ligation-independent cloning of PCR products (LIC-PCR). Nucleic Acids Research, 18:6069-6074.

Bergkessel M, Guthrie C 2013. Colony PCR. Methods in Enzymology, 529:299-309.

Bhagwat AS (1995) [6] - Restriction Enzymes: Properties and Use. In: Wu R, Recombinant DNA Methodology II. Boston: Academic Press, pp. 67-92.

Chaudhary VK, Shrivastava N, Verma V, Das S, Kaur C, Grover P, Gupta A 2014. Rapid restriction enzyme-free cloning of PCR products: a high-throughput method applicable for library construction. PloS One, 9:e111538.

Chen F, Li YY, Yu YL, Dai J, Huang JL, Lin J 2021. Simplified plasmid cloning with a universal MCS design and bacterial in vivo assembly. BMC Biotechnology, 21:24.

Cohen SN, Chang AC, Boyer HW, Helling RB 1973. Construction of biologically functional bacterial plasmids in vitro. Proceedings of the National Academy of Sciences of the United States of America, 70:3240-3244.

Dale JW, Greenaway PJ 1985. The use of alkaline phosphatase to prevent vector regeneration. Methods in Molecular Biology, 2:231-236.

El Qaidi S, Hardwidge PR 2019. ABC cloning: An efficient, simple, and rapid restriction/ligase-free method. MethodsX, 6:316-321.

Engler MJ, Richardson CC (1982) 1 DNA Ligases. In: Boyer PD, The Enzymes. Academic Press, pp. 3-29.

Khan S, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, Hou H 2016. Role of Recombinant DNA Technology to Improve Life. International Journal of Genomics, 2016:2405954.

Langley KE, Villarejo MR, Fowler AV, Zamenhof PJ, Zabin I 1975. Molecular basis of beta-galactosidase alpha-complementation. Proceedings of the National Academy of Sciences of the United States of America, 72:1254-1257.

Li C, Wen A, Shen B, Lu J, Huang Y, Chang Y 2011. FastCloning: a highly simplified, purification-free, sequence- and ligation-independent PCR cloning method. BMC Biotechnology, 11:92.

Maas S 1999. Efficient and Rapid Procedure for Blue-White Screening of Recombinant Bacterial Clones. Biotechniques, 27:1126-1128.

Nambisan P (2017) Recombinant DNA Technology and Genetically Modified Organisms. In: Nambisan P, An Introduction to Ethical, Safety and Intellectual Property Rights Issues in Biotechnology. Academic Press, pp. 83-126.

Scholz J, Besir H, Strasser C, Suppmann S 2013. A new method to customize protein expression vectors for fast, efficient and background free parallel cloning. BMC Biotechnology, 13:12.

Shore D, Langowski J, Baldwin RL 1981. DNA flexibility studied by covalent closure of short fragments into circles. Proceedings of the National Academy of Sciences, 78:4833-4837.

Takita E, Kohda K, Tomatsu H, Hanano S, Moriya K, Hosouchi T, Sakurai N, Suzuki H, Shinmyo A, Shibata D 2013. Precise sequential DNA ligation on a solid substrate: solid-based rapid sequential ligation of multiple DNA molecules. DNA Research, 20:583-592.

Wang Y, Liu Y, Chen J, Tang M, Zhang S, Wei L, Li C, Wei D 2015. Restriction-ligation-free (RLF) cloning: a high-throughput cloning method by in vivo homologous recombination of PCR products. Genetics and Molecular Research, 14:12306-12315.

Zhou MY, Gomez-Sanchez CE 2000. Universal TA cloning. Current Issues in Molecular Biology, 2:1-7.

## *ORAL PRESENTATION*

# An Innovative Approach to the Age-old Probiotic Concept: Postbiotics

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## Abstract

At the beginning of the XX century, Metchnikoff's innovative observation led to the probiotic concept's development. In this Probiotic Century, attention was drawn to live microorganisms that, when administered in adequate amounts, confer a health benefit on the host with the definition of probiotic by FAO/WHO. Scientific studies indicate that the probiotic mechanisms can be distributed as strain-specific and species-specific. Some mechanisms might be widespread among commonly studied probiotic bacteria and yeast genera. The health-promoting effects such as modification of the gut microbiota, improvement of epithelial lining barrier function, modulation of the immune system, and the prevention or treatment of intestinal diseases have been supported by clinical trials. At the point reached today in the probiotic concept, recent evidence indicates that bacterial viability is not necessary to attain health-promoting effects. Two new terms have emerged in the field of probiotics, which imply that bacterial viability is not an essential requirement for health benefits, quite different from what the FAO/WHO has pointed out in its definition: Paraprobiotics and postbiotics. In order to identify the 'paraprobiotics', various terminologies have been suggested in the literature, such as non-viable probiotics, inactivated probiotics, tyndallized probiotics, and ghost probiotics. The term 'postbiotics', also known as metabiotics, pharmabiotics, biogenics, cell-free supernatants; refers the soluble factors (products or metabolic by products include short chain fatty acids, enzymes, teichoic acids, peptidoglycan-derived muropeptides, exo-polysaccharides, cell surface proteins, vitamins, bacteriocins and organic acids), which are secreted by live bacteria or released after bacterial lysis. Both paraprobiotics and postbiotics mimic the proven health benefits of probiotics without the safety risks of probiotics, such as microbial translocation, infection or reducing enhanced inflammatory responses. Unlike probiotics, paraprobiotics and postbiotics do not require stringent production/storage conditions, which makes these new concepts advantageous in the food industry.

**Keywords:** Probiotics, Postbiotics, Paraprobiotics

## INTRODUCTION

The human gastrointestinal system consists of complex intestinal microbiota. As an ecosystem, intestinal microbiota that houses bacteria, archaea, fungi, and viruses is a diverse environment (Rajakovich & Balskus, 2019). The balance of the intestinal microbiota grants essential benefits to human health. Thus, the observation that intestinal microbiota can provide health benefits, led to the development of the probiotic concept. At the beginning of the 1900s, the study of Metchnikoff documented that fermented dairy products such as yoghurt were associated with better health and longevity among people living in Bulgarian villages and signalled the importance of probiotics (Metchnikoff, 1907). The word ‘probiotics’ was used for the first time to refer to ‘essential active substances for the healthy development of life’ in 1953 (Gasbarrini et al., 2016). During five consecutive decades, new definitions for probiotics were proposed. The commonly accepted definition was provided by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 2001 (FAO/WHO, 2001). According to this definition, probiotics are ‘live microorganisms which, when administered in adequate amounts, confer a health benefit on the host’.

Rijkers et al. (2010) classified the beneficial effect of probiotics into three domains. Firstly, probiotics directly interact with the intestinal microbiota or by enzyme activity within the gastrointestinal system. Secondly, probiotics also directly interact with the epithelium and the intestinal mucous layer, influencing the intestinal barrier function and the mucous immune system. Thirdly, outside the gastrointestinal system, probiotics take action to strengthen the immune system in other organs such as the liver and brain.

Despite the precise definition and beneficial effects of probiotics, the mechanisms behind the beneficial effects of probiotics are not particular. The commonly accepted approach relies on the multifactorial effects of probiotics such as modulation of the immune system, adherence to the mucous membrane and epithelium,

strengthening of the intestinal epithelial barrier and hostile effects of probiotics against various microorganisms including the secretion of antimicrobial substances (Bermudez-Brito et al., 2012). The unparticular mechanisms of probiotics may be linked to the viability of bacterial cells. On the other hand, a new approach criticises the viability by recognising some mechanisms and clinical benefits that are not related to live microorganisms. According to this new approach, non-viable bacterial cells, microbial parts or cell debris have beneficial effects on health by promoting additional activity (Adams, 2010; Ananta and Knorr, 2009). Taverniti and Guglielmetti (2011) identified these biomasses as paraprobiotics and postbiotics.

**Paraprobiotics**

Paraprobiotics, also known as "inactivated probiotics" or "ghost probiotics," are characterised as non-viable microbial cells (intact or ruptured), or raw cellular extracts (with complicated chemical composition), which, when delivered (orally or topically) in sufficient proportions, confer a benefit to the human or animal consumer (Taverniti and Guglielmetti, 2011). Therefore, they are microorganisms that had their viability affected as a result of processes that caused structural and metabolic alterations in the bacterial cells. After being exposed to elements that change microbial cell structures, such as DNA filament breaks, cell membrane disruptions, or mechanical damage to the cell envelope, microorganisms that were the source of paraprobiotics, entirely lost their viability. This also includes viability losses brought on by modifications in physiological processes in microbes, such as the inactivation of necessary enzymes or loss of membrane selectivity. After that, paraprobiotics are unable to develop *in vitro*, which may be shown by plating in a suitable culture medium (Lado and Yousef, 2002). Although plating has been the primary method used to examine the cell survival of paraprobiotics, there are certain drawbacks, including the inability to provide results in real-time and the absence of data on cell integrity and metabolic activity. Because wounded and damaged cells can still sustain some metabolic activity that may help to promote health. The methods used to generate paraprobiotics—physical procedures including heat, gamma radiation, UV radiation, high hydrostatic pressure, ultrasonication, and lyophilization—affect their immunomodulatory function (acid deactivation). The loss of viability for the living microorganisms should be occur after exposure to factors that alter microbial cell structure (e.g., DNA break, cell envelope damage) and/or change in their physiological functions (e.g., inactivation of key enzymes or deactivation of membrane selectivity) without disrupting the bacterial membrane and cell wall necessary to maintain the intact structure of the cell (de Almada, et al., 2016). The most effective approach will be determined by the microorganisms being employed and the anticipated clinical benefit. This is because each method has a unique way of accessing the microbial structure and metabolic processes (Almada et al., 2016). In order to maintain the probiotics' benefits, it must employ a strategy that can inactivate the bacteria. Additionally, because the biologic effects and mode of action of each probiotic bacteria are "strain specific," they are not all equally effective. As a result, some phenotypic and functional characteristics are not shared by all strains belonging to the same genus or specie, necessitating the development of further studies to more thoroughly assess the many positive impacts of the different paraprobiotic strains (Deshpande et al., 2018). Paraprobiotics have been introduced with potential pharmaceutical applications in the prevention or treatment of diseases. Several paraprobiotic products with proved effects in the prevention or treatment of some diseases are commercially available as supplements (Cuevas-González et al., 2020) (Table 1).

**Postbiotics**

Postbiotics is a recently coined word that covers a broad spectrum of bioactive substances, including non-viable/inactivated microbial cells, cell components, and any soluble chemicals or metabolic by products coming from probiotics, which, when given to consumers, directly or indirectly mediate favourable biological activity (Tsilingiri and Rescigno, 2013; Mousavi Khaneghah et al., 2020). Because of this, the definition of this concept that was currently in use was "processing of inanimate microorganisms and/or their components that give a health advantage to the host" (Salminen et al., 2021). Postbiotics have therefore been found to imitate the features and operations of probiotics (Patel and Denning, 2013). The postbiotics molecules resulting from extracellular and intracellular probiotic cells can be list as peptidoglycans-derived muropeptides, exopolysaccharide (EPS), teichoic acids, and surface protruding molecules like fimbriae, pili or flagella that constitute cell wall components, secreted proteins/peptides, bacteriocins such as acidophilin, reuterin, and bifidin, cell-free supernatant, organic acids such as lactic acid and acetic acid, vitamins, short-chain fatty acids like butyric acid and propionate, neurotransmitters, biosurfactants, etc. (Bourebaba et al., 2022). Thanks to their stable chemical structures, long storage stability, postbiotics have health advantages both by local effects on specific tissues of the gut epithelium, and by affecting multiple other organs and tissues (Bourebaba et al., 2022). The advantages of postbiotics are *(i)* being more stable than the living bacteria they are derived from *(ii)* having favorable safety profile, as there is no need for the uptake of billions of living

microbes *(iii)* can be applied in a controlled and standardized way, whereas, in the case of the application of living bacteria, the level of the active structure in the intestine is dependent on the number and metabolic activity of the respective strain (Aguilar-Toaláa et al., 2018).

The evidences optained from recent studies demonstrate that postbiotics may be beneficial in metabolic disorders management via several potential effects including anti-inflammatory, antibacterial, immunomodulatory, anti-carcinogenic, antioxidant, antihypertensive (Bourebaba et al., 2022). Several species belonging to *Lactobacillus, Bifidobacterium, Saccharomyces, Bacillus, Streptococcus*, or *Faecalibacterium* were found to have very potent postbiotics, such as cytoplasmic extracts and cell wall components Farhatul-Ain et al., 2018; Jensen et al., 2010; Sokol et al., 2008; Tejada-Simon et al., 1999). The most common probiotics that produce postbiotics are *Lactobacilli* species which include *L. rhamnosus, L. bulgaricus, L. acidophilus, L. reuteri, L. casei, L. fermentum, L. paracasei, L. helveticus, L. johnsonni, and L. gasseri*, as well as *Bifidobacterium* such as *B. bifidum, B. longum subspecies infantus, B. breve, and B. longum* (Choi et al., 2006; Cicenia et al., 2016; Johnson et al., 2019; Kim et al., 2011; Matsuguchi et al., 2003; Tiptiri-Kourpeti et al., 2016; Vidal et al., 2002). *Saccharomyces cerevisiae* yeast, another possible probiotic, is typically employed for the bioproduction of metabolites with postbiotic potentials as anti-inflammatory components. Another potential probiotic, *Saccharomyces cerevisiae* yeast, is usually used for bioproduction of metabolites having postbiotics potentialities as anti-inflammatory components (Jensen et al., 2007). According to the observed physiological benefits (anti-inflammatory, antioxidant, antihypertensive, antiproliferative, antimicrobial, hypocholesterolemic, and immunomodulatory activities) or by their composition, which can come from both bacterial cell compounds and microbial action, postbiotics can be divided into distinct categories (synthesis of metabolites and products from microbial enzymatic activity over the food matrix) (Collado et al., 2019).
Although paraprobiotics have been linked to benefits on health, the majority of the available research indicates that these benefits are only related to their direct ingestion. Thus, using foods as delivery systems for paraprobiotics appears to be a subject that needs to be researched since it offers both potential and obstacles. The selection of appropriate probiotic species and strains is the first problem to be solved for the widespread use of paraprobiotics. These probiotics should exhibit particular health benefits that are reasonably maintained when paraprobiotics are obtained. Additionally, it is unknown if any inactivation method will be suitable for all species, strains, and food applications, despite the fact that a variety of inactivation conditions have been utilised to produce paraprobiotics.
Cell-free preparations obtained from the metabolic products of different beneficial bacteria have been introduced with potential pharmaceutical applications in the prevention or treatment of diseases. Several postbiotic products with proved effects in the prevention or treatment of some diseases are commercially available as supplements (Cuevas-Gonzáleza et al., 2020) (Table 1).

**Table 1** Commercial paraprobiotic and postbiotic products for human use (Cuevas-González et al., 2020)

| **Brand** | **Paraprobiotic** | **Manufacturer** | **Comments** |
|---|---|---|---|
| Pylopass™ | Spray-dried dead of *Lactobacillus reuteri* DSMZ 176-48 | (Novozymes, Germany) | Effective in the control of *Helicobacter pylori* infection. |
| Lacteol® Fort | Non-viable cells of L*actobacillus acidophilus* LB. | Carnot Laboratories (France) | Effective in the treatment of diarrhea |
| **Brand** | **Postbiotic** | **Manufacturer** | **Comments** |
| Bactistatin® | Metabolites of *Bacillus subtilis* VKPM V-2335. | Kraft (Russia) | Restores the microbial ecology of the digestive tract. |
| Pro-Symbioflor® | Lysate and supernatants from *Enterococcus faecalis* DSM 16440 and *Escherichia coli* DSM 17252 | SymbioPharm (Germany) | Effective in helps complaints of stomach- intestinal function, trains the immune system and the development of atopic dermatitis |
| Hylak® Forte | Metabolites (e.g. SCFA, lactic acid an other non-identified metabolites) of *Escherichia coli* DSM 4087, *Streptococcus faecalis* DSM 4086, *Lactobacillus acidophilus* DSM 414, and *Lactobacillus helveticus* DS 4183 | Ratiopharm/Merckle (Germany) | Useful to flatulence, diarrhea and constipation, chronic gastritis, as well as salmonellosis and radiation-induced diarrhea. |
| CytoFlora® | Cell wall of diverse *Lactobacillus, Bifidobacterium, Streptococcus* strains. | BioRay Inc. (USA) | Promotes a balanced immune response, and improve symptoms in autistic children |
| Del-Immune V® | Cell wall peptidoglycans and DNA fragments of *Lactobacillus rhamnosus* | Pure Research Products (USA) | Reduces the severity of gastrointestinal distress in child |
| Colibiogen® | Protein-free filtrate derived from cultures of *Escherichia coli* (strain Laves 1931), containing amino acids, peptides, polysaccharides and fatty acids, | (Laves-Arzneimittel GmbH, Schötz, Switzerland) | Effective in inhibiting *in vitro* both antibiotic resistant and sensitive Salmonella isolates, Effective in the amelioration of murine colitis, Reduce skin lesions in patients with polymorphous light eruptions |

## CONCLUSION

Evidences from recent studies have describe the potential application of postbiotics and paraprobiotics in human food, animal feed, and pharmaceutical industries.

## REFERENCES

Adams CA 2010. The probiotic paradox: Live and dead cells are biological response modifiers. Nutrition Research Reviews, 23: 37-46.

Aguilar-Toaláa, J.E., Garcia-Varelab, R., Garciac, H.S., Mata-Harod, V., González-Córdovaa, A.F., Vallejo-Cordobaa, B., Hernández-Mendozaa, A. (2018). Postbiotics: An evolving term within the functional foods field, Trends in Food Science & Technology, 75: 105–114.

Almada CN, Martinez RCR, Sant'Ana AS 2016. Paraprobiotics: evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods. Trends Food Sci Technol, 58:96-114.

Ananta E, Knorr D 2009. Comparison of inactivation pathways of thermal or high pressure inactivated *Lactobacillus rhamnosus* ATCC 53103 by flow cytometry analysis. Food Microbiology, 26: 542-546.

Bermudez-Brito M, Plaza-Díaz J, Munoz-Quezada S, Gomez-Lorente C, Gil A 2012. Probiotic mechanisms of action. Annals of Nutrition &Metabolism, 61: 160-174.

Bourebaba, Y., Marycz, K., Mularczyk, M., Bourebaba, L. (2022). Postbiotics as potential new therapeutic agents for metabolic disorders management. Biomedicine & Pharmacotherapy, 153: 113138.

Choi SS, Kim Y, Han KS, You S, Oh S, Kim SH 2006. Effects of *Lactobacillus* strains on cancer cell proliferation and oxidative stress *in vitro*, Letters in Applied Microbiology, 42: 452–458.

Cicenia A, Santangelo F, Gambardella L, Pallotta L, Iebba V, Scirocco A, Marignani M, Tellan G, Carabotti M, Corazziari ES, Schippa S, Severi C 2016. Protective Role of Postbiotic Mediators Secreted by

*Lactobacillus rhamnosus* GG Versus Lipopolysaccharide-induced Damage in Human Colonic Smooth Muscle Cells, J. Clin. Gastroenterol. 50: 140–S144.

Collado MC, Vinderola G, Salminen S 2019. Postbiotics: facts and open questions. A position paper on the need for a consensus definition. Beneficial Microbes, 10:711-719.

Cuevas-Gonzáleza, P.F., Liceagab, A.M., Aguilar-Toaláb, J.E. 2020. Postbiotics and paraprobiotics: From concepts to applications. Food Research International 136: 109502.

Deshpande G, Athalye-Jape G, Patole S 2018. Para-probiotics for preterm neonates-the next frontier. Nutrients, 10:871-879.

Farhatul-Ain A, Rubaida M, Sajid H, Muhammad AK, Saqib MK 2018. *Lactobacilli* as Probiotics and their Isolation from Different Sources, British Journal of Research, 05.

Food and Agriculture Organization/World Health Organization (FAO/WHO). 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, report of a joint (FAO/WHO) expert consultation on evaluation of health and nutritional properties of probiotics in food including powed milk with live lactic acid bacteria. Cordoba, Argentina http://www.who.int/ Foodsafety/plubications/fs_management/en/probiotics.pdf

Gasbarrini G, Bonvicini F, Gramenzi 2016. A: Probiotics history. Journal of Clinical Gastroenterology, 50: S116-S119.

Johnson CN, Kogut MH, Genovese K, He H, Kazemi S, Arsenault RJ 2019. Administration of a Postbiotic Causes Immunomodulatory Responses in Broiler Gut and Reduces Disease Pathogenesis Following Challenge, Microorganisms, 7 268.

Jensen GS, Benson KF, Carter SG, Endres JR, Ganeden BC 2010. 30TM cell wall and metabolites: anti-inflammatory and immune modulating effects *in vitro*, BMC Immunology, 11- 15.

Jensen GS, Hart AN, Schauss AG 2007. An anti-inflammatory immunogen from yeast culture induces activation and alters chemokine receptor expression on human natural killer cells and B lymphocytes *in vitro*, Nutrition Research. 27: 327–335.

Kim HG, Lee SY, Kim NR, Lee HY, Ko MY, Jung BJ, Kim CM, Lee JM, Park JH, Han SH, Chung DK 2011. *Lactobacillus plantarum* lipoteichoic acid downregulated Shigella flexneri peptidoglycan-induced inflammation, Mol. Immunol. 48 382–391.

Lado BH, Yousef AE 2002. Alternative food-preservation technologies: Efficacy and mechanisms. Microbes and Infection, 4: 433-440.

Matsuguchi T, Takagi A, Matsuzaki T, Nagaoka M, Ishikawa K, Yokokura T, Yoshikai Y 2003. Lipoteichoic Acids from *Lactobacillus* Strains Elicit Strong Tumor Necrosis Factor Alpha-Inducing Activities in Macrophages through Toll-Like Receptor 2, CVI 10. 259–266.

Metchnikoff II 1907. The prolongation of life: Optimistic studies. Springer Publishing Company.

Mousavi Khaneghah A, Abhari K, Es I, Soares MB, Oliveira RBA, Hosseini H, Rezaei M, Balthazar CF, Silva R, Cruz AG, Ranadheera CS, Sant'Ana AS 2020. Interactions between probiotics and pathogenic microorganisms in hosts and foods: A review, Trends Food Science Technology 95: 205–218.

Rajakovich L J, Balskus EP 2019. Metabolic functions of the human gut microbiota: The role of metalloenzymes. Natural Product Reports, 36(4): 593–625.

Rijkers GT, Bengmark S, Enck P, Haller D, Herz U, Kallioma Ki, M, et al. 2010. Guidance for substantiating the evidence for beneficial effects of probiotics: Current status and recommendations for future research. Journal of Nutrition, 140: 671-676.

Patel RM, Denning PW 2013. Therapeutic Use of Prebiotics, Probiotics, and Postbiotics to Prevent Necrotizing Enterocolitis, Clinics in Perinatology, 40: 11–25.

Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, Sanders ME, Shamir R, Swann JR, Szajewska H, Vinderola G 2021. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics, Nature Reviews Gastroenterology & Hepatology, 18: 649–667.

Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottiere HM, Dore J, Marteau P, Seksik P, Langella P 2008. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients, PNAS. 105: 16731–16736.

Taverniti V, Guglielmetti S 1999. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). Genes & Nutrition, 6:261-274.

Tejada-Simon MV, Pestka JJ 1999. Proinflammatory Cytokine and Nitric Oxide Induction in Murine Macrophages by Cell Wall and Cytoplasmic Extracts of Lactic Acid Bacteria, Journal of Food Protection. 62: 1435–1444.

Tiptiri-Kourpeti A, Spyridopoulou K, Santarmaki V, Aindelis G, Tompoulidou E, Lamprianidou EE, Saxami G, Ypsilantis P, Lampri ES, Simopoulos C, Kotsianidis I, Galanis A, Kourkoutas Y, Dimitrellou D, Chlichlia K 2016. *Lactobacillus casei* Exerts Anti-Proliferative Effects Accompanied by Apoptotic Cell Death and Up-Regulation of TRAIL in Colon Carcinoma Cells, PLoS One, e0147960.

Tsilingiri K and Rescigno M 2013. Postbiotics: what else? Beneficial Microbes, 4: 101–107.

Vidal K, Donnet-Hughes A 2002. Granato D, Lipoteichoic Acids from *Lactobacillus johnsonii* Strain La1 and Lactobacillus acidophilus Strain La10 Antagonize the Responsiveness of Human Intestinal Epithelial HT29 Cells to Lipopolysaccharide and Gram-Negative Bacteria, IAI 70: 2057–2064.

## *ORAL PRESENTATION*

# Isolation and characterization of secondary metabolites from aerial parts of *Cirsium arvense* subsp. *vestitum*

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**Abstract**

The current study includes the isolation and characterization of secondary metabolites from aerial parts of extracts of *Cirsium arvense* subsp. *vestitum* plant. A total of ten compounds were purified from these parts of the plant. Daucosterin and linarin from these compounds were also reported in our previous study. The remaining eight compounds, Nojirimycin, apigenin, myo-inositol, caffeic acid, rutin, apigenin 7-*O*-*a*-D-glucuronide, apigenin 7-*O*-*β*-D-glucuronide methyl ester and apigenin 7-*O*-*β*-D glucuronide were isolated and reported for the first time from this plant.

**Keywords:** *Cirsium arvense* subsp. *vestitum*, Isolation, Phenolic compounds, Seconder metabolites

## INTRODUCTION

*Cirsium arvense* is a plant species belonging to the Asteraceae family, distributed in Western Asia, Europe, North Africa, the eastern United States and Canada. (Nobarinezhad et al., 2020). While there are approximately 481 *cirsium* species in the world, this number is represented by 76 taxa in Turkey. There are two subspecies of the plant Cirsium arvense in Turkey (Güner et al., 2012). *Cirsium* (Asteraceae) species are known as Köygöçüren among the people in our country. 29 species (30 taxa) of Cirsium species distributed in our country are endemic to Turkey. They spread by invading many habitats such as roadsides, cultivated areas such as wheat barley, pastures, embankments, lawns and railways (Carter and Lym., 2017). *Cirsium arvense* is considered to be a harmful plant species because of its allopathic effect, because it generally damages agricultural products, because it grows in these areas uncontrollably and because it does not allow another plant to grow.

Some *Cirsium* species grown in our country are used in the treatment of hemorrhoids, peptic ulcer, cough, eye infections, bleeding piles, skin sores, gonorrhoea, leukaemia, tuberculosis and bronchitis. In the literature review on *Cirsium* species, it has been observed that these species contain flavonoids, tannins, coumarins, phenolic acids, terpenic compounds, steroids and lignans (Dehjurian et al., 2017). Although there are many scientific studies on *Cirsium* species in the world, there are few studies on both endemic and non-endemic *Cirsium* species in Turkey. Except for our previous study on this plant species grown in Turkey, there is no information on phytochemical analysis and isolation on the above-ground parts. Therefore, in this study, secondary component isolation was performed from the methanolic-chloroform extract obtained from the aerial parts of the *Cirsium arvense* subsp. *vestitum* plant.

## MATERIALS AND METHODS

### General Experimental Procedure

The open column chromatography (CC) was carried out using on silica gel, sephadex LH-20. Flash chromatography (MPLC system) was used for C18 packing material using solvent mixtures described in the experimental sections. Fractions were monitored by TLC silica gel 60 F254 after visualizing under UV (254 and 366 nm) and with ethanol-sulfuric acid reagent on silica gel followed by heating at 100 °C. Structures of isolated molecules were determined with 1D and 2D NMR methods using Agilent 600 MHz spectrometers. Solvents were purchased from Sigma-Aldrich (USA). Mass spectra were recorded on an Agilent 6210 L C-TOF/MS equipped with a ZORBAX SB-C18 column (4.6 × 100 mm, 2.7 μm)

**Plant Material**

*Cirsium arvense* (L.) Scop. subsp. *vestitum* (Wimmer et Grab.) Petrak (köy göçüren) plant from the 16th km of Sivas-Karaçayır highway (GPS data: 39 51 29 North, 36 58 48) during the flowering period of June-July with the help of Turkish flora, tubives and botanists who are experts in this field. East 1568 m) was collected in 2015. Species identification of the plant was made by Prof. Dr. Neriman ÖZHATAY, and the witness plant sample is kept in Istanbul University Faculty of Pharmacy Herbarium with the code of ISTE 85428.

**Extraction**

1500 g of the above-ground part of the *C. arvense* plant was crushed in liquid nitrogen. The completely powdered plant was first extracted with hexane and then with methanol/chloroform (1:1) three times under room conditions (Demirtas et al., 2011). The extracts were filtered first through cheesecloth and then through ordinary filter paper. The solvents were evaporated to dryness at 40 ˚C and under reduced pressure (600 mm Hg). The crude extracts obtained at the end of the first, second and third extractions were combined. As a result of this process;

Hexane: 13.7 g
Methanol/Chloroform: 40.1 g

**Isolation**

For open column chromatography, it was decided to do it over isolation using approximately 60 g of sephadex LH 20 column filler. Therefore, 60 g of filler was eluted in a methanol/chloroform (%35/%65) solvent mixture for 24 hours. Then, the eluted sephadex LH 20 was loaded into a column with a Teflon tap, approximately 65 cm long and 4 cm inside diameter. Approximately 5-10 g of the 40 g methanol/chloroform extract obtained previously was reserved for other studies and the remaining 30 g was loaded onto the column at 1 g per load. The methanol/chloroform (35/65) solvent system was used as the isocritical system as the driving phase. Approximately 45 fractions were collected in each column run. TLC images of similar fractions were performed with ceric sulfate and prior to UV light imaging. similar fractions were combined, yielding a total of 10 subfractions (S1-S10). NMR, HPLC-TOF/MS and TLC imaging were performed for the S1 coded fraction. however, no chromatogram could be detected in HPLC, proton and carbon peak in NMR analysis, and no spot in TLC imaging. The color of this part and the fact that it came first in this polarity proved that it was chlorophyll. Separation of the S2 coded fraction was performed using 24 g normal phase silica gel cartridges in flash chromatography. The column system was first started with chloroform as the mobile phase, then the column process was terminated by increasing the polarity with the addition of 5% methanol. Light yellow powder crystals were obtained at the bottom of the tubes within a day or two by reducing the concentration of the solvent by combining similar fractions (**Ca-4**). Separation of the S3 coded fraction was performed again using a 100% methanol solvent over sephadex LH 20 column filler. Similar fractions were combined and a precipitate was observed to form in eight tubes. The precipitate was washed with acetone-methanol-water mixture and a white precipitate was obtained. this compound was isolated in high quantity as the main component (**Ca-5**). Fraction coded S5 was separated into sub-fractions over a mixture of methanol and chloroform in a small open system silica gel column for re-purification and kept in a fume hood to evaporate the solvent. It was observed that there were precipitations in the tubes. The supernatant part of the transparent solution was separated from the precipitate by filtration on blue filter paper, and the crystals were washed with hexane-acetone-methanol solvent. Yellow crystals were obtained (**Ca-2**). The S6 coded fraction was again sub-fractionated on the silica gel stationary phase using an ethylacetate-methanol-acetic acid (65:35:2) solvent mixture in an open system column. Fractions with similar TLC images were combined. Crystals formed in two series of tubes and these crystals were separated from my supernatant portions and crystallized in methanol. Yellow powder crystals (**Ca-8**) were obtained from the first fractions and yellow clear powder (**Ca-9**) crystals were obtained from the lower fractions collected in the last fractions. TLC was examined on the mixture containing the S8 coded fraction $CHCl_3$-MeOH-Acetic acid solution (10:1:0.01) and two spots with Rf values close to each other were observed. When a %10 sulfuric acid solution prepared in ethanol was sprayed on the spots and heated at 135°C, it was observed that one of the two spots gave a yellow color and the other a more orange color. S8 fraction was separated into sub-fractions in MeOH-$CHCl_3$ (20:80) solvent system using 24 g silica gel cartridge in flash chromatography. Two different crystals were obtained in the tubes of the lower fractions. The first crystal was obtained as a white (**Ca-6**) powder and the second crystal was obtained as a yellow colored (**Ca-7**) powder. Black spots were observed in the TLC of the S9 fraction. 40 g polyamide filled flash cartridge was used for S9 fraction flash chromatography. Separation was performed at all wavelengths. Flash chromatography was started using 100% toluene solvent with a flow rate of 20 ml per minute. Then, the separation was continued by increasing the polarity by %5 using methanol solvent. In the toluene-methanol (20:80) solvent system, the substance separated from the column gave

absorbance and was collected in racks in flash chromatography. The column was terminated with %100 methanol solvent. As a result of combining similar tubes, bright transparent and white crystals were obtained in tubes collected in racks in toluene-methanol (20:80) solution (**Ca-1**). As a result of combining similar tubes, the tubes collected in racks in toluene-methanol (5:95) solvent were left to crystallize. The crystal was washed several times with methanol and rounded earthy yellowish crystals were obtained (**Ca-10**). TLC imaging of the fraction collected in waste flasks in 100% methanol solvent was performed and a black spot was seen again. This fraction was allowed to crystallize in its own solvent and clear crystals were observed to form. The crystals were separated from the supernatant and washed several times with a less polar solvent system to obtain crystals (**Ca-3**).

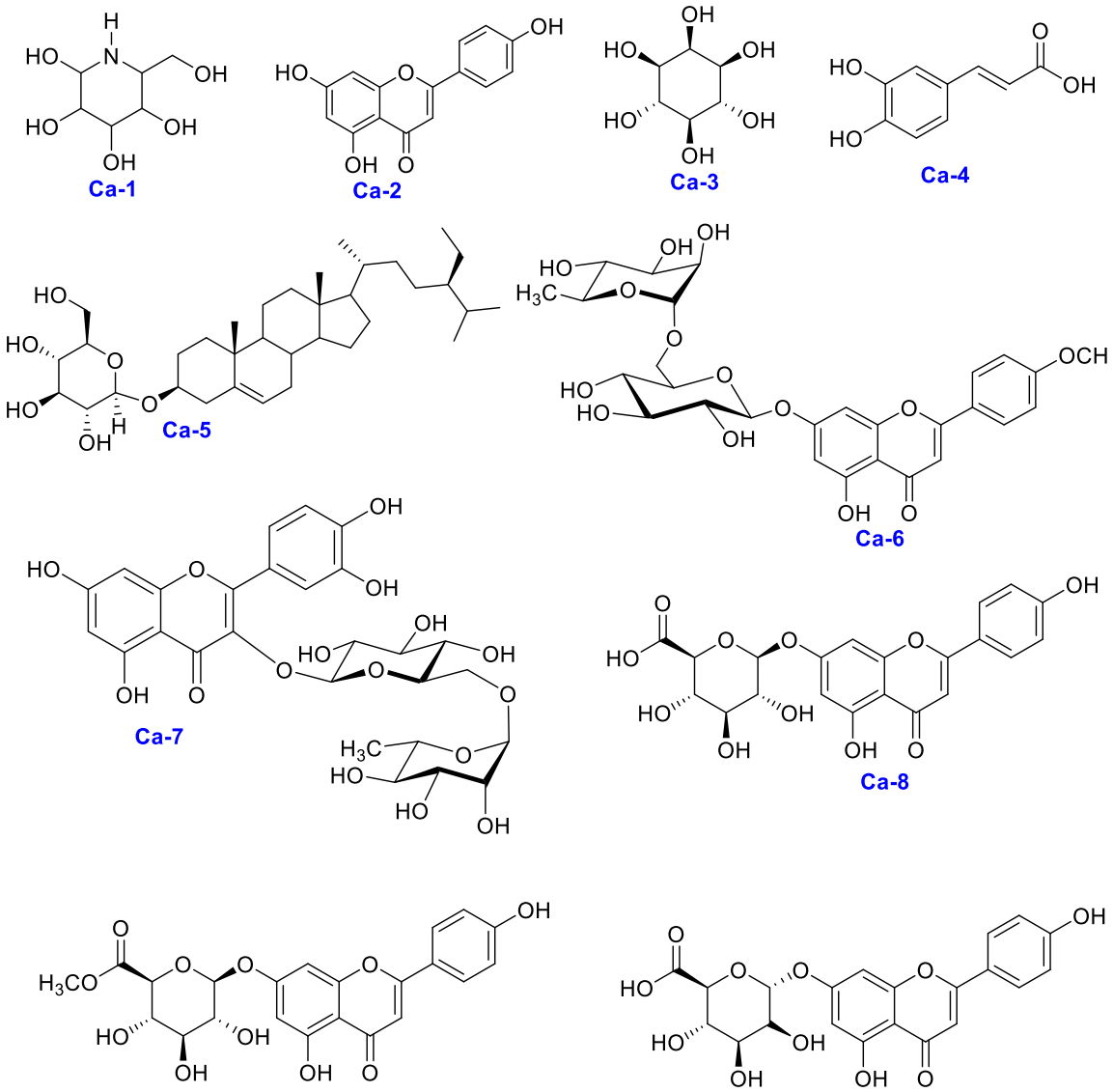


**Figure 1.** Compounds isolated from *Cirsium arvense* subsp. *vestitum*

## RESULTS AND DISCUSSION

As a result of fractionation of the extract obtained from the aerial part of Cirsium arvense subsp vestitum with methanol/chloroform solvent with sephadex LH-20 and isolation from these fractions with both C18 and Silicagel filler, eleven compounds were isolated (Figure 1). The structures of the compounds were evaluated together with 1D and 2D NMR techniques as well as HPLC-TOF/MS analyzes and the structure of each compound was defined. The obtained findings were compared with the literature values and the accuracy of the structure was guaranteed.

**Ca-1:** Ca-1 was obtained as a bright white crystal. HPLC-TOF/MS; m/z $[M-H]^-$ 178.0961. $^1$H NMR (600 MHz, DMSO-$d_6$): 7.13 (d, J= 4.2 Hz, N-H), 5.19 (d, J= 3.42 Hz, H-2), 3.13 (dd, J= 5.73 Hz , 3.42 Hz, H-3), 3.56 (m, H-4), 3.58 (m, H-5), 2.81 (dd, J= 10.5 Hz, 3.35 Hz, H-6), 3.48-3.59 (m, H-7). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 89.36 (C-2), 70.71 (C-3), 72.80 (C-4), 70.23 (C-5), 54.93 (C-6), 61.04 (C-7). According to $^1$H, $^{13}$C-NMR and HPLC-TOF/MS data the compound was determined to be Nojirimycin (Herrera-Gonzalez et al., 2022).

**Ca-2:** Ca-2 was obtained in crystalline form as a yellow powder. HPLC-TOF/MS; m/z $[M-H]^-$ 269.2094. $^1$H NMR (600 MHz, DMSO-$d_6$): 6.76 (s, H-3), 12.93 ( s, -OH), 6.18 (d, J= 1.83 Hz, H-6), 6.46 (d, J= 1.83 Hz, H-8), 7.90 (d, J= 8.81 Hz, H-2'), 6.91 (d, J= 8.81 Hz, H-3'), 6.91 (d, J= 8.81 Hz, H-5'), 7.90 (d, J= 8.81 Hz, H-6'). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 164.19 (C-2), 103.26 (C-3), 182.19 (C-4), 161.90 (C-5), 99.28 (C-6), 164.59 (C-7), 94.41 (C-8), 157.75 (C-9), 104.13 (C-10), 121.62 (C-1'), 128.92 (C-2'), 116.40 (C-3'), 162.90 (C-4'), 116.40 (C-5'), 128.92 (C-6'). According to $^1$H, $^{13}$C-NMR and HPLC-TOF/MS data the compound was determined to be Apigenin (Chaturvedula & Prakash, 2013).

**Ca-3:** Ca-3 was obtained as a white-transparent crystal. $^1$H NMR (600 MHz, DMSO-$d_6$): 3.24 (m, H-1), 3.40 (t, H-2), 3.63 (brs, H-3). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 73.5 (C-1), 71.03 (C-2), 72.52 (C-3). According to $^1$H and $^{13}$C-NMR data the compound was determined to be myo-inositol (Rebecca et al., 2012).

**Ca-4:** Ca-4 obtained as a light yellow powder crystal. $^1$H NMR (600 MHz, DMSO-$d_6$): 6.99 (d, J= 1.86 Hz, H-2), 6.73 ( d, J= 8.29 Hz, H-5), 6.93 (dd, J= 8.29 Hz, J=1.83 Hz, H-6), 7.38 (d, J= 15.88 Hz, H-7), 6.14 (d, J= 15.88 Hz, H-8). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 126.13 (C-1), 115.06 (C-2), 145.95 (C-3), 148.52 (C-4), 116.16 (C-5), 121.58 (C-6), 145.03 (C-7), 115.52 (C-8), 168.30 (C-9). According to $^1$H and $^{13}$C-NMR data the compound was determined to be caffeic acid (Liu et al., 2015, Tosovic and Markovic, 2016).

**Ca-5:** Ca-5 obtained as white powder crystals. $^1$H NMR (600 MHz, DMSO-$d_6$): 1.77-0.97 (H-1), 1.78-1.45 (H-2), 3.43 (m, H-3), 2.34-2.09 (H-4), 5.30 brs (H-6), 1.91-1.46 (m, H-7), 1.31 (m, H-8), 0.88 (d, J=6.45 Hz, H-9), 1.45-1.37 (m, H-11), 1.93-1.15 (m, H-12), 0.95 (m, H-14), 1.51-1.02 (m, H-15), 1.77-1.20 (m, H-16), 1.07 (m, H-17), 0.63 (s, H-18), 0.93(s, H-19), 1.30 (m, H-20), 0.87 (H-21), 1.28-0.97 (H-22), 1.12 (m, H-23), 0.87 (m, H-24), 1.60 (H-25), 0.77 (d, J=7.08 Hz, H-26), 0.88 (d, J=6.6 Hz, H-27), 1.22-1.17 (m, H-28), 0.79 (d, J=7.18 Hz, H-29), 4.19 (d, J=7.87, H-1'), 2.88 (t, J=8.38 Hz, H-2'), 3.11(t, J=8.49 Hz, H-3'), 3.01(t, J=8.49 Hz, H-4'), 3.04 (m, H-5' ), 3.61(d, J=11.41 Hz)-3.39 (H-6'). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 37.28 (C-1), 29.72 (C-2), 77.33 (C-3), 38.77 (C-4), 140.92 (C-5), 121.64 (C-6), 31.87 (C-7), 35.92 (C-8), 50.06 (C-9), 36.67 (C-10), 21.05 (C-11), 39.69 (C-12), 42.32 (C-13), 56.62 (C-14), 24.31 (C-15), 28.23 (C-16), 55.88 (C-17), 12.12 (C-18), 19.55 (C-19), 36.67 (C-20), 19.07 (C-21), 33.79 (C-22), 25.90 (C-23), 45.60 (C-24), 29.16 (C-25), 19.39 (C-26), 19.30 (C-27), 23.06 (C-28), 12.35 (C-29), 101.26 (C-1'), 73.92 (C-2'), 77.24 (C-3'), 70.55 (C-4'), 73.24 (C-5'), 61.53 (C-6'). According to $^1$H-NMR and $^{13}$C NMR data the compound was determined to be daucosterin (Chung et al., 2016, Demirtaş et al., 2017).

**Ca-6:** Ca-6 obtained as white powder crystals**.** $^1$H NMR (600 MHz, DMSO-$d_6$): 6.92 (s, H-2), 12.88 ( s, -OH), 6.43 (d, J= 1.91 Hz, H-6), 6.76 (d, J= 1.85 Hz, H-8), 8.03 (d, J= 8.83 Hz, H-2'), 7.13 (d, J= 8.86 Hz, H-3'), 7.13 (d, J= 8.86 Hz, H-5'), 8.03 (d, J= 8.83 Hz, H-6'), 3.84 (s, -OCH3), 5.04 (d, J= 7.68 Hz, H-1''), 3.25 (t, H-2''), 3.28 (t, H-3''), 3.12 (t, H-4''), 3.57 (m, H-5''), 3.82-3.42 (m, H-6''), 4.51 (d, J= 1.20 Hz, H-1'''), 3.62 (m, H-2'''), 3.42 (m, H-3'''), 3.11 (m, H-4'''), 3.38 (m, H-5'''), 1.03 (d, J= 6.22 Hz, $CH_3$). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 164.41 (C-2), 104.28 (C-3), 182.49 (C-4), 161.61 (C-5), 100.50 (C-6), 163.42 (C-7), 95.24 (C-8), 157.42 (C-9), 105.93 (C-10), 123.15 (C-1'), 128.92 (C-2'), 115.18 (C-3'), 162.89 (C-4'), 115.18 (C-5'), 128.92 (C-6'), 56.03 (-$OCH_3$), 100.41 (C-1''), 73.53 (C-2''), 76.72 (C-3''), 70.06 (C-4''), 76.13 (C-5''), 66.55 (C-6''), 100.98 (C-1'''), 70.81 (C-2'''), 71.21 (C-3'''), 72.52 (C-4'''), 68.78 (C-5'''), 18.41 ($CH_3$). According to $^1$H-NMR and $^{13}$C NMR data the compound was determined to be Linarin (Demirtas et al., 2017; Huynh et al., 2016).

**Ca-7:** Ca-7 obtained as yellow powder crystals. HPLC-TOF/MS; m/z $[M-H]^-$ 609.4094. $^1$H NMR (600 MHz, DMSO-$d_6$): 12.56 ( s, -OH), 6.16 (d, J= 1.74 Hz, H-6), 6.35 (d, J= 1.74 Hz, H-8), 7.50 (d, J= 1.91 Hz, H-2'), 6.81 (d, J= 8.23 Hz, H-5'), 7.51 (dd, J= 8.23 Hz, J= 1.91 Hz, H-6'), 5.31 (d, J= 7.23 Hz, H-1"), 3.19 (t, H-2"), 3.18 (t, H-3"), 3.02 (t, H-4"), 3.22 (m, H-5"), 3.67-3.26 (m, H-6"), 4.35 (s, H-1'"), 3.36 (m, H-2'"), 3.25 (m, H-3'"), 3.04 (m, H-4'"), 3.24 (m, H-5'"), 0.96 (d, J= 6.28 Hz, $CH_3$). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 156.86 (C-2), 133.73 (C-3), 177.77 (C-4), 161.64 (C-5), 99.14 (C-6), 164.68 (C-7), 94.03 (C-8), 157.00 (C-9), 104.33 (C-10), 121.59 (C-1'), 116.67 (C-2'), 145.18 (C-3'), 148.87 (C-4'), 115.65 (C-5'), 122.07 (C-6'), 101.64 (C-1"), 74.50 (C-2"), 76.88 (C-3"), 70.42 (C-4"), 76.34 (C-5"), 67.42 (C-6"), 101.18 (C-1'"), 70.80 (C-2'"), 70.98 (C-3'"), 72.27 (C-4'"), 68.67 (C-5'"), 18.17 ($CH_3$). According to $^1$H-NMR and $^{13}$C NMR data the compound was determined to be rutin (Selvaraj et al., 2013).

**Ca-8:** Ca-8 obtained yellow powder crystals**.** HPLC-TOF/MS; m/z $[M-H]^-$ 445.409. $^1$H NMR (600 MHz, DMSO-$d_6$): 6.74 (s, H-3), 12.93 ( s, -OH), 6.37 (d, J= 1.73 Hz, H-6), 6.73 (d, J= 1.73 Hz, H-8), 7.82 (d, J= 8.80 Hz, H-2'), 6.85 (d, J= 8.80 Hz, H-3'), 6.85 (d, J= 8.80 Hz, H-5'), 7.82 (d, J= 8.80 Hz, H-6'), 5.04 (d, J= 7.67 Hz, H-1"), 3.25 (dd, J= 7.67-8.95 Hz, H-2"), 3.28 (dd, J= 8.95-8.88 Hz, H-3"), 3.19 (dd, J= 9.54-8.88 Hz, H-4"), 3.60 (d, J= 9.54 Hz, H-5"). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 164.71 (C-2), 103.15 (C-3), 182.34 (C-4), 161.38 (C-5), 100.01 (C-6), 163.39 (C-7), 94.98 (C-8), 157.34 (C-9), 105.67 (C-10), 120.85 (C-1'), 128.77 (C-2'), 116.40 (C-3'), 162.30 (C-4'), 116.40 (C-5'), 128.77 (C-6'), 99.93 (C-1"), 73.37 (C-2"), 76.93 (C-3"), 72.28 (C-4"), 74.20 (C-5"), 172.31 (C-6"). According to $^1$H-NMR ve $^{13}$C NMR data the compound was determined to be apigenin 7-*O*-*β*-D-glucuronide (Cheng et al., 2013).

**Ca-9:** Ca-9 obtained yellow powder crystals. HPLC-TOF/MS; m/z $[M-H]^-$ 459.109. $^1$H NMR (600 MHz, DMSO-$d_6$): 6.84 (s, H-3), 12.95 ( s, -OH), 6.45 (d, J= 1.54 Hz, m, H-6), 6.83 (d, J= 1.54 Hz, m, H-8), 7.93 (d, J= 8.43 Hz, H-2'), 6.92 (d, J= 8.43 Hz, H-3'), 6.92 (d, J= 8.43 Hz, H-5'), 7.93 (d, J= 8.43 Hz, H-6'), 5.29 (d, J= 7.40 Hz, H-1"), 3.31 (t, H-2"), 3.28 (t, H-3"), 3.39 (t, H-4"), 4.18 (d, J= 9.79 Hz, H-5"), 3.64 (s, $OCH_3$). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 164.76 (C-2), 103.60 (C-3), 182.43 (C-4), 161.64 (C-5), 99.75 (C-6), 162.86 (C-7), 95.07 (C-8), 157.40 (C-9), 105.93 (C-10), 121.46 (C-1'), 129.08 (C-2'), 116.45 (C-3'), 161.82 (C-4'), 116.45 (C-5'), 129.08 (C-6'), 99.51 (C-1"), 75.86 (C-2"), 73.16 (C-3"), 71.76 (C-4"), 75.60 (C-5"), 169.64 (C-6"), 52.42 (-$OCH_3$). According to $^1$H-NMR ve $^{13}$C NMR data the compound was determined to be apigenin 7-*O*-*α*-D-glucuronide methyl ester (Du et al., 2006; Lin et al., 2003).

**Ca-10:** Ca-10 obtained white cotton crystals. HPLC-TOF/MS; m/z $[M-H]^-$ 445.409. $^1$H NMR (600 MHz, DMSO-$d_6$): 6.82 (s, H-3), 12.94 ( s, -OH), 6.42 (d, J= 1.89 Hz, H-6), 6.81 (d, J= 1.84 Hz, H-8), 7.91 (d, J= 8.69 Hz, H-2'), 6.90 (d, J= 8.69 Hz, H-3'), 6.90 (d, J= 8.69 Hz, H-5'), 7.91 (d, J= 8.69 Hz, H-6'), 5.16 (d, J= 7.40 Hz, H-1"), 3.25 (dd, J= 7.83-8.95 Hz, H-2"), 3.28 (dd, J= 8.95-8.88 Hz, H-3"), 3.29 (dd, J= 9.54-8.88 Hz, H-4"), 3.85 (d, J= 8.10 Hz, H-5"). $^{13}$C-NMR (150 MHz, DMSO-$d_6$): 164.72 (C-2), 103.47 (C-3), 182.43 (C-4), 161.54 (C-5), 99.91 (C-6), 163.13 (C-7), 95.08 (C-8), 157.38 (C-9), 105.80 (C-10), 121.42 (C-1'), 128.99 (C-2'), 116.44 (C-3'), 161.91 (C-4'), 116.44 (C-5'), 128.99 (C-6'), 99.74 (C-1"), 73.32 (C-2"), 76.41 (C-3"), 71.96 (C-4"), 75.22 (C-5"), 170.89 (C-5"). Accordin to $^1$H-NMR ve $^{13}$C NMR data the compound was determined to be apigenin 7-*O*-*β*-D-glucuronide (Yamasaki et al., 2007).

## CONCLUSION

In this study, the isolation and characterization of previously unreported compounds from the plant *Cirsium arvense* subsp. *vestitum* were revealed. Here, as a result of the combination of several chromatographic techniques, a fast and effective isolation process was performed. In the purification process, both normal phase and reverse phase column fillers were used and isolation was performed using open column system and closed medium pressure liquid chromatography. Demonstrating the biological activity of natural product-derived compounds isolated from here sheds light on for a future study. In addition, the rich potential of this plant species, which is thought to be inactive, already has a significant meaning.

## ACKNOWLEDGEMENTS

This study was carried out and was financially supported by Cankırı Karatekin University Scientific Research Projects Unit (Project Number: FF2815B01)

## REFERENCES

Amiri N, Yadegari M, Hamedi B 2018. Essential oil composition of *Cirsium arvense* L. produced in different climate and soil properties. Records of Natural Products, 12(3).

Carter TR., Lym RG 2017. Canada thistle (*Cirsium arvense*) affects herbage production in the Northern Great Plains. Invasive Plant Science and Management, 10(4); 332-339.

Chaturvedula V, Prakash I 2013. Flavonoids from *Astragalus propinquus*. Journal of Chemical and Pharmaceutical Research, 5; 261-265.

Cheng HL, Zhang LJ, Liang YH, Hsu YW, Lee IJ, Liaw CC, Kuo YH 2013. Antiinflammatory and antioxidant flavonoids and phenols from *Cardiospermum halicacabum*. Journal of Traditional and Complementary Medicine, 3(1); 33-40.

Chung MJ, Lee S, Park YI, Lee J, Kwon KH 2016. Neuroprotective effects of phytosterols and flavonoids from *Cirsium setidens* and Aster scaber in human brain neuroblastoma SK-N-SH cells. Life Sciences, 148; 173-182.

Dehjurian A, Lari J, Motavalizadehkakhky A 2017. Anti-bacterial activity of extract and the chemical composition of essential oils in *Cirsium arvense* from Iran. Journal of Essential Oil Bearing Plants, 20(4); 1162-1166.

Demirtas I, Ayhan B, Sahin A, Aksit H, Elmastas M, Telci I 2011. Antioxidant activity and chemical composition of *Sideritis libanotica* Labill. ssp. l*inearis* (Bentham) Borm.(Lamiaceae). Natural Product Research, 25(16); 1512-1523.

Demirtas I, Tufekci AR, Yaglioglu AS, Elmastas M 2016. Studies on the antioxidant and antiproliferative potentials of *Cirsium arvense* subsp. *vestitum*. Journal of Food Biochemistry. 41; e12299.

Du Q, Xu Y, Li L, Zhao Y, Jerz G, Winterhalter P 2006. Antioxidant constituents in the fruits of *Luffa cylindrica* (L.) Roem. Journal of Agricultural and Food Chemistry, 54(12); 4186-4190.

Güner A, Akyıldırım B, Alkayış MF, Çıngay B, Kanoğlu SS, Özkan AM, Öztekin M, and Tuğ GN 2012. Türkçe bitki adları. Şu eserde: Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi Yayınları Flora Dizisi 1 İstanbul.

Herrera-González I, González-Cuesta M, García-Moreno MI, García Fernández JM, Ortiz Mellet C 2022. Stereoselective synthesis of Nojirimycin α-C-glycosides from a bicyclic acyliminium intermediate: A convenient entry to N, C-Biantennary glycomimetics. ACS Omega, 7(26); 22394-22405.

Huynh L, Tran H, Bacher M, Pacher T 2016. Iridoids and flavonoids from *Valeriana hardwickii* Wall. Journal of Pharmacognosy and Phytochemistry, 5(3); 245-249.

Liu Q, Yu J, Liao X, Zhang P, Chen X 2015. One-step separation of antioxidant compounds from *Erythrina variegata* by high speed counter-current chromatography. Journal of Chromatographic Science, 53(5): 730-735.

Nobarinezhad MH, Challagundla L, Wallace LE 2020. Small-scale population connectivity and genetic structure in Canada thistle (*Cirsium arvense*). International Journal of Plant Sciences, 181(4); 473-484.

Rebecca OPS, Boyce AN, Somasundram C 2012. Isolation and identification of myo-inositol crystals from dragon fruit (Hylocereus polyrhizus). Molecules, 17(4); 4583-4594.

Sciubba F, Capuani G, Di Cocco ME, Avanzato D, Delfini M 2014. Nuclear magnetic resonance analysis of water soluble metabolites allows the geographic discrimination of pistachios (Pistacia vera). Food Research International, 62; 66-73.

Selvaraj K, Chowdhury R, Bhattacharjee C 2013. Isolation and structural elucidation of flavonoids from aquatic fern *Azolla microphylla* and evaluation of free radical scavenging activity. International Journal of Pharmacy and Pharmaceutical Sciences, 5(3); 743-749.

Tosovic J, Markovic S 2016. Structural and antioxidative features of chlorogenic acid. Croatica Chemica Acta, 89(4); 1-8.

Yamasaki T, Masuoka C, Nohara T, Ono M. 2007. A new phenylethanoid glycoside from the fruits of *Callicarpa japonica* Thunb. *var. luxurians* Rehd. Journal of Natural Medicines, 61(3); 318-322.

## ORAL PRESENTATION

### Alpha Cypermethrin indüklü toksisiteye karşı kafeik asit fenetil esterin koruyucu etkisi

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**Özet**

Alpha cypermethrin (α-CYP), sentetik piretroid pestisidler grubuna dahil olan bir insektisiddir. Bu çalışmada Spraque-Dawley ırkı erkek sıçanlara subakut uygulanan alpha-cypermethrinin plazma biyokimyasal parametreler üzerine etkilerinin değerlendirmesi amaçlandı. Subakut maruziyet temelli çalışmada yetişkin 48 adet Spraque-Dawley ırkı sıçan kullanıldı. Sıçanlar kontrol (çözücü madde), alpha-cypermethrin grupları (10 mg kg-1, 20 mg kg-1), kafeik asit fenetil ester (CAPE, 10 µmol kg-1) grubu, 10 mg kg-1+CAPE ve 20 mg kg-1+CAPE grupları olmak üzere 6 gruba ayrıldı. Biyokimyasal incelemeler için kan plazma örneklerinde alpha-cypermethrin uygulanan gruplarda kontrol ve CAPE grubuna kıyasla total antioksidan seviyede (TAS) azalma gözlenirken, total oksidan seviye (TOS), malondialdehit (MDA), total sialik asit (TSA) ve nitrik oksit (NO) ve oksidatif stres indeksinde (OSI) ise artış saptandı. Alpha-cypermethrin ile birlikte CAPE uygulanan gruplarda ise CAPE'nin antioksidan özelliğinden dolayı biyokimyasal parametrelerde meydana gelen değişimlerin normal düzeylere yaklaştığı belirlendi. Sonuç olarak, tarım alanında yaygın olarak kullanılan pestisitlerin olumsuz etkilerine karşı kafeik asit fenetil ester gibi antioksidan özelliği bilinen maddelerin kullanımının hem insan sağlığı hem de hayvan sağlığı açısından önemli olduğu kanısındayız.

**Anahtar Kelimeler:** Alpha-cypermethrin, kafeik asit fenetil ester, nitrik oksit, oksidan/antioksidan denge, total sialik asit

### Protective effect of caffeic acid phenethyl ester against Alpha Cypermethrin-induced nephrotoxicity

**Abstract**

Alpha cypermethrin (α-CYP) is an insecticide that belongs to the group of synthetic pyrethroid pesticides. In this study, it was aimed to evaluate the effects of alpha-cypermethrin administered subacute to Spraque-Dawley male rats on plasma biochemical parameters. Forty-eight adult Spraque-Dawley rats were used in the subacute exposure-based study. Rats control (solvent), alpha-cypermethrin groups (10 mg kg-1, 20 mg kg-1), caffeic acid phenethyl ester (CAPE, 10 µmol kg-1) group, 10 mg kg-1+CAPE and 20 mg They were divided into 6 groups as kg-1+CAPE groups. In blood plasma samples for biochemical studies, a decrease in total antioxidant level (TAS) was observed in alpha-cypermethrin applied groups compared to control and CAPE groups, while total oxidant level (TOS), malondialdehyde (MDA), total sialic acid (TSA) and nitric oxide (NO) and oxidative stress index (OSI) was increased. It was determined that the changes in biochemical parameters due to the antioxidant property of CAPE approached normal levels in the groups that were administered CAPE together with alpha-cypermethrin. As a result, we believe that the use of substances with known antioxidant properties such as caffeic acid phenethyl ester against the negative effects of pesticides widely used in agriculture is important for both human and animal health.

**Keywords:** Alpha-cypermethrin, caffeic acid phenethyl ester, nitric oxide, oxidant/antioxidant balance, total sialic acid

## GİRİŞ

Sanayi devrimi sonrası dünya nüfusunun da artmasıyla birlikte tarım ürünleri insan beslenmesi açısından yetersiz gelmeye başlamıştır. Bu tarım ürünlerinin artan insan nüfusuna yetersiz gelmesinin bir diğer nedeni ise tarım ürünlerine zarar veren farklı türdeki zararlılarla gereken mücadelenin yapılamaması sonucu ürün kaybının yaşanmasıdır. Ürün kaybına neden olan bu zararlılara karşı geliştirilen pestisitlerin kullanımıyla birlikte tarım ürünlerinde rekolte artışları meydana gelmiştir. Pestisitler amacına uygun kullanıldığında tarım zararlılarıyla mücadelede oldukça faydalı iken, mesleki maruziyet, toprakta birikim, yeraltı suları ve sucul ekosistemlere karışım neticesinde asıl etkilemesi istenen zararlılar dışındaki canlılar ve çevre sağlığı açısından tehdit oluşturmaktadır (Deveci ve ark. 2015; Deveci ve ark. 2017; Deveci and Karapehlivan, 2018; Dogan ve ark. 2021). Alpha cypermethrin'in (α-CYP) üyesi olduğu piretroidler, tarımsal alanlarda yaygın olarak kullanılmaktadır. En önemli avantajları ise, böceklere karşı oldukça toksik olmasına karşın, memelilerin hızlı metabolizmalarından dolayı düşük toksisiteye sahip olması ve toprakta birikmemesidir (WHO, 2013; Soderlund, 2010). Piretroidlerin seçici toksisitesinde asıl mekanizma, böceklerin sinir sistemine ait sodyum kanallarının piretroidlere yüksek hassasiyet göstermesidir. Zehirlenme belirtileri ve nörofizyolojik araştırmalar, bu grubun sinir sisteminde aksonlardaki iletime etki ettiğini göstermektedir. Sinir hücrelerinin membranlarında bulunan sodyum kanallarının açık kalmasına neden oldukları ve böylece böcekleri felç ederek öldürdükleri düşünülmektedir (Öz ve ark. 2021; Silver, 2014). Alpha cypermethrin tarımsal faaliyetler dışında hayvanlarda ektoparaziter olarak ve insektlerin kontrolü amacıyla halk sağlığı açısından da önem arz etmektedir (İssi ve ark. 2014; Mercan, 2007; Luty ve ark. 1998)
Bu çalışmada tarım alanında yaygın kullanılan bir pestisit olan Alpha cypermethrinin farklı dozlarda hedef organizma dışındaki canlılarda oluşturabileceği toksisiteye karşı CAPE'nin etkinliğinin araştırılması amaçlandı.

## MATERYAL VE METOT

Çalışmada 220-260 gr ağırlığında Spraque-Dawley ırkı toplam 48 adet erkek sıçan kullanıldı. Sıçanlar, ortam sıcaklığı 21 °C olan, günün yarısı aydınlık diğer yarısı karanlık ortamda tutularak, standart sıçan yemi (%21 ham protein içeren) ve musluk suyu ile beslendi. Her grupta 8 sıçan olacak şekilde Kontrol (K), Kafeik asit fenetil ester (CAPE), Alpha cypermethrin 10 mg/kg (α-CYP-10), Alpha cypermethrin 20 mg/kg (α-CYP-20), Alpha cypermethrin 10 mg/kg + Kafeik asit fenetil ester (α-CYP-10+CAPE) ve Alpha cypermethrin 20 mg/kg + Kafeik asit fenetil ester (α-CYP-20+CAPE) olmak üzere 6 grup oluşturuldu. Tüm gruplara yapılan doz uygulamaları aşağıda **Tablo 1**.'de verilmiştir.

**Tablo 1.** Deneysel protokole göre oluşturulan gruplara yapılan uygulamalar

| **K grup** | **CAPE grup** | **α-CYP-10 grup** | **α-CYP-20 grup** | **α-CYP-10 + CAPE grup** | **α-CYP-20 + CAPE grup** |
|---|---|---|---|---|---|
| 1 ml/kg corn oil (Gavaj) | 10 mg/kg α-CYP ((Intraperitoneal) | 10 mg/kg α-CYP (Gavaj) | 20 mg/kg α-CYP (Gavaj) | 10 mg/kg α-CYP + 10 µmol/kg CAPE (Intraperitoneal) | 20 mg/kg α-CYP + 10 µmol/kg CAPE (Intraperitoneal) |

Tüm uygulamalar 30 gün boyunca sürdürüldü. Deney protokolü sonunda sıçanlar intra muskülar (i.m) yolla yapılan ketamin hidroklorür/xylasin (80/10 mg/kg) anestezisi altında intrakardiyak kan örnekleri alındı ve servikal dislokasyon yöntemiyle imha edildi.

**Biyokimyasal Analizler:** Biyokimyasal analizler için alınan kan örnekleri 10 dakika 3000 rpm'de santrifüj edilerek plazmaları elde edildi ve analize kadar -20 °C'de saklandı. Plazma TAS ve TOS analizleri Erel'in (2004; 2005) geliştirdiği metot ile ticari kitler (REL Assay Diagnostics, Gaziantep, Türkiye) kullanılarak spektrofotometrede ölçüldü. OSİ, TOS değerlerinin TAS değerlerine bölünmesiyle elde edildi. TAS sonucu mmol/L cinsinden olduğu için, hesaplamadan önce µmol/L cinsine çevirildi. MDA düzeyleri Yoshoiko ve ark. (1979)'in bildirdikleri metoda gore, TSA ölçümü Sydow'un (1985) bildirdiği metoda göre, nitrik oksit (NO) ölçümü ise Miranda ve ark.'nın (2001) bildirdiği metoda göre spektrofotometrede kolorimetrik olarak ölçüldü.

**İstatistiksel Analiz:** Çalışmamız sonucunda elde edilen bulgular SPSS (Statistical Package for Social Sciences) 22.0 paket programı kullanılarak değerlendirildi. Elde edilen kantitatif değerler istatistiksel veri programında tek yönlü varyans analizi (one-way ANOVA) ile yapıldı. Gruplar arasındaki istatistiksel anlamlılık Tukey HSD çoklu karşılaştırma testi ile değerlendirildi. Anlamlılık eşik değeri olarak $p<0.05$ kullanıldı. Sonuçlar ortalama ±standart sapma olarak verildi.

## BULGULAR

Çalışmadan elde edilen biyokimyasal veriler değerlendirildiğinde, kontrol ve CAPE gruplarındaki değerler normal ve birbirlerine yakın olarak bulundu. Gruplar TAS bakımından incelendiğinde, kontrol ve CAPE grupları arasında fark gözlenmemişken ($p>0,05$), alpha cypermethrin'in 10 ve 20 mg dozunun uygulandığı gruplar ile diğer tüm gruplar arasındaki istatistiki fark önemli ($p<0,01$) olarak tespit edildi. TOS bakımından kontrol, CAPE ve 10 mg. CYP+CAPE grupları ile diğer gruplar arasındaki fark istatistiki olarak önemli bulundu ($p<0,01$). Gruplar arasında MDA, NO, TSA seviyelerinin değerlendirmesi sonucunda TOS'daki durum ile paralel olduğu gözlendi. OSI'nin değerlendirilmesi sonucunda ise, kontrol ve CAPE grubu arasında fark olmadığı gözlenirken, diğer tüm gruplar arasındaki fark istatistiki olarak önemli ($p<0,01$) olduğu tespit edildi. Çalışmadan elde edilen gruplara ait veriler aşağıda **Tablo 2**'de gösterilmiştir.

**Tablo 2.** Gruplara ait TAS, TOS, OSİ, MDA, TSA ve NO düzeyleri ve gruplar arasında istatistiki önem.

| PARAMETRELER | GRUPLAR | | | | | | p< |
|---|---|---|---|---|---|---|---|
| | **Kontrol** (n:8) Mean±SD | **CAPE** (n:8) Mean±SD | **α-CYP-10** (n:8) Mean±SD | **α-CYP-20** (n:8) Mean±SD | **α-CYP-10 + CAPE** (n:8) Mean±SD | **α-CYP-20 + CAPE** (n:8) Mean±SD | |
| **TAS** (mmol Trolax eq./L) | $1,31±0,11^{a}$ | $1,37±0,12^{a}$ | $0,87±0,09^{c}$ | $0,79±0,08^{c}$ | $0,91±0,09^{c}$ | $1,10±0,15^{b}$ | * |
| **TOS** (μmol $H_2O_2$ eq./L) | $6,48±0,75^{c}$ | $6,43±0,54^{c}$ | $7,93±0,83^{a,b}$ | $8,69±0,65^{a}$ | $7,40±0,62^{b,c}$ | $8,03±0,78^{a,b}$ | * |
| **MDA** (μmol/L) | $2,74±0,38^{c}$ | $2,85±0,29^{c}$ | $3,55±0,46^{a,b}$ | $4,04±0,30^{a}$ | $3,18±0,32^{b,c}$ | $3,48±0,40^{b}$ | * |
| **TSA**(mg/dL) | $62,84±6,07^{d}$ | $65,50±5,27^{c,d}$ | $77,70±6,97^{a,b}$ | $83,07±7,24^{a}$ | $73,29±7,35^{b,c}$ | $78,88±5,54^{a,b}$ | * |
| **NO** (μmol/L) | $6,22±0,86^{c}$ | $6,47±0,67^{c}$ | $8,07±1,05^{a,b}$ | $9,17±0,69^{a}$ | $7,23±0,72^{b,c}$ | $7,91±0,91^{b}$ | * |
| **OSI** (Arbitrary Unit) | $0,49±0,03^{d}$ | $0,47±0,04^{d}$ | $0,91±0,06^{b}$ | $1,10±0,08^{a}$ | $0,81±0,10^{c}$ | $0,73±0,04^{b}$ | * |

*: $p<0.01$=İstatistik olarak anlamlı fark, a,b,c,d: Aynı satırda farklı harf taşıyan grup ortalamaları arası fark önemlidir. n: gruptaki denek sayısı, SD: Standart sapma.

## TARTIŞMA

Cypermethrin (CYP), bitkileri haşerelere ve böcek istilasına karşı korumak için yaygın olarak kullanılan önemli bir tip II piretroid pestisittir. Ancak toksisitesi hem insan sağlığı hem de doğadaki diğer canlılar için bir risktir (Alalwani, 2020) Alpha cypermethrin'in üyesi olduğu piretroidler sodyum kanal modülatörleridir. Etkilerini böcekler üzerinde temas ve yutma yoluyla gösterirler. Piretroidler spesifik böcek öldürücüler olsalar da, bazı hedef dışı türler için çok toksiktirler (Corcellas ve ark. 2013).

Cypermethrin'in LD50 dozunun 1/50'si ile ratlar üzerinde yapılan bir çalışmada süperoksit dismutaz (SOD) ve glutatyon S-transferaz (GST) düzeylerinde azalma, malondialdehit (MDA) ve protein karbonil ise önemli bir artışa sebep olduğunu bildirmiştir (Taha ve ark., 2022). Abdou ve ark. (2012)'ları ratlar ile yaptıkları bir çalışmada cypermethrinin MDA, laktat dehidrogenaz (LDH), aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), üre ve kreatinin seviyelerinde artış, glutatyon (GSH)'da ise azalış olduğunu bildirmişlerdir. Ancak susam yağı+cypermethrinin birlikte uygulandığı gruplarda ise oksidatif stresin azalışına bağlı olarak biyokimyasal parametrelerde ve histolojik görünümde iyileşmeler gözlemlemişlerdir (Abdou ve ark., 2012). Cypermethrin'in farklı dozlarının (62.5, 125 ve 250 mg kg-1) uygulandığı bir çalışmada, kontrol grubuna kıyasla cypermethrin uygulanan gruplarda artan doza paralel olarak vücut ve organ ağırlıklarındaki artış yüzdesinde azalma, GSH seviyelerinde düşüş olduğu, AST, ALT, MDA ve kreatinin, seviyelerinde ise

artış olduğu bildirilmiştir (Seven at al., 2022). Eraslan ve ark. (2016), 12 ve 80 mg kg-1 dozda cypermethrin uyguladıkları ratlarda MDA ve NO seviyelerinde artış, SOD, GST, GSH, CAT seviyelerinde ise düşüş gözlemlemişlerdir.

CAPE'nin antioksidan özelliğinin olduğu ve pestisitlerin neden olduğu farklı doku patolojileri üzerinde koruyucu etkiye sahip olduğu bir çok çalışma mevcuttur (Nur ve ark., 2018a; Nur ve ark., 2018b; Özen ve ark., 2004; Yagmurca ve ark., 2004). Cypermethrin uygulamasına bağlı olarak artan CAT ve MDA konsantrasyonuna karşılık cypermethrin+CAPE uygulanan gruplarda ise CAT ve MDA seviyelerinin cypermethrinin yalnız uygulandığı gruba kıyasla azaldığı bildirilmiştir (Aldemir ve ark. 2014). Bizim yaptığımız bu çalışmada da diğer araştırmacıların bulgularını destekler biçimde oksidatif belirteçlerden TOS, MDA, NO, TSA ve OSI değerlerinde artış olduğu, TAS değerlerinde azalış olduğu tespit edildi.

## SONUÇ

Yapılan bu çalışma sonucunda alpha cypermethrin'in özellikle oksidatif stresi indüklediği ve buna bağlı olarak oksidan/antioksidan dengeyi oksidanlar lehine bozduğu tespit edildi. Bundan dolayı alpha cypermethrin sadece etkili olması istenen canlılar üzerine değil aynı zamanda hedef olmayan organizmalarda da toksik özelliğe sahip bir piretroid insektisit olduğu, CAPE'nin ise alpha cypermethrin'in neden olduğu biyokimyasal değişikliklerde koruyucu potansiyelinin olabileceği sonucuna varıldı. Buna göre tarım alanlarında yaygın olarak kullanılan insektisitlerin hedef organizmalar dışındaki canlıları da etkilediği ve bu canlılarda biyokimyasal değişikliklere neden olabileceği unutulmamalıdır.

## KAYNAKLAR

Abdou HM, Hussien H, Yousef MI 2012. Deleterious effects of cypermethrin on rat liver and kidney. Protective role of sesame oil. Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes, 47(4): 306-314.

Alalwani AD 2020. Nephrotoxicity of cypermethrin in rats. Histopathological aspects. Histol Histopathol, 35(12): 1437-1448.

Aldemir OS, Talas ZS, Gulhan MF, Cakır O, Ozdemir I, Dastan SD, Dogan H 2014. Role of Propolis on Oxidative Stress in Various Tissues of Fish. Fresenius Environmental Bulletin, 23(12): 1-5.

Corcellas C, Eljarat E, Barcelo D 2013. Determination of Pyrethroid Insecticides in Environmental Samples by GC-MS and GC-MS-MS. Chapter 9, Comprehensive Analytical Chemistry, Volume 61: 203-230.

Deveci HA, Karapehlivan M, Kaya İ, Kükürt A, Alpay M 2015. Akut klorprifos-etil zehirlenmesine karşı kafeik asit fenetil ester'in koruyucu etkisi. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 62(4): 255-260.

Deveci HA, Karapehlivan M 2018. Chlorpyrifos-induced parkinsonian model in mice: Behavior, histopathology and biochemistry. Pesticide Biochemistry and Physiology, 144:36-41.

Deveci HA, Ünal S, Karapehlivan M, Ayata MK, Gaffaroğlu M, Kaya İ, Yılmaz M, 2017. Effects Of Glyphosate (Herbicide) On Serum Paraoxonase Activity, High Density Lipoprotein, Total Antioxidant and Oxidant Levels in Kars Creek Transcaucasıan Barbs (Capoeta capoeta [Guldenstaedt, 1773]). Fresenius Environmental Bulletin, 26 (5): 3514-3518.

Doğan D, Deveci HA, Nur G 2021. Manifestation of oxidative stress and liver injury in Clothianidin exposed Oncorhynchus mykiss. Toxicology research, 10: 501-510.

Eraslan G, Kanbur M, Siliğ Y, Karabacak M, Soyer Sarica Z, Şahin S 2016. The acute and chronic toxic effect of cypermethrin, propetamphos, and their combinations in rats. Environ Toxicol., 31(11):1415-1429.

Erel O 2005. A new automated colorimetric method for measuring total oxidant status. Clin Biochem, 38:1103-1111.

Erel O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable abts radical cation. Clin Biochem, 37:277-285.

İssi M, Gül Baykalır B, Gül Y 2014. Acute Cypermethrin Intoxication Detected in a Dog. YYU Veteriner Fakültesi Dergisi, 25(1), 19-21.

Luty S, Latuszyńska J, Halliop J, Tochman A, Obuchowska D, Przylepa E, Korczak E 1998. Toxicity of dermally applied alpha-cypermethrin in rats. Ann Agric Environ Med, 5: 109-115.

Mercan U 2007. Adverse Effects of Bioallethrins on Health. YYÜ Vet Fak Derg, 18(2): 73-78.

Miranda KM, Espey MG, Wink DA 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 5: 62- 71.

Nur G, Güven A, Kılıçle PA 2018a. The effects of CAPE on oxidative stress and histopathological values in rats treated with subacute dichlorvos. Journal of Cellular Neuroscience and Oxidative Stress (J Cell Neurosci Oxid Stress), 10(1): 647-655.

Nur G, Husunet MT, Guler I, Deveci A, Koc E, Nur O, Kilicle PA 2018b. The effect of caffeic acid phenethyl ester (CAPE) on hepatic histopathology and oxidative stress in rats treated with malathion. Medicine Science, 7(3): 604-609.

Öz E, Çetin H, Yanıkoğlu A 2021. Investigation of resistance to synthetic pyrethroids in Blattella germanica L., 1767 (Blattodea: Ectobiidae) and Periplanetaamericana L., 1758 (Blattodea: Blattidae) populations in Turkey. Türk. Entomol. Derg., 45 (3): 361-370.

Özen S, Akyol Ö, Iraz M, Söğüt S, Özuğurlu F, Özyurt H, Odacı E, Yıldırım Z 2004. Role of caffeic acid phenethyl ester, an active component of propolis, against cisplatin-induced nephrotoxicity in rats. Journal of Applied Toxicology, 24(1), 27-35.

Seven B, Çavuşoğlu K, Yalçin E, Acar A 2022. Investigation of cypermethrin toxicity in Swiss albino mice with physiological, genetic and biochemical approaches. Sci Rep., 12(1): 11439.

Silver KS, Du Y. Nomura, EE, Oliveira V L, Salgado BS, Zhorov K, Dong. 2014. Voltage-gated sodium channels as insecticide targets. Advances in Insect Physiology, 46: 389-433.

Soderlund DM 2010. Toxicology and Mode of Action of Pyrethroid Insecticides. Chapter 77, Editor(s): Robert Krieger, Hayes' Handbook of Pesticide Toxicology (Third Edition), Academic Press, ISBN 9780123743671, 1665-1686.

Sydow G 1985. A simplified quick method for determination of sialic acid in serum. Biomed Biochim Acta, 44: 1721-1723.

Taha M, Badawy M, Abdel-razik R, Younis H, Abo-el-saad M 2022. Effects of sub-chronic exposure of male albino rats to some insecticides on mitochondrial dysfunction and oxidative stress in the kidney with molecular docking. Journal of Cellular Neuroscience and Oxidative Stress, 13(3).

WHO 2013. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. WHO press, Geneva, 30 pp.

Yagmurca M, Erdogan H, Iraz M, Songur A, Ucar M, Fadillioglu E 2004. Caffeic acid phenethyl ester as a protective agent against doxorubicin nephrotoxicity in rats. Clinica Chimica Acta, 348(1-2): 27-34.

Yoshioka T, Kawada K, Shimada T, Mori M 1979. Lipid peroxidation in maternal and cord blood and protective mechanism against activated oxygen toxicity in the blood. Am. J. Obstet. Gyn., 135: 372-376.

## *ORAL PRESENTATION*

# The current molecular assessments on endangered plants exposed to abiotic stress

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## Abstract

The stress is the condition that limits plant growth and development, reduces fruit yield and quality, in short, adversely affect it. Drought, water scarcity, cold, excessive salinity, UV rays, heavy metals, and anthropogenic activities (such as improper fertilization and urbanization of agricultural areas) are classified as abiotic stress. When plants are faced with any abiotic stress factor, they either develop adaptation by creating a physiological, biochemical or molecular response or are irreversibly negatively affected. Unfortunately, if this situation reaches serious dimensions, the plant may lose their viability. Unfortunately, considering the changing climatic events today, for example, the greenhouse effect of the gases accumulating (methane, carbon dioxide, etc.) in the atmosphere cause the melting of the glaciers and the harsh transitions or shift between the seasons. Moreover, increasing day-by-day forest fires, and thus the resulting deforestation and flood disasters result in the destruction of agricultural lands and drastically declining agricultural yields. All these situations, which cause serious loss of both medicinal and aromatic plants and crop plants, adversely affect many sectors, especially food, and cause an economic crisis. All these situations, which led to the loss of both medicinal and aromatic plants and crop plants, adversely affected many sectors, especially food. The decrease in the quality and safety of the food obtained due to adverse climatic conditions cause the economic crisis. According to a study, it has been observed that up to 70% of the total annual production due to climatic events is adversely affected. In the current review aimed to investigate the molecular studies on stopping or slowing down the serious decrease in yield and quality of plants due to abiotic stress factors that cause the economic problems.

**Keywords:** Climate crisis, greenhouse gas, molecular response, stress genes

## INTRODUCTION

Stress is defined as all adverse conditions that cause slowing or even regression of growth and development in plants, metabolic and physiological deterioration and reduce plant yield and quality (Agar et al., 2022). The negative effects of environmental stress factors such as drought, extreme salinity, extreme cold, chemicals, UV, CO2 emission, heavy metals, which pose a great threat to plants, are seen more dominantly in the 21st century with increasing global warming and industrialization. By 2021, global crop yields and quality are decreasing due to unfavorable climatic conditions and abiotic stresses in response to the increasing food demand of the world population, which is approaching eight billion with a change of ~1%, affecting sustainable agriculture and bringing great challenges such as increasing demand for agricultural production (Fuduka, 2000; Ahuja et al., 2010; Bishnoi et al., 2017). As a result of growing population and increasing anthropogenic activities, the ozone layer in the stratosphere is gradually thinning and many economically and medically valuable plants in the world are exposed to harmful rays and high temperatures. According to a study, nearly 70% of the world's 5.2 billion hectares of agricultural land is already facing drought and salinity problems (Koyro et al., 2011). If the effects of global warming negatively continue as expected, the expansion of drylands will be an inevitable outcome (Park et al., 2018; Sewelan et al., 2020). As a result of all these situations, plants lose water through high evaporation in increasing air temperatures and experience drought stress, and since evaporation also occurs on soil surfaces close to plant roots, the plant also encounters salt stress. This is why plants today have to cope with combined heat, drought and salinity stress (Sewelan et al., 2020). And unfortunately, the detrimental effect of multiple stressors is greater than that of a single stressor. In particular, the simultaneous occurrence of heat and drought is devastating for a plant's yield (Rasmussen et al., 2013; Suzuki et al., 2014; Zinta et al., 2014; Hosseini et al., 2021). Plants subjected to drought and heat stress at the same time showed a decrease in chlorophyll content and photosynthetic rate, a decrease in plant growth and crop yield, a decrease in stomatal conductance, and an increase in peroxide levels, which were

more severe than in the case of either stress (Zinta et al., 2014; Shaar-Moshe et al., 2017; Hosseini et al., 2021). Table 1 shows the yield losses in crops adversely affected by salinity, drought and heat abiotic stresses, singly or in combination, over the last decade.

**Table 1**. Yield losses in crops adversely affected by salinity, drought and heat abiotic stresses, singly or in combination, in the last decade

| Plant Name | Stress Factor | Result | Referance |
|---|---|---|---|
| *Lactuca sativa* L. | Salt | 26.5% decrease in the fresh weight | Franzoni et al., 2022 |
| *Solanum tuberosum* L. | Drought | A reduction of 35 to 60% in root length, 42 to 69% in mean root surface area | Zaki et al., 2022 |
| *Citrus reticulata* Blanco. | Salinity | 30% yield loss | Pagán et al., 2022 |
| *Allium cepa* L. | Drought | 19-28% yield loss, significantly reduced leaf area and onion growth | El–Metwally et al., 2022 |
| *Picea sitchensis* (Bong.) Carr. | Salinity | 50 % loss of xylem conductance | Zhang et al., 2021 |
| *Pinus edulis* Engelm. | Combined heat and drought | Total monoterpene concentrations, ~ 85% and 35% increases in needle and woody tissue | Trowbridge et al., 2021 |
| *Lycopersicon esculentum L.* | Drought and salt | Fresh weight (FW); 69 for drought stress, 67% for salt stress<br>Dry weight (DW); 80% for drought stress, 80% for salt stress | Ors et al., 2021 |
| *Hordeum vulgare L.* | Salinity | Salinity in the environment significantly reduced all yield characteristics, namely number of siblings (53%), ear length (40%) and 100 grain weight (41%) | Noreen et al., 2021 |
| *Coffea* | Drought stress | 41% decrease in yield | Venancio et al., 2020 |
| *Saccharum officinarum* | Drought stress | Total root weight of sugarcane affected by drought by 16.99% | Misra et al., 2020 |
| *Solanum melongena* | Salinity | 50% yield loss | Semiz et al., 2019 |
| *Solanum tuberosum* L. | Salinity | 36.3% yield loss | Abdullah-Al-Mahmud et al., 2018 |
| *Cicer arietinum* L. | Terminal drought | Yield loss up to 35%–64% loss in chickpea-producing regions of India and up to 33% globally | Hajjarpoor et al., 2018 |
| *Zea mays* L. | Drought | Yield loss ranging from 17 to 60% in Southern Africa | Tandzi et al., 2018 |
| *Zea mays* L. | Drought | Average 32% yield loss | Ashok et al., 2018 |
| *Gossypium hirsutum* L. | Drought and salinity | Decline of 31.1% lint yield and 44.4% fresh plant weight | Guo et al., 2018 |
| *Glycine max* (L.) Merr. | Drought | 22% loss of final plant height, Significant seed yield losses of 73–82%, 67% loss of final aboveground dry matter, 61% loss of the leaf area per plant | Wei et al., 2018 |

| | | | |
|---|---|---|---|
| *Triticum aestivum* L. | Drought, water stress (~40% water deficit) | Decrease of 25% yield | Daryanto et al., 2017 |
| *Oryza sativa* L. | Drought, water stress (~40% water deficit) | Decrease of 50% yield | Daryanto et al., 2017 |
| *Populus alba* | Drought stress | Biomass decreased by 20-25% | Lüttschwager et al., 2016 |
| *Abies alba* Mill. | Drought | Decline in the growth of silver fir | Gazol et al., 2015 |
| *Oryza sativa* L. | Salinity | 4.13% yield reduction | Tack et al., 2015 |
| *Gossypium hirsutum* | Drought stress | 35% decrease in lint yield | Chastain et al., 2014 |
| *Oryza sativa* L. | Heat | 28.5 % yield reduction | Aghamolki et al., 2013 |
| *Pinus sylvestris* L. | Drought | Decline of 6.8% of overall pine cover | Vacchiano et al., 2012 |

In 2021, Hosseini et al. applied combined and individual heat and drought stress on lentil plants and using RNA-seq methdou, they found that the genes differentially expressed as a result of transcriptome analysis in multiple stress conditions were not identified as a result of transcriptome analysis in individual stress conditions. In another study, it was observed that a 1°C change in temperatures due to climatic changes caused a 21% decrease in yield, a 2°C change caused a 33% decrease and a 4°C change caused a 50% decrease in yield (Tesfaye et al., 2018). In a study conducted by Lobell et al. in maize plants, it was observed that the yield decreased by 1% due to a 1°C temperature change at temperatures of 30°C and above, while a decrease of up to 40% in yield was reported in the case of combined heat stress and drought stress. In another study in maize, it was found that yield decreased by 58% under drought stress alone and 77% in combination of heat and drought stress (Meseka et al., 2018; Notununu et al., 2022).

In this context, it is understood that it is more accurate to evaluate two or more stress factors in combination rather than elucidating the mechanisms through a single stress factor. In order to reduce or improve all these abiotic stress effects, a better understanding of plant tolerance mechanisms is required. Plants try to adapt to their environment by forming physiological, biochemical or molecular responses to stress factors that they encounter individually or in combination under normal field conditions (Simms, 2000). Metabolically, they perform two basic events. One of them is to prevent water loss by inducing the related osmolyte biosynthesis and the other is to reduce oxidative damage by inducing the biosynthesis of antioxidant compounds (Arbona et al., 2017). To protect against molecular denaturations and osmotic stress, especially during drought, heat and salt stresses, plants increase the synthesis of osmoprotectant molecules through signal transduction pathways. Osmoprotectant molecules prevent denaturation of protein and membrane structure and maintain their stability (Yancey, 2005; Rivero et al., 2014). It has been observed that the expression of genes for osmoprotectant biosynthesis increases under one or a combination of the aforementioned stresses and as a result, stress tolerance is acquired (Delauney and Verma 1993; Kuznestov and Shevyakova 1997; Nuccio et al., 1999; Rivero et al., 2014). It also regulates stomatal mechanisms to prevent water loss, which reduces CO2 uptake-fixation. In this way, photosynthetic events are affected and the rate of ROS production and oxidative damage are balanced (Arbona et al., 2017). Plants also try to adapt biochemically to environmental conditions by synthesizing hormones such as cytokinin (CK), abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), ethylene (ET), gibberellin (GA) and so on. Studies to date have shown that the response at the molecular level consists of stress perception and transmission, transcription and post-transcriptional modification of relevant genes, translation and post-translational modification (Zhang et al., 2022). In short, synthesized hormones or secondary messengers bind to the relevant cis-acting elements in the promoter region of the target gene or interact with transcription factors that will bind to the target gene, thereby activating/inhibiting the gene and ensuring adaptation (Arbona et al., 2017). The perception and transmission of the signal varies according to the type of stress. For example, plants with glutamate receptor-like (GLR) channel protein family and cyclic nucleotide-gated channels (CNGC) family (Swarbreck et al., 2013; Zhu, 2016) increase the

synthesis of free Ca+2 in the cytosol as a stress sensor in salt, heat, cold stress, heavy metals and oxidative stress. This increase enables the perception of the signal (Zhu, 2016). In cold and heat stresses, phospholipids in the cell membrane structure undergo conformational changes and the fluidity of the membrane changes (Sangwan et al., 2002; Zhu, 2016; Zang et a., 2022), which is sensed by channel and transporter molecules in the membrane structure, membrane-bound proteins or through heat shock proteins that bind to misfolded protins due to denaturation (Scharf et al., 2012; Zhu, 2016). After all these sensing, signaling is carried out by a stress-specific (individual or multiple stress) change in the levels of the relevant molecules. The entry of the stress signal into the nucleus triggers the formation of transcripts from the relevant genes. These transcripts undergo post-transcriptional modifications such as splicing, 5'capping, degradation, polyadenylation. Any disruption or defect in these steps has been observed to cause serious problems in abiotic stress response (Hugouvieux et al., 2001; Wu et al., 2010; Zhan et al., 2015; Okamoto et al., 2016). In order for the relevant gene to become functional and play a role in the response to abiotic stress, it undergoes a trans transcription event followed by posttranslational modifications and becomes three-dimensional by folding according to its function. In this way, the plant molecularly creates a response to abiotic stress and tries to survive by providing adaptation.

## CONCLUSION

Unfortunately, the increasing human population and the agricultural areas that will meet the increasing food demand requirement unfortunately cause many agricultural lands to become unusable, especially by causing global warming due to anthropogenic activities related to population growth. In this case, plant stress response mechanisms against climate change need to be properly understood. Unfortunately, there are still large gaps in the existing studies on molecular response. In particular, it may be considered a big mistake to think that molecular mechanisms can be understood through a single stress factor until today. However, compared to individual stressors, combined stresses are more destructive in terms of plant development and crop yield. In the context of elucidation of molecular mechanisms, molecules that are thought to have no role in studies with individual stresses may play an important role in combined stress conditions, since plants are mostly exposed to multiple stress factors rather than a single stress factor under normal living conditions. The fact that the current studies on combined stresses and their molecular mechanisms are very limited and the plant diversity is low in the current studies reveals the necessity to carry out up-to-date studies as soon as possible.

## REFERENCES

Abdullah-Al-Mahmud HM, Akhter S 2018. Screening of CIP potato clones for salinity tolerance in pot and field condition. Advances in Plants & Agriculture Research, 8(6): 573-580.

Agar H, Galatali S, Ozkaya DE, Kaya E 2022. A Primary Study: Investigation of the in vitro salt stress effects on development in *Thymus cilicicus* Boiss. & Bal. Global Journal of Botanical Science, 10: 23-27.

Aghamolki MTK, Yusop MK, Oad FC, Zakikhani H, Jaafar HZ, Kharidah S, Musa M.H 2014. Heat stress effects on yield parameters of selected rice cultivars at reproductive growth stages. Journal of Food, Agriculture and Environment, 12(2): 741-746.

Ahuja I, de Vos RC, Bones AM, Hall RD 2010. Plant molecular stress responses face climate change. Trends in Plant Science, 15(12): 664-674.

Ashok S, Senthil A, Sritharan N, Punitha S, Divya K, Ravikesavan R 2018. Yield potential of small millets under drought condition. Madras Agricultural Journal, 105.

Bishnoi SK, Chauhan RPS, Yadav NK, Bishnoi P 2017. Abiotic stress effects on plants under climate change. International Journal of Bio-resource and Stress Management, 8(6): 863-870.

Chastain DR., Snider J.L, Collins GD, Perry CD, Whitaker J, Byrd SA 2014. Water deficit in field-grown *Gossypium hirsutum* primarily limits net photosynthesis by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis. Journal of Plant Physiology, 171(17): 1576-1585.

Daryanto S, Wang L, Jacinthe PA 2017. Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. Agricultural Water Management, 179: 18-33.

Delauney AJ, Verma DPS 1993. Proline biosynthesis and osmoregulation in plants. The Plant Journal: For Cell and Molecular Biology, 4: 215– 223.

El–Metwally I, Geries L, Saudy H 2022. Interactive effect of soil mulching and irrigation regime on yield, irrigation water use efficiency and weeds of trickle–irrigated onion. Archives of Agronomy and Soil Science, 68(8): 1103-1116.

Franzoni G, Cocetta G, Trivellini A, Garabello C, Contartese V, Ferrante A 2022. Eksojen tuz stresi ve glutamik asit uygulamasının marul (*Lactuca sativa* L.) üzerine etkisi. Scientia Horticulturae, 299: 111027.

Fukuda H 2000. Programmed cell death of tracheary elements as a paradigm in plants. Plant Molecular Biology, 44(3): 245–253.

Gazol A, Camarero JJ, Gutiérrez E, Popa I, Andreu-Hayles L, Motta R, Nola P, Ribas M, Sangüesa-Barreda G, Urbinati C, Carrer M. 2015. Distinct effects of climate warming on populations of silver fir (*Abies alba*) across Europe. Journal of Biogeography, 42(6): 1150-1162.

Guo X, Ullah A, Siuta D, Kukfisz B, Iqbal S 2022. Role of WRKY transcription factors in regulation of abiotic stress responses in cotton. Life, 12(9): 1410.

Hajjarpoor A, Vadez V, Soltani A, Gaur P, Whitbread A, Babu DS., Gumma MK, Diancoumba M, Kholová J 2018. Characterization of the main chickpea cropping systems in India using a yield gap analysis approach. Field Crops Research, 223: 93-104.

Hugouvieux V, Kwak JM, Schroeder JI 2001. An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in Arabidopsis. Cell, 106: 477–487.

Hosseini SZ, Ismaili A, Nazarian-Firouzabadi F, Fallahi H, Nejad AR, Sohrabi SS 2021. Dissecting the molecular responses of lentil to individual and combined drought and heat stresses by comparative transcriptomic analysis. Genomics, 113(2): 693-705.

Koyro HW, Ahmad P, Geissler N 2012. Abiotic Stress Responses in Plants: An Overview. In: Ahmad P, Prasad M (eds), Environmental adaptations and stress tolerance of plants in the era of climate change. New York: Springer, pp. 1-28.

Kuznestov VV, Shevyakova NI 1997. Stress responses of tobacco cells to high temperature and salinity. Proline accumulation and phosphorylation of polypeptides. Physiologia Plantarum, 100: 320– 326.

Lobell DB, Bänziger M, Magorokosho C, Vivek B 2011. Nonlinear heat effects on African maize as evidenced by historical yield trials. Nature Climate Change, 1(1): 42-45.

Lüttschwager D, Ewald D, Atanet Alía L 2016. Consequences of moderate drought stress on the net photosynthesis, water-use efficiency and biomass production of three poplar clones. Acta Physiologiae Plantarum, 38(1): 1-6.

Meseka S, Menkir A, Bossey B, Mengesha W 2018. Performance assessment of drought tolerant maize hybrids under combined drought and heat stress. Agronomy, 8(12): 274.

Misra V, Solomon S, Mall AK, Prajapati CP, Hashem A, Abd_Allah EF, Ansari MI 2020. Morphological assessment of water stressed sugarcane: A comparison of waterlogged and drought affected crop. Saudi Journal of Biological Sciences, 27(5): 1228-1236.

Noreen S, Sultan M, Akhter MS, Shah KH, Ummara U, Manzoor H, Ulfat M, Alyemeni MN, Ahmad P 2021. Foliar fertigation of ascorbic acid and zinc improves growth, antioxidant enzyme activity and harvest index in barley (*Hordeum vulgare* L.) grown under salt stress. Plant Physiology and Biochemistry, 158: 244-254.

Notununu I, Moleleki L, Roopnarain A, Adeleke R 2022. Effects of plant growth-promoting rhizobacteria on the molecular responses of maize under drought and heat stresses: A review. Pedosphere, 32(1): 90-106.

Nuccio ML, Rhodes D, McNeil SD, Hanson AD 1999. Metabolic engineering of plants for osmotic stress resistance. Current Opinion in Plant Biology, 2: 128– 134.

Okamoto M, Matsui A, Tanaka M, Morosawa T, Ishida J, Iida K, Mochizuki Y, Toyoda T, Seki M 2016. Sm-like protein-mediated RNA metabolism is required for heat stress tolerance in arabidopsis. Frontiers in Plant Science, 7: 1079.

Ors S, Ekinci M, Yildirim E, Sahin U, Turan M, Dursun A 2021. Interactive effects of salinity and drought stress on photosynthetic characteristics and physiology of tomato (*Lycopersicon esculentum* L.) seedlings. South African Journal of Botany, 137: 335-339.

Pagán E, Robles JM, Temnani A, Berríos P, Botía P, Pérez-Pastor A 2022. Effects of water deficit and salinity stress on late mandarin trees. Science of The Total Environment, 803: 150109.

Park CE, Jeong SJ, Joshi M, Osborn TJ, Ho CH, Piao S, Chen D, Liu J, Yang H, Park H, Kim BM, Feng S 2018. Keeping global warming within 1.5°C constrains emergence of aridification. Nature Climate Change, 8: 70–74.

Rasmussen S, Barah P, Suarez-Rodriguez MC, Bressendorff S, Friis P, Costantino P, Bones AM, Nielsen HB, Mundy J 2013. Transcriptome responses to combinations of stresses in Arabidopsis. Plant Physiology, 161(4): 1783-1794.

Rivero RM, Mestre TC, Mittler R, Rubio F, Garcia-Sanchez F, Martinez V 2014. The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. Plant, Cell & Environment, 37(5): 1059-1073.

Sangwan V, Orvar BL, Beyerly J, Hirt H, Dhindsa RS 2002. Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways. Plant Journal, 31: 629–638.

Scharf KD, Berberich T, Ebersberger I, Nover L 2012. The plant heat stress transcription factor (Hsf) family: structure, function and evolution. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms, 1819(2): 104-119.

Semiz GD, Suarez DL 2019. Impact of grafting, salinity and irrigation water composition on eggplant fruit yield and ion relations. Scientific Reports, 9(1): 1-9.

Sewelam N, Brilhaus D, Bräutigam A, Alseekh S, Fernie AR, Maurino VG 2020. Molecular plant responses to combined abiotic stresses put a spotlight on unknown and abundant genes. Journal of Experimental Botany, 71(16): 5098-5112.

Shaar-Moshe L, Blumwald E, Peleg Z 2017. Unique physiological and transcriptional shifts under combinations of salinity, drought, and heat. Plant Physiology, 174(1): 421-434.

Simms EL 2000. Defining tolerance as a norm of reaction. Evolutionary Ecology, 14 (4): 563-570.

Swarbreck SM, Colaço R, Davies JM 2013. Plant calcium-permeable channels. Plant Physiology, 163: 514–522.

Tack J, Singh RK, Nalley LL, Viraktamath BC, Krishnamurthy SL, Lyman N, Jagadish KS 2015. High vapor pressure deficit drives salt-stress-induced rice yield losses in India. Global Change Biology, 21(4): 1668-1678.

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R 2014. Abiotic and biotic stress combinations. New Phytologist, 203(1): 32-43.

Tandzi LN, Bradley G, Mutengwa C 2018. Morphological responses of maize to drought, heat and combined stresses at seedling stage. Journal of Biological Sciences, 19(1): 7-16.

Tejedor Garavito N, Newton AC, Golicher D, Oldfield S 2015. The relative impact of climate change on the extinction risk of tree species in the montane tropical Andes. PLoS One, 10(7): e0131388.

Tesfaye K, Kruseman G, Cairns JE, Zaman-Allah M, Wegary D, Zaidi PH, Boote KJ, Rahut D, Erenstein O 2018. Potential benefits of drought and heat tolerance for adapting maize to climate change in tropical environments. Climate Risk Management, 19: 106-119.

Trowbridge AM, Adams HD, Collins A, Dickman LT, Grossiord C, Hofland M, Malone S, Weaver DK, Sevanto S, Stoy PC, McDowell NG 2021. Hotter droughts alter resource allocation to chemical defenses in piñon pine. Oecologia, 197(4): 921-938.

Vacchiano G, Garbarino M, Borgogno Mondino E, Motta R 2012. Evidences of drought stress as a predisposing factor to Scots pine decline in Valle d'Aosta (Italy). European Journal of Forest Research, 131(4): 989-1000.

Venancio LP, Filgueiras R, Mantovani EC, do Amaral CH, da Cunha FF, dos Santos Silva FC, Althoff D, dos Santos RA, Cavatte PC 2020. Impact of drought associated with high temperatures on *Coffea canephora* plantations: a case study in Espírito Santo State, Brazil. Scientific Reports, 10(1): 1-21.

Wei Y, Jin J, Jiang S, Ning S, Liu L 2018. Quantitative Response of Soybean Development and Yield to Drought Stress during Different Growth Stages in the Huaibei Plain, China. Agronomy, 8: 97.

Wu SJ, Wang LC, Yeh CH, Lu CA, Wu SJ 2010. Isolation and characterization of the Arabidopsis heat-intolerant 2 (hit2) mutant reveal the essential role of the nuclear export receptor EXPORTIN1A (XPO1A) in plant heat tolerance. New Phytologist, 186: 833–842.

Yancey P 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. Journal of Experimental Biology, 208: 2819– 2830.

Zaki HE, Radwan KS 2022. Response of potato (*Solanum tuberosum* L.) cultivars to drought stress under in vitro and field conditions. Chemical and Biological Technologies in Agriculture, 9(1): 1-19.

Zhan X, Qian B, Cao F, Wu W, Yang L, Guan Q, Gu X, Wang P, Okusolubo TA, Dunn SL, Zhu JK, Zhu J 2015. An Arabidopsis PWI and RRM motif-containing protein is critical for pre-mRNA splicing and ABA responses. Nature Communications, 6: 8139.

Zhang H, Li X, Wang W, Pivovaroff AL, Li W, Zhang P, Ward ND, Myers-Pigg A, Adams HD, Leff R, Wang A, Yuan F, Wu J, Yabusaki S, Waichler S, Bailey VL, Guan D, McDowell NG 2021. Seawater exposure causes hydraulic damage in dying Sitka-spruce trees. Plant Physiology, 187(2): 873-885.

Zhang H, Zhu J, Gong Z, Zhu JK 2022. Abiotic stress responses in plants. Nature Reviews Genetics, 23(2):104-119.

Zhu JK 2016. Abiotic stress signaling and responses in plants. Cell, 167(2): 313-324.

Zinta G, AbdElgawad H, Domagalska MA, Vergauwen L, Knapen D, Nijs I, Janssens IA, Beemster GTS, Asard H 2014. Physiological, biochemical, and genome-wide transcriptional analysis reveals that elevated CO 2 mitigates the impact of combined heat wave and drought stress in Arabidopsis thaliana at multiple organizational levels. Global Change Biology, 20(12): 3670-3685.

## *ORAL PRESENTATION*

# Biological activities of different parts of *Centaurea antiochia* Boiss. in Turkey

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## Abstract

In the present research, the flowers, and stems of *Centaurea antiochia* BOISS. var. *antiochia* BOISS and *Centaurea antiochia* Boiss. var. *praealta* (Boiss. & Bal.) Wagenitz were colleted in Hatay and Mersin provinces of Turkey, respectively. Antioxidant, and enzyme inhibition activities of the ethyl acetate and ethanolic extracts obtained from *C. antiochia*.var. *antiochia* and *C. antiochia* var. *praealta* flower and stem were performed, respectively. The antioxidant activity of extracts was assessed by DPPH· and $ABTS^{\cdot+}$ radical scavenging and iron chelating assays. Total phenol, and flavonoid contents of extracts were also determined spectrophotometrically. Then *in vitro* enzyme inhibition activities of extracts were investigated against acetyl-butyrylcholine esterase and tyrosinase at different concentrations.

Among these extracts, the EtOAc extract of stem in Mersin has the highest phenolic compound (56.34 ± 0.64 GAEs/g extract) and flavonoid content (195.23±2.67 mg Q/g extract). The ethanolic extract of *C. antiochia* flowers from Mersin showed higher DPPH· radical scavenging activity (inhibition %: 64.40 ± 5.51 µg/mL) than the other extracts, while the ethyl acetate extract of *C. antiochia* stem from Hatay showed the best effect (67.17 ± 2.44%) in iron chelating methods. The highest ABTS activity was detected in ethanol extracts of *C. antiochia* flower from Hatay (83.83 ± 1.89%) In the enzyme results, all extracts exhibited moderate tyrosinase inhibition activity. Ethanol extract of stem of *C. antiochia* from Hatay demonstrated the highest anti-tyrosinase activity (37.98 ± 0.38%). Especially ethyl acetate, and ethanol extracts of *C. antiochia* flower collected from Hatay was showed good and similar activity against acetylcholinesterase enzyme ($IC_{50}$: 23.04 ± 1.70 µg/mL and 22.52 ± 2.61 µg/mL, respectively). Ethyl acetate extract of *C. antiochia* flower ($IC_{50}$: 8.05 ± 5.22 µg/mL) from Hatay had the lowest $IC_{50}$ value against butyrylcholinesterase. In conclusion, it is necessary to identify the phytochemical compounds responsible for the antioxidant and enzyme inhibition activities of *C. antiochia* in future studies.

**Keywords:** *Centaurea antiochia* Boiss. var. *antiochia* Boiss., *Centaurea antiochia* Boiss. var. *praealta* (Boiss. & Bal.) Wagenitz, Antioxidant activities, Enzyme inhibition activities

## INTRODUCTION

*Centaurea* L. (Asteraceae) includes about 500 species with wide distribution in the Mediterranean and Western Asia. *Centaurea* represents Turkey with 194 species, out of which 118 are endemic. Common names for various species are cornflower, knapweed, and star-thistle. Some of the genus are known by different regional names, such as peygamber çiceği, çoban kaldiran, sarıbaş, kötürüm, kotonkıran, and gökbaş in Anatolia (Albayrak et al., 2017; Davis et al., 1988; Erol-Dayi et al., 2011; Kilic et al., 2013; Tekeli et al., 2011).

Some *Centaurea* species are used in popular medicine in some countries and in Turkish traditional medicine to treat different diseases including stomach aches, abscesses, asthma, hemorrhoids, headache, inflammatory

disorders, wound healing, urogenital ailments, endocrine diseases, gastrointestinal symptoms, cardiovascular problems, parasitic and microbial infections, and rheumatic pain (Albayrak et al., 2017; Baharfar et al., 2009; Kilic et al., 2013).

Much research on *Centaurea* indicated the presence of sesquiterpene lactones, flavonoids, lignans alkaloids, simple phenolics, steroids, triterpenes, hydrocarbons, polyacetylenes, and anthocyanins (Baharfar et al., 2009; Ćirić et al., 2011; Dumlu and Gürkan, 2006). Some *Centaurea* has biological activities such as antimicrobial, antifungal, antiplasmodial, cytogenetic, antiulcerogenic activities, antioxidant properties, antiviral, antiprotozoal, anti-colon cancer and cytotoxic activity, anti-inflammatory, neuro-cytotoxic, cardiotonic (Albayrak et al., 2017; Ćirić et al., 2011; Granger et al., 2009; Kilic et al., 2013). *C. antiochia* used in this study has two varieties in Turkey as *C. antiochia var. antiochia*, and *C. antiochia* var. *praealta*. According to the information given in Flora of Turkey (Davis, 1975): Perennial. Stems erect, 30-100 cm, with few long branches, hirsute or sparsely tomentose to subglabrous. Leaves with scattered short hairs, glabrescent, lower interruptedly pinnatifid or pinnatisect with numerous lanceolate lateral segments, entire to dentate or pinnatilobate, terminal segments sometimes slightly larger, median leaves pinnatifid to lyrate. Involucre 20-27 x 18-25 mm, subglobose to ovoid. Appendages small, brown, with 5-13 cilia 2-5 mm on each side, gradually narrowed into terminal spine, 5-25 mm -incl. spine-. Flowers blackish purple. Achenes 5-6 mm; pappus 5-9 mm. FL. 5-6. Macchie, Pinus brutia forest, rocky slopes, 150-500-1000 m. Spine including *C. antiochia* var *antiochia* appendages is 15-25 mm, appendages are 5-10 mm in *C. antiochia* var *praelta*. *C. antiochia* var. *praelta* is endemic plant in Turkey.

The objectives of this research were to: (1) evaluate and compare the *in vitro* antioxidant activities of various extracts from *Centaurea antiochia* from Mersin and Hatay by three bioassays (DPPH and ABTS radical scavenging assay, Iron chelating effect); (2) quantify total phenolic compounds and total flavonoids; (3) investigate acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities; (4) evaluate inhibition activity of extracts on the tyrosinase enzyme.

## MATERIALS AND METHODS

### Plant Collection and Extraction Methods

Plant materials divided two parts as flowers and stems. Then, they were macerated different solvent using with ethanol (EtOH) and ethylacetate (EtOAc) for 3 days. Macerates were filtered through pleated filter paper and evaporated to dryness with a Rotary evaporator at 40°C. The extracts were stored in the refrigerator for biological activity studies. Yield of the extracts were listed Table 1.

**Table 1.** Yield of The Extracts

| Samples (Gathering places, altitude and gathering dates) | Plant parts | Solvent | Extraction yield (%) |
|---|---|---|---|
| ***C. antiochia* var. *antiochia*** (C6: Hatay: Samandağı, Çevlik location, Sloping Slopes, 10m, 30.05.2018) | Flowers | EtOAc | 3.404 |
| | | EtOH | 10.635 |
| | Stems | EtOAc | 1.962 |
| | | EtOH | 8.237 |
| ***C. antiochia* var. *praealta*** (C3: Mersin: Monts Taurus, Güzel Dere, ouvertures, 150m, 21.05.2018) | Flowers | EtOAc | 2.205 |
| | | EtOH | 7.312 |
| | Stems | EtOAc | 3.987 |
| | | EtOH | 6.987 |

### Antioxidant Activity Assay

According to Re et al., ABTS cation radical decolorization activity of the extracts with modest modifications was done (Re et al., 1999). The DPPH radical scavenging activity of the extracts was performed according to the method by Clarke al. (Clarke et al., 2013). The iron chelating activity of the extracts was assessed by the interaction of ferrozin-$Fe^{2+}$ complex, according to Chai et al. ( 2014). The concentration ranges of the extracts used for the antioxidant tests was 0-2 mg/mL. All the analyses were performed in triplicate. Percent inhibition was calculated using the following equation.

% Inhibition = [Absorbance of control – Absorbance of test sample / Absorbance of control] × 100

**Enzyme Inhibition Activity Assay**

This experiment (acetyl-butrylcholin esterase) was conducted out according to the Ellman et al. approach with some modifications ( 1961). Galanthamine was used as a positive control. The spectrophotometric method previously described was used to assess the tyrosinase inhibitory activity of the plant extracts with some minor changes (Jeong et al., 2009). Kojic acid was used as a positive control. The concentration ranges of the extracts used for the antioxidant tests was 0-2 mg/mL. All the analyses were performed in triplicate.

**RESULTS**

**Determination of Antioxidant Properties of *C. antiochia* var. *antiochia* and *C. antiochia* var. *praealta***

Antioxidant activity of various solvents extracts of *C. antiochia* stems and flowers were assessed using ABTS and DPPH radical scavenging, and iron chelating methods, as well as Total Phenol/Flavonoid Content (TPC/TFC) measurements. The results of TPC and TFC were given in Figure 1. According to the analysis results, the TPCs were higher than TFC results in EtOAc extracts of stem, while the ethanol extracts of stem were opposite the results. The TFC of EtOAc extract of the stem in Mersin was the highest flavonoid concentration.

The antioxidant activities results were listed in Table 2. Our results showed that the highest antioxidant activities were shown by ethanol extract of *C. antiochia* var. *praealta* stem from Mersin for DPPH, and by ethanol extract of *C. antiochia* var. *antiochia* stem from Hatay for ABTS, and iron chelating, respectively.

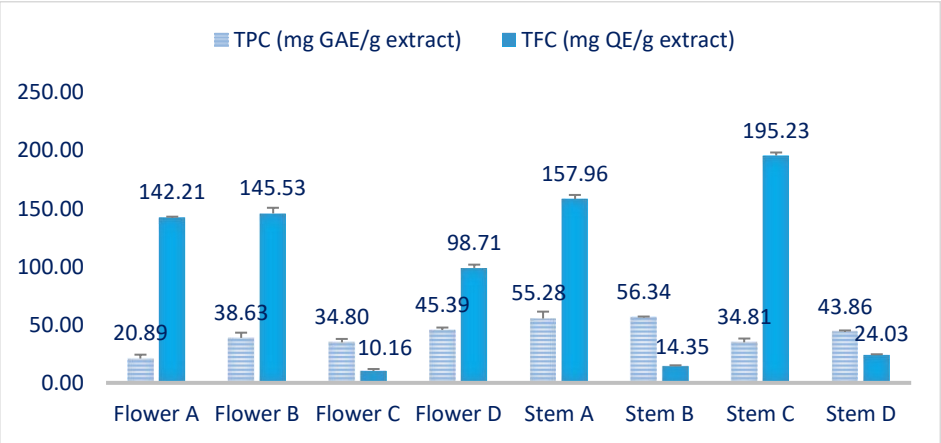


**Figure 1.** TPC and TFC of *C. antiochia* extracts

**Flower A:** EtOAc extract of flower in Hatay; **Flower B:** EtOH extract of flower in Hatay; **Flower C:** EtOAc extract of flower in Mersin; **Flower D:** EtOH extract of flower in Mersin; **Stem A:** EtOAc extract of stem in Hatay; **Stem B:** EtOH extract of stem in Hatay; **Stem C:** EtOAc extract of stem in Mersin; **Stem D:** EtOH extract of stem in Mersin.

**Table 2.** *In vitro* Antioxidant Activities (%) of Ethyl acetate and Ethanol Extracts of *C. antiochia*

| Plant species and Province | Extract | DPPH (percentage±S. D.[a]) 200 µg/mL | ABTS (percentage±S.D.[a]) 333 µg/mL | Iron chelating effect (percentage±S.D[a]) 250 µg/mL |
|---|---|---|---|---|
| ***C. antiochia* var. *antiochia* in Hatay** | EtOAc extract of flower | 28.538 ± 2.53 | 66.400 ± 2.65 | 44.897 ± 0.84 |
| | EtOH extract of flower | 31.168 ± 2.54 | 73.780 ± 1.72<br>$IC_{50}$: 201.15 ± 4.60 µg/mL | 56.960 ± 1.78<br>$IC_{50}$: 123.85 ± 0.07 µg/mL |
| | EtOAc extract of stem | 24.833 ± 0.38 | 75.069 ± 0.47<br>$IC_{50}$: 141.97 ± 4.34 µg/mL | 67.165 ± 2.44<br>$IC_{50}$: 76.453 ± 4.58 µg/mL |
| | EtOH extract of stem | 46.585 ± 2.83 | 83.904 ± 3.69<br>$IC_{50}$: 83.85 ± 1.89 µg/mL | 19.919 ± 0.74 |
| ***C. antiochia* var. *praealta* in Mersin** | EtOAc extract of flower | 27.798 ± 1.98 | 65.551 ± 1.02<br>$IC_{50}$: 214.10 ± 0.85 µg/mL | 9.367 ± 1.88 |
| | EtOH extract of flower | 64.399 ± 5.51 | 84.779 ± 80.87<br>$IC_{50}$: 159.700 ± 4.10 µg/mL | 24.747± 1.51 |
| | EtOAc extract of stem | 13.931 ± 1.00 | 59.772 ± 2.11<br>$IC_{50}$: 217.30 ± 4.38 µg/mL | 60.383 ± 1.90<br>$IC_{50}$:162.40 ± 3.96 µg/mL |
| | EtOH extract of stem | 27.941 ± 1.46 | 76.701 ± 0.98<br>$IC_{50}$: 167.95 ± 4.17 µg/mL | 35.025 ± 5.19 |
| | **Reference** | 86.95 ± 1.26[b] | 91.81 ± 0.04[c] | 87.06±0.34[d] |

**a:** standard deviation; **b:** Trolox; **c:** Gallic acid; **d:** EDTA

**Determination of Enzyme İnhibition Activity of *C. antiochia* var. *antiochia* and *C. antiochia* var. *praealta***

The enzyme inhibition activity results of all extracts are given in Table 3. In results of enzyme inhibition activity, all extracts have moderate anti-TYR activity. *C. antiochia* flower extracts collected from Mersin and Hatay showed similar and remarkable tyrosinase inhibitory activity among themselves. The ethanol extract of *C. antiochia* var. *antiochia* stem was showed the highest anti-TYR activity (37.98%). EtOAc and EtOH of *C. antiochia* var. *antiochia* flower extracts were found to be the highest acetylcholinesterase activity ($IC_{50}$: 23.04 ± 1.70 µg/mL; $IC_{50}$: 22.52 ± 2.61 µg/mL, respectively). EtOAc and EtOH of *C. antiochia* var. *praealta* stem extracts ($IC_{50}$: 52.87 ± 8.16 µg/mL; $IC_{50}$: 29.98 ± 6.23 µg/mL, respectively) were found to have more inhibitory activity than EtOAc and EtOH of *C. antiochia* var. *antiochia* stem extract ($IC_{50}$: 60.95 ± 1.79 µg/mL; $IC_{50}$: 84.45 ± 6.91 µg/mL, respectively).

EtOAc of *C. antiochia* var. *antiochia* flower ($IC_{50}$: 8.05 ± 5.22 µg/mL) and EtOH of *C. antiochia* var. *praealta* stem ($IC_{50}$: 17.19 ± 9.66 µg/mL) extracts showed high anti-butyrylcholinesterase activity. While the EtOAc and EtOH of *C. antiochia* var. *antiochia* flower extracts ($IC_{50}$: 8.05 ± 5.22 µg/mL; $IC_{50}$: 45.63 ± 0.35 µg/mL, respectively) have more inhibition activity than the EtOAc and EtOH of *C. antiochia* var. *praealta* flower extracts ($IC_{50}$: 55.28 ± 2.66 µg/mL; $IC_{50}$: 79.33 ± 2.41 µg/mL, respectively), the EtOAc and EtOH of *C. antiochia* var. *praealta* stem extract ($IC_{50}$: 27.98 ± 5.78 µg/mL; $IC_{50}$: 17.19 ± 9.66 µg/mL,

respectively) is more active than the EtOAc and EtOH of *C. antiochia* var. *antiochia* stem extracts ($IC_{50}$: 52.97 ± 10.53 µg/mL; $IC_{50}$: 45.70 ± 1.61 µg/mL, respectively).

**Table 3.** Enzyme İnhibition Activities of *C. antiochia* Species

| Plant material | Extracts | TYR (percentage ± S.D.[a]) 1 mg/mL[b] | AChE (percentage± S.D.[a]) 0.5 mg/mL[b] | BChE (percentage±S.D.[a]) 0.5 mg/mL[b] |
|---|---|---|---|---|
| ***C. antiochia* var. *antiochia* in Hatay** | EtOAc extract of flower | 31.44 ± 4.72 | 69.14 ± 1.64<br>$IC_{50}$: 23.04 ± 1.70 µg/mL | 79.23 ± 2.66<br>$IC_{50}$: 8.05 ± 5.22 µg/mL |
| | EtOH extract of flower | 31.18 ± 1.96 | 70.30 ± 1.26<br>$IC_{50}$: 22.52 ± 2.61 µg/mL | 60.00 ± 1.78<br>$IC_{50}$: 45.63 ± 0.35 µg/mL |
| | EtOAc extract of stem | 17.04 ± 3.71 | 32.22 ± 3.54<br>$IC_{50}$: 60.95 ± 1.79 µg/mL | 60.00 ± 3.77<br>$IC_{50}$: 52.97 ± 10.53 µg/mL |
| | EtOH extract of stem | 37.98 ± 0.38 | 25.48 ± 11.64<br>$IC_{50}$: 84.45 ± 6.91 µg/mL | 56.92 ± 1.78<br>$IC_{50}$: 45.70 ± 1.61 µg/mL |
| ***C. antiochia* var. *praealta* i n Mersin** | EtOAc extract of flower | 27.39 ± 2.12 | 51.09 ± 1.73<br>$IC_{50}$: 49.09 ± 1.67 µg/mL | 43.08 ± 6.53<br>$IC_{50}$: 55.28 ± 2.66 µg/mL |
| | EtOH extract of flower | 24.07 ± 1.03 | 49.66 ± 1.51<br>$IC_{50}$: 52.77 ± 2.64 µg/mL | 24.62 ± 6.53<br>$IC_{50}$: 79.33 ± 2.41 µg/mL |
| | EtOAc extract of stem | 22.21 ± 4.48 | 48.84 ± 0.24<br>$IC_{50}$: 52.87 ± 8.16 µg/mL | 65.64 ± 7.27<br>$IC_{50}$: 27.98 ± 5.78 µg/mL |
| | EtOH extract of stem | 33.43 ± 4.73 | 64.77 ± 2.53<br>$IC_{50}$: 29.98 ± 6.23 µg/mL | 66.55 ± 3.02<br>$IC_{50}$: 17.19 ± 9.66 µg/mL |
| **Reference** | | 80.96±0.51[c] | 99.10±1.18[d] | 84.34±4.85[d] |

**a:** Standard deviation; **b:** Stock concentration; **c:** Kojic acid; **d:** Galanthamine hydrobromür

## DISCUSSION

Plants produce secondary metabolites in response to environmental stress and increase their chances of survival in this way. The types and amounts of secondary metabolites, which have an important place in the treatment with medicinal plants, may vary according to the changes in environmental factors (such as light irradiation (duration, intensity, quality), temperature, soil water, soil fertility and salinity) (Yang et al., 2018). In this study, antioxidant and enzyme inhibition activities of flower and stem ethanol and ethylacetate extracts of different varietes *C. antiochia* collected from Hatay and Mersin were evaluated. *C. antiochia* is known as "Sultan's thistle" and there are 2 varieties in Turkey: *C. antiochia* var. *antiochia*, *C.antiochia* var. *praealta*. Among these, *C. antiochia* var *praelta* is an endemic plant. Different activity results were obtained from *C. antiochia* var. *antiochia* and *C. antiochia* var. *praealta*. Literature studies on *C. antiochia* include biological

activity studies such as antioxidant, antimicrobial, antibacterial, anti-inflammatory, anticancer and enzyme inhibition activity.

Özsoy *et al.* reported that the total phenol and flavonoid content of extract of *C. antiochia* var. *praealta* aerial parts were found as 3.68 mg GAE/g and 1.94 mg catechin/g, respectively. In this study, *C. antiochia* var. *praealta* extract was found as DPPH radical scavenger ($IC_{50}$ = 5.10 mg/mL).

In a study, the acetylcholinesterase inhibitions of *C. antiochia* var. *praealta* aerial parts collected from Hatay were evaluated. The extract exhibited a moderate anti-AChE activity (46.10%) at 20 mg/mL concentration (Ozsoy et al., 2015). In our study, acetylcholinesterase inhibitions of EtOAc (51.09%) and EtOH (49.66%) extract of *C. antiochia* var. *praealta* flowers exhibited good anti-AChE at lower concentration (stock concentration: 0.5 mg/mL). In enzyme inhibition studies in other *Centaurea* genus, the $IC_{50}$ value of methanol extract of *C. sivasica* aerial parts was found to be 81.62 µg/mL on tyrosinase enzyme inhibition activity (Yırtıcı et al., 2022). In previous studies by Aktumsek et al., the cholinesterase inhibition activities of water and methanol extracts of some *Centaurea* (*C. polypodiifolia var. pseudobehen, C. pyrrhoblephara and C. antalyense*) species were evaluated. AChE and BChE were found be significantly inhibited by methanolic extracts of *C. polypodiifolia* var. *pseudobehen* (24.54 ± 2.23) and *C. antalyense* (21.25 ± 1.94) (Aktumsek et al., 2013). In the study of the enzyme inhibition activity of eight *Centaurea* species by Zengin et al., the chloroform extract of *C. pulchella* exhibited the highest inhibitory activity against both AChE (95.93%) and BChE (95.69%) at the concentration of 2 mg/mL. In the same study, *Centaurea* extracts were found low tyrosinase inhibitory activities (0.91-15.53%, at the concentration of 2 mg/mL) (Zengin et al., 2016). In the present study, all extracts exhibited moderate tyrosinase inhibition activity (17.04-37.98%). Ethanol extract of *C. antiochia* stems demonstrated the highest anti-tyrosinase activity (37.98%). As seen in this study, it can be said that the anti-TYR activity of *C. antioicha* is higher than the *Centaurea* species studied in previous studies. In another study, flowers and stems *C. hypoleuca* collected from the Erikbeli were prepared extracts with different solvents (ethanol, methanol, and ethyl acetate). Ethyl acetate of *C. hypoleuca* flower extract was found to be the most effective than other extracts on both acetylcholinesterase and butrylcholinesterase (Özcan et al., 2019). In this study, EtOAc of *C. antiochia* var. *antiochia* flowers was significantly shown cholinesterase enzyme inhibitory activity.

## CONCLUSION

*C. antiochia* extracts were found to significantly have antioxidant activity. The extracts showed good inhibitory activity against both acetylcholinesterase and butyrylcholinesterase, but moderately inhibited the tyrosinase enzyme. In the next study, the phytochemical contents of *C. antiochia* will be determined and identify which phytochemicals are responsible for the mentioned activities.

The biological activity of *Centaurea antiochia* var. *antiochia* has not been scientifically evaluated. The aerial parts of *C. antiochia* var. *antiochia* has been reported to biological activities. However, the stem and flowers have not been scientifically evaluated.

## REFERENCES

Aktumsek A, Zengin G, Guler GO, Cakmak YS, Duran A 2013. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. Food and Chemical Toxicology, 55: 290-6.

Albayrak S, Atasagun B, Aksoy A 2017. Comparison of phenolic components and biological activities of two *Centaurea* sp. obtained by three extraction techniques. Asian Pacific Journal of Tropical Medicine, 10(6): 599-606.

Baharfar R, Khalilzadeh MA, Gheibi S, Jazayeri O, Azimia R, Tajbakhsha M 2009. Antioxidant and antibacterial activities of the methanolic extract of *Centaurea zuvandica* Sosn. Iranian Journal of Organic Chemistry, 3:172-7.

Chai T, Mohan M, Ong H, Wong F 2014. Antioxidant, iron-chelating and anti-glucosidase activities of *Typha domingensis* Pers (Typhaceae). Tropical Journal of Pharmaceutical Research, 13(1): 67-72.

Ćirić A, Karioti A, Glamočlija J, Soković M, Skaltsa H 2011. Antimicrobial activity of secondary metabolites isolated from *Centaurea spruneri* Boiss. & Heldr. Journal of the Serbian Chemical Society, 76(1): 27-34.

Clarke G, Ting KN, Wiart C, Fry J 2013. High correlation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all

three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. Antioxidants, 2(1): 1-10.

Davis P 1975. Flora of Turkey and the East Aegean Islands, Volume 5 Edinburgh. Edinburgh University Press, 978: 0-8522.

Davis PH, Mill R, Tan K 1988. Flora of Turkey: and the East Aegean Islands.(Supplement), p.

Dumlu MU, Gürkan E, 2006. A new active compound from *Centaurea* species. Zeitschrift für Naturforschung C, 61(1-2): 44-6.

Ellman GL, Courtney KD, Andres Jr V, Featherstone RM 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7(2): 88-95.

Erol-Dayi Ö, Pekmez M, Bona M, Aras-Perk A, Arda N 2011. Total phenolic contents, antioxidant activities cytotoxicity of three *Centaurea* species: *C. calcitrapa* subsp. *calcitrapa*, *C. ptosimopappa C. spicata*. Free Radicals and Antioxidants, 1(2): 31-6.

Granger M, Samson E, Sauvage S, Majumdar A, Nigam P, Nahar L, Celik S, Sarker SD 2009. Bioactivity of extracts of *Centaurea polyclada* DC.(Asteraceae). Arch Biol Sci, 61(3): 447-52.

Jeong SH, Ryu YB, Curtis-Long MJ, Ryu HW, Baek YS, Kang JE, Lee WS, Park KH 2009. Tyrosinase inhibitory polyphenols from roots of *Morus lhou*. Journal of Agricultural and Food Chemistry, 57(4): 1195-203.

Kilic O, Behcet L, Bagci E 2013. Essential oil compounds of three *Nepeta* L. taxa from Turkey and their chemotaxonomy. Asian Journal of Chemistry, 25(14): 8181-3.

Ozsoy N, Kultur S, Yilmaz-Ozden T, Ozbek Celik B, Can A, Melikoglu G 2015. Antioxidant, Anti-Inflammatory, Acetylcholinesterase Inhibitory and Antimicrobial Activities of Turkish Endemic *Centaurea antiochia* var. *praealta*. Journal of Food Biochemistry, 39(6): 771-6.

Özcan K, Acet T, Çorbacı C 2019. *Centaurea hypoleuca* DC: Phenolic content, antimicrobial, antioxidant and enzyme inhibitory activities. South African Journal of Botany, 127: 313-8.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and medicine, 26(9-10): 1231-7.

Tekeli Y, Zengin G, Aktumsek A, Sezgin M, Torlak E 2011. Antibacterial activities of extracts from twelve *Centaurea* species from Turkey. Archives of Biological Sciences, 63(3): 685-90.

Yang L, Wen K-S, Ruan X, Zhao Y-X, Wei F, Wang Q 2018. Response of Plant Secondary Metabolites to Environmental Factors. Molecules, 23(4): 762.

Yırtıcı Ü, Ergene A, Atalar MN, Adem Ş 2022. Phytochemical composition, antioxidant, enzyme inhibition, antimicrobial effects, and molecular docking studies of *Centaurea sivasica*. South African Journal of Botany, 144: 58-71.

Zengin G, Locatelli M, Carradori S, Mocan AM, Aktumsek A 2016. Total phenolics, flavonoids, condensed tannins content of eight *Centaurea* species and their broad inhibitory activities against cholinesterase, tyrosinase, α-amylase and α-glucosidase. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44(1): 195-200.

## ORAL PRESENTATION

### *Isatis tinctoria* (Çivit otu) aracılı gümüş nanopartiküllerin antibakteriyel aktivitesi

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**Özet**

Günümüzde nanoteknolojinin hızla gelişmesiyle birlikte medikal uygulamalarda nanopartiküllerin güvenli kullanımı için toksik olmayan yöntemlerle üretilmesi gerekmektedir. Bu sebeple, basit, temiz, toksik olmayan, uygun maliyetli ve çevre dostu olan yeşil sentezle elde edilen gümüş nanopartiküller tercih edilmektedir. Çalışmamızda, vücutta oluşan yara, leke, ishal, nefes darlığı ile bağışıklık sistemini güçlendirici ve kansere karşı koruyucu etkisi gibi sayısız faydaları sebebiyle çok eski yıllardan beri şifalı bitki olarak kullanılan Çivit otu özütünden yeşil sentez yöntemiyle gümüş nanopartiküller sentezlenmiştir. Sulu gümüş nitrat çözeltisi ile fitokimyasallarca zengin Çivit otu özütünün reaksiyonu sonucu çözeltide oldukça kararlı gümüş nanopartiküllerin oluşumuyla sonuçlanan gümüş iyonlarının indirgenmesi gözlenmiştir. Açık sarı renkten koyu kahve renge dönüşümün gözlendiği biyosentez reaksiyonu sonucu oluşan gümüş nanopartiküllerin karakterizasyonu UV-vis spektroskopisi ve taramalı elektron mikroskobu kullanılarak yapılmıştır. Gram-negatif (*Staphylococcus aureus* ve *Escherichia coli*) ve gram-pozitif (*Bacillus subtilis*) bakterilerine karşı gümüş nanopartiküllerin disk difüzyon yöntemi ile antibakteriyel aktivite gösterdiği tespit edilmiştir.

**Keywords:** Antibakteriyel aktivite, Gümüş nanopartikül, *Isatis tinctoria*, Yeşil sentez.

### Antibacterial activity of silver nanoparticles mediated by *Isatis tinctoria* (woad)

**Abstract**

Today, with the rapid development of nanotechnology, it is necessary to produce nanoparticles with non-toxic methods for safe use in medical applications. For this reason, silver nanoparticles obtained by green synthesis, which are simple, clean, non-toxic, cost-effective, and environmentally friendly, are preferred. In our study, silver nanoparticles were synthesized by the green synthesis method from indigo extract, which has been used as a medicinal plant for many years due to its numerous benefits such as wounds, spots, diarrhea, shortness of breath, immune system strengthening, and protective effect against cancer. Reduction of silver ions was observed resulting in the formation of highly stable silver nanoparticles in the solution as a result of the reaction of aqueous silver nitrate solution and phytochemical-rich indigo extract. The characterization of the silver nanoparticles formed as a result of the biosynthesis reaction, in which the transformation from light yellow to dark brown color was observed, was carried out using UV-vis spectroscopy and scanning electron microscopy. It was determined that silver nanoparticles showed antibacterial activity against gram-negative (*Staphylococcus aureus* and *Escherichia coli*) and gram-positive (*Bacillus subtilis*) bacteria by the disk diffusion method.

**Keywords:** Antibacterial activity, Green synthesis, Silver nanoparticle, *Isatis tinctoria*.

## GİRİŞ

Nano boyutlardaki (1 - 100 nm) malzemeler, bulk materyallere kıyasla dikkate değer farklı özelliklere sahiptir. Bu farklılıklar, fizikokimyasal özellikler ve yüzey/hacim oranındaki farklılıktan dolayı elementin atomlarının, moleküllerinin ve yığın malzemelerinin fiziksel ve yapısal özelliklerinde yatmaktadır (Mody ve ark., 2010). Nanoteknolojideki gelişmeler sayesinde benzersiz özelliklere sahip çok sayıda nanomalzeme ortaya çıkmış

böylece uygulama yelpazesi ve araştırma fırsatları artmıştır (Sharma ve ark., 2009). Yakın geçmişte, gümüş nanopartiküller (AgNP'ler), çok çeşitli mikroorganizmalara karşı olağanüstü savunmaları ve ayrıca yaygın olarak kullanılan antibiyotiklere karşı ilaç direncinin ortaya çıkması nedeniyle araştırmacıların büyük ilgisini çekmiştir (Sharma ve ark., 2009). AgNP'lerin üstün özellikleri onları biyomedikal, ilaç dağıtımı, su arıtma, tarım gibi çeşitli alanlarda uygulanabilir kılmıştır (Chaloupka ve ark., 2010; Prow ve ark., 2011; Dankovich ve Gray 2011; Nair ve ark., 2010). Bunun yanı sıra, AgNP'ler, yüksek iletkenliği nedeniyle mürekkeplerde, yapıştırıcılarda, elektronik cihazlarda, macunlarda uygulanabilir (Park ve ark., 2008). AgNP'leri çok çeşitli fiziksel ve kimyasal yöntemlerle sentezlenmektedir. Fakat, bu sentez yöntemlerinde kullanılan kimyallar toksik etkili olduğu için biyolojik amaçlı kullanımlar için uygun değildir. Ayrıca, yüksek maliyeti, enerji ihtiyacı ve uzun zaman alması dezavantajları arasındadır. Fizyokimyasal yöntemlerin dezavantajları göz önüne alındığında, bitki özleri ve doğal polimerler kullanılarak AgNP sentezinin yeşil sentezi uygun maliyeti ve enerji verimliliği yeni alternatif, indirgeyici ajanlar olarak çok hızlı bir şekilde ortaya çıkmıştır (Song ve Kim 2009; Huang ve Yang 2004). Nanoteknoloji ve yeşil kimya arasındaki ilişki, biyolojik ve sitolojik olarak uyumlu metalik nanopartiküllerin yelpazesini artmıştır (Philip, 2010). AgNP'lerin yeşil sentezinin birincil gereksinimi, gümüş metal iyonu çözeltisi ve indirgeyici biyolojik ajandır. Çoğu durumda hücrelerde bulunan indirgeyici ajanlar veya diğer bileşenler, stabilize edici ve kapaklayıcı ajanlar olarak işlev görür.

Bu çalışmada indirgeyici ajan olarak *Isatis tinctoria* (çivit otu/indigo) ekstraktı kullanılmıştır. *I. tinctoria*, tıbbi özellikleri ve çivit mavisi rengi nedeniyle çok eski zamanlardan beri iyi bilnmektedir. Günümüzde, *I. tinctoria,* geleneksel Çin tıbbında yaygın olarak kullanılmaktadır ve 2011 yılından itibaren Avrupa'da bir farmakope bitkisi olarak tanınmaktadır (Hamburger, 2002; Recio ve ark., 2006; Condurso ve ark., 2006). *I. tinctoria* kozmetik endüstrisinde sabun ve vücut kremlerinin üretiminde kullanılmaktadır. Tohum yağı ve yaprakları (toz/özüt), yumuşatıcı ve nemlendirici özelliklerinden dolayı cilt ve saç bakımı için kozmetik bileşenlerdir (Spataro ve Negri 2008). Kökler (toz/özüt) büzücü ve cilt koruyucu özelliklere sahiptir (Cosing, 2021).

## MATERYAL VE YÖNTEM

**Bitki materyali:** *Isatis tinctoria* (çivit otu/indigo), maliyet etkinliği, bulunabilirlik kolaylığı ve tıbbi özellikleri nedeniyle gümüş nanopartiküller (AgNP'ler) hazırlamak için kullanıldı. Kurutulmuş *Isatis tinctoria* aktardan ticari olarak temin edildi.

**Gümüş Nanopartiküllerinin Sentezi:** Gümüş nanopartiküller (AgNP'ler) *Isatis tinctoria*'nın kaynatılarak elde edilen ekstraktından sentezlendi. Tipik bir reaksiyon prosedüründe, *Isatis tinctoria*'den elde edilen ekstraktlara 0.1 M sulu gümüş nitrat çözeltisi ($AgNO_3$, %99.0; Sigma-Aldrich) karıştırılarak ilave edildi. Bu prosedür, aynı hacim oranında (1:1 ekstrakt/$AgN0_3$ çözeltisi), oda sıcaklığında ve PH 7'de, 24 saat sürekli karıştırılarak gerçekleştirildi. Elde edilen çözelti yeşilden kahverengiye değişti. Elde edilen karışımı saflaştırmak için nanopartiküller 4000 rpm'de 10 dakika santrifüjlendi. Bu işlem 3 kez tekrarlandı ve elde edilen nanopartiküller indirgeyici olmayan iyonları uzaklaştırmak için damıtılmış su ile yıkandı. AgNP'ler dondurularak kurutuldu ve karakterizasyon için 4°C'de saklandı.

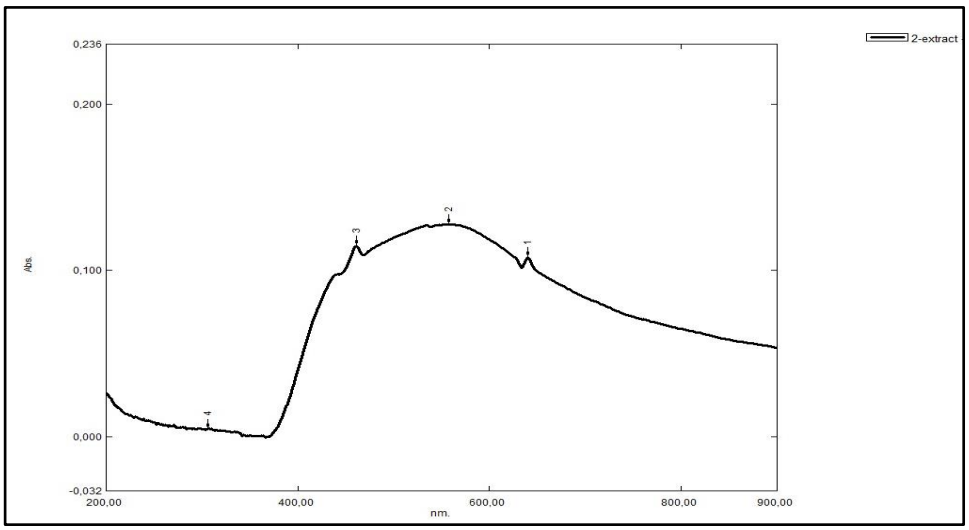
**Sentezlenen gümüş nanopartiküllerin karakterizasyonu:** Gümüş iyonlarının biyolojik indirgenmesi, UV-vis Spektrofotometre (AE-S90-2D) kullanılarak ultraviyole görünür (UV-vis) absorpsiyon ölçümü ile izlendi. Sentezlenen AgNP'lerin SEM mikrografları Hitachi Regulus 8230 FE-SEM kullanılarak elde edildi.

**Antimikrobiyal aktivite:** *Isatis tinctoria* ekstraktı (IT-Exctract) ve Isatis tinctoria ekstraktı ekstraktından sentezlenen gümüş nanopartiküllerin (IT-AgNPs) bakteriler üzerinde etkili olup olmadığını belirlemek için standart disk difüzyon yöntemi kullanılarak antibakteriyel duyarlılık testi yapıldı. Antibakteriyel aktivite için 3 bakteri suşu kullanıldı. Gram negatif *Escherichia coli* ve *Staphylococcus aureus*, gram pozitif *Bacillus subtilis* bakterileri kullanıldı. Pozitif kontrol olarak ampisilin antibiyotik diski (10 µg) ve negatif kontrol olarak boş bir disk üzerinde 20 µL çift damıtılmış su ($ddH_2O$) kullanıldı. Kör disklere antibakteriyel aktivitesi gözlenmek istenen 20 µL IT-AgNP, IT-Extract örnekleri eklendi. Ortam daha sonra 37°C'de 48 saat süreyle inkübe edildi. İnkübasyon sonrasında disk çevresinde oluşan inhibisyon zon çapları petri kaplarının alt yüzeyinden kumpas yardımı ile ölçülmüştür.

## BULGULAR VE TARTIŞMA

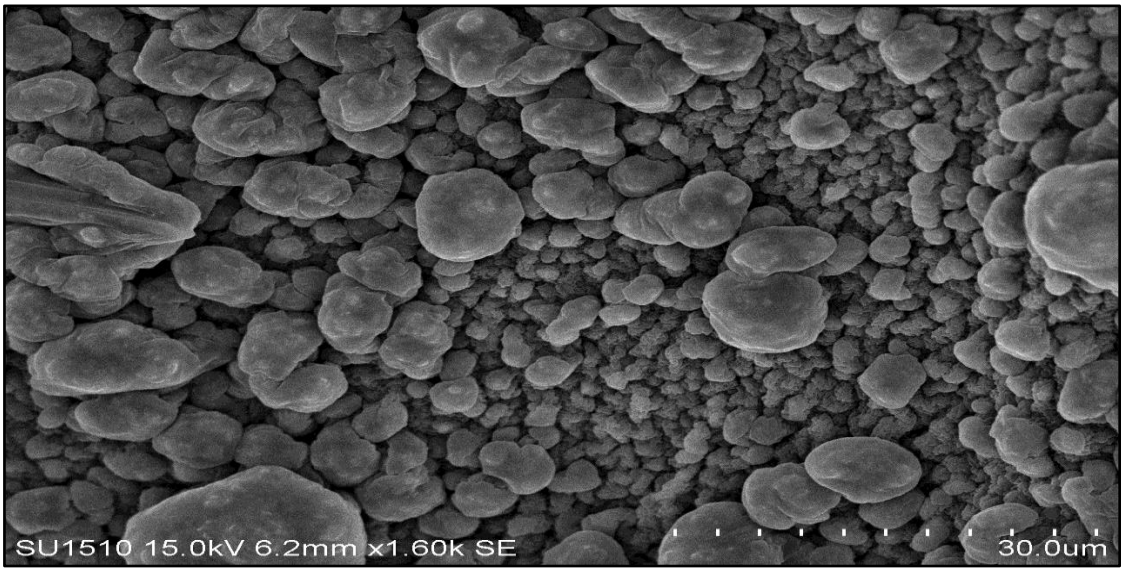
2 mM $AgNO_3$ ve *Isatis tinctoria* ekstraktının farklı hacim fraksiyonları kullanılarak gümüş nanopartiküllerin yeşil sentezi Şekil 1'de gösterilmektedir. UV-Vis spektroskopisi, metal nanoparriküllerin oluşumunu tespit etmek için kullanılan önemli bir tekniktir. Metal nanopartiküller, belirli bir dalga boyundaki ışıkla etkileşime girerek metal nanopartiküllerdeki elektronların toplu salınımından kaynaklanan belirli bir lokalize yüzey

plazmon rezonans fenomeni sergiler (Eustis ve El-Sayed 2006; Padmos ve ark., 2015). SPR, metal nanopartiküllerin oluşumunu tespit etmek için kullanılır (Ramalingam ve ark., 2016). Gümüş nanopartiküller genellikle boyut, şekil ve çevreleyen ortama bağlı olarak 400- 480 nm aralığında bir SPR piki gösterir. Şekil 1'de 3 mL *Isatis tinctoria* ekstraktı ile 2 mM $AgNO_3$ çözeltisi ile karıştırıldığında açık sarıdan koyu kahve rengiye renk değişimi gözlenmiş ve gümüş nanopartikülleri için 427 nm'de karakteristik yüzey plazmon rezonans (SPR) absorpsiyon bandını göstermiştir. Fakat, oluşan gümüş nanopartikülleri (IT-AgNP) düşük yoğunlukta ve az miktarda elde edilmiştir.




**Şekil 1.** Biyojenik gümüş nanopartiküllerinin (IT-AgNP) UV-görünür spektroskopisi

*Isatis tinctoria* ekstraktından sentezlenen nanopartiküllerin partikül boyutu ve morfolojisi, taramalı elektron mikroskobu (SEM) kullanılarak belirlendi. Sentez nanopartiküllerin ortalama 30 nm partikül büyüklüğü ile şekil olarak neredeyse küresel olduğu gözlendi (Şekil 2).




**Şekil 2.** Biyojenik gümüş nanopartiküllerinin (IT-AgNP) UV-görünür spektroskopisi

Ahmad ve ark., 2016 yıllında *Isatis tinctoria* aracılı arttırılmış fotoindüklenmiş antileishmanial aktiviteye sahip amfoterisin B'ye bağlı gümüş nanopartikülleri sentezlemişlerdir. Amfoterisin B ve antileishmanial ilaç sentezledikleri nanopartiküllerin, gümüş nanopartiküller için karakteristik LSPR piklerinin 426, 437 nm ve ortalma boyutunun 10 nm olduğunu kaydetmişlerdir

## SONUÇ

Mevcut yeşil sentez, *Isatis tinctoria*'ın çevreye zarar vermeyen ve yenilenebilir, gümüş nanopartiküllerin sentezi için etkili bir kapatma ve indirgeme maddesi olarak kullanılabileceğini göstermektedir. Yeşil yöntemle sentezlenen gümüş nanopartiküller oldukça kararlıdır. *Isatis tinctoria* gibi yeşil kaynaklar kullanılarak metalik

nanopartiküllerin sentezi, kimyasal senteze güçlü bir alternatiftir, çünkü bu yeni yeşil sentez, gümüş nanopartiküller için toksisite oluşturmayan ve çevre dostu sentetik bir yöntemdir.

## KAYNAKÇA

Ahmad A, Wei Y, Syed F, Khan S, Khan GM, Tahir K, Yuan Q 2016. *Isatis tinctoria* mediated synthesis of amphotericin B-bound silver nanoparticles with enhanced photoinduced antileishmanial activity: a novel green approach. Journal of Photochemistry and Photobiology B: Biology, 161: 17-24.

Chaloupka K, Malam Y, Seifalian AM 2010. Nanosilver as a new generation of nanoproduct in biomedical applications. Trends in Biotechnology, 28(11): 580-588.

Condurso C, Verzera A, Romeo V, Ziino M, Trozzi A, Ragusa S 2006. The leaf volatile constituents of *Isatis tinctoria* by solid-phase microextraction and gas chromatography/mass spectrometry. Planta Medica, 72(10): 924-928.

Cosmetic IngredientDatabase (CosIng). Available online: https://ec.europa.eu/growth/sectors/cosmetics/cosing (accessed on 15 January 2020).

Dankovich TA, Gray DG 2011. Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. Environmental Science & Technology, 45(5): 1992-1998.

Eustis S, El-Sayed MA 2006. Why gold nanoparticles are more precious than pretty gold: noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. Chemical Society Reviews, 35(3): 209-217.

Hamburger M 2002. *Isatis tinctoria*–From the rediscovery of an ancient medicinal plant towards a novel anti-inflammatory phytopharmaceutical. Phytochemistry Reviews, 1(3): 333-344.

Huang H, Yang X 2004. Synthesis of polysaccharide-stabilized gold and silver nanoparticles: a green method. Carbohydrate Research, 339(15): 2627-2631.

Mody VV, Siwale R, Singh A, Mody HR 2010. Introduction to metallic nanoparticles. Journal of Pharmacy and Bioallied Sciences, 2(4): 282.

Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS 2010. Nanoparticulate material delivery to plants. Plant Science, 179(3): 154-163.

Padmos JD, Boudreau RT, Weaver DF, Zhang P 2015. Impact of protecting ligands on surface structure and antibacterial activity of silver nanoparticles. Langmuir, 31(12): 3745-3752.

Park K, Seo D, Lee J 2008. Conductivity of silver paste prepared from nanoparticles. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 313: 351-354.

Philip D 2010. Honey mediated green synthesis of silver nanoparticles. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 75(3): 1078-1081.

Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, Roberts MS 2011. Nanoparticles and microparticles for skin drug delivery. Advanced Drug Delivery Reviews, 63(6): 470-491.

Ramalingam B, Parandhaman T, Das SK 2016. Antibacterial effects of biosynthesized silver nanoparticles on surface ultrastructure and nanomechanical properties of gram-negative bacteria viz. *Escherichia coli* and *Pseudomonas aeruginosa*. ACS Applied Materials & İnterfaces, 8(7): 4963-4976.

Recio MC, Cerdá-Nicolás M, Potterat O, Hamburger M, Ríos JL 2006. Anti-inflammatory and antiallergic activity *in vivo* of lipophilic *Isatis tinctoria* extracts and tryptanthrin. Planta Medica, 72(06): 539-546.

Sharma VK, Yngard RA, Lin Y 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science, 145(1-2): 83-96.

Song JY, Kim BS 2009. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess and Biosystems Engineering, 32(1): 79-84.

Spataro G, Negri V 2008. Adaptability and variation in *Isatis tinctoria* L.: a new crop for Europe. Euphytica, 163(1): 89-102.

## *ORAL PRESENTATION*

# Türkiye'de yetişen endemik bazı *Sideritis* türlerinin *Drosophila melanogaster'in* transheterozigot larvaları üzerine koruyucu etkileri

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## Özet

Eski çağlardan beri geleneksel tıpta hastalıklardan korunma veya hastalık tedavisi için bitkiler kullanılmıştır. İçerdikleri fenolik bileşenlerinin gösterdiği antioksidan özelliklerinden kaynaklanan bu koruyuculuk ve sağaltım sebebiyle insanlar daha güvenli ve ekolojik ürünler aramakta ve bu nedenle doğal antioksidanlara olan eğilim her geçen gün artmaktadır. Bu çalışmada, Türkiye'ye endemik olan *Sideritis* cinsine ait *S. libanotica* ve *S. brevidens* türlerinin toprak üstü kısımlarının farklı konsantrasyonlarda (1,25; 2,5; 5 ve 10 mg/mL) metanol ekstreleri kullanılmıştır. Bir model alkilleyici ajan olarak çok sayıda genotoksisite çalışmasında kullanılan EMS ile indüklenmiş *Drosophila melanogaster*'in transheterozigot larvalarına bitki ekstreleri uygulanarak meyve sineklerinin yaşama yüzdesi belirlenmiş ve total antioksidan ve total oksidan kapasite düzeyleri kolorimetrik olarak ölçülmüştür. Bitki ekstraklarının konsantrasyon artışına bağlı olarak kontrole göre larvaların erginleşme yüzdeleri ile TAS değerlerinin artış gösterirken TOS değerlerinde istatistiksel olarak anlamlı düşüşler olduğu gözlenmiştir ($p<0,05$). Sonuç olarak, bitki ekstrelerinin meyve sineği larvalarının yaşama yüzdesini arttırıp, total oksidan ve antioksidan aktivitelerini düzenleyerek EMS toksisitesine karşı koruyucu etki gösterdiği tespit edilmiştir. Bununla birlikte, bitki ekstreleri arasında ölçülen parametreler açısından istatistiksel olarak önemli bir farklılık olmadığı bulunmuştur ($p>0,05$).

**Anahtar Kelimeler:** *Sideritis*, Total antioksidan aktivite, Total oksidan kapasite, Yaşama yüzdesi

# Protective effects of some endemic *Sideritis* species grown in Türkiye on transheterozygous larvae of *Drosophila melanogaster*

## Abstract

Plants have been used in traditional medicine for the prevention or treatment of diseases since ancient times. Due to the antioxidant properties of the phenolic components they contain, people are looking for safer and more ecological products due to this protection and treatment, and therefore the tendency towards natural antioxidants is increasing day by day. In this study, methanol extracts of different concentrations (1,25; 2,5; 5, and 10 mg/mL) of the aerial parts of *S. libanotica* and *S. brevidens* species belonging to the *Sideritis* genus, which are endemic to Türkiye, were used. The percentage of survival of fruit flies was determined by applying plant extracts to transheterozygous larvae of EMS, which is used as a model alkylating agent in numerous genotoxicity studies, induced *Drosophila melanogaster,* and total antioxidant and total oxidant capacity levels were measured colorimetrically. Depending on the increase in the concentration of plant extracts, it was observed that while the mating percentages and TAS values of the larvae increased compared to the control, there were statistically significant decreases in TOS values ($p<0.05$). As a result, it has been determined that the plant extracts have a protective effect against EMS toxicity by increasing the survival rate of fruit fly larvae and regulating their total oxidant and antioxidant activities. However, it was found that there was no statistically significant difference between the plant extracts in terms of the measured parameters ($p>0.05$).

**Keywords:** Percent survival, *Sideritis*, Total antioxidant activity, Total oxidant capacity

## GİRİŞ

Antioksidanlar, serbest radikallerin oluşumunu engelleyerek veya var olan radikalleri süpürerek hücrenin zarar görmesini engelleyen genellikle fenolik içeriğe sahip özel moleküllerdir (Kahkönen ve ark., 1999) Reaktif oksijen türlerinin çok fazla miktarda üretilmesi durumunda organizmaların doğal antioksidan savunma sistemleri devreye girerek canlıyı bu durumdan kurtarmak üzere antioksidan bileşikleri sentezler (Rice-Evans ve ark., 1997). Sekonder metabolitler olarak isimlendirilen bu bileşikler özellikle bitkiler tarafından çok fazla üretilmekte olup insanlar tarafından beslenme yoluyla alındığında antioksidan etki göstermektedir (Chaudiere ve Iliou, 1999). Bu nedenle son yıllarda besin kaynaklı doğal ve sentetik antioksidanların doğrudan ya da gıda katkı maddesi olarak diyetle alınımı artmıştır (Karagözler ve ark., 2008). Bununla birlikte, sentetik antioksidanların koruyucu özellikleri çok yüksek olmakla beraber doğal olmamaları sebebiyle insan sağlığı açısından tehdit oluşturmaktadır (Pokorny, 2007). Biyolojik ve tıbbi olarak kullanılan çok çeşitli bitkileri bünyesinde barındıran Lamiaceae familyasının bir üyesi olup "dağ çayı" ve "ada çayı" olarak bilinen Akdeniz havzasına endemik olan *Sideritis* cinsi halk arasında bitki çayı şeklinde solunum, sinir ve sindirim sistemi gibi pek çok hastalığın tedavisinde kullanılmaktadır (Davis, 1982; Kırımer ve ark., 1999; Everest ve Öztürk, 2005; Uritu ve ark., 2018). Bu türlerin antispazmodik, antifeedant, gaz giderici, analjezik, sinir sistemi uyarıcı, sedatif, antikonvülsan, antibakteriyel, antiinflamatuar, antimikrobiyal ve antioksidan aktivitelere sahip olduğu bilinmektedir (Yeşilada ve Ezer, 1989; Ezer ve ark., 1992; Ezer ve Abbasoğlu, 1995; Aydın ve ark., 1996; Oztürk ve ark., 1996; Bondi ve ark., 2000; Tunalıer ve ark., 2004). *Sideritis* türleri içerdikleri esansiyal yağlar, yağ asitleri, terpenler ve flavonoidleri sayesinde *in vivo* ve *in vitro* farmakolojik aktivite göstermektedir (Garcia-Granados ve ark., 1985; Baser ve ark., 1997; González-Burgos ve ark., 2011).

Bu bağlamda, Türkiye'de de geleneksel tıbbi amaçlarla yaygın olarak kullanılmakta olan *Sideritis* cinsine ait *S. libanotica* ve *S. brevidens* türlerinin antioksidan ve antigenotoksik aktivitelerinin belirlenmesi amacıyla meyve sineği olarak bilinen *Drosophila melanogaster*'in transheterezigot larvaları üzerinde yaşama yüzdeleri ve antioksidan aktiviteleri araştırılmıştır.

## MATERYAL VE METOD

**Kullanılan organizma:** *Drosophila melanogaster*'in *mwh* (multiple wing hair, *mwh/mwh*) ve $flr^{+3}$ (flare, $flr^{3}$/ *In* (*3LR*), *TM3 BdS)* genlerine sahip iki mutant ırkı kullanılmıştır.

**Kullanılan kimyasallar:** Dimetil sülfoksit Sigma (67-68-5) ve Etil metan sülfonat Sigma 62-50-0) firmasından satın alınmıştır.

**Bitki materyallerinin ekstraksiyonu:** *Sideritis* cinsine ait *S. libanotica* ve *S. brevidens* türlerinin toprak üstü kısımları oda sıcaklığında kurutularak toz haline getirilerek sıvı azot yardımıyla ekstraksiyona hazırlanmıştır. Her bir ekstrakt için 50 g bitki tartılmış ve metanol ile 250 mL'ye tamamlamıştır. Ekstrakte edilen kısımların içerdiği saf metanolün büyük bir kısmı düşük basınç ve 40℃ sıcaklıkta rotary evaporatör yardımıyla uzaklaştırılmıştır (Kotan vd., 2010). Elde edilen ekstraktlardan stok çözeltiler hazırlanmış ve farklı konsantrasyonlarda (1,25; 2,5; 5 ve 10 mg/mL) uygulama gruplarına ilave edilmiştir. Stok çözeltiler +4°C'de buzdolabında saklanmıştır.

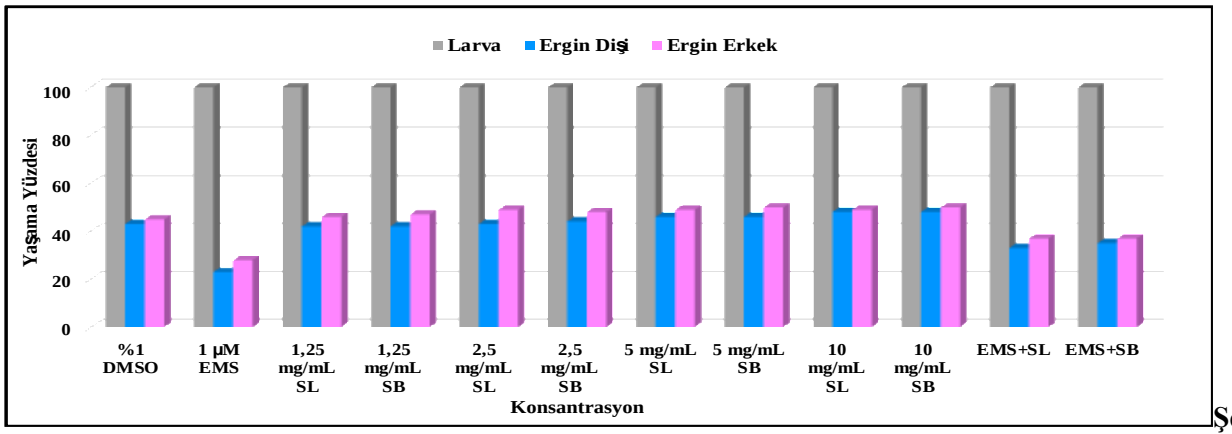
**Yaşama yüzdesi deneyleri:** Larvadan ergine gelişebilen birey sayısını belirten hayatta kalış oranı araştırmak için 1-3 günlük çiftleşmemiş bireylerden elde edilen *D. melanogaster*'in transheterozigot larvaları EMS, SLve SB içeren besiyerlerine alınmıştır. Tüm uygulama gruplarında (*mwh* x $flr^{3}$) çaprazlamasından elde edilen 72±4 saatlik larvalardan ergine gelişebilen bireyler sayılarak hayatta kalış oranları hesaplanmıştır.

**Total oksidan aktivite ve total antioksidan kapasite analizi:** Uygulama gruplarından elde edilen larvalardan erginleşen yetişkin bireylerde gözlenen olası toksik etkinin antioksidan sistemlerin down regülasyonu ile ilişkili olup olmadığını belirlemek için yapılan biyokimyasal analizler için Rel Assay Diagnostics firmasına ait Total Oxidant Status (TOS) Assay Kit ve Total Antioxidant Status (TAS) Assay Kit kullanılarak kalorimetrik ölçümler yapılmıştır. TOS/TAS oranını ifade eden oksidatif stres indeksi (OSI) de hesaplanmıştır.

**İstatistiksel analiz:** Elde edilen verilerle ilgili istatistiksel analizler SPSS 22.0 programı ile yapılmıştır. Kontrol ve uygulama gruplarının yaşama yüzdesi değerlerini karşılaştırmak için tek yönlü varyans analizi (ANOVA) kullanılmıştır. İstatistiksel değerlendirmelerde $p<0.05$ değeri dikkate alınmıştır.

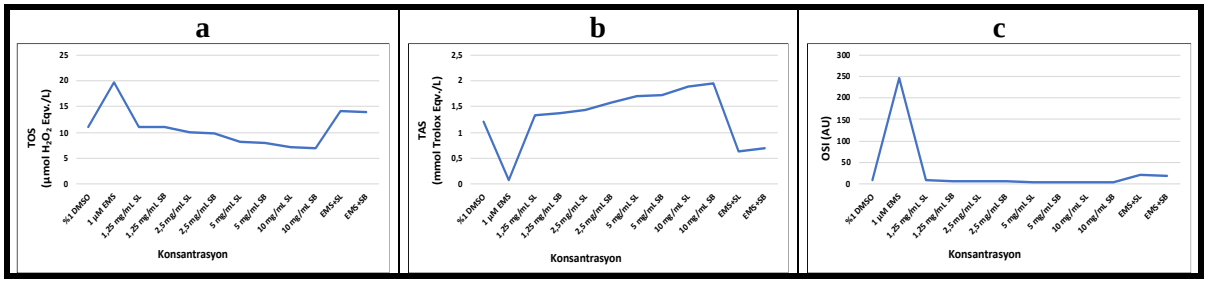
## BULGULAR

EMS'nin toksisitesine karşı SL ve SB bitkilerine ait metanol ekstrelerinin koruyucu etkisi *D. melanogaster*'in transheterozigot larvalarında yaşama yüzdesi deneyleri yapılarak ve oksidatif stres parametreleri ölçülerek araştırılmıştır. Bu amaçla kontrol (%1 DMSO), EMS (1 µM), SL ve SB (1,25; 2,5; 5 ve 10 mg/mL) ve EMS+ SL-SB uygulama gruplarını içeren dört ayrı deney seti hazırlanmıştır. Her deney setinde besiyerlerine aynı yaşlı (72±4 saatlik) transhererozigot larvalar konulmuş ve larvadan ergine gelişebilen bireyler kaydedilerek erginleşebilen bireylerde TAS, TOS ve OSI olmak üzere oksidatif parametreler ölçülmüştür. *Sideritis* bitkilerine ait farklı konsantrasyonlarda metonol eksteresi uygulanan *D. melanogaster* transheterozigot larvalarına ait yaşama yüzdelerine ait sonuçlar Şekil 1'de, oksidatif parametrelere ait sonuçlar ise Şekil 2'de verilmiştir.




**Şekil 1.** Uygulama gruplarına ait *D. melanogaster* transheterozigot larvalarının yaşama yüzdeleri

Çalışma sonucunda, EMS'ye maruz bırakılan larvalarda yaşama yüzdesi ve TAS değerlerinin kontrol grubuna göre düşüş gösterirken TOS değerinin arttığı görülmüştür. SL ve SB ektrelerinin uygulandığı deney setlerine ait 1,25 mg/mL uygulama gruplarında değerlerin kontrole yakın olup konsantrasyon artışıyla birlikte kontrole göre yaşama yüzdesi ve TAS değerlerinin yüksek; TOS değerlerinin ise düşük olduğu saptanmıştır. EMS+SL ve EMS+SB uygulama gruplarında EMS'nin tek başına kullanıldığı gruba göre yaşama yüzdesi ve TAS değerlerinin artarak TOS değerlerinin ise azalarak kontrole yaklaştığı belirlenmiştir. Yaşama yüzdesi ve oksidatif parametre değerleri bakımından kontrole göre gözlenen bu farklılıklar istatistiksel olarak anlamlı bulunmuştur ($p$<0.05).




**Şekil 2.** Uygulama gruplarından elde edilen oksidatif stres parametre verileri

## TARTIŞMA

Hücre ve dokularda serbest radikal oluşumuna bağlı olarak oksidatif stresin artması en hasar verici etkilerden biri olarak bilinmektedir. Antioksidanlar ise oksidatif stres ile meydana gelen radikallerin oluşumunu azaltarak tedavi etkinliğini arttırabilmektedir. Bitkilerin sahip olduğu içerikleri sebebiyle farklı biyolojik etki gösterdiğine ilişkin pek çok literatür mevcuttur (Salameh ve ark., 2018; Pohl ve Lin, 2018). Yapılan bir çalışmada yüksek toplam fenol içeriğine sahip bazı *Sideritis* türlerinin serbest radikalleri süpürücü etki gösterdiği ve lipit peroksidasyonunun önlenmesinde etkili olduğu bildirilmiştir (Tunalıer ve ark., 2004). Yine, *S. leptoclada* bitkisine ait su özütünün DPPH radikallerini giderdiği tespit edilerek *S. leptoclada*'nın potansiyel doğal antioksidan kaynağı olarak gıda takviyesi ve farmasötik endüstrinde bir destek maddesi olarak

kullanılabileceği gösterilmiştir (Ayar-Kayalı ve ark., 2009). *Sideritis* cinsine ait 17 türün liyofilize özütlerinin antioksidan aktiviteleri karşılaştırılmış ve farklı tekniklerle elde edilen tüm ekstrelerin güçlü antioksidan aktivite gösterdiği belirlenmiştir (Güvenç ve ark., 2005). Çalışmamızda bitki ekstrelerinin meyve sineği larvalarının yaşama yüzdesini arttırıp, total oksidan ve antioksidan aktivitelerini düzenleyerek EMS toksisitesine karşı koruyucu etki göstermiş olması literatürü destekler niteliktedir.

## SONUÇ

Temel gıda, gıda katkı maddesi ya da ilaç olarak kullanıma sunulabilmesi için bitki ekstrelerinin sahip olduğu düşünülen aktivitelerinin uygun bilimsel yöntemler ile incelenmesi ve değerlendirilmesi de büyük önem taşımaktadır. Çalışma sonucunda gözlenen bu koruyucu etkiyi *Sideritis* türlerinin terpenler, saponik glikozitler, steroidler, alkaloidler ve flavonoidler gibi biyolojik olarak aktif bileşiklere sahip olmaları ve bu özellikleri sayesinde serbest oksijen radikallerinin oluşumunu engelleyerek ya da ortamdan uzaklaştırarak sağladıkları düşünülmektedir.

## KAYNAKÇA

Aydın S, Oztürk Y, Beis R, Başer KHC 1996. Investigation *of Origanum onites*, *Sideritis congesta* and *Satureja cuneifolia* essential oils for analgesic activity. Phytotherapy Research, 10: 342-344.

Ayar-Kayalı H, Oztürk-Urek R, Nakiboglu M, Tarhan L 2009. Antioxidant activities of endemic *Sideritis leptoclada* and *Mentha dumetorum* aqueous extracts used in Turkey folk medicine. Journal of Food Processing and Preservation, 33(3): 285-295.

Baser KHC, Kırıme, N, Tumen G 1997. Essential oil of *Sideritis scardica* Griseb subsp. *Scardica* Journal Essential Oil Resourch, 9: 205-207.

Bondì ML, Bruno M, Piozzi F, Baser KHC, Simmonds MS 2000. Diversity and antifeedant activity of diterpenes from Turkish species of *Sideritis*. Biochemical Systematics and Ecology, 28(4): 299-303.

Chaudière J, Ferrari-Iliou R 1999. Intracellular antioxidants: from chemical to biochemical mechanisms. Food and Chemical Toxicology, 37(9-10): 949-962.

Davis PH, Mill, R, Tan K 1982. Flora of Turkey and the East Aegean Islands; Edinburgh Univ. 1, 9.

Everest A, Ozturk, E 2005. Focusing on the ethnobotanical uses of plants in Mersin and Adana provinces (Turkey). Journal of Ethnobiology and Ethnomedicine, 1(1): 1-6.

Ezer N, Sakar MK, Rodríguez B, Torre MCD 1992. Flavonoid glycosides and a phenylpropanoid clycoside from *Sideritis perfoliata*. International Journal of Pharmacognosy, 30(1): 61-65.

Ezer N, Akcos Y, Rodriguez B, Abbasoğlu U 1995. An iridoid glucoside from *Sideritis libanotica* Labill. *subsp. linearis* (Bentham) Bornm., and its antimicrobial activity. Hacettepe University Journal of Faculty of Pharmacy, 15: 15-22.

Garcia-Granados A, Martínez A, Onorato ME, Socorro O 1984. Diterpenoids from *Sideritis pusilla* subsp. *flavovirens*. Phytochemistry, 23(3): 607-610.

González-Burgos E, Carretero ME, Gómez-Serranillos MP 2011. *Sideritis spp.*: Uses, chemical composition and pharmacological activities. Journal of Ethnopharmacology, 135(2): 209-225.

Güvenç A, Houghton PJ, Duman H, Coşkun M, Şahin P 2005. Antioxidant activity studies on selected *Sideritis* species native to Turkey. Pharmaceutical Biology, 43(2): 173-177.

Karagözler AA, Erdağ B, Emek YÇ, Uygun DA 2008. Antioxidant activity and proline content of leaf extracts from *Dorystoechas hastata*. Food Chemistry, 111(2): 400-407.

Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry, 47(10): 3954-3962.

Kırımer N, Tabanca N, Tümen G, Duman H, Başer KHC 1999. Composition of Essential Oil of Four Endemic *Sideritis* Species from Turkey, Flavour and Fragrance, 14: 421

Oztürk Y, Aydin S, Oztürk N, Başer KHC 1996. Effects of extracts from certain *Sideritis* species on swimming performance in mice. Phytotherapy Research, 10(1): 70-73.

Pohl F, Lin PKT 2018. The potential use of plant natural products and plant extracts with antioxidant properties for the prevention/treatment of neurodegenerative diseases: *in vitro, in vivo* and clinical trials. Molecules, 23(3283): 1-30.

Pokorný J 2007. Are natural antioxidants better and safer than synthetic antioxidants. European Journal of Lipid Science and Technology, 109(6): 629-642.

Rice-Evans C, Miller N, Paganga G 1997. Antioxidant properties of phenolic compounds. Trends in Plant Science, 2(4): 152-159.

Salameh N, Shraim N, Jaradat N 2018. Chemical composition and enzymatic screening of *Micromeria fruticosa serpyllifolia* volatile oils collected from three different regions of West Bank, Palestine. BioMed. Research International, 6536919: 1-8.

Tunalier, Z., Kosar M, Ozturk N, Baser KHC, Duman H, Kirimer N. 2004. Antioxidant properties and phenolic composition of *Sideritis* species. Chemistry of Natural Compounds, 40(3): 206-210.

Uritu CM, Mihai CT, Stanciu GD, Dodi G, Alexa-Stratulat T, Luca A, Constantin L, Stefanescu R, Bild V, Melnic S, Tamba BI 2018. Medicinal plants of the family Lamiaceae in pain therapy: A review. Pain Research and Management, 2018.

Yeşilada E, Ezer N, 1989. The antiinflammatory activities of some *Sideritis spp*. growing in Turkey. International Journal of Crude Drug Reserarch, 27(11): 38-40.

## *ORAL PRESENTATION*

# Investigation of the effect of protease inhibitors on Chymosin enzyme produced together with *Picihia pastoris* α-mating factor

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**Abstract**

Enzymes that can be produced by microbial cells, animal and plant tissues are generally used in industry; it is widely used in pharmaceutical, food, textile, leather, feed and detergent industries. As a eukaryotic microorganism, the main reasons for choosing Pichia pastoris as a microbial host to recombinantly produce the chymosin enzyme are as follows. It has the advantages of advanced eukaryotic expression systems, such as the orientation of proteins, protein folding, and the ability to easily provide posttranslational modifications (such as phosphorylation and glycosylation). Within the scope of this study, the CYM gene for *P. pastoris* was cloned in E. coli by using the *P. pastoris* heterolog expression system due to the above-mentioned features, and the chymosin enzyme was produced in the form of secretion and purified. Then, the purified enzyme was characterized. Since the pIB4-α plasmid was used in this study, the enzyme could be produced without the need for the use of zeocin antibiotics. After determining the optimum working pH and temperature of the produced enzyme, its responses to 1.0 mM Phenylmethylsulfonyl fluoride (PMSF), Pepsitatin, 1,10-Phenanthroline and Ethylenediaminetetraacetic Acid (EDTA) protease inhibitors were analyzed. Pepstatin, one of the protease inhibitors, inhibited more than all other inhibitors.

**Keywords:** *Pichia pastoris*, Chymosin, Recombinant Enzymes

## INTRODUCTION

Almost all industrial enzymes are biopolymers in protein structure, which are formed by the bonding of amino acids with peptide bonds and catalyse many biochemical or physiological reactions. Enzymes that can be produced by microbial cells, animal and plant tissues are commonly used in the pharmaceutical, food, textile, leather, feed and detergent industries. Excessive use of enzymes specially in the food industry also affects production costs (Spök, 2006). The high costs of the enzymes needed today make these applications difficult. At this point, recombinant enzyme production methods come into play.

The most complex process in cheese making is coagulation of milk. Traditionally, milk coagulation enzymes are obtained from animal sources such as calf abomasum. In recent years, alternative systems have been started to be created regarding milk coagulating enzymes. The use of coagulant enzymes from other animals, plants and microbial sources has begun to develop. In addition, the performance of the coagulating enzyme and the quality of the cheese are directly affected by the source (Broome and Limsowtin, 1998).

As a eukaryotic microorganism, *Pichia pastoris* was chosen as a microbial host (host) to recombinantly produce the chymosin enzyme, due to its advantages of advanced eukaryotic expression systems such as protein folding and ease of posttranslational modifications (such as phosphorylation and glycosylation). In addition to these features, DNA manipulation for this

microorganism is easy because *E. coli* shuttle vectors are used. *Pichia* pastoris is faster, easier and much less expensive than mammalian expression systems. These features make *Pichia* more useful as a protein expression system and one of the favorite expression organisms.

Casein protein makes up about 80% of the nitrogen in bovine milk. Casein alpha S1, alpha S2, β and kappa (κ) is formed by the combination of 4 different proteins in the ratio of 40:10:35:12, respectively. All types of casein contain phosphorus. Alpha S1, Alpha S2 and β subgroups form a precipitate by strongly binding $Ca^{++}$ ions thanks to their high phosphate content. (Foltman, 1987).

Chymosin for industrial use is often referred to as rennet or rennin. Rennet is a type of aspartic protease primarily used in the cheese making industry (Ustunol and Hicks, 1990). These proteases carry two aspartic acid residues, Asp32 and Asp215, in their active sites (Vallejo et al., 2008). The enzyme breaks the peptide bond between the casein protein Phe105-Met106 to form insoluble para-κ-casein, which ultimately enables the milk to form clots (McMahon et al., 1984).

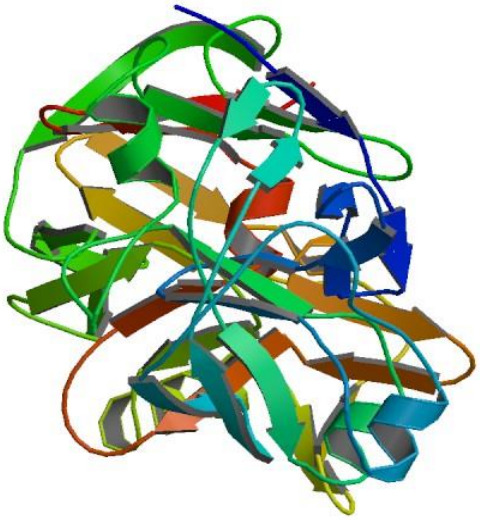


**Figure 1.** 3D structure of chymosin (Gilliland et al., 1990)

Chymosin is synthesized in vivo as preprochymosin, including a 16 amino acid signal peptide. This signal peptide is degraded and disappears as prochymosin is formed. Prochymosin is a propeptide with a molecular weight of 40,777 Da. In the stomach with an acidic pH environment, prochymosin loses the 42 amino acid propeptide part located at the amino terminal end to form the active chymosin molecule. Active chymosin has a molecular weight of 35,600 Da and consists of 323 amino acids (Pedersen et al., 1979). As a result of studies with synthetic substrates, it has been determined that chymosin has a wide optimum pH range ranging from pH 4.2 to 3.7 (Mohanty et al., 1999).

In this study, due to the above-mentioned properties, the CYM gene for *P. pastoris* was cloned in *E. coli* by using the *P. pastoris* heterolog expression system and purified by producing the chymosin enzyme in the form of secretion. Then, the purified enzyme was characterized. Until 2008, the chymosin enzyme was produced in some microorganisms such as *E. coli*, but it could not become very suitable for industry because it could not be secreted. In 2008, chymosin enzyme was produced in the supernatant culture of *P. pastoris* for the first time (Vallejo et al., 2008). However, the use of zeocin antibiotic for the selection of recombinant E. coli and *Pichia pastoris* strains in the production stage has increased the cost excessively. In addition, this production system using zeocin antibiotic in this way does not make large-scale culture applications (fermentor and bioreactor) possible due to its not being economical. Since the pIB4-α plasmid was used in this study, there is no need to use zeocin antibiotics. Because only *P. pastoris* cells carrying the recombinant pIB4-α plasmid can survive in –His broth, selection can be made easily. After determining the optimum working pH and temperature of the produced enzyme, its responses to 1.0 mM Phenylmethylsulfonyl fluoride

(PMSF), Pepsitatin, 1,10-Phenanthroline and Ethylenediaminetetraacetic Acid (EDTA) protease inhibitors were analyzed. Pepstatin, one of the protease inhibitors, inhibited more than all other inhibitors.

## MATERIALS AND METHODS

### Expression of recombinant chymosin in *P. pastoris*

*P. pastoris* transformants were grown in 20 ml freshly prepared buffered minimal glycerol complex medium, BMGY [1% yeast extract), 2% peptone, 100 mM potassium phosphate (pH 6.0), 1.34% YNB, 0.0004% biotin, and 1% glycerol] was grown at 30°C until the OD600 was about 5-6. The cell pellet was then collected and resuspended in minimal methanol medium BMMY [1%yeast extract, 2% peptone, 100 mM potassium phosphate (pH 6.0), 1.34% YNB, 0.0004% biotin and 0.5% methanol] prepared using 100 ml buffer. In this study, P.pastoris GS115 strain was used because it has a mutation in its histidine gene locus. In this way, positive transformants could be easily selected in the -His medium. Addition of absolute methanol to the culture medium was continued every 24 hours until the final concentration was 1% to maintain induction, and the culture supernatant was collected daily by centrifugation. The supernatant was concentrated with Vivacell® protein concentrator tubes, after which the secretion signal protein with 6x His Tag and chymosin enzyme fusion proteins were used for chromatography. The pure protein sample obtained was dialyzed in 5 lt of 20 mM phosphate, 50 mM NaCl pH: 7 buffer and the imidazole in it was removed. Qualitative analysis and purity of proteins were analyzed by 10-12% SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis).

### Total Proteolytic Activity

Total proteolytic activity was determined by the azokazein-based spectrophotometric method (Fernando and Norman, 1993). The reaction was started by adding 20 µl of enzyme solution to 0.5 ml of 1.5% azokazein solution dissolved in Tris-HCl buffer with a pH of 50 mM, pH 7.5. At the end of 15 minutes, the reaction was stopped by adding 0.5 mL of 20% trichloroacetic acid (TCA) to the reaction mixture. The tube containing the reaction mixture was centrifuged at 6500 rpm for 6 minutes and the absorbance of the supernatant was measured at 340 nm. One unit of total proteolytic activity was defined as the absorbance difference created by 1 mL of enzyme solution in one minute, and total proteolytic activity was determined as U/mg protein.

### Effect of pH and Temperature on Proteolytic Activity

In order to determine the optimum pH, total proteolytic activity against azokazeine was measured at 25 ℃ in a range ranging from pH 3-9. Here, 50 mM sodium citrate buffer was used for the pH range of 3-6 and 50-mM Tris-HCl buffer was used for the pH range of 6-9. After determining the optimum pH, the optimum temperature was determined by calculating the proteolytic activity in the temperature range of 20-70 ℃ at the optimum pH (D'Ambrosio et al., 2003).

### Inhibition Studies

In order to determine the effect of inhibitory substances on the chymosin enzyme, the enzyme solution was incubated with the following inhibitory substances at 25 ℃ for 1 hour and then its proteolytic activity against azokazeine was determined as described above (D'Ambrosio et al., 2003).

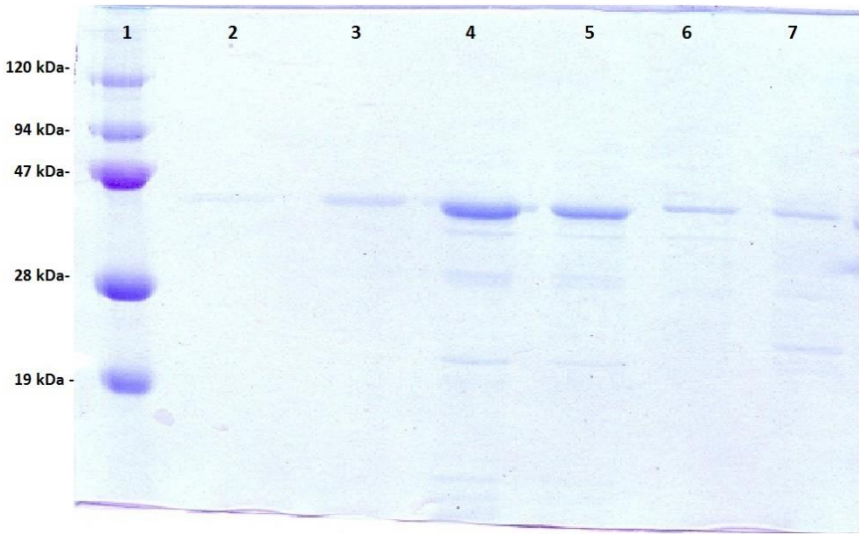
1.0 mM Phenylmethylsulfonyl fluoride (PMSF): Specific inhibitor of serine proteases

0.01 mM Pepsitatin: Aspartic protease specific inhibitor

10 mM 1,10-Phenanthroline and 10 mM Ethylenediaminetetraacetic acid (EDTA): Metallo-proteases specific inhibitor

## RESULTS and DISCUSSION

After purification by affinity chromatography, α-mating factor chymosin fusion protein could be obtained in pure form, as seen in Figure 2. Histidines (6 pieces) added to the amino end of the fusion were attached to the fusion column with the affinity for nickel, other proteins were separated from the column, and thus purification was performed.




**1.** Amresco Blue step Low Range Protein molecular weight marker, **2-7.** Chymosin enzyme purified from the medium taken 2-12 days after induction (with α-mating factor) (40,654 kDa).

**Figure 2.** SDS-PAGE analysis results of the produced chymosin enzyme

**Table 1.** Effect of pH on proteolytic activity

| **pH** | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|
| **% hydrolysis of azokazein** | 38,2 | 51,8 | 60,2 | 86,3 | 83,4 | 79,5 | 61,7 |

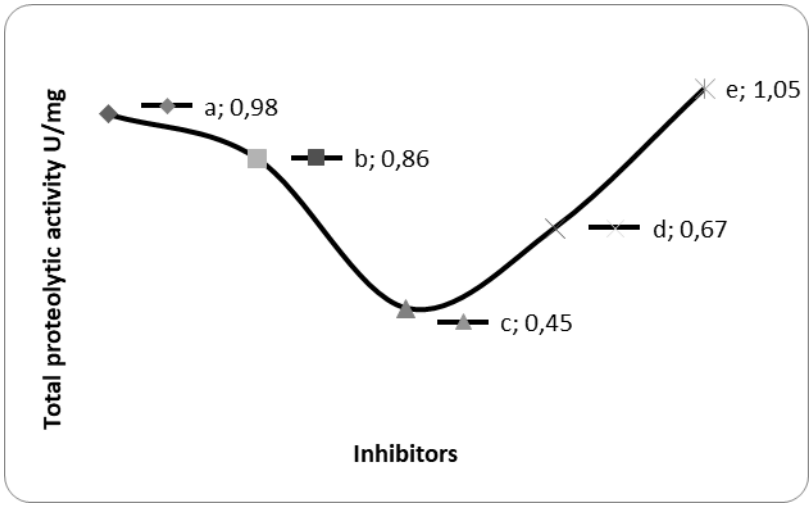
Total proteolytic activity determined by the azokazein-based method was calculated as 0.98 ± 0.01 U/mg protein. Again, the optimum pH was determined as 6.5 and the optimum temperature was determined as 40 °C. Analyzes were performed in 3 repetitions.

**Table 2.** Effect of temperature on proteolytic activity

| **Temperature (°C)** | 20 | 30 | 40 | 50 | 60 | 70 |
|---|---|---|---|---|---|---|
| **% hydrolysis of azokazein** | 37,2 | 56,4 | 85,2 | 81,1 | 39,2 | 23,4 |

The behavior of chymosin enzyme, which was expressed and purified in *Pichia pastoris* organism and showed proteolytic activity against casein, against 4 different protease inhibitors was investigated. In particular, it was observed that the pepsitatin inhibitor inhibited the activity of the

enzyme by more than 50%, while EDTA contributed positively to the protease activity. Here, it can be said that EDTA makes a positive contribution to the shaping of the three-dimensional structure of the enzyme.




**a.**No inhibitor **b.**1,0 mM Phenyl Methyl Sulfonyl fluoride (PMSF) **c.** 0,01 mM Pepsitatin **d.**10 mM 1,10- Phenanthroline **e.** 10 mM EDTA

**Figure 3.** The effect of some inhibitors on the total proteolytic activity of the chymosin enzyme

## CONCLUSION

Although the recombinant chymosin enzyme produced in this study was at a sufficient level in terms of quantity and activity, it could be observed that the yield was low because the organism was cultured only in Erlenmeyer, but the efficiency of the system was fermented. In addition, if the fusion enzyme is separated from its attached fusion, changes may occur in its activity, which may be another factor that increases the yield.

Only two companies meet the recombinant chymosin needs of the cheese industry in the world. In our country, there is currently no supply of recombinant chymosin. Naturally, our industrialists supply their chymosin needs entirely through imports. Currently, rennet used in our country is grouped into three groups. The first of these is renin isolated from calf abomasum, which also faces the raw material problem. As might be expected, it is not possible to meet the chymosin requirement of the worldwide cheese industry in this way. In addition, pepsin pollution may occur during purification from the abomasum, which causes the cheese to become bitter and deteriorate its taste. The second source of coagulants is microbial coagulants, in this way, many by-products are released by the organism along with chymosin during the cheese production process, which cause deterioration in the taste and texture of the cheese. The third source of chymosin is recombinant chymosin, which has been modified to provide the most optimal level of activity and can consistently provide the same quality (Ulusu et al., 2016). Today, the absence of domestic production of recombinant chymosin causes a serious foreign trade deficit for our country. With the industrial production of chymosin, which has been produced as a prototype sample within the scope of this project, our country's imports in this field will be reduced.

## REFERENCES

Broome, MC., Limsowtin, GYK., 1998. Milk coagulants. Aust. J Dairy Technol, 53(3), 188.

D'Ambrosio, A., Rossano, R., Ungaro, N., Riccio, P., 2003. Proteolytic and milk clotting activities in extracts obtained from the crustaceans Munida. Journal of Molecular Catalysis B:Enzymatic, 22, 145-150.

Fernandao Luis, G.C., Norman, F.D., 1993. Characterization of proteinase classes in langostilla (*Pleuroncodes planipes*) and cryfish (*Pasifastacus astacus*) extracts. Journal of Food Biochemistry, 17 (2), 97-113.

Foltman, B., 1987. General and molecular aspects of rennets. In: Fox PF, editor. Cheese: Chemistry. Physics and Microbiology, 1, 37-68.

McMahon, DJ., Brown, RJ., 1984. Enzymic coagulation of milk casein miselles. J. Dairy Sci., 67, 745-748.

Mohanty, AK., Mukhopadhyay, UK., Grover, S., Batish, VK., 1999. Bovine chymosin: Production by rRNA technology and application in cheese manufacture. Biotechnology Advances, 17, 205-217.

Pedersen, VB., Christensen, KA., Foltmann, B., 1979. Investigations on the activation of bovine prochymosin. Eur. J. Biochem., 94, 573-580.

Spök, A., 2006. Safety regulations of food enzymes. Food Technol Biotech, 44 (2), 197-209.

Ulusu, Y., Şentürk, S. B., Kuduğ, H., Gökçe, İ., 2016. Expression, purification, and characterization of bovine chymosin enzyme using an inducible pTOLT system. Preparative Biochemistry and Biotechnology, 46(6), 596-601.

Ustunol, Z., Hicks, CL., 1990. Effect of milk-clotting enzymes on cheese yield. J. Dairy Sci., 73, 8-16.

Vallejo, JA., Ageitos, JM., Poza, M., Villa, TG., 2008. Cloning and expression of buffalo active chymosin in *Pichia Pastoris*. J. Agric. Food Chem., 58, 10606- 10610.

## *ORAL PRESENTATION*

# Molecular docking studies of chalcones from *Astragalus ponticus* Pall.

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## Abstract

In this study, four chalcones, namely 3-deoxysappanchalcone, 4-methoxy-2,3',4'-trihydroxychalcone, isoliquiritigenin and 4',5-dihydroxy-2,2'-dimethoxychalcone, that were isolated from *Astragalus ponticus* Pall. species were subjected to molecular docking studies using some selected human protein structures to analyze ligand-protein interactions. The data was compared to *in vivo* cytotoxicity activity test results against human cervical cancer cell (HeLa) lines, that were obtained in a previous study, and possible target points on the protein active sites were detected to see the interactions which might be responsible for cytotoxicity activities of the chalcones.

**Keywords:** Molecular docking, ligand-protein interaction, cytotoxicity

## INTRODUCTION

Chalcones are phenolic compounds with C6-C3-C6 skeleton, which is seen as a privileged structure by drug design community. These compounds have 1,3-diaryl-2-propene-1-one basic structure and can be found in E or Z configuration, although E isomer is thermodynamically more stable and most of the chalcones from natural sources have the E configuration. Owing to their simple structures and a series of pharmacological activities, chalcones occupy a big space in biological activity research (Rao et al., 2009). Of all the bioactivities they possess, cancer may be counted among the most important ones (Farooq and Ngaini, 2019). According to Anwar et al. (2018), chalcones show their anticancer activities through C-α and C-β unsaturated system, along with the groups on A and B rings.

Drug discovery is a time and energy consuming method to bring out new drug candidate molecules. While in its early times the drug discovery methods relied on in-laboratory testing procedures, this has been changing through time with emergence of new techniques like automated separation/structural analysis, multi sample screening, *in situ* screenings and computer assisted methods (Li and Vederas, 2009). Molecular docking strategy is one of the before mentioned computer assisted methods, which allows the researchers to study possible and potential affinity of a ligand to a target molecule (Mariadoss et al., 2018). This includes determining the character of the interaction, e.g. hydrophilic/hydrophobic, long distance/short distance etc. (Batool et al., 2022). Herein, molecular docking results of four chalcones from *Astragalus ponticus* Pall. were represented.

## MATERIALS AND METHODS

### General

ADME calculations were performed on Swiss ADME website (Daina et al., 2017). UCSF Chimera and AutoDock Vina softwares were used for molecular docking studies (Eberhardt et al., 2021, Pettersen et al., 2004). PyMOL (The PyMOL Molecular Graphics System, Version 2.5.4 Schrödinger, LLC.) and LigPlot (Wallace et al., 1995) softwares were used for the visualization of the molecular interactions.

### Chalcones

Four chalcones, namely 3-deoxysappanchalcone (**B1**), 4-methoxy-2,3',4'-trihydroxychalcone (**B5**), isoliquiritigenin (**B6**) and 4',5-dihydroxy-2,2'-dimethoxychalcone (**B8**) were previously isolated *from Astragalus ponticus* Pall. and were tested against HeLa cells ($IC_{50}$ values can be seen in **Table 2**) (Koldas et al., 2022). Their structures were given in **Figure 1**.

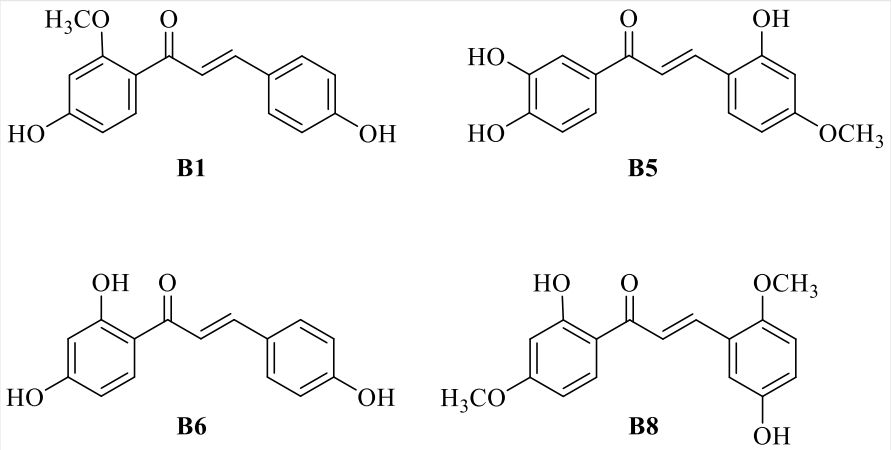


**Figure 1.** Molecular structures of chalcones from *Astragalus ponticus* Pall.

**Preparation of protein structures**

Protein structures for cellular tumor antigen p53 (PDB ID: 1A1U), caspase-3 (PDB ID: 3DEH), mucosal addressin cell adhesion molecule 1 (PDB ID: 4HD9) and NF-kappa-B (PDB ID: 1SVC) were downloaded from RCSB Protein Data Bank (http: //www. pdb. org). Water molecules were deleted and hydrogen atoms were added to the structures before analysis.

Ligand-binding site residues for above mentioned proteins were selected according to the findings of Muthukala et al. (2015) and these were given below.

**Cellular tumor antigen p53**

LEU30,GLN31,ILE32,ARG37,PHE38,GLU39,LYS40,ILE41,ARG42,TYR44,ASN45.

**Caspase-3**

ARG64,SER120,HIS121,GLY122,GLN161,ALA162,CYS163,SER205,TRP206,ARG207,ASN208,SER209,TRP214,MET222,GLN225,TYR226,ARG238,ARG241,LYS242,THR245,GLU246,PHE247,GLU248,SER249,PHE250,SER251,PHE256.

**Mucosal addressin cell adhesion molecule 1**

LEU17,GLY18,TRP38,ARG39,GLY40,LEU41,ASP42,LEU45,GLY46,ALA47,VAL48,LEU57,ASN61,ALA62,SER63,THR69,ARG70,PHE127,SER128,LEU129,LEU130,GLU135,LEU136,GLU137,GLY138,ALA139,ALA141,ASP156,GLU157,ASP158,TRP167.

**NF-kappa B**

THR102,ASN103,GLY104,LYS105,ASN106,HIS108,LEU109,HIS110,LEU154,GLN204,THR205,LYS206, GLU207,MET208,ASP209,VAL212,VAL213.

## RESULTS and DISCUSSION

As an acronym, ADME stands for absorption, distribution, metabolism and elimination of a drug molecule (Hodgson, 2001). In **Table 1**, the ADME calculation results for the chalcones B1, B5, B6 and B8 can be seen. One can understand from the results that all of the analyzed chalcone compounds passes drug likeliness test. The molecules also obey Lipinski's 5 rules and this means the molecules are safe, in case of a use as a drug, to be administered orally.

**Table 1.** ADME calculation results for the chalcones

| Compound | Molecular weight (g/mol) | Molecular Formula | LogP | Number of Rotatable Bonds | H-Bond Donor | H-Bond Acceptor | Lipinski's Rule |
|---|---|---|---|---|---|---|---|
| B1 | 270.28 | $C_{16}H_{14}O_4$ | 2.55 | 4 | 2 | 4 | Y |
| B5 | 286.28 | $C_{16}H_{14}O_5$ | 2.20 | 4 | 3 | 5 | Y |
| B6 | 256.25 | $C_{15}H_{12}O_4$ | 2.37 | 3 | 3 | 4 | Y |
| B8 | 300.31 | $C_{17}H_{16}O_5$ | 2.75 | 5 | 2 | 5 | Y |

In **Table 2**, docking results of the chalcones with four human proteins, along with cytotoxicity results against HeLa cells, can be seen. While there is no correlation with the cytotoxicity results against HeLa cells, chalcones B1, B5, B6 and B8 showed almost similar affinities for cellular tumor antigen p53 protein. The most visible difference in affinities was seen for caspase 3 protein: B6 showed the highest binding energy, where B5 showed the lowest one.

**Table 2.** Docking results of the chalcones with four human proteins and their $IC_{50}$ values against HeLa cells

| | Docking score (kCal/mol) | | | | $IC_{50}$ (µM) |
|---|---|---|---|---|---|
| Compound | 1A1U | 3DEH | 4HD9 | 1SVC | |
| B1 | -5.8 | -7.3 | -6.1 | -5.5 | 116.2 |
| B5 | -5.9 | -7.2 | -6.4 | -6.3 | 36.6 |
| B6 | -5.9 | -7.6 | -6.2 | -5.4 | 105.4 |
| B8 | -6.0 | -6.9 | -5.8 | -5.3 | 20.6 |

As a regulatory protein, transformation-related protein 53 (p53) is a gene that is responsible for tumor suppressor. Activation of this gene stops the generation and development of tumorous tissues. In **Table 2**, the binding score results of four chalcones with 1A1U protein can seen. It can be deduced from the results that there is no big difference in the affinities of compounds towards the protein, being the highest affinity for B8. And according to the LiPlot schemes in **Figure 2**, all the compounds form H bonds to Gln31 residue of the protein with different bond lengths.

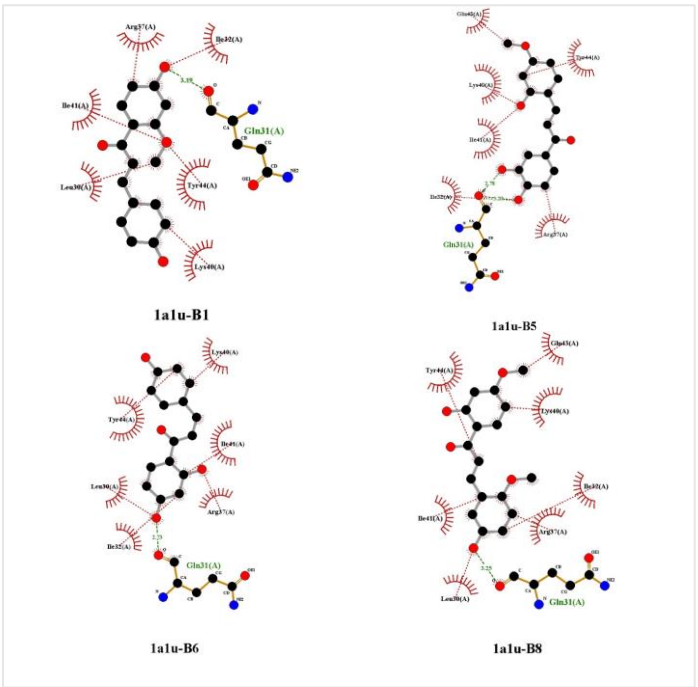


**Figure 2.** Protein-ligand interactions on Cellular tumor antigen p53 active site

Nuclear factor kappa B (NF-kappa B) is another protein, whose over activation is associated with tumor (Batool et al., 2022), accordingly, its suppression in cancer treatments is a key point. Protein-ligand interactions of B1, B5, B6 and B8 can be seen in **Figure 3** and molecular docking results can be seen in **Table 2**. B5 showed the highest affinity towards this protein with -6.3 kCal/mol binding score. B5 forms 4 H bond with the active sites residues of NF-kappa B protein.

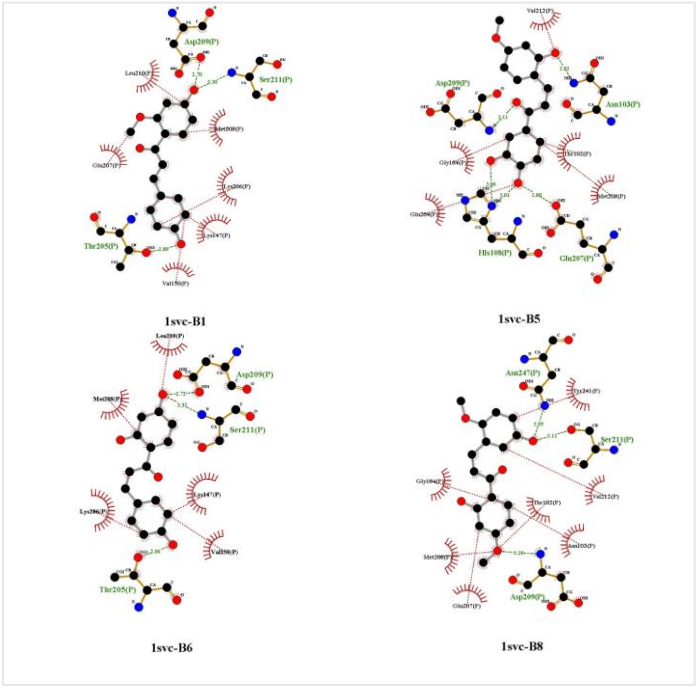


**Figure 3.** Protein-ligand interactions on NF-kappa-B active site

Caspase-3 is a protein which is associated with apoptosis of the cells. Its activation induces some cellular mechanisms that cleave some vital proteins in the cell, subsequently leading to death of the cell. Among all the proteins studied during this research, the chalcone compounds showed the highest binding scores to this protein (**Table 2**), B6 being the best match. Although H bond formation is more for B1 (**Figure 4**), there might be other hydrophobic interactions responsible for this high affinity of B6 towards caspase-3 protein.

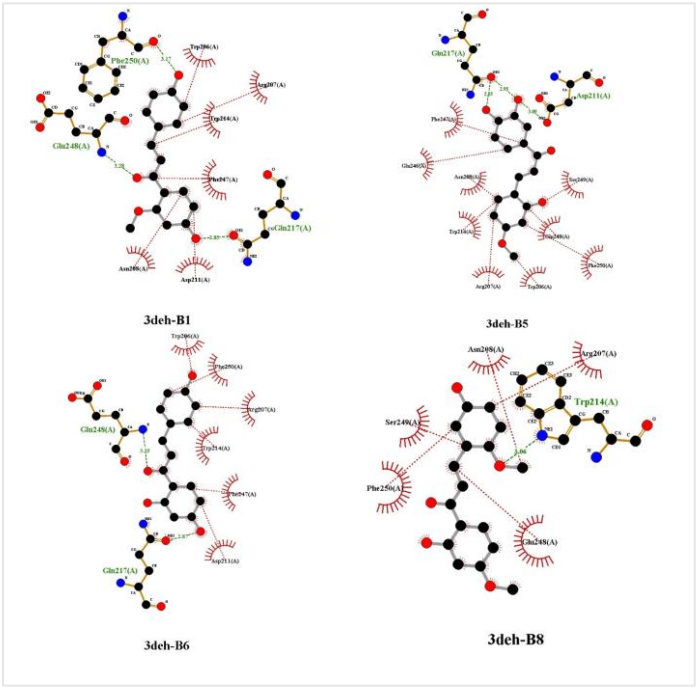


**Figure 4.** Protein-ligand interactions on Caspase-3 active site

Finally, the protein-ligand interaction patterns of the chalcones with mucosal addressin cell adhesion molecule 1 active site (MAdCAM1) can be seen in **Figure 5**. MAdCAM1 protein is linked with cell invasion and metastasis, thus, strategies targeting this protein play important roles in cancer therapies (Janiszewska et al., 2020). B5 showed the highest affinity towards MAdCAM1 protein with -6.4 kCal/mol binding score (**Table 2**). B1 and B5 form highest number H bond with active site residues of the protein (**Figure 5**).

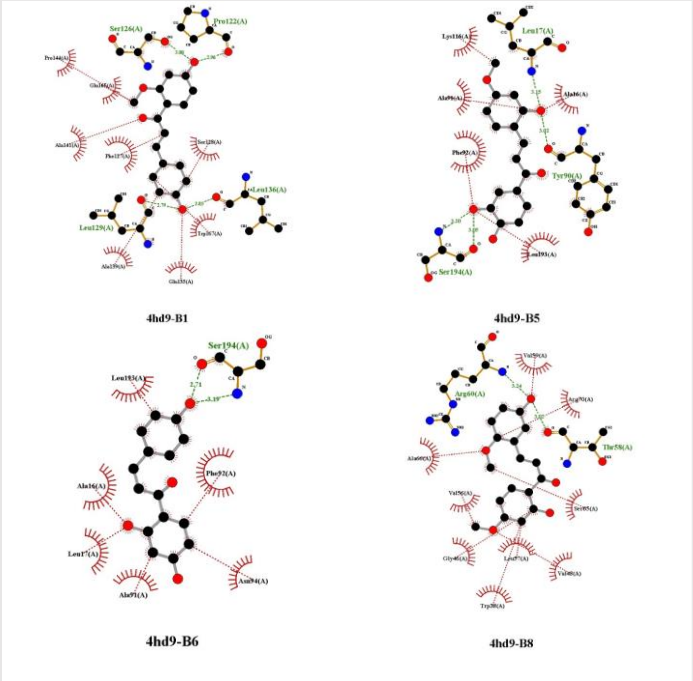


**Figure 5.** Protein-ligand interactions on Mucosal addressin cell adhesion molecule 1 active site

## CONCLUSION

Computer assisted techniques in drug design studies easy the burden of the process by means of time, energy and money. As one of these techniques, molecular docking method helps academia/pharmacy industry by presenting the visualization of interaction or affinity of ligand with target protein/macromolecule. By these data, researchers can select the promising molecules without working on them physically. In this study, ADME calculations of four chalcones from natural sources were done and their affinities towards four human proteins were revealed by molecular docking studies. No direct correlation between *in vivo* cytotoxicity test results and docking scores could be found. Different mechanism of action of the chalcone moieties can be shown as the reason of these findings.

## REFERENCES

Anwar C, Prasetyo YD, Matsjeh S, Haryadi W, Sholikhah EN, Nendrowati N 2018. Synthesis of chalcone derivatives and their in vitro anticancer test against breast (T47D) and colon (WiDr) cancer cell line. Indonesian Journal of Chemistry, 18: 102-107.

Batool A, Miana GA, Alam M, Khan MT, Muddassir M, Zaman W, Saifullah K, Aman A, Khusro A, Arasu MV, Al-Dhabi NA, Choi KC, Sahibzada MUK 2022. Bioassay-guided fractionation and isolation of Arctigenin from Saussurea heteromalla for in vitro and in silico cytotoxic activity against HeLa cells. Physiological and Molecular Plant Pathology, 117: 101749.

Daina A, Michielin O, Zoete V 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports, 7: 42717.

Eberhardt J, Santos-Martins D, Tillack AF, Forli S 2021. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. Journal of Chemical Information and Modeling, 61: 3891-3898.

Farooq S, Ngaini Z 2019. Recent synthetic methodologies for chalcone synthesis (2013-2018). Current Organocatalysis, 6: 184-192.

Hodgson J 2001. ADMET—turning chemicals into drugs. Nature Biotechnology, 19: 722-726.

Janiszewska M, Primi MC, Izard T 2020. Cell adhesion in cancer: Beyond the migration of single cells. Journal of Biological Chemistry, 295: 2495-2505.

Koldas S, Demirtas I, Mutlu D, Atalar MN, Ünver H, Arslan Ş 2022. New cytotoxic chalcone derivatives from Astragalus ponticus Pall. Nat Prod Res, 36: 4666-4674.

Li JW, Vederas JC 2009. Drug discovery and natural products: end of an era or an endless frontier? Science, 325: 161-5.

Mariadoss AVA, Krishnan Dhanabalan A, Munusamy H, Gunasekaran K, David E 2018. In Silico studies towards enhancing the anticancer activity of phytochemical phloretin against cancer drug targets. Current Drug Therapy, 13: 174-188.

Muthukala B, Sivakumari K, Ashok K 2015. In silico docking of Quercetin compound against the Hela cell line proteins. Int J Curr Pharm Res, 7: 13-16.

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE 2004. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem, 25: 1605-12.

Rao YK, Fang S-H, Tzeng Y-M 2009. Synthesis and biological evaluation of 3′, 4′, 5′-trimethoxychalcone analogues as inhibitors of nitric oxide production and tumor cell proliferation. Bioorgan. Med. Chem., 17: 7909-7914.

Wallace AC, Laskowski RA, Thornton JM 1995. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng, 8: 127-34.

## *ORAL PRESENTATION*

# Synthesis of Unsymmetrical Cyclopalladate Complexes with Thiobarbiturate Derivatives and Their Catalytic Application in Suzuki-Miyaura Reaction

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## Abstract

A series of unsymmetrical cyclopalladated complexes with thiobarbiturate derivatives were synthesized. The chelate ligands bearing thiobarbiturate derivatives (**1a-e**) were synthesized by the reaction of aniline derivatives with 1,3-diethyl-4,6-dihydroxy-2-mercaptopyrimidine-5-carbaldehyde. The prepared ligands were treated $[Pd(\mu\text{-}OAc)_2(ppy)]_2$ in methanol and five new Pd(II) complexes (**2a-e**) were synthesized. All ligands and complexes were characterized by elemental analysis and spectroscopic methods. Finally, the performance of Pd(II) complexes **2a-e** was studied on Suzuki-Miyaura reactions of phenylboronic acid with aryl bromides.

**Keywords:** 1,3-diethyl-2-thiobarbituric acid, Cyclopalladate, Suzuki-Miyaura reaction.

## INTRODUCTION

Barbituric and thiobarbituric acids are pyrimidine derivatives (Kulsoom et al., 2022; Figueiredo et al., 2018; Weigand et al., 1998). Pyrimidine-2,4,6-triones have several potential donor atoms such as the oxygen atom of carbonyl groups, the nitrogen atoms of pyrimidine and the carbon atom of active methylene in the ring (Mahmudov et al., 2014; Yilmaz et al., 2009). Barbituric acid and its derivatives can coordinate through one or both of their deprotonated nitrogen atoms, the carbonyl oxygen atoms, and the deprotonated methylene group giving access to a wide range of metal complexes (Muthiah et al., 2007; Karcı and Karcı, 2008; Mahmudov et al., 2012; Mahmudov et al., 2013; Colacio et al., 1994; Calacio et al., 1993; Colacio et al., 1992). Transition metal complexes with barbiturate ligands have shown good biological activities which include antibacterial, antiangiogenic, anticancer, antifungal, immunomodulatory and antioxidant activities whereas they use very rarely in the coupling reaction (Figueiredo et al., 2018; Khan et al., 2016; Garoufis et al., 2009; Salas et al., 2013; Bandyopadhyay et al., 2017; Fırıncı et al., 2017; Fırıncı et al., 2019; Castineiras et al., 2012). Cyclopalladated compounds are the most active catalysts for C-C and C-heteroatom bond-forming reactions (Liu et al., 2010; Kumbhar, 2017; Tsuji, 1995; Maitlis, 1971; Beletskaya and Cheprakov, 2000; Miyaura and Suzuki, 1995; Chinchilla and Najera, 2007; Molnar, 2011; Das, 2016). Palladacycles are one of the most popular and studied classes of organopalladium derivatives (Dupont, 2008; Zim et al., 2008; Dupont and Spencer, 2005; Beletskaya and Cheprakov, 2004; Alacid et al., 2006; Kumbhar, 2019; Balanta et al., 2011; Castillo and Buchwald, 2016; Dorel et al., 2019). Their activities closely depend on both the nature of the ligand and the choice of transition metal (Rajmane et al., 2022; Snelders and Koten, 2011; Navarro et al., 2006). In this context, N,O-bidentate ligands containing complexes can behave as efficient catalysts for coupling reactions. In this study, we reported the synthesis and characterization of thiobarbiturate derivatives and their cyclopalladated complexes. Also, the catalytic activities of new N,O-bidentate donor ligands on palladium-catalyzed on Suzuki-Miyaura reaction were investigated.

## MATERIALS AND METHODS

### Material and Physical Measurement

All the solvents ($CH_3OH$, HOAc, DMF, $CH_2Cl_2$, $Et_2O$, IPA, $HC(OEt)_3$, pentane) and chemical reagents (thiobarbituric acid, 2,6-diisopropylaniline, 2,4,6-trimethylaniline, 2,3,4,5,6-pentafloroaniline, 4-hydroxyaniline, 4-nitroaniline, 2-phenylpyridine, $Pd(OAc)_2$, $Cs_2CO_3$, phenylboronic acid, 4-bromobenzene, 4-bromotoluene and 4-bromoacetophenone) were obtained commercially and used as received. Elemental analyses for C, H and N were carried out on a LECO CHNS-932 elemental analyzer by METU Central Laboratory (Ankara, Türkiye). Infrared spectra were measured with an ATR Spectrum-II, PerkinElmer

spectrometer. The $^{1}$H- and $^{13}$C-NMR spectra were recorded on a Varian 400 spectrometer in DMSO-d6 or $CDCl_3$ and residual signals of the solvent were used as references. Melting points were obtained on a Stuart SMP30 melting point apparatus in open capillary tubes and were uncorrected.

**Synthesis of Ligands** (**1a-e**)

In a flask was charged with 1,3-diethyl-4,6-dihydroxy-2-mercaptopyrimidine-5-carbaldehyde (1.0 g, 1.0 eq), aniline derivatives (2,6-diisopropylaniline, 2,4,6-trimethylaniline, 2,3,4,5,6-pentafloroaniline, 4-hydroxyaniline, 4-nitroaniline) (1.0 eq) and triethyl orthoformate (1.4 eq) in acetic acid (5.0 mL)/dimethyl formamide (1.0 mL) mixture (Figure 1). The solution was stirred at 60 °C for 2h and then distilled water (100.0 mL) was added for precipitation of the product. The residue was filtered and washed with water and pentane. The product was purified by $CH_2Cl_2$/pentane.

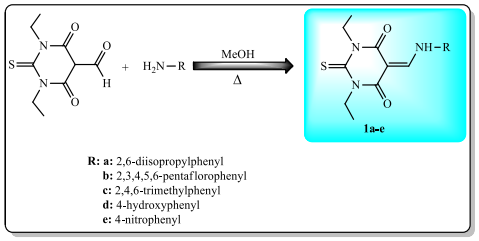



**Figure 1.** Synthesis of ligands

**1a:** Pale pink powder, 1.1 g, Yield: 61.0%, m.p.: 162-164 ºC, IR (ν cm$^{-1}$): 3205, 2947, 2816, 1678, 1590. Anal. Calc. for $C_{21}H_{29}N_3O_2S$: C, 65.08; H, 7.54; N, 10.84; found: C, 65.11; H, 7.52; N, 10.82. $^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm): 1.22 (d, *J*: 2.0 Hz, 6H, $C_6H_3$-2-CH(C*H*$_3$)$_2$), 1.24 (d, *J*: 1.6 Hz, 6H, $C_6H_3$-6-CH(C*H*$_3$)$_2$), 1.29 (t, *J*: 7.0 Hz, 3H, NCH$_2$C*H*$_3$), 1.34 (t, *J*: 7.0 Hz, 3H, NCH$_2$C*H*$_3$), 3.0-3.07 (m, 2H, $C_6H_3$-2-C*H*(CH$_3$)$_2$), 4.51-4.59 (m, 4H, N C*H*$_2$CH$_3$), 7.22 (d, *J*: 7.6 Hz, 2H, $C_6H_3$-6-CH(CH$_3$)$_2$), 7.35 (d, *J*: 7.8 Hz, 1H, $C_6H_3$-6-CH(CH$_3$)$_2$), 8.18 (d, *J*: 14.5 Hz, 1H, C=C*H*), 11.79 (d, *J*: 14.1 Hz, 1H, *H*NCH). $^{13}$C NMR (100 MHz, $CDCl_3$, δ ppm): 12.3 (NCH$_2$*C*H$_3$), 12.4 (NCH$_2$*C*H$_3$), 23.7 ($C_6H_3$CH(*C*H$_3$)$_2$), 28.5 ($C_6H_3$*C*H(CH$_3$)$_2$), 42.3 (N*C*H$_2$CH$_3$), 43.0 (N*C*H$_2$CH$_3$), 93.8 (CH=*C*), 124.2, 129.2, 134.3, 143.9 (*C*$_6$H$_3$-6-CH(CH$_3$)$_2$), 160.9 (*C*H=C), 161.0 (*C*=O), 163.3 (*C*=O), 179.0 (*C*=S).

**1b:** Pale yellow powder, 1.7 g, Yield: 84.0%, m.p.: 133-135 ºC, IR (ν cm$^{-1}$): 3249, 2932, 2821, 1677, 1562. Anal. Calc. for $C_{15}H_{12}F_5N_3O_2S$: C, 45.80; H, 3.08; N, 10.68; found: C, 45.78; H, 3.06; N, 10.64. $^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm): 1.26-1.28 (m, 3H, NCH$_2$C*H*$_3$), 1.29-1.32 (m, 3H, NCH$_2$C*H*$_3$), 3.0-3.07 (m, 2H, $C_6H_3$-2-C*H*(CH$_3$)$_2$), 4.50-4.56 (m, 4H, N C*H*$_2$CH$_3$), 8.70 (d, *J*: 12.9 Hz, 1H, C=C*H*), 12.34 (d, *J*: 12.9 Hz, 1H, *H*NCH). $^{13}$C NMR (100 MHz, $CDCl_3$, δ ppm): 12.2 (NCH$_2$*C*H$_3$), 12.4 (NCH$_2$*C*H$_3$), 42.5 (N*C*H$_2$CH$_3$), 43.2 (N*C*H$_2$CH$_3$), 97.4 (CH=*C*), 155.9 (*C*H=C), 159.9 (*C*=O), 163.2 (*C*=O), 178.7 (*C*=S). $^{19}$F NMR (376 MHz, $CDCl_3$, δ ppm): -159.8 (dd, $J^1$: 21.1 Hz, $J^2$: 15.7 Hz, 2F, *m*- $C_6F_5$), -156.8 (t, *J*: 21.8 Hz, 1F, *p*-$C_6F_5$), - 152.2 (d, *J*: 16.3 Hz, 2F, *o*-$C_6F_5$).

**1c:** Yellow powder, 0.275 g, Yield: 32.0%, m.p.: 179-181 ºC, IR (ν cm$^{-1}$): 3224, 2963, 2815, 1658, 1564. Anal. Calc. for $C_{18}H_{23}N_3O_2S$: C, 62.58; H, 6.71; N, 12.16; found: C, 62.54; H, 6.70; N, 12.14. $^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm): 1.26-1.34 (m, 6H, NCH$_2$C*H*$_3$), 2.28 (s, 6H, $C_6H_2$-2,6-C*H*$_3$), 2.29 (s, 3H, $C_6H_2$-4-C*H*$_3$), 3.88 (s, 3H, $C_6H_2$-6-C*H*$_3$), 4.50-4.58 (m, 4H, NC*H*$_2$CH$_3$), 6.94 (s, 2H, $C_6H_2$-2,4,6-CH$_3$), 8.24-8.28 (d, *J*: 14.5 Hz, 1H, C=C*H*), 11.74-11.78 (d, *J*: 14.1 Hz, 1H, *H*NCH). $^{13}$C NMR (100 MHz, $CDCl_3$, δ ppm): 12.3 (NCH$_2$*C*H$_3$), 12.4 (NCH$_2$*C*H$_3$), 18.3 ($C_6H_2$-2,4,6-*C*H$_3$), 20.9 ($C_6H_2$-2,4,6-*C*H$_3$), 42.3 (N*C*H$_2$CH$_3$), 42.9 (N*C*H$_2$CH$_3$), 93.8 (CH=*C*), 129.8, 132.1, 134.1, 137.0, 152.6, 154.3 (*C*$_6$H$_2$-2,4,6-CH$_3$), 160.1 (*C*H=C), 160.9 (*C*=O), 163.2 (*C*=O), 178.9 (*C*=S).

**1d:** Orange powder, 1.5 g, Yield: 79.0%, m.p.: 195-197 ºC, IR (ν cm$^{-1}$): 3277, 3155, 2907, 2851, 1679, 1588. Anal. Calc. for $C_{15}H_{17}N_3O_3S$: C, 56.41; H, 5.37; N, 13.16; found: C, 56.40; H, 5.35; N, 13.13. $^{1}$H NMR (400 MHz, DMSO-d6, δ ppm): 1.14-1.18 (m, 3H, NCH$_2$C*H*$_3$), 1.18-1.21 (m, 3H, NCH$_2$C*H*$_3$), 4.37-4.44 (m, 4H, NC*H*$_2$CH$_3$), 6.78-6.82 (m, 2H, $C_6H_4$-4-OH), 7.39-7.43 (m, 2H, $C_6H_4$-4-OH), 8.50-8.54 (d, *J*: 14.9 Hz, 1H, C=C*H*), 9.77 (s, 1H, $C_6H_4$-4-O*H*), 12.15-12.18 (d, *J*: 14.9 Hz, 1H, *H*NCH). $^{13}$C NMR (100 MHz, DMSO-d6, δ ppm): 12.2 (NCH$_2$*C*H$_3$), 12.3 (NCH$_2$*C*H$_3$), 41.7 (N*C*H$_2$CH$_3$), 42.3 (N*C*H$_2$CH$_3$), 93.1 (CH=*C*), 116.1, 120.8, 130.1, 153.0 (*C*$_6$H$_4$-4-OH), 156.5 (*C*H=C), 160.0 (*C*=O), 161.7 (*C*=O), 178.0 (*C*=S).

**1e:** Yellow powder, 1.1 g, Yield: 63.0%, m.p.: 244-246 ºC, IR (ν cm$^{-1}$): 3239, 2960, 2882, 1638, 1568. Anal. Calc. for $C_{15}H_{16}N_4O_4S$: C, 51.74; H, 4.63; N, 16.08; found: C, 51.76; H, 4.61; N, 16.04. $^{1}$H NMR (400 MHz, $CDCl_3$, δ ppm): 1.26-1.28 (m, 3H, NCH$_2$C*H*$_3$), 1.30-1.32 (m, 3H, NCH$_2$C*H*$_3$), 4.51-4.57 (qd, $J^1$: 7.0 Hz, $J^2$: 1.4

Hz, 4H, NC$H_2$CH$_3$), 7.79-7.42 (m, 2H, C$_6H_4$-4-NO$_2$), 8.31-8.35 (m, 2H, C$_6H_4$-4-NO$_2$), 8.73 (d, *J*: 13.7 Hz, 1H, C=C*H*), 12.44 (d, *J*: 13.3 Hz, 1H, *H*NCH). $^{13}$C NMR (100 MHz, CDCl$_3$, δ ppm): 12.3 (NCH$_2$*C*H$_3$), 12.4 (NCH$_2$*C*H$_3$), 42.4 (N*C*H$_2$CH$_3$), 43.2 (N*C*H$_2$CH$_3$), 94.2 (CH=*C*), 110.0, 116.9, 120.0, 131.0, 153.0 (*C*$_6$H$_4$-4-NO$_2$), 155.3 (*C*H=C), 161.3 (*C*=O), 163.0 (*C*=O), 178.5 (*C*=S).

**General procedure for synthesis of M(II) complexes** (**2a-e**)

In a flask, the ligand (**1a-e,** 2.0 eq) was reflux with the [Pd($\mu$-OAc)$_2$(ppy)]$_2$ (0.150 g, 1.0 eq) in MeOH (10.0 mL) at 65 ºC for 24 h. At the end of this time, residue formed and the product was filtered in vacuo and washed pentane. The product was purified by CH$_2$Cl$_2$/pentane (Figure 2).

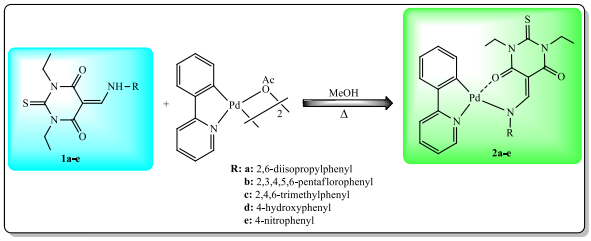



**Figure 2.** Synthesis of Pd complexes

**2a:** White powder, 0.169 g, Yield: 56.0%, m.p.: 280-282 ºC, IR (ν cm$^{-1}$): 3005, 2957, 2836, 1604, 1586. Anal. Calc. for C$_{32}$H$_{36}$N$_4$O$_2$PdS: C, 59.39; H, 5.61; N, 8.66; found: C, 59.40; H, 5.62; N, 8.64. $^1$H NMR (400 MHz, CDCl$_3$, δ ppm): 1.05 (d, *J*: 7.0 Hz, 6H, C$_6$H$_3$-2-CH(C*H*$_3$)$_2$), 1.16 (d, *J*: 7.0 Hz, 6H, C$_6$H$_3$-6-CH(C*H*$_3$)$_2$), 1.31 (t, *J*: 6.9 Hz, 3H, NCH$_2$C*H*$_3$), 1.49 (t, *J*: 6.9 Hz, 3H, NCH$_2$C*H*$_3$), 3.49-3.56 (m, 2H, C$_6$H$_3$-2-C*H*(CH$_3$)$_2$), 4.54-4.59 (q, *J*: 7.0 Hz, 4H, NC*H*$_2$CH$_3$), 5.23 (d, *J*: 8.2 Hz, 1H, pyridyl-C*H*), 6.52-6.56 (td, $J^1$: 7.6 Hz, $J^2$: 1.6 Hz, 1H, C$_6$*H*$_3$-6-CH(CH$_3$)$_2$), 6.92 (t, *J*: 6.9 Hz, 1H, pyridyl-C*H*), 7.19-7.22 (m, 2H, pyridyl-C*H*), 7.29 (d, *J*: 7.0 Hz, 1H, pyridyl-C*H*), 7.33-7.35 (dd, $J^1$: 7.8 Hz, $J^2$: 1.6 Hz, 1H, C$_6$*H*$_3$-6-CH(CH$_3$)$_2$), 7.66 (d, *J*: 7.8 Hz, 1H, pyridyl-C*H*), 7.82-7.86 (td, $J^1$: 7.8 Hz, $J^2$: 1.6 Hz, 1H, C$_6$*H*$_3$-6-CH(CH$_3$)$_2$), 8.35 (s, 1H, C=C*H*), 8.87-8.89 (dd, $J^1$: 5.7Hz, $J^2$: 1.0 Hz, pyridyl-C*H*). $^{13}$C NMR (100 MHz, CDCl$_3$, δ ppm): 12.1 (NCH$_2$*C*H$_3$), 12.5 (NCH$_2$*C*H$_3$), 24.4 (C$_6$H$_3$CH(*C*H$_3$)$_2$), 27.9 (C$_6$H$_3$*C*H(CH$_3$)$_2$), 43.4 (N*C*H$_2$CH$_3$), 43.5 (N*C*H$_2$CH$_3$), 94.3 (CH=*C*), 118.6, 121.4, 123.1, 123.9 (pyridyl-*C*), 124.8, 127.1, 128.2, 129.4, 132.8 (*C*$_6$H$_3$-6-CH(CH$_3$)$_2$), 139.0, 142.1 (pyridyl-*C*), 143.9 (*C*$_6$H$_3$-6-CH(CH$_3$)$_2$), 145.7, 147.5, 148.2, 149.2, 160.6 (pyridyl-*C*), 161.4 (*C*H=C), 165.1 (*C*=O), 165.4 (*C*=O), 177.3 (*C*=S).

**2b:** White powder, 0.282 g, Yield: 92.0%, m.p.: 252-255 ºC, IR (ν cm$^{-1}$): 2987, 2845, 1627, 1569. Anal. Calc. for C$_{26}$H$_{19}$F$_5$N$_4$O$_2$PdS: C, 47.83; H, 2.93; N, 8.58; found: C, 47.82; H, 2.90; N, 8.56. $^1$H NMR (400 MHz, CDCl$_3$, δ ppm): 1.29 (t, *J*: 6.7 Hz, 3H, NCH$_2$C*H*$_3$), 1.43 (t, *J*: 6.9 Hz, 3H, NCH$_2$C*H*$_3$), 4.53-4.58 (q, 2H, *J*: 6.9 Hz, NC*H*$_2$CH$_3$), 4.66-4.71 (m, 2H, NC*H*$_2$CH$_3$), 5.79 (d, *J*: 8.2 Hz, 1H, pyridyl-C*H*), 6.75 (t, *J*: 7.6 Hz, 1H, pyridyl-C*H*), 7.01 (t, *J*: 7.4 Hz, 1H, pyridyl-C*H*), 7.22 (d, *J*: 7.4 Hz, 1H, pyridyl-C*H*), 7.38 (d, *J*: 7.8 Hz, 1H, pyridyl-C*H*), 7.68 (d, *J*: 7.8 Hz, 1H, pyridyl-C*H*), 7.88 (d, *J*: 7.8 Hz, 1H, pyridyl-C*H*), 8.42 (s, 1H, C=C*H*), 8.71 (d, *J*: 5.9 Hz, 1H, pyridyl-C*H*). $^{13}$C NMR (100 MHz, CDCl$_3$, δ ppm): 12.0 (NCH$_2$*C*H$_3$), 12.4 (NCH$_2$*C*H$_3$), 43.5 (N*C*H$_2$CH$_3$), 43.7 (N*C*H$_2$CH$_3$), 96.4 (CH=*C*), 118.9, 121.9, 123.8, 125.2, 129.1, 131.1, 139.3, 145.8, 147.5, 149.5, 161.1 (pyridyl-*C*), 161.2 (*C*H=C), 165.3 (*C*=O), 169.3 (*C*=O), 177.5 (*C*=S).

**2c:** Pale yellow powder, 0.120 g, Yield: 42.0%, m.p.: 280-282 ºC, IR (ν cm$^{-1}$): 2957, 2891, 1622, 1571. Anal. Calc. for C$_{29}$H$_{30}$N$_4$O$_2$PdS: C, 57.57; H, 5.00; N, 9.26; found: C, 57.59; H, 4.98; N, 9.24. $^1$H NMR (400 MHz, CDCl$_3$, δ ppm): 1.26-1.29 (m, 3H, NCH$_2$C*H*$_3$), 1.30-1.32 (m, 3H, NCH$_2$C*H*$_3$), 2.07 (s, 3H, C$_6$H$_2$-4-C*H*$_3$),3.83 (s, 3H, C$_6$H$_2$-2-C*H*$_3$), 3.88 (s, 3H, C$_6$H$_2$-6-C*H*$_3$), 4.52-4.67 (q, *J*: 7.0 Hz, 4H, NC*H*$_2$CH$_3$), 5.30-5.32 (dd, $J^1$: 8.0 Hz, $J^2$: 1.0 Hz, 1H, pyridyl-C*H*), 6.57-6.61 (td, $J^1$: 7.8 Hz, $J^2$: 1.6 Hz, 1H, pyridyl-C*H*), 6.92 (s, 2H, C$_6$*H*$_2$-2,4,6-CH$_3$), 6.93-6.97 (td, $J^1$: 7.4 Hz, $J^2$: 0.8 Hz, 1H, pyridyl-C*H*), 7.19-7.23 (td, $J^1$: 7.3 Hz, $J^2$: 5.8 Hz, $J^3$: 1.4 Hz, 1H, pyridyl-C*H*), 7.35-7.37 (dd, $J^1$: 7.8 Hz, $J^2$: 1.6 Hz, 1H, pyridyl-C*H*), 7.66-7.68 (d, *J*: 7.8 Hz, 1H, pyridyl-C*H*), 7.82-7.86 (td, $J^1$: 7.8 Hz, $J^2$: 1.6 Hz, 1H, pyridyl-C*H*), 8.31 (s, 1H, C=C*H*), 8.86-8.87 (d, $J^1$: 5.0 Hz, $J^2$: 1.0 Hz, 1H, pyridyl-C*H*). $^{13}$C NMR (100 MHz, CDCl$_3$, δ ppm): 12.1 (NCH$_2$*C*H$_3$), 12.5 (NCH$_2$*C*H$_3$), 19.1 (C$_6$H$_2$-2,4,6-*C*H$_3$), 20.9 (C$_6$H$_2$-2,4,6-*C*H$_3$), 43.4 (N*C*H$_2$CH$_3$), 43.6 (N*C*H$_2$CH$_3$), 94.9 (CH=*C*), 118.6, 121.5, 123.2, 124.9, 128.6 (pyridyl-*C*), 129.2, 131.5, 131.6, 135.7 (*C*$_6$H$_2$-2,4,6-CH$_3$), 139.0, 145.8, 147.4, 148.1 (pyridyl-*C*), 148.7, 160.5 (*C*$_6$H$_2$-2,4,6-CH$_3$), 161.6 (*C*H=C), 165.3 (*C*=O), 165.4 (*C*=O), 177.2 (*C*=S).

**2d:** Brown powder, 0.204 g, Yield: 75.0%, m.p.: 264-265 ºC, IR (ν cm$^{-1}$): 3204, 2901, 2875, 1623, 1556. Anal. Calc. for C$_{26}$H$_{24}$N$_4$O$_3$PdS: C, 53.94; H, 4.18; N, 9.68; found: C, 53.92; H, 4.17; N, 9.65. $^1$H NMR (400 MHz, CDCl$_3$, δ ppm): 1.16-1.27 (m, 6H, NCH$_2$C*H*$_3$), 4.37-4.59 (m, 4H, NC*H*$_2$CH$_3$), 5.80-5.82 (d, *J*: 7.8 Hz, 1H, pyridyl-C*H*), 6.56-6.58 (m, 1H, C$_6$*H*$_4$-4-OH), 6.68-6.70 (d, *J*: 9.0 Hz, 1H, pyridyl-C*H*), 6.89-6.93 (d, *J*: 7.0 Hz,

2H, pyridyl-C*H*), 7.25-7.27 (d, *J*: 8.6 Hz, 1H, $C_6H_4$-4-OH), 7.45-7.47 (m, 2H, pyridyl-C*H*), 7.54-7.56 (dd, $J^1$: 7.8 Hz, $J^2$: 1.6 Hz, 1H, $C_6H_4$-4-OH), 7.99- 8.08 (m, 2H, $C_6H_4$-4-OH), 8.28 (s, 1H, C=C*H*), 8.61-8.63 (d, *J*: 5.5 Hz,1H, pyridyl-C*H*), 9.58 (s, 1H, $C_6H_4$-4-O*H*). $^{13}$C NMR (100 MHz, $CDCl_3$, δ ppm): 12.1 ($NCH_2CH_3$), 12.2 ($NCH_2CH_3$), 38.9 ($NCH_2CH_3$), 40.1 ($NCH_2CH_3$), 94.7 (CH=*C*), 115.2, 116.1, 122.9, 123.6 (pyridyl-*C*), 124.5, 125.7, 128.3, 134.0 ($C_6H_4$-4-OH), 140.3, 145.2, 145.6, 147.3, 150.5, 156.0 (pyridyl-*C*), 160.5 (*C*H=C), 163.4 (*C*=O), 164.3 (*C*=O), 176.3 (*C*=S).

**2e:** Yellow powder, 0.203 g, Yield: 71.0%, m.p.: 270-272 ºC, IR (ν $cm^{-1}$): 2988, 2835, 1609, 1558. Anal. Calc. for $C_{26}H_{23}N_5O_4PdS$: C, 51.36; H, 3.81; N, 11.52; found: C, 51.38; H, 3.80; N, 11.51. $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 1.28-1.32 (t, *J*: 7.0 Hz, 3H, $NCH_2CH_3$), 1.40-1.44 (t, *J*: 7.0 Hz, 3H, $NCH_2CH_3$), 4.55-4.60 (q, *J*: 6.8 Hz, 2H, $NCH_2CH_3$), 4.72-4.74 (q, *J*: 6.8 Hz, 2H, $NCH_2CH_3$), 5.79-5.82 (dd, $J^1$: 8.0 Hz, $J^2$: 1.0 Hz, 1H, pyridyl-C*H*), 6.57-6.61 (td, $J^1$: 7.6 Hz, $J^2$: 1.6 Hz, 1H, $C_6H_4$-4-$NO_2$), 6.92-6.96 (td, $J^1$: 7.4 Hz, $J^2$: 0.8 Hz, 1H, pyridyl-C*H*), 7.24-7.28 (m, 2H, pyridyl-C*H*), 7.34-7.36 (dd, $J^1$: 7.6 Hz, $J^2$: 1.4 Hz, 1H, $C_6H_4$-4-$NO_2$), 7.69-7.72 (m, 2H, pyridyl-C*H*), 7.88-7.93 (td, $J^1$: 7.8 Hz, $J^2$: 1.6 Hz, 1H, $C_6H_4$-4-$NO_2$), 8.16- 8.18 (m, 2H, $C_6H_4$-4-$NO_2$), 8.60 (s, 1H, C=C*H*), 8.71-8.72 (m, 1H, pyridyl-C*H*). $^{13}$C NMR (100 MHz, $CDCl_3$, δ ppm): 12.1 ($NCH_2CH_3$), 12.5 ($NCH_2CH_3$), 43.5 ($NCH_2CH_3$), 43.8 ($NCH_2CH_3$), 96.3 (CH=*C*), 118.9, 121.9, 123.4, 124.5 (pyridyl-*C*), 124.8, 126.1, 129.1, 134.6 ($C_6H_4$-4-$NO_2$), 139.4, 145.4, 145.6, 147.5, 150.5, 159.2, 161.4 (pyridyl-*C*), 161.5 (*C*H=C), 165.3 (*C*=O), 165.7 (*C*=O), 177.3 (*C*=S).

## Catalytic activity of complexes (2a-e)

We examined the catalytic activity of **2a-e**, in the Suzuki-Miyaura cross-coupling reaction. Under an argon atmosphere, a two-necked 25 mL flask fitted with a reflux condenser and septum was charged with aryl bromide (0.5 mmol), phenylboronic acid (0.75 mmol), $Cs_2CO_3$ (1.5 mmol), diethyleneglicoldi-*n*-butyl ether (0.3 mmol, internal standard) and the Pd(II) catalyst (1 mol%) in 5 mL of 2-propanol were added. The flask was placed in a preheated oil bath (80 ºC). The reaction was followed by GC at selected period of times (1 h, 2 h, 4 h, 24 h etc.). After the sample was taken from flask, 2-propanol (2.0 mL) was added in a mixture, centrifuged and the solution was transferred to vials. The yields of the product obtained (based on the aryl bromide) were estimated by the area of GC chromatogram peaks.

## RESULTS and DISCUSSION

The FT-IR spectrum of the ligands (**1a-e**) and complexes (**2a-e**) exhibited three marked peaks corresponding to ν(C=O) and ν(C=S). They were observed at 1690-1550 $cm^{-1}$. The ν(N–H) stretching frequencies were assigned between 3200-3280 $cm^{-1}$ for compounds. All IR spectra of **1a-e** and **2a-e** showed sharp peaks at around 3000-2800 $cm^{-1}$ which were assigned to ν(C–H) stretches of the barbiturates. The thiobarbiturate derivatives (**1a-e**) and their cyclopalladated complexes (**2a-e**) were characterized by elemental analysis, $^1$H-, $^{13}$C-NMR and FT-IR spectroscopy. There are two specific peaks on the ligands which are called enamine N*H* and C*H* protons. C*H* protons were observed as doublet between 8.18-8.73 ppm for **1a-e**, respectively. The chemical shifts of enamine N*H* were determined at 11.79, 12.34, 11.76, 12.17 and 12.44 ppm as a doublet. The enamine N*H* proton signals of complexes disappeared in the $^1$H-NMR spectra. The absence of the enamine NH proton peaks at the region 11.00-13.00 ppm can be interpreted as deprotonation of ligands by acetate of Pd salt in the $^1$H-NMR spectrum of complexes. The vinylic C*H* protons of the Pd(II) complexes gave singlets between 8.28-8.60 ppm, respectively. The $^{13}$C-NMR peaks at 93.8, 97.4, 93.8, 93.1 and 94.2 ppm for **1a-e**, 93.0 and 94.3, 96.4, 94.9, 94.7 and 96.3 ppm for **2a-e** were assigned to the vinylic carbon of enamine. The carbonyl carbons peak of compounds was assigned between 159.0 and 166.0 ppm in the $^{13}$C-NMR spectrum. In this work, we examined the catalytic activity of Pd(II) complexes bearing thiobarbiturate derivatives. Synthesized cyclopalladated complexes exhibited moderately catalytic activity when activated aryl bromide was used as substrate. When 4-bromoacetophenone was used as an activated substrate, the reactions produced a high yield at 80 ºC and 24 h. (Table 1, Entry 11-15). Furthermore, the complexes showed low catalytic activity with the other assayed substrates, 4-bromobenzene and 4-bromotoluene, giving low yields of the corresponding biaryl products in 24 h of reaction.

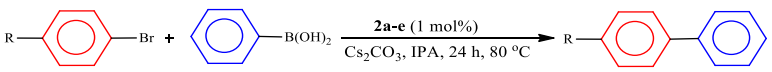
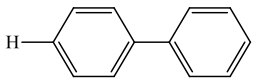
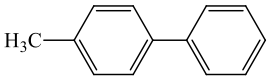
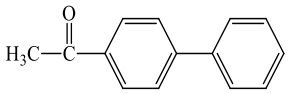


**Table 1.** Suzuki-Miyaura reactions with different substituents catalyzed by Pd(II) complexes

| Entry | Product | [Pd] | Yield [b,c] |
|---|---|---|---|
| 1 |  | 2a | 72 |
| 2 | | 2b | 52 |
| 3 | | 2c | 59 |
| 4 | | 2d | 30 |
| 5 | | 2e | 51 |
| 6 |  | 2a | 30 |
| 7 | | 2b | 35 |
| 8 | | 2c | 45 |
| 9 | | 2d | 21 |
| 10 | | 2e | 35 |
| 11 |  | 2a | 85 |
| 12 | | 2b | 89 |
| 13 | | 2c | 86 |
| 14 | | 2d | 89 |
| 15 | | 2e | 85 |

[a] *Reagents*: an aryl bromide (0.50 mmol), $PhB(OH)_2$ (0.75 mmol), $Cs_2CO_3$ (1.50 mmol), diethyleneglicol di-*n*-butyl ether (0.3 mmol, internal standard), catalyst (1 mol %), and 2-propanol (3.0 mL).
[b] Yields based on the aryl bromide and average of two runs.
[c] All reactions were followed by GC.

## CONCLUSION

In this study, new air- and moisture-stable thiobarbiturate ligands (**1a-e**) and their cyclopalladated complexes (**2a-e**) were synthesized and characterized. The catalytic activities of the complexes were tested for the Suzuki coupling reactions. They reached good yields in the Suzuki-Miyaura coupling reaction of phenylboronic acid with activated aryl bromide within 24 h. The cyclic palladium complexes (**2a-e**) were a less effective catalyst that used inactivated/nonactivated aryl bromide as a substrate for Suzuki-Miyaura coupling reactions.

## ACKNOWLEDGEMENTS

This work was supported by the "Scientific Research Project Unit (BAP) of Aydın Adnan Menderes University" (Project No: FEF-20013).

## REFERENCES

Alacid E, Alonso DA, Botella L, Najera C, Pacheco M.C 2006. Oxime palladacycles revisited: stone-stable complexes nonetheless very active catalysts. Chem. Rec. 6: 117-132.

Balanta A, Godard C, Claver C 2011. Pd nanoparticles for C–C coupling reactions. Chem. Soc. Rev., 40: 4973-4985.

Bandyopadhyay N, Das M, Samanta A, Zhu M, Lu L, Naskar JP 2017. Promising Antimicrobial Activity of an Oxime Based Palladium(II) Complex. ChemistrySelect, 2: 230-240.

Beletskaya IP, Cheprakov A.V 2004. Palladaycycles in catalysis-A critical survey. J. Organomet. Chem., 689: 4055-4082.

Beletskaya IP, Cheprokov A.V 2000. The Heck Reaction as a Sharpening Stone of Palladium Catalysis. Chem. Rev., 100: 3009-3066.

Castineiras A, Fernandez-Hermida N, García-Santos I, Gomez-Rodríguez L 2012. Neutral NiII, PdII and PtII ONS-pincer complexes of 5-acetylbarbituric-4Ndimethylthiosemicarbazone: synthesis, characterization and properties, Dalton Trans. 41: 13486-13495.

Chinchilla R, Najera C 2007. The Sonogashira Reaction: A Booming Methodology in Synthetic Organic Chemistry. Chem. Rev., 107: 874-922.

Colacio E, Dominguez-Vera JM, Costes JP, Kivekas R, Laurent JP, Ruiz J, Sundberg M 1992. Structural and magnetic studies of a syn-anti carboxylate-bridged helix-like chain copper(II) complex. Inorg. Chem., 31: 774-778.

Colacio E, Dominguez-Vera JM, Kivekas R, Ruiz J 1994. Preparation, crystal structures and magnetic properties of a syn-anti carboxylate-bridged dinuclear copper(II) complex and its precursor, a hydrogen-bonded polynuclear copper(II) complex. Inorg. Chim. Acta., 218: 109-116.

Colacio E, Ruiz J, Moreno JM, Kivekas R, Sundberg MR, Dominguez-Vera J.M, Laurent J.P 1993. Structural and magnetic studies of a zigzag chain copper(II) complex and a dipolar spin-coupled copper(II) dimer with the versatile ligand 5-[(2-carboxyphenyl)azo]-1,3-dimethylbarbituric acid. J. Chem. Soc., Dalton Trans., 157-163.

Das P, Linert W 2016. Schiff base-derived homogeneous and heterogeneous palladium catalysts for the Suzuki-Miyaura reaction. Coord. Chem. Rev., 311: 1-23.

Dorel R, Grugel CP, Haydl AM 2019. The Buchwald–Hartwig Amination After 25 Years. Angew. Chem. Int. Ed., 58: 17118-17129.

Dupont J, Consorti C.S, Spencer J 2005. The potential of palladacycles: more than just precatalysts. Chem. Rev., 105: 2527-2571.

Dupont J, Pfeffer M 2008. Palladacycles: Synthesis, Characterization and Applications. Wiley-VCH, Weinheim, 2008.

Figueiredo J, Serrano JL, Cavalheiro E, Keurulainen L, Yli-Kauhaluoma J, Moreira VM, Almeida P 2018. Trisubstituted barbiturates and thiobarbiturates: Synthesis and biological evaluation as xanthine oxidase inhibitors, antioxidants, antibacterial and anti-proliferative agents. Eur. J. Med. Chem., 143: 829-842.

Figueiredo J, Serrano Jl, Cavalheiro E, Keurulainen L, Yli-Kauhaluoma J, Moreira Vm, Almeida P 2018. Tri Substituted Barbiturates And Thiobarbiturates: Synthesis And Biological Evaluation As Xanthine Oxidase Inhibitors, Antioxidants, Antibacterial And Anti-Proliferative Agents. Eur. J. Med. Chem., 143:829.

Fırıncı E, Giziroglu E, Celepci DB, Soyleyici HC, Aygün M 2017. Spiro copper(II)-metallacycles derived from 5-arylaminomethylene-barbituric acids: synthesis and structural characterization. J. Mol. Struct., 1137: 113-118.

Fırıncı R, Fırıncı E, Basbülbül G, Dabanca MB, Celepci BD, Günay ME 2019. Enamines of 1,3-dimethylbarbiturates and their symmetrical palladium(II) complexes: synthesis, characterization and biological activity. Transit. Met. Chem., 44: 391-397.

Garoufis A, Hadjikakou SK, Hadjiliadis N 2009. Palladium coordination compounds as anti-viral, anti-fungal, anti-microbial and anti-tumor agents. Coord. Chem. Rev., 253: 1384-1397.

Karcı F, Karcı F 2008. The synthesis and solvatochromic properties of some novel heterocyclic disazo dyes derived from barbituric acid. Dyes Pigm. 77: 451-456.

Khan H, Badshah A, Said M, Murtaza G, Sirajuddin M, Ahmad J, Butler I.S 2016. Synthesis, structural characterization and biological screening of heteroleptic palladium(II) complexes. Inorg. Chim. Acta., 447: 176-182.

Kulsoom U, Salar U, Khan KM, Chigurupati S, Syed S,·Wadood A, Rehman AU, Fatima B, Saleem F, Taha M,·Felemban SG,·Dachani SR, Perveen S 2022. Synthesis and evaluation of aryl amino methylene substituted barbiturates and thiobarbiturates as novel $\alpha$-amylase inhibitors and radical scavengers. Monatshefte für Chemie - Chemical Monthly, 153:949-960.

Kumbhar A 2017. Functionalized nitrogen ligands for palladium catalyzed cross coupling reactions (part I). J. Organomet. Chem., 848: 22-88.

Kumbhar A 2019. Functionalized nitrogen ligands (C-N) for palladium catalyzed cross-coupling reactions (part II). J. Organomet. Chem., 881: 79-129.

Liu BB, Wang XR, Guo ZF, Lu ZL 2010. Mononuclear versus dinuclear palladacycles derived from 1, 3-bis(N, N-dimethylaminomethyl)benzene:structures and catalytic activity. Inorg. Chem. Commun., 13: 814-817.

Mahmudov KT, Guedes da Silva MFC, Glucini M, Renzi M, Gabriel KCP, Kopylovich MN, Sutradhar M, Marchetti F, Pettinari C, Zamponi S, Pombeiro AJL 2012. Water-soluble heterometallic copper(II)-

sodium complex comprising arylhydrazone of barbituric acid as a ligand. Inorg. Chem. Commun., 22: 187-189.

Mahmudov KT, Kopylovich MN, Maharramov AM, Kurbanova MM, Gurbanov AV, Pombeiro AJL 2014. Barbituric acids as a useful tool for the construction of coordination and supramolecular compounds. Coord. Chem. Rev., 265:1-37.

Mahmudov KT, Kopylovich MN, Pombeiro AJL 2013. Coordination chemistry of arylhydrazones of methylene active compounds. Coord. Chem. Rev., 257:1244-1281.

Maitlis PM 1971. The Organic Chemistry of Palladium, Volume I Metal Complexes. Academic Press, New York and London.

Miyaura N, Suzuki A 1995. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. Chem. Rev., 95: 2457-2483.

Molnar A 2011. Efficient, Selective, and Recyclable Palladium Catalysts in Carbon−Carbon Coupling Reactions. Chem. Rev., 111: 2251-2320.

Muthiah PT, Hemalini M, Bocelli G 2007. Hydrogen-bonded supramolecular motifs in crystal structures of trimethoprim barbiturate monohydrate (I) and 2-amino-4,6-dimethylpyrimidinium barbiturate trihydrate (II). Struct Chem., 18:171-180.

Navarro O, Marion N, Oonishi Y, Kelly RA, Nolan SP 2006. Suzuki−Miyaura, α-Ketone Arylation and Dehalogenation Reactions Catalyzed by a Versatile N-Heterocyclic Carbene−Palladacycle Complex. J. Org. Chem., 71: 685-692.

Rajmane A, Jadhav S, Kumbhar A 2022. N,O-polydentate ligands for palladium-catalyzed cross-coupling reactions (Part III). Journal of Organometallic Chemistry, 957: 122147.

Ruiz-Castillo P, Buchwald SL 2016. Applications of Palladium-Catalyzed C–N Cross-Coupling Reactions. Chem. Rev., 116: 12564-12649.

Salas PF, Herrmann C, Orvig C 2013. Metalloantimalarials. Chem. Rev., 113: 3450-3492.

Shafiq N, Arshad U, Zarren G, Parveen S, Javed I, Ashraf A 2020. A Comprehensive Review: Bio-Potential of Barbituric Acid and its Analogues. Curr. Org. Chem., 24:129-161.

Snelders JMD, van Koten G, Gebbink RJMK 2011. Steric, Electronic, and Secondary Effects on the Coordination Chemistry of Ionic Phosphine Ligands and the Catalytic Behavior of Their Metal Complexes. Chem. Eur J., 17: 42-57.

Tsuji J 1995. Palladium Reagents and Catalysts: Innovation in Organic Synthesis. Wiley, Chichester.

Weigand W, Plener V, Nöth H, Krossing I, Knizek J, Schmidt M 1998. Functionalized 1,1-Ethene Dithiolates as Ligands, Synthesis and Crystal Structure of Palladium(II) and Platinum(II) Complexes with Dithioylidene Barbituric Acid Ligands. Molecular Structure of a 2,6-Diaminopyridine-Platinum(II) Barbiturate Complex. Zeitschrift für Naturforschung, 53 (10): 1135-1143.

Yilmaz VT, Aksoy MS, Sahin O 2009. Different Coordination Modes Of 5,5-Diethylbarbiturate In The Copper(Ii) Complexes With Some Aliphatic Amines: Synthesis, Spectroscopic, Thermal And Structural Studies. Inorg. Chim. Acta., 361: 3703-3707.

Zim D, Nobre SM, Monteiro AL 2008. Suzuki cross-coupling reaction catalyzed by sulfur-containing palladacycles: formation of palladium active species. J. Mol. Catal. A Chem., 28: 16-23.

## *ORAL PRESENTATION*

# Synthesis, Characterization of Investigation of Photophysical Properties of {N′,N′′-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N′′}borane dichloride

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## Abstract

In this study, {N′,N′′-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N′′}borane dichloride was synthesized *via* the reaction of N,N-Dimethylaminoborane dichloride with N′,N′′-Diisopropylcarbodiimide and characterized by using elemental analysis, ATR FTIR and $^{1}H$, $^{11}B$ and $^{13}C$ NMR spectroscopy. The optical features of the prepared compound were examined by using ultraviolet-visible absorption and fluorescence emission spectroscopies. The reactions of {N′,N′′-Diisopropyl-N,N-dimethylguanidinato-κ2N′,N′′}borane dichloride with an excess of Li, Na, K, and Na/K alloy were examined to reduce of the borane dichloride derivative. The reduction product was not obtained from the reactions.

**Keywords:** Boron compound, carbodiimide, spectroscopy.

## INTRODUCTION

N-heterocyclic carbenes (NHC's) have been known since 1970 but they have become the more popular ligands after the first stable carbene was prepared by Arduengo et al. (1991). Numberless studies have been done about main property such as ring size, electronic and steric features of NHC's (Din Reshi and Bera, 2020). Also, NHC's and corresponding metal complexes have been prepared and examined their biological and catalytic activities (Voloshkin et al., 2021). Five-membered NHC's and their metal complexes are widely prepared in organometallic chemistry although the ring size of NHC's expands from five to eight-membered (Dröge and Glorius, 2010). Intense experimental and theoretical studies have been performed to prepare NHC analogues of 13 group elements (Jones et al., 2006; Assay et al., 2011; Zhang and Su, 2021). Although the five-membered NHC analogue of boron has been isolated in 2006 (Segawa et al.), the four and six-membered NHC analogues of boron are still missing (Firinci et al., 2013). Four-membered N-heterocyclic boron compounds, which could be a precursor to prepare a four-membered NHC analogue of boron, can be obtained from the amidinate and guanidinate ligands. Carbodiimides are key reagents to prepare the amidinate and guanidinate derivatives. Boron guanidinate derivatives can be synthesized from both the reaction of boron trihalide with lithium amides and the insertion of carbodiimide into aminoboron dihalides (Findlater et al, 2006, 2008: Pierce et al., 2007, Fırıncı et. al., 2022). The carbodiimide insertions have the advantage of being facile and mostly result in high yields for the preparation of guanidinates. In this work, the guanidinate borane dichloride derivative was prepared from the insert of N′,N′′-Diisopropylcarbodiimide into the N-B bond of N,N-Dimethylaminoborane dichloride and characterized by elemental analysis, ATR FTIR and $^{1}H$, $^{11}B$ and $^{13}C$ NMR spectroscopy. The photophysical properties of the prepared compound were examined by ultraviolet-visible absorption and fluorescence emission spectroscopies. The reactions of {N′,N′′-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N′′}borane dichloride with an excess of Li, Na, K, and Na/K alloy were examined to reduce of the borane dichloride derivative.

## MATERIALS AND METHODS

All experiments were carried out under a dry argon atmosphere using standard Schlenk techniques. The glassware was flame-dried before use. Solvents were distilled from the appropriate drying agent and stored under a dry argon atmosphere. Unless otherwise noted, all starting materials were commercially available and were used without further purification. Tris(dimethylamino)boron and dimethylaminoboron dichloride were prepared according to the given literature procedures (Nöth and Abeler, 1968). The IR spectra were recorded with an ATR Spectrum-II, Perkin Elmer spectrometer. $^{1}H$, $^{11}B$ and $^{13}C$ NMR spectra were measured on a Varian 400 spectrometer (400, 128 and 100 MHz, respectively) in $CDCl_3$. Residual signals of the solvent were used as a reference for $^{1}H$ and $^{13}C$ NMR spectra; $^{11}B$ NMR spectra were referenced according to $Et_2O{\cdot}BF_3$.

Melting points were determined with a Stuart SMP30 melting point apparatus in open capillary tubes and were uncorrected. Elemental analyses were done on a LECO CHNS-932 by METU Central Laboratory (Ankara, Türkiye).

**Experimental Section:**

**1. Synthesis of {N′,N′′-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N′′}borane dichloride**

$Me_2NBCl_2$ (1 mmol, 125 mg,) was added dropwise to a solution of N′,N′′-Diisopropylcarbodiimide (1 mmol, 125 mg) in 10 mL hexane at room temperature, immediately white participate was formed in solution. After filtration and solvent removal, the product was isolated as a white solid (Figure 1).

Yield 226 mg (90%). m. p.: 118-120 °C. IR (ν $cm^{-1}$): 2979, 2938, 1622, 1445, 1426, 1351, 1235, 1128, 1065, 1007, 929, 776, 744, 686, 645. Anal. Calc. for $C_9H_{20}BCl_2N_3$: C, 42.90; H, 8.00; N, 16.68; found: C, 42.87; H, 7.94; N, 16.69. $^1$H NMR (400 MHz, $CDCl_3$, δ ppm): 1.30 (2H, d, *j*= 6,7 *Hz*, $CH(CH_3)_2$), 3.03 (6H, s, $N(CH_3)_2$), 3.74 (2H, m, $CH(CH_3)_2$). $^{13}$C NMR (100 MHz, $CDCl_3$, δ ppm): 46.4 ($CH(CH_3)_2$), 38.3 ($N(CH_3)_2$), 21.2 ($CH(CH_3)$). $^{11}$B NMR (128 MHz, $CDCl_3$, δ ppm): 4.8.

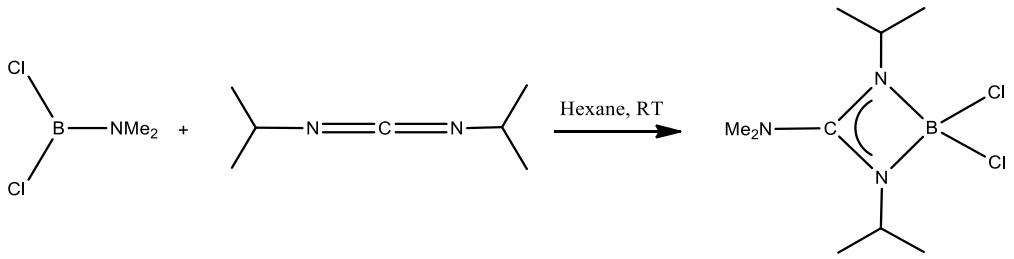


**Figure 1.** Synthesis of {N′,N′′-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N′′}borane dichloride

**RESULTS and DISCUSSION**

The boron guanidinate derivative was characterized with FT-IR, $^1$H, $^{11}$B and $^{13}$C NMR spectroscopy. The FT-IR spectrum of the compound indicates that the ν(C-H) stretching frequencies were observed at 2938 and 2979 $cm^{-1}$. The characteristic C=N frequencies appeared 1618 $cm^{-1}$ (Figure 2).

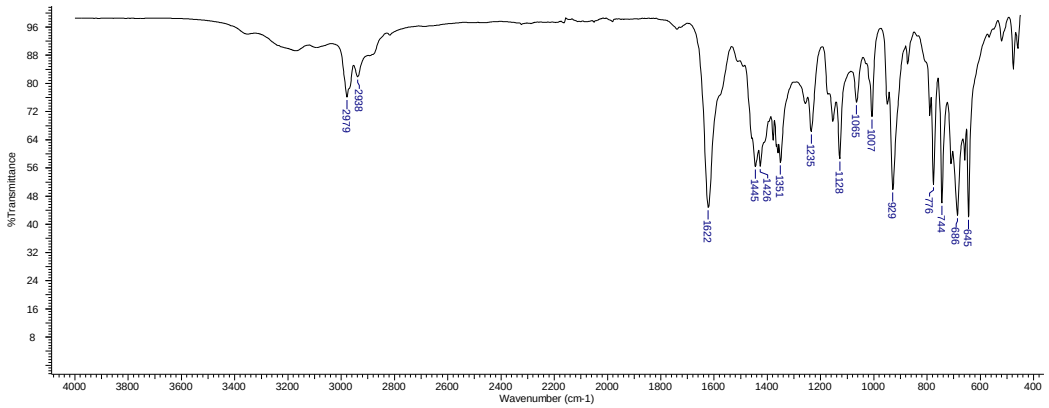


**Figure 2.** IR spectrum of {N′,N′′-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N′′}borane dichloride

In the $^1$H NMR spectrum of {N′,N′′-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N′′}borane dichloride, The doublet peak at 1.30 ppm and the multiplet at 3.74 ppm were assigned as the protons of isopropyl groups. The protons of $NMe_2$ group appeared at 3.03 ppm as a singlet (Figure 3).

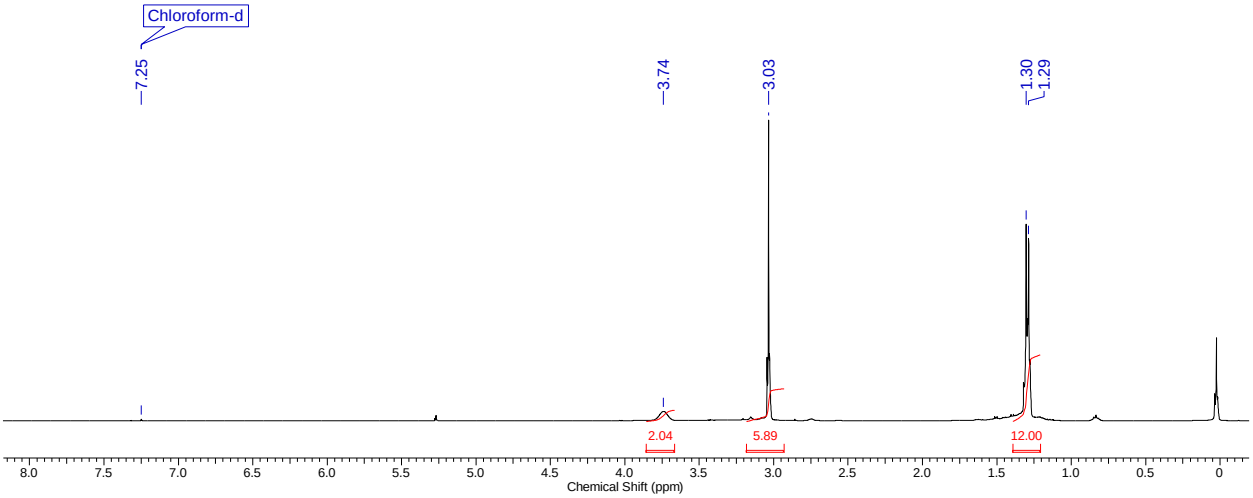


**Figure 3.** $^{1}$H NMR spectrum of {N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ$^{2}$N′,N″}borane dichloride

The *C*H and *C*H$_3$ carbons of isopropyl groups were observed at 46.4 and 21.2 ppm in the $^{13}$C NMR spectra, respectively. The signal at 38.3 ppm was assigned as the carbon atoms of NMe$_2$ group (Figure 4).

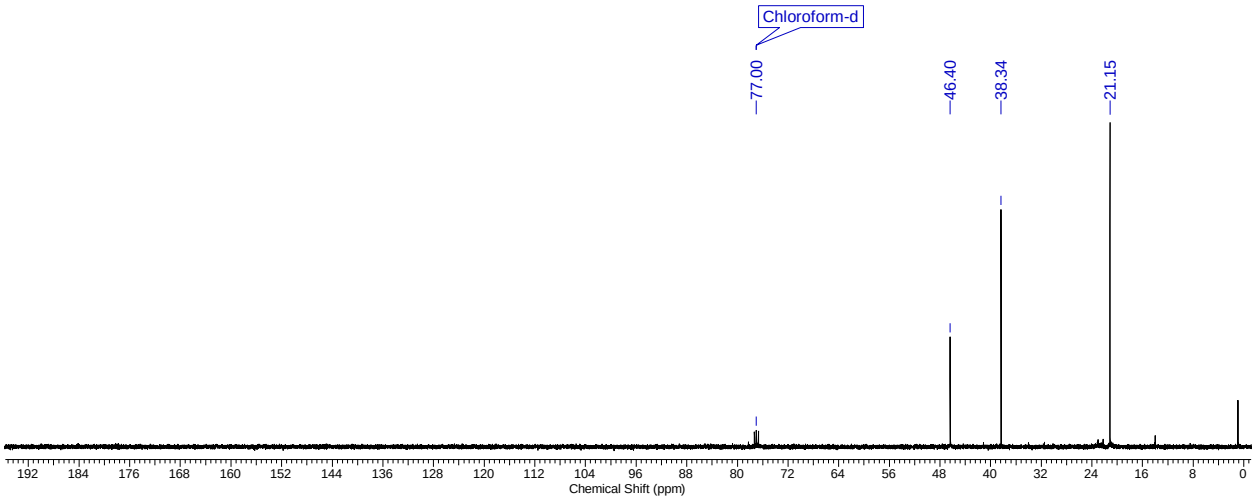


**Figure 4.** $^{13}$C NMR spectrum of {N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ$^{2}$N′,N″}borane dichloride

In the $^{11}$B NMR spectrum of {N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ$^{2}$N′,N″}borane dichloride, a sharp signal appeared at 4.8 ppm (Figure 5). The chemical shift is in a good agreement with four coordinate boron centre. The obtained NMR data are consistent with the related boron amidinate or guanidinate compounds (Findlater et al, 2006, 2008: Pierce et al., 2007, Fırıncı et al., 2022).

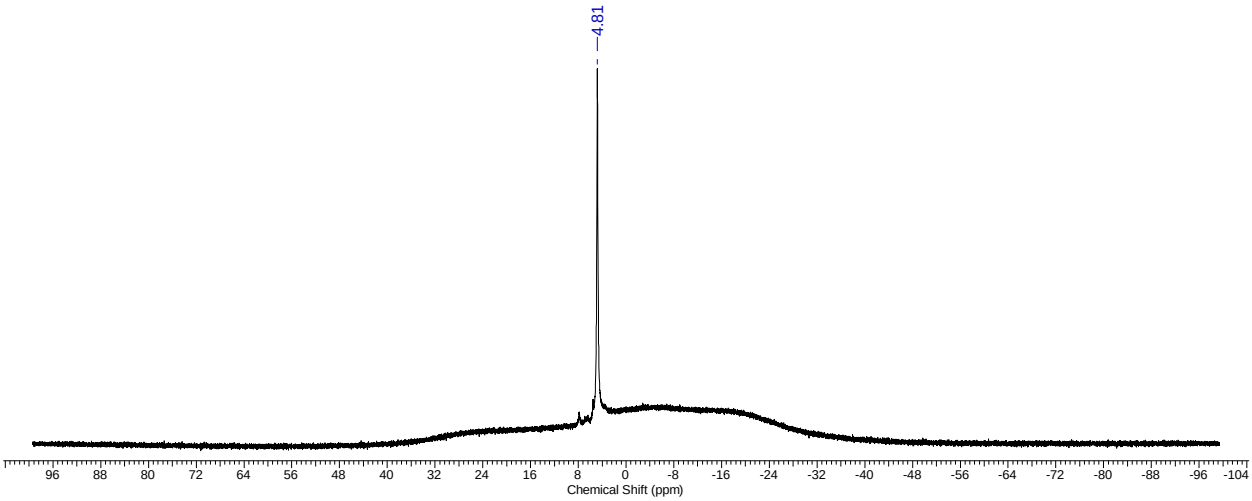


**Figure 5.** $^{11}$B NMR spectrum of {N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ$^2$N′,N″}borane dichloride

The photophysical properties of {N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ$^2$N′,N″}borane dichloride in $CHCl_3$ by UV-vis absorption and fluorescence emission spectroscopy. The absorption and fluorescence emission spectrums are given in Figure 6. The compound presents two main absorption bands ascribed to the π-π* transition. The maximum absorption peak ($\lambda_{abs}$) appeared at 320 nm in the absorption spectrum. The emission spectrum shows a broad emission peak at 400 nm ($\lambda_{ex}$= 320 nm).

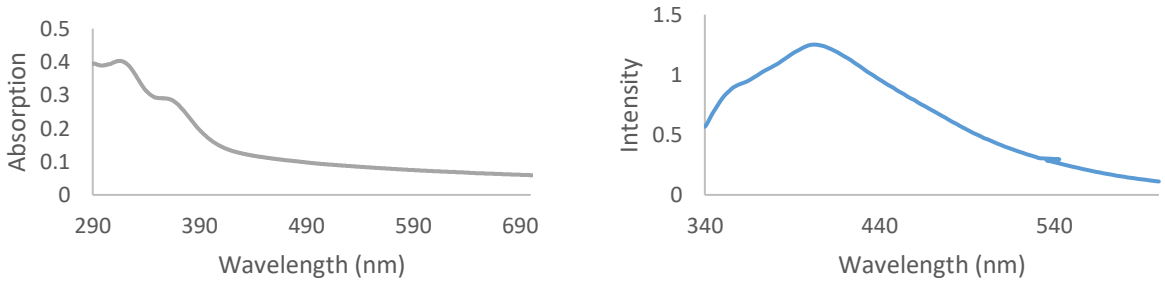


**Figure 6.** Absorption (left) and emission spectra (right) of {N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ$^2$N′,N″}borane dichloride ($\lambda_{ex}$= 320 nm) in $CHCl_3$ at room temperature

To generate four-membered N-heterocyclic borylene or its dimer, N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ$^2$N′,N″}borane dichloride was reacted with an excess of Li, Na, K, and Na/K alloy in diethyl ether, tetrahydrofuran or toluene. The $^{11}$B NMR spectrum showed that the desired products were not obtained from the reduction reactions (Figure 7).

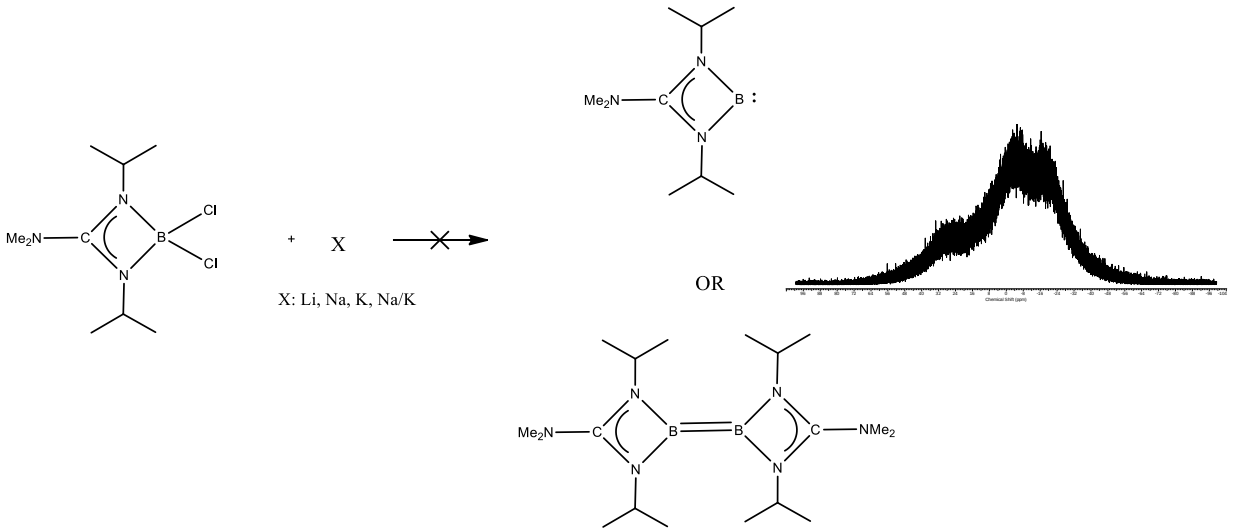


**Figure 7.** Reaction of {N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N″}borane dichloride with Li, Na, K, and Na/K

## CONCLUSION

{N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N″}borane dichloride was prepared from the insert reaction of N′,N″-Diisopropylcarbodiimide and N,N-Dimethylaminoborane dichloride with a good yield at room temperature. The boron compound was characterized by elemental analysis, ATR FTIR and $^{1}H$, $^{11}B$ and $^{13}C$ NMR spectroscopy. The optical features of the prepared compound were examined by using ultraviolet-visible absorption and fluorescence emission spectroscopies Attempts to reduce {N′,N″-Diisopropyl-N,N-dimethylguanidinato-κ²N′,N″}borane dichloride to four-membered N-heterocyclic borylene were not successful.

## ACKNOWLEDGEMENTS

This work was supported financially by The Scientific and Technological Research Council of Turkey (TUBITAK) for financial support (Grant No. 118Z492).

## REFERENCES

Arduengo AJ, Harlow RL, Kline M 1991. A stable crystalline carbene. J. Am. Chem. Soc., 113(1): 361-363.

Asay M, Jones C, Driess M 2011. N-Heterocyclic Carbene Analogues with Low-Valent Group 13 and Group 14 Elements: Syntheses, Structures, and Reactivities of a New Generation of Multitalented Ligands. Chem. Rev., 111(2): 354-396.

Din Reshi NU, Bera JK 2020. Recent advances in annellated NHCs and their metal complexes. Coordination Chemistry Reviews, 422: 213334.

Dröge T, Glorius F 2010. The Measure of All Rings—N-Heterocyclic Carbenes. Angew. Chem. Int. Ed., 49: 6940-6952.

Fırıncı E, Sevinçek R, Fırıncı R, Soyleyici S, Ceyhan E, Aygün M, Sahin Y 2022. Synthesis, characterization and DFT studies of boron guanidinate compounds. Inorganic Chemistry Communications, 140: 109485.

Findlater M, Hill N, Cowley A 2006. Amidinate-substituted boron halides: Synthesis and structure. Polyhedron, 25: 983-988.

Findlater M, Hill N, Cowley A 2008. Synthesis and structure of two new (guanidinate)boron dichlorides and their attempted conversion to boron(I) derivatives. Dalton Trans., 33: 4419-4423.

Firinci E, Bates JI, Riddlestone IM, Phillips N, Aldridge S 2013. Coordinative trapping of the boron β-diketiminato system [B(NMesCMe)2CH] via metal-templated synthesis. Chemical Communications, 49(15): 1509-1511.

Jones C, Junk PC, Platts JA, Stasch A 2006. Four-Membered Group 13 Metal(I) N-Heterocyclic Carbene Analogues: Synthesis, Characterization, and Theoretical Studies. J. Am. Chem. Soc.,128: 2206-2207.

Nöth H, Abeler G 1968. Beitrageazur Chemie des Bors, XL. Derivate des 1.3.2.4.5-Diazatriborols. Chem. Ber., 101: 969-980.

Pierce GA, Coombs ND, Willock DJ, Day JK, Staschb A, Aldridge S 2007. Insertion reactions of dicyclohexylcarbodiimide with aminoboranes,-boryls and –borylenes. Dalton Trans., 39 4405-4412.

Segawa Y, Yamashita M, Nozaki, K 2006. Boryllithium: Isolation, Characterization, and Reactivity as a Boryl Anion. Science, 314(5796):113-5.

Voloshkin VA, Tzourasa NV, Nolan SP 2021. Recent advances in the synthesis and derivatization of N-heterocyclic carbene metal complexes. Dalton Trans., 50: 12058-12068.

Zhang Z-F, Su M-D 2021. Insights into the Factors Controlling the Origin of Activation Barriers in Group 13 Analogues of the Four-Membered N-Heterocyclic Carbenes. ACS Omega, 6(34): 22272-22283.

## *ORAL PRESENTATION*

# Antimicrobial activity and toxicity determination of green synthesized ZnO nanoparticles

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**Abstract**

Considering the environmental and human health effects, cost, applicability and performance perspectives, the disadvantages of traditional and advanced nano-size production methods necessitate the research of new environmentally friendly methods with the same perspectives. Within the scope of this study, it was aimed to investigate the antimicrobial effect and toxicity of green-synthesized ZnO nanoparticles produced in the 10-100 nm size range with green tea *(Camellia sinensis*) leaf extract with high polyphenol content. The antibacterial activity of the green-synthesized ZnO nanoparticles was evaluated with their minimum inhibition concentration (MIC) against *Klebsiella pneumoniae, Escherichia coli, Bacillus cereus, Staphylococcus aureus and Staphylococcus faecalis*. Microtox cytotoxicity test was performed for the suitability of synthesized ZnO nanoparticles for use in environmental applications and ecotoxicity assessment by comparing them with commercial nano and micro sized ZnO particles. With green synthesis, the risks it may pose on the ecosystem and human health was eliminated, and ready-to-use nano-sized ZnO production was provided for various environmental applications.

**Keywords:** green production, nano-sized ZnO, environmental applications, antimicrobial activity, toxicity

## INTRODUCTION

Green synthesis has become a frequently used method today due to its advantages such as simple and economical processes, reducing toxic substances usage, and suitability for commercial production in a wide range. Green synthesized nanoparticles (NPs) are preferred to nanomaterials produced by physical or chemical methods because green synthesized NPs do not cause large capital, use of toxic solvents, formation of dangerous by-products, deterioration of the surface structure and many other problems such as reactivity, toxicity affecting human health and the environment in a harmful way (Drummer et al. 2020). NPs produced by the biogenic reduction of metal precursors can be used for environmentally friendly, sustainable, chemical-polluting, inexpensive and mass production. The green production of zinc oxide (ZnO) NPs using plants, microorganisms, algae and enzymes is ecologically favorable and sustainable compared to physical and chemical approaches for nanomaterial production. This environmentally friendly synthesis approach is an inexpensive and useful technique suitable for large-scale commercial production (Şenkal et al., 2019).

NPs can be produced from various plants, such as tea coffe, mint, moss, banana, eucalyptus, pineapple and mango, extracts containing polyphenols obtained by different methods (Demissie et al. 2020). In recent years, nano-sized ZnO has received increasing attention in potential environmental applications due to its unique optical, electrical and chemical properties (Mahalakshmi et al. 2019). The presence of a wide variety of nanostructures makes nano-sized ZnO an ideal material for optoelectronic and piezoelectric nanogenerators, biotechnology and antimicrobial processes for environmental applications (Ardakani et al. 2021). Various metal compound are mixed different plant extracts by determined volumetric ratio to produce green ZnO nanoparticles. Zinc compounds used in the synthesis of G-ZnO-NPs are zinc (II) nitrate, zinc sulphate and zinc(II) acetate (Ogunyemi et al. 2019). The percentage of Zn compound in total mix solution depends on polyphenols fraction in plant extract, and thus the volumetric ratio of Zn solution and plant extract must be investigated every plant extract to achieve nano-sized ZnO particle (Obied and Abd-Ali 2020). Considering the environmental-human health effects, cost, applicability and performance perspectives, the disadvantages of traditional and advanced nano-size production methods necessitate the research of new methods with the same perspectives. The most recent method of green metal oxide nano-particle synthesis is using of vegetable, fruit, and organic waste extracts by mixing certain proportion of an iron compound without the energy and any toxic chemical requirements (El-Belely et al. 2021). The green metal oxide nanoparticles production method

occurs with special reaction, which are defined as the specific color change between metal source with polyphenols found in plant extract in very short time at room temperature. Green metal oxide nano-particles are produced by this method from 10 to 100 nm size distribution. In comparison to published reports on the green production of metal oxide NPs and physical and chemical properties, very limited information is available on the antibacterial properties of metal NPs. In literature, green production of zinc oxide (ZnO) NPs using plants, microorganisms, algae and enzymes is ecologically favorable and sustainable compared to physical and chemical approaches for nanomaterial production.

Within the scope of this proposed study, it was aimed to investigate the antimicrobial effect and toxicity of green ZnO nanoparticles produced in the 10-100 nm size range with green tea *(Camellia sinensis)* leaf extract with high polyphenol content. The present study was carried out for synthesis and characterization of green zinc oxide nanoparticles (G-ZnO-NPs) using green tea leaves extracts. The antibacterial activity of G-ZnO-NPs was evaluated for the disinfection in the water and wastewater treatment. This study was aimed to investigate the antimicrobial and toxic effect of G-ZnO-NPs produced by green tea (*Camellia sinensis*) leaf extract.

## MATERIALS AND METHODS

### Minimum Inhibition Concentrations (MIC) of G-ZnO-NPs

The antimicrobial activity of synthesized G-ZnO-NPs was determined by the disc diffusion method. Antibacterial studies were performed in Hereous KSP-18 class II sterile cabinet in order to prevent external microbiological contaminations. All equipment and solutions have been sterilized within the Nuve 40 autoclave for a period of 20 min at 121°C. Gram-negative *Klebsiella pneumoniae, Escherichia coli* and Gram-positive *Bacillus cereus, Staphylococcus aureus and Staphylococcus faecalis* bacterial strains were used for antibacterial testing. Approximately 150 -200 µL bacterial solution with $2x10^6$ CFU/mL initial bacteria concentration was inoculated to these plates.

400 µg of commercial nano-sized ZnO (N- ZnO-NPs), micro-sized (M- ZnO, ) and synthesized green (G-ZnO-NPs) were weighed and placed in 1 ml distilled water obtaining 400 µg/mL stock solution. A serial dilution was obtained by dilution by half to 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL and 25 µg/mL. Then, using the Agar well diffusion method, 7 wells with a diameter of 10 mm were opened on the surface of the petri dish, on which plate count agar (PCA, Merck) is inoculated into solid nutrient medium. This procedure was repeated for each bacterium, adding 100 microliters of the positive control containing liquid nutrient broth, the negative control containing dimethyl sulfoxide (DMSO), and the dilutions containing ZnO solutions to each well. After inoculation of the samples, the plates were incubated at 37°C in Innova-42 shaker series incubator with 18–24 h. Lastly, the dimension of the bacterial inhibition zone occurred in the surface of plates was measured for the investigation of antibacterial activity. Three independent experiments were performed and the average results were given to illustrate the antibacterial activity. The concentration around which a zone of non-bacterial growth is observed will be reported as MIC.

### Toxicity of G-ZnO-NPs

Microtox cytotoxicity test was used to determine the toxicity level of commercial nano-sized ZnO (N-ZnO), micro-sized (M- ZnO) and synthesized green ZnO (G-ZnO-NPs). The test consists of the lyophilized bacterial reagent *Vibrio fischeri*, (NRRL number B-111177) as the test microorganism, the solutions required for the test run, and the Model 500 Toxicity Analyzer. In this bacterial toxicity experiment, which gave results in a short time, experimental studies were carried out according to the device procedure, which constitutes the instrument and the method, by taking double samples for each sample. Each test was constructed from a control sample and four serial dilutions of the sample. The toxic effects of the test result (with 95% confidence interval) will be recorded according to the light emission reduction percentage value of *Vibrio fischeri* incubated at 15°C, after 5 minutes and 15 minutes of exposure.

## RESULTS and DISCUSSION

Antibacterial disc diffusion test result of commercial M-ZnO and MIC value varying bacteria strain was given in the Table 1.

**Table 1.** Disc diffusion test result of M-ZnO and MIC value varying bacteria strain

| **Inhibition zone diameter (mm)/ ZnO Concentration** | **Gram negative** | | **Gram positive** | | |
|---|---|---|---|---|---|
| | ***Klebsiella pneumoniae*** | ***Escherichia coli*** | ***Bacillus cereus*** | ***Staphylococcus aureus*** | ***Staphylococcus faecalis*** |
| **25 µg/mL** | - | - | 18±1 | - | - |
| **50 µg/mL** | 15±1 | 12±1 | 22±2 | 12±1 | - |
| **100 µg/mL** | 18±1 | 13±1 | 24±2 | 14±1 | - |
| **200 µg/mL** | 20±2 | 14±1 | 24±2 | 15±1 | 12±1 |
| **400 µg/mL** | 22±2 | 15±1 | 26±2 | 16±1 | 14±1 |
| **MIC (µg/mL)** | 200 | >400 | 50 | >400 | >400 |

The commercial M-ZnO showed high antibacterial activity against gram-negative and gram-positive bacteria. The inhibition zone diameters of commercial M-ZnO, large zone diameters were observed against all bacteria strain referring high antibacterial effect because the zone diameters were bigger than 10 mm. MIC values of commercial M-ZnO were obtained as 200 µg/mL, >400 µg/mL, 50 µg/mL, >400 µg/mL and >400 µg/mL for Gram-negative *Klebsiella pneumoniae, Escherichia coli* and Gram-positive *Bacillus cereus, Staphylococcus aureus and Staphylococcus faecalis* bacterial strains respectively. Antibacterial disc diffusion test result of commercial N-ZnO-NPs and MIC value varying bacteria strain was given in the Table 2.

**Table 2.** Disc diffusion test result of commercial N-ZnO-NPs and MIC value varying bacteria strain

| **Inhibition zone diameter (mm)/ ZnO Concentration** | **Gram negative** | | **Gram positive** | | |
|---|---|---|---|---|---|
| | ***Klebsiella pneumoniae*** | ***Escherichia coli*** | ***Bacillus cereus*** | ***Staphylococcus aureus*** | ***Staphylococcus faecalis*** |
| **25 µg/mL** | 20±2 | - | 14±1 | - | 12±1 |
| **50 µg/mL** | 20±2 | 12±1 | 20±2 | 12±1 | 14±1 |
| **100 µg/mL** | 22±2 | 14±1 | 22±2 | 14±1 | 16±1 |
| **200 µg/mL** | 22±2 | 16±1 | 24±2 | 15±1 | 18±1 |
| **400 µg/mL** | 22±2 | 18±1 | 26±2 | 18±1 | 20±2 |
| **MIC (µg/mL** | 25 | 400 | 50 | 400 | 400 |

The commercial N-ZnO-NPs with 10-20 nm particle size showed high antibacterial activity against gram-negative and gram-positive bacteria. The inhibition zone diameters of N-ZnO-NPs, large zone diameters were observed against all bacteria strain referring high antibacterial effect because the zone diameters were bigger than 10 mm. MIC values of commercial N-ZnO-NPs were obtained as 25 µg/mL, 400 µg/mL, 50 µg/mL, 400 µg/mL and 400 µg/mL for Gram-negative *Klebsiella pneumoniae, Escherichia coli* and Gram-positive *Bacillus cereus, Staphylococcus aureus and Staphylococcus faecalis* bacterial strains respectively. It was stated that the MIC values of the commercial N-ZnO-NPs were lower than the commercial M-ZnO for both *Klebsiella pneumoniae* and *Bacillus cereus* strains Selvam et al. (2021), were studied Gram positive bacteria (*Staphylococcus aureus and Bacillus sp.*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella enterica* and *Vibrio sp.*) applications in antimicrobial activities in the study on the synthesis of CdO/ZnO-eggshell nanocomposites using eggshell as a reducing and stabilizing agent. In this study, CdO/ZnO- eggshell nanocomposites were successfully synthesized using eggshell as bio-template, reducing and stabilizing agents. The antimicrobial activity of the prepared CdO/ZnO-eggshell nanocomposites was tested against both Gram positive and Gram-negative bacteria by agar well diffusion method. Their study correlated the results obtained in this study for the commercial N-ZnO-NPs.

Antibacterial disc diffusion test result of G-ZnO-NPs and MIC value varying bacteria strain was given in the Table 3. The G-ZnO-NPs with 10-100 nm particle size showed high antibacterial activity aainst gram-negative and gram-positive bacteria. The inhibition zone diameters of G-ZnO-NPs, large zone diameters were observed against all bacteria strain referring high antibacterial effect because the zone diameters were bigger than 10 mm. MIC values of G-ZnO-NPs were obtained as 200 µg/mL, 400 µg/mL, 100 µg/mL, 400 µg/mL and 400 µg/mL for Gram-negative *Klebsiella pneumoniae, Escherichia coli* and Gram-positive *Bacillus cereus, Staphylococcus aureus and Staphylococcus faecalis* bacterial strains respectively. It was stated that the MIC values of the commercial G-ZnO-NPs were lower than the commercial N-ZnO- NPs for both Klebsiella *pneumoniae* and *Bacillus cereus* strains.

**Table 3.** Disc diffusion test result of G-ZnO-NPs MIC value varying bacteria strain

| **Inhibition zone diameter (mm)/ ZnO Concentration** | **Gram negative** | | **Gram positive** | | |
|---|---|---|---|---|---|
| | ***Klebsiella pneumoniae*** | ***Escherichia coli*** | ***Bacillus cereus*** | ***Staphylococcus aureus*** | ***Staphylococcus faecalis*** |
| **25 µg/mL** | - | - | 12±1 | - | - |
| **50 µg/mL** | 17±1 | - | 18±1 | 12±1 | 12±1 |
| **100 µg/mL** | 18±1 | 14±1 | 20±2 | 14±1 | 15±1 |
| **200 µg/mL** | 20±2 | 17±1 | 24±2 | 18±1 | 18±1 |
| **400 µg/mL** | 25±2 | 19±1 | 26±2 | 20±2 | 23±2 |
| **MIC (µg/mL** | 200 | 400 | 100 | 400 | 400 |

The precipitation method was used to prepare nano-sized ZnO, which is the easiest method to produce nanomaterials. ZnO was prepared by base hydrolysis of zinc acetate in 2-propanol medium, as well as a precipitation method using Zn $(NO_3)_2$ and NaOH. Bacteriological tests such as minimum inhibitory concentration (MIC) and disk diffusion were performed by a standard microbial method using different concentrations of ZnO on solid agar plates and liquid broth systems. In the study, the relative antibacterial activity of ZnO suspensions of 12 nm, 45 nm and 2 µm particles against E. coli was investigated in aqueous LB broth qualitatively by disc diffusion and quantitatively in terms of MIC and MBC. MBC is the lowest concentration (µg/ml) at which a compound will kill more than 99% (2 log) bacteria, and lower MIC corresponds to higher antibacterial activity (Amutha and Sridhar 2018). Hussain et al. (2016), used that mint leaf extract for ZnO green synthesis with microwave radiation (2.45GHz and 1000 W). They reported that the optimum ratio of mint leaf extract and zinc (II) nitrate solution was 20:80 at a volumetric ratio at pH 10. Final light brown precipitated ZnO- NPs was separated from the suspension by centrifugation at 500 rpm for 8 minutes. It was then washed repeatedly with distilled water and ethanol and dried in an oven at 60 °C for 24 hours (Hussain et al. 2016). Mohammadi-Aloucheh et al. (2018) was obtained by the same procedure for the synthesis of ZnO NPs resulting white precipitate. In comparison to published reports on the green production of metal oxide NPs and physical and chemical properties, very limited information is available on the antibacterial properties of metal NPs. The antibacterial activity of ZnO nanoparticles with various particle sizes was investigated in the study of Padmavathy et al. (2008). Antibacterial disc diffusion test result of commercial nano-sized ZnO (N- ZnO-NPs), micro-sized (M- ZnO) and synthesized green (G-ZnO-NPs) and MIC value varying bacteria strain and the observed inhibition zones against all bacteria strain were illustrated in the Figure 1.

Figure 1 showed that G-ZnO-NPS have large inhibition zone diameters against all bacteria strain referring high antibacterial effect because the zone diameters were bigger than 10 mm comparing commercial micro-sized and nano-sized ZnO particles. The cytotoxicity test results of the commercial nano-sized ZnO (N- ZnO-NPs), micro-sized (M- ZnO) and synthesized green (G-ZnO-NPs) was given in the Table 4. Cytotoxicity test toxic levels were given in the Table 5.

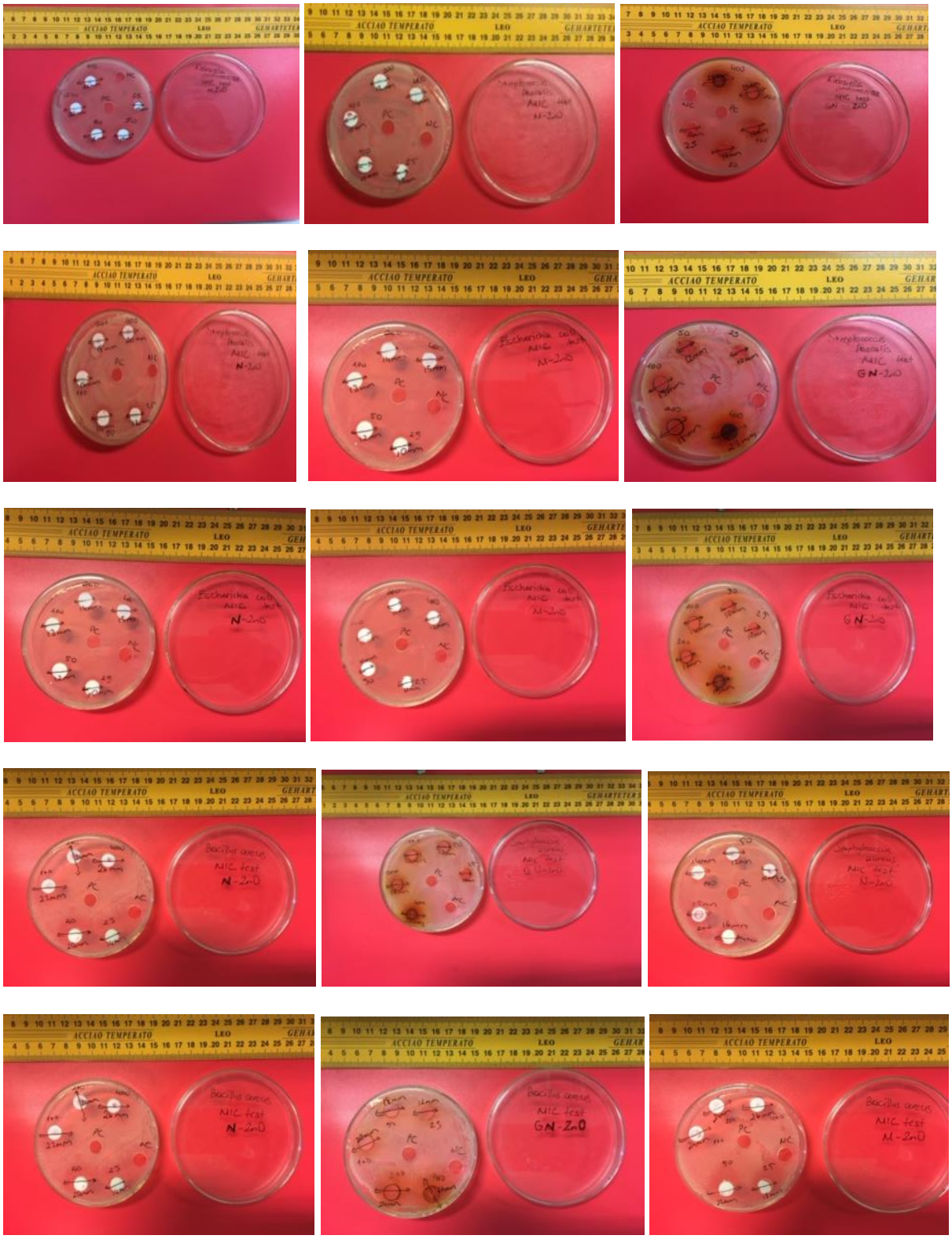

**Figure 1**. The antibacterial inhibition zone images of commercial nano-sized ZnO (N- ZnO-NPs), micro-sized (M- ZnO) and synthesized green (G-ZnO-NPs)

**Table 4.** Cytotoxicity test results of the commercial nano-sized ZnO (N- ZnO-NPs), micro-sized (M- ZnO) and synthesized green (G-ZnO-NPs)

| **Bacteria strain** | *Vibrio fischeri* | | |
|---|---|---|---|
| **Contact time** | t=5 min | t=15 min | Non-cytotoxic |
| **Positive control ($LD_{50}$, %v/v)** | 98±2 | 98±2 | Non-cytotoxic |
| **Negative control ($LD_{50}$, %v/v)** | 24±1 | 23±1 | Highly-cytotoxic |
| **M- ZnO ($LD_{50}$, %v/v)** | 26±2 | 25±2 | Highly-cytotoxic |
| **N- ZnO-NPs ($LD_{50}$, %v/v)** | 20±2 | 20±2 | Highly-cytotoxic |
| **G-ZnO-NPs ($LD_{50}$, %v/v)** | 45±1 | 46±1 | Modarately-cytotoxic |

**Table 5.** Cytotoxicity test toxic levels

| **$LD_{50}$ (% v/v)** | **Result** |
|---|---|
| 0-25 | Highly-cytotoxic |
| 26-50 | Modarately-cytotoxic |
| 51-75 | Low-cytotoxic |
| 76-100 | Non-cytotoxic |

The $LD_{50}$ (% v/v) value showed that the commercial nano-sized ZnO (N- ZnO-NPs) and micro-sized ZnO (M-ZnO) were highly cytotoxic effect while synthesized green (G-ZnO-NPs) were moderately cytotoxic. Since the final G-ZnO-NPs product is tend to agglomerate and creates bigger amorphous particles, they might have lower toxic effect.

## CONCLUSION

Environmentally friendly, green manufacturing process eliminates the cost and toxic substance consumption for the metal oxide NPs production. The minimum inhibition concentration of G-ZnO-NPs were determined to support antibacterial studies. Toxicological investigation of G-ZnO-NPs were performed to ensure the minimization of treatment method risk. Since the final product is tend to agglomerate, it may need to be distributed with a technique, such as sonication, to take advantage of the nano size feature before usage. The G-ZnO-NPs with 10-100 nm particle size showed high antibacterial activity against gram-negative and gram-positive bacteria. The inhibition zone diameters of G-ZnO-NPs, large zone diameters were observed against all bacteria strain referring high antibacterial effect because the zone diameters were bigger than 10 mm. MIC values of G-ZnO-NPs were obtained as 200 µg/mL, 400 µg/mL, 100 µg/mL, 400 µg/mL and 400 µg/mL for Gram-negative *Klebsiella pneumoniae, Escherichia coli* and Gram-positive *Bacillus cereus, Staphylococcus aureus and Staphylococcus faecalis* bacterial strains respectively. It was stated that the MIC values of the commercial G-ZnO-NPs were lower than the commercial N-ZnO- NPs for both *Klebsiella pneumoniae* and *Bacillus cereus* strains. Additional characterization studies such as SEM, EDX and XRD are required to better elucidate the physical, chemical and morphological properties of G-ZnO-NPs.

## REFERENCES

Amutha, S, and Sridhar S,2018. Green synthesis of magnetic iron oxide nanoparticle using leaves of Glycosmis mauritiana and their antibacterial activity against human pathogens. Journal of Innovations in Pharmaceutical and Biological Sciences 5.2: 22-26.

Ardakani, LS, et al., 2021.Green synthesis of iron-based nanoparticles using Chlorophytum comosum leaf extract: methyl orange dye degradation and antimicrobial properties. Heliyon 7.2: e06159.

Demissie, MG, et al. 2020.Synthesis of zinc oxide nanoparticles using leaf extract of lippia adoensis (koseret) and evaluation of its antibacterial activity. Journal of Chemistry (2020).

Drummer, S, Tafirenyika M, and Mahabubur C,2021. Green Synthesis of Transition-Metal Nanoparticles and Their Oxides: A Review. Materials 14.11: 2700.

El-Belely, EF., et al.,2021.Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using Arthrospira platensis (Class: Cyanophyceae) and evaluation of their biomedical activities. Nanomaterials 11.1:95.

Hussain M, Singh. Ajey Singh. Himani NB, Singh SC,2016.Grenn Synthesis of Nanoparticles and Its Potantial Application, 38:545 – 560

Mahalakshmi, S, Hema N, and Vijaya PP, 2019. In vitro biocompatibility and antimicrobial activities of zinc oxide nanoparticles (ZnO NPs) prepared by chemical and green synthetic route—A comparative study. BioNanoScience, 2019: 1-10.

Mohammadi-Aloucheh R, et al., 2018. Green synthesis of ZnO and ZnO/CuO nanocomposites in Menthalongifolia leaf extract: characterization and their application as antibacterial agents. 29:13596–13605.

Obied, NJ and Abd-Ali NK,2020. Bıosynthesıs Of ZnO Nanopartıcle in Presence of Red Onıon Extract. Plant Archives Volume 20(2):7854-785.

Ogunyemi, SO, et al.,2019. Green synthesis of zinc oxide nanoparticles using different plant extracts and their antibacterial activity against Xanthomonas oryzae pv. oryzae. Artificial cells, nanomedicine, and biotechnology, 47.1: 341-352.

Padmavathy, N, and Vijayaraghavan, R, 2008. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Science and technology of advanced materials.

Selvam, PSS., Chinnadurai, GS, Ganesan, D, Perumal, P, and Kandan, V., 2021. Cadmium oxide-zinc oxide nanocomposites synthesized using waste eggshell membrane and its in-vitro assessments of the antimicrobial activities and minimum inhibitory concentration. Journal of Inorganic and Organometallic Polymers and Materials, 31(2), 816-835.

Şendal.KÜ, Özgur.M,2019. Cynarae Folium Yapraklarının Sulu Ekstresi ile ZnO Nanopartiküllerin Biyosentezi ve Karakterizasyonu.

Yusof, HM, Rosfarizan M, and Uswatun HZ.,2019. Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. Journal of animal science and biotechnology, 10.1: 1-22.

Zhou, G, et al. 2021.Biosynthesis and Characterization of Zinc Oxide Nanoparticles and Their Impact on the Composition of Gut Microbiota in Healthy and Attention-Deficit Hyperactivity Disorder Children. Frontiers in Microbiology 12:1-12.

## *ORAL PRESENTATION*

# A waste reduction study: suitability of thermal power plant ash as sludge conditioner

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## Abstract

The usability of the thermal power plant ash has been investigated in the waste stabilization and wastewater treatment although most of these ashes are utilized in the waste landfills. Expanding alternative uses of thermal power plant ashes is important in terms of industrial waste management. The usability of these ashes in the sludge dewatering process in wastewater treatment plants was investigated to expand their usage areas instead of disposing in the waste fields. In this study, their use as a conditioner in wastewater sludge and their effect on dewatering efficiency were evaluated after characterization both fly ash and bottom ash obtained from a thermal power plant. Their suitability for dewatering has been investigated for domestic sewage sludge. Promising results were obtained for the thermal power plant bottom ash and fly ash on the conditioning resistance compared to the commercial sludge conditioners currently in use.

**Keywords:** thermal power plant ash, waste reduction, sludge treatment, conditioning resistance.

## INTRODUCTION

The thermal power plant ashes are formed as an industrial waste when coal is burned, and their ingredient are the mineral substances in the coal. These mineral substances are divided into internal minerals found in the core of the coal and secondary minerals that are later attached to the content of the coal. Natural mineral substances, which are composed of chemical elements in the vegetation where coal is formed and elements chemically bonded to coal during its formation, are organically combined with coal. The second minerals, which include the materials carried in the air and water that settle in the coal bed during or after the formation of the coal, are foreign materials to the organic structure of the coal. They usually consist of mineral forms associated with clay, shale, sandstone, or limestone, and contain fragments ranging in size from microscopic to thick layers. Other foreign materials may also be involved in the mining process. Coal ash is divided into two categories according to its chemical composition. Lignite ash is formed when the ash contains more CaO and MgO than $Fe_2O_3$. Bituminous ash is defined as ash with more $Fe_2O_3$ than the sum of CaO and MgO (Tokyay et al. 1990).

The organic part of the coal is consumed by burning because of the combustion of pulverized coal in thermal power plants, while the inorganic part remains suspended in the flue gas or accumulated in the bottom. The part of the burnt coal in the flue gas creates ash particles with different chemical structures and different sizes. It is possible to classify these ashes as bottom ash and fly ash. Bottom ash is the portion of fuel ash that falls to the bottom of the boiler or from the stoker drain. In coal-fired cyclone furnace boilers, bottom ash consists of slag that falls from the bottom of the furnace into a slag tank for solidification. This ash, which cannot be transported with flue gases, are larger than fly ash. Fly ash is the finer ash material carried to the rear end of the boiler by the flue gas from the furnace; falls in the economizer and air heater funnels or is collected by the particle control equipment. In a pulverized coal-fired system, the total produced ashes contain 15-40% bottom ash, 5% economizer ash, 5% heater ash, and 50-75% fly ash from the particle collector (Kitto and Stultz 2005). Although the amount varies according to the combustion system and the type of coal, there are mainly $SiO_2$, $Al_2O_3$, $Fe_2O_3$ and CaO compounds and small amounts of MgO, $SO_3$, alkali oxides in the fly ash (Akyazılı,

2009). Solid and molten ashes in the flue gases, which cannot be transported with these gases because they have relatively large grains and precipitated into the collection system under the boiler, are called bottom ash. While the ashes collected at the base are lighter, the slags are darker and brighter in color. In addition, the color of the ash darkens as the unburned carbon ratio increases. There are very small (0.5-150 micron) particles in the flue gases formed because of the combustion of pulverized coal in thermal power plants, which are carried by these gases and collected in particulate matter control systems such as cyclones or electro-filters. These particles, called fly ash, constitute the largest part of the ash formed as a result of combustion in the power plant (Türker et al. 2009). The chemical components of fly ash vary according to the type of coal used in combustion, its combustion efficiency, the non-combustible materials in the coal and the amount of these materials. In general, the amount of $Fe_2O_3$ is higher in hard coal fly ash, while the amount of CaO is higher in lignite coal fly ash. In addition, the amount of unburned carbon in lignite fly ashes is less, so the color is lighter (Öksüz, 2006). Fly ash creates an alkaline reaction in aqueous solutions due to the calcium and sulfate they contain. The water solubility of $SiO_2$, $Fe_2O_3$, $Al_2O_3$ and MgO in the ash content is quite low. Fly ash, which has 2-3% solubility in water, can dissolve more with the increase in temperature. The densities of fly ash vary depending on the mineral content and the fineness of the ash (Karahan, 2006).

According to the EPA, about 70% of all coal ash generated in the United States is stored on-site, and two-thirds of the ashes thus stored are in the form of aqueous storage of coal ash, with the remainder predominantly in the form of landfills. Landfills are used for approximately 95% of all off-site disposal in the US. The ocean disposal method, which was mentioned in the EPA's 1988 congressional report as being at the research and development stage, is not widely used today. The fly ash dump method was widely used in the UK, in the North Sea, but was discontinued in 1992 (Zhang, 2014). It is also stated that backfilling of coal ash into mines can improve the structural integrity of the embankments in mine reclamation, while also providing neutralization potential to combat acid mine discharge. These ashes are used as raw materials in the production of concrete and cement, and are also used in road foundations, soil and waste stabilization, and mining applications. Due to the adsorption property of fly ash, studies have been carried out on its use as an adsorbent in many different areas. Color removal from textile industry wastewater, heavy metal removal from aqueous media, treatment of sugar industry wastewater and phenol and lignin removal from paper mill wastewater are some of these studies (Polat et al. 2004).

Conditioning is a process used to increase the solids concentration in the sludge and reduce the volume of the sludge by reducing some of the water. Conditioned sludge is fluid and can be easily pumped. The purpose of reducing the volume of sludge with the thickening process is to increase the efficiency of the processes to be applied to the sludge and to reduce the cost. Due to its high volume and low solids content, it is important to concentrate the waste activated sludge. Dewatering is the process of removing water from the sludge to achieve a volume reduction much greater than that achieved in the condensation process. With sludge dewatering, a solid or semi-solid material (cake) is obtained which is easier to handle and transport. Sludge Conditioning refers to the process of increasing the water release of sludge. Conditioning is an essential part of mechanical thickening and sludge dewatering. Conditioning can be applied using inorganic or organic chemicals, fly ash from power plants and sludge incinerators, or by physical methods such as heating, freezing, and melting (TBB, 2015). The factors affecting the sludge conditioning efficiency are sludge type, solids concentration, pH, alkalinity, particle size and distribution, surface charge and degree of hydration. Traditionally, excess sludge is conditioned to improve its dewatering properties and then dewatered by mechanical force. In practice, chemical conditioning is most applied. The mechanism of chemical conditioning is to destroy the colloidal framework of the sludge and use calcium oxide, ferric chloride, polyacrylamide, etc. to agglomerate the sludge clots with additional conditioners. However, conditioning chemicals are expensive and add significantly to the overall sludge management cost.

The benefits of using ash as sludge conditioner include reducing or eliminating the use of commercial chemical conditioners, increasing dryness in the sludge cake, and providing a significant increase in filtration quality. The disadvantage of using ash is the high amount of inert material added to the sludge and the useful use of these added waste materials is limited. If the sludge is to be sent to the landfill after dewatering, the use of ash can be evaluated as it will increase the total solid content. The use of ash provides drier sludge, but if the sludge is to be burned, it will not contribute to the fuel value. The ash has no thermal value and lowers the volatile solids content in the sludge; therefore, the need for external fuel increases in the combustion process (Chen et al. 2010).

In this paper, the usability of these ashes in the sludge dewatering process in wastewater treatment plants was investigated to expand their usage areas instead of disposing in the waste fields. In this study, their use as a

conditioner in wastewater sludge and their effect on dewatering efficiency were evaluated both fly ash and bottom ash obtained from a thermal power plant.

## MATERIALS AND METHODS

In this study, both fly ash and bottom ash obtained from a thermal power plant were used as sludge conditioner for domestic wastewater sludge comparing with $FeCI_3.7H_2O$ (Merck) and $AlSO_4.18H_2O$) for 0.4 g/L, 1.2 g/L and 2.0 g/L dosage. The characteristic of domestic wastewater sludge was given in the Table 1 and dewatering experiment setup was illustrated in the Figure 1.

**Table 1**. Characteristic of domestic wastewater sludge

| Parameters | Value |
|---|---|
| pH | 6.45 |
| Conductivity (µS/cm) | 2585 |
| Temperature (° C) | 10.3 |
| Total Solids (mg/L) | 892 |
| Total Suspended Solids (mg/L) | 712 |
| Alkalinity (mg/L) | 31.6 |



**Figure 1.** Experimental setup

The sludge samples were dewatered an experimental ambient temperature using the stock chemical conditioners were prepared in the to be 100 mL volumetric flask according to chemical dosing program. Buchner funnel was used to filter each sludge samples after conditioning. The net area of the filter paper was recorded, and the filter paper was moistened before the funnel was attached to ensure complete sticking. Filtration was provided for 2 min only to provide a uniform cake formation by gravity and the filtrate volume was recorded from vacuuming began initially, every 15 s, afterwards these intervals were extended to read the filtrate volume and vacuum pressure. Dewatering was continued until all the filtrate was collected and filtration data registered in table as t / V ($s/m^3$) and V ($m^3$) plotting t/V values against the filtrate volume (V) to determine the slope (m) of the linear line passed through the points on the graph (linear regression). The specific filtration resistances of domestic wastewater sludge domestic wastewater sludge and optimum conditioner dosage for dewatering were determined with Eqn. (1) below.

$$R = \left( \frac{2\Delta P A^2}{\mu w} \right) m \qquad (1)$$

Here,

R: The specific filtration resistance (m/kg)
ΔP: vacuum pressure ($N/m^2$)
m: graph slope of V against t/V for the recorded value from dewatering experiment (s)
A: filter area ($m^2$)
w: dry weight on filter paper per filtrate volume ($kg/m^3$)

**RESULTS and DISCUSSION**

The effect of $FeCI_3.7H_2O$ on the specific filtration resistances (R) of the domestic wastewater sludge for 0.4 g/L, 1.2 g/L and 2.0 g/L dosage was given in the Figure 2. The optimum conditioner dosage for dewatering was determined as 0.4 g/L for $FeCI_3.7H_2O$ obtaining %99.5 reduction on the specific filtration resistances (R) of the domestic wastewater.

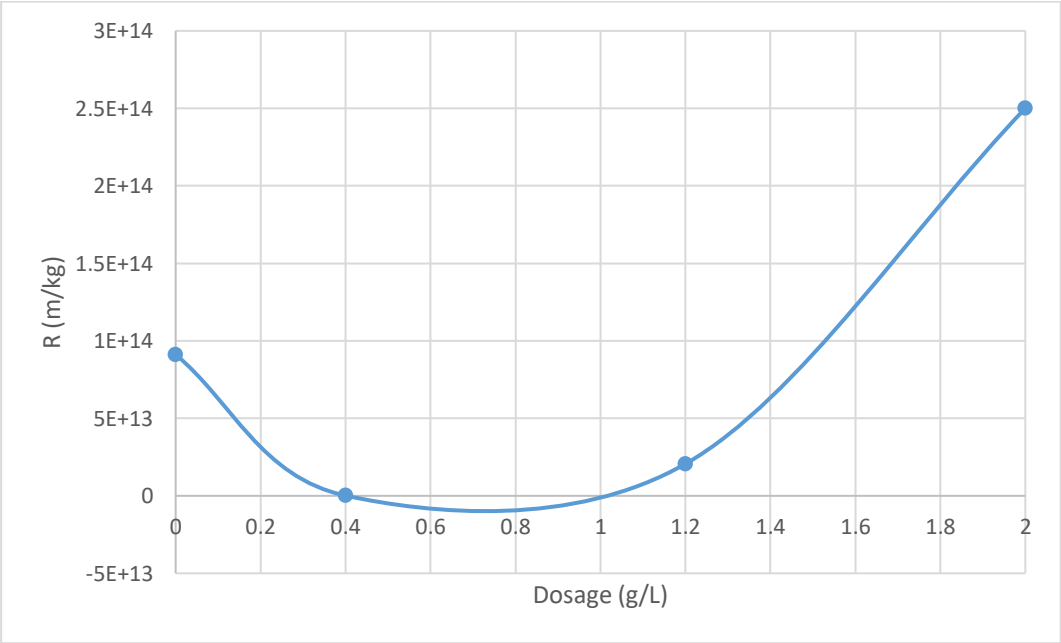


**Figure 2.** The effect of $FeCI_3.7H_2O$ on the specific filtration resistances of domestic wastewater sludge for 0.4 g/L, 1.2 g/L and 2.0 g/L dosage

The optimum conditioner dosage for dewatering was determined as 0.4 g/L for $FeCI_3.7H_2O$ obtaining %99.5 reduction on the specific filtration resistances (R) of the domestic wastewater. The effect of $AlSO_4.18H_2O$ on the specific filtration resistances (R) of the domestic wastewater sludge for 0.4 g/L, 1.2 g/L and 2.0 g/L dosage was given in the Figure 3.

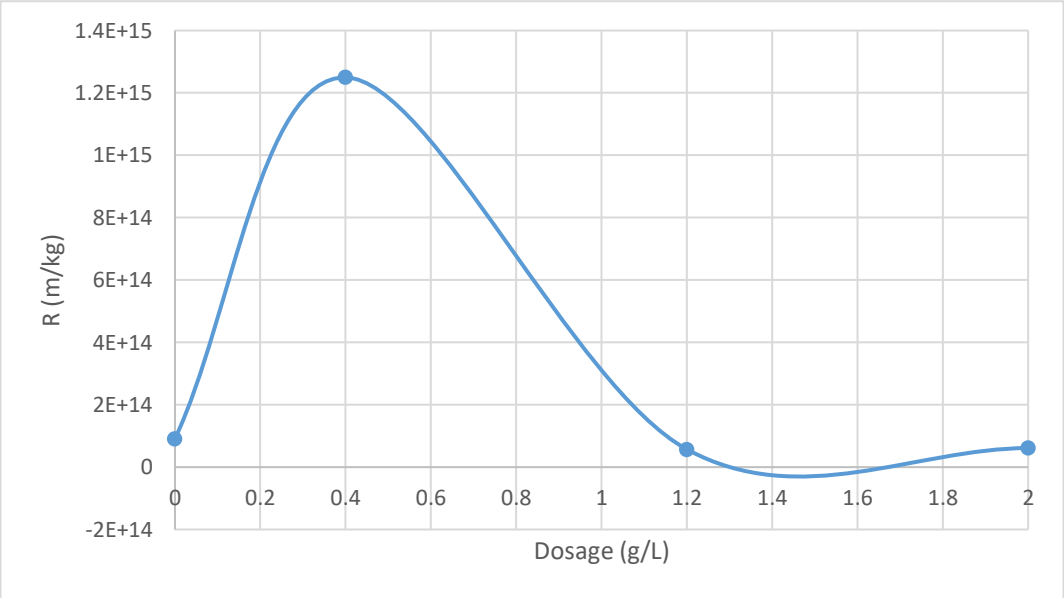


**Figure 3.** The effect of $AlSO_4.18H_2O$ on the specific filtration resistances of domestic wastewater sludge for 0.4 g/L, 1.2 g/L and 2.0 g/L dosage

The optimum conditioner dosage for dewatering was determined as 1.2 g/L for $AlSO_4.18H_2O$ obtaining %37.8 reduction on the specific filtration resistances (R) of the domestic wastewater. The effect of bottom ash on the specific filtration resistances (R) of the domestic wastewater sludge for 0.4 g/L, 1.2 g/L and 2.0 g/L dosage was given in the Figure 4.

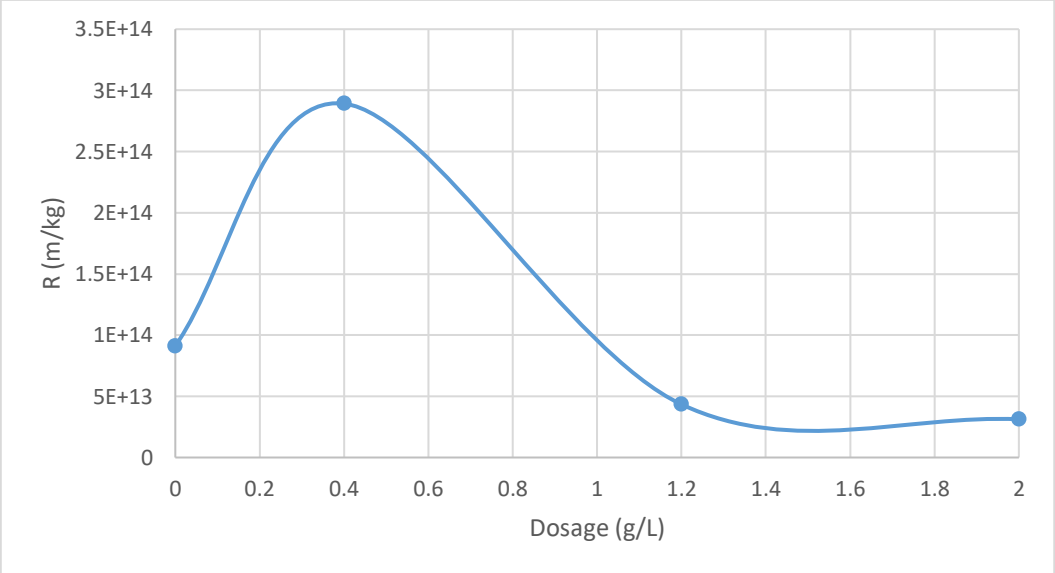


**Figure 4.** The effect of bottom ash on the specific filtration resistances of domestic wastewater sludge for 0.4 g/L, 1.2 g/L and 2.0 g/L dosage

The optimum conditioner dosage for dewatering was determined as 2 g/L for bottom ash obtaining %65.7 reduction on the specific filtration resistances (R) of the domestic wastewater. The effect of fly ash on the specific filtration resistances (R) of the domestic wastewater sludge for 0.4 g/L, 1.2 g/L and 2.0 g/L dosage was given in the Figure 5.

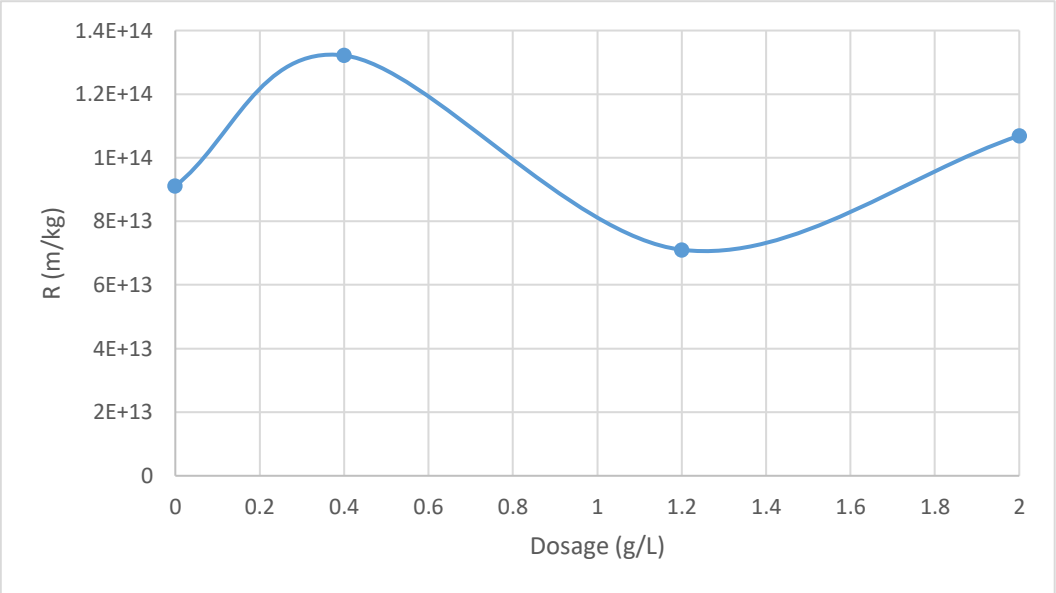


**Figure 5.** The effect of fly ash on the specific filtration resistances of domestic wastewater sludge for 0.4 g/L, 1.2 g/L and 2.0 g/L dosage

The optimum conditioner dosage for dewatering was determined as 1.2 g/L for bottom ash obtaining %22.3 reduction on the specific filtration resistances (R) of the domestic wastewater. The effect of conditioner type on the specific filtration resistances (R) of the domestic wastewater sludge was summarized in the Table 2.

**Table 2**. The effect of conditioner type on the specific filtration resistances (R) of the domestic wastewater sludge

| **Specific filtration resistances (R) (m/kg) /Conditioner dosage (g/L)** | **Conditioner type** | | | |
|---|---|---|---|---|
| | Bottom ash | Fly ash | $AlSO_4.18H_2O$ | $FeCI_3.7H_2O$ |
| 0.00 | 9.11E+13 | 9.11E+13 | 9.11E+13 | 9.11E+13 |
| 0.40 | 2.89E+14 | 1.32E+14 | 1.25E+15 | 1.38E+11 |
| 1.20 | 4.34E+13 | 7.11E+13 | 5.71E+13 | 2.06E+13 |
| 2.00 | 3.12E+13 | 1.07E+14 | 6.13E+13 | 2.50E+14 |

Their suitability for dewatering has been investigated for domestic sewage sludge. Promising results were obtained for the thermal power plant bottom ash and fly ash on the conditioning resistance compared to the commercial sludge conditioners currently in use. In the literature, thermal power plant bottom ash and fly ash have been investigated for environmental treatment as alternative solution. In a study conducted by Öksüz, (2006) in which fly ash was used as adsorbent in the removal of phenol and lignin from paper mill wastewater, %81 lignin and %65 phenol removal were achieved. It was observed that an effective color removal was achieved in the removal of reactive Chemactive D Black N dyestuff by adsorption on the fly ash . Chen et al. (2010) investigated the possibility of using coal fly ash in wastewater sludge treatment in their study. In this study, the use of coal fly ash modified with sulfuric acid was investigated by determining the optimal conditions and related mechanisms of conditioning and dewatering, the improvement of the dewaterability of wastewater sludge with sulfuric acid modified coal fly ash was investigated. In this study, acid concentration, ratio of acid to coal fly ash and soaking time were accepted as the most important factors for coal fly ash modification. As a result of the experiments, coal fly ash modified with sulfuric acid showed a much stronger conditioning capacity than raw coal fly ash. The ratio of acid to coal fly ash, 5:1; If the soaking time was 3 hours, maximum efficiency was obtained and it was observed that the moisture of the filter cake decreased by %40. In other study, Fang, et al. (1999) investigated carbon, nitrogen and phosphorus cycles during the composting of wastewater sludge with coal fly ash, and the degree of effect of fly coal ash on sewage sludge composting was tried to be determined. The composted product can be used as a fertilizer or soil conditioner due to its large organic matter content. As a result of the studies, it has been suggested to use 25% or less volatile coal to make compost together with wastewater sludge. In addition, this study showed that the addition of fly ash significantly reduced the presence of heavy metals in wastewater sludge.

The composition of dewatered sludge and permeate water must be investigated after conditioned by thermal power plant ash. Bhangare, et al. (2011) made analyzes to determine the elemental concentrations of coal and ash samples collected from five thermal power plants of different capacities in India. Cr, Co, Fe, Li, Mg, Mn, Ni, Zn arsenic and Hg, lead, Cd and Cu were analyzed. In the analyzes performed, the concentration of 13 elements in fly ash was higher than that of bottom ash and coal obtained from five different coal plants in India. found to be. It has been determined that fly ash is richer in elements than bottom ash. The ratio of high volatile elements such as As, Hg and Zn for fly ash and bottom ash was found to be minimum compared to coal, indicating that these elements escaped into the atmosphere with flue gas. Volatile elements such as Mg, Cd and Li are more concentrated in fly ash compared to bottom ash while non-volatile elements are less in fly ash than in other groups. These metals and heavy metals can enter the dewatered sludge and permeate water. Senapati (2011) included research on the beneficial uses of these ashes. In the study, which mentioned the necessity of proper control of large amounts of waste fly ash from thermal power plants, it was suggested that fly ash can be considered as a potential growth promoter, which can ensure the uptake of necessary nutrients / minerals (Ca, Mg, Zn, Mo, S and Se) by crops and plants. On the other hand, some metals and trace element found in the ash can be used in the appropriate field.

## CONCLUSION

Their suitability for dewatering has been investigated for domestic sewage sludge. Promising results were obtained for the thermal power plant bottom ash and fly ash on the conditioning resistance compared to the commercial sludge conditioners currently in use. In the literature, thermal power plant bottom ash and fly ash have been investigated for environmental treatment as alternative solution. The composition of dewatered sludge and permeate water must be investigated after conditioned by thermal power plant ash while some metals and trace element found in the ash can be used in the appropriate field.

## REFERENCES

Akyazılı, S., 2009. Soma Termik Santral Cüruflarından Yanmamış Karbonun Kazanılması, yüksek lisans tezi, İ.T.Ü., İstanbul, Türkiye.

Bhangare R.C., Ajmal P.Y., Sahu S.K., Pandit G.G., Puranik V.D. 2011. Distribution of trace elements in coal and combustion residues from five thermal power plants in India, Int. J. Coal Geol. 86, 349-356.

Chen, C., Zhang, P., Zeng, G., Deng,J., Zhou, Y., Lu, H. 2010. Sewage sludge conditioning with coal fly ash modified by sulfuric acid, Chemical Engineering Journal.

Fang M. et. all. 1999. Co-composting of Sewage Sludge and Coal Fly Ash:Nutrient Transformations, Bioresource Technology 67.

Karahan, O., 2006. Liflerle Güçlendirilmiş Uçucu Küllü Betonların Özellikleri , doktora tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü , Adana, Türkiye.

Kitto J.B., S.C. Stultz (Eds.) 2005. Steam: Its generation and use, fourty-first ed., The Babcock& Wilcox Company.

Öksüz, K., 2006. Afşin – Elbistan Termik Santralı Uçucu Külünün Zemin Stabilizasyonunda Kullanımı, yüksek lisans tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü , Adana, Türkiye.

Polat, H., Vengosh, A., Pankrotav. I. ve Polat, M. 2004. A New Technology for Reomoval of Boron from Water by Coal and Fly Ash, Destilation, Destilation Research and Technology, 164, pp. 173-188.

Senapati, M. R. 2011. Fly ash from thermal power plants – waste management and overview, Current Science ,.

Tokyay, M., Erdoğan, T., Yeğinobalı, A., 1990. Termik Santral Küllerinin Çimento ve Beton Katkı Maddesi Olarak Kullanılma İmkanlarının Etüd ve Envanteri Prosesi Ara Raporu, ODTÜ, İnşaat Mühendisliği, Ankara.

Türker, P., Erdoğan, B., Katnaş, F., Yeğinobalı, A. 2009. Türkiye'deki Uçucu Küllerin Sınıflandırılması ve Özellikleri. Türkiye Çimento Müstahsilleri Birliği (TÇMB), AR-GE Enstitüsü, Ankara.

Türkiye Belediyeler Birliği Atıksu Arıtma Çamurlarının İşlenmesi ve Bertarafı (El Kitabı), Ankara, Mayıs 2015.

Zhang X. 2014. Management of coal combustion wastes. IEA Clean Coal Centre.

## ORAL PRESENTATION

# Salisilik asidin soğuk stresine maruz kalmış sarıçam tohumlarında çimlenme üzerine etkilerinin araştırılması

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## Özet

Salisilik asit (SA) bitkiler tarafından sentezlenen, hem biyotik hem de abiyotik stres etkenlerine karşı tolerans yanıtı oluşturan ve sinyal bileşiği olarak görev yapan organik bir moleküldür. Bu çalışmada soğuk stresine maruz bırakılmış sarıçam tohumlarına farklı konsantrasyonlarda dıştan uygulanan SA'nın çimlenme üzerine etkilerini araştırmak amaçlanmıştır. Bu amaçla sarıçam tohumlarına 24 saat boyunca sekiz farklı SA konsantrasyonu (0- 5000 µM) uygulanmıştır. Uygulama sonunda petri kaplarına aktarılan tohumlar iklim kabininde 20 gün boyunca 16°C düşük sıcaklıkta çimlenmeye bırakılmıştır. 20 günlük fidelerde; çimlenme sayısı, kök boyu uzunluğu, nispi su içeriği (NSİ), toplam klorofil ve karotenoid miktarı, lipit peroksidasyonu (TBARS), hidrojen peroksit ($H_2O_2$), prolin, süperoksit dismutaz (SOD) ve guaikol peroksidaz (GPX) enzim aktiviteleri ölçülmüştür. Bulgulara göre, konsantrasyonlar arasından 50 ve 100 µM SA uygulamasının soğuk stresi koşullarında (16°C) diğer konsantrasyonlara göre sarıçam fidelerinde çimlenme oranını, kök uzunluğunu, NSİ, fotosentetik pigment ve prolin içeriğini arttırdığı, ancak TBARS ve $H_2O_2$ seviyeleriyle, SOD ve GPX enzim aktivitelerini önemli ölçüde azalttığı belirlenmiştir. Bulgular ışığında, 50 ve 100 µM SA uygulamalarının özellikle prolin gibi sinyal ara bileşiklerin/ozmolitlerin metabolizmasını düzenleyerek ve su alınımını sürdürerek sarıçam tohumlarında soğuk toleransını arttırmasına yönelik tohumlarda çimlenme üzerine olumlu etkisi olduğu tespit edilmiştir.

**Anahtar Kelimeler:** Salisilik asit, soğuk stresi, sarıçam, tohum çimlenmesi.

# Investigation of the effects of salicylic acid on germination in scotch pine seeds exposed to cold stress

## Abstract

Salicylic acid (SA) is an organic molecule that is synthesized by plants and which acts as a signaling compound and has a tolerance response to both biotic and abiotic stressors. In this study, it was aimed to investigate the effects of exogenous applied SA at different concentrations on the germination of scots pine seeds exposed to cold stress. For this purpose, eight different SA concentrations (0-5000 µM) were applied to scotch pine seeds for 24 hours. At the end of the application, the seeds transferred to petri dishes were left to germinate in the climate cabinet for 20 days at a 16 °C low temperature of 20 days old seedlings; germination rate, root length, relative water content (RWC), total chlorophyll and carotenoid content, lipid peroxidation (TBARS), hydrogen peroxide ($H_2O_2$), proline, total protein amount, activities of superoxide dismutase (SOD) and guaiacol peroxidase (GPX) enzymes were measured. According to the findings, compared to other SA concentrations applications of 50 and 100 µM SA increased the germination rate, root length, RWC, photosynthetic pigment, and proline content, but TBARS, $H_2O_2$ levels, SOD and GPX enzyme activities. was found to be significantly reduced in scotch pine seedlings under cold stress (16°C). In the light of the findings, it was determined that 50 and 100 µM SA applications can a positive effect on the seed germination especially by regulating the metabolism of osmolytes /signal intermediate compound such as proline, maintaining water intake and thus increasing the cold tolerance in scots pine seeds.

**Keywords:** Salicylic acid, cold stress, scots pine, seed germination.

# GİRİŞ

Bitkiler yaşamları boyunca soğuk, sıcaklık, kuraklık, sel ve tuz stresi gibi abiyotik; virüs, mantar ve bakteriler gibi çeşitli biyotik streslerin etkenlerine maruz kalmaktadırlar. Dünya genelinde bitkisel ürün kayıplarına sebep olan etkenlerin %50'den fazlası abiyotik stres çeşitlerinden kaynaklanmaktadır (Bray vd., 2000). Bunlar arasından soğuk stresi özellikle yarı tropik ve tropik bölgelerde yaşayan bitkilerde verim miktarını, ürün kalitesini etkileyen abiyotik stresler arasında yer almaktadır (Yang vd., 2005). Çimlenme aşamasında soğuk stresine maruz kalan bitkiler, gecikmiş ve daha düşük çimlenme yüzdesi gösterirken (Zhang ve ark., 2014) vejetatif aşamada semptomlar bitki büyüme hızının yavaşlaması, yaprak boyutlarında indirgenme, senesens, klorofil yıkımı, hücre zarı bütünlüğünün bozulması ve bunlara bağlı olarak bitkide ölüm durumları ortaya çıkar (Saltveit ve Morris, 1990; Kratsch ve Wise, 2000; Mahajan ve Tuteja, 2005; Bertamini ve ark., 2007; Rymen ve ark., 2007). Soğuk stresinin neden olduğu reaktif oksijen türlerinin (ROT), malondialdehit (MDA), yapısal proteinler ve enzimlerin aşırı birikimi nedeniyle membran lipid kompozisyonunun değiştirilmesi gibi çeşitli hücresel bileşenlerde ciddi hasara neden olabilir (Song ve ark., 2011). Bu gibi olumsuz etkilere yanıt olarak diğer stres etkenlerinde olduğu gibi bitkinin savunma mekanizması devreye girer (Kratsch ve Wise, 2000; Einset ve ark., 2007; Zhao ve ark., 2013; Ruelland, 2017). Üşüme kaynaklı hasarı en aza indirmek için bitkiler, enzimatik ve enzimatik olmayan antioksidanlar gibi farklı temizleyici sistemleri düzenleyebilir (Gill ve Tuteja, 2010). Ayrıca, bitkiler çeşitli bitki büyüme düzenleyicilerini (salisilik asit gibi) ve ozmoprotektanları (prolin gibi) sentezleyebilir (Gautam ve Singh, 2009; AbdElgawad ve ark., 2016; Tabassum ve ark., 2017).

Salisilik asit (SA) birçok bitki türünde fazlaca tanınan önemli fenolik bileşik grubudur. Bitki büyümesi, gelişmesi, olgunlaşması ve özellikle abiyotik stres cevabının düzenlenmesinde önemli bir rol oynar (Rivas-San Vicente ve Plasencia, 2011; Hara ve ark., 2012). Genel olarak, düşük SA konsantrasyonları bitkilerde antioksidan kapasiteyi artırabilir, ancak yüksek konsantrasyonları hücre ölümüne veya abiyotik streslere karşı duyarlılığa neden olabilir (Hara ve ark., 2012). Yapılan pek çok çalışma soğuk stresi altında dıştan uygulanan SA'nın stres tolerasını arttırdığını rapor etmiştir (Kang ve ark., 2003; Korkmaz, 2005; Mora-Herrera ve ark., 2005; Senaratna ve ark., 2000; Wang ve ark., 2012; Yang ve ark., 2012). Bununla birlikte, abiyotik stres altında dıştan uygulanan SA'nın farklı angiosperm tohumlarında (buğday (*Triticum aestivum* L. cv. *Roshan*), mürdümük (*Lathyrus sativus* L.) çimlenmeyi nasıl teşvik ettiği hakkında çalışmalar rapor edilmiştir (Dolatabadian ve ark., 2009). Ancak, dıştan uygulanan SA'nın soğuk stresi altında gymnospoerm tohumlarının çimlenmesi üzerine etkisinin ele alındığı ayrıntılı bir araştırmaya rastlanmamıştır.

Sarıçam (*Pinus sylvestris* L.) dünyada en geniş yayılış alanına sahip konifer türüdür (Boratynski, 1991). Türkiye'deki doğal yayılışı Kuzeydoğu Anadolu'da 2700 metrelere kadar çıkar. Trabzon'un Of ve Sürmene ilçeleri arasında, adını verdiği Çamburnu Mevkiindeki yayılışına ilave olarak Artvin-Arhavi-Sugören ile yine adını verdiği Çamlı köyleri arasında da sahile inen iki ayrı doğal toplu yayılışı bulunur. Farklı abiyotik stres uygulamalarının sarıçam tohumlarının çimlenmesi üzerine etkisi ile ilgili birkaç çalışma mevcuttur (Turna ve Bilgili, 2006; Zhu ve ark., 2006). Ancak, soğuk stresi altında SA uygulamasının sarıçam tohumlarında çimlenme yanıtlarını nasıl düzenlediğine dair yapılan herhangi bir çalışmaya rastlanmamıştır. Bu nedenle mevcut çalışmada, soğuk stresi koşullarında dıştan uygulanan sekiz farklı SA uygulamasının (0-5000 µM) yerel orjinli (Trabzon-Vakfıkebir) sarıçam tohumlarında çimlenme üzerine etkisinin araştırılması amaçlanmıştır. Bu amaç doğrultusunda, sarıçam tohumlarında çimlenme sayısı, çimlenen kotiledonların kök uzunluğu, nispi su içeriği (NSİ), toplam klorofil ve karotenoid içeriği, lipid peroksidasyonu (TBARS), hidrojen peroksit ($H_2O_2$), prolin, süperoksit dismutaz (SOD) ve guaikol peroksidaz (GPX) antioksidanların aktivitelerindeki değişimler ortaya koyulmuştur.

## MATERYAL VE METOT

### Bitki Materyalinin Hazırlanması, Deney Tasarımı ve Uygulamalar

Mevcut çalışmada deney materyali olarak Vakfıkebir orjinli sarıçam tohumları (*Pinus sylvestris* L.) Trabzon-Of fidanlığı tarafından temin edildi. Tohumların 0 (kontrol) µM grubu saf su ile 1, 25, 50, 100, 500, 1000 ve 5000 µM sekiz farklı salisilik asit çözelti içerisinde 24 saat süreyle inkübe edildi. İnkübasyon sonunda, her bir petride 50'şer adet tohum şekilde ekim gerçekleştirildi. Tohumlar soğuk stresi olarak 16 °C iklimlendirme kabininde 20 gün boyunca çimlenmeye bırakıldı. 20 günün sonunda çimlenen tohumlar analizler için hasat edildi.

**Kök Uzunluğunun Belirlenmesi**
Mevcut çalışmanın 20'nci gününde çimlenen tohumlarda kök gelişimi takip edildi. Kök boylarının ölçülebilmesi için radikula uzunluğunun 2 mm'den uzun olmasına dikkat edildi. Ölçüm dijital kumpas yardımıyla gerçekleştirildi.

**Nispi Su İçeriği (NSİ) Ölçümü**
Mevcut çalışmada bitki su durumunu belirlemek için nispi su içeriği (NSİ) belirlendi. Bu amaçla çimlenen tohumların hipokotillerinden 0,1 g taze numune örneği alındı. Numunelerde taze ağırlık (TA), turgid ağırlık (TU) ve kuru ağırlık (KA) ölçümleri yapılarak elde edilen sonuçlar Smart ve Bingham (1974) formülünde yerine konuldu ve nispi su içeriği % olarak hesaplandı.

**Pigment Tayini**
Fotosentetik pigmentlerin tayini Arnon (1949) ve Witham ve ark., (1971)'a göre yapıldı. Bu amaçla çimlenen tohumların hipokotillerinden 0,1 g taze numune sıvı azot ile homojenize edilerek üzerine 5 ml soğuk aseton (%80) eklendi. Homojenat 10 dakika süre ile 3.000 rpm'de ve +4 °C 'de santrifüj edildi. Süpernatantın absorbansı 450 645 ve 663 nm dalga boylarında absorbans değerleri spektrofotometrede ölçüldü. Toplam klorofil ve karotenoid miktarı Lichtenthaler (1987) methoduna göre belirlenmiştir.

**Lipit Peroksidasyonu Tayini (TBARS)**
Çalışmada Heath ve Packer (1968)'e göre lipit peroksidasyonu (TBARS) tayini yapıldı. Örneklerin spektrofotometre ile 532 (spesifik) ve 600 (spesifik olmayan) nm dalga boylarında absorbansları ölçüldü. Delta absorbans değerleri, $\Delta=\text{Ɛ.c.l}$ formülünde yerine konularak MDA miktarı hesaplandı ($\Delta=A_{532}-A_{600}$, Ɛ: Absorbsiyon katsayısı, 155 $mmol^{-1}$ $cm^{-1}$, c: konsantrasyon).

**Hidrojen Peroksit ($H_2O_2$) Tayini**
Hidrojen peroksit ölçümü Velikova vd. (2000) yöntemi ile gerçekleştirildi. Çimlenen tohumların hipokotillerinden alınan 0,25 g taze örneklerin süpernatantlarından 1000 µl alınarak üzerine 1000 µl 10 mM potasyum fosfat tamponu ve 1500 µl 1 M KI ilave edildikten sonra oluşan sarı renk 390 nm'de spektrofotometrede kayıtlı standart grafikten hesaplandı.

**Prolin Tayini**
Prolin tayini Bates vd., (1973) yöntemine göre gerçekleştirildi. Bu amaçla çimlenen tohumların hipokotillerinden 0,1 gr taze örnek alındı ve 1,8 ml %3 süfosalisilik asit eklendi. Örnekler 15000 g'de 10 dakika ve +4 $^oC$'de santrifüj edildi. Süpernatantın 1 ml'lik kısmı alındı ve 1 ml asit ninhidrin çözeltisi eklendi. Örnekler 100 $^oC$'de 1 saat boyunca inkübe edildi. Sonrasında örneklere 3 ml tolüen eklendi ve karışım vortekslendi. Oluşan karşımın üst fazı spektrofotometrede 520 nm dalga boyundaki absorbans değerleri cihaza girilmiş standartlara göre mg $ml^{-1}$ taze ağırlık$^{-1}$ olarak belirlendi.

**Süperoksit Dismutaz (SOD) Aktivitesi**
Süperoksit dismutaz (EC 1.15.1.1) aktivitesi, Beauchamp ve Fridovich (1971) metodu kullanılarak belirlendi. Potasyum fosfat tamponu (50 mM, pH 7,8), 0,1 mM EDTA, 13 mM metiyonin, 75 µM nitrobluetetrazolyum ve 50 µl ekstrakt içeren 1 ml reaksiyon ortamına 2 µM riboflavin eklenerek reaksiyon oluşturuldu ve daha sonra bu karışım 10 dakika süre 375 µmol $m^{-2}s^{-1}$ şiddetinde beyaz ışık altındaki inkübasyonun ardından 560 nm'de absorbans değerleri belirlendi.

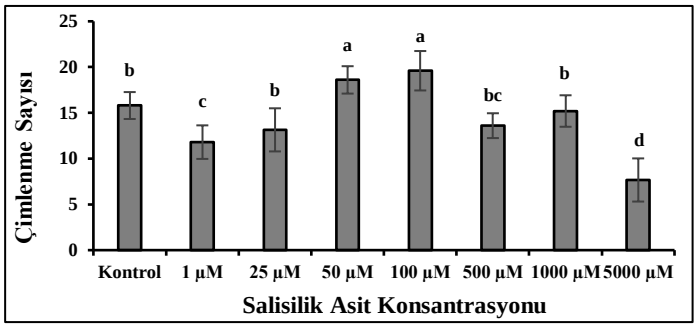
**Guaikol Peroksidaz (GPX) Aktivitesi**
Guaikol peroksidaz (EC 1.11.1.7) aktivitesi Urbanek ve ark., (1991) yöntemine göre belirlendi. Bu amaçla enzim aktivitesi, 50 µl enzim ekstraktının eklendiği reaksiyon ortamında (potasyum fosfat tamponu 100 mM, pH 7; EDTA 0,1 mM; guaiakol 5 mM; $H_2O_2$ 15 mM) son hacim 1 ml olacak şekilde 470 nm dalga boyunda bir dakika süreyle ölçüldü. GPX aktivitesi $\text{Ɛ}=26,6$ $mM^{-1}cm^{-1}$ tükeniş katsayısının kullanılarak hesaplandı.

**İstatistik Analizler**
Üç tekrarlı olarak oluşturulan analizlerle belirlenen ortalamalar SPSS 22.0 paket istatistik programı ile OneWayAnova testi ve gruplar arasındaki farkı belirleyebilmek için Duncan testi kullanıldı. Barlar ortalamalara ait standart sapmayı göstermektedir. Sütunlar üzerindeki farklı harfler, tüm uygulamalar arasındaki önemli farkları ($p<0.05$) göstermektedir.

## Bulgular

### Çimlenme Sayısı

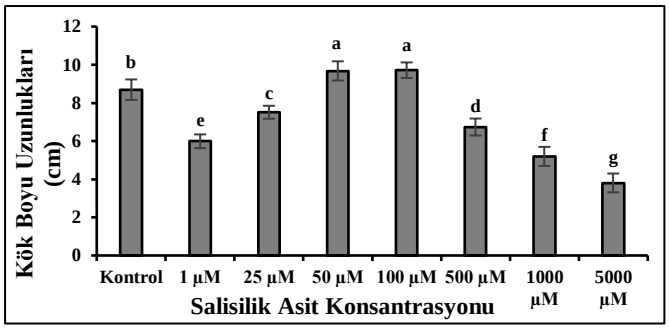
Her bir SA konsantrasyonu için 50'şer tohum üzerinden çimlenen tohumların sayıları kaydedilmiştir. Soğuk stresi altında kontrol grubuna göre en yüksek çimlenme sayısı 50 ve 100 µM SA uygulanmış gruplarda tespit edildi. En düşük çimlenme sayısı ise 5000 µM SA uygulamasında görüldü (Şekil 1).




Şekil 1. Soğuk stresi altında sarıçam tohumlarında farklı salisilik asit uygulamalarının çimlenme sayıları üzerine etkisi.

### Kök Boyu Uzunlukları

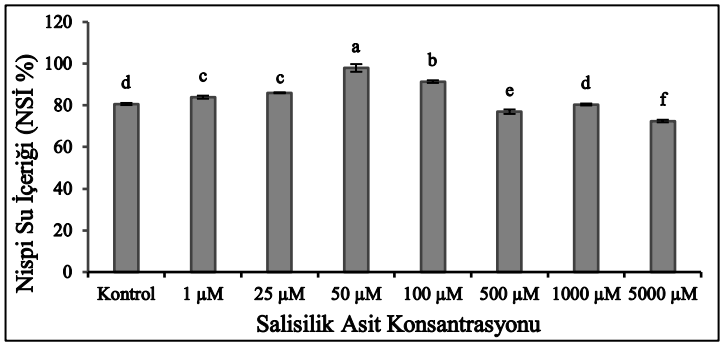
Soğuk stresi koşullarında kontrol grubuna göre en yüksek kök uzunluğu 50 ve 100 µM SA uygulanmış gruplarda görüldü. En düşük kök boyu uzunluğu 5000 µM SA uygulanmış grupta tespit edildi (Şekil 2).




Şekil 2. Soğuk stresi altında sarıçam tohumlarında farklı salisilik asit uygulamalarının kök boyu uzunluğu üzerine etkisi.

### 3.3. Nispi Su İçeriği

Soğuk stresi koşullarında kontrolle karşılaştırıldığında, en yüksek NSİ sırasıyla 50 ve 100 µM SA uygulanmış gruplarda gözlenirken, en düşük NSİ ise 5000 µM SA uygulamasında olduğu belirlendi (Şekil 3).

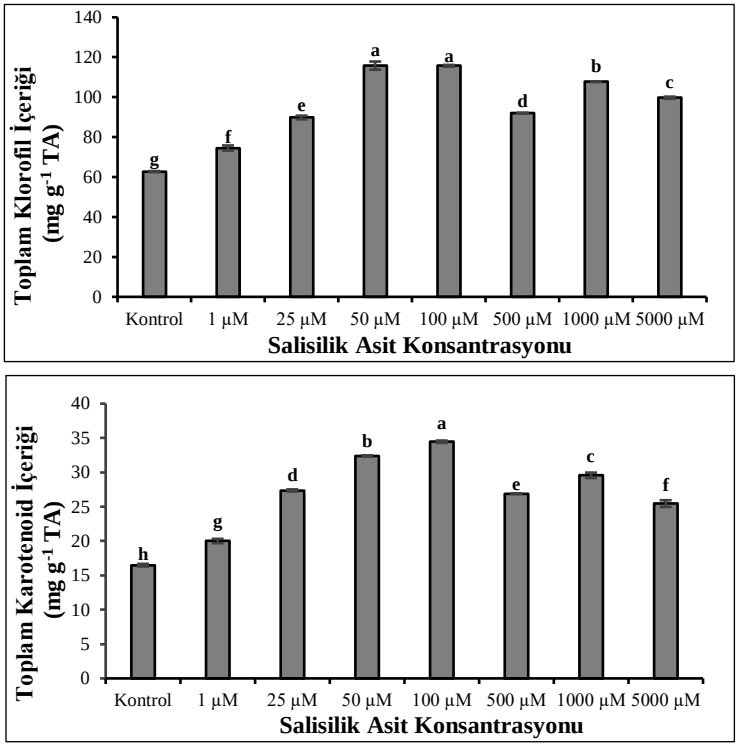



Şekil 3. Soğuk stresi altında sarıçam tohumlarında farklı salisilik asit uygulamalarının nispi su içeriği üzerine etkisi.

### Toplam Klorofil ve Karotenoid İçeriği

Kontrol grubuna göre en yüksek klorofil içeriği 50 ve 100 µM SA uygulanmış gruplarda görüldü. Farklı olarak, en düşük klorofil içeriğinin ise SA uygulaması olmayan kontrol grubunda görüldü (Şekil 4). Soğuk stresi koşullarında en yüksek karotenoid içeriğine sırasıyla 100 ve 50 µM SA uygulanmış gruplarda

rastlandı. Bununla beraber, en düşük karotenoid içeriğinin ise kontrol grubunda olduğu tespit edildi (Şekil 4)

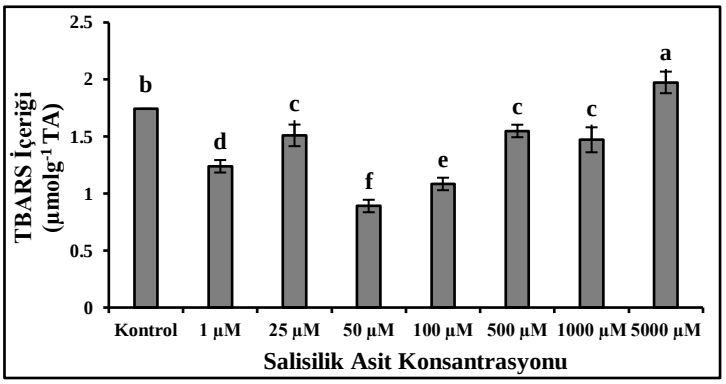



Şekil 4. Soğuk stresi altında sarıçam tohumlarında farklı salisilik asit uygulamalarının toplam klorofil ve toplam karotenoid içeriği üzerine etkisi.

A

**Lipid Peroksidasyonu (TBARS) Tayini**

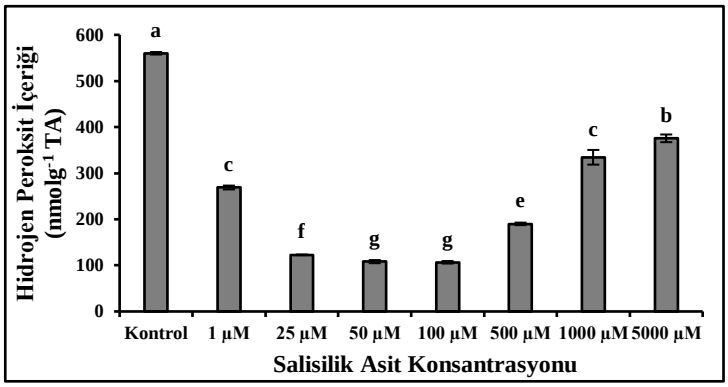
Çalışma bulgularına bakıldığında soğuk stresi koşullarında kontrole göre en düşük TBARS içeriği sırasıyla 50 ve 100 µM SA uygulanmış gruplarda belirlenirken, en yüksek TBARS içeriği ise 5000 µM SA uygulamasıyla tespit edildi (Şekil 5).




Şekil 5. Soğuk stresi altında sarıçam tohumlarında farklı salisilik asit uygulamalarının TBARS içeriği üzerine etkisi.

**Hidrojen Peroksit ($H_2O_2$) Tayini**

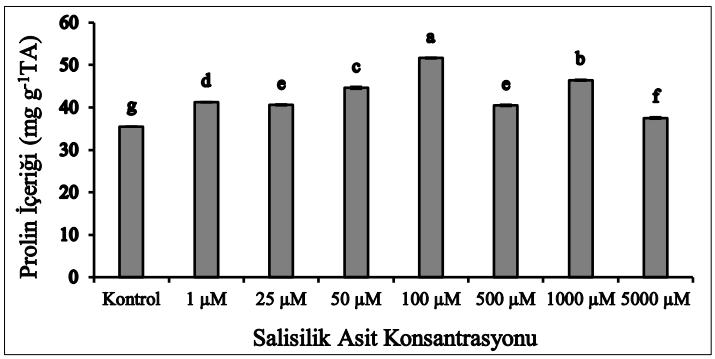
Gruplar arasında en düşük $H_2O_2$ içeriğinin 50 ve 100 µM SA uygulanmış gruplarda belirlendi. En yüksek $H_2O_2$ içeriği ise SA uygulanmamış kontrol grubunda olduğu tespit edildi. (Şekil 6).




Şekil 6. Soğuk stresi altında sarıçam tohumlarında farklı salisilik asit uygulamalarının $H_2O_2$ içeriği üzerine etkisi.

**Prolin Tayini**

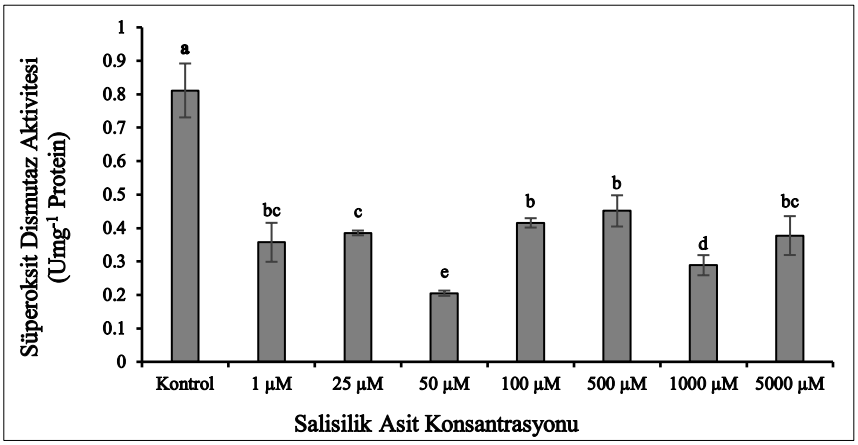
Mevcut çalışmada soğuk stresi altında kontrole kıyasla en yüksek prolin içeriği 100 µM SA uygulama grubunda tespit edildi. Bununla birlikte, en düşük prolin içeriği ise kontrol grubunda belirlendi (Şekil 7).




Şekil 7. Soğuk stresi altında sarıçam tohumlarında farklı salisilik asit uygulamalarının prolin içeriği üzerine etkisi.

**Süperoksit Dismutaz (SOD) Aktivitesi**

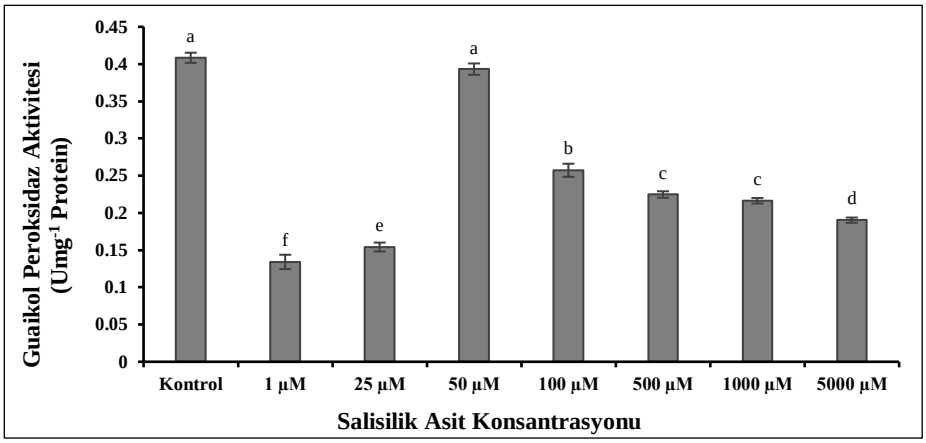
Sonuçlara göre, soğuk stresi altında kontrol grubuna kıyasla en düşük SOD aktivitesi 50 µM SA uygulanmış gruplarda tespit edildi. En yüksek SOD aktivitesi ise SA uygulanmamış kontrol grubunda belirlendi (Şekil 8).




Şekil 8. Soğuk stresi altında sarıçam tohumlarında farklı salisilik asit uygulamalarının SOD aktivitesi üzerine etkisi.

**Guaikol Peroksidaz (GPX) Aktivitesi**

Gruplar arasında en yüksek aktivitenin, salisilik asit uygulanmayan kontrol ve 50 µM SA grubunda olduğu görülürken, en düşük GPX aktivitesinin de 1 µM salisilik asit uygulamasında olduğu tespit edildi (Şekil 9).




Şekil 9. Soğuk stresi altında sarıçam tohumlarında farklı salisilik asit uygulamalarının GPX aktivitesi üzerine etkisi.

## TARTIŞMA

Düşük sıcaklıklarda çimlenme oranı genellikle düşüktür. Salisilik asitin kesin rolü ve ilgili moleküler mekanizmalar tam olarak açıklanmamış olmasına rağmen, stresli koşullar altında çimlenmede de rol oynadığı yapılan çalışmalarda belirtilmiştir. (Borsani ve ark., 2001; Rajjou ve ark., 2006; Alonso-Ramírez ve ark., 2009). Bulgularımıza benzer olarak yapılan çalışmalarda soğuk stresi altında SA uygulamalarının bitkilerde çimlenmeyi teşvik ettiği yönündedir. Örneğin, Korkmaz (2005) salisilik asidin biber tohumlarının düşük sıcaklıktaki (15 °C) çimlenme dereceleri üzerine etkisini incelediği bir çalışmada, çimlenme ortamına ilave edilen 0,1 mM salisilik asidin, tohumlarda %91 çimlenme oranını teşvik ettiğini bildirmiştir. Bu bilgiler ışığında, 50 µM ve 100 µM SA uygulamalarının soğuk stresi koşullarında sarıçam tohumlarının çimlenmesini teşvik ettiğini söylemek mümkün olabilir.

Düşük sıcaklık, kök gelişimini de etkilemektedir. Bitki kök uzunluğu düşük optimum sıcaklığa duyarlı olduğundan, kök dallanmasının ve kök yüzey alanının azalmasına neden olur. Sonuç olarak, sınırlı kök yüzey alanı, bitkinin su ve besin alınımını engeller (Hussain ve ark., 2018). Farklı araştırmacılar tarafından elde edilen sonuçlar, dıştan uygulanan SA'nın artan kök uzunluğu ile ilişkili olduğunu göstermiştir (Hanan, 2007). Bulgulara göre, 50 ve 100 µM SA'nın soğuk stresi ile başa çıkabilmek için kök büyümesini iyileştirdiği söylenebilir.

Soğuk stresine maruz kalan bitkiler, su alımının ve su kaybının soğuğa bağlı inhibisyonu nedeniyle sıklıkla su stresi belirtileri gösterir. Nispi su içeriği (NSİ) bitkilerin su durumunun nicel bir göstergesidir. Yapılan birçok çalışma, SA'nın ozmotik stres kaynaklı su kayıplarına karşı bitkiyi koruduğunu göstermiştir (Marcinska ve ark., 2013). Bulgular ışığında, 50 µM ve 100 µM SA uygulamalarının soğuk stresi altında sarıçam kotiledonlarında su durumunu devam ettirerek soğuk stresine karşı bitkiyi koruyabileceğini göstermektedir.

Soğuk stresi sadece fotosentetik pigmentlerde önemli bir hasara neden olmaz, aynı zamanda tilakoid membranda bozulmayada yol açar (Keshavarz ve ark., 2016). Uzunlu (2006) yaptığı bir çalışmada, soğuk stresi altında tohumdan ve yapraktan uygulanan SA'nın kavun fidelerinde kontrole göre klorofil içeriğini arttırdığını tespit etmiştir. Başka bir araştırmada ise yine dışarıdan uygulanan SA'nın soğuk stresi koşullarında fotosentezi koruduğu bildirilmiştir (Liu ve ark., 2009). Hem mevcut çalışma verileri hem de literatür verileri ışığında 50 ve 100 µM SA'nın soğuk stresi koşullarında bitkide pigment miktarını arttırdığını yada korunmasına yardımcı olduğunu söylemek mümkündür.

TBARS içeriği, membran stabilitesinin hayati bir göstergesi olup, oksidatif hasarın belirtecidir (Hu ve ark., 2016; Kaya ve İnan, 2017). Oksidatif hasar hemen hemen tüm stres çeşitlerinin sonunda meydana gelir ve bitkinin strese maruz kaldığını gösteren önemli bir parametre olarak kabul edilir (Bhattacharjee, 2012; Fayez ve Bazaid, 2014). Mevcut çalışmada, SA'nın TBARS seviyesini azaltarak soğuk stresi altında membran hasarını azaltmaya yardımcı olduğuyla açıklanabilir. Bulgularımızı destekler nitelikte Güneş ve ark., (2007) mısır bitkisinde yaptıkları bir çalışmada, SA'nın TBARS seviyesini ve hücre zarı geçirgenliğini azalttığını ve böylece bitki büyümesini teşvik ettiğini rapor etmiştir.

Hidrojen peroksit, bitkilerde çeşitli stres koşullarında (kuraklık, üşüme, yüksek sıcaklık ve ışık gibi) oksidatif stres sonucu oluşan başlıca ROT'tur (Das ve Roychoudhury, 2014). Bulgular ışığında, soğuk stresi altında dıştan SA uygulaması, $H_2O_2$ artışını engellediği, bu da SA'nın sarıçamda soğuk kaynaklı oksidatif stresi azalttığını göstermektedir. Sonuçlarımıza benzer olarak, soğuk stresi altında SA uygulamasının ROT içeriğini azalttığıyla ilgili Mutlu ve ark., (2013) arpada ve Wang ve ark., (2009) pirinçte rapor etmişlerdir.

Prolin stres sırasında redoks dengesini ve hücresel homeostazı korur ve ROT'un zararlı etkilerini azaltabilir. Dıştan uygulanan SA'nın prolin gibi ozmolitlerin birikimini uyardığı bilinmektedir (Gharib, 2006). Buna göre, prolinin soğuğa toleransta önemli rol oynayan ana ozmolitlerden biri olmasına göre, düşük sıcaklıkta SA seviyesinin artması, buğday fidelerinin soğuğa toleransının daha yüksek olmasına neden olduğunu göstermiştir. Mevcut çalışmada, elde edilen veriler doğrultusunda sarıçam kotiledonlarında serbest prolin birikimi soğuk stresinin neden olduğu ozmotik strese yanıt olarak su dengesinin korunması şeklinde olabileceği yönündedir.

Bitkilerin ROT'tan korunmasında antioksidan enzimler, onları nötralize etme kabiliyeti nedeniyle kritik bir rol oynamaktadır (Pal ve ark., 2013). Mevcut çalışmada farklı konsantrasyonda SA uygulamalarıyla birlikte SOD ve GPX enzim aktivitelerideki azalışın muhtemelen bir diğer antioksidan özelliğe sahip olan prolin içeriğinin artışından dolayı olduğu düşünülmektedir. Genel olarak yapılan çalışmalarla stres koşullarında antioksidan enzim aktivitelerinde artışlar meydana geldiği rapor edilmiştir (Bowler ve ark., 1992; Fu ve Huang, 2001; Zhao ve ark., 2010). Mevcut çalışmada kontrol grubundaki (soğuk stresi altında büyüyen bitkiler) enzim artışlarının bu yönüyle literatürle uyumlu olduğu görülmüştür. Örneğin SA'nın soğuk stresine karşı etkilerinin araştırıldığı bir çalışmada, kışlık buğday yapraklarında soğuk koşullarında yüksek miktarda SA tespit edildiği rapor edilmiştir (Taşgın ve ark., 2003). Aynı çalışmayla, yapraklara dışarıdan uygulanan 100 µM SA'nın soğuk toleransını arttırdığı belirtilmiştir. Araştırmacılar söz konusu tolerans artışını antioksidan enzim etkinliğindeki artışa bağlamışlardır. Raporlardaki sonuçların aksine mevcut çalışmada farklı konsantrasyonda SA uygulamalarıyla birlikte SOD ve GPX enzim aktivitelerideki azalışın muhtemelen bir diğer antioksidan özelliğe sahip olan prolin içeriğinin artışından dolayı olduğu düşünülmektedir. Burada antioksidan olarak oksidatif stres hasarının iyileştirilmesinde prolinin rolü üstlendiği ve ROT'ların temizlenmesinde, membran hasarının iyileştirilmesinde önemli etkiye sahiğ olduğu açıktır. Literatür bilgileride prolinin ve SA'nın antioksidan rollerinin olduğunu desteklemektedir (Raskin, 1992; Fariduddin ve ark., 2003; Muthulakshmi ve Lingakumar, 2017). Bu nedenle hücrede stres kaynaklı biriken ROT'ların hem salisilik asit aracılıklı prolin seviyesindeki artışla temizlendiğini söylemek muhtemeldir.

Sonuç olarak, soğuk stresi altında 50 ve 100 µM SA'nın çimlenme sayısını, kök boyu uzunluğunu, NSİ, toplam klorofil ve karotenoid içeriği ile prolin miktarını arttırdığı, TBARS, $H_2O_2$ ile SOD ve GPX aktivitesini ise azalttığı tespit edildi. Bu değerlerin dışında kalan SA konsantrasyonları genellikle bu parametreler üzerinde ya etkisiz ya da olumsuz etki yaptığı görüldü. Çalışmada 50 ve 100 µM SA aracılıklı prolin artışı hem ozmolit olarak bitkide su durumunun korumasını sağlamasının yanında antioksidan olarak $H_2O_2$ gibi ROT'ların seviyesini ve membran hasarını azalttığı belirlendi. Dıştan uygulanan 50 ve 100 µM SA'nın soğuk stresine tolerans sağlayan (16 °C) sarıçam tohumlarının yüksek oranda çimlenme ve çıkış performanslarını arttırdığı ve bu artışın prolin birikiminin SA tarafından teşvik edilmesinden (SA-prolin karşılıklı etkileşim) kaynaklandığı ortaya koyulmuştur.

## KAYNAKLAR

AbdElgawad H, Zinta G, Hegab MM, Pandey R, Asard H, Abuelsoud W 2016. High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Frontiers in Plant Science, *7*, 276.

Alonso-Ramirez A, Rodriguez D, Reyes D, Jimenez JA, Nicolas G, Lopez-Climent M, Gomez-Cadenas A, Nicolas C 2009. Evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in *Arabidopsis* seeds. Plant Physiol, 150(3): 1335-1344.

Arnon DI 1949. Copper Enzymes in Isolated Chloroplasts, Polyphenoxidase in *Beta vulgaris*. Plant Physiology, 24: 1-15.

Bates LS, Waldren RP, Teare ID 1973. Rapid Determination of Free Proline for Water Stress Studies. Plant and Soil, 39: 205-207.

Beauchamp C, Fridovich I 1971. Superoxide Dismutase: Improved Assays and An Assay Applicable to Acrylamide Gels. Analytical Biochemistry, 44: 276-287.

Bertamini M, Zulini L, Muthuchelian K, Nedunchezhian N 2007. Low night temperature effects on photosynthetic performance on two grapevine genotypes. Biologia Plantarum, 51: 381-385.

Bhattacharjee S 2012. An inductive pulse of hydrogen peroxide pretreatment restores redox-homeostasis and oxidative membrane damage under extremes of temperature in two rice cultivars. Plant Growth Regulation, 68: 395-410.

Boratynski A 1991. Range and natural distribution, In: Genetics of Scotspine: (Ed: Giertych, M., Matyas, C.), Elsevier, Amsterdam, pp. 19-30.

Borsani O, Valpuesta V, Botella MA, 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. Plant Physiol, 126(3): 1024–1030.

Bowler C, Van Maontague M, Inze D 1992. Superoxide Dismutase and Stress Tolerance. Annual Review of Plant Physiology and Plant Molecular Biology., 43: 83-116.

Bray EA, Bailey-Serres J, Weretilnyk E 2000. Responses to a biotic stress. Biochemistry and Molecular Biology of Plants, In: Gruissem, W., Buchannan, B., Jones, R. (ed.), American Society of Plant Biologists, Rockville, pp. 158-1249.

Das K, Roychoudhury A 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science, 2: 53.

Dolatabadian A, Modarres Sanavy SAM, Sharifi M 2009. Effect of salicylic acid and salt on wheat seed germination. Acta Agriculturae Scandinavica Section B: Soil and Plant Science, 59(5): 456–464.

Einset J, Winge P, Bones A 2007. ROS signaling pathways in chilling stress. Plant Signal Behav, 2: 365–367.

Fariduddin Q, Hayat S, Ahmad A 2003. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. Photosynthetica, 41: 281–284.

Fayez KA, Bazaid SA 2014. Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. Journal of the Saudi Society of Agricultural Sciences, 13: 45-55.

Fu J, Huang B 2001. Involvement of Antioxidants and Lipid Peroxidation in the Adaptation of Two Cool-Season Grasses to Localized Drought Stress. Environmental and Experimental Botany, 45: 105-114.

Gautam S, Singh PK 2009. Salicylic acid-induced salinity tolerance in corn grown under NaCl stress. Acta Physiol Plant, 31: 1185.

Gharib FA 2006. Effect of salicylic acid on the growth, metabolic activities and oil content of basil and marjoram. Int. J. Agr. Biol, 4: 485-492.

Gill SS, Tuteja N 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in cropplants. Plant Physiol Biochem, 48: 909–930.

Güneş A, Inal A, Alpaslan M, Eraslan F, Bagci EG, Cicek N 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. Journal of Plant Physiology, 164: 728-736.

Hanan ED 2007. In fluence of salicylic acid on stress tolerance during seed germination of Triticumaestivum and mordeumvulgare. Biological Research, 1:40-48.

Hara M, Furukawa J, Sato A, Mizoguchi T, Miura K 2012. Abiotic stress and role of salicylic acid in plants. Abiotic Stress Responses in Plants, 235-251.

Heath RL, Packer L 1968. Photoperoxidation in Isolated Chloroplast. I. Kinetics and Stoichiometry of Fatty Acid Peroxidation, Archives of Biochemistry and Biophysics, 125: 189-198.

Hu Z, Fan J, Xie Y, Amombo E, Liu A, Gitau MM., Khaldun ABM, Chen L, Fu J 2016. Comparative photosynthetic and metabolic analyses reveal mechanism of improved cold stress tolerance in bermudagrass by exogenous melatonin. Plant Physiology and Biochemistry, 100: 94-104.

Hussain HA, Hussain S, Khaliq A, Ashraf U, Anjum SA, Men S, Wang L 2018. Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. Frontiers in Plant Science, 9: 393.

Kang G, Wang C, Sun G, Wang Z 2003. Salicylic acid changes activities of $H_2O_2$-metabolizing enzymes and increases the chilling tolerance of banana seedlings. Environ. Exp. Bot. 50: 9–15.

Kaya A, İnan M 2017. Tuz (NaCl) stresine maruz kalan reyhan (*Ocimum basilicum* L.) bitkisinde bazı morfolojik, fizyolojik ve biyokimyasal parametreler üzerine salisilik asidin etkileri. Harran Tarım ve Gıda Bilimleri Dergisi, 21: 332-342.

Keshavarz H, Sanavy SAMM, Moghadam RSG 2016. Impact of foliar application with salicylic acid on biochemical characters of canola plants under cold stress condition. Notulae Scientia Biologicae, 8(1): 98-105.

Korkmaz A 2005. Inclusion of acetyl salicylic acid and metyl jasmonate into the priming solution improves love-tempature germination and emergence of sweet pepper. Horticultural Science, 40: 197-200.

Korkmaz A 2005. Inclusion of acetyl salicylic acid and metyl jasmonate into the priming solution improves love-tempature germination and emergence of sweet pepper. Horticultural Science, 40: 197-200.

Kratsch HA, Wise RR 2000. The ultrastructure of chilling stress. Plant, Cell and Environment, 23(4): 337-350.

Liu W, Ai XZ, Liang WJ, Wang HT, Liu SX, Zheng N 2009. Effects of salicylic acid on the leaf photosynthesis and antioxidant enzyme activities of cucumber seedlings under low temperature and light intensity. Chinese J. Appl. Ecol., 20: 441–445.

Mahajan S, Tuteja N 2005. Cold, salinity and drought stresses: an over- view. Arch Biochem Biophys, 444:139–158.

Marcińska I, Czyczylo-Mysza I, Skrzypek E, Grzesiak MT, Janowiak F, Filek M, Cyganek K, 2013. Alleviation of osmotic stress effects by exogenous application of salicylic or abscisic acid on wheat seedlings. International journal of molecular sciences, 14(7): 13171-13193.

Mora-Herrera ME, López-Delgado H, Castillo-Morales A, Foyer CH, 2005. Salicylic acid and $H_2O_2$ function by independent pathways in the induction of freezing tolerance in potato. Physiol. Plant, 125: 430–440.

Muthulakshmi S, Lingakumar K 2017. Role of salicylic acid (SA) in plants-a review. Int J Appl Res, 3(3): 33-37.

Mutlu S, Karadağoğlu Ö, Atici Ö, Nalbantoğlu B 2013. Protective role of salicylic acid applied before cold stress on antioxidative system and protein patterns in barley apoplast. Biologia Plantarum, 57(3): 507-513.

Pal O, Gondor OK, Janda T 2013. Role of salicylic acid in acclimation to low temperature. Acta Agron Hung., 61(2):161-172.

Rajjou L, Belghazi M, Huguet R, Robin C, Moreau A, Job C, Job D 2006. Proteomic investigation of the effect of salicylic acid on Arabidopsis seed germination and establishment of early defense mechanisms. Plant Physiol, 141(3): 910–923.

Raskin I 1992. Salicylate, A New Plant Hormone. Plant Physiology, 99: 799-803.

Rivas-San Vicente M, Plasencia J 2011. Salicylic acid beyond defence: its role in plant growth and development. Journal of Experimental Botany, 62(10): 3321-3338.

Ruelland E 2017. Plant responses to chilling temperatures. Plant Stress Physiology, CABI, Wallingford, Oxfordshire, UK, pp: 97-137.

Rymen B, Fiorani F, Kartal F, Vandepoele K, Inzé D, Beemster GTS 2007. Cold nights impair leaf growth and cellcycle progression in maize through transcriptional changes of cell cycle genes. Plant Physiology, 143: 1429-1438.

Saltveit ME, Morris LL 1990. Overview on chilling injury of horticultural crops. Chilling Injury of Horticultural Crops, 3-15.

Senaratna T, Touchell D, Bunn E, Dixon K 2000. Acetyl Salicylic Acid Induce Multiple Stress Tolerance in Bean and Tomato Plants. Plant Growth Regulation, 30: 157-161.

Smart RE, Bingham GE 1974. Rapid estimates of relative water content. Plant Physiology, 53: 258-260.

Song SY, Chen Y, Chen J, Dai XY, Zhang WH 2011. Physiological mechanisms underlying OsNAC5-dependent tolerance of rice plants to abiotic stress. Planta, 234: 331-34.

Tabassum T, Farooq M, Ahmad R 2017. Seed priming and trans generational drought memory improves tolerance against salt stress in breadwheat. Plant Physiol Biochem, 118: 362–369.

Taşgın E, Atıcı Ö, Nalbantoğlu B, 2003. Effects of salicylic acid and cold on freezing tolerance in winter wheat leaves. Plant Growth Regulation, 41: 231-236.

Turna I, Bilgili E 2006. Effect of heat on seed germination of Pinus sylvestris and *Pinus nigra* ssp. *pallasiana*. International Journal of Wildland Fire, 15(2): 283-286.

Urbanek H, Kuzniak-Gebarowska E, Herka K 1991. Elicitation of defense responses in bean leave by botrytis cinerea polygalacturanase. Acta Physiologiae Plantarum, 13: 43-50.

Uzunlu M 2006. Aspirinin kavun fidelerinin değişik abiyotik stres koşullarına karşı toleranslarının artırılması üzerine etkileri. Yüksek Lisans Tezi, Fen Bilimleri Enstitüsü, Bahçe Bitkileri Anabilim Dalı, Kahramanmaraş.

Velikova V, Yordanov I, Edreva A, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective roles of exogenous polyamines. Plant Science, 151: 59-66.

Wang DH, Li XX, Su ZK, Ren HX 2009. The role of salicylic acid in response of two rice cultivars to chilling stress. Biologia Plantarum, 53(3), 545.

Wang Y, Hu J, Qin G, Cui H, Wang Q 2012. Salicylic acid analogues with biological activity may induce chilling tolerance of maize (*Zea mays*) seeds. Botany, 90: 845–855.

Witham FH, Blaydes BF, Devlin RM, 1971. Chlorophyll absorption spectrum and quantitative determination. Experiments in Plant Physiology, 167-200.

Yang MT, Chen SL, Lin CY, Chen YM 2005. Chilling stress suppresses chloroplast development and nuclear gene expression in leaves of mung bean seedlings. Planta, 221(3): 374-385.

Yang Z, Cao S, Zheng Y, Jiang Y 2012. Combined salicyclic acid and ultrasound treatments for reducing the chilling injury on peach fruit. J. Agric. Food Chem. 60: 1209–1212.

Zhang Q, Chen Q, Wang S, Hong Y, Wang Z 2014. Rice and cold stress: methods for its evaluation and summary of cold tolerance-related quantitative trait loci, Rice, 7: 1-12.

Zhao S, Liu Q, Qi Y, Duo L 2010. Responses of root growth and protective enzymes to copper stress in Turfgrass. Acta Biologica Cracoviensia Series Botanica, 52: 7-11.

Zhao XQ, Wang WS, Zhang F, Zhang T, Zhao W, Fu BY, Li ZK 2013. Temporal profiling of primary metabolites under chilling stress and its association with seedling chilling tolerance of rice (*Oryza sativa* L.). Rice, 6: 23.

Zhu J, Kang H, Tan H, Xu M 2006. Effects of drought stresses induced by polyethylene glycol on germination of *Pinus sylvestris* var. *mongolica* seeds from natural and plantation forests on sandy land. Journal of Forest research, 11(5): 319-328.

## *ORAL PRESENTATION*

# Hydrogen peroxide applications in agricultural production

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## Abstract

The main purpose in plant production is to obtain the highest yield and quality with minimum input per unit area. Today, there are important stress factors on plants, mainly water and soil quality, other environmental conditions and climate-related stress factors. In order to reduce or eliminate the mentioned stress factors, many different applications are made in the production process. These applications can also cause some positive or negative effects on yield and quality. In recent years, Hydrogen peroxide ($H_2O_2$) has been applied to irrigation waters in order to reduce/prevent the negative effects of abiotic stress factors. Application of $H_2O_2$ together with irrigation water to the root zone of the plant can increase plant yield and quality by increasing the oxygen ($O_2$) concentration in the root zone. In this study, the studies on the use of $H_2O_2$ in agricultural production and its effects were examined and its advantages and disadvantages were examined.

**Keywords:** Plant, $H_2O_2$, Agricultural Use

## INTRODUCTION

Outdoor crop production is a form of production completely dependent on climatic conditions. It is a vital issue to reduce the negative effects of other abiotic stress factors, especially climatic fluctuations, on plants, and even if possible, to eliminate them. The basic logic in crop production is to obtain the highest yield, quality and therefore income with minimum input per unit area. Every application made in herbal production can cause some positive or negative effects on yield and quality. When the literature is examined, it is reported that $H_2O_2$ application has positive effects on plant growth, especially in low quality irrigation water and soil conditions. When the studies are examined, there are many studies reporting that the application of $H_2O_2$ to irrigation water improves both the yield and quality of the plant by increasing the $O_2$ concentration of the root zone of the plant. In particular, there are studies that changing the frequency, volume and position of aeration affect different chemical and physical properties of the rhizosphere soil and increase the number of microorganisms, enzyme activity and the availability of nutrients in the soil. When the temperature and humidity of the soil are suitable and aeration is sufficient, aerobic microorganisms in the soil are more active and more nutrients that plants can get from the soil are given to the environment (Gyaneshwar et al., 2002). Root zone oxygen concentration is an extremely important parameter for plant growth as explained. Reducing the oxygen concentration in the soil affects plant physiological processes such as nutrient and water intake, respiration, the redox potential of soil elements as well as the activity of soil microorganisms. There is a very close relationship between the soil air and the biological properties of the soil. Soil respiration, soil enzyme activity and soil biomass are the leading biological activities of the soil. Soil respiration is a measure of the carbon dioxide ($CO_2$) released from the soil by the decomposition of soil organic matter by microorganisms, from plant roots or by respiration of soil organisms. Soil respiration is an important indicator of soil health and is affected by excessive tillage, soil compaction, anaerobic conditions or other factors that reduce soil health. The respiration rate of the soil is an indicator of the nutrients contained in the organic matter (for example, phosphate as $PO_4$, nitrate nitrogen as $NO_3$, and sulfate as $SO_4$).
In this study, the possible advantages and disadvantages of $H_2O_2$ application to plants will be revealed in the light of the researches.

## What is Hydrogen Peroxide

Hydrogen peroxide is a chemical compound composed of water and oxygen. Small amounts of gaseous hydrogen peroxide occur naturally in the air. The chemical formula of hydrogen peroxide is $H_2O_2$. It was first recognized as a chemical compound in 1818, and in 1894 Richard Wolffenstein finally discovered how to produce pure $H_2O_2$ and made it widely commercially available for the first time. $H_2O_2$ can be used in multiple environments to kill 99.9% of all microorganisms. Depending on its concentration, it can be used as a low-level as well as high-level disinfectant, and as a sterilizer in homes, personal properties and commercial environments such as schools, and in-hospital rooms and equipment. It is mostly safe and environmentally friendly for humans and pets. The extra oxygen in $H_2O_2$ attacks and destroys a microorganism's membrane lipids, DNA, and other cell components that often act as a defense mechanism. Due to its ability to attack the defenses of microorganisms and relative safety for humans, pets and the environment, it is an excellent antimicrobial disinfectant cleaner and disinfectant.
The CDC report on chemical disinfectants includes protocols for the use of hydrogen peroxide as low as 0.05% for a regular disinfecting cleaner. As a high-level disinfectant, the user should use 7.5% hydrogen peroxide for 30 minutes at 20 degrees Celsius. To use as a high-grade sterilizer, the user needs to use the same concentration for 6 hours at 20 degrees Celsius. It can be used for 21 days and has a stable shelf life of 2 years. The most common and safe commercially is 3%. In high concentrations, it can be used as a bleach for textiles and paper, as a component of rocket fuels, and to produce foam rubber and organic chemicals. Much higher concentrations are commercially available, from 35% to 50% and 90%. The higher the strength, the more volatile the liquid and the more dangerous it is with more bleaching and burning effects. Hydrogen peroxide is an inexpensive, effective disinfectant and sanitizing agent with relatively few disadvantages. Among the different disinfectants and cleaners available on the market, hydrogen peroxide is time and again far superior at disinfecting and sterilizing an environment.

## Efforts to Increase Yield

Mansouri and Ali (2021), conducted a experiment in the winter season of 2020-2021 to investigate the effect of foliar spraying with hydrogen peroxide and foliar application of zinc on some growth, yield and antioxidant enzymes of the wheat crop. The experiment consists of three different applications, including the control subject. As a control subject, only distilled water was sprayed on the plant leaves. As an application, three different concentrations of $H_2O_2$ (25, 50 and 75 ppm) and three different concentrations of zinc (50, 100 and 150 mg $L^{-1}$) were sprayed. Hydrogen peroxide ($H_2O_2$) was sprayed twice, once during tillering and stem elongation and second when flag leaf started to form. Zinc; It was applied by spraying twice, the first at the head stage (where the head would come out of the stem) and the second at the flowering stage. As a result of the applications, high concentrations of hydrogen peroxide spraying on wheat plants led to a significant reduction in plant height, yield and components. However, it led to a significant increase in the activity of antioxidant enzymes (SOD and CAT) and proline content. In addition, it was determined that spraying wheat plants with high concentrations of zinc resulted in a significant increase in all parameters examined.

Bhattarai et al., (2004), investigated the effects of $H_2O_2$ and air injection application in subsurface drip irrigation on yield and yield components of pumpkin, soybean and cotton plants. In the study, two subjects, which are applied and not applied hydrogen peroxide, are discussed. For $H_2O_2$ application, 1 ml/L of $H_2O_2$ was mixed into the irrigation water. While normal irrigation water contains oxygen concentration between 3-8 ppm, a concentration as high as 42 ppm was obtained in aerated irrigation water. As a result, it was stated that the applied $H_2O_2$ increased the fruit yield by 25% (16.53 t $ha^{-1}$) compared to the control subject (13.59 t $ha^{-1}$).
Jafariyan and Zarea (2016), set up an experiment in a semi-arid climate region of Iran to investigate whether the pretreatment of wheat seeds with $H_2O_2$ affects the stimulating effect of *Azospirillum* on the plant. Experiment; it consists of three subjects: seeds inoculated with *Azospirillum* only, seeds not treated with *Azospirillum*, and seeds soaked with hydrogen peroxide at four concentrations (0.25, 50 and 80%) and inoculated with *Azospirillum*. Soaking the seed in various concentrations of $H_2O_2$ solution caused an increase in stomatal density, an increase in leaf length and histological components. In this study, pre-sowing application of $H_2O_2$ increased grain yield of wheat through modulation of leaf histological components, increase of photosynthetic pigments, induced activity of catalase and ascorbate peroxidase. In addition, *Azospirillum* vaccine improved the grain yield of wheat and it was observed that the grain yield increasing effect was more pronounced when used together with $H_2O_2$ pretreatment. Soaking the seed in 80% $H_2O_2$

solution and then grafting it with *Azospirillum* resulted in the highest grain yield. In addition, it has been observed that *Azospirillum* affects some features of leaf anatomical components.

Farouk et al. (2018), the role of pre-sowing seeds soaking in hydrogen peroxide (0.2% and 4%) and/or foliar application and folic acid (0, 10 and 20 mg/l) alone in the Experimental Farm of the Department of Agricultural Botany of Mansoura University conducted two field experiments to evaluate the effects of pea plants (Pisum sativum L. Cv Master-B) on growth, yield, some biochemical components and anatomical characters, both alone and/or in combination. Experiment; untreated control plant, seed pre-soak in 2% hydrogen peroxide ($H_2O_2$), seed pre-soak in 2% $H_2O_2$ plus 10 mg/L folic acid (FA) spray to leaves, 2% $H_2O_2$ seed pre-soak plus 20 mg/L FA foliar spray, seed pre-soak in 2% hydrogen peroxide ($H_2O_2$), seed pre-soak in 2% $H_2O_2$ plus 10 mg/L folic acid (FA) spray to leaves, 2% $H_2O_2$ seed pre-soak plus 20 mg/L FA foliar spray , Seed pre-soak in 4% $H_2O_2$, Seed pre-soak in 4% $H_2O_2$ plus 10 mg/L FA foliar spray, 4% $H_2O_2$ seed pre-soak plus 20 mg/L FA foliar spray, 10 mg/L FA foliar application and 20 mg /L FA consists of nine processes, including foliar application. As a result of the applications; stated that seed soaking with 2% hydrogen peroxide plus 20 mg/L folic acid foliar application before planting can be recommended to increase the growth, yield and seed quality of pea plants. They emphasized that further experiments are needed to better understand the relationships between folic acid concentration, hydrogen peroxide and/or folic acid administration in pea seed treatment.

Ahmad et al.,(2014) conducted two different experiments (pot and field) to investigate the role of exogenous application of ascorbic acid (ASA), salicylic acid (SA) and $H_2O_2$ on the morphology, biochemical properties and grain yield of first crop maize. The pot experiment was carried out in pots filled with sand in the net house of the Department of Crop Physiology, Agricultural University of Pakistan, Faisalabad. The field experiment was carried out at the Application Station. Hi Sawn 9697 hybrid maize cultivar was used as experiment material. In the pot experiment, ASA, SA and $H_2O_2$ (0, 20 and 40 $mgL^{-1}$ each) were applied as a foliar spray at 3 leaf stages (18 days after planting). At 35 days after sowing, five uniform seedlings were harvested from each pot; the seedlings were analyzed for vigor and antioxidant activity. In the field experiment, three treatments were applied by applying ASA, SA and $H_2O_2$ as a seed coat or as a foliar spray. In the results of the study; in the pot experiment, application of ASA, SA and $H_2O_2$ to the leaves at each concentration improved seedling growth, leaf juice, chlorophyll b content, membrane stability and enzymatic antioxidant activities in maize. In the field experiment, the application of these substances via seed preparation or foliar spray improved the morphological, yield-related characteristics and grain yield of maize. However, they concluded that seed coating was more effective than foliar application. As a result, they stated that the yield of maize can be increased by seed preparation method with ASA, SA and $H_2O_2$ under low temperature stress.

Mahmoud (2015) conducted a study to evaluate the effects of hydrogen peroxide on the salt tolerance response of two wheat cultivars [Gemaiza 9 (G9) and Sakha 93 (S93)] in a greenhouse, in which 18.0 g of seeds (average 372 g seeds) were placed in 40 separate plots of 1 $m^2$ filled with alluvial soil were planted. G9 variety is sensitive to salinity, while S93 is highly salinity tolerant variety. Two different H2O2 levels (0.1 and 0.2 mM) were applied to different irrigation water salinity (0.65, 10 and 15 dS/m) levels. Addition of $H_2O_2$ to salt water made G9 wheat plants produce more soluble sugars compared to salt-stressed plants without $H_2O_2$ treatment, whereas S93 had no significant effect. It was determined that the addition of $H_2O_2$ to the irrigation water increased both the biomass and grain yield of G9 at all irrigation water salinity levels, while it decreased the yield of the tolerant variety S93. The addition of $H_2O_2$ to brine at early growth stages is thought to increase plant growth and yield of salt-sensitive wheat varieties.

**Efforts to Increase Quality**

Ijaz Ahmad et al., (2015) conducted a study in the Department of Crop Physiology, Agricultural University, Pakistan, Faisalabad, Pakistan, on the improvement of seedling growth of spring corn at the lowest temperature by seed coating with ASA, SA and $H_2O_2$. The experiment consists of three applications as seed coating with different levels (0, 20 and 40 mg $L^{-1}$) of ASA, SA and $H_2O_2$. As a result of the experiment; It was determined that seed coating with all three applications improved the growth of seedlings at low temperatures. Likewise, seed preparation with ASA, SA and $H_2O_2$ has been found to improve seedling formation by triggering the antioxidant defense system and nutrient homeostasis. 20 mg $L^{-1}$ or 40 mg $L^{-1}$ $H_2O_2$ provided maximum seed germination in maize by stimulating the seed preparation superoxide dismutase (SOD) activity with ASA and SA solution, improving the nutrient content in roots and shoots. According to the results of this study, it was concluded that 20 mg $L^{-1}$ $H_2O_2$, ASA and SA is the appropriate concentration for seed preparation.

Kucerová et al., (2021), investigated the effect of plasma active water on lettuce plant (Lactuca sativa L. Var.) and compared it with the effect of $H_2O_2$ and/or $NO_3^-$ solutions at various concentrations to evaluate their role in plasma active water. In the study, lettuce plants previously grown in pots were irrigated with plasma activated water (PAW), $H_2O_2$ and $NO_3$ solutions obtained from tap water and after 5 weeks, growth parameters, leaf number and quality, fresh and dry weights of plants, photosynthetic pigment (chlorophyll a+b) content, photosynthetic rate and activities of antioxidant enzymes (superoxide dismutase, SOD) were evaluated. In the results of working; It was observed that lettuce plants irrigated with PAW had similar dry weight compared to chemically equivalent $H_2O_2$ and $NO_3$ solutions. At the same time, PAW resulted in higher photosynthetic pigment content, higher photosynthetic rate and lower SOD activity. $NO_3$ mainly contributed to the increase in dry weight, photosynthetic pigment content and photosynthetic rate, and overall better appearance of the plants. $H_2O_2$ contributed to the increase of dry weight and induction of SOD activity. In general, it has been observed that $H_2O_2$ and $NO_3$ can stimulate plant growth and affect physiological properties at appropriate concentrations.

**Studies Against Diseasest and Pests**

Tenientea et al.,(2018) The effect of exogenous application of $H_2O_2$ on the induction of resistance to infection in the pepper plant of Papper Golden Mosaic Virus (PepGMV), widely found in Mexico, accepted as the main viral pathogen of pepper (*Capsicum annuum* L.) and transmitted by whitefly they examined. As the first stage of the study, $H_2O_2$ was applied to plants with 4-6 leaves at three different concentrations (6, 14 and 18 mM) every two weeks while growing the plants. Next, positive disease control plants were irrigated with tap water and PepGMV inoculated immediately after the first $H_2O_2$ application. Negative disease control plants were biolistically mock-inoculated using the pBluescript plasmid, irrigated with tap water or applied $H_2O_2$ at the same concentration applied every five days. Another negative disease control group was evaluated by applying only three concentrations of $H_2O_2$ without vaccination. In conclusion, applications of $H_2O_2$ at the three concentrations evaluated showed significant symptomatic reduction in plants during all trials in a dose-dependent manner. These results revealed that external foliar applications of $H_2O_2$ protected pepper plants against PepGMV infection and stimulated the plant host defense system.

Moore et al.,(2011), conducted a study to evaluate the efficacy of different antimicrobial plant extract-concentrated formulations on four types of organic leafy greens (organic lettuce, iceberg, adult bunched spinach and baby spinach) inoculated with S. Enterica serovar Newport. In the study, each leaf sample was washed, inoculated with Salmonella Newport (106 CFU/ml) and dried. Then, olive extract, apple, mallow and hydrogen peroxide concentrates prepared in certain proportions were applied and the results were evaluated. According to these results; it has been observed that the antimicrobial activity of natural plant extract applications tested against Salmonella Newport is dependent on both concentration and time. In addition, the immersion process is similar to traditional fresh produce washing processes in which the product is immersed in chlorinated water. They also noted that immersion showed the greatest reduction in bacteria in other studies.

Sichela et al., (2009) conducted a study to show for the first time how disinfection concentrations of $H_2O_2$ can be significantly reduced using the synergistic lethality of $H_2O_2$ and sunlight for fungi and disinfection. In this study, experiments were carried out on spores of the ubiquitous python and human pathogenic fungus Fusairum solani. 35% by weight $H_2O_2$ was used and diluted directly in the reactor water. These studies were carried out using natural well water so that the chemical environment in which inactivation takes place is as similar as possible to irrigation water that phytopathogenic fungi can produce. As a result of the study; Combined solar and $H_2O_2$ disinfection were found to be effective against F. Solani even under low solar irradiation at laboratory and CPC reactor scale. This finding has been proven not only in distilled water, but also in real well water. They emphasized that the sensitivity of F. Solani spores to $H_2O_2$+solar UV is the first proof of the suitability of this method for fungal disinfection.

Alakhdar and Shoala (2021), experiment with the two-spotted spider mite *Tetranychus urticae* to examine the performance of $H_2O_2$ application in three cultivars (Nebraska, Paulista and Valentino) of Common Bean (*Phaseolus vulgaris* L.) for morphological characteristics and yield under field infestation. Experimental design was planned as a randomized full block with three repetitions. $H_2O_2$ was applied exogenously to three different bean varieties at concentrations of 1 mM and 1.5 mM. Exogenous application of $H_2O_2$ improved the signaling of Reactive Oxygen Species (ROS), which subsequently improved the cellular level and the

resistance of the whole plant. It has been observed that ROS or hydrogen peroxide increase in cells in response to exogenous application of $H_2O_2$ can be toxic to mites, affecting their feeding and then reproduction of mites. Exogenous application of $H_2O_2$ at a certain concentration was found to induce interactions in increasing crop yield and resistance to spider mites.

El-Saedy et al. (2019) studied the nematicidal effects of abamectin, boron, chitosan, hydrogen peroxide, and Bacillus thuringiensis against citrus nematodes in Valencia orange trees in successive 2017 and 2018 seasons. The study was carried out in a Valencia orange orchard infested with citrus nematode in Nubaria, in the Egyptian province of El-Behera. The applications were applied twice a season with the drip irrigation system installed at a depth of 10 cm from the surface. The first application was made at the beginning of the research (in April) and the second application was made 3 months later (in August). In the results of study; it was observed that abamectin, boron and other tested compounds had significant nematicidal activity against citrus nematode. It was also found that these treatments significantly improved fruit yield and fruit quality of orange trees compared to untreated trees (control experiment).

**Studies on the Use of Low Quality Irrigation Water**

Tonegawa et al., (2003) used additives to facilitate the removal of 2,4-dichlorophenol (2,4-DCP) from water by using chopped horseradish (*Armoracia rusticana*) as a carrier of peroxidase activity. The use of additives such as polyethylene glycol (PEG), selected surfactants, chitosan gel or activated carbon has been shown to improve the enzymatic treatment of water contaminated with organic compounds. Triple samples of contaminant solutions (5 or 10 mL) were modified with different additives, minced horseradish roots (or horseradish pieces sealed between two closed chitosan films) and incubated with $H_2O_2$. As a result of the study; Chopped horseradish, $H_2O_2$ and additives has proven to be a promising combination for removing phenols from aqueous solutions and at the same time improving the quality of purified water. However, it has been seen that the selection of additives and the development of application methods for certain situations (eg. combinations of pollutants, environmental conditions, amount of pollution, etc.) require further studies.

Taş et al., (2016) subjected wastewater treatment plant effluent to different hygiene treatments (control, activated carbon treatment (AC), AC+ $H_2O_2$ and ozone) in a study they conducted under greenhouse conditions. As a result of the research, they reported that the plant height of the subject applied $H_2O_2$ was higher than the other applications. They stated that this was followed by ozone and AC + $H_2O_2$ applications. They attributed the reason for this to the fact that the oxygen in the $H_2O_2$ structure increased the oxygen content of the wastewater. In addition, they emphasized that the application of $H_2O_2$ significantly improves the oxygen concentrations of wastewater and that such studies will also prevent biofilm formation and dripper clogging, especially in surface and subsurface drip irrigation applications.

**Studies on Limited Irrigation**

Orabi et al. (2018) conducted a pot experiment in Egypt, Dokki, National Research Center greenhouse in 2013/2014 winter season to evaluate the effect of $H_2O_2$ spraying on growth, yield and antioxidant defense system of canola plants grown at different irrigation water levels. This experiment included 9 treatments, including all combinations between three irrigation interval levels (D4, D8 and D12) and three external applications of $H_2O_2$ (H0, H1 and H2). In the results of study; By expanding irrigation intervals, $H_2O_2$ was increased as water stress, free radical and lipid peroxidation, but this situation negatively affected growth and yield. However, the application of low concentrations of $H_2O_2$ resulted in the highest growth and yield, as well as acting as a signaling molecule to improve the antioxidative defense system. Therefore, it was concluded that the application of low concentration of $H_2O_2$ can help plants resist drought stress caused by long periods between irrigation of canola plants.

Sarıyev et al., (2020), in a study they conducted in Adana, used subsurface drip irrigation method and different water constraints (100% and 70%) and $H_2O_2$ (0 ppm, 250 ppm, 500 ppm) injection in maize plant yield and yield examined the effect on their properties. In the study, irrigations were applied to the field capacity (100%) when 50% of the available soil moisture was consumed, with full water restriction (70%). $H_2O_2$ was applied 3 times during the growing season in the corn plant according to the development periods. As a result of the study, they stated that the highest corn yield was obtained from the subject of full water and the application of 250 ppm $H_2O_2$. As a result of the study, they reported that even though the $H_2O_2$ concentration was different

in limited water conditions, when injected into the soil through irrigation water, plant growth, yield parameters and water use efficiency were optimally improved.

**Studies Against Other Stress Factors**

Sohag et al., (2020), in this study, investigated the relative effectiveness of exogenous $H_2O_2$ and SA in providing drought tolerance in rice (*Oryza sativa* L.). The experiment was repeated twice, first in the hydroponic system and second in the soil. In the hydroponic system experiment, seeds were soaked in $H_2O_2$ (5 and 10 mmol/L) or SA (0.5 and 1 mmol/L) solution in the dark for 24 hours and then incubated in Petri dishes containing 20 mL water or 15% PEG-6000 for 7 days. At the seedling stage, 7-day-old seedlings were exposed to nutrient solution (Cooper 1996) containing 15% PEG-6000 and H2O2 (5 and 10 mmol/L) or SA (0.5 and 1 mmol/L) were applied to leaves (10 mL/L) at 1-day intervals. spray/pot) applied. In the soil-based study, they planted the germinated seeds and grew them with good irrigation for 10 days, then stopped the irrigation and applied $H_2O_2$ or SA (10 mL/spray/pot) at 1 day intervals. As a result of these studies, it was revealed that drought inhibited germination indices, seedling growth, photosynthetic pigments and water content, but increased proline content. For all that, they observed that exogenous $H_2O_2$ or SA treatment effectively attenuated oxidative damage in rice seedlings in both hydroponic and soil systems by up-regulating antioxidant enzymes. They also stated that exogenous $H_2O_2$ or SA protects photosynthetic pigments from oxidative damage, which can help maintain normal photosynthesis under drought. Finally, their findings indicated that exogenous $H_2O_2$ or SA could be equally effective on varying growth rates of rice seedlings under drought conditions.

Abd Elhady et al., (2021), in a study they conducted, showed that subsurface drip irrigation and different water constraints (full and restricted irrigation) and $H_2O_2$ applications (0, 300, 600 ppm) alleviate drought stress, improve growth, examined yield characteristics and its effect in potato plants. In the experiment, $H_2O_2$ application started from the sprouting of potato tubers (21 days after planting) and continued until the ripening period. In addition, they injected $H_2O_2$ in each irrigation until the end of the irrigation period. They reported that they achieved the highest yield value with both 300 and 600 ppm at full irrigation level, and 600 ppm $H_2O_2$ injection under limited irrigation alleviated the inhibitory effects of drought stress on plant growth. In the study, they reported that the number of tuber was the highest at 600 ppm $H_2O_2$ level in the subject where the restriction was applied, and a higher tuber number was obtained at the 300 ppm level compared to 0 ppm. They also examined the effect on irrigation water use efficiency in potato plant and reported that the highest Irrigation Water Use Efficiency (IWUE) was achieved at 600 ppm $H_2O_2$ application at restricted irrigation level.

Ashfaque et al., (2014) applied different concentrations (0, 50, 100 nM) of $H_2O_2$ and 100 mM NaCl to bread wheat varieties grown under controlled conditions 1 week after germination in a study they conducted in India. It has been determined that $H_2O_2$ application provides protection against stress conditions by increasing the proline content in 30-day-old wheat seedlings. They also determined that the increase in proline also increased the water potential and osmotic potential of the plants. Likewise, they found that there was an improvement in the decrease in the pigment content of plants under stress with $H_2O_2$ application. They reported that plant growth parameters were improved in both groups with and without salt application, and $H_2O_2$ application reduced the pressure caused by salt stress, but increased the proline content, nitrogen assimilation, water holding capacity and the effectiveness of photosynthetic pigments.

Silva et al.,(2019) conducted a study on the use of $H_2O_2$ in basil (*Ocimum basilicum* L.) acclimation to salt stress. The aim of this study was to examine the effects of $H_2O_2$ application on basil's salt stress tolerance and essential oil yield. The applications in the study were control plants T1 – 0 mM NaCl and 0 µM $H_2O_2$; T2 - 80 mM NaCl and 0 µM $H_2O_2$; seedling stage T3 - 80 mM NaCl and 1000 µM $H_2O_2$/24 hours; seedling stage T4 - 80 mM NaCl and 1 µM $H_2O_2$/48 hours; T5 - 80 mM NaCl and 1000 µM $H_2O_2$/24 hours during the seedling and flowering period; were carried out in a completely random design with 6 treatments and 5 repetitions, including T6 – 80 mM NaCl and 1 µM $H_2O_2$/48 h during the seedling and flowering period. According to the results of the study, application of 1 µM $H_2O_2$/48 hours in the nutrient solution during the seedling period and its reapplication in the pre-blooming period of basil cv. they stated that 'Gennaro de Menta' was the most effective strategy for increasing salt tolerance. In this study, they showed that the application of $H_2O_2$ in nutrient

solution for basil cultivation in hydroponic systems using salt water is a suitable technique to promote biomass or essential oil yield.

Capitulinoa et al., (2022) conducted a study to evaluate $H_2O_2$ administration patterns as an attenuator of salt stress on gas exchange and growth of soursop seedlings. In working greenhouse conditions, five different electrical conductivity levels ECw (0.6 - control, 1.2, 1.8, 2.4 and 3.0 dS m-1) and four $H_2O_2$ application modes (M1 - without $H_2O_2$ application, M2 - application with seed soak, M3 - foliar spraying and M4 - seed wetting plus foliar spraying). In the study, they used 20 µM $H_2O_2$ concentration in different application forms. As a result of the study; They observed that irrigation water salinity above 0.6 dS m-1 reduced gas exchange at 145 days after planting and the growth of soursops plants at 85 and 145 days after planting. They also observed that foliar spray $H_2O_2$ application method reduced the effect of salt stress on stomatal conductivity, transpiration and $CO_2$ assimilation rate, and seed soaking and $H_2O_2$ application method reduced the detrimental effects of salt stress on the growth of soursops 85 days after sowing.

Semida (2016) conducted a study to examine the effect of $H_2O_2$ application on the leaves of two onion (*Allium cepa* L.) cultivars on salt stress under two different soil salinity (ECe = 7.94 and 8.81 dS/m) conditions. The study was conducted in the Sennoris Region of Fayoum Southwest Cairo, Egypt, in the 2013/14 and 2014/15 production seasons. The plants were irrigated with drip irrigation method at 2-day intervals under full irrigation conditions. $H_2O_2$ was applied to plant leaves at three different levels (0, 1.0 and 2.0 mM) by spraying at 20, 40 and 60 days after planting the seedlings. As a result of the study; It was determined that onion plants (Giza Red or Giza 20 variety) provided positive increases in plant growth, productivity and water use efficiency under salt stress conditions. In addition, it has been suggested by the researcher that the application of $H_2O_2$ to plants can activate the photosynthetic system and improve physiological properties in plants grown under adverse conditions of newly reclaimed saline soils.

Hemalatha et al. (2017); A study was conducted to evaluate the effects of $H_2O_2$ application on seeds of salt-tolerant, sensitive and moderately tolerant rice varieties under salt stress conditions. While maximum germination was achieved in all cultivars, the germination performance of the salt sensitive rice cultivar (ADT (R) 49) was improved under higher NaCl concentrations.

**Efforts to Increase Water Use Efficiency**

Pilar et al., (2009) reported that the biomass of the air in the root part of the plant and the water use efficiency increased significantly by injecting $H_2O_2$ into the irrigation water in their study on the avocado plant. In the study, $H_2O_2$ was applied to the soil with subsurface drip irrigation and the soil moisture was kept close to the field capacity. In order to keep the soil oxygen content at 16%, they injected 1 mg kg-1 $H_2O_2$ (50%) solution into the soil through the irrigation system. They planned according to the methodology described by Bhattarai et al., (2004), with the injection time corresponding to 10% of the total irrigation time. The oxygen diffusion in the soil was measured with oxygen electrodes placed at a depth of 15 cm before and after irrigation. As a result, it was found that injecting $H_2O_2$ into heavy clay soil managed at water content close to field capacity for four months resulted in higher WUE (water use efficiency) as well as an increase in the biomass of the aerial part of avocado trees.

Researchers (Gill et al., 2009, Schaffer et al., 2002) reported that the application of $H_2O_2$ to the soil through the irrigation system significantly increases the water use efficiency, ultimately the growth and development of plants. A few researchers (Gill et al., 2009, Kozlowski 1997, Hsu et al., 1999) reported that a decrease in the xylem/phloem ratio, which changes the physiological process of the plant, was observed in plants in low oxygen soils, but that the oxygen content of the $H_2O_2$ injected into the soil with the SDI (subsurface drip irrigation) system was found in the plant root zone. They reported that it increased the xylem/phloem ratio in this case.

**Studies on Different Irrigation Methods**

Ben-Noah and Friedman (2016), in a study on the applicability of $H_2O_2$ in different irrigation methods (subsurface and surface drip irrigation), stated that surface drip irrigation is more effective than surface drip

irrigation in increasing soil oxygen concentration. In addition, they stated that the most important advantage of adding $H_2O_2$ to irrigation water is to create a favorable atmosphere in the root zone. In the experiment, the oxygen storage capacity of the peroxide solution was higher than that of deoxygenated water, for example, the amount of oxygen in 1 L of 800 ppm $H_2O_2$ solution was measured as approximately 375 mg, which corresponds to the amount of oxygen in 1.56 L of atmospheric air or 46.8 L of water in equilibrium with atmospheric air (8 ppm $O_2$) stated that they are equivalent.

**Studies to Increase Frost Tolerance**

Wang et al., (2018); In this study, they conducted a research on the roles and interactions of $H_2O_2$ and ABA in SA-induced frost tolerance in wheat plants. In the study, winter wheat (*Triticum aestivum* L. Cv. Yangmai 16) was used as a plant. In the study, 0, 10, 100, and 1000 mM solution was sprayed on wheat leaves three times at 12-hour intervals at the four-leaf stage to determine the optimum exogenous SA concentration. Freezing stress was applied 12 hours after salic acid pretreatment and the plants were tested for 24 hours at -2ºC/400 mmol $m^{-2}$ $s^{-1}$. To investigate the exogenous source of $H_2O_2$ induced by SA, leaves were treated with plants containing 100 mM salic acid either 5 mM SHAM (cell wall peroxidase inhibitor) or 100 mM DPI (NADPH oxidase inhibitor) for 8 hours before application. To assess crosstalk between $H_2O_2$ and abscisic acid in SA-induced freezing stress, leaves were pretreated with 2 mM DMTU (a scavenger of $H_2O_2$ and OH) or 1 mMFlu (an abscisic acid synthesis inhibitor) 8 hours before 100 mm salic acid treatment. After 12 hours of salic acid treatment, the plants were frozen as described above. As a result of these applications, they observed that both endogenous $H_2O_2$ and abscisic acid (ABA) play important roles in salic acid (SA) induced freezing tolerance. They observed that $H_2O_2$ and ABA could form a positive feedback loop to mediate SA induced freeze tolerance in wheat plants. Finally, they have stated to increased $H_2O_2$ and ABA separately trigger defense pathways to upregulate the expression of cold sensitive genes and the activities of antioxidant enzymes to reduce electrolyte leakage and cell membrane peroxidation and ultimately improve the photosynthetic capacity (Fv/Fm and ETR) of wheat under freezing stress.

## CONCLUSION

In this review article, studies on $H_2O_2$ have been examined, and information on various $H_2O_2$ applications in agricultural areas has been summarized. However, considering the studies conducted, it has been determined that the studies on this subject are extremely inadequate. In case of a large number of studies covering a wide range of different applications, the inadequacy in this area can be partially eliminated.
As a result of the articles examined, it has been determined that the use of $H_2O_2$ in agricultural areas is very effective in increasing plant yield and quality, and reducing the stress factors that occur in plants for various reasons. In addition, since hydrogen peroxide has a positive effect on the disinfection of wastewater and has an effect on increasing water use efficiency, it is thought that it will be a very useful solution in eliminating the negative effects of plants due to water shortage, which is the biggest problem of today. These positive effects are directly related to the concentrations and application forms of hydrogen peroxide. If it is used in high doses, it can cause serious toxicity in plants, especially in the soil in the root zone. A microorganism attacks and destroys membrane lipids, DNA, and other cell components that often act as a defense mechanism. It has the ability to attack the defenses of microorganisms. As a result of the studies, it is stated that the most effective application method is the application types made on the plant surface (external). Similarly, there are findings indicating that the applications together with the subsurface drip irrigation system increase the yield and quality of the plants. However, such studies need to be diversified and brought to the literature in order to reach a full conclusion.

## REFERENCES

Abd Elhady S. A., Abd El-Gawad H.G., Ibrahim M.F.M., Mukherjee S., Elkelish A., Azab E., Gobouri A.A, Farag R., Ibrahim H. and Abu El-Azm N. (2021). Hydrogen Peroxide Supplementation in IrrigationWater Alleviates Drought Stress and Boosts Growth and Productivity of Potato Plants. Sustainability 2021, 13, 899. https://doi.org/10.3390/su13020899.

Ahmad I., Basra S.M.A., and Wahid A., 2014. Exogenous Application of Ascorbic acid, Salicylic acid and Hydrogen peroxide Improves the Productivity of Hybrid Maize at Low Temperature Stress. International Journal Of Agriculture & Biology, Online: 1814-9596.

Ahmad I, Basra S.M.A., Hussain S., Hussain S.A., Rehman H., Rehman A., and Ali A., 2015. Priming with Ascorbic Acid, Salicylic Acid and Hydrogen Peroxide improves Seedling Growth of Spring Maize at Suboptimal Temperature. Journal Of Environmental and Agricultural Sciences, 3: 14-22.

Alakhdar H.H., Shoala T., (2021). Exogenous application of hydrogen peroxide in different resistant bean cultivars of *Phaseolus vulgaris* to *Tetranychus urticae* (Acari: Tetranychidae). Arthropod-Plant Interactions (2021) 15: 439–445. https://doi.org/10.1007/s11829-021-09829-1.

Al-Mansouri M.A., Ali I.M., 2021. Effect of Hydrogen Peroxide and Zinc Spraying on the Growth, Yield and Antioxidant Enzyme of Wheat *Triticum aestivum* L. *IOP Conf. Ser.: Earth Environ. Sci.* 904 012071.

Ashfaque F., Iqbal M., Khan R. and Khan N. A. (2014). Exogenously Applied H2O2 Promotes Proline Accumulation, Water Relations, Photosynthetic Efficiency and Growth of Wheat (Triticum aestivum L.) Under Salt Stress. Annual Research & Review in Biology, 4(1), 105-120.

Ben-Noah I. and Friedman S.P. (2016). Oxygation Of Clayey Soils By Adding Hydrogen Peroxide To The İrrigation Solution: Lysimetric experiments. 2452-2198/& 2016 Elsevier B.V. All rights reserved. *http://dx.doi.org/10.1016/j.rhisph.*2016.08.002.

Bhattarai S.P., Huber S. and Midmore, D.J. (2004). Aerated Subsurface İrrigation Water Gives Growth And Yield Benefits To Zucchini, Vegetable Soybean And Cotton İn Heavy Clay Soils. Ann. appl. Biol. (2004), 144:285-298 Printed in UK.

Capitulinoa J.D., Limaa G.S., Azevedoa C.A.V., Silvaa A.A.R., Velosoa L.L.S.A., Fariasa M.S.S., Soaresb L.A.A., Gheyia H.R., and Limaa V.L.A., (2022). Gas exchange and growth of soursop under salt stress and H2O2 application methods. Brazilian Journal of Biology, 2022, vol. 82, e261312.

Cooper A. (1996): ABC of Nft: Nutrient Film Technique: The World's First Method of Crop Production Without a Solid Rooting Medium (181P). Chicago, International Specialized Book Services.

El-Saedy MAM., Hammad SE., Awd Allah SFA., (2019). Nematicidal effect of abamectin, boron, chitosan, hydrogen peroxide and Bacillus thuringiensis against citrus nematode on Valencia orange trees. J Plant Sci Phytopathol. 2019; 3: 111-117.

Farouk S., Qados Abdul A.M.S., (2018). Enchancing seed quality and productivity as well as physio-anatomical responses of pea plants by folic acid and/or hydrogen peroxide application. Scientia Horticulturae 29-37.

Gill M.P.M., Ferreyra E. R., Barrera M. C., Zúñiga E. C., Gurovich R. L. (2009). Effect Of İnjecting Hydrogen Peroxide İnto Heavy Clay Loam Soil On Plant Water Status, Net CO2 Assimilation, Biomass, and Vascular Anatomy Of Avocado Trees. Chilean Journal Of Agricultural Research, 69(1), 97–106.

Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245: 83-93.

Hemalatha G., Renugadevi J., and Eevera T., (2017). Studies on Seed Priming with Hydrogen Peroxide for Mitigating Salt Stress in Rice. International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 6 Number 6 (2017) pp. 691-695.

Hsu Y., Tseng M., Lin C. (1999). The Fluctuation Of Carbohydrates And Nitrogen Compounds İn Flooded Wax-Apple Trees. Botanical Bulletin of Academia Sinica, 40, 193–198.

Jafariyan T., Zarea M.J., 2016. Hydrogen Peroxide Affects Plant Growth Promoting Effects of Azospirillum. J. Crop Sci. Biotech. 2016 (June), 19 (2) : 167-175.

Kuˇcerová, K.; Henselová, M.; Slováková, L'.; Baˇcovˇcinová, M.; Hensel, K. Effect of Plasma Activated Water, Hydrogen Peroxide, and Nitrates on Lettuce Growth and Its Physiological Parameters. Appl. Sci. 2021, 11, 1985. *https://doi.org/ 10.3390/app11051985.*

Kozlowski T.T. (1997). Response Of Woody Plants to Flooding and Salinity. Tree Physiol. Monograph 1:1-29.

Mahmoud A.H., (2015). Applied H2O2 to Saline Water Improves Wheat (Triticum aestivum) Yield. Advances in Environmental Biology, 9(24) November 2015, Pages: 374-381.

Moore K.L., Patel J., Jaroni D., Freidman M., and Ravishankar S., (2011). Antimicrobial Activity of Apple, Hibiscus, Olive, and Hydrogen Peroxide Formulations against Salmonella enterica on Organic Leafy Greens. Journal of Food Protection, Vol. 74, No. 10, 2011, P:1676–1683.

Orabi, S.A., Hussein, M.M., S. zaki, S., and Sharara F.A., 2018. Influence of hydrogen peroxide on growth, yield and biochemical constituents of canola plants grown under different irrigation intervals. Current Science International, P: 407-418.

Pilar M., Gil M., Raúl Ferreyra E., Cristián Barrera M., Carlos Zúñiga E., and Luis Gurovich R. (2009). Effect Of Injecting Hydrogen Peroxide in to Heavy Clay Loam Soil on Plant Water Status, Net Co2n Assimilation Biomass, and Vascular Anatomy of Avocado Trees. CHILEAN JOURNAL OF AGRICULTURAL RESEARCH 69 (1): 97-106.

Sariyev A., Barutcular C., Acar M., Hossain A. ve Sabagh E.A. 2020. Sub-Surface Drip Irrigation in Associated with H2O2 Improved the Productivity of Maize under Clay-Rich Soil of Adana. Phyton-International Journal of Experimental Botany. Phyton, 2020, vol.89, no.3 DOI: 10.32604/phyton.2020.09142.

Semida W.M., (2016). Hydrogen Peroxide Alleviates Salt-Stress in Two Onion (Allium cepa L.) Cultivars. American-Eurasian J. Agric. & Environ. Sci., 16 (2): 294-301.

Sichela C., Ferna´ndez-Iba´n˜eza P., Carac M., Telloc J., (2009). Lethal synergy of solar UV-radiation and H2O2 on wild Fusarium solani spores in distilled and natural well water. Elsevier Ltd. All rights reserved, Science Direct Water Research (2009): 1841-1850.

Silva H., Neto A.A., Menezes R., Silva P., Gheyi H., (2019). Use of hydrogen peroxide in acclimation of basil (*Ocimum basilicum* L.) to salt stress. Turkish Journal of Botany: Vol. 43: No. 2, Article 7. *https://doi.org/10.3906/bot-1807-80* Available at: *https://journals.tubitak.gov.tr/botany/vol43/iss2/7*.

Sohag A.A.M., Tahjib-Ul-Arif M., Brestič M., Afrin S., Sakil M.A., Hossain M.T., Hossain M.A., Hossain M.A. (2020): Exogenous salicylic acid and hydrogen peroxide attenuates drought stress in rice. Plant Soil Environ., 66: 7–13.

Taş İ., Yıldırım Y.E., Özkay F., Yeter T., Görgişen C. (2016). Morphologıcal Characterıstıcs Of Tomato Irrıgated Wıth Wastewaters Wıth Dıfferent Oxygen Concentratıons. Current Trends in Natural Sciences. Vol. 5, Issue 9, pp. 187-193, 2016. http://www.natsci.upit.ro.

Tenientea, L.M., Floresa B.A.D., Pachecoa, I.T., Chavirac, M.M.G., Bustamanted R.F.R., Pereza, A.A.F., Ramíreze, I.P., Guzmánf, N.E.R., Camachoe, R.R., Gonzáleza, R.G.G., (2018). Hydrogen peroxide protects pepper (Capsicum annuum L.) against pepper golden mosaic geminivirus (PepGMV) infections. Physiological and Molecular Plant Pathology 106 (2019): 23-29.

Tonegawa M., Dec J., and Bollag J.M., 2003. Use of Additives to Enhance the Removal of Phenols from Water Treated with Horseradish and Hydrogen Peroxide. Published in J. Environ. Qual. 32:1222–1227.

Wang X., Zhang J., Song J., Huang M., Cai J., Zhou Q., Dai T. & JiangD., (2020). Abscisic acid and hydrogen peroxide are involved in drought priming-induced drought tolerance in wheat (*Triticum aestivum* L.). Plant Biology 22 (2020) 1113–1122.

Wang W., Wang X., Huang M., Cai J., Zhou Q., Dai T., Cao W. and Jiang D., (2018). Hydrogen Peroxide and Abscisic Acid Mediate Salicylic Acid-Induced Freezing Tolerance in Wheat. Front. Plant Sci. 9:1137. doi: 10.3389/fpls.2018.01137.

## *ORAL PRESENTATION*

# Theoretical chemical calculations and molecular docking studies of epigallocatechin gallate

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## Abstract

In recent years, there are many studies in which green tea has been evaluated in vitro, in vivo, and in silico due to its widespread potential biological effects. Epigallocatechin gallate (EGCG), a pharmacologically active polyphenol found in green tea, using the 6-31G** basic set and the density functional theory (DFT/B3LYP) method, the highest occupied molecular orbital (HOMO) - the lowest unoccupied molecular orbital (LUMO), the molecular electrostatic potential (MEP), and electronic properties (total energy, dipole moment, electronegativity, chemical hardness and softness) were analyzed with Spartan '10 software. Also, in silico studies were carried out using web-based tools. The binding energy for the EGCG molecule - acetylcholinesterase complex has been calculated as -11.4 kcal/mol.

**Keywords:** Epigallocatechin gallate, Spartan '10, Molecular docking, 1EVE.

## INTRODUCTION

Nowadays, neurodegenerative diseases are the most common reason for demise and disablement worldwide (Benhamú et al., 2014). Alzheimer's disease (AD) is the most extensive neurodegenerative disease, which is defined by progressive memory loss, a decrease in language skills and has a major clinical and socioeconomic effect (Wu et al., 2018). Symptoms ultimately increase intense enough to interrupt in daily dutys. Alzheimer's disease affects commonly older people who are 65 years old and over (Khanam et al., 2016). The exact mechanism of the illness is yet not obvious. Diverse hypotheses on the formation of the AD are suggest. Some of these hypotheses and the most important are amyloid cascade hypothesis (Tolar et al., 2019), cholinergic hypothesis (El-Mezayen et al., 2022), oxidative stress (Pratap et al., 2022), tau hypothesis (Du et al., 2018), inflammation hypothesis, and metabolic hypothesis (Folch et al., 2016). Various nominee targets were determined including acetylcholinesterase (ACh), butyrylcholinesterase (BuCh), cyclooxygenase-1 (COX-1), nitric oxide synthase (NOS), cyclooxygenase-2 (COX-2), β-Site amyloid precursor protein cleaving enzyme 1 (BACE1), and etc. in these hypotheses (Srivastava et al., 2021). The key enzyme in the hydrolysis of the neurotransmitter acetylcholine is Acetylcholinesterase Enzyme (AChE). AChE is the target of most of the clinically utilized medicines for the therapy of AD (Anand et al., 2012; Rees and Brimijoin, 2003). It has a critical role in many various physiological processes such as attention, learning ability, and stress (Ferreira-Vieira et al., 2016).

Computational methods play a crucial role in silico drug design, where computer techniques are used to develop new drug molecules. In silico drug design software used in computers is used for molecular modeling of genes, protein sequence analysis, and 3D structure of proteins, and these are of great importance in target identification and design of new drugs (Jorgensen, 2004; Sliwoski et al., 2014). In silico approaches and bioinformatics have assisted discover new compounds to solve biological problems and combat many critical diseases (Malathi and Ramaiah, 2018). It is known that many natural products have different therapeutic and disease-protective roles. Tea flavonoids have been shown to be potent antitoxicant, antioxidant, and anti-inflammatory agents. Catechins flavonoids, are found in green tea extract (GTE) and are identified as active components of green tea due to their therapeutic properties. The antitumorigenic role of catechin derivatives, especially EGCG (Figure 1), has been definitively demonstrated by many laboratories. Numerous in vitro and in vivo studies have been reported so far regarding the role of EGCG in the treatment of AD disorder (Lee et al., 2013; Chen et al., 2021; Zhang et al., 2017). As a result of these studies, it is seen that EGCG plays a neuroprotective role and can potentially be used as a therapeutic agent for AD disease (Lee et al., 2013).

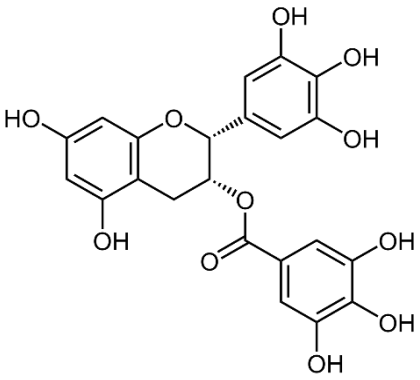



**Figure 1.** Chemical structure of EGCG

This study, it was investigated the molecular properties of the EGCG compound via theoretical, and in silico biological methods. Frontier molecular orbitals (FMOs), MEP map, some drug-likeness properties, ADMET, and the AChE inhibitory activity. Theoretical calculations were calculated utilizing the DFT/B3LYP method with the 6-31G** basis set by using Spartan '10 packet program. The data acquired from the theoretical calculations were used to compute the global reactive descriptors such as HOMO, LUMO, $E_{HOMO}$-$E_{LUMO}$ gap, chemical hardness (η), electronic chemical potential (μ), electronegativity (χ), electrophilicity index (ω), and global softness. The MEP determined nucleophilic and electrophilic sites, which may interact with biological targets. In silico studies such as DFT calculations, ADME/T predictions, and molecular modeling, have been performed and discussed to achieve more understanding of the structure-activity relationship of the EGCG molecule. Molecular docking calculation was carried out for the AChE of AD to explore the inhibitory activity of the EGCG molecule.

## MATERIALS AND METHODS

FMOs and MEP map calculations were carried out by using the Spartan '10 software (Spartan 10, Wavefunction) with DFT: B3LYP/6-31G** method. The data computed using the same program were imaged.

Drug-likeness and ADMET analysis of the EGCG molecule was performed using SwissADME (Daina et al., 2017), Pro Tox-II (Banerjee and Ulker, 2022), SwissTargetPrediction (Thomas et al., 2020), ADMETlab (http://admet.scbdd.com), and admetSAR (https://admetmesh.scbdd.com/) prediction tools. The molecular docking experiment was performed using Autodock Vina (molecular docking software) (Voiculescu et al., 2022) and UCSF Chimera (molecular visualization software) (Pettersen et al., 2021). PubChem was utilized for downloading the 3D SDF format of the EGCG molecule (Kim, 2021).

## RESULTS AND DISCUSSION

The difference between HOMO-LUMO energy values is an important stability index. A large HOMO-LUMO energy gap represents that the molecule is more stable and less reactive, while a small HOMO-LUMO energy gap represents that the molecule is less stable and more reactive (Kim et al., 2005). HOMO and LUMO energy values with the HOMO-LUMO energy range are used to estimate parameters such as electronegativity, hardness, softness, electrophilicity index, and chemical potential. The reduction in the energy gap usually leads to a decrease in stiffness and easier polarization of the molecule (Mandal et al., 2003). The molecule optimization was performed using DFT with functional B3LYP/6-31 G** basis set in the gas phase and the HOMO-LUMO energies were calculated. The computed HOMO-LUMO energy (a.u.), and band gap value for the EGCG molecule are -0.205 eV, -0.039 eV, and 0.166 eV, respectively. Also, it was calculated electronic properties and the global reactivity parameters of the EGCG molecule and the calculated results were given in Table 1. To the energy band gap given in Table 1 is less than 1.5 eV indicating that the molecule is not stable and durable in terms of thermodynamics. It also means that the molecule can react with itself, dimerization, and polymerization can occur. The electrophilicity index value of the studied molecule was calculated as 0.276 eV. The electrophilicity index (ω) scale lets the grading of organic molecules as marginal electrophiles with $\omega < 0.8$ eV. 3D plots of FMOs and the energies of the FMO gaps (ΔE) were shown in Figure 2.

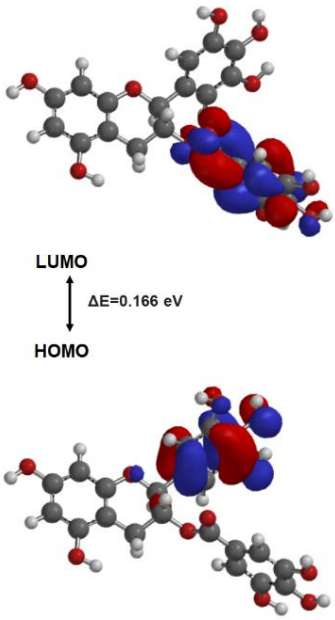



**Figure 2.** 3D pictures of HOMO, and LUMO orbitals, and the energies of the FMO gaps (ΔE) for the EGCG molecule

Molecular electrostatic potential (MEP) is a useful method used to explain the reactivity, structure-activity, and hydrogen bonding of molecular behavior. The green-colored parts are the regions where the electrostatic potential is zero. In MEP maps, the electrostatic potential increases in the order of Red<Orange<Yellow<Green<Blue (Alkorta and Perez, 1996; Politzer and Truhlar, 2013).

**Table 1.** Global reactivity descriptors of the EGCG molecule

| Parameters | Values (eV) |
|---|---|
| $E_{HOMO}$ | -0.205 |
| $E_{LUMO}$ | -0.039 |
| Energy band gap ($\Delta E = E_{LUMO}-E_{HOMO}$) | 0.166 |
| Ionization potential ($I = -E_{HOMO}$) | 0.205 |
| Electron affinity ($A = -E_{LUMO}$) | 0.039 |
| Electronegativity ($\chi = (I + A)/2$) | 0.122 |
| Chemical hardness ($\eta = (I-A)/2$) | 0.083 |
| Chemical softness ($\sigma = 1/2\eta$) | 6.024 |
| Chemical potential ($\mu = -(I + A)/2$) | -0.122 |
| Electrophilicity index ($\omega = \mu^2/2\eta$) | 0.089 |

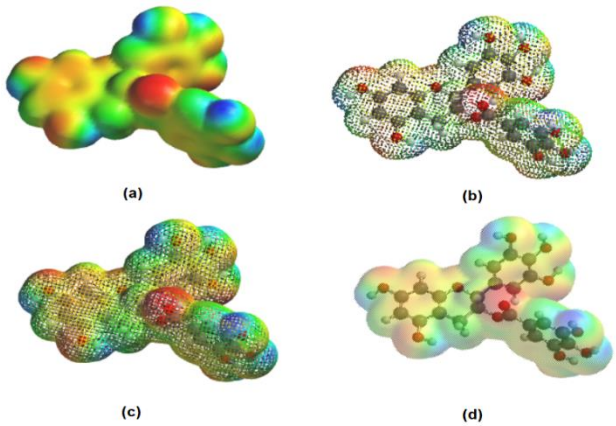



**Figure 3.** The illustration of MEP maps of the molecule, (a) *solid, (b) dots, (c) mesh and (d) transparent*

As seen in Figure 3, since the negative potential regions in the map are distributed over the oxygen atoms and carbonyl group, these regions represent high electrophilic attack efficiency. The positive regions are located on the hydrogen atoms and are suitable for nucleophilic attacks.

Molecular docking analyses provide to know the molecular interactions of reactions between enzymes and compounds. Docking calculations ease the conception of the mechanism of action of target compounds, interactions, and suitable binding regions. As a result of the docking analysis, the interaction of EGCG molecule and AChE with each other was carried out. The docking outcome supported that the EGCG molecule indicated a good energy value (-11.4 kcal/mol). Ligand-receptor 3D interactions were visualized owing to Biovia Discovery Studio Visualizer program (D.S.J.S.D. Biovia, CA, USA, Discovery Studio Visualizer). The 3D structure of AChE (PDB code: 1EVE) at 2.50 Å resolution was acquired via the RSCB PDB website. EGCG molecule formed secondary interactions (hydrogen bonds, van der Waals, π–π stacking) with the AChE complex. These interactions were shown in Figure 4.

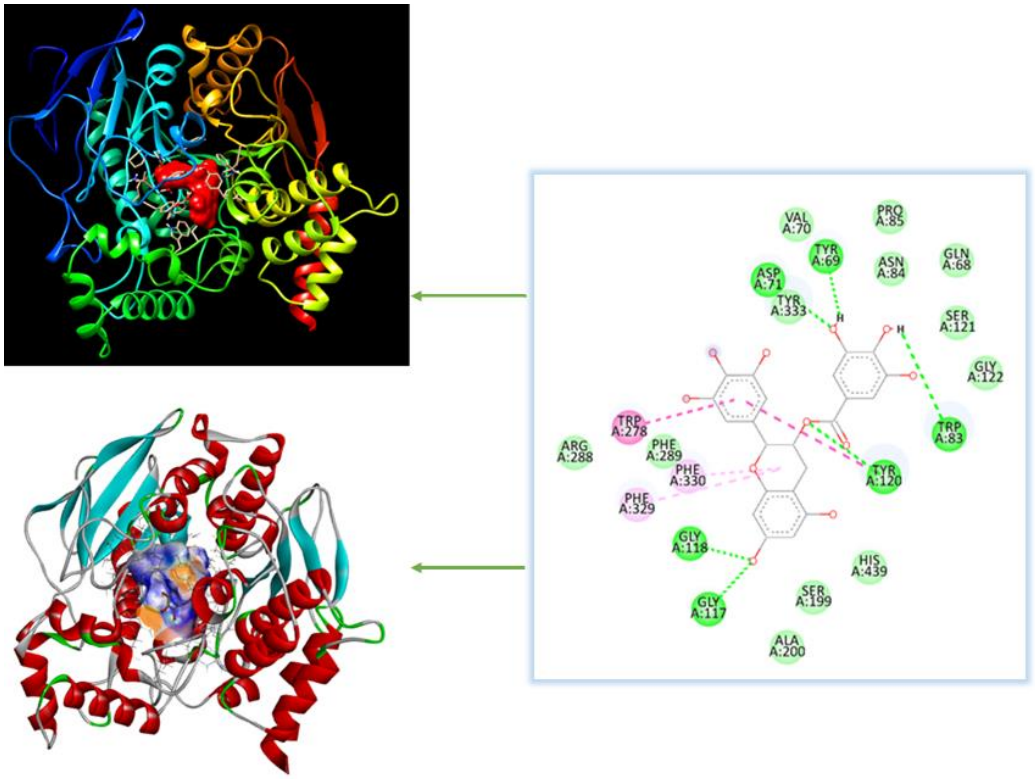



**Figure 4.** Diagrams of the AChE-EGCG interactions for the most stable complex

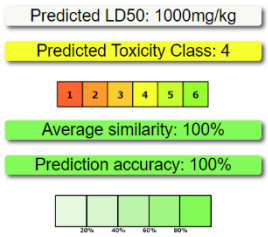
The EGCG-AChE protein complex was stabilized by hydrogen bonds, van der Waals, π–π stacking interactions. In EGCG–1EVE complex, six hydrogen bonds were formed between residues TYR69, ASP71, TRP83, TYR120, GLY117, and GLY118 at the distance, 2.31, 2.94, 2.68, 2.16, 2.35, and 2.33 respectively. Furthermore, four π–π stacking interactions were found in the TYR120, TRP278, PHE329, and PHE330 residues at 3.09, 3.85, 3.93, and 3.96, respectively.

Drug-like properties refer to the molecule's physicochemical, and ADMET properties. By virtue of in vivo and in vitro analyses being costly and time-consuming, in silico processes have been extensively used to estimate the ADME properties of molecules (Pelkonen et al., 2011). It was used web-based various in silico tools to identify the ADMET properties of the EGCG molecule such as SwissADME, ADMETlab, admetSAR, SwissTargetPrediction, and ProTox-II. The obtained results were compared as given in Table 2.

**Table 2.** Some ADMET properties estimated by different web tools for the ECGC molecule

| | **SwissADME** | **ADMETlab** |
|---|---|---|
| | **Physicochemical Properties** | |
| **Formula** | $C_{22}H_{18}O_{11}$ | $C_{22}H_{18}O_{11}$ |
| Molecular weight | 458.37 g/mol | 458.08 g/mol |
| Number heavy atoms | 33 | - |
| Number aromatic heavy atoms | 18 | - |
| Fraction $Csp^3$ | 0.14 | 0.136 |
| Number rotatable bonds | 4 | 4 |
| Number hydrogen bond acceptors | 11 | 11 |
| Number hydrogen bond donors | 8 | 8 |
| Molar refractivity | 112.06 | - |
| TPSA | 197.37 $Å^2$ | 197.37 $Å^2$ |
| Lipophilicity | | |
| $LogP_{o/w}$ | 1.17 | 4.244 |
| **Water Solubility** | | |
| LogS | -3.56 | -3.805 |
| Solubility | 1.27e0.1 mg/ml | - |
| **Absorption** | | |
| GI absorption | Low | - |
| **Distribution** | | |
| BBB permeation | No | No |
| P-gp substrate | No | No |
| **Metabolism** | | |
| CYP1A2 inhibitor | No | No |
| CYP2C19 inhibitor | No | No |
| CYP2C9 inhibitor | No | + |
| CYP2D6 inhibitor | No | No |
| CYP3A4 inhibitor | No | No |
| $LogK_p$ (skin permeation) | -8.27 cm/s | - |
| **Drug-likeness** | | |
| Lipinski | No; 2 violation: NorO>10, NHorOH>5 | No |
| Ghose | Yes | - |
| Veber | No; 1 violation: TPSA>140 | - |
| **Medicinal Chemistry** | | |
| PAINS | 1 alert: catechol_A | 1 alert |
| Brenk | 1 alert: catechol | - |
| **Leadlikeness** | No; 1 violation: MW>350 | - |
| **Bioavailability Score** | 0.17 | - |

The toxicity prediction was performed owing to the Pro-Tox II web server. The predicted outcomes have been given in Figure 5. As regards the toxicity outcomes, it can achieve some estimates for the EGCG molecule: The EGCG molecule displays a median toxicity skeleton. The molecule isn't cytotoxic, carcinogenic, immunotoxic, or mutagenic. According to Pro Tox-II, the molecule is categorized as toxicity class 4.




Toxicity Model Report

| Classification | Target | Shorthand | Prediction | Probability |
|---|---|---|---|---|
| Organ toxicity | Hepatotoxicity | dili | Inactive | 0.70 |
| Toxicity end points | Carcinogenicity | carcino | Inactive | 0.54 |
| Toxicity end points | Immunotoxicity | immuno | Inactive | 0.89 |
| Toxicity end points | Mutagenicity | mutagen | Inactive | 0.70 |
| Toxicity end points | Cytotoxicity | cyto | Inactive | 0.82 |
| Tox21-Nuclear receptor signalling pathways | Aryl hydrocarbon Receptor (AhR) | nr_ahr | Inactive | 0.85 |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor (AR) | nr_ar | Inactive | 0.96 |
| Tox21-Nuclear receptor signalling pathways | Androgen Receptor Ligand Binding Domain (AR-LBD) | nr_ar_lbd | Inactive | 0.95 |
| Tox21-Nuclear receptor signalling pathways | Aromatase | nr_aromatase | Inactive | 0.98 |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Alpha (ER) | nr_er | Inactive | 0.87 |
| Tox21-Nuclear receptor signalling pathways | Estrogen Receptor Ligand Binding Domain (ER-LBD) | nr_er_lbd | Inactive | 0.89 |
| Tox21-Nuclear receptor signalling pathways | Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma) | nr_ppar_gamma | Inactive | 0.92 |
| Tox21-Stress response pathways | Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) | sr_are | Inactive | 0.98 |
| Tox21-Stress response pathways | Heat shock factor response element (HSE) | sr_hse | Inactive | 0.98 |
| Tox21-Stress response pathways | Mitochondrial Membrane Potential (MMP) | sr_mmp | Inactive | 0.79 |
| Tox21-Stress response pathways | Phosphoprotein (Tumor Supressor) p53 | sr_p53 | Inactive | 0.93 |
| Tox21-Stress response pathways | ATPase family AAA domain-containing protein 5 (ATAD5) | sr_atad5 | Inactive | 0.97 |

**Figure 5.** The toxicity computation of EGCG molecule by Pro-Tox II web tool

## CONCLUSION

The quantum chemical descriptors of the EGCG molecule have been determined and analyzed at DFT level employing B3LYP/6-31G** basis set. As seen from the results, the EGCG molecule has a small energy gap (ΔE), which indicates it is not a hard molecule. The molecule is soluble. The predicted binding energy is -11.4 kcal/mol. The docking result showed that this molecule was inhibited through interactions including hydrogen bonds, van der Waals, π–π stacking interactions. The bioavailability score of 0.17 predicts poor oral bioavailability. It is not classified as risky in terms of toxicity. The EGCG molecule exposed a suitable drug-like property with no immunotoxicity, carcinogenicity, mutagenicity, or cytotoxicity.

## REFERENCES

Alkorta I, Perez JJ 1996. Approximations in the computation of molecular polarization maps. Electronic Journal of Theoretical Chemistry, 1(1): 26-37.

Anand P, Singh B, Singh N 2012. A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease. Bioorganic & Medicinal Chemistry, 20(3): 1175-1180.

Banerjee P, Ulker OC 2022. Combinative ex vivo studies and in silico models ProTox-II for investigating the toxicity of chemicals used mainly in cosmetic products. Toxicology Mechanisms and Methods, 1-7.

Benhamú B, Martín-Fontecha M, Vázquez-Villa H, Pardo L, López-Rodríguez ML 2014. Serotonin 5-HT6 receptor antagonists for the treatment of cognitive deficiency in Alzheimer's disease. Journal of Medicinal Chemistry, 57(17): 7160-7181.

Chen TS, Liou SY, Lin HH, Hung MY, Lin CC, Lin YM, Lin KH, Padma VV, Yao CH, Kuo WW, Huang CY 2021. Oral administration of green tea Epigallocatechin-3-gallate reduces oxidative stress and enhances restoration of cardiac function in diabetic rats receiving autologous transplantation of adipose-derived stem cells. Archives of Physiology and Biochemistry, 127(1): 82-89.

Daina A, Michielin O, Zoete V 2017. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. Nature Publishing Group; 7: 1–13.

Du X, Wang X, Geng M 2018. Alzheimer's disease hypothesis and related therapies. Translational neurodegeneration, 7(1): 1-7.

D.S.J.S.D. Biovia, CA, USA, Discovery Studio Visualizer, 2017, 936.

El-Mezayen NS, Abd el Moneim RA, El-Rewini SH 2022. Vitamin B12 as a cholinergic system modulator and blood brain barrier integrity restorer in Alzheimer's disease. European Journal of Pharmaceutical Sciences, 174: 106201.

Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM 2016. Alzheimer's disease: targeting the cholinergic system. Current Neuropharmacology, 14(1): 101-115.

Folch J, Petrov D, Ettcheto M, Abad S, Sánchez-López E, García ML, Olloquequi J, Beas-Zarate C, Auladell C, Camins, A 2016) Current research therapeutic strategies for Alzheimer's disease treatment. Neural Plasticity, 2016: 1-16.

Jorgensen WL 2004. The many roles of computation in drug discovery. Science, 303(5665): 1813-1818.

Khanam H, Ali A, Asif M 2016. Neurodegenerative diseases linked to misfolded proteins and their therapeutic approaches: A review. European Journal of Medicinal Chemistry, 124: 1121-1141.

Kim KH, Han YK, Jung J 2005. Basis set effects on relative energies and HOMO–LUMO energy gaps of fullerene C36. Theoretical Chemistry Accounts, 113: 233-237.

Kim S 2021. Exploring chemical information in PubChem. Current Protocols, 1(8): e217.

Lee YJ, Choi DY, Yun YP, Han SB, Oh KW, Hong JT 2013. Epigallocatechin-3-gallate prevents systemic inflammation-induced memory deficiency and amyloidogenesis via its anti-neuroinflammatory properties. The Journal of Nutritional Biochemistry, 24(1): 298-310.

Malathi K, Ramaiah S 2018. Bioinformatics approaches for new drug discovery: a review. Biotechnology and Genetic Engineering Reviews, 34(2): 243-260.

Mandal K, Chattopadhyay T, Nandi PK, Bhattacharyya SP 2003. Theoretical study of the variation of first hyperpolarizability in a number of push-pull benzene, thiophene and trans butadiene molecule. Indian Journal of Chemistry, 42 A: 449-459.

Pratap GK, Nagaraju PG, Danagoudar A, Joshi CG, Poornima PCG, Mohammed YHI, Koodlur L, Shantaram M 2022. Neuroprotective, lifespan, memory enhancing potential, and molecular docking studies of natural compound from Curculigo orchioides Gaertn.: A study on Alzheimer's disease model of Appl-GAL4 Drosophila melanogaster. South African Journal of Botany, 149: 60-66.

Pelkonen O, Turpeinen M, Raunio H 2011. In vivo-in vitro-in silico pharmacokinetic modelling in drug development. Clinical pharmacokinetics, 50(8): 483-491.

Pettersen EF, Goddard TD, Huang CC, Meng EC, Couch GS, Croll TI, Ferrin TE 2021. UCSF ChimeraX: Structure visualization for researchers, educators, and developers. Protein Science, 30(1): 70-82.

Politzer P, Truhlar DG 2013. Chemical applications of atomic and molecular electrostatic potentials: reactivity, structure, scattering, and energetics of organic, inorganic, and biological systems: Springer Science & Business Media.

Rees TM, Brimijoin S 2003. The role of acetylcholinesterase in the pathogenesis of Alzheimer's disease. Drugs Today (Barc), 39(1): 75-83.

Sliwoski G, Kothiwale S, Meiler J, Lowe EW 2014. Computational methods in drug discovery. Pharmacological Reviews, 66(1): 334-395.

Spartan 10, Wavefunction, Inc., Irvine, USA, http://wavefun.com, 2010.

Srivastava S, Ahmad R, Khare SK 2021. Alzheimer's disease and its treatment by different approaches: A review. European Journal of Medicinal Chemistry, 216: 113320.

Thomas L, Mathew S, Johnson S 2020. In-silico prediction of role of chitosan, chondroitin sulphate and agar in process of wound healing towards scaffold development. Informatics in Medicine Unlocked, 20: 100406.

Tolar M, Abushakra S, Sabbagh M 2019. The path forward in Alzheimer's disease therapeutics: reevaluating the amyloid cascade hypothesis. Alzheimer's & Dementia, 16(11): 1553-1560.

Voiculescu DI, Roman DL, Ostafe V, Isvoran A 2022. A cheminformatics study regarding the human health

Wu Y, Li Z, Huang YY, Wu D, Luo HB 2018. Novel Phosphodiesterase Inhibitors for Cognitive Improvement in Alzheimer's Disease: Miniperspective. Journal of Medicinal Chemistry, 61(13): 5467-5483.

Zhang ZX, Li YB, Zhao RP 2017. Epigallocatechin Gallate attenuates β-Amyloid generation and oxidative stress involvement of PPARγ in N2a/APP695 cells. Neurochemical Research, 42(2): 468–480.

## ORAL PRESENTATION

### Çörek otu (*Nigella sativa L.*) yağının in vitro sitotoksisitesinin belirlenmesi

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**Özet**

*Nigella sativa*, halk arasından bilinen adıyla çörek otu, genellikle Akdeniz kıyılarında yetişen otsu bir bitkidir. Son zamanlarda bitki antioksidanları yan etkilerinin az olması sebebiyle, besin takviyesi olarak sıkça tercih edilir hale gelmiş ve insanlar tarafından sağlığı desteklemek amacıyla kullanılmıştır. Bu çalışmada, *Nigella sativa* türünden soğuk sıkım (SK) ve süper kritik ekstraksiyon (SFE) yöntemleri ile elde edilen çörek otu yağının, CCD18-Co (sağlıklı kolon epiteli) hücreleri üzerindeki sitotoksik etkilerinin MTT (3-(4,5-dimetiltiazol-2-YL)-2,5-difeniltetrazolyum bromür) test yöntemiyle belirlenmesi amaçlanmıştır. Bu amaçla hücreler, %10 fetal bovine serum (FBS) içeren EMEM (Eagle's Minimum Essential Medium) besiyerinde 37°C'de %5 $CO_2$ içeren ortamda kültüre edilmiştir. Gerekli yoğunluğa ulaşan hücrelere farklı konsantrasyonlarda (100-50-25-12,5-6,25-3,125-1,56 µg/mL) çörek otu yağı ile 24 saat boyunca muamele edilmiştir. Hücre canlılığı, Canlılık (%) = ortalama deneysel (optik yoğunluk) OD değeri / ortalama kontrol OD değeri) x %100 formülü kullanılarak ölçülmüştür. Sonuçta çörek otu yağının, uygulanılan dozlarda CCD18-Co hücreleri üzerinde hücre canlılığında azalmaya neden olmadığı gözlenmiş ve çörek otu yağının in vitro şartlarda sağlıklı kolon epiteli hücre hatlarında sitotoksik etkisi olmadığı sonucuna ulaşılmıştır. Elde edilen verilerden çörek otu yağının takviye edici gıda olarak güvenle kullanılabilecek bir ürün olduğu ve üzerinde daha fazla araştırma yapılması gerektiği düşünülmektedir.

**Anahtar Kelimeler:** Çörek otu yağı, MTT, sitotoksisite, kolon epiteli

### Determination of in vitro cytotoxicity of black cumin (*Nigella sativa L.*) oil

**Abstract**

*Nigella sativa*, popularly known as black cumin, is a herbaceous plant that usually grows on the Mediterranean coast. Recently, plant antioxidants have become frequently preferred as nutritional supplements due to their low side effects and have been used by people to support health. In this study, the cytotoxic effects of black cumin oil obtained from *Nigella sativa* species by cold pressing (SK) and supercritical extraction (SFE) methods on CCD18-Co (healthy colon epithelium) cells were investigated in MTT (3-(4,5-dimethylthiazol-2). -YL)-2,5-diphenyltetrazolium bromide) test method. For this purpose, cells were cultured in EMEM (Eagle's Minimum Essential Medium) medium containing 10% fetal bovine serum (FBS) at 37°C in a medium containing 5% $CO_2$. Cells that reached the required density were treated with black cumin oil at different concentrations (100-50-25-12.5-6.25-3.125-1.56 µg/mL) for 24 hours. Cell viability was measured using the formula Viability (%) = mean experimental (optical density) OD value / mean control OD value) x 100%. As a result, it was observed that black cumin oil did not cause a decrease in cell viability on CCD18-Co cells at the applied doses, and it was concluded that black cumin oil did not have a cytotoxic effect on healthy colon epithelial cell lines in vitro. From the data obtained, it is thought that black cumin oil is a product that can be used safely as a food supplement and more research should be done on it.

**Keywords:** Black seed oil, MTT, cytotoxicity, colon epithelium

## GİRİŞ

Bitkiler, insanlık tarihinde geleneksel ilaçların temeli olarak uzun süredir kullanılmaktadır ve aynı zamanda modern ilaçların kaynağı olarak da işlev görmektedir. Dünya Sağlık Örgütü'ne göre, sınırlı kaynaklara sahip ülkelerdeki toplulukların dörtte üçünden fazlası, temel sağlık ihtiyaçları için tıbbi bitkilere güvenmektedir. Beslenme alanındaki yeni gelişmelere paralel olarak, günümüzde bitkilerin gıda ve ilaç kaynağı olarak kullanımına olan ilgi yeniden canlanmaktadır. Sadece kolay erişilebilirlikleri ve düşük maliyetleri nedeniyle değil, aynı zamanda doğal ilaçların sentetik ilaçlara kıyasla daha az zararlı etkiye sahip oldu inancından dolayı çok sayıda hastalık için fito-tıbbın kullanımı çarpıcı biçimde artmıştır (Adib ve ark., 2018).

Çörek otu (*Nigella sativa)* tohumları, Orta Doğu, Avrupa ve Asya ülkelerinde sağlığın teşviki ve birçok rahatsızlığın tedavisi için sıklıkla kullanılmakta olan bir bitkidir (Dalli ve ark., 2021). Yaygın olarak "siyah kimyon" veya "siyah tohumlar" olarak bilinen bu bitki yaklaşık 60 cm yüksekliğe sahip yıllık otsu hermafrodit bir bitkidir. Bitki, yeşilden koyu yeşile değişen bir renkle karakterize edilir. Yaprakları yaşla birlikte kırmızıya dönen yeşil bir renge sahiptir. Bitki çiçeklenmesi, nisan ayından ağustos ayına kadar başlar ve esas olarak 20 ila 35 mm çapında beş yapraktan oluşur. Çiçekler, genç yaşta yeşil bir renkle karakterize edilir ve olgunlaşınca maviye dönüşür. Bitkinin meyveleri üç ila altı karpelden oluşur ve her biri tohum içerir. Tohumlar olgunlaştıktan ve havaya maruz kaldıktan sonra renkleri siyahlaşır (Dalli ve ark., 2021).

Çörek otu mucizevi bitki olarak tanımlanan bir bitki olup, en eski bitki uzmanları tarafından “cennetten gelen bitki” olarak kabul edildi (Ahmad ve ark., 2013). Muhammed (sav) çörek otunun iyileştirici güçlerini “Bu çörek otunu kullanmaya devam edin, çünkü ölüm dışında her hastalığa çaresi vardır” şeklinde açıklamıştır (Bukhari 2018). Ayrıca “Tıbbın Kanunu” adlı kitabıyla tanınan 10. yüzyılın ünlü hekimi İbni Sina, vücudun enerjisini artırmak, yorgunluk ve ruhsuzluktan kurtulmayı desteklemek için çörek otu tohumlarının kullanılmasını tavsiye etmiştir (Yimer ve ark., 2019). Son zamanlarda, çörek otunun güçlü antikanser özelliğinin anlaşılmasına yönelik artan yoğun araştırmalar vardır. Bu araştırmalarda çörek otunun karaciğer, kolon, meme, servikal, akciğer, pankreas, prostat kanseri vb. gibi çeşitli kanser türlerine karşı önemli antiproliferatif etkiler gösterdiği belirtilmiştir (Ansary ve ark., 2021).

## MATERYAL VE METOT

Çörek otu yağının olası sitotoksik etkisi MTT (3-(4,5-dimetiltiyazol-2-il)-2,5-difenil tetrazolyum bromür) testi kullanılarak CCD18-Co sağlıklı kolon epiteli hücrelerinde değerlendirilmiştir. MTT testi Mossman’ın metoduna göre gerçekleştirilmiştir (Mosman 1983). Buna göre, CCD18-Co hücreleri %10 Fetal Bovine Serum (FBS), %1 penisilin/streptomisin içeren EMEM (Eagle's Minimum Essential Medium) içerisinde, %5 CO2 bulunan 37ºC’ye sabitlenmiş etüvde hücre kültür flasklarında üretilmiştir. Gerekli yoğunluğa ulaşan hücreler 96 kuyulu platelere kuyu başına 104 hücre gelecek şekilde ekilmiştir. Kuyuların tabanına tutunan hücrelere farklı konsantrasyonlarda (100-50-25-12,5-6,25-3,125-1,56 µg/mL) çörek otu yağı ile 24 saat boyunca muamele edilmiştir. Ardından muamele yapılan kuyucuklara MTT uygulaması yapılmıştır ve 3 saat inkübasyona bırakılmıştır. İnkübasyon süresi sonunda tüm kuyucuklara DMSO eklenerek MTT uygulaması ile oluşan formazan kristallerinin çözünür hale geçmesi sağlanmıştır. Elisa cihazı ile aborbans değerleri belirlenmiş ve Hücre canlılığı, Canlılık (%) = ortalama deneysel (optik yoğunluk) OD değeri / ortalama kontrol OD değeri) x %100 formülü kullanılarak belirlenmiştir.

## BULGULAR ve TARTIŞMA

Çörek otu yağının, uygulanılan dozlarda CCD18-Co hücreleri üzerinde hücre canlılığında azalmaya neden olmadığı gözlenmiş ve çörek otu yağının *in vitro* şartlarda sağlıklı kolon epiteli hücre hatlarında sitotoksik etkisi olmadığı sonucuna ulaşılmıştır. Aşağıdaki tablo 1’de bulunan sonuçlar gösterilmiştir.

**Tablo 1.** Çörek otu yağının farklı dozlarda kullanımının sağlıklı kolon hücreleri üzerindeki antisitotoksik etkisi

| | **Doz** | **% Canlılık** |
|---|---|---|
| Hücre Kontrol | 3,8759 | 100 |
| (+) Kontrol | 1,7136 | 44,21166697 |
| 100 ppm | 3,9681 | 102,3788023 |
| 50 ppm | 3,9184 | 101,0965195 |
| 25 ppm | 3,8775 | 100,0412807 |
| 12,5 ppm | 3,9193 | 101,1197399 |
| 6,25 ppm | 3,8693 | 99,82971697 |
| 3,12 ppm | 3,8769 | 100,0258005 |
| 1,56 ppm | 3,8627 | 99,65943394 |
| DMSO | 3,7657 | 97,15678939 |

Timokinon, kanser tedavisi ve önlenmesinde terapötik bir potansiyele sahip olan çörek otunun aktif bileşenidir. Şakalar ve arkadaşlarının yapmış olduğu bir çalışmada timokinonun negatif meme kanseri hücre hattında sitotoksisite ve apoptozu indüklediği ve yara iyileşmesini engellediği gösterilmiştir (Şakalar ve ark., 2016). Ayrıca bu çalışmada timokinonun hücre kültüründe ve farelerde kanser büyümesini inhibe etti açıkça belirtilmiştir. Meme kanseri ile alakalı Dastjerdi ve arkadaşlarının yapmış olduğu araştırmada da timokinonun MCF-7 hücrelerinde apoptozu indükleyebileceği gösterilmiştir (Dastjerdi ve ark., 2016). Yapılan bir başka çalışmada timokinonun akciğer kanseri tedavisi için potansiyel yeni bir terapötik ajan olabileceğini öne sürüldü. Bu çalışmada timokinon A549 insan akciğer tümörü hücrelerinde canlılığı azalttı ve apoptotik hücre ölümünü arttırdı (Samarghandian ve ark., 2019). Khalife ve arkadaşlarının yapmış olduğu çalışmada ise timokinonun kolorektal kanser hücrelerinde antitümör aktivitesini desteklediği belirtilmiştir (Khalife ve ark., 2016).

## SONUÇ

Tüm çabalara rağmen, kanser yıllık ölüm oranı bir milyonu aşan günümüz hastalıkların başında gelmektedir. Çörek otu gibi doğal ürünler, yeni antikanser stratejilerinin keşfedilmesine katkıda bulunmak ve kanserle mücadele etmek için umut verici adaylardır. Yaptığımız çalışmada çörek otu yağının, uygulanılan dozlarda CCD18-Co hücreleri üzerinde hücre canlılığında azalmaya neden olmadığı gözlenmiş ve çörek otu yağının in vitro şartlarda sağlıklı kolon epiteli hücre hatlarında sitotoksik etkisi olmadığı sonucuna ulaşılmıştır. Elde edilen verilerden çörek otu yağının takviye edici gıda olarak güvenle kullanılabilecek bir ürün olduğu düşünülmektedir. Bu tür çalışmalardan elde edilen sonuçlar, çeşitli kanser türlerinin önlenmesi için alternatif ilaçlar alanında yeni bir yol açabilir. Bu nedenle, birçok kanserin etkili tedavisi için çörek otunun mevcut kemoterapötik ajanlarla uygun kombinasyonlarda antikanser etkisini incelemek için daha fazla araştırma yapılması gerekmektedir.

## KAYNAKLAR

Adib-Hajbaghery M, Rafiee S 2018. Medicinal plants use by elderly people in Kashan, Iran. Nursing and Midwif Studies, 7(2):67-73.

Ahmad I, Tripathi J, Manik S, Umar L, Rabia J 2013. Preliminary phytochemical studies of the miracle herb of the century, Nigella sativa L. (black seed). Indo American Journal of Pharmaceutical Research, 3(4):3000-3007.

Ansary J, Giampieri F, Forbes-Hernandez TY, Regolo L, Quinzi D, Gracia Villar S, Garcia Villena E, Tutusaus Pifarre K, Alvarez-Suarez JM, Battino M, Cianciosi D 2021. Nutritional Value and Preventive Role of

Nigella sativa L. and Its Main Component Thymoquinone in Cancer: An Evidenced-Based Review of Preclinical and Clinical Studies. Molecules, 7;26(8):2108.

Bukhari AA 2018. Available at: http://quranx.com/Searchq=Black+cumin&context=Hadith.

Dalli M, Bekkouch O, Azizi SE, Azghar A, Gseyra N, Kim B 2021. Nigella sativa L. Phytochemistry and Pharmacological Activities: A Review (2019-2021). Biomolecules, 12(1):20.

Dastjerdi MN, Mehdiabady EM, Iranpour FG, Bahramian H 2016. Effect of Thymoquinone on P53 Gene Expression and Consequence Apoptosis in Breast Cancer Cell Line. Int J Prev Med, 14;7:66.

Khalife R, Hodroj MH, Fakhoury R, Rizk S 2016.Thymoquinone from Nigella sativa Seeds Promotes the Antitumor Activity of Noncytotoxic Doses of Topotecan in Human Colorectal Cancer Cells in Vitro. Planta Med, 82(4):312-21.

Mossman T 1983. Rapid colometric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65, 55-63.

Samarghandian S, Azimi-Nezhad M, Farkhondeh T 2019. Thymoquinone-induced antitumor and apoptosis in human lung adenocarcinoma cells. J Cell Physiol, 234(7):10421-10431.

Şakalar Ç, İzgi K, İskender B, Sezen S, Aksu H, Çakır M, Kurt B, Turan A, Canatan H 2016. The combination of thymoquinone and paclitaxel shows anti-tumor activity through the interplay with apoptosis network in triple-negative breast cancer. Tumour Biol, 37(4):4467-77.

Samarghandian S, Azimi-Nezhad M, Farkhondeh T 2019. Thymoquinone-induced antitumor and apoptosis in human lung adenocarcinoma cells. J Cell Physiol, 234(7):10421-10431.

Yimer EM, Tuem KB, Karim A, Ur-Rehman N, Anwar F 2019. Nigella sativa L. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses. Evid Based Complement Alternat Med, 12;2019:1528635.

## *ORAL PRESENTATION*

# Antioxidant activity of *Thymus vulgaris* and *Citrus limon* essential oils widely used in the winter

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**Abstract**

Secondary metabolites produced in large quantities by medicinal plants have pharmacological and biological properties. These substances are created as a reaction to various environmental stresses. Terpenes, phenolics, and alkaloids are the primary phytochemicals that are bioactive. The biological and pharmacological effects of medicinal plants that have been investigated the most include their antibacterial, antioxidant, anticancer, anti-inflammatory, and hypoglycaemic properties. In Adana region, the people can easily reach *Thymus vulgaris* and *Citrus limon* plants with these characteristics. It also prepares and consumes drugs (essential oils, infusions, cooking spices, colognes, etc.) obtained from these plants. While infusions of lemon and thyme are prepared and consumed in the summer for their cooling effect, their essential oils are consumed especially in the treatment of upper respiratory tract infections (URTIs) for their breath-opening and immune-strengthening effects. Since this effect was thought to be due to the antioxidant activity, the antioxidant properties of the essential oils of these plants were analyzed by the DPPH method. In this study, vitamin C (ascorbic acid), one of the natural antioxidants, was used as the control group. The percentage of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was calculated. According to the results obtained, when the $IC_{50}$ values were compared, the antioxidant activity of *Thymus vulgaris* essential oil was found to be stronger than *Citrus limon* essential oil.

**Keywords:** *Thymus vulgaris*, *Citrus limon*, Essential oils, Antioxidant activity, DPPH

## INTRODUCTION

Upper respiratory tract infections can be caused by a variety of viruses and bacteria. Numerous patient illnesses, such as acute bronchitis, the common cold, influenza, and respiratory distress syndromes, are brought on by them. The majority of those patient disorders are challenging to define. Since, upper respiratory tract infections (URTIs) sometimes have overlapping manifestations and share a same etiology. URTIs are characterized by self-limiting irritation and swelling of the upper airways together with a cough, but without evidence of pneumonia, the presence of another illness to explain the patient's symptoms, or a history of chronic obstructive pulmonary disease (COPD), emphysema, or chronic bronchitis (Wenzel and Fowler, 2006). The nose, sinuses, pharynx, larynx, and lung airways are all affected by URTIs.

In an outpatient setting, medical professionals frequently deal with URTIs. The infections can range from a simple flu to a potentially fatal condition as acute epiglottitis. In general, URTIs cause a significant level of temporary impairment. It's normal for people to miss work and school; in addition, the symptoms might be bothersome, and being extremely exhausted is the typical. Encourage patients to stay hydrated, get plenty of rest, quit smoking, and take their medications as directed (Le Maréchal et al., 2018; Thomas and Bomar, 2022).

Around the Mideast, herbal remedies are frequently used to treat respiratory conditions going back centuries (Lev, 2006). Some ethnobotanical studies carried out over the recent years have clarified the use of many aromatic plants for the treatment of respiratory problems (Ali-Shtayeh et al., 2000; Said et al., 2002). Traditional, complementary, and alternative medicine all frequently use herbal medicine. Herbal medicine is being used more frequently and is becoming more accepted in both developed and developing nations worldwide (Bardia et al., 2007; Ben-Arye et al., 2011; Chen et al., 2007).

Combining medicinal plants, plant extracts, or isolated compounds can generate the required therapeutic impact and minimize any potential negative consequences. An even more mixture is quite helpful for a variety

of disorders because it covers a wide spectrum of symptoms. An example of this would be the use of herbal remedies to treat viral infections, minor bacterial disorders, and colds and flu-related coughs, colds, and high temperatures. Viruses (echoviruses, adenoviruses, and rhinoviruses) are mostly responsible for the respiratory tract inflammation known as tracheobronchitis, which is more common in children. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are the main other culprits. Bacterial infection often leads to viral infection, causing exacerbation of diseases. The combination of antimicrobial and antiviral effects, as well as medicinal herbs with expectorant and immune system strengthening effects, can be very beneficial for the patient and accelerate recovery (Pauk, 2011). There are numerous immune-boosting herbs that can be used to treat upper respiratory tract flu. Although the constituent parts of these plants are well recognized, substantial in vitro and in vivo research is still being done on their activities (R Cundell, 2014; Šmejkal and Rjašková, 2016; Tan and Vanitha, 2004).

The antioxidant capacity of herbs from the Mediterranean region has recently been studied. Herbal extracts have been proven to reduce lipid peroxidation in cultivated lung cells exposed to ozone or iron. Cold and flu prevention and treatment traditionally involve specific blends of fragrant herbs (Lionis et al., 1998). The biological affects, bioactivities, and antimicrobial qualities of essential oils have been documented in the scientific literature (Cowan, 1999).

For these reasons, it was decided to work with lemon and thyme, which are regularly used in the public to treat upper respiratory tract disorders throughout the winter and are generally available to people in the Mediterranean region. This study compared the antioxidant capacity of vitamin C, a naturally occurring and potent antioxidant, by examining the antioxidant activity of *Thymus vulgaris* and *Citrus limon* essential oils.

## MATERIALS AND METHODS

### Chemicals

2,2-diphenyl-1-picrylhydrazine (DPPH), vitamin C (vit C, as a positive control), and ferric chloride were purchased from Sigma-Aldrich (USA). We bought methanol (MeOH, as a solvent and a negative control), chloroform ferric chlorid and sulfuric acid from Merck (Germany). Each and every chemical was analytic reagent grade.

### Essential Oil Samples

Thyme essential oil (*T. vulgaris*) and limon essential oil (*C. limon*) were acquired from Adana, Türkiye, local market for use in this study. All samples of essential oils were verified and stored in the Analytical Chemistry Laboratory of the Faculty of Pharmacy, Cukurova University in Türkiye.

### Extract Screening for Bioactive Agent

Following are some key constituents of the plants' essential oil samples that were subjected to phytochemical screening utilizing in their respective qualitative methods descriptions (Richardson, 1990; Runde et al., 2015).

### Test for Terpenoids

To dissolve the 2 mL of the plants' essential oil samples, 2 mL of chloroform was employed, and the mixture was then allowed to dry. After heating for approximately 2 minutes, another 2 mL of concentrated sulfuric acid was added. After adding, the sulfuric acid was heated for about 2 minutes. Terpenoids were evident because of the development of a greenish colour (Richardson, 1990).

### Test for Essential Oils

A little amount of the extracts was dissolved in 90% alcohol, and two drops of $FeCl_3$ were added. There was a greenish color that also suggested the presence of essential oils (Runde et al., 2015).

### DPPH Assay

An assay for free radical scavenging activity uses 1,1-diphenyl-2-picrylhydrazine (DPPH). The decrease in the stable free radical DPPH's absorbance at 517 nm can be used to assess the scavenging capacity of natural compounds. Scavenger combines with the purple free radical to produce the colourless product DPPH. A 0.1 M stock solution of DPPH in methanol was prepared. In order to prevent deterioration, this solution is stored in the refrigerator wrapped in foil. By adapting the methods of Brand-Williams et al. (1995) (Brand-Williams et al., 1995), and Blois (1958) (Blois, 1958), the DPPH test of the essential oil samples was investigated (2019). The essential oil samples were diluted with MeOH in twelve stages. A 96-well plate was loaded with 120 µL

of the diluted essential oil samples and vit C. In order to start the reaction, 40 µL of 0.1 M DPPH in MeOH were added. A UV spectrophotometer was used to test the reaction mixture's absorbance at 517 nm after it had stood at room temperature for 45 minutes (Thermo Scientific, Multiskan Sky Microplate Spectrophotometer, Waltham, MA, USA). The mean expression was calculated using three observations of the data. From the drop in absorbance, the % DPPH radical scavenging activity of samples and standards was determined. It was determined what percentage of DPPH was removed (($A_{Control}$ $A_{Sample}$)/$A_{Control}$)x100). The value of the half-maximal inhibitory concentration ($IC_{50}$) is then determined. The values of effective concentration ($EC_{50}$), antiradical power (ARP), and ascorbic acid equivalent antioxidant capacity (AEAC)were determined using the $IC_{50}$, respectively (Aynur et al., 2020; Güven et al., 2021; Karahüseyin et al., 2022; Kayiran et al., 2022; Yamali et al., 2022).

### Statistical Analysis

The mean and standard deviation (mean ± SD) of the $IC_{50}$ were used to express all data. The data were compared using a one-way ANOVA analysis, and all tests were deemed statistically significant at $p < 0.005$.

## RESULTS

### Test for Phytochemical Screening

Results of phytochemical screening tests on *Thymus vulgaris* and *Citrus limon* essential oils samples showed that they contain terpenoids and essential oils. Results are given Table 1.

**Table 1.** Phytochemical study of samples.

| | **Terpenoids** | **Essential oil** |
|---|---|---|
| ***Thymus vulgaris* samples** | + | + |
| ***Citrus limon* samples** | + | + |

### Essential Oils Antioxidant Activities

The antioxidant capacity of the samples of essential oils rose as the $IC_{50}$ value declined. The $IC_{50}$ value displays the antioxidant activity when it is lower. If an antioxidant has low $IC_{50}$-$EC_{50}$ and high ARP-AEAC values, it is known to be more potent. It was discovered that thyme and limon also showed antioxidant activity after statistical analysis, so that results are given Table 2. The essential oils were ranked in terms of antioxidant potency based on antioxidant criteria: *Thymus vulgaris* EO (IC50: 0.007 ± 0.002 mg/mL)> Vit C (IC50: 0.0111 ± 0.001 mg/mL)> *Citrus limon* EO (IC50: 4.894 ± 0.054 mg/mL).

**Table 2.** Antioxidant properties of the samples of essential oils.

| | **$IC_{50}$ (mg/mL)** | **$EC_{50}$ (mg/mL)** | **ARP (mL/mg)** | **AEAC** |
|---|---|---|---|---|
| **Vitamin C** | 0.0111 ± 0.001 | 0.289 | 345.260 | - |
| ***Thymus vulgaris* EO** | $0.007 \pm 0.002^{a}$ | 0.204 | 489.220 | 152349.349 |
| ***Citrus limon* EO** | $4.894 \pm 0.054^{b}$ | 122.543 | 0.816 | 227.405 |

$EC_{50}$: Effective concentration, ARP: Antiradical power, AEAC: Ascorbic acid equivalent antioxidant capacity. $IC_{50}$ values expressed are means ± standard deviation of three measurements. The values of the superscript-containing the essential oil samples differed considerably ($p \leq 0.005$).

## DISCUSSION

Ever since the dawn of time, people have looked to nature as a medicine. The use of medicinal plants has historically been dependent on natural inclination and practical knowledge. Therapeutic methods have been developed during past, involving medicinal plants that developed from the empirical procedure that, before the discovery of chemical pharmaceuticals, constituted solely feasible remedy for specific disorders. As science and engineering have continued to advance, more synthetic pharmaceuticals have been created, and herbal treatments have fallen out of favor. With scientific evidence of the therapeutic benefits of medicinal plant products, its reputation is regaining ground and it provides a powerful substitute for conventional treatment.

The use of medicinal plants in the treatment of URTIs is helpful. URTIs is common illnesses connected to seasonal immune system deterioration. As a result, plant-based medications having a generalized

immunomodulating action are frequently employed (Involving *Echinacea* and American ginseng). Combining *Thymus* and *Pelargonium* species are used as two medicinal herbs with antibacterial and antiseptic properties. They can decrease the course of the illness and lessen the requirement for antibiotic therapies to treat URTIs (Komaravelli and Casola, 2014).

Wide range of medicinal plants have antioxidant properties, and consuming antioxidants regularly is known to reduce the oxidative stress and damage to which organisms are attracted. This is done in part by promoting the production of new endogenous antioxidants. Phenols, flavonoids, tocopherols, ascorbic acid (vit C), and other antioxidant compounds are abundant in many medicinal plants [36]. Vit C affects immunity to infections, and since the 18$^{th}$ century, medicinal plants have been linked to immune system regulation. [8] Supplemental vit C has been offered for both medicinal and preventative purposes.

It is common to use thyme and lemon together for URTI during the winter months within the scope of the ethnobotanical studies conducted in the Adana region. Therefore, in this study, the radical scavenging effect of thyme and lemon essential oil was investigated by the DPPH method. It has been found that thyme essential oil has higher antioxidant capacity than lemon essential oil. However, it has been observed that thyme essential oil has a more potent antioxidant capacity than vit C, which is known to be a natural and powerful antioxidant.

The health and delicious and unique perfume of the individual are greatly influenced by the lemon essential oils. It's a really adaptable option for the entire body. The essential oils may be applied topically, ingested internally, diluted, or used in the following way: inhaled as fragrances.

A living plant belonging to the Rutaceae family is the *Citrus*. The genus *Citrus* contains over 1300 species and 140 taxa. The word "limon" for the lemon is a remnant of prehistoric French. The main components of the chemical components include flavonoids, acids, caffeine, pectin, and minerals. Limonene, which is a large component of essential *Citrus* oil, gives the oil its distinctive aroma. Lemon is antibacterial, antifungal, anti-inflammatory, anti-cancer, depurative, and antiscorbutic, among other properties (Al-Qudah et al., 2018).

*T. vulgaris* L. is an important medicinal plant which belongs to the Lamiaceae family; it has been used for centuries as spice, home remedy, drug, perfume and insecticide. In medicine, it is used as antispasmolytic, antibacterial, antifungal, secrotolytic, expectorant, antiseptic, anthelmintic and antitussive as reported by other authors (Nadia and Rachid, 2013; Özgüven and Tansi, 1998).

## CONCLUSION

In conclusion, we investigated the antioxidant activities of essential oils obtained from lemon and thyme species, which are the indispensable products of the winter months and grow in abundance in the Adana region. The results showed that thyme essential oil has the highest antioxidant activity. Thyme can be used as a convenient source of natural antioxidants in this context for branded foods and medications.

## REFERENCES

Al-Qudah TS, Zahra U, Rehman R, Majeed MI, Sadique S, Nisar S, Tahtamouni R, Tahtamouni RW 2018. Lemon as a source of functional and medicinal ingredient: A review. International Journal of Chemical and Biochemical Science 14:55-61.

Ali-Shtayeh MS, Yaniv Z, Mahajna J 2000. Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants. Journal of Ethnopharmacology 73(1-2):221-232.

Aynur S, Özsoy N, Karahüseyin S 2020. Taraxacum farinosum Hausskn. & Bornm. bitkisinin antioksidan aktivite yönünden incelenmesi. Sağlık Bilimlerinde İleri Araştırmalar Dergisi 3(1):13-19.

Bardia A, Nisly NL, Zimmerman MB, Gryzlak BM, Wallace RB. (2007). Use of herbs among adults based on evidence-based indications: findings from the National Health Interview Survey. Paper presented at the Mayo Clinic Proceedings.

Ben-Arye E, Dudai N, Eini A, Torem M, Schiff E, Rakover Y 2011. Treatment of upper respiratory tract infections in primary care: a randomized study using aromatic herbs. Evidence-Based Complementary and Alternative Medicine 2011

Blois MS 1958. Antioxidant determinations by the use of a stable free radical. Nature 181(4617):1199-1200.

Brand-Williams W, Cuvelier M-E, Berset C 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology 28(1):25-30.

Chen FP, Chen TJ, Kung YY, Chen YC, Chou LF, Chen FJ, Hwang SJ 2007. Use frequency of traditional Chinese medicine in Taiwan. BMC Health Services Research 7(1):1-11.

Cowan MM 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12(4):564-582.

Güven UMK, Serpil Demirci, Aygül A, Nenni M, Kirici S 2021. Design of microemulsion formulations loaded Scutellaria salviifolia Benth, Sideritis libanotica Labill. subsp. linearis (Bentham) Bornm, and Ziziphora clinopodioides Lam. extracts from Turkey and in vitro evaluation of their biological activities. Turkish Journal of Botany 45(8):789-799.

Karahüseyin S, Aynur S, OZSOY N 2022. Antioxidant activity and three phenolic compounds from the roots of Taraxacum gracilens Dahlst. Istanbul Journal of Pharmacy 52(1):69-72.

Kayiran SD, ÖZKAN EE, Kara EM, BEKTAŞ NY, Özden T, Nenni M 2022. Determination of the chemical composition, antioxidant potential of Sambucus ebulus L.(dwarf elder) fruit extracts and investigation of antimicrobial activity on Trichophyton rubrum (Castell.) Sabour and some microorganisms. Istanbul Journal of Pharmacy 52(2):148-155.

Komaravelli N, Casola A 2014. Respiratory viral infections and subversion of cellular antioxidant defenses. Journal of Pharmacogenomics & Pharmacoproteomics 5(4)

Le Maréchal M, Tebano G, Monnier AA, Adriaenssens N, Gyssens IC, Huttner B, Milanič R, Schouten J, Stanić Benić M, Versporten A 2018. Quality indicators assessing antibiotic use in the outpatient setting: a systematic review followed by an international multidisciplinary consensus procedure. Journal of Antimicrobial Chemotherapy 73(Suppl_6):vi40-vi49.

Lev E 2006. Ethno-diversity within current ethno-pharmacology as part of Israeli traditional medicine–A review. Journal of Ethnobiology and Ethnomedicine 2(1):1-12.

Lionis C, Faresjö Å, Skoula M, Kapsokefalou M, Faresjö T 1998. Antioxidant effects of herbs in Crete. The lancet 352(9145):1987-1988.

Nadia Z, Rachid M 2013. Antioxidant and antibacterial activities of Thymus vulgaris L. Medicinal and Aromatic Plant Research Journal 1(1):5-11.

Özgüven M, Tansi S 1998. Drug yield and essential oil of Thymus vulgaris L. as in influenced by ecological and ontogenetical variation. Turkish Journal of Agriculture and Forestry 22(6):537-542.

Pauk MDN 2011. Terapie akutní bronchitidy. Interní medicína pro praxi:327-328.

R Cundell D 2014. Herbal phytochemicals as immunomodulators. Current Immunology Reviews 10(2):64-81.

Richardson PM 1990. Phytochemical methods: a guide to modern techniques of plant analysis. Brittonia 2(42):115-115.

Runde M, Kubmarawa D, Maina H 2015. Compositional Analysis and Anti-Oxidant Assesment of Essential Oil of some Aromatic Plants Obtained from North-Eastern Nigeria. Research Journal of Chemical Sciences ISSN 2231:606X.

Said O, Khalil K, Fulder S, Azaizeh H 2002. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. Journal of Ethnopharmacology 83(3):251-265.

Šmejkal K, Rjašková V 2016. Use of plant extracts as an efficient alternative therapy of respiratory tract infections. Ceska a Slovenska farmacie: casopis Ceske farmaceuticke spolecnosti a Slovenske farmaceuticke spolecnosti 65(4):139-160.

Tan BK, Vanitha J 2004. Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review. Current Medicinal Chemistry 11(11):1423-1430.

Thomas M, Bomar PA 2022. Upper Respiratory Tract Infection. StatPearls Publishing, Treasure Island (FL).

Wenzel R, Fowler A 2006. Acute Bronchitis. Clinical practice. N. Engl. Journal of Medicine 355:2125.

Yamali C, Gul HI, Sakarya MT, Saglik BN, Ece A, Demirel G, Nenni M, Levent S, Oner AC 2022. Quinazolinone-based benzenesulfonamides with low toxicity and high affinity as monoamine oxidase-A inhibitors: Synthesis, biological evaluation and induced-fit docking studies. Bioorganic Chemistry 124:105822.

## ORAL PRESENTATION

# Dirençli Nişastanın Gıda Teknolojisi ve Sağlık Açısından Önemi

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**Özet**

Dirençli nişasta (DN), sindirim enzimlerine dirençli olması ve kalın bağırsak florasında bulunan mikroorganizmalarca fermente edilebilmesi sebebiyle diyet lifi olarak kabul edilen bir nişasta türüdür. DN işlem görmemiş tahıl, baklagil, patates ve yeşil muz gibi bitkisel kaynaklarda doğal olarak bulunabildiği gibi, zengin nişasta içeriğine sahip bu ürünlere uygulanan çeşitli işlemler neticesinde sonradan da oluşturulabilmektedir. Hem besleyicilik hem de teknolojik açıdan sahip olduğu niteliklerden dolayı, yeni fonksiyonel gıdaların tasarlanmasında başvurulan, diyet lifi kaynaklarından biri olarak artan ilgiye sahiptir. Tahıl ürünlerinin tercih edilirliği büyük ölçüde ürünün duyusal ve tekstürel kalitesine bağlıdır. Diğer besinsel lif kaynaklarının aksine, eklendiği gıdada varlığının tekstürel açıdan çok belirgin olmaması nedeniyle, DN'nın gıdalarda kullanımı yaygınlaşmaktadır. DN gıdalarda; jel oluşturma, su bağlama ve şişme kapasitesini arttırma gibi teknolojik niteliklerinden dolayı tekstürel kaliteyi arttırmaya yönelik bir katkı olarak değerlendirilmektedir. Yapılan çalışmalarda; DN ilavesinin, kaplamalı ürünlerin ve kahvaltılık tahılların gevrekliğini arttırma, hamur reolojik özelliklerini iyileştirme ve iyi ekstrüzyon oluşturma gibi etkilere sahip olduğu belirtilmiştir. DN'nın yağ ikame edici özelliği de gıda formülasyonlarında önemli faydalar sağlamaktadır. Yağ yerine DN kullanılarak, yağların kazandırdığı spesifik özelliklerden ödün vermeksizin yağ miktarı azaltılabilmektedir. Ayırt edilebilir tadının olmaması, beyaz renkli olması, vizkoz yapıyı güçlendirmesi DN'yı gıda sanayinde ön plana çıkaran diğer vasıflarındandır. Bununla birlikte, DN tüketiminin, kişilerin hastalıklardan korunması ve fizyolojik durumlarının iyileştirilmesinde de etkili olduğu bilinmektedir. DN'nın, ince bağırsakta düşük oranda sindirilebilmesi sebebiyle kandaki glukoz miktarını düşürdüğü rapor edilmiştir. Bu durum kandaki glisemik indeks düzeyinin iyileşmesini sağlamakta ve tokluk hissini arttırmaktadır. Ayrıca DN, bağırsak mikroflorası üzerindeki olumlu etkilerinden dolayı prebiyotik olarak kabul edilmektedir. Bu özellikleri nedeniyle başta kolon kanseri olmak üzere pek çok sindirim sistemi merkezli matabolik hastalıkların önlenmesinde de rol oynamaktadır. Bu çalışmada, gerek gıda sanayindeki kullanım alanları gerekse de insan sağlığı üzerinde sahip olduğu potansiyel fizyolojik faydaları nedeniyle son yıllarda dikkat çeken bir bileşen olan DN'nın önemli fonksiyonlarına ve gıdalarda kullanım şekillerine yer verilmiştir.

**Anahtar Kelimeler:** Dirençli nişasta, diyet lifi, fonksiyonel özellikler, prebiyotik, fizyolojik etki

# Importance of resistant starch for food technology and human health

**Abstract**

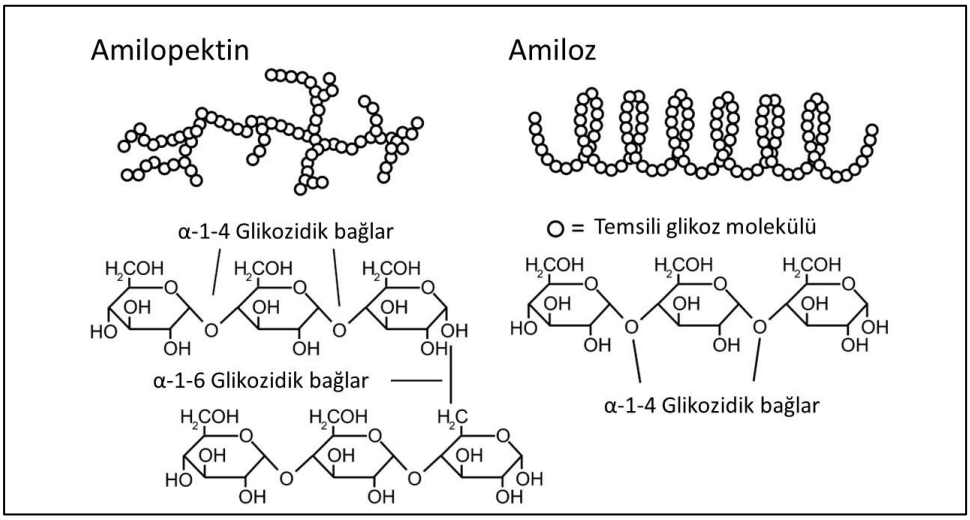
Resistant starch (RS) is a type of starch that is accepted as a dietary fiber because it is resistant to digestive enzymes and can be fermented by microorganisms in the large intestine flora. RS can be naturally found in unprocessed grains, legumes, potatoes and green bananas, as well as being formed later as a result of various processes applied to these products with rich starch content. Due to its nutritional and technological characteristics, it has gained increasing attention as one of the sources of dietary fiber used in the design of new functional foods. The preference for cereal products largely depends on the sensory and textural quality of the product. Contrary to other dietary fiber sources, the use of RS in foods is becoming more widespread, since its presence in the food to which it is added is not very pronounced in terms of texture. RS in foods; it is considered an additive to increase textural quality due to its technological properties, such as gel formation, water binding and increasing swelling capacity. In the studies; it has been stated that the addition of RS has effects such as increasing the crispness of coated products and breakfast cereals, improving the dough's rheological properties and forming good extrusion. The fat replacer feature of RS also provides important benefits in food formulations. By using RS instead of oil, the amount of oil can be reduced without compromising the specific properties of oils. Lack of distinguishable taste, being white in colour and strengthening the viscous structure are other characteristics that make RS stand out in the food industry.

Moreover, it is known that RS consumption is also effective in protecting people from diseases and improving their physiological conditions. It has been reported that RS reduces the amount of glucose in the blood due to its low digestibility in the small intestine. This situation improves the glycemic index level in the blood and increases the feeling of satiety. In addition, RS is considered a prebiotic due to its positive effects on intestinal microflora. Due to these properties, it also plays a role in the prevention of many digestive system-centered metabolic diseases, especially colon cancer. In this study, the important functions of RS, which have attracted attention in recent years due to its use in the food industry and its potential physiological benefits on human health, and its use in foods are included.

**Keywords:** Resistant starch, dietary fiber, functional properties, prebiotic, physiological effect

## 1. GİRİŞ

Nişasta mısır, patates, pirinç ve buğday gibi birçok bitkisel ürünün ana bileşenidir. Nişasta granülleri soğuk suda çözünmemektedir. Nişatalar amiloz ve amilopektinden meydana gelmektedir. Amiloz düz zincir şeklinde olup α-1,4-glikozidik bağına sahiptir. Amilopektin ise dallanmış zincirlerden oluşmakta moleküller arasında α-1,6-glikozidik bağı oluşturmaktadır (Özdemir ve Sıdalı., 2013). Amiloz ve amilopektin moleküllerinin kimyasal yapıları ve bağlanma şekilleri Şekil 1'de verilmiştir. Nişasta bitki dokularında (mısırda %65-75; pirinçte %70-80; buğdayda %60-70) depo maddesi olarak bulunur (Savlak ve Türker, 2019) ve insan beslenmesinde oldukça önemli bir karbonhidrat kaynağıdır (Ratnayake and Jackson, 2008). Bir çok bitki dokusunda yer almakta ve bitkinin çeşidine göre genelde 1 ile 100 µm çapında olduğu görülmektedir (Fuentes-Zaragoza ve ark., 2010).




**Şekil 1.** Nişastayı oluşturan Amiloz ve Amilopektin moleküllerinin kimyasal ve sembolik gösterimleri (Willfahrt ve ark., 2019'dan adapte edilmiştir)

Nişasta hızına ve sindirimine göre 3 ayrı gruba ayrılır. Bunlar; yavaş sindirilen nişasta, hızlı sindirilen nişasta ve dirençli nişastadır (Mir ve ark., 2013). Dirençli nişastalar enzim ile sindirilmeyen ve ince bağırsakta absorbe olmayan nişastadır.

Fonksiyonel olarak ele alındığında; DN1, DN2, DN3, DN4 ve DN5 olmak üzere 5 gruba ayrılmaktadır (Zhu ve ark., 2013). Dirençli nişastalar doğal olarak tahıl tanelerinde, ısıl işlem gördükten sonra soğutulan nişasta içeren gıdalarda ve tohumlarda bulunmaktadır. (Homayouni ve ark., 2013).

Normal nişasta molekülü insan vücudunda sindirilerek enerji kaynağı olarak kullanılabiliyorken, dirençli nişastaların sindirilememesinin üç farklı nedeni vardır. Bunlar;

i. DN'nın molekül yerleşimi sebebiyle sindirim enzimlerinin etkin olmaması

ii. Nişasta granüllerinin hücre duvarı ile korunuyor olması

iii. Nişasta partiküllerinin jelatinizasyon sonrasında soğutulması ve retrograde nişasta meydana gelmesidir (Arıbaş, M. 2020).

Dirençli nişasta, normal nişasta gibi amiloz ve amilopektinden meydana gelmesine rağmen sindirime dirençli olduğu için diyet lifleri grubunda ele alınmaktadır (Burdurlu ve Karadeniz, 2003).

DN gıdalarda doğal olarak bulunabildiği gibi, Normal nişasta yapılarının çeşitli yöntemlerle işlenmesi sonucu sonradan da elde edilebilmektedir. Bu yöntemler; ısı-nem uygulamaları (mikrodalga ve kondüktiv ısıtma), yüksek hidrostatik basınç, ekstrüzyon, hidroliz enzimleri ile muamele etme, gama ışıması ve ultrasonikasyon olarak çeşitli çalışmalarda belirtilmiştir (Altan, 2015; Demirekin, 2019; Erbaş ve ark., 2016; Şimsek, 2012; Zhou ve ark., 2014).

## 2. DİRENÇLİ NİŞASTANIN YAPISI VE SINIFLANDIRILMASI

Dirençli nişasta fiziksel ve kimyasal özelliklerine göre DN(1,2,3,4,5) olmak üzere 5 fraksiyona ayrılmaktadır. DN tipleri, kaynakları ve sindirim hızları Tablo 1'de, Bazı gıdaların DN miktarları Tablo 2'de verilmiştir.

**Tablo 1.** Dirençli Nişasta Tipleri, Kaynakları ve Sindirim Hızları (Birt ve ark., 2013; Homayouni ve ark., 2013, Hasjim, Ai ve Jane, 2013)

| **Tip** | **Açıklama** | **Kaynak** | **Sindirim Hızı** |
|---|---|---|---|
| DN1 | Hücre duvarları ile fiziksel olarak korunan DN | Öğütülememiş veya kısmen öğütülmüş tohumlar, tahıllar, baklagiller | Yavaş |
| DN2 | Kristal yapılı, doğal granül nişasta | Çiğ patates, olgunlaşmamış muz, yüksek amiloz içeren nişastalar | Çok yavaş |
| DN3 | Retrograde Nişasta | Pişirilmiş ve retrograde olmuş nişasta içeren gıdalar | Yavaş |
| DN4 | Kimyasal olarak modifiye edilmiş nişasta | Lif takviyesi ile modifiye edilmiş fonksiyonel gıdalar | Hidrolize karşı dirençli |
| DN5 | Amiloz-Lipid kompleksi | Nişasta ve lipid içeren gıdalar | Yavaş |

**Tablo 2.** Çeşitli dirençli nişasta içerikleri (%) (Holt ve ark., 1995;Murphy ve ark., 2008)

| Gıda | DN | Gıda | DN | Gıda | DN | Gıda | DN |
|---|---|---|---|---|---|---|---|
| Mercimek | 25,4 | Patlamış buğday | 6,2 | Pizza hamuru (pişmiş) | 2,8 | Mısır unu | 1,7 |
| Barbunya | 24,6 | Muz | 4 | Patates püresi | 2,4 | Pirinç unu | 1,6 |
| Börülce | 17,7 | Çavdar ekmeği | 3,2 | Patlamış pirinç | 2,3 | Pişmiş makarna | 1,4 |
| Mısır unu | 11 | Mısır gevreği | 3,2 | Ekmek hamuru | 2,1 | Ekmek (beyaz ekmek) | 1,2 |
| Yulaf | 7,2 | Tortilla (mısır) | 3 | Buğday unu | 1,7 | Pişmiş beyaz pirinç | 1,2 |

## 3. DİRENÇLİ NİŞASTANIN GIDA ENDÜSTRİSİNDE KULLANIM ALANLARI

Tahılların beslenmeye katkısı ve rolü toplumun yapısına göre farklılıklar oluşturmaktadır. Ayırt edilebilir tadının olmaması, yüksek su tutma kapasitesi, jelleşerek vizkoz yapıyı güçlendirmesi, beyaz renkte görünmesi, meyveler, kepek veya tam tahıl gibi geleneksel lif kaynakları ile incelendiğinde küçük partikülleri nedeniyle ürün dokusuna etkisinin düşük olması ve doğal kaynaklardan elde ediliyor olması, gıda sanayinde dirençli nişastanın önemini açıklamaktadır (Demirekin, 2019).

Bustos ve ark,. (2011), yulaf kepeğininin formülasyonuna DN2 ve DN4'nın ilave edilmesiyle makarna üretimi yapılan çalışmada, glisemik indeksin ciddi oranda azalması ve maltozun uzun sürede salınması ve beslenme anlamında iyileşme sağlamışlardır. Bir başka çalışmada DN4 ilavesinin makarnanın pişme kaybında düşüşe, DN4 ilavesinin oranı arttıkça pişme kaybında artış gözlendiği belirtilmiştir (Sözer ve ark., 2007; Demirekin, A. 2019).

Ekmek üretiminde farklı oranlarda ticari DN'ların kullanıldığı bir çalışmada fazla oranlarda ilave edilen DN'nın, ekmeğin gözenek oluşumunda, renginde ve dış görünüşünde istenmeyen bir duruma neden olmadığı, sadece ilave oranı arttıkça ekmeğin hacminde azalma olduğu görülmüştür (Köksel ve ark., 2009;Baghurst, Baghurst ve Record, 1996). Başka bir çalışmada ise farklı oranlarda DN4 ilave edilen ekmek üretimi yapılmıştır. DN4'ün oranının artmasıyla ekmeklerin diyet lifi miktarında artış olduğu belirlenmiştir (Sajilata,

Singhal ve Kulkarni, 2006;Öztürk, Köksel ve Ng, 2009;Sanz-Penella ve ark., 2010;Van Hung ve Morita, 2004).

DN2 ve DN3'ün yüksek su tutma kapasiteli, doğal kaynaklı, beyaz renkli, lezzetli, laksatif etkisi ve ince partikül boyutlu olması fakat jelatinizasyon sıcaklığının (JS) yüksek olması gibi fonksiyonel özellikleri eklendiği gıdada olumlu etkiler meydana getirmektedir. Yapılan diğer çalışmalarda DN'nın gevrekliği arttırma, fonksiyonel bileşen olarak kullanılabilme, hamur reolojik özelliklerini olumlu etkileme, etkin film oluşturma ve ekstrüzyon özellikleri ve geleneksel lifli ürünlerden daha yüksek su tutma kapasitesi sağlama gibi avantaj oluşturduğu görülmüştür (Thomson, 2000;Sajilatave ark., 2006; Mikulíková ve ark., 2008;Tharanathan, 2002;Younes ve ark., 2005, Thomson, 2000; Kahraman ve Köksel, 2006; Heydari ve ark., 2011; Taş ve El 2000; Homayouni ve ark., 2013). DN'nın gıdalarda kullanılmasına dair çalışmalar son yıllarda artış göstermektedir. Proses koşullarına daha fazla uyum gösterdiğinden, DN ilave edildiğinde ürün formülasyonunda ve üretim koşullarında daha az modifikasyon uygulanmaktadır (Savlak ve Türker B., 2015;Sharma ve Yadav, 2008).

## 4. DİRENÇLİ NİŞASTANIN SAĞLIK ÜZERİNE ETKİLERİ

DN'nın kardiyovasküler hastalıkların, Tip2 diyabetin ve obezitenin ortaya çıkmasında risk düşürücü rol oynadığı bilinmektedir. DN, ince bağırsakta düşük sindirim miktarına sahip olması sebebiyle kandaki glukoz miktarını azaltmaktadır. Bu durum kandaki glisemik ve insülin yanıtlarının iyileşmesine neden olmaktadır. Çözünür lifler sindirim sisteminde bulunan safra asitlerini kendi yapısına dahil ederek serum kolesterol miktarının düşmesine olanak sağlamaktadır. Genetik bakımından zayıf, obez ve diyabetik sıçanlar üzerinde yapılan çalışmalarda DN'nın plazma kolesterol miktarını düşürdüğü tespit edilmiştir. DN, bağırsak mikroflorası üzerindeki etkisinden dolayı prebiyotik olarak kabul görmektedir (Balamurugan ve ark., 2019;Gibson ve Roberfroid, 1995). Prebiyotikler insan metabolizmasında sindirime uğramayan ancak bağırsaklarda bulunan probiyotik mikroorganizmaların fermente edebildiği ve böylece probiyotik bakterilerin enerji kaynağı olarak kullanabildiği karbonhidrat türevleridir (Nugent, 2005;Arıbaş, 2020).

Yapılan diğer çalışmalarda dirençli nişasta miktarı fazla olan gıdalarla beslenen farelerin çinko, magnezyum, bakır, magnezyum ve demirin absorbsiyonunda artışa raslandığı belirlenmiştir (Mikušová ve ark., 2009;Reader ve ark., 2002;Younes ve ark., 2001). DN ilave edilmiş ürünler ile beslenen farelerin kalın bağırsağında yapılan çalışmalarda, dışkısında kütle artışı ve dışkı pH'sının azalmasının yanı sıra daha çok kısa zincirli yağ asidinin meydana geldiği, böylece kolon kanseri oranını düşürdüğü saptanmıştır (Rahman ve ark., 2007; Ferguson ve ark., 2000;Tharanathan ve Mahadevamma, 2003). Özellikle DN3 tüketiminin kan insulin-glukoz seviyesini azalttığı görülmüştür (Prado-Silva ve Azavedo 2014). DN ihtiva eden gıdaların yemek sonrasında kan şekerini düşürdüğü ve diyabetin kontrol altına alınmasında etkili olabileceği de tespit edilmiştir (Sajilata ve ark., 2006; Prado-Silva ve Azavedo 2014). Tip 2 diyabet hastalarına yapılan incelemelerde yemek sonrasında kan insulin-glukoz seviyeleri üzerine, üç farklı üründe tüm nişaasta tiplerinin akut etkileri değerlendirilmiş ve vücuda alımından 1 saat sonra, enerji bar ve şekerleme barının kan glikoz seviyesindeki artışları benzerken, dirençli nişasta içeren bardan oldukça yüksektir (Reader ve ark., 2002;Ashwar ve ark., 2016). Amerika ve Avrupa'dan çeşitli ülkelerde safra taşı oluşum sayısının, DN tüketim miktarının fazla olduğu Asya ülkeleriyle kıyaslandığında; çok daha yüksek olduğu belirtilmiş ve DN'nın bir etkisi olarak yorumlanmıştır (Sajilata ve ark., 2006). Dirençli nişastanın sağlık faydaları ile ilgili yapılmış çeşitli çalışmalar Tablo 3'de verilmiştir.

**Tablo 3.** Dirençli nişastanın sağlık üzerine faydaları (Sharma ve ark., 2008;Türker ve Savlak, 2015)

| **Sağlık Etkisi** | **Açıklama/Muhtemel Mekanizma** |
|---|---|
| Diyabeti önleme | Yemek sonrası daha düşük insülin tepkisi, artan insülin duyarlılığı, yemek sonrası daha düşük glikoz tepkisi, insülin direncinin geç başlaması (Öztürk, 2008; Nestel ve ark., 2006) |
| Toplam karaciğer kolesterolünü azaltma | Kolesterol ve safra asitlerinin yüksek oranda salgılanması (Torres-Zapata ve ark., 2012; Wong ve ark., 2009) |
| Mineral emilimi | Kalsiyum ve magnezyum emilimi artırmak (Hashimoto ve ark., 2006; Younes ve ark., 2001) |
| İshali önleme | DN tarafından teşvik edilen probiyotikler yoluyla rotavirüs ishal süresinin kısalması (Scholz-Ahrens ve ark., 2007; Raghupathy ve ark., 2006) |
| Obeziteyi önleme | Düşük kalori değeri nedeniyle azalan enerjinin, iştahta azalma ile sonuçlanması (Ramakrishna ve ark., 2000; Brown, 2004) |
| Prebiyotik ajan | Seçilerek bifidobakteriler tarafından kullanılma; Laktobasillerin ve bifidobakterilerin büyümesini teşvik etme; mikroenkapsülasyon içindeki probiyotiklerin canlılığını arttırma (Bodinham ve ark., 2010; Ziar ve ark., 2012) |
| Kolon kanserinin önlenmesi | DN'nın fermentasyonu ile anaerobik bakteriler tarafından üretilen SCFA (Kısa zincirli yağ asitleri) üretiminin artması (Sharma, Yadav ve Yadav, 2008) |
| Bağırsak sağlığını geliştirmesi | Fermente olabilmesi; artan SCFA üretimi; ikincil safra asitlerinin azalması; kolon transit süresinin azalması (Sharma, Yadav ve Yadav, 2008) |

## 5. SONUÇ

Dirençli nişasta konusu üzerine yapılan çalışmalar değerlendirildiğinde,hem teknolojik özellikleri hem de sağlık üzerine olumlu etkileri nedeniyle son ürün özelliklerini olumlu yönde etkilediği görülmektedir. Bu özellikleri bakımından yeni fonksiyonel gıdaların üretilmesinde kullanılma potansiyeli yüksek bir diyet lifi kaynağı olarak bilinmektedir. Literatürde bakıldığında; çeşitli yöntemlerle nişastanın dirençli nişastaya dönüştürülmesine yönelik çalışmalar çoğunluktadır. Bunların yanı sıra gıda sanayi artıklarında doğal olarak bulunan dirençli nişasta yapılarının ekstrakte edilmesine yönelik çalışmalara ağırlık verilmesinde fayda bulunmaktadır. Fonksiyonel gıdalar; gıda sektörü içinde tüketici bazında her geçen yıl artan ilgiye sahip olan ve gıda üreticilerin ürün yelpazelerini geliştirme noktasında ar-ge çalışmalarını yoğunlaştırdığı bir alt disiplin olarak dikkat çekmektedir. Bu bağlamda diyet lifleri de sahip oldukları sağlık faydalarına paralel olarak yeni fonksiyonel gıdaların üretiminde önemli bir fonksiyonel gıda bileşenidir. Ancak gıdaların diyet lifince zenginleştirme çalışmaları daha çok selüloz gibi suda çözünmeyen diyet lifi kaynakları ile yapılmaktadır. Suda çözünebilen tipte diyet lifleri hem getirdikleri ilave sağlık faydaları hem de gıda tekstürünü suda çözünmeyen diyet liflerine nazaran daha az olumsuz etkiledikleri için fark yaratmaktadır. Dirençli nişasta hem suda çözünebilen hem de çözünmeyen diyet liflerinin sağlık açısından sahip oldukları faydaları sağlayabilmesinin yanı sıra gıda teksürü bakımından da adapte edilmesi çok uygun bir diyet lifi fraksiyonu olduğu için bu alanda yapılacak inovatif yeni ürün formülasyonlarında kullanımı en uygun fonksiyonel gıda bileşeni olarak ön plana çıkmaktadır.

## KAYNAKLAR

Ashwar, B. A., Gani, A., Shah, A., Wani, I. A. and Masoodi, F. A., Starch, 68 (2016) 287

Altan, H. 2015. Hidrotermal İşlemlerin Karabuğday Nişastasının Fizikokimyasal Özellikleri ve Dirençli Nişasta Miktarı Üzerindeki Etkisi. İstanbul Teknik Üniversitesi. Fen Bilimleri Enstitüsü. YL Tezi. 109s. İstanbul.

Arıbaş M. 2020. Tip 4 Enzime Dirençli Nişasta İlavesiyle Üretilen Ekmek ve Makarna Örneklerinde Tahmini Glisemik İndeks Değerlerinin ve Bazı Fonksiyonel Özelliklerinin İncelenmesi. Hacettepe Üniversitesi. Doktara Tezi. 112s. Ankara

Baghurst, P. A., Baghurst, K. I. and Record, S. J., 1996. Food Australia, 48 (1996)

Balamurugan, R., Pugazhendhi, S., Balachander, G. M., Dharmalingam, T., Mortimer, E. K., Gopalsamy, G. L., and Ramakrishna, B. S., 2019. International Journal of Environmental Research and Public Health, 16 (2019) 3922.

Birt, D. F., Boylston, T., Hendrich, S., Jane, J.-L., Hollis, J., Li, L., and Whitley, E. M., Advances in Nutrition, (2013) 4-587

Bodinham, C.L., Frost, G.S., Robertson, M.D., 2010. Acute ingestion of resistant starch reduces food intake in healthy adults. Br. J. Nutr. 103: 917–922.

Burdurlu, H.S., Karadeniz, F., 2003. Gıdalarda Diyet Lifinin Önemi. Gıda Mühendisliği Dergisi 7(15): 18-25.

Bustos, M.C., Perez, G.T., León, A.E., 2011. Sensory and nutritional attributes of fibre-enriched pasta. LWT – Food Sci. Technol. 44: 1429–1434.

Bustos, M. C., Perez, G. T. and León, A. E., 2013. International Journal of Food Science & Technology, 48 (2013) 309

Brown, I.L., 2004. Applications and uses of resistant starch. J. AOAC Int. 87: 727–732.

Demirekin, A., 2019. Pirinç Nişastasından Çeşitli Yöntemler ile Elde Edilen Enzime Dirençli Nişastanın Karakterizasyonu ve Ekmek Kalitesi Üzerine Etkisi. Süleyman Demirel Üniversitesi, YL Tezi, 82s, Isparta.

Englyst, H.N., Wiggins, H.S., Cummings, J.H., 1982. Determination of the nonstarch polysaccharides in planty foods by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst 107: 307-318.

Erbaş, M. Ark., 2016. Enzime Dirençli Nişasta Üretim Yöntemleri ve Gıda Endüstrisinde Kullanım Amaçları. Akdeniz Üniversitesi Mühendislik Fakültesi G›da Mühendisli¤i Bölümü, GIDA (2016) 41 (6): 419-426. Antalya

Ferguson, L.R., Tasman-Jones, C., Englyst, H., Harris, P.J., 2000. Comparative effects of three resistant starch preparations on transit time and short-chain fatty acid production in rats. Nutrition & Cancer 36: 230–237.

Fuentes-Zaragoza, E., Riquelme-Navarrete, M.J., Sánchez-Zapata, E., Pérez-Álvarez, J.A., 2010. Resistant starch as functional ingredient: A review. Food Research International 43: 931–942.

Gibson, G. R. and Roberfroid,1995. M. B., The Journal of Nutrition, 125 (1995) 1401

Hashimoto, N., Ito, Y., Han, K. H., Shimada, K., Sekikawa, M., Topping, D. L., 2006. Potato pulps lowered the serum cholesterol and triglyceride levels in rats. Journal of Nutritional Science and Vitaminology 52: 445–450.

Hasjim, J., Ai, Y. and Jane, J., 2013. Novel Applications of Amylose-Lipid Complex as Resistant Starch Type 5. Resistant Starch, Shi, Y. C. and Maningat, C. C. (Eds), John Wiley ve Sons, New Jersey, 79–94,

Heydari, S., Mortazavian, A.M., Ehsani, M.R., Mohammadifar, M.A., 2011. Biochemical, microbiological and sensory characteristics of probiotic yogurt containing various prebiotic compounds. Italian J. Food Sci. 23. 153–163.

Holt, S.H.A.; Brand Miller, J.C.; Petcoz, P.; Farmakalidie, E.A. Satiety Index of Common Food. Eur. J. Clinical Nutr. 1995, 49, 675–690.

Homayouni, Aziz, Amini, A., Keshtiban, A. K., Mortazavian, A. M., Esazadeh, K. and Pourmoradian, S., Starch, 66 (2013) 102

Jiang, G., Liu, Q., 2002. Characterization of residues from partially hydrolyzed potato and high amylose corn starches by pancreatic α-amylase. Starch/Stärke 54: 527-533.

Jyothsna, E. and Hymavathi, T. W., 2017. Journal of Pharmacognosy and Phytochemistry, 6 (2017) 67

Kahraman, K., Köksel, H., 2006. Enzime dirençli nişasta üretimi ve fonksiyonel özelliklerinin incelenmesi. Hububat Ürünleri Tekn. Kong., 7-8 Eylül, Gaziantep.

Koksel, H., Masatcioglu, T., Kahraman, K., Ozturk, S., Basman, A., 2008. Improving effect of lyophilization on functional properties of resistant starch preparations formed by acidhydrolysis and heat treatment. J. Cereal Sci. 47, 275–282.

Mikulíková, D., Masár, S., Kraic, J., 2008. Biodiversity of legume health-promoting starch. Starch 60: 426–432

Mikušová, L., Šturdík, E., Mošovská, S., Brindzová, L., Mikulajová, A., 2009. Development of new bakery products with high dietary fibre content and antioxidant activity for obesity prevention. In Proceedings of 4th international dietary fibre conference (p. 185). Vienna, Austria: International association for cereal science and technology (ICC)

Mir, J. A., Srikaeo, K. and García, J. 2013. Effects of amylose and resistant starch on starch digestibility of rice flours and starches. International Food Research Journal. 20:1329-1335.

Murphy, M.M., J.S. Douglass and A. Birkett. 2008. Resistant starch intakes in the United States. J. Am. Diet. Assoc. 108: 67-78.

Nestel, P.J., Noakes, M., Clifton, P., McIntosh, G., 1996. High amylose starch on bowel function, insulin sensitivity and lipids. FASEB J. 10:1-5.

Nugent, A.P., 2005. Health properties of resistant starch. Nutrition Bulletin 30: 27–54.

Özdemir A. ve Sıdalı U. 2013. Streptomyces Sp. Mc10 suşunun alfa amilaz üretim kabiliyetinin belirlenmesi. Celal Bayar Üniv. Fen Bilimleri Dergisi. 9:39 – 46.

Öztürk S., 2008. Farklı nişasta kaynaklarından enzime dirençli nişasta üretimi ve karakterizasyonu. Doktora Tezi. Hacettepe Üniversitesi, Fen Bilimleri Enstitüsü, Ankara.

Öztürk, S., Köksel, H. and Ng, P. K. W., 2019. International Journal of Food Sciences and Nutrition, 60 (2009) 449.

Prado-Silva L., Azavedo L., 2014. Sesame and resistant starch reduce the colon carcinogenesis and oxidative stress in 1,2-dimethylhydrazine- induced cancer in Wistar rats. Food Research International 62: 609–617.

Raghupathy, P., Ramakrishna, B.S., Oommen, S.P., Ahmed, M.S.,2006. Amylase-resistant starch as adjunct to oral rehydration therapy in childrenwith diarrhea. J. Pediatr. Gastroenterol. Nutr. 42: 362–368.

Rahman, S., Bird, A., Regina, A., Li, Z., Ral, J.P., McMaugh, S., Topping, D., Morell, M., 2007. Resistant starch in cereals: Exploiting genetic engineering and genetic variation. J. Cereal Sci. 46: 251-260.

Ramakrishna, B.S., Venkataraman, S., Srinivasan, P., Dash, P., 2000. Amylase-resistant starch plus oral rehydration solution for cholera. New Engl. J. Med. 342: 308–313.

Raigond, P., Ezekiel, R., Singh, B., Dutt, S., Joshi, A. and Rinki., Potato Journa, 42 (2015) 81.

Ratnayake, W.S., Jackson, D.S., 2008. Thermal behavior of resistant starches RS2, RS 3, and RS 4. Journal of Food Science 73(5): 356–366.

Reader, D., Johnson, M.L., Hollander, P., Franz, M., 1997. Response of resistant starch in a food bar vs. two commercially available bars in persons with type II diabetes mellitus. Diabetes 46(1): 254.

Reader, D., O'connell, B., Johnson, M., Franz, M., 2002. Glysmic and insulinemic response of subjects with type 2 diabetes after consumption of three energy bars. Journal of the American Dietetic Association 102(8): 1139-1142.

Sajilata, M.G., Singhal, R.S., Kulkarni, P.R., 2006. Resistant starch-a review. Comprehensive Reviews in Food Science and Food Safety 5: 1-17

Sanz-Penella, J.M., Wronkowska, M., Soral- Smietana, M., Collar, C., 2010. Impact of the addition of resistant starch from modified pea starch on dough and bread performance. Eur. Food Res. Technol. 231: 499–508.

Savlak, N., Türker, B. 2015. Dirençli Nişasta: Tipleri, Kaynaklan, Fizyolojik Etkileri ve Fonksiyonel Özellikleri. Celal Bayar Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü. Akademik Gıda 13(4) (2015) 354-359. Manisa

Scholz-Ahrens, K. E., Ade, P., Marten, B.,Weber, P., 2007. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J. Nutr. 137: 838S–846.

Sharma, A., Yadav, B.S., Ritika, 2008. Resistant starch: Physiological roles and food applications. Food Reviews International 24: 193–234.

Sözer, N., Dalgıç, A.C., Kaya, A., 2007. Thermal, textural and cooking properties of spaghetti enriched with resistant starch. Journal of Food Engineering 81: 476-484

Şimşek, S. and El, S. N., Carbohydrate Polymers, 90 (2012) 1204.

Taş, A. A. and El, S. N., 2000. Food Chemistry, 70 (2000) 493.

Tharanathan, R.N., 2002. Food-derived carbohydrates: Structural complexity and functional diversity. Critical Reviews in Biotechnology 22(1): 65–84.

Tharanathan, R.N., Mahadevamma, S., 2003. Grain legumes: A boon to human nutrition. Trends in Food Science & Technology 14: 507–518.

Thompson, D.B., 2000. Strategies for the manufacture of resistant starch. Trends Food Sci. Technol. 11, 245–253.

Torres-Zapata, A.E., Aparicio-Trápala, M.A., Blé- Castillo, J.L., Corzo-Sosa, C.A., 2012. Glycemic and insulinic response of patients with type 2 diabetes to the consumption of pumpkin soup creole (Cucúrbita pepo L.) enriched with banana starch. Inf. Tecnol. 23: 71–86.

Van Hung, P. and Morita, N., 2004. Food Research International, 37 (2004) 461

Willfahrt, A., Steiner, E., Hötzel, J., Crispin, X., 2019. Materials science & Proseccing. Printable acid-modifed corn starch as non-toxic, disposable hydrogel-polymer electrolyte in supercapacitors. Applied Physics A (2019) 125:474

Wong, J.M.W., De Souza, R., Kendall, C.W. C.,Emam, A., 2006. Colonic health: Fermentation and short chain fatty acids. J. Clin. Gastroenterol. 40:235–243

Younes, H., Levrat, M.A., Demige, C., Remesy, C., 1995. Resistant starch is more effective than cholestyramine as a lipid-lowering agent in the rat. Lipids 30: 847–853.

Younes, H., Coudray, C., Bellanger, J., Demigné, C., 2001. Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. Br. J. Nutr. 86: 479– 485.

Ziar, H., Gérard, P., Riazi, A., 2012. Calcium alginate-resistant starch mixed gel improved the survival of Bifidobacterium animalis subsp. lactis Bb12 and Lactobacillus rhamnosus LBRE-LSAS in yogurt and simulated gastrointestinal conditions. Int. J. Food Sci. Technol. 47: 1421– 1429.

Zhou, Y., Meng, S., Chen, D., Zhu, X., Yuan, H., 2014. Structure characterization and hypoglycemiceffects of dual modified resistant starch fromindica rice starch. Carbohydr Polym, 103, 81-86

Zhu F., Wang S. and Wang Y. 2013. Physical properties and enzyme susceptibility of rice and highamylose maize starch mixtures. Journal Science Food Agriculture. 93:3100–3106

## *ORAL PRESENTATION*

# Optical Spectroscopy for the Structural Investigation of Protein Nanofibers

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## Abstract

Protein nanofibers obtained from plant and animal proteins through innovative techniques such as electrospinning are promising for various applications. Once produced, protein nanofibers should be well-characterized to better understand their morphological and physicochemical behavior. Thus, in correlation with these properties, the most appropriate treatments and applications can be achieved. Fundamental knowledge regarding the structural features is critical for that purpose. Optical spectroscopy serves a great opportunity to determine the molecular structure of nanoparticles including protein nanofibers. It deals with the responses revealed through electromagnetic radiation with atoms and molecules. Infrared (IR), Raman and circular dichroism (CD) spectroscopy techniques are commonly used successful techniques for the structural investigation of proteins and protein-based nanostructures. The stability, surface properties and binding capacity of the nanofibers can be identified both qualitatively and quantitatively. In this study, current research works presenting conformational modifications and molecular interactions in protein nanofiber structures through the mentioned optical spectroscopy methods were reviewed.

**Keywords:** protein, nanofiber, electrospinning, spectroscopy, structure

## INTRODUCTION

Proteins are complex molecules with significant biological and nutritional properties. They are widely used in industry due to their functional properties including texturizing, gelation, foaming and emulsification, binding and surfactant properties. Proteins obtained from animal, plant and microbial sources are also occupied to produce various talented matrices and functional micro- and nano-scale particles for encapsulation, transport and delivery purposes. Protein hydrogels, micro/ nanocapsules and nanofibers are some well-recognized examples.

Due to their superior properties, nanofibers fabricated via electrospinning are widely used in many industrial areas such as electronics, textile, pharmaceutical and food. Currently, peptide- and protein-based nanofibers are getting attention since they are promising for biocompatible and biodegradable matrice opportunities quite well corresponded in biomedical and food applications (Yıldız et al., 2022). Animal-based proteins such as collagen/gelatin, keratin, albumin, silk fibroin and whey proteins, and plant-based proteins such as corn zein, gluten and soy proteins are popular proteins used to produce biopolymer nanofibers.

To better evaluate the nanofiber web for designing the proper application, morphological and structural characteristics should be well identified. Electron and force microscopy techniques are the most powerful tools for the morphological determination of nanofibers. Optical spectroscopy techniques such as Fourier Transform Infrared (FT-IR), Raman, and Circular Dichroism (CD) Spectroscopy are commonly occupied tools for detailed analysis of protein structures (Bürck et al., 2012; Tarhan et al., 2014). In the presented study, structural features of proteins, protein based nanofibers and optical spectroscopy used to analyze the chemical structure of proteins through nanofiber formation have been discussed.

## STRUCTURAL FEATURES OF PROTEINS

The building blocks of proteins are amino acids which are attached through peptide bonds and thus form a long polypeptide chain. The final shape or confirmation of protein is closely related to its molecular structure which has four hierarchical levels as primary, secondary, tertiary and quaternary (Sun et al., 2004). The first level is the primary structure defined as the linear amino acid sequence of a polypeptide chain. The uniqueness

and higher levels of proteins are mainly determined by the primary amino acid sequence of proteins. The local spatial conformation of the main polypeptide chain (by dihedral angles and H-bondings) forms the secondary structure commonly include α-helices, β-sheets and turns, loops and random coils. Tertiary structure is associated with the three-dimensional arrangement of the secondary structure elements through further folding. The spatial arrangement of more than two polypeptide chains as subunits within the complex protein refers to the quaternary structure. The complex structure of proteins are stabilized by H-bondings, electrostatic, van der Waals and hydrophobic interactions.

Proteins are not rigid molecules. Due to the formation of target protein particles or matrices, different treatments are applied to intact protein, leading to disruption of quaternary and tertiary structure. This results in alteration of the structural conformation and thus protein functionality. It might be desirable or undesirable depending on the target production. Structural investigation of proteins through spectroscopy during these treatments can provide significant information promoting proper design and application.

## NANOFIBERS

Nanofibers are unique nano-scale materials possessing a fiber diameter below 1000 nm (Kample et al., 2017). They have high importance due to their excellent features such as extremely high surface-to-volume ratio, special dimensions, flexibility in surface functionalities and superior mechanical performance. Nanofiber materials are highly promising for many industrial fields including catalysis in the chemical industry, scaffolding in tissue engineering, protective clothing in the textile industry, filtration in process industries, biomedical technology, optical electronics, and food technology. They are produced through different techniques such as phase separation, template synthesis, melt-blown technology, and electrospinning which is the most commonly occupied one. Although there are different methods of electrospinning, basically a high voltage is applied to a polymer solution to induce the formation of liquid jets then form solid nanofibers over a grounded collector while evaporating the solvent (Baji et al., 2010). Solution parameters such as viscosity, conductivity and molecular weight of polymer; process parameters such as electrical force, the distance between nozzle and collector and feed rate; ambient parameters such as temperature and humidity are critically affecting the electrospinning process and the properties of the resultant nanofibers (Eskitoros-Togay et al., 2019).

## PROTEIN BASED NANOFIBERS

Proteins such as collagen/ gelatin, silk, keratin, elastin, zein, soy and whey proteins can be processed into nanofibers with unique physicochemical and functional properties. Selecting solvent for proteins to be electrospun is an important point since the protein can be degraded in different solvents eligible for electrospinning. In order to overcome this, increase their spinnability and improve the morphology and stability of the resultant nanofibers desirably, synthetic polymer carriers such as polylactic acid (PLA), poly-L-lactic acid (PLLA), polycaprolactone (PCL), and polyethylene oxide (PEO) can be mixed with proteins. Cross-linkers such as glutaraldehyde, genipin and proanthocyanidin can be used for the same purposes (Bhardwaj and Kundu, 2010).

Besides conventional electrospinning methods, protein nanofibers are formed by co-axial electrospinning, wet-spinning and emulsion electrospinning (Yıldız et al., 2020). Since they are biocompatible and biodegradable protein based nanofibers are preferred for biomedical, pharmaceutical and food applications.

## OPTICAL SPECTROSCOPY TECHNIQUES

Spectroscopy, refers to the measurement of the interaction between light and matter. The amount of light absorbed, emitted and scattered provide information about the chemical structure of the analyzed materials. Fourier Transform Infrared Spectroscopy (FT-IR) is a particular type of infrared (IR) spectroscopy that uses an interferometer to produce an IR spectrum. It is commonly used for the identification of the materials in nanoscale matrices, interaction mechanisms of analytes and molecular arrangements (Tarhan et al., 2014; Tarhan and Harsa 2016; Alessio et al, 2017; Gözler et al., 2021). IR spectroscopy measures the amount of IR light absorbed.

Raman spectroscopy is used to characterize the nanomaterials based on molecular vibration due to excitement and interpreting the interaction afterward (Tarhan et al., 2014; Tarhan and Harsa 2016). It uses inelastic scattering of light occurring when the matter is irradiated by light.

CD spectroscopy is an absorption spectroscopy method based on the differential absorption of left and right circularly polarized light. These techniques are complementary in analyzing nano-structured materials including nanofibers. Conformational determination of the secondary and tertiary structure of proteins are investigated by the given optical spectroscopy techniques.

## OPTICAL SPECTROSCOPY FOR THE STRUCTURAL ANALYSIS OF PROTEIN NANOFIBERS

Proteins are subjected to different treatments for producing nanomaterials. These treatments caused structural alterations in proteins and those can be determined using optical spectroscopy. During nanofiber formation, native protein chain conformation is manipulated and protein aggregation is encouraged (Dror et al., 2008). Besides the alteration of electrostatic charge, the reduction of self-disulfide bonding and promotion of spontaneous new cross-linkings can be achieved. Moreover, molecular orientation regarding interactions of other structural elements, eg. helix-helix, can be enhanced. All these changes might result in protein nanofibers with desired mechanical and barrier properties relevant to the specific application.

As stated before, the functionality of protein nanofibers is closely related to their molecular structure. Therefore, the assessment of chemical structure, conformational alterations in structural levels of proteins and confirmation of particular molecules existing/involved in nanofiber structure have great importance. These features are successfully analyzed through the mentioned optical spectroscopy techniques as demonstrated in many literature reports (Ki et al. 2005; Bürck et al., 2012; Jalaja et al., 2016; Kebede et al., 2018).

## CONCLUSION

Electrospun nanofibers, due to their large specific area and high porosity, have attracted much attention across various fields. Keratin, collagen, silk, elastin, zein, whey and soy proteins are some of the most common proteins used in nanofiber fabrication. Confirmation of chemical constituents, conformational changes and arising interactions in molecular structure during protein nanofiber formation are determined by optical spectroscopy. Identification of these properties are critical in effective designing the application purposes of relevant nanofibers.

## REFERENCES

Alessio P, Aoki PH, Furini LN, Aliaga AE, Constantino CJL 2017. Spectroscopic techniques for characterization of nanomaterials. In Nanocharacterization techniques (pp. 65-98). William Andrew Publishing.

Baji A, Mai YW, Wong SC, Abtahi M, Chen P 2010. Electrospinning of polymer nanofibers: Effects on oriented morphology, structures and tensile properties. Composites Science and Technology, 70(5): 703-718.

Bürck J, Heissler S, Geckle U, Ardakani MF, Schneider R, Ulrich AS, Kazanci M 2013. Resemblance of electrospun collagen nanofibers to their native structure. Langmuir, 29(5): 1562-1572.

Bhardwaj N, Kundu SC 2010. Electrospinning: a fascinating fiber fabrication technique. Biotechnology Advances, 28(3): 325-347.

Dror Y, Ziv T, Makarov V, Wolf H, Admon A, Zussman E 2008. Nanofibers made of globular proteins. Biomacromolecules, 9(10): 2749-2754.

Eskitoros-Togay ŞM, Bulbul YE, Tort S, Korkmaz FD, Acartürk F, Dilsiz N 2019. Fabrication of doxycycline-loaded electrospun PCL/PEO membranes for a potential drug delivery system. International Journal of Pharmaceutics, 565: 83-94.

Gözler M, Yardımcı, A İ, Tarhan Ö 2021. Structural and Thermal Properties of Electrospun Whey Protein/PEO Nanofibers. Bulletin of Biotechnology, 2(2): 32-37.

Jalaja K, Naskar D, Kundu SC, James NR 2016. Potential of electrospun core–shell structured gelatin–chitosan nanofibers for biomedical applications. Carbohydrate polymers, 136: 1098-1107.

Kamble, P., Sadarani, B., Majumdar, A., & Bhullar, S. (2017). Nanofiber based drug delivery systems for skin: A promising therapeutic approach. Journal of Drug Delivery Science and Technology, 41: 124-133.

Kebede TG, Dube S, Nindi MM 2018. Fabrication and characterization of electrospun nanofibers from Moringa stenopetala seed protein. Materials Research Express, 5(12): 125015.

Ki CS, Baek DH, Gang KD, Lee KH, Um IC, Park YH 2005. Characterization of gelatin nanofiber prepared from gelatin–formic acid solution. Polymer, 46(14): 5094-5102.

Sun PD, Foster CE, Boyington JC 2004. Overview of protein structural and functional folds. Current Protocols in Protein Science, 35(1): 17-1.

Tarhan Ö, Tarhan E, Harsa Ş 2014. Investigation of the structure of alpha-lactalbumin protein nanotubes using optical spectroscopy. Journal of Dairy Research, 81(1): 98-106.

Tarhan Ö and Harsa Ş 2014. Nanotubular structures developed from whey-based α-lactalbumin fractions for food applications. Biotechnology progress, 30(6): 1301-1310.

Yıldız A, Kara AA, Acartürk F (2020). Peptide-protein based nanofibers in pharmaceutical and biomedical applications. International Journal of Biological Macromolecules, 148: 1084-1097.

*ORAL PRESENTATION*

# Biyoetanolün üretimi ve saflaştırılmasında yenilikçi ve ekonomik proses: pervaporasyon membran biyoreaktör

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## Özet

Bu çalışmada Saccharomyces cerevisiae kullanılarak, pervaporasyon membran biyoreaktörde melastan biyoetanol sentezi incelenmiştir. Membran malzemesi olarak poliviniliden florür (PVDF) polimeri seçilmiştir. Membran yapısı, bir taramalı elektron mikroskobu (SEM), Fourier-dönüşümlü kızılötesi spektroskopisi (FTIR), Termogravimetrik Analiz (TGA) kullanılarak araştırılmıştır. Proses verimliliği, etanol konsantrasyonunun, toplam akının ve etanolün ayırma faktörünün bir fonksiyonu olarak belirlenmiştir. Pervaporasyon membran biyoreaktör sonuçları, sistem verimliliğini değerlendirmek için kesikli fermantasyon sonuçları ile karşılaştırılmıştır. Pervaporasyon membran biyoreaktör, kesikli fermantasyon reaktörüne kıyasla üstün performansa sahiptir.

**Anahtar Kelimeler:** Biyoetanol, melas, membran, pervaporasyon membran biyoreaktör

# Innovative and economical process for the production and purification of bioethanol: pervaporation membrane bioreactor

## Abstract

In this study bioethanol synthesis from molasses by using Saccharomyces cerevisiae has been investigated in pervaporation membrane bioreactor. Polyvinylidene fluoride (PVDF) polymer has been chosen in membrane material. The membrane structure has been investigated using a scanning electron microscope (SEM), Fourier-transform infrared spectroscopy (FTIR), Thermogravimetric analysis (TGA). Process efficiency has been determined as a function of ethanol concentration, total flux, and ethanol separation factor. Pervaporation membrane bioreactor results have been compared with batch fermentation results in order to evaluate system efficiency. Pervaporation membrane bioreactor has superiority performance compared to batch fermentation reactor.

**Keywords:** Bioethanol, membrane, molasses, pervaporation membrane bioreactor

## GİRİŞ

Dünyanın giderek artan enerji talebinin karşılanmasında kullanılan temel kaynakların fosil kaynaklar olması önemli problemleri de beraberinde getirmektedir. Fosil kaynakların tükeniyor olması, talep artışından dolayı petrol fiyatlarının son zamanların en yüksek değerine ulaşması ve karbondioksit emisyonunun iklim üzerindeki etkileri sebebiyle alternatif enerjiye olan ilgi artmıştır. Bu kapsamda biyoyakıt üzerine yapılan çalışmalar hız kazanmıştır (Olsson ve Hahn, 1996). Canlı organizmalar ya da onların metabolik yan ürünlerden üretilen enerji olarak tanımlanan biyoyakıt, doğadaki karbon dengesini bozmaması, yenilenebilir ve biyobozunur olması sebebiyle tercih edilen bir temiz enerji türüdür (Nguyen ve Saddler, 1991). Biyoyakıtlar arasında biyoetanol şeker pancarı, mısır, buğday, seker kamışı, tatlı sorgum, patates, odunsular gibi bitkilerden elde edildiği gibi tarımsal atıklar ve selüloz içerikli evsel atıklardan da elde edilebilen, benzinle hatta son yıllarda motorinle

karıştırılarak kullanılabilen bir biyoyakıt türüdür. Ülkemizde biyoetanol üretimi yapan firmalarda bulunmaktadır.

Ülkemizde şeker fabrikasında şeker kamışı ve şeker pancarının atığı olarak ortaya çıkan melastan biyoetanol üretimi gerçekleşmektedir. Biyoetanol üretimi sırasında saflaştırma aşamasında distilasyon prosesi kullanılmakta olup, bu proses oldukça fazla enerji sarf etmekte ve istenilen saflıkta biyoetanol eldesi söz konusu olmamaktadır. Biyoetanolü tek bir distilasyon prosesi ile %99 ve üzeri saflıkta elde etmek oldukça güçtür. Bu hususlardan dolayı bu projede çevre dostu, ilave kimyasal gerektirmeyen, düşük maliyetli ve düşük enerji tüketimi sağlayan membran destekli ayırma prosesi olan pervaporasyon prosesi önerilmiştir.

Pervaporasyon, geleneksel yöntemlerle ayrılması mümkün olmayan sıvı-sıvı karışımları ayıran membran destekli bir ayırma prosesidir. Bu proseste ayırma işlemi distilasyondaki gibi uçuculuk farkına bağlı değildir, bu nedenle azeotrop karışımların, yakın kaynama noktalı bileşenleri içeren çözeltilerin ya da ısıl duyarlılığa sahip karışımların pervaporasyon prosesi ile ayrılması mümkündür (Feng ve Huang, 1997; Hoof ve ark., 2004).

Literatürde etanolün saflaştırılması için pervaporasyonun kullanıldığı uygulamalar mevcuttur. Ancak yapılan çalışmalar ağırlıkla etanol-su karışımlarının dehidrasyonuna yöneliktir. Bu çalışmada fermantasyon ve ayırmanın eş zamanlı gerçekleşeceği pervaporasyon membran biyoreaktör uygulaması gerçekleştirilecektir. Pervaporasyon membran biyoreaktörler, reaksiyon ve ayırmanın eş zamanlı gerçekleştiği sistemlerdir. Pervaporasyon membran biyoreaktör prosesinde hazırlanan melas çözeltisi doğrudan membran biyoreaktöre beslenerek membran hücresinin üst tarafında fermantasyon işlemi gerçekleşirken, oluşan etanol fermantasyon karışımından membran ile sürekli olarak ayrılmaktadır. Böylece fermantasyon sonrası distilasyon prosesi ile gerçekleştirilen saflaştırma işlemi pervaporasyon membran biyoreaktör prosesi ile tek bir adımda yüksek ayırma verimi ile gerçekleştirilmektedir. Oluşan etanolün membrandan ayrılarak saf olarak elde edilmesi için etanole seçici hidrofobik karakterde PVDF polimeri tercih edilmiştir. Kesikli reaktörde elde edilen etanol konsantrasyonu ile pervaporasyon membran biyoreaktör uygulamasından elde edilen etanol konsantrasyonu karşılaştırılmıştır.

## MATERYAL VE METOT

### PVDF Membranın Hazırlanması

Ağırlıkça %5 PVDF, N,N-Dimetilformamid içerisinde çözülerek polimerik membran çözeltisi hazırlanmıştır. Oda sıcaklığında homojen karışım elde edilinceye kadar karıştırılmıştır. Homojen karışım elde edildikten sonra kuruması için cam yüzeye dökülerek 120$^{o}$C'de 3 saat etüvde kurutularak membranlar hazırlanmıştır (Nigiz ve Hilmioglu, 2017).

### Membran Karakterizasyonu

Hazırlanan membranların karakterizasyonu SEM, FTIR ve TGA kullanılarak yapılmıştır. Hazırlanan membranların morfolojik yapısı SEM analizi ile belirlenmiştir. Membranın kimyasal bağ yapıları FTIR analizi ile belirlenmiştir. Membranın ısıl dayanımı ise TGA kullanılarak incelenmiştir.

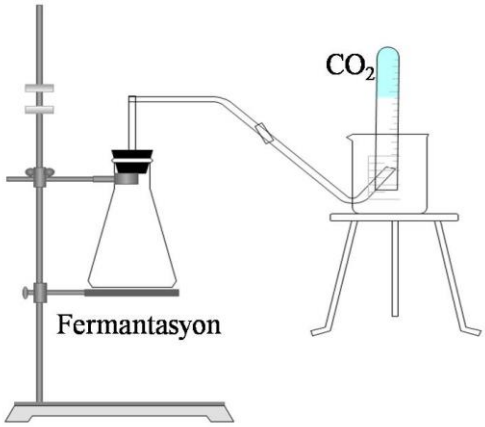
### Biyoetanol Sentezi

### Melas çözeltisinin hazırlanması

Şeker kamışı melası, şeker içeriği 180-200 g/L olacak şekilde suyla seyreltilmiştir. %10 v/v sülfürik asit ile ortamın pH değeri 4 değerine ayarlanmıştır. Yaklaşık 30 dakika kaynatılarak, süzülmüş ve santrifüj edilmiştir (Goksungur ve Zorlu, 2001).

### Kesikli fermantasyon

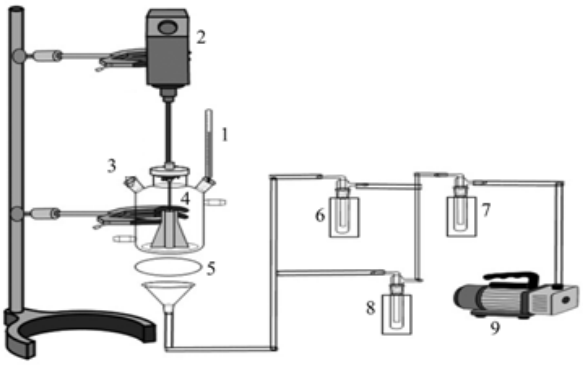
Fermentasyon deneyleri oda sıcaklığında 7 saat süreyle gerçekleştirilmiştir. Toplam şeker ağırlığına göre ağırlıkça %10 Saccharomyces cerevisiae mayası melas çözeltisine eklenmiştir. Fermantasyon oksijensiz ortamı ve $CO_2$ çıkışı Şekil 1'de görülen deney düzeneği ile sağlanmıştır. Etanol konsantrasyonu, refraktometre ile belirlenmiş ve gaz kromatografisi ile de doğrulanmıştır.




**Şekil 1.** Kesikli fermantasyon deney düzeneği

**Pervaporasyon Membran Biyoreaktörde Biyoetanol Üretimi**

Fermantasyon ve ayırmanın eş zamanlı gerçekleşmesi için membran biyoreaktör prosesi kullanılmıştır. Tek adımlı fermantasyon-ayırma sistemi pervaporasyon membran biyoreaktör prosesi Şekil 2'de gösterilmiştir.




**Şekil 2.** Pervaporasyon Membran Biyoreaktör Prosesi: (1) termometre, (2) mekanik karıştırıcı, (3) gaz çıkışı, (4) membran biyoreaktör, (5) membran, (6−8) soğutucu kapan, (9) vakum pompası (Unlu ve Hilmioglu, 2016).

Pervaporasyon membran biyoreaktör prosesinde hazırlanan melas çözeltisi doğrudan membran biyoreaktöre beslenmiştir. Toplam şeker ağırlığına göre ağırlıkça %10 Saccharomyces cerevisiae mayası melas çözeltisine eklenmiştir. Membran hücresinin üst tarafında fermantasyon işlemi gerçekleşirken, oluşan etanol fermantasyon karışımından membran ile sürekli olarak ayrılmıştır. Böylelikle reaksiyon ve ayırma eş zamanlı olarak gerçekleştirilmiştir. Membran biyoreaktör tarafından (üst akım, besleme tarafı) numune alınarak üretilen etanol miktarı, membranın alt akımından numune alınarak da etanolün saflık derecesi belirlenmiştir. Membran biyoreaktörün ayırma performansı da Eşitlik 1 ve Eşitlik 2'de verilen akı ve ayırma faktörü ile belirlenmiştir.

$$J = \frac{m}{A.t} \qquad (1)$$

Eşitlik (1)'deki J; akı (kg/$m^2$.h) m; kapanlarda biriken numune miktarı (kg), A; membran alanı ($m^2$), t; zaman (saat) değeridir.

$$\alpha = \frac{C_{a_p}/C_{b_p}}{C_{a_f}/C_{b_f}} \qquad (2)$$

$C_a$ ve $C_b$ karışımdaki istenen ve istenmeyen bileşenlerinin konsantrasyonlarıdır. p ve f sırasıyla geçen akım ve beslemeyi tanımlamaktadır. Hidrofobik membran etanole seçici olduğu için etanolün ayırma faktörü hesaplanmıştır.

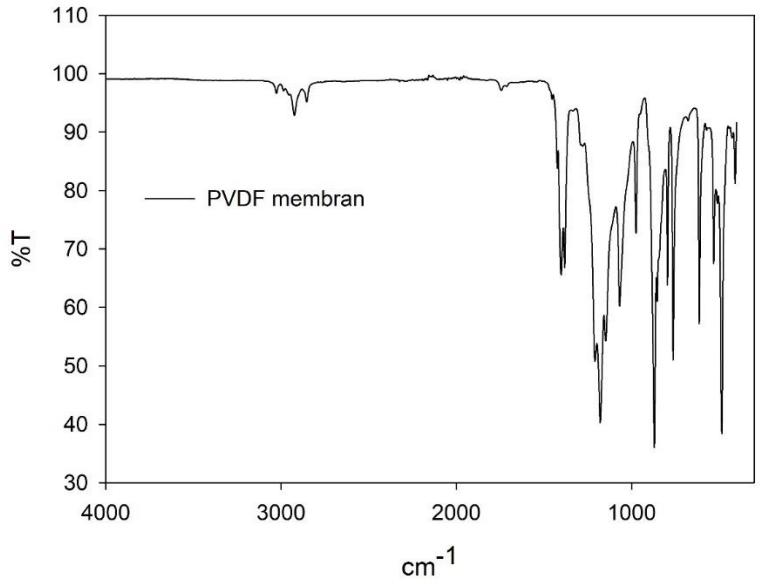
Kesikli fermantasyon sonrası gerçekleştirilen membran destekli etanol saflaştırma prosesi ile membran biyoreaktör performansı karşılaştırılmıştır.

## BULGULAR ve TARTIŞMA

### Membran Karakterizasyon Sonuçları

### FT-IR analizi

Sentezlenen PVDF membranın kimyasal bağ yapıları FTIR ile analiz edilmiştir. Şekil 3'te PVDF membranın FTIR analiz sonucu görülmektedir.

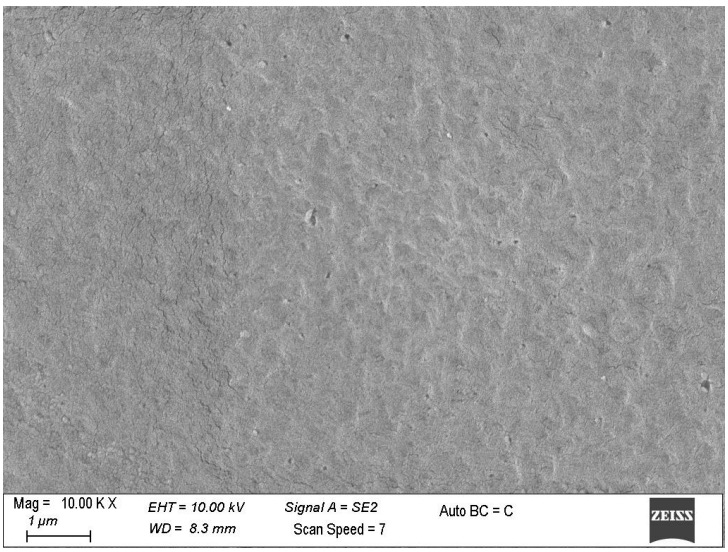



**Şekil 3.** PVDF membranın FTIR analizi

1168 $cm^{-1}$ ve 1405 $cm^{-1}$'deki karakteristik tepe noktaları, sırasıyla PVDF'nin $CF_2$ ve $CH_2$ gerilme modlarına karşılık gelir. 877 $cm^{-1}$'deki tepe noktası, C-C bağının iskelet titreşimidir (Zhang ve ark., 2013; Yu ve ark., 2015).

### SEM Analizi

Şekil 4'te hidrofobik PVDF membranın SEM yüzey görüntüsü görülmektedir.

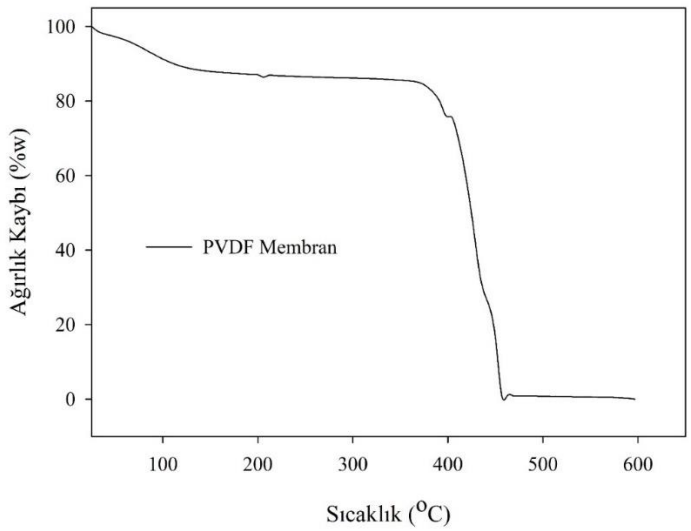



**Şekil 4.** Hidrofobik PVDF membranın SEM yüzey görüntüsü

Sentezlenen PVDF membranın yüzey görüntüsüne bakıldığında membranın gözeneksiz bir membran olduğu, yoğun, homojen bir yapı sergilediği açıkça görülmüştür. Pervaporasyonda gözeneksiz membran kullanıldığı için hedeflenen yapıya ulaşıldığı açıkça görülmektedir.

## TGA Analizi

Hazırlanan membranların termal dayanımları TGA ile belirlenmiştir. Şekil 5'te PVDF membranın TGA analiz sonucu eğrisi verilmiştir.

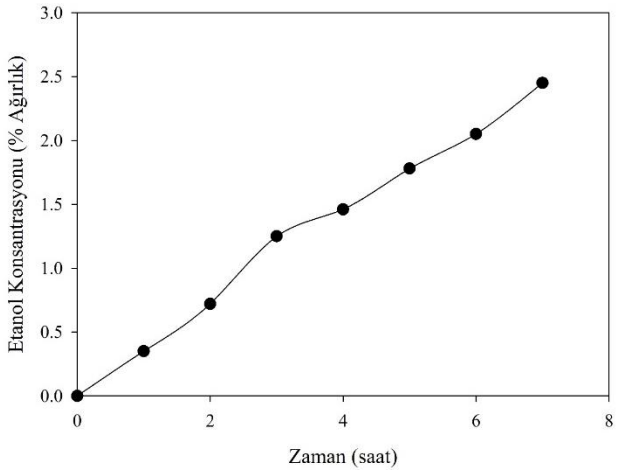



**Şekil 5.** PVDF membranın TGA eğrisi

TGA eğrisine bakıldığında termal bozunmanın 400°C civarında başladığı görülmüştür. Bu da PVDF membranın yüksek termal dayanıma sahip olduğunu göstermektedir (Soloukipour ve ark., 2016).

## Etanol Üretimi

Etanol üretimi, birim zaman başına üretilen etanol miktarı olarak tanımlanmıştır. Fermantasyon ünitesinde oda sıcaklığında 7 saat boyunca gerçekleştirilen fermantasyon deneyinde saat başı numune alınarak etanol konsantrasyonu belirlenmiştir. Şekil 6'da etanol konsantrasyonunun zamanla değişimi verilmiştir.

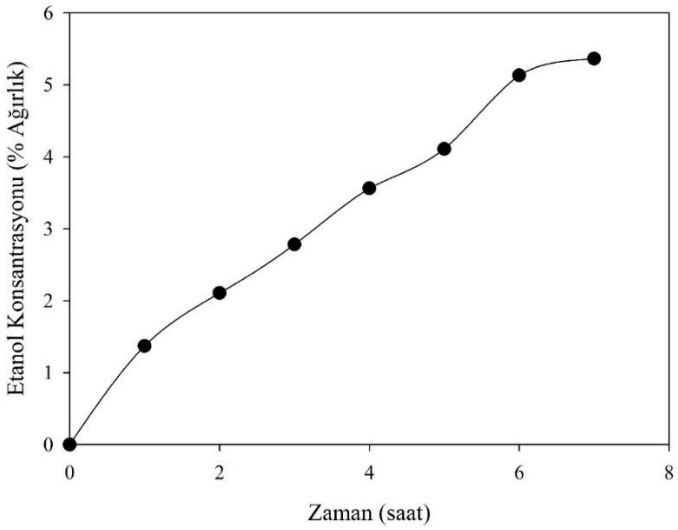



**Şekil 6.** Fermantasyon ünitesinde etanol konsantrasyonu

Zamana bağlı olarak etanol konsantrasyonunun arttığı gözlenmiştir. Ancak beklendiği gibi fermantasyon sonucunda biyoetanol seyreltik olarak olarak elde edilmiştir. 7 saatin sonunda ağırlıkça %2.45 etanol konsantrasyonu elde edilmiştir.

**Pervaporasyon Membran Biyoreaktörde Biyoetanol Üretimi**

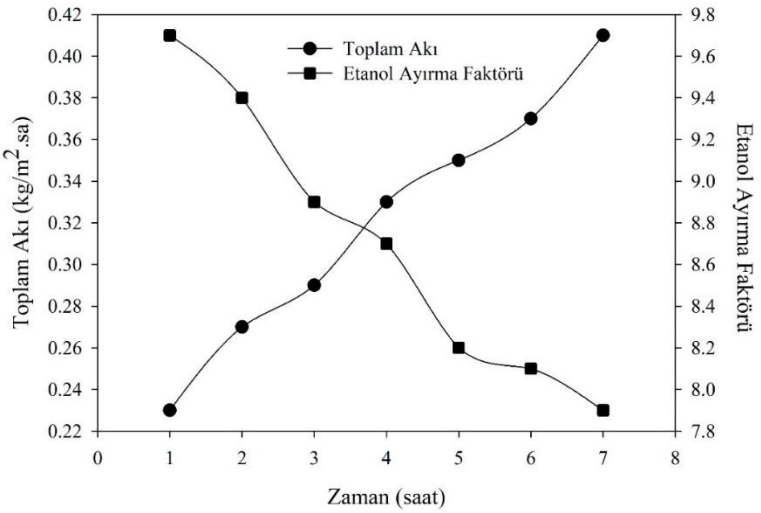
Pervaporasyon membran biyoreaktör, fermantasyon ve ayırmanın eş zamanlı gerçekleştirildiği proseslerdir. Pervaporasyon membran biyoreaktör prosesinde hazırlanan melas çözeltisi doğrudan membran biyoreaktöre beslenmiştir. Pervaporasyon membran biyoreaktör ve kesikli reaktörde aynı koşullar altında fermantasyon gerçekleştirilmiştir. Pervaporasyon membran biyoreaktörde membran biyoreaktörün üst akımında fermantasyon işlemi gerçekleşirken, oluşan etanol fermantasyon karışımından membran ile sürekli olarak ayrılmıştır. Şekil 7'de etanol konsantrasyonunun zamanla değişimi verilmiştir.




**Şekil 7.** Pervaporasyon membran biyoreaktör prosesinde etanol üretimi

Pervaporasyon membran biyoreaktör prosesinde zamana bağlı olarak etanol konsantrasyonunun arttığı gözlenmiştir. 7 saatin sonunda ağırlıkça %5.36 etanol konsantrasyonu elde edilmiştir. Kesikli fermantasyonda ise bu değer %2.45 olarak bulunmuştur. Ortamdaki etanol konsantrasyonu, kesikli reaktörünkinden daha yüksek elde edilmiştir. Bu konsantrasyon artışının nedeni fermantasyon ve ayırmanın eş zamanlı olarak gerçekleşmesi, fermantasyon sonucu oluşan etanolün sürekli hidrofobik PVDF membrandan çekilerek uzaklaştırılmasıdır. Ayrıca fermantasyon sonucu oluşan etanolün sürekli uzaklaştırılması etanolün maya üzerindeki inhibisyon etkisini de ortadan kaldırmaktadır. Bu da fermantasyon verimi için önemli bir avantajdır. Mayanın hem aktivitesi hem de dayanımı daha uzun süre korunabilir. Ayrıca ürünün uzaklaştırılması substrat-maya temas alanını arttırarak daha fazla etanol üretimi ile sonuçlanmaktadır.

Şekil 8'de pervaporasyon membran biyoreaktörün ayırma performansı verilmektedir.




**Şekil 8.** Pervaporasyon membran biyoreaktörün ayırma performansı

Pervaporasyon membran biyoreaktörde ortamdaki etanol konsantrasyonu zamanla arttıkça akı değeri de artış göstermiştir. Hidrofobik PVDF membran tercihen etanol moleküllerini sorplamış ve su moleküllerini itmiştir. Ancak zamanla PVDF membranının şişmesi, geçen akımda su buharının mol fraksiyonunda da artışla sonuçlanmış ve etanol ayırma faktörü azalmıştır. 7 saatin sonunda 0.41 kg/m$^2$.sa akı ve 7.9 etanol ayırma faktörü değeri elde edilmiştir.

## SONUÇ

Bu çalışmada biyoetanol endüstrisinde ayırma prosesi olarak sıklıkla kullanılan distilasyon prosesine alternatif, enerji tüketimi ve maliyeti düşük, çevreci ve yüksek ayırma verimine sahip bir proses önerilmiştir. Bu amaçla pervaporasyon membran biyoreaktör, distilasyona alternatif olarak sunulmuştur. Pervaporasyon membran biyoreaktörde etanol, kesikli reaktöre göre hem yüksek konsantrasyonda hem de saf olarak elde edilmiştir. Pervaporasyon membran biyoreaktörde 7 saatin sonunda ağırlıkça %5.36 etanol konsantrasyonu elde edilmiştir. Ayırma performansı olarakta 7 saatin sonunda 0.41 kg/m$^2$.sa akı ve 7.9 etanol ayırma faktörü değeri elde edilmiştir. Elde edilen sonuçlar, pervaporasyon membran biyoreaktörün kesikli prosese önemli bir alternatif olduğunu göstermiştir.

## TEŞEKKÜR

Bu çalışma, TÜBİTAK tarafından 2209-A projesi kapsamında maddi olarak desteklenmiştir.

## KAYNAKLAR

Feng X, Huang RYM 1997. Liquid Separation by Membrane Pervaporation: A Review. Industrial & Engineering Chemistry Research, 36(4):1048–1066.

Goksungur Y, Zorlu N 2001. Production of ethanol from beet molasses by Ca-Alginate immobilized yeast cells in a packed-bed bioreactor. Turkish Journal of Biology, 25: 265–275.

Nguyen QA, Saddler JN 1991. An integrated model for the technical and economic evaluation of an enzymatic biomass conversion process. Bioresource Technology, 35(3): 275–282.

Nigiz FU, Hilmioglu ND 2017. Pervaporative desalination of seawater using a polyvinylidene fluoride based membrane. Water Science and Technology: Water Supply, 18:1674-1681.

Olsson, L, Hahn HB 1996. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme and Microbial Technology, 18:312-331.

Soloukipour S, Saljoughi E, Mousavi SM, Pourafshari CM 2016. PEBA/PVDF blend pervaporation membranes: preparation and performance. Polymers for Advanced Technologies, 28(1): 113–123.

Unlu, D., & Durmaz Hilmioglu, N. (2016). Synthesis of Ethyl Levulinate as a Fuel Bioadditive by a Novel Catalytically Active Pervaporation Membrane. Energy & Fuels, 30(4), 2997–3003.

Van HV, Van den AL, Buekenhoudt A, Dotremont C, Leysen R 2004. Economic comparison between azeotropic distillation and different hybrid systems combining distillation with pervaporation for the dehydration of isopropanol. Separation and Purification Technology, 37(1):33–49.

Yu Z, Pan Y, He Y, Zeng G, Shi H, Di H 2015. Preparation of a novel anti-fouling β-cyclodextrin–PVDF membrane. RSC Advances, 5(63): 51364–51370.

Zhang F, Zhang W, Yu Y, Deng B, Li J, Jin J 2013. Sol–gel preparation of PAA-g-PVDF/TiO2 nanocomposite hollow fiber membranes with extremely high water flux and improved antifouling property. Journal of Membrane Science, 432: 25–32.

## *ORAL PRESENTATION*

# Cleavage activity analysis of thrombin enzyme on fusion TolAIII-UnaG protein

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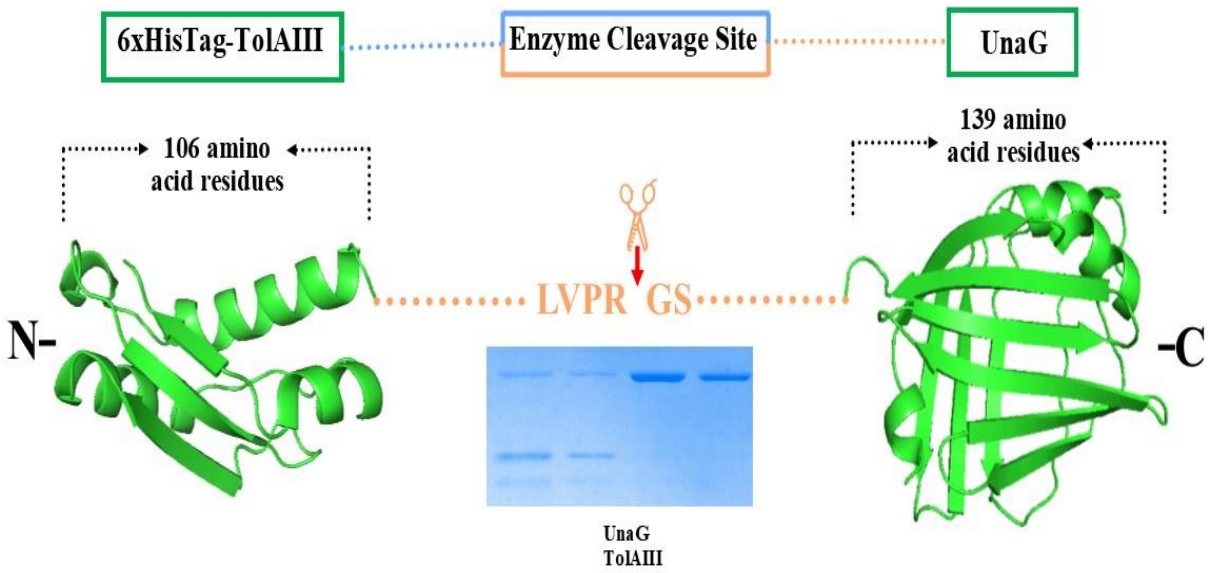
**Abstract**

Fusion proteins are obtained by expression of the recombinant construct formed by fusing two or more genes; they can be used to enhance the biophysical properties of proteins, provide a label to aid in the purification step, target and deliver a protein or biomolecule as a signal molecule or carrier. In this study, the TolAIII protein was used as the fusion partner of the UnaG protein which becomes fluorescent when combined with unconjugated bilirubin (UC-BR). A plasmid was designed by Thrombin enzyme cleavage site (LVPRGS) between the two proteins and expression/purification studies of the proteins were made. Thrombin enzyme cleavage activity was observed on this purified fusion protein. The thrombin enzyme failed in separating the fusion protein as TolAIII and UnaG from the cleavage site because it could not reach the protein cut region.

**Keywords;** Cleavage enzyme, Thrombin protease, UnaG




## Introduction

Recombinant fusion protein technology is one of the commonly used methods in applications with its many functions such as stabilizing the protein by enhancing its biophysical properties, providing a tag to assist in the purification step, targeting and delivering a protein or biomolecule as a signal molecule or carrier (Jenny et al. 2003). Also, while this technology can be used in imaging and tracking by providing fluorescence to the fusion protein, it has been reported that recombinant protein production positively affects its yield and, in some cases, also supports proper folding and increases its solubility (Waugh 2011, Chen et al. 2013). Fusion proteins, obtained by the expression of the recombinant construct formed by fusing two or more genes by genetically, require suitable linkers such as cleavage sites, helical linkers and specific peptide linkers to bind together (Chen et al. 2010). The cleavage site as one single polypeptide chain is used generally to separate fusion proteins from each other, while helical linkers and specific peptide linkers are used to get proper protein folding for flexibility, to improve protein stability, to achieve higher expression levels of soluble proteins or to reach the highest activity in cell proliferation (Arai et al. 2001, Wriggers et al. 2005, Bai and Shen 2006, Amet et al. 2009). Thrombin enzyme is a serine protease and also a highly sequence-specific cleavage site enzyme frequently used for controlled separation of fusion proteins.

In this study, the TolAIII protein (the third domain of the periplasmic protein TolA, expressed in high yields as a soluble protein in the cytoplasm of *Escherichia coli* (Anderluh et al. 2003)) was used as the fusion partner of the UnaG protein which becomes fluorescent when combined with unconjugated bilirubin (UC-BR) (Kumagai et al. 2013). A pToLT-UnaG plasmid was designed to contain a 6xHis-Tag at their N-terminus for easier purification by adding a Thrombin enzyme cleavage site (LVPRGS) between the two proteins. TolAIII-UnaG fusion protein was expressed by the transformation of plasmids into *E. coli* C41 competent cells and then fused protein was purified with using immobilized metal affinity chromatography (IMAC). Separation studies of TolAIII and UnaG proteins were performed with using commercial Thrombin cleavage enzyme under optimal conditions.

## Material and Method

### Cell line

The bacterium *Escherichia coli* and its reconstructed improved versions are the most widely used host to efficiently produce proteins. For the production of TolAIII-UnaG fusion protein, *E. coli C41(DE3)* cell strain was chosen as the host cell according to our previous study on strain optimization (Eczacioglu et al. 2022). The *E. coli C41(DE3)* cell strain is frequently used to production of toxic membrane proteins from organisms such as bacteria, mammals, yeast, viruses and plants. It contains phenotypically selected genetic mutations that suppress basal expression of T7 RNA polymerase before induction (under the control with the addition of inducer isopropyl β-d-1- thiogalactopyranoside) to overcome the toxicity associated with overexpressed recombinant proteins. This strain also provides plasmid stability for toxic proteins (Dumon-Seignovert et al. 2004).

### Construction of fusion protein plasmid containing the cleavage site

In order to perform the expression of UnaG protein with TolAIII in *E. coli C41(DE3)* cell, the recombinant plasmid was constructed including the Thrombin enzyme cleavage site. The gene encoding 6xHis-tagged TolAIII-UnaG with cleavage site was purchased from Thermo Fisher Scientific (GeneArt™ Strings™ DNA Fragments) and cloned into pTolT plasmid with using In-Fusion® HD Cloning Kit (Clontech® Laboratories).

### Expression of fusion proteins

Protein expression and purification studies were performed as in our previous experiments (Eczacioglu et al. 2020, 2022). In brief, first the transformation with the heat shock method of the plasmid to a competent *E. coli* host strain stably was performed. Transformed cells were grown on the solid LB medium presence of the +Amp (Ampicillin, w:w 1:1000). One of the positive clone cell spot was transferred into a LB liquid medium containing +Amp and scaled up at 37 °C. During the exponential phase of the cells (corresponds to 0.5-0.6 $OD_{600}$), isopropyl β-d-1-thiogalactopyranoside (IPTG, 0.1 mM final concentration) induction was done to begin the expression of the protein. Three hours of expression of the target protein were carried out at 37 °C, and then cells were harvested by centrifugation at 5000 rpm for 30 min.

After mechanical (ultra-sonication on ice for 1 hour, Sonics Vibracells, %40 amplitude, 20 kHz) and enzymatic (lysozyme, Thermo Fisher) degradation of the harvested *E. coli* cell pellets, cell debris was removed from the soluble fraction by ultracentrifugation (Hitachi Himac CP100WX ultracentrifuge, P45AT rotor) at 40000 rpm, 4 °C for 1 hour. The protein purification process was performed by passing the supernatant containing the protein mixture through the immobilized metal affinity chromatography column (Ni-NTA agarose resin, QIAGEN). The column previously equilibrated with washing buffer (Tris-HCl, pH:7.5). Then the soluble fraction was passed through the column. The column was operated a series of washes with wash buffer supplemented with different concentration (1mM to 30 mM) of imidazole. Bound proteins were eluted with elution buffer containing 300mM imidazole. 12% The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was carried out to identify the proteins and determine the degree of purity of the chromatography fragments. Purified protein was dialyzed in Tris-HCl, pH:7.5 buffer with using an 8-10 MWCO membrane from Spectra/Por™ and so ready to use for cleavage experiments.

### Thrombin enzyme cleavage

The thrombin enzyme was attempted to separate the TolAIII-UnaG fusion protein from TolAIII by cutting from the cleavage site as LVPRGS (*Fig.1*).

Merck (Novagen) 69671 | Thrombin Restriction Grade kit was used to cleavage according to the manufacturer's recommended procedure and the extent of cleavage of the proteins was analysed by SDS-PAGE. According

to the results obtained, optimization studies were tried to make in the use of the kit. The same concentration of Thrombin enzyme was used at two different temperatures, 20 ºC and room temperature (RT). Samples were taken as 10 ml aliquots into 10 ml 2X SDS sample buffer after 2, 4, 8 and 16 h for in the first trial and analysed by SDS-PAGE. In the second trial, different concentrations of Thrombin enzyme in the range of 2 to 10 units were used at two different temperatures (20 ºC and RT) and at the 2, 4, 8 and 16 h samples were taken for SDS-PAGE (*Fig.2* inner gel image).

Then, the cleavage study was designed in two groups as pre-cut and post-cut boiled TolAIII-UnaG fusion protein for the Thrombin enzyme cleavage. TolAIII-UnaG fusion protein was boiled at 95 ºC for 5 minutes and cooled to room temperature. Thrombin enzyme concentration in the range of 2 to 10 units was used for cleavage of boiled and non-boiled protein and 2, 4, 8 and 16 h samples were collected for SDS-PAGE (*Fig.2*).

## Result and Discussion

### TolAIII - UnaG fusion protein production

TolAIII is a periplasmic protein that plays a role in maintaining the integrity of the inner membrane in *E. coli* encoded by the pTolT expression plasmid. Some proteins may have a toxic effect on the host cell. This plasmid is used in the production of toxic proteins that prevent the formation of inclusion bodies by directing heterologous proteins to the periplasmic region (Ulusu 2019).

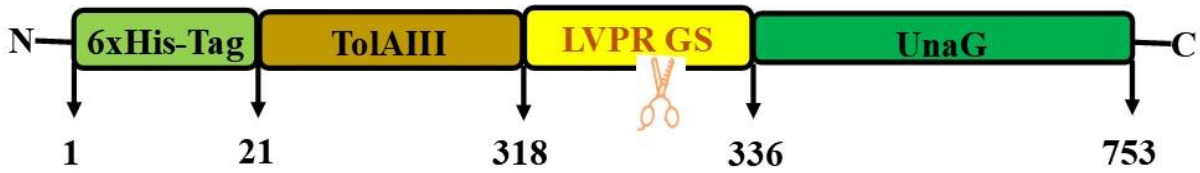



***Fig. 3*** Linear plasmid map based on the nucleotide sequence of the pTolT construct to produce the TolAIII-UnaG fusion protein

Since UnaG protein originates from a highly structured organism, expression studies were designed as a fusion with TolAIII protein in the E. coli pTolT expression system in case of the possibility of inclusion body formation or toxicity. Recombinant TolAIII-UnaG fusion protein was expressed with TolAIII as a fusion protein to examine separation by attempting the Thrombin enzyme cleavage site. The SDS-PAGE showed that the TolAIII-UnaG fusion protein was expressed in soluble form and reached electrophoretic purity after IMAC separation.

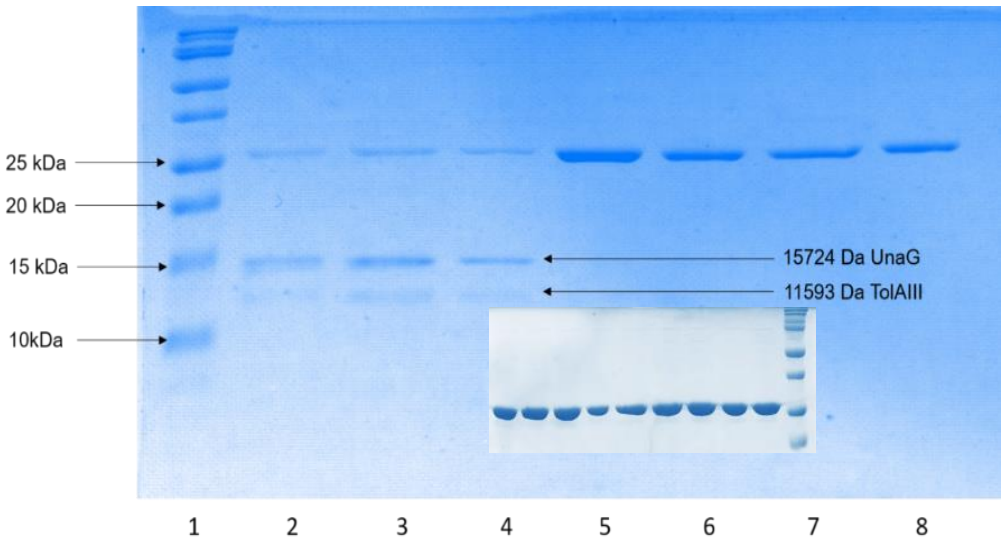



**Fig. 4** Thrombin enzymes cleavage studies; 1. BioRad Presicion Plus Protein Marker, 2. Pre-boiled 10 µL 0.5 mg/mL TolAIII-UnaG fusion protein + 2 units of thrombin, 3. Pre-boiled 10 µL 0.5 mg/mL TolAIII-UnaG fusion protein + 5 units of thrombin, 4. Pre-boiled 10 µL 0.5 mg/mL TolAIII-UnaG fusion protein + 10 units of thrombin, 5. Post-boiled 10 µL 0.5 mg/mL TolAIII-UnaG fusion protein + 2 units of thrombin, 6. Post-boiled 10 µL 0.5 mg/mL TolAIII-UnaG fusion protein + 5 units thrombin, 7. Post-boiled 10 µL 0.5 mg/mL TolAIII-UnaG fusion protein + 10 units thrombin, 8. Control (TolAIII-UnaG fusion protein), inner SDS-PAGE

image showed the trial of different temperatures, duration and concentrations of Thrombin enzymes application on TolAIII-UnaG protein.

Thrombin enzyme is a site-specific protease (LVPRGS) that exhibits very low non-specific cleavage under many conditions. One thrombin enzyme unit is defined as the amount of enzyme required to cleave 1 mg of the protein in optimal conditions (temperatures, incubation times, thrombin enzyme concentrations etc.) in the presence of standard buffers. In the cutting studies with the thrombin enzyme, we tested several temperatures (as recommended 20 ºC, and RT), incubation times (2, 4, 8 and 16 h) and/or thrombin enzyme concentrations (2 to 10 units) avoiding the presence of serine protease inhibitors to optimize specificity and efficiency of cleavage. Thrombin enzyme cleavage specificity and efficiency can be easily monitored by testing for the removal of TolAIII part from fusion proteins upstream of the cleavage site. It was seen that from the SDS-PAGE gel image, the fusion protein was unable to be cleaved into two parts as TolAIII and UnaG (*Fig.2*).

## Conclusion

These results estimated that the thrombin enzyme does not reach its cleavage site because the protein was fully folded. So, we denatured the fusion protein by boiling and its configuration get linearized. In this way, the thrombin enzyme was able to reach the its cleavage site and divided the protein into two parts as TolAIII (11593 Da) and UnaG (15724 Da) (*Fig.2*). In conclusion, it has been demonstrated TolAIII-UnaG fusion protein can be cleaved after linearization by boiling (pre-cut boiled) of it.

## Acknowledgements

This study was supported by The Scientific and Technological Research Council of Turkey (Grant Number 215Z700) and British Council of UK (Grant Number RES/0120/7785).

## References

Amet, N., Lee, H.F. & Shen, W.C. 2009. Insertion of the designed helical linker led to increased expression of Tf-based fusion proteins. Pharm. Res. 26:523–8.

Anderluh, G., Gökçe, I. & Lakey, J.H. 2003. Expression of proteins using the third domain of the Escherichia coli periplasmic-protein TolA as a fusion partner. Protein Expr. Purif. 28:173–81.

Arai, R., Ueda, H., Kitayama, A., Kamiya, N. & Nagamune, T. 2001. Design of the linkers which effectively separate domains of a bifunctional fusion protein. Protein Eng. 14:529–32.

Bai, Y. & Shen, W.C. 2006. Improving the oral efficacy of recombinant granulocyte colony-stimulating factor and transferrin fusion protein by spacer optimization. Pharm. Res. 23:2116–21.

Chen, X., Bai, Y., Zaro, J.L. & Shen, W.C. 2010. Design of an in vivo cleavable disulfide linker in recombinant fusion proteins. Biotechniques. 49:513–8.

Chen, X., Zaro, J.L. & Shen, W.C. 2013. Fusion protein linkers: Property, design and functionality. Adv. Drug Deliv. Rev. 65:1357–69.

Dumon-Seignovert, L., Cariot, G. & Vuillard, L. 2004. The toxicity of recombinant proteins in Escherichia coli: A comparison of overexpression in BL21(DE3), C41(DE3), and C43(DE3). Protein Expr. Purif. 37:203–6.

Eczacioglu, N., Ulusu, Y., Gokce, İ. & Lakey, J.H. 2022. Investigation of mutations (L41F, F17M, N57E, Y99F_Y134W) effects on the TolAIII-UnaG fluorescence protein's unconjugated bilirubin (UC-BR) binding ability and thermal stability properties. Prep. Biochem. Biotechnol. 52:365–74.

Eczacioglu, N., Yilmaz, B., Ulusu, Y. & Bayrakci, M. 2020. Recovery and Reusability of ApoUnaG Fluorescence Protein from the Unconjugated Bilirubin Complex Structure. J. Fluoresc. 30:497–503.

Jenny, R.J., Mann, K.G. & Lundblad, R.L. 2003. A critical review of the methods for cleavage of fusion proteins with thrombin and factor Xa. Protein Expr. Purif. 31:1–11.

Kumagai, A., Ando, R., Miyatake, H., Greimel, P., Kobayashi, T., Hirabayashi, Y., Shimogori, T. et al. 2013. A bilirubin-inducible fluorescent protein from eel muscle. Cell. 153.

Ulusu, Y. 2019. Escherichia coli Ekspresyon Plazmiti pTolT ' nin Nokta Mutasyonu Yöntemiyle Moleküler Modifikasyonu Molecular Modification of Escherichia coli Expression Plasmid pTolT by Point Mutation Method. 1–8.

Waugh, D.S. 2011. An overview of enzymatic reagents for the removal of affinity tags. Protein Expr. Purif. 80:283–93.

Wriggers, W., Chakravarty, S. & Jennings, P.A. 2005. Control of protein functional dynamics by peptide linkers. Biopolym. - Pept. Sci. Sect. 80:736–46.

## *ORAL PRESENTATION*

# Synthesis of mesoporous organosilica nanoparticles functionalized with chiral urea-amine ligand and investigation of their effectiveness in heterogeneous asymmetric transfer hydrogenation

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**Abstract**

The aim of this study is to attach chiral 1-((1*R*,2*R*)-2-amino-1,2-diphenylethyl)-3-(3-(triethoxysilyl)propyl) **1**, which shows high catalytic activity in the asymmetric transfer hydrogenation of aromatic ketones, to a support material to ensure its reusability in the same reaction type. Two different synthesis methods were used for heterogenization. In this way, the effect of the synthesis method applied in the heterogenization process on the catalytic efficiency was also examined. In the first method, heterogeneous catalyst **1@mMCM41** was prepared by covalent grafting of chiral ligand **1** on magnetic MCM41 (**mMCM-41)**. In this method, homogeneous attachment of the chiral ligand on the support material cannot be achieved. In order to ensure the homogeneous distribution of the chiral ligand throughout the support material, the co-condensation method was chosen as the second heterogenization method. Using this method, mesoporous organosilica (**MON-A**) material was prepared in the reaction of **1** with tetraethyl orthosilicate (TEOS) in the presence of triblock copolymer cetyltrimethylammonium bromide (CTAB) as a template in basic solution. Finally, the catalytic activities of the obtained catalysts were investigated in asymmetric transfer hydrogenation of aromatic ketones. The catalytic activities of the heterogeneous catalysts were compared both with each other and with the catalytic activities of the homogeneous catalyst. Heterogeneous catalyst **MON-A**, with homogeneous distribution of chiral ligand **1** in the pores of the mesoporous material, showed higher catalytic activity but lower enantioselectivity than **1@mMCM-41**, which had a heterogeneous distribution of chiral ligand **1** in the pores of the mesoporous material (98% vs 40%, 24h) In all trials, **MON-A** showed higher catalytic activity compared to **1@MCM-41**. These results show us that better catalytic activity can be obtained if the chiral ligand is homogeneously distributed on the walls and surfaces of the mesoporous structure.

**Keywords:** Asymmetric transfer hydrogenation, heterogeneous asymmetric catalysis, mesoporous materials, MCM-41

## INTRODUCTION

Chiral drugs continue to be a significant force in the global pharmaceutical market as chirality significantly influences a drug's biological and pharmacological properties. Stereochemistry impacts decisions made in the absorption, distribution, metabolism, excretion, and toxicity stages of drug discovery (Sekhon, 2013). While one of the *enantiomers* contained in drugs that are not pure stereoisomers has the desired effect in terms of the organism, the other *enantiomer* may cause negative effects on the organism, so it has begun to be desired that the active ingredients of modern drugs be single *enantiomers*. For economic, environmental, and social reasons, the trend toward the application of optically pure compounds is undoubtedly increasing. Among the various methods to selectively produce a single *enantiomer*, asymmetric catalysis is the most attractive method from the atom-economic point of view (Song et al., 2003). Many different approaches have been tried and continue to be tried in order to use chiral catalysts with high selectivity in more favorable conditions in asymmetric catalysis. The most important of these approaches is the fixation of the homogeneous chiral catalyst by attaching it to a support material with the help of covalent bonds. Thus, it is possible to recover and reuse the heterogeneous catalyst used in the reaction medium and to easily separate the product from the environment (Kragl et al., 2001). The newly developed immobilization methods make it possible for preparing the robust heterogeneous chiral catalysts while still maintaining the high enantioselectivity. In particular, recent progress made in the heterogeneous asymmetric catalysis shows that a quite number of catalysts result in the enantioselectivity being even higher than their corresponding homogeneous catalysts. Mesoporous organosilicas (MONs) are ordered mesoporous hybrids with organic groups inside the framework. The organic

groups of MONs are homogeneously distributed by co-condensation of monosilylated organic groups with TEOS (TMOS), thus making the access of substrate to the active site easy (Li, 2005). Mesoporous organosilica nanospheres (MONs) were further shown to be excellent supports for bifunctional catalysts that exhibit interesting cooperative catalytic activities (Mihalcik et al., 2009). In recent years, instead of the synthesis approach of new chiral ligands, it has come to the fore to obtain suitable heterogeneous catalysts with fine adjustments made during the attachment (MSN) or addition (MON) of homogeneous chiral catalysts to the support material.

## MATERIALS AND METHODS

1-((1*R*,2*R*)-2-amino-1,2-diphenylethyl)-3-(3-(triethoxysilyl)propyl) **1**, magnetic MCM-41 and **1@mMCM41** urea were previously synthesized by our group (Gök et al., 2020). Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), (1*R*, 2*R*)-1,2-diphenylethane-1,2-diamine and all other reagents were obtained from commercial suppliers (Merck or Fluka). All the materials used during the experiments were of analytical grade. Drying and purification of solvents used in this study were performed by following the procedure in Perrin and Armarego (Perrin and Armerago, 1989). Infrared spectroscopy was performed using Fourier transform infrared spectroscopy (FT-IR), the spectrum 65 Fourier infrared spectrometer from Perkin Elmer using KBr pellet technique. The X-ray diffraction (XRD) measurement was carried out on a PANalytical Empyrean X-ray diffractometer using Cu-Kα radiation (λ = 0.154056 nm) within the scattering angle 2θ range of 1°–10°, and 10°–80°, respectively. Brunauer–Emmett–Teller (BET) method was used to calculate the surface area of mesoporous materials, whereas Barrett, Joyner, and Halenda (BJH) method was used for the calculation of pore size distribution. Enantiomeric excess was determined on a Shimadzu Prominence LC-20A with DAD detection using YMC Chiral ART Amylose-C column and on Shimadzu GC-2010 Plus using chiral column (HP-Chiral-20B).

### Synthesis of mesoporous organosilica material (MON-A)

Briefly, CTAB (0.385 g) and dd$H_2O$ (203 mL) were placed in a three-necked balloon and mixed at 40 °C. Then 2 M NaOH (1.4 mL) was added to the mixture under stirring. The temperature was raised to 80° C. 1-((1R,2R)-2-amino-1,2-diphenylethyl)-3-(3-(triethoxysilyl) propyl) urea **1** (0.459 g) was dissolved with 2 mL of ethanol. TEOS (2.08 g) and **1** were dripped simultaneously for 20 minutes from two separate syringes. Mixture was then stirred at 80 °C for 3 hours. After 3 hours, the mixing stopped. The reaction contents were allowed to mature for 2 days at 80 °C. The solid powder portion was filtered off. It was washed with distilled water and dried in an oven at 60 °C under vacuum. 200 mL of ethyl alcohol and 3.4 mL of HCl were added per 1 g of mesostructured material and left to stir at 60°C for 7 hours. After this time, the solid was filtered and washed with water and ethyl alcohol. Oven dried at 60°C. Then, the mixture was stirred with 0.01 M NaOH (300 mL) for 30 minutes. It was filtered again, washed and dried under vacuum. 0.715 g of mesoporous organosilica material **MON-A** was obtained.

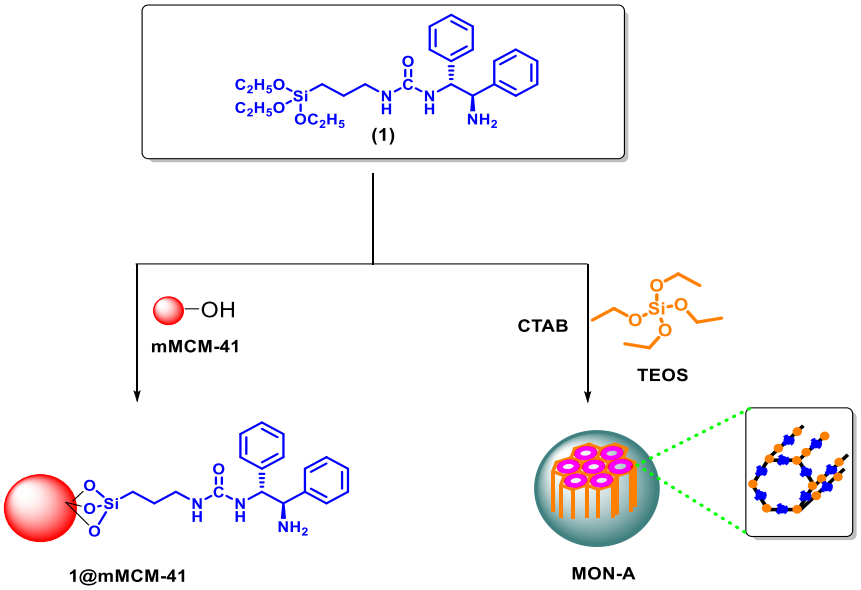
### Screening of catalytic activity of heterogeneous catalyst MON-A in enantioselective transfer hydrogenation of aromatic ketones.

In order to compare the catalytic activity of **MON-A** in ATH of aromatic ketones with the results obtained from homogeneous counterpart **1** and heterogeneous counterpart **1@mMCM41** in our previous study, the protocol for the reactions of asymmetric transfer hydrogenation was applied as same as in the previously published study (Gök et al., 2020). The general conditions for the reactions are as follows. $[Ru(p\text{-}cymene)Cl_2]_2$ was added to the solution of **MON-A** in 7 ml of isopropanol under inert atmosphere at 25 °C. Then, the temperature was brought to 82 °C, stirred for 2h. After 2h, the aromatic ketone was added to the reaction flask at 25 °C, and then the temperature of reaction mixture was adjusted to 82 °C. Base catalyzed ATH was initiated by adding NaOH to the reaction mixture. To screen the conversions and enantiomeric excess, a few drops of reaction mixture were taken from the flask, diluted with isopropanol, and then filtered through microfilter. The filtrate was loaded on GC equipped with a chiral column. The conversions and enantiomeric excess were calculated from the GC measurements by using the area under the peak corresponding to the ketone and its alcohol product screening of the catalytic activity **MON-A** in enantioselective transfer hydrogenation of aromatic ketones.

## RESULTS AND DISCUSSION

### Characterization of mesoporous organosilica nanocatalyst (MON-A)

The preparation of mesoporous silica and organosilica nanoparticles supported chiral urea-amine bifunctional catalysts **1@mMCM41** and **MON-A,** respectively, were performed by following the steps described in Scheme 1.




**Scheme 1**. Synthesis of mesoporous silica nanoparticles supported chiral urea-amine bifunctional catalysts

FT-IR spectroscopy was applied as the first step to characterize **MON-A** nanoparticles. The spectrum of **MON-A** was represented in figure 1a. Observation of the main three characteristic peaks at 1.078, 802, and 455 $cm^{-1}$, corresponded to the antisymmetric, symmetric Si-O stretching and deformation mode of $SiO_4$ tetrahedral in the silica framework confirmed the successful synthesis of **MON-A**. The presence of the additional bands at 1635 $cm^{-1}$ for C=O group, around 2935 $cm^{-1}$ for -$CH_2$ group, and 3030 $cm^{-1}$ for aromatic ring in the spectrum of **MON-A** can be taken as clear evidence that **MON-A** is successfully synthesized as a result of condensation of **1** and TEOS.

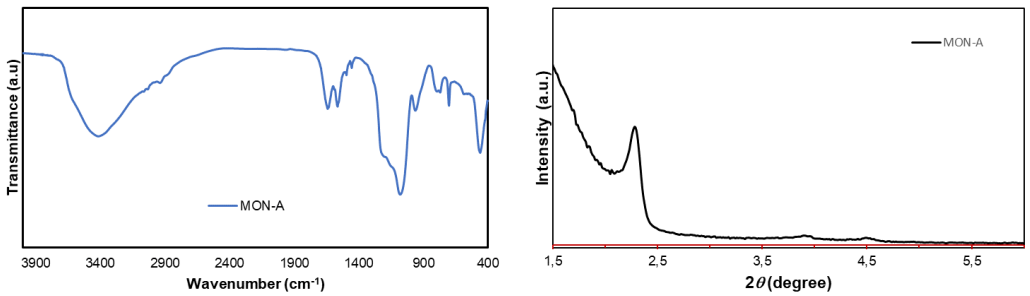



**Figure 1**. a) FT-IR spectrum of MON-A mesoporous structure, b) Low-angle X- ray diffraction (XRD) patterns of MON-A

The low angle powder X-ray diffraction pattern for the **MON-A** is shown in figure 1b. In the low angle XRD dust pattern, obtained a very strong signal at 2θ = 2.2° and two low-intensity signals appearing at 2θ = 3.8 and 4.4° indicate the hexagonal arrangement for **MON-A**. The $N_2$ sorption isotherms of **MON-A** contain a capillary condensation step and these isotherms correspond to the type IV isotherm according to the IUPAC classification. This type of curve indicates that the material is uniformly mesoporous. The BJH (Barrett-Joyner-Halenda) method was used to calculate the pore size distribution from the adsorption curve. The BET surface area of the **MON-A** mesoporous silica structure was calculated as 415 $m^2/g$ and the average pore size (DBJH) of 2.6 nm.

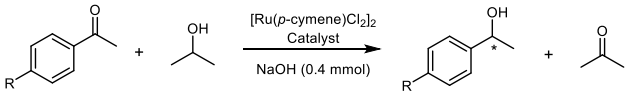
## Catalytic studies

The catalytic activity of **MON-A** was tested in heterogeneous asymmetric transfer hydrogenation of various aromatic ketones under the reaction conditions where homogeneous catalyst **1** showed the best catalytic activity. The results obtained in this study for **MON-A** are summarized in Table 1 in comparison with the results of heterogeneous catalyst **1@mMCM41** and homogeneous catalyst **1**.

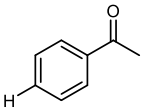
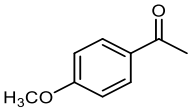
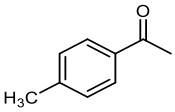
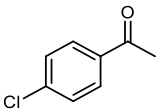
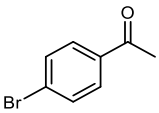
The catalytic activities of homogeneous catalyst **1** and its heterogeneous form **1@mMCM41** were investigated in detail by our group in asymmetric transfer hydrogenation reactions of aromatic ketones (Gök et al., 2020). In the experiments, the best reaction conditions were determined as 0.4 mmol NaOH, 250/1 substrate catalyst ratio and 1:2 Ru-ligand ratio. The preparation method of the heterogeneous catalyst **MON-A** used in this study is different from the preparation of the heterogeneous catalyst **1@mMCM41** used in our previous study. **1@mMCM41** was prepared by covalently grafting 1 to the silica support mMCM41. **MON-A** was prepared by co-condensation of chiral ligand **1** with silyl ends and TEOS. The biggest difference is that in the attachment method, the chiral ligand exhibits a heterogeneous distribution on the support material, while in the condensation method, the chiral ligand exhibits a homogeneous distribution throughout the mesoporous.

When the results obtained in the asymmetric transfer hydrogenation reactions of aromatic ketones in the presence of homogeneous catalyst **1**, heterogeneous catalysts **1@mMCM41** and **MON-A** were examined as a whole, it was determined that the highest catalytic activity and enantioselectivity were obtained in the presence of homogeneous catalyst **1** (entries 1, 4, 7, 10, 13). The conversion values obtained were in the range of 46-99% and enantioselectivity values in the range of 18-38% *ee*. In reactions using heterogeneous catalyst **1@mMCM41** (entries 2, 5, 8, 11, 14), a decrease in catalytic activity and enantioselectivity was observed. While the catalytic activity values obtained decreased to the range of 11-56%, the enantioselectivity values were observed in the range of 5-37% *ee*. Considering the 24-h period, **1@mMCM41** exhibited comparable enantioselectivity but lower catalytic activity than their homogeneous counterpart **1**. The poor catalytic activity of **1@mMCM41** can be explained by irregularly distributed **1** onto the mMCM41, which makes the access of substrate to the active site difficult (Liu et al., 2010a; Liu et al., 2010b; Jiang et al.,2006).

**Table 1.** Ru-catalyzed enantioselective transfer hydrogenation of ketones with chiral urea-amine bifunctional catalysts **1** and their heterogeneous counterparts **1@mMCM-41** and **MON-A** [a]




R = H, $OCH_3$, $CH_3$, Cl, Br  R = H, $OCH_3$, $CH_3$, Cl, Br

| Entry | Substrate | Catalyst | Conversion (%)[b] | % *ee* | Configuration[c] |
|---|---|---|---|---|---|
| **1**[d] |  | **1** | 89 | 33 | (*R*) |
| **2**[d] | | **1@mMCM-41** | 18 | 37 | (*R*) |
| **3** | | **MON-A** | 72 | - | *ras* |
| **4**[d] |  | **1** | 46 | 25 | (*R*) |
| **5**[d] | | **1@mMCM-41** | 11 | 5 | (*R*) |
| **6** | | **MON-A** | 27 | - | *ras* |
| **7**[d] |  | **1** | 70 | 38 | (*R*) |
| **8**[d] | | **1@mMCM-41** | 22 | 31 | (*R*) |
| **9** | | **MON-A** | 45 | - | *ras* |
| **10**[d] |  | **1** | 99 | 18 | (*R*) |
| **11**[d] | | **1@mMCM-41** | 56 | 20 | (*R*) |
| **12** | | **MON-A** | 94 | 3 | (*R*) |
| **13**[d] |  | **1** | 99 | - | *ras* |
| **14**[d] | | **1@mMCM-41** | 40 | 15 | (*R*) |
| **15** | | **MON-A** | 98 | 4 | (*R*) |

[a] Reactions were performed by using 1 mmol acetophenone in 7 mL of $^i$PrOH with Ru:ligand = 1:2; Substrate/catalyst ratio was 250/1 for all entries; reaction time was 24 hours for all entries. [b] Determined by GC (HP-Chiral-20B). [c] Absolute configuration was confirmed by comparing the retention times of the enantiomers on the GC traces with that reported in the literature. [d] The results were taken from published study (Gök et al., 2010)

When the results of the catalytic activity of the heterogeneous catalyst **MON-A** under the same reaction conditions were examined (entries 3, 6, 9, 12 and 15), It was observed that the conversion values obtained were lower than the conversion values obtained in the presence of homogeneous catalyst, but higher than the conversion values given by heterogeneous catalyst **1@mMCM41.** The fact that the catalytic activity of **MON-A** is higher than the catalytic activity of heterogeneous catalyst **1@mMCM41** and approaches the activity of homogeneous catalyst **1** can only be explained by the homogeneous distribution of chiral ligand **1** throughout the mesoporous. These results show us that better catalytic activity can be obtained if the chiral ligand is homogeneously distributed on the walls and surfaces of the mesoporous materials. When the results are evaluated in terms of enantioselectivity, it is seen that homogeneous ligand distribution throughout the mesoporous, which causes an increase in catalytic activity, does not show the same effect in terms of enantioselectivity. The highest enantioselectivity value obtained was only 4% *ee*.

## CONCLUSION

Synthesis of mesoporous organosilica nanoparticles functionalized with chiral urea-amine ligand and investigation of their effectiveness in heterogeneous asymmetric transfer hydrogenation was discussed in this study. The obtaining results showed that the catalytic activities of mesoporous organosilica systems (MON) in which the organic chiral ligand is homogeneously distributed throughout the heterogeneous catalyst skeleton are higher than the mesoporous silica systems (MSN) where the organic chiral ligand cannot be homogeneously distributed. However, it is necessary to carry out studies involving different approaches to increase enantioselectivity as well as catalytic activity. The studies in this direction are continued by our group.

## ACKNOWLEDGMENTS

The chiral homogeneous catalyst 1-((1*R*,2*R*)-2-amino-1,2-diphenylethyl)-3-(3-(triethoxysilyl)propyl) 1 and heterogeneous catalyst 1@mMCM41 used in this study were synthesized in the TUBITAK project numbered 118Z523. We would like to thank TUBITAK for their support to the project numbered 118523. Mert Akgün from Science and Technology Application and Research Centre, Çanakkale Onsekiz Mart University is gratefully acknowledged for his technical assistance.

## REFERENCES

Gök Y, Aykut IT, Gök HZ 2020. *Readily Accessible Mesoporous Silica Nanoparticles Supported Chiral Urea-Amine Bifunctional Catalysts for Enantioselective Reactions.* Applied Organometallic Chemistry, 34: e6015.

Jiang D, Yang Q, Wang H, Zhu G, Yang J, Li C 2006. *Periodic mesoporous organosilicas with trans-(1R,2R)-diaminocyclohexane in the framework: A potential catalytic material for asymmetric reactions.* Journal of Catalysis, 239(1): 65–73.

Kragl U, Dwars T 2001. *The development of new methods for the recycling of chiral catalysts*. Trends Biotechnol. 19(11): 442-9.

Li C 2005. *Chiral synthesis on catalysts immobilized in microporous and Mesoporous materials*. ChemInform, 36(29).

Liu X, Wang P, Zhang L 2010(b). *Chiral Mesoporous Organosilica Nanospheres: Effect of Pore Structure on the Performance in Asymmetric Catalysis*. Chemistry - A European Journal, 16: 12727-35.

Liu X, Wang P, Zhang, L 2010(a), *(R)-(+)-Binol-Functionalized Mesoporous Organosilica as a Highly Efficient Pre-Chiral Catalyst for Asymmetric Catalysis,* Chemistry – An Asian Journal, 5: 1232-9.

Mihalcik D, Lin W 2009. *Mesoporous silica nanosphere-supported chiral ruthenium catalysts: Synthesis, characterization, and asymmetric hydrogenation studies*. ChemCatChem, 1(3):406-413.

Perrin, DD, Armarego, WFL 1989. *Purification of Laboratory Chemicals, Second ed., Pergamon, Oxford, UK* 1989.

Sekhon BS 2013. *Exploiting the Power of Stereochemistry in Drugs: An Overview of Racemic and Enantiopure Drugs.* J. Mod. Med. Chem., 1: 10-36.

Song CE, Lee S 2003. *Supported chiral catalysts on inorganic materials.* ChemInform, 34(3).

## *ORAL PRESENTATION*

# Activity of Polyphenol oxidase from peel and pulp of ripe and unripe Anamur bananas

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## Abstract

Polyphenol oxidase (PPO) oxidizes phenolic compounds found in fresh fruits and causes unwanted browning during storage and food processing. In this study, PPO was extracted from the peel and pulp of ripe and unripe Anamur banana grown in Turkey. The peels of bananas were separated from the pulp. Five grams of the husk and pulp samples were homogenized in 150 mL of 0.1 M phosphate buffer solution (pH 7.1) in a blender at 4 °C for 3 minutes. The extracts were then centrifuged at 6000 rpm for 10 minutes at 4 °C and the supernatant was used as the crude enzyme solution to estimate PPO activity. Pyrocatechol was used as a substrate for PPO activity. In a continuous monitoring system, a jacketed reaction chamber was connected to the flow cell placed in the spectrophotometer by polypropylene tubing and a peristaltic pump. Serial concentrations of 20 mL pyrocatechol were used in the jacketed reaction chamber at 30 °C as a substrate. Then, 150 µL of crude enzyme solution was added to the substrate. The solution in the chamber was continuously passed through a flow cell. Absorbance values were recorded at 415 nm every 30 seconds for 20 minutes. PPO activity kinetic parameters (Km and Vmax) were calculated by Lineweaver-Burk linearization. Specific activity was calculated according to the protein content in PPO extract. There wasn't any PPO activity in the peel of the unripe banana. Km and Vmax values were calculated as 29.426 mM and 739.2 Units/g in the ripe banana peel, respectively. While Vmax values were determined as 1056 Units/g and 1408.75 Units/g for unripe and ripe banana pulp samples, respectively, Km values were determined as 8.647 mM and 12.545 mM. Both Vmax and specific activity increased with maturation.

**Keywords:** Polyphenol oxidase (PPO), Anamur banana, enzyme activity

## INTRODUCTION

As a result of the consumer's increasing awareness, it is demanded that the foods consumed are suitable both for the environment and human health, as well as the taste. For this reason, new methods of production and marketing are being developed in the field of food industry. Some color changes occur in fruits and vegetables due to mechanical damage and processes such as striking, cutting, peeling and slicing. These color changes in different shades from pink to blue-black are called "browning". In many fruit and vegetable products, these color changes are desired to some extent, but often cannot be stopped at the desired level. This reaction, called enzymatic browning, is related to both the activity of the polyphenol oxidase (PPO) enzyme and the phenolic substance concentration. Browning caused by the PPO enzyme not only causes discoloration of the product, but also reduces its flavor and quality.

The most noticeable feature of banana ripening is the color change from green to yellow. This discoloration is largely due to the breakdown of chlorophyll found in unripe bananas, resulting in carotenoids. This chlorophyll destruction during ripening can be explained enzymatic activities, i.e. peroxidase, lip oxygenase , chlorophyll oxidase and polyphenol oxidase. Coloration is due to an oxidation of polyphenols in particular of dopamine (John and Marchal, 1995). This compound is a substrate of PPO. The tannin and polyphenol content, which depends on the fruit culture, affects the ripeness of the fruits (Chang and Hwang, 1990). During ripening, dopamine content has declined but synthesis of certain phenolic components, such as catechin, chlorogenic acid and coumarin has synthesized (Qu et al., 2013). These are potential substrate of PPO. In the peel PPO activity is practically assumed zero (John and Marchal, 1995). It was found that in the pulp PPO activity has increased until full ripening then it has fallen (Jayaraman et al., 1987). The increase in permeability of the

tissues during ripening allows the polyphenol oxidase to react with the polyphenolic compounds and tannins to migrate from the peel towards to pulp (Chang and Hwang, 1990).

In this study, it was aimed to extract the PPO enzyme in the unripe and ripe peel and pulp parts of Anamur banana and to examine its activity. A continuous enzyme activity measurement system has been developed for fast, accurate and reliable data collection. In addition, the quantitative distribution of the PPO enzyme in the peel and pulp of Anamur banana and the change of PPO enzyme activity during the ripening period were investigated.

## MATERIALS AND METHODS

### Materials

The unripe banana sample was supplied from the commercial establishment and stored at -25°C until the study. The fresh and ripe banana sample was purchased from the local market. Pyrocatechol (Sigma-Aldrich) was used as a substrate for PPO activity. Bovine serum albumin (BSA) (Sigma-Aldrich) was used as a standard in Lowry method for protein determination.

### Methods

### Protein extraction

The peel and fruit pulp were separated and homogenized with a 0.1 M, pH 7.1 phosphate buffer solution at 4 °C and 5 grams/150 mL for 3 minutes with a Waring 700 G series blender. After the extracts were passed through 4-layer cheesecloth, they were centrifuged for 10 minutes at 6000 rpm with a Hettich Micro 220 R microcentrifuge at 4 °C. The supernatant was used as crude enzyme solution to estimate PPO activity.

### Enzyme activity assay and developing continuous measurement system

In the determination of PPO activity, pyrocatechol (Sigma-Aldrich) was used as a substrate. Serial dilutions of pyrocatechol (0.5-10 mM) were prepared and 20 mL of the solutions were taken into the jacketed reaction chamber (T=30 °C), and 150 µL of the banana-enzyme extract solutions were added to the chamber. The solution in the chamber was passed through a 6 mm ID polypropylene tubing with a peristaltic pump into a 500 µL flow cell placed in the spectrophotometer. Absorbance values were recorded at 415 nm every 30 seconds for 20 minutes. The schematic of the designed continuous monitoring system is shown in Figure 1.

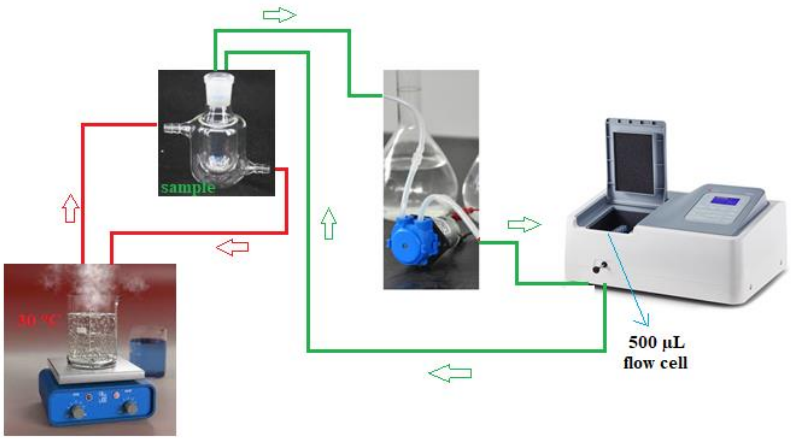


**Figure 1.** The continuous flow cell system

Michaelis-Menten equation was used to determine the enzyme activity. The reaction rate given equation 1, contains two parameters, $V_{max}$ and $K_m$.

$$V = \frac{V_{max} q_S}{(q_S + K_m)} \quad (1)$$

It is possible to estimate $V_{max}$ and $K_m$.by first taking the reciprocal of Eq.1. Lineweaver-Burk was used for linearization (Eq.2).

$$\frac{1}{V} = \frac{K_m}{V_{max}}\left(\frac{1}{q_S}\right) + \frac{1}{V_{max}} \quad (2)$$

By plotting 1/V against $1/q_s$, a straight line results with the ordinate-intercept as $1/V_{max}$ and the slope as $K_m$.

**Protein assay**

Lowry protein assay was used to determine the protein concentration in the extract. Serial dilutions of BSA solutions (between 40-500 µg/mL) were prepared to obrain a BSA calibration curve. 1 N NaOH, 1% copper sulfate ($CuSO_4.5H_2O$), 2% sodium tartrate ($KNa\text{-}C_4H_4O_6.4H_2O$), 2% sodium carbonate ($Na_2CO_3$) and 1 N Folin-Ciocalteu reagents were prepared. On the day of analysis, copper sulfate, tartrate and sodium carbonate reagents were mixed. 5 mL of this solution was added to the tube containing 0.5 mL of protein/or enzyme solution, mixed vigorously and then let stand for 10 min. Afterwards, 0.5 mL 1N Folin reagent was added to the tube and absorbance value was measured with spectrophotometer at 660 nm (Pomory, 2008).

**RESULTS and DISCUSSION**

**PPO Activity**

Enzyme activity was calculated from the graphs obtained when the absorbance values of the prepared ripe and unripe banana pulp and peel solutions were read in the spectrophotometer against time.

The absorbance values recorded for 20 minutes at 415 nm at different substrate concentrations of the unripe banana pulp sample are given in Figure 2. The enzyme activities corresponding to the substrate concentration of these samples were determined by Michaelis-Menten and Lineweaver-Burk plots and given in Figure 3a and Figure 3b, respectively.

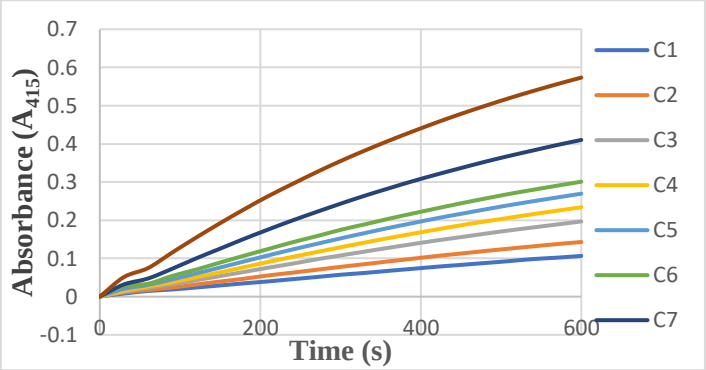


**Figure 2.** Enzyme activity of unripe banana pulp for different concentrations of pyrocatechol.

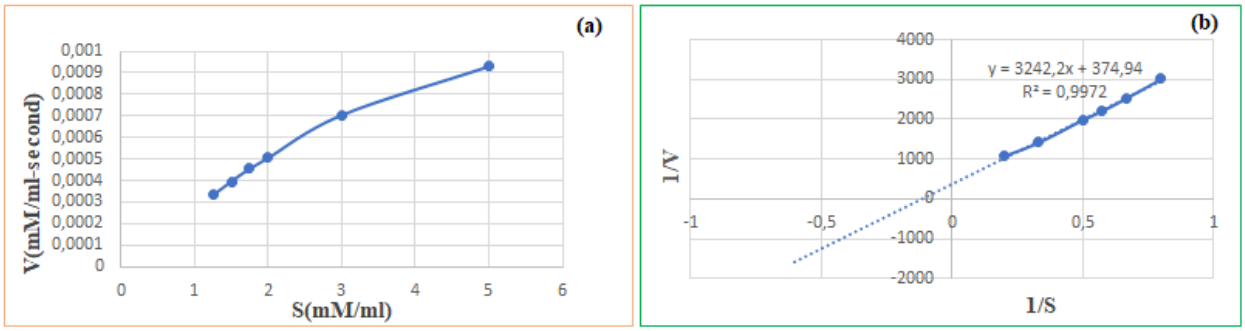


**Figure 3.** For the unripe banana pulp sample; (a) Effect of pyrocatechol substrate concentration on enzyme activity rate (Michaelis-Menten plot), (b) Lineweaver-Burk plot for catechol pyrocatechol

The Km/Vmax (slope) value obtained from the Lineweaver-Burk plot was determined as 3242.2 and the 1/Vmax value as 374.94. From these values, Km and Vmax were calculated as 8.64 mM and 0.002667 Abs/ml-second, respectively.

In the spectrophotometric analysis performed to determine the enzyme activity in unripe banana peel samples, no absorbance value at 415 nm could be obtained. This result showed that there is little or no PPO enzyme in the unripe banana peel. The absorbance values recorded at 415 nm for 20 minutes for different substrate concentrations of the ripe banana pulp sample were given in Figure 4.

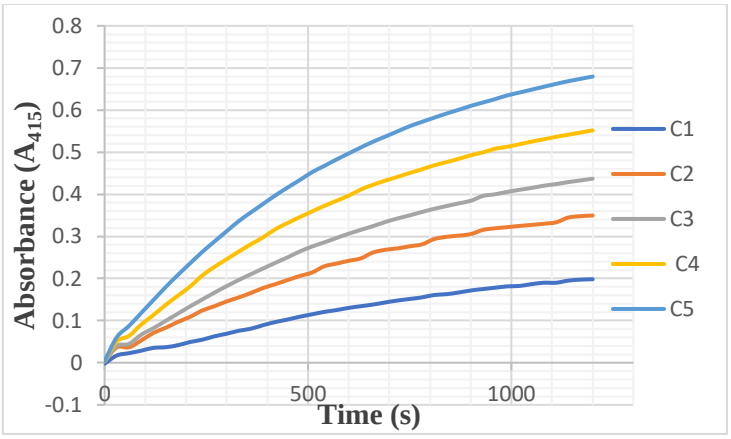


**Figure 4.** Enzyme activity of ripe banana pulp for different concentrations of pyrocatechol.

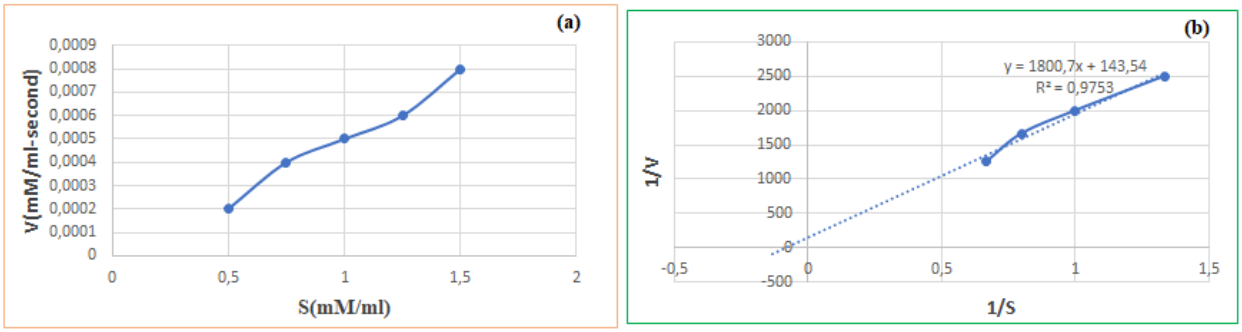


**Figure 5.** For the ripe banana pulp sample; (a) Effect of pyrocatechol substrate concentration on enzyme activity rate (Michaelis-Menten plot), (b) Lineweaver-Burk plot for catechol pyrocatechol

The Km/Vmax (slope) value obtained from the Lineweaver-Burk plot (Figure 5b) was determined as 1800.7 and the 1/Vmax value as 143.54. Depending on these values, Km and Vmax were calculated as 12.54 mM and 0.006967 Abs/ml-second, respectively. The absorbance values recorded at 415 nm for 20 minutes for different substrate concentrations of the ripe banana peel sample were given in Figure 6.

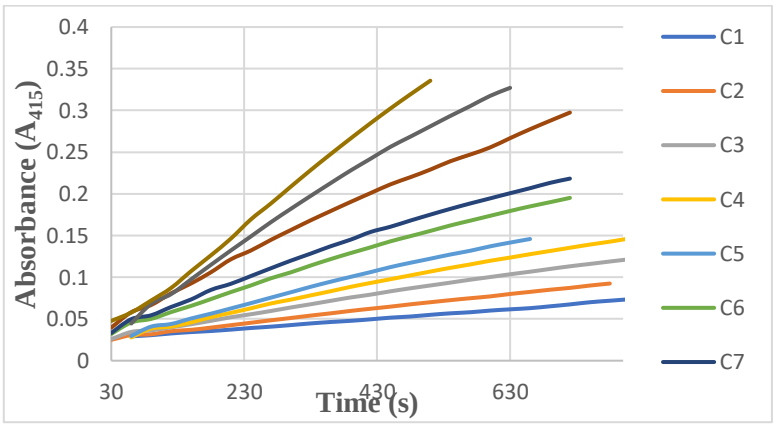


**Figure 6.** Enzyme activity of ripe banana peel for different concentrations of pyrocatechol.

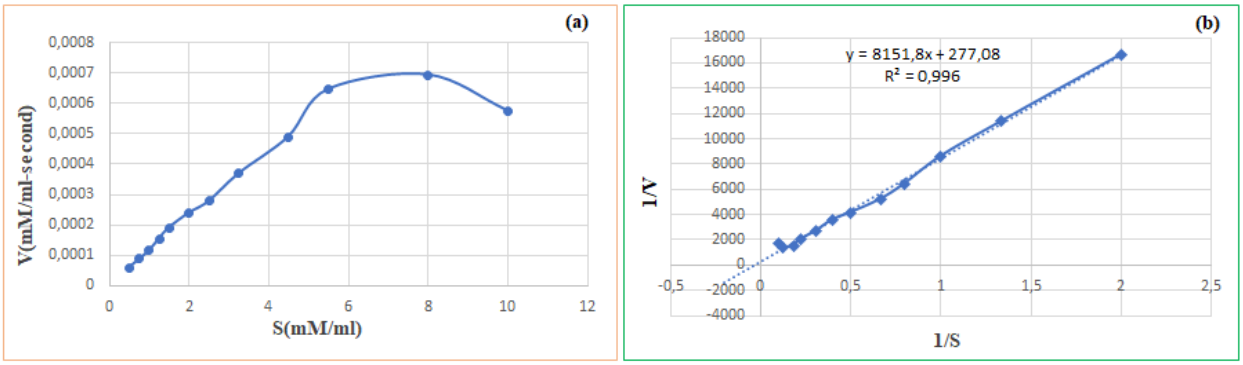


**Figure 7.** For the ripe banana peel sample; (a) Effect of pyrocatechol substrate concentration on enzyme activity rate (Michaelis-Menten plot), (b) Lineweaver-Burk plot for catechol pyrocatechol

From the Lineweaver-Burk plot, the Km/Vmax (slope) value was determined as 8151.8 and the 1/Vmax value as 277.08. Depending on these values, Km and Vmax were calculated as 29.42 mM and 0.00361 Abs/ml-second, respectively.

**Total Protein Determination**

Total protein amount in ripe and unripe Anamur banana pulp and peel enzyme solutions was determined using the Lowry method. The results were evaluated using the calibration curve of BSA standard (Figure 8).

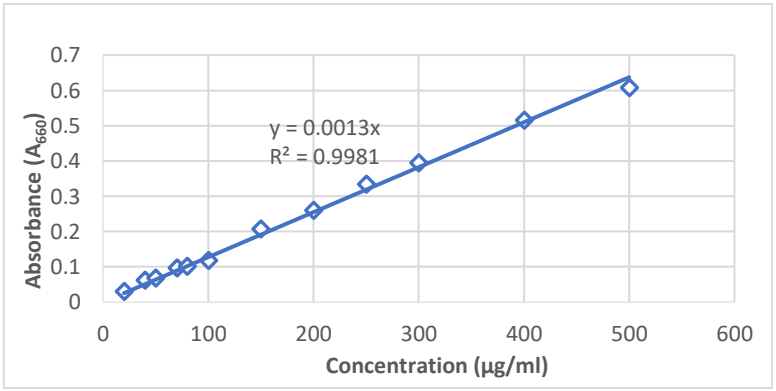


**Figure 8.** Calibration curve of BSA standard

The total protein amounts found for each of the banana samples were given in Figure 9. It was determined that the amount of protein in the pulp was higher in the unripe Anamur banana, and the amount of protein in the peel was higher in the ripe Anamur banana.

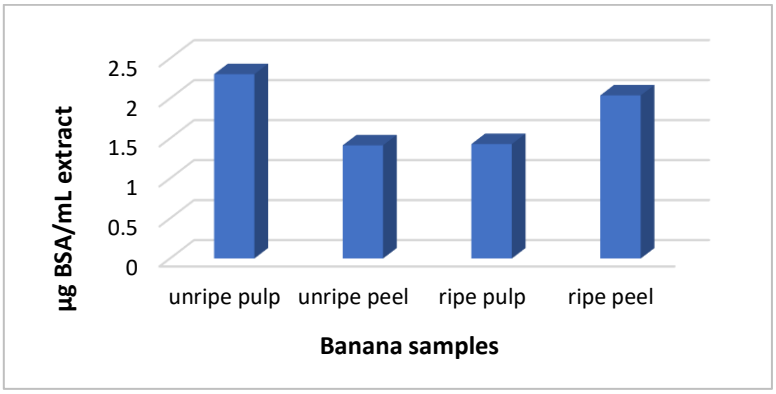


**Figure 9.** Protein amounts of Anamur banana samples

Vmax and specific activity values increase with maturation. Specific activity was calculated according to the protein content in PPO extract.

**Table 1.** Michaelis-Menten constant (Km), maximum rate (Vmax) and specific activity results of the reaction of polyphenol oxidase with pyrocatechol substrate in banana samples

| Sample | $K_m$ (mM) | $V_{max}$ (*Units/g banana) | PPO specific activity (Units/g protein) |
|---|---|---|---|
| Ripe banana pulp | 125,445 | 1408,75 | 984,93 |
| Ripe banana peel | 29,426 | 739,2 | 362,62 |
| Unripe banana pulp | 86,473 | 1056 | 458,37 |

*One unit (U) of enzyme activity corresponds to a change in absorbance value of 0.001 under test conditions.

Maximum PPO activity was found in the ripe banana pulp sample. The activity of ripe banana pulp was higher than that of ripe banana peel. Aziz and Al-Saady (2015) stated in their study that they investigated the extraction conditions of polyphenol oxidase from banana peel, that the specific activity of polyphenol oxidase obtained from banana pulp was higher than that of banana peel.

The Km and Vmax values obtained in this study were given in Table 1. In the previous study, the purified PPO enzyme from Anamur banana grown in Turkey was investigated for its characteristic properties and the Km and Vmax values were reported as 8.5 mM and 0.754 OD410 $min^{-1}$, respectively (Unal, 2007). In another study, PPO enzyme was purified from banana fruit using Sepharose 4B-L-tyrosine-p-aminobenzoic acid affinity gel and its Km and Vmax values were reported as 13.8 mM and 37887 U/mLmin, respectively (Erik, 2016). When the results of the present study were compared with the literature, it was seen that the Km values were very similar to these studies. While Unal (2007) found the Km value of the free PPO enzyme in bananas to be 8.5 mM, the Km value of the unripe banana pulp sample was found to be 8.6 mM in the present study. Erik (2016) found the Km value of the PPO enzyme in bananas to be 13.8 mM, whereas the Km value of the ripe banana pulp sample in the present study was found to be 12.5 mM in our study. All the results were consistent with the literature.

## CONCLUSION

PPO enzyme activity was observed in all samples except for the unripe Anamur banana peel sample. It was determined that the PPO specific activity and Vmax increased with ripening in banana pulp. It was determined that the amount of protein in the pulp was higher than in the unripe Anamur banana, and the amount of protein in the peel was higher than in the ripe Anamur banana. The "continuous flow cell" system designed in the present study could be used effectively. Thanks to this system, continuous and reliable data collection under controlled working condition was achieved successfully.

## REFERENCES

Aziz, GM, AL-Saady, AJR 2015. Extraction conditions of polyphenol oxidase from banana peel. Biotechnology Department, College of Science, University of Baghdad, Iraq.

Chang WH, Hwang YJ 1990. Effect of ethylene treatment onthe ripening, polyphenol oxidase activity and water-soluble tannin content of Taiwan northern banana at different maturity stages and the stability of banana polyphenol oxidase. Acta Horticulturae, 275: 603–610.

Erik, S, 2016. Immobilization of banana polyphenol oxidase enzyme and investigation of some properties. Master's Thesis, Balıkesir University Graduate School of Natural and Applied Sciences, Department of Chemistry.

Jayaraman KS, Ramanuja MN, Vijayaraghavan PK 1987. Studies on the purification of banana polyphenol oxidase. Food Chemistry, 24(3): 203-217.

John P, Marchal J 1995. Ripening and Biochemistry of the fruit. In: Bananas and Plantains. New York: Springer, pp. 434-468.

Pomory CM 2008. Color development time of the Lowry protein assay. Analytical biochemistry, 378: 216-217.

Qu LZ, Gu X, Jin SC, Wang FJ, Wang JZ 2013. Polyphenols in banana (*Musa acuminata* Colla (AAA)) pulp act as polyphenol oxidase substrates. Journal of Food Agriculture and Environment, 11(3): 162-167.

Unal, MU 2007. Properties of Polyphenol Oxidase from Anamur Banana (*Musa cavendishii*). Food Chemistry, 100(3):909-913.

## ORAL PRESENTATION

# İn-vitro inflamatuar bağırsak hastalığı modelinde mirisetinin infliximab ile birlikte kullanılabilme potansiyelinin araştırılması

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## Özet

Günümüzde önemi giderek artan İnflamatuar Bağırsak Hastalığı'nın (IBD) tedavisinde TNF-alfa inhibitörleri yaygın olarak kullanılmakla birlikte hastaların tedavisinde istenilen başarıya ulaşılamamış olması ve uzun süreli TNF- alfa inhibitörlerinin kullanımının neden olduğu ciddi olumsuzluklar yeni tedavi yöntemlerinin geliştirebilmesine ihtiyaç oluşturmuştur. Anti-inflamatuar, anti-oksidan ve anti-kanserojenik etkilere sahip bir flavonoid olan mirisetinin TNF-alfa inhibitörlerinin olumsuz etkilerini önlemedeki rolü bilinmemektedir. Mevcut çalışmada, in-vitro IBD ortamında mirisetinin TNF-alfa inhibitörü ile birlikte kullanımının etkinliğinin araştırılması ve bu flavonoidin IBD'li hastalarda TNF-alfa inhibitörleri ile birlikte kullanılabilirliğinin test edilmesine yönelik yeni ileri in-vivo projelerin yapılandırılmasına ön veri oluşturulması hedeflenmektedir. Kolon adenokarsinom hücre hattı olan HT-29, E. Coli endotoksini olan Lipopolisakkarit (LPS) ve TNF-alfa ile inflamasyon gelişimi uyarılarak in-vitro IBD ortamı oluşturuldu. Mirisetin'in tek başına ve IBD'li hastaların tedavisinde kullanılan TNF-alfa inhibitörü olan infliximab ile kombin halde kullanımıyla hücre proliferasyonu XCelligence yazılımıyla değerlendirildi. % 70'ten fazla canlılık sağlayabilen mirisetin dozlarının tek başına ve infliximab ile kombinasyon halindeki muamelelerinin HT-29 hücrelerinin invazyon yeteneği üzerine olan etkileri yara iyileşmesi deneyiyle analiz edilerek ImageJ yazılımıyla değerlendirildi. Mirisetin'in HT-29 hücre hattı üzerinde hücre proliferasyonu testi sonucunda 24h için LD50 dozu 39,56 µM, % 70'ten fazla canlılık sağlayabilen dozu ise 12,5 µM olarak belirlendi. Mirisetin'in 12,5 µM'lık öldürücü olmayan dozu ile gerçekleştirilen yara iyileşmesi deneyi sonucunda kontrol yara alanında % 29,24 kapanmaya, mirisetin % 18,63 kapanmaya, infliximab % 11,69 kapanmaya, mirisetin + infliximab % 17,08 kapanmaya neden olduğu gözlendi ($p<0.0001$). Bulgularımız mirisetinin ölümcül olmayan dozlarının HT-29 hücrelerinde infliximab ile benzer etkiye sahip olabileceğini göstermiştir. Bu sebeple mirisetinin IBD tedavisine yönelik ileri in-vivo projelerin yapılandırılmasında kullanılabilirliğinin olduğu öngörülmüştür.

**Anahtar Kelimeler:** İnflamatuar bağırsak hastalığı, İnflamasyon, İnfliximab, Mirisetin

## Abstract

TNF-alpha inhibitors are widely used in the treatment of Inflammatory Bowel Disease (IBD), which is becoming increasingly important today. The lack of success in the treatment of patients and the serious problems caused by the long-term use of TNF-alpha inhibitors have created a need to develop new treatment methods. The role of myricetin, a flavonoid with anti-inflammatory, anti-oxidant and anti-carcinogenic effects, in preventing the adverse effects of TNF-alpha inhibitors is unknown. In the current study, it is aimed to create preliminary data for the construction of new advanced in-vivo projects to investigate the efficacy of myricetin combined with TNF-alpha inhibitor in the in-vitro IBD environment and to test the usability of this flavonoid with TNF-alpha inhibitors in patients with IBD. In-vitro IBD environment was created by stimulating the development of inflammation with colon adenocarcinoma cell line HT-29, E. Coli endotoxin Lipopolysaccharide (LPS) and TNF-alpha. Cell proliferation was evaluated with XCelligence software with the use of myricetin alone or in combination with infliximab, a TNF-alpha inhibitor used in the treatment of patients with IBD. The effects on the invasive ability of HT-29 cells of treatment with doses of myricetin alone and in combination with infliximab, which can provide more than 70% viability, were analyzed by wound healing assay and evaluated with ImageJ software. As a result of the cell proliferation test of myricetin on the

HT-29 cell line, the LD50 dose was determined as 39,56 µM for 24h, the dose that could provide more than 70% viability was 12.5 µM. As a result of the wound healing experiment performed with a 12.5 µM non-lethal dose of myricetin, 29.24% closure in the control wound area, 18.63% closure with myricetin, 11.69% closure with infliximab, and 17.08% closure with myricetin + infliximab. was observed ($p<0.0001$). Our findings showed that non-lethal doses of myricetin can have a similar effect to infliximab in HT-29 cells. For this reason, myricetin has been predicted to have utility in structuring advanced in vivo projects for the treatment of IBD.

**Keywords:** Inflammatory bowel disease, Inflammation, Infliximab, Myricetin

## GİRİŞ

İnflamatuar bağırsak hastalığı (IBD), çeşitli faktörlerden kaynaklanan kronik tekrarlayan bağırsak iltihabını temsil etmektedir (Abraham ve ark., 2017). Crohn hastalığı ve ülseratif kolit iki ana IBD tipi olarak tanımlanmıştır (Rademakers ve ark., 2017). IBD, erkeklerde kadınlardan daha sık görülmekte ve günümüzde bu hastalığın morbidite oranında artış görülmektedir (Thia ve ark., 2008). Son zamanlarda IBD, en yaygın sindirim sistemi hastalıklarından biri haline gelmiştir. IBD'nin etiyolojisi belirsizliğini korusa da, IBD'nin patogenezinde son zamanlarda ilerlemeler kaydedilmiştir. Genetik duyarlılık ve bakteri enfeksiyonu da dahil olmak üzere belirli çevresel faktörler arasındaki etkileşimden kaynaklanan, değişmiş immünolojik fonksiyonun, gastrointestinal sistemin mukozal inflamatuar yanıtlarının gelişimine katkıda bulunduğu kuvvetle ileri sürülmektedir (Khor ve ark., 2011). IBD ve bu proinflamatuar sitokinler, süper oksijen ürünleri, kemokinler, proteinazlar ve doku hasarı ve gelişimi ile sonuçlanan sitokinler dahil olmak üzere toksik moleküller üretmek için geri bildirim olarak bağışıklık hücrelerini daha da aktive etmektedir (Kamada ve ark., 2005). Aktif makrofajlar ve T lenfositler tarafından serbest bırakıldığında, TNF aşağıdaki çoklu biyolojik reaksiyonları başlatmaktadır; (1) immün hücre fonksiyonunu modüle eder, (2) adaptif immün yanıtları harekete geçirir, (3) epitel apoptozu tetikler ve (4) epitel bariyerini kırar, hücreler arası yapışma molekülü 1 (ICAM1) gibi adezyon moleküllerini eksprese eden endoteli indükler. Klinik çalışmalar, TNF protein ve mRNA düzeylerinin serumda, bağırsak dokusunda, aktif IBD'nin dışkısında hastalık aktivitesi ile bağlantılı olarak yükseldiğini göstermiştir (Papadakis ve ark., 2000; Komatsu ve ark., 2001; Wang ve ark., 2005). TNF üretiminin klinik inhibisyonu, hastalık remisyonu, iyileştirilmiş yaşam kalitesi ve nüksün önlenmesi ile ilişkilendirilmiştir, bu arada IBD'nin klinik tedavisinin başarısızlığı, immün hücreler tarafından TNF salgılama kapasitesinin erken reaktivasyonuna bağlanmıştır (Nikolaus ve ark., 2000; Rutgeerts ve ark., 2004). Bulgular, TNF'nin hastalık gelişimi için kritik olduğunu göstermektedir. İltihaplı mukozada TNF üretimini inhibe etmek, IBD yönetiminin önemli hedeflerinden biridir. Anti-TNF-α tedavisi, TNF-α'nın etkilerini antagonize etmeyi amaçlamaktadır. IBD'nin klinik ortamında kullanılan veya kullanılmış olan anti-TNF-α tedavilerinin örnekleri infliximab (IFX), adalimumab, golimumab, sertolizumab, etanercept, onercept ve CDP571'dir (Gareb ve ark., 2020). Bu biyolojikler, TNF-α 'yı nötralize eden antikorlar veya çözünür TNF-α reseptörleridir. Ana etki mekanizması TNF-α antagonizması olmasına rağmen, bu ilaçlar kısmen moleküler yapıdaki varyasyonlardan dolayı tek tek bileşiğe özgü olan farklı farmakodinamik profillere sahiptir. Bu nedenle, IBD'de farklı anti-TNF-α tedavilerinin gözlenen etkinliği değişken olduğu ve eşdeğer olmadığı saptanmıştır (Mitoma ve ark., 2018; Levin ve ark., 2016; Peake ve ark., 2013; Naviglio ve ark., 2018; Slevin ve ark., 2015; Adegbola ve ark., 2018; Olesen ve ark., 2016). Aynı zamanda, infliximab gibi anti-TNF-α ajanları olarak kullanılan ilaçlar ile sürekli tedavi, fırsatçı enfeksiyon ve kötü huylu tümör riskini artırmaktadır. Bu bağlamda, antienflamatuar aktivite sergileyen doğal bileşikler üzerine yapılan araştırmalar, IBD için etkili terapötik stratejiler geliştirmeye yeni bir yaklaşım olabileceği öngörülmüştür (Linshan Duan, 2021). Flavonoidlerin, antioksidan ve antienflamatuar aktivitelerinin aracılık ettiği insan sağlığına yarar sağladığı öne sürülmüştür. Mirisetin (3,3',4',5,5',7-heksahidroksiflavon, MYR), meyveler, otlar ve sebzeler gibi doğal bileşenlerden ekstrakte edilen bir flavonoid bileşiğidir (Guo ve ark., 2019). Birikmiş raporlar, mirisetin'in anti-inflamatuar, antioksidan, anti-proliferatif ve anti-kanserojen etkiler gibi birçok biyolojik aktiviteye sahip olduğunu göstermiştir (Fu ve ark., 2013; Li ve ark., 2016). Bu nedenle mirisetin, hastalık tedavisi için giderek daha fazla kullanılmaktadır. Anti-inflamatuar etkilerle ilgili olarak, bazı çalışmalar, mirisetin (10 mM) ile 24 saat ön tedavinin, IL-1β ile uyarılan SW982 sinovyal hücrelerinde MAPK'ları inhibe ederek inflamatuar mediatörleri azalttığını ortaya koymuştur (Lee ve ark., 2010). İlerleyen çalışmalarda, mirisetin, azoxymethane/Dextran sulfate sodium ile indüklenen ülseratif kolit ve kolorektal tümör oluşumu üzerinde, kolonik dokulardaki inflamatuar faktörleri azaltma mekanizması yoluyla inhibisyon gösterdiği belirlenmiş ve mirisetinin ülseratif kolit tedavisinde terapötik bir ilaç olarak kullanılabileceği öngörülmüştür (Zhang ve ark., 2017). Ek olarak mirisetinin IBD ile yapılan çalışmasında, Dextran sulfate sodium ile uyarılan IBD'li farelerde iltihabı iyileştirebileceği ve antioksidan

etkisinden dolayı IBD'nin iki alt tiplerinden biri olan ülseratif kolit'in yeni bir terapötik maddesi olarak kullanılabileceği öngörülmüştür (Zhao ve ark., 2013). Ancak IBD tedavisinde anti-TNF-α ajanı olarak kullanılan infliximab ile flavonoid olan mirisetinin kombinasyon halinde bu hastalığın inflamasyon sürecine olan etkisi ve tümör hücrelerinin agresifliğinin baskılaması yönündeki mekanizma üzerine etkisi bilinmemektedir.

## MATERYAL VE METOT

### Mirisetinin Tek Başına Ve Infliximab İle Kombinasyonlarının HT-29 Hücre Hattında Hücre Proliferasyonu Testi

Hücre Hattı Ve Malzemelerin Temini
Projemizde çalışılması planlanan insan Kolon tümör hücre hattı olan HT-29 hücre hattı BUÜ Tıbbi Biyoloji AD stoklarından temin edilmiştir. Mirisetin, TNF-α ve infliximab ticari olarak firmadan temin edilmiştir.

İn-vitro IBD Modelinin Yapılandırılması
Gastrointestinal sistemde gram-negatif bakterilerin baskınlığı göz önüne alındığında, endotoksinin (lipopolisakkarit; LPS) IBD'li hastalarda bağışıklığın aktivasyonu ve hastalık komplikasyonlarındaki rolünün aydınlatılması önemlidir (Pasternak ve ark., 2010). Bu sebeple çalışmamızda HT-29 hücrelerinde inflamasyon durumunun oluşturulması için kültür ortamında ticri olrk alınn LPS kullnılmıştır. HT-29 hücrelerinin TNF-α ile uyarılması: İnflamasyon ortamı oluşturulmuş HT-29 hücrelerinde TNF-α blokajı olarak infliximab'ın kullanılmasından dolayı ortama ilaç tedavisinden 1 gün önce 10 ng/mL TNF-α 37°C'de 24 saat süreyle maruz bırakılacaktır (Rodriguez P ve ark., 1995).

İlaç Tedavisi Altında Hücre Proliferasyonunun Gerçek Zamanlı Analizi
HT-29 hücreleri %10 oranında fetal bovine serum (FBS), %1 Antibiotic/Antimycotic solution, 2mM L-glutamin ve 1mM sodium pyruvate içeren Dulbecco's Modified Eagle's Medium (DMEM)'dan oluşan besiyeri ortamında, uygun kültür kaplarında; 37˚C sıcaklık ve %5 $CO_2$ oranı sağlayan inkübatörde 48 saat boyunca üretilecektir. 30,000 hücre/200µL HT-29 hücresi 16 kuyulu E-plate'e ekilecek ve xCELLigence sistemi (RTCA DP, Agilent Technologies, Santa Clara, CA, ABD) içerisinde 3 tekrarlı olarak Mirsetinin 0, 12.5, 25, 37,5, 50 µM/ infliximab'ın 0,5, 5, 10, 15, ve 20 µg/ml arası konsantrasyonlarına maruz bırakılmıştır. E-plate zeminine yerleşik sensörler aracılığıyla hücre büyümesi 96 saat boyunca her 5dk da bir ölçülerek, hücre büyüme eğrisi oluşturulacak ve mirisetinin hücre proliferasyon hızını %50 azaltan konsantrasyonu ve inkübasyon süresi belirlenmiştir. Daha sonra, HT-29 hücreleri E-platelere ekilerek xCELLigence sistemi içerisinde belirlenen inkübasyon süresi boyunca mirisetinin ve infliximab'ın etken dozuları ile farklı kombinler halinde maruz bırakılmıştır ve her 5 dk da bir yapılacak ölçümlerle hücre proliferasyonundaki değişim gerçek zamanlı olarak belirlenecektir. Deneyler her bir doz için 3 tekrarlı olarak gerçekleştirilmiştir.

### Mirisetinin Tek Başına Ve Infliximab İle Kombinasyon Halinde Hücreler Üzerindeki İnvazyon Etkisinin Belirlenmesi

Mirisetinin tek başına ve infliximab ile kombinasyon halinde hücreler üzerindeki invazyon etkisi yara iyileşmesi yöntemi ile belirlenmiştir. HT-29 hücrelerin her biri % 10 FBS içeren altı kuyucuklu plakaların üzerine tek-katman olacak şekilde ekilmiştir. Hücrelerin birleşme oranı yaklaşık %80'ne ulaştığında, tek tabaka 200µl plastik bir uç yardımı ile çizilecek ve hücre artıklarını çıkarmak için PBS ile yıkanmıştır. Hücrelere medyum eklenerek kültür plakaları 37°C etüvde inkübe edilmiştir. Çizik boyunca oluşan yara iyileşmesi farklı zamanlarda gözlenmiştir ve çizik hatları fotoğraflanmıştır. Yara kapanışı, ters bir mikroskop kullanılarak beş rastgele alanda değerlendirilecektir (Mutlu ve ark., 2021).

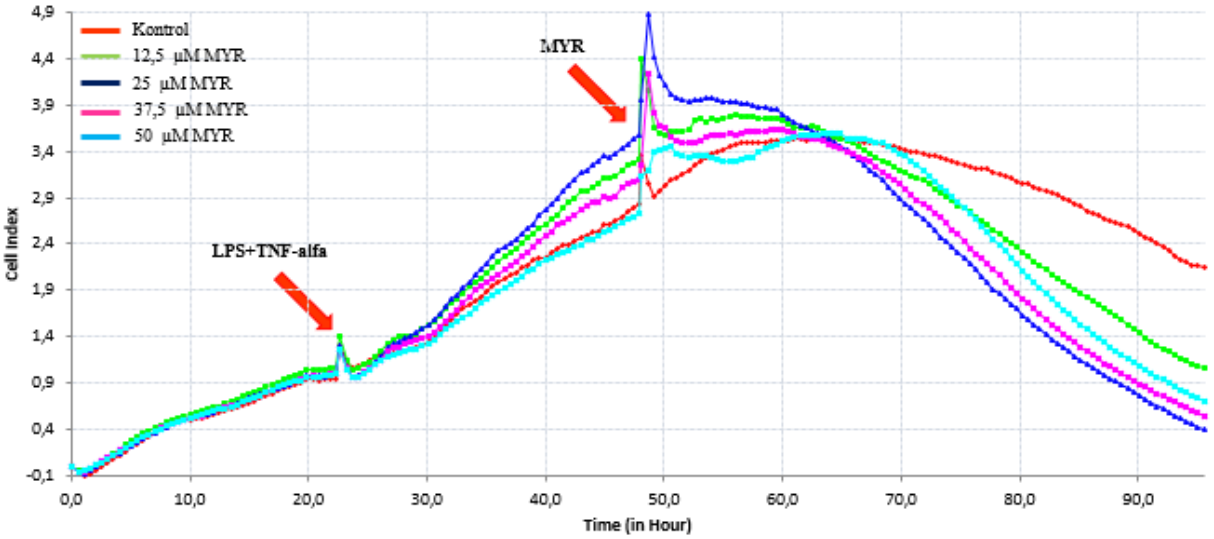
### İstatistiksel Analizler

İn-vitro verilere ait tamamlayıcı istatistikler sayı, yüzde, ortalama ve standart sapma olarak gösterilecek ve gruplar arasındaki farklılıklar one way ANOVA ile gösterilmiştir. İstatistiksel analizler SPSS(20.0) yazılımı ve GRAPHPAD Prism (16.0) programlarından yararlanılarak yapılmıştır. 0.05'ten küçük P değeri istatistiksel olarak anlamlı kabul edilmiştir. Her bir analiz aşaması en az üç tekrarlı olarak gerçekleştirilmiştir.

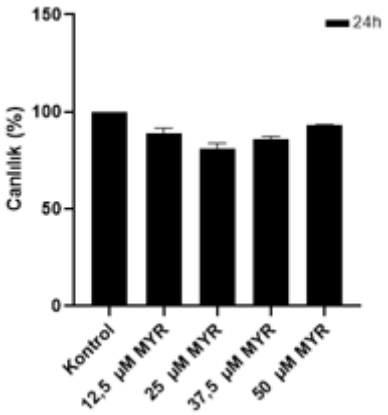
## BULGULAR ve TARTIŞMA

### Hücre Proliferasyonu Deneyi

HT-29 hücre hattında mirisetinin % 70'ten fazla canlılık sağlayabilen dozu 12,5 µM olarak belirlendi (Şekil 1, 2). Mirisetin'in HT-29 hücre hattı üzerinde hücre proliferasyonu testi sonucunda 24h için LD50 dozu 39,56 µM olarak belirlendi.

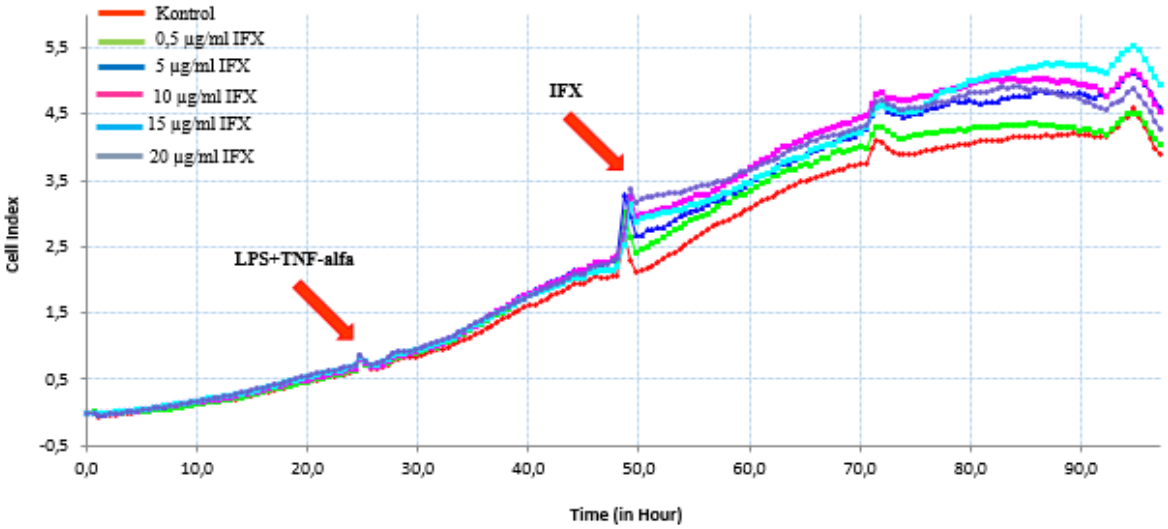



**Şekil 1.** HT-29 hücrelerinde MYR'nin hücre proliferasyonuna etkisinin analizi

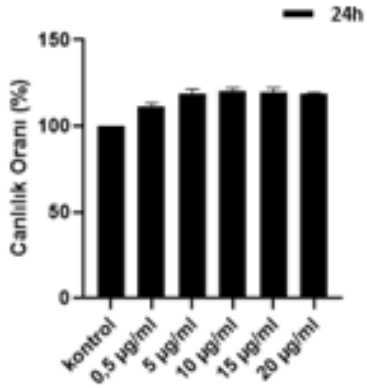



**Şekil 2.** HT-29 hücrelerinde MYR'nin canlılık üzerine etkisi

HT-29 hücre hattında İnfliximabın ölümcül etki yaratmayan ve literatüre uyumlu olan dozu 15 µg/ml olarak belirlendi (Şekil 3, 4).

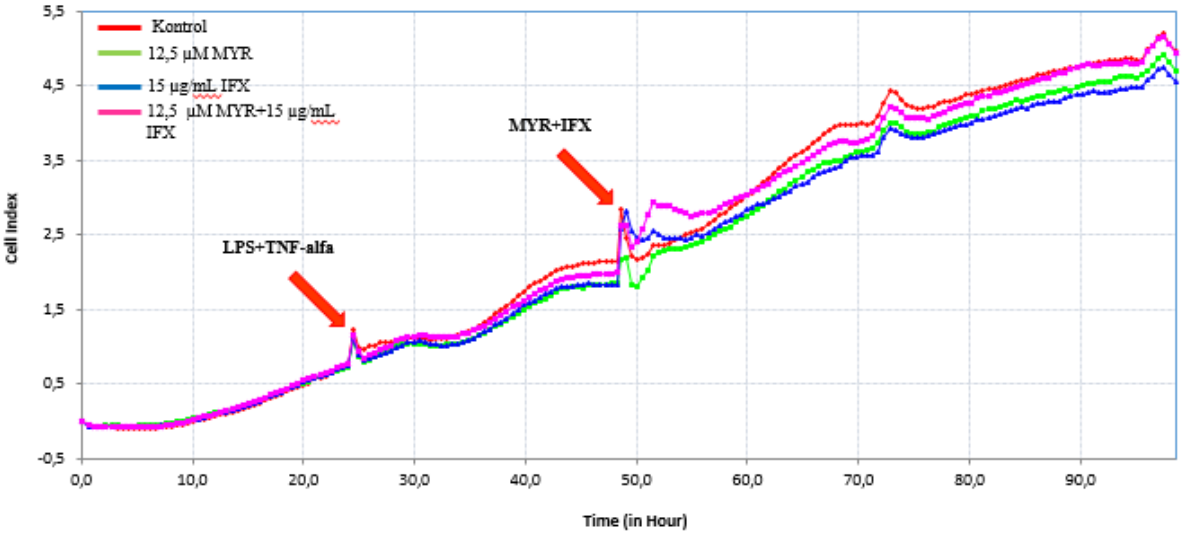



**Şekil 3.** HT-29 hücrelerinde IFX'in hücre proliferasyonuna etkisinin analizi

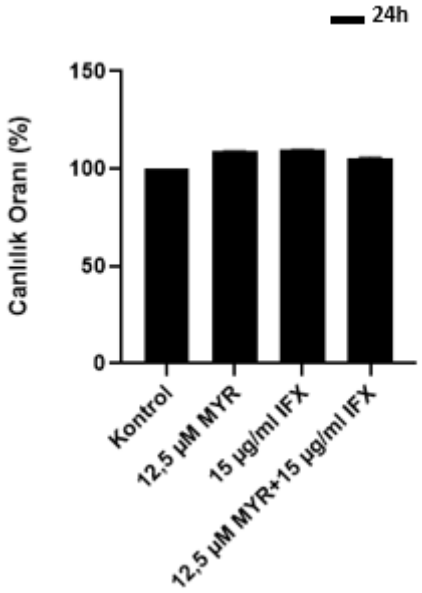



**Şekil 4.** HT-29 hücrelerinde IFX'in canlılık üzerine etkisi

Mirisetin ve İnfliximab'ın seçilen dozlarıyla tek başlarına ve birlikte muamelesi HT-29 hücre hattında ölümcül bir etki yaratmamıştır (Şekil 5, 6).



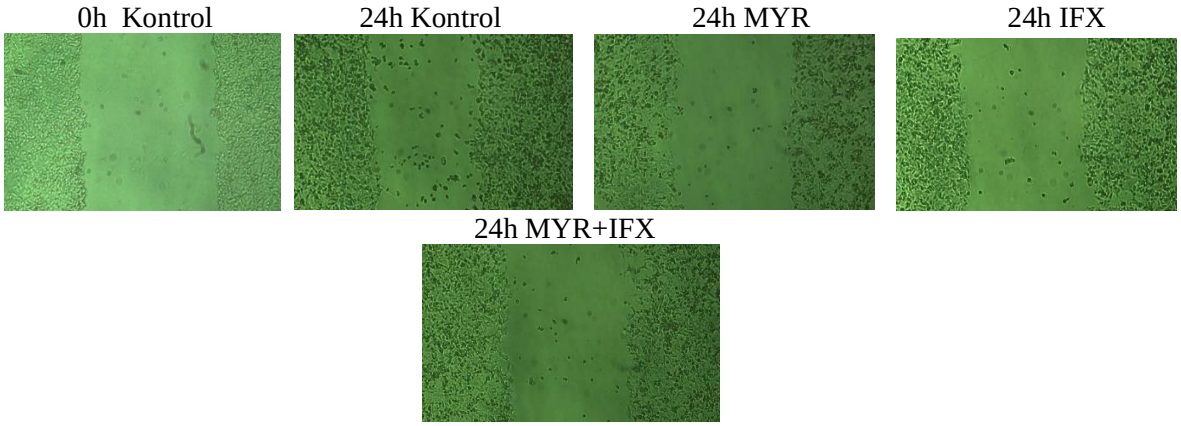

**Şekil 5.** HT-29 hücrelerinde MYR+IFX'in hücre proliferasyonuna etkisinin analizi



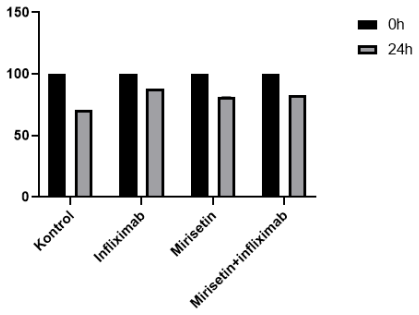

**Şekil 6.** HT-29 hücrelerinde MYR+IFX'in canlılık üzerine etkisi

**Yara İyileşmesi Deneyi:**

Mirisetin'in 12,5 µM'lık öldürücü olmayan dozu ile gerçekleştirilen yara iyileşmesi deneyinin ImageJ yazılımı ile değerlendirilmesi sonucunda, kontrol yara alanında % 29,24 kapanmaya neden olurken, mirisetin % 18,63 kapanmaya ($p<0.0001$), infliximab % 11,69 kapanmaya ($p<0.0001$), mirisetin + infliximab % 17,08 kapanmaya neden olduğu gözlendi ($p<0.0001$) (Şekil 7, 8)




**Şekil 7.** HT-29 hücrelerinde MYR ve IFX'ın yara analizi deneyi ile invazyon üzerine etkileri




**Şekil 8.** HT-29 hücrelerinde MYR ve IFX'ın alan kapanma oranları (%)

Mirisetin'in hem in-vitro hem in-vivo çalışmalarda anti-inflamatuar, anti-oksidan, anti-proliferatif ve anti-kanserojen gibi birçok biyolojik aktiviteye sahip olduğunu göstermiştir. Bu nedenle mirisetin, hastalık tedavisi için giderek daha fazla kullanılmaktadır (Fu ve ark., 2013). Anti-TNF-α tedavisi olarak kullanılan İnfliximab'ın hem UC hem CD için etkili bir tedavi yöntemi olduğu bilinmektedir (Gareb ve ark., 2020). Ancak mirisetin ve İnfliximab'ın birlikte kullanılabilme potansiyeli ve etki mekanizması bilinmemektedir. Bazı anti-

TNF-alfa ajanlarının uzun süreli kullanımı ciddi enfeksiyona neden olabilir ve malign tümör riskini artırabilir. Bu bağlamda, belirli doğal bileşikler ve bunların sentetik türevleri, potansiyel bir araştırma yönünü temsil edebilir. Bu tür doğal bileşiklerin IBD'yi önleme ve tedavi etme potansiyellerinin ortaya çıkarılması ve IBD için yeni aday ilaçları belirlenmesinde önem taşımaktadır (Duan ve ark., 2021). Mevcut çalışmada mirisetin ve İnfliximab'ın birlikte kullanılabilme potansiyelinin bulunduğu ortaya konmuştur.

## SONUÇ

Bulgularımız, mirisetinin ölümcül olmayan dozlarının HT-29 hücrelerinde İnfliximab ile benzer etkiye sahip olabileceğini göstermiştir. Bu bilgiler ile mirisetinin LD50 dozunun değerlendirildiğinde invazyon etkisini en az IFX kadar etkili olabileceği yönünde öngörü oluşturulmuştur ve mirisetinin IBD tedavisine yönelik ileri in-vivo projelerin yapılandırılmasında kullanılabilirliğinin olduğu öngörülmüştür.

## TEŞEKKÜR

Bu çalışmayı kapsayan THIZ-2022-748 numaralı projeye mali destek sağlayan Bursa Uludağ Üniversitesi BAP birimine teşekkürlerimizi sunarız.

## KAYNAKLAR

Abraham BP, Ahmed T, Ali T 2017. Inflammatory bowel disease: pathophysiology and current therapeutic approaches. Handb Exp Pharmacol, 239:115-146.

Adegbola SO, Sahnan K, Warusavitarne J, Hart A, Tozer P 2018. Anti-TNF Therapy in Crohn's Disease. Int. J. Mol. Sci, 19:2244.

Duan L, Cheng S, Li L, Liu Y, Wang D, Liu G 2021. Natural Anti-Inflammatory Compounds as Drug Candidates for Inflammatory Bowel Disease. Front Pharmacol, 14;12:684486.

Franken NA, Rodermond HM, Stap J, Haveman J, van Bree C 2006. Clonogenic assay of cells in vitro. Nat Protoc, 1(5):2315-9.

Fu RH, Liu SP, Chu CL, Lin YH, Ho YC, Chiu SC, Lin WY, Shyu WC, Lin SZ 2013. Myricetin attenuates lipopolysaccharide-stimulated activation of mouse bone marrow-derived dendritic cells through suppression of IKK/NF-kappaB and MAPK signalling pathways. J Sci Food Agric, 93:76–84.

Gareb B, Otten AT, Frijlink HW, Dijkstra G, Kosterink JGW 2020. Review: Local Tumor Necrosis Factor-α Inhibition in Inflammatory Bowel Disease. Pharmaceutics, 12(6):539.

Guo C, Xue G, Pan B, Zhao M, Chen S, Gao J, Chen T, Qiu L 2019. Myricetin Ameliorates Ethanol-Induced Lipid Accumulation in Liver Cells by Reducing Fatty Acid Biosynthesis. Mol Nutr Food Res, 63(14):e1801393.

Hering NA, Andres S, Fromm A, van Tol EA, Amasheh M, Mankertz J, Fromm M, Schulzke JD 2011. Transforming growth factor-β, a whey protein component, strengthens the intestinal barrier by upregulating claudin-4 in HT-29/B6 cells. J Nutr, 141(5):783-9.

Kamada N, Hisamatsu T, Okamoto S, Sato T, Matsuoka K, Arai K, Nakai T, Hasegawa A, Inoue N, Watanabe N, Akagawa KS, Hibi T 2005. Abnormally differentiated subsets of intestinal macrophage play a key role in Th1-dominant chronic colitis through excess production of IL-12 and IL-23 in response to bacteria. J Immunol, 175(10):6900-8.

Khor B, Gardet A, Xavier RJ 2011. Genetics and pathogenesis of inflammatory bowel disease. Nature, 474(7351):307-17.

Komatsu M, Kobayashi D, Saito K, Furuya D, Yagihashi A, Araake H, Tsuji N, Sakamaki S, Niitsu Y, Watanabe N 2001. Tumor necrosis factor-alpha in serum of patients with inflammatory bowel disease as measured by a highly sensitive immuno-PCR. Clin Chem, 47(7):1297-301.

Lee YS, Choi EM 2010. Myricetin inhibits IL-1beta-induced inflammatory mediators in SW982 human synovial sarcoma cells. Int Immunopharmacol, 10:812–814.

Levin AD, Wildenberg ME, van den Brink GR 2016. Mechanism of Action of Anti-TNF Therapy in Inflammatory Bowel Disease. J. Crohn's Colitis, 10:989–997.

Li Y, Cui SX, Sun SY, Shi WN, Song ZY, Wang SQ, Yu XF, Gao ZH, Qu XJ 2016. Chemoprevention of intestinal tumorigenesis by the natural dietary flavonoid myricetin in APCMin/+ mice. Oncotarget, 7:60446–60460.

Mitoma H, Horiuchi T, Tsukamoto H, Ueda N 2018. Molecular mechanisms of action of anti-TNF-α agents–Comparison among therapeutic TNF-α antagonists. Cytokine, 101:56–63.

Mutlu M, Tunca B, Ak Aksoy S, Tekin C, Egeli U, Cecener G 2021. Inhibitory Effects of Olea europaea Leaf Extract on Mesenchymal Transition Mechanism in Glioblastoma Cells. Nutr Cancer, 73(4):713-720.

Naviglio S, Giuffrida P, Stocco G, Lenti MV, Ventura A, Corazza GR, Di Sabatino A 2018. How to predict response to anti-tumour necrosis factor agents in inflammatory bowel disease. Expert Rev. Gastroenterol. Hepatol, 12:797–810.

Nikolaus S, Raedler A, Kühbacher T, Sfikas N, Fölsch UR, Schreiber S 2000. Mechanisms in failure of infliximab for Crohn's disease. Lancet, 356(9240):1475–1479.

Olesen CM, Coskun M, Peyrin-Biroulet L, Nielsen OH 2016. Mechanisms behind efficacy of tumor necrosis factor inhibitors in inflammatory bowel diseases. Pharmacol. Ther, 159:110–119.

Papadakis KA, Targan SR 2000. Role of cytokines in the pathogenesis of inflammatory bowel disease. Annual Review of Medicine, 51:289–298.

Pasternak BA, D'Mello S, Jurickova II, Han X, Willson T, Flick L, Petiniot L, Uozumi N, Divanovic S, Traurnicht A, Bonkowski E, Kugathasan S, Karp CL, Denson LA 2010. Lipopolysaccharide exposure is linked to activation of the acute phase response and growth failure in pediatric Crohn's disease and murine colitis. Inflamm Bowel Dis, 16(5):856-69.

Peake STC, Bernardo D, Mann ER, Al-Hassi HO, Knight SC, Hart AL 2013. Mechanisms of Action of Anti–tumor Necrosis Factor α Agents in Crohn's Disease. Inflamm. Bowel Dis, 19:1546–1555.

Rademakers G, Vaes N, Schonkeren S, Koch A, Sharkey KA, Melotte V 2017. The role of enteric neurons in the development and progression of colorectal cancer. Biochim Biophys Acta Rev Cancer, 1868:420-434.

Rodriguez P, Heyman M, Candalh C, Blaton MA, Bouchaud C 1995. Tumour necrosis factor-alpha induces morphological and functional alterations of intestinal HT29 cl.19A cell monolayers. Cytokine, 7(5):441-8.

Rutgeerts P, Van Assche G, Vermeire S 2004. Optimizing anti-TNF treatment in inflammatory bowel disease. Gastroenterology, 126(6):1593–1610.

Slevin SM., Egan LJ 2015. New Insights into the Mechanisms of Action of Anti–Tumor Necrosis Factor-α Monoclonal Antibodies in Inflammatory Bowel Disease. Inflamm. Bowel Dis, 21:2909–2920.

Thia KT, Loftus EV Jr, Sandborn WJ, Yang SK 2008. An update on the epidemiology of inflammatory bowel disease in Asia. Am J Gastroenterol, 103(12):3167-82.

Wang J, Fu YX 2005. Tumor necrosis factor family members and inflammatory bowel disease. Immunological Reviews, 204:144–155.

Zhang MJ, Su H, Yan JY, Li N, Song ZY, Wang HJ, Huo LG, Wang F, Ji WS, Qu XJ, Qu MH 2018. Chemopreventive effect of Myricetin, a natural occurring compound, on colonic chronic inflammation and inflammation-driven tumorigenesis in mice. Biomed Pharmacother, 97:1131-1137.

Zhao J, Hong T, Dong M, Meng Y, Mu J 2013. Protective effect of myricetin in dextran sulphate sodium-induced murine ulcerative colitis. Mol Med Rep, 7(2):565-70.

## *ORAL PRESENTATION*

# Investigation of the effects of fisetin with the combination of chemotherapy drug temozolomide to the biological behaviour of glioblastoma cells

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**Abstract**
Glioblastoma (GB) (stage IV) is the most common and aggressive primary brain tumor in adults and, even with the standard treatment with the chemotherapy drug Temozolomide (TMZ), the median survival of patients is still limited to only 12.6 months. Therefore, natural compounds such as flavonoids that can be used to treat GB can improve the success of TMZ treatment. Our aim is to examine the effects of fisetin, which is widely used in various cancers, on the treatment of GB cells as combined with TMZ. In the present study, cell viability of TMZ and fisetin in T98G cells was determined by real-time measurements via high-tech xCELLigence analysis. In addition, to evaluate the effects of fisetin alone and in combination with TMZ on GB cell aggressiveness; its invasive feature with wound healing test and its effects on adhesion-independent cell proliferation with colony formation test were performed. Fisetin alone reduced the cell proliferation and the IC50 value of fisetin was 16,40 µmol/L at 24h. Fisetin alone and combined with TMZ suppressed invasive ability in the T98G cell line. At the end of 24h of wound scratches, the percentages of wound widths were plotted in TMZ, Fisetin, and TMZ+Fisetin groups as 75.56% ($p<0,0001$), 90.13% ($p<0,0001$), 98.03% ($p<0,0001$), respectively while the wound width of the control group was only 13.95%. Moreover, the colony formation and the number of colonies were inhibited more than a hundred times with the TMZ+Fisetin combination treatment compared to untreated cells ($p<0,0001$). Our study highlights that fisetin and combination therapy can reduce tumor aggressiveness by inhibiting the invasion and colony ability of GB. Overall, it has been predicted that fisetin may be a new drug candidate by improving the life quality of patients and increasing the effectiveness of TMZ in the GB treatment with further studies.
**Keywords:** Glioblastoma, Therapy, Temozolomide, Fisetin, Tumor aggressiveness

## INTRODUCTION

Glioblastoma (GB) is the most common and most aggressive malignant brain tumor (Wen and Kesari, 2008). The World Health Organization defines GB as a group of stage IV tumors characterized as malignant, mitotically active, and prone to necrosis. GB has a 5-year survival rate of 4-5% and has a rather poor prognosis (McLendon and Halperin, 2003). Standard treatment of GB consists of surgical resection, radiotherapy, and chemotherapy. Despite this treatment protocol, patients have a median survival of only 12.6 months (Louis et al., 2007). Maximum surgical resection is an effective treatment method that prolongs the survival of this disease. However, due to the invasive nature of GB and the location of the tumor in the brain, maximum resection cannot be performed in many cases, and this is largely associated with recurrence (Stupp et al., 2009). One of the biggest causes of failure in GB treatment is the presence of the blood-brain barrier (BBB). TMZ, an alkylating agent, is a chemotherapy drug used for the treatment of GB, which can exceed the BBB and is effective in oral use. In addition to the limited use of the chemotherapy drug, the severe side effects of the drug and the resistance of the patients to the drug over time are the most important factors that complicate the treatment. Nowadays, natural compounds have been frequently used in the determination of new treatment methods for many cancer types, and efforts have been made to facilitate existing treatments as more effective and to reduce the resulting cytotoxic effects (Kammerud et al., 2021). In addition, studies expressing that the use of natural compounds with certain therapeutic properties together with chemotherapeutic agents increases the effectiveness of the chemotherapeutic agent by creating a synergistic effect, thus reducing the dose of the available agent in the treatment and reducing the side effects of this agent (Cragg and Newman, 2006 and Li et al., 2010). Flavonoids are natural constituents of the polyphenolic secondary metabolite class, mainly found

in fruits and vegetables. Flavonoids, which have been determined to have neuroprotective and anti-tumor effects by research, are classified within themselves and the most widely used subgroup of flavonoids in human food intake is flavonol. Flavonols are 3-hydroxy derivatives of flavanones (Panche et al., 2016). Fisetin is a flavonol found in strawberries, apples, onions, wine, and tea. Fisetin exhibits a variety of bioactivities, including antioxidant, anti-inflammatory, and anticancer. One of the most important goals is to reduce the effective dose of the drug by using flavonoids together with the drug, to reduce the side effects and increase the effectiveness of the drug, and also to postpone the resistance gained against the drug. Therefore, there is a fundamental need to develop new therapeutic strategies for the treatment of GB. In the literature, it is found that fisetin increases the expression of caspase3, caspase 9, caspase 8 and Bax, decreases the expression levels of Bcl-2 and survivin, and negatively affects the MAPK/ERK pathway in the GB cell line T98G. Based on these results, it is argued that fisetin will serve as the basis for future *in-vitro* and *in-vivo* studies, with its apoptotic effect on GB cells (Pak and Vatan, 2019). Studies in different cancer types have shown that fisetin inhibits growth in human epidermoid carcinoma A431 cells, stops the cell cycle in G2/M, and induces apoptosis (Pal et al., 2013). In addition, it has been found that it inhibits the cell cycle in bladder tumors and induces apoptosis with p53 activation (Li et al., 2011). Furthermore, PI3K, MAPK, and HIF-1α pathways, which are of great importance in TMZ resistance in GB cells, are also targeted by fisetin (Singh et al., 2021 and Zhang et al., 2016). Due to the presence of common pathways involved in TMZ resistance and targeted by fisetin (Kammerud et al., 2021), fisetin has been predicted to be a guide for obtaining positive results and creating a synergistic effect in TMZ effectiveness and resistance. However, the effects of fisetin in combination with TMZ on biological processes in GB cells and tumor aggressiveness are unknown. There is a need to understand more about the efficacy of fisetin on cell aggressiveness caused by TMZ and explain the drug resistance mechanisms better in TMZ-exposed and resistant cell lines. In the current project, it was aimed to investigate the effects of fisetin on cell viability, migration, invasion, and colony formation in GB cells in combination with TMZ in order to determine its efficacy for use in the treatment of GB.

## MATERIALS AND METHODS

### Cell Proliferation Assay in T98G and L929 Cell Line with Fisetin Alone and Combinations with TMZ

Procurement of cell lines and materials

T98G, the TMZ-resistant human GB tumor cell line planned to be studied in our project, and L929 cell line to be used as a control is procured from the University of Bursa Uludag, Department of Medical Biology stocks. Fisetin and TMZ are supplied commercially by the company and our stocks in Department, respectively. T98G and L929 cells are incubated in appropriate culture well plates in a medium consisting of Dulbecco's Modified Eagle's Medium-F12 (DMEM-F12) containing 10% fetal bovine serum (FBS), 1% Antibiotic/Antimycotic solution, 2mM L-glutamine and 1mM sodium pyruvate for 48 hours in an incubator that provides 37˚C temperature and 5% $CO_2$ rate.

Real-time analysis of Cell Proliferation Under Drug Treatment

10,000 cells/100µL of T98G and L929 cells are seeded into a 16-well E-plate and exposed to fisetin in concentrations between 1-200µmol/L in 3 replicates in the xCELLigence system (RTCA DP, Agilent Technologies, Santa Clara, CA, USA). Cell growth is measured every 5 minutes for 72 hours by using the RTCA (Real Time Cell Analyzer) biosensor system by making real-time measurements with e-plates with high-density gold electrode arrays on the specially designed e-plate base. A cell growth curve is created to determine the incubation time and the concentration of fisetin, which reduces the cell proliferation rate by 50% (IC50). Then, cells are seeded on 16-well e-plates, and incubation period is determined in the xCELLigence system. During incubation, cells are exposed in different combinations with the active dose of fisetin and the effective TMZ concentrations, determined in our previous studies. The change in cell proliferation is recorded in real-time with measurements that are made every 5 minutes. Experiments will be performed in 3 replicates for each dose.

### Determination of Invasive Effect of Fisetin on Cells Alone and in Combination with TMZ

The invasive effect and migration ability of fisetin alone and in combination with TMZ in T98G cells are investigated by the wound healing method. T98G cells are seeded in a single-layer onto six-well plates containing 10% FBS. When the confluence of cells reaches approximately 80%, the monolayer is scratched with a 10µl plastic tip and washed with PBS to remove cell debris. Fresh media is added to the cells and culture plates are incubated in a 37°C incubator. Wound healing along the scratch is observed at different times (0-6-

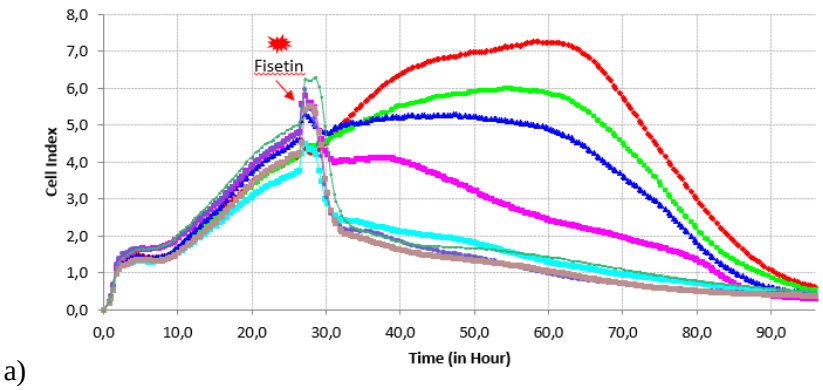
18-24h) and the lines of the scratch are photographed. Wound closure is evaluated in five random fields using an inverted microscope. Triplicate wells are examined for each condition and each experiment is repeated in 3 replicates.

**Determination of the Effect of Fisetin Alone and in Combination with TMZ on Colony Formation in T98G Cell Line**

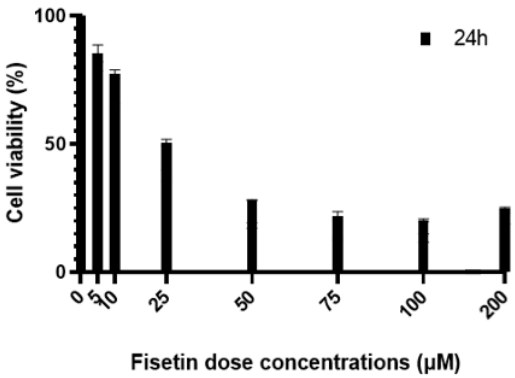
The CellMAXTM Colonogenic Assay Kit (BioPioneer, USA) is used to examine the effects of fisetin and TMZ, alone or in combination, on colony formation ability in T98G cells. T98G cells are seeded into 6-well cell culture dishes at $25x10^4$ cells per well. Prior to the treatment, the culture plates are incubated in an incubator at 37°C temperature and 5% $CO_2$ until they reach 70% density. The determined active doses of fisetin and TMZ, alone and in combination, are given to cells that reach 70% density in the culture plates and are left to incubate for 24 hours in an incubator. At the end of 24 hours, the cells are removed by trypsinization and replanted in 6-well cell culture plates with $1x10^3$ cells per well according to experimental groups and left to incubate for 10 days. At the end of the incubation, the medium in the wells is withdrawn and washed with 1X PBS. 1 ml of Fixation Buffer is added to the cells and left at room temperature for 15 min. Then, 1 ml of Staining Solution is poured into the cells and left at room temperature for 45 minutes. In the final step, cells are washed with 1X PBS after staining. Colonies stained with blue color are counted under 20X magnification with an inverted microscope. The experiment is carried out in triplicates.

**RESULTS**

Initially, the cytotoxicity of fisetin on T98G cells was evaluated via a high-tech xCELLigence system. Due to the dose-dependent inhibition of cell proliferation, the cell index data of fisetin is determined (Table 1). The IC50 values calculated after 24 and 48 h of incubation were 16,40 µmol/L and 12,26 µmol/L for T98G, respectively. Consequently, fisetin deters cell proliferation in a dose-dependent manner (Figure 1).




a)



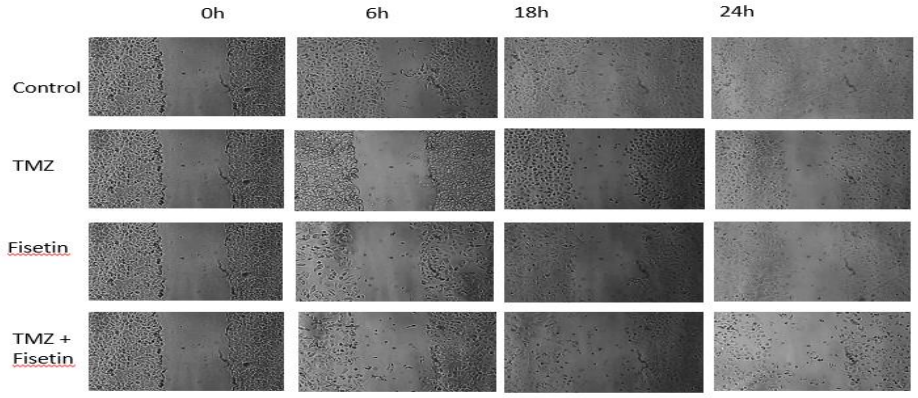

b)

**Figure 1.** a) Raw data of the effect of fisetin dose concentration on T98G cell proliferation b) Graphical representation of fisetin dose-dependent cell viability (%) on T98G cell line.

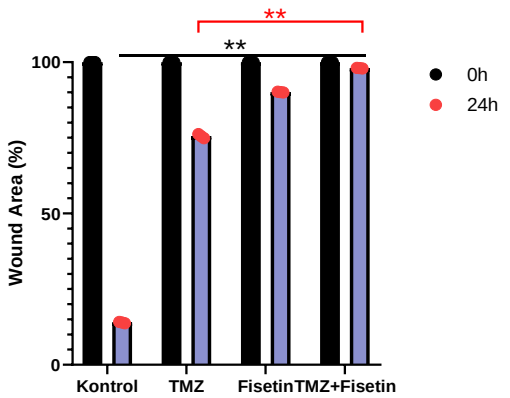
**Table 1.** Digital data of cell index after 24h of fisetin treatment on T98G cell line.

| | Cell Index (24h) |
|---|---|
| Control | 6.83 |
| 5 µmol/L | 5.84 |
| 10 µmol/L | 5.28 |
| 25 µmol/L | 3.46 |
| 50 µmol/L | 1.92 |
| 75 µmol/L | 1.50 |
| 100 µmol/L | 1.39 |
| 200 µmol/L | 1.69 |

Later, the scratch wound healing assay was done and T98G cells were treated with effective doses of TMZ and fisetin for 24h. Images of the wounded sub-confluent monolayers of T98G cells treated with the effective dose of fisetin were taken at 100 × at 0-6-18-24h (represented) and percentage wound widths were plotted. The number of cells/field is shown. The data shown is representative of three independent experiments. Control cells are untreated cells with high metastatic and wound-closure (86,05%) ability after 24h. At the end of 24h, the open wounded areas in the TMZ-only, Fisetin-only, and TMZ+Fisetin groups were 75,56%, 90,13%, and 98,03%, respectively (Table 2). According to this, it means that only 1,97% of the wounded area is closed for the TMZ+Fisetin treatment. As a result, fisetin suppresses cell migration and invasion ability alone (9,87%) and is even better in combination therapy with TMZ (1,97%) (**p<0,0001) (Figure 2).




a)




b)

**Figure 2.** The effect of TMZ, Fisetin, and TMZ+Fisetin on cell migration and invasion with wound healing assay on T98G cell line a) Representative images b) Graphical representation of the percentages of cells in each wound area.

**Table 2.** Digital data of the rates of wound closure with TMZ, Fisetin, and TMZ+Fisetin treatment on T98 cell line.

| | Control | TMZ | Fisetin | TMZ + Fisetin |
|---|---|---|---|---|
| **0h** | 100% | 100% | 100% | 100% |
| **24h** | 13,95% | 75,56% | 90,13% | 98,03% |

During the follow-up, the effects of TMZ and Fisetin treatment on adhesion-independent cell proliferation is tested via colony formation assay. TMZ alone decreased colony number by 98,73%. On the other hand, the combined TMZ+Fisetin therapy inhibited the number of 99,58% colonies formed (235,3 fold more compared to the untreated cells; $p<0,0001$) (Figure 3).

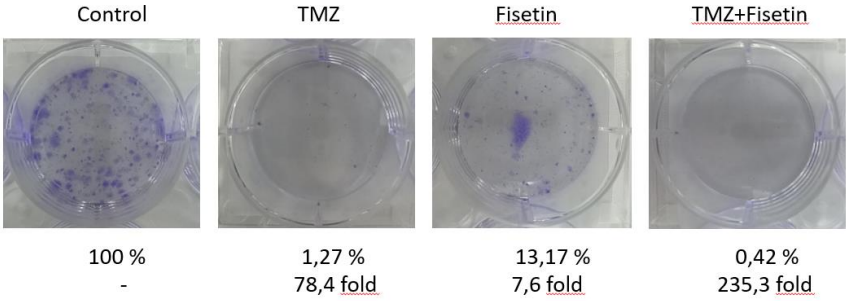



**Figure 3.** The ability of inhibition of colony formation with TMZ, Fisetin, and TMZ+Fisetin combined treatment on T98G cell line.

## DISCUSSION

In the literature, the effect of fisetin on apoptosis with its senolytic effect in the T98G cell line (Pak and Vatan, 2019) and, also the effect of fisetin on GB senescence in another GB cell line (Beltzig et al., 2022) were mentioned, but its effects on cell aggressiveness in combination with TMZ were not shown. There are very valuable resources to provide preliminary findings for the studies we have done and will do. However, the effect of fisetin combined with TMZ in the TMZ-resistant T98G cell line on the biological behavior and cell aggressiveness of the TMZ-resistant GB cell line will contribute to the literature and future studies.

## CONCLUSION

Overall, the results showed that fisetin treatment with the combination of TMZ in GB could reduce the tumor aggressiveness which can pave a path to us to understand the anti-cancer activity of a widely used natural product, fisetin better. TMZ+Fisetin treatment significantly weakened the migration and invasion by T98G cells. Consequently, important findings have been obtained with TMZ+Fisetin combined therapy in order to lower the effective dose of the drug, thereby reducing the side effects and enhancing the therapeutic efficiency of the drug, as well as postponing resistance to the drug. Thus, it will be possible to structure new *in-vitro* and *in-vivo* projects for the development of a drug candidate that will contribute to improving the survival and quality of life of patients. Furthermore, the findings of our current study may shed light on the development of new strategies that are more effective, more economical, and with fewer side effects for the treatment of GB in the future.

## ACKNOWLEDGEMENTS

We thank all colleagues who assisted in this study. This study was funded and supported by the Scientific Research Projects Coordination Unit (BAP) at the University of Bursa Uludag (BUÜ) (THIZ-2021-747).

## REFERENCES

Beltzig L, Christmann M, Kaina B 2022. Abrogation of Cellular Senescence Induced by Temozolomide in Glioblastoma Cells: Search for Senolytics. Cells. 11(16):2588. doi: 10.3390/cells11162588.

Cragg GM, Newman DJ 2006. Plants as a Source of Anti-Cancer Agents. J Ethnopharmacol, 22;100(1-2):72-9. Kammerud SC,

Metge BJ, Elhamamsy AR, Weeks SE, Alsheikh HA, Mattheyses AL, Shevde LA, Samant RS 2021. Novel role of the dietary flavonoid fisetin in suppressing rRNA biogenesis. Lab Invest. 101(11):1439-1448.

Li J, Cheng Y, Qu W, Sun Y, Wang Z, Wang H, Tian B 2011. Fisetin, a dietary flavonoid, induces cell cycle arrest and apoptosis through activation of p53 and inhibition of NF-kappa B pathways in bladder cancer cells. Basic & Clinical Pharmacology & Toxicology, 108(2), 84– 93. Li, J. Liu, H. Ramachandran, S. Waypa GB, Yin JJ, Li CQ, Han M, Huang HH, Sillard WW, Vanden Hoek TL, Shao ZH 2010. Grape seed proanthocyanidins ameliorate Doxorubicin-induced cardiotoxicity. Am J Chin Med., 38(3):569-84. Louis DN, Ohgaki H,

Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P 2007. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 114:97–109. McLendon RE, Halperin EC 2003. Is the long-term survival of patients with intracranial glioblastoma multiforme overstated? Cancer, 98:1745–1748.

Pak F, Vatan PO 2019. Fisetin effects on cell proliferation and apoptosis in glioma cells. Z Naturforsch C J Biosci. 2019 Nov 26;74(11-12):295-302.

Pal HC, Sharma S, Elmets CA, Athar M, Afaq F 2013. Fisetin inhibits growth, induces $G_2$ /M arrest and apoptosis of human epidermoid carcinoma A431 cells: role of mitochondrial membrane potential disruption and consequent caspases activation. Exp Dermatol. 22(7):470-475.

Panche AN, Diwan AD, Chandra SR 2016. Flavonoids: an overview. Journal of Nutritional Science 5, 1–15.

Singh N, Miner A, Hennis L, Mittal S 2021. Mechanisms of temozolomide resistance in glioblastoma - a comprehensive review. Cancer Drug Resist 4:17-43.

Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO 2009. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10, 459–466.

Wen PY, Kesari S 2008. Malignant gliomas in adults. N. Engl. J. Med. 359, 492–507.

Zhang XJ, Jia SS 2016. Fisetin inhibits laryngeal carcinoma through regulation of AKT/NF-κB/mTOR and ERK1/2 signaling pathways. Biomed Pharmacother. 83:1164-1174.

## ORAL PRESENTATION

# 2,4-D Isooctyl ester herbisitinin sulardan elektrooksidasyon yöntemi ile giderimi ve toksisitesinin belirlenmesi

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## Özet

Tarımsal faaliyetler sonucu ortaya çıkan su kirliliği, içme ve kullanma temininde kullanılan ve yüzeysel sularının kalitesini etkileyen sebeplerin başında gelmektedir. Sudaki pestisit kalıntıları, bozunma veya dönüşüm ürünlerinin çevresel etkileri pestisit ile kontamine olmuş suların arıtımını zorunlu hale getirmektedir. Çalışma kapsamında tarımsal üretimden kaynaklanan herbisit kirliliğine odaklanılarak yaygın kullanıma sahip 2,4-D Isooctyl ester herbisitinin model atıksudan elektrooksidasyon yöntemi ile giderimi sağlanmıştır. Ru/Ir kaplı metal oksit (MMO) elektrotlar kullanılarak gerçekleştirilen elektrooksidasyon çalışmalarında akım yoğunluğunun etkisi (50, 75, 100 ve 125 mA/cm$^2$), destek elektrolit türü ve miktarının etkisi (5, 10, 15 mM $NaNO_3$ ve 5, 10, 15 mM $Na_2SO_4$) ve başlangıç pH'ının (pH 3, doğal pH ve pH 9) sistem verimine etkisi incelenmiştir. 125 mA/cm$^2$ akım yoğunluğunda, 5 mM $Na_2SO_4$ destek elektrolit ilavesi ve doğal pH' da gerçekleştirilen çalışmada %89,66 KOİ giderim verimi elde edilmiştir. Elde edilen bu optimum koşullarda toksisite analizleri gerçekleştirilmiştir. Elde edilen sonuçlar doğrultusunda 2,4-D Isooctyl ester herbisitinin model atıksudan MMO elektrooksidasyonu ile etkin bir şekilde gidirebildiği görülmüştür.

**Anahtar Kelimeler:** 2,4-D Isooctyl ester, pestisit, herbisit, elektrooksidasyon, MMO elektrot.

## Abstract

Water pollution resulting from agricultural activities is one of the leading causes that affect the quality of surface waters used for drinking and utilization. The environmental effects of pesticide residues, degradation or transformation products in the water make it necessary to treat pesticide-contaminated water. In this study, the herbicide pollution caused by agricultural production was focused on and the widely used 2,4-D Isooctyl ester herbicide was removed from the model wastewater by electrooxidation method. In studies using Ru/Ir coated metal oxide (MMO) electrodes, the effect of current density (50, 75, 100 and 125 mA/cm$^2$), the effect of supporting electrolyte type and concentration (5, 10, 15 mM $NaNO_3$ and 5, 10, 15 mM $Na_2SO_4$), and the effect of initial pH (pH 3, natural pH and pH 9) on system efficiency were investigated. In the study carried out at 125 mA/cm$^2$ current density, 5 mM $Na_2SO_4$ supporting electrolyte addition and natural pH, 89.66% COD removal efficiency was obtained. Toxicity analyzes were carried out under these optimum conditions. According to the results obtained, it was observed that 2,4-D Isooctyl ester herbicide could be effectively removed from the model wastewater by MMO electrooxidation.

## GİRİŞ

Tahıl üretimi ülkemiz ekonomisinin önemli bir parçasıdır. Artmakta olan dünya nüfusu, gelişen sanayi ve kentleşme nedeniyle tarım alanlarının azalması birim alandan daha yüksek miktar ve verimde ürün elde etmeye yönelik tarım uygulamalarını gündeme getirmektedir. (Solmaz vd., 2010; Yadav vd., 2015; Sağlam, 2008). Ülke nüfusunun artması, tarım ve sanayi faaliyetlerinin mevcut büyüme hızlarına paralel artan su ihtiyacı, su kaynaklarının sürdürülebilir kullanıma dikkat çekmektedir (Polat,2013). Bu sebeple çeşitli faaliyetler sonrasında oluşacak su kirliliğinin azaltılması, oluşan atıksuların yeniden kullanımının maksimum düzeye çıkarılması gerekmektedir. Tarımsal faaliyetler sonucu ortaya çıkan su kirliliği, içme ve kullanma temininde kullanılan ve yüzeysel sularının kalitesini etkileyen sebeplerin başında gelmektedir. Sudaki pestisit kalıntıları, bozunma veya dönüşüm ürünlerinin çevresel etkileri pestisit ile kontamine olmuş suların arıtımını zorunlu hale getirmektedir. Pestisitlerin yüksek kimyasal kararlıkta olması, biyolojik parçalanmasının zor olması ve giderim yöntemlerinin her tür pestisit için etkin olmaması nedeniyle daha etkin giderim yöntemlerine ihtiyaç duyulmaktadır **(**Solmaz vd., 2010; Oller vd., 2006; Bruggen vd., 1998; Huang vd., 2007; Rodrigo vd., 2014). Bu sebeple elektrokimyasal yöntemler pestisit içeren su ve atıksuların arıtımında yüksek giderim verimliliği

nedeniyle tercih edilmektedir. Çalışma kapsamında tarımsal üretimden kaynaklanan herbisit kirliliğine odaklanılarak yaygın kullanıma sahip 2,4-D Isooctyl ester herbisitinin model atıksudan elektrooksidasyon yöntemi ile giderimi sağlanmıştır. Ru/Ir kaplı metal oksit (MMO) elektrotlar kullanılarak gerçekleştirilen çalışmalarda elektrooksidasyona etki eden deneysel parametreler incelenmiştir. Elde edilen sonuçlar doğrultusunda 2,4-D Isooctyl ester herbisitinin model atıksudan MMO elektrooksidasyonu ile etkin bir şekilde giderimi sağlanmış ve elde edilen optimum koşullarda toksisite analizleri gerçekleştirilmiştir.

## MATERYAL VE METOT

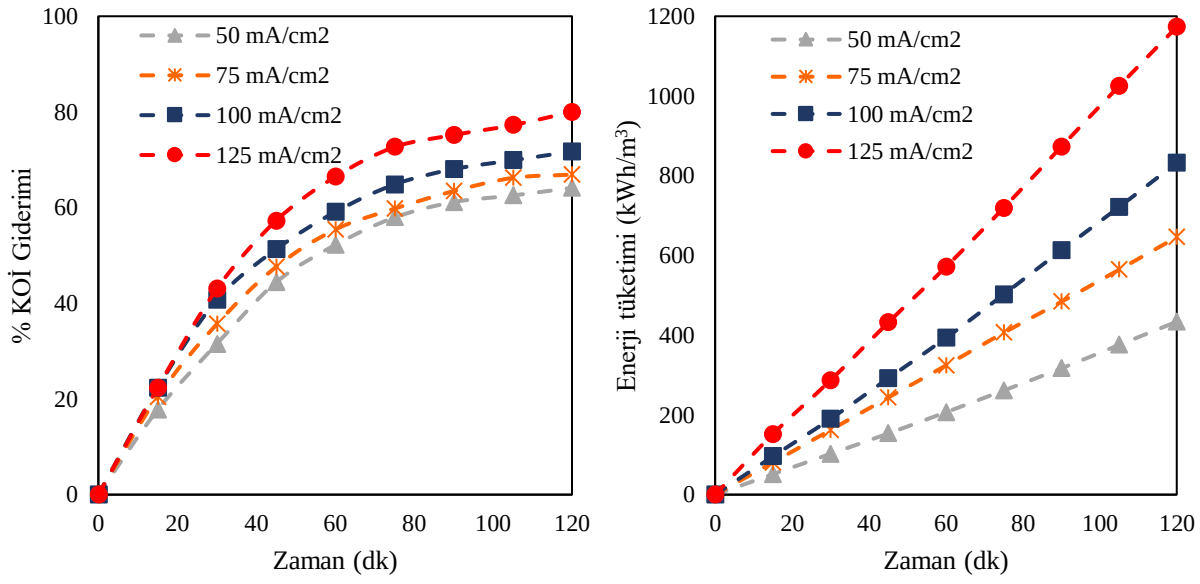
Bu çalışmada 2,4-D Isooctyl ester herbisitinin MMO elektrooksidasyon yöntemi kullanılarak model atıksudan giderimi araştırılmıştır. Elektrooksidasyon yöntemine etki eden parametreler ve bu parametrelerin giderim verimine etkisi incelenmiştir. Elektrooksidasyon çalışmaları 300 ml numune hacminde, 200 rpm karıştırma hızında, MMO elektrotlar kullanılarak gerçekleştirilmiştir. MMO elektrot demetleri 1 mm kalınlığında 5x5 cm olarak boyutlandırılmış 2 adet plakadan oluşmaktadır. Elektrooksidasyon çalışmaları basit kesikli reaktörde gerçekleştirilmiştir. Çalışma düzeneği güç kaynağı (Statron, Type 3262), multimetre (Fluke, 26-III), pH metre (Thermo Scientific, Orion STAR A215) ve manyetik karıştırıcıdan (Heidolph, MR3001) oluşmaktadır. Çalışma başlangıcında (t=0) ve çalışmalar süresince belirli aralıklarla reaktörden numuneler alınmış ve bu numunelerde KOİ analizleri TS 2789 Su kalitesi – Kimyasal Oksijen İhtiyacı Tayini kodlu Türk Standardına göre gerçekleştirilmiştir. Her sistem için akım yoğunluğu, destek elektrolit türü, miktarı ve başlangıç pH' ı gibi parametrelerin sistem verimine etkileri incelenmiş ve en yüksek giderim verimi elde edilen şartlar belirlenmiştir. Belirlenen optimum şartlarda 2,4 D Isooctyl ester herbisitinin toksisitesinin belirlenmesi amacıyla sitotoksisite çalışmaları yapılmıştır.

## BULGULAR ve TARTIŞMA

MMO elektrotlarla gerçekleştirilen elektrooksidasyon çalışmalarında akım yoğunluğunun etkisi (50, 75, 100 ve 125 mA/cm$^2$), destek elektrolit türü ve miktarının etkisi (5, 10, 15 mM $NaNO_3$ ve 5, 10, 15 mM $Na_2SO_4$) ve başlangıç pH'ının (3, doğal pH ve pH 9) sistem verimine etkisi incelenmiştir.

### Akım yoğunluğunun sistem verimine etkisi

MMO elektrotlarla gerçekleştirilen elektrooksidasyon çalışmalarında akım yoğunluğunun sistem verimine etkisi, 5 mM $NaNO_3$ destek elektrolit ilavesi ile 50, 75, 100 ve 125 mA/cm$^2$ akım yoğunluğunda incelenmiştir. Akım yoğunluğunun sistem verimine etkisinin incelenmesine ait giderim ve enerji tüketim grafikleri Şekil 1'de verilmiştir.




**(a)** **(b)**

**Şekil 1.** (a) Akım yoğunluğunun %KOİ giderimine etkisi ($C_0$=500 mg/L 2,4-D Isooctyl ester, 5mM $NaNO_3$, doğal pH), (b) Akım yoğunluğunun enerji tüketimine etkisi

50, 75, 100 ve 125 mA/cm$^2$ akım yoğunluğunda gerçekleştirilen çalışmalarda zamana bağlı %KOİ giderimi sırasıyla %64,14, %66,90, %71,72 ve %80 olarak ve enerji tüketimi sırasıyla 433,76, 645,87, 832,48 ve 1173,04 kWh/m$^3$ olarak bulunmuştur.

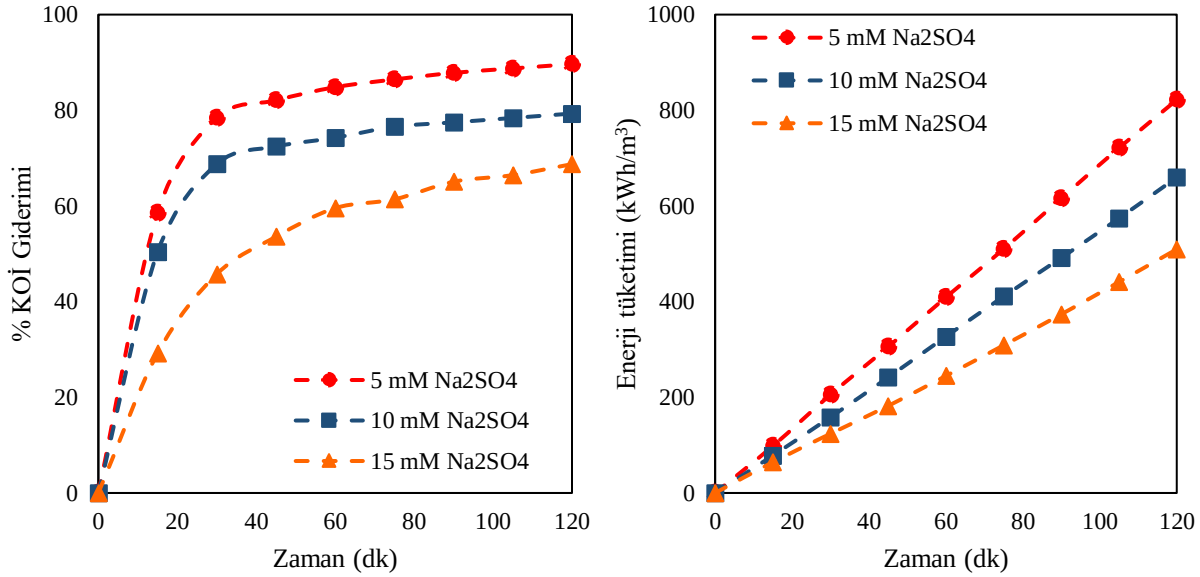
**Destek elektrolit türü ve miktarının sistem verimine etkisi**

Destek elektrolit türü ve miktarının sistem verimine etkisi, 5 mM, 10 mM ve 15 $NaNO_3$; 5 mM, 10 mM ve 15 $Na_2SO_4$ destek elektrolit ilavesi ile 125 mA/cm$^2$ akım yoğunluğunda incelenmiştir.

$NaNO_3$ destek elektrolitinin sistem verimine etkisi, 5 mM, 10 mM ve 15 $NaNO_3$ destek elektrolit ilavesi ile 125 mA/cm$^2$ akım yoğunluğunda gerçekleştirilen çalışmalarda zamana bağlı %KOİ giderimi sırasıyla %80, %68,74 ve %50,34 olarak ve enerji tüketimi sırasıyla 1173,04, 869,23 ve 667,14 kWh/m$^3$ olarak bulunmuştur.

$Na_2SO_4$ destek elektrolitinin sistem verimine etkisi, 5 mM, 10 mM ve 15 $Na_2SO_4$ destek elektrolit ilavesi ile 125 mA/cm$^2$ akım yoğunluğunda gerçekleştirilen çalışmalarda zamana bağlı %KOİ giderimi sırasıyla %89,66, %79,31 ve %68,74 olarak ve enerji tüketimi sırasıyla 821,71, 659,59 ve 509,46 kWh/m$^3$ olarak bulunmuştur.

$Na_2SO_4$ destek elektrolitinin sistem verimine etkisinin incelenmesine ait grafikler Şekil 2'de verilmiştir.




**(a)** **(b)**

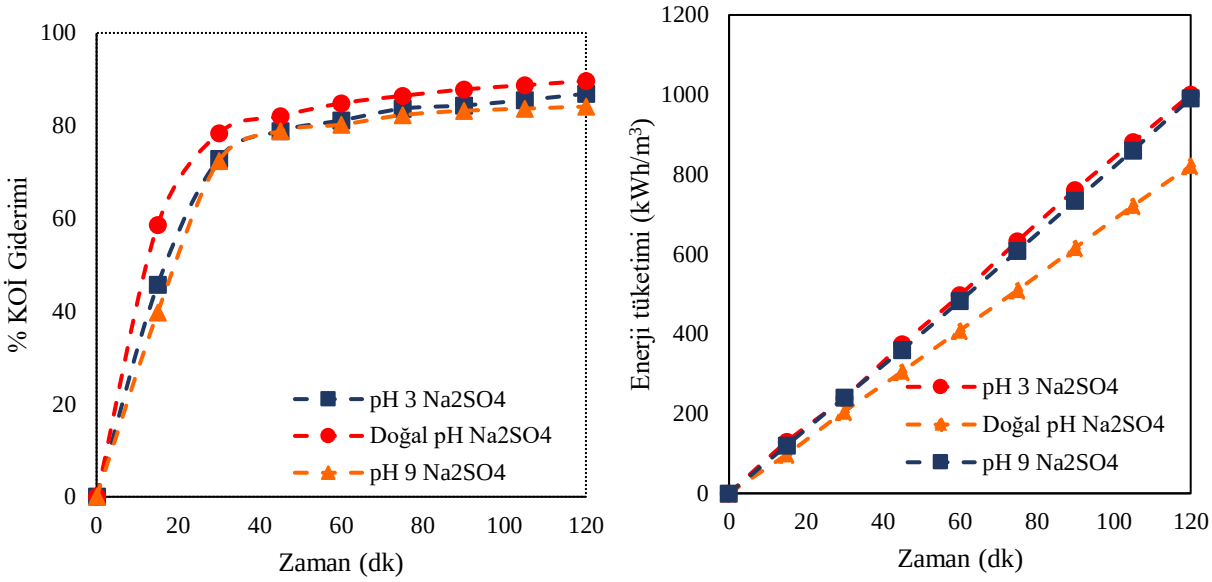
**Şekil 2.** (a)$Na_2SO_4$ destek elektrolitinin %KOİ giderimine etkisi ($C_0$=500 mg/L 2,4-D Isooctyl ester, i=125 mA/cm$^2$, doğal pH) (b)$Na_2SO_4$ destek elektrolitinin enerji tüketime etkisi

**Başlangıç pH'ının sistem verimine etkisi**

Başlangıç pH'ının sistem verimine etkisi, pH 3, doğal pH ve pH 9'da 5 mM $NaNO_3$ ve 5 mM $Na_2SO_4$ destek elektrolit ilavesi ile 125 mA/cm$^2$ akım yoğunluğunda incelenmiştir.

Başlangıç pH'ının sistem verimine etkisi, 5 mM $NaNO_3$ destek elektrolit ilavesi ile 125 mA/cm$^2$ akım yoğunluğunda gerçekleştirilen çalışmalarda zamana bağlı %KOİ giderimi sırasıyla %72,18, %80 ve %64,60 olarak ve enerji tüketimi sırasıyla 1119,74, 1173,04 ve 1251,66 kWh/m$^3$ olarak bulunmuştur.

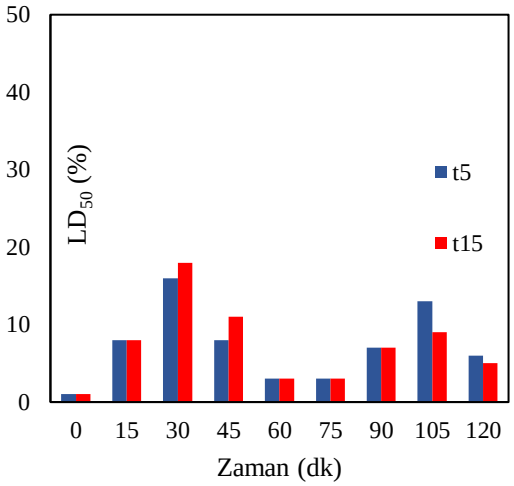
Başlangıç pH'ının sistem verimine etkisi, 5 mM $Na_2SO_4$ destek elektrolit ilavesi ile 125 mA/cm$^2$ akım yoğunluğunda gerçekleştirilen çalışmalarda zamana bağlı %KOİ giderimi sırasıyla %86,90, %89,66 ve %84,14 olarak ve enerji tüketimi sırasıyla 1000,26, 821,71 ve 990,94 kWh/m$^3$ olarak bulunmuştur. Başlangıç pH'ının 5 mM $Na_2SO_4$ destek elektrolit ilavesi ile sistem verimine etkisinin incelenmesine ait grafikler Şekil 3'te verilmiştir.




**(a)** **(b)**

**Şekil 3.** (a) Başlangıç pH'ının sistem verimine etkisi ($C_0$=500 mg/L 2,4-D Isooctyl ester, 125mA/cm$^2$, 5mM $Na_2SO_4$) (b) Başlangıç pH'ının enerji tüketimine etkisi

2,4 D Isooctyl ester herbisitinin MMO elektrotlarla gerçekleştirilen elektrooksidasyon çalışmalarında KOİ giderimi üzerinden belirlenen optimum koşullarda 120 dakikalık arıtım süresince 15 dakika aralıklarla alınan örneklerinde *V. fischeri* bakterisi üzerindeki toksik etkileri $LD_{50}$ derişimleri belirlenerek incelenmiştir. Şekil 4'te görüldüğü gibi arıtımla birlikte $LD_{50}$ derişimlerinin arttığı görülmektedir.




**Şekil 4.** 2,4 D Isooctyl ester- MMO elektrooksidasyon toksisitesi

**SONUÇ**

MMO elektrotlarla gerçekleştirilen elektrooksidasyon çalışmalarında 50, 75, 100 ve 125 mA/cm$^2$ akım yoğunluğunda gerçekleştirilen çalışmalarda zamana bağlı %KOİ giderimi sırasıyla %64,14, %66,90, %71,72 ve %80 olarak ve enerji tüketimi sırasıyla 433,76, 645,87, 832,48 ve 1173,04 kWh/m$^3$ olarak bulunmuştur. Akım yoğunluğunun artması ile KOİ gideriminde ve enerji tüketiminde büyük ölçüde artış görülmüştür. (Şekil 1).

$NaNO_3$ destek elektrolitinin sistem verimine etkisi, 5 mM, 10 mM ve 15 $NaNO_3$ destek elektrolit ilavesi ile 125 mA/cm$^2$ akım yoğunluğunda gerçekleştirilen çalışmalarda zamana bağlı %KOİ giderimi sırasıyla %80, %68,74 ve %50,34 olarak ve enerji tüketimi sırasıyla 1173,04, 869,23 ve 667,14 kWh/m$^3$ olarak bulunmuştur. $Na_2SO_4$ destek elektrolitinin sistem verimine etkisi, 5 mM, 10 mM ve 15 $Na_2SO_4$ destek elektrolit ilavesi ile 125 mA/cm$^2$ akım yoğunluğunda gerçekleştirilen çalışmalarda zamana bağlı %KOİ giderimi sırasıyla %89,66,

%79,31 ve %68,74 olarak ve enerji tüketimi sırasıyla 821,71, 659,59 ve 509,46 kWh/$m^3$ olarak bulunmuştur. Destek elektrolit miktarının artmasıyla KOİ gideriminde azalma olduğu her iki destek elektrolit türünde gözlenmiştir. Destek elektrolit miktarının artmasıyla enerji tüketiminde görülen düşüş her iki destek elektrolit türünde görülmüştür. Destek elektrolit türünün incelenmesinde $Na_2SO_4$ destek elektrolitinin daha yüksek KOİ giderimi sağlaması ve daha düşük enerji tüketimi göstermesi nedeniyle daha avantajlı olduğu gözlenmiştir.
Başlangıç pH'ının sistem verimine etkisi, 5 mM $NaNO_3$ destek elektrolit ilavesi ile 125 mA/$cm^2$ akım yoğunluğunda gerçekleştirilen çalışmalarda zamana bağlı %KOİ giderimi sırasıyla %72,18, %80 ve %64,60 olarak ve enerji tüketimi sırasıyla 1119,74, 1173,04 ve 1251,66 kWh/$m^3$ olarak bulunmuştur. Başlangıç pH' ının sistem verimine etkisi incelendiğinde doğal pH değerinde gerçekleştirilen çalışmalarda en yüksek giderim verimi elde edilmiştir. Enerji tüketimi açısından incelendiğinde tüm pH değerlerinin enerji tüketimlerinin yakın olduğu görülmüştür. Böylelikle sistem pH' ının ayarlanmasına gerek olmadığı sonucuna varılmıştır.
MMO elektrotlarla gerçekleştirilen elektrooksidasyon çalışmalarında Şekil 4'te görüldüğü gibi arıtımla birlikte $LD_{50}$ derişimlerinin dalgalanarak arttığı görülmektedir. Gözlemlenen bu dalgalanmaların elektrooksidasyon çalışmalarında oluşabilecek muhtemel toksik yan reaksiyonlar ve toksik yan ürünlerden kaynaklanabileceği düşünülmektedir.
Elde edilen sonuçlar doğrultusunda 2,4-D Isooctyl ester herbisitinin model atıksudan MMO-elektrooksidasyonu ile etkin bir şekilde giderilebildiği görülmüştür.

## TEŞEKKÜR

Bu çalışma Eskişehir Teknik Üniversitesi BAP Komisyonu tarafından kabul edilen 1704F104 no.'lu proje kapsamında desteklenmiştir.

## KAYNAKLAR

Bruggen B.V., Schaep J., Maes W., Wilms D. ve Vandecasteele C. (1998). Nanofiltration as a treatment method for the removal of pesticides from ground waters. Desalination, 117,139-147.

Huang X., He J., Sun J., Pan J., Sun X.ve Li S. (2007). Isolation and characterization of a metsulfuron-methyl degrading bacterium *Methylopila sp*. S113. International Biodeterioration & Biodegradation, 60, 152–158.

Oller I., Gernjak W., Maldonado M.I., Estrada L.A.P, (2006). Solar photocatalytic degradation of some hazardous water-soluble pesticides at pilot-plant scale. Journal of Hazardous Materials B138, 507–517.

Rodrigo M. A., Oturan N. ve Oturan M. A. (2014). Electrochemically assisted remediation of pesticides in soils and water: a review. Chemical Reviews, 114, 8720−8745.

Sağlam H. (2008). Melen Havzasında Pestisit Uygulamaları ve Pestisitlerin Biyolojik Bozunma, Yüzeysel Akış ve Sızma Yüzdelerinin Tahmini, Doktora tezi, İstanbul Teknik Üniversitesi, Istanbul.

Solmaz S.K.A., Azak H., Üstün G.E. ve Morsünbül T. (2010). Pestisit gideriminde fenton proseslerinin kullanımına yönelik bir envanter çalışması. Uludağ Üniversitesi Mühendislik-Mimarlık Fakültesi Dergisi, 15(1), 179-194.

Polat, A. (2013). Su kaynaklarının sürdürülebilirliği için arıtılan atıksuların yeniden kullanımı. Türk Bilimsel Derlemeler Dergisi, (1), 58-62.

Yadav I.C., Devi N.L., Syed J.H., Cheng Z., Li J., Zhang G. ve Jones K.C. (2015). Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: a comprehensive review of India. Science of The Total Environment, 511, 123–137.

*ORAL PRESENTATION*

# Tekstil endüstrisi atıksularından katyonik nonwoven adsorban ile boyarmadde giderimi

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## Özet

Tekstil prosesleri sonucu oluşan boyarmadde içerikli atıksuların doğrudan alıcı ortama deşarjı çevre ve insan sağlığı açısından önemli sorunlar oluşturmaktadır. Bu çalışma kapsamında katyonik nonwoven tekstil malzemesinin endüstriyel prosesler sonucu açığa çıkan boyarmadde içerikli atıksulardan boyarmadde gideriminde adsorban olarak kullanılabilirliği araştırılmıştır. Adsorpsiyon etki eden parametreler araştırılmış ve boyarmadde giderim verimine etkisi incelenmiştir. Sıcaklık ile giderimin veriminin arttığı görülmüş ve doğal pH, 50 ºC'de 5 g adsorban ile %98,64 boyarmadde giderim verimi sağlanmıştır. Elde edilen veriler doğrultusunda katyonik nonwoven adsorpsiyonunun Langmuir izoterm modeline uygun olduğu görülmüştür.

**Anahtar Kelimeler:** Katyonik nonwoven tekstil malzemesi, atıksu arıtımı, adsorpsiyon, boyarmadde, Langmuir izotermi.

## Abstract

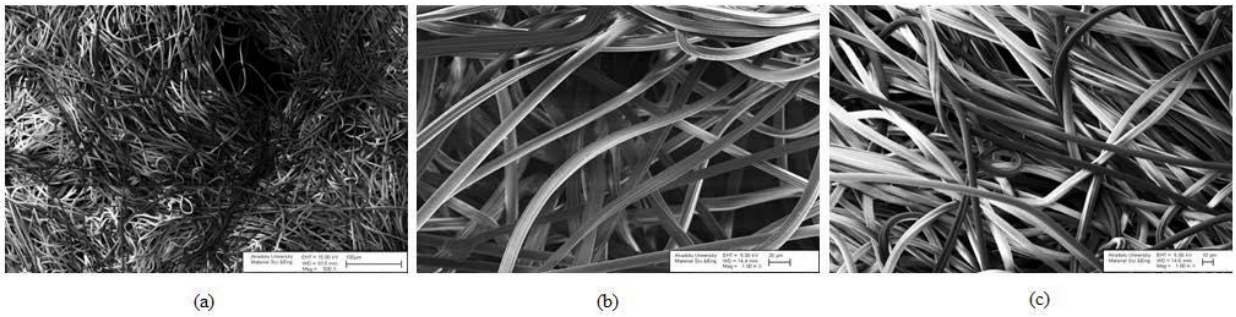
The direct discharge of dyestuff-containing wastewater, which is formed as a result of textile processes, to the receiving environment can cause serious problems in terms of the environment and human health. In this study, the usability of cationic nonwoven textile material as an adsorbent in the removal of dyestuffs from the dyestuff-containing wastewater produced as a result of industrial processes was investigated. The effects of the parameters affecting the adsorption on the dye removal efficiency were investigated. It was observed that the removal efficiency increased with temperature and dyestuff removal efficiency was 98.64% achieved with 5 g adsorbent at natural pH, 50 ºC. According to the data obtained, the cationic nonwoven adsorption was fit for the Langmuir isotherm model.

## GİRİŞ

Endüstriyel prosesler sonucu oluşan ve deşarj edilen boyarmadde içerikli atıksular, çevre ve insan sağlığı açısından önemli sorunlar oluşturmaktadır (Mittal ve ark.,2009; Attia ve ark., 2008). Üretim çeşitliliği ve miktarının artmasının sonucu, endüstriyel atıksularda çeşitli tür ve miktarlarda kompleks olan yapılar meydana gelmektedir (Alinsafi ve ark., 2007). Tekstil endüstrisinde, boyama ve apreleme proseslerinden kaynaklanan atıksuların hacmi ve kirletici içerikleri göz önüne alındığında, bu atıksuların arıtımının gerekliliği ön plana çıkmaktadır. Tekstil proseslerinde yoğun su kullanımı, boyarmadde fiksasyonu için kullanılan tuzlar, boyarmaddelerin ağır metal içeriği ve fikse olmayan yüksek dozlardaki boyarmaddelerin atıksuya karışması tekstil endüstrisi proseslerini çevresel açıdan oldukça önemli kılmaktadır (Yagub ve ark.,2014; Al-Ghouti ve ark., 2003). Atıksulardaki boyarmadde türüne bağlı olarak boyarmadde giderim veriminin farklılık göstermesi, atıksulardan boyarmadde giderimi için en uygun yöntemin seçimini zorlaştırmaktadır. Bu atıksuların arıtımında adsorpsiyon diğer arıtım yöntemlerine kıyasla maliyetinin düşük olması ve yüksek giderim verimliliği sağlaması gibi avantajlarından dolayı tercih edilmektedir (Singh ve ark., 2011; Crini, 2006). Bu çalışmada mevcut adsorbanlara alternatif olarak endüstriyel atıksulardan boyarmadde gideriminde adsorban olarak katyonik nonwoven tekstil malzemesinin kullanılabilirliği araştırılmıştır. Maliyeti düşük ve kullanımı kolay olan katyonik nonwoven tekstil malzemesinin etkinliğinin belirlenmesinde adsorpsiyona etki eden parametreler ve Langmuir izoterm modeli incelenmiştir. Bu çalışmada etkinliği belirlenen ürünün boyarmadde giderimiyle ilgili çevre sorunları için ekonomik bir çözüm niteliğinde olacağı ve sürdürülebilir çevre kavramına katkıda bulunacağı düşünülmüştür.

## MATERYAL VE METOT

Katyonik nonwoven ile gerçekleştirilen adsorpsiyon çalışmaları kesikli sistemde gerçekleştirilmiştir. Çalışmalarda tekstil endüstrisi boyama prosesinden çıkan gerçek atıksu gerekli seyreltmeler yapılarak kullanılmıştır. Katyonik nonwoven tekstil malzemesi EGD Tekstil A.Ş., Türkiye'den temin edilmiştir. Katyonik nonwoven tekstil malzemesine ait SEM görüntüleri Şekil 1'de verilmiştir.




**Şekil 1.** Katyonik nonwoven malzemesinin SEM görüntüleri (a) 500X, (b) 1.00KX ve (c) 1.00KX

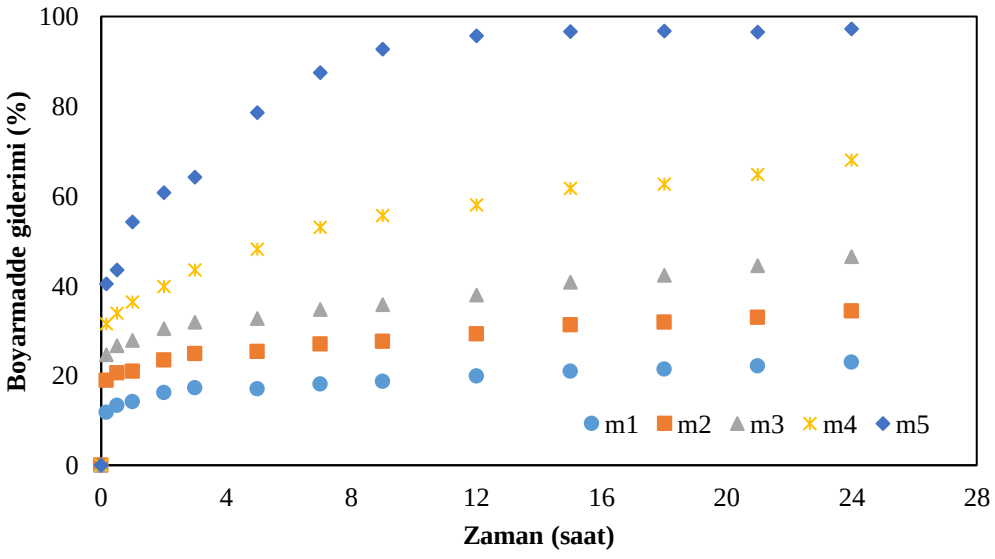
Çalışma kapsamında adsorpsiyona etki eden parametreler olarak adsorban miktarı, atıksu başlangıç pH'ı ve sıcaklığın etkisi incelenmiştir. Kesikli sistemde gerçekleştirilen çalışmalar 100 ml atıksu hacminde, doğal pH'da (5,15) ve 125 rpm karıştırma hızında çalkalamalı inkübatör (Zhicheng ZHWY-2102C) kullanılarak 24 saatte gerçekleştirilmiştir. İzoterm çalışmaları farklı miktarlarda (1-5g) adsorban eklenerek belirtilen sıcaklıklarda çalkalamalı inkübatörde gerçekleştirilmiştir. Numuneler 506 nm dalga boyunda UV-Vis spektrofotometre (Shimadzu UV1700) kullanılarak analiz edilmiştir.

## BULGULAR ve TARTIŞMA

Katyonik nonwoven adsorban ile gerçekleştirilen çalışmalarda adsorban miktarının etkisi (1, 2, 3, 4 ve 5g), başlangıç atıksu pH'ının etkisi (3, doğal pH ve pH 10), ve sıcaklığın (10, 25 ve 50 ºC) sistem verimine etkisi incelenmiştir.

### Adsorban miktarının etkisi

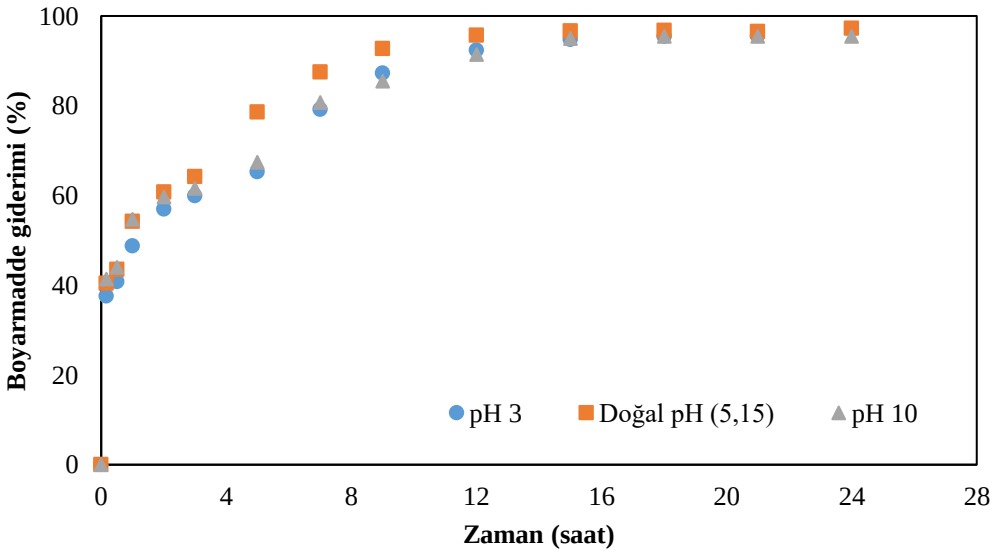
Adsorban miktarının sistem verimine etkisi 1-5 g nonwoven ilavesi ile 25 ºC ve doğal pH' da gerçekleştirilen çalışmalarda boyarmadde giderimleri sırasıyla %22,91, %34,37, %46,50, 67,96 ve %97,22 olarak hesaplanmıştır. Adsorban miktarının boyarmadde giderim verimine etkisinin incelendiği grafik Şekil 2'de verilmiştir.




**Şekil 2.** Adsorban miktarının boyarmadde giderim verimine etkisi

**Başlangıç pH'ının etkisi**

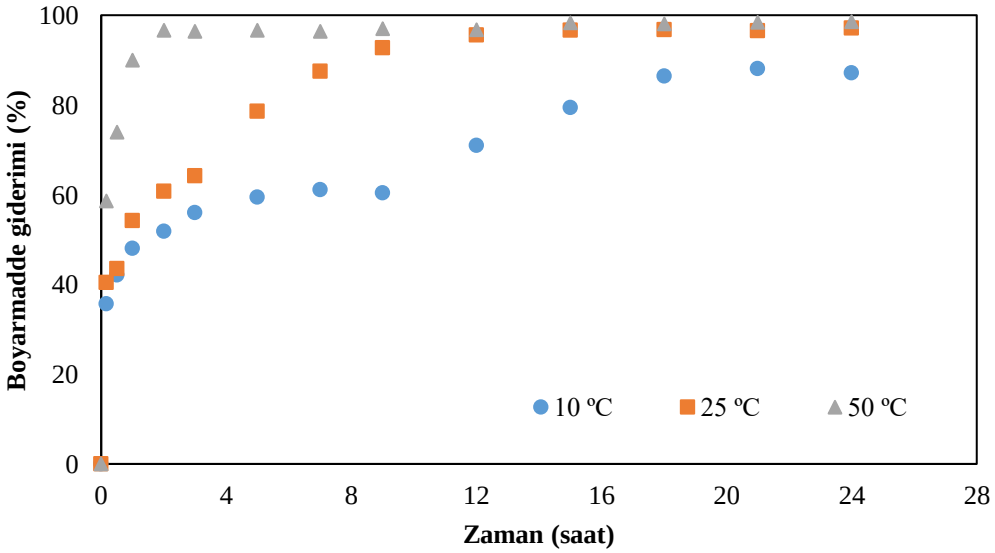
Atıksu başlangıç pH'ının sistem verimine etkisi 5 g nonwoven ilavesi ile 25 ºC'de ve pH 3, doğal pH ve pH 10'da gerçekleştirilen çalışmalarda boyarmadde giderimleri sırasıyla %95,77, %97,22 ve %95,52 olarak hesaplanmıştır. Başlangıç pH'ının giderim verimine etkisinin incelendiği grafik Şekil 3'te verilmiştir.




**Şekil 3.** Başlangıç pH'ının boyarmadde giderim verimine etkisi

**Sıcaklığın etkisi**

10, 25 ve 50°C sıcaklıklarında 5 g adsorban ilavesi ile doğal pH'da gerçekleştirilen çalışmalarda boyarmadde giderim verimleri sırasıyla %87,22, %97,22 ve %98,64 olarak bulunmuştur. Sıcaklığın sisteme etkisi gösteren boyarmadde giderim verimine ait grafik Şekil 4'te verilmiştir.




**Şekil 4.** Sıcaklığın boyarmadde giderim verimine etkisi

**Adsorption izotermi**

Adsorpsiyon izotermleri, adsorpsiyon prosesinin analiz ve dizayn mekanizmasının anlaşılabilmesi açısından oldukça önemli rol oynamaktadır. Nonwoven adsorpsiyonunun Langmuir izoterm modeline uygunluğu adsorpsiyona ait denge verileri kullanılarak incelenmiştir. Langmuir izoterm modeline ait grafik Şekil 5'te verilmiştir.

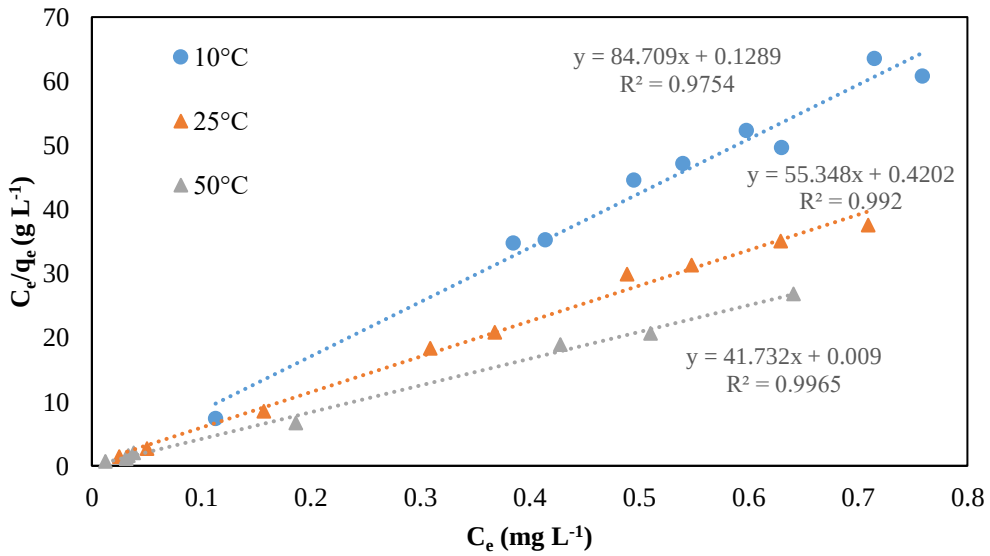
Langmuir eşitliğinin doğrusallaştırılmasıyla Eşitlik 1 elde edilir (Chen ve ark.,2010; Foo ve Hameed, 2010).

$$\frac{C_e}{q_e}=\frac{C_e}{q_m}+\frac{1}{K_L q_m} \quad (1)$$

Burada;

$q_m$: Maksimum adsorpsiyon kapasitesi (mg/g)

$K_L$: Langmuir adsorpsiyon sabiti (L/mg)'dır.




**Şekil 5.** Langmuir izoterm modeli

Langmuir izoterm modelinde ait Ce/qe 'ye karşı $C_e$ grafiği Şekil 5'te gösterilmiştir. Langmuir izoterm sabitleri olan $q_m$ ve $K_L$ değerleri çizilen bu grafikten hesaplanmış ve 10, 25 ve 50°C sıcaklıkları için hesaplanan maksimum adsorpsiyon kapasiteleri 0,0118 mg/g, 0,0181 mg/g, 0,0240 mg/g ve $K_L$ değerleri 657,17 L/mg, 131,72 L/mg, 4636,89 L/mg olarak bulunmuştur. Korelasyon katsayılarının 10, 25 ve 50°C sıcaklıkları için sırasıyla 0.9754, 0.992 ve 0.9965 olması Langmuir izoterm modeline uygunluğu ifade etmektedir.
Langmuir izoterminin esas karakteristiği denge faktörü olan $R_L$ gibi boyutsuz denge parametresi ile ifade edilmektedir.

$$R_L=\left(\frac{1}{1+K_L C_0}\right) \quad (2)$$

$R_L$ değerinin 1'den büyük çıkması durumunda adsorpsiyon prosesi elverişsiz, 1'e eşit olması durumunda lineer, 0 ile 1 arasında bir değer olması durumunda elverişli ve 0 olması durumunda ise tersinmez olmaktadır (Foo ve Hameed, 2010). 10, 25 ve 50°C sıcaklıkları için Eşitlik 2 kullanılarak hesaplanan $R_L$ değerleri sırasıyla

0,0017, 0,0085 ve 0,0002 ve bu değerlerin 0 ile 1 arasında olması adsorpsiyonun elverişli olduğunu göstermektedir.

## SONUÇ

Tekstil endüstrisinden kaynaklı boyarmadde içerikli atıksuların arıtımı tekstil sektörü için önemli bir konudur. Bu atıksuların arıtımı için etkin ve ekonomik giderim yöntemlerinin geliştirilmesine ihtiyaç duyulmaktadır. Bu çalışma kapsamında mevcut yöntemlere alternatif ve ekonomik boyarmadde tutabilen katyonik nonwoven tekstil malzemesinin etkinliği incelenmiştir. Adsorpsiyona etki eden parametreler olarak adsorban miktarının, başlangıç pH'ının ve sıcaklığın etkisi kesikli sistemde incelenmiştir. Adsorban miktarının etkisi incelendiğinde 1-5 g nonwoven ilavesi ile 25 ºC ve doğal pH' da gerçekleştirilen çalışmalarda boyarmadde giderimleri sırasıyla %22,91, %34,37, %46,50, 67,96 ve %97,22 olarak bulunmuştur. Elde edilen veriler doğrultusunda adsorban miktarının artması ile boyarmadde giderim veriminin arttığı görülmektedir. Atıksu başlangıç pH'ının sistem verimine etkisi incelendiğinde doğal pH'da gerçekleştirilen çalışmalarda giderim veriminin en yüksek olduğu görülmektedir. Bu sebeple atıksuyun pH'ının ayarlanmasına gerek olmadığı görülmüştür. Sıcaklığın sistem verimine etkisi incelendiğinde sıcaklık ile giderimin veriminin arttığı görülmüş ve doğal pH, 50 ºC'de 5 g adsorban ile %98,64 boyarmadde giderim verimi sağlanmıştır. Katyonik nonwoven adsorpsiyonunun Langmuir izoterm modellerine uygunluğu incelenmiş ve yüzeyde adsorplanan moleküllerin tek tabaka halinde adsorplandığı Langmuir izoterm modeline uygun olduğu görülmüştür.

## KAYNAKLAR

Al-Ghouti, M. A., Khraisheh, M. A. M., Allen, S. J. ve Ahmad, M. N. (2003). The removal of dyes from textile wastewater: a study of the physical characteristics and adsorption mechanisms of diatomaceous earth. Journal of environmental management, 69(3), 229-238.

Alinsafi, A., Evenou, F., Abdulkarim, E. M., Pons, M. N., Zahraa, O., Benhammou, A. ve Nejmeddine, A. (2007). Treatment of textile industry wastewater by supported photocatalysis. Dyes and Pigments, 74(2), 439-445.

Attia, A. A., Girgis, B. S. ve Fathy, N. A. (2008). Removal of methylene blue by carbons derived from peach stones by $H_3PO_4$ activation: batch and column studies. Dyes and pigments, 76(1), 282-289.

Chen, S., Zhang, J., Zhang, C., Yue, Q., Li, Y. ve Li, C. (2010). Equilibrium and kinetic studies of methyl orange and methyl violet adsorption on activated carbon derived from *Phragmites australis*. Desalination, 252(1-3), 149-156.

Crini, G. (2006). Non-conventional low-cost adsorbents for dye removal: a review. Bioresource technology, 97(9), 1061-1085.

Foo, K. Y. ve Hameed, B. H. (2010). Insights into the modeling of adsorption isotherm systems. Chemical engineering journal, 156(1), 2-10.

Mahmoodi, N. M., Hayati, B., Arami, M. ve Lan, C. (2011). Adsorption of textile dyes on pine cone from colored wastewater: kinetic, equilibrium and thermodynamic studies. Desalination, 268(1-3), 117-125.

Mittal, A., Mittal, J., Malviya, A. ve Gupta, V. K. (2009). Adsorptive removal of hazardous anionic dye "Congo red" from wastewater using waste materials and recovery by desorption. Journal of colloid and interface science, 340(1), 16-26.

Singh, K. ve Arora, S. (2011). Removal of synthetic textile dyes from wastewaters: a critical review on present treatment technologies. Critical reviews in environmental science and technology, 41(9), 807-878.

Yagub, M. T., Sen, T. K., Afroze, S., & Ang, H. M. (2014). Dye and its removal from aqueous solution by adsorption: a review. Advances in colloid and interface science, 209, 172-184.

## *ORAL PRESENTATION*

# Nar kabuğu biyokütlesinin torefikasyon özelliklerinin incelenmesi

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## Özet

Küresel olarak fosil kaynaklı yakıtlara alternatif sürdürülebilir ve yenilenebilir enerji kaynaklarının arayışı her geçen gün artmaktadır. Enerji üretiminde fosil kaynaklı olmayan ve $CO_2$ nötr hammaddeler daha fazla kullanılmalıdır. Bu amaçla kullanılabilecek malzemeler olarak biyokütleler karşımıza çıkmaktadır. Tarımsal ve endüstriyel faaliyetler sonucu oluşan atık biyokütleler torefikasyon prosesi sonrasında yakıt veya adsorbent olarak kullanılabilmektedir. Isparta ilinde yoğun meyve üretimi neticesinde oldukça fazla meyve atığı ortaya çıkmaktadır. Bu sebeple bu çalışmada tarımsal biyokütle olarak nar kabuğu seçilmiştir. Nar kabuğu torefikasyon işlemi 60 dakika çalışma süresinde ve 250 °C, 260 °C, 270 °C, 280 °C ve 290 °C olmak üzere beş farklı sıcaklıkta gerçekleştirilmiştir. Nar kabuğunun beş farklı sıcaklıkta torefiye edilmesinden sonra sabit karbon içeriği sırasıyla % 8,82, % 15,47, % 9,71, % 5,30 ve % 8,66 olarak tespit edilmiştir. Nar kabuğu numunesinin torefikasyonunda karbon içeriği en yüksek 260 °C torefikasyon sıcaklığında elde edilmiştir.

**Anahtar Kelimeler:** Biyokütle, nar kabuğu, torefikasyon

## Abstract

Globally, investigation for alternative sustainable and renewable energy sources to fossil fuels is increasing day by day. In energy production, non-fossil and $CO_2$ neutral raw materials should be mostly used. Biomass could be used for this purpose. Waste biomass formed as a result of agricultural and industrial activities can be used as fuel or adsorbent after the torrefaction process. As a result of intensive fruit production in Isparta province, large amount of fruit waste occurs. For this reason, pomegranate peel was chosen as the agricultural biomass in this study. The pomegranate peel torrefaction process was carried out at five different temperatures, 250 °C, 260 °C, 270 °C, 280 °C and 290 °C, for 60 minute. After the pomegranate peel was torrefied at five different temperatures, the fixed carbon content was determined as 8.82%, 15.47%, 9.71%, 5.30% and 8.66%, respectively. In the torrefaction of the pomegranate peel sample, the carbon content was obtained at the highest torrefaction temperature of 260 °C.

**Keywords:** Biomass, pomegranate peel, torrefaction

## GİRİŞ

Dünya enerji gereksinimini öncelikli olarak fosil yakıtlardan karşılamaktadır (Safar ve ark., 2019). Fosil kaynaklı yakıtların kullanılması atmosfere yüksek miktarlarda sera gazlarının yayılmasına yol açmaktadır (Tumuluru ve ark., 2011; Acharya ve ark., 2015). Bu nedenle küresel olarak fosil kaynaklı yakıtlara alternatif sürdürülebilir ve yenilenebilir enerji kaynaklarının arayışı her geçen gün artmaktadır. Sera gazlarının emisyonunu azaltmak için enerji üretiminde fosil kaynaklı olmayan ve $CO_2$ nötr hammaddelerin oranının arttırılması gereklidir (Cahyanti ve ark., 2020). 'Karbon nötr' ya da 'net sıfır' ifadeleri insan aktiviteleri nedeniyle atmosfere salınan $CO_2$'in denk bir miktarının giderilmesi anlamına gelmektedir (International Energy Agency, 2020). Biyokütle, yenilenebilir doğası ve $CO_2$'nin kapalı bir döngüde dolaşımı nedeniyle böyle bir malzemedir (Eia, 2020). Biyokütle, global enerjinin % 10-15'ini karşılamaktadır (Chandra ve ark., 2012; Siddiqui ve ark., 2019). Biyokütle, bitkilerden ve hayvanlardan elde edilen organik maddelerdir ve yenilebilir enerji kaynağı olarak kullanılabilir. Bitkiler fotosentez prosesi ile güneşten aldıkları radyasyon enerjisini glikoz ya da şeker olarak kimyasal enerji olarak depolar.

TÜİK Bitkisel Üretim İstatistikleri verilerine göre ülkemizde 2021 yılı nar üretimi 647676 ton olarak gerçekleşmiştir (TUİK, 2022). En fazla nar üretimi yapılan bölgelerimiz ise iklim özellikleri narın yetişmesine uygun olan Akdeniz, Ege ve Güneydoğu Anadolu Bölgesi'dir (Şahin, 2020). Her geçen gün faydalı özellikleri nedeniyle nar ürünlerinin üretimi artmakta ve üretim sonrasında önemli oranda atık nar yan ürünleri oluşmaktadır (Okumuş, 2016). Torefikasyon 200-300 °C aralığında inert bir ortamda 15-60 dakika süreyle yürütülen, biyokütlenin termokimyasal dönüşümünü sağlayan bir ön arıtım metodudur. Bu metotla, biyokütle kalitesi artırılırken fiziksel ve kimyasal sorunlar önlenmiş olur (Bates ve Ghoniem, 2012). Torefiye biyokütle ham biyokütle ile karşılaştırıldığında daha parlak, kırılgan, öğütülmesi daha kolay ve az enerji gerektiren hidrofobik bir yapıya ulaşır ve daha iyi bir yakıt karakteristiği sergiler. Bu özellikleri ile ham biyokütlenin aksine depolanması ve taşınması kolaylaşmaktadır (Btg, 2020). Bu amaçlar doğrultusunda, bu çalışmada nar kabuğu biyokütlesinin torefikasyon özellikleri incelenmiştir.

## MATERYAL VE METOT

### Nar kabuğu temini ve ön işlemler

Bu çalışmada biyokütle olarak nar kabuğu atığı incelenmiştir. Nar kabuğu seçilmesinin sebebi, Akdeniz Bölgesi'nde nar üretiminin yaygın olması ve nar ve buna bağlı olarak yüksek miktarlarda atık nar kabuğu ortaya çıkması sebebiyledir. Bununla birlikte, ülkemizde nar kabuğunun torefikasyon çalışmalarında daha önce kullanılmamış olması da bu çalışmanın orijinalliğini ortaya koymaktadır. Nar kabuğu (Şekil 1) sezonunda üreticilerden temin edilerek, laboratuar ortamında doğal kurutmaya bırakılmıştır.



**Şekil 1** Kurutulan nar kabukları

Deneysel çalışmalarda kullanmak üzere temin edilen nar kabuğu biyokütlesi, Isparta il merkezinde bulunan değirmende öğütülmüştür. Öğütülen biyokütleden 500 g'lık temsili numuneler alınmış ve 500 µm elek (Retsch) ile elenmiştir. 500 µm elek altında biyokütle, kuru olmasına rağmen nem içerebileceği göz önüne alınarak etüvde (FN 500, Nüve) 60 °C'de 24 saat kurutulmuştur. Bu makalenin ilerleyen bölümlerinde kolay takip edilebilmesi amacıyla ham nar kabuğu numunesi (HNK), torefiye nar kabuğu numunesi (TNK) olarak kısaltılmıştır.

### Torefikasyon çalışmaları

Bu makale kapsamında torefikasyon çalışmaları 250, 260,270, 280 ve 290 °C olmak üzere beş farklı sıcaklıklarda incelenmiştir. Torefikasyon çalışmalarında 50 $cm^3$ 'lük kapaklı seramik krozeler kullanılmıştır. 2 gram HNK biyokütlesi folyo içerisine sarılıp, krozelerin içine hava almayacak şekilde yerleştirilmiş ve krozelerin kapakları kapatılarak tekrardan folyoya sarılmıştır. Kül fırınına (MF 110, Nüve) (Şekil 2) yerleştirilerek torefikasyon işlemi gerçekleştirilmiştir.



**Şekil 2** Kül fırınında torefiye edilen HNK numuneleri

Torefikasyon işlemi 60 dakika çalışma süresinde ve 250 °C, 260 °C, 270 °C, 280 °C ve 290 °C olmak üzere beş farklı sıcaklıkta gerçekleştirilmiştir (Gan ve ark., 2018). Tüm torefikasyon çalışmaları üçlü olarak yürütülmüştür. Torefiye edilen malzemeler, nem almaması için kilitli poşetlere konularak desikatörde saklanmıştır. Torefiye edilen tüm TNK malzemesinin proksimit (yaklaşık, kısa) analizleri; nem analizi, uçucu madde analizi, kül analizi yapılarak ve karbon içeriği hesaplanarak belirlenmiştir.

**Analizler**

**Nem miktarı**

Torefiye edilen numunenin nem içerikleri ASTM E-871-82 standardına göre yapılmıştır. Krozelere yaklaşık 2 gram numune konularak, numuneler 103±2 °C'ye getirilen etüvde 3 saat bekletilmiştir. 3 saatin sonunda krozeler çıkarılıp desikatörde soğumaya bırakılmıştır. Soğuyan numunelerin tartımı yapılıp, Denklem 1'den yararlanılarak nem miktarı hesaplanmıştır.

$$\%\, Nem = \frac{HN-NN}{HN}\, x\, 100 \quad (1)$$

HN: Numunelerin ilk tartım ağırlığı (g)

NN: Numunelerin103 °C'de kurutulmasından sonraki (kuru) ağırlığı (g)

**Uçucu madde miktarı**

Torefiye edilen numunenin uçucu madde içeriklerinin belirlenebilmesi için ASTM E-872 standardı kullanılmıştır. 2 g örnek alınarak üstü kapalı bir krozeye yerleştirildikten sonra 950 °C'de 7 dakika kül fırınında (Nüve, MF 140) tutulmuştur. İşlem süresinin sonunda çıkarılan numuneler desikatörde soğumaya bırakılmış, soğuma işlemi tamamlanmış numuneler tartılmıştır ve Denklem 2 kullanılarak uçucu madde miktarları belirlenmiştir.

$$\%\, Uçucu\ Madde = \frac{HN-UN}{HN} - \%\, nem\ x\ 100 \quad (2)$$

HN: Numunelerin ilk tartım ağırlığı (g)

UN: Numunelerin 950 °C'de ısıtma sonrası ağırlığı (g)

**Kül miktarı**

Yaklaşık 2 g olarak tartılan numuneler ASTM D 1102-84 standardına göre 1 saat süresince 600 °C'de kül fırınında bekletilmiştir. İşlem sonrası numuneler desikatörde soğutulmuş, soğuma işlemi tamamlandıktan sonra tartım işlemi yapılarak, Denklem 3 yardımı ile numunelerin kül miktarları belirlenmiştir.

$$\%\, Kül\ = \frac{HN-KN}{HN} x\ 100 \quad (3)$$

HN: Numunelerin ilk tartım ağırlığı (g)

KN: Numunelerin 600 °C'de ısıtma sonrası ağırlığı (g)

**Sabit karbon içeriği**

Numunelerin sabit karbon içeriği (sabit C) Denklem 4 yardımıyla hesaplanmıştır (Taşar, 2021).

$$Sabit\ C\ (\%) = \ 100\ -\ [\%\ nem + \%\ uçucu\ madde + kül] \quad (4)$$

**Analitik metodlar**

HNK ve 270 °C'de torefiye edilen TNK numunesinin elementel analizi Leco TruSpec Micro cihazı ile Antalya Büyükşehir Belediyesi Hava Kirliliği Laboratuvarı ve Recep Tayyip Erdoğan Üniversitesi Merkezi Araştırma Laboratuvarı'nda (MERLAB) yapılmıştır.

**Bulgular ve Tartışma**
**Farklı çalışma sıcaklıklarında torefikasyon sonuçları**

Torefiye biyokütlelerin fizikokimyasal özelliklerinin iyileştirilmesi amacıyla, sıcaklık ve torefiye süresi gibi termal proses koşulları araştırılabilir veya torefiye edilmesi istenen farklı özelliklerde biyokütleler dikkate alınabilir (McCaffrey ve ark., 2021). Bu çalışmada, nar kabuğu biyokütlesinin farklı çalışma sıcaklıklarda torefikasyonu değerlendirilmiştir.

Çalışılan HNK biyokütlesi ile yapılan torefikasyon deneylerinde nem içeriği yaklaşık % 8,4 olarak bulunmuştur. Nar kabuğu, ağaç ve orman atığı, tarım atıklarından oluşan odunsu biyokütle olması sebebiyle, odunsu biyokütlelerin düşük nem içeriği, yüksek yoğunluk ve yüksek kalorifik değerlerine sahip oldukları bilinmektedir (Danish ve Ahmad, 2018). Biyokütlelerin düşük nem içeriklerine sahip olması, biyokarbon üretimi için daha az enerji ve daha az süre gerektirmesi sebebiyle avantaj oluşturmaktadır (Amalina ve ark., 2022).

HNK biyokütlesinin torefikasyon işlemi 60 dakika çalışma süresinde ve 250 °C, 260 °C, 270 °C, 280 °C ve 290 °C olmak üzere beş farklı sıcaklıkta gerçekleştirilmiştir (Gan ve ark., 2018). Ham nar kabuğu ve farklı sıcaklıklardaki torefikasyon işlemi sonrasındaki nar kabuğu numunelerinin yaklaşık analiz sonuçları Tablo 1'de verilmiştir.

**Tablo 1.** Farklı sıcaklıklarda torefikasyon sonuçları

| | Nem (%) | Uçucu madde (%) | Kül (%) | Sabit karbon (%) |
|---|---|---|---|---|
| Ham | 8,97 | 66,92 | 18,71 | 5,41 |
| | 8,92 | 66,97 | 16,80 | 7,32 |
| | 7,43 | 68,99 | 17,81 | 5,76 |
| Ortalama | 8,44±0,48 | 67,63±1,18 | 17,77±0,96 | 6,16±1,02 |
| 250 °C | 1,63 | 58,77 | 22,65 | 16,94 |
| | 1,85 | 57,43 | 36,31 | 4,41 |
| | 1,78 | 58,42 | 34,69 | 5,11 |
| Ortalama | 1,75±0,46 | 58,21±0,69 | 31,22±7,46 | 8,82±7,04 |
| 260 °C | 1,11 | 57,10 | 26,68 | 15,11 |
| | 1,30 | 55,36 | 25,37 | 17,96 |
| | 1,59 | 55,50 | 29,58 | 13,33 |
| Ortalama | 1,34±0,66 | 55,99±0,97 | 27,21±2,15 | 15,47±2,34 |
| 270 °C | 0,84 | 75,65 | 14,47 | 9,04 |
| | 1,25 | 66,93 | 20,83 | 10,98 |
| | 1,35 | 72,56 | 16,97 | 9,12 |
| Ortalama | 1,15±0,49 | 71,71±4,42 | 17,43±3,20 | 9,71±1,10 |
| 280 °C | 0,96 | 62,85 | 30,65 | 5,53 |
| | 1,68 | 67,45 | 25,97 | 4,90 |
| | 1,78 | 60,75 | 31,99 | 5,48 |
| Ortalama | 1,47±0,47 | 63,69±3,43 | 29,54±3,16 | 5,30±0,35 |
| 290 °C | 1,87 | 64,89 | 24,10 | 9,14 |
| | 2,04 | 63,70 | 25,30 | 8,96 |
| | 2,62 | 60,31 | 29,19 | 7,88 |
| Ortalama | 2,18±0,59 | 62,96±2,38 | 26,20±2,66 | 8,66±0,68 |

Ham nar kabuğunun yaş ağırlıkça nem içeriği % 61,42'dir (Tabloda verilmemiştir). Sharma ve ark. (2018) çalışmalarında yaş ağırlıkça nar kabuğunun % 69,7 nem, % 5,6 kül içerdiğini belirtmişlerdir. Ham nar kabuğunun kuru ağırlıkça nem içeriği % 8,44, UM içeriği % 67,63, kül içeriği % 17,77 ve sabit karbon içeriği % 6,16 olarak bulunmuştur. Elde edilen veriler literatür ile uyumludur. Rowayshed ve ark. (2013), nar kabuğunun yaklaşık analizinde kuru ağırlıkça nem içeriğini % 13,70, kül içeriğini % 3,30 bulmuştur. Literatür verileri ile kıyasla nar kabuğunun sabit karbon değeri daha düşük bulunmuştur. Siddiqui ve ark. (2019)'nin yapmış olduğu çalışmada, ham nar kabuğu biyokütlesinin sabit karbon içeriği % 25,37 olarak bulunmuştur.

Farklı torefikasyon sıcaklıklarında nar kabuğu numunesinin nem içeriğinin % 1,15-% 2,18 aralığında, UM içeriğinin % 55,99-% 62,96 aralığında, kül içeriğinin % 17,43-% 31,22 ve sabit karbon içeriğinin % 5,30-% 15,47 aralığında değiştiği görülmektedir. Yaklaşık analiz sonuçlarından elde edilen verilerde sıcaklığa bağlı olarak artış ya da azalışla ilgili bir eğilim yoktur. Literatürde nar kabuğunun torefikasyonu ile ilgili bir çalışmaya rastlanamamıştır.

Torefikasyon sonrasında nem içeriğinin oldukça azaldığı Tablo 1'de görülmektedir. Ham ve torefiye biyokütlelerin yaklaşık analiz sonuçları karşılaştırıldığında nem içeriğinin % 8,44'den 270 °C'de torefikasyon sonrası % 1,15'e düştüğü görülmektedir. % 67,63 olan UM içeriği ise çalışılan sıcaklıklarda büyük oranda azalma eğilimi göstermiştir. Kül içeriği, ham nar kabuğu biyokütlesine oranla artmıştır. Sabit karbon değerlerinin ise % 6,16'dan 260 °C'de torefikasyon sonrası % 15,47 çıkarak, 260 °C'de en yüksek değere ulaştığı görülmektedir.

Tablo 2'de HNK ve TNK'nun elementel analiz sonuçları gösterilmektedir.

**Tablo 2.** Elementel analiz sonuçları

| | Azot (%) | Karbon (%) | Hidrojen (%) | Sülfür (%) | Oksijen (%) |
|---|---|---|---|---|---|
| H | 0,25 | 41,35 | 4,57 | - | 36,05 |
| T | 0,51 | 53,27 | 3,67 | - | 25,11 |

* Oksijen miktarı,100-(Azot+Karbon+hidrojen+Kükürt+Kül) farkından hesaplanmıştır.

Tablo 2'de, H, ham nar kabuğunun, T ise torefiye edilmiş nar kabuğunun elementel analizlerini belirtmektedir. Elementel analiz sonuçlarına göre nar kabuğu numunesinin torefikasyon sonrasında karbon içeriğinin arttığı, hidrojen ve oksijen içeriklerinin azaldığı görülmektedir. Biyokütleyi oluşturan lignin, selüloz ve hemiselüloz, iskelet ana molekülünde hidroksil gruplarında bağlı büyük miktarda oksijene sahiptir. Bu gruplar, malzeme ısıtıldığında ayrışır ve oksijen içeren karboksilik asitler, özellikle formik asit ve asetik asit oluşturur, bunlar daha yüksek sıcaklıklarda su ve karbondioksite ayrışır (Arnsfeld ve ark., 2014).

Torefikasyon sonrasında ham nar kabuğu karbon içeriği % 41,35'den % 53,27'ye yükselmiştir. Ham biyokütlelerin yapılarında bulunan oksijen miktarları, torefikasyon sonrasında oluşan oksijen oranını etkileyen en temel nedendir. Oksijen içeriği artan sıcaklıkla büyük ölçüde tükenir (Dhaundiyal ve ark., 2021). Pehlivan, 2017'nin yapmış olduğu çalışmada, ham nar posası % 1,75 azot, % 48,80 karbon, % 6,96 hidrojen, % 42,49 oksijen içerdiği bulunmuştur.

## Sonuçlar

Tarımsal ve endüstriyel faaliyetler sonucu oluşan atık biyokütleler, torefikasyon prosesi sonrasında adsorbent olarak pek çok alanda kullanılabilir. Nar kabuğu torefikasyon işlemi 60 dakika çalışma süresinde ve 250 °C, 260 °C, 270 °C, 280 °C ve 290 °C olmak üzere beş farklı sıcaklıkta gerçekleştirilmiştir. Nar kabuğunun beş farklı sıcaklıkta torefiye edilmesinden sonra sabit karbon içeriği sırasıyla % 8,82, % 15,47, % 9,71, % 5,30 ve % 8,66 olarak tespit edilmiştir. Nar kabuğu numunesinin torefikasyonunda karbon içeriği en yüksek 260 °C torefikasyon sıcaklığında elde edilmiştir. Torefikasyon çalışmalarında karbon içeriğini arttırmak amacıyla, farklı atıkların kombinasyonu araştırılabilir. Torefiye biyokütlelerin mekanizmaların da belirlenebilmesi amacıyla biyokütlelerin detaylı karakterizasyonları, hemiselüloz, lignin içerikleri tespit edilebilir.

## Teşekkür

Bu makale Ecem Portakal'ın yüksek lisans tez çalışmasının bir bölümünden üretilmiştir. Bu makale, FYL-2021-8426 No`lu SDÜ-BAP Yüksek Lisans Projesi ile desteklenmiştir. Destekleri için SDÜ-BAP Yönetim Birimi Başkanlığı'na teşekkür ederiz.

## KAYNAKLAR

Acharya, B., Dutta, A., Minaret, J., 2015. Review on comparative study of dry and wet torrefaction. Sustainable Energy Technologies and Assessments 12 (2015) 26–37. https://www.sciencedirect.com/science/article/abs/pii/S221313881500048X

Amalina, F., Razak, A. S. A., Krishnan, S., Zularisam, A.W., Nasrullah, M., 2022. A comprehensive assessment of the method for producing biochar, its characterization, stability, and potential applications in regenerative economic sustainability – a review. Cleaner Materials, 3, 100045. https://www.sciencedirect.com/science/article/pii/S2772397622000053

Arnsfeld, S., Senk, D., Gudenau, H.-W., 2014. The qualification of torrefied wooden biomass and agricultural wastes products for gasification processes. Journal of Analytical and Applied Pyrolysis, 107, 133-141. https://www.sciencedirect.com/science/article/pii/S0165237014000370?via%3Dihub

Bates, R. B., Ghoniem, A. F., 2012. Biomass torrefaction: Modeling of Volatile and Solid Product Evolution Kinetics. Bioresource Technology, 124, 460–469.

Btg, 2020. Erişim Tarihi: 19.11.2021. https://www.btgworld.com/en/

Cahyanti, M. N., Doddapaneni, T.R. K. C., Kikas, T., 2020. Biomass torrefaction: an overview on process parameters, economic and environmental aspects and recent advancements. Bioresource Technology, 301, 122737. https://www.sciencedirect.com/science/article/pii/S0960852420300067

Chandra, R., Takeuchi, H., Hasegawa, T., 2012. Methane production from lignocellulosic agricultural crop wastes: a review in context to second generation of biofuel production. Renewable and Sustainable Energy Reviews, 16(3), 1462-1476. https://www.sciencedirect.com/science/article/pii/S1364032111005818

Chen, W.-H., Liu, S.-H., Juang, T.-T., Tsai, C.-M., Zhuang, Y.-Q., 2015. Characterization of solid and liquid products from bamboo torrefaction. Applied Energy, 160, 829-835. https://www.sciencedirect.com/science/article/pii/S0306261915003062

Chen, W.H., Huang, M.Y., Chang, J.-S. Chen, C.-Y. 2015. Torrefaction operation and optimization of microalga residue for energy densification and utilization. Applied Energy, 154, 622–630. https://www.sciencedirect.com/science/article/abs/pii/S0306261915007011?via%3Dihub

Danish, M., Ahmad, T., 2018. A review on utilization of wood biomass as a sustainable precursor for activated carbon production and application. Renewable and Sustainable Energy Reviews, 87, 1-21. https://www.sciencedirect.com/science/article/pii/S1364032118300236

Dhaundiyal, A., Singh, S. B., Atsu, D., Toth, L., 2021. Comprehensive analysis of pre-treated austrian pine. Fuel, 287, 119605. https://www.sciencedirect.com/science/article/pii/S0016236120326016

Eia, 2020. Erişim Tarihi: 16. 09. 2020. https://www.eia.gov/energyexplained/biomass/

Gan, Y.Y., Ong, H. C., Show, P. L., Ling, T. C., Chen, W.H., Yu, K. L., Abdullah, R., 2018. Torrefaction of microalgal biochar as potential coal fuel and application as bio-adsorbent. Energy Conversion and Management, 165, 152–162. https://www.sciencedirect.com/science/article/abs/pii/S0196890418302759?via%3Dihub

McCaffrey Z., Torres L., Chiou B.-S., Ferreira S. R., Silva L. E., Wood D. F., Orts W. J., 2021. Torrefaction of almond and walnut byproducts. Frontiers in Energy Research, 9.

Okumuş, G., 2016. Nar (punica granatum l.) Kabuk ve Çekirdeklerinin Antioksidan Kapasitelerinin Belirlenmesi. Uludağ Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği Anabilim Dalı, Yüksek Lisans Tezi. Bursa. https://acikerisim.uludag.edu.tr/bitstream/11452/10644/1/459171.pdf

Pehlivan, E., 2017. Meyvesuyu endüstrisi katı atığından üretilen aktif karbonun sulu çözeltilerden reaktif kırmızı (procion red mx-5b) adsorpsiyonu için kullanımı. Pamukkale Üniversitesi Mühendislik Bilim Dergisi, 23(7), 912-918. https://dergipark.org.tr/tr/pub/pajes/issue/33120/379541

Rowayshed, G., Salama, A., Abul-Fadl, M., Akila-Hamza, S., Emad, A. Mohamed, 2013. Nutritional and chemical evaluation for pomegranate (punica granatum l.) fruit peel and seeds powders by products. Middle East Journal of Applied Sciences, 3(4), 169-179. https://www.curresweb.com/mejas/mejas/2013/169-179.pdf

Safar, M., Lin, B-J., Chen, W-H., Langauer, D., Raclavska, H., Petrissans, A., Petrissans, M., 2019. Effects of impregnated potassium on biomass torrefaction. Energy Procedia. 158, 55-60. https://www.sciencedirect.com/science/article/pii/S187661021930044X

Sharma K, Akansha, Chauhan E.S., 2018. Comparative studies of proximate, mineral and phytochemical compositions of pomegranate (punica granatum) in peel, seed and whole fruit powder. International Journal of Food Science and Nutrition, 3(2), 192-196. https://www.researchgate.net/publication/265052028_Proximate_Composition_Minerals_Content_Antibacterial_and_antifungal_Activity_Evaluation_of_Pomegranate_Punica_granatum_L_Peels_Powder

Siddiqui, M.T.H., Nizamuddin, S., Mubarak, N.M., Shirin, K., Aijaz, M., Hussain, M., Baloch, H. A., 2019. Characterization and process optimization of biochar produced using novel biomass, waste pomegranate peel: a response surface methodology approach. Waste Biomass Valor, 10, 521–532. https://link.springer.com/article/10.1007/s12649-017-0091-y

Şahin A., 2020. Nar Yetiştiriciliği. T.C. Tarım ve Orman Bakanlığı, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü Batı Akdeniz Tarımsal Araştırma Enstitüsü. Antalya, Mayıs. https://arastirma.tarimorman.gov.tr/batem/Belgeler/Kutuphane/Teknik%20Bilgiler/Nar%20Yeti%C5%9Ftiricili%C4%9Fi.pdf

Taşar, Ş., 2021. Biyokütle kaynaklarının proximate bileşimleri ile üst ısı değerleri arasında yeni bir denklem geliştirilmesi ve denklemin başarısının araştırılması. Artvin Çoruh Üniversitesi, Orman Fakültesi Dergisi, 22(1), 42-51. http://ofd.artvin.edu.tr/tr/pub/issue/62326/744752

Türkiye İstatiksel Kurumu (TÜİK), 2022. Erişim Tarihi: 02.03.2022. https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2021-37249#:~:text=Buna%20g%C3%B6re%20%C3%BCretim%20miktarlar%C4%B1%202021,9%20milyon%20ton%20olarak%20ger%C3%A7ekle%C5%9Fti.

Tumuluru, J. S., Sokhansanj, S., Hess, J. R., Wright, C. T., and Boardman R. D., 2011. A review on biomass torrefaction process and product properties for energy applications. Industrial Biotechnology, October, 7(5), 384-401. https://www.researchgate.net/publication/228083732_A_Review_on_Biomass_Torrefaction_Process_and_Product_Properties_for_Energy_Applications

*ORAL PRESENTATION*

# Synthesis, computational calculations, and anti-cancer activity of the novel 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide

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## Abstract

The discovery of the important effect of sulfone compounds in biological applications providing the development of new sulfone-containing compounds and their derivatives has become one of the popular studies in organic chemistry. In this regard, there are many sulfones having a wide range of biological activities such as anticancer, antibacterial, and anti-HIV. In this study, it is aimed to synthesize, perform the computational calculations, and determination of anti-cancer activity of the novel 2-(2- Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide. In this context, 2-chloro benzhydrazide and hexyl isothiocyanate were mixed with the proper solvent and synthesized the novel 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide. IR, 1H, and 13C data of the compound were given proper to it's chemical structure. Visualization of chemical reaction calculations was conducted with Gaussian 09 and GaussView 5.0 program. Moreover, anti-cancer activity was performed by using MTT methods. It is figured that the electron density is concentrated on heteroatoms in HOMO orbitals whereas it is concentrated on the aromatic ring in LUMO orbitals. While the most negative atoms are sulphur and oxygen appear in the red region, hydrogens bounded to nitrogens appear in the blue region due to being the most positively charged atoms according to MEP analysis. While the active dose 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide was found to be 200 µM in HCT-116 colon cancer cells and MD-MB-231 breast cancer cells, it was found that the active dose was 100 µg/ml when the cells were treated with cisplatin under the same conditions. This study sheds light on organic reactions containing sulfone compounds for biological applications as medication, especially in cancer.

**Keywords:** Hydrazine carbothioamides, DFT, Anti-cancer activity

## 1. Introduction

Nowadays, with the discovery of the important effect of sulfone compounds in biological applications, the development of new sulfone-containing compounds and their derivatives has become one of the popular studies in organic chemistry(Yang et al., 2019). In this regard,there are many sulfones having a wide range of biological activities such as anticancer, antimicrobial, and anti-HIV(Ahmed et al.,2019). One of these compounds is hydrazinecarbothioamides. In the simplest definition;it is considered as one of the most important chemical compounds containing nitrogen and sulfur which is obtained from the formation of heterocyclizationand reaction with several π-deficient compounds (Hassan et al.,2011).Hydrazinecarbothioamides have a significant place in various transformations of chemical reactions.The uses of this compound in organic synthesis especially play an important role in the formation of heterocyclic compounds. The reactions which are prepared with a high yield thanks to chemicals containing C=O and C=N groups are aimed to synthesize biologically active compounds such as pyrazole, thiazole, triazole, thiadiazole, oxadiazole, triazine and tridiazine (Jafari et al.,2021). Besides, these precursors and their derivatives, which are widely used in heterocyclic synthesis, have a great value for the development of chemicals with the pharmaceutical effect in terms of biological activity(Yehye et al.,2012). Especially, when their biologically active derivatives are synthesized, there are several possible ways such as the interaction of carbohydrazides with aryl isothiocyanates under different conditions, carbon disulfide with base-catalyzed nucleophilic addition of arylamines and heating carbohydrazides with ammonium thiocyanate in aceton(Basavaraja et al.,2010).One of these hydrazine derivatives is benzohydrazides, which have recently gained great importance with their manifold biological properties.These compounds, also known as pharmacors, have been widely used as reagents in the derivation of carbonyl compounds in organic synthesis.

Moreover, they are multifunctional molecules since they contain C=O, -NH and -NH2 structures(Prasan,2013). Isothiocyanates, another sulfone compound, is a very popular molecule with its biological activity and has gained great importance with its anti-cancer properties.In this study, it is aimed to synthesize, perform the computational calculations, and show anti-cancer activity of the novel 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide.

## 2. Materials and Method

### *2.1. Synthesis*

#### *2.1.1.Synthesisof2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide*

The chemicals used in the experiments were obtained from Sigma-Aldrich (USA). First of all, 4.77 g of 2-chloro benzhydrazide as a white solid and 4.75 mL of colorless liquid hexyl isothiocyanate were mixed in a glass flask. Then, approximately 20 mL ofliquidtetrahydrofuron was added to the mixture as a solvent and refluxed together at 150°C for 7 hours.While the color of the liquid in the glass flask was yellow before the experiment started, it turned light yellow after 7 hours. At the end of the experiment, the mixture was first cooled.Then the mixture was evaporated so that it's solvent was removed. IR (ATR, $cm^{-1}$): 3220 (stretching vibration, aromatic N-H), 3160 (stretching vibration, aromatic C-H), 2820 (stretching vibration, aliphatic C-H), 1670 (stretching vibration, C=O), 1630 (stretching vibration, C=C), 1490 (bending vibration, N-H). $^{1}H$ NMR(300 MHz, $CHCl_3$): 0.85 ($CH_3$), 1.23 (m, 4H, $CH_2$), 1.53 (p, 2H, $CH_2$), 3.56 (q, 2H, N-$CH_2$), 7.49-7.31 (aromatic C-H), 7.70 (S-N-H), 7.73 (C-N-H), 9.92 (N-H). $^{13}C$ NMR (75 MHz, $CDCl_3$): 132.3, 134.6, 130.1, 133.5, 126.9, 128.9, 164.8, 184.2, 44.9, 30.2, 27.0, 31.5, 22.7, 14.1.

### *2.2. Computational Methods*

The visualization of chemical reaction calculations was carried out with Gaussian 09 and GaussView 5.0 programs (Frisch et al.,2009). For optimized molecule structure **(Figure 1)**, DFT method (B3LYP/6-311þ G(d,p)) (Becke's three-parameter hybrid model using the Lee–Yang–Parr correlation functional) (Becke, 1992; Lee & Yang, 1988) was engaged to the study. The distribution of HOMO –LUMO and molecular electrostatic potential (MEP) analysis were implemented by using Crystal Explorer 3.1 (Wolff et al., 2012).

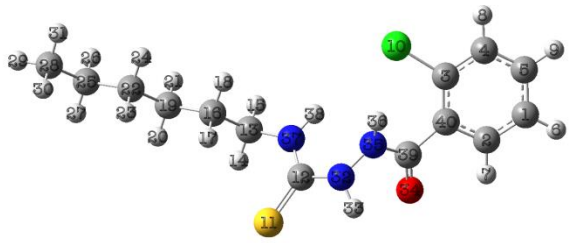


**Figure 1.**The optimized structure of 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide

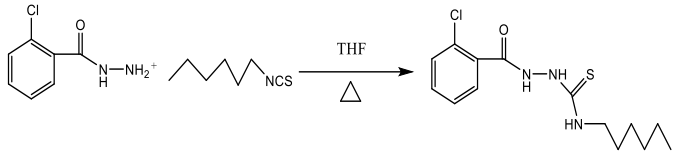
### *2.3. Anti-cancer activity*

Anticancer activity of 2-(2- Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide was determined with the help of the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] method. For the MTT analysis, HCT-116 colon cancer cells and MDA-MB-231 triple negative breast cancer cells were used.

## 3. Results

### *3.1.Synthesis of 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide*

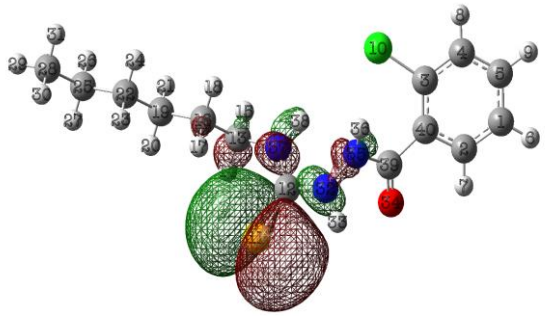
When 2-chloro benzhydrazide and hexyl isothiocyanate are mixed with proper solvent,2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamideoccurs as a product. It's chemical reaction is shown in **Scheme 1**.



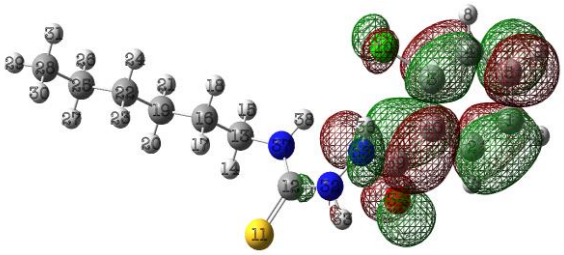

**Scheme 1.** Synthesis of 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide

### *3.2. HOMO-LUMO analysis*

HOMO and LUMO, which are types of molecular orbitals represents for the highest occupied molecular orbitals,HOMO, and the lowest unoccupied molecular orbitals, LUMO. They were calculated for the novel chemical compound by using the B3LYP functional and the 6-311þþG(d,p) basis set in chloroform. In this compound, as shown in **Figure 2**, the electron density is concentrated on heteroatoms, which are nitrogen and sulfur atoms, in HOMO orbitals whereas it is concentrated on the aromatic ring in LUMO orbitals. Here, it can be said that the flow of electron charge is from the heteroatoms to the aromatic ring.



HOMO (-6.1263576137513eV)



LUMO (-*2.*2000355915066eV)

**Figure 2.**Calculated Frontier molecular orbitals of the title compound with DFT/B3LYP-G(d,pþþ).

### *3.3. Molecular electrostatic potential (MEP) analysis*

MEPanalysis is a map in which electron density of the chemical compound is indicated in the particular regionswith specific colors. This map shows the distribution of charge three-dimensionally. In the map, the color scale takes place in between red and blue colors in the particular regions. Accordingly, red color means compound has the highest electron density whereas the blue has the lowest electron density. As illustrated in **Figure 3**, while sulfur and oxygen atoms of the compound appear in the red region due to high electron density, which means the most negative atoms are sulfur and oxygen, hydrogens that are bounded to nitrogens appear in the blue region because of low electron density meaning that these hydrogens are the most positively charged atoms. Furthermore, the benzene ring and the hexyl group of the compound are located in between yellow and green so that they have a medium electron density.

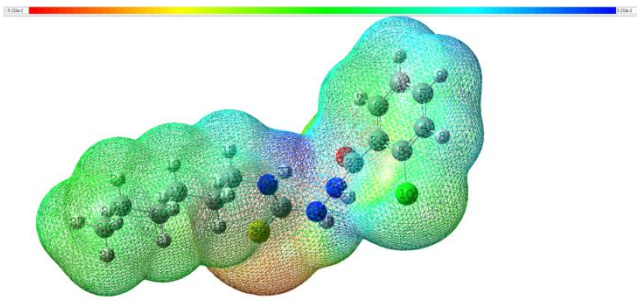


**Figure 3.** MEP maps of the title compound calculated at (B3LYP/6311þG(d,p)).

### *3.4. Anti-cancer activity*

The anticancer activity of the newly synthesized 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazinecarbothioamide compound was determined in colon and breast cancer cells. The active dose was found to be 200 µM in HCT-116 colon cancer cells. In the same conditions, cisplatin showed activity at a concentration of 100 µg/ml. Similar results were found in MDA-MB-231 cells as in HCT-116 cells (**Figure 4**).

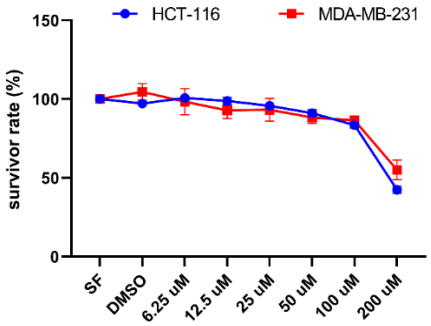



**Figure 4.** Effect of 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide compound on cell viability in HCT-116 and MDA-MB-231 cells

## 4. Discussion

In this study, 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide was synthesized from mixing of 2-chloro benzhydrazide and hexyl isothiocyanate with the proper conditions. DFT method was used to optimize molecule structure. By using Crystal Explorer 3.1, it is obtained that the electron density is concentrated on heteroatoms in HOMO orbitals whereas it is concentrated on the aromatic ring in LUMO orbitals. While the most negative atoms having high electron density placed in the red region, the most positively charged atoms having low electron density placed in the blue region with the use of MEP analysis in order to show the distribution of charge three-dimensionally. Furthermore, the anticancer activity of the chemical compound was determined in colon and breast cancer cells via MTT method. In this respect, Zangrando et al. (2017) informed that Escherichia coli was successfully eradicated by hydrazine carbothioamides derivatives namely from S-hexyldithiocarbazate and 4-methylbenzaldehyde. Kongot et al. (2019) discovered that acopper(II) combination made of 2-hydroxy-5-(phenyldiazenyl)benzaldehyde and S-benzyldithiocarbazate had remarkable biological activity against a human cervical cancer line. Stefan et al. (2020) synthesized hydrazinocarbothioamide derivatives being screened for antihyperglycemic activity in diabetic mice. Results showed that all treated groups had a significant decrease in blood glucose levels. Ashraf et al. (2022) improved that antiapoptotic action results from the transformation of opening hydrazinecarbothioamides into cyclized thiadiazol. In this way, these compounds act as an inhibitor for the cancer.

## 5. Conclusion

In this study, the novel 2-(2-Chlorobenzoyl)-N-(1-Hexyl)hydrazine carbothioamide was synthesized and visualized with chemical reaction calculations programs. The synthesized compound was optimized by using DFT (B3LYP/6311þ G(d,p)) methods. The distribution of HOMO–LUMO and MEP analysis were implemented by using Crystal Explorer 3.1. The anti-cancer activity of the novel compound with colon and breast cancer cells.

## Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Authors' contributions:** AyçaBayhan: investigation, methodology, data curation, and writing-original draft, writing-review & editing. Murat Genç: investigation, methodology, data analysis and curation, supervision, writing-review & editing. Esra Bozgeyik: methodology, data analysis and curation, writing-review & editing.

**Conflict of interest disclosure:**

The writers stated that they had no competing interests in this work. We certify that none of our associations or business interests pose a conflict of interest with respect to the work that has been submitted.

## References

1- Ahmed N, Konduru NK, Owais M. 2019. Design, Synthesis and Antimicrobial Activities of Novel Ferrocenyl and Organic Chalcone based Sulfones and bis-Sulfones. Arabian J. Chem. 12, 1879.

2- Aly AA, Hassan AA, Makhlouf MM, AlshammariMB, Hafez SMNA, Marwa MMR, Bräse S, Nieger M, Ramadan M. 2020. Design and synthesis of hydrazinecarbothioamide sulfones as potential antihyperglycemic agents. Archiv der Pharmazie.

3- Basavaraja HS, Jayadevaiah KV, Mumtaz MH, Vijay Kumar MM, Basavaraj P. 2010. Synthesis, crystal structure and Hirshfeldsurface analysis of (1*H*-benzimidazol-2-yl)(morpholin-4-yl)methanethione. J Pharm. Sci. Res. 2,5-12.

4- Becke AD. 1992. Density-functional thermochemistry. I. The effect of the exchange-only gradient correction. Journal of Chemical Physics. 96(3), 2155–2160.

5- Devi Prasan O, Kandikere RP. 2013. Benzohydrazides: As potential bio-active agents.J Org Chem. 78(23):12136-12143.

6- Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Petersson GA, Nakatsuji H, Li X, Caricato M, Marenich A, Bloino J, Janesko BG, Gomperts R, Mennucci B, Hratchian HP, Ortiz JV, Fox DJ. 2009. Gaussian 09. Revision C.01, Gaussian, Inc.

7- Hassan AA, Abdel-Latif FF, El-Din AMN, Abdel-Aziz M, Mostafa SM, Bräse S. 2011. Synthesis of novel spiro(indolone-3,2′-[1,3,4]thiadiazol)-2-ones and evaluation of their antidepressant and anticonvulsant activities. J. Heterocycl. Chem. 48, 1050.

8- Hassanzadeh F, Jafari E, Shojaei F, and Sadeghi-Aliabadi H. 2021. Synthesis and cytotoxic activity evaluation of some new 1, 3, 4-oxadiazole, 1, 3, 4-thiadiazole and 1, 2, 4- triazole derivatives attached to phthalimide. Res Pharm Sci.

9- Kongot M, Reddy D, Singh V, Patel R, Singhal NK, Kumar A. 2019.Potent drug candidature of an ONS donor tethered copper (II) complex: Anticancer activity, cytotoxicity and spectroscopically approached BSA binding studies. Spectrochim. Acta A 212, 330-342.

10- Mostafa SM, Aly AA, Bräse S, Nieger M, Sara Mohamed Naguib Abdelhafez SMN, Abdelzaher WY, Abdelhafez EMN.2022.Synthesis, Characterization, and In Vivo Study of Some Novel 3,4,5-

Trimethoxybenzylidene-hydrazinecarbothioamides and Thiadiazoles as Anti-Apoptotic Caspase-3 Inhibitors. Molecules. 27(7):2266.

11- Wolff SK, Grimwood DJ, McKinnon JJ, Turner MJ, Jayatilaka D, Spackman MA. 2012. Crystal Explorer University of Western Australia. Version 3.1.

12-Yang HB, Feceu A, Martin DBC, Catal ACS. 2019. Catalyst-Controlled C–H Functionalization of Adamantanes Using Selective H-Atom Transfer. 9, 5708.

13- Yang LC, W & Parr RG. 1988. Development of the Colle–Salvetti correlation-energy formula into a functional of the electron density. Physical Review B, 37, 785–789

14-Yehye, WA, Rahman NA, Alhadi AA, Khaledi H, WengNgS, Ariffin A. 2012. Butylated hydroxytoluene analogs: Synthesis and evaluation of their multipotent antioxidant activities. Molecules 17, 7645–7665.

15- Zangrando E, Begum MS, Sheikh MC, Miyatake R, Hossain MM, Alam MM, Hasnat MA, Halim MA, Ahmed S, Rahman MN, Ghosh A. 2017. Synthesis, characterization, density functional study and antimicrobial evaluation of a series of bischelated complexes with a dithiocarbazate Schiff base ligand. Arabian J. Chem. 10,172-184.

## *ORAL PRESENTATION*

# İzmir ili Ayvalık zeytin (*Olea europaea L. cv.* Ayvalık) plantasyonlarından izole edilen arbüsküler mikoriza sporlarının dağılımı ve bazı toprak özellikleri ile ilişkileri

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**Özet**

Bu makale, TUBİTAK tarafından desteklenen "Gemlik zeytin (*Olea europaea* L. cv. Gemlik) fidanlarının kuraklık stresini azaltmalarında arbüsküler mikoriza uygulamalarının etkisi" adlı projenin ön deneme sonuçlarını içermektedir. Bu ön çalışmada amaç, İzmir ili sınırları içinde doğal koşullarda yaşamlarını sürdüren kurak koşullara dayanıklı Ayvalık zeytin çeşidinin yetiştiği farklı zeytin plantasyonlarından 11 farklı toprak ve bitki kökü örneği alınarak bu örneklerde arbüsküler mikoriza spor sayımları, enfeksiyon oranları ve toprakların fiziko-kimyasal özellikleri arasındaki ilişkilerin saptanmasıdır. En yüksek spor sayısı ve enfeksiyon oranı Çeşme-Germiyan'daki zeytin topraklarında, en düşük spor sayısı ve enfeksiyon oranı ise Menemen-Süleymanlı'dan alınan topraklarda saptanmıştır. İncelenen parametreler arasındaki korelasyonlar belirlenmiş ve spor sayısı ile mikorizal enfeksiyon oranı, toprağın elektriksel geçirgenliği ve alınabilir mangan içeriği arasında istatistiksel olarak önemli pozitif, mikorizal enfeksiyon oranı ile alınabilir fosfor ve çinko miktarları arasında ise önemli negatif korelasyonlar tespit edilmiştir.

**Anahtar Kelimeler:** Arbüsküler mikoriza, Ayvalık zeytin çeşidi, mikorizal enfeksiyon, toprak özellikleri

# Distribution of arbuscular mycorrhizal spores isolated from Ayvalık olive (Olea europaea L. cv. Ayvalık) plantations in İzmir province and their relationship with some soil properties

**Abstract**

This article includes the pre-experiment results of the project named "The influence of arbuscular mycorrhiza applications to reduce drought stress of Gemlik olive (*Olea europaea* L. cv. Gemlik) plantlets" supported by TUBITAK. The aim of this study is to determine arbuscular mycorrhiza spore counts, infection rates and the relationships between these parameters and physico-chemical properties of soils. 11 different soil and root samples were taken from different olive plantations where the Ayvalık olive variety, which survives in natural conditions and is resistant to dry conditions in İzmir province. The highest number of spores and infection rate were found in the olive soils of Çeşme-Germiyan, the lowest number of spores and infection rates were found in the soils taken from Menemen-Süleymanlı. The significant positive correlations were found between the number of spores and mycorrhizal infection rate, electrical permeability of the soil and available manganese content, and significant negative correlations were found between mycorrhizal infection rate and available phosphorus and zinc amounts.

**Keywords:** Arbuscular mycorrhiza, Ayvalık olive variety, mycorrhizal infection, soil properties

## GİRİŞ

Akdeniz ülkelerinde çok eski çağlardan beri süregelen zeytin (*Olea europaea* L.) yetiştiriciliği günümüzde önemli ve geleneksel bir agro-endüstriyel faaliyet durumundadır. En sağlıklı yağ asitleri arasında yer alan oleik asiti yüksek düzeyde içermesinden dolayı zeytin ve zeytinyağına olan talep tüm dünyada gittikçe artmaktadır (Orlandi ve ark., 2003). TUİK verilerine göre zeytin ağacı varlığımız 2016/2017 sezonunda yaklaşık 174 milyon adet, toplam zeytin üretimimiz ise 1 milyon 730 bin ton civarındadır. Dünya sofralık zeytin ve zeytinyağı üretiminde İspanya birinci konumda iken, Türkiye ise sofralık zeytin üretiminde üçüncü, zeytinyağı üretiminde ise beşinci sıradadır (TUİK, 2018).

Toprakta AMF varlığı, zeytin ağaçlarının kuraklığa tepkisinde önemli bir anahtar faktör olarak karşımıza çıkmaktadır (Calvo-Polanco ve ark., 2016). Bitkiler AMF ile sembiyotik bir ilişkiye girdiklerinde strese toleransı artıran morfoloik ve fizyolojik değişime uğramakta ve suyun hücre düzeyindeki korunumu artmaktadır (Porcel ve ark., 2006). Su eksikliği köklerin absorbsiyon gücünü azaltmakta fakat AMF ile kolonizasyon, bu durumu kompanse etmektedir (Barea ve Jeffries, 1995; Ruiz-Lozano ve ark., 1996). Kuraklık stresinin baskın olduğu ve bu baskının daha da artması beklenen Akdeniz ülkelerinde, zeytin bitkisinin bu stresi yenmesinde mikoriza kullanımı ile ilgili çalışmalara ilgi son yıllarda gittikçe artmaktadır.

Yaklaşık 85 milyon zeytin ağacı ile ülkenin toplam zeytin ağacı varlığının yarısını içinde barındıran Ege Bölgesinde, kuraklık toleransı yüksek olan zeytin çeşitlerinin seçilmesi ve bu toleransın güçlendirilmesi, zeytindeki verimin korunması ve artırılması açısından son derece önem arz etmektedir.

TUBİTAK tarafından desteklenen bu projenin ilk aşamasını içeren bu çalışmada; kurak koşullara daha dayanıklı bir çeşit olan Ayvalık zeytin ağaçlarının rizosfer bölgelerinden kök ve toprak örnekleri alınmış ve en yüksek spor sayısı ve mikorizal enfeksiyon yüzdesine sahip olan topraklar belirlenmiştir. İkinci aşamada ise bu topraklarda üçgül ve sorgum bitkileri ile bir tuzak bitki denemesi yürütülmüş ve mikorizal sporların zenginleştirilmesi sağlanmıştır.

## MATERYAL VE METOT

Çalışmanın materyalini İzmir ili sınırları içinde hiçbir şekilde sulamanın yapılmadığı susuz koşullarda yaşamlarını sürdüren Ayvalık zeytin çeşidinin yetiştiği farklı zeytin plantasyonlarından alınan 11 toprak ve bitki kök örneği oluşturmuştur. Çalışma örneklerinin alındığı yerler Tablo 1'de verilmiştir.

Tablo 1. Araştırma materyalini oluşturan toprak/bitki örneklerinin alındığı yerler

| Örnek No | Örneğin alındığı İlçe-Köy/Belde |
|---|---|
| 1 | Dikili-Bademli |
| 2 | Bergama-Zeytindağ |
| 3 | Bergama-Tekkedere |
| 4 | Çeşme-Germiyan |
| 5 | Çeşme-Germiyan Organik |
| 6 | Torbalı-Karaot |
| 7 | Aliağa-Aşağı Şakran |
| 8 | Buca-Kaynaklar |
| 9 | Bornova-Gökdere |
| 10 | Foça-Bağarası |
| 11 | Menemen-Süleymanlı |

Toprak örnekleri arazideki zeytin ağaçlarının taç izdüşümünden, toprak üstündeki bitkisel kalıntılar belle sıyrıldıktan sonra 0-20 cm derinlikten zeytin ağacının ince bitki köklerini de kapsayacak şekilde alınmıştır. Laboratuvara getirilen toprak örneklerinin bir kısmı hava kurusu hale getirildikten sonra 2 mm'lik elekten elenerek fiziksel ve kimyasal toprak analizleri gerçekleştirilmiştir. Toprakların geri kalan kısmı ile mikoriza spor sayısını artırmak amacıyla tuzak denemesi kurulmuştur. Tuzak bitki denemesinde, test bitkisi olarak mikoriza bağımlılığı yüksek olan üçgül (*Trifolium resipinatum*) bitkisinin yanında fotosentez hızı yüksek ve sıcaklığa dayanıklı bir C4 bitkisi olan sorgum (*Sorghum bicolor* L. *Moench*) da kullanılmıştır. Tuzak bitki denemesine 11 toprak x 2 bitki x 3 tekerrür=66 saksı ile başlanmıştır. Saksı denemesinde kullanılmak üzere dere kumu temin edilmiş ve 120 °C/30 dk da birer gün ara ile toplam 3 gün süre ile otoklav cihazında sterilize edilmiştir. Daha sonra 3 lt'lik saksılara 1:1 oranında kum: toprak karışımı konulmuştur. Üçgül tohumları saksı başına 20 adet, sorgum tohumları ise saksı başına 6 adet olmak üzere ekilmiştir. Tohumlar yaklaşık 1 cm derinliğe ekilmiş ve can suyu verilerek tohumların çimlenmesi ve gelişmesi sağlanmıştır. Denemede kullanılan üçgül tohumları Acem çeşidi, sorgum tohumları ise Rox çeşididir. Tuzak bitki saksı denemesi Zeytincilik

Araştırma Enstitüsü serasında tesadüf parselleri deneme desenine göre yürütülmüş ve saksı toprakları daha önce saptanan Tarla Kapasitesi nem değerlerinin %80'i olacak şekilde çeşme suyu ile düzenli olarak sulanmıştır. Üç ay sonra bitkiler hasat edilerek saksı topraklarında spor sayımı ve mikorizal enfeksiyon oranları belirlenmiştir.

## BULGULAR

Çalışma kapsamında alınan 11 toprak örneğinde yapılan fiziksel ve kimyasal analiz sonuçları Tablo 2a ve 2b'de verilmiştir. İşba değerlerine göre yapılan bünye analizi sonucu toprakların killi tın ve tın bünyeye sahip oldukları ve nötr reaksiyona sahip 7. ve 10. örnekler dışındaki diğer toprak örneklerinin hafif alkalin reaksiyonda oldukları belirlenmiştir. Tuzluluk problemi olmayan toprakların kireç içerikleri 3 ve 7 numaralı örnekler dışında orta ve yüksek düzeylerdedir. Toprakların organik madde içerikleri genelde düşük (%1.02-2.35 aralığında) ve toplam N miktarları ise çok düşükten çok yükseğe geniş bir dağılım göstermiştir. Toprakların yarayışlı P miktarları 3, 8 ve 9 numarada yeterli miktarlarda iken diğer topraklarda çoğunlukla çok düşük düzeylerde (4-6 mg $kg^{-1}$) olduğu görülmektedir. Toprakların yarayışlı K miktarları 6 no'lu toprak hariç diğerleri yeterli düzeydedir. Toprakların yarayışlı Ca, Mg ve Fe düzeyleri iyi, Mn, Cu ve B düzeyleri yeterlidir. Toprakların Zn düzeylerinin kritik değerin (0.5 mg $kg^{-1}$) üzerinde olduğu belirlenmiştir.

Tablo 2a. Toprak örneklerinin fizikokimyasal özellikleri

| Örnek No | Bünye | E.C. (mmhos/cm) | pH | Kireç (%) | Org. Mad. (%) | Top. N (%) | Yar. P (mg $kg^{-1}$) | Yar. K (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | Killi tın | 0.78 | 7.76 | 45.41 | 2.35 | 0.27 | 5.41 | 609.99 |
| 2 | Tın | 0.45 | 7.56 | 57.18 | 1.88 | 0.15 | 6.14 | 197.62 |
| 3 | Tın | 0.44 | 7.77 | 4.99 | 1.31 | 0.07 | 18.50 | 271.01 |
| 4 | Killi tın | 1.15 | 7.59 | 6.91 | 1.35 | 0.13 | 5.37 | 442.13 |
| 5 | Killi tın | 0.98 | 7.61 | 5.30 | 1.02 | 0.08 | 4.35 | 445.78 |
| 6 | Tın | 0.39 | 7.66 | 7.75 | 1.30 | 0.06 | 5.35 | 77.48 |
| 7 | Tın | 0.58 | 7.15 | 2.85 | 1.44 | 0.14 | 5.61 | 629.31 |
| 8 | Tın | 0.60 | 7.64 | 49.76 | 1.58 | 0.10 | 18.67 | 301.44 |
| 9 | Killi tın | 0.56 | 7.66 | 24.84 | 2.27 | 0.12 | 12.53 | 453.67 |
| 10 | Killi tın | 0.54 | 7.32 | 19.28 | 2.21 | 0.15 | 4.74 | 323.83 |
| 11 | Killi tın | 0.49 | 7.66 | 8.02 | 1.76 | 0.12 | 6.60 | 453.64 |

Tablo 2b. Toprak örneklerinin fizikokimyasal özellikleri (Devam)

| Örnek No | Yarayışlı (mg kg$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Ca** | **Mg** | **Fe** | **Mn** | **Zn** | **Cu** | **B** |
| 1 | 5830 | 997 | 4.62 | 10.07 | 1.42 | 1.35 | 1,66 |
| 2 | 4847 | 415 | 5.82 | 6.71 | 1.40 | 0.95 | 1,10 |
| 3 | 3530 | 447 | 5.55 | 13.10 | 1.20 | 2.03 | 1,19 |
| 4 | 7229 | 531 | 8.15 | 24.29 | 0.74 | 2.43 | 0,85 |
| 5 | 8177 | 628 | 6.30 | 16.98 | 1.45 | 1.42 | 0,83 |
| 6 | 3664 | 378 | 7.77 | 9.91 | 0.84 | 1.18 | 1,21 |
| 7 | 3843 | 1056 | 5.83 | 18.52 | 1.03 | 1.48 | 2,93 |
| 8 | 5253 | 515 | 5.48 | 10.49 | 2.34 | 3.85 | 2,08 |
| 9 | 5359 | 679 | 8.56 | 20.00 | 1.86 | 1.19 | 1,41 |
| 10 | 5190 | 591 | 11.00 | 14.50 | 2.61 | 0.77 | 1,50 |
| 11 | 5096 | 865 | 9.12 | 9.47 | 1.99 | 32.07 | 2,13 |

Tuzak bitki denemesi sonunda saptanan ortalama spor sayıları ve mikorizal enfeksiyon oranlarıTablo 3'de verilmiştir. Bu sonuçlara göre 1, 4 ve 7 numaralı saksı topraklarının diğerlerine oranla daha yüksek spor sayılarına ulaştığı görülmektedir. Mikorizal enfeksiyon yüzdeleri, spor sayımlarını teyit etmiş ve en yüksek mikorizal enfeksiyon yüzdeleri yine 1, 4 ve 7 numaralı örneklerde saptanmıştır.

Tablo 3. Tuzak bitki saksı denemesi sonunda saptanan mikorizal spor sayıları

| **Örnek No** | **Ortalama spor sayısı (10 gr$^{-1}$)** | | **Mikorizal enfeksiyon oranı (%)** | |
|---|---|---|---|---|
| | **Üçgül** | **Sorgum** | **Üçgül** | **Sorgum** |
| 1 | 76 ± 29 | 62 ± 25 | 86,7 | 98,3 |
| 2 | 46 ± 21 | 31 ± 15 | 65,0 | 66,7 |
| 3 | 50 ± 11 | 37 ± 26 | 68,3 | 55,0 |
| 4 | 100 ± 37 | 39 ± 12 | 93,3 | 98,3 |
| 5 | 63 ± 18 | 62 ± 29 | 80,0 | 73,3 |
| 6 | 57 ± 7 | 61 ± 31 | 81,7 | 83,3 |
| 7 | 98 ± 53 | 77 ± 29 | 96,7 | 90,0 |
| 8 | 70 ± 9 | 35 ± 15 | 58,3 | 85,0 |
| 9 | 48 ± 11 | 31 ± 13 | 68,3 | 81,7 |
| 10 | 50 ± 11 | 34 ± 7 | 75,0 | 78,3 |
| 11 | 31 ± 22 | 58 ± 38 | 55,0 | 85,0 |

## TARTIŞMA

Topraklardaki fungus popülasyonu, arbüsküler mikoriza fungus (AMF) ile enfekte olmuş bitkiler ile birlikte artmaktadır (Giovanetti, 1985). AMF spor yoğunluğu, farklı toprak tipleri ve bitki çeşitlerine göre değişebilmektedir. Çalışmamızda da farklı lokasyonlardan alınan toprakların fizikokimyasal özelliklerine bağlı olarak AMF spor miktarı ve enfeksiyon oranları farklılık göstermiştir. Bu farklılık üzerine etkili toprak özellikleri elektriki geçirgenlik, alınabilir P, Mn ve Zn miktarları olmuştur (Tablo 4). Mikorizal enfeksiyon

oranı ile topraktaki alınabilir P miktarları arasında % 5 önem düzeyinde önemli negatif bir ilişki ortaya çıkarken, mikoriza spor sayısı ile alınabilir P miktarı arasında negatif fakat önemli olmayan bir korelasyon belirlenmiştir. Topraklardaki AMF spor sayısı ve enfeksiyon oranının topraktaki yarayışlı P miktarındaki artışa bağlı olarak azaldığı (Isobe ve ark., 1993, Mohammad ve ark., 2004, Lekberg ve Koide, 2005, Isobe ve ark., 2008) bu çalışmamızda da önemli bir bulgu olarak ortaya çıkmıştır. Benzer bir korelasyon topraktaki alınabilir Zn miktarları ile de bulunmuştur. Mikoriza enfeksiyon oranı ile topraktaki alınabilir Zn miktarları arasında % 5 önem düzeyinde önemli negatif bir ilişki ortaya çıkarken, mikoriza spor sayısı ile alınabilir Zn miktarı arasında negatif fakat önemli olmayan bir korelasyon belirlenmiştir. Bi ve ark (2008) yüksek dozda Zn (400 mg/kg) uygulanmış topraklarda düşük bir AMF kök kolonizasyonu saptarlarken, düşük dozda Zn (50 mg/kg) uygulanmış topraklarda ise yüksek düzeyde bir AMF kök kolonizasyonu belirlemişlerdir. Araştırıcılar AMF'nin bitkinin fazla Zn alınımına karşı bitkiyi koruduklarını bildirmişlerdir. Çalışmada, mikoriza spor sayısı ile mikorizal enfeksiyon oranları arasında % 1 düzeyinde önemli pozitif bir ilişki ortaya çıkmıştır. Bu sonuçlar mikorizayla yapılan diğer araştırma sonuçları ile paralellik göstermektedir (Tawaraya ve ark., 1995; Wuen ve ark., 2002; Mohammad ve ark., 2004; Isobe ve ark., 2008). AMF fungusları, bitki köklerini enfekte ettiğinde spor oluşumu başladığı için AMF fungusları tarafından enfekte olan bitki köklerinin artışı ile spor miktarı da artmaktadır.

Tablo 4. Toprağın kimyasal özellikleri ile mikoriza spor sayıları ve enfeksiyon yüzdesi arasındaki korelasyonlar

| | | Mikoriza spor sayısı | Mikorizal enfeksiyon oranı |
|---|---|---|---|
| Mikoriza spor sayısı | | - | 1.714** |
| EC | | 0.593* | 0.522* |
| pH | | -0.215 | -0.308 |
| Kireç | | -0.92 | -0.368 |
| Organik Madde | | -0.089 | -0.206 |
| Toplam N | | 0.351 | 0.271 |
| Alınabilir | P | -0.491 | -0.534* |
| | K | 0.494 | 0.411 |
| | Ca | 0.191 | 0.175 |
| | Mg | 0.282 | 0.320 |
| | Fe | -0.274 | -0.138 |
| | Mn | 0.575* | 0.564* |
| | Zn | -0.304 | -0.642* |
| | Cu | -0.465 | -0.510 |

## SONUÇ

Sonuç olarak; İzmir ili sınırları içinde hiçbir şekilde sulamanın yapılmadığı susuz koşullarda yaşamlarını sürdüren Ayvalık zeytin çeşidinin yetiştiği farklı zeytin plantasyonlarınının rizosfer bölgesinden alınan toprak örneklerinde yapılan AMF spor sayımı ve enfeksiyon oranlarının, toprakların kimyasal özelliklerine göre değişim gösterdiği ve topraklardaki alınabilir P ve Zn miktarlarının düşük olduğu yerlerde yetişen zeytin ağacı köklerinin daha yüksek AMF spor yoğunluğuna ve enfeksiyon oranlarına sahip olduğu belirlenmiştir.

## TEŞEKKÜR

Bu bildiri makalesi halen yürürlükte olan 120 o 289 no'lu 1001 TUBİTAK projesinin 1.aşama sonuçlarını içermektedir. Verdiği maddi destek için TUBİTAK'a çok teşekkür ederiz.

## KAYNAKLAR

Barea J. M, Jeffries P. 1995. "Arbuscular mycorrhizasin sustainable soilplantsystems. Editör: Varma A., Hock B., Mycorrhiza: structure, function, molecular biology and biotechnology", Heidelberg, Springer, 521-59.

Bi YL, Li XL, Christie P 2003. Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus soil amended with zinc and phosphorus. *Chemosphere, 50*(6), 831-837.

Calvo-Polanco, M., Sánchez-Castro I., Cantos, M., García, J. L., Azcón, R., Ruiz-Lozano J. M., Beuzón, C. R., Aroca, R. 2016. "Effects of different arbuscular mycorrhizal fungal backgrounds and soils on olive plants growth and water relation properties under well-watered and drought conditions", Plant Cell Environ, 39 (11), 2498-2514.

Giovannetti M 1985. Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. Trans. Br. Mycol. Soc. 84 : 679-684.

Isobe K, Fujii H, Tsuboki Y 1993. Studies on behavior of VA-mycorrhizal fungi in soybean culture. Jpn. J. Crop Sci. 62:351-358.

Isobe K, Sugimura H, Maeshima T, Ishii R 2008. Distribution of arbuscular mycorrhizal fungi in upland field soil of japan: 2. spore density of arbuscular mycorrhizal fungi and infection ratio in soybean and maize fields. *Plant production science*, *11*(2), 171-177.

Lekberg Y, Koide RT 2005. Arbusclar mycorrhizal fungi, rhizobia, available soil P and nodulation of groundnut (*Arachis hypogaea*) in Zimbabwe. Agric. Ecosyst. Environ. 110 : 143-148.

Mohammad A, Mitra B, Khan AG 2004. Effects of sheared-root inoculum of Glomus intraradices on wheat grown at different phosphorus levels in the field. Agric.Ecosyst. Environ. 103: 245-249.

Orlandi, F., Ferranti, F., Romano, B., Fornaciari, M. 2003. "Olive pollination: flowersand pollen of two cultivars of *Olea europaea*", N. Z. J. Crop Hortic. Sci, 31, 159-168.

Porcel R., Aroca R., Azcón R., Ruiz-Lozano J. M. 2006. "PIP aquaporin gene expression in arbuscular mycorrhizal Glycine max and Lactuca sativa plants in relation to drought stress tolerance", Plant Molecular Biology, 60, 389-404.

Ruı´z-Lozano J. M., Azco´n R, Go´mez M. 1996. "Alleviation of salt stress by arbuscular-mycorrhizal Glomus species in *Lactuca sativa* plants", Physiol Plant, 98, 767-72.

Tawaraya K, Kunii Y, Wagatsuma T 1995. Effect of arbuscular mycorrhizal inoculation and phosphate application on phophorus uptake and growth of white clover (*Trifolium repens* L.) and onion (*Allium cepa* L.) in Andosols. Jpn. J. Soil Sci. Plant Nutr. 66 : 48-53.

TÜİK 2017. "Türkiye İstatistik Yıllığı", Türkiye istatistik Kurumu Matbaası, Ankara.

Wuen Saito K, Sato S, Sugawara K 2002. Arbuscular mycorrhizal colonization and sporulation in rhizosphere of common species on native and sown grasslands. Grassland Sci. 48 : 248-253.

## *ORAL PRESENTATION*

# Green synthesis of $Ag_2S$ and its photocatalytic hydrogen production under visible irridation

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**Abstract**

This study reports on the green synthesis of silver sulfide nanoparticles using sweet cherry fruit extract. The crystal structure and band gap energy of the silver sulfide nanoparticles were characterized by X-ray powder diffraction (XRD) and UV–vis diffuse reflectance spectroscopy (UV–DRS). The silver sulfide nanoparticles were investigated in an aqueous solution in the presence of three different sacrificial agent for photocatalytic hydrogen evolution under visible light. The hydrogen evolution rate of sulfide nanoparticles were analyzed by gas chromatograph and the amount of $H_2$ production of sulfide nanoparticles were found as 490 μmol $g^{-1}$ for 180 min under visible light irridation.

**Keywords:** green synthesis, $Ag_2S$, photocatalytic, hydrogen production.

## INTRODUCTION

As the energy crisis and environmental disruptions grow, the need for clean and sustainable energy has become a matter facing human society (Güy, 2020). The intense use of fossil fuels such as oil, coal and natural gas in the world, especially in recent years, and effects such as ozone layer depletion, acid rain, and global warming will and will continue to expose the world to an environmental pollution that may be difficult to return (Jiang et al., 2014). Today, it is accepted by all scientists that the most advanced and only energy source that can provide the world's increasing energy needs without polluting the environment and sustainably is the solar-hydrogen system. Hydrogen ($H_2$) gas can be obtained by one of the methods described later, as well as wind, wave and biomass, which are considered as solar energy or its derivatives (Atacan et al., 2020). The sun is almost entirely composed of $H_2$, and its energy is composed of the fusion of $H_2$ atoms. $H_2$ is usually found on earth in the form of water combined with oxygen (Belakehal et al., 2022). Therefore, oceans, lakes, rivers are our hydrogen "mines". If $H_2$ is to be used as energy source, especially water will be utilized. Therefore, $H_2$ used as fuel will produce water again. $H_2$ is a clean-burning molecule, meaning that it can help to decarbonize a range of sectors that have proved hard to clean up in the past. But today, most $H_2$ is produced from $CO_2$-emitting fossil fuels. $H_2$ produced from renewable electricity, known as green hydrogen, could be the solution to cutting our carbon footprint. But first, it must overcome a number of challenges (Güy et al., 2022).

Recently, the biosynthesis of metal nanoparticles is highly preferred as an alternative to chemical methods as cost-effective and environmentally friendly (Saidu et al., 2019). Among them, the synthesis of metal nanoparticles using plant extracts and fruits was found to be relatively faster than extracts from fungi and bacteria. In addition, for the production of well-dispersed metal nanoparticles at the industrial level, the use of a common by-product reducing potential (such as orange, lemon, and pomegranate), their safety, and synthesis from industrial waste materials provide an extra advantage (Nishanthi et al., 2019). Silver sulfide ($Ag_2S$), as a direct semiconductor with a band gap of approximately 0.9-2.5 eV, has a relatively large absorption coefficient, leading to its wide application in photovoltaic cells and photocatalytic applications (Jiang et al., 2014). In order to obtain $Ag_2S$ nanoparticles (NPs), physicochemical methods such as molecular beam and lithography techniques, single source precursor method, microwave heating, hydrothermal synthesis, which are very labor-intensive and not environmentally friendly methods are used (Borovaya et al., 2020). They also have disadvantages such as using dangerous toxic chemicals and requiring high temperatures and pressures (Sibiya

and Moloto, 2018). Therefore, an alternative and harmless approach to obtain $Ag_2S$ nanoparticles are the biological or green synthesis of nanoparticles (Borovaya et al., 2020).

The aim of this study is to prepare $Ag_2S$ from sweet cherry by green synthesis method and to investigate its effect on photocatalytic hydrogen production. Firstly, the $Ag_2S$ nanoparticles were synthesized by the green method. Secondly, the photocatalytic $H_2$ evolution performance of $Ag_2S$ nanoparticles was evaluated and also its reusability has been examined.

## MATERIALS AND METHODS

### Materials and characterizations

All the chemicals were used in analytical grade. The sweet cherry fruit was obtained from local market. NaOH, ethylene glycol, $Na_2S$, $AgNO_3$, $Na_2SO_3$, methanol, lactic acid, and ethanol were purchased from Merck. Deionized water (DW) has been used throughout the chemical reactions.

X-ray diffraction (XRD) pattern was performed through a Bruker D8 Discover diffractometer (Bruker, USA) with filtered Cu-Kα radiation between $2\theta = 20^{o}$ and $80^{o}$. The diffuse reflectance of the photocatalyst was measured by using a UV–vis spectrophotometer fitted with a diffuse reflectance attachment. The band gap energy of the photocatalyst was determined by the Kubelka–Munk function, F (R) and by extrapolating the [F(R)hv] ½ versus photon energy (hv). $H_2$ evolved was analyzed on a thermal conductivity detector (TCD) gas chromatograph (GC, Agilent-6890, nitrogen as carrier gas).

### Preparation of $Ag_2S$ nanoparticles

In order to produce $Ag_2S$ NPs, $AgNO_3$ (silver nitrate) solution was prepared by mixing $AgNO_3$ salt in distilled water. The sweet cherry fruit was extracted and filtrated. Then, the clear filtrate was added into a flask containing $AgNO_3$ solution. After that $Na_2S$ was slowly added to the flask (Ayodhya and Veerabhadram, 2016). Solution immediately changed color to dark. Then the synthesized nanoparticles were centrifuged at 4000 rpm for 5 min. The obtained $Ag_2S$ NPs were filtered and dried 70 $^{o}$C for overnight.

### Photocatalytic $H_2$ production

PC $H_2$ evolution was performed in a 100 mL quartz photoreactor in which the outlet was closed with a silicone rubber septum (Güy et al., 2022). In a typical experiment, the photocatalyst powder (50 mg) was dispersed by a magnetic stirrer in 100 mL of 0.5 M $Na_2S/Na_2SO_3$ aqueous solution as a sacrificial agent. Three different sacrificial agents were used in this study ($Na_2S/Na_2SO_3$, methanol, lactic acid). The mixture was dispersed under ultrasound irradiation for 10 min. After replaced with nitrogen gas, the flask was vacuumized several times to remove air completely. Then the flask was placed in the photocatalytic test device to evaluate the $H_2$ evolution performance of $Ag_2S$ photocatalyst. The reaction liquid was irradiated under vigorous stirring conditions by a Xenon lamp (300 W) with a UV cutoff filter ($\lambda \geq 420$ nm) (He et al., 2022). Gas chromatograph (GC, Agilent-6890, nitrogen as carrier gas) equipped with a thermal conductivity detector (TCD) was used for on-line monitoring of the generated $H_2$ gas.

## RESULTS AND DISCUSSION

The crystalline structures of the $Ag_2S$ NPs were characterized by using X-ray powder diffraction (XRD), as illustrated in Figure 1 The majority of the characteristic peaks can be well-indexed as acanthite phase while the peaks at 45° and 64° can be attributed to the reflections of elemental silver (Ayodhya and Veerabhadram, 2016).

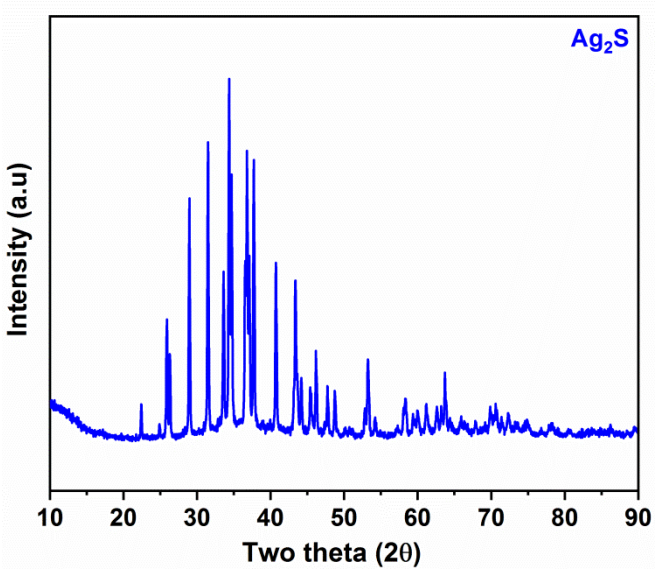


**Figure 1.** XRD pattern of $Ag_2S$

UV-vis diffuse reflectance spectroscopy (DRS) was imposed to peruse the photoabsorption characteristics of photocatalysts. The band gap energies of the photocatalyt was identified by the Kubelka Munk function and exhibited in Figure 2. Band gap energy determined by Kubelka Munk function graph is consistent with absorbance spectrum. The determined band gap energy of $Ag_2S$ is 2.16 eV.

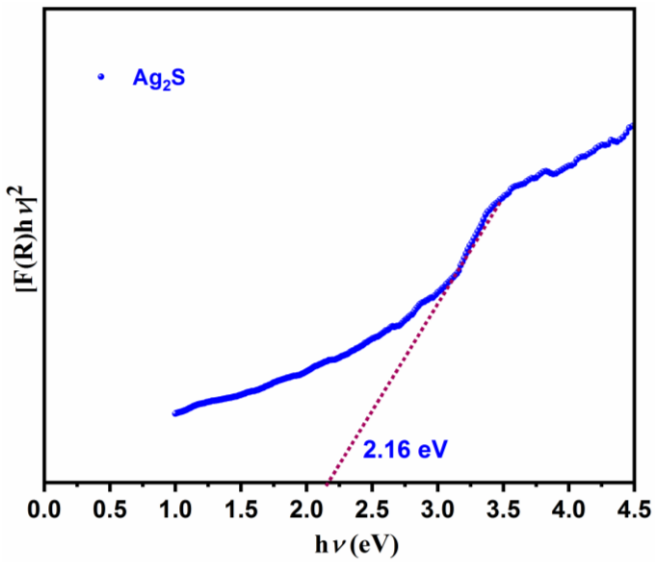


**Figure 2.** The plot of the transformed Kubelka–Munk function versus the band gap energy of $Ag_2S$

The amounts of $H_2$ versus irradiation time of the photocatalysts are delineated in Figure 3 (a). As can be depicted from Figure 3 (a), $Ag_2S$ with a value of 490 µmol $g^{-1}$ displays the greatest $H_2$ production activity, after 180 min using $Na_2S/Na_2SO_3$ as a sacrificial agent. The yields of $H_2$ production activity are found 350 µmol $g^{-1}$ and 175 µmol $g^{-1}$ using lactic acid and methanol sacrificial agents, respectively. As shown in Figure 3 (b), the $Ag_2S$ NPs (163.4 µmol $g^{-1}$ $h^{-1}$) exhibit higher photocatalytic $H_2$ evolution rates in $Na_2S/Na_2SO_3$ than the other sacrificial agent. As shown in Figure 3(c), the amount of $H_2$ evolution appears as a linear function of the reaction time. At the end of 5 cycles, the $H_2$ evolution ratio of $Ag_2S$ was detected to be 95% (484.16 µmol $g^{-1}$). The results show a high photocatalytic stability and reusability of $Ag_2S$.

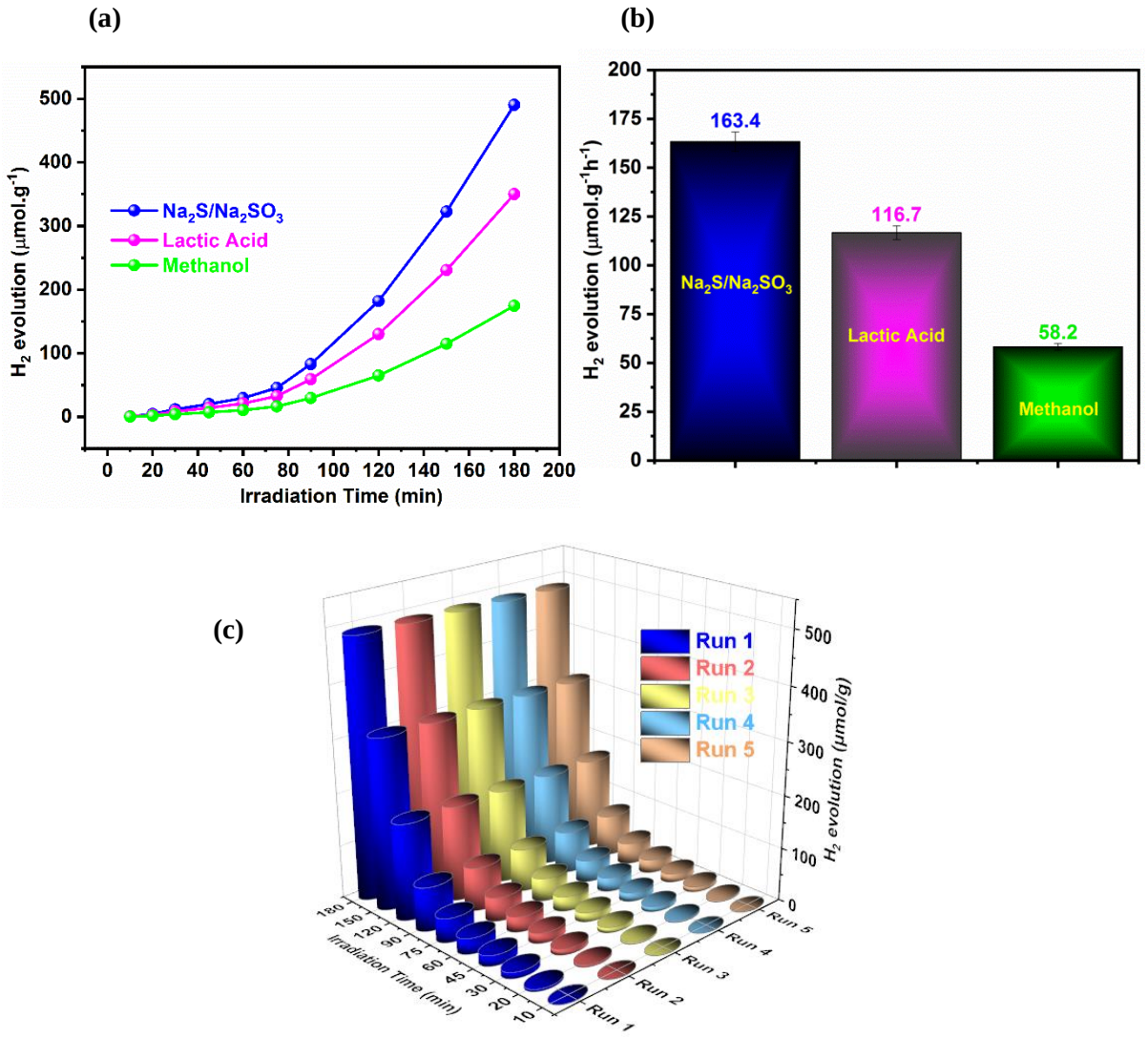


**Figure 3. (a)** The time courses of $H_2$ evolution of $Ag_2S$, **(b)** The $H_2$ evolution rates of the $Ag_2S$ and **(c)** $H_2$ evolution stability analysis of the $Ag_2S$ under visible light (l > 420 nm).

## CONCLUSION

In this study, $Ag_2S$ NPs were successfully prepared by green synthesis method from sweet cherry. $Ag_2S$ NPs were characterized and found the yield of PC $H_2$ production as 490.16 μmol $g^{-1}$ for 180 h. The fact that the $H_2$ production yield of $Ag_2S$ did not change considerably even after five continuous cycles evidences its superb recycling stability. Thus, we have developed an eco-friendly and easy reproducible technology for green synthesis of $Ag_2S$ NPs using the sweet cherry fruit.

## ACKNOWLEDGEMENTS

This study covers a part of Aydın Barış Şimşir's master's thesis. This work was supported by the financial support from the Sakarya University of Applied Sciences, Akyazı Vocational School of Health Services (Project No:083-2022). The authors extend our thanks to the Sakarya University, Biomedical, Magnetic and Semiconductor Materials Application and Research Center (BIMAS-RC) for $H_2$ production (GC-TCD) measurement. Also, we would like to thank Assoc. Prof. Nuray GÜY for her helping in this study.

## REFERENCES

Atacan K, Güy N, Boutra B, Özacar M 2020. Enhancement of photoelectrochemical hydrogen production by using a novel ternary $Ag_2CrO_4$/GO/$MnFe_2O_4$ photocatalyst. International Journal of Hydrogen Energy, 45: 17453–17467.

Ayodhya D, Veerabhadram G 2016. Green synthesis, characterization, photocatalytic, fluorescence and

antimicrobial activities of Cochlospermum gossypium capped $Ag_2S$ nanoparticles. Journal of Photochemistry & Photobiology, B: Biology, 157: 57–69.

Belakehal R, Atacan K, Güy N, Megriche A, Özacar M 2022. Fabrication of heterostructured CdS/g-$C_3N_4$/$ZnFe_2O_4$ nanocomposite synthesized through ultrasonic-assisted method for efficient photocatalytic hydrogen production. Applied Surface Science, 602: 154315.

Borovaya M, Naumenko A, Horiunova I, Plokhovska S, Blume Y, Yemets A 2020. "Green" synthesis of $Ag_2S$ nanoparticles, study of their properties and bioimaging applications. Applied Nanoscience, 10: 4931–4940.

Güy N 2020. Directional transfer of photocarriers on CdS/g-$C_3N_4$ heterojunction modified with Pd as a cocatalyst for synergistically enhanced photocatalytic hydrogen production, Applied Surface Science, 522: 146442.

Güy N, Atacan K, Özacar M 2022. Rational construction of p-n-p CuO/CdS/$CoWO_4$ S-scheme heterojunction with in fl uential separation and directional transfer of interfacial photocarriers for boosted photocatalytic $H_2$ evolution. Renewable Energy, 195: 107–120.

He W, Liu L, Ma T, Han H, Zhu J, Liu Y, Fang Z, Yang Z, Guo K 2022. Controllable morphology $CoFe_2O_4$/g-$C_3N_4$ p-n heterojunction photocatalysts with built-in electric field enhance photocatalytic performance. Applied Catalysis B: Environmental, 306: 121107

Jiang D, Chen L, Xie J and Chen M 2014. $Ag_2S$/g-$C_3N_4$ composite photocatalysts for efficient Pt-free hydrogen production. The co-catalyst function of Ag/$Ag_2S$ formed by simultaneous photodeposition, Dalton Transactions, 43: 4878.

Nishanthi R, Malathi S, John Paul S, Palani P 2019. Green synthesis and characterization of bioinspired silver, gold and platinum nanoparticles and evaluation of their synergistic antibacterial activity after combining with different classes of antibiotics. Materials Science & Engineering C-Materials for Biological Applications, 96: 693–707.

Saidu FK, Mathew A, Parveen A, Valiyathra V, Thomas GV 2019. Novel green synthesis of silver nanoparticles using clammy cherry (*Cordia obliqua Willd*) fruit extract and investigation on its catalytic and antimicrobial properties. SN Applied Sciences 1: 1368.

Sibiya PN, Moloto MJ 2018. Green Synthesis of $Ag_2S$ nanoparticles: Effect of pH and capping agent on size and shape of NPs and their antibacterial activity. Digest Journal of Nanomaterials and Biostructures, 13: 411–418.

## *ORAL PRESENTATION*

# Use of vinegar waste as a low cost and environmentally friendly biosorbent: response surface methodology approach

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## Abstract

Water is one of the most important natural resources necessary for living things to survive. As the world's growing population increases the need for water, water conservation becomes just as important. The rapid development of technology brings along many problems such as industrial, domestic and agricultural wastes and these problems play a major role in water pollution. Industrial wastes produced in large quantities as a result of industrial activities have no commercial cost. Such wastes have the potential to be used as biosorbent to remove heavy metals from aqueous media. Heavy metals are one of the environmental pollutants with carcinogenic, mutagenic effects and high toxicity properties that cause serious damage to the ecological system and human health. Hg(II) ions are a potential pollutant for aqueous media that must be removed from water/wastewater due to their harmful effects on the environment and human health, even at trace concentrations. This makes the use of such biosorbents in water treatment excellent alternatives for the removal of Hg(II), which unfortunately is not biodegradable in nature, while promoting the recycling and use of such wastes. This study aims to remove mercury from aqueous solutions by using grape vinegar waste, which is environmentally friendly and cost-effective. In this study, the use of response surface methodology (RSM) was investigated to remove Hg(II) ions from water/wastewater using vinegar waste. Adsorption experiments and optimization results proved that vinegar waste has a significant affinity for Hg(II) and is a good alternative as a biosorbent due to its rapid removal and capacity.

**Keywords:** adsorption, vinegar waste, Hg(II), RSM

## INTRODUCTION

Because of the vital importance of water to humanity, its quality needs to be improved and maintained. Domestic and agricultural activities, especially industrial wastes, environmental and global changes are the main sources of water pollution. In most cases, the discharge of wastewater into the environment without treatment poses a danger to ecosystems because it contains different and numerous pollutants (İnce and Kaplan İnce, 2019). The effects of heavy metals on the organism and the environment can be seriously toxic and harmful. even in low concentrations. Many technologies are used in physicochemical and biological treatment techniques used for heavy metal removal from water/wastewater in industry. Techniques such as ion exchange, extraction, membrane filtration, chemical precipitation and especially adsorption have been widely applied to remove heavy metals (Soliman and Moustafa, 2020). Many of these technologies are expensive, require high energy and reagent costs, involve the production of toxic secondary sludge, and are ineffective in treating waters with low metal concentrations (Fabre et al., 2021). In addition, the adsorption is generally one of the most preferred methods due to its simplicity, non-toxicity, cost-effectiveness and suitability for the removal of toxic heavy metals from aqueous media (Imran-Shaukat et al., 2021). Also, natural adsorbents have been used in this technique as a preferred option for many researchers (Soliman and Moustafa, 2020). Activated carbon is the most used adsorbent, but its cost is a limiting factor. Therefore, it is an important issue to search for new materials with lower cost in water treatment processes. By-products from agricultural or industrial processes have attracted attention due to their low cost and advantages due to their specific chemical compositions (Indah et al., 2022).

However, optimization studies using RSM to removal heavy metals by investigating various parameters are limited. Although there are many studies on heavy metal removal by using different materials in the literature, there are few studies on the application of wastewater treatment using RSM approach. Classical/conventional

methods cannot show all combinations of factors affecting process. On the other hand, the experimental design is a suitable technique to develop, improve and optimize the process. It explores the mutual relationship between various factors for optimal conditions of the process, which helps to identify interactions between optimized parameters (Montgomery, 2008; Bezerra et al., 2008). Limitations can be overcome by using an experimental design that collectively optimizes all effective parameters. RSM with fewer experiments is widely used for modeling process parameters in various processes, especially adsorption. (Çetintaş et al., 2022; Özkaraaslan et al., 2021; Öztürk et al., 2021; Güler et al., 2021; Çetintaş and Bingöl, 2018; Bingöl et al., 2015; Tekin et al., 2015; Bingol et al., 2010).

The aim of this study was to test and compare their ability to remove Hg(II) from waste water with waste grape pomace as a biosorbent using a statistical technique, RSM. Mercury was chosen because of its emissions and emissions that cause worldwide concern, causing bio-growth through the chain of bonds, and hazardous effects on human health and the environment.

## MATERIALS AND METHODS

Grape vinegar waste (GVW) samples were collected from a local vinegar factory in Kocaeli. The wastes were washed with ultrapure water, dried at 333 K, ground and then used for experimental studies.

Hg(II) stock solution (1 g $L^{-1}$) was freshly prepared from mercury(II) acetate ($Hg(CH_3COO)_2$. Hg(II) amount was determined by using the diphenylcarbazide method (Bingöl et al., 2015).

Hg(II) amount remaining in solution after adsorption were analyzed at $\lambda_{max}$ (530 nm) using a UV/VIS Spectrophotometer (T80 + UV-VIS, PG Instruments Ltd.). Removal efficiency (R, %) were calculated by considering Equation 1.

$$R\ (\%) = \frac{(C_o - C_e)}{C_o} \times 100 \quad (1)$$

here; $C_0$ (mg $L^{-1}$) and $C_e$ (mg $L^{-1}$) are the concentration of Hg(II) in the initial and equilibrium solution, respectively.

RSM is an effective method for optimizing multiple variables to find the optimum response with a minimum number of experiments using a designed set of experiments. The optimization process is formulated as a quadratic model, including linear terms, of the relationship between response, factors, and interactions (Equation 2).

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_1^2 + \sum_{1 \le i \le j}^{k} \beta_i x_i x_j + \varepsilon \quad (2)$$

where $\beta_0$, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ are regression coefficients as constant term, linear effect term, quadratic effect term, interaction effect term, respectively; $x_i$ and $x_j$ are factors; ε is random error and y is the response of the factors (Montgomery, 2008).

In the present study, the effects of two independent variables (analyte concentration and adsorbent amount) on the response function (R%) were investigated using RSM with central composite design (CCD), and optimum conditions were determined by maximizing the percentage of Hg(II) removal.

## RESULTS

The factors and levels used in CDD design are presented in Table 1. The predictive ability of the model used to optimize the Hg(II) adsorption process was determined by ANOVA analysis. Table 2 shows ANOVA results for percent Hg(II) removal (R%) in 95% confidence level. The reduced model had high correlation values ($R^2$ > 99%). This meant that only ~1% of the total variation could not be explained by the model. According to the results obtained, initial concentration of Hg(II) ($C_0$) and adsorbent amount ($m$) were found to be important factors at the 5% significance level ($P < 0.05$). However, the effects of m*m square interaction with $P$ values above 0.05 were eliminated. $C_0$*$C_0$ square and $C_0$*$m$ interaction effects were also found to be effective parameters for the adsorption of Hg(II) onto GVW. Then, a suitable regression model with a determination

coefficient ($R^2$) of 0.95 ($P < 0.05$) for the adsorption system was proposed. According to the second order model, the amount of adsorbent for R% has a synergistic effect, while Hg(II) concentration has an antagonistic effect. When the effect of a factor is positive, the R% increase by changing the value of a factor (such as *m*) from low to high. However, if the effects are negative, changing the higher level to a lower level such as $C_0$ for R% produces a reduction in removal efficiency. It was shown that the amount of GVW (*m*) was more effective on the removal efficiency of Hg(II) adsorption during the adsorption process. This effect can be attributed to the increase in available adsorption sites that remain unsaturated during the removal process. The presence of interaction means that factors can affect the response factor (R%) interactively, but not independently. Combined effects may be greater or less than would be expected for direct addition of effects. These synergistic effects cannot be detected in a univariate optimization of the system (Zolgharnein et al., 2017). These effects were observed in the response surface and contour plots.

**Table 1**. CCD matrix

| Factors | Symbol | Levels of factors | | | | |
|---|---|---|---|---|---|---|
| | | –α (–1,414) | –1 | 0 | +1 | +α (+1,414) |
| $C_0$ (mg $L^{-1}$) | $X_1$ | 100 | 159 | 300 | 441 | 500 |
| *m* (g) | $X_2$ | 0.0100 | 0.0232 | 0.0550 | 0.0868 | 0.1000 |

| Run | Coded variable levels | | Actual variable levels | |
|---|---|---|---|---|
| | $X_1$ | $X_2$ | $C_0$ (mg $L^{-1}$) | *m* (g) |
| 1 | -1 | -1 | 159 | 0.0232 |
| 2 | 1 | -1 | 441 | 0.0232 |
| 3 | -1 | 1 | 159 | 0.0868 |
| 4 | 1 | 1 | 441 | 0.0868 |
| 5 | -1,414 | 0 | 100 | 0.0550 |
| 6 | 1,414 | 0 | 500 | 0.0550 |
| 7 | 0 | -1,414 | 300 | 0.0100 |
| 8 | 0 | 1,414 | 300 | 0.1000 |
| 9-13 | 0 | 0 | 300 | 0.0550 |

*(N=2)

The response surface and the contour plots were graphical representations of the quadratic equation derived from the model. To estimate the Hg(II) adsorption efficiency over the independent variables, the response surface and the contour plots were represented in Figure 1 (a and b), respectively. Figures shows the effects of GVW amount and initial Hg(II) concentration on adsorption efficiency. It was shown that the adsorption efficiency increased with the decrease in the Hg(II) concentration and the increase in the amount of GVW. The positive increase in sorption efficiency is attributed to the increase in the usable surface area and adsorption sites due to the increase in the amount of adsorbent.

**Table 2**. ANOVA results

| Source | DF | Adj SS | Adj MS | F-Value | P-Value | VIF |
|---|---|---|---|---|---|---|
| Model | 5 | 9150.64 | 1830.13 | 150.28 | 0.000 | |
| Linear | 2 | 7949.54 | 3974.77 | 326.40 | 0.000 | |
| $C_0$ | 1 | 3601.65 | 3601.65 | 295.76 | 0.000 | 1.00 |
| $m$ | 1 | 4347.89 | 4347.89 | 357.04 | 0.000 | 1.00 |
| Square | 2 | 1045.25 | 522.63 | 42.92 | 0.000 | |
| $C_0$*$C_0$ | 1 | 937.18 | 937.18 | 76.96 | 0.000 | 1.02 |
| $m$*$m$ | 1 | 39.86 | 39.86 | 3.27 | 0.085 | 1.02 |
| 2-Way Interaction | 1 | 155.85 | 155.85 | 12.80 | 0.002 | |
| $C_0$*$m$ | 1 | 155.85 | 155.85 | 12.80 | 0.002 | 1.00 |
| Error | 20 | 243.55 | 12.18 | | | |
| Lack-of-Fit | 3 | 140.73 | 46.91 | 7.76 | 0.002 | |
| Pure Error | 17 | 102.83 | 6.05 | | | |
| Total | 25 | 9394.19 | | | | |

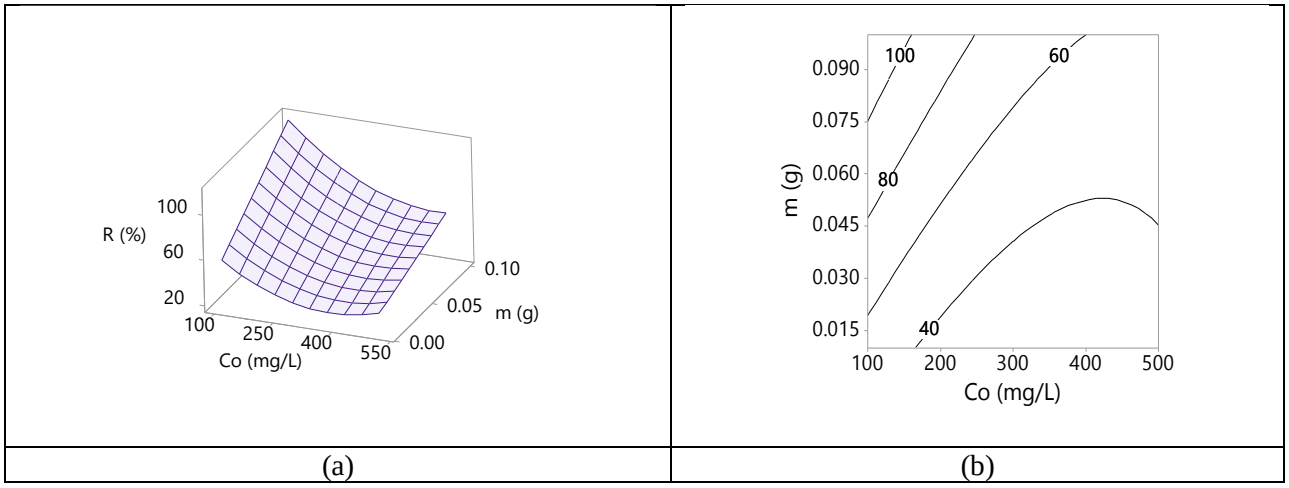
*Regression Equation in Coded Units*

R (%) = 47.51 - 0.2984 $C_0$ + 996 $m$ + 0.0004 $C_0$*$C_0$ - 0.981 $C_0$*$m$

*Reduced Model Summary*

$S$= 1.38 $R^2$= 99.56% $R^2(adj)$= 99.48% $R^2(pred)$= 99.36%

DF: total degrees of freedom, F-value: Test statistic, P-value: Probability,
Adj SS: Adjusted sum of squares, Adj MS: Adjusted mean squares,
VIF: Variance inflation factor, S: Standard deviation,
$R^2$: Coefficient of determination, $R^2(adj)$: Adjusted coefficient of determination,
$R^2(pred)$: Predicted coefficient of determination




(a) (b)

**Figure 1.** (a) Response surface plot and (b) Contour plot for Hg(II) removal

## DISCUSSION

The results revealed that the selected model had good precision and good agreement between the predicted and experimental data. Optimum removal of Hg(II) was achieved as 96.2%±1.4 under the optimum conditions of adsorbent amount of 0.090 g and analyte concentration of 150 mg $L^{-1}$. Experimentally obtained percent removal was 96.9%±0.5 with standard deviation (N=3). The resulting model can be successfully applied to predict experimental data. According to the validation experiments, the predicted and the experimental results obtained under the optimum conditions were found to be quite compatible. As an adsorbent, vinegar wastes can be effectively used for Hg(II) removal from aqueous solutions.

## CONCLUSION

The results presented above clearly showed that vinegar wastes can be successfully applied as a high efficiency adsorbent in the removal of Hg(II) from aqueous solutions. Adsorption conditions were optimized and the effects of experimental factors were evaluated with RSM. Quantitative results for Hg(II) removal from aqueous

solutions were obtained under the adsorption conditions determined by CCD. The adsorption process was presented as a quadratic model with a limited number of experiments using the RSM approach.
Removal of Hg(II) from industrial effluents using vinegar wastes represent many features where, grape vinegar wastes, considered as a promising and inexpensive alternative concept for wastewater treatment via environmentally-friendly way. The vinegar wastes offering potential advantages as a low-cost, availability, high efficiency, and green alternative source.

## REFERENCES

Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA 2008. Response Surface Methodology (RSM) as a Tool for Optimization in Analytical Chemistry. Talanta, 76:965-977.

Bingöl D, Saraydin D, Özbay DŞ 2015. Full Factorial Design Approach to Hg(II) Adsorption onto Hydrogels. Arab J Sci Eng, 40: 109–116.

Bingol D, Tekin N, Alkan M 2010. Brilliant Yellow dye adsorption onto sepiolite using a full factorial design. Applied Clay Science, 50(3): 315-321.

Cetintas S, Ergül HA, Öztürk A, Bingöl D 2022. Sorptive Performance of Marine Algae (Ulva lactuca Linnaeus, 1753) with and without Ultrasonic-Assisted to Remove Hg(II) Ions from Aqueous Solutions: Optimization, Equilibrium and Kinetic Evaluation. International Journal of Environmental Analytical Chemistry, 102(6): 1428-1451.

Çetintaş S, Bingöl D 2018. Optimization of Pb(II) Biosorption with Date Palm (*Phoenix Dactylifera* L.) Seeds Using Response Surface Methodology. Journal of Water Chemistry and Technology, 40: 370-378.

Fabre E, Vale C, Pereira E. et al. 2021. Sustainable Water Treatment: Use of Agricultural and Industrial Wastes to Remove Mercury by Biosorption. Water Air Soil Pollution, 232:284.

Güler M, Çetintaş S, Bingöl D 2021. Cinnamon bark as low-cost and eco-friendly adsorbent for the removal of indigo carmine and malachite green dyestuffs. International Journal of Environmental Analytical Chemistry, 101(6): 735-757.

Imran-Shaukat M, Wahi R, Ngaini Z 2022. The application of agricultural wastes for heavy metals adsorption: A meta-analysis of recent studies. Bioresource Technology Reports, 17:100902.

Ince M, Ince OK 2019. Heavy Metal Removal Techniques Using Response Surface Methodology: Water/Wastewater Treatment.In: Ince M., Ince OK, Ondrasek G (eds.), Biochemical Toxicology - Heavy Metals and Nanomaterials, IntechOpen. London: 10.5772/intechopen.88915.

Indah S, Helard D, Primasari B, Edwin T, Andeslin S 2022. Performance Evaluation of Physically and Chemically Modified Pumice on Removal of Cu(II) from Aqueous Solution. Trends in Sciences, 19(4): 2585.

Montgomery DC 2008. Design and Analysis of Experiments, seventh ed., John Wiley & Sons, New York.

Özkaraaslan H, Çetintaş S, Bingöl D 2021. A novel composite derived from carbonized hawthorn waste pulp/marble waste powder by ball milling: preparation, characterization, and usability as bifunctional adsorbent. Biomass Conversion and Biorefinery, Available at: https://doi.org/10.1007/s13399-021-01412-1

Öztürk A, Çetintaş S, Bingöl D 2021. The use of pomegranate seed activated by mechanochemical process as a novel adsorbent for the removal of anionic dyestuffs: response surface method approach. Chemical Engineering Communications, 208(9): 1279-1300.

Soliman NK, Moustafa AF 2020. Industrial solid waste for heavy metals adsorption features and challenges; a review. Journal of Materials Research and Technology, 9(5): 10235-10253.

Tekin N, Şafaklı A, Bingöl D 2015. Process modeling and thermodynamics and kinetics evaluation of Basic Yellow 28 adsorption onto sepiolite. Desalination and Water Treatment, 54(7): 2023-2035.

Zolgharnein J, Dalvand K, Rastgordani M, Zolgharnein P 2017. Adsorptive removal of phosphate using nano cobalt hydroxide as a sorbent from aqueous solution; multivariate optimization and adsorption characterization. Journal of Alloys and Compounds, 725: 1006-1017.

*ORAL PRESENTATION*

# Toxicity assessment of domestic effluents for reuse in irrigation

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## Abstract

One of the measures that can be taken against water scarcity and droughts due to global climate change is the use of alternative water resources in agricultural irrigation. Treated wastewater is one of those alternative water sources. However, many hazardous substances that are likely to be present in treated wastewaters at undetectable concentrations may exhibit toxic, ecotoxic and/or phytotoxic effects. Therefore, in addition to the physicochemical characterization, it is also of great importance to assess the aquatic toxicity, ecotoxicity and phytotoxicity of effluents prior to reuse. In this study, a test battery comprised of three different bioassays was utilized to assess the toxic, ecotoxic, and phytotoxic impacts of four streams sampled from a BioWWTP treating domestic wastewater. Experimental results implied that the plant was effective in reducing the toxic effects of the incoming wastewater and discharging an effluent with no aquatic toxicity, thus pointing to the potential of reusing the treated stream for irrigation.

**Keywords:** Toxicity, ecotoxicity, phytotoxicity, irrigation

## Introduction

Water scarcity is one of the main consequences of climate change and the quality of freshwater resources keep declining due to increasing pollution pressures. The agricultural sector has the highest share in water consumption with approximately 75% of freshwater resources being used for agricultural irrigation. One of the remedial measures is the use of alternative water sources in agricultural irrigation and other possible sectors and treated wastewater is one of the promising alternatives for that purpose.

For the use of treated wastewater in agriculture, and in addition to the physico-chemical properties, it is also important to display the risks of toxicity to plants and aquatic ecosystems. Although the concentrations of many potentially dangerous substances in treated wastewater might be low, and the amounts of those that are restricted in the regulations may not exceed the specified limits, those type of dangerous substances may have toxic, ecotoxic and phytotoxic effects when they are together. Therefore, besides the physico-chemical characterization of treated wastewater, it is also of great importance to assess the aquatic toxicity, ecotoxicity and phytotoxicity of waste streams/effluents if to be considered for reusing in irrigation.

The effectiveness of wastewater treatment plants (WWTPs) needs to be evaluated in terms of protecting the ecosystem components from negative effects by reducing the pollutants in wastewater before discharging to the receiving environment or by providing the possibility of recycling and reuse of treated water; i.e. for irrigation in agriculture. A combination of bioassays, in other words a test battery, is a convenient tool for assessing the toxicity characteristics of wastewaters/effluents. Moreover, toxicity tests are not as expensive and laborious as the high-tech instrumental analysis, hence it is more cost-effective and practical to perform a toxicity test battery to evaluate the possibility of using treated wastewater in agriculture and to perform subsequent physicochemical analysis if appropriate results are obtained. This study aims at using such a test battery to determine the toxic, ecotoxic, and phytotoxic impacts of the waste streams sampled from a BioWWTP treating domestic wastewater.

## Material and Methods

Samples were collected from the influent and effluent of a Bio-WWTP treating domestic wastewater. Additionally, supernatant from the sludge thickener and inlet to the EBPR unit were also sampled. Toxic, eco-toxic and phytotoxic effects in those samples were assessed using a test battery comprised of respirometric

tests (ISO 8192:2007, oxygen uptake rate measurements) for toxicity, in vitro tests with bioluminescent bacteria *Vibrio fischeri* (ISO 11348-3:2008, AboaTox-Watertox™) for ecotoxicity, and tests for the determination of the growth-inhibiting response of duckweed (*Lemna minor*) (ISO 20079:2006) for phytotoxicity.

## Results and Discussion

In the experiments where four samples taken from different points of a BioWWTP were tested, aquatic toxicity assessment was done by monitoring the oxygen uptake rates (0, 30, 180 min) of an acclimated heterotrophic biomass exposed to three different dilutions of each sample. In ecotoxicity measurements, reconstituted *Vibrio fischeri* were exposed to test samples, which were chosen from a combination of two geometric dilution series (D = 2, 4, 8, 16 etc., and D = 3, 6, 12, 24 etc.) where 2% NaCl solution was used as the diluent. Luminescence intensity measurements after 0, 15, and 30 min contact time (at +15°C) were used to calculate the % inhibition (%INH). For phytotoxicity measurements, *Lemna minor* duckweed cultures were exposed to a series of different dilutions of the samples for 7 days. Phytotoxicity was calculated by evaluating growth inhibition at the end of 7 days. Results of the ecotoxicity tests are given below in Table 1 to exemplify the toxicity effects recorded in the tested samples. The highest ecotoxicity was recorded in the influent, followed by the BioP inlet stream, whereas there was no ecotoxic effect in the supernatant and effluent streams. Accordingly, the BioWWTP was found to be effective in reducing the ecotoxic effects of the incoming wastewater and discharging an effluent which might be considered to be reused as a water resource for irrigation.

**Table 1** Ecotoxicity effects of the influent, supernatant, BioP inlet, and effluent streams of the BioWWTP assessed by the *Vibrio fischeri* inhibition test*

| | | | %INH in BioWWTP streams | | | |
|---|---|---|---|---|---|---|
| **Dilution rate** | **Dilution series** | **%Conc.** | **Influent** | **Supernatant** | **BioP Inlet** | **Effluent** |
| - | - | 80% | - | - | 48.7 | none |
| - | 2 | 50% | 48.6 | none | 39.7 | none |
| 2:3 | 3 | 33% | 42.8 | none | 32.1 | none |
| 1:2 | 2 | 25% | 37.0 | none | 22.9 | none |
| 2:6 | 3 | 16.5% | 24.9 | - | 7.8 | none |
| 1:4 | 2 | 12.5% | - | none | - | - |
| 2:12 | 3 | 8.25% | none | none | none | - |

**Validity check with 52.3 mg/L of potassium dichromate in the final test suspension: 43.7% inhibition after 30 min contact time (within the recommended range of 20 to 80%)*

## Conclusions

The experimental results showed that the test battery applied in this study was useful in assessing the toxicity characteristics of the tested waste streams, and particularly the effluent from the BioWWTP for possible reuse in irrigation.

## References

BS EN ISO 8192:2007. Water quality — Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation

BS EN ISO 11348-3:2008. Water quality - Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) - Part 3: Method using freeze-dried bacteria

BS EN ISO 20079:2006. Water quality - Determination of the toxic effect of water constituents and waste water on duckweed (Lemna minor) - Duckweed growth inhibition test)

## *ORAL PRESENTATION*

# Investigation of Photocatalytic $H_2$ Production of g-$C_3N_4$ Supported Bimetallic Sulfide Nanocomposites

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## Abstract

Photocatalytic hydrogen ($H_2$) evolution is a very promising strategy for the production of $H_2$ from water using solar energy. In applications of visible-light-driven hydrogen evolution, photocatalysts with proper band energy levels have come into particular focus. Herein, $Cd_{0.6}Zn_{0.4}S$ nanocomposites containing different amounts of g-$C_3N_4$ were successfully obtained, and their photocatalytic $H_2$ production was analyzed. When their activities were compared, it was determined that the activities of 10 wt% g-$C_3N_4$/ $Cd_{0.6}Zn_{0.4}S$ nanocomposites with the value of 43124.25 μmol/g were higher than both pure g-$C_3N_4$ and $Cd_{0.6}Zn_{0.4}S$. Furthermore, the stability of the $Cd_{0.6}Zn_{0.4}S$ was remarkably boosted due to the rapid hole exhaustion by $Na_2S/Na_2SO_3$ via sulfur vacancies. This study will shed light on the design of carbon-supported bimetallic sulfide nanocomposites for efficient photocatalytic $H_2$ production applications.

**Keywords:** Bimetallic sulfide, g-C3N4, photocatalytic $H_2$ production.

## INTRODUCTION

In recent years, the concept of energy has become the most substantial theme in our lives. With the development of industrialization and the rapid increment in population, the need for energy is increasing (Liu et al., 2021). Traditional energy sources utilized to meet this need negatively affect the environment and human health. With the intense consumption of fossil energy, studies on clean, sustainable, and renewable alternative energy sources that will replace traditional energy sources have increased (Güy et al., 2022). Hydrogen ($H_2$) energy, one of the renewable energy sources, has become a superb energy source due to its low pollutant, high combustion efficiency, and sustainability (Atacan et al., 2020; Güy et al., 2022; Liu et al., 2021; Xiang et al., 2019; Xue et al., 2016). K. Honda and A. Fujishima discovered in 1972 that they could, for the first time, split water into hydrogen and oxygen ($O_2$)under ultraviolet light utilizing $TiO_2$ single-crystal electrodes, indicating that hydrogen could be produced through solar photolysis. Photocatalytic $H_2$ production through water separation offers a carbon-free renewable source for clean and sustainable economic advancement (Tang et al., 2019; Xiang et al., 2019).

Numerous visible-light-driven photocatalysts have been evolved to utilize solar energy fully. CdS, one of the most significant transition metal sulfides, has been one of the most extensively investigated photocatalysts in photocatalytic $H_2$ production with its narrow band gap and relatively negative conduction band potential (Liu et al., 2021; Tang et al., 2019; Xiang et al., 2019; Xue et al., 2016). However, the handicaps of CdS, such as rapid recombination and photo corrosion, restrict its photocatalytic applications (Liu et al., 2021; Tang et al., 2019; Xiang et al., 2019; Xue et al., 2016). In this context, solid solutions of $Cd_xZn_{1-x}S$ have been extensively studied due to their controllable band structures and better activities as a result of their twinning nature (Xiang et al., 2019). g-$C_3N_4$ is a semiconductor photocatalyst with a narrow band gap (~2.7 eV) and graphene-like structure, and it also has a relatively negative conduction band (CB) potential, which can effectively reduce $H^+$ to $H_2$ (Feng et al., 2021). Carbon-based nanomaterials such as g-$C_3N_4$ can offer continuous ways to increase kinetics for electrons and ions and serve as a capacity provider with surface adsorption/desorption to improve the specific capacity further. Moreover, Nanocomposites containing g-$C_3N_4$ can form a Z- or S-scheme

heterostructured systems that can attain significantly advanced photocatalytic performance compared to single material systems.

In this study, we successfully prepared g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ nanocomposites containing g-$C_3N_4$ with different weights via the microwave method. Compared with bare g-$C_3N_4$, bare $Cd_{0.6}Zn_{0.4}S$, and g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ nanocomposites with the different weights of g-$C_3N_4$, it has been found that 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ has the greatest the photocatalytic hydrogen production efficiency. The efficiency of $Cd_{0.6}Zn_{0.4}S$ with good photocatalytic $H_2$ production results in a remarkable increase with the combination of g-$C_3N_4$, as the recombination of photocarriers, is delayed and the number of active sides is increased. This work will dispense a new perspective on booming bimetallic sulfide-based nanocomposites for efficient photocatalytic $H_2$ production applications.

## MATERIALS AND METHODS

Cadmium acetate ($Cd(Ac)_2{\cdot}2H_2O$), zinc acetate ($Zn(Ac)_2.2H_2O$) and thiourea ($CH_4N_2S$), ethylenediamine **(**$C_2H_4(NH_2)_2$) and melamine ($C_3H_6N_6$) were utilized without further purification. Reaction solutions were prepared by utilizing deionized water.

g-$C_3N_4$ was obtained considering the synthesis procedure in the study reported by Nuray. The synthesis procedure is summarized in Figure 1 (Güy, 2020).

The twinning $Cd_{0.6}Zn_{0.4}S$ nanoparticles were synthesized by a microwave method (Li et al., 2010). In the $Cd_{0.6}Zn_{0.4}S$ synthesis procedure, 0.48 g $Cd(Ac)_2{\cdot}2H_2O$, 0.264 g $Zn(Ac)_2.2H_2O$, and $CH_4N_2S$ were dissolved in the mixture of 20 mL ethylenediamine and 10 mL water. The resulting mixture was heated to 140 °C in a microwave oven (CEM Mars model) with a controlled power of 600 W for 10 min and then cooled to room temperature naturally. For the x% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ nanocomposites, it was utilized $Cd_{0.6}Zn_{0.4}S$ synthesis procedure. The x indicates different ratios of g-$C_3N_4$ in the sample.

Photocatalytic $H_2$ production experiments were carried out considering the experimental procedure in the study reported by Nuray et al (Güy et al., 2022).

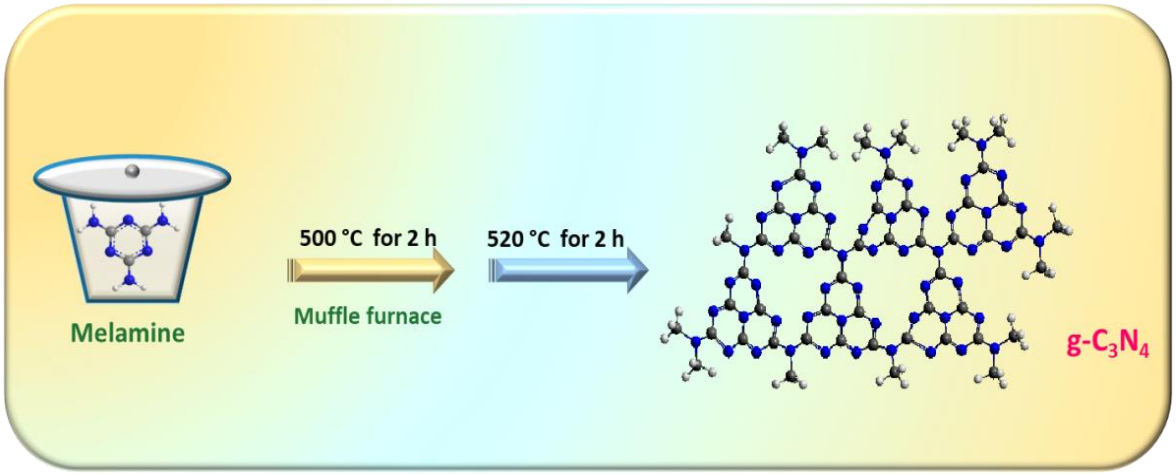


**Figure 1.** The synthesis of g-$C_3N_4$

## RESULTS and DISCUSSION

First, the photocatalytic $H_2$ production activities of g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ nanocomposites containing different weights of g-$C_3N_4$ such as 5% wt, 10% wt, and 15% wt were investigated in $Na_2S/Na_2SO_3$ electron donor solution and the results were represented in Figure 2. When $H_2$ production efficiency was compared, it was found that nanocomposites' $H_2$ production efficiency ratios were greater than pure $Cd_{0.6}Zn_{0.4}S$ and g-$C_3N_4$. Considering the effect of g-$C_3N_4$ content in the nanocomposite on photocatalytic hydrogen production, 10% by wt g-$C_3N_4$ content was detected as the optimum ratio. The $H_2$ production rates of g-$C_3N_4$, 5 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$, 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ and 15 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ are 18.9, 5425.79, 43124.25 and 18754.6 µmol/g, respectively.

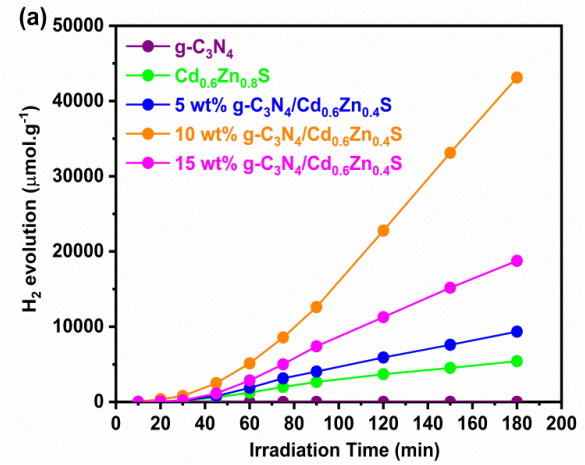


**Figure 2.** The time courses of $H_2$ production of photocatalysts under visible light (λ > 420 nm).

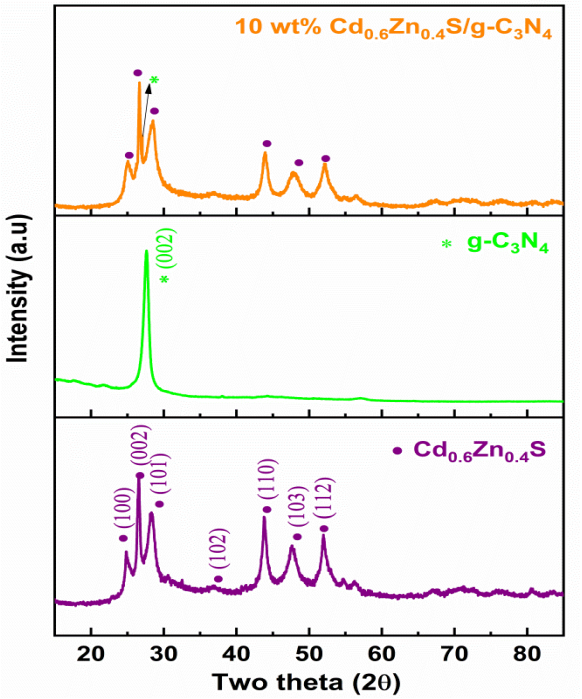


**Figure 3.** XRD patterns of $Cd_{0.6}Zn_{0.4}S$, g-$C_3N$, and 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ photocatalyts.

Figure 3 delineates the XRD patterns of photocatalysts to characterize their structure composition and crystal phase. In the XRD pattern of pure $Cd_{0.6}Zn_{0.4}S$, observed the diffraction peaks correspond well to the (100), (002), (101), (102), (110), (103) and (112) crystal planes of $Cd_{0.6}Zn_{0.4}S$, respectively (Liu et al., 2021; Xiang et al., 2019; Xue et al., 2016). The pure g-$C_3N_4$ has a characteristic peaks at 27.4°, which is imputed to the (002) planes of hexagonal structure of g-$C_3N_4$ (JCPDS file No. 87-1526) (Güy, 2020). For 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ nanocomposite, the XRD pattern is like to that of $Cd_{0.6}Zn_{0.4}S$ and g-$C_3N_4$. The poor peak intensity of g-$C_3N_4$ indicates the low content of g-$C_3N_4$ in the nanocomposite.

The optical features and band gap energies of the photocatalysts were inspected by UV-vis DRS to understand the reasons for the boosted $H_2$ production performance. The band gap energies of the products were specified by the Kubelka Munk function (Güy, 2020). As depicted in Figure 4, band gap energies of all the products correspond to visible region. As the g-$C_3N_4$ content increases, the band gap energies of the products also increase.

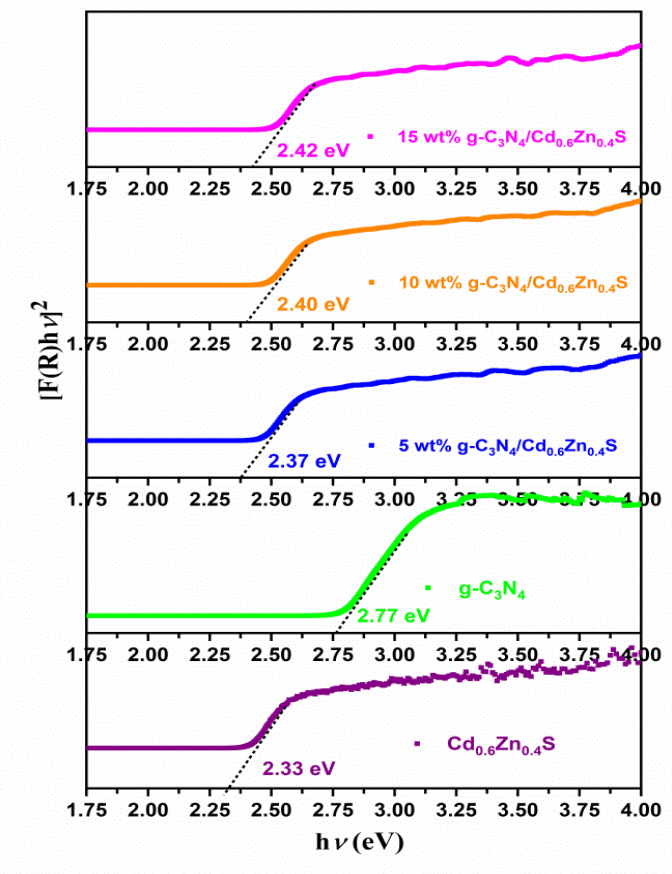


**Figure 4.** The band gap energies plots of photocatalyts.

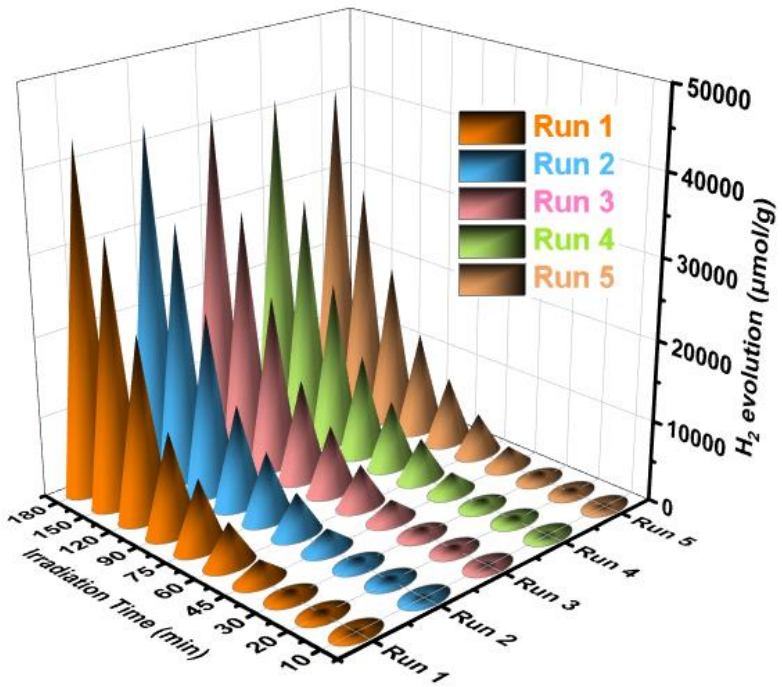


**Figure 5.** Cycling analysis of 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ nanocomposite in the $H_2$ production under visible light ($\lambda > 420$ nm).

Another considerable parameter for photocatalytic applications is reusability stability. Therefore, reusability tests were performed for 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$, and the results are delineated in Figure 5. Considering the results, it was observed that there was no substantial change in the $H_2$ production performance of 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ S even after five cycles, and its stability was guarded.

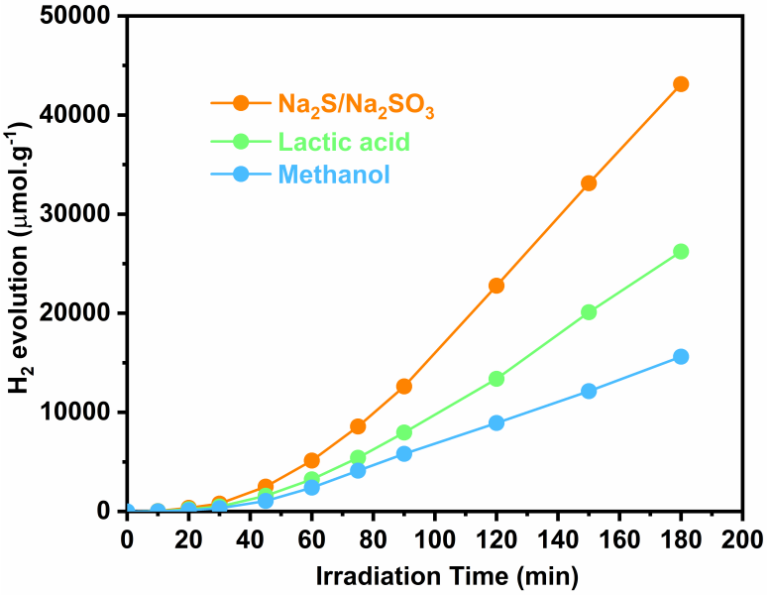


**Figure 6.** Photocatalytic hydrogen production performance of 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ in the presence of different hole scavengers.

The effect of different photocatalytic hole scavengers in the presence of 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ under visible light irradiation on the current photocatalytic $H_2$ production process was perused, and obtained results are represented in Figure 6. The hydrogen production yields of hole scavengers in an aqueous solution system followed an order of $Na_2S/Na_2SO_3$ > lactic acid > methanol.

In light of the literature information, considering the conduction band and valence band potentials of $Cd_{0.6}Zn_{0.4}S$ and g-$C_3N_4$, it is concluded that the photocatalytic $H_2$ production mechanism fits the S-Scheme (Bibi et al., 2019). Under visible light, both semiconductor materials are excited; valance band (VB) electrons are excited to conduction band (CB). According to the S-scheme, the CB electrons of g-$C_3N_4$ tend to recombine at the composite interface with the VB electrons of $Cd_{0.6}Zn_{0.4}S$. The active CB electrons of $Cd_{0.6}Zn_{0.4}S$ are also involved in $H_2$ production.

## CONCLUSION

In summary g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ nanocomposites were synthesized by microwave method and their $H_2$ production yields executed. Compared to the $H_2$ production performances of g-$C_3N_4$, $Cd_{0.6}Zn_{0.4}S$, 5 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$, 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$, and 15 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$, 10 wt% g-$C_3N_4$/$Cd_{0.6}Zn_{0.4}S$ indicates the greatest performance with 43124.25 μmol/g. With the synergistic interaction between g-$C_3N_4$and $Cd_{0.6}Zn_{0.4}S$, the lifetime of the charge carriers is extended, and the charge separation efficiency and light absorption capacity increase, resulting in boosted $H_2$ production efficiency. To sum up, hhis study will shed light on the design of bimetallic sulfide nanocomposites with carbon-containing support material for efficient photocatalytic $H_2$ production applications.

## ACKNOWLEDGEMENTS

This study covers part of Serpil Yıldız's master's thesis. Financial support for this work has been provided by the Scientific Research Projects Commission of Sakarya University (Project number: 2022-7-24-142).

## REFERENCES

Atacan K, Güy N, Boutra B, Özacar M 2020. Enhancement of photoelectrochemical hydrogen production by using a novel ternary $Ag_2CrO_4$/GO/$MnFe_2O_4$ photocatalyst. Int. J. Hydrogen Energy, 45, 17453–17467.

Bibi T, Manzoor S, Azhar S, Saeed M, Raheem, MA 2019. Dual-vacancies design on Z-scheme g-

$C_3N_4/Zn_{0.3}Cd_{0.7}S$ heterojunctions for boosting photocatalytic $CO_2$ reduction. Available at: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4226947 [22 Sep 2022].

Feng C, Chen Z, Jing J, Sun M 2021. Significantly enhanced photocatalytic hydrogen production performance of g-$C_3N_4$/CNTs/CdZnS with carbon nanotubes as the electron mediators. Journal of Materials Science & Technology, 80, 75–83.

Güy N 2020. Directional transfer of photocarriers on CdS/g-$C_3N_4$ heterojunction modified with Pd as a cocatalyst for synergistically enhanced photocatalytic hydrogen production. Appl. Surf. Sci., 522, 146442.

Güy N, Atacan K, Özacar M 2022. Rational construction of p-n-p CuO/CdS/$CoWO_4$ S-scheme heterojunction with influential separation and directional transfer of interfacial photocarriers for boosted photocatalytic $H_2$ evolution. Renew. Energy, 195, 107–120.

Li W, Li D, Zhang W, Hu Y, He Y, Fu X 2010. Microwave synthesis of $Zn_xCd_{1-x}$ S nanorods and their photocatalytic activity under visible light. J. Phys. Chem. C, 114, 2154–2159.

Liu Q, Jian C, Ji Y, Liu Y, Liu C, Che G, Wang D, Junming C, Li W, Liu X 2021. The direct Z-scheme $Cd_xZn_{1-x}S$ nanorods-$Fe_2O_3$ quantum dots heterojunction/reduced graphene oxide nanocomposites for photocatalytic degradation and photocatalytic hydrogen evolution. Appl. Surf. Sci., 570, 151085.

Tang Y, Zhang D, Qiu X, Zeng L, Huang B, Li H 2019. Fabrication of a $NiCo_2O_4/Zn_{0.1}Cd_{0.9}S$ p-n heterojunction photocatalyst with improved separation of charge carriers for highly efficient visible light photocatalytic $H_2$ evolution. J. Alloys Compd., 809, 151855.

Xiang J, Wang H, Wang X, Chen X, Wu T, Wan H 2019. Colloidal $Cd_xZn_{1-x}S$ nanocrystals as efficient light irradiation, 4001–4007.

Xue F, Fu W, Liu M, Wang X, Wang B 2016. Growth mechanism and their application for photocatalytic hydrogen production. Int. J. Hydrogen Energy 41, 20455–20464.

## *ORAL PRESENTATION*

# Synthesis, theoretical calculations, and investigation of anti-tumor activities of the novel 3-(2-(4-Ethyl-5-thion-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl)coumarin

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## Abstract

Compounds of triazole derivatives are widely used in medicine and pharmacology. Likewise, the use of compounds containing coumarin ring in these areas is quite common. In this study, these two biologically active rings were designed and synthesized compound was illuminated by IR, NMR, LCMS-MS. Moreover, Gaussian calculations was performed. Anti-cancer activity of HCT-116 and HT-29 colon cancer and MDA-MB-231 breast cancer cells was studied by MTT method.

**Keywords:**Triazole, DFT, Anti-cancer activity

## INTRODUCTION

Heterocycles in which a majority of biologically active pharmaceuticals are synthesized are the largest class in organic chemistry. Up to now, a wide range of heterocyclic compounds are explored and enhanced for the human health. Among these compounds, triazoles are one of the most significant heterocycles showing significant pharmacological activity because of being a fundamental chemical compound for the livings (Michael et al., 2012). Triazole, which is basic aromatic chemical is a five-membered heterocyclic ring being has three nitrogen atoms in 1,2 and 4 positions. The properties of triazoles have been used in several aspects for years in pharmacological medicine for their biological activity such as antitumor, antimicrobial, fungicidal, antiviral, antioxidant, and antitubercular. In this sense, one of the triazole derivatives is coumarin, which includes 2H-chromen-2-one structure is commonly found in plants and various oil types from the nature (Cao et al.,2019). Coumarins take a main place in the synthesis of many pharmaceutical products, especially in the pharmaceutical industry (Yang et al., 2021). These compounds have an intense interest due to their many biological activities such as anticancer, antifungal, anti-HIV, antimicrobial, and antioxidant activities (Duangdee et al., 2020). Therefore, many types of coumarin derivatives being synthesized exhibit different pharmacological activities and are widely used in medicine. In this study, it is aimed to synthesize, show the theoretical calculations, and indicate anti-tumor activities of the novel 3-(2-(4-Ethyl-5-thion-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl)coumarin.

## MATERIALS AND METHODS

### *2.1. Synthesis*

#### *2.1.1.Synthesis of 3-(2-(4-Ethyl-5-thion-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl)coumarin*

40 ml of tetrahydrofuran (THF) was added to the 50 ml flask in the magnetic stirrer and 0.19 ml of triethylamine (TEA) was added. 0.23 g of 4-ethyl-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol was added to the resulting mixture, after 60 minutes, 0.3 g of 3-(bromoacetyl)coumarin was added to the mixture. The mixture turned into a light yellow color. After boiling for 24 hours, filtration was carried out. The liquid portion was precipitated in the beaker. The precipitated solid was 0.425 g of 3-(2-(4-Ethyl-5-thion-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl)coumarin, has yellow color and a melting point of 96°C.IR (ATR, $cm^{-1}$): 3057-3030 (stretching vibration, aromatic C-H), 2977-2924 (stretching vibration, aliphatic C-H),1721 (stretching vibration, coumarin-C=O), 1680 (stretching vibration, acetyl-C=O), 1606 (stretching vibration, aromatic C=C), 1223(stretching vibration, C-O). $^1$H NMR(300 MHz, $CHCl_3$): 1.17 (t,$CH_3$), 2.40 (s, phenyl-$CH_3$), 2.88

(q, 2H, $CH_2$), 4.84 (s, coumarin-$CH_2$), 7.70-7.30 (m,aromatic C-H), 8.60 (s,1H,aromatic c-C-H). $^{13}$C NMR (75 MHz, $CDCl_3$):190.84, 167.36, 159.25, 155.78, 149.76, 140.33, 134.81, 130.45, 129.73, 129.63, 128.46, 128.38, 125.52, 125.18, 124.26, 123.56, 118.26, 116. 67.97, 45.72, 21.44, 9.67.According to LCMS-MS, which is a mass spectrometer device provided from central research laboratory, it is stated that the chemicals get +1 for positive ionization and -1 for negative ionization. In this compound, it was found that when the compound got positive ionization, it had 406 g. When it got negative ionization, it had 404 g. The molecule weight of this compound has already 405 g.

### *2.2. Computational Methods*

The applications Gaussian 09 and GaussView 5.0 were used to visualize chemical reaction calculations (Frisch et al.,2009). DFT approach (B3LYP/6-311 G(d,p)) (Becke's three-parameter hybrid model employing the Lee-Yang-Parr correlation functional) (Becke, 1992; Lee & Yang, 1988) was applied to the investigation for the optimized molecular structure **(Figure 1).** Utilizing Crystal Explorer 3.1, the distribution of HOMO-LUMO and molecular electrostatic potential analyses were carried out (Wolff et al., 2012).

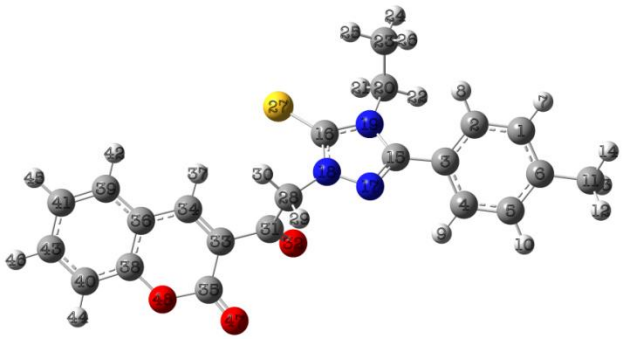


**Figure 1.** The optimized structure of 3-(2-(4-Ethyl-5-thion-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl)coumarin

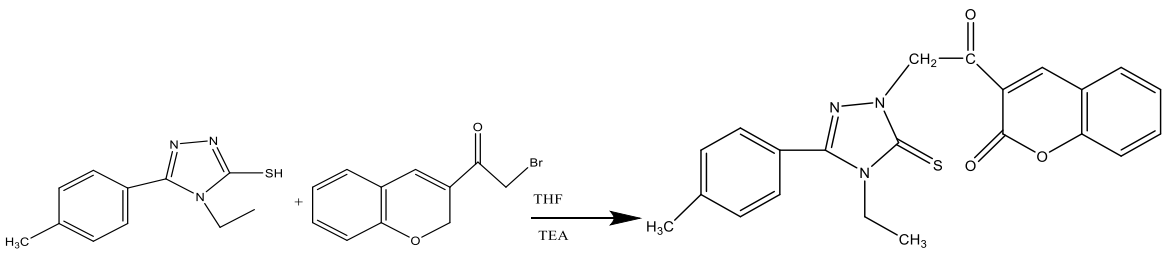
### *2.3. Anti-cancer activity*

The MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] technique was used to assess the anticancer activity of 3-(2-(4-Ethyl-5-thion-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl)coumarin. HCT-116 and HT-29 colon cancer cells and MDA-MB-231 triple negative breast cancer cells were employed for the MTT analysis.

## 3. Results

### *3.1.Synthesis of 3-(2-(4-Ethyl-5-thion-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl)coumarin*

When 4-ethyl-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol, 3-(bromoacetyl)coumarin, and TEA(triethylamine)are mixed with the solvent. As a consequence, 3-(2-(4-Ethyl-5-thion-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl)coumarin is synthesized. It's chemical reaction is given in Scheme 1.

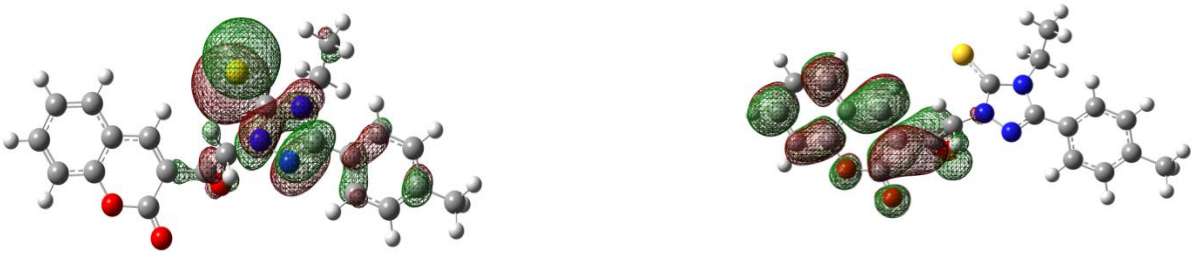



**Scheme 1.** Synthesis of 3-(2-(4-Ethyl-5-thion-3-(p-tolyl)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl)coumarin

### *3.2. HOMO-LUMO analysis*

The molecular orbital types HOMO and LUMO stand for the highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbitals, respectively. They were determined using the B3LYP functional and

the 6-311G(d,p) basis set in chloroform for the novel chemical compound. Here, in this chemical, as seen in **Figure2**, it is deduced that the electron density is accumulated in the sulphur and nitrogen atoms in HOMO while it is concentrated on the coumarin ring in LUMO. Therefore, it is asserted that the charge flows from the triazole and toluene ring to the coumarin ring.



HOMO (-5.8142437209302eV) LUMO (-0.25581366228514 eV)

**Figure 2.** Calculated Frontier molecular orbitals of the title compound with DFT/B3LYP-G(d,pþþ).

### *3.3. Molecular electrostatic potential (MEP) analysis*

A map known as a molecular electrostatic potential analysis (MEP) reveals the electron density of a chemical molecule in various regions with different colors. The distribution of charge is depicted in three dimensions on this map. The color scale on the map is situated between the red and blue in the specific locations. As a result, blue has the lowest electron density while red indicates that the chemical has the maximum electron density. As seen in **Figure 3**, oxygens of the coumarin ring and the carbonyl group attached to it are in the red region meaning that the electron density is high whereas the nitrogen and sulphur atoms are in yellow and green region meaning of medium electron density level.

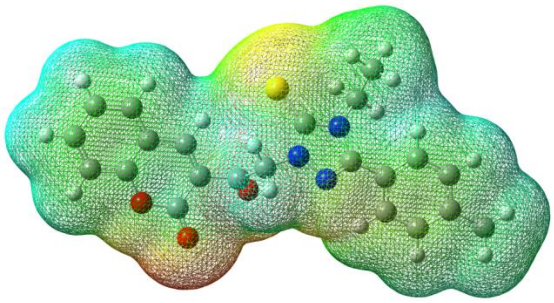


**Figure 3.** MEP maps of the title compound calculated at (B3LYP/6311þG(d,p)).

### *3.4. Anti-cancer activity*

The active dose of 4-ethyl-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol3-(bromoacetyl)coumarin compound was found to be 100 µM in HCT-116 cells, 200 µM in HT-29 cells, and 50 µM in MDA-MB-231 cells. Under the same conditions, the active dose of cisplatin was 100 µg/ml in HCT-116 cells, 50 µg/ml in HT-29 cells, and 150 µg/ml in MDA-MB-231 cells (**Figure 4**). These findings are especially promising for the development of new molecules that can be used in the treatment of breast cancer.

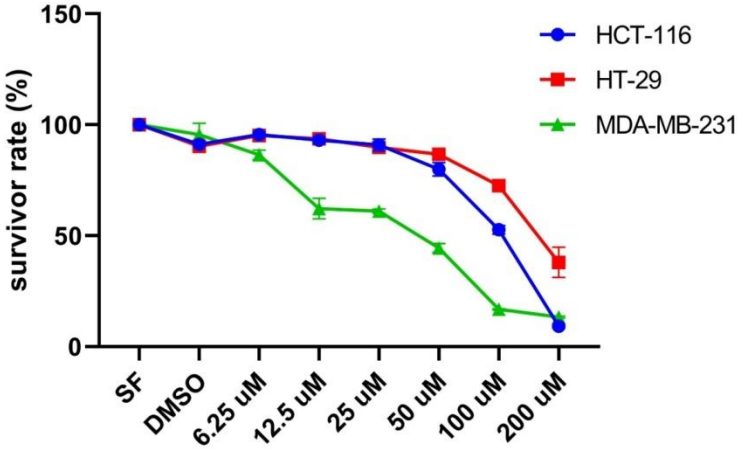



**Figure 4.** Effect of 4-ethyl-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol 3-(bromoacetyl)coumarincompound on cell viability in HCT-116, HT-29, and MDA-MB-231 cells.

## 4. DISCUSSION

In this study, 4-ethyl-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol 3-(bromoacetyl)coumarin was synthesized from mixing of 4-ethyl-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol and 3-(bromoacetyl)coumarin with the aid of TEA. Molecule structure optimization was done using the DFT technique. The electron density is found to be centered on heteroatoms in HOMO orbitals as compared to the aromatic ring in LUMO orbitals using Crystal Explorer 3.1. Using MEP analysis, it was possible to illustrate the distribution of charge in three dimensions, with the most negatively charged atoms having high electron density presented in the red zone and the most positively charged atoms having low electron density presented in the blue region. Additionally, using the MTT method, the chemical compound's anticancer activity was assessed breast cancer cells. In this regards, Areche et al. (2021) indicated that the chemicals containing coumarin acts as an inhibitor for SARS-CoV-2. Önder (2020) stated that the cytotoxic activity closely related to anti-cancer activity of coumarinsplay a fundamental role in cancer prevention and treatment. Three synthetic coumarins made from triphenylethylene (TCHs) were shown to have anticancer properties by Cui et al. (2020) on breast cancer cell lines via inhibiting angiogenesis.Capasso et al. (2020) demonstrated that esculetin, a coumarin molecule, has a variety of pharmacological actions connected to cell proliferation and antitumor effectiveness.

## 5. CONCLUSION

The novel4-ethyl-5-(p-tolyl)-4H-1,2,4-triazole-3-thiol 3-(bromoacetyl)coumarin was synthesized in this work and visualized using tools that calculate chemical reactions. The chemical that was synthesized was improved using DFT (B3LYP/6311 G(d,p)) techniques. Utilizing Crystal Explorer 3.1, the distribution of HOMO-LUMO and MEP analysis was implemented. The unique compound's anti-cancer effects on breast cancer cells.

### Acknowledgements

This study was supported by Adiyaman University Scientific Research Projects Coordination Unit (Project No: FEFYL\2022-002).

**Authors' contributions:** Mehmet Akşimşek:investigation, methodology, data curation, and writing-original draft, writing-review & editingAyçaBayhan: investigation, methodology, data curation, and writing-original draft, writing-review & editing. Murat Genç: investigation, methodology, data analysis and curation, supervision, writing-review & editing. Esra Bozgeyik: methodology, data analysis and curation, writing-review & editing.

**Conflict of interest disclosure:** No conflicting of interests in this work.

## REFERENCES

Areche C, Yañez O, Osorio MI, Uriarte E, Tiznado W, Pérez-Donoso JM, García-Beltrán O, González-Nilo F. 2021. In Silico Study of Coumarins and Quinolines Derivatives as Potent Inhibitors of SARS-CoV-2 Main Protease. Frontiers in Chemistry.

Becke AD. 1992. Density-functional thermochemistry. I. The effect of the exchange-only gradient correction. Journal of Chemical Physics. 96(3), 2155–2160.

Cao D. 2019. Coumarin-based small-molecule fluorescent chemosensors. Chemical Reviews 119(18): 10403-10519.

CapassoR, Akkol EK, Genç, Y, Karpuz B, Sobarzo-Sánchez E. 2020. Coumarins and Coumarin-Related Compounds in Pharmacotherapy of Cancer. PubMed Central. 1959.

Cui N, Lin DD, Shen Y, Shi JG, Wang B, Zhao MZ, Zheng L, Chen H, Shi JH. 2020. Triphenylethylene-Coumarin Hybrid TCH-5c Suppresses Tumorigenic Progression in Breast Cancer Mainly Through the Inhibition of Angiogenesis. Anti. Cancer Agents Med. Chem.

Duangdee N. 2020. Design synthesis and anti-proliferative activity of some new coumarin substituted hydrazide–hydrazone derivatives. Journal of Chemical Sciences 132(1): 1-12.

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Petersson GA, Nakatsuji H, Li X, Caricato M, Marenich A, Bloino J, Janesko BG, Gomperts R, Mennucci B, Hratchian HP, Ortiz JV, Fox DJ. 2009. Gaussian 09. Revision C.01, Gaussian, Inc.

Micha: A study of 1H- and 1-methyl-1,2,4-triazole by vacuum ultraviolet photoabsorption and ultraviolet photoelectron spectroscopy and a comparison with ab initio configuration interaction computations. J. Chem. Phys. 136, 094310-21el HP, Philip JC Søren VH, Nykola CJ, Ashley RH, Dennis LL. 2012. The electronic states of 1,2,4-triazoles.

Önder A. 2020. Coumarins: Coumarins are phenolic substances made of fused benzene and a-pyrone rings. In Studies in Natural Products Chemistry.

Wolff SK, Grimwood DJ, McKinnon JJ, Turner MJ, Jayatilaka D, Spackman MA. 2012. Crystal Explorer University of Western Australia. Version 3.1.

Yang G. 2021. The synthesis of coumarin thiazoles containing a trifluoromethyl group and their antifungal activities. Arabian Journal of Chemistry 14(1): 102880.

Yang LC, W & Parr RG. 1988. Development of the Colle–Salvetti correlation-energy formula into a functional of the electron density. Physical Review B, 37, 785–789.

## *ORAL PRESENTATION*

# Effects of coagulase negative staphylococci on proteolytic activity in fermented meat products and meat products made from whole pieces of meat

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**Abstaract**

Proteolysis is the main degradation mechanism that takes place during the ripening of fermented meat products. In dry cured meat products meat products made from whole pieces of meat, proteolysis is carried out by endogenous enzymes and exogenous enzymes originating from microorganisms. *Staphylococci* are used as starter cultures in fermented meat products and have significant effects on the technological properties of the product. These microorganisms mainly contribute to the desired red color formation and stability through their nitrate reductase activity. They are also effective in the development of organoleptic properties such as texture and flavor. These functions are performed by special enzyme systems involved in protein and lipid metabolisms. During the processing of fermented meat products, peptides, free amino acids and free fatty acids are produced by the activity of the muscle and microbial enzyme systems (proteolytic and lipolytic). These compounds are the leading components of both flavor and other volatile aroma compounds that affect the flavor of dry cured meat products. *Staphylococ* species are more effective on flavor and texture of fermented meat products compared to lactic acid bacteria. *Staphylococcus* species used as starters and isolated from fermented meat products such as *Staphylococcus equorom*, *S. xylosus* and *S. saprophyticus* play an important role in improving the sensory properties of meat products made from whole pieces of meat and fermented meat products by producing flavor and aroma compounds with their protease and lipase activities. In this current review, the proteolytic activities of staphylococci, which are effective in providing the desired taste and aroma in fermented meat products and dry cured meat products, will be emphasized.

**Key Words:** *Staphylocococ*, Proteolytic Activity, Starter Culture

## GİRİŞ

Proteazlar, proteinlerin peptit bağlarını daha küçük boyutlu gruplara hidrolize eden enzimlerdir. Fermantasyon ve olgunlaşma sırasında fermente et ürünlerinde karbonhidratlar, yağlar ve proteinlerde çeşitli değişiklikler olmaktadır. Ayrıca fermantasyon ve olgunlaşma sırasında tat ve koku gelişimi üzerine önemli derecede etkili olan proteolitik parçalanmalar gerçekleşmektedir. Kasa ait proteolitik enzimler fermente et ürünlerinde fermantasyon sırasında gelişen proteoliz üzerine etkili olurken, bakterilerin proteolitik enzim aktiviteleri ise olgunlaşma sırasında oluşan proteolitik parçalanmalardan sorumludur ( Serdaroğlu ve Tömek, 1995). Proteolitik enzimler, özellikle et için endojen olanlar, lezzet için büyük önem taşır. Et proteazları, özellikle katepsin D-benzeri enzimler, fermantasyon sırasında proteoliz ve peptit oluşumundan sorumlu iken, mikrobiyal enzimler daha çok olgunlaşmanın sonraki aşamalarında salınan oligopeptitler üzerine etki eder (Leroy ve ark., 2006). Bu durum oligopeptitlerin tam hidrolizi için hem endojen hem de bakteriyel peptidazların gerekli olduğu hipotezine yol açmaktadır (Casaburi ve ark., 2008).Et proteinlerine karşı mikrobiyal proteolitik aktivite, fermente edilmiş sosislerde bulunan koşullar altında düşüktür, ancak belirli bir aktivite, küçük ölçüde ve suşa bağlı bir şekilde de olsa, başlangıçtaki protein parçalanmasına kısmen katkıda bulunabilir. Ayrıca kas proteolizi ile üretilen peptitler, onları hücre içinde amino asitlere bölen ve aroma bileşenlerine dönüştüren bakteriler tarafından alınabilir (Leroy ve ark., 2006).

## FERMENTE ET ÜRÜNLERİNDE PROTEOLİZİS

Fermente et ürünleri, uzun bir geçmişe ve paha biçilemez bir gastronomik değere sahip geleneksel ürünlerdir. Bu ürünlerin dayanıklılığı genellikle, dehidrasyon ve tuz ilavesi dahil olmak üzere su aktivitesini düşürme

teknikleri kullanılarak fermentasyon ile elde edilir (Aro Aro ve ark., 2010). Fermente et ürünlerinde lezzet, ilave edilen baharatların yanı sıra protein, yağ ve karbonhidratların parçalanmasıyla oluşmakta (Serdaroğlu ve Tömek, 1995, Aro Aro ve ark., 2010) ve parçalanma ürünleri, fermente etlerin karakteristik aromasına ve dokusuna katkıda bulunmaktadır (Aro ve ark., 2010). Organik bileşiklerde meydana gelen yıkım, fermantasyon ve olgunlaşma sırasında kasa ve mikrobiyal floraya ait enzimlerin aktivitelerinden ileri gelmektedir. Fermente et ürünlerinde ana biyokimyasal reaksiyonlardan biri olan proteolizis (Candoğan ve ark., 2009) endojen lysosomal enzimler veya katepsin B ve D'ye benzer özellik gösteren bakteri proteazları ile gerçekleştirilmektedir ( Serdaroğlu ve Tömek, 1995, Candoğan ve ark., 2009). Et proteinleri, önce katepsinler ve kalpainler gibi endojen kas enzimleri tarafından polipeptitlere ve daha sonra peptidazların etkisiyle daha küçük peptitlere hidrolize olmaktadır. Aminopeptidazlar tarafından peptitlerden serbest amino asit üretimi, proteoliz olayındaki son adımdır ve etin kendisinde bulunan enzimlerin yanı sıra bakteriyel enzimlere de atfedilmiştir (Candoğan ve ark., 2009). Olgunlaşma sırasındaki proteolizden *Micrococ* cinsine ait bakteriler sorumludur. Laktik asit bakterilerinin ise proteolitik aktivitesi oldukça düşüktür ( Serdaroğlu ve Tömek, 1995).

Fermente sosislerin olgunlaşması sırasında meydana gelen biyokimyasal ve fiziksel değişiklikler son ürünün tat ve kokusunun şekillenmesinde önemlidir. Bu değişiklikler esas olarak fermantasyonun sonucu olarak asitleşme, pH'da azalma, başlangıç mikroflorasının değişimi, nitratın nitrite dönüşümü ve nitrosomyoglobin oluşumu, miyofibriler ve sarkoplazmik proteinlerin çözülmesi ve jelleşmesi, proteolizis, lipolizis, oksidatif olaylar ve dehidrasyondur. Fermente sosislerin olgunlaşması sırasında proteinleri etkileyen esas parçalanma mekanizması proteolizisdir (Dalmış ve Soyer, 2008, Aro Aro ve ark., 2010). Proteolizis ile üretilen serbest aminoasitler fermente sosislerin temel tatlarının oluşumunda doğrudan sorumlu iken aynı zamanda çok sayıda aroma maddesinin öncüleri olduğundan dolaylı olarak karakteristik aroma oluşumuna da katkı sağlamaktadırlar (Candoğan ve ark., 2009, Singh ve ark., 2019, Xiao ve ark., 2020). Serbest aminoasit gibi ürünler daha sonrasında dekarboksilasyon ve deaminasyon ile sırasıyla aminler ve organik asitlerin oluşumuyla sonuçlanan diğer metabolitlere dönüştürülebilir. Bu aşamada üretilen çok sayıda son ürün aroma verici etkide önemli bir rol oynar veya bunun için bir öncüldür ( Singh et al 2019). Ayrıca bazı dallı zincirli amino asitler (örneğin valin, lösin ve izolösin), fermente etlerin tipik aromasını sağlayan uçucu maddelere dönüştürülebilir (Xiao ve ark., 2020). Nitekim amino asitlerin ileri derece katabolizmasından kaynaklanan yoğun aroma özelliklerine sahip aldehitler ve ketonlar, lezzet gelişiminde önemli bir role sahiptir (Candoğan ve ark., 2009). Genelde katepsinler başta olmak üzere endojen proteinazların fermente etlerde proteolizi başlattığı kabul edilirken, mikrobiyal enzimler olgunlaşma sürecinin sonraki aşamalarında daha önemli hale gelmektedir (Xiao ve ark., 2020).

Lezzet bileşenlerinin üretimde mikrobiyal enzimler de önemli rol üstlenmektedir. Nitekim fermente sosislerden izole edilen *Micrococcaceae* familyasına ait bazı suşlar proteolitik aktivite sergilemektedir. Bu proteolitik etki kuru fermente sosislerin florasında yer alan *Micrococ* ve laktik asit bakterilerindeki proteaz enzimlerinden ileri gelmektedir. Ancak *Lactobacillus* ve *Pediococcus* gibi laktik asit bakteri cinsine ait birkaç tür protein hidrolizi üzerine sınırlı bir etkiye sahiptir. (Singh ve ark., 2019). Fermente sosislerde laktik asit bakterilerinin en önemli katkısı organik asit, bakteriyosin ve $H_2O_2$'yi içeren antibakteriyel metabolitleri ile patojenleri baskılamasından ileri gelmektedir (Chen ve ark., 2021). Ancak bununla birlikte sarkoplazmik protein ekstrakları üzerine *Pediococcus acidilactici, Lactobacillus sakei, L. curvatus, Streptomyces griseus, S. xylosus* ve *S. carnosus*'un proteolitik aktivitesinin incelendiği bir çalışmada en yüksek proteolitik aktiviteyi gösteren tür *L. sakei* olarak tespit edilmiş olup bu türü *L. curvatus* ve *S. carnosus* takip etmiştir. *S. xylosus*'un proteolitik aktivitesi ise araştırılan türler içinde en düşük değeri vermiştir (Candoğan ve Acton, 2004).

Koagülaz negatif koklar fermente edilen et ürünlerinin doğal florasında yaygın bir şekilde bulunmaktadır. Starter kültür ilave edilmeden üretilen kuru fermente sosislerde de nispeten yüksek sayılara ulaşabilmektedirler (Casaburi ve ark., 2005). Bu mikroorganizmalar aktif bir şekilde gelişen asidik bakterilere kıyasla zayıf rekabet etme özelliği göstermekte olup genellikle olgunlaşma sırasında 1 log cfu/g'dan fazla gelişme gösteremezler (Leroy ve ark., 2006). Koagülaz negatif koklar ve *Kocuria* proteolizis ve lipolizis yoluyla lezzet ve aroma bileşikleri üreterek, peroksit kompozisyonu ile ransiditeyi öneleyerek, nitratı nitrite indirgeyerek ve daha sonrasında nitroz oksit oluşumu ile fermente sosislerin duyusal özelliklerinin gelişmesinde önemli rol üstlenmektedirler (Casaburi ve ark., 2005, Leroy ve ark., 2006, Chen ve ark., 2021). Fermente sosislerde baskın tür olarak *S. xylosus, S. saprophyticus* ve *S. carnosus* izole edilmektedir. (Casaburi ve ark., 2005, Leroy

ve ark., 2006). Ayrıca bazen *Kocuria varians* da doğal olarak fermente edilen sosislerden düşük miktarlarda izole edilebilmektedir (Leroy ve ark., 2006). Fermente bir et ürünü olan sucukta da *S. xylosus* ve *S. saprophyticus* baskın olarak izole edilen türlerdir. (Kaban, 2013). Ayrıca bazı sosis çeşitlerinde baskın olan türlerin yanında *S. haemolyticus, S. warneri, S. equorum, S. cohnii, S. epidermidis, S. hominis, S. capitis, S. intermedius* gibi koagülaz negatif stafilokok türleri de izole edilebilmektedir. *Staphylococcus* türleri yaygın bir şekilde fermente sosislerde starter olarak kullanılmaktadır (Casaburi ve ark., 2005). *S. xylosus* ve *S. carnosus* ile inoküle edilen sosislerde, üretilen 3-metil butil aldehit, lösin, izolösin, valin ve fenilalanin aminoasitlerinin parçalanması ile oluşan ve fermente et ürünlerine özgü lezzeti veren önemli bir bileşiktir (Wang ve ark., 2019). Wang ve ark. (2019) tarafından yapılan kuru fermente sosisin fiziko-kimyasal özellikleri üzerine farklı starter kültür kombinasyonlarının etkisinin incelendiği çalışmada *Lactobacillus sakei* ve *S. carnosus*'un lezzet gelişimini etkileyen serbest aminoasitlerin salınımında esas rol oynayan proteolitik aktiviteye sahip suşlar olduğu tespit edilmiştir.Ayrıca Candoğan ve Acton (2004) tarafından da *S. xylosus* ile fermente edilen sosislerin duyusal lezzeti ve lezzet ile ilişkili bileşikler arasında bir bağlantı olabileceği belirtilmiştir. Bunun yanında proteolitik ve lipolitik aktiviye sahip *S. xylosus* ve *L. curvatus* karışık starteri, yalnızca *S. xylosus* starteri ve startersiz olarak üretilen üç grup fermente sosiste de proteolizisin gözlemlendiği ve bu durumun hem mikrobiyal lipaz hem de pH'daki düşmeye paralel aktivite olan endojen proteazlardan ileri gelebileceği Casaburi ve ark. (2008) tarafından belirtilmiştir.

Sarkoplazmik proteinler kas endojenleri ve mikrobiyal enzimler tarafından yıkıma uğratılabilmektedir. Starter kültürler tarafından üretilen eksojen proteazlar ve endojen proteinler fermente sosislerin olgunlaştırılması sırasında proteolizisde önemli rol oynamaktadır (Wang ve ark., 2019).Olgunlaşma sırasında gerçekleşen proteolizis fermente et ürünlerinin duyusal ve besinsel özelliklerinden sorumludur (Chen ve ark., 2021). Dalmış ve Soyer (2008) tarafından proses metodları ve starter kültürlerin (*Staphylococcus xylosus* ve *Pediococcus pentosaceus)* olgunlaşma ve depolama sırasında sucukta meydana gelen proteolitik değişiklikler üzerine etkisi incelenmiş ve hem starter kültür ile inoküle edilen hem de startersiz üretilen sucuklarda proses sırasında proteolitik aktivitenin olduğu belirlenmiştir. Ayrıca sarkoplazmik ve miyofibriler proteinlerin starter ilavesi, fermantasyon, kurutma ve ısıtma işlemlerinden etkilendiği, fermantasyon sırasında starter ilave edilen ve edilmeyen sucuk örneklerinin hem geleneksel hem de ısı işleminin dahil olduğu üretim prosesinde yoğun proteolizisin meydana geldiği tespit edilmiştir. Bu durum fermente sosislerde lezzet gelişiminde önemli süreçlerden biri olan proteolizisi (Xiao ve ark., 2020), ürün formülasyonu, proses şartları ve starter kültür gibi değişkenlerden etkilendiğini de göstermektedir (Aro Aro ve ark., 2010).

## KURU KÜR EDİLMİŞ ET ÜRÜNLERİNDE PROTEOLİZİS

Kuru kür edilmiş et ürünlerinin çoğu 1 ile 24 aylık olgunlaştırma süresinin sonunda elde edilir (Martin ve ark., 2001). Kuru kür edilmiş et ürünleri prosesi sırasında ette gerçekleşen kompozisyonel ve yıkıcı değişiklikler proteinler ve lipitlerde gerçekleşen değişikliklerden ileri gelmektedir. Bu değişiklikler özellikle olgunlaşma sırasında gerçekleşir ve yeterli bir tekstür ve karakteristik lezzet oluşumuna katkı sağlar (Kaban, 2009). Olgunlaştırma süresinde sarkoplazmik ve miyofibriler proteinlerde ürünün lezzet ve tekstürünü etkileyen önemli düzeyde proteolitik değişiklikler meydana gelmektedir (Martin ve ark., 2001). Bu değişiklikler üzerinde hem endojen hem de mikrobiyal enzimler etkili olmaktadır (Martin ve ark., 2001, Aro Aro ve ark., 2010). Tüm ham prosesi sırasında endojen enzimlerden katepsin B, H ve L kısmen aktif durumdadır (Martin ve ark., 2001). Kasda mevcut olan proteolitik mekanizmalar; kalpain sistemi, lizozomal katepsinler ve proteazomlardır. Proteazomlar multikatalitik proteinaz kompleksi olarak da isimlendirilmektedir. Kalpain sistemi ölüm sonrası meydana gelen proteolizis ve buna bağlı olarak gerçekleşen yumuşama olayında esas rolü üstlenmektedir. Kalpainler uygun sıcaklık ve pH koşullarında etkili bir şekilde miyofibriler proteinlerin ayrışmasını gerçekleştirmektedir. Kalpainler yoluyla kas proteinlerinin proteolizisi Z çizgisi, titan ve kostamerik proteinlerin bozulmasından kaynaklanır. Etin olugunlaşması sırasında gerçekleşen yumuşama sürecinde yapısal düzeyde bir takım değişiklikler meydana gelmektedir. En yaygın olarak miyofibrillerin parçalanması, I-bandı ve Z-çizgisi alanındaki değişiklikler ve T troponinler, I troponinler, titinler, nebulinler, desminler vb. gibi miyofibriler proteinlerin bozulması gözlemlenmektedir. Katepsinler ise ölüm sonrasında gerçekleşen proteolizis olayından ziyade sonrasında gerçekleşen bozulma reaksiyonlarında etkili olmaktadır. Multikatalitik proteinaz kompleksi ise etin yumuşaması üzerine önemli bir rol oynamamaktadır. ( Singh ve ark., 2019).

Kuru kür edilmiş et ürünlerinde proteolizis esasen endojen enzimler tarafından gerçekleştirilmektedir (Toldra ve ark., 1992). Ancak et proteinleri üzerine proteolitik aktivite gösterebilen mikroorganizmalar tarafından özellikle oligopeptit ve küçük peptitlerin ikincil hidrolizi gerçekleştirilebilmektedir (Mauriello ve ark., 2002). Kür edilmiş çiğ et ürünlerinde önemli bir mikroorganizma olarak identifiye edilen *Staphylococcus* türleri kas enzimlerinden sonra iyi bir lezzet ve renk oluşumunun yanı sıra aromanın gelişmesinde önemli bir role sahiptir (Landeta et al. 2011). Rodriguez ve ark. (1998) kuru kür edilmiş ham olarak adlandırılan üründe valin, metionin, lösin, histidin, fenilalanin ve tiramin gibi amino asitlerin ürünün iç kısmına göre yüzeyde daha fazla miktarda bulunmasının ürün yüzeyindeki proteolitik mikroorganizmalar ile ilgili olduğunu ileri sürmüş ve yürüttükleri çalışmada küflerin ve kokların, miyosin içeren sıvı besiyerinde yüksek proteolitik aktivite gösterdiğini, myofibriler ve sarkoplazmik proteinlerdeki en önemli değişikliklerin sırasıyla *Penicillium chryosengum* ve *Staphylococcus xylosus* suşları tarafından gerçekleştirildiğini rapor etmişlerdir. Ayrıca Casquete ve ark. (2012) tarafından yapılan çalışmada *S.vitulinus* RS34 suşunun 'H-meromyosine' karşı yoğun aktivite gösterirken, aktine karşı herhangi bir aktivite gözlenmediği belirtilmiştir. Kuru kür edilmiş ham kaynaklı *S.xylosus* suşunun çok zayıf lösin arilamidaz aktivitesi gösterdiği ise konu ile ilgili diğer bir araştırmada rapor edilmiştir (Molina ve Toldra, 1992). Bonomo ve ark. (2009) ise 23 stafilokok suşunun (14 *S.xylosus*, 3 *S. equorom*, 3 *S. pulvereri/S. vitulinus*, 2 *S. succinus* ve 1 *S. pasteuri*) sarkoplazmik protein fraksiyonlarını hidrolize edebilmesine karşın, myofibriler proteinler üzerinde ise sadece bu suşların %30,4'ünün etkili olduğunu bildirmişlerdir.

Laktik asit bakterileri ve katalaz pozitif koklar kür edilmiş çiğ et ürünü olan pastırmadaki iki önemli mikroorganizma grubudur. Katalaz pozitif koklar katalaz aktivitesi yanında nitrat redüktaz aktivitesine de sahiptir. Proteolitik ve lipolitik aktivitelerinin yanı sıra bu mikroorganizmalar renk oluşum ve stabilizasyonunda, oksidasyonun geciktirilmesinde ve aroma oluşumunda önemli rol oynamaktadırlar. Laktik asit bakterileri ise proteolitik aktivitelerinin yanı sıra asit üretimleri ile ürünün duyusal ve tekstürel özelliklerini etkilemektedir. Pastırma üretimi sırasında stafilokok ve laktik asit bakterilerinde önemli gelişme söz konusudur. Katalaz pozitif koklar aside hassas mikroorganizmalar olup uygun pH'dan dolayı pastırmada (Kaban, 2013) vb kür edilmiş çiğ et ürünlerinde (Bosse ve ark., 2018) önemli gelişme gösterirler (Kaban 2013, Bosse ve ark., 2018). Pastırmadaki katalaz pozitif kokların büyük çoğunluğu stafilokoklardan oluşmaktadır. *S. saprophyticus* ve *S. xylosus* pastırmadaki stafilokok türleri arasında baskın iki tür olarak izole edilmektedir. Ayrıca *Staphylococcus simulans, Staphylococcus warneri, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus gallinarum* and *Staphylococcus vitulinus* gibi stafilok türleride de identifiye edilen diğer türlerdir (Kaban, 2013). Kür edilmiş çiğ et ürünlerinde de *S. equorum, S. saprophyticus, S. sciuri, S. gallinarum, S. cohnii, S. intermedius, S. capitis, S. epidermidis, S. simulans*, ve *S. warneri* biyokimyasal metodlar ile düşük miktarlarda belirlenmelerine rağmen en fazla identifiye edilen tür *S. xylosus*'tur (Bosse ve ark., 2018). Kuru kür edilmiş et ürünü olan pastırma üretim prosesinin başlangıcında pH'da hafif bir düşme söz konusu iken takip eden aşamalarda muhtemelen proteolizisin bir sonucu olarak bazik bileşiklerin oluşumundan dolayı pH'da hafif bir artış meydana gelmektedir (Kaban, 2013). Nitekim proteolitik *S. carnosus* LTH 3838 ile inoküle edilen kür edilmiş et ürünü olan loin'lerde aroma ile ilgili uçucu bileşikler (3-metilbütanol, benzaldehit, asetofenon, 1-octen-3-ol), duyusal kabul edilebilirlik, koku ve tatta önemli artış tespit edilmiş olup starter kültür ilave edilmeyen kontrol grubuna kıyasla pH'da da artış söz konusu olmuştur (Bosse ve ark., 2017).

Pastırma gibi kür edilmiş çiğ et ürünlerinde starter kültür kullanımı son ürünün kalite özelliklerininin gelişmesi açısından önem arz etmektedir. *Satphylococcus carnosus, S. xylosus* ve *Micrococcus varians* gibi bazı *Micrococcaceae* familyasının üyeleri et ürünlerinde starter kültür olarak kullanılmaktadır. Bu mikroorganizmalar proteolitik ve lipolitik aktiviteleri açısından oldukça önemlidirler. *Lactobacillus plantarum, L. sakei, L. curvatus ve L. pentosus* gibi laktik asit bakterileri de starter olarak kullanılmakta ancak *Micrococcaceae* familyasına kıyasla daha zayıf lipolitik ve proteolitik aktivite sergilemektedirler (Aktaş ve ark., 2005). *S. carnosus* ve *S. equorom* suşları ile inoküle edilen kür edilmiş loin ve ham'ler de uçucu bileşikler, duyusal kabul, mikrobiyal güvenlik ve renk oluşumunu önemli ölçüde etkilenmektedir. Bu durum stafilokok suşlarının karbonhidratların, aminoasitlerin ve yağ asitlerinin parçalanmasında rol oynayarak aroma ile ilgili uçucu bileşikler ve/veya öncüleri üretmesinden kaynaklanmaktadır (Bosse ve ark., 2017). Kür edilmiş çiğ ham'in mikrobiyal florası, farklı işleme aşamaları sırasında değişir ve nihai ürünün çeşitli kalite parametreleri için oldukça önemlidir. Nitrat indirgemesinin yanı sıra mikrobiyal flora lipoliz, lipid oksidasyonu, proteoliz ve

amino asit parçalanması gibi farklı enzimatik aktivitelerle, ürünün tadını, aromasını ve rengini etkiler. Bu parametreler, üretiminde uzun olgunlaştırma sürelerinin olduğu ham'in lezzeti üzerine önemli etkiye sahip olan proteolitik ve lipolitik enzimler gibi etin kendi yapısında bulunan endojen enzimler tarafından da önemli ölçüde etkilenmektedir (Bosse ve ark., 2018).

## SONUÇ

Fermente et ürünlerinde endojen enzimler fermantasyonun başlangıcında etkili olurken olgunlaşma sırasında ise mikrobiyal kaynaklı proteolitik enzimler etkili olmaktadır. Uzun süre olgunlaştırılan kuru kür edilmiş et ürünlerinde ise proteolizis esasen endojen enzimler tarafından gerçekleşmekte ancak et proteinleri üzerine proteolitik aktivite gösterebilen mikroorganizmalar tarafından özellikle küçük peptitlerin ikincil hidrolizi gerçekleştirilebilmektedir. Mevcut bu derlemede fermente et ürünleri ve kuru kür edilmiş et ürünlerinde ki iki önemli mikroorganizma grubundan biri olan stafilokokların proteolitik aktivitesi üzerinde durulmuştur. Fermente sosiste yaygın starter kültür olarak kullanılan ve izole edilen *S. xylosus* ve *S. carnosus*'un bazı suşlarının et proteinleri üzerine etki ederek aromaya katkı sağlamaktadırlar. Ayrıca kuru kür edilmiş et ürünlerinde de bazı *staphylococcus* (*S. vitulinus, S. xylosus, S. equorom* vd) suşlarının et proteinleri üzerine farklı proteolitik aktivite sergilediği de derlemede belirtilmiştir. Kuru kür edilmiş et ürünlerinde starter kullanımı son ürünün teknolojik özellikleri üzerine olumlu etkide bulunmaktadır ve *S. carnosus* ve *S. equorom* suşları ile inoküle edilen kür edilmiş çiğ et ürünlerin duyusal özelliklerinin gelişmeside söz konusudur. Ayrıca kuru sosis ve kür edilmiş bir et ürünü olan ham'in olgunlaşma prosesi sırasında katepsin aktivitesinden kaynaklı yoğun proteolizis oluşumu da söz konusudur. Gerek fermente et ürünleri gerekse de kuru kür edilmiş et ürünlerinde proteolizis, ürün formülasyonu, proses şartları, starter kullanımı gibi faktörlere de bağlı olarak hem endojen hem de mikrobiyal kaynaklı enzimler tarafından gerçekleşmektedir.

## KAYNAKLAR

Aktaş, N., Aksu, M. I., Kaya, M. 2005. Changes in myofibrillar proteins during processing of pastirma (Turkish dry meat product) produced with commercial starter cultures. *Food chemistry*, *90*(4), 649-654.

Aro, J. M. A., Nyam-Osor, P., Tsuji, K., Shimada, K. I., Fukushima, M., Sekikawa, M. 2010. The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. *Food Chemistry*, *119*(1), 279-285.

Bonomo, M.G., Ricciardi, A., Zotta, T., Sico, M.A., Salzano, G., 2009. Technological and safety characterization of coagulase negative *Staphylococci* from traditionally fermented sausage of Basilicata region. Meat Science, 83, 15-23.

Bosse, R., Müller, A., Gibis, M., Weiss, A., Schmidt, H., Weiss, J. 2018. Recent advances in cured raw ham manufacture. *Critical reviews in food science and nutrition*, *58*(4), 610-630.

Bosse, R., Wirth, M., Gibis, M., Schmidt, H., Weiss, J. 2017. Kinetics of volatile marker compounds during ripening of cured loins inoculated with Staphylococcus carnosus. *Journal of the Science of Food and Agriculture*, *97*(9), 3050-3057.

Candogan, K., Acton, J. C. 2004. Proteolytic activity of bacterial starter cultures for meat fermentation. *Journal of Muscle Foods*, *15*(1), 23-34.

Candogan, K., Wardlaw, F. B., Acton, J. C. 2009. Effect of starter culture on proteolytic changes during processing of fermented beef sausages. *Food Chemistry*, *116*(3), 731-737.

Casaburi, A., Blaiotta, G., Mauriello, G., Pepe, O., Villani, F. 2005. Fermente sucuklardan izole edilen Staphylococcus carnosus ve Staphylococcus simulans suşlarının teknolojik aktiviteleri. *Et Bilimi*, *71* (4), 643-650.

Casaburi, A., Di Monaco, R., Cavella, S., Toldrá, F., Ercolini, D., Villani, F. 2008. Proteolitik ve lipolitik başlatıcı kültürler ve bunların geleneksel fermente sosislerin olgunlaşması ve duyusal özellikleri üzerindeki etkileri. *Gıda Mikrobiyolojisi* , *25* (2), 335-347.

Casquate , R., Benito, M.J., Martín, A., Ruiz-Moyano, S., Aranda, E., Córdoba, M.G., 2012. Use of autochthonous *Pediococcus acidilactici* and *Staphylococcus vitulinus* starter culturesin the production of "Chorizo" in 2 different. Journal of Food Science, 71, 70-79

Chen, X., Mi, R., Qi, B., Xiong, S., Li, J., Qu, C., Wang, S. 2021. Çin Dong fermente edilmiş domuz etinden (Nanx Wudl) izole edilen proteolitik başlangıç kültürünün kuru fermente sosislerdeki mikrobiyolojik, biyokimyasal ve organoleptik özellikler üzerindeki etkisi. *Gıda Bilimi ve İnsan Sağlığı* , *10* (1), 13-22.

Dalmış, Ü.,Soyer, A. 2008. Türk sucuklarında (sucuk) olgunlaşma ve depolama sırasındaki proteolitik değişiklikler üzerine işleme yöntemlerinin ve başlatıcı kültürün (Staphylococcus xylosus ve Pediococcus pentosaceus) etkisi. *Et bilimi , 80* (2), 345-354.

Kaban, G. 2009. Changes in the composition of volatile compounds and in microbiological and physicochemical parameters during pastırma processing. *Meat Science*, *82*(1), 17-23.

Kaban, G. 2013. Sucuk and pastırma: Microbiological changes and formation of volatile compounds. *Meat science*, *95*(4), 912-918.

Landeta, G., Reverón, I., Carrascosa, A. V., de las Rivas, B., Muñoz, R. 2011. Use of recA gene sequence analysis for the identification of Staphylococcus equorum strains predominant on dry-cured hams. *Food microbiology*, *28*(6), 1205-1210.

Leroy, F., Verluyten, J., De Vuyst, L. 2006. Functional meat starter cultures for improved sausage fermentation. *International journal of food microbiology*, *106*(3), 270-285.

Martín, A., Córdoba, J. J., Rodríguez, M. M., Núñez, F., Asensio, M. A. 2001. Evaluation of microbial proteolysis in meat products by capillary electrophoresis. *Journal of Applied Microbiology*, *90*(2), 163-171.

Mauriello, G., Casaburi, A., Villani, F., 2002. Proteolytic activity of *Staphylococcusxylosus* strains on pork miyofibrillar and sarcoplasmic proteins and use of selected strains in the production of 'Naples type' salami. Journal of Applied Microbiology, 92, 482-490.

Molina, I. Toldra F., 1992. Detection of proteolytic activity in microorganisms isolated from dry cured ham. Journal of Food Science, 57(6), 1308-1310.

Rodríguez, M., Núñez, F., Córdoba, J. J., Bermúdez, M. E., Asensio, M. A. 1998. Evaluation of proteolytic activity of micro-organisms isolated from dry cured ham. *Journal of Applied Microbiology*, *85*(5), 905-912.

Serdaroğlu, M., Tömek, S. 1995. Fermente Et Ürünlerinde Proteolitik Enzimlerin Önemi. *Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi*, *1*(2), 89-94.

Singh, P. K., Shrivastava, N., Ojha, B. K. 2019. Enzymes in the meat industry. In *Enzymes in Food Biotechnology* (pp. 111-128). Academic Press.

Toldrá, F., Arıstoy, M. C., Part, C., Cerveró, C., Rıco, E., Motılva, M. J., Flores, J. 1992. Muscle and adipose tissue aminopeptidase activities in raw and dry-cured ham. *Journal of Food Science*, *57*(4), 816-818.

Wang, D., Zhao, L., Su, R., Jin, Y. 2019. Effects of different starter culture combinations on microbial counts and physico-chemical properties in dry fermented mutton sausages. *Food Science & Nutrition*, *7*(6), 1957-1968.

Xiao, Y., Liu, Y., Chen, C., Xie, T., & Li, P. (2020). Effect of Lactobacillus plantarum and Staphylococcus xylosus on flavour development and bacterial communities in Chinese dry fermented sausages. *Food Research International*, *135*, 109247.

## *ORAL PRESENTATION*

# Adsorption of Hazelnut Skin Bioactive Compounds on Silk Fibroin

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## Abstract

The hazelnut skin is the brown pericarp tissue covering the surface of the hazelnut kernel as a thin layer. The bioactive components present in the hazelnut skin play a role in protection from diseases such as heart diseases, diabetes and cancer. In this study, adsorption process was used to selectively separate bioactives of hazelnut skin. A comparative evaluation was performed for adsorption kinetics and isotherms of a polyhenolics and flavonoids by using silk fibroin. For this purpose, polyphenolic compounds and flavonoids were analyzed by Folin-Ciocalteu and total-flavonoid assays, respectively. Continuous spectrophotometric measurement system was set up to monitor instantaneous change in solute concentration during the study. In this system, a jacketed reaction chamber was connected to the flow cell placed in the spectrophotometer by polypropylene tubing and peristaltic pump. A stone filter was used to keep the adsorbent particles in the reaction chamber and to eliminate the turbidity caused in the solution. In this system, sorption working conditions such as solid/liquid ratio, working volume, temperature, mixing speed were kept constant. Hazelnut skin bioactive components were extracted from ground hazelnut skin with 70% ethyl alcohol for 2 hours at a ratio of 1/10 (w/v). The solution was separated from the pulp by centrifugation and concentrated by rotary evaporator. Total phenol and flavonoid content of the concentrated extract was determined as 437 mg GA/g and 110 mg quarcetin/g of hazelnut skin, respectively. Serial dilutions were prepared to obtain solutions having different initial concentration. In order to investigate the mechanisms involved in the interactions between hazelnut skin bioactives and silk fibroin, five sorption isotherm models, including Freundlich, Langmuir, Tempkin, Dubinin-Radushkevich, and Redlich-Petersen models, were performed in this study. The sorption equilibrium data were best fitted to the Langmuir isotherm ($R^2 > 0.99$) indicating monolayer coverage.

**Keywords:** adsorption, bioseparation, silk fibroin, hazelnut, bioactives

## INTRODUCTION

In recent years, there is great interest in recovering biologically active compounds from different industrial by-products. Hazelnut skin is one of the food processing by-products and presents potential source of polyphenols. The skin represents 3% of the nut weight and may be valorized due to its distinctive polyphenol compounds present in it. While more than 95% of total polyphenols are comprised flavan-3-ols, flavones and dihydrocalchones were also found about 3.5% (Del Rio et al., 2011). Recently, it has shown that favonols exhibit different bioactivities and pharmacological activities (Ding et al., 2012). Flavonoids as a subclass of flavan-3-ols, possess various beneficial effects, including anticancer, antioxidant, anti-inflammatory and antiviral properties. These bioactivities depend on the type and amount of each individual compounds present and their bioavailabilities with its mode of action (Ullah et al., 2020). In order to benefit from their biological activities, flavonoids must be effectively separated from the liquid environment and obtained in high purity.

Adsorption process is very useful low-cost separation technique to selectively separate targeted compounds from the crude extract. Both synthetic and natural adsorbents have been used for the sorption of different bioactive compounds, such as Amberlite XAD series, activated carbon, diatomaceous earths (as synthetic ones) and cellulose, collagen, fibers, silk fibroin (as biosorbents) (Bayçın et al., 2007). Homogeneous structure, easy availability, predefined known adsorption mechanisms are the main advantages of the synthetic adsorbents.

But, after the adsorption process, the desorption process is also required. Thus, recently biosorbents have been studied extensively.

Silk fibroin, as a biopolymer, is an edible protein with a β-plate structure, mainly containing the amino acids glycine and alanine. Silk fibroin is effectively used in many biotechnological applications such as biomaterials, tissue engineering, controlled drug delivery systems and adsorption processes (Bayçın et al, 2007; Mandal and Kundu, 2008; Wani and Veerabhadrappa, 2018; Nguyen et al., 2019).

Experimental studies should be carefully designed for the adsorption studies. The mechanisms of the sorption process and the mass transfer resistances controlling it can only be accurately explained with sufficient data that can be collected with good experimental planning and kinetic measurements. Therefore, in adsorption studies, both dependent and independent variables such as temperature, solute concentration, mixing speed, adsorbent/liquid ratio need to be studied in detail with intensive experimental studies. For this reason, it is necessary to establish a practical experimental setup that can facilitate the process, have a low error rate, obtain accurate data, and control the parameters.

The aim of this study is to design a system providing stable working conditions with a continuous spectrophotometric measurement for the adsorption of hazelnut skin bioactive components on silk fibroin. As far as we know, there is no study in the literature on the adsorption of hazelnut skin polyphenols on silk fibroin. This study also aims to reveal the affinity of the adsorbent to the hazelnut skin polyphenolic compounds and to explain the adsorption mechanism.

## MATERIALS AND METHODS

### Material and Reagents

Hazelnut skin was kindly supplied from Fiskobirlik Integrated Hazelnut Processing Facilities. The hazelnut skins were used after grinding in a coffee grinder to a particle size of less than 50 µm. All reagents used in the study are of analytical grade. Ultrapure water was used in adsorption studies. Silk fibroin from silk Biochemical Co. Ltd. (Hangzou, China) was used as adsorbent. Folin Ciocalteu reagent (Merck) and sodium carbonate (Sigma) were used for total phenol content analysis. Aluminium chloride ($AlCl_3$) and sodium nitrite ($NaNO_2$) were used for total flavonoid assay.

### Extraction of Hazelnut Skin Polyphenols

The extraction of hazelnut skin was performed with 70% ethanol for 2 h at room temperature in an orbital shaker. After filtration of the solution, supernatant was centrifuged at 5000 rpm for 5 minutes. Then the solvent of the extract was evaporated and concentrated by using rotary evaporator at 42 °C, under vacuum. Concentrated extract was centrifuged again in order to remove insoluble components at 3000 rpm for 10 minutes. Final extract solution was used for the adsorption studies.

### Total Phenol Content assay

Folin Ciocalteu assay was used to determine the total phenol content. In this assay, 0.5 mL of the properly diluted sample was mixed with 0.5 mL of 1/10 diluted Folin reagent and was allowed to stand for 5 min. After that, 1.25 mL 20% sodium carbonate solution was added to the solution, was mixed and allowed 40 min to obtain stabilized blue color. The absorbance of the solution was measured at 760 nm. Results were expressed as gallic acid equivalent. All measurements were carried out in triplicate.

### Total flavonoid assay

The spectrophotometric assay based on aluminium complex formation was used to determine total flavonoid content of the extract solution according to the study of Pekal and Pyrznska, 2014. In this procedure, 0.25 mL of sample, 1.25 mL of water and 75 µL of $NaNO_2$ (5%, w/v) were shaken in a 2.5mL reaction tube. Mixture was allowed to stand 6 min at room temperature. Then, 150 µL of $AlCl_3$ (10%, w/v) was added. After 5 min, 0.5 mL of 1M NaOH solution and 275 µL of water was added to the solution. Finally, absorbance of the solution was measured at 510 nm by UV-spectrophotometer.

### Designing experimental setup for adsorption study

Continuous spectrophotometric measurement system was set up to monitor instantaneous change in solute concentration during the study. In this system, a jacketed reaction chamber was connected to the 500 µL quartz flow cell placed in the spectrophotometer by 0.6 mm ID polypropylene tubing and peristaltic pump (Figure 1).

A stone filter was used to keep the adsorbent particles in the reaction chamber and to eliminate the turbidity caused in the solution.

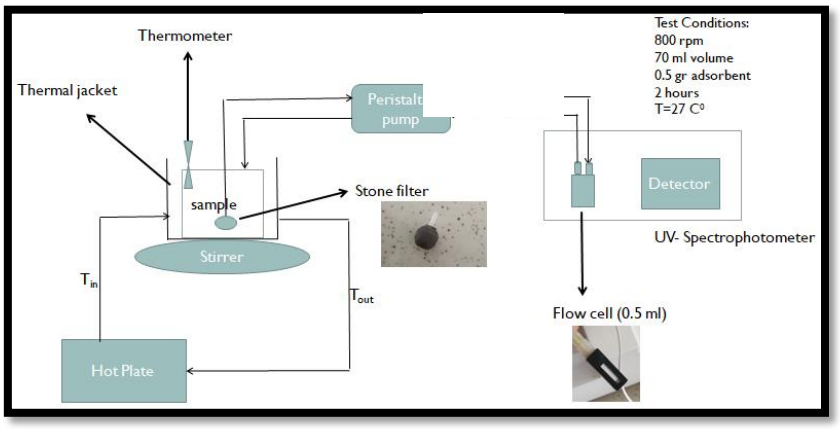


**Figure 1.** Experimental setup for the adsorption studies

## Adsorption of Hazelnut Skin Polyphenols

Hazelnut skin concentrated extract was serially diluted to the different initial concentrations (Ci). After wavelength scanning, the polyphenol content of the solutions was determined by measuring the absorbance values directly at 460 nm. The accuracy of monitoring absorbance values at 460 nm was determined by analyzing the correlation between the $A_{460}$ values and both the total phenol and total flavonoid contents of the solutions.

Solutions with various Ci concentrations were prepared and 70 mL of these solutions were used in adsorption studies. 0.5 grams of silk fibroin was added to the solutions so that the solid-liquid ratio was adjusted to a constant value of 1/140. During the study, stirrer speed and temperature were kept constant at 200 rpm and 25 °C, respectively. Continuous monitoring was achieved by direct measurement of the absorbance value of the solution at 460 nm through flow cell. For control purposes, samples were taken from the working chamber at predetermined times, and the $A_{460}$ value, total phenol and flavonoid content of each sample were measured.

## Statistical Evaluations

Assays were performed in triplicate and mean values were used for the evaluation. Parameters for isotherms models were determined linear least-squares methods using Microsoft Excel 2010.

## RESULTS and DISCUSSION

### Total phenol and flavonoid content of hazelnut skin

Total phenol content and flavonoid content of the hazelnut skins were determined as 437 mg GA and 110 mg quercetin equivalent per gram of hazelnut skin. Total phenol content value is similar with reported values of 400-700 mg GA/g for 10% and 96% ethanol extracts, respectively (Ivanovic et al., 2020). Taş and Gökmen, 2015 reported that depending on the origin of the hazelnuts, their skin's total phenol contents were varied between 52-203 mg GA/g. Flavonoid contents were reported in different equivalents as 27.2 mg/g rutin equivalent (Lelli et al., 2021); 550 mg/g catechin equivalent (Ivanovic et al., 2020) and 31-112 mg/g CE (Taş and Gökmen, 2015). Different values may be caused by origin nad type of the hazelnut varieties, different solvents and extraction methods. It has a fairly high values compared to most fruits and vegetables. Therefore it is necessary to recover the major bioactive components from the hazelnut skin. This waste, which is a by-product in significant amounts in hazelnut processing plants, can be utilized in different fields such as food, chemistry, medical and pharmaceutical industries due to the polyphenols it contains.

### Efficiency of the measurement system

According to the trial studies experimental setup was developed. The major challange was the selection of suitable filter. Various filters, such as laboratory microfilter (milipore), filter pipettes, gasoline filter, and stone filters were tried and stone filter was the most suitable one. During the adsorption study, the stone filter was used successfully. The filter made it possible to transfer a clear, particle-free solution to the flow cell. Another adjustment was made with a peristaltic pump. The flow rate was adjusted at a level that would not cause

foaming in the flow cell. After these adjustments, the reaction chamber was first loaded with 70 mL of hazelnut skin extract, then flow was maintained throughout the system. After the solution temperature stabilized, the adsorbent was added to the medium and kinetic measurements were started.

During the experiment, continuous absorbance measurements were carried out at 460 nm. Correlation study was also performed to confirm that this measurement was due to polyphenolic components. For this study, different dilutions of hazelnut skin extract solutions were prepared and total phenol and flavonoid analyzes were performed in all these solutions. Correlation graphs are given in Figure 2.

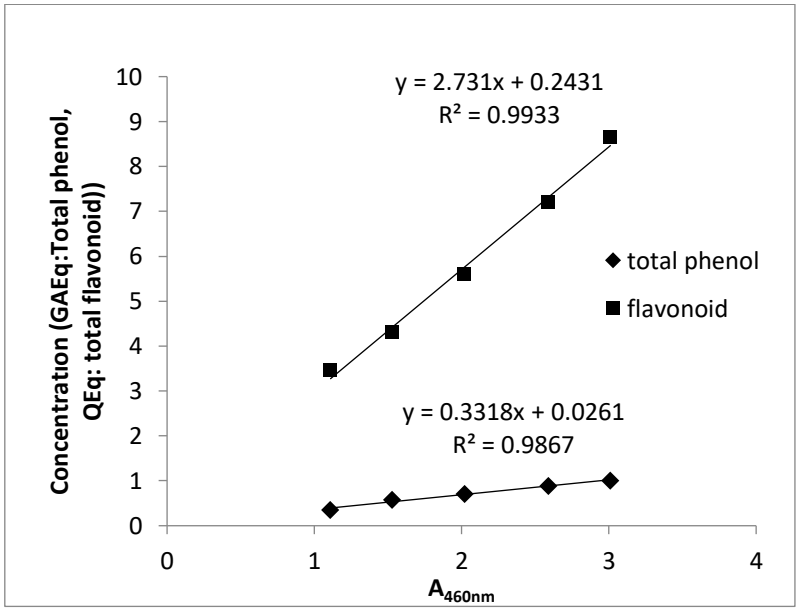


**Figure 2.** Correlation lines between A460 and total phenol- flavonoid contents, GAEq: Gallic acid equivalent, QEq: Quercetin equivalent

Figure 2 shows a high correlation between direct 460 nm absorbance values and total phenol and flavonoid content. This shows that the solution concentration can be followed in the adsorption study with the flow cell.

The kinetic data from the sorption study carried out with a solution having a pre-determined initial concentration of hazelnut skin polyphenol is given in Figure 3. Large and sequential collection of data is important in order to explain the sorption process correctly. When Figure 3 is examined, the variation of polyphenolic component concentration in the solution has been effectively demonstrated.

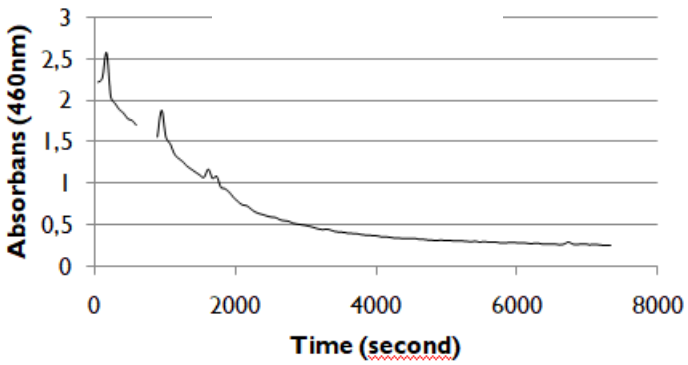


**Figure 3.** Polyphenolic compounds concentration kinetic change, $A_{460i}$ : 2.59

## Adsorption isoterms and model equations

In order to investigate mechanism involved in the interactions between hazelnut skin polyphenols with silk fibroin, different adsorption isotherms were analyzed. Model equations and their lineer forms are presented in Table 1.

Table 1. Isotherm models used in this study

| Isotherm model | Equation | Linear form | Plot |
|---|---|---|---|
| **Langmuir** | $q_e = \frac{K_L C_e}{1 + a_L C_e}$ | $\frac{C_e}{q_e} = \frac{a_L}{K_L} C_e + \frac{1}{K_L}$ | $\frac{C_e}{q_e}\ vs\ C_e$ |
| **Freundlich** | $q_e = K_F C_e^{n_f}$ | $logq_e = logK_f + n_f logC_e$ | $logq_e\ vs\ logC_e$ |
| **Tempkin** | $q_e = \frac{RT}{b_T} \ln (A_T C_e)$ | $q_e = \frac{RT}{b_T} lnA_T + \frac{RT}{b_T} lnC_e$ | $q_e\ vs\ lnC_e$ |
| **Dubinin-Radushkevich** | $q_e = q_s \exp(-B_\epsilon^2)$ | $lnq_e = lnq_s - B_\epsilon^2$ | $lnq_e\ vs\ \epsilon^2$ |
| **Redlich-Petersen** | $q_e = \frac{K_R\ C_e}{1 + a_R C_e^\beta}$ | $\ln\left[K_R \frac{C_e}{q_e} - 1\right] = lna_R + \beta lnC_e$ | $\ln\left[K_R \frac{C_e}{q_e} - 1\right]\ vs\ lnC_e$ |

The Freundlich isotherm model assumes that the adsorbent has a heterogeneous surface and that both monolayer and multilayer sorption processes are involved. (Yang, 1998). On the other hand, Langmuir isotherm explains that the adsorbent surface is homogeneous and solute molecules interact with the surface to form a monolayer. The molecules attach to the active groups on the surface, turn off the available active groups, and there is no further adsorption to these specific sites. Maximum adsorption capacity ($q_{max}$ _mg/g adsorbent) is calculated with the Langmuir constants; $K_L$ (L/g) and $\alpha_L$ (L/mg). The Tempkin model, on the other hand, explains that as the sorbent sorbate interaction continues, the sorption energy decreases linearly, and the surface active regions are covered. Dubinin isotherm with pore-filling mechanism includes qs (mg/g) and B model constants. The Redlich-Petersen isotherm, which features both the Freundlich and Langmuir isotherm models, includes three parameters in the equation; $K_R$ (L/g), $\alpha_R$ (L/mg), β (Foo and Hameed, 2010).

For isotherm analysis, solutions with different initial concentrations (all other parameters were kept constant at certain values) were studied and a graph of equilibrium concentrations against Ci values was drawn (Figure 4a). Langmuir constants can be calculated from the curve between Ce and Ce/qe obtained by applying the Langmuir isotherm equation (Fig. 4b). Accordingly, the maximum adsorption amount was calculated as 357.14 mg total polyphenol/g adsorbent.

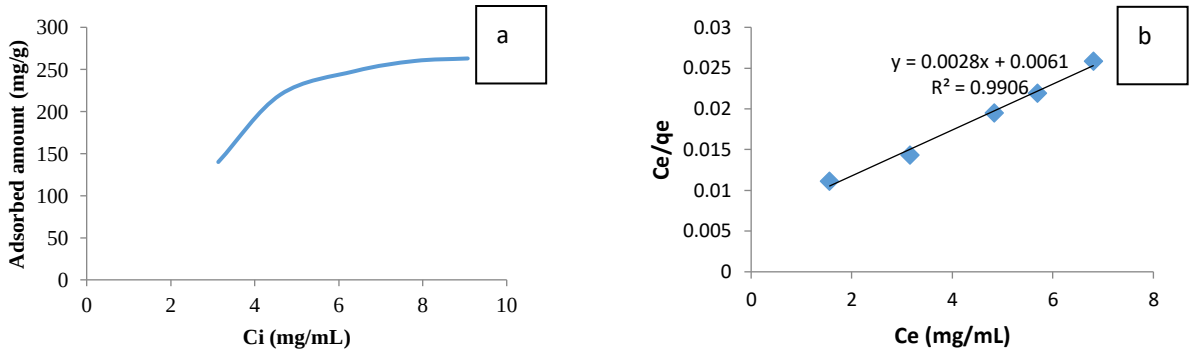


**Figure 4.** Adsorption isotherm and Langmuir isotherm model equation application

## CONCLUSION

Since hazelnut skin exhibits a distinctive feature with its high polyphenolic and flavonoid content, this waste, which is a food process by-product, should be valorized in food, pharmaceutical and medical fields. However,

the bioactive polyphenolic components must be separated from the solution. Silk protein is an important substance for this process with its features such as being natural, containing amino acid structures, being edible and giving high sorption capacity values. With the experimental system created in the studies, all parameters that may affect the sorption process can be controlled. Each parameter can be studied separately so that the adsorption mechanism can be understood and optimization studies can be carried out to reach the maximum adsorption capacity. With further detailed studies, the interactions of silk fibroin-hazelnut skin polyphenols should be understood and especially which components should be selectively adsorbed.

**ACKNOWLEDGEMENTS**

This study was financially supported by Giresun University Scientific Research Project Office with the consumables purchased with the scope of the project (Project no: FEN-BAP-A-150219-03). The authors thank the Giresun University BAPKO unit. Authors are also grateful to Fiskobirlik Integrated Hazelnut Processing Facilities in Giresun for providing hazelnut skins.

**REFERENCES**

Bayçın D, Altiok E, Ülkü S, Bayraktar O 2007. Adsorption of olive leaf (*Olea europaea* L.) antioxidants on silk fibroin. Journal of Agricultural and Food Chemistry, 55: 1227-1236.

Del Rio D, Calani L, Dall'Asta M, Brighenti F 2011. Polyphenolic composition of hazelnut skin. Journal of Agricultural and Food Chemistry, 59: 9935-9941.

Ding P, Liao X, Shi B 2013. Adsorption chromatography separation of the flavonols kaempferol, quercetin and myricetin using cross-linked collagen fibre as the stationary phase. Journal of the Science of Food and Agriculture, 93: 1575-1583.

Foo KY, Hameed BH 2010. Insights into the modeling of adsorption isotherm systems. Chemical Engineering Journal, 156: 2-10.

Ivanovic S, Avramovic N, Dojcinovic B, Trifunovic S, Novakovic M, Tesevic V, Mandic B 2020. Chemical composition, total phenols and flavonoids contents and antioxidant activity as nutritive potential of roasted hazelnut skins (*Corylus avellana* L.). Foods, 9: 430-445.

Lelli V, Molinari R, Merendino N, Timperio AM 2021. Detection and comparison of bioactive compounds in different extracts of two hazelnut skin varieties, Tonda Gentile Romana and Tonda Di Giffoni, using a metabolomics approach. Metabolites, 11: 296-317.

Mandal BB, Kundu SC 2008. Non-bioengineered silk fibroin protein 3D scaffold potential biotechnological and tissue engineering application. Macro Molecular Bioscience, 8: 807-818.

Nguyen TP, Nguyen QV, Nguyen VHL, Huynh VQN, Vo DN, Trinh QT, Kim SY, Le QV 2019. Silk Fibroin-Based Biomaterials for Biomedical Applications: A Review. Polymers, 11: 1-25.

Pękal, A, Pyrzynska, K 2014. Evaluation of aluminium complexation reaction for flavonoid content assay. Food Analytical Methods, 7: 1776–1782.

Taş NG, Gökmen V 2015. Bioactive compounds in different hazelnut varieties and their skins. Journal of Food Composition and Analysis,43: 203-208.

Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Laremko M 2020. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules, 25: 5243-5250.

Wani SUD, Veerabhadrappa GH 2018. Silk Fibroin Based Drug Delivery Applications: Promises and Challenges. Current drug targets, 19: 1177-1190.

Yang C, 1998. Statistical mechanical study on the Freundlich isotherm equation. Colloid. Journal of Colloid Interface Science, 208: 379-387.

## *ORAL PRESENTATION*

# Fabrication and characterization of electrospun ellagic acid-loaded Poly (lactic acid)-polyethylene glycol nanofibers for biomedical application

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**Abstract**

Ellagic acid (dilactone of hexahydroxydiphenic acid, 2,3,7,8-tetrahydroxy-1-benzopyran-5,4,3-cde-1-benzopyran-5,10-dione, EA) is one of the natural polyphenolic antioxidants present in numerous fruits (pomegranate, persimmon, raspberry, black raspberry, strawberry, blueberry, peach, plumes, almonds, walnuts, mango kernel), vegetables and nuts to the tune of 100–1900 mg/kg and contributes significantly to the dietary consumption as nutraceuticals or functional foods. EA demonstrates a broad array of biological roles including anti-inflammatory, anti-carcinogenic, anti-fibrosis, anti-diabetic, anti-ulcer, anti-viral, anti-microbial, anti- apoptotic, anti-mutagenic and cardio-, hepato-renal and neuro-protective together with defense against oxidative stress, lipid peroxidation and free radical damage. Because of its multi-target pharmacological properties endowed with multiple mechanisms of action, can serve as an innovative option for several applications, such as the treatment of bacterial infections and diabet.

The use of natural compounds without undesired side effects, in tissue engineering applications, is highly preferred compared to chemical drugs. In recent years, many studies have been carried out to produce nanofibrous materials by electrospinning method. Electrospun nanofibers have very large surface area, controllable pore size and tunable drug release profiles. Several biocompatible polymers with excellent biocompatibility and biodegradability including poly ethylenglycol (PEG) and poly lactic acid (PLA) have been widely used for the synthesis of nanofibers using the electrospun technique.

In this study, nanofibers were obtained by adding of EA at different concentration into PLA by electrospinning technique. Morphological (SEM), molecular interaction (FT-IR), thermal analysis (DSC), antioxidant activity and physical analysis were carried out after production process. The resulting structures of nanofibers are suitable for biomedical applications.

**Keywords:** Polylactic acid (PLA), nanofiber, ellagic acid, electrospinning

## INTRODUCTION

Polyphenols are secondary plant metabolites. They are naturally occurring compounds found in many foods, including fruits, vegetables, grains, and beverages (Rahman et al., 2022). Polyphenolic compounds represent possible treatments for many diseases in the developing world. (Rahman et al., 2022; Montes et al., 2016).

Ellagic acid (EA), is a natural polyphenolic antioxidants, found in fruits (Bidanchi et al., 2022). EA has attracted great interest recently, as several research studies have proven that it has therapeutic effects (Zuccari et al., 2020; Ceci et al., 2020). Because of these effects, EA is considered a high-value antioxidant, anti-inflammatory and chemopreventive agent (Aguilar-Zarate et al., 2018). EA is often consumed therapeutically in the form of capsules, powder, and liquid for the treatment of disease (Pirzadeh-Naeeni et al., 2020). However, its low water solubility and low oral bioavailability limit the clinical applications of EA. Furthermore, little has been reported about effective strategies to increase the solubility and poor oral bioavailability of EA (Ceci et al., 2020). Based on this, EA delivery formulation, including nanocarriers and polymer matrices, has been proposed to improve the pharmacokinetic properties and therapeutic efficacy of EA (Ceci et al., 2020). Micro or nanoparticle systems containing EA such as microspheres (Ogawa et al., 2002), nanoparticles (De Souza Tavares et al.,2021; Singh Hallan et al., 2020), pH-dependent microassemblies (Barnaby et al., 2011), zinc-layer hydroxide nanohybrid (Hussein et al., 2011), and nanogels (Behl et al., 2011). have been developed by researchers. Electrospun nanofibers have large surface area, controllable pore size and tunable drug release profiles (Contreras-Cáceres et al., 2019 ).

Based on this background, in this study, nanofiber structure were formed by adding different concentrations of EA to PEG/PLA by electrospinning technique to prepare a new nanodrug delivery systems containing EA. After this stage, morphological, molecular interaction, thermal analysis, antioxidant activity analyzes were performed.

## MATERIALS AND METHODS

**Preparation of electrospinning solutions** PLA solution was prepared by dissolving PLA granules of in 20 ml chloroform by magnetic stirring for 2 h at room temperature. 3 wt % Tween 80 was added into the PLA solution. Afterward 1 wt % PEG added to the 8 wt% PLA solution by stirring for 10 min. Following that, different contents of EA ie., 1.0, 1.5 and 2.5 mg were added to the PLA (w/v) solution and mixed for one hour.

**Production of nanofibers with the electrospinning method**
Each polymeric solution was poured into 10 ml disposable syringe. The solutions were delivered to the electrospinning device at a constant flow rate of 5 ml/h using a syringe pump. The voltage was subjected to about 24 kV and collector was positioned 120 mm away from the tip needles.

**Characterization of nanofibers**

The chemical properties of the nanofibers were recorded Fourier transform infrared spectroscopy with a scanning rate between 4000–400 $cm^{-1}$. The surface morphologies of the nanofibers was observed by SEM.. The average fiber diameter and their distribution were measured by using image software Mechanical properties of the obtained samples were determined with uniaxial tensile testing equipment . All samples were subjected to test the speed of 5 mm/min until the breaking point at room temperature (23 $^0$C). Melting temperatures (Tm) and glass transition temperatures (Tg) were recorded on DSC. Tm and Tg were recorded for polymers as the maximum peak temperature from DSC curves.Total antioxidant activity of samples (DPPH assays) and total phenolic contents (Folin–Ciocalteu method) were determined by following method of Seyhan et al., 2020.

## RESULTS and DISCUSSION

**FT-IR Analysis**

The results of FTIR analysis for the electrospun nanofiber of pure PLA/PEG and nanofibers with three different concentration of EA are indicated in Figure 1. PEG characteristic infrared bands were observed C–H stretching at 2890 $cm^{-1}$ and the C–O (ether) stretching at 1125 $cm^{-1}$. PLA the main absorption peaks were observed C––O vibration peak at 1749 $cm^{-1}$, $CH_3$ asymmetrical scissoring at 1453 $cm^{-1}$, C–O, C–O–C stretching at 1080 $cm^{-1}$, C–$CH_3$ stretching at 1042 $cm^{-1}$ and C–COO stretching peak at 867 $cm^{-1}$. Compared to PLA/PEG, the EA spectrum, absorbsiyon peaks at 3580 $cm^{-1}$, 3480 $cm^{-1}$, and 3360-2840 $cm^{-1}$ were assigned to the stretching vibrations of the OH group.

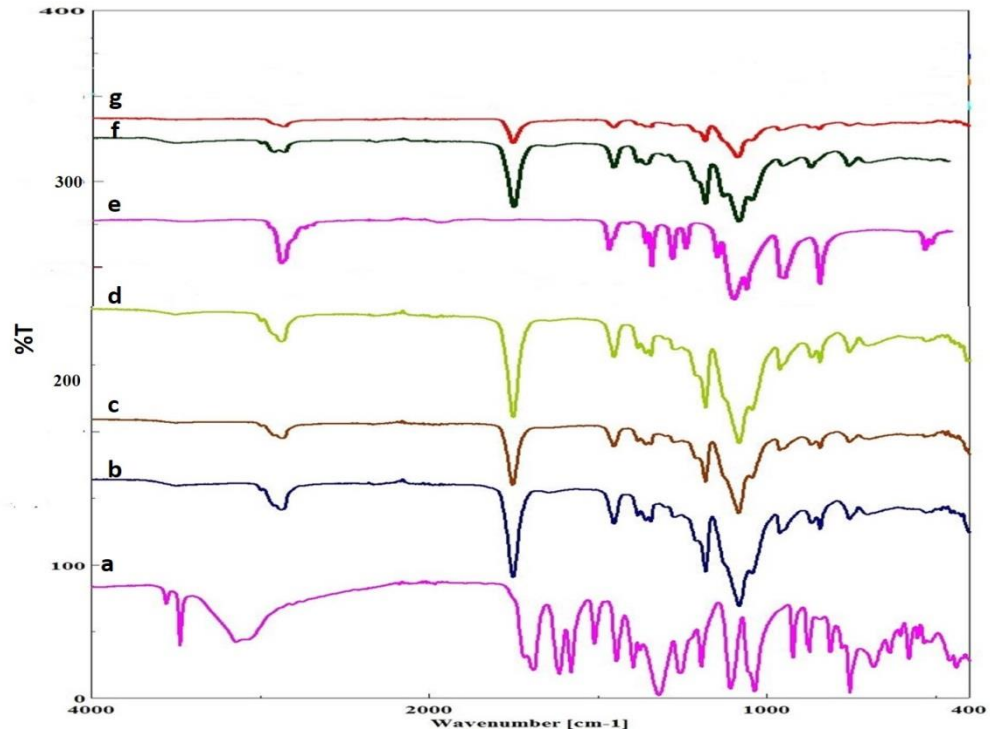


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**Figure 1.** FTIR spectra of the (a) pure EA, (b) PLA/PEG/1,0 mg EA, (c) PLA/PEG/1.5 mg EA, (d) PLA/PEG/2.5 mg EA (e) PEG, (f) PLA and (g) PLA/PEG/Tween 80 nanofiber

**SEM analysis**

Fiber sizes and morphologies of nanofibers were analyzed by using SEM. Due to EA uniformly distributed within the polymer matrices, the obtained EA-loaded nanofibers homogeneously dispersed. As can be seen in Figure 2d, the 2.5 mg EA-loaded nanofiber provided smaller fiber diameter (Figure 2a)

**DSC analysis**

DSC analyzes revealed that the decreased cold-crystallinity is associated with the increase of Ellagic acid ratio and It was observed that EA loading on PLA/PEG nanofiber changed the thermal behavior of the fibers (Figure 3 ).

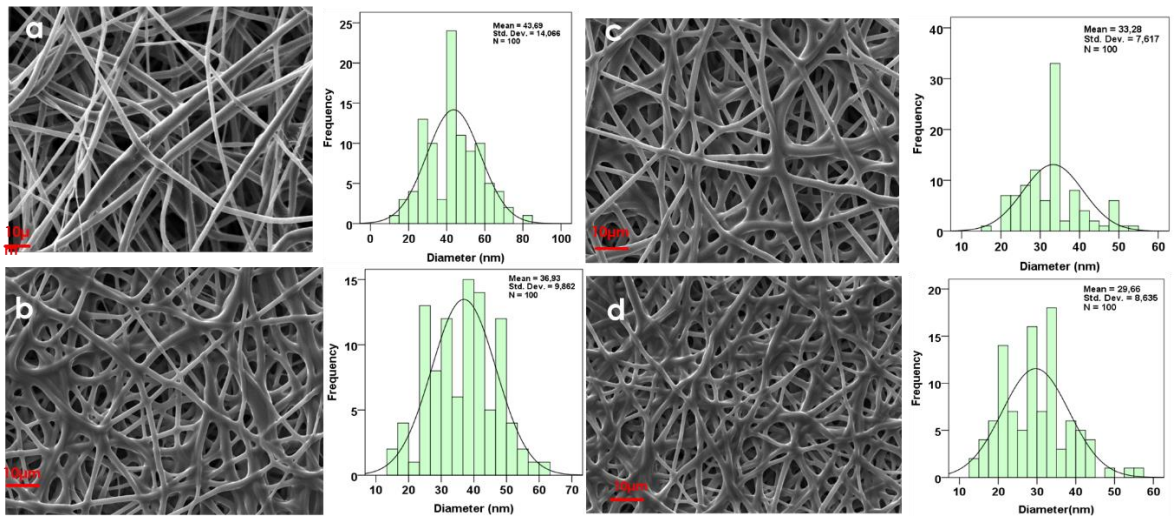


**Figure 2.** SEM images and fiber diameter distribution of (a) Pure PLA/PEG (b) PLA/PEG/1.0 mg EA (c) PLA/PEG/1.5 mg EA (d) PLA/PEG/2.5 mg EA

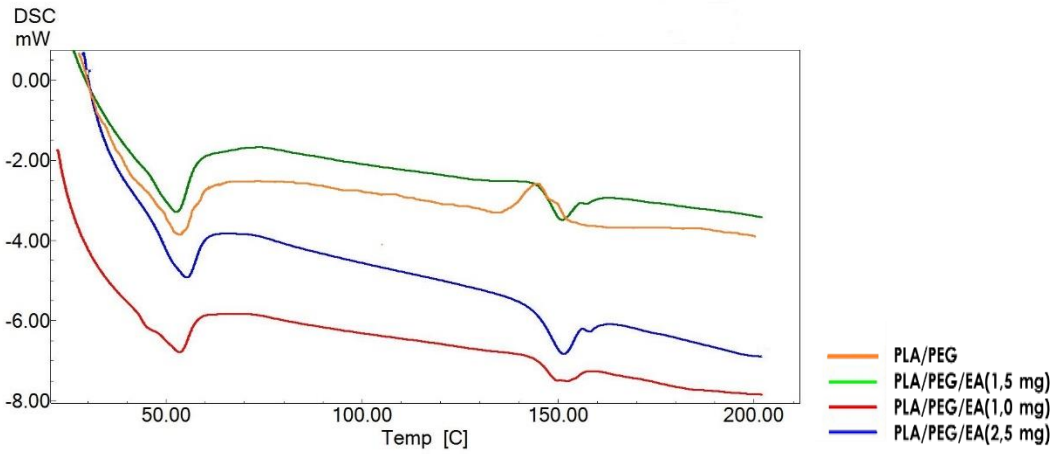


**Figure 3.** DSC curves of PLA/PEG, PLA, and PEG solution fibers without and with EA

## Tensile Mechanical Properties

According to the tensile strength and strain at break measurement results, it was observed that when ellagic acid was added to the PLA/PEG combination, strain at break values decreased proportionally with the concentration of ellagic acid and the tensile strength values also decreased proportionally with the concentration of ellagic acid.

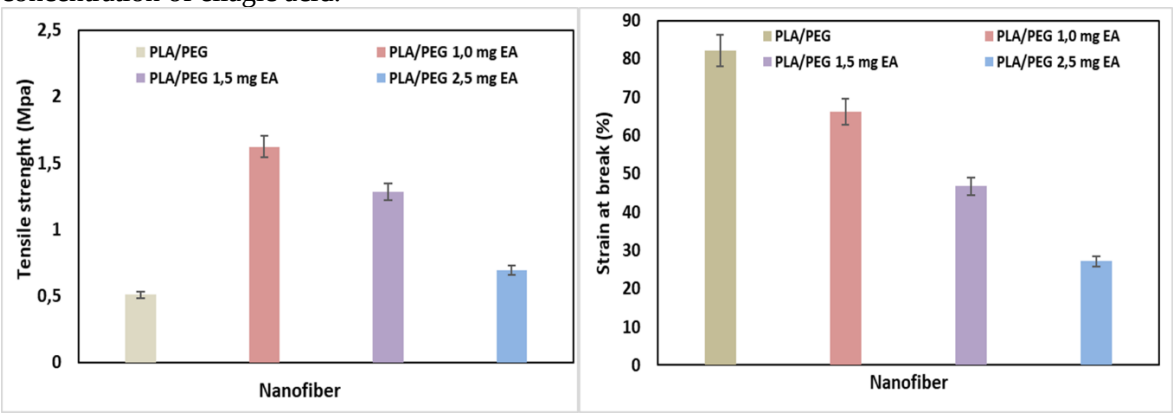


**Figure 4.** Tensile properties of nanofibers PLA/PEG, PLA/PEG/ EA

## Antioxidant activity

Total amount of phenolic substance in EA nanofibers was determined by using Folin-Ciocalteu reagent and free radical scavenging activity of the nanofibers was determined by using DPPH radical. The figure shows that the DPPH antioksidant activity and the reducing capacity of the PLA/PEG nanofiber was very weak, while the PLA/PEG/EA fibers exhibited pronounced antioxidant activity. As seen from the results, the pure PLA/PEG did not show any antioxidant effect; however, it acted as protection system for the antioxidants.

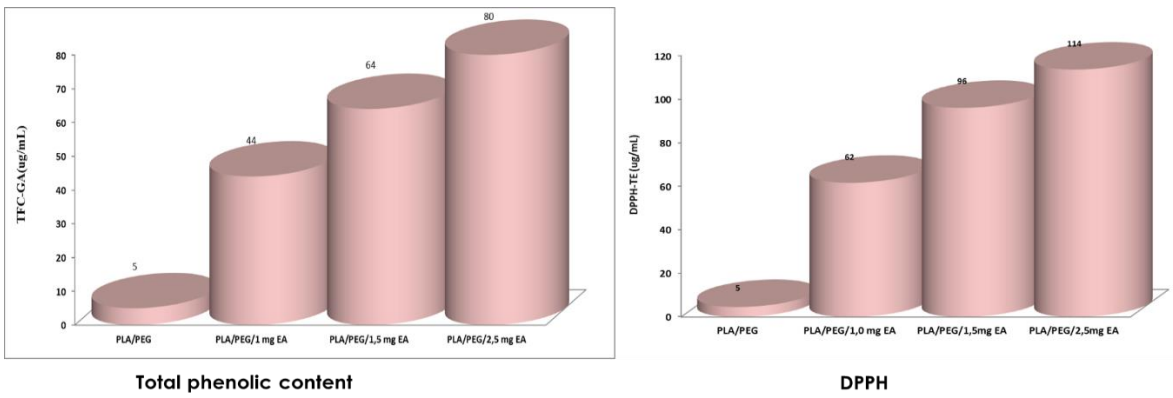


**Figure 5**. Antioxidant activity

## CONCLUSION

It is highlighted that, elagic acid extract nanofibers have antioxidant activity and can be useful for their synergistic effect for treatment of disease. Therefore, further studies are needed for understanding their synergistic effects in therapeutic applications. Thus, the synergistic antioxidative activity of the mixture can be optimized as an alternative therapeutic support for the treatment of various diseases.

## REFERENCES

Aguilar-Zarate P, Wong-Paz JE, Buenrostro-Figueroa JJ, Ascacio JA, Contreras-Esquivel JC and Aguilar CN 2018. Ellagitannins: bioavailability, purification and biotechnological degradation Mini-Reviews Med. Chem. 18: 1244–52.

Barnaby SN, Yu SM, Tsiola A, Fath KR and Banerjee IA 2011. pH dependent spontaneous growth of ellagic acid assemblies for targeting HeLa cells J. Nanosci. Nanotechnol. 11: 7579–86.

Behl G, Sharma M, Dahiya S, Chhikara A and Chopra M 2011 Synthesis, characterization, and evaluation of radical scavenging ability of ellagic acid-loaded nanogels J.Nanomater. 1–9.

Bidanchi RM, Lalrindika L.; Khushboo M, Bhanushree B, Dinata R, Das M Nisa N, Lalrinzuali S, Manikandan B, Saeed-Ahmed 2022. Antioxidative, Anti-Inflammatory and Anti-Apoptotic Action of Ellagic Acid against Lead Acetate Induced Testicular and Hepato-Renal Oxidative Damages and Pathophysiological Changes in Male Long Evans Rats. Environ. Pollut. 302: 119048.

Ceci C, Graziani G, Faraoni I, Cacciotti I 2020. Strategies to improve ellagic acid bioavailability: from natural or semisynthetic derivatives to nanotechnological approaches based on innovative carriers. Nanotechnology 31:382001

Contreras-Cáceres R, Cabeza L, Perazzoli G, Díaz A, Lopez-Romero JM, Melguizo C, Prados J 2019. Electrospun Nanofibers: Recent Applications in Drug Delivery and Cancer Therapy. Nanomaterials. 24, 9(4):656.

De Souza Tavares W, Pena GR, Martin-Pastor M, de Sousa FFO 2021. Design and characterization of ellagic acid-loaded zein nanoparticles and their effect on the antioxidant and antibacterial activities. J. Mol. Liq. 341: 116915.

Hussein MZ, Al Ali SH, Zainal Z and Hakim MN 2011. Development of antiproliferative nanohybrid compound with controlled release property using ellagic acid as the active agent Int. J. Nanomedicine 6 :1373–83

Montes A, Wehner L, Pereyra C, Martínez de la Ossa EJ 2016. Generation of microparticles of ellagic acid by supercritical antisolvent process. Journal of Supercritical Fluids, 116: 101–110.

Ogawa Y, Kanatsu K, Iino T, Kato S, Jeong Y, Shibata N,Takada K and Takeuchi K 2002. Protection against dextran sulfate sodium-induced colitis by microspheres of ellagic acid in rats Life Sci. 71: 827–39

Pirzadeh-Naeeni S, Mozdianfard MR, Shojaosadati SA, Khorasani AC, Saleh T 2020.A comparative study on schizophyllan and chitin nanoparticles for ellagic acid delivery in treating breast cancer. Int. J. Biol. Macromol. 144:380–388

Rahman MM, Rahaman MS, Islam MR, Rahman F, Mithi FM, Alqahtani T, Almikhlafi MA, Alghamdi SQ, Alruwaili AS, Hossain MS et al. 2021.Role of phenolic compounds in human disease: current knowledge and future prospects. Molecules, 27(233):1-36

Seyhan SA, Yilmaz Z, & Yilmaz DC 2020. The influence of extraction method on antioxidant potential of Tilia argentea flowers and bracts. Ovidius University Annals of Chemistry, 31(1): 14–17.

Singh Hallan S, Sguizzato M, Pavoni G, Baldisserotto A, Drechsler M, Mariani P, Esposito E, Cortesi R 2020. Ellagic Acid Containing Nanostructured Lipid Carriers for Topical Application: A Preliminary Study. Molecules 25,1449:1-15.

Zuccari G, Baldassari S, Ailuno G, Turrini F, Alfei S, Caviglioli G 2020. Formulation strategies to improve oral bioavailability of ellagic acid. Applied Sciences,10:3353:1-27.

## *ORAL PRESENTATION*

# Screening and comparison of antioxidant activity of solvent extracts of medicinal plants grown in Turkey

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## Abstract

In the developing world, the tendency towards natural antioxidants has increased due to the harmful effects of synthetic antioxidant use on the health. Hence, the researches on the antioxidant components in root, leaf and fruit of plant material are increasing day by day. Flavonoids and other phenolic compounds of plant origin have been reported as scavenger ROS, thus they are viewed as promising therapeutic drugs for free radical pathologies. The antioxidant activity of plant origin is dependent on the type and polarity of the extracting solvent as well as on the test system and the substrate to be protected by the antioxidant.
The present study was undertaken to perform the screening of antioxidant properties of 3 medicinal plants (L*ycium barbarum* L,*Morus nigra* L and *Inula helenium* L grown in Turkey, and we selected 70% methanol and water as extract solvents which permits comparison of the antioxidant properties among the polarity based solvent extracts of medicinal plants. We used different extraction techniques, such as magnetic and ultrasonic stirrer, and the total phenolic content of the L*ycium barbarum* L., *Morus nigra* L. and *Inula helenium* L. extracts was determined by modified Folin-Ciocalteu method. The reducing power and radical scavenging activity of the extracts were also evaluated.

**Keywords***: Lycium barbarum L., Morus nigra L., Inula helenium L.,* extraction, antioxidant activity

## INTRODUCTION

Nature has been a source of traditional medicinal practices since ancient time. In this way many of natural products have become current drug candidates for treating diseases which are often associated with the termination of free radical propagation in biological systems. Due to the radical acceptor role of phenolic compounds in natural products ,the use and consumption of natural antioxidants from plant material has become an important to prevent from disease.Hence, this study is aimed to give an overview of plant materials due to their potential medicinal uses.
*Morus nigra L.(*MN) is frequently consumed by peple both as food and to alleviate many ailments due to its rich antioxidant content and flavor (Kostic et al., 2013). For example, various parts of MN (such as fruit, root and bark) are used in the treatment of sore throat, anemia, iron deficiency, tonsillitis, wound healing, hypertension, diabetes and arthritis; It is used in traditional medicine as an antipyretic and diuretic to protect the liver, to treat vision difficulties, to reduce blood pressure and to prevent cardiovascular diseases (Jiang 2011). It has also been demonstrated by scientific studies that this plant contains significant amounts of bioactive substances and exhibits many different biological activities such as antimicrobial, anti-inflammatory, antioxidative, antidiabetic, antiatherogenic, antiaging, antiobesity and neuroprotective (Hojjatpanah et al 2011, Diab et al 2020, Akmese et al 2020).
*Lycium barbarum* L (LB.) is one of the important plant species used in traditional Chinese medicine due to its medicinal properties. Flavonoids and phenolic acids found in LB are the main components that cause LB. to exhibit antioxidant and antimicrobial activity (Soner et al 2019; Amagase & Farnsworth 2011; Kulczyński & Gramza-Michałowska 2016).

*Inula helenium L*(INH) have widesprad medical use. This genus is known for its biological activities such as anticancer, antibacterial, antitumor, cytotoxic, hepatoprotective. There are almost few studies on the bioactivity of Inula species belonging to the flora of Turkey (Iguță et al 2014, Koc and et al 2018,Aybastier O 2021). The main component of INH is alantolactone and has a strong antibacterial effect. INH is rich in sesquiterpene, essential oil, lactones and some phenolic substances, and its roots are used as diuretic and expectorant (Konishi et al 2002).

Extraction is the main process for plant materials. The aim of extraction process is to obtain the highest activity of these extracts. The extraction activity of the sample extract is not only affected by the extraction technique but also by the extraction solvent that is recognized as one of the most important parameters (Truong et al 2019). Also polarity of the extraction solvents is another parameter because it could determine the level of the extract.

In this study, the sample extracts obtained from methanol and water solvents were studied for their total phenoic content and antioxidant activity by using FCR, DPPH and FRAP assays. It is important to evaluate and quantify effective antioxidant principles of medicinally viable plant materials. The plant-derived antioxidant compounds with their mechanisms of antioxidant defenses that can help in the prevention of these diseases. In this reason the antioxidant properties of roots, leaves and fruits of natural medicinal plants that support the treatment in disease were determined according to the part with the highest antioxidant properties. Hence in the present study the most effective solvent/technique was investigated for extracting antioxidant compounds from different parts of selected medicinal plants native to Turkey. For this reason, obtaining the findings about the parts of the plants that show the highest antioxidants and the optimum conditions of the related methods and numerically expressing the analysis results of these plants will guide future studies.

## MATERIALS and METHODS

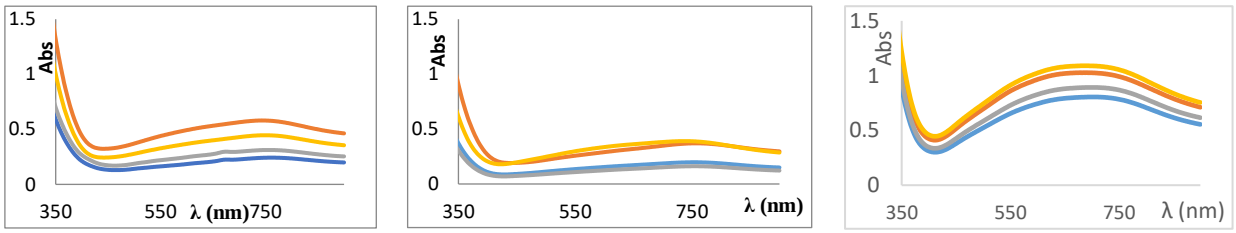
### Plant Material and Preparation of Extract

Different parts of samples were purchased from a local market in Istanbul, Turkey. 1.000 g of each sample from our dried and pulverized sample was weighed. The sample was first extracted with 10.00 mL of solvent for 60 minutes, with a second 10.00 mL for 60 minutes, and with 10.00 mL for 60 minutes in an ultrasonic bath (U) and heated magnetic stirrer(M) at °C in a closed glass shot bottle in three stages. These three extracts were combined to make up to 30.00mL with solvent. Water and methanol (70%) were used as solvents. After the extraction process, the extracts were centrifuged at 2500 rpm for 20 minutes. The supernatant was separated and stored at -20 ˚C until analysis.

### Total phenolic substance content

The total amount of phenolic substances in the extracts was determined using the Folin-Ciocalteu reagent (FCR). 100 µL of the prepared extracts was taken and 4 mL of distilled water was added. This mixture was incubated with 100 µL of FCR and 5 min at 30°C and 200 rpm. Then, 800 µL of 6% $Na_2CO_3$ solution was added. The mixture was incubated at 30°C and 200 rpm for 30 min. The absorbance of the resulting blue color was measured in the wavelength range of 685-760 nm. The experiment was repeated twice and the absorbance was averaged.

In order to obtain the standard linear equation of gallic acid, 25 mg/25 mL stock solution of gallic acid was first prepared with methanol. By diluting this solution with methanol, standard solutions with concentrations of 62.5, 125, 300, 500 and 1000 µg/mL were obtained. The total phenolic content of the prepared solutions was determined using the Folin-Ciocalteau reagent. The absorbances of the solutions were measured at 685-760 nm. The total phenolic content of the extracts was determined as µg gallic acid equivalents using the line equation obtained from the standard gallic acid graph. The equation of calibration curve was $y=0.0019x-0.0419$, where $R^2 =0.9931$.




MN b. INH c.LB

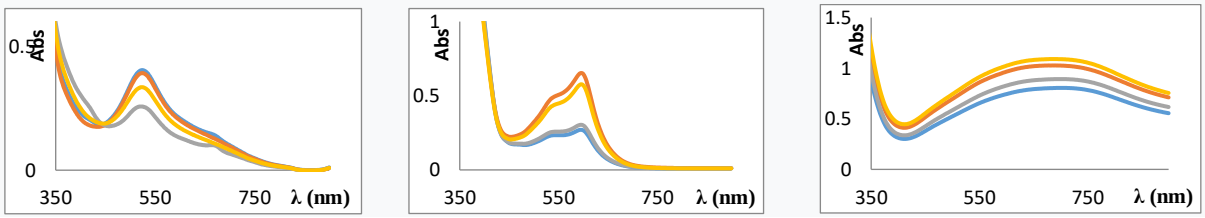
**Figure 1: Spectrum of extracts prepared by Folin -Ciocalteau method**

UMet UW MMet MW

**Iron (III) ion reducing antioxidant capacity method (FRAP)**

FRAP reagent was prepared by mixing 250 mL of 300 mM NaAc-AA buffer (pH 3.6), 25 mL of TPTZ solution (solution of 10 mM TPTZ in 100 mM HCl) and 25 mL of 20 mM FeCl3.6H2O. Then, 100 µL of each sample was added to 3 mL of FRAP solution and incubated for 4 minutes in an orbital shaker at 37 °C. The absorbance of the solutions was measured after 4 minutes, and the maximum absorbance value was measured in the wavelength range of 580-600 nm.

In order to obtain the correct equation of Trolox, firstly, a 1000 µM 25 mL stock solution of Trolox was prepared with distilled water. By diluting this solution with water, solutions of 75, 250, 500, 750 and 1000 µM concentrations were obtained. The absorbance of the colors formed after the FRAP method was applied to the solutions was recorded at 580-600 nm. The FRAP values of the extracts were determined from the Trolox curve y=0.0016x+0.0412 ($R^2$ =0.9916)




**a. MN b. INH c.LB**

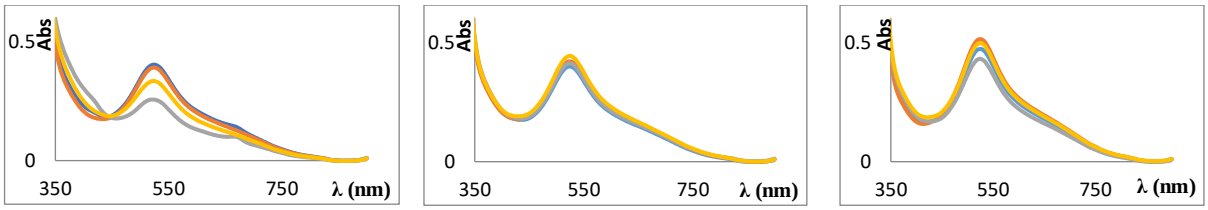
**Figure 2:** Spectrum of extracts prepared by FRAP

UMet UW MMet MW

**DPPH Radical Scavenging Activity**

To prepare 100 mL of 100 µM DPPH solution, 0.0039 g of DPPH is weighed and dissolved in methanol. Then, 1.5 mL of each extract was taken and 1.5 mL of 100 µM DPPH solution was added. After mixing the DPPH solution with the added extracts, it was incubated for 15 minutes at 30 $^0$C and 200 rpm in an orbital shaker. The absorbances of the samples were recorded at 515-528 nm in a spectrophotometer. Each experiment was repeated twice and absorbances were averaged.

y = -0.0129 x + 0.5498 ($R^2$: 0.9982)




a.MN b. INH c. LB

Figure 3: Spectrum of extracts prepared by DPPH method

UMet UW MMet MW

## RESULTS AND DISCUSSION

In this study, antioxidant activities of MN leaf, LB dried fruit and INH root were determined based on the extractions in heated magnetic stirrer and ultrasonic assisted bath. The antioxidant activities of the samples were analyzed by 3 different spectrophotometric methods and the calculated values were recorded.When the percentages of antioxidant activity obtained by different analysis methods are compared, It has been seen that the analysis methods are not 100% parallel with each other (Figure 4). Total phenolic content in different parts plants was determined with Folin-Ciocalteu method. The results were expressed as mg gallic acid equivalent per 1g sample. the experimental results revealed that aqueous solvent extracts of INH and MN prepared by both ultrasonic assisted extraction and magnetic stirrer techniques, exhibited better antioxidant activities and higher phenolic contents. However, when methanol was used, the antioxidant activity was low for INH and MN. Regarding the antioxidant activity of the plant extracts, there is not specific approach due to the structural diversity of compounds . It is thought that it would be a more accurate assay method to give the data according to each feature (Yilmaz et al 2019). On the other hand, it was concluded that the methanolic extract of the fruit LB showed more potent in vitro antioxidant activity, than the aqueous extract of the fruit (Table 1) .While methanol is a suitable solvent for extracting polar compounds, water is selectively preferred for extracting highly polar compounds. In this case, it was accepted that flavonoid structures were present for INH and MN, and anthocyanin groups were present for LB under the conditions in the study.As can be observed the antioxidant capacity results, both the type of solvent and concentration in the medium is important factor for extraction condition. Hence, it should be considered that antioxidant compounds may respond differently to different radical or oxidant compounds.

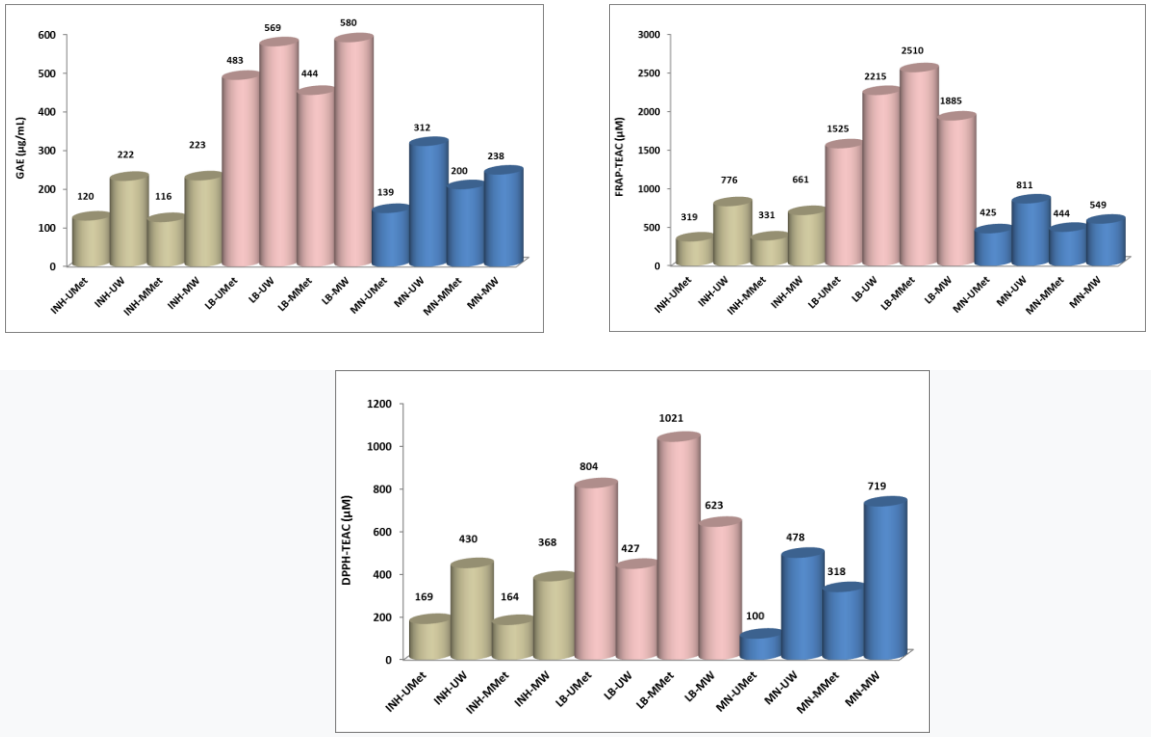



**Figure 4: a.** Total phenolic content in the extracts expressed in terms of GAE.
**b.** FRAP values for extracts
**c.** .DPPH scavenging activity of extracts.

Due to the differences in the extraction conditions of phenolic compounds in the Folin-Ciocalteu method, the total phenolic contents were calculated in µg gallic acid equivalent (GAE) units respectively (Okan 2013). Among plant part derived from different plants, the maximum reducing capacity was corresponded to LB samples obtained in methanolic fruit extracts with the highest ferricreducing potential. Lastly, the aqueous extracts had lower radical scavenging activities for INH and MN whereas methanolic extract had higher for LB.

## CONCLUSION

The plants is governed by a complex combination. Therefore, the abundance of phytochemicals in different plant parts are mportant in determining the plant species with maximum photochemical yield and greater antioxidant potential. Considering this aspect, different parts of plants extracts contain an effective antioxidant activity that has been supported by different in vitro assays including DPPH radical scavenging activity, reducing power assay and total antioxidant capacity. It is highlighted that ,in this study leaf and root had antioxidant activity as high as fruıt extracts and may be useful for their synergistic effect for treatment of disease. In fact, further studies are needed for understanding their synergistic effects in therapeutic applications. Thus, the synergistic antioxidative activity of the mixture can be optimized as an alternative therapeutic support for the treatment of various diseases.

## REFERENCES

Akmese O Acet T, Ozcan K, Gustıj G 2020. Determination of Antioxidant and Antimicrobial Activities and Synergistic effect with Antibiotics of Morus nigra L. Extracts Grown in Elazig Province 10 (4): 983-995

Amagase H & Nance DM 2008. A randomized, doubleblind, placebocontrolled, clinical study of the general effects of a standardized Lycium barbarum (goji) juice, GoChi. Journal of Alternative and Complementary Medicine, 14:403-412

Aybastıer O 2021. Efficacy of methanol-water extract of Inula helenium root against oxidative DNA damage J Tradit Chin Med41(2):293-300

Diab KA, Fahmy MA, Hassan EM, Hassan ZM, Omara EA ve Abdel-Samie NS. 2020. Inhibitory Activity of Black Mulberry (Morus Nigra) Extract Against Testicular, Liver and Kidney Toxicity Induced by Paracetamol in Mice. Molecular Biology Reports, 47:1733- 1749.

Engin MS, Kalkan S, Otag MR 2019. Goji berry (Lycium barbarum L.) Meyvesinin Farklı Çözgenlerden Elde Edilen Ekstratlarının Toplam Fenolik İçerikleri ile Antioksidan ve Antimikrobiyal Aktivitelerinin Karşılaştırılma Journal of Anatolian Environmental and Animal Sciences, 4( 3),:359-365

Hojjatpanah G, Fazaeli M ve Emam-Djomeh Z 2011. Effects of Heating Method and Conditions on the Quality Attributes of Black Mulberry (Morus nigra) Juice Concentrate. International Journal of Food Science and Technology, 46(5):956-962.

Iguță C, Cornea CP, Ionıță L, Brîndușe E, Farcaș N,Bobıt D, Matеı F 2014. Studies on antimicrobial activity of Inula helenium L Romanian cultivar Romanian Biotechnological Letters. 19: 5,…..

Jiang H, Xu L., Liu JC ve Huang XZ, 2011. Research Progress on Active Ingredients and Pharmacological Functions of Black Mulberry (Morus nigra L.). Science of Sericulture, 1: 20.

Kaur C and Kapoor HC 2001, Antioxidants in fruits andvegetables-themillennium'shealth. International Journal of Food Science and Technology, 36: 703-725.

Koc K, Ozdemir O, Ozdemir A,Dogru U, Turkez H 2018. Antioxidant and anticancer activities of extract of Inula helenium L. in human U-87 MG glioblastoma cell linear : Journal of Cancer Research and Therapeutics. 14( 3): 658-661

Konishi T, Shimada, Y, Nagao T., Okabe H and Konoshima T (2002). Antiproliferative Sesquiterpene Lactones from the Roots of Inula helenium. Biol. Pharm. Bull., 25 (10): 1370-1372.

Kostic DA, Dimitrijevic DS, Mitic SS, Mitic MN, Stojanovic GS ve Zivanovic AV 2013. A Survey on Macro- and Micro-Elements, Phenolic Compounds, Biological Activity and Use of Morus Spp. (Moraceae). Fruits, 68(4) :33-47

Kulczyński B & Gramza-Michałowska A 2016. Goji berry (Lycium barbarum): composition and health effects–a review. Polish Journal of Food and Nutrition Sciences, 66(2) :67-76.

Truong DH , Nguyen DH, Anh Ta NT, Vo Bui A, Tuong Ha Do, and Nguye HC. 2019. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. Journal of Food Quality 1-9.

Yılmaz DC , Ozdoğan O , Bulut G , Seyhan SA. 2019.Comparison uf Antioxidant Activities of Two Thyme Species (Thymbra Spicata Var. Spicata And Origanum Onites) ) Internatıonal Journal of Eastern Anatolia Science Engineering and Design 1(2):296-306.

## *ORAL PRESENTATION*

# Harşit Çayı (Gümüşhane, Türkiye) *Alburnus derjugini* populasyonuna ait boy-ağırlık ve boy-boy ilişkileri

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## Özet

Bu çalışmada, Harşit Çayı'nda Mayıs 2021 tarihinde örneklenen 91 adet (40 ♀, 51 ♂) *Alburnus derjugini* bireyinin boy-ağırlık ve boy-boy ilişkilerinin belirlenmesi amaçlanmıştır. *A. derjugini* örneklerinin total boyları 6,4 ile 19,2 cm, ağırlıkları 2,49 ile 47,14 g arasında değişmiştir. Boy-ağırlık ilişkilerinin b değerleri populasyon geneli, dişi ve erkekler bireyler için sırasıyla 2,993, 2,812, 3,193 şeklindedir. Analizler sonucunda elde edilen b değerlerinin 3'ten önemli derecede farklı olmadığı ($P>0,05$) ve türün izometrik büyüme gösterdiği belirlenmiştir. Harşit Çayı'nda yaşayan *A. derjugini* bireylerinin total boy-çatal boy, total boy-standart boy ve çatal boy-standart boy arasında kuvvetli ilişkiler olduğu tespit edilmiştir ($P<0,001$, $r^2>0,95$). Tüm hesaplamalar populasyon geneli, dişi ve erkek bireyler için ayrı ayrı gerçekleştirilmiştir. Bu araştırmada *A. derjugini* türünün bu habitattaki boy-ağırlık ve boy-boy ilişkileri ilk defa rapor edilmiştir.

**Anahtar Kelimeler:** Boy-ağırlık ilişkisi, boy-boy ilişkisi, *Alburnus derjugini*, Harşit Çayı, Gümüşhane

# Length-weight and length-length relationships of the *Alburnus derjugini* population of Harşit Stream (Gümüşhane, Turkey)

## Abstract

In this study, it was aimed to determine the length-weight and length-length relationships of 91 (40 ♀, 51 ♂) *Alburnus derjugini* individuals sampled in Harşit Stream in May 2021. The total lengths of *A. derjugini* samples ranged from 6.4 to 19.2 cm, and their weights ranged between 2.49 and 47.14 g. The b values of the length-weight relationships are 2.993, 2.812, and 3.193 for the general population, female and male individuals, respectively. It was determined that the b values obtained as a result of the analyses were not significantly different from 3 ($P>0.05$) and the species showed isometric growth. It has been determined that there are strong relationships between total length-fork length, total length-standard length, and fork length-standard length of *A. derjugini* individuals living in Harşit Stream ($P<0.001$, $r^2>0.95$). All calculations were carried out separately for the general population, female and male individuals. In this study, the length-weight and length-length relationships of *A. derjugini* in this habitat were reported for the first time.

**Keywords:** Length-weight relationship, length-length relationship, *Alburnus derjugini*, Harşit Stream, Gümüşhane

## GİRİŞ

Sucul ekosistemin sürdürülebilirliğinin en iyi şekilde sağlanması ve maksimum verimin elde edilebilmesi için bu habitatlarda yayılış gösteren balık stoklarının düzenli olarak incelenmesi ve takibinin yapılması hem balıkçılık yönetimi ve hem de balıkçılık biyolojisi çalışmalarında önemli bir yer tutmaktadır (Avşar, 1996). Bu sebeple son yıllarda gerçekleştirilen birçok çalışmada balıklarda boy-ağırlık ilişkileri ve boy-boy ilişkileri oldukça büyük bir önem arz etmektedir (Tran ve ark., 2021; Apaydın Yağcı ve ark., 2022; Pessali ve ark., 2022). Boy-ağırlık ilişkisi verileri, farklı habitat ve/veya bölgelerde yaşayan balık populasyonları arasındaki türler arası karşılaştırmaların gerçekleştirilmesi ve yaşam döngüsü çalışmalarının ortaya çıkarılmasına da katkı sağlar (Petrakis ve Stergiou, 1995; Gonçalves ve ark., 1997). Diğer morfometrik ölçümler gibi, boy-ağırlık

ilişkileri de balıkların metamorfoz, büyüme ve eşeysel olgunluk başlangıcı gibi biyolojik özellikleri sırasında değişim gösterebilmektedir (Le Cren, 1951). Bununla birlikte boy-ağırlık ilişki parametreleri olan a ve b değerleri farklı habitatlarda yayılış gösteren popülasyonların morfolojileri ve balık türlerinin yaşam süreçlerinin karşılaştırılmasına olanak sağlar (Yılmaz ve ark., 2010; Yedier ve ark., 2019).Ayrıca balıkçılık biyolojisi çalışmalarında kullanılan önemli bir parametre olarak değerlendirilen boy-boy ilişkileri, aynı türün farklı habitatlardaki stoklarının karşılaştırılmasında yararlı olmanın yanı sıra ya da total boyu zarar görmüş bir balığın standart boyundan total boyunun tahmin edilmesi gibi farklı amaçlar için de sıklıkla kullanılmaktadır (Moutopoulos ve Stregiou, 2002).

En son gerçekleştirilen taksonomik çalışmalar ile Leuciscidae familyasına dahil edilen *Alburnus* cinsine ait 18'i endemik, 27 tür Türkiye'de saptanmıştır (Mangıt ve Yerli 2018; Çiçek vd. 2018; Bayçelebi, 2020). Bu türlerden *Alburnus derjugini* Berg, 1923 ülkemiz sularında özellikle Çoruh Havzası'ndan bildirilen ve bununla birlikte Harşit Çayı üzerinde de kayıtlara geçen bir balık türüdür (Bayçelebi ve ark., 2015).

Bu çalışma ile Harşit Çayı'nda dağılım gösteren *A. derjugini* türünün (i) boy-ağırlık ve boy-boy ilişkilerinin ilk kez belirlenmesi, (ii) boy dönüşümlerinin gerçekleştirilmesi ve böylece türün faklı populasyonları ile yapılacak diğer araştırmalarla karşılaştırma olanağı sağlayabilecek verilerin elde edilmesi amaçlanmıştır.

## MATERYAL VE METOT

Araştırma materyali olan *Alburnus derjugini* örnekleri Mayıs 2021 tarihinde Gümüşhane İli, Torul İlçesi'nde bulunan Harşit Çayı'ndan elektroşoker yardımıyla örneklenmiştir. Gümüşhane'nin akarsu ağını Harşit Çayı (Doğankent) ve Kelkit Çayı ile bunların kolları oluşturmaktadır. Harşit Çayı dar ve derin vadilerden geçerek, Torul ve Kürtün ilçelerini kat ettikten sonra Tirebolu'dan denize dökülmektedir (Doğru ve ark., 1999). Torul ilçesindeki kolundan örneklenen balıklar Ondokuz Mayıs Üniversitesi Fen Fakültesi İhtiyoloji Araştırma Laboratuvarı'na getirilmiş tür tayinleri gerçekleştirilmiştir (Çiçek ve ark., 2020; Fricke ve ark., 2022). Balıkların total boy, çatal boy ve standart boyları ölçülmüş (±0,1 cm), ağırlıkları tartılmıştır (±0,01 g). Balıkların eşeyleri gonadların makroskobik incelenmesi ile tayin edilmiştir.

Boy-ağırlık ilişkisinin hesaplanmasında aşağıdaki formül kullanılmıştır (Bagenal ve Tesch, 1978).

$$W=aL^b \quad (1)$$

Eşitlikteki W, gram cinsinden balık ağırlığını, a ve b ilişki sabitlerini, L, total boyu ifade etmektedir. a ve b ilişkisi parametreleri Log W= Log a + b Log TL doğrusal regresyon eşitliği ile tespit edilerek, b değerinin 3'ten farklı olup olmadığı t-testi ile test edilmiş türün büyüme şekli ortaya çıkarılmıştır (Zar, 1999). Ayrıca b değerinin güven aralığı (%95CI) belirlenmiştir. Boy-boy ilişkileri doğrusal regresyon analizi kullanılarak hesaplanmıştır. Regresyon analizi denklemi şu şekildedir;

$$y=ax\pm b \quad (2)$$

Boy-ağırlık ve boy-boy ilişkileri dişi, erkek ve tüm bireyler için ayrı ayrı hesaplanmıştır. Dişi ve erkek bireylerin total boy ve ağırlık bakımından aralarında fark olup olmadığı independent-t testi ile test edilmiştir. İstatistiksel analizlerde, SPSS (IBM 21), Minitab paket programları kullanılmıştır.

## BULGULAR ve TARTIŞMA

Örnekleme sonucunda 51 erkek (%56,1), 40 dişi (%43,9), olmak üzere toplam 91 *Alburnus derjugini* bireyi elde edilmiştir. Populasyon genelinde total boylar 6,4-19,2 cm, standart boylar 6,0-17,5 cm, çatal boylar 4,9-15,4 cm ağırlıklar ise 2,490-47,140 g arasında değişmiştir. Harşit Çayı *A.derjugini* türünün populasyon geneli, dişi ve erkek bireyler için balık boyu ve ağırlık verilerinin tanımlayıcı istatistikleri Tablo 1'de sunulmuştur.

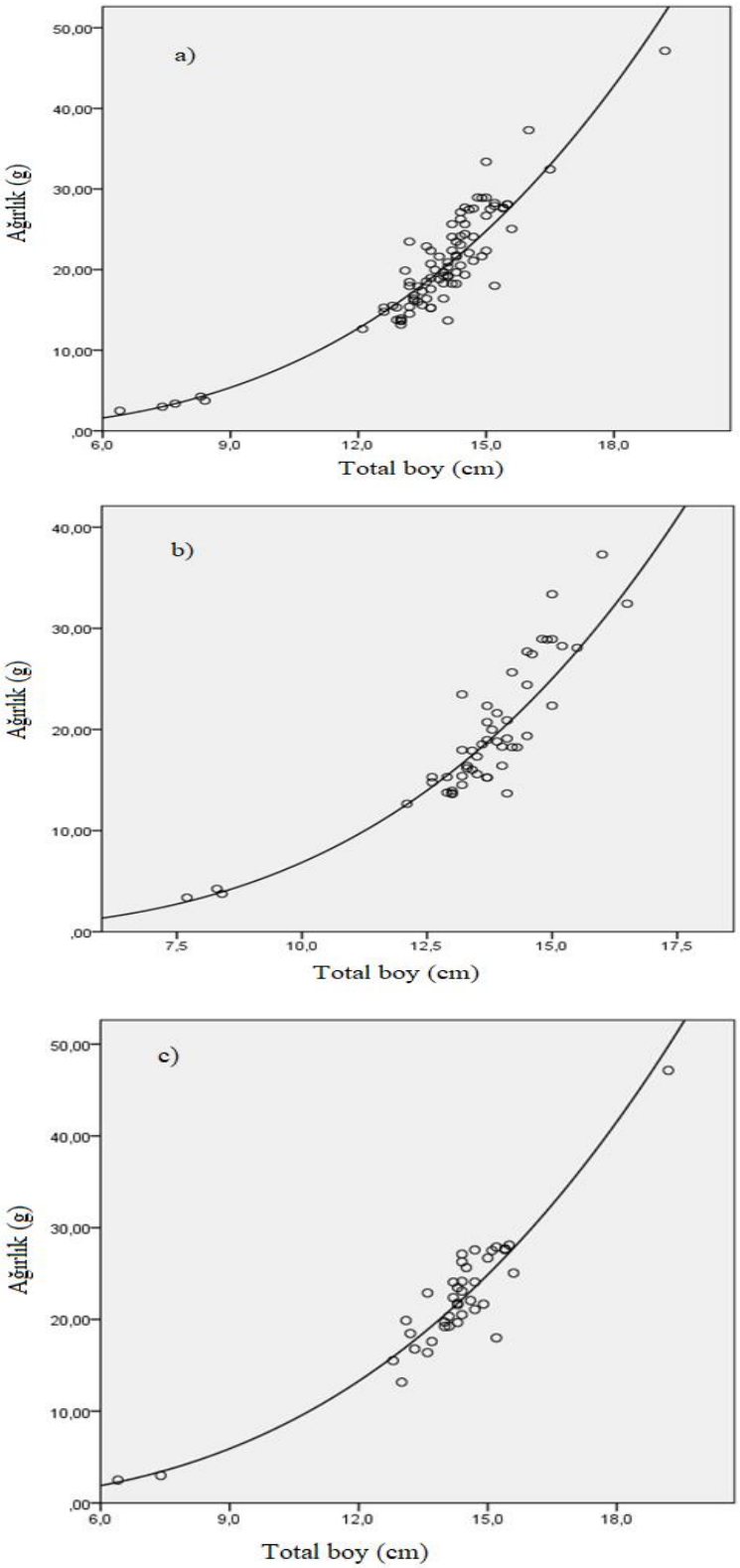
**Tablo 1.** Harşit Çayı *Alburnus derjugini* populasyonuna ait boy ve ağırlık verilerinin tanımlayıcı istatistik verileri

| | Değişken | Minimum | Maksimum | Ortalama | Standart hata | Standart sapma |
|---|---|---|---|---|---|---|
| Tüm populasyon | Total boy | 6,4 | 19,2 | 13,802 | 0,188 | 1,793 |
| | Çatal boy | 6,0 | 17,5 | 12,718 | 0,173 | 1,652 |
| | Standart boy | 4,9 | 15,4 | 11,075 | 0,158 | 1,503 |
| | Ağırlık | 2,49 | 47,14 | 20,451 | 0,748 | 7,135 |
| Erkek | Total boy | 7,7 | 16,5 | 13,576 | 0,228 | 1,628 |
| | Çatal boy | 7,1 | 15,5 | 12,573 | 0,217 | 1,547 |
| | Standart boy | 6,0 | 14,2 | 10,947 | 0,197 | 1,410 |
| | Ağırlık | 3,37 | 37,31 | 19,305 | 0,988 | 7,054 |
| Dişi | Total boy | 6,4 | 19,2 | 14,090 | 0,311 | 1,966 |
| | Çatal boy | 6,0 | 17,5 | 12,903 | 0,281 | 1,780 |
| | Standart boy | 4,9 | 15,4 | 11,238 | 0,256 | 1,618 |
| | Ağırlık | 2,49 | 47,14 | 21,91 | 1,12 | 7,05 |

Dişi ve erkek bireyler total boy (t testi; P=0,187) ve ağırlık (t testi; P=0,084) bakımından karşılaştırıldığında istatistiki açıdan aralarında önemli derecede fark olmadığı belirlenmiştir. Harşit Çayı'nda yayılış gösteren *A.derjugini* bireylerinin boy ve ağırlık değerleri arasında kuvvetli ilişkiler mevcuttur (P<0,001, $r^2$>0,90, Tablo 2). Türün boy-ağırlık ilişki denkleminden elde edilen b değeri 2,812 ile 3,193 arasında değişmiştir. Dişi, erkek ve populasyon geneli için hesaplanan b değerinin 3'ten önemli derecede farklı olmadığı tespit edilmiştir (t test, P>0,05). Analiz sonuçlarına göre Harşit Çayı'ndaki *A.derjugini* türünün dişi, erkek ve tüm bireylerinin izometrik büyüme gösterdiği belirlenmiştir. Boy-ağırlık ilişkisi grafikleri Şekil 1'de sunulmuştur. b değeri balıklarda yaş, büyüme, üreme, eşey, beslenmeye göre değişiklik göstermektedir (Bagenal ve Tesch, 1978). Bu değer 2,5 ile 3,5 arasında değişiklik göstermektedir (Froese, 2006). Harşit Çayı *A.derjugini* örneklerinden elde edilen b değeri bu aralıktadır.

**Tablo 2**. Harşit Çayı *A.derjugini* bireylerinin boy-ağırlık ilişkisi parametreleri ve büyüme şekli

| | Boy-Ağırlık İlişkisi Parametreleri | | | $r^2$ | P değeri |
|---|---|---|---|---|---|
| | a | b | % 95 güven aralığı | | |
| Tüm populasyon | 0,007 | 2,993 | 2,810-3,178 | 0,922 | <0,001 |
| Erkek | 0,004 | 3,193 | 2,898-3,488 | 0,906 | <0,001 |
| Dişi | 0,012 | 2,812 | 2,594-3,030 | 0,947 | <0,001 |




**Şekil 1:** Harşit Çayı *A.derjugini* populasyonu için boy-ağırlık ilişkisi grafikleri a) Populasyon geneli b) erkek bireyler c) dişi bireyler

Harşit Çayı *A.derjugini* bireyleri için boy-boy dönüşümleri arasında kuvvetli ilişkiler tespit edilmiştir ($P<0.001$, $r^2>0.95$). Total boy-çatal boy, total boy-standart boy, çatal boy-standart boy ilişki denklem parametreleri Tablo 3'te sunulmuştur. Regresyon ilişki katsayısının yüksek olması balık boylarının birbirlerine dönüşümünde güvenilir bir şekilde kullanılabileceğini göstermektedir.

**Tablo 3.** Harşit Çayı *A.derjugini* populasyonu bireylerinin boy-boy ilişkisi denklem ve parametreleri

| | Denklem | a | b | $r^2$ | P |
|---|---|---|---|---|---|
| Tüm populasyon | TB = a + b ÇB | 0,147 | 1,073 | 97,9 | <0,001 |
| | TB = a + b SB | 0,797 | 1,174 | 97,0 | <0,001 |
| | ÇB = a + b SB | 0,701 | 1,085 | 97,4 | <0,001 |
| Erkek | TB = a + b ÇB | -0,003 | 1,092 | 97,8 | <0,001 |
| | TB = a + b SB | 0,506 | 1,208 | 98,9 | <0,001 |
| | ÇB = a + b SB | 0,664 | 1,089 | 97,9 | <0,001 |
| Dişi | TB = a + b ÇB | 0,441 | 1,044 | 98,6 | <0,001 |
| | TB = a + b SB | 1,232 | 1,127 | 95,2 | <0,001 |
| | ÇB = a + b SB | 0,749 | 1,079 | 96,8 | <0,001 |

## SONUÇ

Harşit Çayı'nda yaşayan *Alburnus derjugini* populasyonunun boy-ağırlık ve boy-boy ilişkilerinin ortaya çıkarıldığı herhangi bir araştırma bulunmamaktadır. Bu çalışma ile ilk kez Harşit Çayı'ndaki *A.derjugini* türünün boy-ağırlık ilişki denklemleri çıkarılmış, büyüme şekli belirlenmiş ve boy-boy dönüşümleri sunulmuştur. Elde edilen verilerin tür ile ilgili gerçekleştirilecek yaş, büyüme, üreme ve biyolojik özellerini kapsayan çalışmalara kaynak oluşturacağı düşünülmektedir.

## KAYNAKLAR

Apaydın Yağcı M, Alp A, Yağcı A, Uysal R, Bilgin F, Cesur M, Yeğen V 2022. Length-weight relationships of ten fish species from Lake Eğirdir, Turkey. Journal of Anatolian Environmental and Animal Sciences, 7(2): 105-109.

Avşar D 1996. Balıkçılık biyolojisi ve populasyon dinamiği. Çukurova Üniversitesi, Su Ürünleri Fakültesi No:5, Baki Kitap ve Yayınevi, 303s.

Bagenal TB, Tesch FW 1978. Age and growth. In: Methods for assessment of fish production in freshwaters, (Bagenal, T.B., Ed), Oxford, UK, Blackwell Science Publication, pp. 101-136.

Bayçelebi E, Turan D, Japoshvili B 2015. Fish Fauna of Çoruh River and Two First Record for Turkey. Turkish Journal of Fisheries and Aquatic Sciences, 15(4): 777-788.

Bayçelebi E. 2020. Distribution and diversity of fish from Seyhan, Ceyhan and Orontes River Systems. Zoosystematics and Evolution. 96(2): 747-767.

Çiçek E, Fricke R, Sungur S, Eagderi S 2018. Endemic freshwater fishes of Turkey. FishTaxa. 3(4);1-39.

Çiçek E, Sungur S, Fricke R 2020. Freshwater lampreys and fishes of Turkey; a revised and updated annotated checklist 2020. Zootaxa, 4809: 241-270.

Doğru E, Kural A, Pir H, Aydın CA, Bulgur İ 1999. Cumhuriyet'in 75. yılında Gümüşhane, Ekspres Ofset, İstanbul.

Fricke R, Eschmeyer WN, van der Laan R 2022. Eschmeyer's catalog of fishes: genera, species, references. Available at: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmail.asp [25.10.2022]

Froese R 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology, 22: 241-253.

Gonçalves JMS, Bentes L, Lino PG, Ribeiro J, Canário AVM, Erzini K 1997. Weight-length relationships for selected fish species of the small-scale demersal fisheries of the south and south-west coast of Portugal. Fisheries Research, 30: 253-256.

Le Cren ED 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). The Journal of Animal Ecology, 201-219.

Mangıt F, Yerli SV 2018. Systematic evaluation of the genus *Alburnus* (Cyprinidae) with description of a new species. Hydrobiologia. 807: 297–312.

Moutopoulos DK, Stregiou KI 2002. Length-weight and length-length relationships of fish species from the Aegean Sea (Greece). Journal of Applied Ichthyology, 18(3): 200-203.

Pessali TC, Salvador GN, Alves CBM, Drager DHC, Zawadzki CH, Prata EG, ... & Freitas TMDS 2022. Length–weight relationships of 78 fish species from São Francisco River basin, Brazil. Journal of Applied Ichthyology, 38: 344-351.

Petrakis G, Stergiou KI 1995. Weight-length relationships for 33 fish species in Greek waters. Fisheries Research, 21(3-4): 465-469.

Tran HD, Nguyen HH, Ha LM 2021. Length–weight relationship and condition factor of the mudskipper (*Periophthalmus modestus*) in the Red River Delta. Regional Studies in Marine Science, 46, 101903.

Yedier S, Kontaş S, Bostancı D 2019. Marmara Denizi'nde Yaşayan *Pagellus acarne* (Risso, 1827)'nin kondisyon faktörü, boy-boy ve boy-ağırlık ilişkileri. Journal of Anatolian Environmental and Animal Sciences, 4(2): 82-88.

Yılmaz S, Polat N, Yazıcıoğlu O 2010. Samsun ili iç sularında yaşayan sazan (*Cyprinus carpio* L.,1758)'ın boy-ağırlık ve boy-boy ilişkileri. The Black Sea Journal of Sciences, 1: 39-47.

Zar, JH 1999. Biostatistical analysis. 4th ed. Prentice-Hall, New Jersey. 663 pp.

## *ORAL PRESENTATION*

# A rare case of pneumonia due to *Saprochaete capitata* developed as a result of coronary artery by-pass operation

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**Abstract**

*Saprochaete capitata* is isolated from environmental sources, and also it can be found in the skin, respiratory and gastrointestinal tract microbiota in humans and can cause infection in immunocompromised individuals. We report a rare case of pneumonia due to *Saprochaete capitata*, which developed as a result of coronary artery by-pass operation. A 57-year-old male patient was admitted to our emergengy department with the complaint of exertional chest pain that was relieved by rest for the past week. The patient with known hypertension and peptic ulcer disease has a family history of cardiac disease. Physical examination of the patient is unremarkable, Electrocardiography (ECG): SR V3-6 ST depressed, Heart Rate (HR): 75/min, Blood Pressure (BP): 170/80 mmHg. Echocardiography (ECHO) in the emergency revealed Ejection Fraction (EF): 40%, lateral midbasal, septum midbasal, inferior midbasal hypokinetic. The patient was taken to cardiac angiography with the diagnosis Non-ST-Segment Elevation Myocardial Infarction (NSTEMI). Coronary Artery Bypass Grafting (CABG) decision was taken in the council with the preliminary diagnosis of three vessel disease according to the angiography results. The patient underwent coronary artery bypass surgery. From the patient was intubated in the intensive care unit (ICU), on the second postoperative day, blood, urine and endotracheal aspirate cultures (ETA) were taken because of fever and respiratory distress. In ETA cultures of the patients *S. capitata* grown and number>100.000. Pleural effusion was observed in the direct chest X-ray of the patient who was followed up in the ward on the postoperative 13th day. Pleuroken was inserted in the patient under ICU conditions, and 1200 cc of fluid was drained and the patient's breathing was relieved. Since the patient's condition was good and he did not have any complaints, he was discharged after medical treatment was arranged.

**Keywords:** Pneumonia, Saprochaete capitata, coronary artery bypass operation

## INTRODUCTION

*Saprochaete capitata* is found in enviroonmental sources such as air, water, soil, plants and dairy products. It is also part of the skin, respiratory and gastrointestinal tract microbiota in humans. These yeasts, which are opportunistic pathogens, have been increasing recently and can cause infection in immunocompromised individuals (Parkan et al., 2017; Pamidimukkala et al., 2017). It has been reported in a wide range of clinical conditions such as fungemia, pneumonia, urinary tract infection, onychomycosis, endocarditis, osteomyelitis, brain abscess, spondylodiscitis and hepatosplenic infection caused by these yeasts (Parkan et al., 2017; Pamidimukkala et al., 2017). We report a rare case of pneumonia due to *Saprochaete capitata*, which developed as a result of coronary artery bypass operation.

## CASE REPORT

A 57-year-old male patient, who was diagnosed with inferior and posteroinferior myocardial infarction (MI), and stenting placed in the CXpl and DX arteries six years ago, and was under full medical follow-up due to occlusion in the distal LAD, was admitted to our emergency department with the complaint of exertional chest pain that was relieved by rest for the past week. He has a known history of hypertension and peptic ulser disease and cardiac family history. The drugs he uses are beloc 25 mg 2x1, ator 40mg 1x1, brilinta 90 mg 1x1, coraspin 100 mg 1x1 and delix 10 mg 1x1. Physical examination is unremarkable, Electrocardiography (ECG): SR V3-6 ST depressed, Heart Rate (HR): 75/min, Blood Pressure (BP):170/80 mmHg. In the emergency Echocardiography (ECHO), Ejection Fraction (EF) was 40%, lateral midbasal, inferior midbasal hypokinetik were detected. The patient was taken to cardiac angiography with the diagnosis of Non-ST-Segment Elevation Myocardial Infarction (NSTEMI), and LAD PROXIMAL subtotal, RCA PROXIMAL CTO, CX-PL subtotal stenosis were detected. With the preliminary diagnosis of three vessel disease, Coronary Artery Bypass Grafting (CABG) decision was taken in the council. After the procedure, the patient was taken to the coronary intensive care unit (ICU), monitored, nitrate treatment was started, and ECG follw-ups were performed. Bedside ECHO revealed lateral midbasal hypokinetic and mild inferior basal hypokinetic MI. The patient was operated on the next day with the final diagnoses of atherosclerotic heart disease, chronic obstructive pulmonary disease (COPD), hyperlipidemia and hypertension. He underwent coronary artery by-pass surgery. The patient, who was followed intubated on the post-operative 1st day after the operation, was receiving dopamine (6.6 mcg) perfusion in the ICU follow-ups, while the BP: 110/70 mmHg, the rhythm was sinus. On the second post-op day, the patient's urine was stopped and dialysis was started, and in the following hours, the patient became hypothermic and in blood gases, the glucose decreased to 76 mg/dL, and the lactate level increased to 5.9 mmol/L. Blood, urine and Endotracheal Aspirate (ETA) cultures were taken due to the patient's fever and respiratory distress. Meropenem 500 mg 2x1 and teicoplanin 800 mg 1x1 IV as the first loading dose were started, then teicoplanin 400 mg 1x1 was continued in the following days. No tamponade was detected in bedside ECHO, and EF and valve functions were evaluated as normal. The patient was reintubated under elective conditions. He did not have any cardiac problems, but fever and respiratory distress continued for 10 days, while White Blood Cell (WBC) and C Reactive Protein (CRP) remained high. The renal dose of dopamine (3.3 mcg) and antibiotic therapy were continued. There was no growth in the blood and urine cultures of the patient, but in ETA cultures, cream-colored yeast colonies that could grown at $45^0$C were detected on blood agar and Sabouraud Dextrose Agar, urease activity was negative, and the number was >100.000. Identification was done with API 20C AUX (bioMerieux, France) and determined as *Saprochaete capitata*. Antifungal was not given to the patient. On the 6th post-op day, the patient receives renal dose dopamine, hemodynamics is stable, BP:115/70 mmHg, HR:80 min, total urine in 24 hours: 6750 cc, balance:2448, blood gases are good, respiration is relieved. First, the patient, who was taken to the continuous positive airway pressure (CPAP) mode, was extubated. There was no deterioration in oxygenation in the control blood gas. The patient was transferred to the service on the 8th post-op day. The patient's service follow-ups were routine, the patient was mobilized. There was no deterioration in oxygenation in the control blood gas. Pleural effusion was observed in the direct chest X-ray of the patient, who was followed up in the service conditions on the 13th post-op day, and pleuroken was inserted in the intensive care conditions. 1200 cc of fluid was drained. The patient's breathing was relieved and since no complications developed, it was decided to continue his follow-up in the service conditions. Afterwards, these values started to decrease gradually and the patient started to recover. After the patient's general condition was good and he did not have any complaints, his medical treatment was lasix 2x1, crestor 10 mg 1x1, augmentin BID 1000 mg 2x1, diltizem-SR 120 mg 1x1, coraspin 100 mg 1x1, panto 40 mg 1x1, plavix 75 mg 1x1. He was discharged with salah and was referred to the chest disease polyclinic.

## DISCUSSION

In recent years, infection caused by rare opportunistic yeasts such as *S. capitata* occur in immunocompromised patients (Parkan et al., 2017; Pamidimukkala et al., 2017). The clinical specturum of *S. capitata* infections is often confused with infections caused by Candida species. The biggest difference is that lung involvement is very common in *S. capitata* infections, unlike disseminated candidiasis where lung involvement has been reported (Garcia-Ruiz et al., 2013). The first systemic infection with *S. capitata* was reported in 1964 (Chang and Buerger, 1964). Opportunistic infections caused by *Saprochaete* are increasing worldwide. Most cases were initially reported from the Mediterranean Region and Cenrtal Europe (Garcia-Ruiz et al., 2013; Birrenbach et al., 2012; Duran Graeff et al., 2017). *S. capitata* is considered intrinsically resistant to echinocandins, with variable susceptibility to fluconazole and itraconazole (Schuermans et al., 2011; Chittick et al., 2009). Monotherapy or combined regimens are usually effective in treatment. Studies have reported that it has good activity against liposomal amphotericin B, flucytosine and voriconazole (Schuermans et al., 2011; Chittick et al., 2009; Ulu-Kilic et al., 2015 ).

In our patient with COPD, who was followed up in the ICU in the post-operative period, whose three vessels were changed with coronary bypass surgery, blood, urine and ETA cultures were taken 48 hours later due to fever and respiratory distress, and *S. capitata* was grown in ETA cultures. However, 13 days after the operation pleural effusion was observed in the chest X-ray of the patient, he was relieved after 1200 cc of fluid was drained by inserting pleuroken under intensive care conditions.

Based on a limited number of in vitro and clinical studies, many authors recommend high-dose fluconazole or voriconazole with amphotericin B or flucytosine in combination, or amphotericin B (Garcia-Ruiz et al., 2013; Arrendup et al., 2014; Mazzocato et al., 2014).

Studies on infections caused by *S. capitata* are reported sporadically in our country (Parkan et al., 2017; Ulu-Kilic et al., 2015; Koç et al., 2016; Celik et al., 2009; Aydın Kaynar et al., 2016).

In conclusion, inability to distinguish *Candida* species and *S. capitata* from more common infections may lead to inappropriate treatments with echinocandins to which *S. capitata* is naturally resistant. Early diagnosis and treatment of *S. capitata* infections is very important by carefully examining the epidemological, clinical and microbiological aspects.

## REFERENCES

Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. Clin Microbiol Infect, 20(s3): 76-98.

Aydın Kaynar L, Özkurt ZN, Ülküden B, Güzel Tunçcan Ö, Aygencel G, Akyürek N, Kalkancı A 2016. A rare and mortal infection agent in patients with hematological malignancies: *Saprochaete capitata*. Mikrobiyol Bul, 50(4): 613-620.

Birrenbach TN, Bertschy S, Aebersold F, Mueller NJ, Achermann Y, Muehlethehaler K, Zimmerli S 2012. Emergence *Blastoschizomyces capitatus* yeast infections, Central Europe. Emerg Infect Dis, 18(1): 98-101.

Celik AD, Ozaras R, Kantarcıoglu S, Mert A, Tabak F, Ozturk R 2009. Spondylodiscitis due to an emergent fungal pathogen: *Blastoschizomyces capitatus*, a case report and review of the literature. Rheumatol Int, 29(10): 1237-1241.

Chang WW, Buerger L 1964. Disseminated geotrichosis: case report. Arch Intern Med, 113(3): 356-360.

Chittick P, Palavecino EL, Delashmitt B, Evans J, Peacock JE Jr 2009. Case of total *Blastoschizomyces capitatus* infection occuring in a patient receiving empiric micafungin therapy. Antimicrob Agents Chemother, 53(12): 5306-5307.

Duran Graeff L, Seidel D, Vehreschild MJ, Hamprecht A, Kindo A, Racil Z, Demeter J, De Hoog S, Aurbach U, Ziegler M, Wisplinghoff H, Cornely OA, FungiScope Group 2017. Invasive infections due to

*Saprochaete* and *Geotrichum* species: report of 23 cases from the FungiScope Registry. Mycoses, 60(4): 273-279.

Garcia-Ruiz JC, Lopez-Soria L, Olazabal I, Amutio E, Arrieta-Aguirre I, Velasco-Benito V, Ponton J, Moragues MD 2012. Invasive infections caused by *Saprochaete capitata* in patients with haematological malignancies: report of five cases and review of the antifungal therapy. Rev Iberoam Micol, 30(4): 248-255.

Koç AN, Atalay MA, Timur D, Demir G, Kaynar L 2016. Molecular epidemiology and antifungal susceptibility of *Saprochaete capitata* (*Blastoschizomyces capitatus*) isolates causing nosocomial infection in Kayseri/Turkey. Infect Dis (Lond), 48(8): 596-603.

Mazzocato S, Marchionni E, Fothergill AW, Sutton DA, Staffolani S, Gesuita R, Skrami E, Fiorentini A, Manso E, Barchiesi F 2014. Epidemiology and outcome of systemic infections due to *Saprochaete capitata*: case report and review of the literature. Infection, 43(2): 211-215.

Pamidimukkala U, Kancharla A, Sudhaharan S, Gundeti S, Mandarapu S, Nagalla VK, Raju SB, Karanam SD 2017. Isolation of the rare opportunistic yeast *Saprochaete capitata* from clinical samples-experience from a tertiary care hospital in Southern India and a brief review of the literature. J Clin Diagn Res, 11(9): 36-42.

Parkan ÖM, Atalay MA, Koç AN, Pala Ç, Aydemir G, Kaynar L 2017. Fungemia and septic arthritis caused by *Saprochaete capitata* in a patient with Fanconi aplastic anemia: a case report. Mikrobiyol Bul, 51(1): 87-93.

Schuermans C, van Bergen M, Coorevits L, Verhaegen J, Lagrou K, Surmont I, Jeurissen A 2011. Breakthrough *Saprochaete capitata* infection in patients receiving echinocandins: case report and review of the literature. Med Mycol, 49(4): 414-418.

Ulu-Kilic A, Atalay MA, Metan G, Cevahir F, Koç N, Eser B, Çetin M, Kaynar L, Alp E 2015. *Saprochaete capitata* as an emerging fungus among patients with haematological malignencies. Mycoses, 58: 491-497.

## ORAL PRESENTATION

# Hidroksitirosol ve Tirosol'ün tek başına ve kemoterapi ilacı olan Temozolomide ile kombinasyon halinde Glioblastoma hücrelerinin biyolojik davranışlarına olan etkilerinin araştırılması

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## Özet

Glioblastoma (GB), yüksek proliferasyon ile karakterize merkezi sinir sisteminin en sık görülen malign tümörüdür ve tedaviye rağmen GB hastalarının ortalama sağkalımı 14-15 aydır. Bu nedenle GB tedavisinde kemoterapi ilacı olan Temozolomide (TMZ)'nin etkinliğini artırabilecek ve neden olduğu hücresel toksisiteyi azaltabilecek yeni tedavi süreçlerine ihtiyaç vardır. Mevcut çalışmada, etkili flavonoidler olan Hidroksitirosol (HT) ve Tirosol (TYR)'ün tek başına ve TMZ ile kombinasyonlarının T98G hücrelerindeki in-vitro biyolojik davranış ve agresiflik üzerine etkilerinin incelenmesi amaçlanmıştır. Çalışmada HT ve TYR'nin T98G (insan GB hücresi) hücrelerindeki hücre canlılığı WST-1 (Roche, Germany) kiti ile, hücre ölümü ve hücre döngüsü analizleri sırasıyla Muse®Annexin V&Dead Cell Assay Kit ve Muse™ Cell Cycle Kit (MilliporeSigma, ABD) ile Muse®Cell Analyzer (Millipore, ABD) cihazında, gerçekleştirildi. Hücre ölümünün morfolojik analizi ise Acridin Orange/Propidium Iodide boyaması ile floresan mikroskobunda (EVOS M5000, Thermo Fisher Scientific, ABD) gerçekleştirdi. Flavonidlerin T98G hücrelerindeki invazyon-migrasyona olan etkisi yara iyileştirme tahlili ile yapıldı (Proje no: BUÜ, BAP: THIZ-2021-550). Bulgular doğrultusunda, HT ve TYR'nin IC50 değerleri sırasıyla; 91.4 µM ve 350 µM olarak belirlendi. HT-tedavisi, T98G hücrelerinde TMZ'den daha yüksek bir apoptoz oranıyla sonuçlanırken ($p<0.0001$), TYR-tedavisi, muamelesiz hücrelere kıyasla apoptozu arttırdı ($p<0.0001$), ancak TYR'nin bu etkisinin TMZ'nin apoptotik etkisinden daha az olduğu gözlendi. TYR ve HT'lerin TMZ ile kombinasyonunda TMZ'ye göre daha yüksek oranda apoptoza yol açtıkları belirlendi ($p<0.0001$). Hücre ölümünün morfolojik analizi Annexin analizi sonuçlarını destekler nitelikte olduğu görüldü. HT'nin G0/G1 fazlarındaki hücre sayısını azalttığı ($p<0.0001$), ancak TYR'nin hücre döngüsü dağılımını etkilemediği görüldü. T98G hücrelerinin invazyon yeteneğini ortaya koyan yara iyileşme analizinde, muamelesiz hücreler, 24 saat içinde yara alanını %75.84' ünü kapatırken, HT ve TRY yara iyileşmesini %21.4 ve %22.66'e gerileterek hücrelerin invazyon yeteneğini azalttı ($p<0.0001$). HT ve TYR'nin TMZ ile kombinasyonunda tek başına TMZ'ye göre yara alanını açtığı gözlendi ($p<0.0001$). Bulgularımız, GB'ye karşı daha ileri ilaç geliştirme çalışmalarına dahil etmek için HT'nin uygun bir aday flavonoid olabileceğini vurgulamaktadır.

**Anahtar Kelimeler:** Glioblastoma, Tedavi, Flavonoidler

# Investigation of the effects of Hydroxytyrosol-only and Tyrosol-only or in combination with the chemotherapy drug Temozolomide on the biological behavior of Glioblastoma cells.

## Abstract

Glioblastoma (GB) is the most common malignant tumor of the central nervous system characterized by high proliferation, and the mean survival of GB patients is 14-15 months despite treatment. Therefore, there is a need for new treatment processes that can increase the efficacy of the chemotherapy drug Temozolomide (TMZ) and reduce the cellular toxicity it causes in the treatment of GB. In the current study, it was aimed to examine the effects of the effective flavonoids Hydroxytyrosol (HT)-only and Tyrosol (TYR)-only or in combination with TMZ on in-vitro biological behavior and aggressiveness in T98G cells. In the study, cell viability of HT and TYR in T98G (human GB cell) cells were analyzed with the WST-1 (Roche, Germany) kit, cell death and cell cycle assays, respectively, with the Muse®Annexin V&Dead Cell Assay Kit and Muse™ Cell Cycle Kit (MilliporeSigma, USA) on the Muse®Cell Analyzer (Millipore, USA). Morphological

analysis of cell death was performed under a fluorescent microscope (EVOS M5000, Thermo Fisher Scientific, USA) with Acridin Orange/Propidium Iodide staining. The effect of flavonides on invasion-migration in T98G cells was determined by wound healing assay (Project no: BUU, BAP: THIZ-2021-550). In line with the findings, the IC50 values of HT and TYR are respectively; 91.4 µM and 350 µM. HT-treatment resulted in a higher apoptosis rate in T98G cells than TMZ ($p<0.0001$), while TYR-treatment increased apoptosis compared to untreated cells ($p<0.0001$). However, this effect of TYR was observed to be less than the apoptotic effect of TMZ. It was determined that TYR and HT in combination with TMZ caused apoptosis at a higher rate than TMZ-only ($p<0.0001$). Morphological analysis of cell death was found to support the results of Annexin analysis. It was observed that HT decreased the number of cells in the G0/G1 phases ($p<0.0001$), but TYR did not affect the cell cycle distribution. In the wound healing analysis demonstrating the invasive ability of T98G cells, untreated cells covered 75.84% of the wound area within 24 hours, while HT and TRY decreased wound healing to 21.4% and 22.66% ($p<0.0001$). In the combination of HT and TYR with TMZ, it was observed that the wound area opened up compared to TMZ alone ($p<0.0001$). Our findings highlight that HT may be a suitable candidate flavonoid for inclusion in further drug development studies against GB.

**Keywords:** Glioblastoma, Treatment, Flavonoids

## GİRİŞ

Glioblastoma (GB), yüksek proliferasyon ve invazyon ile karakterize edilen merkezi sinir sisteminin en sık görülen primer evre IV malign tümörüdür (McNeill, 2016). Mevcut GB tedavisi maksimum cerrahi rezeksiyon, radyoterapi ve kemoterapiden oluşmaktadır (DeAngelis, 2001). Maksimum cerrahi rezeksiyon, bu hastalığın yaşam süresinin uzatılmasını sağlayan efektif tedavi yöntemidir. Ancak GB'nin invaziv özelliğinden ve tümörün beyin içerisindeki konumundan dolayı maksimum rezeksiyon birçok durumda gerçekleştirilememekte ve tedaviden sonra tümör tekrardan nüksedebilmektedir. Alkilleyici bir ajan olan Temozolomid (TMZ), GB'nin en çok kabul gören standart kemoterapi tedavisidir (Laws ve ark., 2003). Bu tedavi protokollerine rağmen GB'nin yaşam süresi 14-15 ay'dır ve 5 yıllık sağkalım oranı %4-5'tir (McLendon ve Halperin, 2003). TMZ ile kemoterapi tedavisi alan GB hastalarında bulantı, kusma, yorgunluk ve hematolojik toksisite gibi çeşitli yan etkiler görülebilmektedir (Omar ve Mason, 2010). Standart GB tedavisindeki bu yetersizlikler göz önüne alındığında, TMZ'nin terapötik etkisini arttırabilecek ve bu tedavinin neden olduğu hücresel toksisiteyi azaltabilecek yeni tedavi yaklaşımına ihtiyaç duyulmaktadır. Olea europaea, dünya mahsulünün yaklaşık %98'ini kapsayan, Akdeniz'de en çok yetiştirilen ağaçlardan biridir (Omar ve Mason, 2010). Zeytin yaprağı uzun zamandır şifalı bir bitki olarak kabul edilmektedir. Birçok bireysel çalışma grubu, bağışıklık sistemini desteklemede, genel sağlığı korumada ve öksürük, soğuk algınlığı ve grip gibi hastalıkları tedavi etmede terapötik etkilerini göstermiştir (Kermanshah ve ark., 2020; De Cicco ve ark., 2022). Zeytin yaprağı ekstraktı, ekstrakta farklı terapötik özellikler kazandırdığı düşünülen sekoiridoidler, flavonoidler ve basit fenoller gibi birçok farklı bileşik içermektedir (Markhali ve ark., 2020). OLE'nin ana bileşeni (~%17-%23) sekoiridoidler adı verilen bir bileşik olan oleuropein (OL)'dir ve OL'ün antimikrobiyal, antioksidan, antienflamatuar ve kalp koruyucu özelliklere sahip olduğu bildirilmiştir (Afaneh ve ark., 2015). OL'nin hidrolizi ile oluşan Hidroksitirosol (HT), zeytin yaprağı ekstraktında yaklaşık 2,28 mg içerir ve genel olarak antioksidan, anti-kanserojen ve anti-inflamatuar etkilere sahiptir (Fri ve ark., 2020). Zeytin yaprağında eser miktarda bulunan Tirosol (TYR; kuru bazda yaklaşık 0,0007 mg/g), diğer flavonoidlere göre nispeten daha düşük antioksidan güce sahiptir (De Cicco ve ark., 2022). Bitki ekstraktı türevi olan fenolik bileşikler, kemoterapötik ajanlar üzerinde katkılı veya tamamlayıcı etkiler göstermesi ve bunların sitotoksik yan etkilerini hafifletmesi ile dikkat çekmiştir (Zhang ve ark., 2021). Son on yıldaki çalışmalarımız, OLE'nin TMZ'nin etkisini arttırdığından GB hücre hatları ve primer GB tümör hücreleri üzerindeki baskılayıcı etkisine dair kanıtlar sağlamıştır (Mutlu ve ark., 2021). Bununla birlikte, 2019'daki çalışmamız, GB hücrelerine karşı TMZ ile birlikte verilen kombin tedavide sadece OL ve OLE'nin farklı düzeyde etkisine işaret etti (Tezcan ve ark., 2019). Bu bulgu, GB'ye karşı en destekleyici tedavi yaklaşımının geliştirilmesinde ileriye doğru bir adım atmak için OLE içindeki flavonoidlerin bireysel etkilerinin açıklığa kavuşturulması gerekliliğini göstermektedir. Mevcut çalışmada, OLE'de bulunan etkili flavanoidler olan HT ve TRY'nin tek başına ve TMZ ile kombinasyonlarının GB hücre hattı olan T98G hücrelerindeki in-vitro biyolojik davranış üzerine etkilerinin incelenmesi amaçlanmıştır.

## MATERYAL VE METOT

### Hücre Hatları ve Reaktifler

İnsan GB hücre hattı, T98G Koç Üniversitesi öğretim üyelerinden Dr. Tuğba Bağcı Önder tarafından temin edildi. Bu hücreler, %10 fetal Bovin Serumu (FBS; BIOCHROME, Berlin, Almanya), 1 mM sodyum piruvat, 100 µg/mL streptomisin ve 100 U/ml penisilin ile takviye edilmiş L-glutamin ile takviye edilmiş Dulbecco Modifiye Eagle Medium-F12 (DMEM-F12; HyClone, Utah, ABD) kullanılarak 37°C ve %5 $CO_2$'de inkübe edildi. TMZ (Kat no: T2577), HT (Kat no: PHL80152) ve TYR (Kat no: 188255) Sigma'dan (St. Louis, MO, ABD) satın alınmıştır. TMZ, HT ve TYR dimetil sülfoksit (DMSO) içinde çözüldü.

### Hücre Çoğalması ve Canlılığı

Fenolik bileşiklerin T98G hücrelerinin çoğalma hızı üzerindeki etkisini belirlemek için hücre çoğalma reaktifi WST-1 (Roche Applied Sciences, Mannheim, Almanya) kullanıldı. Kısaca, $2x10^4$ hücre sayısında 6 kuyulu plakalara ekilen T98G hücresi, 24 saat sonunda TMZ, HT ve TYR'nin IC50 konsantrasyonları ile ön işleme tabi tutuldu. Ek olarak, muamele edilmemiş hücreler, %0.1 konsantrasyonu aşmayan ve hücre büyümesini etkilemeyen fenolleri çözmek için kullanılan DMSO'nun tam konsantrasyonu ile muamele edildi. Fenol muamelelerinden sonra hücreler, 37°C'de 2 saat süreyle 10 µL önceden karıştırılmış WST-1 hücre çoğalma reaktif maddesine maruz bırakıldı. Hücrelerin proliferasyon hızının kantitatif değerlendirmesi, 450nm absorbansta bir mikroplaka okuyucu (Tecan, İsviçre) tarafından saptandı. Hücre büyümesindeki inhibisyon oranı, daha önce açıklandığı gibi tespit edildi (Tunca ve ark., 2012).

### Gerçek Zamanlı Hücre Çoğalması İzleme

T98G hücreleri ($15x10^3$), standart ortam içinde bir E-Plate 16 (ACEA Biosciences, San Diego, CA, ABD) üzerine kaplandı. Hücreler, TMZ, HT ve TYR'nin WST-1 analizi ile belirlenen IC50 dozlarıyla muamele edildi. xCELLigence biyosensör hücre analiz sistemi (ACEA Biosciences, ABD), 96 saat boyunca her 30 dakikada bir hücre proliferasyon indeksini saptamak için elektrik empedansını ölçtü.

### Apoptoz Analizi

TMZ ve fenolik bileşiklerin T98G hücrelerindeki apopotoz analizi, Muse®Annexin V&Dead Cell Assay Kit (Millipore; Burlington, MA, ABD) kullanılarak, üreticinin talimatları izlenerek gerçekleştirildi. $15x10^4$ hücre, ayrı ayrı ve TMZ ile kombinasyon halinde HT ve TYR'nin IC50 dozları ile muamele edildi. 24 saat sonra hücreler toplandı ve karanlıkta 20 dakika Annexin V ve Ölü Hücre reaktifi ile işlendi. Akış sitometri analizini, Muse®Cell Analyzer (Millipore; Burlington, MA, ABD) cihazında gerçekleştirdi. Yalnızca annexin-V için pozitif olan hücreler erken apoptotik olarak tanımlanırken, Annexin V ve PI için pozitif olan hücreler geç apoptotik olarak sayıldı. Yalnızca PI için pozitif olan hücreler nekroz veya apoptotik olmayan hücre ölümü göstermiştir (Geretto ve ark., 2018). Tüm deneyler üç tekrarda gerçekleştirildi.

### Acridin Orange/Propidium Iodide (AO/PI) Floresan Boyama

$5x10^4$ T98G hücreleri, 24 oyuklu bir plakaya ekildi ve 24 saat boyunca TMZ, HT, TYR ve fenolik bileşiklerin TMZ ile kombinasyonları ile dozlandı. GB hücreleri, 5 dakika boyunca 10 µg/ml AO ve 5 µl PI (Sigma, St. Louis, MO) içeren bir ikili floresan boyama solüsyonuna (100 µl) maruz bırakıldı. Apoptotik hücrelerin morfolojisi, bir flüoresan mikroskobu (EVOS M5000, Thermo Fisher Scientific, CA, ABD) kullanılarak incelendi.

### Hücre Döngüsü Dağılım Analizi

Hücre döngüsünün analizi, üreticinin önerileri izlenerek Muse™ Hücre Döngüsü Kiti (MilliporeSigma, St. Louis, MO, ABD) kullanılarak yapıldı. $15x10^4$ fenolle önceden işlenmiş hücreler, buz gibi soğuk, %70 etanol içinde toplandı ve -20°C'de 3 saat inkübe edildi. Daha sonra hücreler, karanlıkta, oda sıcaklığında 30 dakika boyunca 200 uL Muse® Hücre Döngüsü reaktifi ile boyandı. Muse®Cell Analyzer (Millipore; Burlington, MA, ABD) cihazı hücre döngüsü dağılımını analiz etti.

### Yara İyileştirme Testi

6 oyuklu bir plakada T98G hücreleri %95'e yakın doluluk oranına ulaştığında, ince zar boyunca 100 uL'lik bir pipet ucunun sürüklenmesiyle çizik alanı oluşturuldu ve hücre döküntüsünü çıkarmak için iki kez PBS içinde yıkandı. Yaralı tek tabakalar, 24 saat boyunca TMZ, HT,TYR ve fenolik bileşiklerin TMZ ile kombinasyonları ile dozlandı. Yaralanmadan hemen sonra ve 24. saatin sonunda tüm yaralı bölgenin mikroskobik görüntüleri alındı. Yara boyutundaki değişiklikler, ImageJ v1.53s yazılımı (Ulusal Sağlık Enstitüleri, Bethesda, MD, ABD) kullanılarak ölçüldü. Her deney, üç tekrarlı olarak gerçekleştirildi.

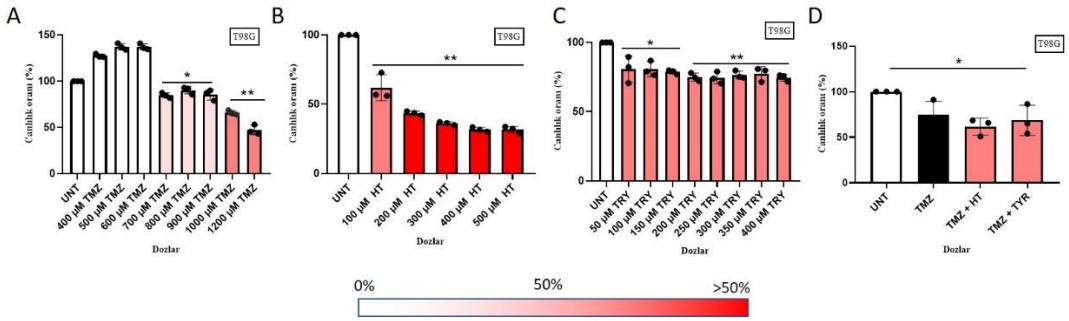
### İstatistiksel Analiz

İstatistiksel analiz, One-Way ve Two-Way ANOVA testleri, IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, ABD) ve GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, Kaliforniya) kullanılarak bağımsız örneklem T testi kullanılarak yapıldı. İstatistiksel anlamlılık p değeri $<0.05$ olarak ayarlandı. Tüm deneyler üç tekrarlı olarak gerçekleştirildi ve veriler ortalama ± SE olarak sunuldu.

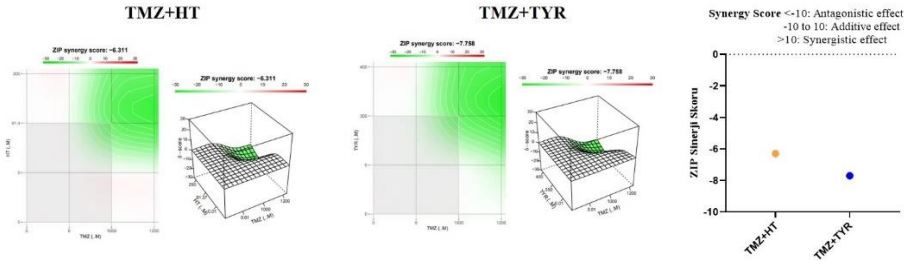
## BULGULAR

### OLE'deki aktif fenolik bileşikler GB hücre proliferasyonunu inhibe etti

T98G hücreleri, TMZ (400µM-1200µM), HT (100µM-500µM) ve TRY (50µM-400µM) ile 24 saat boyunca muamele edildi. 1000 µM TMZ, T98G hücrelerinin çoğalmasını %65,9 oranında azaltırken, fenolik bileşiklerden HT'nin 100 µM dozu %61,94, TYR'nin 350 µM dozu ise %77,35 oranında canlılığı azalttı (Şekil 1A-C). Hücre çoğalmasını önleme kapasitelerine göre, TMZ, HT ve TYR'nin IC50 değerleri sırasıyla; 1000 µM, 91,4 µM, ve 350 µM olarak tespit edildi. T98G hücrelerinde HT ve TRY'nin TMZ ile eş zamanlı muamele edilmesinde hücre canlılığını sırasıyla %61.7 ve %68.5'e düşürdü (Şekil 1D). SynergyFinder programında hesaplanan TMZ ile kombinasyon sıfır etkileşim potansiyeli (Zero-interaction potency; ZIP) sinerji puanı $HT_{SZIP}$ = -6.3, $TYR_{SZIP}$ = -7.7 olarak bulundu (Şekil 2). Bu değerler doğrultusunda, OLE fenoliklerinden HT ve TRY, TMZ ile kombinasyon halinde aditif bir etki gösterdi.



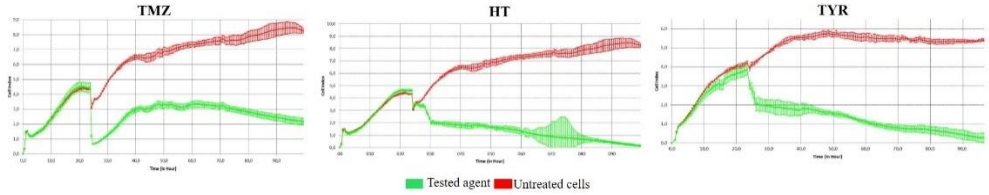

Şekil 1. TMZ (A), HT (B), TYR (C) ve fenolik bişeşiklerin TMZ kombinasyonları (D) doza bağlı olarak T98G hücre proliferasyonunu azalttı. p değerleri one-way ANOVA and Tukey's posthoc tests kullanılarak hesaplandı (n =3). Veriler, Ortalama ± SD olarak sunudu. *:p<0.05; **:p<0.0001. **UNT:** Tedavi edilmemiş, **TMZ:** Temozolomid, **HT:** Hidroksitirosol, **TYR:** Tirosol.




Şekil 2. TMZ'nin HT ve TYR ile birleşik etkisi. TMZ+HT ve TMZ+TYR'in neden olduğu ZIP sinerji puanları. **TMZ:** Temozolomid, **HT:** Hidroksitirosol, **TYR:** Tirosol.

### OLE'deki aktif fenolik bileşiklerin GB hücre proliferasyonunu azalttığı X-celligence cihazı ile doğrulandı

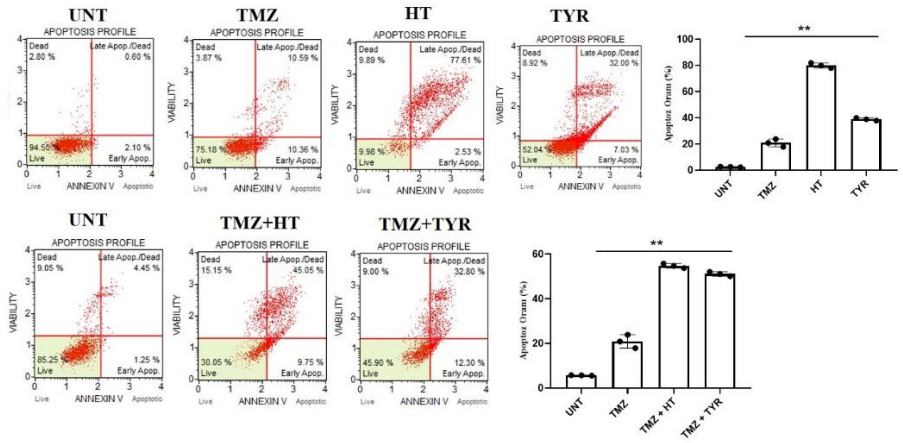
Tek başlarına TMZ, HT ve TRY'nin IC50 konsantrasyonlarının T98G hücreleri üzerindeki etkisi, bu hücrelerin hücre büyüme kinetiklerinin 90 saat boyunca gerçek zamanlı izlenmesiyle doğrulandı (Şekil 3). Muamelesiz T98G hücrelerinde hücre çoğalması görülürken, TMZ, HT ve TRY ile dozlu hücrelerinde hücre indeksinde azalış gösterdi.




Şekil 3. TMZ, HT ve TRY'nin IC50 dozlarının T98G hücrelerinin hücre çoğalma indeksi üzerindeki etkisi. **UNT:** Tedavi edilmemiş, **TMZ:** Temozolomid, **HT:** Hydroxytyrosol, **TYR:** Tyrosol.

**HT ve TRY GB hücrelerini apoptoza yönlendirdi**

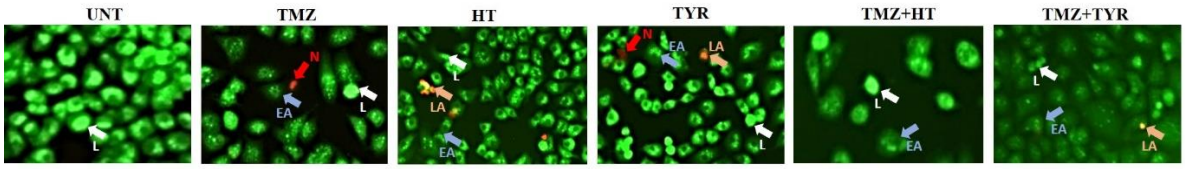
Annexin V analizine göre, T98G hücrelerinde tedavi edilmemiş hücrelere kıyasla HT ve TYR muamelesi hücre apoptozunu istatistiksel olarak anlamlı şekilde arttırdı ($p<0.0001$, Şekil 4). Ayrıca HT ve TYR'nin apoptozu tetikleyen etkisi, yalnızca TMZ'den daha yüksekti ($p<0.0001$), Şekil 4). HT ve TYR'nin TMZ ile kombinasyon etkisi, hem tedavi edilmemiş hem de tek başına TMZ ile tedavi edilmiş hücrelere kıyasla daha fazlaydı (Şekil 4).




Şekil 4. HT ve TYR'nin tek başına ve TMZ ile kombin muamelesi T98G hücrelerinde apoptozu indükledi. p değerleri one-way ANOVA and Tukey's posthoc tests kullanılarak hesaplandı (n=3). **$p<0.0001$. **UNT:** Tedavi edilmemiş, **TMZ:** Temozolomid, **HT:** Hidroksitirosol, **TYR:** Tirosol.

**HT ve TRY'nin GB hücrelerindeki hücre ölümüne etkisi florasans mikroskonunda doğrulandı**

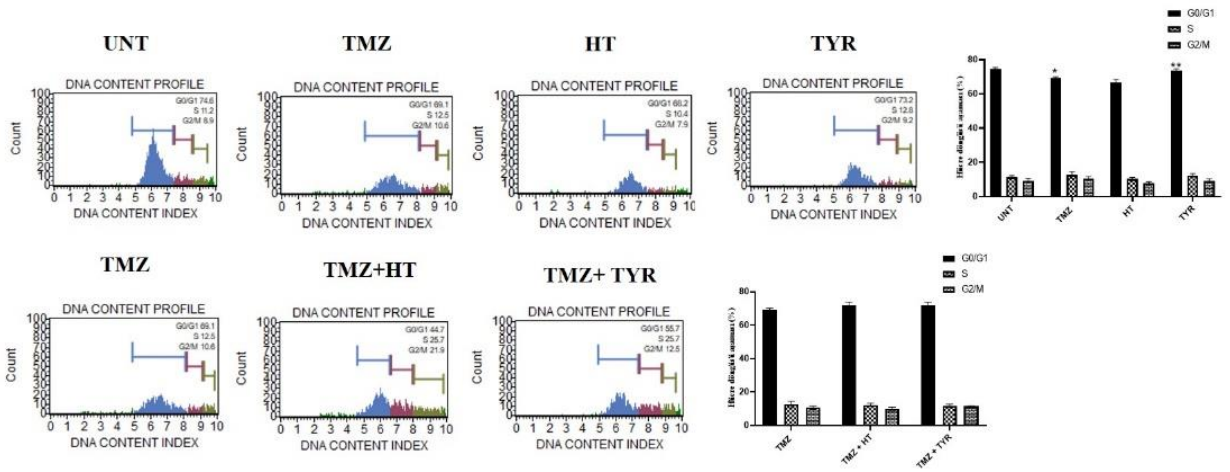
Muamele edilmemiş T98G hücreleri, merkezde tekdüze dairesel bir çekirdeğe sahipti ve bu hücreler canlı hücre olarak adlandırıldı. Bununla birlikte, TMZ, HT ve TRY ve fenolik bileşiklerin TMZ ile kombinasyonları, hücre çekirdeklerinin morfolojisini, apoptoz sırasında ortaya çıkan nükleer parçalanmanın bir işareti olan at nalı şeklinde değiştirdi (Şekil 5).




Şekil 5. T98G hücrelerine HT ve TYR'nin tek başına ve TMZ ile kombin muamelesinden sonraki AO/PI boyaması. Renkli oklar hücreleri şu şekilde gösterir: canlı hücreler beyaz, erken apoptoz mavi, geç apoptoz turuncu ve nekroz kırmızı. **UNT:** Tedavi edilmemiş, **TMZ:** Temozolomid, **HT:** Hidroksitirosol, **TYR:** Tirosol.

**HT ve TYR GB hücrelerindeki hücre döngüsünü inhibe etti**

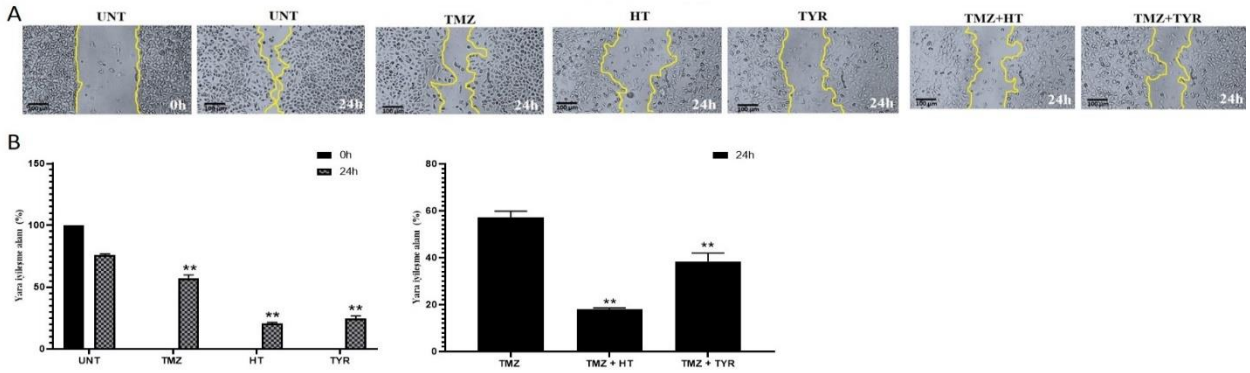
TMZ, T98G hücrelerinin hücre döngüsü içindeki dağılımında G0/G1 fazındaki hücre yoğunluğunu azaltırken ($p<0.05$), TYR, bu fazdaki hücre yoğunluğunu arttırdı (Şekil 6; $p<0.0001$). T98G hücrelerinde HT ile muamele hücre döngüsü içindeki dağılıma etki etmedi. Fenolik bileşiklerin TMZ ile kombinasyonu tek başına TMZ'ye göre herhangi bir etki göstermedi (Şekil 6).




Şekil 6. T98G hücrelerinin G0/G1, S ve G2/M faz oranları (n=3). Veriler ortalama ± SD olarak sunuldu. p değeri, Two-Way ANOVA testi kullanılarak muamele edilmemiş ve tek başına TMZ ile muamele edilmiş hücrelere kıyasla hesaplandı. * $p<0.05$ ** $p<0.0001$. **UNT:** Tedavi edilmemiş, **TMZ:** Temozolomid, **HT:** Hidroksitirosol, **TYR:** Tirosol.

**HT ve TRY tek başına ve TMZ ile birlikte GB hücrelerindeki invazyonu engelledi**

OLE fenoliklerinin T98G hücrelerinin iki boyutlu göç kapasitesi üzerindeki etkisi, bu fenolikler ile tedaviden sonraki yara iyileşme hızı ölçülerek tespit edildi. Tedavi edilmeyen T98G hücrelerinin yaralı alanın %75,84'ü 24 saat içinde kapandı. TMZ-tedavisi ise yara iyileşme oranını %56,96'ya yavaşlattı (Şekil 7A). OLE fenolikleri, HT ve TRY, T98G hücrelerinde yaranın sırasıyla %21,4 ve %22,66'ısını kapattı (Şekil 7A). Böylece tek başına HT ve TRY, tek başına TMZ'ye göre yara alanını daha çok koruyarak invazyonu azalttı ($p<0.0001$; Şekil 7B).OLE fenolikleri ile kombine tedavide HT+TMZ ve TRY+TMZ tedavisi yara alnını sırasıyla %18.31 ve %35.38 oranında kapattı (Şekil 7A). Bu iki kombin tedavi tek başına TMZ'ye göre istatistiksel olarak anlamlı olsa da HT+TMZ tedavisinin invazyonu daha yüksek oranda engellediği görüldü ($p<0.0001$; Şekil 7B).




Şekil 7. HT ve TRY'nin GB hücrelerinin migrasyon kapasitesi üzerindeki aditif etkisi. **(A)** Fenolik bileşiklerin tek başına ve TMZ ile kombinasyonu tedavi edilmemiş hücrelere kıyasla hücrelerin göçünü azalttı. Yaralı bölgenin boyutu, 40x büyütmede tedaviden 24 saat sonra analiz edildi. Yaralı bölgenin boyutu ImageJ yazılımı ile ölçüldü. p değerleri, bağımlı bir örneklem T-testi kullanılarak hesaplandı **(B)**. Veriler ortalama ± SD olarak gösterilmiştir. **$p< 0.0001$. **UNT:** Tedavi edilmemiş, **TMZ:** Temozolomid, **HT:** Hidroksitirosol, **TYR:** Tirosol.

## TARTIŞMA

OLE'nin ikinci ve üçüncü majör yapısında olan HT ve TRY'nin anti-proliferatif, pro-apoptotik ve anti-enflamatuar kapasitesi çeşitli çalışmalarla kanıtlanmıştır (Laghezza ve ark., 2022; Visioli ve ark., 1998). Bu çalışmada HT ve TRY, GB hücre hattında anti-proliferatif etki gösterdi. Aynı zamanda TMZ'ye additif etki göstererek TMZ kombinasyonlarında tek başına TMZ'ye göre GB hücrelerinde daha yüksek etki göstermesini sağladı. HT ve TRY düşük konsantrasyonda, GB'ye karşı güçlü önleme kapasitesini, hücreleri apoptoza yönlendirerek kanıtladı. Bunun yanında HT ve TRY'ye göre çizik alanını açık tutarak GB hücrelerinin invazyonunu engellese de TMZ ile kombin tedavide HT+TMZ tedavisinin daha etkili olduğu görüldü.

## SONUÇ

Sonuç olarak, bu çalışma ilk önce OLE fenoliklerinden HT ve TRY'nin GB hücreleri üzerindeki etkisini ve bunların TMZ tedavisine katkı potansiyelini araştırdı. Çalışma ekibinin daha önceki çalışmaları ile karşılaştırıldığında OLE'de bulunan fenolik bileşik olan HT ve TRY'nin GB hücrelerinde OLE kadar etkili olduğu bulundu. Bu fenolik bileşikler TMZ'nin etkisini artırabilecek ilave etkiler gösterdiğinden, bulgular, GB'ye karşı bireysel bir antikanser ilacı olma potansiyelini netleştirmek için daha fazla araştırmanın yapılabilmesinin gerekliliğini ortaya koymaktadır.

## TEŞEKKÜR

GB hücre hatlarını tarafımıza sağladığı için Koç Üniversitesi'nden Dr. Tuğba Bağcı Önder'e teşekkür etmek isteriz. Bu çalışma TÜBİTAK (Proje No.121S624) ve Bursa Uludağ Üniversitesi BAP (Proje no: TOA-2021-578 ve THIZ-2021-550) tarafından desteklenmiştir.

## KAYNAKLAR

Afaneh I, Yateem H, Al-Rimawi F 2015. Effect of olive leaves drying on the content of oleuropein. American Journal of Analytical Chemistry. 6:3.

De Cicco P, Ercolano G, Tenore, GC ve ark., 2022. Olive leaf extract inhibits metastatic melanoma spread through suppression of epithelial to mesenchymal transition. Phytotherapy Research. 36(10):4002-4013.

DeAngelis LM 2001. Brain tumors. N Engl J Med., 344:114–123.

Fri I, Sayadi S, Mahmoudi A ve ark., 2020. Comparative Study on Beneficial Effects of Hydroxytyrosol-and Oleuropein-Rich Olive Leaf Extracts on High-Fat Diet-Induced Lipid Metabolism Disturbance and Liver Injury in Rats. Biomed Res. Int. 1–15.

Geretto M, Ponassi M, Casale M ve ark., 2018. A novel calix[4]pyrrole derivative as a potential anticancer agent that forms genotoxic adducts with DNA. Sci Rep. 23;8(1):11075.

Kermanshah Z, Samadanifard HOSEIN, Moghaddam OM ve ark., 2020. Olive leaf and its various health-benefitting effects: a review study. Pakistan Journal of Medical & Health Sciences, 14(2), 1301-1312.

Laghezza Masci V, Bernini R, Villanova N v earl., 2022. In Vitro Anti-Proliferative and Apoptotic Effects of Hydroxytyrosyl Oleate on SH-SY5Y Human Neuroblastoma Cells. Int J Mol Sci. 23(20):12348.

Laws ER, Parney IF, Huang W ve ark., 2003. Survival following surgery and prognostic factors for recently diagnosed malignant glioma: data from the Glioma Outcomes Project. J Neurosurg. 99:467–473.

Markhali FS, Teixeira JA, Rocha CM 2020. Olive tree leaves—A source of valuable active compounds. Processes, 8(9), 1177.

McLendon RE, Halperin EC 2003. Is the long-term survival of patients with intracranial glioblastoma multiforme overstated?. Cancer, 98:1745–1748.

McNeill KA 2016. Epidemiology of brain tumors. Neurol Clin., 34(4):981–998.

Mutlu M, Tunca B, Aksoy SA ve ark., 2021. Olea europaea leaf extract decreases tumour size by affecting the LncRNA expression status in glioblastoma 3D cell cultures, Eur J Integr Med 45. 101345.

Omar AI, Mason WP 2010. Temozolomide: The evidence for its therapeutic efficacy in malignant astrocytomas. Core evidence, 4, 93–111.

Tezcan G, Aksoy SA, Tunca B ve ark., 2019. Oleuropein modulates glioblastoma miRNA pattern different from Olea europaea leaf extract. Hum Exp Toxicol. 38(9):1102-1110.

Tunca B, Tezcan G, Cecener G ve ark., 2012. Olea europaea leaf extract alters microRNA expression in human glioblastoma cells. J Cancer Res Clin Oncol. 138(11): 1831–1844.

Visioli F, Galli C 1998. The effect of minor constituents of olive oil on cardiovascular disease: new findings. Nutr Rev. 56(5 Pt 1):142-147.

Zhang Z, Shi J, Nice EC ve ark., 2021. The Multifaceted Role of Flavonoids in Cancer Therapy: Leveraging Autophagy with a Double-Edged Sword. Antioxidants (Basel).19;10(7):1138.

## ORAL PRESENTATION

# *Cordyceps militaris* mantarının antioksidan aktivitesi üzerine analitik çalışmalar

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## Özet

Mantarlar, klorofil içermeyen ve sporlar yoluyla parazit veya saprofit olarak üreyen canlı organizmalardır. Bir Cordyceps cinsi olan Cordyceps militaris, geleneksel Çin tıbbında yüzyıllardır terapötik olarak kullanılmaktadır. Cordyceps militaris, biyolojik ve farmakolojik aktiviteleri olan cordycepin, adenozin, cordycepic asit, süperoksit dismutaz enzimi ve cordyceps polisakkaritlerini içerir. Cordycepin, biyolojik olarak aktif geniş spektrumlu bir nükleosid analoğudur ve ilk olarak Cordyceps militaris'ten elde edilmiştir. Çalışmamızda Cordyceps militaris'in antioksidan aktivitesi ve içerdiği biyoaktif bileşiklerin tayini ile ilgili analitik çalışmalar incelenerek bir derleme oluşturulmuştur. İnceleme çalışmasında Cordyceps militaris'in antioksidan özellikleri ve fenolik bileşenleri Springer, PubMed, Web of Science, Science Direct ve Scopus veri tabanları ile literatür taranarak elde edilmiştir. Çeşitli spektrofotometrik yöntemlerle elde edilen bilgilerden, DPPH(1,1-Difenil-2-pikrilhidrazil), $ABTS^{\bullet+}$(2,2`azinobis (3-etilbenzotiazolin-6-sülfonik) asit), FRAP(demir (III) iyonu indirgeyici antioksidan güç yöntemi), Oksijen Radikal Absorbans Kapasitesi (ORAC) Yöntemi, Hidroksi radikali yakalama aktivitesi ve Folin-Ciocalteu reaktifi (FCR), TEAC(Trolox eşdeğeri antioksidan kapasitesi) yöntemleri incelenmiştir. Verilere bakıldığında Cordyceps militaris'in önemli biyoaktif özelliklere sahip, antiviral, antioksidan, antienflamatuar, antitümör, nöroprotektif, antihipertansif ve immünomodülatör olduğu bilinen polisakkaritleri içerdiği belirlenmiştir. Bu polisakkaritlerin karaciğer koruyucu, böbrek koruyucu, nöroprotektif, immünomodülatör, antidiyabet, antikanser, antioksidan etkileri olduğu kanıtlanmıştır. Çin'deki birçok sağlık ürünü Cordyceps militaris içerir. Cordyceps militaris bitkisinden üretilen ilaçların böbrek, akciğer sağlığı, yaşlanmayı geciktirme ve uyku düzenleyici olarak kullanıldığı bilinmektedir. Bu veriler göz önünde bulundurularak tıpta destekleyici tedavide doktor kontrolünde kullanılması önerilebilir.

**Anahtar Kelimeler:** Cordyceps militaris, Cordycepin, Antioksidan Aktivite Yöntemleri, Biyoaktif bileşikler.

# Analytical Studies on the Antioxidant Activity of Cordyceps militaris Mushroom

## Abstract

Fungi are living organisms that do not contain chlorophyll and reproduce as parasites or saprophytes via spores. Cordyceps militaris, a genus of Cordyceps, has been used therapeutically for centuries in traditional Chinese medicine. Cordyceps militaris contains cordycepin, adenosine, cordycepic acid, superoxide dismutase enzyme and cordyceps polysaccharides with biological and pharmacological activities. Cordycepin is a biologically active broad-spectrum nucleoside analogue and was originally derived from Cordyceps militaris. In our study, a review was created by examining the analytical studies on the antioxidant activity of Cordyceps militaris and the determination of the bioactive compounds it contains. In the review study, the antioxidant properties and phenolic components of Cordyceps militaris were obtained by searching the literature with Springer, PubMed, Web of Science, Science Direct and Scopus databases. From the information obtained by various spectrophotometric methods, DPPH(1,1-Diphenyl-2-picrylhydrazil), $ABTS^{\bullet+}$(2,2`azinobis (3-ethylbenzothiazoline-6-sulfonic) acid), FRAP(iron (III) ion reducing antioxidant power method), Oxygen Radical Absorbance Capacity (ORAC) Method, Hydroxy radical capture activity and Folin-Ciocalteu reagent (FCR), TEAC (Trolox equivalent antioxidant capacity) methods were investigated. Looking at the data, it was determined that Cordyceps militaris contains polysaccharides with important bioactive properties, which are known to be antiviral, antioxidant, anti-inflammatory, antitumor, neuroprotective, antihypertensive and immunomodulatory. These polysaccharides have been proven to have liver-protective, kidney-protective, neuroprotective, immunomodulatory, antidiabetes, anticancer, and antioxidant effects. Many health products in China contain Cordyceps militaris. It is known that drugs produced from the Cordyceps militaris plant are

used for kidney, lung health, delaying aging and sleep, regulators. Considering these data, it can be recommended to be used in supportive treatment in medicine under the control of a doctor.

**Keywords:** Cordyceps militaris, Cordycepin, Antioxidant Activity Methods, Bioactive compounds.

## GİRİŞ

Doğa canlılığın başladığı ilk günden itibaren tıp için önemli bir ilham kaynağı olmuştur. Evrim boyunca doğa, çeşitli hastalıkların buna kanser tedavisi de dahil olmak üzere muazzam terapötik potansiyele sahip çok çeşitli biyolojik olarak aktif maddeler üretir. Doğal ürünler dediğimiz bu ürünler yaygın olarak kullanılan bir dizi bileşik üretmişken, bu tür ürünlerin halk tıbbında ve geleneksel tıpta uygulanması her zaman terapötik potansiyele sahip yeni bileşik kaynaklarına işaret eden önemli bir ipucu olmuştur (Blagodatski ve ark. 2018).

Mantarlar doğal ekosistemlerde önemli doğal kaynaklar olup, klorofil içermeyen sporla üreyen, parazit veya saprofit olarak yaşayan canlılardır (Baytop, 1996). Hücre duvarlarının bulunması, sporla çoğalmaları, hareket etme yeteneklerinin olmaması nedeniyle bitki olarak değerlendirilmişlerdir fakat kök, gövde, yapraklarının bulunmaması ve klorofil içermemeleri nedeniyle de bitkilerden ve hayvanlardan çok farklı olup ayrı bir alem olarak sınıflandırılmıştır. Mantarlara ait ilk sistematik çalışma 1836 yılında Fries yapmıştır. Bugünde bu sistematik çalışma kullanılmaktadır (Alkın, 2017).

Yeryüzündeki mantar sayısının 140.000 olduğu tahmin edilmekte olup yaklaşık olarak sadece %10'u adlandırılmıştır. Mantarlar veya mantar ekstraktları protein ve karbonhidrat içeriği bakımından zenginken, lipid içerikleri düşüktür. İnsan vücudundaki amino asitlerin ihtiyaçlarını karşılamaya yardımcı olan esansiyel amino asitler, nükleotidler, önemli mineraller, vitaminler, mineraller ve enzimleri bol miktarda içerdikleri, özellikle ve en önemlisi, modern tıp için, antitümör ve immün sistemi uyarıcı özelliklere sahip sınırsız bir polisakkarit kaynağı oldukları için geleneksel bitkisel ilaçlar olarak tedavide Çin ve Japonya'da yüzyıllardır kullanılmaktadır. Mantarlar, bitkilerden farklı olarak klorofilden yoksundur, bu nedenle lignin, selüloz ve diğer önemli karbonhidratlar açısından zengin çürümüş organik maddeler üzerinde büyürler. Ekonomik olup, ekimi kolaydır, az kaynak ve alan gerektirir ve dünyanın her yerinde yetiştirilebilirler (Uhrinová and Poľančíková 2018, Wasser 2010).

Eşzamanlı olarak, modern kimyanın gelişimi, günümüzde birçok ilacın sentezi için ilaç veya başlangıç maddesi olarak hizmet etmiş şifalı bitkilerden kimyasalların izolasyonuna olanak tanımıştır. İlk olarak şifalı mantarlar da dahil olmak üzere şifalı bitkilerden izole edilen kimyasalların etki mekanizmalarına ilişkin çalışmalardan elde edilen bilgilerin bir sonucu olarak birçok modern ilaç sentezlenmiştir. Böylece şifalı bitkiler modern tıbbın gelişmesinde büyük rol oynamış ve orijinal halleriyle yaygın olarak kullanılmaya devam etmektedir. Yenilebilir mantarlar ve tıbbi mantarlar, besleyici, antienfektif, stres giderici ve antioksidan özellikleri nedeniyle gıda, ilaç ve kozmetik endüstrilerinde değerli kaynaklar olarak kullanılmaktadır. (Das ve ark. 2010)

### Cordyceps türleri

Cordyceps spp. Ascomycota, Pyrenomycetes, Hypocreales ve Clavicepitaceae familyalarına ait ve 750'den fazla tanımlanmış türle, tür sayısı, morfolojisi ve konakçısındaki varyasyon açısından Clavicipitaceae familyasında sınıflandırılan en büyük ve en çeşitli genelde böcek, eklem bacaklılar ve diğer mantarların üzerinde yaşayan bir endoparazittir. Cordyceps cinsi, böcekler üzerindeki mantarlara verilen ismiyle varlığı M.Ö. 2000 yılından beri Çin'de ise 300 yılı aşkın süredir bilinmekte ve ilaç olarak kullanılmaktadır. İlk olarak 1694 yılında Wang Ang tarafından Ben-Cao-Bei-Yao'da kaydedilmiştir. Genellikle Himalaya platosundaki yüksek rakımlarda bulunan ve geleneksel Çin tıbbında kullanılan, doğal olarak oluşan nadir bir entomopatojenik mantardır. Cordiseps esas olarak Kuzey Amerika, Avrupa ve Asya'da bulunmuştur. Hindistan'da, yaygın olarak "Keera Ghas"(Böcek otu) olarak bilinen Himalayalar'da dağlık bölgelerde belirgin bir şekilde bulunur. (Panda ve Swain 2011; Singh ve ark. 2010).

Cordyceps türünün ilk yazılı kaydı MS 620 yılında, Tang zamanında Çin'den gelmektedir. Tibet bilginleri tarafından 15. ve 18. yy boyunca mantar/bitki olarak yazılı kaynaklarda geçerken 1757'de yazar Wu-yiluo tarafından Ben Cao Congxin'de ("Yeni Derleme Materia Medica"), Qing Hanedanlığı döneminde bilimsel

olarak tasviri yapılmıştır. Latince kelime birleşimi, stroma ve meyve gövdesi genellikle Himalaya Hayalet Güvesi Thitarodes armoricanus (= Hepialis armoricanus) gibi böcek larvalarının mumyalanmış karkaslarından uzanan bu kulüp mantarlarının görünümünü doğru bir şekilde tanımlar. Cordyceps türlerinin tıbbi bilgisi 1726'da Paris'te bilimsel bir toplantıda tanıtılarak batılı dinleyicilere ulaşmıştır. İlk örnekler Fransa ile Çin İmparatoru mahkemesinde bir Cizvit rahibi tarafından Cordyceps mantarı ile ilgili deneyimlerini anlatmasıyla başlamıştır. Nadir, Çin efsanelerine göre bu değerli şifalı mantar ve onun bukalemun gibi özellikleri binlerce yıl öncesine uzanır (Holliday ve Cleaver, 2008).

Cordyceps, Batı toplumuna ilk olarak 17. yüzyılda tanıtılmıştır. 1878'de, bir İtalyan bilim adamı olan Saccardo, Cordyceps adını Çin'de resmi olarak Cordyceps sinensis (Berk.) Sacc. olarak türetmiştir ve bu terminoloji günümüze kadar benimsenerek gelmiştir (Shashidhar ve ark. 2013). Cordyceps adı, Yunanca "kulüp" anlamına gelen "kordyle" ve "kafa" anlamına gelen Latince "-ceps" kökünden türetilmiştir. Tüm Cordyceps türleri benzer yaşam döngüsüne sahiptir ve konakçılarını istila etmek ve üzerlerinde büyümek için mekanizmalar geliştirmiştir. En büyük farkları büyüdükleri yer ve enfekte ettikleri konakçı böcektir. Cordyceps türleri, bir konakçıyla temasa geçene kadar toprakta uykuda kalır. Morfolojik çeşitliliklerinin yanı sıra, Cordyceps türleri böcekleri, eklembacaklıları ve diğer mantarları istila eder ve konağın bağışıklık sisteminden kaçarak, konağın yaşam döngüsünü hayatta kalma mekanizması ve üreme niyetiyle uyumlu hale getirir ve konak ile etkileşimleri, diğerleri arasında kordisepin, adenozin, guanozin, kordimin, γ-aminobutirik asit (GABA), ekzopolisakkaritler, kordisin A-E gibi farklı benzersiz ikincil metabolitler üretmelerini sağlar (Olatunji ve ark, 2018).Yaklaşık 20 Cordyceps türü Elapomyces cinsi üzerinde parazitlenirken, bazısı yine bir güve türü olan Hepialus armoricanus Oberthur'un (Lepidoptera) larvasının kafasında yaşar, Kalan türler Arachnida, Coleoptera, Hemiptera, Hymenoptera, Isoptera ve Lepidoptera sınıfına ait böcekler ve diğer eklembacaklılar üzerinde parazitlenir. Cinsteki türlerden birkaçı, geniş bir konakçı yelpazesini (birkaç familyadaki veya eklembacaklıların takımlarındaki türler) enfekte etme yeteneğine sahiptir. Bununla birlikte, çok sayıda Cordyceps türü, yalnızca tek bir konak türü veya birkaç yakından ilişkili türü enfekte etmekle sınırlıdır. (Shashidhar ve ark, 2013).

Cordyceps türleri en çok nemli ılıman iklimlerde bulunur. Kuzey Amerika'da geniş bir dağılıma sahip olan cins; tropikal ormanlarda Avrupa, Doğu ve Güneydoğu Asya ülkeleri, özellikle Çin, Japonya, Nepal, Vietnam, Bhutan, Kore ve Tayland'da Dünya çapında dağılım göstermiştir. Hindistan'da, esas olarak Kumaon Himalaya ve Garhwal Himalaya (yüksek rakımlarda) gibi ormanlık bölgelerde bulunur. Diğer birçok türler dünyanın diğer bölgelerinde değişen habitatlarda tanımlanmıştır. (Olatunji ve ark., 2018, (Maity, 2013; Chakraborty ve ark, 2014).

Tüm tıbbi mantar ürünlerinin yaklaşık %80-85'i meyve gövdelerinden çıkarılırken sadece %15'i miselyum kültüründen elde edilir (Lindequist ve ark. 2005). Cordyceps'in meyve gövdesi çok küçük bıçak benzeri bir yapıdır, bu da toplanmasını zor ve pahalı hale getirir. Tıbbi mantar biyo-metabolitlerine büyük bir gereksinim olduğundan, miselyum biyokütlesinin yapay olarak yetiştirilmesi gereklidir ve bunun için birçok araştırma grubu tarafından ekimi için çeşitli yöntemler önerilmiştir (Masuda ve ark, 2006; Das ve ark, 2008, 2010). Cordyceps miselyum, farklı besin içeren ortamlarda büyüyebilir, ancak geçmişte ticari fermantasyon ve yetiştirme için böcek larvaları (ipekböceği kalıntısı) ve çeşitli tahıl taneleri kullanılmıştır. Tutarlı bir şekilde hem böcek larvalarından hem de tahıl tanelerinden mantarın meyve gövdesinin neredeyse karşılaştırılabilir tıbbi özelliklerle elde edilebileceği görülmüştür (Tuli ve ark, 2013)

Bu nadir ve ilginç şifalı mantarın yayılımı ise yağmur, hava ve böcekler aracılığıyla gerçekleşir; tüm yaşam döngüsünde enfeksiyon, parazitlik ve saprofitizm olmak üzere üç evrede bulunur (Pal ve Misra, 2018). İlk aşamada, Cordyceps spp. konakçıyı larva aşamasında askosporlar (yaz aylarında ve sonbaharın başlarında olgun meyve gövdelerinden havaya salınan) yoluyla enfekte eder ve çimlenir. Bazı durumlarda enfeksiyon, Cordyceps ile kontamine olmuş gıdaların yenilmesiyle oluşur. Parazit evre enfeksiyondan sonra ortaya çıkar ve bu evrede Cordyceps spp. konağın bağırsağından beslenir. Mantar hücreleri vücuda yayılır ve kış aylarında hızla çoğalarak larvanın tüm iç organlarını tüketerek dış iskeleti sağlam bırakır. Daha sonra mantar hücresi larva gövdesinde beyaz bir kütleye dönüşür (endosclerotium) Bu süreçte çevre koşulları oldukça sert olup, mantar kar ve soğuğa karşı direnmek zorundadır. Bahar aylarında dış sıcaklık arttığında, endosklerotyum çimlenir ve konağın ağız boşluğundan dışarı çıkar, yazın olgunlaşır, meyve veren gövdeyi oluşturur ve

askosporları salmaya başlar (saprofitik aşama). Bu mevsimde mantar toplama işlemi yapılmaktadır. (Tuli ve diğerleri, 2013)

Bazı yazarlara göre Cordycepsler güve larvalarına saldırı sonucu sporla çoğalan ajan olarak kabul edilmektedir. Cordycepin entomopatojenitesi tartışmalı olsa da dikkate değerdir. Birçok önde gelen araştırmacı Cordyceps sinensis'in aslında konakçı ile simbiyotik bir ilişkisi olduğunu ve bağlantının patojenik olmaktan çok karşılıklı olarak yararlı olduğunu öne sürmektedir. Doğa, bir parazite karşı seçim yapma eğilimindedir çünkü bir parazit genellikle konağın ölümüyle sonuçlanır. Bir böcek ve bu mantar arasındaki benzersiz çiftleşme için belki daha mantıklı bir açıklama, bunun karşılıklı olarak yararlı bir ortak yaşam olması ve böylece güve, vücudunda yaşayan Cordycepslerden bir enerji artışı veya başka bir fayda elde etmesi olabilir (Holliday ve Cleaver., 2008).

Cordyceps'in bütün türlerinin farklı farklı yararlı özellikleri vardır. Anti-kanser, anti-proliferatif, anti-anjiyojenik, antimetastasis, apoptoz indüksiyonu, anti-inflamatuar, antioksidan, anti-fibrotik, anti-arterioskleroz, anti-hipertansif, anti-osteoporotik, anti-trombotik, anti-sıtma, anti-fungal, hipolipidemik, anti-diyabetik, hipoglisemik, anti-astmatik, anti-fertilite, anti-viral, yaşlanma karşıtı, steroidogenez, spermatojenik, böbrek fonksiyonlarını düzenleyici ve immünomodülatör etki dahil olmak üzere dikkate değer klinik sağlık etkilerine sahiptir (Liu ve ark. 2015; Jin ve ark, 2018).

**Cordycepin, Cordyceps militaris ve antioksidan aktivitesi**

Cordyceps sp.'den elde edilen, çeşitli farmakolojik olarak aktif bileşikler içinde en önemli olan "Cordycepin" geniş spektrumlu biyolojik aktivitesi yüksek olan bir bileşik olup tıbbi değeri ve nutrasötik potansiyeli nedeniyle üzerinde en çok çalışılan aktif bileşendir ve büyük ilgi görmektedir. Cordycepin ilk olarak Cordyceps militaris'den ardından Cordyceps sinensis'ten ve Cordyceps kyushuensis'ten elde edilmiştir. Cordyceps'ten, cordycepin ve türevleri, cordycepic asit, ergosterol, polisakkaritler, nükleosidler, proteinler, amino asitler, vitaminler ve mineraller dahil olmak üzere çeşitli bileşikler ekstrakte edilmiş ve saflaştırılmıştır. Cordycepin (3-deoxyadenosine) biyolojik etkinliği olan bir geniş spektrumlu bir nükleosit analoğudur (Masuda ve ark., 2006; Patel ve Ingalhalli, 2013). Farmasötik biyotekniklerdeki son gelişmeler nedeniyle, Cordyceps'ten biyoaktif bileşikleri izole etmek ve onu toz ve kapsül formunda elde etmek mümkündür. Çeşitli biyokimyasal ve moleküler süreçlerle pürin biyosentezi, DNA/RNA sentezi ve mTOR (memelilerde rapamisin hedefi) sinyal iletimine müdahale ettiği bilinmektedir (Tuli ve ark., 2014). Cordysepin yapı olarak adenosine çok benzer ve nükleosid analog gibi rol oynar. Kemoterapi direnci ve metastaz nedeni ile kanser önleme ve tedavi uygulamalarında birçok alternatif tedavi yönteminde önerilmiştir, geleneksel Çin tıbbında Cordycepin kanser tedavisinde uzun süredir kullanılmaktadır ayrıca immünolojik, hepatik, renal, kardiyovasküler sistemlerde birçok biyolojik ve farmakolojik etkiye sahip, çeşitli nutrasötik ve terapötik potansiyel özellikleri ile bilinir. Cordycepin tedavisinin tümördeki işlevi, tümör tiplerine ve konsantrasyonuna bağlıdır (Rao ve ark. 2010).

Son yıllarda Cordyceps'in önemli ölçüde popülerliğinin artması doğal oluşumunun sömürülmesi ile azalmasına neden olmuştur. Bu yüzden mantarı fermentasyon teknikleriyle kültürünü yetiştirme yöntemlerine başvurulmuştur. Tıbbi mantar ürünlerinin yaklaşık %80–85'i meyve veren gövdesinden çıkarılırken, sadece %15'i miselyum kültüründen türetilmiştir. Cordyceps'in meyve veren gövdesi çok küçük bir bıçak benzeri yapısı, koleksiyonunu zor ve pahalı hale getirir. Çok büyük bir tıbbi mantar ihtiyacı olduğu için biyo-metabolitler, miselyum biyokütlesinin yetiştirilmesi gereklidir. Yapay olarak yetiştiriciliği için çeşitli yöntemler birçok araştırma grubu tarafından önerilmiştir (Tuli ve ark., 2014).

Geleneksel Çin tıbbında ve Asya'da yaygın olarak ham ilaç ve fonksiyonel gıda olarak kullanılan ve yenilebilir tıbbi mantar olan Cordyceps militaris (Şekil 1), cordyceps türleri arasında araştırılan ikinci en popüler türdür. Genellikle Yong Chong Cao ve Bei Dong Chong Xia Cao olarak adlandırılır. Ascomycota filumuna ve Pyrenomycetes sınıfına ait, Cordyceps sinensis ile benzer farmakolojik etkiye sahip olduğu kanıtlanmıştır (Shi ve ark., 2019). Bu türün kanda cordycepin, adenosin, cordycepin gibi biyolojik ve farmakolojik aktivitelere sahip bol miktarda özel doğal bileşenler içermesi nedeniyle glikoz kontrolü, hipolipidemik, anti-tümör, anti-mikrobiyal, anti-viral, antiprotozoal, anti-inflamatuar, nöroprotektif, antioksidan ve immün-koruyucu aktiviteler dahil olmak üzere başta Çin olmak üzere birçok Asya ülkesinde yüzlerce yıldır ilaç olarak

kullanılmaktadır. Bu nedenle, Cordyceps militaris çeşitli rahatsızlıkların tedavisi için önemli bir aday olarak kabul edilebilir. (Phull ve ark. 2022).



Şekil 1. Cordyceps militaris

## MATERYAL VE METOD

Yaptığımız derleme çalışmasında; Cordyceps türleri, Cordyceps militaris, Cordycepin, biyoaktif bileşikler, biyolojik aktiviteler, antioksidan özellikler kelimeleri kullanılmıştır. İncelenen veri tabanları; Web of Science, US National Library of Medicine (PubMed), Google Scholar, Scopus, Medical Literature Analysis and Retrieval System Online (MEDLINE) ve EMBASE, gibi akademik veri tabanlarındaki bulunan makalelerin incelenmiştir. Çalışmamızın seçim kriterleri arasında çalışmanın derlemeyle ilgili olması, yapılmış ve sonuçlanmış çalışmalar olması, çalışmanın hakemli bir dergide yayımlanmış olması ve çalışmanın 2022 yılına kadar yayınlanmış olması dikkate alınmıştır.

## BULGULAR VE TARTIŞMA

Cordyceps militaris'in antioksidan aktivitesini DPPH yöntemi ile Shi ve ark., 201 tayin etmişlerdir. DPPH konsantrasyonunu başlangıç konsantrasyonunun %50 sine ($IC_{50}$) düşürmek için gereken antioksidan konsantrasyonu, antioksidan aktiviteyi belirlemek için bir parametredir. $IC_{50}$ ve DPPH süpürme aktivitesi arasında negatif bir korelasyon vardır. Sonuçlara bakıldığında, farklı öğütme süreleriyle işlenen toz numunelerin sulu ekstraktlarının 10 saat boyunca sırasıyla DPPH radikal süpürme yeteneğinin 1.54 ve 2.42 ml/ml'den 0.32 ve 0.34 ml/ml'ye öğütme süresindeki artışla birlikte düştüğünü, sulu ekstraktlarının antioksidan aktivitesinin, parçacık boyutunun azalmasıyla önemli ölçüde arttığını tespit etmişlerdir. DPPH radikal süpürme yeteneğinin cordycepin'in verimi ile pozitif bir korelasyon içinde olduğunu göstermişlerdir.

Chen ve ark, 2013, kültüre alınmış Cordyceps militaris'in in vitro antioksidan aktivitesi için DPPH, hidroksil radikali ve süperoksit radikal yakalama yöntemlerini kullanmışlardır. 3 farklı örnek için DPPH yöntemi ile $IC_{50}$'si sırasıyla W-CBP50, W-CBP50 I ve W-CBP50 II için 0.042, 0.019 ve 0.135 mg/ml, hidroksil radikali süpürme aktivitesi $IC_{50}$'si sırasıyla W-CBP50 I ve W-CBP50 için 1,485 ve 0,081 mg/ml, süperoksit radikali süpürme aktivitesini ise $IC_{50}$'si sırasıyla W-CBP50 ve W-CBP50 I için 0,144 ve 0,035 mg/ml olarak tayin etmişlerdir. Üçü arasında DPPH, hidroksil radikali ve süperoksit radikali yakalama aktivitelerinde W-CBP50I' in süpürme gücünü diğerlerinden daha güçlü bulmuşlardır. W-CBP50 II'nin DPPH üzerindeki süpürme gücü en düşük bulunmuştur. Bunun nedeni, saflaştırma işlemi sırasında kaybedilen bazı nedenlerden dolayı olabileceğini söylemişlerdir. Ayrıca, W-CBP50 II'nin her iki süperoksit radikali üzerindeki süpürme gücünü liyofilizasyon sonrasında çözünürlüğünün oldukça düşük olmasından dolayı tespit edememişlerdir.

Kim ve ark. 2015, Kore'de Üniversiteden kültürünü aldıkları Cordyceps miseli'nin antioksan aktivite tayinini yapmışlardır. Biyoaktivitedeki değişiklikleri incelemek için Cordyceps militaris miselini kullanarak U. pinnatifida'yı fermente etmişler U. pinnatifida, Cordyceps militaris miseli ve FUCM ekstraktlarının verimleri sırasıyla %15.22, %2.15 ve % 51.40 olmuştur. Cordyceps'in U. pinnatifida ve FUCM'un DPPH, hidroksil ve alkil radikal süpürme aktivitesi, ABTS ve FRAP yöntemleri ile antioksidan özelliklerini incelemişlerdir. Buldukları değerler sırasıyla U. Pinnatifida, Cordyceps ve FUCM; $IC_{50}$, 0.773 ± 0.032 0.318 ± 0.021ve 0.022 ± 0.002 $IC_{50}$, mg/ml bulmuşlardır. FRAP'a göre ekstraktın değerinin konsantrasyona bağlı olarak arttığını görmüşlerdir. FUCM özütünün (0,443±0,052 mM $FeSO_4$ eş./mg), U. pinnatifida özleri (0,125±0,040 mM $FeSO_4$ eş./mg) ve Cordyceps özlerinden (0,109±0,010 mM $FeSO_4$ eq./mg) ekstraktın 4 kat daha yüksek FRAP

değerine sahip olduğunu göstermiştir. Yine numunenin ABTS radikali süpürme aktivitesini, standart miktarda Trolox ile karşılaştırılmış ve ekstrakt FUCM için (0,396 ± 0,002 mM Trolox eq./mg ekstrakt); U. pinnatifida özlerininkine (0,413 ± 0,006 mM Trolox eq./mg özü) ve Cordyceps militaris özütlerinden daha yüksek (0,204 ± 0,002 mM Trolox eq./mg ekstresi) bulunmuştur. FUCM, U. pinnatifida ve Cordyceps militaris özlerinin ORAC değerleri sırasıyla 63,19 ± 2,12; 56,09 ± 1,19 ve 52,55 ± 2,57 uM TE/mg olarak bulunmuştur. Hidroksil radikali süpürme aktivitesi FUCM özlerinde (IC50, 0.475 ± 0.018 mg/ml), U. pinnatifida (IC50, 7.752 ± 0.221mg/ml) ve Cordyceps militaris ($IC_{50}$, 0.515 ± 0.016mg/ml) daha yüksek hidroksil radikali yakalama aktiviteleri göstermiştir. $Fe^{2+}/H_2O_2$ sisteminde üretilen hidroksil radikalleri, FUCM özleri ($IC_{50}$, 0.475 ± 0.018 mg/ml), U. pinnatifida ($IC_{50}$, 7.752 ± 0.221 mg/ml) veya Cordyceps militaris'ten($IC_{50}$, 0.515 ± 0.016mg/ml) daha yüksek hidroksil radikali süpürme aktiviteleri gösterdiğini tespit etmişlerdir.

Wu ve ark., 2012 de katı pirinç ortamı ve ipekböceği pupası üzerinde yetiştirilen kültür Cordyceps militaris'in meyve gövdelerinden, sıcak su, etanolle çöktürme ve etanol ile ekstrakte edilen kültüre edilmiş Cordyceps militaris'in meyve gövdelerinden elde edilen asidik polisakkaritler arasında yapısal karakterizasyon, antioksidan aktivite açısından farklılıklar olup olmadığını araştırmışlardır. ORAC değeri (mmol Trolox/g), TEAC değeri (µmol Trolox/g) OH süpürme aktivitesini hesaplamışlardır. Katı pirinç ortamı üzerinde yetiştirilen Cordyceps militaris'ten ekstrakte edilen polisakkaritin antioksidan aktivitesi, ipekböceği pupası üzerinde yetiştirilen Cordyceps militaris'ten biraz daha yüksek bulmuşlardır.

Prommaban ve ark. 2022, laboratuvarda üretilen kültür Cordyceps militaris mantarının antioksidan aktivitesi ile ilgili yaptıkları çalışmada konsantrasyona bağlı bir şekilde $ABTS^{\bullet+}$, $DPPH^{\bullet}$ ve lipid peroksidasyonunu inhibe ettiğini bulmuşlardır. $ABTS^{\bullet+}$radikali için sırasıyla meyvede ($IC_{50}$ değeri (mg/mL)) olarak; 0.24 ± 0.02 miselde 0.46 ± 0.01; $IC_{50}$; DPPH için meyvede 1.64 ± 0.35 miselde 1.20 ± 0.18; lipid peroksit inhibisyonunu meyvede 0.38 ± 0.08 ve miselde 5.53 ± 1.83 olarak bulmuşlardır. Ekstrelerin kimyasal bileşenleri ile antioksidan aktiviteleri arasındaki ilişkiyi, adenozin içeriğini lipid peroksidasyonu inhibe edici aktiviteyi güçlü bir şekilde etkileyen bir faktör olarak göstermiştir. Çalışmadan elde ettikleri bulguları, toplam fenolik içeriğin $ABTS^{\bullet+}$ inhibisyonu ve DPPH• inhibisyonu ile yüksek bir korelasyona sahip olması nedeni ile ekstraktların radikal süpürme aktivitelerinden sorumlu başlıca kimyasal bileşenler olarak fenolik bileşikleri göstermiştir. Negatif korelasyonların ise daha yüksek fenolik içeriğin daha düşük bir $IC_{50}$ değeri ile ilişkili olduğunu ortaya çıkarmıştır; %50 inhibisyon için daha düşük bir konsantrasyon gerektiğinden daha güçlü radikal yakalama aktivitesi olduğunu, fenolik bileşiklerin, hidroksil grupları yoluyla serbest radikal temizlemeyi kolaylaştıran temel kimyasal bileşenler olduğunu gösteren önceki araştırmalarla uyum içinde olduğunu söylemişlerdir. Ayrıca, bu çalışmada orta düzeyde bir korelasyonla yüksek indirgeme kapasiteli indirgeyicilerin varlığı, potansiyel antioksidan aktivite ile yüksek oranda ilişkili olduğundan flavonoidlerin sorumluluğunda $Fe^{3+}$ yı, $Fe^{2+}$ ye indirgeme yeteneklerinin olduğu belirtmişlerdir.

## SONUÇ

Günümüzde, çeşitli destek tedavi yaklaşımlarının doğal maddeler ve fonksiyonel gıdalar alınarak yapıldığı bilinmektedir. Avrupa Konsensüsüne göre en doğru tanım, "bir gıda, sağlığın iyileştirilmesine ve hastalık riskinin en aza indirilmesine katkıda bulunacak şekilde vücuttaki hedef işlevleri makul ölçüde geliştiriyorsa, yeterli besleyici etkilerinin ötesinde işlevsel olarak kabul edilebilir" şeklindedir. Fonksiyonel gıdalar doğal maddelerdir; bununla birlikte, sağlığı iyileştirmek veya cinsiyet ve yaşa dayalı olarak bireysel veya tanımlanmış gruplarda hastalıkları önlemeye yardımcı olmak için biyoteknolojik prosedürler yoluyla farklı bileşenler dahil edilebilir veya bunlardan çıkarılabilir (Phull ve ark. 2022). Burada derlemesini yaptığımız Cordyceps militaris'in ile yapılan antioksidan aktivite tayin yöntemleri incelenmiştir. İncelemelerden; Cordyceps militaris'in içeriğindeki bileşenlerden dolayı kuvvetli antioksidan aktiviteye sahip olduğu görülmektedir. Chaturvedi ve ark., 2018, son yıllara kadar yapılan çalışmaların çoğu hayvanlarda veya hücre kültürü modellerinde yapıldığını ve insan deneysel çalışmaları çok daha az olduğunu ve her zaman in vitro çalışmaları desteklemediğini söylemişlerdir. Bu nedenle antioksidan özelliklerini ve sağlık üzerindeki olumlu etkilerini düşündüğümüz ve bildiğimiz Cordyceps militaris'i tedavide kesinlikle doktor kontrolünde kullanılmasının gerekli olduğunu söyleyebiliriz.

## KAYNAKLAR

Alkın M, 2017, Türkiye'de Kültürü Yapılan ve Doğal Yenilebilir Mantarların Antioksidan ve Antimikrobiyal Aktivitelerinin Belirlenmesi. Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Isparta (Danışman: Prof. Dr. Atıf Can Seydim)

Baytop A, 1996, Farmasötik Botanik. 6.baskı. İstanbul: İstanbul Üniversitesi Yayınları No:3637, Eczacılık Fakültesi Yayınları No:58.

Blagodatski A, Yatsunskaya M, Mikhailova V, Tiasto V, Kagansky A, Katanaev VL 2018, Medicinal mushrooms as an attractive new source of natural compounds for future cancer therapy. Oncotarget. 2018; 9: 29259-29274.

Chakraborty S, Chowdhury S ve Nandi, G, 2014, Review on yarsagumba (Cordyceps sinensis)-an exotic medicinal mushroom. Int. J. Pharm. Phytochem. Res. 6, 339–346.

Chaturvedi VK, Agarwal S, Gupta KK 2018, Ramteke PW, and Sing MP, Medicinal mushroom: boon for therapeutic applications, Medicinal mushroom: boon for therapeutic applications, Biotech, 8(8): 334.

Chen X, Wu G, Huang Z, 2013, Structural analysis and antioxidant activities of polysaccharides from cultured Cordyceps militaris International Journal of Biological Macromolecules. 58;18–22.

Das G, ve ark., 2021, Cordyceps spp.: A Review on Its Immune-Stimulatory and Other Biological Cordyceps spp.: PotentialsFrontiers in Pharmacology. 11: 1-30.

Holliday J, Cleaver M, 2008, Medicinal Value of the Caterpillar Fungi Species of the Genus Cordyceps (Fr.) Link (Ascomycetes). A Review. Int J Med Mushr.10.3:219–234.

Jin Y, Meng X, Qiu Z, Su Y, Yu P, Qu P, 2018, Anti-tumor and anti-metastatic roles of cordycepin, one bioactive compound of Cordyceps militaris. Saudi J. of Biological Sciences. 25:5:991-995.

Kim SY, Kim EU, Hwang JW, Han YK, Kim ES, Jeong JH, Moon SH, Jeon BT, Park PJ, 2015, Radical Scavenging Activities of Undaria pinnatifida Extracts Fermented with Cordyceps militaris Mycelia. J. Microbiol. Biotechnol. 25(6), 820–827.

Liu Y, Wang J, Wang W, Zhang H, Zhang X, Han C, 2015, The Chemical Constituents and Pharmacological Actions of Cordyceps sinensis. Evidence-Based Complementary and Alternative Medicine. 1–12.

Maity D, 2013, A study on ethnomedicinal uses of yartshagumba, Cordyceps sinensis (berk.) sacc. (cordycipitaceae), by the trinal communities of North Sikkim and its conservation. Explore. Anim. Med. Res. 3, 95–101.

Masuda M, Urabe E, Sakurai A, Sakakibara M, 2006. Production of cordycepin by surface culture using the medicinal mushroom Cordyceps militaris. Enzyme and Microbial Technology 39:641–646.

Panda AK, Swain KC, 2011.Traditional uses and medicinal potential of Cordyceps sinensis of Sikkim. J Ayurveda Integr Med 2:9–13.

Olatunji OJ, Tang J, Tolac A, Auberond F, Oluwaniyie O, Ouyangb Z, 2018, The genus Cordyceps: An extensive review of its traditional uses, phytochemistry and pharmacology, Fitoterapia. 129:293–316.

Patel KJ, Ingalhalli S, (2013). Cordyceps militaris (L.: Fr.) Link – An Important Medicinal Mushroom. Journal of Pharmacognosy and Phytochemistry. 2 :1, 315-319.

Phull AR, Ahmed M, ve Park HJ, 2022, Cordyceps militaris as a Bio Functional Food Source: Pharmacological Potential, Anti-Inflammatory Actions and Related Molecular Mechanisms. Microorganisms 10, 405.

Rao YK, Fang SH, Wu WS ve Tzeng YM, 2010. Constituents isolated from Cordyceps militaris suppress enhanced inflammatory mediator's production and human cancer cell proliferation. J. Ethnopharmacol. 131, 363–367.

Prommaban A, Sriyab S, Marsup P, Neimkhum W, Sirithunyalug J, Anuchapreeda S, To-anun C, ve Chaiyana W, 2022, Comparison of chemical profiles, antioxidation, inhibition of skin extracellular matrix degradation, and anti-tyrosinase activity between mycelium and fruiting body of Cordyceps militaris and Isaria tenuipes, Pharm Biol. 60(1): 225–234.

Shashidhar MG, Giridhar P, Sankar KU, Manohar B, 2013, Bioactive principles from Cordyceps sinensis: A potent food supplement - A review. J Funct Foods . 2013 Jul;5(3):1013-1030.

Shi H, Zhang M, Bhandari B, Wang Y, Yi S, 2019, Effects of superfine grinding on the properties and qualities of Cordyceps militaris and its spent substrate, J Food Process Preserv. 2019;43:e14169.

Singh N, Pathak R, Singh AK, Rautela D, Dubey A (2010) Collection of Cordyceps sinensis (Berk.) Sacc. in the interior villages of Chamoli district in Garhwal Himalaya (Uttarakhand) and its social impacts. J Am Sci 6:5–9

Tuli HS, Sandhu SS, Sharma AK, 2014, Pharmacological and therapeutic potential of Cordyceps with special reference to Cordycepin. Biotech 4:1–12.

Uhrinová, A, Poľančíková, N, 2018, Antioxidant Activity Of The Fungus Cordyceps sinensis grown on two different media, Folia Veterinaria. 62,3: 68-73.

Wasser PS, 2010, Medicinal Mushroom Science:History, Current, Status, Future trends, and unsolved Problems. Applied Microbiology and Biotechnology. 12(1): 1-16.

Wu F, Yan H, Ma X, Jia J, Zhang G, Guo Z ve Gui Z, 2012, Comparison of the structural characterization and biological activity of acidic polysaccharides from Cordyceps militaris cultured with different media. World Journal of Microbiology and Biotechnology volume 28:2029–2038.

## *ORAL PRESENTATION*

# Biyolojik Silah ve Biyoajanlar

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## Özet

Biyolojik savaş ve biyoterörizm, esas olarak silah olarak kullanılabilecek birçok ajan ve çevreye ve nüfusa yayılmanın çok çeşitli yolları nedeniyle çok karmaşık konulardır. Eitzen 1997 yılında, biyolojik bir ajanı potansiyel bir biyolojik silah yapan özellikleri tanımlamıştır. İdeal olarak, bir biyolojik silahın bulunması veya üretilmesi kolay olmalıdır. İdeal biyolojik silahın ayrıca etkilenenleri etkisiz hale getirme kapasitesinin yüksek olması veya alternatif olarak son derece ölümcül olması gerekir. Ani veya gecikmeli etkilerin gerekip gerekmediğine bağlı olarak inkübasyon süresi olan bir ajan seçilmesi uygundur. Biyolojik bir silahın diğer önemli özellikleri, bulaşma yolu ve dolayısıyla uygun bir dağıtım yöntemiyle yayılma kolaylığıdır. Son olarak, sistemin istikrarı özellikle büyük miktarların belirsiz sürelerle depolanması gerektiğinde ajan değerlendirilmelidir.
ABD Hastalık Kontrol ve Önleme Merkezleri, biyoterörizm saldırısını "insanlarda, hayvanlarda veya bitkilerde hastalığa veya ölüme neden olmak için kullanılan virüslerin, bakterilerin veya diğer mikropların (ajanların) kasıtlı olarak salınması" olarak tanımlar. Biyolojik ajanları üç kategoriye ayırır:
1. Kategori A: Kişiden kişiye kolayca yayılabilen veya iletilebilen ajanlar. Yüksek ölüm oranlarına neden olurlar ve önemli halk sağlığı etkisi potansiyeline sahiptirler..
2. Kategori B: Yayılması orta derecede kolay olan ajanlar. Orta derecede morbidite oranları ve düşük mortalite ile sonuçlanırlar.
3. Kategori C: Kütle için tasarlanabilen gelişmekte olan ajanlar kullanılabilirliklerinden dolayı gelecekte yaygınlaştırılacaktır. Üretilmesi ve yayılması kolaydır. Potansiyel olarak yüksek morbidite ve mortalite oranları ve önemli sağlık etkileri ile bağlantılıdırlar.
Biyolojik ajanlar, diğer konvansiyonel olmayan silahlarla (kimyasal veya radyolojik) karşılaştırıldığında benzersiz bir özelliğe sahiptir; toksinler hariç, konakta çoğalabilir ve sırayla diğer bireylere bulaşabilirler. Bu nedenle, daha fazla yayılmayı kontrol altına almak için uygun sınırlama önlemlerinin alınması için bir biyolojik saldırının derhal tanımlanması esastır. Bu nedenle, biyolojik ajanların hem kasıtlı hem de kasıtsız salınması durumunda derhal harekete geçmek için biyolojik ajanları uzun menzilli tespit etmek için yeni teknolojilerin geliştirilmesine açık bir ihtiyaç vardır.

**Anahtar Kelimeler:** Biyolojik silah, biyolojik ajan, mikroorganizma

# Biological Weapons and Bioagents

## Abstract

Biological warfare and bioterrorism are very complex issues, mainly due to the many agents that can be used as weapons and the wide variety of ways they can spread to the environment and population. Eitzen described the properties that make a biological agent a potential biological weapon in 1997. Ideally, a biological weapon should be easy to find or manufacture. The ideal biological weapon should also have a high capacity to incapacitate those affected, or alternatively be extremely deadly. It is appropriate to choose an agent with an incubation time depending on whether immediate or delayed effects are required. Other important characteristics of a biological weapon are its mode of transmission and thus ease of spread with an appropriate method of distribution. Finally, the stability of the system should be evaluated, especially when large quantities need to be stored for indefinite periods of time.

The U.S. Centers for Disease Control and Prevention defines a bioterrorism attack as "the deliberate release of viruses, bacteria, or other microbes (agents) used to cause disease or death in humans, animals, or plants." It divides biological agents into three categories:

1. Category A: Agents that can spread or be transmitted easily from person to person. They cause high mortality rates and have the potential for significant public health impact.

2. Category B: Agents that are moderately easy to spread. They result in moderate morbidity rates and low mortality.

3. Category C: Emerging agents that can be designed for mass will be expanded in the future due to their availability. It is easy to produce and spread. They are associated with potentially high morbidity and mortality rates and significant health effects.

Biological agents have a unique property when compared to other non-conventional weapons (chemical or radiological); Except for toxins, they can multiply in the host and in turn infect other individuals. Therefore, prompt identification of a biological attack is essential so that appropriate containment measures are taken to contain its further spread. Therefore, there is a clear need to develop new technologies for long-range detection of biological agents to take immediate action in case of both deliberate and unintentional release of biological agents.

**Keywords:** Biological weapon, biological agent, microorganism

## GİRİŞ

DaSilva (1999) biyolojik savaşı, insanlar, çiftlik hayvanları ve mahsuller arasında hastalık ve ölüm meydana getirmek için genellikle mikrobiyal, bitki veya hayvan kaynaklı mikroorganizmaların ve toksinlerin kasıtlı kullanımı olarak tanımlamıştır.Biyolojik savaş ve biyoterörizm, esas olarak silah olarak kullanılabilecek birçok ajan ve çevreye ve nüfusa yayılmanın çok çeşitli yolları nedeniyle çok karmaşık konulardır. Biyolojik bir olay, en az iki aktörün varlığını sağlar: bir veya daha fazla patojen (bakteri, virüs veya toksin) ve bunların yayılması için bir araç. Potansiyel biyolojik ajanların yüksek yayılma kapasitesi ve öldürücülüğüne ek olarak, görünmezlikleri ve son derece zor kısa süreli tespitleri, müteakip enfeksiyonlardaki artışa kadar hemen tanı koymayı imkansız kılmaktadır.Aslında, çoğu biyolojik silah (örneğin, toksinler ve bakteri sporları hariç), diğer geleneksel olmayan silahların (kimyasal ve radyolojik gibi) sahip olmadığı benzersiz bir kaliteye sahiptir; biyolojik ajanlar konakçı organizmada çoğalabilir ve sırayla yeni konakçılara bulaşabilir, bu şekilde hem kurban sayısı hem de coğrafi yayılım açısından popülasyon üzerinde öngörülemeyen etkiler yaratır ( Zalini, 2010; Vogel, 2012; Tucker, 2013).

## BİYOLOJİK AJANLAR

ABD Hastalık Kontrol ve Önleme Merkezleri ise (CDC), biyoterörizm saldırısını “insanlarda, hayvanlarda veya bitkilerde hastalığa veya ölüme neden olmak için kullanılan virüslerin, bakterilerin veya diğer mikropların (ajanların) kasıtlı olarak salınması” olarak tanımlar. Özette açıklandığı gibi biyolojik ajanları A,B ve C olmak üzere üç kategoride sınıflandırır.

Genel olarak biyolojik ajanlar (biyo-silah olarak kullanılanlar dahil), sağlığa yönelik tehlikeyi tanımlayan belirli özelliklere göre daha fazla sınıflandırılabilir:

a. Enfektivite: Bir ajanın konakçıya nüfuz etme ve çoğalma yeteneği.

b. Patojenite: Etkenin vücuda girdikten sonra hastalığa neden olma yeteneği.

c. Bulaşabilirlik: Etkenin enfekte bir kişiden sağlıklı bir kişiye bulaşma yeteneği

d. Nötralize etme yeteneği: Önleyici araçlara ve/veya tedavi amaçlı amaçlara sahip olma anlamına gelir.

Biyolojik ajanlar bir veya daha fazla yolla bulaşabilir (La Placa, 2010):

a. Parenteral: Vücut sıvıları veya kan yoluyla bulaşan ajanlar.

b. Havayolu (damlacıklar): Enfekte kişiler tarafından yayılan ve sonra çevredeki insanlar tarafından solunmalıdır.

c. Temas: Enfekte organizmanın yüzeyinde bulunan ajanların başka bir organizmayı enfekte edebildiği.

d. Oral-fekal yol: Enfekte hastaların dışkılarıyla kontamine olan nesneler, yiyecekler veya diğer maddeler veya cinsel temas yoluyla.

## BİYOLOJİK SİLAH OLARAK KULLANILABİLECEK BİYOLOJİK AJANLAR

İnsanlarda, hayvanlarda ve bitkilerde hastalıklara neden olan çok sayıda patojen (bakteri, virüs ve toksin) varken, çok azı biyolojik silah olma özelliklerine sahiptir. Eitzen (1997), biyolojik bir ajanı potansiyel bir biyolojik silah yapan özellikleri tanımladı. İdeal olarak, bir biyolojik silahın bulunması veya üretilmesi kolay olmalıdır. Hassas hedeflere veya popülasyona yönelik biyolojik bir saldırı geliştirmek için aslında çok miktarda biyolojik ajan gereklidir; bir hedefte hastalık oluşturabilmek için çok sayıda biyolojik ajana (veya belirli miktarda toksine) sahip olmanın gerekli olduğu düşünülmelidir. İdeal biyolojik silahın ayrıca etkilenenleri etkisiz hale getirme kapasitesinin yüksek olması veya alternatif olarak son derece ölümcül olması gerekir. Ani veya gecikmeli etkilerin gerekli olup olmamasına bağlı olarak inkübasyon süresi olan bir ajan seçilmesi uygundur. Biyolojik bir silahın diğer önemli özellikleri, bulaşma yolu ve dolayısıyla uygun bir dağıtım yöntemiyle yayılma kolaylığıdır. Son olarak, özellikle büyük miktarların belirsiz sürelerle saklanması gerektiğinde, ajanın kararlılığı değerlendirilmelidir (Kortepeter ve Parker, 1999).

## BAKTERİLER

**Bacillusanthracis:** Bacillusanthracis, genellikle bir kapsül ile çevrili Gram pozitif, hareketsiz, fakültatif anaerobik endospor oluşturan bir bakteridir. En sık olarak otçulların epizootik veya enzootiklerininkontamine toprakla doğrudan temastan sporlar edindikten sonra enfekte olması durumunda ortaya çıkan şarbonun etiyolojik ajanıdır. İnsanlarda hastalık, enfekte hayvanlara, enfekte hayvanlardan alınan dokulara veya yüksek konsantrasyonlarda şarbon sporlarına maruz kaldığında ortaya çıkabilir. Şarbon endosporları ölçülebilir bir metabolizmaya sahip değildir, bölünmezler ve kurumaya, ısı-ultraviyole ve iyonlaştırıcı radyasyona, kimyasal dezenfektanlara ve diğer stres biçimlerine karşı dirençlidirler, çevrede yıllarca kalırlar (Bhalla&Warheit, 2004), toprakta hayatta kalma süresi 200 yıla kadar rapor edilmektedir (Yuen, 2001).

**Clostridiumbotulinum:** Clostridiumbotulinum, doğal yaşam alanı olan topraktan izole edilebilen, botulizmin etiyolojik ajanı olan, spor oluşturan ve zorunlu anaerob bir maddedir. C. botulinum'un farklı genomları ve ortak özellikleri ile karakterize edilen dört türü bilinmektedir. Botulinum toksini. ek olarak, yedi farklı antijenik tipte botulinum toksini (A-G), çapraz nötralizasyonun olmaması ile tanımlanır. Toksin hastalıktan sorumludur ve bir iki zincirli polipeptiddir: 100 KDa'lık bir ağır zincir, asetilkolin içeren veziküllerin terminal membranla kaynaşmasını engelleyen çinko içeren endopeptidaz olan 50 KDa'lık bir hafif zincire tek bir disülfid bağıyla birleştirilir. motor nöronun gevşemesi, gevşek kas felci ile sonuçlanır (Arnon ve diğerleri, 2001). Botulinum toksini bilinen en öldürücü toksindir ve yedi türün tümü benzer şekilde hareket eder. Ölüm genellikle faringeal ve diyafragma kaslarının felci ve ardından solunum durması sonucu meydana gelir (Bhalla ve Warheit, 2004).

**Yersiniapestis:**Yersiniapestis, hem aerobik hem de anaerobik koşullarda yetişen, Gram negatif hareketsiz, spor oluşturmayan bir kokobasildir. Nemli toprakta veya suda günlerce canlı kalabilir, ancak doğrudan güneş ışığına maruz bırakılarak öldürülür (WHO, 2004). Bakteri, insanları ve hayvanları etkileyebilen bir hastalık olan vebanın etiyolojik ajanıdır. (La Placa, 2010). Yabani kemirgenler patojen taşıyıcılardır ve diğer hayvanlara bulaşma pireler, enfekte hayvan dokuları, kontamine toprak veya solunum damlacıklarına maruz kalma yoluyla gerçekleşir. Endemik kırsal alanlarda, Y. pestis'in yabani kemirgen konakçılarıyla temas eden

kişiler, iki şekilde görülen vebadan etkilenebilirler; hıyarcıklı ve pnömonik vebalar (WHO, 2004). Bubonik veba, maruziyetten 2-6 gün sonra kuluçka süresi olan pirelerin hastalık taşıyıcısı olarak kullanılması durumunda ortaya çıkar. Lenf düğümlerinin şişmesi (hıyarcıklar), ateşin başlaması, titreme, baş ağrısı ve ardından mide bulantısı ve kusma ile birlikte oluşur. Tedavi edilmeyen hıyarcıklı veba septisemiye neden olur. Pnömonik veba, solunan organizmalardan veya enfekte kana maruz kalmaktan oluşabilir.Kanlı balgamlı üretken öksürük, öksürük yoluyla kişiden kişiye yayılabilen tipik bir pnömonik veba belirtisidir (La Placa, 2010).

**Francisellatularensis:** Francisellatularensis küçük, Gram negatif, hareketsiz, fakültatif hücre içi, aerobik kokobasildir. Zoonotik bir hastalık olan tularemiden sorumludur. İki bakteri alt türü mevcuttur; F. tulariensistulariensis (Tip A) ve F. tularensispalaearctica (Tip B). Tip A, Tip B'den daha öldürücüdür (WHO, 2004). Organizma toprakta, suda, samanda ve toprakta birkaç haftaya kadar hayatta kalabilir. Birçok vahşi hayvan (tavşan, kunduz, misk sıçanı, yabani tavşan, tarla faresi) patojen taşıyıcılarıdır. İnsanlar, eklembacaklılar tarafından ısırıldığında, kontamine yiyecek ve suyun yutulmasıyla ve kontamineaerosollerin solunmasıyla enfekte olabilir. Enfekte hayvanlarla doğrudan temas da insanlar için tehlikelidir, ancak kişiden kişiye bulaşma gözlemlenmemiştir (Bhalla ve Warheit, 2004). Enfeksiyondan sonra kuluçka süresi genellikle 3-5 gündür ancak 14 güne kadar uzayabilir. Hastalığın semptomları, bulaşıcı ajanın virülansına bağlıdır. İki farklı klinik belirti mevcuttur; ülseroglandüler (vakaların %75'i) ve tifo (vakaların %25'i) tularemi. İlki, giriş yerinde yavaş ülser ve lokal lenf bezlerinin ağrılı şişmesi ile karakterizedir; "Tifo tularemi" ifadesi, görünürde birincil enfeksiyon bölgesi olmayan sistemik hastalığı belirtir. Ağrılı farenjit ve servikallenfadenit, kontamine yiyecek veya suyun yutulması yoluyla enfeksiyondan kaynaklanır (Bhalla ve Warheit, 2004).

## VİRÜSLER

**Variolamajor ve Poxviridae:** Poxviridae, yalnızca omurgalı veya omurgasız hücrelerinin sitoplazmasında çoğalan, genetik olarak ilişkili, büyük, zarflı DNA virüslerinin bir ailesini içerir. Yalnızca çiçek hastalığı, maymun çiçeği, aşı ve sığır çiçeği içeren Orthopoxvirus cinsinin üyesi insanları enfekte edebilir. Bunlardan sadece çiçek hastalığı kolayca tükürük veya burun salgısı damlacıkları ve kontamine nesneler yoluyla kişiden kişiye bulaşır (Moss, 2007).Hastaların çoğunda ekimoz, mukozal kanama ve hematemez gibi açık hemorajik belirtiler de mevcuttur. Ölümler tipik olarak semptomların başlamasından 8-16 gün sonra meydana gelir ve ölüm genellikle şiddetli yaygın neden olur. Koagülopati, çoklu organ yetmezliği, şok ve koma (Brauburger ve ark., 2012). Filoviral enfeksiyonlara karşı spesifik bir tedavi yoktur ve semptomları sınırlamak için destekleyici bakım sağlanır (Clark ve ark., 2012). Yüksek öldürücülük ve enfektivite ile birlikte onaylanmış terapötiklerin veya aşıların olmaması nedeniyle, Filoviridae ile çalışmak yüksek içerikli BSL-4 laboratuvarlarıyla sınırlıdır (DHHS, 2009).

**Arenaviridae:** Arenaviridae ailesi, kemirgenlerde (hayvanlarda) kronik enfeksiyonlara ve insanlarda zoonotik olarak edinilmiş hastalıklara neden olan zarflı, negatif sarmallı, iki parçalı RNA virüslerinden oluşur (Salvato ve ark., 2011). Arenavirus cinsi, genetik ve coğrafi verilere göre iki gruba ayrılan 22 viral tür içerir;

Eski Dünya (OW) ve Yeni Dünya (KB) kompleksleri. OW kompleksi, insanlarda akut aseptik meningosefalite neden olan dünya çapında yayılmış Lenfositikkoriomenenjit virüsünü (LCMV) ve Afrika kıtasına endemik olan Lassa (LASV) ve Lujo (LUJV) virüslerini içerir.

hemorajik ateş (HF). Daha büyük NW arenavirüs grubu ayrıca üç bölüme ayrılmıştır; A, B ve C. Clade B, Güney Amerika'da HF'ye neden olan arenavirüslerin çoğunu içerdiğinden, insan patolojisi açısından daha alakalıdır (Charrel& de Lamballerie, 2003).

**Filoviridae:** Filoviridae ailesi (Latince terim filumdan, virion şekline atıfta bulunur), insanlarda ve insan dışı primatlarda ciddi zoonotik kanamalı ateşe neden olan zarflı, negatif sarmallı RNA virüslerinden oluşur. Aile iki ayrı cins içerir; Marburg virüsü ve Ebola virüsü. Marburgvirus cinsi şunları içerir: Marburg (MARV) ve Ravn (RAVV) virüsleri olmak üzere iki üyesi olan Marburgmarburgvirus. Ebolavirus cinsi, her biri tek bir

üyeye sahip olan beş tür içerir; Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), TaïForestebolavirus (TAFV), Bundibugyoebolavirus (BDBV) ve Restonebolavirus (RESTV) (Adams &Carstens, 2012).

## TARTIŞMA

Biyolojik ajanların biyolojik silah olarak kullanımının kökleri, bakteri, toksin veya virüs kavramlarının henüz bilinmediği eski çağlara dayanmaktadır. 2000 yılı aşkın bir süre önce, biyolojik savaşın ilkel teknikleri, insanlar arasındaki ilk anlaşmazlıkları çözdü. Modern bilimin evrimi ile birlikte (özellikle 18. yüzyılda) biyolojik ajanların biyolojik silah olarak kullanılma olasılığı rafine edilmiştir. Son birkaç on yılda, yenilikçi biyoteknoloji tekniklerinin gelişimi, daha agresif biyolojik silahlar yaratma bilgisini sağlamıştır.

Bu yeni organizmalar, en tehlikeli vahşi tip biyolojik ajanlarla aynı seviyede, hatta onlardan daha yüksek, yıkıcı ve tamamen beklenmedik etkiler üretebildikleri için büyük endişe yaratıyor. Uluslararası sözleşmeler biyolojik ajanların saldırı amaçlı kullanımını yasaklasa da birçok terörist grubun biyolojik ajanların biyolojik silah olarak olası kullanımı konusunda araştırmalarını sürdürdüğü bilinmektedir. Mağdurlar, hem nesnel zorluklardan potansiyel bir saldırının tespitinde. Biyolojik ajanlar, diğer konvansiyonel olmayan silahlarla (kimyasal veya radyolojik) karşılaştırıldığında benzersiz bir özelliğe sahiptir; toksinler hariç, konakta çoğalabilir ve sırayla diğer bireylere bulaşabilirler. Bu nedenle, daha fazla yayılmayı kontrol altına almak için uygun sınırlama önlemlerinin alınması için bir biyolojik saldırının derhal tanımlanması esastır. Bu nedenle, biyolojik ajanların hem kasıtlı hem de kasıtsız salınması durumunda derhal harekete geçmek için biyolojik ajanları uzun menzilli tespit etmek için yeni teknolojilerin geliştirilmesine açık bir ihtiyaç vardır.

## REFERANSLAR

Acheson, N.H. (2011). *Fundamentals of Molecular Virology.* Wiley, New York.

Adams, M..J. &Carstens, E.B. (2012). Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses. *Arch. Virol.m***157**:1411–1422.

Aldhous P. (1990). Biological warfare: Gruinard Island handed back. *Nature,* **344**:801.

Arnon, S.S., Schechter, R., Inglesby, T.V., Henderson, D.A., Bartlett, J.G., Ascher, M.S., Eitzen, E., Fine, A.D., Hauer, J., Layton, M., Lillibridge, S., Osterholm, M.T., O'Toole, T., Parker, G., Perl, T.M., Russell, P.K., Swerdlow, D.L. & Tonat, K. (2001). Botulinum toxin as a biological weapon: medical and public health management. JAMA, 285:1059-1070.

Bente, D., Gren, J., Strong, J.E. &Feldmann, H. (2009). Disease modeling for Ebola And Marburg viruses. *Dis. Model. Mech.* **2**:12-17.

Bhalla, D.K. &Warheith, D.B. (2004). Biological agents with potential form isuse: A historical perspective and defense measures. *Toxicol. Appl. Pharm.*,**199**:71-84.

Brauburger, K.,Hume, A.J., Mühlberger, E. &Olejnik, J. (2012). Forty-five years of Marburg virus research. *Viruses*, **4**:1878-1927.

Briese, T.,Paweska, J.T., McMullan, L.K., Hutchison, S.K., Street, C., Palacios, G.,
Khristova, M.L.,Weyer, J., Swanepoel, R., Egholm, M., Nichol, S.T. &Lipkin, W.I. (2009). Genetic detection and characterization of Lujovirus, a new hemorrhagic fever-associated arena virus from Southern Africa. *PLoSPathog.* **5**: e1000455.

Buchmeier, M.J., de la Torre J.-C. &Peters C.J. (2007). Arenaviridae: The Viruses and their Replication. *In:* Knipe D.M. and Howley P.M. (Eds.), *FieldsVirology*. Lippincott Williams &Wilkins, Philadelphia, PA, USA, pp.1791–1827.

Bhalla, D.K. & Warheit, D.B. (2004). Biological agents with potential for misuse: a historical perspective and defensive measures. Toxicol Appl Pharmacol., 199:71-84.

Burri, D.J., da Palma, J.R., Kunz, S. &Pasquato A. (2012). Envelopeglycoprotein ofarenaviruses. *Viruses*, **17**: 2162-2181.

Christopher, G.W.,Cieslak, T.J., Pavlin J.A. &Eitzen, E.M. (1997). Biological warfare: a historical perspective. *JAMA*, **278**: 412-417.

Charrel, R.N. & de Lamballerie, X. (2003). Arenaviruses other than Lassa virus. Antiviral Res., 57: 89-100.

Clark, D.V.,Jahrling, P.B. &Lawler, J.V. (2012). Clinical management of filovirus infected patients. *Viruses*, **4**:1668-1686.

Cronin, A.K. (2004). Terrorist motivations for chemical and biological weapons use: Placing the threat in context. *Defense Secur. Anal.*, **20**:313-320.

DaSilva, E. (1999). Biological warfare, bio terrorism, bio defence and toxin weapons convention. *EJB*, **2**:99-129.

Davis, C.J. (1999). Nuclear blindness: An overview of the biological weapons programs of the former Soviet Union and Iraq. *Emerg. Infect. Dis.*,**5**: 509-
512.

Dembek, Z.F. (2006). The history and threat of biological weapons and bio terrorism. *In*McIsaac J.H. (ed.), *Hospital Preparation for Bioterror: A Medical and Biomedical Systems Approach.* Elsevier, London.

Dennis, D.T.,Inglesby, T.V., Henderson, D.A., Bartlett, J.G., Ascher, M.S., Eitzen, E., Fine, A.D., Friedlander, A.M., Hauer, J.,Layton, M., Lillibridge, S.R., McDade, J.E., **18.**Osterholm, M.T., O'Toole, T., Parker, G., Perl, T.M., Russell, P.K. &Tonat, K. (2001). Tularemia as a biological weapon – Medical and public health management. *JAMA*, **285**: 2763–2773.

Department of Health and Human Services (DHHS) (2009). *Biosafety in Microbiological and Biomedical Laboratories, 5th ed.* U.S. Department of Health and Human Services (DHHS), Washington, D.C.Department of Health and Human Services (DHHS) (2010).

Eitzen M.S., E., Fine, A.D., Hauer, J., Layton, M., Lillibridge, S., Osterholm, M.T., O'Toole, T., Parker, G., Perl, T.M., Russell, P.K., Swerdlow, D.L. &Tonat, K. (2001). Botulinum toxin as a biological weapon: medical and public health management. *JAMA*, **285**:1059-1070.

Gonzalez, J.P.,Lukashevich, I.S., Peters, C.J. &Romanowski, V. (2011). Arenaviridae. *In*

Harris, S. (2002). *Factories of Death: Japanese Biological Warfare, 1932-1945, and*
*The American Cover-Up*. Routledge, London. Hugh-Hones, M. (1992). Wickham Steed and German biological warfare research. *Intell. Nat. Secur.*,**7**:379-402.

King, A.M.Q., Adams, M.J., Carstens, E.B., and Lefkowitz, E.J. (Eds). *Virus Taxonomy Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses.* Elsevier-AcademicPress, Oxford, pp. 715–724.

Knipe, D.M.,Howley, P.M., Esposito, J.J. &Fenner, F. (2001). Pox viruses. *In:* Knipe D.M. and Howley P.M. (Eds.), *Fields Virology*. Lippincott Williams&Wilkins, Philadelphia, PA, USA, pp. 2885–2921. Kortepeter, M.G. &Parker, G.W. (1999). Potential biological weapons threats. *Emerg. Infect. Dis.* **5**:523– 527.

Kortepeter, M.G. & Parker, G.W. (1999). Potential biological weapons threats. Emerg. Infect. Dis. 5:523– 527.

Kuhn J.H.,Dodd, L.E., Wahl-Jensen, V., Radoshitzky, S.R., Bavari, S. &Jahrling,P.B. (2011). Evaluation of perceived threat differences posed by filo virus variants. *Biosecur. Bioterror.* **9**:361–371. La Placa, M. (2010). *Principles of Medical Microbiology*. Esculapio, Lahore.

La Placa, M. (2010). Principles of Medical Microbiology. Esculapio, Lahore.

Moss, B. (2007). Poxviridae: The viruses and the irreplication. *In:* Knipe D.M. and Howley P.M. (Eds.), *Fields Virology*. Lippincott Williams &Wilkins, Philadelphia, Pennsylvania, pp. 2905–2946.

Salvato, M.S., Clegg, J.C.S., Buchmeier, M.J., Charrel, R.N., Gonzalez, J.P., Lukashevich, I.S., Peters, C.J. & Romanowski, V. (2011). Arenaviridae. In King, A.M.Q., Adams, M.J., Carstens, E.B., and Lefkowitz, E.J. (Eds). Virus Taxonomy Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier-Academic Press, Oxford, pp. 715–724.

Sanchez, A.,Geisbert, T.W. &Feldmann, H. (2007). Filoviridae: Marburgand Ebola Viruses. *In* Knipe D.M. and Howley P.M. (Eds.), *Fields Virology*. Lippincott Williams &Wilkins, Philadelphia, Pennsylvania, pp. 1409–48.

Schattner, M.,Rivadeneyra, L., Pozner, R.G. &Gómez, R.M. (2013). Pathogenic Mechanisms involved in the hematological alterations of arenavirus-induced Hemorrhagic fevers. *Viruses*, **5**:340-351.

Sokolski, H.D. & James M. Ludes. (2001). *Twenty-First Century Weapons Proliferation*. Routledge, London. Stochkholm International Peace Research Institute (SIPRI) (1971a). *The Problem of Chemical and Biological Warfare, Vol. 1: The Rise of CB Weapons*.

Stochkholm International Peace Research Institute (SIPRI) (1973). *The Problem of Chemical and Biological Warfare, Vol. 3: CBW and the Law of War*. Humanity Press. New York.

Tucker, J.B. (2013). The current bio weapons threat. *In* Hunger, I., Radosavljevic, V.,Belojevic, G. &Rotz, L.D. (Eds.), *Biopreparedness and Public Health*.Springer, Amsterdam, pp. 7-16.

Vela, E. (2012). Animal models, prophylaxis, and therapeutics for arenavirus infections. *Viruses*, **4**:1802-1829.

Vogel, K.M. (2012). *Phantom Menaceor Looming Danger: A New Framework for Assessing Bioweapons Threats*. JHU Press, New York.

Warfield, K.L.,Swenson, D.L., Demmin, G., &Bavari, S. (2005). Filovirus-like particles as vaccines and discovery tools. *Expert Rev. Vaccines*, **4**:429-440.

Weber, D.J. &Rutala, W.A. (2001). Risks and prevention of nosocomial transmission of rare zoonotic diseases. *Clin Infect Dis.*, **32**:446-456.

Weelis, M. (2002). Biological warfare at the 1346 siege of Caffa. *Emerg. Infect. Dis.*,**8**: 971-975.

World Health Organization (WHO) (2004). *Public Health Response to Biological And Chemical Weapons*. World Health Organization (WHO), Geneva, Switzerland.

World Health Organization (WHO) (2007). *WHO Guidelines on Tularaemia*. World Health Organization (WHO), Geneva, Switzerland. Yuen, E.C.P. (2001). Biological warfare: The facts. *Hong Kong J. of Emerg. Med.***8**:232-240.

Yuen, E.C.P. (2001). Biological warfare: The facts. Hong Kong J. of Emerg. Med. 8:232-240.

Zalini, Y. (2010). Combating and reducing the risk of biological threats. *J. Defence Secur.*,**1**: 1-15.

## *ORAL PRESENTATION*

# Biyoteknolojinin Biyosavunmadaki Önemi

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## Özet

Hastalığı bir silah olarak kullanma kavramı yüzyıllardır var olmuştur. Biyolojik araştırmadaki gelişmeler, muhtemelen yeni etkiler ortaya çıkarmak için tasarlanmış yeni bir gelişmiş biyolojik savaş ajanları sınıfının geliştirilmesine izin verecektir. Buna ek olarak, biyoteknoloji gelişmiş biyolojik savaş ajanları'nın silahlandırılmasını, yayılmasını ve dağıtımını destekleyen uygulamalara sahip olacaktır. Bu tür yeni aracılar ve dağıtım sistemleri, biyolojik savaşlardaki paradigma'yı genişleterek çeşitli yeni kullanım seçenekleri sağlayacaktır. Biyoteknoloji biyolojik silahlar için biyolojik ajanların geliştirilmesi, silahlandırılması ve sınırlamalarının altında yatan kavramlarda yenilik yaratma potansiyelini açısından iyi bir araçtır. Moleküler biyolojik teknikler biyolojik savaşa uygulandığında biyoteknolojinin, geleneksel ajanlar tarafından sunulan tehditlere karşı koymayı hedefleyen biyolojik savunma çabalarına karşı nasıl mücadele ettiği önem kazanmaktadır. Rekombinant DNA teknolojisinin ortaya çıkmasıyla birlikte, araştırmacılar bir organizmanın genetik yapısını değiştirmek için standart metodolojiler geliştirdiler. Geleneksel biyolojik savaş ajanlarını geliştirmek için bu teknolojinin uygulanması, genetiği değiştirilmiş biyolojik silah ajanlarının ayrı bir biyolojik silah ajan kategorisi olarak sınıflandırılmasına yol açmıştır. Potansiyel modifikasyon örnekleri arasında antibiyotik direnci, artan aerosol stabilitesi veya yüksek patogenez sayılabilir. Önemli olarak, genetik modifikasyonlar, tespit ve teşhis için kullanılan epitopları veya dizileri değiştirebilir, bu sistemlere birden fazla referans noktasının dahil edilmesini gerektirebilir ve biyolojik tespit stratejilerine ilişkin güvenlik ihtiyacını vurgulayabilir. Bununla birlikte, genetiği değiştirilmiş biyolojik silah ajanları, genetik düzeyde ana ajanla yakından ilişkili kalacak ve geleneksel teşhis kullanılarak genel olarak tanımlanabilir olmalıdır. Uygulanan modifikasyonlar, biyolojik savaş kullanımına uygun doğal özellikleri tehlikeye atabilecek bir şekilde ebeveyn organizmayı önemli ölçüde manipüle etmeden, geleneksel bir BW ajanının etkinliğini arttırmaya veya hedef popülasyonun biyomedikal savunma stratejisinin bilinen yönlerine karşı koymaya hizmet eder.
Sonuç olarak, gelecekteki zorlukların üstesinden gelmek için iyi bir konumda olabilmek için, biyolojik savunmanın tüm alanlarındaki bazı kaynakların, ortaya çıkan tehditlere karşı koymaya yetecek yeni nesil yaklaşımların geliştirilmesine yönlendirilmesi gerekir.
**Anahtar Kelimeler:** Biyolojik silah, Biyoteknoloji, Rekombinant DNA teknolojisi

# Importance of Biotechnology in Biodefense

## Abstract

The concept of using disease as a weapon has existed for centuries. Advances in biological research will likely allow the development of a new class of advanced biological warfare agents designed to elicit new effects. In addition, biotechnology will have applications that support the weaponization, dissemination and distribution of advanced biological warfare agents. Such new agents and delivery systems will expand the paradigm in biological warfare and provide a variety of new use options. Biotechnology is a good tool for biological weapons in terms of its potential to innovate in the concepts underlying the development, weaponization and limitations of biological agents. When molecular biological techniques are applied to biological warfare, it becomes important how biotechnology combats biological defense efforts aimed at countering the threats presented by conventional agents. With the advent of recombinant DNA technology, researchers have developed standard methodologies for modifying an organism's genetic makeup. The application of this technology to develop conventional biological warfare agents has led to the classification of genetically

modified biological weapons agents as a separate biological weapon agent category. Examples of potential modifications include antibiotic resistance, increased aerosol stability, or increased pathogenesis.Importantly, genetic modifications may alter epitopes or sequences used for detection and diagnosis, require the inclusion of multiple reference points in these systems, and emphasize the need for security regarding biological detection strategies. However, genetically modified biological weapons agents will remain closely related to the parent agent at the genetic level and should be generally identifiable using conventional diagnostics. Applied modifications serve to increase the efficacy of a conventional BW agent or to counter known aspects of the target population's biomedical defense strategy without significantly manipulating the parent organism in a way that could compromise natural characteristics suitable for biological warfare use.
Consequently, to be well positioned to meet future challenges, some resources in all areas of biological defense need to be diverted to the development of next-generation approaches sufficient to counter emerging threats.

**Keywords:** Biological weapon, Biotechnology, Recombinant DNA technology

## GİRİŞ

Tarihsel olarak, biyolojik savaş ajanları, geleneksel BW ajanları olarak adlandırılan, nispeten seçilmiş bir patojen ve toksin grubunu içermiştir. Geleneksel BW ajanlarının tümü doğal olarak oluşan organizmalar veya bunların toksik ürünleridir. Bir biyolojik savaş bilimcisinin bakış açısına göre, geleneksel BW ajanları tesadüfen seçilmiş bir grup özelliği geliştirmiştir: toksisite, kararlılık ve üretim kolaylığı. Bununla birlikte, son on yıllara kadar, araştırmacılara seçili organizmaları seçmede yardımcı olan şans, BW uygulamalarını mevcut ajanların özellikleriyle de sınırladı. Örneğin çevresel stabilite, bulaşıcı doz, etki süresi, klinik ilerleme ve öldürücülük, biyolojik savaş için faydalarını sınırlayabilen aday geleneksel ajanlara özgü özelliklerdir (Alibek, 1999).

## BİYOTEKNOLOJİ

Biyoteknoloji biyolojik savaşta en önemli silah olma önemine sahiptir. Biyoteknolojinin biyolojik savaş için biyolojik ajanların geliştirilmesi, silahlandırılması açısından potansiyeli yüksektir. Biyolojik savaşa uygulandığında biyoteknolojinin, geleneksel ajanlar tarafından sunulan tehditlere karşı koymayı hedefleyen biyolojik savunma çabalarına karşı, çoğalma, tespit ve tıbbi karşı önlemlerin geliştirilmesi önem konusudur. Amaç olarak, gelecekteki zorlukların üstesinden gelmek için iyi bir konumda olabilmek için, biyolojik savunmanın tüm alanlarındaki bazı kaynakların, ortaya çıkan tehditlere karşı koymaya yetecek yeni nesil yaklaşımların geliştirilmesine yönlendirilmesi gerekir.

## GENETİK OLARAK DEĞİŞTİRİLMİŞ BİYOLOJİK SİLAH AJANLARI

Tarihsel olarak, biyolojik savaş ajanları, geleneksel biyolojik silah ajanları olarak adlandırılan, nispeten seçilmiş bir patojen ve toksin grubunu içermiştir. Geleneksel biyolojik silah ajanlarının tümü doğal olarak oluşan organizmalar veya bunların toksik ürünleridir. Bir biyolojik savaş bilimcisinin bakış açısına göre, geleneksel biyolojik silah ajanları tesadüfen seçilmiş bir grup özelliği geliştirmiştir: toksisite, kararlılık ve üretim kolaylığı (Kwik, 2003; Frasen, 2001).

Bununla birlikte, son on yıllara kadar, yöntemler araştırmacılara seçili organizmaları seçmede yardımcı oldu, ayrıca biyolojik silah uygulamalarını mevcut ajanların özellikleriyle sınırladı. Örneğin çevresel stabilite, bulaşıcı doz, etki süresi, klinik ilerleme ve öldürücülük, biyolojik savaş için faydalarını sınırlayabilen aday geleneksel ajanlara özgü özelliklerdir. Rekombinant DNA teknolojisinin ortaya çıkmasıyla birlikte, araştırmacılar bir organizmanın genetik yapısını değiştirmek için standart metodolojiler geliştirdiler. Geleneksel biyolojik savaş ajanlarını geliştirmek için bu teknolojinin uygulanması, genetiği değiştirilmiş BW ajanlarının ayrı bir BW ajan kategorisi olarak sınıflandırılmasına yol açmıştır (Block, 1999; Hood, 2003; Crampton, 1999). Potansiyel modifikasyon örnekleri arasında antibiyotik direnci, artan aerosolstabilitesi veya yüksek patogenez sayılabilir. Önemli olarak, genetik modifikasyonlar, tespit ve teşhis için kullanılan epitopları veya dizileri değiştirebilir, bu sistemlere birden fazla referans noktasının dahil edilmesini gerektirebilir ve

biyolojik tespit stratejilerine ilişkin güvenlik ihtiyacını vurgulayabilir (James, 2001). Bununla birlikte, genetiği değiştirilmiş BW ajanları, genetik düzeyde ana ajanla yakından ilişkili kalacak ve geleneksel teşhis kullanılarak genel olarak tanımlanabilir olmalıdır. Sonuç olarak, bu modifikasyonlar, biyolojik savaş kullanımına uygun doğal özellikleri tehlikeye atabilecek bir şekilde ebeveyn organizmayı önemli ölçüde manipüle etmeden, geleneksel bir BW ajanının etkinliğini arttırmaya veya hedef popülasyonun biyomedikal savunma stratejisinin bilinen yönlerine karşı koymaya hizmet eder (Kissel, 1997; Stephens, 2002).

## YENİ BİYOLOJİ SİLAHLAR VE KULLANIMLARI

İleri biyolojik silah ajanlarında tasarlanabilecek geniş etki yelpazesi, istihdam seçeneklerini önemli ölçüde genişletecek ve nihayetinde biyolojik savaş kullanımı için mevcut eşiği düşürebilir. Bu yeni kullanım seçenekleri arasında, örneğin, minimum atıf riski ile stratejik etki için bir sivil nüfusu gizlice hedefleme fırsatı olabilir. İleri biyolojik silah ajanlarının geliştirilmesini destekleyen diğer özellikler aşağıdakileri içerebilir:

-Gelişmiş ajan geliştirmenin özelleştirilebilir yönleri, ajanın serbest bırakılmasının ardından öngörülebilir, istenen sonuçlara izin verebilir.

-Olağandışı klinik görünüm, biyolojik bir savaş saldırısının doğal bir salgın olarak yanlış karakterize edilmesine ve tespit edilmeden kalmasına izin verebilir.

-Daha önce tıp camiasında bilinmeyen yeni ajanların geliştirilmesi, teşhis edilmesi ve tedavi edilmesi zor olan BW ajanlarına yol açacaktır. Bunu atlatmak için gelişmiş ajanlar geliştirilebilir.

-Geleneksellere karşı koymak için tasarlanmış aşılar veya tedaviler ajanlar, aracılar belirli bir popülasyonu hedef alacak şekilde uyarlanabilir

-Sterilize edici, onkojenik (kansere neden olan) veya zayıflatıcı ajanlar, uzun vadeli etkiler için hedef popülasyona karşı stratejik bir silah olarak kullanılmak üzere oluşturulabilir.

Biyolojik savaşı asimetrik bir silah olarak sürdürmeye yönelik geleneksel motivasyonlarla (düşük maliyet-etki oranı, birkaç teknik engel, kuvvet çarpanı vb.) birleştiğinde, bu yeni kullanım seçenekleri muhtemelen BW'yi daha çekici hale getirecektir. Bu nedenle, biyoteknoloji araştırmalarındaki ilerlemeler, teknolojik açıdan yetkin haydut uluslar ve muhtemelen karmaşık terör örgütleri için BW gelişiminde yaklaşan bir devrime yol açabilir (Shirtliff, 2002; Hu, 2000; Wassef, 1994)

## BİYOSAVUNMADAKİ ZORLUKLAR

İleri biyolojik silah ajanlarının gelişimini destekleyerek, ortaya çıkan biyoteknolojiler, biyosavunma stratejilerine yeni zorluklar sunacaktır. Şu anda, biyosavunma ile ilgili kavramların kökleri büyük ölçüde kanıtlanmış tıbbi tedavilere ve profilaktiklere dayanmaktadır. Bu stratejiler, acil biyolojik tehditleri ele almak için kritik olsa da, ABW ajanlarına karşı yetersiz olacaktır. Bu nedenle, teknolojinin yanlış kullanımından kaynaklanabilecek daha geniş tehditlerle başa çıkmak için tasarlanan yeni girişimlerin, mevcut ve planlanan programlarla paralel olarak sürdürülmesi gerekmektedir. Bu girişimler, ulusal biyosavunma altyapısının her bir bileşenine göre uyarlanmalıdır (Uherek, 2000; Cello 2002).

## GELECEKTE BİYOTEKNOLOJİ VE EKİPMAN

Gelecekte biyoteknolojinin biyolojik savaşı destekleyen ekipman ve uzmanlık üzerinde etkisi olacaktır. Yeni ekipman ve üretim stratejileri muhtemelen BW etkinliğiyle ilişkili imzaları değiştirecek ve bunları meşru araştırmanın arka plan imzalarıyla harmanlayacaktır. Ayrıca, gelişmiş laboratuvar teknikleri için teknik kitlerin ve ayrıntılı protokollerin geliştirilmesi yoluyla ABW ajanlarının mühendisliğinin önündeki teknik engellerin azaltılması, yüksek düzeyde eğitimli bilim adamlarına olan ihtiyacı azaltacaktır (Fraser, 2001). Nükleer silahların yayılmasını önleme uzmanlarının, yüksek ikili kullanım potansiyeline sahip son teknoloji

biyoteknolojilerle ilişkili ekipman ve tekniklerin farkında olmaları ve temeldeki bu teknolojilerin ve yeteneklerin yabancı edinimlerini daha dikkatli bir şekilde izlemeleri gerekecektir (Wassf, 1994).

Gelişmekte olan dünyanın birçok alanında hızla büyüyen meşru biyoteknoloji araştırma ve üretim faaliyetleri arka planının ortasında bunu yapmak giderek zorlaşacaktır. Ek olarak, ulusal güvenlik topluluğunun, gelişen teknolojilerin BW faaliyetleriyle ilişkili standart imzalar üzerindeki etkilerini hesaba katması ve stratejilerini buna göre ayarlaması gerekecektir.Bugüne kadar insan, meyve sineği, nematod, bakteri ve birçok virüs dahil olmak üzere birçok organizmanın genomları belirlenmiştir. Tüm bu bilgiler, şu anda İnternet üzerinden atfedilemeyen bir şekilde yaygın olarak erişilebilen dijital veri dosyalarında saklanır. DNA sentez teknolojisindeki gelişmelerle birleştiğinde, virüsleri genomik dijital veri dosyalarından yeniden yapılandırmak giderek daha fazla mümkün hale geliyor ve bu süreç "dijital çoğalma" olarak giderek daha fazla kabul görüyor. İleri biyolojik silah ajanları oluşturmak içinTicari DNA sentezi işletmelerinin sayısı ve bu teknolojinin daha küçük laboratuvarlardaki yaygınlığı arttıkça, CDC seçili ajan listesindeki organizmaların yayılmasını ve edinilmesini izlemeye yönelik standart yaklaşımlar daha az etkili hale gelecektir (Block, 1999).

## ÇEVRESEL DEDEKTÖRLER

Hali hazırda, biyolojik savunma, ilgili biyolojik ajanlar için gerçek zamanlı çevresel dedektörlerin yokluğu nedeniyle zorlanmaktadır. Şu anda geliştirilmekte olan algılama sistemleri, büyük ölçüde boyut, antijen tanıma veya nükleik asit dizisine göre tehlikeli biyoaerosolleri saptamaya odaklanmaktadır. Duyarlılık, seçicilik, yanlış pozitif oranlar ve bu sistemlerin kullanımı için metodoloji ile ilgili bir takım teknik hususların ele alınması gerekmesine rağmen, bunlar nihayetinde seçilmiş bir grup geleneksel biyolojik savaş tehdidi ajanının tespitine izin verecek temel yaklaşımlara dayanmaktadır. Ne yazık ki, ABW ajanlarının otomatik olarak tasarlanma doğası, geleneksel ajanların oluşturduğu tehditleri ele almak için şu anda geliştirilmekte olan sistemleri atlatmalarına izin verecektir. Bu nedenle, daha genel tespit stratejilerinin geliştirilmesine yönelik sınırlı miktarda kaynak tahsis etmek ihtiyatlı olacaktır (Whitney, 2003).

## YENİ AJANLARLA TIBBİ MÜCADELE

Gelişmiş biyolojik savaş ajanları, uygun tıbbi karşı önlemlerin geliştirilmesinde en büyük zorluğu oluşturacaktır. Biyolojik silah ajanları için tıbbi teşhis, profilaksi ve terapötikler için mevcut stratejiler, ortaya çıkan İleri biyolojik silah tehditlerini ele almak için yetersiz olabilir. Bu, büyük ölçüde, farklı fiziksel niteliklere dayalı olarak bireysel ajanları ele alma çabalarına odaklanan temel ve uygulamalı araştırmalardaki standart paradigmalardan kaynaklanmaktadır. Bu süreç, amaçlanan organizmaya karşı koruma sağlayan, bazen mükemmel bir özgüllükle karşı önlemler sağlasa da, yeni tehdit ajanlarına yönelik temel ilkeleri uygulamak için tekerleğin sürekli olarak yeniden icat edilmesi gerekir. İleri biyolojik silah ajanlarının standart tıbbi karşı önlemleri atlatmak için tasarlanabileceği yaklaşan ortamda, askeri ve sivil nüfusu bilinmeyen özelliklere ve kökene sahip ajanlardan korumak için yeni stratejiler üzerine araştırmalara artan bir ihtiyaç olacaktır (Hood, 2003). Hali hazırda, terapötikler, ya ajan patogenezi için kritik olan spesifik moleküler yolaklara veya etkileşimlere karşı tasarlanır ya da hedeflenen ajan üzerinde inhibitör etkisi olan biri tanımlanana ve daha fazla çalışma için doğrulanana kadar bileşik kitaplıklarının yüksek verimli taranması yoluyla tanımlanır. Bu yaklaşımlar, biyolojik bir savaş veya biyoterör saldırısı meydana geldikten sonra yanıt verecek yeni bileşikler elde etmek için geçerli kalmalıdır. Bununla birlikte, ABW ajanlarının insan biyo-düzenleyici genlerini entegre etme veya başka bir şekilde gen ekspresyonunu etkileme potansiyeli tarafından gündeme getirilen terapötik gelişmeyle ilgili ana soru şudur: "Genomdaki bir saldırı nasıl tedavi edilir?" RNA enterferansı için hücresel yolları incelemek için tasarlanmış ön çalışmalar (RNAi)25 ve kısa firkete RNA (shRNA) moleküllerinin26 geliştirilmesi, bazı ABW ajanlarının etkilerini hızla hafifletmek için tasarlanmış genelleştirilmiş terapötik stratejilere yol açabileceğinden, bu soruyu ele alma potansiyeline sahiptir. Ek olarak, gen terapisi araştırması, terapötikshRNA dizileri için uygun dağıtım vektörleri hakkında fikir verebilir. Bununla birlikte, bu ön çalışmalar, konuşlandırılabilir bir terapötik vektörden çok uzaktır; Bu ve özellikle biyolojik savunmaya ayrılmış diğer araştırmaların yönlerini desteklemek için kaynaklar tahsis edilmelidir (Cummings, 2002).

## BİYOSAVUNMA İÇİN YENİ NESİL YAKLAŞIMLARA İHTİYAÇ VARDIR

Biyolojik savunma yeteneklerimizin ortaya çıkan tehditlere karşı yeterli koruma sağladığından emin olmak için çeşitli adımlar atılmalıdır. İlk olarak, ABW ajan gelişimini destekleyebilecek ve bu ajanlar tarafından sunulan tehditlere öncelik verebilecek yeni ortaya çıkan biyoteknolojilerin değerlendirilmesine izin vermek için kaynaklar tahsis edilmelidir. İstihbarat uzmanlarının hem geleneksel hem de ABW ajanlarına ilişkin sınıflandırılmamış tavsiyeleri, araştırma önceliklerini belirlerken dikkate alınmalıdır. Daha da önemlisi, bu değerlendirmeler öncelikle yabancı teknolojik yeteneklere dayanmalıdır. Yabancı biyolojik savaş niyetlerine dayanan yeni karşı önlemleri keşfetmek, araştırma topluluğunu sürekli olarak ortaya çıkan tehditler konusunda eğrinin gerisinde kalmaya zorlayarak biyolojik savunma çabalarını engelleyebilir.

İkincisi, deneysel olarak federal olarak finanse edilen bir mekanbiyoteknoloji tehdit değerlendirmelerinin doğrulanması gerekir. Biyosavunma kaynaklarının uygun şekilde tahsisi, genel biyobilim topluluğu için sınırlı etkileri olan ancak hain bilim adamları için önemli uygulamaları olan bazı araştırmalar gerektirecektir. Bu araştırma konsolide edilmeli ve tek bir federal tesiste yürütülmelidir. Araştırma bulguları, genel araştırma için fon tahsislerine öncelik verilmesinde de hesaba katılabilir. Ayrıca, müttefikleri ve diğer uluslararası gözlemcileri, bu tür biyolojik savunma tehdidi değerlendirme araştırmalarının anlaşma ihlallerini örtbas etmek için genel yayınlardan alıkonulmadığı konusunda temin etmek için adımlar atılması gerekecektir.Üçüncüsü, çevresel tespit, tıbbi teşhis, profilaktik ve terapötikler için yeni nesil sistemlerin geliştirilmesini teşvik etmek için bazı federal biyobilim araştırma fonları tahsis edilmelidir. Bu tür sistemlerin, ilgili ajanların daha geniş analizini ve tanımlanmasını sağlaması gerekecektir (Hu, 2000; Cello, 2002).

Bu yeni nesil sistemlerin gelişiminin altında yatan temel özellikleri araştıran araştırmacılar, bir gösterge panelinin varlığına dayalı olarak ajanları tanımlamaya odaklanmalıdır.

Daha da önemlisi, biyolojik bilim araştırma topluluğu ve ulusal güvenlik toplulukları, biyolojik savunmaya yönelik önemli uygulamalarla teknolojik atılımlar konusunda şeffaflığı sağlamak için daha yakından çalışmalıdır.

## REFERANSLAR

Alibek K. *Biohazard:The Chilling True Story of the Largest Biological Weapons Program in the World*. NewYork: Random House; 1999.

Kwik G, Fitzgerald J, Inglesby TV, O'Toole T. Biosecurity: Responsible stewardship of bioscience in an age of catastrophic terrorism. *Biosecurity and Bioterrorism: Biodefense*

*Strategy, Practice, and Science* 2003;1(1):27–36..

Fraser CM, Dando MR. Genomics and future biological weapons: The need for preventive action by the biomedical community. *Nature Genetics* 2001;29(3):253–256.

Block SM. Living night mares: Biological threats enabled by molecular biology. In: Drell S et al. eds. *The New Terror*. Stanford, CA: Hoover Inst. Press; 1999:39–75.

Hood L. Systemsbiology: Integrating technology, biology and computation. *Mech Ageing Dev* 2003;124(1):9–16. 8.

James E, Lee JM. The production of foreign proteins from Genetically modified plant cells. *Adv Biochem Eng Biotechnol* 2001;72:127–156.

Crampton JM, Stowell SL, Karras M, Sinden RE. Model systems to evaluate the use of transgenic haematophagous insects to deliver protective vaccines. *Parassitologia*

1999;41(1–3):473–477.

Kissel T, Koneberg R, Hilbert AK, Hungerer KD. Micro encapsulation of antigens using biodegradable polyesters: Facts and phantasies. *Behring Inst Mitt* 1997; (98):172–183.

Stephens C. Microbiology: Breaking down biofilms. *CurrBiol*2002 Feb 19;12(4):R132–134.

Shirtliff ME, Mader JT, Camper AK. Molecular interactions in biofilms. *Chem Biol* 2002; 9(8):859–871.

Hu WS, Pathak VK. Design of retroviral vectors and hepler cells for gene therapy. *Pharmacol Rev*2000;52(4):493– 511.

Wassef NM, Alving CR, Richards RL. Liposomes as carriers for vaccines. *Immunomethods* 1994;4(3):217–222.

Uherek C, Wels W. DNA-carrier proteins for targeted gene delivery. *Adv Drug Deliv Rev* 2000 Nov 15;44(2–3):153–166.

Cello J, Paul AV, Wimmer E. Chemical synthesis of polioviruscDNA: Generation of infectious virus in the absence of natural template. *Science* 2002 Aug 9;297(5583):
1016–1018.

Whitney AR, Diehn M, Popper SJ, Alizadeh AA, BoldrickJC, Relman DA, Brown PO.Individuality and variation in gene expression patterns in human blood. *Proc Natl Acad*
*Sci U S A* 2003;100(4):1896–1901.

Cummings CA, Relman DA. Genomics and microbiology. Microbialforensics—"cross-examining pathogens." *Science*2002 Jun 14;296(5575):1976–1979.

## *ORAL PRESENTATION*

# Yeşilırmak havzası sulak alanlarında fitoplankon komünitesinin ekolojik değerlendirilmesi

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## Özet

Bu çalışma Yeşilırmak Havzasındaki sulak alanlarda 2021 yıllarında iki dönem fitoplankton örneklemesi şeklinde gerçekleştirilmiştir. Fitoplankton örneklemesi hem kalitatif hem de kantitatif olarak iki yöntemle alınmıştır. Kalitatif örnekler 20 µm göz açıklığına sahip plankton kepçesi yardımıyla vertikal (dikey) ve horizontal (yatay) çekim metotları uygulanarak toplanmıştır. Kantitatif örnekler ise yüzeyden alınan 1 L su örneğinin lügol solüsyonu ile fikse edildikten sonra laboratuvarda sedimantasyon ile 10 ml hacme kadar konsantre edilmiştir. Bölgedeki lotik sistemlerden (Abdal nehri ve mansabı, Yeşilırmak nehri ve mansabı) ve lentik sistemlerden (Simenit Gölü) alınan fitoplankton örneklerinde Avrupa Birliği Su Çerçeve direktifine ve Türkiye Yerüstü ve Yeraltı Su Kalite Yönetmeliğine göre ekolojik durum değerlendirmesi gerçekleştirilmiştir. Bölgede belirlenen fitoplankton türlerinin bolluğuna ve niteliğine göre örnek alınan noktalar ötrofik karakterli olup, yoğun antropojenik baskı altında olduğu gözlenmiştir.

**Anahtar Kelimeler:** Fitoplankton, Yeşilırmak, WFD, SÇD, EKO, Türkiye

## Abstract

This study was carried out via two term phytoplankon sampling in 2021 in the waterlands of Yeşilırmak Basin. Phtyoplankton samles were collected by qualitative and quantitative sampling methods. Qualitative samples were taken verticaly and horizontaly via a plankton net with 20 micrometer mesh size. Quantitative samples, however, were concentrated into 10 ml volume in lab after one liter samples were collected from the surface water and were fixed via lugol's iodine solution. The phytoplankton samples which were taken grom the lotic (Abdal Creek, River Yeşilırmak) and lentic systems (Simenit Lake), an ecological classification system of Türkiye Water Quality Directive and Water Framework Directive of European Union as applied on the data. The sampling stations were in an eutrophic state and were onserved to be under anthropogenic stress according to the abundance and quality of phytoplankton species.

## Giriş

Türkiye sulak alanlar açısından Avrupa ve Ortadoğu ülkeleri arasında büyük öneme sahiptir. Batı Palearktik Bölgede bulunması ile farklı topoğrafik yapı ve iklim koşullarının oluşturduğu zengin sucul ekotonlara sahiptir [1]. Türkiye'nin en büyük sulak alanlarından biri olan Yeşilırmak Deltası, Çarşamba taşkın ovasının mansabı, Karadeniz'in de kıyısında yer alan sediman birikimleri ve yüzey akımlarının oluşturduğu bir lagünler sisteminin parçasıdır [2]. Bölgedeki sulak alanlarda genellikle sazan ve kefal avcılığı yapılmaktadır. Yeşilırmak Deltası Sulak Alanı'ndaki olta balıkçılığı faaliyetlerinin var olduğu bilinmekle birlikte, olta balıkçılığı miktarı ve potansiyeli ise bilinmemektedir. Tarım ve ormancılık bölgelerinin en yakınında olmasının yanı sıra ve sulak alanlardaki balıkçılık faaliyetlerinin yanında, göl, akarsu ve sulama kanallarında hayvancılık ve sazcılık faaliyetleri yapılmaktadır. Bu gibi faaliyetlerin sucul ekosisteme etkileri besin zincirinin ilk basamağında yani birincil üretimde dahi görülmektedir [3].

Fitoplankton sucul ekosistemdeki birincil üretimde ve biyojeokimyasal döngüde kilit bir rol oynamaktadır. Bulunduğu ortamdaki değişikliklere karşı gösterdiği hassasiyet ve tepkiler göz önünde bulundurulduğunda, su kalitesindeki değişikliklere karşı iyi bir indikatör olarak kullanılabilir [4]. Fitoplankton, yerleşim, fabrikalar ya da noktasal olmayan kaynaklar gibi dış yüklemelerden etkilenen ve ötrofikasyon baskısına tepki veren ilk biyolojik topluluktur ve su sütunundaki nütrient konsantrasyonlarında bulunan biyolojik kalite unsurlarının en doğrudan göstergesi olarak düşünülmektedir [5], [6].

SÇD'nin 2000 yılında Avrupa'da yürürlüğe girmesinden bu yana, fitoplankton yüzey sularının ekolojik durumunu değerlendirmek üzere kullanılması önerilen dört kalite unsurundan biri olarak kullanılmaktadır[7]. Tüm Avrupa içsu, kıyı ve geçiş sularının miktar ve kalite açısından iyi su durumuna ulaşması ve bozulmaların önlenmesi amaçlanmıştır. Bu kapsamda SÇD'ye göre nehirlerin ve havzalarının şimdiki ve gelecek kuşaklar için çok yönlü kullanımının devam ettirilmesi ve sürdürülebilirliğinin sağlanması hedeflenmektedir. İnsan faktörünün sucul canlılar üzerine etkisini tanımlamak amacıyla Avrupa Birliği Su Çerçeve Direktifi (WFD, 2000/60/EC)'nde Ekolojik statü sınıflandırması terimi kullanılmıştır. Buna göre bir su kütlesinde yaşayan biyolojik topluluklar beş temel statüde değerlendirilmiştir (Çok iyi, iyi, Orta, Zayıf, Kötü). Bu statüler sucul ortamın bozulmamış durumdan bozulmuşa doğru değişimini yansıtmaktadır.

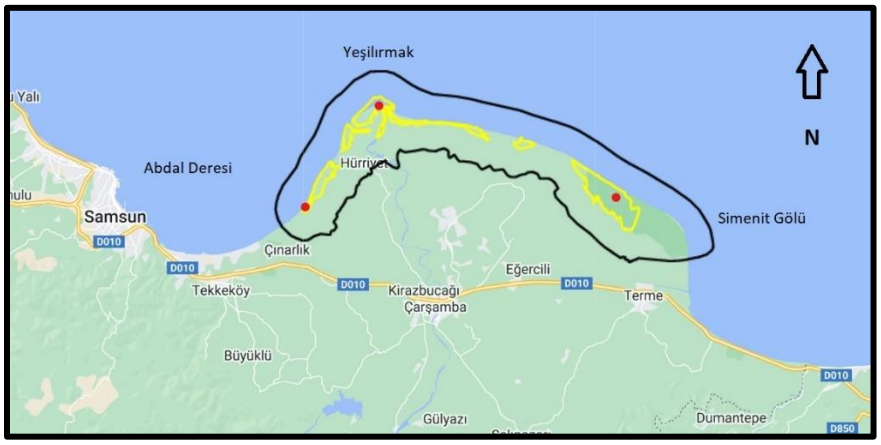
Su Çerçeve Direktifi, su kaynaklarının durumunu iyileştirme çalışmalarında biyoindikatörlere göre belirlenen ―Ekolojik Kalite Oranı (EQR) kavramını getirmiştir. Ekolojik Kalite Oranı, ekolojik sınıflandırma için Su Çerçeve Direktifinde belirtilen üç ana gereksinimi bir araya getirir; çalışılan sucul ekosistemin tipolojisi, referans durumu ve sınıf sınırları. Biyolojik izlemelerin sonucunda, çalışılan istasyonların ekolojik durumlarını belirlemek için sayısal bir değer kullanmak gerekir. Bu değer de gözlenen değerin beklenen değere oranıdır. Burada beklenen değerden kastedilen referans istasyonun değeridir (eğer yapılan çalışmada birden çok referans istasyon varsa, belirtilen hesaplamaların ortalamaları alınarak ortalama referans değerler elde edilir [8].

Ekolojik Kalite Oranı (EQR)=Gözlenen Değer/Beklenen (Referans) Değer

Bu çalışmanın amacı, SÇD ve 2012 tarihli Resmi gazetede yayınlanan ve 2017 de değişiklik yapılan ülkemiz Yeraltı ve Yerüstü Su Kalite Yönetmeliği uyarınca, Ramsar koruma bölgesi ilan edilen ve çeşitli koruma statüleri verilen Yeşilırmak Deltasındaki sulak alanlarda fitoplankton kalite bileşenine göre ekolojik durumun belirlenmesidir.

## METOT

Yeşilırmak Deltası Sulak Alanı fitoplanktonunu belirlemek amacıyla Mayıs 2021 ve Haziran 2021 tarihlerinde 2 lotik ve 1 lentik sistem olmak üzere toplam 3 lokasyondan (Abdal Nehri, Yeşilırmak ve Simenit Gölü) örnek alma çalışmaları gerçekleştirilmiştir (**Şekil 1**). Fitoplankton örneklemesi hem kalitatif hem de kantitatif olarak iki yöntemle alınmıştır. Kalitatif örnekler 20 µm göz açıklığına sahip plankton kepçesi yardımıyla vertikal (dikey) ve horizontal (yatay) çekim metotları uygulanarak toplanmıştır. Kantitatif örnekler ise yüzeyden alınan 1 L su örneğinin lügol solüsyonu ile fikse edildikten sonra laboratuvarda sedimantasyon ile 10 ml hacme kadar konsantre edilmiştir. Elde edilen örnekler Utermohl yöntemine göre invert mikroskop altında incelenmiş ve fotoğraflanmıştır [9]. Alglerin taksonomik teşhisinde; [10], [11], [12], [13], [14], [15] kullanılmıştır.

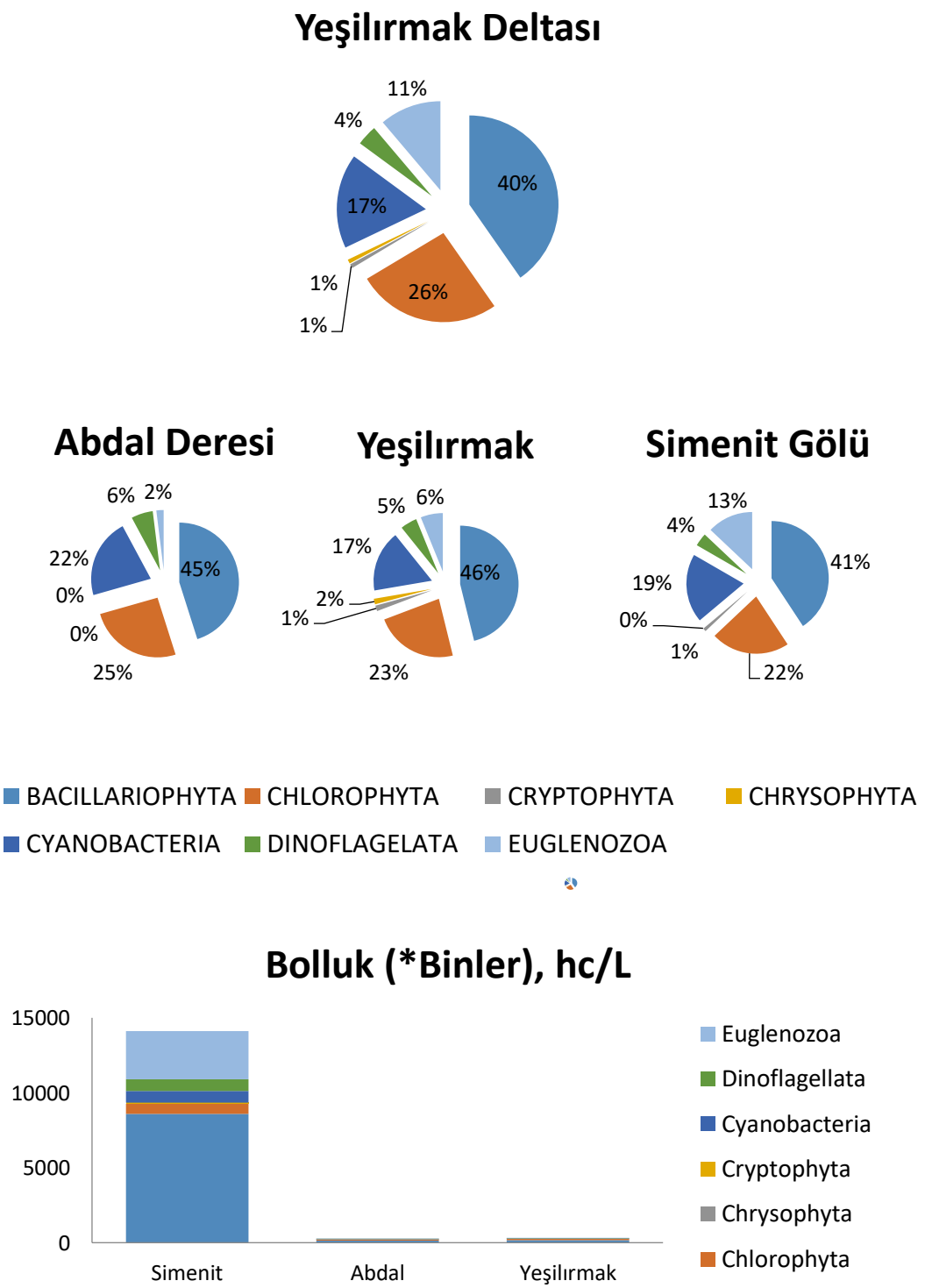



**Şekil 1**. Yeşilırmak deltası sulak alanlarında fitoplankton örneklemelerinin yapıldığı istasyonlar (Kırmızı noktalar).

Fitoplankton ait ekolojik durumun ortaya konulması için Ulusal Su Bilgi Sisteminde yer alan Sondergaard metriği kullanılmıştır [16]. Ayrıca bu metriğe ek olarak Yerüstü Su Kalitesi Yönetmeliği trofik durum değerlendirmesi ve Carlson trofik indeksi [17] kullanılarak karşılaştırması yapılarak nihai durumun ortaya konulmuştur. Carlson trofik indeksi (TSI) sınıflandırması aşağıda olduğu gibi gösterilmekte ve hesaplanmaktadır.

## BULGULAR VE TARTIŞMA

Bu çalışmada Toplam 7 divizyoya ait 134 takson tespit edildi. Literatür taramaları sonucunda ise bu bölgeden toplamda 301 fitoplankton taksonu rapor edilmiştir. İncelenen tüm istasyonlarda en fazla tür sayısı sırasıyla Bacillariophyta, Chlorophyta ve Euglenozoa divizyolarında görüldü (**Şekil 2a**). Hücre sayısı bakımından en yüksek bolluk görülen istasyon Simenit istasyonu ve sonrasında Yeşilırmak oldu (**Şekil 2b**).




**Şekil 2**. A) Yeşilırmak Deltası ve istasyonlarda belirlenen fitoplankton tür gruplarının divizyolara göre dağılımı. B) İstasyonlardaki Fitoplankton Bolluk değerleri

Yeşilırmak Deltasındaki Sulak Alanlarda, T.C. Orman ve Su İşleri Bakanlığı Doğa Koruma ve Milli Parklar Genel Müdürlüğü 11. Bölge Müdürlüğü, Samsun Şube Müdürlüğü Ulusal Biyolojik Çeşitlilik Envanter ve İzleme Projesi gerçekleştirilmiştir [2]. T.C. Orman ve Su İşleri Bakanlığı Devlet Su İşleri Genel Müdürlüğü tarafından Yeşilırmak Havzasında Su Kalitesi İzleme Projesi gerçekleştirilmiştir [3]. Ersanlı vd., (2006), 1997-1998 yıllarında Akgöl fitoplanktonun aylık değişimini çalışmışlar ve toplamda 119 taksa rapor etmişlerdir [18]. 2000-2001 yılları arasında ise Simenit Gölünde fitoplanktonun mevsimsel değişimi araştırılmış ve toplamda 175 fitoplanton taksonu teşhis edilmiştir [19]. Simenit gölü için 2006 yılında 167 fitoplankton taksonunun aylık değişimi incelenmiştir [20].

Su örneklerinde oldukça yoğun olarak belirlenen fitoplanktonik Cyclotella, Cocconeis, Anabaena, Trachelomonas ve Euglena cinslerine ait türler ötrofik karakterdeki sulak alanlarda çok yoğun aşırı üremeler gerçekleştirmektedir. Ayrıca ötrofik bölgelerde çok yoğun toksik aşırı üremeler gösteren Anabaena flos-aquae, Aphanocapsa sp., Microcystis aeruginosa gibi siyanobakteriler Yeşilırmak Deltası'ndaki durgun su kütlelerinde oldukça bol gözlenmektedir. Ayrıca lotik bölgelerde gözlenmiş olan Euglena viridis ve E. oxyuris türleri organik kirliliğin olduğuna işaret etmektedir. Bölgede belirlenen fitoplankton türlerinin bolluğuna ve niteliğine göre örnek alınan noktalar ötrofik karakterli olup, yoğun antropojenik baskı altında olduğu gözlenmiştir.

Fitoplankton ait ekolojik durumun ortaya konulması için hesaplanan Sondergaard metriği ve Carlson trofik indeksi (TSI) sınıflandırmasına göre Simenit gölü kötü ekolojik durumda tespit edilmiştir (**Tablo 1)**. Bu metrikler ülkemizde lentik (durgun) su sistemlerine göre kalibre edilmiş olup lotik (akar) su sistemlerinde kullanılması mümkün değildir. Bununla birlikte deltadaki lotik sulak alanlarda tespit edilen siyanobakteri türleri yavaş akan nehirlerde veya debisinin azaldığı dönemlerde de toksik aşırı üremelere sebep olabilmektedir [10].

**Tablo1**. Sondergaard ve TSI metriklerinin değerleri, referans değerleri, ekolojik kalite oranları ve ekolojik durumları. * Ersanli ve Gönülol (2006) verilerinden hesaplanmıştır.

| | | | Sondergaard | | TSI |
|---|---|---|---|---|---|
| Referans Değer* | | | 3,00 | | 68 |
| Ortalama Indeks Değeri | | | 3,80 | | 75 |
| Ekolojik Kalite Oranı-EKO | | | 0,25 | | 0,14 |
| Ekolojik Durum | | | Zayıf | | Kötü |
| Sonuç | | | KÖTÜ | | |
| EKO Sınıf Sınır Değerleri | 0,8 ÇOK İYİ | 0,6 İYİ | 0,4 ORTA | 0,2 ZAYIF | 0,01 KÖTÜ |

DSİ tarafından temin edilen Simenit su derinliği verilerine göre Şubat/2020 – Şubat/2021 dönemini kapsayan 1 yıllık süreç içindeki ortalama su derinliği 40 cm olarak ölçülmüştür. Yıl içinde su derinliği 1 m ile 1 cm arasında değişen seviyeler almaktadır. Göl su seviyesi yaklaşık 40 cm olan su kotu ortalamasının altına düştüğünde, sudaki besleyici element konsantrasyonu artacağından, uygun ekolojik şartlarda hiperötrofik olaylar gözlenebilir. Halihazırda sığ ve ötrofik olan Akgöl ve Simenit gölleri fitoplanktonu için daha önceden yapılan çalışmalara göre [19][20], yaz aylarında tür kompozüsyonunun önemli bir kısmını oluşturan siyanobakteriler aşırı üreme gösterebilir.

## SONUÇ

Bazıları toksik maddeler salgılayan siyanobakteriler gölde bulunan özellikle balıklar ve dip çamurunda bulunan omurgasız canlıları zehirleyerek ya da göl suyunda oksijenin tükenmesine yol açarak kitlesel ölüme yol açabilir. Aşırı üreme ile su kalitesinin önemli ölçüde düşmesinden dolayı (örneğin bulanıklılığın artması, hipoksia, vb) gölün ekolojik yaşamı olumsuz etkilenecektir. Bu sebeple göl uygun olan su seviyesi kotunda tutulmalıdır. Deltadaki sulak alanlarda, avcılık, tarım aktivitelerinin baskısı ve kirlilik faktörleri doğal yapının bozulmasına neden olmaktadır.

## TEŞEKKÜR

Bu Çalışma T. C. Orman ve Su İşleri Bakanlığı XI. Bölge Müdürlüğü Yeşilırmak Deltası Sulak Alan Yönetim Planı Projesi Yeşilırmak Deltası Sulak Alan Alt Havzası Biyolojik Çeşitlilik Araştırma Alt Projesi Kapsamında gerçekleştirilmiştir.

## KAYNAKLAR

1-Anonim, 2012. Yeşilırmak Deltası Sulak Alan Yönetim Planı Projesi, Yeşilırmak Deltası Sulak Alan Alt Havzası Biyolojik Çeşitlilik Araştırma Alt Projesi. T.C. Orman ve Su İşleri Bakanlığı, XI. Bölge Müdürlüğü, Samsun.

2-Anonim, 2014. Samsun İlinin Karasal Biyolojik Çeşitlilik ve İç Su Ekosistemleri Biyolojik Çeşitlilik Envanter ve İzleme İşi raporu, Orman ve Su İşleri Bakanlığı, Ankara.

3-Anonim, 2018. Yeşilırmak Deltası Sulak Alan Yönetim Planı Projesi Yeşilırmak Deltası Sulak Alan Alt Havzası Biyolojik Çeşitlilik Araştırma Alt Projesi. T. C. Orman ve Su İşleri Bakanlığı XI. Bölge Müdürlüğü SAMSUN.

4- [5] Vaulot, D. (2001). Phytoplankton. *Encyclopedia of Life Sciences*.

5-Carvalho, L., Dodkins, I., Carse, F., Dudley, B., & Maberly, S. (2006). Phytoplankton classification tool for UK lakes. Environment Agency.

6-Ilmavirta, V. (1982). Dynamics of phytoplankton in Finnish lakes. *Developments in Hydrobiology, 7*, 11-20.

7- Katsiapi, M., Moustaka-Gouni, M., Michaloudi, E., & Kormas, K. A. (2011). Phytoplankton and water quality in a Mediterranean drinking-water reservoir (Marathonas Reservoir, Greece). *Environmental Monitoring Assessment, 181*, 563-575.

8- European Commission, (2005). Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance on the intercalibration process 2004-2006. Luxembourg, Office for official publications of the European Communities.

9- Lund, J.W.G., Kipling, C.D. and Le Cren, E. 1958. The Inverted Microscope Method of Estimating Algal Numbers and Statistical Basis of Estimations by Coutung, Hydrobiol, 11: 143-170. Ordnunug Chlorococcales, E. Schweizerbarth'scheVerlagsbuchhandlug (Nagele u. Obermiller), Stuttgart,1044 p.

10- John D.M., Whitton B.A., Brook A., 2003. The Freshwater Algal Flora of the British Isles, An Identification Guide to Freshwater and Terrestrial Algae.

Cambridge, Cambridge University Press, 702 p.

11- Krammer, K. and Lange-Bertalot, H. 1986. Süßwasserflora von Mitteleuropa. Bacillariophyceae, Band 2/1, 1. Teil: Naviculaceae. 1-876. Berlin: Spectrum

Academicher Verlag.

12- Krammer, K. and Lange-Bertalot, H. 1991a. Süßwasserflora von Mitteleuropa. Bacillariophyceae, Band 2/3, 3. E. Ersanlı and A. Gönülol / Turk. J. Fish. Aquat. Sci. 3: 29-39 (2003) 39 Teil: Centrales, Fragillariaceae, Eunoticeae. 1-576. Stuttgart: Gustav Fischer Verlag.

13- Krammer, K. and Lange-Bertalot, H. 1991b. Süßwasserflora von Mitteleuropa. Bacillariophyceae, Band 2/4, 4. Teil: Achnanthaceae. Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis. 1-436. Stuttgart: Gustav Fischer Verlag.

14- Krammer, K. and Lange-Bertalot, H. 1999. Süßwasserflora von Mitteleuropa. Bacillariophyceae, Band 2/2, 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. 1- 610. Berlin: Spectrum Academicher Verlag.

15- Komarek, J., Eloranta, P. and Lhotsky, P. 1998. Cyanobacteria / Cyanophyta. Morphology, taxonomy, ecology. Proceedings of the 14th Symposium of the International Association for Cyanophyte Research (IAC), Lammi (Finland). J. Komarek, P. Eloranta and P. Lhotsky (Eds.). (Algological Studies, 94/ Archiv

für Hydrobiologie, Supplementbande 129). 382 p.

16-Søndergaard, M., S. E. Larsen, T. B. Jørgensen & E. Jeppesen, 2011. Using chlorophyll a and cyanobacteria in the ecological classification of lakes. Ecological Indicators 11:1403–1412.

17- Carlson, R. E., 1977. A trophic state index for lakes. Limnology and Oceanography 22: 361–369.

18- Ersanli, E., Gönülol, A., Şehirli, H., & Baytut, Ö. (2006). The phytoplankton of lake Akgöl, Turkey. Jornal of Freshwater Research, 523-525.

19-Ersanli, E., Gönülol, A. (2003). Study On The Phytoplankton And Seasonal Variation Of Lake Simenit (Terme - Samsun, Turkey) . Turkish Journal of Fisheries and Aquatic Sciences, (3) 1-19.

20- Ersanli, E., Gonulol, A. (2006). A study on the phytoplankton of Lake Simenit, Turkey. Cryptogamie-Algologie, 27(3), 289.

## *ORAL PRESENTATION*

# ZIF-8 Synthesis, Characterization and Modification with Gallic Acid as a Drug Delivery System

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## Abstract

Metal-organic structures (MOFs), consisting of metal ions and organic coordination molecules, have become a promising biomedical material due to their superior properties such as large surface area, large pore volume, flexible functionality and superior performance for drug loading. In this study, nanoporous ZIF-8 MOF structure was synthesized as a support material in drug delivery system. After the modification of ZIF-8 with gallic acid, a phenolic compound, 5-FU drug loading studies have been carried out successfully. The results were confirmed by characterization studies.

**Keywords:** MOF, ZIF-8, Gallic acid, 5-FU

## INTRODUCTION

Metal-organic frameworks (MOFs), a type of inorganic-organic hybrid material, have attracted great interest since they were first introduced. Bio-imaging, storage of catalysis materials, detection, purification and drug delivery etc. It has been used in various fields including MOFs are endowed with many unusual features in the delivery mechanisms of drugs compared to conventional products. The properties of MOFs such as specially designed surfaces, large pores and large surface areas increase the loading efficiency of drugs or other guest molecules, while properties such as water solubility, simple functionality, biodegradability and good biocompatibility increase the efficacy and bioavailability of drugs in the body. Also, drugs can be physically encapsulated or involve van der Waals forces, hydrogen bonds, π-π interactions between aromatic rings, coordination bonds, electrostatic interactions, covalent bonds, etc. It can be chemically conjugated in many carriers via various bonds such as Biomolecules are commonly incorporated into MOFs in different ways, such as surface attachment, pore encapsulation, and covalent bonding (Al-jabbar et al., 2022).

Zeolitic imidazolate framework-8 (ZIF-8) is a sub-MOF type synthesized through coordination between $Zn^{2+}$ ions and 2-methylimidazole. Fluorescein has been used in controlled transport systems for a variety of molecules such as enzymes, drugs, and DNA. Since ZIF-8 is stable at 7.4, the body's natural physiological pH, and can decompose under the acidic conditions of many cancer cells, it is an extremely good candidate for the creation of a pH-sensitive drug delivery system (Özsoy et al., 2021).

Gallic acid (3,4,5-trihydroxybenzoic acid) (GA) is one of the main phenolic compounds naturally found in plants (gallnut, blueberry, tea leaf, black tea and other herbs).Gallic acid (GA) has many biological activities, including anti-inflammatory, antimutation, antioxidation and antifree radical (Ye et al., 2020).

In this work, GA was used both to bind 5-Fluorouracil (5-FU), an anticancer drug, to ZIF-8, and to contribute to cancer treatment by showing a synergistic effect. The synthesized ZIF-8 was modified with GA, bound to 5-FU and characterized by FTIR analysis.

## MATERIALS AND METHODS

### ZIF-8 Synthesis

ZIF-8 was synthesized using a method detailed in the literature (Fu et al., 2018). According to this method, 2.7 g Zn(NO3)2.6H2O and 1.5 g 2-methylimidazole solutions were prepared separately in 60 mL methanol. The synthesis was carried out by adding 2-methylimidazole solution dropwise into Zn(NO3)2.6H2O solution at room temperature and stirring for 1 hour. ZIF-8 crystals were separated by centrifugation (4000 rpm, 15 min) and washed repeatedly with 3x30 mL methanol. The sample was dried overnight.

### ZIF-8@GA Synthesis

ZIF-8@GA synthesis was performed with a ZIF-8:GA mass ratio of 1:5. Solutions of 0.287 g of ZIF-8 and 1.435 g of GA in 60 mL of methanol were prepared separately to coat GA on the synthesized ZIF-8. The synthesis was carried out by adding GA solution dropwise into ZIF-8 solution at room temperature and stirring for 2 hours. ZIF-8@GA crystals were separated by centrifugation (4000 rpm, 15 min) and washed repeatedly with 3x30 mL methanol. The sample was dried overnight.

### ZIF-8@GA@5-FU Synthesis

The ideal mole ratio for the synthesis of ZIF-8@GA@5-FU was compared with the information in the literature (Zhang et al., 2018); It was determined as 0.05 mol of 5-FU for 1 mol of GA. 5 mg of 5-FU was dissolved in 10 mL of ultrapure water and stirred in a magnetic stirrer until dissolution occurred. 25 mg of ZIF-8@GA was added to it in powder form and mixed in a magnetic stirrer for 3 days. After 3 days, the synthesized sample was left to dry overnight.

## RESULTS and DISCUSSION

### Characterization of ZIF-8

The synthesized ZIF-8 was characterized by XRD, SEM and TGA analysis and the results are given in Figure 1-3.

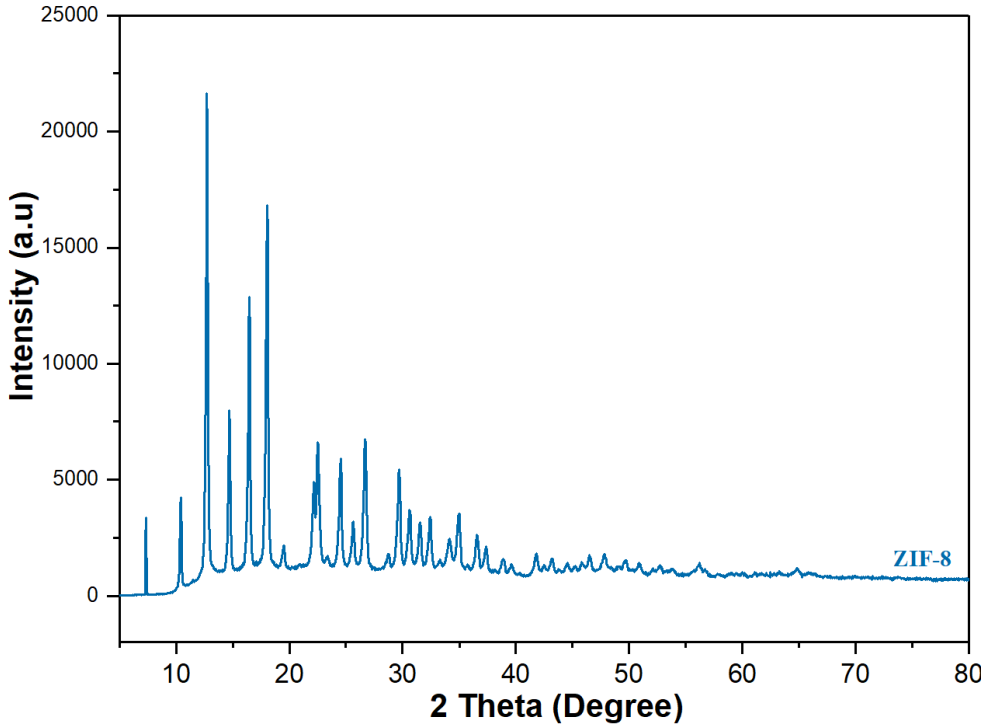


Figure 1. XRD pattern of ZIF8

The crystal phase was examined by XRD and is shown in Figure 1. The presence of strong peaks at 2θ = 7.34, 10.41, 12.73, 14.72, 16.45 and 18.05◦ corresponds to the planes (011), (002), (112), (022), (013) and (222), respectively, the results being prepared It shows the high crystallinity of ZIF-8 (Özsoy et al., 2021).

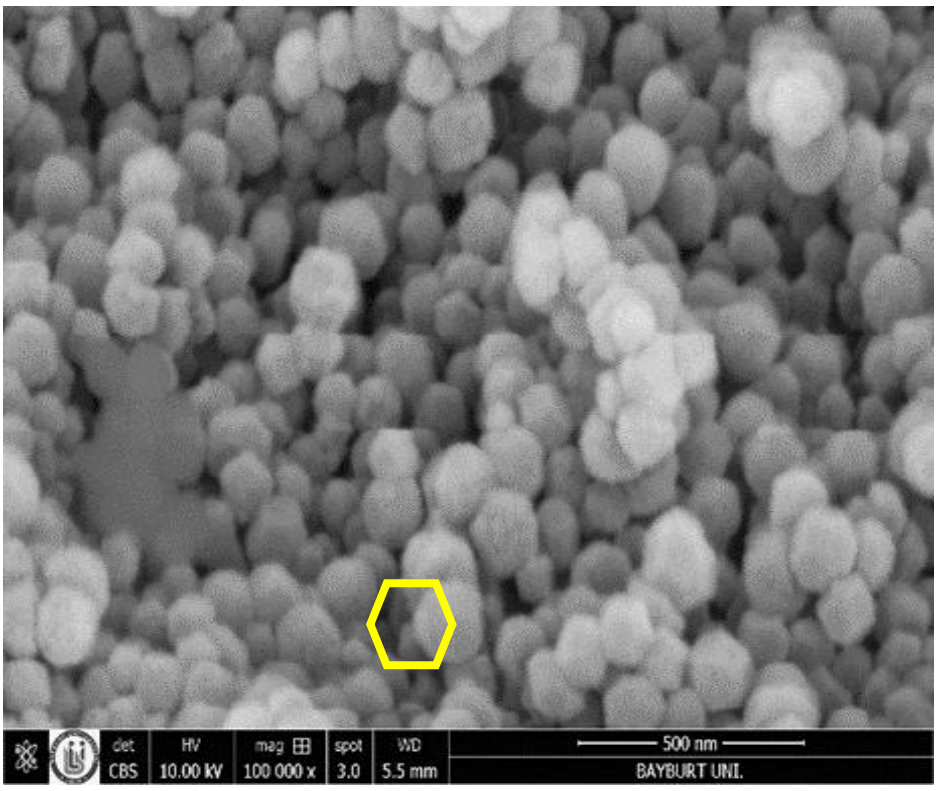


Figure 2. SEM image of ZIF-8

The microstructure of the synthesized ZIF-8 was examined by SEM and shown in Figure 2. A hexagonal shape with typical ZIF-8 morphology was observed (Al-jabbar et al., 2022).

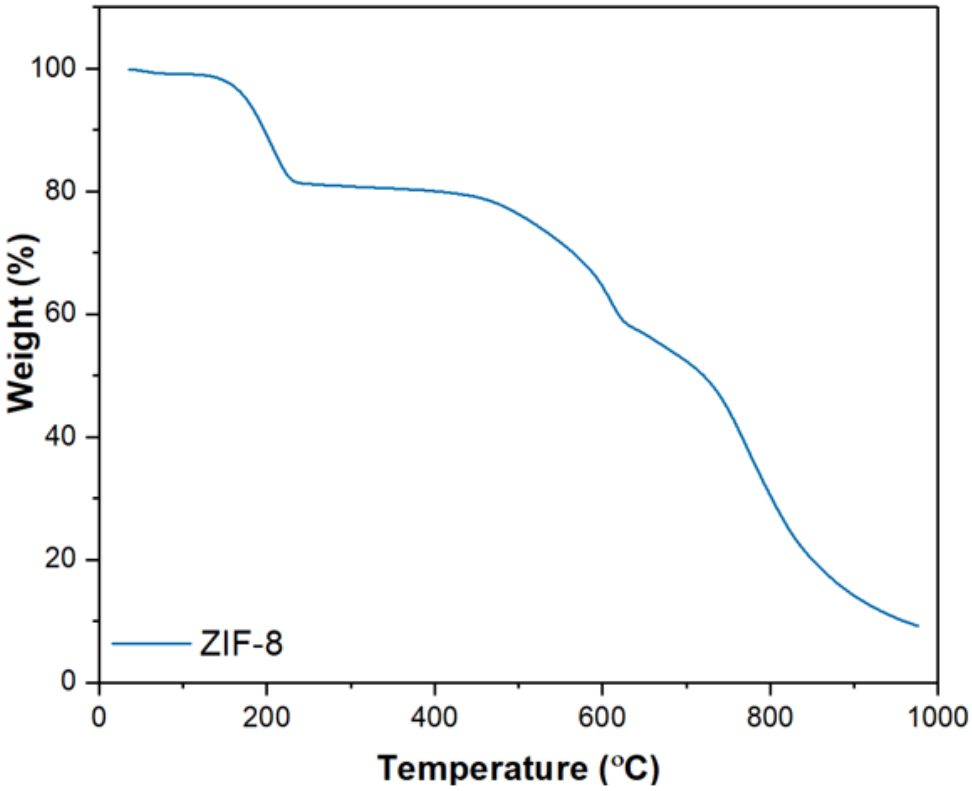


Figure 3. TGA curve of ZIF8.

The TGA curve obtained for ZIF-8 expressed in Figure 3 showed a weight loss of 20% in the sample. It is suitable for removing solvent molecules in the range of 180-250 ◦C. Afterwards, the weight loss of ZIF-8 was found to be 40% up to 650 ◦C, 40% between 650-850◦C and 10% between 850 and 950 ◦C. At high temperatures, thermal decomposition of ZIF-8 leads to decomposition of 2-methylimidazole, resulting in the emergence of ZnO as the final calcination component of ZIF-8 crystals (Özsoy et al., 2021).

Characterization of ZIF-8@GA and ZIF-8@GA@5-FU

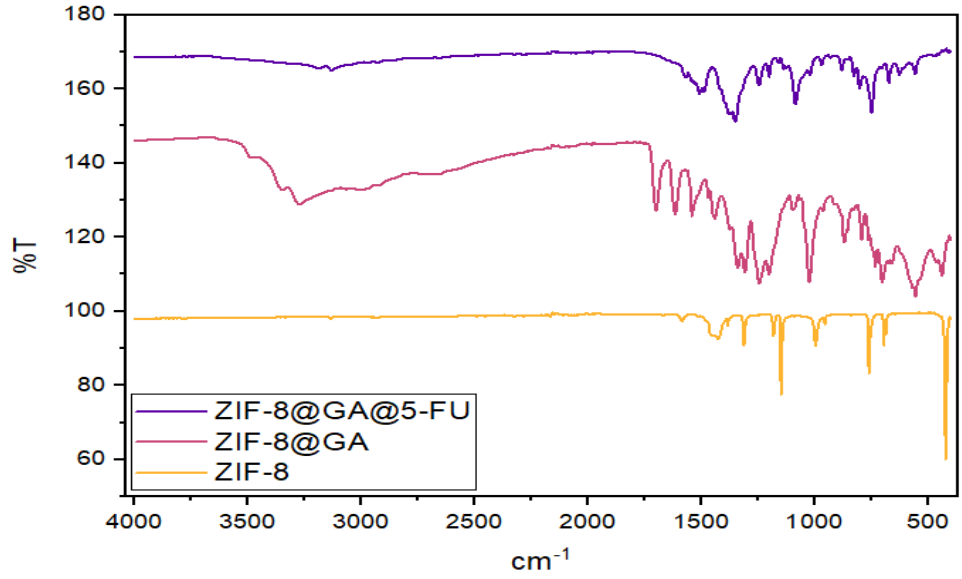


Figure 4. FTIR spectra for ZIF-8, ZIF-8@GA, ZIF-8@GA@5-FU

FTIR spectra of ZIF-8, ZIF-8@GA, ZIF-8@GA@5-FU samples are shown in Figure 4. The peaks at 1567 cm-1 and 1409 cm-1 are related to the C=N and C-N stretching modes, respectively, while the peaks at 1341 cm-1 are related to the overall ring stretching. Bands in the range of 1112, 1019 and 928 cm-1 can be attributed to in-plane bending of the ring, furthermore, the peaks at 622 and 653 cm-1 are related to aromatic sp2 N-H bending. The band at 483 cm-1 is attributed to Zn-N stretching, as the zinc atoms in the structure of ZIF-8 are associated with the nitrogen atoms of the 2-methylimidazole linker during ZIF-8 formation. The peaks at 3411 cm-1, 1702 cm-1 and 1048 cm-1 are the peaks of gallic acid, and the observed changes in the ZIF-8@GA peaks compared to ZIF-8 indicated successful GA-modification of ZIF-8. The peaks at 1610 cm-1 and 1235 cm-1 are attributable to 5-FU, and the observed changes in the ZIF-8@GA@5-FU peaks compared to ZIF-8@GA indicated successful loading of 5-FU into the system.

## CONCLUSION

In our work, nanoporous ZIF-8 MOF structure was synthesized as a support material in the drug delivery system. After the modification of ZIF-8 with gallic acid, a phenolic compound, 5-FU drug loading studies have been carried out successfully. The results were confirmed by characterization studies. Further studies on the drug delivery system continue.

## REFERENCES

Al-jabbar SA, Atiroğlu V, Hameed RM, Güney Eskiler G, Atiroğlu A, Deveci Özkan A, Özacar M 2022. Fabrication of dopamine conjugated with protein @metal organic framework for targeted drug delivery: A biocompatible pH-Responsive nanocarrier for gemcitabine release on MCF-7 human breast cancer cells. Bioorganic Chemistry, 118: 105467, https://doi.org/10.1016/j.bioorg.2021.105467.

Chang Y, Rui W, Zhang M, Zhou S, Qiu L, Cui P, Hu H, Jiang P, Du X, Ni X, Wang C, Wang J 2022. Facile preparation of copper-gallic acid nanoparticles as a high reproducible and drug loading platform for doxorubicin. Journal of Drug Delivery Science and Technology, 76: 103686, https://doi.org/10.1016/j.jddst.2022.103686.

Fu F, Zheng B, Xie LH, Du H, Du S, Dong Z 2018. Size-Controllable Synthesis of Zeolitic Imidazolate Framework/Carbon Nanotube Composites. Crystals, 8: 367. https://doi.org/10.3390/cryst8100367.

Özsoy M, Atiroğlu V, Güney Eskiler G, Atiroğlu A, Deveci Özkan A, Özacar M 2021. A protein-sulfosalicylic acid/boswellic acids @metal–organic framework nanocomposite as anticancer drug delivery system. Colloids and Surfaces B: Biointerfaces, 204: 111788. https://doi.org/10.1016/j.colsurfb.2021.111788.

Ye C, Chen X, Xu J, Xi H, Wu T, Deng D, Zhang J 2020. Highly sensitive detection to gallic acid by polypyrrole-based MIES supported by MOFs-Co2+@Fe3O4. Ganhui Huang Journal of Electroanalytical Chemistry, 859: 113839. http://dx.doi.org/10.1016/j.jelechem.2020.113839

Zhang L, Wan SS, Li CX, Xu L, Cheng H, Zhang XZ 2018. An Adenosine Triphosphate-Responsive Autocatalytic Fenton Nanoparticle for Tumor Ablation with Self-Supplied H2O2 and Acceleration of Fe(III)/Fe(II) Conversion. Nano Lett., 18: 7609−7618. https://doi.org/10.1021/acs.nanolett.8b03178.

Zhang X, An D, Zhang R, Huang Y, Liu Z 2022. Preparation of carbon nanotubes and polyhedral oligomeric-reinforced molecularly imprinted polymer composites for drug delivery of gallic acid. International Journal of Pharmaceutics, 615: 121476. https://doi.org/10.1016/j.ijpharm.2022.121476.

## *ORAL PRESENTATION*

# Süt ineklerinde bazı dışkı parametreleri kullanılarak uygun düzeyde fiziksel olarak etkili nötr deterjan lifi içeren toplam rasyon karışımının etkinliğinin değerlendirilmesi

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## Özet

Bu çalışma, standart düzeyde peNDF (%21,3) içeren toplam rasyon karışımı (TRK) ile beslenen süt ineklerinde fekal pH ve fekal skordaki değişimlerin belirlenmesi amacıyla yapılmıştır. Çalışmada hayvan materyali olarak laktasyonun 90. gününe kadar %21.3 peNDF'li TRK tüketen 6 Holstein ırkı süt ineği kullanılmıştır. TRK'nın parçacık boyutu dağılımı, Penn State Partikül Separatörü (PSPS) ile ölçülmüştür. Laktasyon periyotunun 90. gününde art arda 2 gün fekal pH ve skor ölçümü yapılmıştır. Fekal pH tayini için örnekler, sabaha ilk beslemeye göre 4 farklı zaman noktasından (0, 4, 8 ve 12. saatlerde) her bir ineğin rektumundan tek kullanımlık palpasyon eldiveni kullanılarak toplanmıştır ve portatif bir pH metre kullanılarak ölçülmüştür. Fekal skor, 5 seviyeli bir puanlama ölçeği kullanılarak sabah ve akşam belirlenmiştir. Normal sınırlar içerisinde peNDF'ye sahip TRK ile beslenen süt sığırlarında fekal pH'ın gün boyunca (6,73-6,84) önemli bir değişim göstermediği, fekal skorun ise ideal olduğu saptanmıştır. Fekal pH ve skorun rasyonun peNDF etkinliğinde kullanılıp kullanılamayacağını değerlendirmek için daha fazla hayvan materyalinin kullanıldığı çalışmalara ihtiyaç vardır.

**Anahtar Kelimeler**: Süt sığırı, fiziksel etkili NDF fekal pH ve skor

# Evaluation of the effectiveness of the total mixed ration containing an appropriate level of physically effective neutral detergent fiber using some fecal parameters in dairy cattle

## Abstract

This study was carried out to determine the changes in fecal pH and fecal score in dairy cows fed with a total mixed ration (TMR) containing an appropriate level of physically effective neutral detergent fiber (peNDF). In the study, 6 Holstein dairy cows consuming TMR with 21.3% peNDF until the 90th day of lactation were used as animal material. The particle size distribution of TMR was measured with the Penn State Particle Size Separator (PSPS). On the 90th day of the lactation period, fecal pH and score were measured for 2 consecutive days. The fecal pH determination samples were collected from each cow's rectum at 4 different time points (0, 4, 8, and 12 hours) from the first feeding in the morning, using a disposable palpation glove, and measured using a portable pH meter. The fecal score was determined in the morning and evening using a 5-level scoring scale. It was determined that fecal pH did not change significantly during the day (6.73-6.84) in dairy cattle fed with TMR with peNDF within normal limits, and the fecal score was ideal. Studies using more animal materials are needed to evaluate whether the fecal pH and score can be used in the peNDF effectiveness of the diet.

**Keywords:** Dairy cattle, physically effective NDF, fecal pH and scor

## GİRİŞ

Süt sığırları, enerji ve besin madde gereksinimlerinin önemli bir kısmı lifli bitki materyalinin rumendeki mikrobiyal fermantasyonundan karşılanır. Son yıllarda ekonomik, sosyal ve çevresel nedenlerden dolayı süt ineklerinin yüksek kaba yem içeren rasyonlar ile beslenmesi ilgi çekmektedir (Martin ve ark., 2017). Bu nedenle, son araştırmalar, süt ineği rasyonlarında lifi optimize etmeye ve lifin hem kimyasal hem de fiziksel özelliklerinin tüketim ve laktasyon performansını nasıl etkilediğini anlamaya odaklanmıştır. NDF analizi, yem lifinin fiziksel veya sindirilebilirlik özelliklerini hesaba katmaz (Van Soest, 1994). Mertens (1997) tarafından fiziksel olarak etkili NDF'nin (peNDF) geliştirilmesiyle lifin parçacık boyutu NDF sistemine dahil edilmiştir. peNDF, çiğneme davranışını uyaran, rumen sindirim matını oluşturan ve rumenden geçişe dirençli, 1,18 mm'den (Mertens, 1997) veya 4 mm'den (Penn State Particle Separator; Cotanch ve ark., 2010) daha büyük bir parçacık boyutuna sahip rasyon lifi fraksiyonudur. Bu ölçüm, yem maddesinin hem fiziksel hem de kimyasal özelliklerini hesaba katmaktadır. Rasyonda peNDF içeriğinin ve sindirilebilir karbonhidratların optimize edilmesi, ineğin rumen ve metabolik sağlığının desteklenmesi (Zebeli ve ark., 2012), süt verimi ve süt bileşenlerinin iyileştirilmesi için kritik öneme sahiptir. Laktasyonun farklı dönemlerindeki süt ineklerinde fekal parametreler tüketilen rasyonların ve yem maddelerinin fermentasyon ve sindirim bölgesi ve süresi hakkında önemli bilgiler sağlayabilir (Cunha ve ark., 2021; Humer ve ark., 2018; Mgbeahuruike, 2007). Ancak, standart peNDF seviyesine sahip rasyonla beslenen süt sığırlarında fekal skor ve pH'a ilişkin veriler yetersizidir. Bu nedenle, bu çalışmada, TRK'nın standart peNDF içeriğinin süt ineklerinde gün boyunca farklı zaman noktalarında fekal pH değişimi ve skoru üzerindeki etkileri değerlendirilmiştir.

## MATERYAL VE METOT

Bu çalışma, 1-3 Ekim-2022 tarihleri arasında Samsun ili Atakum ilçesinde faaliyet gösteren 150 baş kapasiteli özel süt sığırı işletmesinde yürütülmüştür. Çalışma, padoklarda serbest sistemde tutulan ve kuru madde (KM) bazında %56 ve 44 oranında kaba ve konsantre yem içeren TRK (KM: %52, HP: %18, HY: %2,5, HK: %10, Nişasta: %17) ile bir önceki günlük tüketimin %110'u oranında artırılarak günde iki kez (07:30 ve 19:30 h) besleme programı uygulanan 6 adet laktasyon dönemindeki Holstein inekte (laktasyonun ortalama 90. günü ve süt verim ortalamaları 27 kg/gün) yürütülmüştür. Taze fekal örnekleri (yaklaşık 500 g, ıslak ağırlık), sabah 07:30'deki ilk beslemeye göre 4 farklı zaman noktasından (0, 4, 8 ve 12. saatlerde) her bir ineğin rektumundan tek kullanımlık palpasyon eldiveni kullanılarak toplanmıştır. Fekal pH, portatif bir pH metre kullanılarak bir cam şişede 100 ml damıtılmış su ve 15 g taze dışkı karıştırılarak numune alındıktan hemen sonra 3 paralel olarak belirlenmiştir (Cunha ve ark., 2021). Fekal skor, hayvanlar sabah ve akşam sağımından sonra yem kilitlerinde iken değerlendirilmeye alınmıştır. Muayeneler aynı saatlerde ve aynı kişi tarafından yapılmıştır. Puanlama 1-5 puan arasında verilmiştir. 1 çok sulu, 5 ise çok katı forma sahiptir (Mendelez ve Roy, 2016). İdeal dışkı skoru 2,5-3 olarak kabul edilmektedir (Zaaijer ve Noordhuzen, 2001). Yem numuneleri, KM içeriğini ölçmek için 48 saat boyunca 65°C'de etüvde kurutuldu ve daha sonra 0,5 mm'lik bir elekli laboratuvar değirmeninde öğütülmüştür. NDF analizleri Van Soest ve ark. (1991)'e göre yapılmıştır. TRK numunelerinin partikül boyutu dağılımı, 19, 8 ve 1,18 mm'lik elekler ve bir alt tava içeren Penn State Partikül Separatörü kullanılarak belirlenmiştir. Pef (0 ile 1 arasında değişen) ilk üç elekte (≥ 1,18 mm) tutulan parçacıkların oranının (KM bazında) toplamı olarak belirlendi. Rasyonun peNDF içeriği, fiziksel etkinlik faktörü (pef) ve rasyon NDF içeriği çarpılarak hesaplanmıştır. TMR'nin partikül boyutu dağılımı Tablo 1'de gösterilmiştir.

**Tablo 1.** Rasyonun Parçaçık büyüklüğü dağılımı, fiziksel etkinlik faktörleri ve fiziksel olarak etkili nötr deterjan lif içeriği (% KM)

| PPPS elek çapı | TRK |
|---|---|
| >19 mm | 10,18 |
| 8-19 mm | 32,3 |
| 8- 1.18 mm | 21,1 |
| <1.18 mm | 36,4 |
| pef ≥1.18 mm | 0,64 |
| NDF, % KM'de | 34,00 |
| peNDF ≥1.18 mm, % KM'de | 21,63 |

Pef: Fiziksel etkinlik faktörü; NDF: Nötral detergent fibre; peNDF: Fiziksel olarak etkili NDF

### İstatistiksel analiz

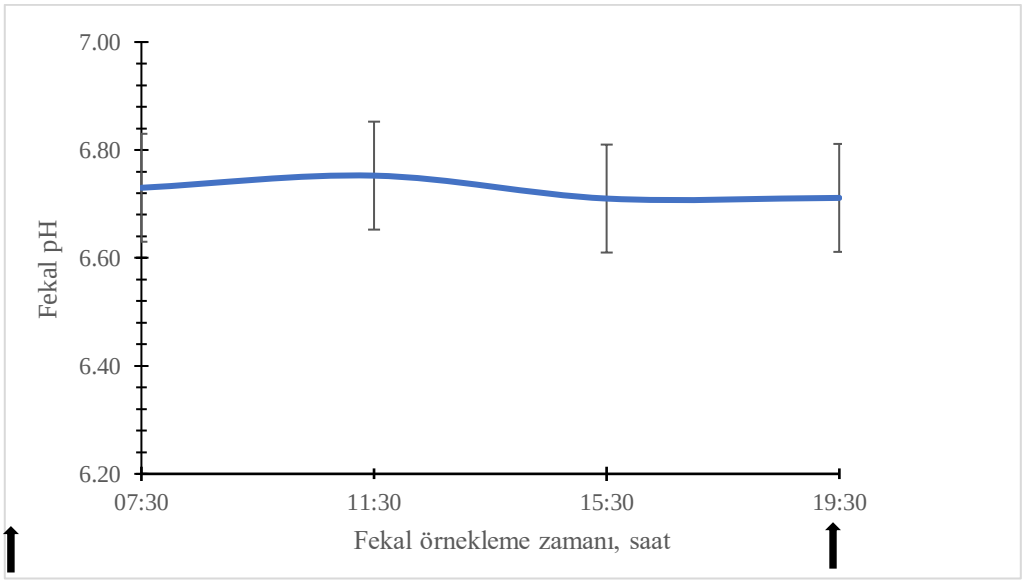
Elde edilen veriler Statistical Package for the Social Sciences (SPSS, Inc., Chiago, II, ABD) istatistik paket programı kullanılarak değerlendirilmiştir. Elde edilen veriler arasında istatistiksel farklılığın olup olmadığını one-way ANOVA testi ile saptanmıştır ve P < ,05 değeri anlamlı fark olarak kabul edilmiştir.

## BULGULAR VE TARTIŞMA

Ticari bir süt sığırı işletmesi koşullarında altında yürütülen ve fiziksel ve kimyasal özellikleri belirlenen TRK'nı tüketen hayvanlar tarafından üretilen dışkıların pH ve skorunu belirlemeyi amaçlayan bu çalışmada, diyetin partikül boyutu dağılımı, üst, orta, alt elekler ve alt tavada değişiklik göstermiştir ve >19 mm eleklerdeki partiküllerin %10,18 olarak ölçülmüştür (Tablo 1). Bu çalışmada, rasyonun çiğneme aktivitesini ve tükürük salgısını uyaran fraksiyonu olan penNDF>1,18 değerleri Mertens (1997) tarafından önerilen %21'e yakın bulunmuştur. Bu düzey, ruminal pH'ı 6,2'de tutmak, subklinik rumen asidoz riskini azaltmak ve KM tüketimi ve süt üretimi üzerinde herhangi bir olumsuz etki göstermeden süt yağı depresyonunu önlemek için yeterli olduğu düşünülmektedir (Zebelli ve ark., 2012).

**Tablo 2** : Standart peNDF'e göre fekal pH ve skor

| | Fekal pH | | |
|---|---|---|---|
| Zaman,saat | Minimum | Maximum | Ortalama |
| 0 | 6,61 | 6,81 | 6,73 ± 0,08 |
| 4 | 6,66 | 6,89 | 6,76 ± 0,06 |
| 8 | 6,55 | 6,88 | 6,71 ± 0,12 |
| 12 | 6,50 | 6,95 | 6,71 ± 0,15 |
| P | | | 0,84 |
| | Fekal skor | | |
| Sabah | 2,00 | 3,00 | 2,83 ± 0,40 |
| Akşam | 3,00 | 4,00 | 3,16 ± 0,40 |
| P | | | 0,18 |




**Şekil 1.** Farklı zaman noktalarında fekal pH değişimi

Bu çalışmada, standart peNDF düzeyindeki rasyonu tüketen ineklerin normal skorlu dışkıya sahip olduğu gözlemlenmiştir (Tablo 2). Bu gözlemin nedeni, rasyon bileşiminin ruminasyon, ruminal fermentasyon ve sindirim özelliklerini iyileştirmesi olabilir (Lyu ve ark., 2022). Aksine, yem partiküllerinin ince ve kalın bağırsağa geçmesi durumunda gevşek ve köpüklü bir kıvamda dışkıya neden olabilirdi (Mendelez ve Roy, 2016).

Çalışmamızda beslenmeden sonra her 4 saatte bir fekal örneklerin pH'ında (ortalama 6,72) önemli bir farklılık saptanmamıştır (Tablo 2, Şekil 1). Bu sonuç, rasyonun peNDF düzeyinden kaynaklandığı düşünülmektedir.

Bunun nedeni, peNDF düzeyinin zaman içinde sabit bir dışkı tutarlılığını koruyan daha kararlı bir sindirim fonksiyonunu indüklemesi olabilir. Diğer bir ifadeyle kaba yem partikül boyutu, rumende lif yıkımlanmasını teşvik etmek ve rasyonun tekdüzeliğine katkı sağlamış olabilir. Bu durum, yemlerin daha az seçimine ve ineklerin yeme davranışlarının iyileşmesi ile sonuçlanmasından dolayı önemlidir. Buna ek olarak, gün içinde iyi bir dağılım gösteren peNDF tüketiminden dolayı rumende uygun pH seviyesinin korunması (Zebeli ve ark., 2012) ve/veya kalın bağırsak asidozunun görülmemesi olabilir (Lyu ve ark., 2022). Bu sonuçların aksine, Palladino ve ark. (2022)'nın çalışmasında, fekal pH, beslenmeden sonraki 6 saat içinde en yüksek seviyeye yükseldiğini ve ardından sonraki 3 saatte hızla düştüğünü, 24 saat boyunca 5,8-6,8 arasında değişim gösterdiğini ve günlük ortalama 6,2 olduğunu bildirmişlerdir. Genel olarak, daha yüksek fekal pH'ın düşük fekal nişasta içeriğiyle ilişkilendirme eğilimi vardı (Zınn ve ark., 2007). Nişasta açısından zengin diyetler ile beslemede rumen fermantasyonu ve ince bağırsak sindirimini atlayarak daha büyük miktarlarda nişastanın kalın bağırsağa geçerek fermente olmasına ve sonuçta fekal pH'nın düşmesine neden olabilir. Bu nedenle, dışkı pH'sı her durumda olmasa da yüksek konsantre diyetlerle beslenen hayvanlarda nişasta sindiriminin yararlı bir göstergesi olabilir (Da Silva ve ark., 2012). Benzer şekilde, Degregórıo ve ark. (1982), pH'ın, fermantasyondan kaynaklanan asitlik derecesini yansıttığı için kalın bağırsaktaki fermente nişasta miktarının en basit göstergesi olduğunu bildirmiştir. Khorrami ve ark (2022)'nın yakın tarihli bir çalışmasında, yüksek konsantre besleme sırasında, dışkı pH'ı (6,26–6,41) beslemeden önce en düşük düzeyde olduğunu ve gün içinde keskin bir şekilde arttığını ($P < 0.05$), beslemeden 8 ve 12 saat sonra en yüksek değerlere ulaştığını bildirmiştir. Bu çalışmada, fekal pH'nın gün boyunca önemli bir değişiklik göstermemesinin muhtemel diğer bir nedeni de kullanılan diyetin nişasta içeriği yaklaşık %17 gibi düşük düzeyde olmasıdır.

## SONUÇ

Sonuçlarımız, %54 kaba ve %46 konsantre yem içeren %17 nişasta ve %21,3 düzeyinde peNDF içeren TRK ile beslene hayvanlarda gün boyunca fekal skor ve pH'da dinamik bir değişim olmadığını göstermiştir. Bu durumun, rasyonun standart peNDF düzeyinde, rumen ortamının stabilitesi ve arka bağırsak sağlığının korunduğunu düşündürmektedir. Fekal pH'ın rasyonun peNDF etkinliğini doğrudan ölçmek için uygulanır bir yöntem olup olmadığını değerlendirmek için farklı peNDF düzeylerinde ve daha fazla hayvan materyalinin kullanıldığı çalışmalara ihtiyaç vardır.

## TEŞEKKÜR

Bu çalışmanın yürütülmesine katkı sağlayan YUSİF Süt Sığırı İşletmesi yetkililerine ve Veteriner Hekim Mehmet Yıldız'a teşekkür ederiz

## KAYNAKLAR

Cotanch KW, Darrah JD, Ballard CS, Grant RJ 2010. Modification of the Penn State Particle Separator with 3.18- or 4.76-mm perforated steel sieves to measure physically effective fiber. Journal of Dairy Science, 88(E Suppl. 1):147. (Abstr.)

Cunha GSP, Maia MDA, Fernandes LMG, Geraseev LC, Chaves AS 2021. Physically effective fiber changes nutrient levels of total mixed ration and fecal parameters in beef feedlots. Ciência Rural, 51;5:1-6.

Da Sılva HL, França AFDS, Castro FG 2012. Fecal indicators of Nellore bovines fed high concentrate diets. Ciência Animal Brasileira, 13:145-156.

DeGregorıo RM, Tucker RE, Mıtchell GEJR, Gıll WW 1982. Carbohydrate fermentation in the large intestine of lambs. Journal of Animal Science, Savoy, 54:4855-862.

Humer E, Aschenbach JR, Neubauer V, Kröger I, Khiaosa-Ard R, Baumgartner W, Zebeli Q 2018. Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. Journal of Animal Physiology and Animal Nutrition, 102;2:380-392.

Khorrami B, Kheirandish P, Zebeli Q, Castillo-Lopez E 2022. Variations in fecal pH and fecal particle size due to changes in dietary starch: Their potential as an on-farm tool for assessing the risk of ruminal acidosis in dairy cattle. Research in Veterinary Science, 152;20:678-686.

Lyu J, Yang Z, Wang E, Liu G, Wang Y, Wang W, Li S 2022. Possibility of using by-products with high NDF content to alter the fecal short chain fatty acid profiles, bacterial community, and digestibility of lactating dairy cows. Microorganisms, 10;9:1731.

Martin NP, Russelle MP, Powell JM, Sniffen CJ, Smith S., Tricarico JM, Grant RJ 2017. Invited review: Sustainable forage and grain crop production for the US dairy industry. Journal of Dairy Science. 100:9479-9494.

Melendez P, Roy E 2016. The association between total mixed ration particle size and fecal scores in holstein lactating dairy cows from florida, USA. American Journal of Animal and Veterinary Sciences, 11;1:33-40.

Mertens DR 1997. Creating a system for meeting the fiber requirements of dairy cows. Journal of Dairy Science, 80;7:1463-1481.

Mgbeahuruike AC 2007. Fecal characteristics and production of dairy cows in early lactation. MSc Thesis, Swedish University of Agricultural Sciences, Skara, Sweden

Palladino RA, Olmeda MF, Juliano N, Bargo F, Ipharraguerre IR 2022. Daily fecal pH pattern and variation in lactating dairy cows. JDS Communications, 3;2;106-109.

Van Soest PJ, Robertson JB, Lewis BA 1991. Method for Dietary Fiber, Neutral Detergent Fiber and Nostarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Scince, 74:3583-3597.

Zaaijer D, Noordhuizen JPTM 2001. Dairy cow monitoring in relation to fertility performance. Cattle Practice 9:205-210.

Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN, Drochner W 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. Journal of Dairy Science, 95;3:1041-1056.

Zinn RA, Barreras A, Corona L, Owens FN, Ware RA 2007. Starch digestion by feedlot cattle: Predictions from analysis of feed and fecal starch and nitrogen. Journal of Animal Science, 85;7:1727-1730.

## *ORAL PRESENTATION*

# Essential oil composition of *Ziziphora clinopodioides* (Kırnanesi) from Erzincan region, Turkey

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**Abstract**

Plants have been used by societies for the prevention of infectious diseases for years. Especially the antibacterial, antifungal, and antimicrobial effects of essential oils give special importance to plants. *Ziziphora clinopodioides* (Lamiaceae), which grows in different regions of Turkey, has medicinal aromatic plant properties due to its rich essential oil content. In our study, we revealed the essential oil composition of *Z. clinopodioides* species grown in Erzincan using the GC-MS method. In the essential oil profile of this species, 30 compounds were determined and identified. Among these compounds, pulegone (29.70%), eucalyptol (19.23%), menthol (12.97%), and *p*-menthone (10.17%) were determined as the major compounds of essential oil. Compared to other studies in Turkey, eucalyptol is found only in *Z. clinopodioides* from Erzincan. Parameters such as climate, altitude and geographical conditions that differ in Erzincan compared to other places caused the species to contain eucalyptol. As a result, this species grown in Erzincan chemotaxonomically differs from other *Z. clinopodioides* species collected in different locations in Turkey.

**Keywords:** *Essential oil, GC-MS, Ziziphora clinopodioides, lamiaceae, eucalyptol*

## INTRODUCTION

Infectious diseases show widespread all over the world thanks to various organisms. The impacts of infectious illnesses, which harm human health and country economies, cannot entirely be averted despite significant efforts. Although antimicrobials are widely used to eliminate this problem, these agents are insufficient in treatment and lead to the formation of multi-resistant strains with gene mutations of microorganisms. Plants have been more important in recent years as a natural, secure, and alternative treatment for the prevention of resistant strains, particularly as a result of unintentional antibiotic usage. (Razavi et al., 2016) Plants with a variety of biological activities serve as a compass for the creation of novel therapies because of the phytochemical active compounds they contain. Especially essential oils obtained from plants play an important weapon role against bacterial, fungal, and microbial effects due to the varying amounts of biologically active compounds in their structure.(Ait Sidi Brahim et al., 2015)

Aromatic plants with rich essential oil and biologically active compound content provide important benefits to people in terms of health and diet. Essential oils rather than the extracts of these plants are generally used in the treatment of infectious pathologies on the respiratory system, urinary tract, gastrointestinal and biliary systems, and the skin. For years, many civilizations have given special attention to plants from the Lamiaceae family as an alternative form of treatment. Many fragrant medicinal plants with rich essential oil profiles in monoterpenoid care are found in the Lamiaceae family, which is well-known around the world for its flavor and therapeutic benefits. The amount of essential oils in these plants varies depending on their growing environment's geographic and climatic parameters. Due to their various effects such as anti-inflammatory, cytotoxic, antioxidant, and antibacterial properties, the essential oils of Lamiaceae plants have been the subject of a lot of research to date (Hazrati et al., 2020).

According to the literature, plants of the Lamiaceae family are particularly valuable as a source of essential oils. The *Ziziphora* genus (Kakouti Kouhi in Persian), which belongs to the Lamiaceae family, has represented four species worldwide, especially widespread in Iran and Turkey. These species are *Z. clinopodioides L., Z. capitata L., Z. persica*, and *Z. tenuior L.* The Eastern Anatolia Region is the homeland of a lot of *Z. clinopodioides*, which have antibacterial, anthelmintic, antifungal, and antiviral properties. (Ghavam, 2022)

The leaves, flowers, and stem parts of *Z. clinopodioides*, which is widely growing in Central and Eastern Anatolia, are used in culinary flavoring. In Turkey, *Z. clinopodioides* is commonly used for wound healing and an antibacterial effect topically, as well as for gastrointestinal system diseases and gas relief in the form of decoction or infusion. (Kilic and Bagci, 2013)
Several investigations on the essential oil composition of *Z. clinopodioides* were reported previously. *Z. clinopodioides* collected in Azerbaijan, Tajikistan, and Iran were investigated (Amiri, 2009; Baser et al., 2011; Behravan et al., 2013; Salehi et al., 2005; Sharopov and Setzer, 2011; Sonboli et al., 2010). Also, the essential oil composition of this species that is grown in Turkey was determined. For example, Erzurum (Ozturk and Ercisli, 2007) and Van (2900 altitude) (Okut et al.,2016), Coruh valley (Alp et al., 2016), Ermenek (Mediterranean Region) (Maral et al., 2015) and Southeastern Anatolia Region (Kilic and Bagci, 2013).
As is evident, no study has been done to evaluate the essential oil composition of *Z. clinopodioides* grown in and around Erzincan, which is located in the Eastern Anatolia Region at an altitude of 2200. In this study, we aimed to determine the essential oil profile of the *Z. clinopodioides* plant grown in Erzincan using the GC-MS method.

## MATERIALS AND METHODS

### *Plant material and essential oil distillation*

The plant materials were collected from Pöske Mountain (2200 altitude), Erzincan/Turkey, in June 2022. It was authenticated by senior taxonomist Prof. Dr. Ali Kandemir and a specimen was deposited in the EBYU Herbarium (Department of Science and Art Faculty, Erzincan Binali Yıldırım University, Erzincan, Turkey).

Air-dried aerial parts (100 g) were subjected to the hydrodistillation method with a Neo-Clevenger apparatus for 3 hours to obtain the essential oil. The essential oils were dehydrated with anhydrous sodium sulfate and stored in the dark at +4°C until analysis.

### *GC-MS Analysis of the essential oil samples*

A Thermo Scientific Trace 1310 GC-MS system with an HP-5MS capillary column (30 m x 0.25 mm and 0.25 µm film thickness) was used for GC-MS studies. In split mode by 50:1, helium was employed as a carrier gas with a constant flow rate of 1.2 mL/min. The temperature of the mass transfer line and the injection site was fixed at 280 °C. The temperature of the column oven was configured to begin at 60 °C, hold for 3 minutes, ramp to 200 °C at a rate of 3 °C/min, hold for 0 minutes, and then ramp to 240 °C at a rate of 5 °C/min, hold for 5 minutes. Conditions for the mass spectrometer were as follows: Ionization energy in the EI mode was 70 eV, and the ion source temperature was 280 °C. Using the Van den Dool and Kratz equation and the homolog n-alkane series retention periods, the retention indexes (RI) for each compound were computed. The identities of the compounds were verified using the Wiley and NIST2004 MS libraries. Based on the MS chromatograms, the relative peak area percentages of each chemical were determined.

## RESULTS AND DISCUSSION

The retention times and relative percentage of the compounds of the essential oil obtained by hydrodistillation of *Z. clinopodioides* are given in Table 1. Major compounds are shown in the GC chromatogram. (Figure 1)

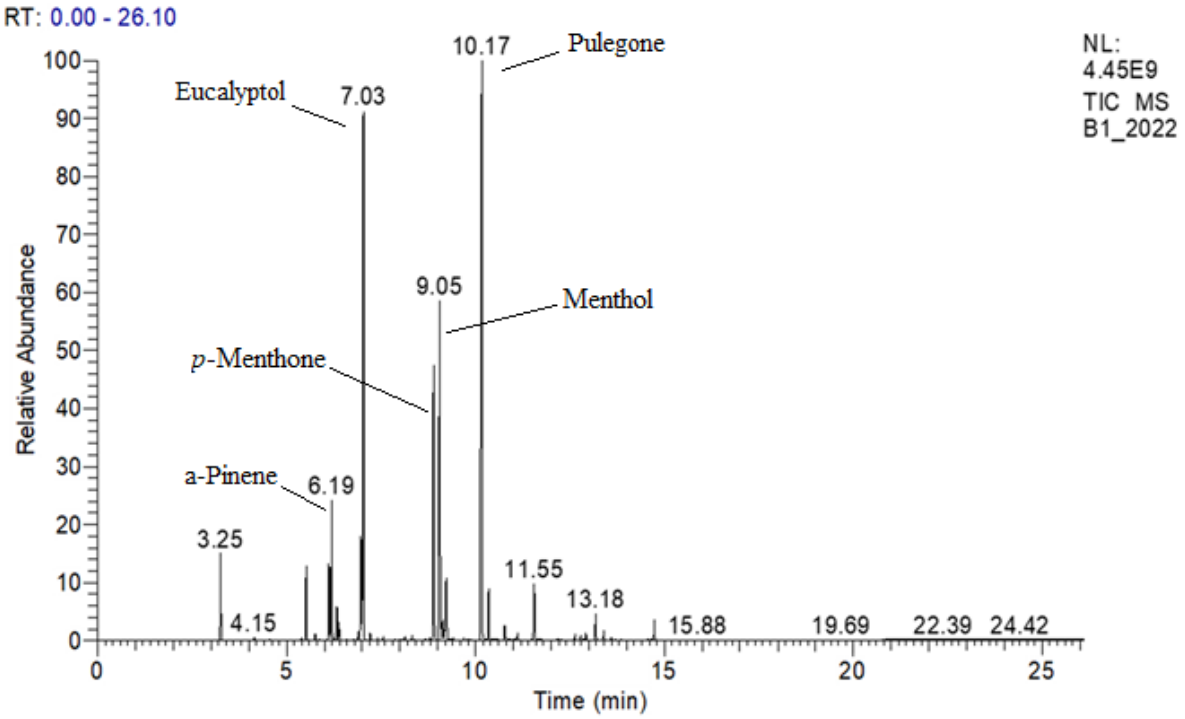


**Figure 1.** GC chromatogram showing separation and retention times of essential oil compounds.

**Table 1-** Retention times and percentage amounts of compounds identified from the essential oil of *Z. clinopodioides.*

| Retention Time (min) | Compounds | Composition % |
|---|---|---|
| 5.52 | *α*-Pinene | 2.36 |
| 5.76 | Camphene | 0.24 |
| 6.12 | Sabinene | 2.83 |
| 6.19 | *α*-Pinene | 4.58 |
| 6.33 | *α*-Myrcene | 1.04 |
| 6.38 | 3-Octanol | 0.61 |
| 6.89 | Cymene | 0.29 |
| 6.97 | Limonene | 4.00 |
| **7.03** | **Eucalyptol** | **19.23** |
| 7.21 | *Cis*-Ocimene | 0.25 |
| 7.56 | *Trans*-Sabinene hydrate | 0.12 |
| 8.13 | 1-Octen-3-yl acetate | 0.12 |
| 8.32 | 3-Octanyl acetate | 0.17 |
| **8.89** | ***p*-Menthone** | **10.17** |
| **9.05** | **Menthol** | **12.97** |
| 9.15 | Gama-Terpineol | 0.71 |
| 9.22 | Isopulegone | 2.98 |
| 9.42 | Terpineol | 0.11 |
| **10.16** | **Pulegone** | **29.70** |
| 10.35 | Piperitone | 1.76 |
| 10.77 | *p*-Cymenol | 0.67 |
| 11.12 | Isopulegyl acetate | 0.25 |
| 11.55 | Verbenone | 2.11 |

| | | |
|---|---|---|
| 12.64 | *Trans*-Caryophyllene | 0.20 |
| 12.79 | Citronellic acid | 0.27 |
| 12.92 | *Trans*-a-Farnesene | 0.25 |
| 13.18 | Exo-Isocamphonone | 0.90 |
| 13.39 | Germacrene-D | 0.32 |
| 13.59 | Bicyclogermacrene | 0.13 |
| 14.73 | *Cis*-caran-trans-3-Ol | 0.66 |

In the GC-MS analysis of *Z. clinopodioides* essential oil, 30 different compounds were identified. When the results of the analysis were examined, it was determined that the monoterpene content of the essential oil was high. Pulegone is the most abundant major compound in essential oil with a rate of 29.70%. Other major compounds included eucalyptol (19.23%), menthol (12.97%), and *p*-menthone (10.17%), in order from high to low.

Although our analyses confirm other research on the essential oil profiles of *Z. clinopodioides*, the climatic and geographic setting of Erzincan resulted in the existence of a different main compound that was unlike the other investigations. Similar to our study, the essential oil profiles of plants in Erzurum and Van contain pulegone (31.86%-29.31%) and menthon (6.73%-21.79%), respectively. Menthol (9.13%) and $\alpha$-pinene (3.17%) were also found in plants from Erzurum and Van, respectively. While *Z. clinopodioides* collected from Erzincan had a higher amount of menthone than Erzurum, both Erzurum and Van had a similar profile with Erzincan in terms of pulegone, menthol, and $\alpha$-pinene content. (Okut et al., 2016; Ozturk and Ercisli, 2007) In parallel with our study, pulegone, menthol, and $\alpha$-pinene were detected as major compounds from Ermenek, Coruh Valley, and Southeast Regions. (Kilic and Bagci, 2013, 2013; Maral et al., 2015). Eucalyptol (19.23%) was also detected in *Z. clinopodioides* in the Erzincan region, unlike major compounds in plants from other regions.

## CONCLUSION

According to our research, eucalyptol-containing Z. *clinopodioides* was first reported. The ecological, seasonal, and geographic conditions can be affected the composition of essential oil. These findings have chemotaxonomic importance for this species. Z. *clinopodioides* samples collected from Pöske Mountain in Erzincan province in Turkey can be suggested as a new chemotype containing eucalyptol-menthol-menthone-pulegone.

## ACKNOWLEDGEMENTS

I would like to thank Hüseyin Akşit and Samed Şimşek for their assistance in the collection of *Z. clinopodioides.* For providing a detailed description of the plant samples, Prof. Dr. Ali Kandemir also acknowledged.

## REFERENCES

Ait Sidi Brahim M, Fadli M, Hassani L, Boulay B, Markouk M, Bekkouche K, Abbad A, Ait Ali M, Larhsini M 2015. Chenopodium ambrosioides var. ambrosioides used in Moroccan traditional medicine can enhance the antimicrobial activity of conventional antibiotics. Ind. Crops Prod. 71: 37–43.

Alp S, Ercisli S, Dogan H, Temim E, Leto A, Zia-Ul-Haq M, Hadziabulic A, Aladag H 2016. Chemical composition and antioxidant activity *Ziziphora clinopodioides* ecotypes from Turkey. Rom. Biotechnol. Lett. 21.

Amiri H 2009. Influence of growth phase on the essential oil composition of *Ziziphora clinopodioides* Lam. 23: 601–606.

Baser KHC, Sezik E, Tümen G 2011. Composition of the Essential Oil of *Ziziphora clinopodioides* Lam. 3: 237–239.

Behravan J, Ramezani M, Hassanzadeh MK, Eskandari M, Kasaian J, Sabeti Z 2013. Composition, Antimycotic and Antibacterial Activity of *Ziziphora clinopodioides Lam*. Essential Oil from Iran. 10: 339–345.

Ghavam M 2022. In vitro biological potential of the essential oil of some aromatic species used in Iranian traditional medicine. Inflammopharmacology 30: 855–874.

Hazrati S, Govahi M, Sedaghat M, Beyraghdar Kashkooli A 2020. A comparative study of essential oil profile, antibacterial and antioxidant activities of two cultivated *Ziziphora* species (*Z. clinopodioides* and *Z. tenuior*). Ind. Crops Prod. 157: 112942.

Kilic O, Bagci E 2013. Essential oils of three *Ziziphora L.* taxa from Turkey and their chemotaxonomy. Asian J. Chem. 25: 7263–7266.

Maral H, Taghikhani H, Kaya A, Kirici S 2015. The Effect of Different Levels of Altitutes on Composition and Content of Essential Oils of *Ziziphora Clinopodioides* in Southern of Turkey. Int. J. Agric. Wildl. Sci. 1: 1–6.

Okut N, Selcuk N, Yagmur M, Yildirim B 2018. Chemical diversity of essential oil from leaves of *Ziziphora clinopodioides* growing in Van, Turkey. Fresenius Environ. Bull. 27: 2785-2790

Ozturk, S., Ercisli, S., 2007. Antibacterial activity and chemical constitutions of *Ziziphora clinopodioides*. Food Control 18: 535–540.

Razavi N, Molavi Choobini Z, Salehian Dehkordi M, Saleh RS, Salehian D, Mahboobeh MCS 2016. Overview of the antibacterial properties of essential oils and extracts of medicinal plants in Iran. J. Shahrekord Univ. Med. Sci. 17: 41–52.

Salehi P, Sonboli A, Eftekhar F, Nejad-Ebrahimi S, Yousefzadi M 2005. Essential Oil Composition, Antibacterial and Antioxidant Activity of the Oil and Various Extracts of *Ziziphora clinopodioides subsp. rigida* from Iran. Biol. Pharm. Bull. 28: 1892–1896.

Sharopov FS, Setzer WN 2011. Chemical Diversity of *Ziziphora Clinopodioides*: Composition of the Essential oil of *Z. clinopodioides* from Tajikistan. 6: 695–698.

Sonboli A, Atri M, Shafiei S 2010. Intraspecific Variability of the Essential Oil of *Ziziphora clinopodioides ssp. rigida* from Iran. Chem. Biodivers. 7: 1784–1789.

## *ORAL PRESENTATION*

# Evaluation of the Effects of Telomerase Inhibition on MMP-9 in Anaplastic Thyroid Cancer

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## Abstract

**Introduction:** Anaplastic thyroid cancer (ATC) is the type with the worst prognosis of thyroid cancers. In studies conducted to date, it has been determined that 73% of ATCs have TERT mutations and 25% have BRAF mutations. It is presumed that these mutations, singly or in combination, are responsible for the aggressive profile in ATC. Due to this aggressive profile, it invades the surrounding tissues intensively and frequently metastasizes to distant tissues. MMP-9 promotes tumor growth by degrading the matrix barriers of cancer cells and increasing angiogenesis. In this study, it was predicted that the presence of a link between TERT and MMP-9 could control the aggressive nature of anaplastic thyroid cancer.
BIBR1532 is a non-nucleocytic synthetic compound targeting human telomerase containing human telomerase reverse transcriptase and telomerase RNA components and is widely used in cancer research to inhibit active telomerase in tumors.
In this study, it was foreseen that treating ATCs carrying TERT mutations with BIBR1532 could be a targeted approach since telomerase is not active in healthy tissues. It was aimed to reveal the effect of TERT inhibition with BIBR1532 on MMP-9 with has an essential role in ATC metastasis.
**Method:** The cytotoxic effect of BIBR1532 on ATC cells was evaluated with WST-1 test in a micro plate reader at 450 nm and 620 nm wavelengths. In order to evaluate the effect of BIBR1532 on MMP-9 protein concentration, total protein isolation was performed with RIPA buffer. Bradford method was used for quantification of the obtained proteins. MMP-9 concentration in total protein was measured by Sandwich-elisa test at a wavelength of 595 nm in a micro plate reader.
**Result:** The $IC_{50}$ value of BIBR1532 in SW1736 cells (ATC model) was found as 189 µM (r: 0.83) at 24$^{th}$ hour, 236.6 µM (r: 0.63) at 48$^{th}$ hour, and 39.5 µM (r: 0.96) at 72$^{nd}$ hour. After SW1736 cells were treated with the $IC_{50}$ value of BIBR1532 found at 72$^{nd}$ hour, the MMP9 protein density decreased from 4.18 ng/mL to 3.27 ng/mL with a 22.3% reduction compared to the untreated control group.
**Conclusion:** Anaplastic thyroid cancer cells SW1736 were targeted with Telomerase inhibitor BIBR1532 for the first time in this study. We think that BIBR1532 can be a game changer in the creation of new treatment strategies for the treatment of ATC.

**Keywords:** Anaplastic thyroid cancer, BIBR1532, SW1736, TERT, MMP-9

## INTRODUCTION

### Thyroid Cancer

Thyroid carcinomas are the most common endocrine system cancers and constitute approximately 90% of them (Jameson JL, 2005). Thyroid cancers are caused by two main epithelial cells. According to their cell origin and histological characteristics, thyroid cancers are generally divided into follicular thyroid cancer (FTC), papillary thyroid cancer (PTC), medullary thyroid cancer (MTC) and anaplastic thyroid cancer (ATC) (Hurng-Seng WJ, 2000; M., 2000).
Anaplastic thyroid carcinomas are the most aggressive type of all thyroid carcinomas, accounting for approximately 1-4% of them. It tends to metastasize by hematogenous and lymphogenous route in a very fast time and has a 2-year survival rate of 0-17% (Hundahl, Fleming, Fremgen, & Menck, 1998). It is also accepted that all patients with a naplastic thyroid cancer are stage IV (Pstrag, Ziemnicka, Bluyssen, & Wesoly, 2018) and is resistant to all known treatment methods and has a low chance of treatment. It is known that anaplastic thyroid cancers have a high mutation load and approximately 45% of them harbor an activating BRAF (V600E) mutation.In addition to the patient motif formed by RAS and EIF1AX, promoter mutations of BRAF, RAS and TERT gene encoding human telomerase reverse transcriptase play a key role in the microevolution of

tumors and are distinctly different from other types in the majority of patients (Hurng-Seng WJ, 2000; M., 2000).

**Telomere**

Telomeres are heterochromatic DNA sequences containing "TTAGGG" sequence repeat at the ends of linear chromosomes (U., 2007).

Telomerase enzyme (telomer terminal transferase, telomere deoxynucleotidyl transferase) is a holoenzyme responsible for telomere maintenance, elongating telomeres, the terminal DNA sequences of chromosomes (Deng, Chan, & Chang, 2008). This function is mostly performed by the catalytic subunit TERT, which works by adding TTAGGG repeats to telomeric DNA using an internal RNA template (TERC) (Deng et al., 2008).

hTERT is an important component of human telomerase, which lengthens the end of chromosomes and maintains chromosomal stability, resulting in cellular immortalization and tumor formation (Stewart et al., 2002). In somatic cells, the TERT gene is not copied and telomerase is not active; therefore, telomeric ends shorten in each cell copy until they reach a critical erosion point after a programmed number of replications (Deng et al., 2008). Instead, cancer cells tend to maintain a high rate of proliferation; therefore, it is not surprising that TERT expression and telomerase activity (TA) represent a common feature of most human malignancies (Low & Tergaonkar, 2013).

TERT modulates the expression of genes that affect cell migration and invasion, thus playing a role in epithelial-mesenchymal transition and metastasis (Low & Tergaonkar, 2013).

**BIBR1532**

BIBR1532 (2-[(E)-3-naphthalen-2-yl-but-2-enoylamino]-benzoic acid) is a widely used, non-nucleocytic, non-competitive, small molecule telomerase inhibitor that acts directly via the hTERT active site. inhibits telomerase activity. (S. Kim et al., 2016).

**MMP9**

MMP-9, one of the most widely researched matrix metalloproteinases (MMPs), is an important protease that plays vital roles in many biological processes (Gialeli, Theocharis, & Karamanos, 2011). MMP-9 can break down many extracellular matrix (ECM) proteins to regulate the restructuring of the ECM. MMP-9 has been widely found to be related to the pathology of cancers, including but not limited to invasion, metastasis, and angiogenesis (Huang, 2018). Since MMP-9 promotes cancer development and progression in most cases and is an important target for some MMP-9-related diseases, targeting MMP-9 is of very high value.

## MATERIALS AND METHODS

**Cytotoxicity Test**

The cytotoxic effect of BIBR1532, which we gave to the SW1736 cell line at different doses using the Wst-1 preservative, was evaluated and the IC50 value was targeted.The experiment was performed in 3 replicates by adding 2x103 cells to each well in 96-well plates.

After 24 hours of incubation, cells were incubated for 24, 48, 72 hours with increasing probabilities of BIBR1532 in the range of 1.56-100 µM.WST-1 (10 µl) solution was added to each well for the specified time, and absorbance values were measured with a Multiskan FC microplate reader (Thermo Fisher Scientific) at 450 and 620 nm wavelengths at 30 min intervals. IC50 values were calculated with Calcusyn Software v.2.1.

**Protein isolation and Sandwich-elisa test**

In order to determine the change in the amount of MMP9 protein in SW1736 cells after BIBR1532 treatment, total protein extraction, protein concentration by Bradford method, and then Sandwich-elisa test were performed.After SW1736 cells were inoculated into 25 cm2 flasks, BIBR1532 was given to the dose group and fresh medium to the control group.At the end of 72 hours, the cells were removed with trypsin-EDTA and washed twice.For total protein isolation, 500 µl of RIPA Lysis and Extraction Buffer (Thermo Scientific; Cat. No: PI89900) and 50 µl of Protease inhibitor cocktail (Roche; Cat. No: 4693124001) were added to each sample and vortexed at 18,000 rpm at +4oC. It was centrifuged for 25 min. The samples were taken on ice after centrifugation, the supernatants were discarded and the protein concentration was determined with the Bradford Reagent. Bovine Serum Albumin Standard Set was used for protein quantification. The absorbance of each sample and standards in the concentration range of 1.25 µg/ml – 25 µg/ml was measured on a Multiskan FC microplate reader at 595 nm wavelength.

The absorbance values of the blank control were subtracted from each sample and standard, then the protein concentration of each sample was found by plotting the standard curve and multiplied by the dilution factor.Each sample and standard was studied in triplicate and absorbance measurements were made in triplicate. MMP9 (Elabscience; Cat. No: E-EL-H6075) was added to the plate coated with protein-specific antibodies, adding total protein from the sample to the final concentration in each well in the range of 200 µg - 300 µg and incubated for 90 minutes at 37oC, then incubated in each well. The biotinylated detection antibody solution supplied by the manufacturer was added and the samples were incubated at 37oC for 60 minutes. After washing three times with wash buffer, the samples were added to the HRP conjugate and left to incubate in the incubator for 30 minutes. Afterwards, after 15 minutes of incubation with substrate solution at 37oC, enzyme substrate reaction was stopped with stop solution and measurements were made in a microplate reader at 450 nm wavelength in order to determine the optical density.

**Statistical Analysis**

All tests are studied in three repetitions and the graphs created show the average of the three-repetition studies together with the standard deviation.

## RESULTS

**BIBR1532 has cytotoxic effect on SW1736 cells**

A proliferation test was performed to determine the amount of SW1736 cell line to be used in the cytotoxicity test and to give the appropriate absorbance value. 3 repeated experiments, the cell concentration giving the desired absorbance value was determined as 2x104 cells/ml. When the cytotoxic effect of BIBR1532 on SW1736 cells was evaluated by WST-1, the IC50 value was found to be 189 µM (r: 0.83) at the 24th hour, 236.6 µM (r: 0.63) at the 48th hour and 39.5 µM (r: 0.96) at the 72nd hour (Figure.1; Figure.2, Figure.3).

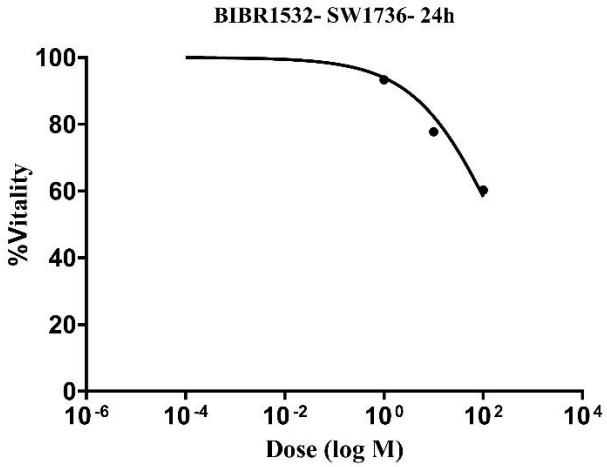


Figure 1. BIRB1532 on SW1736 cells 24 hour Cytotoxic effect
($IC_{50}$: 189,0 - $R^2$: 0,8288)

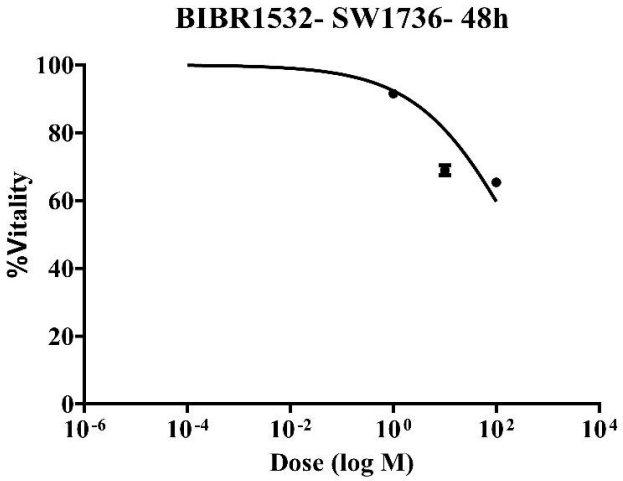


Figure 2. BIRB1532 on SW1736 cells 48 hour Cytotoxic effect
($IC_{50}$: 236,6 - $R^2$: 0,6318)

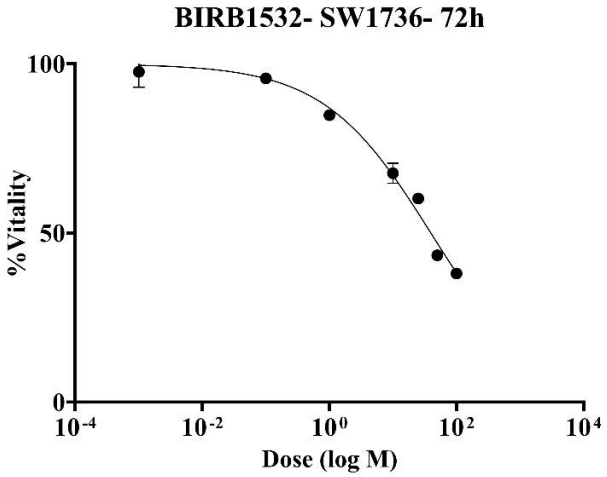


Figure 3. BIRB1532 on SW1736 cells 72 hour Cytotoxic effect
($IC_{50}$: 39,55 µM - $R^2$=0,9684)

**BIBR1532 decreases the expression of MMP9 at the protein level**

As a result of targeting telomerase with BIBR1532 of SW1736 cells, the concentration of MMP9 protein, which is responsible for metastasis, was 1.18 ng/mL in the control group, while it decreased to 0.91 ng/mL in the dosed group, resulting in a 22.3% reduction (Figure4.).

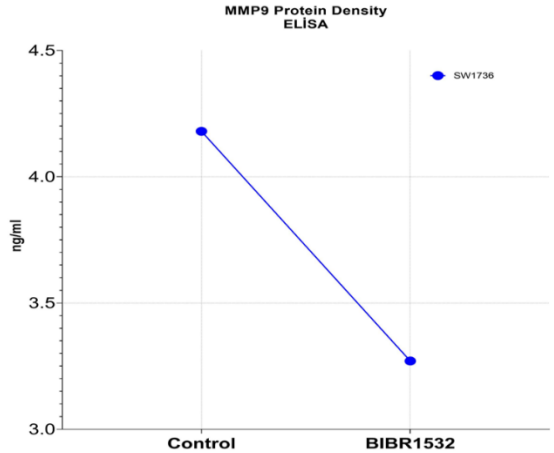


Figure 4. Effect of BIBR1532 on MMP9 protein density

## DISCUSSION

Anaplastic thyroid cancer (ATC) is among the most aggressive cancers ever seen in human history, known as an unusual type in the thyroid cancer subclass with a mortality rate close to 100% (Molinaro et al., 2017). Given that ATC is not common, to date there is no standard or effective treatment for prolonging the survival of patients with ATC (Cavalheiro et al., 2016; Ferrari et al., 2020; Lim et al., 2016). Therefore, there is an urgent need to identify new biological targets that can be translated into therapeutic approaches.
In the thyroid, as in other cancers, TERT promoter mutations are markers of malignant transformation, as no evidence has been reported so far of their occurrence in benign thyroid lesions (de Biase et al., 2015). Also, overexpression of TERT has been reported in thyroid carcinomas, lymph node metastases, and ATCs (Maggisano et al., 2017; Muzza et al., 2015). Interestingly, in the vast majority of published studies, TERT promoter mutations correlate with the B-Raf proto-oncogene, serine/threonine kinase (BRAF) V600E mutation, which is considered the major oncogenic determinant of TC. Therefore, hTERT has been proposed as an optimal target for the treatment of thyroid cancer, and in vitro studies have demonstrated the effectiveness of silencing and inhibiting hTERT. Studies suggest that inhibition of hTERT significantly reduces the proliferation, invasion and migration abilities of ATK cells. (Maggisano et al., 2017).
Treatment of many types of cancer cells with BIBR1532 has been shown to effectively suppress telomerase activists, leading to growth arrest and cell death. (Saraswati et al., 2019). BIBR1532 has also been observed to have a strong ability to reduce the growth of various cancer cells including breast, leukemia, lung, ovarian, chondrosarcoma and germ cell tumors (Bashash, Ghaffari, Mirzaee, Alimoghaddam, & Ghavamzadeh, 2013; Damm et al., 2001; Meng, Taylor, Ray, Shevde, & Rocconi, 2012; Mueller et al., 2007).
In our study, the cytotoxic effect of BIBR1532 on SW1736 cell lines was examined with the WST-1 test and the IC50 dose was found to be 39.55 μM at 72 hours. We think that the high cytotoxic effect of BIBR1532 on SW1736 cells may be effective in the usability of the active substance against cancer cells. For this reason, the cytotoxic effect of BIBR on anaplastic thyroid cancer cell, which is the most aggressive type of thyroid cancer, suggests that it may be effective against thyroid cancers.
Matrix metalloproteinases (MMPs) are important links of cell invasion in various forms of malignant cancer(E. S. Kim et al., 2011). At the same time, previous studies have shown that MMP9 increases the invasion of thyroid cancer cells (Rajoria et al., 2011; Zhang, Lu, Jiao, Chen, & Wu, 2012). Through analysis of the protein-protein (PPI) Interaction Network was demonstrated by Gong et al. in a 2022 publication that included MMP9 among 10 central genes that may be strongly associated with the development of carcinogenesis and ATC (Gong, Xu, Deng, & Peng, 2022). Although MMP-9 has been found to be a potential cancer biomarker in various types of cancer, it still needs many improvements in this area of research.
As a result of the elisa experiment, MMP9 protein density was reduced by 22.3% compared to the control group.
In studies, treatment with BIBR1532 has been shown to reduce MM9 protein expression in different cancer cells through down-regulation of TERT (Kong et al., 2015), and this is the first time anaplastic thyroid cancer has been studied, and our results will contribution to the literature.

## CONCLUSION

The $IC_{50}$ value of BIBR1532 in SW1736 cells (ATC model) was found as 189 μM (r: 0.83) at 24$^{th}$ hour, 236.6 μM (r: 0.63) at 48$^{th}$ hour, and 39.5 μM (r: 0.96) at 72$^{nd}$ hour. After SW1736 cells were treated with the $IC_{50}$ value of BIBR1532 found at 72$^{nd}$ hour, the MMP9 protein density decreased from 4.18 ng/mL to 3.27 ng/mL with a 22.3% reduction compared to the untreated control group.

In this study**, at the first time**, anaplastic thyroid cancer cell SW1736 was targeted with telomerase inhibitor BIBR1532. We think that BIBR1532 could be a game changer in the creation of new treatment strategies for the treatment of ATC.

## REFERENCES

Bashash, D., Ghaffari, S. H., Mirzaee, R., Alimoghaddam, K., & Ghavamzadeh, A. (2013). Telomerase inhibition by non-nucleosidic compound BIBR1532 causes rapid cell death in pre-B acute lymphoblastic leukemia cells. *Leuk Lymphoma, 54*(3), 561-568. doi:10.3109/10428194.2012.704034

Cavalheiro, B. G., Matos, L. L., Leite, A. K., Kulcsar, M. A., Cernea, C. R., & Brandao, L. G. (2016). Surgical treatment for thyroid carcinoma: retrospective study with 811 patients in a Brazilian tertiary hospital. *Arch Endocrinol Metab, 60*(5), 472-478. doi:10.1590/2359-3997000000209

Damm, K., Hemmann, U., Garin-Chesa, P., Hauel, N., Kauffmann, I., Priepke, H., . . . Schnapp, A. (2001). A highly selective telomerase inhibitor limiting human cancer cell proliferation. *EMBO J, 20*(24), 6958-6968. doi:10.1093/emboj/20.24.6958

de Biase, D., Gandolfi, G., Ragazzi, M., Eszlinger, M., Sancisi, V., Gugnoni, M., . . . Ciarrocchi, A. (2015). TERT Promoter Mutations in Papillary Thyroid Microcarcinomas. *Thyroid, 25*(9), 1013-1019. doi:10.1089/thy.2015.0101

Deng, Y., Chan, S. S., & Chang, S. (2008). Telomere dysfunction and tumour suppression: the senescence connection. *Nat Rev Cancer, 8*(6), 450-458. doi:10.1038/nrc2393

Ferrari, S. M., Elia, G., Ragusa, F., Ruffilli, I., La Motta, C., Paparo, S. R., . . . Antonelli, A. (2020). Novel treatments for anaplastic thyroid carcinoma. *Gland Surg, 9*(Suppl 1), S28-S42. doi:10.21037/gs.2019.10.18

Gialeli, C., Theocharis, A. D., & Karamanos, N. K. (2011). Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *FEBS J, 278*(1), 16-27. doi:10.1111/j.1742-4658.2010.07919.x

Gong, Y., Xu, F., Deng, L., & Peng, L. (2022). Recognition of Key Genes in Human Anaplastic Thyroid Cancer via the Weighing Gene Coexpression Network. *Biomed Res Int, 2022*, 2244228. doi:10.1155/2022/2244228

Huang, H. (2018). Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. *Sensors, 18*(10), 3249.

Hurng-Seng WJ, Y. M., Clark O. . (2000). Tiroid kanserlerine genel bakış. İşgör A. (ed). *Tiroid Hastalıkları ve Cerrahisi, 1.baskı, Avrupa Tıp Kitapçılık, İstanbul, 367-78*.

Jameson JL, W. A. (2005). Jameson JL, Weetman AP. Disorders of the thyroid gland. In: Kasper DL, Fauci AS,

Long o DL, Braunwald E, Hauser SL, Jameson JL, (eds). Harrison's. *o DL, Braunwald E, Hauser SL, Jameson JL, (eds). Harrison's*

*Principles ofInternal Medicine, 16 th Edition, New York: The McGraw Hill*

*Companies 2005; 320: 2014 2126.*

Kim, E. S., Kim, J. S., Kim, S. G., Hwang, S., Lee, C. H., & Moon, A. (2011). Sphingosine 1-phosphate regulates matrix metalloproteinase-9 expression and breast cell invasion through S1P3-Galphaq coupling. *J Cell Sci, 124*(Pt 13), 2220-2230. doi:10.1242/jcs.076794

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., . . . Bryant, S. H. (2016). PubChem Substance and Compound databases. *Nucleic Acids Res, 44*(D1), D1202-1213. doi:10.1093/nar/gkv951

Kong, W., Lv, N., Wysham, W. Z., Roque, D. R., Zhang, T., Jiao, S., . . . Zhou, C. (2015). Knockdown of hTERT and Treatment with BIBR1532 Inhibit Cell Proliferation and Invasion in Endometrial Cancer Cells. *J Cancer, 6*(12), 1337-1345. doi:10.7150/jca.13054

Lim, A. M., Taylor, G. R., Fellowes, A., Cameron, L., Lee, B., Hicks, R. J., . . . Rischin, D. (2016). BRAF Inhibition in BRAFV600E-Positive Anaplastic Thyroid Carcinoma. *J Natl Compr Canc Netw, 14*(3), 249-254. doi:10.6004/jnccn.2016.0030

Low, K. C., & Tergaonkar, V. (2013). Telomerase: central regulator of all of the hallmarks of cancer. *Trends Biochem Sci, 38*(9), 426-434. doi:10.1016/j.tibs.2013.07.001

M., Ö. (2000). Tiroid kanseri ve onkogenleri. . *Tiroid Fizyolojisi. 1.baskı; İstanbul Üniversitesi cerrahpasa tıp Fak. Yayınları, 325*.

Maggisano, V., Celano, M., Lombardo, G. E., Lepore, S. M., Sponziello, M., Rosignolo, F., . . . Bulotta, S. (2017). Silencing of hTERT blocks growth and migration of anaplastic thyroid cancer cells. *Mol Cell Endocrinol, 448*, 34-40. doi:10.1016/j.mce.2017.03.007

Meng, E., Taylor, B., Ray, A., Shevde, L. A., & Rocconi, R. P. (2012). Targeted inhibition of telomerase activity combined with chemotherapy demonstrates synergy in eliminating ovarian cancer spheroid-forming cells. *Gynecol Oncol, 124*(3), 598-605. doi:10.1016/j.ygyno.2011.11.018

Molinaro, E., Romei, C., Biagini, A., Sabini, E., Agate, L., Mazzeo, S., . . . Elisei, R. (2017). Anaplastic thyroid carcinoma: from clinicopathology to genetics and advanced therapies. *Nat Rev Endocrinol, 13*(11), 644-660. doi:10.1038/nrendo.2017.76

Mueller, S., Hartmann, U., Mayer, F., Balabanov, S., Hartmann, J. T., Brummendorf, T. H., & Bokemeyer, C. (2007). Targeting telomerase activity by BIBR1532 as a therapeutic approach in germ cell tumors. *Invest New Drugs, 25*(6), 519-524. doi:10.1007/s10637-007-9063-6

Muzza, M., Colombo, C., Rossi, S., Tosi, D., Cirello, V., Perrino, M., . . . Fugazzola, L. (2015). Telomerase in differentiated thyroid cancer: promoter mutations, expression and localization. *Mol Cell Endocrinol, 399*, 288-295. doi:10.1016/j.mce.2014.10.019

Rajoria, S., Suriano, R., George, A., Shanmugam, A., Schantz, S. P., Geliebter, J., & Tiwari, R. K. (2011). Estrogen induced metastatic modulators MMP-2 and MMP-9 are targets of 3,3'-diindolylmethane in thyroid cancer. *PLoS One, 6*(1), e15879. doi:10.1371/journal.pone.0015879

Saraswati, A. P., Relitti, N., Brindisi, M., Gemma, S., Zisterer, D., Butini, S., & Campiani, G. (2019). Raising the bar in anticancer therapy: recent advances in, and perspectives on, telomerase inhibitors. *Drug Discov Today, 24*(7), 1370-1388. doi:10.1016/j.drudis.2019.05.015

Stewart, S. A., Hahn, W. C., O'Connor, B. F., Banner, E. N., Lundberg, A. S., Modha, P., . . . Weinberg, R. A. (2002). Telomerase contributes to tumorigenesis by a telomere length-independent mechanism. *Proc Natl Acad Sci U S A, 99*(20), 12606-12611. doi:10.1073/pnas.182407599

U., E. (2007). Investigation of telomerase activitiy in diagnosis of endometrial and cervical cancer. *Odtü Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi*.

Zhang, X. G., Lu, X. F., Jiao, X. M., Chen, B., & Wu, J. X. (2012). PLK1 gene suppresses cell invasion of undifferentiated thyroid carcinoma through the inhibition of CD44v6, MMP-2 and MMP-9. *Exp Ther Med, 4*(6), 1005-1009. doi:10.3892/etm.2012.729

## ORAL PRESENTATION

# Ruxolitinib Inhibits Hippo Pathway Effector YAP1 in Chronic Myeloid Leukemia Cells

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## Abstract

A novel signaling pathway, Hippo signalling pathway, has played a crucial role in maintaining organ size by regulating cell proliferation and death. The human Hippo pathway is based on a kinase signaling cascade including MST1 and MST2, as well as LATS1/2, SAV1, and MOB1. When the Hippo pathway is inactive, unphosphorylated YAP/TAZ enters the nucleus and interacts with TEA DNA-binding proteins (TEAD1-4), then target genes regulated by this complex.
Several hematological malignancies have been linked to abnormal expression or genetic deficiencies in the Hippo signaling pathway, including acute leukemia and lymphoproliferative neoplasms. Ruxolitinib is the first agent used in myelofibrosis treatment with its potent JAK/STAT inhibitory effect. In this research, we aimed to study the anti-leukemic effect of Ruxolitinib on Hippo pathway components in K-562 human chronic myeloid leukemia cells compared to NCI-BL 2171 human healthy B lymphocyte cells.
The cytotoxic effect of Ruxolitinib was determined by using WST-1 assay in K-562 and NCI-BL 2171 cells. To determine the Hippo pathway-related gene expression changes, total RNA was isolated from K-562 and NCI-BL 2171 cells treated with Ruxolitinib and untreated cells as a control group. A reverse transcription procedure was performed for cDNA synthesis, and gene expressions were shown by RT-qPCR.
IC50 values for K-562 and NCI-BL 2171 cells were defined as 20 and 23.6 µM at the 48th hour, respectively. Ruxolitinib treatment caused a notable decrease in the expression of YAP1, TEAD1/2, JAK2, and STAT3 genes in K-562 cells, compared to control cells.
Ruxolitinib is a promising agent in chronic myeloid leukemia treatment by blocking JAK/ STAT and Hippo pathways. This is the first study that reveals the relationship between ruxolitinib and YAP1 inhibition in chronic myeloid leukemia.

**Keywords:** Hippo Signaling Pathway, Ruxolitinib, Chronic Myeloid Leukemia

## INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal disorder characterized by BCR/ABL, an activated tyrosine kinase generated from the 9 and 22 reciprocal translocation chromosomes. BCR/ABL activates multiple pathways involved in the regulation of apoptosis and cell proliferation including PI3K-AKT, MEK-ERK, and STAT5. Patients diagnosed with CML are usually treated with imatinib BCR/ABL inhibitor. Nevertheless, tyrosine kinase inhibitors-resistance has come out as a significant clinical matter (H. Li et al., 2016). Ruxolitinib as a Janus tyrosine kinase (JAK1/JAK2) inhibitor is the first FDA-approved for CML treatment. JAKs dimerization modulates crucial biological processes via the activation of signal transducer and activation of transcription factors (STATs), which increases the target gene expressions (Chen et al., 2017). The Src family kinases have been proposed to be involved in BCR-ABL-stimulated transformation. The Src family kinases can phosphorylate and activate YAP a member of the Hippo signaling pathway, which has been shown in some tumors (P. Li et al., 2016).

The hippo signaling pathway, at first discovered in Drosophila, is evolutionarily maintained in mammals. Hippo pathway plays a significant role in controlling organ size, cancer initiation, and cancer progression. First, the mammalian STE20-like protein kinase 1/2 (Mst1/2) composes a complex with Salvador homologue 1 (Sav1), and this complex phosphorylates large tumor suppressor 1/2 (Lats1/2). Thus, activated Lats1/2 phosphorylates YAP and initiates its cytoplasmic holding and next degradation by ubiquitin-proteasome system (Meng, Moroishi, & Guan, 2016; Yu & Guan, 2013). The highly expression of YAP has also been

found to act as a stem cell regulator. It has been disclosed that the YAP expression was higher in chronic lymphoblastic leukemia patients. Even though it has been shown that YAP- stimulated apoptosis was regulated by the aberrant presence of ABL1 in the nucleus in multiple myeloma cells, in CML cells where ABL1 was mostly translocated, the function of YAP was uncertain. Thinking, the role of YAP in the pathogenesis of CML may be quirky to discover (Cottini et al., 2014; H. Li et al., 2016).

In this study, we aimed to study the anti-leukemic effect of Ruxolitinib on Hippo pathway components in K-562 human chronic myeloid leukemia cells compared to NCI-BL 2171 human healthy B lymphocyte cells.

## MATERIALS AND METHODS

Ruxolitinib was obtained from Incyte Corporation. K-562 (CCL-243) human CML cell line and NCI-BL-2171 human normal B lymphocyte were used in this study. For the culture of the desired K-562 and NCI-BL-2171 cell lines, RPMI-1640 medium with a volume of 500 ml and 50 ml of Fetal Bovine Serum (FBS), 1% 2 mM L-glutamine, and 1% penicillin-streptomycin was prepared and were inoculated at 37$^{\circ C}$, and 5% $CO_2$ conditions.
NCI-BL 2171 and K-562 cells were incubated at a density of $6.25 \times 10^4 - 2 \times 10^6$ cells/mL in 1 mL of the medium by using 96-well plates. 10 µL/well WST-1 solution was used to determine optimum cell concentration for a 72 h incubation period.
Cytotoxic effects of ruxolitinib in NCI-BL 2171 and K-562 cells were determined by WST-1 assay. Cells were incubated at a concentration of $1 \times 10^6$ cells/mL medium, and ruxolitinib was treated to cells with different concentrations from 10 nM to 10 mM in 96-well plates for 24-, 48-, and 72-h incubation periods. WST-1 assay was used to determine the cytotoxic effects of ruxolitinib. $IC_{50}$ doses of ruxolitinib on K-562 and NCI-BL 2171 cells were calculated via GraphPad Prism 5.0 software.
Gene expression analysis changes in the expression level of genes involved in the Hippo signal pathway in NCI-BL 2171 and K-562 cells treated with Ruxolitinib were determined by real-time PCR at 48 h. Total RNA was isolated from the cells using the RNeasy® Mini Kit and cDNA synthesis was realized using RT$^2$ First Strand Kit. Expression changing of the genes associated with the Hippo signaling pathway was examined using 2x Master Mix Green and Light Cycler 480.
Fold changes of the genes were calculated by the $2^{-\Delta\Delta Ct}$ method, and values $p < 0.05$ were considered as significant.

## RESULTS and DISCUSSION

The $IC_{50}$ value of ruxolitinib on the cells was determined as 20 µM, and 23,6 µM for K-562 and NCI-BL 2171 cells at the 48th hour, respectively.

In this study, no significant changes were found in the expression of Hippo signaling pathway genes in NCI-BL2171 healthy cells compared to an untreated control group. We found that ruxolitinib treatment caused a significant decrease in the expression of YAP1, TEAD1/2, JAK2, and STAT3 genes in K-562 cells compared to the control cells. Also, Lats1/2 and MOBKL1A/1B were down-regulated in K-562 cells compared to the control cells (Table 1; Fig 1).

The Hippo signaling pathway has a special ability to promote cancer. To treat cancer, a complete understanding of the Hippo signaling pathway system will be needed. Moreover, interaction with other pathways such as Wnt, mTOR, Notch, AMPK, JNK, TGF-β, Ras/MAP, and JAK2/STAT3 pathways can lend to carcinogenesis. YAP and TAZ phosphorylation could lead to leukemogenesis, which this process could be modulated through other signaling pathways (Noorbakhsh, Hayatmoghadam, Jamali, Golmohammadi, & Kavianpour, 2021; Xie et al., 2019). Recent studies have demonstrated dysregulated Yap expression in acute leukemias and myelodysplastic syndromes (Yuen et al., 2013). The study of Marsola et al. investigated the expression of the Hippo signaling pathway genes (*YAP*, TAZ, and *LATS1*/*LATS2*) in CML patients resistant or sensitive to imatinib and different stages of the disease. They showed *LATS2* overexpression in patients with CML, TAZ overexpression in CML patients at advanced phases, and upregulation of TAZ in CML imatinib-resistant (Marsola et al., 2018). Safari et al. demonstrated YAP1 and MST1/2 gene expression in acute myeloid leukemia (Safari et al., 2014). YAP as an oncoprotein and the nuclear effector of the Hippo signaling pathway

participates in the progression of various cancers. Also, few reports have investigated the effect of YAP inhibition in human leukemia cells. Chen et al. showed that YAP inhibition suppresses cell proliferation and increases apoptosis in leukemia cells. Therefore, pharmacological inhibition of YAP could be established for acute promyelocytic leukemia (Chen et al., 2017). The our results showed that treatment with ruxolitinib caused a significant decrease in the expression of YAP1 genes in K-562 cells compared to the control cells.

## CONCLUSION

Hippo pathway. This is the first study that reveals the relationship between ruxolitinib and YAP1 inhibition in chronic myeloid leukemia. Therefore, pharmacological inhibition of YAP could be established for acute promyelocytic leukemia and a novel treatment strategy for CML.

**Table 1.** Gene expression changes in K562 cells after Ruxolitinib treatment

| Symbol | K562- Ruxolitinib | | |
|---|---|---|---|
| | Fold Change | Log2- Fold Change | p-value |
| LATS1 | 0.10 | -3.32 | 0.0000 |
| TEAD1 | 0.14 | -2.84 | 0.0022 |
| LATS2 | 0.21 | -2.25 | 0.0100 |
| TEAD2 | 0.22 | -2.18 | 0.0003 |
| MOBKL1A | 0.17 | -2.56 | 0.0020 |
| STAT3 | 0.04 | -4.64 | 0.0005 |
| MOBKL1B | 0.001 | -9.97 | 0.0030 |
| JAK2 | 0.08 | -3.64 | 0.0016 |
| MST1 | 0.10 | -3.32 | 0.0001 |
| YAP1 | 0.26 | -1.94 | 0.0020 |
| MST2 | 0.08 | -3.64 | 0.0014 |
| NF2 | 0.03 | -5.06 | 0.0003 |
| SAV1 | 0.35 | -1.51 | 0.0033 |

| Symbol | NCI-BL- Ruxolitinib | | |
|---|---|---|---|
| | Fold Change | Log2- Fold Change | p-value |
| LATS1 | **0.30** | -1.74 | 0.060 |
| TEAD1 | 0.69 | -0.54 | 0.400 |
| LATS2 | **0.50** | -1.00 | 0.200 |
| TEAD2 | 1.92 | 0.94 | **0.003** |
| MOBKL1A | 0.91 | -0.14 | 0.006 |
| STAT3 | 1.95 | 0.96 | 0.300 |
| MOBKL1B | 0.88 | -0.18 | 0.900 |
| JAK2 | 1.18 | 0.24 | 0.100 |
| MST1 | **0.22** | -2.18 | 0.700 |
| YAP1 | **4.35** | 2.12 | 0.300 |
| MST2 | **0.49** | -1.03 | 0.800 |
| NF2 | **2.25** | 1.17 | 0.110 |
| SAV1 | **0.35** | -1.51 | 0.250 |

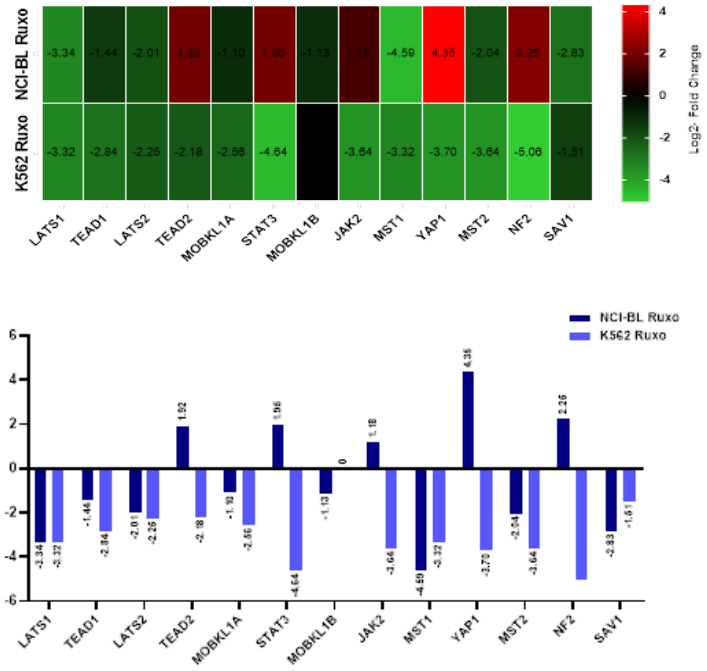


**Figure 1.** Heatmap and graph of Hippo pathway gene expression changes in K562 and NCI-BL 2171 cell line after ruxolitinib treatment

**REFERENCES**

Chen, M., Wang, J., Yao, S. F., Zhao, Y., Liu, L., Li, L. W., . . . Liu, B. Z. (2017). Effect of YAP Inhibition on Human Leukemia HL-60 Cells. *Int J Med Sci, 14*(9), 902-910. doi:10.7150/ijms.19965

Cottini, F., Hideshima, T., Xu, C., Sattler, M., Dori, M., Agnelli, L., . . . Tonon, G. (2014). Rescue of Hippo coactivator YAP1 triggers DNA damage-induced apoptosis in hematological cancers. *Nat Med, 20*(6), 599-606. doi:10.1038/nm.3562

Li, H., Huang, Z., Gao, M., Huang, N., Luo, Z., Shen, H., . . . Feng, W. (2016). Inhibition of YAP suppresses CML cell proliferation and enhances efficacy of imatinib in vitro and in vivo. *J Exp Clin Cancer Res, 35*(1), 134. doi:10.1186/s13046-016-0414-z

Li, P., Silvis, M. R., Honaker, Y., Lien, W. H., Arron, S. T., & Vasioukhin, V. (2016). αE-catenin inhibits a Src-YAP1 oncogenic module that couples tyrosine kinases and the effector of Hippo signaling pathway. *Genes Dev, 30*(7), 798-811. doi:10.1101/gad.274951.115

Marsola, A. P. Z. C., Simões, B. P., Palma, L. C., Berzoti-Coelho, M. G., Burin, S. M., & de Castro, F. A. (2018). Expression of Hippo signaling pathway and Aurora kinase genes in chronic myeloid leukemia. *Medical Oncology, 35*(3), 26. doi:10.1007/s12032-018-1079-6

Meng, Z., Moroishi, T., & Guan, K. L. (2016). Mechanisms of Hippo pathway regulation. *Genes Dev, 30*(1), 1-17. doi:10.1101/gad.274027.115

Noorbakhsh, N., Hayatmoghadam, B., Jamali, M., Golmohammadi, M., & Kavianpour, M. (2021). The Hippo signaling pathway in leukemia: function, interaction, and carcinogenesis. *Cancer Cell Int, 21*(1), 705. doi:10.1186/s12935-021-02408-7

Safari, S., Movafagh, A., Zare-Adollahi, D., Ghadiani, M., Riazi-Isfahani, S., Safavi-Naini, N., & Omrani, M. D. (2014). MST1/2 and YAP1 gene expression in acute myeloid leukemia. *Leukemia & Lymphoma, 55*(9), 2189-2191. doi:10.3109/10428194.2013.867493

Xie, L., Wu, S., Cao, D., Li, M., Liu, J., Nie, G., . . . Yang, H. (2019). Huyang yangkun formula protects against 4-Vinylcyclohexene diepoxide-induced premature ovarian insufficiency in rats via the Hippo–JAK2/STAT3 signaling pathway. *Biomedicine & Pharmacotherapy, 116*, 109008. doi:https://doi.org/10.1016/j.biopha.2019.109008

Yu, F. X., & Guan, K. L. (2013). The Hippo pathway: regulators and regulations. *Genes Dev, 27*(4), 355-371. doi:10.1101/gad.210773.112

Yuen, H. F., McCrudden, C. M., Huang, Y. H., Tham, J. M., Zhang, X., Zeng, Q., . . . Hong, W. (2013). TAZ expression as a prognostic indicator in colorectal cancer. *PLoS One, 8*(1), e54211. doi:10.1371/journal.pone.0054211

## ORAL PRESENTATION

# Efficacy Of Entomopathogenic Fungal Formulations Against Some Stored-Product Pests

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**Abstract**

Two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium robertsii*, were formulated as powder, and the mortality effects of the formulations were tested on five important stored product pests (*Sitophilus oryzae*, *Rhyzopertha dominica*, *Plodia interpunctella*, *Oryzaephilus surinamensis* and *Trigoderma granarium*). A total of four formulations (BbTF1, BbTF2, MrTF1, and MrTF2), two for each fungus, were prepared and tested. Along with the formulations, unformulated fungi (*B. bassiana* 5-4 and *M. robertsii* S3 isolates) were tested at $2x10^{10}$ spores/kg wheat concentration. Formulations without fungi (TF1 and TF2) were also tested. The experiments were conducted in darkness at 65±5% relative humidity at 25±2 and 30±2°C temperatures with five replications. Wheat kernels (50 g) were treated by mixing with the formulations and twenty adults were released for each replication. Insect mortalities were recorded after 7 and 14 days. In comparison to unformulated fungi and non-fungus formulations, formulated fungi demonstrated significantly higher mortality on days 7 and 14 at both temperatures, with the exception of *S. oryzae*. Compared to BbTF2 and MrTF2, TF1 caused higher mortality of *S. oryzae* adults. The mortality due to the treatments was not significantly different at two tested temperatures. The results showed that the most resistant species among the pests was *T. granarium*, and BbTF1 and MrTF1 were the most successful formulations against all the tested stored product pests. As a result, formulations of both fungi with increased efficacy were achieved for the control of stored product pests.

**Keywords:** Entomopathogenic fungi, Formulation, Stored-product pests, Mortality

**Özet**

*Beauveria bassiana* ve *Metarhizium robertsii* entomopatojen fungus türleri toz olarak fomüle edilmiştir ve formülasyonların ölüm etkileri beş önemli depolanmış ürün zararlısına(*Sitophilus oryzae*, *Rhyzopertha dominica*, *Plodia interpunctella*, *Oryzaephilus surinamensis* ve *Trigoderma granarium*) test edilmiştir. Her fungus türü için iki adet olmak üzere toplam dört formülasyon (BbTF1, BbTF2, MrTF1 ve MrTF2) hazırlanmış ve test edilmiştir. Formülasyonlarla birlikte, formüle edilmemiş funguslar (*B. bassiana* 5-4 ve *M. robertsii* S3 izolatları) $2x10^{10}$ spor/kg buğday konsantrasyonunda test edilmiştir. Fungus içermeyen formülasyonlar (TF1 and TF2) da test edilmiştir. Denemeler, %65±5 nispi nemde 25±2 ve 30±2°C sıcaklıklarda karanlıkta beş tekerrürlü olarak yürütülmüştür. Buğday taneleri (50 g) formülasyonlarla ile karıştırılmıştır ve her tekerrür içerisine yirmi adet birey salınmıştır. Böcek ölümleri 7. ve 14. günlerde sayılmış ve kaydedilmiştir. Formüle edilmemiş funguslar ve fungus içermeyen boş formülasyonlar karşılaştırıldığında, formüle edilmiş funguslar, *S. oryzae* hariç diğer zararlılara karşı her iki sıcaklıkta da 7. ve 14. günlerde önemli ölçüde daha yüksek ölüm oranı göstermiştir. TF1, *S. oryzae*'ye BbTF2 ve MrTF2'ye kıyasla daha yüksek ölüme neden olmuştur. Test edilen iki sıcaklıkta, uygulamaların neden olduğu ölümler önemli farklılık göstermemiştir. Bu sonuçlar, zararlılar arasından en dayanıklı böcek türünün *T. granarium* olduğunu ve tüm zararlılara karşı en başarılı formülasyonların MrTF1 ve BbTF1 olduğunu göstermiştir. Sonuç olarak, depolanmış ürün zararlılarının mücadelesi için her iki fungus türünün de formülasyon ile daha etkili hale getirilebileceği ortaya konmuştur.

**Keywords:** Entomopatojen funguslar, Formülasyon, Depolanmış ürün zararlıları, Ölüm etkisi

## GİRİŞ

Buğday besin ihtiyacını karşılama açısından önemli bir konuma sahip olan tahıl ürünüdür. Bu nedenle, ülkemizde ve dünyada buğday en çok üretilen tahıllar arasında yer almaktadır (FAO, 2021; TÜİK, 2021). Yüksek miktarlarda üretilmesi nedeniyle buğdaylar hemen tüketilememektedir ve büyük bir kısmı depolanmaktadır.

Depolanmış olan buğdaylar depolanma sürecinde birçok zararlı böcek türüne karşı savunmasız kalmaktadırlar. Bu zararlı böcekler ürünün ağırlık, besin değeri ve çimlenme gibi özelliklerinde kayba neden olarak ciddi ekonomik kayba neden olmaktadırlar. Bu nedenle, depolanmış ürün zararlılarına karşı mücadele etmek büyük önem taşımaktadır ve yaygın olarak sentetik insektisitler ve fumigantlar kullanılmaktadır (Arthur, 1996). Fakat, kimyasallarla mücadele yöntemi zararlılarda dayanıklılık problemi, üründe kalıntı bırakması, hedef dışı canlılara etkisi, çevreye ve memelilere toksik etkisi gibi birçok olumsuz etkilere yol açmaktadır (Hagstrum ve Flinn 1994; Moore et al. 2000; Batta 2016). Bu negatif durumu gidermek veya azaltmak için zararlılara karşı çevre dostu mücadele yöntemlerin arayışına girilmiştir ve bu arayışa biyolojik mücadele önemli bir cevap olmuştur (Wakil, 2015).

Biyolojik mücadelede kullanılan yöntemler arasından da depolanmış ürün zararlıları ile mücadelede mikrobiyal mücadele etmenlerinden birisi olan entomopatojen funguslar diğer etmenlere kıyasla ön plana çıkmıştır (Moore et al. 2000; Batta 2016). Entomopatojen funguslar arasından da *Beauveria bassiana* ve *Metarhizium anisopliae* depolanmış ürün zararlılarına karşı yoğun olarak çalışılmış olan ve önemli sonuçlar elde edilmiş türlerdir. Fakat, yapılan çalışmalar sonucu potansiyeli bilinen izolatların daha efektif hale getirilebilmesi için formüle edilmesi bir ihtiyaç haline gelmiştir.

Başarılı bir biyopestisit, depo şartlarına dayanıklı, kolay uygulanabilir ve zararlıya karşı etkinliği yüksek olmalıdır (Wraight ve ark., 2001). Depolar şartlarında zararlılarla mücadelede depolanmış ürüne toz formülasyonların uygulanmasının daha uygun olduğu bilinmektedir. Bu amaçla, toz formülasyonların geliştirilmesinde talk tozu, kaolin, kil, kül, silika içermeyen maddeler, sodyum silkat ve diatom gibi birçok dolgu maddesi kullanılmıştır (Golob ve Webley, 1980) ve entomopatojen fungus ile birlikte kullanımı sonucu zararlılara etkileri birçok çalışma ile değerlendirilmiştir (Lord 2005; Vassilakos et al. 2006; Athanassiou ve Steenberg, 2007; Wakil et al. 2011).

Yapılan çalışmada, 5-4 nolu *B. bassiana* ve S3 nolu *M. robertsii* izolatlarının ve bu izolatlara ait 4 farklı toz formülasyonun, 5 farklı depolanmış buğday zararlısına karşı ölüm etkileri değerlendirilmiştir.

## MATERYAL ve METOD

### Böcek Kültürleri

Biyolojik testlerde, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Oryzeaphilus surinamensis* ile *Trogoderma granarium* ve *Plodia interpunctella* 'nın ise larvaları kullanılmıştır.

*R. dominica* ve *S. oryzae* (26±2ºC) ekmeklik buğdayda, *O. surinamensis* yumuşak ekmeklik buğday, yulaf ezmesi ve kuru mayadan oluşan 100-200g karışım besinde, *T. granarium*' un köpek maması, yulaf ezmesi ve buğday rüşeymi birleşimi besinde, *P. interpunctella* ise buğday unu kepeği, mısır unu, gliserin, glikoz şurubu ve maya karışımı besinde 30±2ºC sıcaklık ve %65±5 nem koşullarında karanlık ortam şartlarında yetiştirilmiştir.

### Entomopatojen Funguslar

Biyolojik testlerde kullanılan *Metarhizium robertsii*'ye ait izolat (S3) ve *Beauveria bassiana*'ya ait izolat (5-4) K.S.Ü Ziraat Fak. Bitki Koruma Bölümü Entomopatojen fungus koleksiyonunda mevcut olan izolatlardır. *M. robertsii* S3 nolu izolatı, Gaziantep'te bir fıstık bahçesinden alınmış bir toprak örneğinden izole edilmiş $F_{17\text{-}2\text{-}1}$ nolu izolata ait bir tek spor kültürüdür (Barış, 2022). *B. bassiana* 5-4 nolu izolatı, Şanlıurfa'da bir buğday deposundan alınmış *Rhyzopertha dominica* ergininden izole edilen 151138 nolu izolata ait bir tek spor kültürüdür (Er ve ark. 2016).

### Entomopatojen fungus sporlarının çoğaltılması ve çimlenme güçlerinin belirlenmesi

Biyolojik testlerde kullanılan entomopatojen fungus sporları kitlesel üretim yöntemi ile pirinç kullanılarak elde edilmiştir (Barış, 2021). Kitlesel olarak üretilmiş olan sporlar, pirincin yüzeyinden ayrılacak seviyeye kadar kurutulduktan sonra 500 µm'lik elekle elenmiştir. İzolatlara ait kitlesel üretim sonucu elde edilmiş olan sporlar ile $10^6$ spor/ml konsanstrasyonda süspansiyonlar hazırlanmış ve süspansiyon içerisinden 100 µl örnek alınıp Patates dekstroz agar üzerine yayılmıştır. Yayma işlemi tamamlanmış olan petriler 30±2°C sıcaklıkta 24 sa. inkübe edilmiş ve bu süre sonunda her bir petride 3 farklı nokta belirlenip 100 spor sayılmıştır. Bu işlem her izolat için üç tekerrürlü olarak gerçekleştirilmiştir.

### Toz formülasyonların içerikleri ve biyolojik testler için hazırlanışı

İki farklı izolata ait sporların eleme sonrası elde edilmesinden sonra 25±2°C sıcaklıkta kurumaya bırakılmıştır. Bunun yanı sıra, dolgu maddeleri de 70°C sıcaklıkta üç gün boyunca etüvde kurutulup tüm

maddeler desikatörde 48 saat bekletilmiştir. Bu süre sonunda desikatörden çıkarılan materyallerin su aktiviteleri Novasina (ms1 set-aw) cihazı ile üç tekerrürlü olarak belirlenmiştir. Formülasyonlarda kullanılan sporların ve dolgu maddelerinin su aktivite değerleri Tablo 1'de verilmiştir.

Tablo 1. Toz formülasyonlarda kullanılan sporların ve dolgu maddelerinin su aktiviteleri

| **Materyaller** | **Kimyasal yapısı veya türü** | **Su aktiviteleri ($a_w$)** |
|---|---|---|
| 5-4 | *Beauveria bassiana* | 0.128±0.001 |
| S3 | *Metarhizium robertsii* | 0.128±0.001 |
| K16 | Diatom toprağı | 0.098±0.004 |
| Kaolin | China Clays | 0.158±0.006 |

Elde edilmiş olan materyaller ile Tablo 2'de belirtilen oranlarda toz formülasyonlar hazırlanmıştır. Hem tek başına izolatların hem de toz formülasyonların, %90'ın üzerinde çimlenme gücüne sahip olduğu tespit edilmiştir.

Tablo 2. Toz formülasyonların içerikleri (kg ürün)

| Formülasyon isimleri | AKTİF MADDE | DOLGU MADDESİ | ADJUVANT |
|---|---|---|---|
| BbTF1 | %20 *B. bassiana* ($2.4x10^{13}$ spor) | %75 Diatom Toprağı(K16) | %5 Silikon Dioksit |
| BbTF2 | %20 *B. bassiana* ($2.4x10^{13}$ spor) | %75 Kaolin | %5 Silikon Dioksit |
| MrTF1 | %42.9 *M. robertsii* ($1.7x10^{13}$ spor) | %53.6 Diatom Toprağı(K16) | %3.5 Silikon Dioksit |
| MrTF2 | %42.9 *M. robertsii* ($1.7x10^{13}$ spor) | %53.6 g Kaolin | %3.5 Silikon Dioksit |

**Biyolojik testler**

Tablo 2'de belirtilen toz formülasyonlar, boş formülasyonlar ve entomopatojen fungus izolatlarına ait sporlar *R. dominica, S. oryzae, O. surnamensis T. granarium* ve *P. interpunctella'*ya karşı 25±2°C ve 30±2°C sıcaklıkta %65±5 nispi nemde ve karanlık ortam şartlarında test edilmiştir. *B. bassiana* içerikli toz formülasyonlar 1g/kg buğday, *M. robertsii* içerikli formülasyonlar 1.4g/kg buğday konsantrasyonunda uygulanırken boş formülasyonlar içerisinde spor içermeyen haliyle uygulanmıştır. Tek başına izolatlar ise toz formülasyonların içeriğinde belirtilmiş olan etkili madde miktarı ($2x10^{10}$ spor/kg buğday) kadar buğdaylara uygulanmıştır. Kontrol uygulamalarında kullanılmış olan buğdaylara herhangi bir uygulama yapılmamıştır. Biyolojik testlerde, içerisinde 40 g makarnalık buğday bulunan 50 ml kapasiteli falkon tüpleri kullanılmıştır. Her bir uygulama için falkon tüpüne 20 ergin böcek bırakılmıştır ve %65±5 nispi nemde, 25±2°C ve 30±2°C sıcaklık sağlayan iklim odalarına bırakılmıştır. Ölümler, 7. ve 14. günlerde yapılan sayımlar ile belirlenmiştir ve kaydedilmiştir. Yedinci ve ondördüncü gün yüzde ölüm verilerine arcsine transformasyonu uygulanmış ve ANOVA testine tabi tutulmuştur. Her bir uygulamanın farklı böcek türlerinde neden olduğu ölüm ortalamaları ve her bir böcekte farklı uygulamalar arasındaki ölüm ortalamaları arasındaki farklılıklar TUKEY çoklu karşılaştırma testi ile belirlenmiştir**.**

**BULGULAR**

Her bir uygulamanın, 25±2°C sıcaklıkta 7. ve 14. günde beş farklı depolanmış ürün zararlısına karşı ölüm ortalamaları arasında ve her bir zararlıya farklı uygulamaların neden olduğu ölüm ortalamaları arasında istatistiksel olarak farklılık olduğu tespit edilmiştir. *S. oryzae* hariç diğer zararlılara, tüm formülasyonların formüle edilmemiş uygulamalara kıyasla daha iyi ölüm etkisi gösterdiği tespit edilmiştir. BbTF1 ve MrTF1, 14. günde her iki sıcaklıkta da *T. granarium* hariç diğer zararlılara %100 oranda ölüm etkisi göstermiştir.

Her bir uygulamanın, 30±2°C sıcaklıkta 7. ve 14. günde beş farklı depolanmış ürün zararlısına karşı ölüm ortalamaları arasında ve her bir zararlıya farklı uygulamaların neden olduğu ölüm otalamaları arasında istatistiksel olarak farklılık olduğu tespit edilmiştir. Yapılan uygulamalar sonucunda 30±2°C sıcaklıkta elde edilmiş olan sonuçlar 25±2°C sıcaklıkta elde edilmiş olan sonuçlarla paralellik göstermiştir.

Tüm sonuçlar değerlendirildiğinde BbTF1 beş farklı depo zararlısına karşı da en etkili formülasyon olmuştur. Depolanmış ürün zararlıları arasından yapılan uygulamalara karşı en dayanıklı türün *T. granarium,* en hassas türün ise *P. interpunctella* olduğu tespit edilmiştir. *T. granarium*'a karşı en etkili uygulama olarak ön plana çıkan BbTF1 formülasyonu ile %100 ölüm elde edilememiştir (14. gün için, 25°C sıcaklıkta %87, 30°C sıcaklıkta %95). Fakat, 7. günde her iki sıcaklıkta da *T. granarium* hariç diğer böceklerde %100 ölüm etkisi gösteren tek uygulama BbTF1 olmuştur. İzolatların neden olduğu ölümlere nazaran formüle edilmiş olan uygulamaların neden olduğu ölümlerin önemli derecede yükseldiği tespit edilmiştir.

Bu çalışma ile izolatların formüle edilmesi ile depolanmış ürün zararlılarına karşı daha etkili hale getirilebileceği ortaya konmuştur. Sadece depolarda değil diğer tarımsal alanlarda da mücadelede biyolojik kökenli preparatların formüle edilmesiyle birçok özelliği bakımından daha iyi hale getirilebileceği sonucuna varılmıştır.

**KAYNAKLAR**

Arthur FH, 1996. Grain protectants: current status and prospects for the future. Journal of Stored Products Research, 32: 293-302.

Wraight SP, Jackson MA, Kock SL, 2001. Production, stabilization and formulation of fungal biocontrol agents. In: Butt, T. M., Jackson, C. W.and Magan, N. (eds.), Fungi as biocontrol agents: progress, problems and potential, CABI Publishing, Wallingford, pp 253–287.

FAO, 2021. Food and Agriculture Organization of the United Nations. (Erişim tarihi: 01/05/2021)

Wakil W, Riasat T, Ghazanfar MU, Kwon YJ, Shaheen FA, 2011. Aptness of *Beauveria* bassiana and enhanced diatomaceous earth (DEBBM) for control of *Rhyzopertha dominica* F. Entomological Research, 41: 233-241.

Lord JC, 2005. Low humidity, moderate temperature and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae). Biological Control, 34: 180-186.

Batta YA, 2016. Recent advances in formulation and application of entomopathogenic fungi for biocontrol of stored grain insects. Biocontrol Sciense Technology 26:1171–1183.

Arthur FH, 1996. Grain protectants: current status and prospects for the future. Journal of Stored Products Research, 32: 293-302.

Hagstrum DW, Flinn PW, 1994. Survival of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat under fall and winter temperature conditions. Environmental Entomology, 23: 390–395.

TUİK, 2021. Türkiye İstatistik Kurumu. (Erişim tarihi: 01.05.2021) http://www.tuik.gov.tr/.

Vassilakos TN, Athanassiou CG, Kavallieratos NG, Vayias BJ, 2006. Influence of temperature on the insecticidal effect of *Beauveria bassiana* in combination with diatomaceous earth against *Rhyzopertha dominica* and *Sitophilus oryzae* on stored wheat. Biological Control, 38: 270–281.

Golob P, Webley DJ, 1980. The Use Of Plants and Minerals as Traditional Protectants of Stored Products. Report Tropical Product Institute, G138.

Barış C 2021. Depolanmış Ürün Zararlılarına Karşı Entomopatojen Fungusların Kullanım Yönteminin Geliştirilmesi, Kahramanmaraş Sütçü İmam Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Ana Bilim Dalı, 146 S.

Athanassiou CG, Steenberg T, 2007. Insecticidal Effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) in Combination with Three Diatomaceous Earth Formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Biological Control 40, 411-416.

Er MK, Tunaz H, Işıkber AA, 2016. Improving the Virulence Of A Native *Beauveria bassiana* Isolate against *Rhyzopertha dominica* Adults. 7th International Scientific Agriculture Symposium (Agrosym 2016). 6-9 October 2016, Jahorina, Bosnia And Herzegovina. Pp: 1464–1469

Moore D, Lord JC, Smith SM, 2000. "Pathogens, 193-227". In: Alternatives To Pesticides in Stored-Product IPM (Eds: B. H. Subramanyam & D. W. Hagstrum). Kluwer Academic Publishers, Dordrecht Netherlands, 437 Pp.

## *ORAL PRESENTATION*

# Anticancer activity of melatonin on triple-negative breast cancer in hypoxic conditions

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## Abstract

Melatonin is a pleiotropic molecule with many biological activities. It regulates anticancer mechanisms such as inhibition of cell proliferation, side effects associated with chemotherapy and radiotherapy, and reduction of drug resistance. Hypoxia is known as oxygen deficiency. Cancer cells turn many mechanisms in their favor to increase their chances of survival under hypoxic conditions. They induce angiogenesis by overexpressing growth factors, especially by altering energy metabolism and promoting cell proliferation. This study investigated the effects of melatonin, known to have anticancer activity, on the amino acid profile of triple-negative breast cancer (MDA-MB-231) cells under hypoxic conditions. MDA-MB-231 cells were treated with increasing doses of melatonin (1 and 2 mM) and incubated for 16 hours under 1% hypoxia. After incubation, cell lysates were prepared, and intracellular free amino acid determination was performed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). The significance levels of amino acids were analyzed using GraphPad Prism 9 programs. Arginine, glutamine, and glutamic acid amino acids were significantly decreased in melatonin-treated breast cancer cell groups compared to the control group (***$p<0.001$); taurine amino acid was found to increase significantly (***$p<0.001$). Melatonin is known to have anticancer activity. However, its effect on many survival mechanisms that cancer cells change to survive under hypoxia is unknown. In our study, the decrease in arginine, glutamine, and glutamic acid amino acids in the group treated with melatonin indicates that melatonin shows anticancer activity in hypoxic conditions. In addition, increasing taurine amino acid shows that apoptosis is triggered in cells. According to these results, melatonin showed anticancer activity by inhibiting hypoxia-induced cancer cell survival and energy metabolism.

**Keywords:** Triple Negative Breast Cancer, MDA-MB-231, Melatonin, Hypoxia, Amino Acid Profiles

## INTRODUCTION

Cancer is a significant cause of morbidity and mortality in developed and developing countries (Siegel, Miller, Fuchs, & Jemal, 2021). Cancer cells have characteristics such as persistent proliferation and insensitivity to growth suppressors, sustained DNA replication, impaired energy metabolism, and supported angiogenesis, invasion, and metastasis to pave the way for tumor progression (Herceg et al., 2018). Hypoxia has an essential role in tumor progression through overexpression of growth factors and cellular adaptation genes. These changes promote cell survival, proliferation, invasion, metastasis, angiogenesis, and energy metabolism in favor of cancer development. (Marin-Hernandez, Gallardo-Perez, Ralph, Rodriguez-Enriquez, & Moreno-Sanchez, 2009). Hypoxia also plays a central role in determining the resistance of tumors to chemotherapy (Wilson & Hay, 2011). Melatonin (N-acetyl-5-methoxytryptamine), a multifunctional molecule secreted by the pineal gland, has many functions, such as regulation of biological rhythms, antioxidant actions, sleep induction, vasoregulation and immunomodulation (Simko & Pechanova, 2009; Veneroso, Tuñón, González-Gallego, & Collado, 2009). In addition, it has been shown by studies that melatonin has an anticancer effect (Chao et al., 2019; Chuffa et al., 2015; Estaras et al., 2021). Interestingly, studies have shown that melatonin modulates hypoxia-induced tumorigenesis, and the administration of melatonin in combination with other therapeutic compounds increases the efficacy of these treatments (Chen et al., 2017; Park et al., 2010). More than 90 years ago, Otto Warburg's groundbreaking discovery of aerobic glycolysis established metabolic reprogramming as one of the first hallmarks of cancer (Warburg, Wind, & Negelein, 1927). The field of

cancer metabolism then focused on central carbon metabolism, including the citric acid cycle and the pentose phosphate pathway, revealing additional metabolic changes in cancer. However, recent reports have shown significant non-carbon metabolism contributions to cancer cell viability and growth (Li et al., 2016). Amino acids, nutrients vital to the survival of all cell types, have been the evidence pointing to reprogrammed metabolism in cancer. Amino acids are molecules responsible for intracellular regulation by promoting cancer growth and metastatic potential or producing derivatives with anticancer activity (Choi & Coloff, 2019). This molecule has critical functions in the cell, such as changing the chromatin structure (Maddocks et al., 2017), modulating cell proliferation (Pegg, 2009), and controlling unlimited proliferation (Keenan & Chi, 2015) by changing the intracellular redox exchange and energy metabolism (Vaughn & Deshmukh, 2008). With a full understanding of the metabolic flexibility and diversity of amino acid utilization in cancer cells, it is possible to provide more insight into metabolic dependencies and liabilities that can be exploited early diagnosis or therapeutically. In this context, the effects of melatonin, which is known to have anticancer activity, on the amino acid profile of triple-negative breast cancer (MDA-MB-231) cells under hypoxic conditions were investigated.

## MATERIALS AND METHODS

### Cell Culture

In the study, the MDA-MB-231 (breast adenoma cancer cell) cell line obtained from ATCC and stocked in liquid nitrogen and cultured in RPMI-1640 (Gibco) medium supplemented with 10% FBS, 100 µg/mL streptomycin/100IU/mL penicillin, in a humid atmosphere, 5% CO2 in a 37 ºC incubator.

### Melatonin Treatment Under Hypoxia Condition

MDA-MB-231 cells were cultured by seeding in 6 well ($2x10^5$) plates. After approximately 1 day, it was treated with increasing melatonin dose (1 and 2 mM) and incubated for 16 hours in 1% hypoxic environment.

### Measurement of Intracellular Free Amino Acids by LC-MS/MS

MDA-MB-231 cells treated with melatonin were removed from the medium on ice after incubation and the cells were scraped with a cell scraper in PBS and taken into clean tubes. The homogenized cells were centrifuged at 14,000 rpm for 5 minutes, and the supernatants obtained were used for amino acid determination. Intracellular free amino acid analysis was performed using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) (Shimadzu-8045) according to the kit protocol (Bome Trivitron, Trimaris-BR130030, Turkey). We used a derivative method in the analysis based on the kit protocol. 50 µL of sample is mixed with an internal standard solution consisting of 20 amino acids with C13 and N15 labeled atoms prepared in 0.1 M HCl. Essential organic buffer components were added for pH balance. A chloroform/isooctane mixture containing 5% alkyl chloroformate as an active ingredient was added to the sample mixture and incubated at room temperature for 3 minutes. Derived amino acids were removed by centrifugation from the supernatant containing organic solvents. The signal of the MS device is increased in parallel with the increased molecular weight of esterified amino acids. Trimaris Amino Acid LC-MS/MS column (250mm x 2mm, 3µM) with C18 phase filler was used for the chromatographic separation, and multiple reaction monitoring (MRM) mode was preferred to analyze.

### Statistical Analysis

GraphPad prism 9 (GraphPad Software, Inc, San Diego, USA) package program was used for the analysis of continuous data. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine whether there was a difference between the distribution of continuous data and the normal distribution. One-Way ANOVA test was used for three independent group comparisons of normally distributed data, while Kruskal-Wallis H test was used for three-group comparisons with non-normally distributed data. $p<0.05$ was considered statistically significant.

## RESULTS and DISCUSSION

### Melatonin altered the amino acid profile of breast cancer cells under hypoxia

The effects of melatonin, which is known to have anticancer activity, on the amino acid profile of triple negative breast cancer (MDA-MB-231) cells under hypoxia were investigated. MDA-MB-231 cells were treated with increasing dose of melatonin (1 and 2 mM) and incubated for 16 hours under 1% hypoxia conditions. After incubation, cell lysates were prepared and intracellular free amino acid determination was performed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Arginine, glutamine and glutamic acid amino acids were significantly decreased in melatonin-treated breast cancer cell groups compared to the control group (***p<0.001); taurine amino acid was found to increase significantly (***p<0.001) Figure 1.

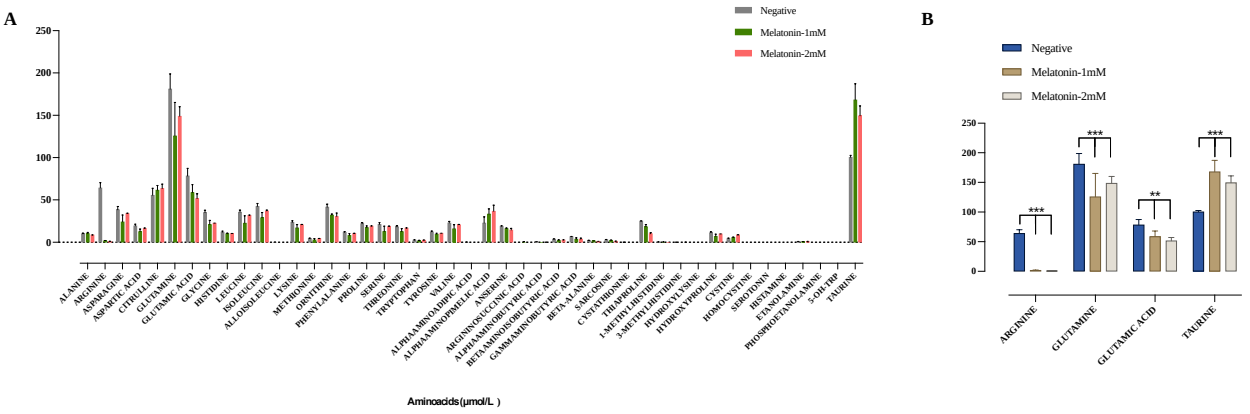


**Figure 1. Comparison of free amino-acid levels between negative and melatonin treatment groups in MDA-MB-231 cell lines. (A)** Free amino acid levels in MDA-MB-231 cell line after treatment with the melatonin at doses of 1 and 2 mM. **(B)** Significantly changed amino acids in MDA-MB-231 cell lines. All experiments were performed in triplicate. (*p<0.05, **p<0.01, ***p<0.001).

Melatonin is known to have anticancer activity. However, its effect on many survival mechanisms that cancer cells change in order to survive under hypoxia is unknown. In our study, the decrease in arginine, glutamine and glutamic acid amino acids in the group treated with melatonin indicates that melatonin shows anticancer activity in hypoxic conditions.

Arginine is an amino acid critically involved in multiple cellular processes including the syntheses of nitric oxide and polyamines, and is a direct activator of mTOR, a nutrient-sensing kinase strongly implicated in carcinogenesis. Decreased arginine amino acid in MDA-MB-231 cells after melatonin treatment indicates decreased proliferation in cells. Metabolic reprogramming of energy metabolisms by cancer cells is essential for continuous proliferation and increased survival. Therefore, the cancerous cells adapt to rearrange the energy metabolism when exposed to nutrient starvation and hypoxia. Glutamine is a derivative of glutamic acid and is formed in the body from glutamic acid and ammonia in an energy-requiring reaction catalyzed by glutamine synthase. Glutamine is firstly used as the glucose precursor in glucose deprivation, and in this way, the cells ensure energy production under catastrophic conditions. In this study, the reduction of glutamine and glutamic acid amino acids in cells treated with melatonin altered energy metabolism in MDA-MB-231 cells. Recent studies have shown that breast cancer cells treated with taurine amino acid trigger apoptosis in cells by activating pro-apoptotic proteins and caspase cascade. The level of amino acid taurine increased in the cell groups treated with melatonin. According to these results, melatonin showed anticancer activity by inhibiting hypoxia-induced cancer cell survival and energy metabolism.

## CONCLUSION

With a complete understanding of the metabolic flexibility and diversity of amino acid utilization in cancer cells, it is possible to provide more insight into metabolic dependencies and liabilities that can be exploited early diagnosis or therapeutically.

## REFERENCES

Chao, C.-C., Chen, P.-C., Chiou, P.-C., Hsu, C.-J., Liu, P.-I., Yang, Y.-C., . . . Tang, C.-H. (2019). Melatonin suppresses lung cancer metastasis by inhibition of epithelial–mesenchymal transition through targeting to Twist. *Clinical Science, 133*(5), 709-722.

Chen, X., Wang, Z., Ma, H., Zhang, S., Yang, H., Wang, H., & Fang, Z. (2017). Melatonin attenuates hypoxia-induced epithelial-mesenchymal transition and cell aggressive via Smad7/CCL20 in glioma. *Oncotarget, 8*(55), 93580.

Choi, B.-H., & Coloff, J. L. (2019). The diverse functions of non-essential amino acids in cancer. *Cancers, 11*(5), 675.

Chuffa, L. G. A., Fioruci-Fontanelli, B. A., Mendes, L. O., Ferreira Seiva, F. R., Martinez, M., Fávaro, W. J., . . . Martinez, F. E. (2015). Melatonin attenuates the TLR4-mediated inflammatory response through MyD88-and TRIF-dependent signaling pathways in an in vivo model of ovarian cancer. *BMC cancer, 15*(1), 1-13.

Estaras, M., Gonzalez-Portillo, M. R., Fernandez-Bermejo, M., Mateos, J. M., Vara, D., Blanco-Fernandez, G., . . . González, A. (2021). Melatonin induces apoptosis and modulates cyclin expression and MAPK phosphorylation in pancreatic stellate cells subjected to hypoxia. *International journal of molecular sciences, 22*(11), 5555.

Herceg, Z., Ghantous, A., Wild, C. P., Sklias, A., Casati, L., Duthie, S. J., . . . Koturbash, I. (2018). Roadmap for investigating epigenome deregulation and environmental origins of cancer. *International journal of cancer, 142*(5), 874-882.

Keenan, M., & Chi, J.-T. (2015). Alternative fuels for cancer cells. *Cancer Journal (Sudbury, Mass.), 21*(2), 49.

Li, J., Sung, C. Y. J., Lee, N., Ni, Y., Pihlajamäki, J., Panagiotou, G., & El-Nezami, H. (2016). Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. *Proceedings of the National Academy of Sciences, 113*(9), E1306-E1315.

Maddocks, O. D., Athineos, D., Cheung, E. C., Lee, P., Zhang, T., van den Broek, N. J., . . . Kruiswijk, F. (2017). Modulating the therapeutic response of tumours to dietary serine and glycine starvation. *Nature, 544*(7650), 372-376.

Marin-Hernandez, A., Gallardo-Perez, J. C., Ralph, S. J., Rodriguez-Enriquez, S., & Moreno-Sanchez, R. (2009). HIF-1α modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms. *Mini reviews in medicinal chemistry, 9*(9), 1084-1101.

Park, S. Y., Jang, W. J., Yi, E. Y., Jang, J. Y., Jung, Y., Jeong, J. W., & Kim, Y. J. (2010). Melatonin suppresses tumor angiogenesis by inhibiting HIF-1α stabilization under hypoxia. *Journal of pineal research, 48*(2), 178-184.

Pegg, A. E. (2009). Mammalian polyamine metabolism and function. *IUBMB life, 61*(9), 880-894.

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2021). Cancer statistics, 2021. *Ca Cancer J Clin, 71*(1), 7-33.

Simko, F., & Pechanova, O. (2009). Potential roles of melatonin and chronotherapy among the new trends in hypertension treatment. *Journal of pineal research, 47*(2), 127-133.

Vaughn, A. E., & Deshmukh, M. (2008). Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c. *Nature cell biology, 10*(12), 1477-1483.

Veneroso, C., Tuñón, M. J., González-Gallego, J., & Collado, P. S. (2009). Melatonin reduces cardiac inflammatory injury induced by acute exercise. *Journal of pineal research, 47*(2), 184-191.

Warburg, O., Wind, F., & Negelein, E. (1927). The metabolism of tumors in the body. *The Journal of general physiology, 8*(6), 519.

Wilson, W. R., & Hay, M. P. (2011). Targeting hypoxia in cancer therapy. *Nature Reviews Cancer, 11*(6), 393-410.

## *ORAL PRESENTATION*

# Investigation of amino acid profile in lung cancer and normal cells

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**Abstract**
Lung cancer is a complex disease that varies in molecular and histological characterization. The emergence of large-scale molecular profiling has enabled the identification of new molecular targets that can be applied in the treatment of lung cancer and the detection of biomarkers with the potential to be used in the early diagnosis of the disease. Studies conducted in recent years have shown that metabolites have the potential to be used as a disease diagnosis and treatment target. Amino acids, a metabolite, have become a remarkable biomarker with varying levels, especially in metabolic diseases. Altered metabolic restructuring in cancerous cells has been accepted as the distinguishing feature of the disease. This study investigated the potential of using amino acids as early detection markers in the early diagnosis of lung cancer. Cell lysates prepared using lung cancer (A549) and normal (Beas-2B) cells were measured by intracellular free amino acid measurement using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Significance levels of amino acids varying between two cells were analyzed using GraphPad Prism 9 programs. Among the 45 amino acids, the levels of arginine, glutamine, and glutamic acid amino acids in lung cancer cells were found to be significantly increased compared to normal cells (***$p<0.001$). Arginine is an amino acid playing a critical role in multiple cellular processes, including the synthesis of nitric oxide and polyamines. It acts as a direct activator of the nutrient-sensing kinase mTOR, which is strongly involved in carcinogenesis. Glutamine and glutamic acid are required in redox homeostasis, the construction of macromolecules, and the regeneration of TCA cycle intermediates. This study suggests that these amino acids can be used in the early diagnosis of lung cancer and may be a therapeutic target.

**Keywords:** Lung Cancer, Amino acid, LC-MS/MS, A549

## INTRODUCTION

Lung cancer is one of the most common types of cancer worldwide and is the leading cause of cancer death worldwide. Recently, the incidence and mortality rates have increased significantly with gender and geographical differences (Gao et al., 2020). The research reported about 2.2 million new cases of lung cancer and 1.8 million deaths worldwide in 2020 (Sung et al., 2021). Despite the frequently used treatments for lung cancer, satisfactory clinical results have not been observed due to early metastasis of the tumor (Hu et al., 2020). Therefore, there is a need for rapid and reliable early diagnosis methods that can be used in the early diagnosis of the disease.

Amino acids are essential signaling molecules that regulate metabolic pathways, protein translation, and many other functions and are indispensable for the growth of cancer cells (Bröer, 2020).
Metabolic reprogramming has been widely recognized as a feature of cancer progression. The uptake and metabolism of amino acids are known to be abnormally excessive in many cancers dependent on specific amino acids. Amino acids facilitate the survival and proliferation of cancer cells under cellular stress (Wei, Liu, Cheng, Yu, & Yi, 2021).

In this context, amino acids ranging between lung cancer and normal cells were identified, and their potential for use for early diagnosis was investigated.

## MATERIALS AND METHODS

### Cell Culture

A549 (lung adenoma cancer cell) and Beas-2B (normal lung cell) cell lines were obtained from ATCC and stocked in liquid nitrogen, and cultured in RPMI-1640 (Gibco) medium supplemented with 10% FBS, 100 µg/mL streptomycin/100IU/mL penicillin, in a humid atmosphere, 5% $CO_2$ in a 37 ºC incubator.

### Measurement of Intracellular Free Amino Acids by LC-MS/MS

After the A549 and Beas-2B cell lines were 80-90% confluent in the flasks, they were placed on ice, and the growth mediums were removed and washed with PBS. It was scraped with a cell scraper in cold PBS and taken into clean tubes. The homogenized cells were centrifuged at 14,000 rpm for 5 minutes, and the supernatants obtained were used for amino acid determination. Intracellular free amino acid analysis was performed using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) (Shimadzu-8045) according to the kit protocol (Bome Trivitron, Trimaris-BR130030, Turkey). We used a derivative method in the analysis based on the kit protocol. 50 µL of sample is mixed with an internal standard solution consisting of 20 amino acids with C13 and N15 labeled atoms prepared in 0.1 M HCl. Essential organic buffer components were added for pH balance. A chloroform/isooctane mixture containing 5% alkyl chloroformate as an active ingredient was added to the sample mixture and incubated at room temperature for 3 minutes. Derived amino acids were removed by centrifugation from the supernatant containing organic solvents. The signal of the MS device is increased in parallel with the increased molecular weight of esterified amino acids. Trimaris Amino Acid LC-MS/MS column (250mm x 2mm, 3µM) with C18 phase filler was used for the chromatographic separation, and multiple reaction monitoring (MRM) mode was preferred to analyze.

### Statistical Analysis

GraphPad Prism 9 (GraphPad Software, Inc, San Diego, USA) package program was used to analyze continuous data. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine whether there was a difference between the distribution of continuous data and the normal distribution. The One-Way ANOVA test was used for three independent group comparisons of normally distributed data. In contrast, the Kruskal-Wallis H test was used for three-group comparisons with non-normally distributed data. $p<0.05$ was considered statistically significant.

## RESULTS and DISCUSSION

### Amino Acid Profile Between Lung Cancer and Normal Cells

Intracellular free amino acid profiling of A549 and Beas-2B cells was performed. Among the 45 amino acids, the levels of arginine, glutamine, and glutamic acid amino acids in lung cancer cells were found to be significantly increased compared to normal cells (***$p<0.001$).

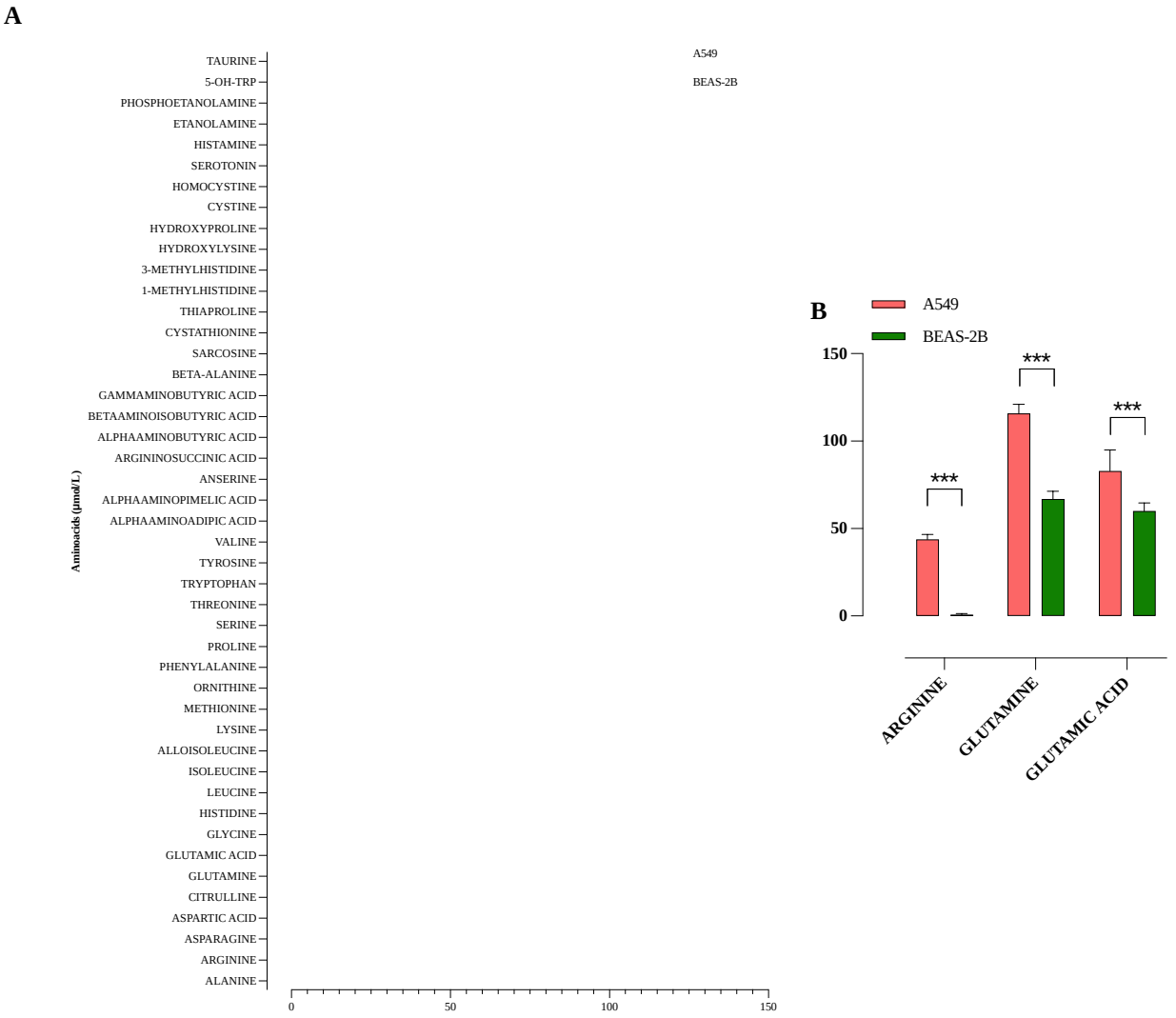


**Figure 1. Comparison of free amino-acid levels between A549 and Beas-2B cell lines. (A)** Free amino acid levels in A549 and Beas-2B cells. **(B)** Significantly changed amino acids in cell lines. All experiments were performed in triplicate. (*p<0.05, **p<0.01, ***p<0.001).

Arginine is an amino acid playing a critical role in multiple cellular processes, including the synthesis of nitric oxide and polyamines. It acts as a direct activator of the nutrient-sensing kinase mTOR, which is strongly involved in carcinogenesis (Zou, Wang, Liu, Ke, & Xu, 2019). In cancer, there is a depletion of glutamine, as cancer cells consume glutamine at a large scale (Kulkarni, Kulkarni, & Hamsa, 2005). Glutamine plays a crucial role in tumor cells (Dutta, Ray, & Nagarajan, 2013). In many cancer cells, glutamine and glutamic acid are required in redox homeostasis, the construction of macromolecules, and the regeneration of TCA cycle intermediates (Wise & Thompson, 2010). In the study, we observed that the amino acids determined in lung cancer cells increased. Lung cancer cells regulate metabolic reprogramming to ensure the unlimited proliferation and to support energy metabolism.

## CONCLUSION

It is suggested that these amino acids can be used in the early diagnosis of lung cancer and might be a therapeutic target.

## REFERENCES

Bröer, S. (2020). Amino acid transporters as targets for cancer therapy: why, where, when, and how. *International Journal of Molecular Sciences, 21*(17), 6156.

Dutta, S., Ray, S., & Nagarajan, K. (2013). Glutamic acid as anticancer agent: An overview. *Saudi Pharmaceutical Journal, 21*(4), 337-343.

Gao, S., Li, N., Wang, S., Zhang, F., Wei, W., Li, N., . . . He, J. (2020). Lung cancer in People's Republic of China. *Journal of Thoracic Oncology, 15*(10), 1567-1576.

Hu, H., Wang, F., Wang, M., Liu, Y., Wu, H., Chen, X., & Lin, Q. (2020). FAM83A is amplified and promotes tumorigenicity in non-small cell lung cancer via ERK and PI3K/Akt/mTOR pathways. *International journal of medical sciences, 17*(6), 807.

Kulkarni, C., Kulkarni, K., & Hamsa, B. (2005). L-Glutamic acid and glutamine: Exciting molecules of clinical interest. *Indian journal of pharmacology, 37*(3), 148.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians, 71*(3), 209-249.

Wei, Z., Liu, X., Cheng, C., Yu, W., & Yi, P. (2021). Metabolism of amino acids in cancer. *Frontiers in cell and developmental biology, 8*, 603837.

Wise, D. R., & Thompson, C. B. (2010). Glutamine addiction: a new therapeutic target in cancer. *Trends in biochemical sciences, 35*(8), 427-433.

Zou, S., Wang, X., Liu, P., Ke, C., & Xu, S. (2019). Arginine metabolism and deprivation in cancer therapy. *Biomedicine & Pharmacotherapy, 118*, 109210.

## *ORAL PRESENTATION*

# Chemical synthesis of heterocycle benzimidazole: practical application

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## Abstract

Benzimidazoles have attracted many researchers worldwide in assessing their potential therapeutic importance. This heterocycle is found in anti-helmintic molecules such as albendazole, mebendazole or proton pump inhibitor molecules such as esomeprazole, pantoprazole or as angiotensin II receptor antagonists type 1 (AT1), telmisartan and azilsartan are the best examples.The work aims to realize the chemical synthesis, in the laboratory, of this heterocycle by one of the methods described. This synthesis is followed by infrared spectral identification with an IR spectrophotometer at fourier transform (FT-IR), melting point measurement with Kofler bench and purity estimation by thin layer chromatography.
IR analysis has proven its importance in the identification of the functional groups of the structure of the synthesized product. The CCM has concluded that the purity is very satisfactory for it.

**Keywords:** benzimidazole, activity, synthesis, identification.

## INTRODUCTION

Benzimidazoles are known for their crucial role in the treatment of many diseases through various mechanisms. Substitution of the benzimidazole ring is an essential step in the process of drug discovery. The objective of the work is to achieve a chemical synthesis of heterocycle benzimidazole and its identification. This basic nucleus can be substituted on the nitrogen or on the C2 by starting with a non-monocarbon acid or aldehyde.

A literature review found that o-phenylenediamines readily react with most carboxylic acids to give 2-substituted benzimidazoles, generally with very good yield. The reaction is carried out by heating the reagents together in a steam bath under reflux or at elevated temperature, or by heating them in a sealed tube [4]. Rithe et al. reported that the synthesis yield of these compounds, in the presence of ammonium chloride as catalyst, was excellent (72 to 90%), (Cf. FIG. 1) [5].

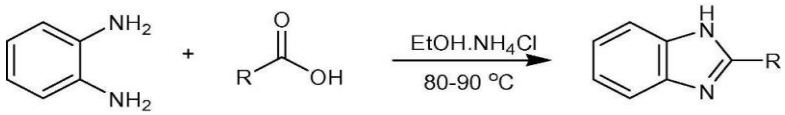


**Figure 1.** Synthesis of 2-substituted benzimidazole derivatives from o-phenylenediamine and carboxylic acids.

Under correct conditions, aldehydes can react with o-phenylenediamines to give benzimidazoles substituted in position 2 (Cf. FIG. 2) [3].

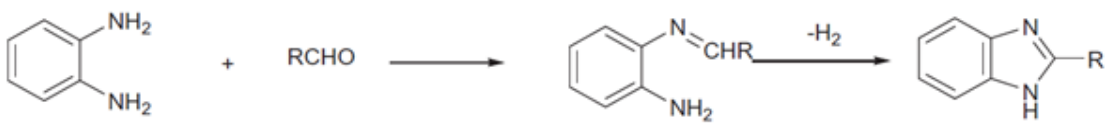


**Figure 2.** Synthesis of 2- alkylebenzimidazole derivatives from o-phenylenediamine and aldehydes.

Benzimidazoles-2-carbamates are synthesized by reduction of derivatives of o-nitroarylamines. First the 1-carbamate-2-aminobenzimidazole is produced, which is further heated between 60-80°C, giving an excellent yield of corresponding benzimidazole derivatives (Cf. FIG. 3). This procedure is used in industries for the production of large quantities of benzimidazole because its yield is very high [6].

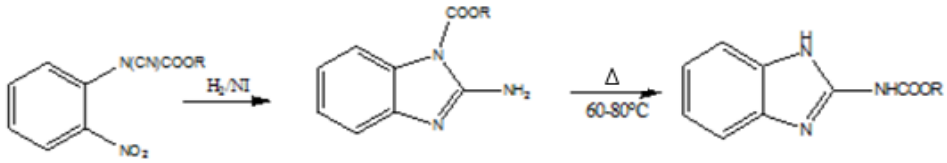


**Figure 3.** Synthesis of benzimidazoles-2-carbamates.

## MATERIALS AND METHODS

### 1. Synthesis of 1*H*-benzimidzole

In a 250 ml two-necked flask, introduce 5.4 g of purified o-phenylenediamine with 3.2 ml (3.46 g) of 98% formic acid. The mixture is heated in a water bath between 102 and 103° C for two hours. After cooling, a 10% sodium hydroxide solution is added slowly, until the pH paper turns to 10 (about 4 ml).

The impure benzimidazole is collected in a Büchner, by vacuum filtration, followed by rinsing cold distilled water (about 25 ml), and this after having let it crystallize in an ice bath.

Purification is carried out by hot and then cold recrystallization of the product obtained, it consists in dissolving the impure benzimidazole in 75 ml of boiling distilled water in a 150 ml beaker. The solution is digested for fifteen minutes with about 2 g of activated charcoal until completely dissolved and then quickly filtered while hot. The filtrate is cooled between 10 and 15°C then filtered again under vacuum. The solid product obtained is rinsed with 10 ml of cold water and finally dried at 100°C.

### 2. Identification of 1*H*-benzimidzole

The synthetic product is identified by an infrared spectral analysis (IR), by measurement of the melting point and its purity is estimated by a separation analysis (TLC) and measurement of the melting point.

The precise determination of the melting point of the sample is done after calibration of the Kofler bench with one or two standard substances.

The IR analysis is done by an IR spectrophotometer at fourier transform (FT-IR). The perfectly anhydrous sample is prepared as a KBr pellet, a support which does not absorb in the mid-IR. The acquisition of the absorption spectra of the sample is done after elimination of the background noise spectrum. They are recorded in transmittance.

Separative analysis is performed by TLC. The eluent is a mixture of two solvents n-hexane/AcOEt, 3:7 (v/v). The o-phenylenediamine (starting reagent) and the benzimidazole are dissolved in ethyl acetate and spotted on a silica gel plate (TLC Silica gel 60 F254). The plates are examined in the visible light and under the UV lamp (254 nm).

## RESULTS and DISCUSSION

### 1. Synthesis of 1*H*-benzimidzole

The appearance of impure benzimidaole and purified benzimidazole is shown in the figure 4.



**Figure 4.** Comparative aspect between impure benzimidazole (left) and purified benzimidazole (right).

The purified product has the appearance of a powder of fine, needle-shaped white crystals.

According to the bibliographic reference data, benzimidazole is in the form of a substantially white crystalline powder [2, 1]. The result obtained is then in accordance with the literature data.

The yield is calculated from the average of the weighings of the mixture of the two products, resulting from the two tests, carried out under the same conditions and by the same team. It is equal to 67.79%.

Dr. Phillips and co-workers synthesized 1*H*-benzimidazole using the same reagents (OPD and formic acid), at a temperature of 80 to 120 ºC for 2 to 4 hours, with HCl (4N) as the recrystallization solvent and resulted in a yield of 80–90% [6].

The synthetic yield is 67.79%, it is less than that resulting from the work previous ones. This is probably due to the nature of the recrystallization solvent (distilled water), the use of activated carbon in the purification or the quantity of distilled water used for rinsing the product.

## 2. Identification of 1*H*-benzimidzole

- **Melting point measurement**

The melting point of the product measured is 170° C, that cited in reference [7] is 170.5° C. The product synthesized is then 1H-benzimidazole, subject to the IR results.

- **Infrared analysis**

The IR spectrum of the synthesized product and that of the reference of benzimidazole, taken from the SDBS are reported in Figures 5 and 6.

The attribution of the peaks of the two spectra in table 1 was carried out by using the reference tables.

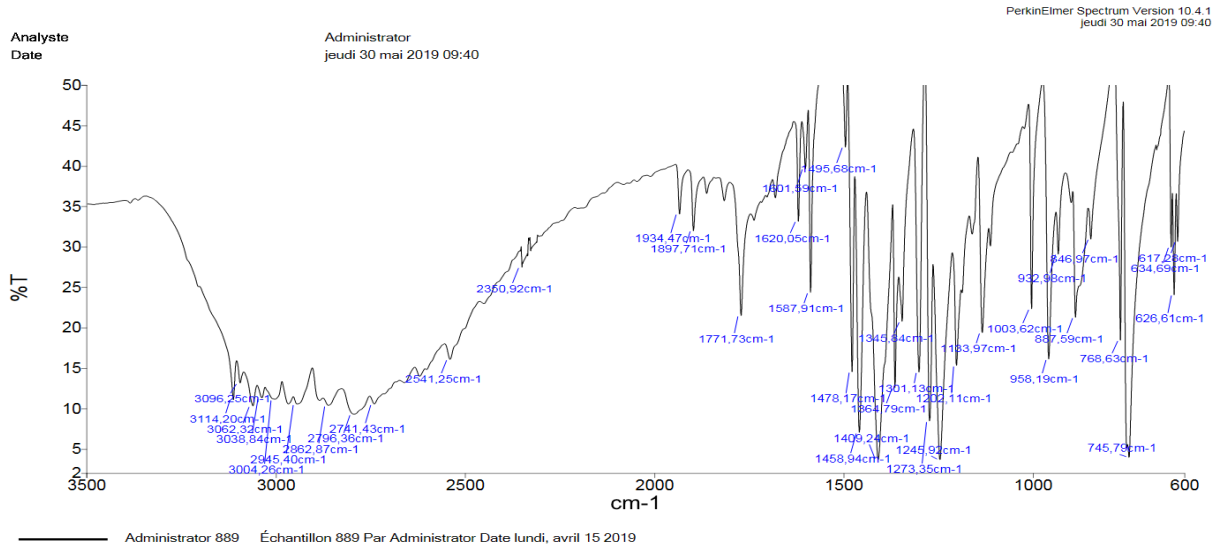


**Figure 5.** Infrared spectrum of the synthesized product.

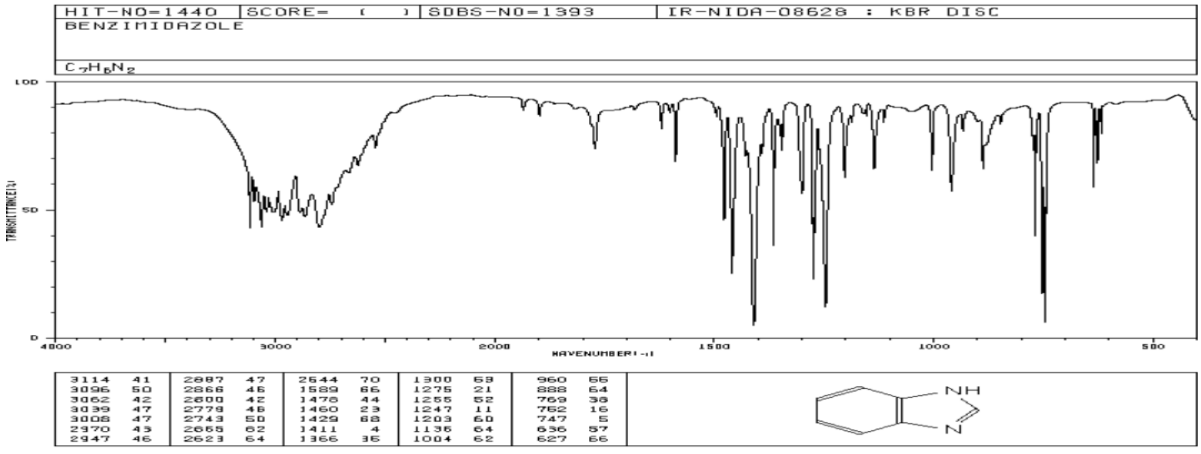


**Figure 6.** Infrared spectrum of 1*H*-benzimidazole according to SDBS of AIST.

**Table 1**. Comparative analysis of the IR spectrum of the synthesized product with the spectrum of 1*H*-benzimidazole.

| Reference spectrum ($cm^{-1}$) | Practical spectrum ($cm^{-1}$) | Attribution of peaks |
|---|---|---|
| 3114 | 3114 | =C-H elongation vibration |
| 3096 | 3096 | N-H elongation vibration |
| 3039 | 3039 | =C-H elongation vibration, multi-band [1,2-disubstituted, 4H adjacent] |
| 1589 | 1587 | C=N elongation vibration combined with C=C |
| 1476 | 1478 | N-H deformation vibration |
| 1366 | 1364 | C-N elongation vibration (secondary aromatic) |
| 1300 | 1301 | C-N elongation vibration (secondary aromatic), out of plane |
| 1203 | 1201 | C-N aromatic vibration |
| 1136 | 1132 | C-H deformation vibration [1,2-bisubstituted, adjacent 4H] |
| 960 | 957 | C-H out-of-plane deformation vibration |

The presence of characteristic bands on the practical spectrum confirmed the existence of functional groups present in benzimidazole such as: C=N (1587 cm-1), C-H (3114 cm-1), -NH large around 3096 $cm^{-1}$. The superposition of the practical spectrum with the reference spectrum (Cf. Table. 1) shows an analogy, which confirms the identity of the synthesized product: 1*H*-benzimidazole.

- **Separation analysis TLC**

Examination of the TLC plate (Cf. FIG. 7) shows that the o-phenylenediamine and the synthesized product each have a single spot, which proves that they are pure.

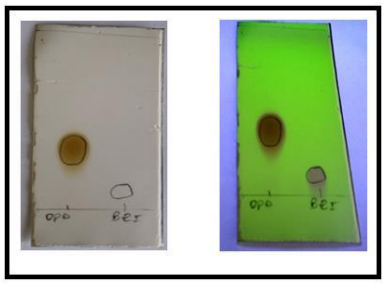

**Figure 7.** CCM plate of o-phenylenediamine and the synthesized product examined in the visible then under UV.

The result of CCM is shown in table 2.

**Table 2**. TLC results of o-phenylenediamine and the synthesized product in the eluent.

| Product | Migration (cm) | Rf | Color | |
|---|---|---|---|---|
| | | | Visible | UV (254 nm ) |
| **O-phenylenediamine** | 1.8 | 0.3 | Brown copper | Fluorescent dark purple |
| **Synthesized product** | 0.6 | 0.1 | - | Fluorescent dark Blue |

The Rf of the reagent used is different from that of the product obtained, the reaction is therefore incomplete. The fluorescence points to the presence of aromaticity.

## CONCLUSION

The products to be synthesized could be obtained efficiently in the laboratory. Spectroscopic analysis, separation analysis and measurement of a physical parameter (melting point) confirmed the identity of the product and concluded that the purity was acceptable or even satisfactory.

These results could be refined by NMR and HPLC analysis.

## REFERENCES

David R, Lide 2005. CRC Handbook of Chemistry and Physics, Internet Version 2005, Press.

John Wiley & Sons 2016. Hawley's Condensed Chemical Dictionary Sixteenth Edition. 1565 pp.

Kiumars Bahrami 2008, Mild and Highly Efficient Method for the Synthesis of 2-Arylbenzimidazoles and 2-Arylbenzothiazoles. J. Org. Chem. 3 pp.

Rathod, C.P., Rajurkar, R. M., Thonte, S.S. 2013. Indo American Journal of and Pharmaceutical Research. 3(2).

S.R. Rithe 2015. One pot synthesis of substituated benzimidazole derivatives and their caracterization. J. Chem. (8)02, pp. 213 –217.

Singh VK and Parle 2019. The intriguing benzimidazole: a review. Int J Pharm Sci & Res. 10(4): 1540-52.

Wright, J.B. 1951. THF : Chemistry of the benzimidazoles. 143 pp.

## *ORAL PRESENTATION*

# Method of value added recovery of organic wastes (tea pulp) by converting heavy metal parameters to natural cycle: *E. foetida* vermicomposting

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## Abstract

Intensive use of chemical fertilizers and pesticides, which pose a great risk in terms of human and environmental health and safety, deplete the soil quality of agricultural lands and increase pathogen resistance, cause serious concerns about the future of natural resources. For this reason, one of the methods that is useful in terms of providing added value to nature in terms of sustainability in terms of sustainability of organic-based residues and wastes is the vermicompost process, which is one of the compost models. Recently, the vermicompost technique, which has superior properties compared to the conventional compost process, has become widespread and has found application and use very quickly. Thus, the process of preparing Eisenia foetida vermicompost by mixing tea pulp, which cannot be used directly for agricultural purposes, and a certain amount of cattle manure, providing their nutrition and ultimately obtaining vermicompost, and investigating the possibilities of obtaining 100% water-soluble form biologically by liquefying solid granular vermicompost and Examination was the aim of the study. Heavy metal parameters determined in the wastes subjected to vermicomposting process for two weeks were analyzed in the samples taken. In terms of heavy metal parameters that were liquefied and analyzed at the end of vermicomposting, no parametric heavy metal was found in vermicompost products, which are the waste of worms fed with chemical fertilizers and pesticides. In vermicomposting, which allows the conversion of organic matter and nutrients required in the food chain: it sheds light on the usability and sustainability of traditional composting or vermicomposting methods. In this study, as a result of vermicompost production methods and analyzes, it is predicted that a sustainable value-added agricultural production model with high economic value and reliable in terms of human and environmental health and safety will be supported.

**Keywords:** Vermicompost, Organic agriculture, E.foetida, Sustainable agriculture, Heavy metal.

## INTRODUCTION

In this study, the production and detailed characterization of vermicompost material was chosen as the organic material and the working trend. Bioreactors in the pilot plant we designed to determine the characteristic parameters of the nano-bio-preparation, which will be obtained by working the optimum condition with the maceration and digestion model in the laboratory environment before the organic structure diversity in the content of the Vermicompost material and the coelom material, and then again with the large-scale maceration and digestion model It is aimed to obtain nanoscale with

Within the scope of the study, in order to evaluate the vermicompost sample in terms of characterization, after the Maceration (after the sample is brought to the appropriate size by micronized grinding, keeping it at room temperature in a closed container and mixing processes) method, Digestion (at least 40 $^{o}$C range of temperature) was applied to reduce the toxicological effects and prevent its deterioration. The maceration process using solvent in a temperature environment) method was applied.

## Subject and Scope

The need for the nutritional needs of human beings continues in the forward direction in parallel with the increasing speed of the population. In order to increase agricultural production efficiency, the use of environmentally harmful chemical fertilizers is increasing. Due to the use of these chemical fertilizers, the decrease in soil fertility, increase in soil pollution, deterioration of beneficial microorganisms in the soil, weakening of soil ecology over time, accumulation of toxic substances (such as heavy metals and cyanide derivatives) in plants, decrease in organic substances (such as organic carbon) in the soil and sustainable agriculture cannot be done. negative effects such as For this reason, the need and orientation to organic origin fertilizers is considered as an important point. Organic-based agricultural materials are obtained by natural methods and are materials that trigger the cation exchange capacity of the soil and its water holding ability, support the soil with oxygen and nitrogen, prevent the harmful effects of toxic substances in the soil and plants, increase the mass of beneficial bacteria for the soil and plants, and improve the soil quality.

## The Original Value at Work

The results revealed in this study, the methodology of reducibility of organic materials to sub-dimensions, and characterization studies for small-scale use of the obtained bio-material were carried out. In this direction, scientific value-added working methods have been completed in detail in the fields of soil improvement and increasing plant growth with the application of vermicompost, a new plant nutrition element whose heavy metal content is not detected. Simultaneously with the investigation studies of the fertilizer material on a small scale, both the product quality and the effectiveness of the method were evaluated by designing the pilot plant.

## Source Research

The amount of supply and demand is increasing simultaneously with the increasing human population. Due to these increases, it is especially important that agricultural activities are more efficient, longer-term and more sensitive in terms of human and environmental health. Fertilizer use has a key role in controlling agriculture. The fertilizer used affects many agricultural performance factors such as the strengthening of the plant root structure in the soil, the development of the plant, and the improvement and protection of the soil. However, if the origin of the fertilizer used is inorganic, it causes deterioration of soil quality over time, accumulation of unwanted materials (such as heavy metals) in plants, deterioration of water resources, and air pollution due to the micro and reactivity of toxic components (such as Hg, cyanide derivatives and sulfur compounds). For this reason, researches and applications related to fertilizer have turned to organic fertilizers, which have no harm to nature and humans.

## Eisenia Foetida (*E. foetida*)

Worms in the family of invertebrates consist of 3 types: Polychaeta, Hirunidea and Oligochaeta (Tomlin and Miller, 1988). The most known is; It is the Oligochaeta class, which includes land worms (Kalaç, 1997). Eisenia foetida is one of 8 species of the Lumbricidae family of the ringed terrestrial worms. This variety, whose Latin name is Eisenia foetida; It was named as "California Red Worm" because it was first crossed at the California Institute (USA). Eisenia foetida is a genus of worms belonging to the Lumbricid family and is in the group known as "earthworms" in English. E. foetida, also called “compost worm”, is 7-8 cm long and generally 2-5 mm in diameter (Reinecke and Viljoen, 1991).

These nerves in E. foetida provide the worm's mobility and communication of organs. Worms perform their respiratory function with their outer surfaces and carry hemoglobin as respiratory pigment as in humans. They can also renew their broken, cut and broken parts by repairing them. Therefore, the way to live a long life is paved (Tomlin and Miller, 1988; Zhu et al., 1996). Body anatomy of an adult individual; mouth, anus, clitellum, consists of segments and cilia (Figure 1.).

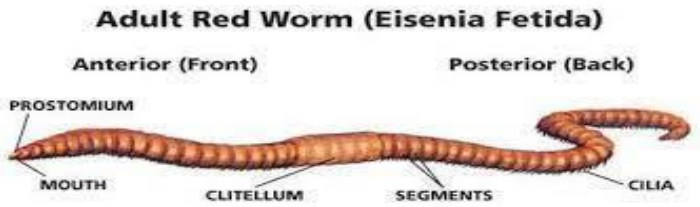


**Figure 1.** E. foetida body anatomy (Podolak et al., 2020)

One of the most important features of California, which has a short life-life cycle like all worms, is its rapid reproduction, and adult worms produce 4-5 cocoons in 25-30 days, and each cocoon contains an average of 6 worm eggs (Figure 2.).

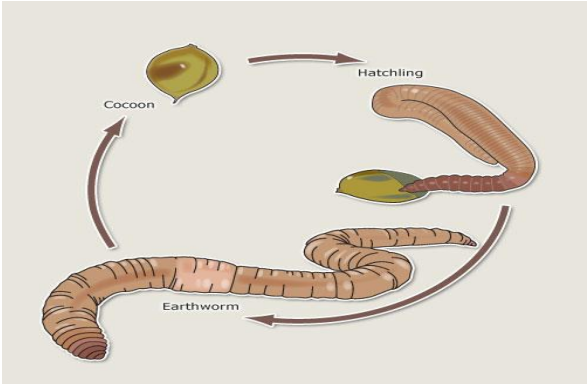


**Figure 2.** Life cycle of worms (Podolak et al., 2020)

Their cocoons (Figure 3) are 2.4-5.2 mm long and 2.3-4.4 mm in diameter (Sims and Gerard, 1985; Podolak et al., 2020).




**Figure 3.** E. foetida cocoons (cocoon) (Podolak et al., 2020)

California worms are hermaphrodites, meaning they are androgynous. When mating is provided in suitable conditions, it takes place all night without any problems up to an average of 27 ° C. As a result, cocoons with eggs are formed. E. foetida provides rapid reproduction in this way. They can produce almost 20-25 times cocoons in a year. In about 1 month from the eggs and about 1.5-2 mm in length offspring are formed (Edwards and Bohlen, 1996; Kalaç, 1997).

**Vermicompost**

Worms have been living in nature and performing their duties since prehistoric times.
(Lehtonen et al., 2001) dried the coal powder and micronized it and ground the powdered coal to pass through the sieve. Powdered coal was mixed with dilute chloroform throughout the day, and some of this sample was left to stand for a while in separate containers with 0.1 M KOH. The resulting yields were collected and centrifuged at high speed for 2 hours. The obtained liquid was dosed with acid up to Ph 4-5, and yield was achieved. Before starting the experiments, the peat sample contained 48% C and 1.4% N on a dry basis. The yield was 44%. In a study conducted at Ohio University, it was observed that vermicomposts contribute to plant growth. It has been determined that many ornamental plants flower more quickly with vermicompost

application compared to professional cultivation (Edwards and Burrows, 1988). In applied studies, it has been reported in the literature that the amount of organic C in the soil structure increases with the use of vermicompost, the pH value becomes stable, the porosity and water holding capacity are regulated (Scott, 1988, Maheswarappa et al., 1999).

## MATERIAL and METHOD

The steps in this study are given below. These;

• First of all, in order to obtain the bio-preparation we have determined, first of all, the optimum condition study related to the quality of the material (such as temperature, solubility, mixing speed of the material and determination of the effects that will ensure the optimal efficiency of other inputs) is carried out,

• After obtaining the optimum conditions to increase the performance of the material in the laboratory environment, the production of concentrated vermicompost bio-prepare from vermicompost material with the help of the venturi effect in the bioreactor with the logic of mechanical cavitation, together with the formation of chaos in the pilot plant,

• Elemental content analysis (micro/macro) to determine the characterization of the molecules that make up the structure of nanoparticles, and

• Finally, heavy metal analysis of the obtained vermicompost materials and their derivatives.

### Elemental Content Properties of Vermicomposts: Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

The ICP/MS device used in elemental studies is an advanced technology product that allows analytical testing of many elements in samples. With the ICP/MS technique, solid and liquid samples can be analyzed simultaneously at very low concentrations sensitively. ICP/MS is a method based on breaking the intermolecular bonds by sending the samples to an argon plasmic environment at advanced temperatures and separating them into ions of atoms. The sample is sent to the nebulizer in the system as a solution. In this process, the sample is fogged with rapidly flowing argon gas. Very small droplets are transported to the argon plasma, other non-adherent parts decompose as waste. At high temperature, plasma dissociates, ionizes and evaporates the solution into its ions. The ionic flow passes from the ambient pressure to the high vacuum environment through the columns beekeeping. Then, by focusing on the quadrupole by means of ionic flux lenses, they are separated according to the mass-charge balance and are detected and measured. Figure 4. shows the schematic of the device (ICP/MS).

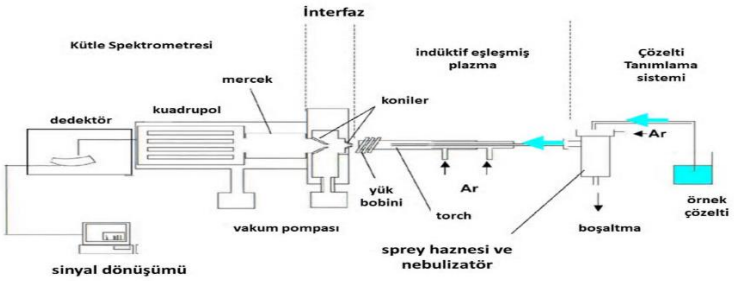


**Figure 4.** Inductively coupled plasma mass spectrometry (ICP/MS) schematic.

ICP/MS gives advanced results primarily in trace element determinations. For multiple measurements, the measurement limit is in ppb. The operating band of ICP/MS devices is very wide (ppm/ppb) compared to other techniques. It has been determined that vermicompost contains nutrients necessary and sufficient for plant nutrition, and the distribution and amount of nutrients in its structure vary depending on the raw material of vermicompost (Agarwal, 1999).

It has been determined that vermicompost contains 22.70-70 mg.100g-1 Calcium and Magnesium, 2-9.3 mg.kg-1 Copper, 5.7-11.5 mg.kg-1 Zinc and 128-548 mg.kg-1 available Sulfur. According to the research conducted within the scope of this thesis, although there are many studies on the content of vermicompost in the literature, according to the knowledge of the author and group researchers, no study has been found on the effect of heat treatment applications (Kale et al., 1992).

**First Step: Preparation of samples for optimum condition to be used in the pilot plant in the laboratory environment**

After Europe and America, in the last ten years, professionally established and produced in our country as worm production centers, large-mass vermicompost samples obtained from raw vermicomposts produced in Konya region are brought to the laboratory in the form of heaps in the open environment, and they are turned into fine grains with the help of a mixer (Figure 5). has been brought. The samples, which came to the fine grain size, were micronized in small size and separated into small particles. In this way, the smaller particle structure is directly proportional to the yield of nano-sized molecules and the amount of fragmentation in the dissolution event. Moisture percentages of the dissected samples were kept in an oven environment and their moisture was removed and brought to constant weighing.




**Figure 5.** Tiller mixer

In order to speed up the process in order to adjust the moisture content of the raw products, the determined amount was taken immediately after the samples were ground into small particles and left to dry in the oven, which was adjusted for approximately two hours at approximately 105°C -110°C. The moisture content was calculated after this step.

The following calculation equations were used for moisture determination:

Humidity (%) =[(m1-m2).m-1] x 100 (3.1)

m1: The weight of the sample taken + the weight of the drying container brought to constant weighing (g.)

m2: Dried sample + weight of the drying vessel brought to constant weighing (g.)

m: Weight of sample taken (g.)

In order to make the samples dehumidified in the oven environment suitable for nano scale, the grain sizes were micronized by the ring discs (Figure 6) in the ring grinder (Figure 7), and operations were carried out according to the process order of obtaining nanoparticles from top to bottom.



**Figure 6.** Ring grinder



**Figure 7.** Grinder discs

**Extraction: KOH Extraction**

In the extraction method given in Figure 3.6, KOH solution prepared in different concentrations was added to the raw vermicompost samples. The adjusted concentrations of KOH solutions used in the bio-preparation preparation were prepared at different concentrations such as 0.5, 1 and 2 mol/L, respectively. KOH added solutions were processed in a heating magnetic stirrer (Figure 8). The effect of temperature on the yield was also investigated for the samples of each adjusted concentration. Operating temperatures were chosen as 60, 70 and 80°C (Figure 9). $C_6H_8O_7$ was added to the obtained high concentration raw vermicompost

solution to adjust the pH band and because its macro/micro elements are a very good chelating agent. The solution was then left to stand. Thus, exemplary samples were obtained (Figure 10).



**Figure 8.** Magnetic stirrer with heater



**Figure 9.** Heating-Stirring process

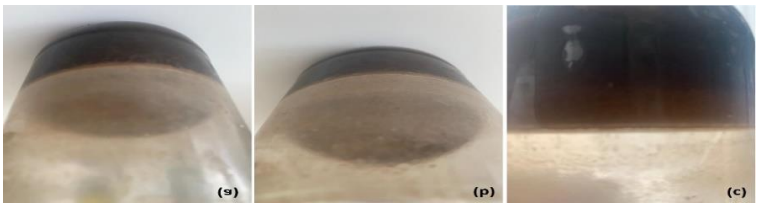


**Figure 10.** Samples of the recovered sample (a: Sample recovered from the first application b: Sample recovered from the second application c: Sample recovered from the third application)

**Double Step Extraction**

In the literature, it has been determined that the pre-oxidation step in organic materials and humate substances increases with the amount of organic functional groups that increase due to the conversion of methoxy (-OCH3) groups in the organic structures and especially the humate source to the carboxylic acid (-COOH) form. For this reason, a flash oxidation step with $C_6H_8O_7$ was applied to increase the yield in the hand method. Afterwards, the samples oxidized with $C_6H_8O_7$ were treated with KOH and organic structures, namely methoxy groups, carboxylic groups and humate substances were dissolved and obtained as filtrate. The resulting filtrate was washed and then dried and weighed. The filtrate was removed by evaporation of water, kept at room temperature in the laboratory, and dried. The samples were stored under suitable conditions for testing and analysis (Figure 11).

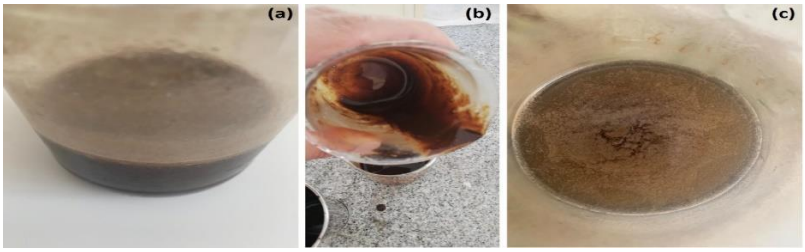


**Figure 11.** Obtained vermicompost forms (a: Concentrated vermicompost b: Filtrate vermicompost c: Solid vermicompost)

**Instantaneous Oxidation Step**

In general, in all organic-based materials studied, some structures are released in both alkaline and acidic environments and dissolve. However, some organic structures dissolve only in acidic or only alkaline environments and become free, that is, they pass into solution and can be easily extracted. For this reason, in most of the academic studies, while specific organic substances are obtained from bioorganic substances, alkaline extraction is applied directly in the process step, and then the mixtures obtained are acidified with $C_6H_8O_7$ and the desired organic structures are precipitated or the desired materials are obtained by separating the solids and liquid phases with phase formation. Since this study is a characterization study and there will be a general organic material degradation, both acidic and alkaline media steps were used rather than the principles used in obtaining a specific material. Based on the principle used here, the priorities for the double-step extraction process are alkaline medium and then $C_6H_8O_7$ is added to the medium to create an acidic

medium. For this reason, different concentrations of $C_6H_8O_7$ were used for the flash oxidation process. For these selected acid concentrations, the raw vermicompost samples were shaken at 60, 70 and 80°C for 3 hours to obtain concentrated vermicompost from the samples.

## FINDINGS and DISCUSSION

In this study, organic origin vermicompost material, which was heat treated according to the legislation of the Ministry of Agriculture, was used. Characteristics such as chemical contents and physical structures of the vermicompost product obtained from the Red California Worm vary from region to region and nutritional diversity. Therefore, it is inevitable that different results will emerge in the analyzes of this product. These different results should not mislead researchers.

Since the findings obtained as a result of the research are considered to be a large-scale study that the thesis will bring to the field of agriculture, advanced material technology and nano-scale research, each parameter has been delicately presented and discussed under separate headings.

### Elemental Content Analysis of Vermicomposts

The elemental content of the vermicompost sample, which was obtained in nano size by applying heat treatment in accordance with the legislation, then with the maceration and digestion model, venturi effect and cavitation process steps, is shown in Table 4.2, and the heavy metal content in Table 1. In addition, heavy metal limitations according to the legislation are given in Table 2.

In vermicompost samples, all process steps and applied thermal firing did not affect the trace element and heavy metal content of the vermicompost.

It has been stated that vermicompost contains 22.70-70 mg100g-1 calcium and magnesium, 2-9.3 mgkg-1 copper, 5.7-11.5 mgkg-1 zinc and 128-548 mgkg-1 available sulfur (Kale, 1996). Although there are many studies on the elemental structure of vermicompost, no research has been found on the effect of heat treatment applications.

**Table 1.**Heavy metal content of heat treated vermicompost sample

| Numune Kodu | Analiz Parametresi | Birimi | Miktarı |
|---|---|---|---|
| **1** | As | µg/l | 0,00 |
| **2** | Cd | µg/l | 0,00 |
| **3** | Pb | µg/l | 0,00 |
| **4** | Hg | µg/l | 0,00 |

**Table 2.** Heavy metal upper limits according to legislation (mg/kg)

| Element | Sembol | Miktarı |
|---|---|---|
| Kadmiyum | Cd | 3 |
| Bakır | Cu | 450 |
| Nikel | Ni | 120 |
| Kurşun | Pb | 150 |
| Çinko | Zn | 1100 |
| Civa | Hg | 5 |
| Krom | Cr | 350 |
| Kalay | Sn | 10 |

## CONCLUSION

As a result of the study, a micro-sized organic material was produced using nano technology. advanced material that is more efficient, more effective and more resistant to environmental conditions by reducing the production has been achieved. Optimal process steps for the production of this material After the determination of these steps, pilot mechanical production systems were designed. Within this designed mechanism, the optimum condition determined in laboratory conditions The steps are integrated into the mechanization in the

pilot plant designed in accordance with the industry. In addition, worm feeding (waste tea pulp) of organic material obtained through tests and analyzes. As a result, the absence of heavy metals was also detected.

## REFERENCES

Edwards, C. A. ve Burrows, I., 1988, The potential of earthworms composts as plant growth media. In: Edward, C.A. and Neuhauser, E.F. Eds., 'Earthworms in Waste and Environmental Management', *SPB Academic Publishing, The Hague*, 21-32.

Edwards, C. A. ve Bohlen, P. J., 1996, Biology and Ecology of Earthworms, Chapman and Hall, New York, Springer, p.

Kalaç, Y., 1997, Dendrobaena venata (Oligochaeta lumbricidae)'da Bakteri Enfeksiyonuna Hücresel ve Humoral Yanıt, *İstanbul Üniversitesi, Fen Bilimleri Enstitüsü*, 25-69.

Lehtonen, K., Hanninen, K. ve Ketola, M., 2001, Structurally bound lipids in peat humic acids, *Organic Geochemistry*, 32, 33-43.

Maheswarappa, H. P., Nanjappa, H. V. ve Hegde, M. R., 1999, Influence of organic manures on yield of arrowroot, soil physico-chemical and biological properties when grown as intercrop in coconut garden, *Annals of Agricultural Research*, 20 (3), 318-323.

Podolak, A., Kostecka, J., Mazur-Pączka, A., Garczyńska, M., Pączka, G. ve Szura, R., 2020, Life cycle of the Eisenia fetida and Dendrobaena veneta earthworms (Oligohaeta, Lumbricidae), *Journal of Ecological Engineering*, 21 (1), 40-45.

Reinecke, A. J. ve Viljoen, S. A., 1991, A comparison of the biology of Eisenia fetida and Eisenia andrei (Oligochaeta), *Biology and Fertility of Soils*, 11, 295-230.

Scott, M. A., 1988, The use of worm-digested animal waste as a supplement to peat in loamless composts for hardy nursery stock. In Earthworms in Environmental and Waste., *SPB Academic Publishing*, 221-229.

Sims, R. W. ve Gerard, B. M., 1985, Earthworms: Keys and Notes for the identification and Study of the Species (Synopses of the British Faunano). , Backhuys Publishers, p.

Tomlin, A. D. ve Miller, J. J., 1988, Impact of ring-billed gull (Larus delawarensis ord.) foraging on earthworm populations of southwestern Ontario agricultural soils, *Agriculture, Ecosystems & Environment*, 20 (3), 165-173.

Zhu, H., Ownby, D. W., Riggs, C. K., Nolasco, N. J. ve Stoops, J. K., 1996, Assembly of the gigantic hemoglobin of the earthworm Lumbricus terrestris, *The Journal of Biological Chemistry*, 271 (47), 30007-30021.

## *ORAL PRESENTATION*

# Chemical synthesis and identification of 1,2,4-triazole derivative

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## Abstract

The introduction of triazole into medicinal chemistry comes just after the success that the imidazoles had. Indeed, triazoles are the isosteres of imidazoles in which the carbon atom is isosterically replaced by a nitrogen. The objective is to carry out the synthesis and identification of 1,2,4-triazole-3-thiol by cyclization in a basic medium after its prior formylation with formic acid. Infrared analysis confirmed the presence of characteristic product bands, 1-formyl-3-thiosemicarbazide and 1,2,4-triazole-3-thiol. TLC chromatographic analysis showed a single-spot profile for the two synthesized products, this purity was confirmed by measuring the melting point.

**Keywords:** 1,2,4-triazole-3-thiol, synthesis, identification, infrared spectroscopy.

## INTRODUCTION

Research in heterocyclic chemistry has been oriented in recent years towards the discovery of new flexible and modular synthetic methodologies allowing obtaining biologically active molecules.

The objective of this work is to synthesize and then identify an aza-heterocycle of therapeutic interest, 1,2,4 triazole-3-thiole.

This heterocycle is found in several active ingredients, such as the antiviral ribavirin, the anticancer letrozole, anastrozole and vorozole or even as an antifungal in fluconazole and posaconazole.

## MATERIALS AND METHODS

The experimental study concerns the chemical synthesis of 1,2,4-triazole-3-thiol (P3B). This product is first obtained from a formylated derivative of thiosemicarbazide, it will be designated by P3A (Cf. FIG. 1).

P3A and P3B products are identified by measurement melting point and infrared analysis. The estimation of P3B purity is done by thin layer chromatography (TLC).

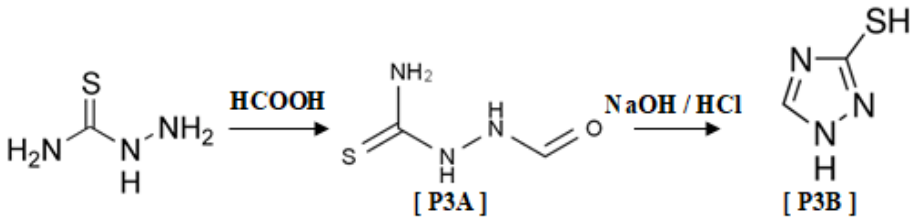


**Figure 1.** General diagram of synthesis carried out.

- **Chemical synthesis**
  - ♦ Synthesis of 1-formyl-3-thiosemicarbazide

Heat under reflux, in a water bath, 3.67 ml of 98% formic acid, introduced into a 100 ml single-neck flask, for 15 minutes at 80°C, then add 1.82 g of purified thiosemicarbazide. Shake until this reagent is completely dissolved. The heating is continued for 30 minutes during which the crystals of 1-formyl-3 thiosemicarbazide separate. Isolation and purification are done by adding 6 ml of boiling distilled water to the cooled mixture, the milky solution obtained is filtered through a fluted filter paper. After allowing to stand for 1 h, the filtrate is cooled in an ice bath and the 1-formyl-3-thiosemicarbazide which separates is collected by filtration under vacuum and then dried.

- Synthesis of 1,2,4-triazole-3-thiol

In a single neck flask carried on a reflux assembly, dissolve with agitation magnetic 0.3 g of sodium hydroxide in 10 ml of distilled water. Then introduce 0.89 g of 1-formyl-3-thiosemicarbazide and heat it in a water bath at 80°C for 1 hour. The solution is then cooled in an ice bath and then treated with 2 ml of concentrated hydrochloric acid. The reaction mixture is cooled again for two hours. The 1,2,4-triazole-3-thiol precipitate is collected by vacuum filtration.
The triazole derivative is dissolved in 4 ml. of boiling water and the solution is filtered through fluted filter paper. The filtrate is cooled in an ice bath for 1 hour, then the thiol is collected by vacuum filtration and dried.

- **Caracterisation**

The determination of the melting point of P3A and P3B is done with the Kofler bench after calibration.

The IR analysis is realized by an IR spectrophotometer with fourier transform (FT-IR) PerkinElmer after preparation of the two samples in the KBr pellet.

For TLC, the eluent is a mixture of two solvents n-hexane/AcOEt, 6:4 (v/v). The Thiosemicarbazide (starting reagent) and P3B are dissolved in ethyl acetate and spotted on a silica gel plate (TLC Silica gel 60 F254). The plates are examined in the visible light then under the UV lamp (254 nm).

## RESULTS and DISCUSSION

- **Chemical synthesis**

The appearance of the synthesized powders is shown in Figure 2.

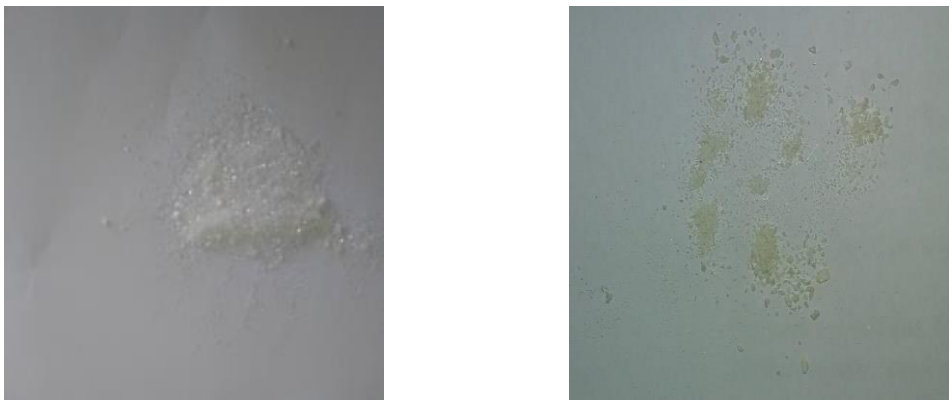

**Figure 2.** Appearance of synthesized P3A (left) and P3B (right)

The yield of synthesis of P3A is 52.74%. That of P3B synthesis is 42.66%.

C. Ainsworth, used the same protocol with his reagents to arrive at 1-formyl-3-thiosemicarbazide with a yield of 79 to 81%, a relatively better value compared to the obtained result. He continued his work under the same conditions to cyclize 1-formyl-3-thiosemicarbazide and to have 1,2,4-triazole-3-thiol with a yield of 78% [2]. The yield values obtained (52.74 and 42.66%.) are moderate, probably due to excessive use of the solvent during purification.

- **Caracterisation**
  - Melting point (M.P) determination

The melting point measurement results are summarized in Table 1.

**Table 1**. Results of melting point measurement of P3A and P3B.

| Product | P3A | 1-formyl-3-thiosemicarbazide (reference) [2] | P3B | 1,2,4-triazole-3-thiol (reference) [1] |
|---|---|---|---|---|
| M.P (° C) | 176 | 177-178 | 221 | 220-222 |

For P3A, a slight difference (1°C) between the two values can lead to the expected product but confirms at the same time the presence of impurities.
For P3B the results are identical. It can be ensured that the P3B product is identified, it is 1,2,4-triazole-3-thiol, subject to IR results.

- TLC

The separative analysis results (TLC) are clarified in figure 3.

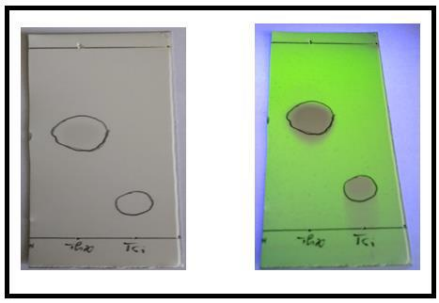

**Figure 3.** TLC plate of thiosemicarbazide (Thio) and P3B (Tri)examined in the visible then under UV in the eluent n-hexane/AcOEt, 6:4 (v/v).

The height of the solvent front is equal to 6 cm.

The TLC result is shown in Table 2.

**Table 2**. TLC Results of thiosemicarbazide and P3B.

| Product | Migration (cm) | RF | Color | |
|---|---|---|---|---|
| | | | Visible | UV lamp (254 nm) |
| **Thiosemicarbazide** | 3.3 | 0.5 | Grey | Purple |
| **P3B** | 1.1 | 0.2 | - | purplish blue |

The TLC plate shows that the P3B presents a single spot at different RF which proves that it is pure and that the reaction was complete.

- IR spectrum

The infrared spectrum of the product P3A, P3B and that of 1,2,4-triazole-3-thiol (reference) according to SDBS are shown in figure 4,5 and 6.

The interpretation of the practical spectrum of P3A is summarized in table 3.

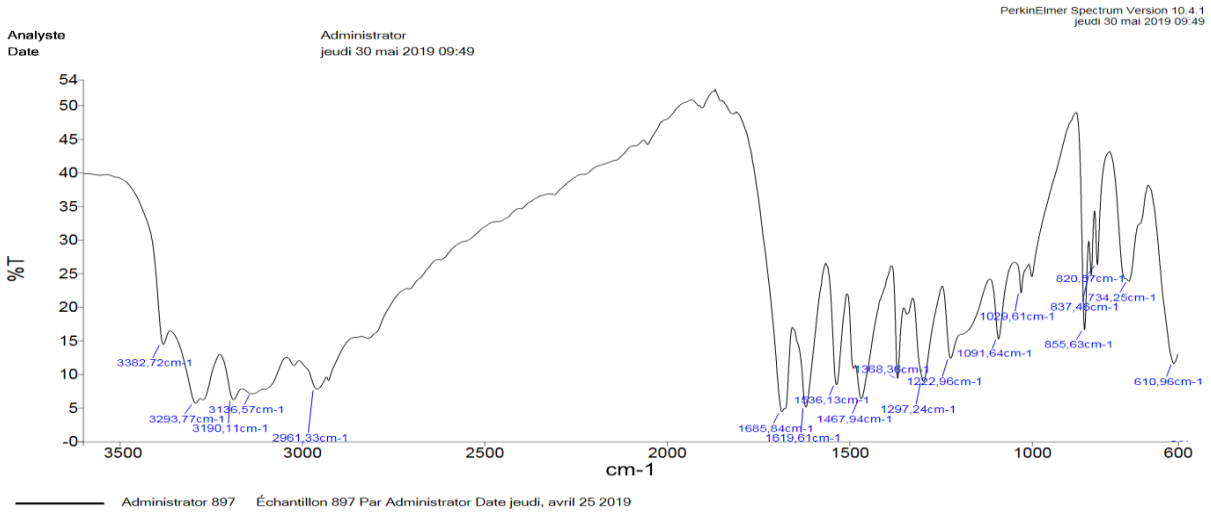


**Figure 4.** Infrared spectrum of the product (P3A).

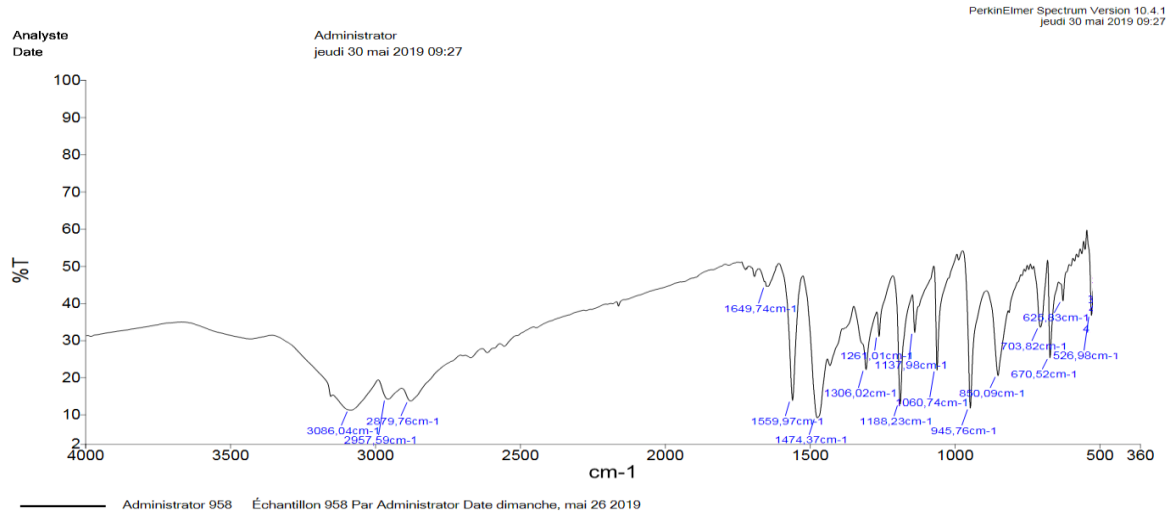


**Figure 5.** Infrared spectrum of the product (P3B).

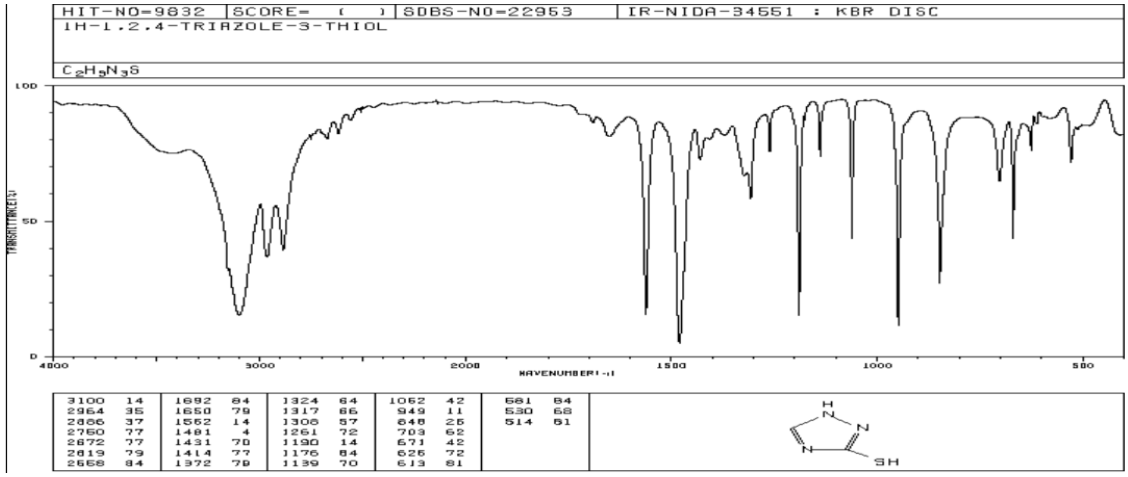


**Figure 6.** Infrared spectrum of 1,2,4-triazole-3-thiole according to SDBS of AIST.

**Table 3**. Attribution of peaks of P3A infrared spectrum.

| Practical spectrum ($cm^{-1}$) | Attribution of peaks |
|---|---|
| 3382 | N-H primary elongation vibration asymmetrical |
| 3284 | N-H primary elongation vibration symmetrical |
| 2954 | C-H in-plane deformation vibration |
| 1682 | C=O elongation vibration |
| 1618 | N-H secondary deformation vibration |
| 1090 | C-N elongation vibration |

The assignment of the peaks of the IR spectrum of P3B and the reference 1,2,4-triazole-3-thiol of are made in the table 4.

**Table 4**. Attribution of peaks of P3B and 1,2,4-triazole-3-thiol infrared spectrum (according to reference tables IR).

| Reference spectrum ($cm^{-1}$) | Practical spectrum ($cm^{-1}$) | Attribution of peaks |
|---|---|---|
| 3100 | 3087 | N-H valence vibration |
| 1650 | 1645 | Association of valence vibration C=N with N-H deformation vibration |
| 1552 | 1560 | N-H deformation vibration |
| 1306 | 1306 | vibration of the C-N bond (amine aromatic secondary) |
| 1139 | 1137 | valence vibration of the C=S bond (form tautomer) |

The presence of the characteristic bands on the practical spectrum P3A confirms the existence of the characteristic functional groups on 1-formyl-3-thiosemicarbazide, which directs towards obtaining the product expected.

The presence of some characteristic bands on the practical P3B spectrum confirms the existence of functional groups present in 1,2,4-triazole-3-thiol such as C=S (tautomeric form), C-N (aromatic secondary amine), Association of vibration of C=N valence with N-H deformation vibration (tautomerism). The analogy of the results with the reference spectrum confirms the identity of the P3B product.

## CONCLUSION

The products to be synthesized could be obtained efficiently in the laboratory. Spectroscopic analysis, separation analysis and measurement of a physical parameter (melting point) confirmed the identity of the product and concluded that the purity was acceptable or even satisfactory. These results could be refined by NMR and HPLC analysis.

## REFERENCES

Li, J.J. 2013, Hetrocyclic Chemistry in Drug Discovery.

Reid, J. R. 1976; Heindel, N. D. J. Heterocycl. Chem. 13, 925.

## ORAL PRESENTATION

# Investigation of carnitine metabolism in melatonin-treated MDA-MB-231 cells Melatonin uygulanmış MDA-MB-231 hücrelerinde karnitin metabolizmasının incelenmesi

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**Özet :**
Meme kanseri en sık görülen kanser türlerinden bir tanesidir. Deneysel ve klinik çalışmalardan melatoninin kanseri önlemek ve tedavi etmek için kullanılabileceğini düşündüren önemli kanıtlar bulunmaktadır. Yapılan çalışmalar melatoninin karsinogenezi doğrudan etkileyerek ve sirkadiyen döngüyü dolaylı olarak bozarak kanser patogenezini azalttığını göstermektedir. Karnitin, lipid metabolizmasında ve ara metabolik yollarda önemli bir fizyolojik rol oynamaktadır. Uzun zincirli yağ asitlerinin sitoplazmadan mitokondriyal matrikse taşınmasına yardımcı olmaktadır. Karnitinin en önemli biyolojik işlevi, açilkarnitin oluşturmak için bir süreç olan β-oksidasyonda yağ asitlerini mitokondriye taşımaktır. Yapılan çalışmalara bakıldığında, karnitinin mitokondriyi koruyarak karsinogenezi inhibe ettiğini göstermektedir. Çalışmada MDA-MB-231 meme kanseri hücre hattında, hipoksi ortamında, melatonin uygulanmış hücrelerde, melatoninin tedavi edici özelliğini araştırmak ve karnitin metabolizması açısından normal hücrelerle tedavi edilmiş hücrelerin arasındaki farkları belirlemektedir. Bu kapsamda MDA-MB 231 hücreleri çoğaltıldı. Çoğalan hücrelere belirli dozlarda melatonin uygulanarak %1 lik hipoksik koşullarda 16 saat inkübe edildi. İnkübasyon sonrası hücreler kazınarak toplandı. Yaklaşık 50 µl gutria kağıdına hücre süspansiyonu damlatılarak 2-3 saat 22-24 °C'de kurutuldu. Uygun prosedürde Sıvı Kromatografisi-Kütle Spektrofotometresi (LC-MS/MS) cihazı kullanılarak karnitin seviyeleri ölçüldü. Veriler, SPSS 22.0 ve GraphPad Prism 9 programları kullanılarak analiz edildi. Melatonin uygulanarak tedavi edilmiş MDA-MB 231 gruplarında toplam 27 karnitin arasından serbest karnitin (C0) (***p<0.001) ve Asetil L-karnitin (C2) (**p<0.01) düzeyleri anlamlı olarak azaldığı bulundu. Diğer karnitin türevleri için istatistiksel olarak anlamlı bir fark tespit edilmedi. Serbest karnitin ve asetil L-karnitinin, yağ asitlerini β-oksidasyon için mitokondriyal matrise taşımaktır. Yağ asidi β-oksidasyonu, farklı koşullara bağlı olarak karsinomun ilerlemesini destekleyebilir veya engelleyebilir. Hipoksik koşullarda tetiklenen HIF-1'nın yağ asidi oksidasyonunun baskılanmasında ve kanser progresyonu için kritik öneme sahiptir. Bu çalışmada, melatonin ile tedavi edilen grupta C0 ve C2 'nin anlamlı düzeyde azaldığı bulunmuştur. Melatoninin hipoksik koşullarda karnitin metabolizmasını etkileyerek karsinomun ilerlemesi ile negatif ilişkili olduğu düşünülmektedir.

**Anahtar Kelimeler:** Melatonin, MDA-MB-231, hipoksi, karnitin.

**Abstract:** Breast cancer is one of the most common types of cancer. There is substantial evidence from experimental and clinical studies suggesting that melatonin can be used to prevent and treat cancer. Studies show that melatonin reduces cancer pathogenesis by directly affecting carcinogenesis and indirectly disrupting the circadian cycle. Carnitine plays an important physiological role in lipid metabolism and intermediate metabolic pathways. It helps to transport long-chain fatty acids from the cytoplasm to the mitochondrial matrix. The most important biological function of carnitine is to transport fatty acids to mitochondria in β-oxidation, a process to form acylcarnitine. Studies show that carnitine inhibits carcinogenesis by protecting mitochondria. In this study, we investigate the therapeutic properties of melatonin in MDA-MB-231 breast cancer cell line, hypoxia environment, melatonin-treated cells and determine the differences between normal cells and treated cells in terms of carnitine metabolism. In this context, MDA-MB 231 cells were grown. Proliferating cells were incubated for 16 hours under 1% hypoxic conditions by applying certain doses of melatonin. After incubation, cells were harvested by scraping. Approximately 50 µl of cell suspension was dropped onto gutria paper and dried at 22-24 °C for 2-3 hours. Carnitine levels were measured using a Tandem Mass Spectrophotometry (MS/MS) device in the appropriate procedure. Data were analyzed using SPSS 22.0 and GraphPad Prism 9 programs. Free carnitine (C0) (***p<0.001) and Acetyl L-carnitine (C2) (**p<0.01) levels were found to be significantly decreased among 27 carnitines in MDA-MB 231 groups treated with melatonin. No statistically significant difference was detected for other carnitine derivatives. Free carnitine and acetyl L-

carnitine transport fatty acids to the mitochondrial matrix for β-oxidation. Fatty acid β-oxidation can promote or inhibit the progression of carcinoma depending on different conditions. It is critical for suppressing fatty acid oxidation of HIF-1 triggered in hypoxic conditions and for cancer progression. In this study, C0 and C2 were found to be significantly reduced in the group treated with melatonin. Melatonin is thought to be negatively related to the progression of carcinoma by affecting carnitine metabolism in hypoxic conditions.
**Key Words:** Melatonin, MDA-MB-231, hypoxia, carnitine.

## GİRİŞ

Meme kanseri, kötü prognozu, yüksek mortalite oranı ve yeni teşhis edilen vakaları nedeniyle en önemli sağlık sorunlarından biri olarak kabul edilmektedir. Kanser teşhis ve tedavisindeki tüm gelişmelere rağmen her yıl çok sayıda ölüm bildirilmektedir. Bu durum daha etkili yaklaşımlara yönelik yoğun bir ihtiyaç olduğu anlamına gelir. Melatonin birçok hücresel ve moleküler süreçte rol oynayan çok değerli bir bileşiktir ve bu nedenle kanser gibi birçok hastalığın tedavisi için uygun bir aday olarak görülmektedir. Melatoninin yapısı ve fonksiyonları sebebi ile bilim insanlarını özellikle meme kanseri üzerindeki etkilerinin araştırmaya yöneltmiştir (Sadoughi ve ark,. 2022). Canlı varlıklarda önemli rol oynayan birçok fizyolojik molekül arasında yer alan melatonin, evrimde milyarlarca yıl önce ortaya çıkmış ve bulunduğu bütün canlılarda evrimsel olarak korunmuştur (Manchester ve arkd,. 2015). Melatonin hücreler için son derece toksik reaktif oksijen türlerinin (ROS) üretilmesinden sorumlu olduğu bilinmektedir. Bu nedenle, melatoninin ortaya çıkmasının bir antioksidan ve serbest radikal temizleyicisi olarak hareket ederek oksidatif stresi kontrol edebilen bir moleküle olan ihtiyaçtan kaynaklandığı öne sürülmüş ve bazı bilim insanları melatonini atadan kalma bir antioksidan olarak tanımlamaktadır (Tan ve ark,. 2013). Hücrelerden melatonin salınımı sirkadiyen bir ritim sergiler. Sirkadiyen ritim ve güçlü bir antioksidan olmasının yanı sıra, melatoninin onkostatik ve onkolitik aktivite gösterdiği, metastatik ilerlemeyi azalttığı bilinmektedir. Çeşitli klinik bozukluklar ve kanser türlerinin ortaya çıkması bozulmuş sirkadiyen ritim ile ilişkilendirilmiştir (Davis ve ark,. 2012). Melatoninin kansere ve metastazına karşı savaşmak için indüklediği çoklu mekanizmaların yanı sıra, melatoninin kemoterapi veya radyoterapiye dirençli kanserleri bir terapiye dönüştürme konusunda yüksek bir yeteneğe sahip olduğu bilinmektedir. Tümöral hücrelerde metabolik ritimleri değiştirerek onların dış uyaranlara ve ilaçlara tepkilerini değiştirmelerine yol açmaktadır (Reiter-Russel ve arkd,. 2017). Melatoninin antioksidan fonksiyonu, hipoksi ile indüklenebilir faktör 1α (HIF1A) varlığında ROS'a bağlı Akt sinyal yolunun inaktivasyonunu, siklin D1, PCNA ve Bcl-2'nin aşağı regülasyonunu ve Bax'ın yukarı regülasyonunu sağlamaktadır. Bu etkiler, metastatik tümör hücreleri tarafından indüklenen hücre proliferasyonunun sınırlamalarını koşullandırır (Liu ve ark,. 2018). Melatonin ile tedavi otofajiyi indükleyerek MT2/mTORC1/TFE3 sinyal yollarını tetikliyor gibi görünmektedir (Sung ve ark,. 2020). Yağ asidi β-oksidasyonu, farklı koşullara bağlı olarak karsinomun ilerlemesini destekleyebilir veya engelleyebilir. Hipoksik koşullarda tetiklenen HIF-1'nın yağ asidi oksidasyonunun baskılanmasında ve kanser progresyonunda kritik öneme sahiptir. Karnitin metabolizması, uzun zincirli yağ asidinin oksidasyon için sitoplazmadan mitokondriye iletilmesinden sorumludur; burada karnitinler yağ asidi oksidasyonunun hız sınırlayıcı adımını katalize etmektedir. Yağ asidi oksidasyonunun kanserdeki kritik rolünün anlaşılmasıyla birlikte, karnitinler kanser metabolik mekanizmasında çok önemli bir aracı olarak yeniden çalışılmaya başlanmıştır. Karnitinler, yağ asidi oksidasyonunu aktive ederek kanser metabolizması adaptasyonunun önemli bir parçasını oluşturan ATP ve NADPH üretimi yoluyla kanser büyümesini hızlandırmaktadır (Qu ve ark., 2016). Bu çalışma ile MDA-MB-231 meme kanseri hücre hattının hipoksik koşullarda melatonin ile tedavisinin karnitin metabolizması üzerindeki etkileri araştırılmıştır.

## MATERYAL VE METOT

Hücre Kültürü ve Koşulları

Sterilizasyon

Hücreler açılmadan ve besiyeri hazırlanmadan önce laminar kabin sterilizasyonu için 30 dk UV ve %70'lik etil alkol (Sigma Aldrich, ABD) kullanılmıştır. Steril edilen laminar kabinde kullanılacak malzemeler %70 alkolle silinerek içeri alınmıştır. Hücre kültürü çalışmaları için temin edilen sarf malzemeler 0,22 µm ve 0,45 µm por çapına sahip filtreler (Sartorius AG, Almanya) kullanılarak sıvı malzemelerin (Fetal Bovine Serum, Penicillin/Streptomycin ve L-Glutamine) (Sigma Aldrich, ABD) sterilizasyonu sağlanmıştır.

Besiyerinin Hazırlanması

MDA-MB-231 hücre hattı için besiyeri ortamı RPMI-1640 Hücreleri kültür kaplarında büyütmek için gerekli olan kimyasallar (50 ml %10 FBS, 5 ml %1 Penicillin/Streptomycin, 5 ml %1 L-Glutamin kullanılmıştır.

Hücrelerin Çoğaltılması

Çalışmada ATCC'den temin edilip stoklanan MDA-MB-231 belirtilen hücreler kullanılmıştır. Sıvı azot tankından çıkarılan donmuş haldeki hücreler 37 °C'de çözülerek laminar kabin içerisine alınmıştır. Hücrelerin büyümesi için hazır hale getirilen RPMI 1640 (Sigma Aldrich, ABD) besiyerine hücreler aktarılarak 1200 rpm'de 5 dakika santrifüj edilip süpernatant uzaklaştırılmıştır. Pellet el yardımı ile hafifçe vurularak homojenize edilip 4 ml besiyerinde süspanse hale getirilmiştir. Süspansiyondan alınan hücreler (10µl) ve %5'lik Tripan-Blue (10µl) (Sigma Aldrich, ABD) karıştırılarak thoma lamında (Marienfeld, Almanya) hücre canlılığı değerlendirilerek 12 kuyucuklu hücre kültürü plakasına ekim yapılmıştır. Her bir kuyucuğa 1 ml RPMI 1640 besiyeri eklenip 37 °C' de %5 $CO_2$ ve %95 nemli ortam içeren ortamda inkübasyona bırakılmıştır. Çoğalan hücrelere 3 tekrarlı ve negatif kontrol, 1mM ve 2 mM olarak üç farklı dozda melatonin uygulanarak %1 lik hipoksik koşullarda 16 saat inkübe edilmiştir. İnkübasyon sonrası hücreler scaper ile kazınarak bir ependorfa aktarılmıştır.

Hücre süspansiyonunda karnitin profilinin LC-MS/MS ile incelenmesi

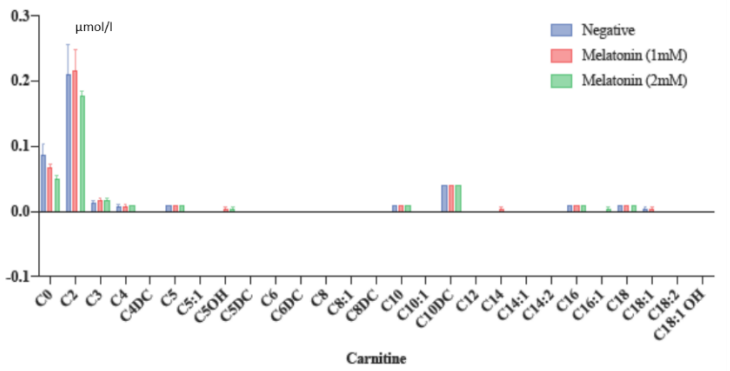
Ependorflara aktarılan hücre süspansiyonunun pipetlenerek homojen bir karışım olması sağlandıktan sonra yaklaşık 50 µl gutria kağıdına damlatılmış ve 2-3 saat 22-24 °C'de absorbe edilmesi sağlanmıştır. 200 µl NBS internal standart eklendikten sonra süpernatan yeni plate'e alınarak azot gazı altında 30 dk kurutulmuştur. 60 µl DRVT eklenerek 60 C de 15 dk inkübasyona bırakılarak türevlendirilmiştir. Daha sonra azot gazı altında 30 dk kurutulmuştur. 100 µl mobil faz (acetonitril: metanol: formicacid) solüsyonu eklenerek LC-MS-MS enjekte edilerek cihazda pik sonuçları alınmıştır.

İstatistiksel Analizler

Deneyler sonrası elde edilen verilerin analizi için GraphPad prism 9 (GraphPad Software, Inc, San Diego, ABD) paket programı kullanılmıştır. Sürekli verilerin dağılımı ile normal dağılım arasında fark olup olmadığı Kolmogorov-smirnov ve Shapiro-Wilk testleriyle araştırılmıştır. Normal dağılan verilerin üç bağımsız grup kıyaslamalarında One-Way ANOVA testi kullanılırken, normal dağılmayan üç gruplu kıyaslamalarda Kruskal-Wallis H testi kullanılmıştır. $p<.05$ istatistiksel olarak anlamlı kabul edilmiştir.

## BULGULAR ve TARTIŞMA

Melatonin uygulanarak tedavi edilmiş MDA-MB 231 gruplarında toplam 27 karnitin arasından serbest karnitin (C0) (***$p<0.001$) ve Asetil L-karnitin (C2) (**$p<0.01$) düzeyleri anlamlı olarak azaldığı bulundu. Diğer karnitin türevleri için istatistiksel olarak anlamlı bir fark tespit edilmedi.




**Şekil 1.** Çeşitli dozlarda melatonin ile tedavi edilmiş MDA-MB-231 hücre hatlarının karnitin profili

Yağ asidi β-oksidasyonu, farklı koşullara bağlı olarak karsinomun ilerlemesini destekleyebilir veya engelleyebilir. Hipoksik koşullarda tetiklenen HIF-1'nın yağ asidi oksidasyonunun baskılanmasında ve kanser progresyonunda kritik öneme sahiptir (Wang ve ark., 2022). Bu çalışmada, melatonin ile tedavi edilen grupta serbest karnitin ve asetil L-karnitinin anlamlı düzeyde azaldığı bulunmuştur.

## SONUÇ

Hipoksik koşullarda melatonin ile tedavi edilmiş MDA-MB-231 hücre hattında karnitin metabolizması etkilenerek, karsinomun ilerlemesine azalttığı düşünülmektedir. Karnitin metabolizmasının, MDA-MB-231 hücre hattının ilerlemesi ile negatif korelasyona sahip olduğu düşünülmektedir.

**KAYNAKLAR**

Davis S., Mirick D.K., Chen C., Stanczyk F.Z. Night shift work and hormone levels in women. *Cancer Epidemiol. Biomark. Prev.* 2012;21:609–618. doi: 10.1158/1055-9965.EPI-11-1128.

Liu R., Wang H.L., Deng M.J., Wen X.J., Mo Y.Y., Chen F.M., Zou C.L., Duan W.F., Li L., Nie X. Melatonin inhibits reactive oxygen species-driven proliferation, epithelial-mesenchymal transition, and vasculogenic mimicry in oral cancer. *Oxid. Med. Cell. Longev.* 2018;2018:3510970. doi: 10.1155/2018/3510970.

Manchester L.C., Coto-Montes A., Boga J.A., Andersen L.P.H., Zhou Z., Galano A., Vriend J., Tan D.X., Reiter R.J. Melatonin: An ancient molecule that makes oxygen metabolically tolerable. *J. Pineal Res.* 2015;59:403–419. doi: 10.1111/jpi.12267.

Reiter-Russel J., Rosales-Corral S.A., Tan D.X., Acuna-Castroviejo D., Lilan Q., Yang S.F., Xu K. Melatonin, a full service anti-cancer agent: Inhibition of initiation, progression and metastasis. *Int. J. Mol. Sci.* 2017;18:843. doi: 10.3390/ijms18040843.

Sung E.S., Kim J.Y., Ahn Y.T., Lee I.W., Choi S.W., Jang H.B. Melatonin exerts anticancer effects in human tongue squamous cell carcinoma cells by promoting autophagy. *Anticancer Res.* 2020;40:6295–6303. doi: 10.21873/anticanres.14650

Tan D.X., Manchester L.C., Liu X., Rosales-Corral S.A., Acuña-Castroviejo D., Reiter R.J. Mitochondria and chloroplasts as the original sites of melatonin synthesis: A hypothesis related to melatonin's primary function and evolution in eukaryotes. *J. Pineal Res.* 2013;54:127–138. doi: 10.1111/jpi.12026.

Qu Q.,Zeng F., Liu X., Wang Q.J., Deng F. Fatty acid oxidation and carnitine palmitoyltransferase I: emerging therapeutic targets in cancer *Cell Death Dis* 2016 may 19;7(5):e2226. doi: 10.1038/cddis.2016.132.

Sadoughi F., Dana P. M., Asemi Z., Shafabakhash R., Mohammadi S., Heidar Z., Mirzamoradi M., Targhazeh N., Mirzaei H. Molecular and cellular mechanisms of melatonin in breast cancer. *Biochimie*. 2022; March 25. doi: 10.1016/j.biochi.2022.03.005

Wang J., Zhou Y., Zhang D., Zhao W., Lu Y., Liu C., Lin W., Zhang Y., Chen K., Hui Wang H., Zhao L. CRIP1 supresses BBOX-1mediated carnitine metabolism to promote stemness in hepatocelular carcinoma. *The Embo Journal.* 41:e110218|2022.

## *ORAL PRESENTATION*

# Sarıseki-Dörtyol Arasında Yer Alan Tarım Arazilerinde Temel Toprak Özelliklerinin Yersel Dağılımının Modellenmesi ve Haritalanması

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## Özet

Toprak özelliklerinin bilinmesi, hem tarımın sürdürülebilirliği hem de çevre koruma açısından son derece önemlidir. Bu çalışmada, Sarıseki-Dörtyol (Hatay) arasında kalan tarım arazilerde toprakların temel özelliklerinin saptanması ve bu özelliklerin çalışma alanındaki dağılımlarının modellenerek haritalarının oluşturulması amaçlanmıştır. Bu amaçla, araştırma alanındaki toprakları temsil edecek şekilde rastgele örnekleme yöntemine göre 0-30 cm derinlikten toplam 42 adet bozulmuş toprak örneği alınmıştır. Toprak örneklerinin alındığı noktaların coğrafi koordinatları küresel konumlama cihazı (GPS) kullanılarak kayıt edilmiştir. Toprak örneklerinde pH, elektriksel iletkenlik (EC), organik madde (OM), kireç bünye analizleri yapılmıştır. Toprak özelliklerinin yersel dağılımının belirlenmesi ve haritalanması için jeoistatistiksel yöntemler kullanılmıştır. Çalışma alanındaki toprakların verimlilik ile ilgili özelliklerinin dağılımlarının oldukça farklı sınırlar içinde değiştiği belirlenmiştir. Toprak örneklerinin pH değerleri 6.80 ile 7.81, EC 151 ile 1337 µS $cm^{-1}$, kireç içerikleri % 0.03 ile % 39.97 ve organik madde içerikleri % 0.39 ile % 13.39, arasında değişmiştir. Toprakların temel özellikleri içerisinde varyasyon katsayısı (VK) en düşük olanı pH (% 3.2) en yüksek olanı ise kireç içeriği olup (% 169.8), bunu % 65.8 ile EC değerleri izlemektedir. En uygun variyogram modeli; EC, kum ve silt, değerleri için küresel; organik madde ve kil için Gaussian ve pH ve kireç için ise üssel olarak belirlenmiştir.

**Anahtar Kelimeler:** Sarıseki-Dörtyol bölgesi, Toprak özellikleri, Jeoisatistik

# Modeling and mapping of the spatial distribution of the basic soil properties in the agricultural lands between Sariseki-Dörtyol

## Abstract

Knowing the soil properties is extremely important for both the sustainability of agriculture and environmental protection. In this study, it is aimed to determine the basic properties of the soils in the agricultural lands between Sarıseki-Dörtyol (Hatay), and to model the distribution of these properties in the study area and to create maps. For this purpose, a total of 42 degraded soil samples were taken from 0-30 cm depth according to the random sampling method to represent the soils in the research area. Geographical coordinates of the points where soil samples taken were recorded using a global positioning device (GPS). Soil samples were analyzed for pH, electrical conductivity (EC), organic matter (OM), lime and texture. Geostatistical methods were used to determine and map the terrestrial distribution of soil properties. It has been determined that the distribution of the properties of the soils in the study area varies within quite different limits. The pH, EC, lime and organic matter contents of the soil samples varied between 6.80 and 7.81, 151 and 1337 µS $cm^{-1}$, 0.03% and 39.97%, 0.39% and 13.39%, respectively. The lowest coefficient of variation (VK) among the basic properties of soils is pH (3.2%) and the highest one is lime content (169.8%), followed by EC values with 65.8%. The most suitable semivariogram model were found to be Spherical for EC, sand and silt, Gaussian for organic matter and clay and Exponential for pH and lime content.

**Key Words**: Sarıseki-Dörtyol district, Soil properties, Geostatistics

## GİRİŞ

Topraklar; sağladığı faydalar nedeniyle her geçen gün önemi daha iyi anlaşılan değerli doğal kaynaklarımızdandır. İnsan yaşamının devamlılığı için gerekli olan toprak, artan nüfusun gereksinimlerini karşılamakta yetersiz kalmaktadır. Bu yüzden toprak verimliliği ön plana çıkmaktadır.
Bunun yanında toprağın sürdürülebilir olması tarım faaliyetlerinin ve insan yaşamının devamlılığı için koruma altına alınması gerekmektedir. Tarımsal üretimde verimliliğin ve üretimin sürdürülebilir olması toprak yapısı ve özellikleri ile önemli derecede ilişkilidir. Bu nedenle toprak yapısı ve özelliklerinin belirlenmesi, belirlenen özelliklerin yersel dağılımının nasıl değiştiği verimlilik, sürdürülebilirlik, yaşamın devamlılığı ve sonraki yapılacak olan çalışmalara kolaylık sağlayacağından dolayı son derece önemlidir.
Aynı arazi içerisinde toprakların birçok fiziksel, kimyasal ve biyolojik özelliği heterojen bir yapıya sahiptirler. Klasik istatistiksel yöntemler, toprakların yersel olarak gösterdiği değişkenliği belirlemede yetersizdir. Ayrıca, toprağa ait bilgileri toplamak için yapılan arazi çalışmaları ve alınan örneklerin analizleri kolay ve ekonomik değildir. Bu nedenle, toprağa ait değişkenliklerin önemli ölçüde var olup olmadığını değerlendirmek ve daha detaylı araştırmalar için yatırım yapmadan önce düşük maliyetli yöntemlere ihtiyaç duyulmaktadır. Toprak özelliklerinin yersel değişkenliklerinin modellenerek alansal tahmin haritalarının üretilmesi, daha düşük maliyet ile geniş alanların toprak özelliklerinin belirlenmesinde kullanılan yaygın yöntemlerin başında gelmektedir (Turgut ve Öztaş, 2012). Bu işlem de jeoistatistikle kolay bir şekilde yapılabilmektedir.
Bu çalışmada, Sarıseki-Dörtyol bölgesinde yer alan tarım arazilerindeki toprakların temel özellikleri belirlenmiş ve bu özelliklerin çalışma alanındaki dağılım haritaları oluşturulmuştur.

## MATERYAL VE YÖNTEM

### Materyal

Sarıseki-Dörtyol bölgesindeki çalışma alanı 36º40'12" - 36º49'48" kuzey enlemleri ile 36º09'36" - 36º15'00" doğu boylamları arasında yer almaktadır. Araştırma alanının doğusunda Amanos Dağları, Batısında ise Akdeniz bulunmaktadır. Çalışma alanının büyük bir kısmını tarım alanları, az bir kısmı ise orman ve fundalık alanlar oluşturmaktadır. Çalışma alanında kışları ılık ve yağışlı, yazları ise sıcak ve kurak olan Akdeniz iklimi hâkimdir (Anonim, 2004).

### Yöntem

Bu araştırmada, çalışma alanını temsil edecek şekilde rastgele örnekleme yöntemine göre 0-30 cm derinlikten toplam 42 adet bozulmuş toprak örneği alınmıştır (Şekil 1). Ayrıca her bir örnekleme noktasının UTM sistemine göre coğrafi koordinatları GPS cihazı ile belirlenmiştir.
Alınan toprak örnekleri laboratuvarda kurutulduktan sonra elenerek analize hazır hale getirilmiştir. Analize hazır hale getirilen toprak örneklerinde; pH, elektriksel iletkenlik (EC), kireç, organik madde (OM) ve bünye analizleri yapılmıştır. Örneklerde pH tayini saturasyon çamurunda pH-metre ile EC tayini ise saturasyon ekstraktında EC-metre ile ölçülmüştür (Richards, 1954). Organik madde Walkey-Black yaş yakma yöntemi ile (Allison, 1965), kireç miktarı ise Allison ve Moodie (1965)'e göre belirlenmiştir. Bünye analizi ise hidrometre yöntemine (Bouyoucos, 1951) göre yapılmıştır.
Toprak özelliklerin tanımlayıcı istatistik analizleri (ortalama, en düşük ve en yüksek değerler, standart sapma, varyasyon katsayısı, çarpıklık (skewness), basıklık (kurotsis) vb.) yapılmıştır. İstatistik analizler için Windows uyumlu SPPS 21 istatistik paket programı kullanılmıştır.
Daha sonra her bir parametrenin dağılım modellemesi ve iki boyutlu dağılım haritası oluşturulmuştur. Toprak özelliklerinin dağılımlarının modellenmesi ve haritalanmasında Windows uyumlu GS+ (sürüm 10) jeoistatistik programı kullanılmıştır.




Şekil 1. Çalışma alanının coğrafi konumu ve örnekleme noktaları

Toprak özelliklerinin yersel bağımlılıklarının değerlendirilmesinde nugget yarıvaryansın (Co) toplam variyansa (Co + C) olan oransal yüzdesi kıstas olarak kullanılmıştır. Hesaplanan olan bu oran %25 veya daha düşük ise özelliklerdeki yersel bağımlılık kuvvetli, %25-75 arasında ise orta ve %75'den büyük ise zayıf olarak değerlendirilmiştir (Cambardella ve ark., 1994).

## BULGULAR ve TARTIŞMA

### Temel Toprak Özellikleri

Araştırma konusu torakların temel özelliklerine ait analiz sonuçları Tablo ’de verilmiştir. Toprak örneklerine ait pH değerleri 6.80 ile 7.81 arasında değişmekte olup ortalama pH değeri 7.38 olarak belirlenmiştir. Özdel (2010) tarafından aynı bölgede yapılan çalışmalarda pH değerleri biraz daha yüksek bulunmuştur. Bu durum büyük olasılıkla pH tayin yöntemlerinin farklı olmasından, ya da zamana bağlı olarak pH değişiminden kaynaklanmıştır. Toprakların elektriksel iletkenlik (EC) değeri ise 151 µS $cm^{-1}$ ile 1337 µS $cm^{-1}$ arasında olup, ortalama EC değeri 439 µS$cm^{-1}$ olarak saptanmıştır.

Çalışma alanındaki toprakların kireç içerikleri % 0.03 ile %39.97, organik madde içerikleri %0.39 ile %13.39 arasında değişmiş olup, ortalama %5.79 olarak belirlenmiştir. Sonuçlardan da görüldüğü gibi toprakların kireç içerikleri çok geniş sınırlar içinde değişmektedir. Bu durum büyük olasılıkla çalışma alanının büyük bir kısmının; değişik ana materyallerden gelen aluviyal depozitlerin oluşmasından kaynaklanmıştır. Zira toprakların oluştuğu alanların çoğu, kuvartener dönemi temsil eden oluşuklar; geniş alanda yayılım gösteren bazaltlar, moloz örtüler ve alüviyonlardan oluşmuştur. Bu alanda ayrıca, kireç taşları ve dolomitik kireç taşları, konglomeralar, serpantinleşmiş ultra bazik kayaç parçaları ve serpantin arenitleri de yer almaktadır (Koç ve Değer, 1991).

Toprakların organik madde içerikleri % 0.39 – 13.49 gibi geniş sınırlar içinde değişmesine rağmen, ortalama organik madde içeriği %3.92 olarak belirlenmiştir. Topraklarda organik maddeyi yükselten birkaç noktanın (9, 24, 28 ve 36 nolu örneklerin allandığı alanlar) işlenmemiş olan ormanlık alanda ve maki bitki örtüsü altında olmasından kaynaklanmaktadır. Doğal bitki örtüsü altında organik madde, işlenmiş alanlara göre daha uzun sürede parçalanmaktadır.

Tablo 1. Araştırma konusu toprakların tanımlayıcı istatistik analiz sonuçları

| | pH | EC ($\mu S\ cm^{-1}$) | Kireç (%) | Organik madde (%) | Tane irilik dağılımı (%) Kum | Silt | Kil |
|---|---|---|---|---|---|---|---|
| **EK** | 6.80 | 151 | 0.03 | 0.39 | 10.4 | 16.1 | 7.7 |
| **EY** | 7.81 | 1337 | 39.97 | 13.49 | 73.6 | 59.1 | 48.5 |
| **Ort.** | 7.38 | 439.3 | 5.79 | 3.92 | 40.8 | 32.3 | 26.9 |
| **SS** | 0.24 | 289.2 | 9.83 | 2.38 | 15.3 | 10.6 | 8.7 |
| **VK** | 3.2 | 65.8 | 169.8 | 60.7 | 37.5 | 32.8 | 32.3 |
| **Çarpıklık** | -0.45 | 1.78 | 2.43 | 1.81 | 0.16 | 0.68 | 0.21 |
| **Basıklık** | -0.10 | 2.54 | 5.59 | 5.19 | -0.20 | 0.31 | 0.83 |

EK: en küçük değer, EY: en yüksek değer, Ort.: ortalama değer, SS: standart sapma, VK: varyasyon katsayısı

Kum içerikleri %10.4 ile %73.6, silt içerikleri %16.1 ile %59.1, kil içerikleri ise %7.7 ile %48.5 arasında değişmiştir (Tablo 1). Özdel (2010) yaptığı çalışmada da aynı bölge topraklarının organik madde, kum, silt ve kil dağılımlarını bu çalışmadakilerle benzer bulmuştur. Aradaki küçük farklılıklar toprakların heterojenliğinden kaynaklanmıştır.

Toprakların temel özellikleri içerisinde VK değeri en düşük olanı pH (%3.2), en yüksek olanı ise kireç içeriği olup (%169.8), bunu % 65.8 ile EC değerleri izlemektedir. Mohamed ve ark. (2021) tarafından yapılan bir çalışmada da bu konuda benzer sonuçlar elde edilmiştir. Abdennour ve ark. (2020) tarafından yapılan bir çalışmada ise en düşük VK pH değerlerinde bulunmuş, ancak EC değerlerinin VK diğer parametrelerin VK değerlerine göre daha düşük bulunmuştur. Bu durum araştırma alanında pH değerlerinin çok homojen, kireç içeriklerinin ise heterojen dağıldığını göstermektedir. Bu durumu çarpıklık değerleri de teyit etmektedir. Çarpıklık değerleri ne kadar küçükse, o özelliğin araştırma alanındaki dağılımı da o kadar homojen olmaktadır. VK değeri en yüksek olan kireç içeriğinin aynı zamanda çarpıklık değeri de en yüksektir.

Zhou ve ark. (2011)'e göre variasyon katsayısı %10'dan düşük ise değişkenlik düzeyi de düşük, %10-100 arasında ise değişkenlik düzeyi orta ve %100'den büyük ise değişkenlik düzeyi yüksektir. Bu kıstaslara göre, toprak özellikleri içerisinde; sadece pH değerlerinin çalışma alanındaki dağılımı çok homojen, kireç içeriklerinin dağımı ise çok heterojen olduğu saptanmıştır. Diğer parametrelerin homojenlik düzeylerinin ise orta düzeyde olduğu belirlenmiştir.

**Toprak özelliklerinin yersel dağılımının modellenmesi**

Toprak özelliklerinin yersel dağılımın modellenmesinde, çarpıklık değerlerinin düşük olması nedeniyle, pH, kum, silt ve kil içeriklerine ait veri setlerine jeoistatistiksel modellemelerden önce herhangi bir dönüşüm yapılmamıştır. Kireç ve EC değerlerine çarpıklık değerlerinin yüksek olması nedeniyle logaritmik, organik madde içeriği veri setine ise karekök dönüşüm uygulandıktan sonra modellemede kullanılmıştır. Toprak özelliklerine ait yarıvariyograma parametreleri Tablo 2'de verilmiştir. Çalışma alanı topraklarının pH, Organik madde ve kil içerikleri için en uygun yarıvaryogram modeli Guassian, EC, kum ve silt içerikleri için Küresel ve kireç içerikleri için ise Üssel olarak belirlenmiştir (Tablo 2). Değişik araştırıcılar yaptıkları çalışmalarda temel toprak özellikleri için en uygun yarıvaryogram modellerini belirlemişlerdir. Örneğin, Mondal ve ark. (2021) yaptıkları çalışmada; EC değerleri için en uygun modelin küresel, Sharma ve ark. (2020) pH değerleri için en uygun modelin Üssel olduğunu belirlemişlerdir. Yine Li ve ark. (2011) tarafından Çin'de yapılan bir çalışmada toprakların organik madde içirikleri için en uygun modeli Küresel, Reza ve ark. (2015) ve Ahmad ve ark. (2018) tarafından yapılan çalışmalarda ise toprakların kum içerikleri için en uygun modelin Üssel olduğu belirlenmiştir. Sun ve ark. (2003) ise toprak özelliklerinin yersel değişkenliklerinin modellenmesinde en fazla uygunluk gösteren yarıvaryogram modellerin Küresel, Üssel ve Gaussian olduğu belirtmişlerdir (Gürel, 2020).

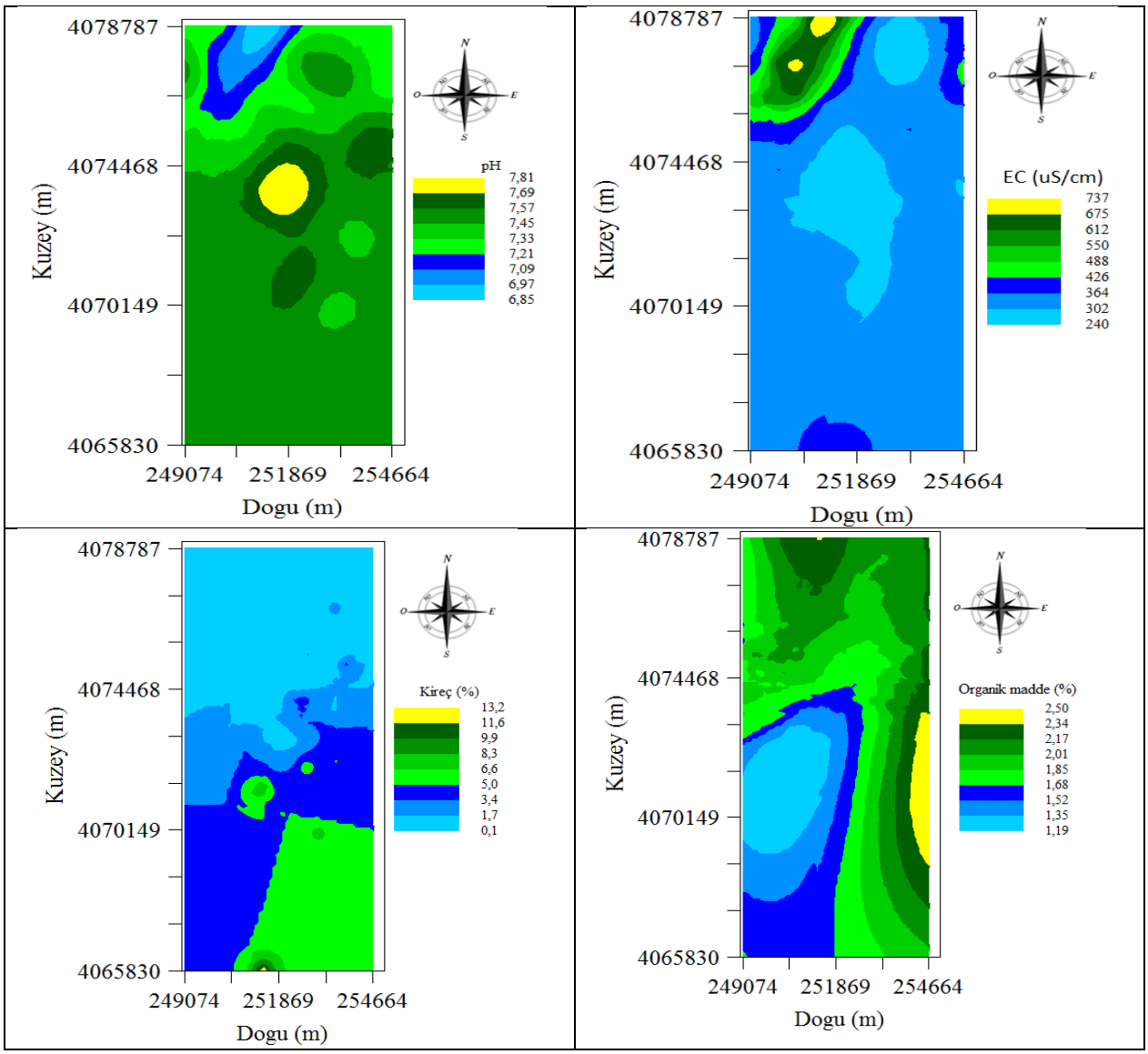
Tablo 2. Toprak özelliklerine ait yarıvariyogram parametreleri

| Özellik | Model | Ao (m) | Nugget ($C_0$) | Sill ($C_0$+C) | ($C_0$)/ ($C_0$+C)*100 | $r^2$ |
|---|---|---|---|---|---|---|
| pH | Gaussian | 2930 | 0.015 | 0.087 | 17.2 | 0.839 |
| EC | Küresel | 2620 | 0.151 | 0.303 | 49.8 | 0.489 |
| Kireç | Üssel | 1210 | 2.555 | 5.111 | 50.0 | 0.580 |
| Org. Mad. | Gaussian | 14590 | 0.229 | 1.539 | 14.9 | 0.822 |
| Kum | Küresel | 4360 | 113.3 | 226.7 | 50.0 | 0.681 |
| Silt | Küresel | 4040 | 59.9 | 119.9 | 50.0 | 0.410 |
| Kil | Gaussian | 10890 | 43.8 | 298.5 | 14.7 | 0.831 |

Jeoistatistikte $A_0$ (range) değeri; herhangi bir değişken için yersel bağımlılığın devam ettiği maksimum mesafeyi belirtmektedir (Isaaks ve Sriastava, 1989). Bu mesafeden sonra noktalar arasında herhangi bir yersel bağımlılık bulunmamaktadır. Araştırma alanındaki toprak özelliklerinin range değerleri 1210 m (Kireç) ile 14590 m (organik madde) arasında değişmiştir. Bu durum toprak özelliklerindeki yersel bağımlılığın çok geniş sınırlar içinde değiştiğini göstermektedir. Bu konuda da birçok araştırıcı farklı sonuçlar bulmuştur. Örneğin, Reza ve ark. (2015) toprakların kil içirikleri için $A_0$ değerinin 2352 m, Doğan ve ark. (2020) Arsuz ovasında yaptıkları çalışmada pH için A0 değerini 700 m, Sharma ve ark. (2020) ise EC değerleri için $A_0$ değerini 1599 m olarak belirlemişlerdir. Bu çalışmada silt içeriklerinin 4040 m (Tablo 2) olmasına rağmen Ahmad ve ark. (2018) ise silt içeriklerinin $A_0$ değerini oldukça yüksek (31470 m) bulmuşlardır. Bu farklılık büyük olasılıkla araştırma alanlarının genişliğinin farklı olmasında kaynaklanmış olabilir.
Toprak özelliklerinin Nugget/Sill oranlarına göre (Cambardella ve ark., 1994); pH, organik madde ve kil içeriklerinin kuvvetli: kireç, EC, silt ve kum içeriklerinin ise orta düzeyde yersel bağımlılık göstermiştir.

**Toprak özelliklerinin Haritalanması**

Toprakların temel özelliklerinin haritalanmasında ordinary Kriging enterpolasyon yöntemi kullanılmıştır. Bu yöntem kullanılarak oluşturulan yersel dağılım haritaları Şekil 2'de verilmiştir. Çalışma alanı topraklarının pH değerlerinin dağılım haritasına incelendiğinde, dağılımın ağırlıklı olarak 7.21 - 7.57 değerleri arasında olduğu, kuzeye doğru gidildikçe pH değerlerinin düştüğü gözlenmektedir. En yüksek pH değerlerine ise çalışma alanının orta kısımlarında rastlanmıştır. Topraklarının EC değerlerinin dağılımının ağırlıklı olarak 302 – 240 µS $cm^{-1}$ değerleri arasında olduğu ve kuzeybatı yönünde ilerledikçe arttığı ve en yüksek değerlere ulaştığı görülmüştür. En düşük EC değerlerinin (0 - 240 µS $cm^{-1}$ ) ise çalışma alanının orta kesimlerinde olduğu belirlenmiştir (Şekil 2).
Topraklarının kireç içeriklerinin çalışma alanındaki dağılımına bakıldığında, en düşük değerlerin kuzeyde, en yüksek değerlerin ise güneydoğu kısmında olduğu görülmektedir. Topraklarında organik madde içeriklerinin dağılım haritası incelendiğinde alanın doğu kesimlerinde yüksek olduğu ve batı ve kuzey kesimlerine doğru azaldığı görülmektedir. Doğu kesiminde yüksek olması bu bölgedeki ormanlık alanlardan kaynaklanmıştır. Alanda en düşük organik madde içeriği ise batı kesimlerinde görülmektedir. (Şekil 2).
Topraklarının kum içeriklerine alandaki dağılımı incelendiğinde, dağılımın ağırlıklı olarak güneyde yoğunluk gösterdiği, kuzeybatıya doğru gidildikçe bu değerlerin düştüğü ve en düşük değerlerin kuzey batı kesiminde olduğu gözlemlenmiştir. Toprakların silt değerlerinin dağılım haritası incelendiğinde, kuzey batı kesimlerinde yüksek, güneydoğu kesimlerinde ise düşük olduğu görülmektedir. Toprakların kil içerikleri dağılımına bakıldığında, ağırlıklı olarak çalışma alanının güne kesimlerinde düşük, kuzey kesimlerinde ise yüksek olduğu görülmektedir (Şekil 2).



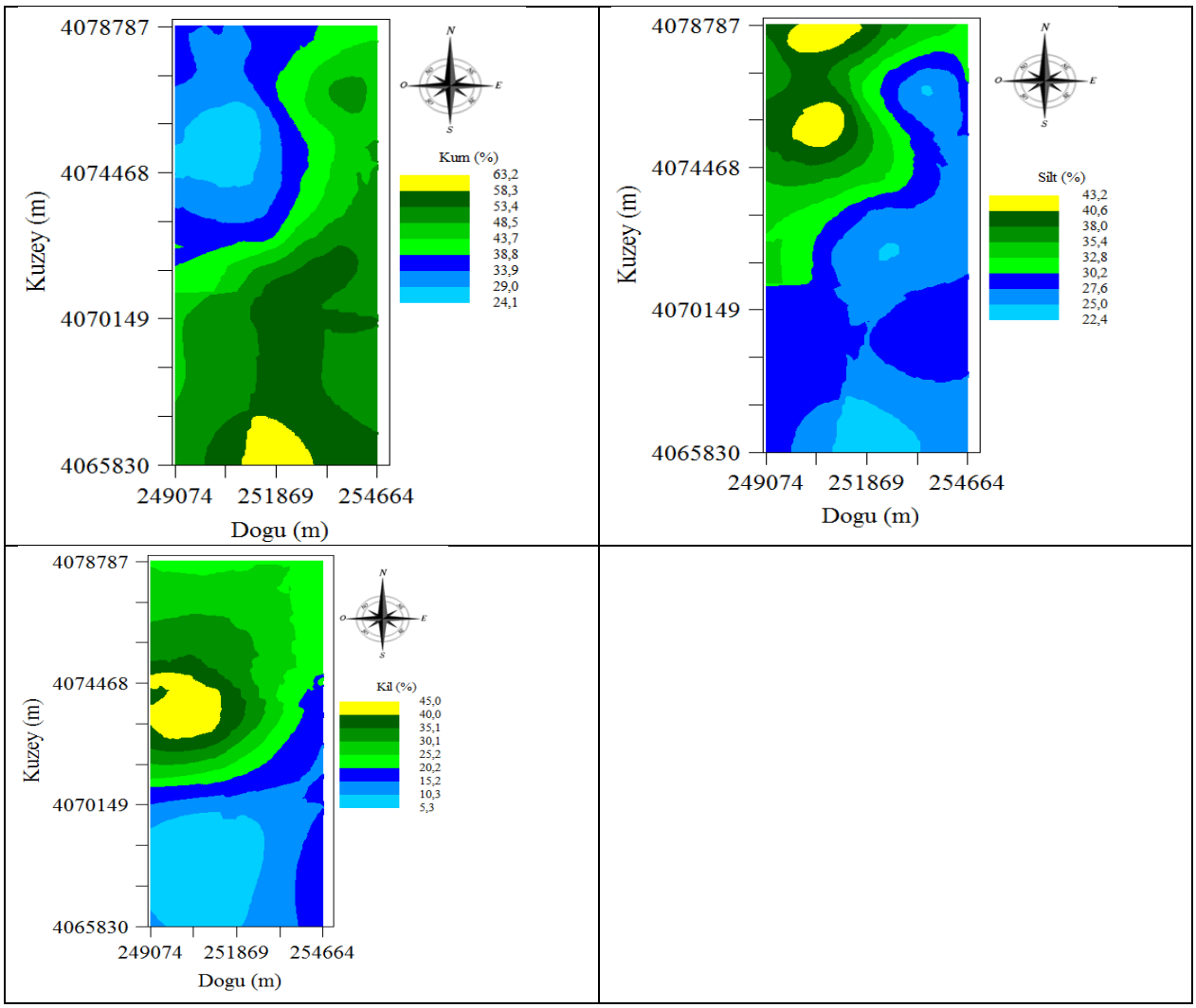

Şekil 2. Çalışma alanı toprak özelliklerinin yersel dağılım haritaları

## SONUÇ

Bu çalışmada topraklarının verimlilik potansiyelini belirlenmesinde önemli kıstaslardan olan temel özelliklerinin yersel dağılımını modelleyerek, her özelliğin alandaki yersel dağılım haritalarını oluşturmaktır. Sarıseki-Dörtyol bölgesindeki toprakların özellikleri ile ilgili yeterli araştırma bulunmamaktadır.

Çalışma alanındaki toprak özelliklerinin dağılımlarının oldukça farklı sınırlar içinde değiştiği belirlenmiştir. Alanda en homojen olarak dağılan parametrenin pH, en heterojen dağılanın ise kireç içeriğinin olduğu belirlenmiştir

Toprakların kireç içerikleri çok geniş sınırlar içerisinde değişmiştir. Toprakların çoğunluğu orta (tın) bünyelidir. Toplam 42 adet toprak örneğinin 34 tanesi orta bünyeli (tın, kumlu tın, kumlu killi tın ve killi tın) iken, sadece 8 tanesi ince (kil) bünyelidir. Dolayısıyla bu toprakların besin elementi ve su tutma kapasiteleri iyidir.




Şekil 2.(Devam) Çalışma alanı toprak özelliklerinin yersel dağılım haritaları

Toprakların reaksiyonu nötrden orta bazikliğe kadar değişmektedir. Organik madde içerikleri çok geniş sınırlar içerisinde değişmiştir. Ancak, genellikle doğusundaki ormanlık alanlarda yüksek, düz alanlarda ise düşük olduğu belirlenmiştir. Organik madde içeriği düşük olan alanlara azotlu gübre uygulaması ile birlikte çiftlik gübresi de verilerek organik madde içeriği yükseltilmelidir. Kireç içeriği yüksek olan topraklara azotlu gübre olarak amonyum sülfat veya üre gibi asit içerikli gübreler verilmesi daha uygundur.
Bu çalışma sonuçları, bölgede bundan sonra yapılacak olan daha detaylı çalışmalara ışık tutacak ve bölge toprakları hakkındaki bilgi birikimine önemli katkılar sağlayacaktır.

## TEŞEKKÜR

Bu bildiri, Yunus KARAKAYA'nın Hatay Mustafa Kemal Üniversitesi, Bilimsel Araştırma Projeler Koordinatörlüğü (BAP) tarafından **20.YL.007** nolu proje ile desteklenen Yüksek Lisans tezinin bir bölümünden hazırlanmıştır. Yazarlar, maddi destekleri nedeniyle BAP koordinatörlüğüne teşekkür ederler.

## KAYNAKLAR

Abdennour MA, Douaoui A, Barrena J, Pullido M, Bradai A, Bennacer A, Piccini C, Alfonso-Torreño A 2020. Geochemical characterization of salinity of irrigated soils in arid regions (Biskra, SE Algeria). Acta Geochim, Available at: https://doi.org/10.1007/s11631-020-00426-2.

Ahmad M, Muhammad D, Mussarat M, Naseer M, Khan M A, Khan AA, Shafi MI 2018. Spatial variability pattern and mapping of selected soil properties in hilly areas of Hindukush range northern, Pakistan. Eurasian J Soil Sci, 7 (4) 355–364.

Allison, E. l965. Organic Carbon (C.A. Black, Editör), Methods of Soil Analysis. Part 2. Agronomy Series. No. 9, ASA. pp. l367-1378. Wisconsin.

Allison. E, Moode CD,1965. Carbonate. (C.A. Black, Editör), Methods of Soil Analysis. Part 2. Agronomy Series. No. 9, ASA. pp. 1379-1396, Wisconsin.

Anonim 2004. Hatay ili tarım master planı. Tarım ve Köyişleri BakanlığıAraştırma Planlama ve Koordinasyon Kurulu Başkanlığı Hatay Tarım İl Müdürlüğü, 232 s. Hatay.

Bouyoucos GJ 1951. A recalibration of the hydrometer method for making mechanical analyisis of soils. Agronomy Journal, 43:434-438.

Cambardella CA, Moorman TB, Parkin TB, Karlen DL, Novak JM, Turco RF, Konopka AE 1994. Field-scale variability of soil properties in central Iow a soils. Soil Sci. Soc. Am. J., 58: 1501-1511.

Doğan K, Ağca N, Keçecioğlu F, Benice A. Tuğba T 2020. Spatial distribution of microbial activities in Arsuz plain soils (Hatay, Turkey). Arabian Journal of Geosciences, 13:581.

Gürel F 2020. Uludağ göknarı ve Uludağ göknarı-sarıçam meşcerelerinde bazı toprak özelliklerinin uzaysal değişkenliği. Çankırı Karatekin Üniversitesi Fen Bilimleri Enstitüsü, Orman Mühendisliği Anabilim Dalı Yüksek Lisans Tezi. Çankırı

Isaaks HE, Srivastava RM 1989. Applied geostatistics. Oxfort University press, Inc. 561 pp.

Koç Ş, Değer MA 1991. Payas (Hatay) bölgesi boksitli demir cevherleşmesinin oluşumu. MTA Dergisi, 113: 113-126

Liu W, Su Y, Yang R, Yang Q, Fan G 2011. Temporal and spatial variability of soil organic matter and total nitrogen in a typical oasis cropland ecosystem in arid region of Northwest China, Environ Earth Sci, 64:2247–2257.

Mohamed A E, AbdelRahman E, Zakarya MY, Metwaly MM, Koubouris G 2021. Deciphering soil spatial variability through geostatistics and ınterpolation techniques. Sustainability, 13: 194.

Mondal BP, Sekhon BS, Rahul Sadhukhan R 2021. Geostatistical assessment of spatial variability of soil organic carbon under different land uses of Northwestern India. Agric Res., 10 (3): 407–416.

Özdel E 2010 Sarıseki – Dörtyol yöresi topraklarında ağır metallerin yersel dağılımının jeoistatistiksel yöntemlerle belirlenmesi. Mustafa Kemal Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi. Hatay.

Reza SK, Nayaka DC, Mukhopadhyaya S, Chattopadhyaya T, Singh SK 2018. Characterizing spatial variability of soil properties in alluvial soils of India using geostatistics and geographical information system. Archives of Agronomy and Soil Scıence, 63 (11): 1489–1498.

Richards LA 1954. Diagnosis and improvement of saline and alkali soils. USDA Agriculture Handbook. No: 60.

Sharma RP, Chattaraja S, Vasua D, Karthikeyana K, Tiwarya P, Naitama R, Dasha, B, Tiwaria G, Jangira A, Daripaa A, Singhb, SK, Anantwara S G, Nimkar AM 2020. Spatial variability assessment of soil fertility in black soils of central India using geostatistical modelling. Archives of Agronomy and Soil Science, Available at: https://doi.org/10.1080/03650340.2020.176667.

Turgut B, Öztaş T 2012. Bazı toprak özelliklerine ait yersel değişimin jeoistatistiksel yöntemlerle belirlenmesi. Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi, 7 (2): l0-22.

Zhou XH, Obuchowski NA, McClish DK, 2011. Statistical methods in diagnostic medicine, 2nd Edition. ISBN: 978-0-470-18314-4, 592 pp.

*ORAL PRESENTATION*

# The effect of using pekmez and honey as sugar substitutes in sponge cake and cookie formulation on the rheology of batter and cookie dough

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**Abstract**

The use of honey or pekmez in the production of bakery products as an alternative to sucrose has been gaining popularity in recent years. The use of honey or pekmez in bakery products enriches the product with nutritional components such as minerals, vitamins, and phenolic compounds, and can also contribute to prolonging the shelf life. However, this application can also cause a decrease in the quality of the product, such as the formation of thermal process contaminants, deterioration in sensorial properties, and changes in dough rheology. Hence, this study investigates the rheological properties of the sponge cake batter and cookie dough samples produced by using pekmez or honey as sugar substitutes. Oscillation tests were performed for the measurement of the structure of cookie dough without deformation. Within the linear viscoelastic range, the frequency sweep test was performed and the storage modulus (G') and loss modulus (G") values were recorded and plotted as a function of angular frequency. The flow behavior of batter samples was analyzed by varying the shear stress depending on the shear rate. And the apparent viscosity values were plotted as a function of shear rate. The data were fitted well with the Power Law model having high $R^2$ values ($R^2 > 0.99$). Consistency index values of the batter samples containing pekmez at different rates did not differ from the control sample significantly ($P>0.05$), although there was a significant difference between the control sample and the batter samples containing honey at different rates ($P<0.05$).

**Keywords:** rheology, cake, cookie, pekmez, honey

## 1. INTRODUCTION

Rheology is the science of the flow and the deformation of materials (Kasapis and Bannikova, 2017). In the food industry; the rheological properties of the substances are one of the most important features to be considered in terms of production and quality. Today, food rheology is not just a definition based on measuring the viscosity of food. There is also the scope for understanding the microstructure of foods and its connection to the texture and rheology of food. In this way, textural defects can be reduced and consumer satisfaction can be increased (Ahmed et al., 2017).

Bakery products such as sponge cakes and cookies are products that have gained popularity and have become the part of the daily diet due to their variety and cost-effectiveness (Tsatsaragkou et al., 2021). In cookies and sponge cakes, the main reformulation challenge lies in mimicking the structure and texture of the product ( Renzetti and van der Sman, 2022; Şahin et al., 2019). Hence, the reformulations made are very important in determining the structure, texture, shelf life and sensorial properties of bakery products (Şahin, 2019). One of the common features of cookies and sponge cakes is the use of sucrose as the main sweetening ingredient (Renzetti et al., 2021). In addition to providing sweetness in cakes, sucrose has many roles such as increasing the foam stability by means of increasing the viscosity, controlling the cake volume by affecting the starch gelatinization temperature and protein denaturation temperature (Renzetti and van der Sman, 2022; Tsatsaragkou et al., 2021). In cookies, sucrose not only affects the flavor, size, color and texture (Gallagher et al., 2003) but also contributes to the spreading process during baking and increases surface cracking due to recrystallization (Sahin et al., 2019).

High consumption of sugar is known to cause chronic diseases based on malnutrition such as obesity, diabetes, cholesterol, coronary heart disease, etc. In this context, reducing the amount of sugar in the formulations of

commonly consumed sugary bakery products such as sponge cakes and cookies stands out as the best way to obtain healthier and low-calorie products (Şahin et al., 2019). For this reason, the addition of honey or pekmez as sugar substitutes in the production of bakery products has been gaining popularity in recent years. In order to avoid the harmful effects of refined sugar, the use of honey or pekmez as sugar substitutes is increasing both in industrial applications and home-type products.

Adding honey or pekmez to the formulation is a positive practice in terms of enriching the bakery products with nutritional components such as minerals, vitamins and phenolic compounds (Bornare and Ajaz Khan, 2015; Demir and Kılınç, 2019; Gunderson et al., 2018; Ertaş and Çoklar, 2008), and can also contribute to prolonging the shelf life of the product (Ertaş and Çoklar, 2008; Aghamohammadi et al., 2012). However, it has also the potential for negative consequences such as the formation of thermal process contaminants (Kazancı, 2021), deterioration in sensorial and textural properties of the products (Bornare and Ajaz Khan, 2015; Demir and Kılınç, 2019) and changes in dough rheology (Bilgiçli and Akbulut, 2010). The effect of the use of pekmez and honey as sugar substitutes on the quality characteristics and the acrylamide content of sponge cakes and cookies were investigated in detail in our previous study (Kazancı, 2021). However, the effect of pekmez and honey addition on the rheological properties of batter and dough were not studied. In the production of bakery products such as cookies and sponge cakes, the analysis of the rheological properties of the dough and batter is very important because it directly affects the quality of the final product and gives information about the dough structure. Hence, the aim of this study was to investigate the effects of using honey or pekmez as substitutes for refined sugar in cookies and sponge cakes on dough and batter rheology.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Powdered sugar, honey, grape pekmez, egg white powder, skimmed milk powder, ammonium bicarbonate, sodium bicarbonate, salt, and baking powder used in the production of cakes and cookies were obtained from local markets. Pastry margarine (Alba) used in the production of cakes and cookies were obtained from Unipro (Turkey). The multi-purpose additive-free flour used in the formulations was provided from Eksun Flour Factory (Eksun Food and Agriculture Industry Inc., Turkey).

### 2.2. Sponge cake and cookie production

Samples containing pekmez at percentages of 100 %, 80% and 60% and honey at percentages of 100 %, 80% and 60% as sugar substitutes in the batter and dough formulations were prepared. Samples of sponge cake and cookies were produced using saccharose as the sole sugar source to obtain control samples. Sponge cake production was carried out according to AACC (American Association of Cereal Chemists) Method 10.90.01 with slight modifications (Kazancı, 2021) as given in Table 1. The water content given in the AACC (10.90.01) batter formulation was modified by taking the water content of the sugar source (namely honey and pekmez) into account. The sugar and/or honey or pekmez in the formulation were first mixed with water, and this was called the aqueous sugar solution. After mixing all dry ingredients except oil in a bowl, 60% of the oil and remaining aqueous sugar solution was added. It was mixed at low speed for 30 seconds and then at medium speed for 4 minutes using a mixer (KitchenAid, USA). Then, half of the remaining aqueous sugar solution was added and mixed for 30 seconds at low speed, then mixed for 2 more minutes at medium speed, then all the remaining solution was added and mixed again for 2 minutes after 30 seconds at low speed to obtain a homogeneous mixture. For each cake, 150 g of cake batter was put into cake molds and baked in an industrial oven (Sveba Dahlen, Sweden) at 170°C for 30 minutes. The baked cakes were removed from the oven and cooled at room temperature for 1 hour.

**Table 1.** Formulations used in sponge cake production

| Ingredients (g) | Control | %100 pekmez | %80 pekmez | %60 pekmez | %100 honey | %80 honey | %60 honey |
|---|---|---|---|---|---|---|---|
| Code | K | A | B | C | D | E | F |
| Wheat flour | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Powdered sugar | 250 | - | 50 | 100 | - | 50 | 100 |
| Pekmez | - | 357 | 286 | 214 | - | - | - |
| Honey | - | - | - | - | 305 | 244 | 183 |
| Egg white powder | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| Nonfat dry milk | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| Shortening | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Salt | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Baking powder | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Distilled water* | 250 | 143 | 164 | 186 | 195 | 206 | 217 |

**The amount of water in all formulations was adjusted by taking the water content of the sugar source (namely honey and pekmez) into account.*

Cookie production was carried out according to AACC Method 10.54.01 with slight modifications (Kazancı, 2021) as given in Table 2. The water content given in the AACC (10.54.01) formulation was modified by taking the water content of the sugar source (namely honey and pekmez) into account. After mixing the margarine, salt, and milk powder in the mixer (KitchenAid, USA) at medium speed for 3 minutes, ammonium bicarbonate, sodium bicarbonate, and aqueous sugar solution were mixed in a separate bowl and added to the mixture and then mixed for 1 more minute. Finally, flour was added to this mixture, and the dough was kneaded by hand for 5 minutes. The dough was divided into equal pieces of 45 g and each piece was placed into a special mold having a diameter of 8 cm in order to obtain identical cookie discs. Cookies were baked in the oven (Sveba Dahlen, Sweden) at 205 °C for 20 minutes. The baked cookies were removed from the oven and cooled at room temperature for 1 hour.

**Table 2.** Formulations used in cookie production

| Ingredients (g) | Control | %100 pekmez | %80 pekmez | %60 pekmez | %100 honey | %80 honey | %60 honey |
|---|---|---|---|---|---|---|---|
| Code | N | G | H | I | J | L | M |
| Wheat flour | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Powdered sugar | 43.5 | - | 8.7 | 17.4 | - | 8.7 | 17.4 |
| Pekmez | - | 62.1 | 49.7 | 37.3 | - | - | - |
| Honey | - | - | - | - | 53.0 | 42.4 | 31.9 |
| Margarin | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| Nonfat dry milk | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Ammonium bicarbonate | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Sodium bicarbonate | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Salt | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| Distilled water* | 22 | 3.4 | 7.1 | 10.81 | 12.46 | 14.4 | 16.3 |

**The amount of water in all formulations was adjusted by taking the water content of the sugar source (namely honey and pekmez) into account.*

### 2.3. Rheological Analysis of Batter and Cookie Dough Samples

The rheological properties of the batter samples were determined using Discovery Hybrid Rheometer-2 (TA Instruments, DE, USA). 40 mm standard peltier parallel-plate configuration was used as the geometry. The measurements were conducted at a 1000 µm gap distance at 25 °C. Flow curves were obtained under steady shear conditions, in which the shear rate ranged from 0.1/s to 100/s in 2 min. The data of shear rate–viscosity and shear rate–shear stress were recorded throughout the experiment (Uysal et al., 2019). The data were fitted to Power Law rheological model to determine the flow behavior characteristics as shown in Equation 1, where, $\tau$, K, $\gamma$ and n represent shear stress (Pa), consistency index (Pa.s$^n$), shear rate (s$^{-1}$), and flow behavior index, respectively.

$$\tau=K\cdot\gamma^{n} \quad (1)$$

Model parameters and $R^2$ values were determined by the TA instrument software (TA Instruments, DE, USA). The consistency index and flow index values of cake batters were determined by using the model equations.

Oscillation tests were performed for the measurement of the structure of cookie dough without deformation (Kurt and Gençcelep, 2018). Within the linear viscoelastic range, the frequency sweep test was performed within a frequency range of 0.1 and 100 rad/s at 25°C. The storage modulus (G') and loss modulus (G") values were recorded and plotted as a function of angular frequency.

All analyses were performed in duplicate and two parallels, and all the results were given as mean ± standard error. Analysis of variance (One-way-ANOVA) was applied to determine whether there was a significant difference among the means. The significance of the differences among the means was tested at the $p<0.05$ level. Significant sources of variation were subjected to the Duncan test. Statistical analyses were performed using SPSS 17.0 (SPSS Inc. Chicago, IL).

## 3. RESULTS and DISCUSSION

### 3.1 Rheological Properties of Batter

Batter viscosity is a very important parameter for the quality and especially for the volume of sponge cakes. Batters with very low viscosity cannot hold air bubbles in their matrix and products such as sponge cakes collapse in the oven, whereas with the batters having high viscosity it is possible to obtain a porous cake structure that does not collapse during baking (Tsatsaragkou et al., 2021).

The analysis of the rheological properties of the batter samples is important in terms of giving information about the sponge cake quality. The flow behavior of batter samples was analyzed by varying the shear stress depending on the shear rate. And the apparent viscosity values were plotted as a shear rate function, as seen in Figure 1.

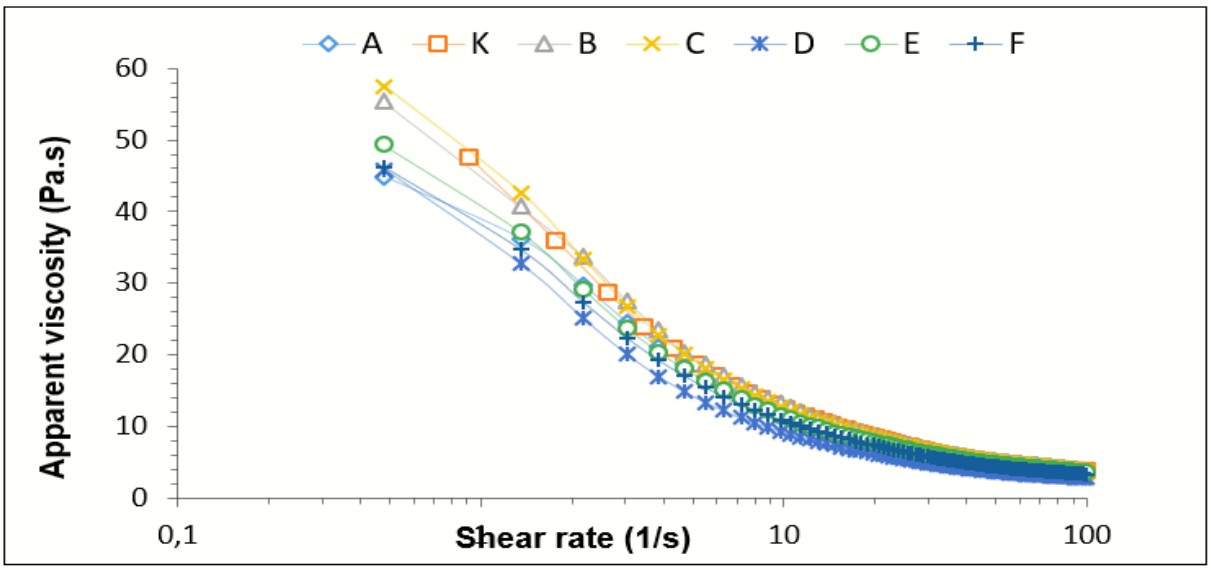


**Figure 1.** Flow curve of batter samples (Lines represent Power-Law model)

Figure 1 shows, the apparent viscosity of batter samples containing honey (D, F and E) and the sample having pekmez in highest concentrations (A) were lower than the control sample (K) and the samples B and C (samples containing pekmez at percentages of 80% and 60 %, respectively). The decrease in apparent viscosity with increasing shear rate is expressed as shear thinning behavior (pseudo-plastic shear thinning behavior). For batter samples in the present study, viscosity decreases with increasing shear rate. This indicates that the samples show less resistance to flow. The results obtained are consistent with the results obtained in other studies with batter samples (Bilgiçli and Akbulut, 2010; Tsatsaragkou et al., 2021; Turabi et al., 2008; Uysal et al., 2019).

**Table 3.** Flow behavior characteristics of batter samples

| Sample | K (Pa.s$^n$) | n | $R^2$ |
|---|---|---|---|
| K (control) | 38.71±1.9a | 0.48±0.02b | 0.99 |
| A | 36.84±1.03a | 0.49±0.00a | 0.99 |
| B | 39.16±0.81a | 0.49±0.00a | 0.99 |
| C | 37.93±0.08a | 0.49±0.00a | 0.99 |
| D | 27.1±0.68c | 0.5±0.01a | 0.99 |
| E | 32.12±2.58b | 0.50±0.00a | 0.99 |
| F | 31.81±0.77b | 0.5±0.00a | 0.99 |

*Values are given as mean ± standard error Mean values in the same row not followed by a common letter differ significantly (P < 0.05). 'K' is the consistency index and 'n' is the flow behavior index.*

The data fits well with the Power Law model having high $R^2$ values ($R^2$>0.99). The flow behavior characteristics calculated using the model were given in Table 3. The flow behavior index gives information whether the flow is shear thinning or shear thickening (Sakıyan et al., 2004). It is known that according to n value, the fluids can be characterized as pseudoplastic (shear thinning, n< 1), dilatant (shear thickening, n>1) or Newtonian (n=1). The n values were determined to be lower than 1 (n< 1), indicating the pseudoplastic flow behavior of the batter samples. In the study of Psimouli and Oreopoulou (2011), the effects of mannitol, maltitol, sorbitol, lactitol, fructose, oligofructose and polydextrose instead of sucrose in cake formulation on starch gelatinization and dough rheology and physical and sensory properties of cake were investigated and all samples were determined to show pseudoplastic flow behavior. Bilgiçli and Akbulut (2010) also reported that batter samples showed a pseudoplastic flow behavior when different types of pekmez were added to the cake formulations.

It can be stated that all batter samples showed non-Newtonian flow behavior since the consistency depends on the shear rate and the n values were below 1. Non-Newtonian flow behavior is a fluid in which the applied shear stress and shear rate do not change linearly. Moreover consistency index (K) values of the A, B and C batter samples did not differ from the control sample (K) significantly (P>0.05), although there is a significant difference between the K and the D, E and F samples (P<0.05). The consistency index ranged from 27.1 to 39.16 Pa.s$^n$ with batters containing pekmez and honey. Batters with honey samples showed less resistance to flow (low K) and the addition of pekmez and honey to the batter showed less shear rate dependent behavior (high n) compared to the other samples. Similar results were reported in a study investigating the rheological properties of the use of inulin in dough to reduce sugar in cake and cookie products (Tsatsaragkou et al., 2021).

### 3.2 Rheological Properties of Cookie Dough

The elastic modulus, known as the storage modulus (G'), is a measure of the structure of matter. The viscosity modulus, also known as the loss modulus (G''), is a measure of the flow properties of the material (Kasapis and Bannikova, 2017; Kor-Ulukut, 2010). According to a study, higher storage and loss module values prevented the development of the cake structure and led to a lower volume (Gómez et al., 2010). In the cookie dough samples, the frequency sweep tests were carried out in the linear viscoelastic range, in the frequency range of 0.1 and 100 rad/s at 25°C. The storage (G') and loss (G'') modulus values of the samples are given in Figures 2 and 3 depending on the frequency (rad/s).

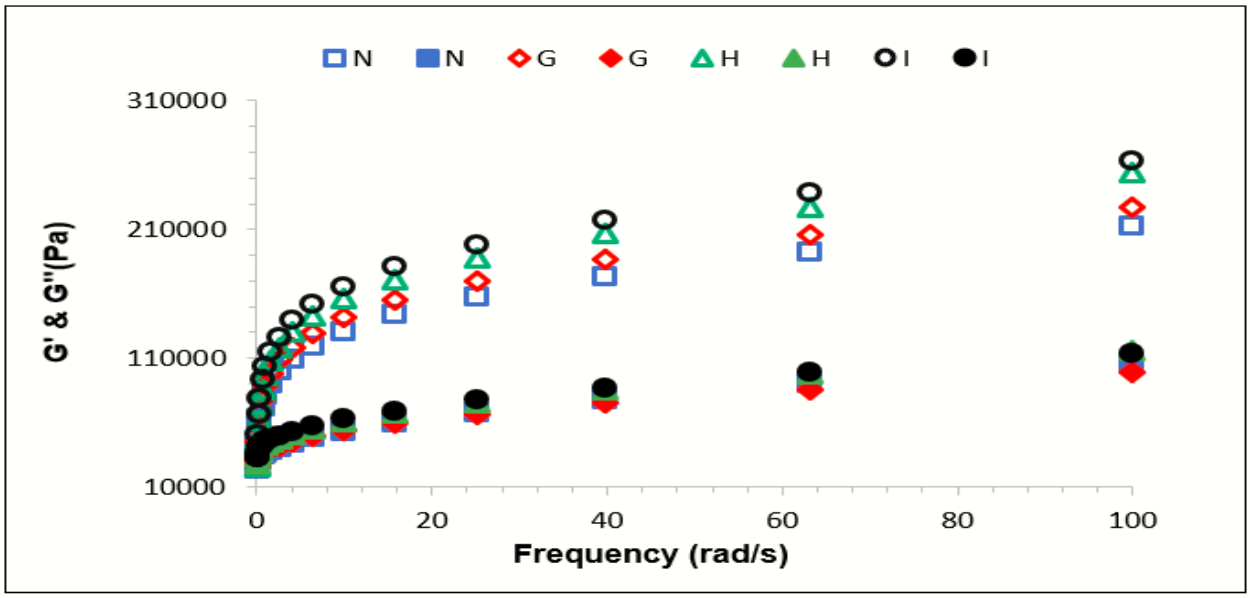


**Figure 2.** Change of Storage (G'), and Loss (G") modulus of the pekmez cookie dough samples with frequency (*Empty symbols represent storage modulus (G') and full symbols represent loss modulus (G") values).*

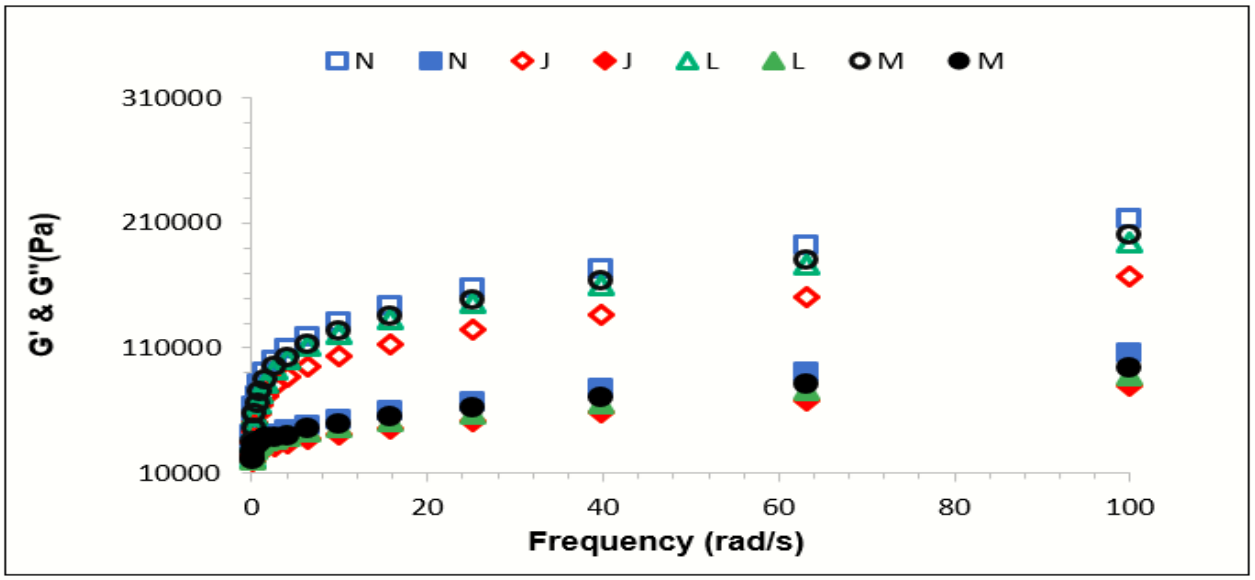


**Figure 3.** Change of Storage (G'), and Loss (G") modulus of the honey cookie dough samples with frequency (*Empty symbols represent storage modulus (G') and full symbols represent loss modulus (G") values).*

Figure 2 and 3 represent the storage modulus (G') and loss modulus (G") of the seven different doughs. For all samples, G' was higher than G" over the frequency range, thus explaining that all of them had a rather elastic-like behavior. The fact that the G' values of the pekmez containing cookie dough samples I, H and G were higher than the G' values of the control sample (N) indicate that these cookie dough samples have better viscoelastic properties than the control sample (Figure 2). Gluten development contributes to dough elasticity, so it can be said that in the dough samples containing pekmez, gluten development is better than the control sample containing sugar (Tsatsaragkou et al., 2021). It is seen that the G' and G" values of cookie dough samples containing honey are lower than the control group. This shows that the viscoelastic property of honey products are lower than the control group (Figure 3). It was determined that the storage modulus (G') values decreased as the amount of honey increased in the cookie dough. As the frequency increased, the storage and loss modulus values of the samples increased. Sugar affects the spread of the cookie during baking. With increasing dough temperature, fat and sugar melts. This leads to the release of water and reduces the viscosity of the dough, causing it to spread. The degree of spread of the cookie is positively related to the amount of added sugar. The highest G" value was obtained by adding 60% pekmez to the cookie formulation which is I (Figure 2). This indicates that the degree of spread of the cookie will be lower while it is being baked (Sahin et al., 2019).

## 4. CONCLUSION

There are many factors affecting dough rheology. The analysis of the rheological properties of the dough is very important because it directly affects the quality of bakery products such as sponge cakes and cookies, and also gives information about the dough structure. Sucrose affects flavor, size, color and texture of the product to which it is added as well as the rheology of the dough. For this reason, it is important to investigate the rheological properties of bakery products to be produced by adding different ingredients instead of sucrose. A statistically significant difference was observed between the control (K) and honey batter samples (D, E and F) ($P<0.05$). All batter samples showed pseudoplastic flow behavior. Storage and Loss values of honey cookie dough samples (J, L, and M) were determined to be lower than the control group (N).

## ACKNOWLEDGEMENTS

The authors are thankful to the R&D officials of Eksim R&D Holding Company, Turkey.

**Funding:** This study was supported by the Research Fund of Tekirdağ Namık Kemal University. Project number: NKUBAP.03.GA.20.234.

## REFERENCES

AACC. 2000. Approved methods of the American Association of Cereal Chemists. (10th ed.) Methods 44-15A, 02-52, 76-21, 10-90, 10-91, 10– 05 St. Paul, MN.

AACC. 2000. American Association of Cereal Chemists, Approved Methods of the AACC, 10th Ed., The Association, St. Paul, MN.

Aghamohammadi B. Ghıassı T. B. Honarvar M. Delkhosh B. 2012. The Effects of Using Molasses as a Replacement for Sugar on Dough Properties and Volume and Color of Shortened Cake. Journal of Food Science and Technology , 4(2): 37–45.

Ahmed J. Ptaszek P, Basu S. 2017. Food Rheology: scientific development and importance to food industry. in advances in food rheology and its applications. Elsevier Ltd. 1-4.

Akbulut M. Bilgicli N. 2010. Effects of different pekmez (fruit molasses) types used as a natural sugar source on the batter rheology and physical properties of cakes. Journal of food process engineering, 33(2): 272-286.

Bilgiçli N. Akbulut M. 2009. Effects of Different Pekmez (fruit molasses) Types on Chemical, Nutritional Content and Storage Stability of Cake. Journal of Food Quality, 32(1): 96–107.

Bornare D. T. Ajaz Khan K. S. 2015. Physical and sensory evaluation of cookies incorporated with oats and honey. International Journal of Engineering Research And, V4(08): 407–411.

Demir M. K. Kılınç, M. 2019. Effect of honey powder substitution on cake quality. Necmettin Erbakan University Journal of Science and Engineering, 1(1): 53–58.

Ertaş N. Çoklar H. 2008. The Effect of Different Types of Pekmez as Natural Source of Sugar on Cake Dough and Cake Characteristics. Selçuk Üniversitesi Ziraat Fakültesi Dergisi, 22(46): 51–54.

Gallagher E. O'Brien C. Scannell A. Arendt E. 2003. Evaluation of sugar replacers in short dough biscuit production. J. Food Eng. 56: 261–263.

Gómez M. Moraleja A. Oliete B. Ruiz E. Caballero P. A. 2010. Effect of fibre size on the quality of fibre-enriched layer cakes. LWT-Food Science and Technology, 43(1): 33-38.

Gunderson K. Coate K. Terry P. 2018. The effects of replacing granulated sugar with locally farmed honey on the physical, nutritional and sensory characteristics of sugar cookies. Journal of the Academy of Nutrition and Dietetics, 118(9): A94.

Kasapis S. Bannikova A. 2017. Rheology and food microstructure. advances in food rheology and its applications:7-46.

Kazancı M. 2021. Formülasyonunda şeker yerine pekmez ve bal kullanılarak üretilen bazı fırıncılık ürünlerinde akrilamid oluşumunun araştırılması. Yüksek Lisans Tezi. Tekirdağ Namık Kemal Üniversitesi, Türkiye

Kor-Ulukut A. G. 2010. Çezerlik mısır hamurunda nohut unu, yerfıstığı unu ve kırmızıbiber tohum unu eklenmesi sonucu ısıl ve reolojik özelliklerinde meydana gelen değişimin incelenmesi. Yüksek Lisans Tezi. İstanbul Teknik Üniversitesi, Türkiye.

Kurt A. Gençcelep H. 2018. Enrichment of meat emulsion with mushroom (Agaricus bisporus) powder: Impact on rheological and structural characteristics. Journal of Food Engineering, 237: 128-136.

Psimouli V. Oreopoulou V. 2012. The effect of alternative sweeteners on batter rheology and cake properties. Journal of the Science of Food and Agriculture, 92(1): 99-105.

Renzetti S. van den Hoek I. A. van der Sman R. G. 2021. Mechanisms controlling wheat starch gelatinization and pasting behaviour in presence of sugars and sugar replacers: Role of hydrogen bonding and plasticizer molar volume. Food Hydrocolloids, 119: 106880.

Renzetti S. van der Sman R. G. 2022. Food texture design in sugar reduced cakes: Predicting batters rheology and physical properties of cakes from physicochemical principles. Food Hydrocolloids, 131: 107795.

Sahin A. W. Zannini E. Coffey A. Arendt E. K. 2019. Sugar reduction in bakery products: Current strategies and sourdough technology as a potential novel approach. Food Research International, 126, 108583.

Sakiyan O. Sumnu G. Sahin S. Bayram G. 2004. Influence of fat content and emulsifier type on the rheological properties of cake batter. European Food Research and Technology, 219(6): 635-638.

Tsatsaragkou K. Methven L. Chatzifragkou A. Rodriguez-Garcia J. 2021. The functionality of inulin as a sugar replacer in cakes and biscuits; highlighting the influence of differences in degree of polymerisation on the properties of cake batter and product. Foods, 10(5): 951.

Turabi E. Sumnu G. Sahin S. 2008. Rheological properties and quality of rice cakes formulated with different gums and an emulsifier blend. Food Hydrocolloids, 22(2): 305–312.

Uysal R. S. Sumnu G. Boyaci I. H. 2019. Effects of heat-treated liquid whole egg on cake batter rheology and the quality of baked cake. Journal Food Process Engineering, 42:e12977.

*ORAL PRESENTATION*

# Trabzon Akçaabat Yöresi Fındık Bahçesi Topraklarının Temel Özellikleri ve Bazı Besin Element İçeriklerinin Belirlenmesi

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## Özet

Bu araştırma Trabzon İli Akçaabat İlçesinde yetiştirilen fındık bahçeleri topraklarının temel özellikleri ve bazı bitki besin elementi içeriklerini ortaya koymak amacıyla yapılmıştır. Bu amaçla 19 bahçeden, 0-30 cm derinlikten toprak örnekleri alınarak analiz edilmiştir. Toprak örneklerinde; pH, kireç, elektriksel iletkenlik (EC), organik madde (OM), alınabilir fosfor (P); alınabilir potasyum (K), kalsiyum (Ca), magnezyum (Mg) demir (Fe), bakır (Cu), mangan (Mn) ve çinko (Zn) analizleri yapılmıştır. Toprak örneklerinin pH, EC ve kireç içerikleri sırasıyla 5.5 ile 7.4, 72 ile 613 µS $cm^{-1}$ ve % 0.42 ile %4.95 arasında değişmiştir. Yine incelenen toprakların sırasıyla OM, alınabilir P, K, Ca ve Mg içeriklerinin % 0.34 ile 3.76, 7.27 ile 98.34 mg $Kg^{-1}$, 41.5 ile 1294.5 mg $Kg^{-1}$, 6700 ile 20200 mg $Kg^{-1}$ arasında olduğu belirlenmiştir. Topraklardaki alınabilir mikro elementlerden Fe, Zn, Mn ve Cu içerikleri ise sırasıyla 2.75 ile 95.85 mg $Kg^{-1}$, 0.44 ile 2.95 mg $Kg^{-1}$, 4.37 ile 62.60 mg $Kg^{-1}$, 0.68 ile 4.70 mg $Kg^{-1}$arasında değiştiği belirlenmiştir. Araştırma bulguları, besin elementlerinin çoğunun bitkiler için yeterli ve fazla düzeyde olduğunu ortaya koymuştur.

**Anahtar sözcükler:** Fındık, toprak, makro elementler, mikro elementler

# Determination of basic properties and some nutrient content of hazelnut orchard soils in Trabzon Akçaabat Region

## Abstract

This research was carried out to reveal the basic properties and some plant nutrient content of the soils of hazelnut orchards grown in Trabzon Akçaabat District. For this purpose, soil samples were taken from 0-30 cm depth from 19 orchards and analyzed. In soil samples; pH, lime, electrical conductivity (EC), organic matter (OM), available phosphorus (P); Available potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) analyzes were made. The pH, EC and lime contents of the soil samples varied between 5.5 and 7.4, 72 and 613 µS $cm^{-1}$, and 0.42% and 4.95%, respectively. Again, it was determined that the OM, available P, K, Ca and Mg contents of the investigated soils were between 0.34% and 3.76, 7.27 and 98.34 mg $Kg^{-1}$, 41.5 and 1294.5 mg $Kg^{-1}$, 6700 and 20200 mg $Kg^{-1}$, respectively. Fe, Zn, Mn and Cu contents of the available microelements in the soils were determined to vary between 2.75 and 95.85 mg $Kg^{-1}$, 0.44 and 2.95 mg $Kg^{-1}$, 4.37 and 62.60 mg $Kg^{-1}$, 0.68 and 4.70 mg $Kg^{-1}$, respectively. Research findings have revealed that most of the nutrients are sufficient or in excess for plants.

**Key words:** Hazelnut, soil, macro elements, micro elements

## GİRİŞ

Fındık, Türkiye ekonomisi ve Karadeniz bölgesi için büyük önem taşımakta ve yaklaşık 2,5 milyon üreticinin geçim kaynağını oluşturmaktadır. Fındık geleneksel ihraç ürünlerimiz içerisinde tütün ve pamuktan sonra 3. sırada yer almaktadır. Türkiye, gerek üretim alanı ve gerekse üretim miktarı bakımından dünya fındık üreticisi ülkelerin başında gelmektedir. Bununla birlikte dekara verimin en az olduğu ülke konumundadır (Genç, 1976). Fındık nemli, ılıman iklim bitkisi olup, toprak istekleri açısından seçici değildir. Bununla birlikte besin

maddeleri ve humusça zengin, tın bünyeli topraklarda iyi gelişim göstermekte, ağır ve fazla su tutan, taban suyu seviyesi yüksek topraklarda gelişimi gerilemekte ve az meyve vermektedir.

Verim düşüklüğünün sayılabilecek birçok nedeni arasında topoğrafik yapının düzensizliği, işlenebilir toprak derinliğinin azlığı, toprak verimliliğinin düşüklüğü, kültürel uygulamaların yeterince yapılmaması, gübrelemeye yeterince yer verilmemesi veya bilinçli bir şekilde yapılmaması ilk akla gelenlerdir.
Fındık bitkisinin beslenmesi ve gübrelenmesi ile ilgili çalışmaların son yıllarda yoğunlaştığı izlenmektedir. Bu bağlamda fındığın gübrelenmesinin verim ve kaliteye olan önemli etkileri dikkate alınarak ülkemizde ve yurt dışında çok sayıda çalışmalar yapılmıştır (Painter, 1963; Ayfer, 1967; Stebbins, 1969; Genç, 1971;Türüdü, 1971; Fregoni ve Zioni, 1972; Kowalenko, 1984).
Fındık bitkisi 1200 kg/ha ürün ile topraktan 19 kg N/ha, 9 kg $P_2O_5$/ha, 12 kg $K_2O$/ha ve 16 kg CaO/ha kaldırmaktadır. Bu bağlamda fındığın gübrelenmesinde N'lu ve K'lu gübreleme büyük önem taşımaktadır (Genç, 1976).
Carlone (1968), fındığın N ve K gereksiniminin yüksek, P gereksiniminin ise oransal olarak düşük olduğunu ve gübrelemede N, $P_2O_5$, $K_2O$ oranının 1:0.5:1.0 olması gerektiğini belirtmektedir.
Painter ve Hammer (1962) N, P, K Mg ve B ile gübrelemenin fındıkta; verim, gelişme ve yaprakların makro ve mikro besin elementi konsantrasyonuna etkilerini 6 yıl süren bir tarla denemesi ile incelemişlerdir. Araştırıcılar verim, sürgün gelişimi ve yaprakların N kapsamı üzerine azotlu gübrenin büyük etkisinin olduğunu, K ve Mg'lu gübrelemenin yaprak K ve Mg kapsamını artırdığını, P'lu gübrelemenin ise yaprakların P kapsamına bir etkisinin bulunmadığını belirlemişlerdir.
Akçaabat yöresi 9150 hektar fındık dikim alanı ve 3660 ton üretim miktarı ile bölgenin önemli fındık üretim merkezlerinden biridir (Anonim, 1995). Bu bölgede diğer kültürel ve teknik işlemlerin yanında, gübrelemenin de etkin ve bilimsel olarak yapılmadığı izlenmektedir. Bu çalışma bölgenin potansiyeli dikkate alınarak, gelecekte yapılacak gübreleme programlarına yön vermesi açısından fındık bahçelerinin temel toprak özelliklerinin ve toprakların verimlilik düzeyini ortaya koymak amacı ile yapılmıştır.

## MATERYAL ve YÖNTEM

### Materyal

Araştırma materyalini Akçaabat yöresi ve çevresinde fındık yetiştiriciliğinin yoğun şekilde yapıldığı yerleri temsilen seçilen 19 bahçeden ve 0-30 cm derinlikten alınan toprak örnekleri oluşturmaktadır. Toprak örneklerinin alındığı yerler Tablo 1'de verilmiştir. Örneklerin alındığı bahçelerin büyük çoğunluğunda Giresun Tombul Fındık çeşidi (*Corylus maxima* L.) yetiştirilmektedir. Bu fındık çeşidi iri meyveli olup, varyeteleri Lambert veya Kan fındığı adı altında yetiştirilmektedir (Özbek, 1981).

### Yöntem

Toprak örneklerinde pH, elektriksel iletkenlik (EC), organik madde (OM), kireç, değişebilir katyonlardan potasyum (K), bitkilerce alınabilir fosfor (P) ve mikro element (Fe, Cu, Mn, Zn) analizleri yapılmıştır. Topraklarda pH, saturasyon çamurunda pH-metre (Jackson 1958), toplam çözünebilir tuzlar ise saturasyon ekstrakıtının elektriksel iletkenlik değerlerinden belirlenmiştir (Richards, 1954). Kireç tayini, Scheibler kalsimetre (Allison ve Moode, 1965), organik madde tayini ise Walkey Black yaş yakma yöntemine (Allison, 1960) göre yapılmıştır. Alınabilir K, Ca ve Mg içerikleri 1N amonyum asetat ekstraksiyon yöntemi ile (Richards, 1954), alınabilir P miktarları ise Olsen ve ark. (1954)'na göre, belirlenmiştir. Toprakların alınabilir demir (Fe), bakır (Cu), mangan (Mm) ve çinko (Zn) içeriklerinin belirlenmesinde DTPA-TEA exstraksiyon yöntemi (Lindsay ve Norwell 1978) kullanılmıştır.

Tablo 1. Toprak örneklerinin alındıkları yerler

| Örnek no | Köy | Örnek No | Köy |
|---|---|---|---|
| 1 | Kaleönü | 11 | Kaleönü |
| 2 | Kaleönü | 12 | Kaleönü |
| 3 | Dürbinar | 13 | Kavaklı |
| 4 | Kaleönü | 14 | Gümüşlü |
| 5 | Kaleönü | 15 | Gümüşlü |
| 6 | Özdemirci | 16 | Cevizli |
| 7 | Kavaklı | 17 | Kaleönü |
| 8 | Cevizli | 18 | Kirazlık |
| 9 | Gümüşlü | 19 | Kaleönü |
| 10 | Ortamahalle | | |

Tüm parametrelerinin tanımlayıcı istatistiksel analizleri (en küçük, en büyük ve ortalama değer, standart sapma) Windos uyumlu SPSS (sürüm 21) istatistik programı ile yapılmıştır. Varyasyon katsayısı ise standart sapma ve ortalama değerlerden hesaplanmıştır. Ayrıca toprak özellikleri ve besin elementlerinin içerikleri, sınır değerleri ile karşılaştırılarak değerlendirilmiştir.

## BULGULAR VE TARTIŞMA

Toprak özellikleri ve besin elementi içeriklerine ait tanımlayıcı istatistik analiz sonuçları Tablo 2'de verilmiştir. Toprak özelliklerinin varyasyon katsayıları incelendiğinde, en düşük varyasyon katsayısının (VK) pH (% 8), en yüksek varyasyon katsayısının ise kireç değerlerinde (% 103.1) olduğu görülmektedir (Tablo 2). pH değerlerinde varyasyon katsayısının düşük olması, pH değerlerinin çalışma alanında çok homojen dağıldığını göstermektedir. Toprak özelliklerinin varyasyon katsayıları arttıkça, çalışma alanındaki dağılımlarının homojenliği azalmaktadır. Zhou ve ark. (2011)'e göre varyasyon katsayısı %10'dan düşük ise değişkenlik düzeyi de düşük, %10-100 arasında ise değişkenlik düzeyi orta ve %100'den büyük ise değişkenlik düzeyi yüksektir. Bu kıstaslara göre, pH değerlerinin dağılımı homojen, kireç içeriklerinin dağılımı içe çok heterojendir. Diğer parametrelerin homojenlik düzeylerinin ise orta olduğu görülmektedir.

Toprak örneklerinin pH değerleri 5.5 ile 7.4 arasında değişmekte olup ortalama 6.5 olarak belirlenmiştir (Tablo 2). Ülgen ve Yurtsever (1995)'e göre, toprak örneklerinin 11 tanesi nötr (6.6-7.5), 7 tanesi hafif asit (5.6 – 6.5), 1 tanesi ise orta kuvvetli asit (4.6-5.5) reaksiyonludur. Bu sonuçlar toprakların genelde nötr, az bir kısmında da asidik reaksiyona sahip olduğunu göstermektedir. Fındığın hafif asit reaksiyonlu topraklarda daha iyi yetiştiği, kuvvetli asit ve bazik reasiyonlu toprakları tercih etmediği ve gerektiğinde pH'yı istenilen seviyeye getirmek için kireçlemenin yapılması gerektiği belirtilmektedir. (Genç ve Sarıhan,1976; Özbek, 1981). Topraklarda tuz içeriğinin bir göstergesi olan EC değerlerinin 72-411 µS/cm arasında değişmekte olup tamamı 4000 µS/cm'den daha düşüktür. Richards (1954)' e göre, bu durum toprakların tamamında tuzluluk sorunu bulunmamaktadır.

Tablo 2. Tanımlayıcı istatistik analiz sonuçları (n = 19)

| Parametre/ istatistik | Birim | En küçük | En yüksek | Ortalama | Standart sapma | Varyasyon katsayısı % |
|---|---|---|---|---|---|---|
| pH | | 5.5 | 7.4 | 6.5 | 0.52 | 8.0 |
| EC | µS $cm^{-1}$ | 72.0 | 613.0 | 239.6 | 161.6 | 67.4 |
| Kireç | % | 0.42 | 4.95 | 0.96 | 0.99 | 103.1 |
| Organik madde | % | 0.34 | 3.76 | 1.94 | 0.86 | 44.3 |
| K | mg $Kg^{-1}$ | 41.5 | 1294.5 | 397.3 | 384.7 | 96.8 |
| Ca | mg $Kg^{-1}$ | 6700 | 20200.0 | 10996.8 | 3233.6 | 29.4 |
| Mg | mg $Kg^{-1}$ | 104.8 | 2056.2 | 1078.6 | 515.3 | 47.8 |
| P | mg $Kg^{-1}$ | 7.27 | 98.34 | 39.12 | 23.41 | 59.8 |
| Fe | mg $Kg^{-1}$ | 2.75 | 95.85 | 45.85 | 29.04 | 63.34 |
| Cu | mg $Kg^{-1}$ | 0.68 | 4.70 | 2.59 | 1.02 | 39.38 |
| Mn | mg $Kg^{-1}$ | 4.37 | 62.60 | 27.41 | 12.91 | 47.10 |
| Zn | mg $Kg^{-1}$ | 0.44 | 2.95 | 2.17 | 0.64 | 29.5 |

Toprak örneklerinde kireç içeriği % 0.42 ile 4.95 arasında değişmiştir. Ortalama kireç içeriği ise % 0.96 olarak belirlenmiştir. Toprakların hemem hemen tamamının (17 örnekte) az kireçli (<%1) olduğu belirlenmiştir (Ülgen ve Yurtsever, 1995). Carleone (1968), fındık yetiştirilen kireçsiz topraklara, toprak pH'sı dikkate alınarak aralıklı olarak 500 kg kireç verilmesini önermektedir. İncelenen toprakların organik madde içerikleri % 0.34 - 3.76 arasında değişmektedir. Ortalama organik madde içeriği ise % 1.94 olarak belirlenmiştir (Tablo 2). Ülgen ve Yurtsever (1995)'e göre, toprakların 2 tanesi çok az, 9 tanesi az, 6 tanesi orta ve sadece 2 tanesi yeterli organik madde içeriğine sahiptir. Örneklerin çoğunda organik medde içiriğinin düşük olması, topraklara organik madde uygulaması gerekebileceğini ortaya koymaktadır.

Toprak örneklerinin alınabilir fosfor içerikleri 7.27 ile 98.34 mg $Kg^{-1}$ arasında değişmektedir. Ortalama P içeriği ise 39.12 mg $Kg^{-1}$ olarak hesaplanmıştır. Olsen ve ark. (1954)'e göre, toprakların fosfor (P) içerikleri 1 örnekte az (2.5-8.0 mg $Kg^{-1}$), 5 örnekte yeterli (8.0-25 mg $Kg^{-1}$), 12 örnekte fazla (25.0-80.0 mg $Kg^{-1}$) ve 1 örnekte çok fazla(>80 mg $Kg^{-1}$) olarak değerlendirilmiştir. Bu sonuçlar sadece bir örnekte P içeriğinin düşük olduğunu göstermektedir. Diğer örneklerde ise yeterli veya fazladır.

Toprakların alınabilir potasyum değerleri 41.5 ile 1294.5 mg $Kg^{-1}$ arasında değişmekte olup, ortalama değer ise 397.3 mg $Kg^{-1}$ olarak hesaplanmıştır. FAO (1990)'a göre, alınabilir potasyum 1 örnekte çok az (< 50 mg $Kg^{-1}$), 4 örnekte az (50-140 mg $Kg^{-1}$) , 7 örnekte yeterli (140-370 mg $Kg^{-1}$) , 4 örnekte fazla (370-1000 mg $Kg^{-1}$), 3 örnekte ise çok fazladır (>1000 mg $Kg^{-1}$). Bu sonuçlar toprakların % 26'sında yetersiz düzeyde olduğunu göstermektedir. Toprak örneklerinin alınabilir kalsiyum konsantrasyonları 6700 ile 20200 mg $Kg^{-1}$ arasında değişmiştir. Ortalama Ca içeriği ise 10996.8 mg $Kg^{-1}$ olarak hesaplanmıştır. Bu sonuçları, bütün topraklarda alınabilir kalsiyum düzeyinin fazla (3500-10000 mg $Kg^{-1}$) ve çok fazla (>10000 mg $Kg^{-1}$) olduğunu göstermiştir (FAO,1990). Toprakların alınabilir magnezyum içerikleri 104.8 ile 2056.2 mg $Kg^{-1}$ arasında değişmektedir. Ortalama Mg içeriği ise 1078.6 mg $Kg^{-1}$ olarak hesaplanmıştır. Alınabilir Mg ile ilgi tüm sonuçlar incelendiğinde, FAO (1990)'a göre toprakların sadece % 5.3'ünde Mg'un yetersiz olduğu görülmüştür.

İncelenen topraklardaki alınabilir demir içerikleri 2.75 ile 95.85 mg $Kg^{-1}$ arasında belirlenmiş, ortalama Fe içeriği ise 45.85 mg $Kg^{-1}$ olarak hesaplanmıştır. Buna göre toprakların %95'inde alınabilir demir içerikleri iyi (>4.5 mg $Kg^{-1}$) durumda bulunmaktadır (Lindsay ve Norvell,1978).Toprakların alınabilir çinko içerikleri 0.44 ile 2.95 mg $Kg^{-1}$ arasında değişmekte olup ortalama Zn içerikleri 2.17 mg $Kg^{-1}$ olarak hesaplanmıştır. Buna göre alınan örneklerin % 10.53'ünde yetersiz (<0.6 mg $Kg^{-1}$ )bulunmuştur. Diğer topraklarda ise Fe yeterli veya fazla düzeylerdedir (Lindsay ve Norvell,1978). Toprakların alınabilir mangan içerikleri 4.37 ile 62.60 mg $Kg^{-1}$ arasında değişmektedir. Ortalama Mn içeriğ ise 27.41 mg $Kg^{-1}$. Toprakların tamamında Mn içerikleri yeterli (3.5-7.0 mg $Kg^{-1}$) veya yüksek (>7.0 mg $Kg^{-1}$) düzeydedir (Lindsay ve Norvell,1978).İncelenen toprakların alınabilir bakır içerikleri 0.68 ile 4.70 mg $Kg^{-1}$ arasında değişmiştir. Toprakların ortalama Cu içeriği ise 2.59 mg $Kg^{-1}$ olarak belirlenmiştir. Bu sonuçlar göre tüm toprakların alınabilir bakır içerikleri yeterli (0.4-0.8 mg $Kg^{-1}$) veya fazla (>1.6 mg $Kg^{-1}$) olduğunu göstermektedir (Lindsay ve Norvell,1978).

## SONUÇ

Yapılan toprak analizleri ile bu analizlerin ilgili referans değerleri karşılaştırılması; çoğu besin elementlerinin bitkiler için yeterli veya fazla düzeyde olduğunu ortaya koymuştur. Ancak az da olsa bazı bölgelerde P, K, Mg ve Fe eksikliğine rastlanmıştır. Bu yörelerde yetiştirilen fındık bahçelerinde bu besin elementlerinden verilmesi gereken miktarları saptamak için kalibrasyon denemelerinin kurulması yararlı görülmektedir. Bazı yöredeki fındık bahçelerinde toprak reaksiyonunun aşırı asit reaksiyon göstermesi, yüksek verim ve kalite açısından kireçlemenin gerekebileceğini, bu yönüyle de kalibrasyon denemelerinin kurulması gerekliliğini işaret etmektedir. Kireç içeriklerinin çok düşük olması da bu kanıyı güçlendirmektedir.

## TEŞEKKÜR

Bu çalışmayı destekleyen Hatay Mustafa Kemal Üniversitesi Bilimsel Araştırma Projeleri (BAP) koordinatörlüğüne teşekkür ederiz.

## KAYNAKLAR

Allison LE 1960. Wet combustion apparatus and procedure for organic and inorganic carbon in soil. Soil Science Society of American Journal 24:36–40.

Allison E, Moode CD 1965. Carbonate. (C.A. Black, Editör), Methods of Soil Analysis. Part 2. Agronomy Series. No. 9, ASA. 1379-1396, Wisconsin

Anonim 1995. Tarımsal yapı ve üretim 1995. Başbakanlık D.İ.E. yayınları, Yayın No: 2031, Ankara.

Ayfer M 1967. Fındık yetiştiriciliğimiz ve problemleri. A. Ü. Ziraat Fak. Meyve Yetiştirme ve Islahı Kürsüsü, Meyvecilik sempozyumu tebliği.

FAO 1990. Micronutrient, Assessment at the Country Level: An International Study. FAO Soil Bulletin by Sillanpaa. Rome.

Fergoni M, Zioni E 1972. The effect of increasing rates of fertilizer element on yield, fruit quality and foliar nutrition in hazelnut. Hort. Abst., 42(4): 7412.

Genç Ç 1976. Giresun tombul fındık çeşidinde gübrelemenin verim ve kaliteye etkisi üzerinde bir araştırma. Doktora tezi (basılmamış).

Genç Ç 1971. Gençlik devresi ticari gübre denemesi. Giresun Zirai Araştırma Enstitüsü. 1970-1971 yılı F/6 no'lu proje raporu.

Genç Ç, Sarıhan S 1976. Fındıkta dikimden önce bir defada verilen normal ve aşırı miktarlardaki kireç ve slam'ın fındığın verim ve kalitesine etkileri üzerinde bir araştırma. Gıda Tarım ve Hayvancılık Bakanlığı, Tarımsal Araştırma Genel Müdürlüğü. Proje No: 111 035-I-280. Giresun.

Jackson ML 1958. Soil chemical analyses. Prentice-Hall, Inc. Englwood Clifs, New Jersey. USA. 498 pp.

Kowalenko N V 1984. Derivation of nutrient requirements of filberts using orchard surveys. Canadion-Journal of-Soil-Science, 64 (1): 115-123.

Lindsay W L, Norwell WA 1978. Development of a DTPA soil test for Zinc, Iron, Manganese and Copper. Soil Sci. Soc. Amer. Jour., 42 (3).

Özbek N 1981. Meyve ağaçlarının gübrelenmesi. Tarım Bakanlığı Yayınları. S.244- 254. Ankara.

Painter J H 1963. A recent leaf analysis service development of importance nut growers in Oregon. Nut. Gro. Soc. of Oregon and Washington, 49: 6-8.

Painter J H, Hammer, HE1962. Effect of differential applications of nitrogen, K, Mg, B and P on their concentration in leaves of filbert trees.

Reuter DJ, Robinson JB 1986. Plant Analysis. An Interpretation Manual. Inkata Press. Melbourne Sydney. Proc. Amer. Soc. Hort. Sci., 80: 315-326.

Richards LA 1954. Diagnosis and improvement of saline and alkali soils. USDA Agriculture Handbook. No: 60.

Stebbins R L 969. The concept of plant analysis and how to take a leaf sample. OSV. Fr. 118. Revised.

Ülgen N, Yurtsever N 1995. Türkiye gübre ve gübreleme rehberi (4. Baskı). T.C. Başbakanlık Köy Hizmetleri Genel Müdürlüğü Toprak ve Gübre Araştırma Enstitüsü Müdürlüğü Yayınları, Genel Yayın, (209), 230.

Türüdü ÖA 1971. Grundlagen und anwendung der blattananlyse im- Haselnussanbau. Hanover Teknik Üniversitesi Doktor Tezi.

Zhou XH, Obuchowski NA, McClish D K, 2011. Statistical methods in diagnostic medicine, 2nd Edition. ISBN: 978-0-470-18314-4, 592 pp.

## *ORAL PRESENTATION*

# Network Based Integration of Multi-Omic Profiles to Purpose New Biomarker Candidates in Pancreatic Cancer

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## Abstract

Due to its late diagnosis, poor survival rates, and high incidence of metastasis, pancreatic cancer is one of the most fatal malignancies. Considering the uncertainties in its molecular mechanism, in the present study, we aimed to provide shed more light on the molecular regulatory signatures of circRNAs in PDAC progression and a different perspective to identify potential biomarkers for PDAC by network-based integrative analysis. PDAC expression profiles for the mRNA, miRNA, and circRNA were downloaded nine Gene Expression Omnibus microarray datasets. The statistical analysis of the microarray datasets established the differentially expressed mRNAs (DEmRNAs), miRNAs (DEmiRNAs), and circRNAs (DEcircRNAs). Utilizing a combination method of circRNA-miRNA pairs with miRNA-mRNA pairs, the competitive endogenous RNA (ceRNA; DEcircRNA-DEmiRNA-DEmRNA) regulatory network was created. The pathway and functional enrichment analysis were carried out using the Metascape, and hub genes were identified using the MCODE plugin. An overall, 110 DEcircRNAs, 10 DEmiRNAs, and 248 DEmRNAs were detected. The ceRNA network including 46 circRNAs, 10 miRNAs, and 31 mRNAs specific to PDAC was established. In ceRNA network, seven hubgenes were found (SUZ12, IGF2BP2, PPP1CB, FN1, ACTN4, TP53, and PRKAA1) by using the protein-protein interaction network and module analysis. The functional annotation of the DEmRNAs in the ceRNA regulatory network showed that these DEmRNAs were considerably augemented in focal adhesion, regulation of muscle tissue development and assembly of collagen fibrils and other multimeric structures. In conclusion, this research improve present knowledge of the ceRNA regulatory mechanisms in the regulation of PDAC carcinogenesis and provide novel circRNAs as candidate biomarkers.

**Keywords:** biomarkers, pancreatic cancer, network biology, multi-omic data

## INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant tumor, becoming the second leading cause of cancer death worldwide by 2030 (Siegel et al., 2019, 2015). The only available therapeutic option as of now is surgery, although the 5-year overall survival (OS) remains unsatisfying (Miller et al., 2019). Challenges in early diagnosis and failure of exact prognostic biomarkers make it difficult to intervene in postoperative relapses, and cause an increase in the mortality rate of patients who cannot reach a sufficiently accurate diagnosis and treatment (Maeda et al., 2019; Zhang et al., 2016). Thus, it is critical to comprehend the potential molecular mechanisms driving the PDAC carcinogenesis and to identify novel biomarkers in order to create more potent therapeutic strategies.

Circular RNAs (CircRNAs) are continuous and have covalently closed circular structures without 3' and 5' ends, increasing their stability compared to numerous linear RNAs (Du et al., 2017). According to the competing endogenous RNAs (ceRNAs) hypothesis proposed by Salmena et al. (Salmena et al., 2011), transcript RNAs act as natural miRNA sponges through interacting with miRNA response elements (MREs) to regulate the gene expression (Qi et al., 2015). Albeit the presence of a large amount of conserved MREs in circRNAs has emerged in recent years, emphasizing the importance of circRNAs in ceRNA networks (Zhong et al., 2018), the molecular mechanisms underlying the circRNAs in PDAC require further delination.

For our present study, the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih. gov/geo/) was used to obtain the mRNA, miRNA and circRNA expression profiles. The flowchart for this procedure is presented in Fig. 1. Differentially expressed mRNAs (DEmRNAs), miRNAs (DEmiRNAs), and circRNAs

(DEcircRNAs) were determined and ceRNA (DEcircRNA-DEmiRNA- DEmRNA) regulatory network were established successfully. In addition to these, a set of analyses which includes functional enrichment analyses, heatmap analyses, and protein-protein interactions were conducted. In addition to these, a set of analyses which includes functional enrichment analyses, heatmap analyses, and protein-protein interactions were conducted. The present study aims to understand the correlation between circRNAs and pathogenesis of pancreatic ductal adenocarcinoma (PDAC).

## MATERIALS AND METHODS

### 1. Microarray Data Processing

In order to identify robust biomarkers for diagnosis at the early stages of PDAC, we particularly collected publicly available datasets consisting tissue samples from from two different phenotypes (control vs. disease). Three miRNA expression profiles (GSE60978, GSE32678, and GSE41369), four mRNA expression profiles (GSE15471, GSE41368, GSE71989, and GSE62165) and two circRNA expression profiles (GSE69362 and GSE79634) were obtained from the Gene Expression Omnibus (NCBI-GEO) database (Barrett et al., 2013), containing data from PDAC tissues and non-tumor tissues. More information on these datasets is in Table 1.

**Table 1.** List of mRNA/miRNA/circRNA study samples from GEO

| Detected RNA Type | Data source | Author | Platform | Samples (N:PC) | Tissue | Year | PMID |
|---|---|---|---|---|---|---|---|
| Messenger RNA | GSE15471 | Liviu Badea | GPL570 | 39:39 | PDAC Tissue | 2009 | 19260470;28881803 |
| | GSE41368 | Teresa Colombo | GPL6244 | 6:6 | PDAC Tissue | 2013 | 24120476 |
| | GSE71989 | Thomas Schmittgen | GPL570 | 8:14 | PDAC Tissue | 2015 | 27363020 |
| | GSE62165 | Rekin's Janky | GPL13667 | 13:118 | PDAC Tissue | 2016 | 27520560 |
| MicroRNA | GSE60978 | Vandana Sandhu | GPL15159 | 6:51 | PDAC Tissue | 2016 | 25579086 |
| | GSE32678 | Linh My Tran | GPL7723 | 7:25 | PDAC Tissue | 2011 | 22261810; 25846727 |
| | GSE41369 | Teresa Colombo | GPL16142 | 9:9 | PDAC Tissue | 2013 | 24120476 |
| Circular RNA | GSE69362 | Shibin Qu | GPL19978 | 6:6 | PDAC Tissue | 2015 | 27997903;26484292 |
| | GSE79634 | ShiXiang Guo | GPL19978 | 20:20 | PDAC Tissue | 2016 | 29620241 |

GEO, Gene Expression Omnibus; PC, pancreatic cancer; PMID, Pubmed ID.

### 2. Differential Expression Analysis

For differential expression analysis of mRNA, miRNA, and circRNA, the online statistical tool GEO2R, which incorporates R/Bioconductor and Limma package v3.26.8, was used (Barrett et al., 2013; Ritchie et al., 2015; Smyth et al., 2014). Following the processing of microarray datasets, we established the principal standards of $p<0.05$ to identify DEGs, DEMs, and DECs that are indicated statistically significant difference. The direction of gene expression was determined due to logFC<0 as down-regulated or logFC>0 as up-regulated. Hierarchical clustering analysis of the gene expression data was conducted using R package "pheat-map" (https:// cran.r-project.org/web/packages/pheatmap/index.html).

### 3. Functional Enrichment Analysis of mRNAs

Functional and pathway enrichment analysis were carried out using Metascape (Zhou et al., 2019), in order to identify the primary function of DEGs. Gene Ontology (GO) (Harris et al., 2006) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway (Kanehisa et al., 2008) databases were selected as data source biological characteristics and functional annotations. $P<0.05$ was regarded as a significant threshold.

### 4. Construction of Protein-Protein Interaction (PPI) Network and Screening of Hub Genes

The protein protein interaction (PPI) network was constructed through Cytoscape (v3.7.0) software (Shannon et al., 2003). In order to determine hub proteins, the topological analysis were carried out using Cytohubba plug-in (Chin et al., 2014) according to the dual metric approach (i.e. degree as a local metric, and betweenness centrality as a global metric). The Molecular Complex Detection (MCODE) plug-in was used to screen modules of hub genes in PDAC. The top three modules which showed strong associations were further analyzed.

### 5. Construction of the ceRNA (DemRNA-DEmiRNA- DEcircRNA) Regulatory Network

Based on the outcomes of the differential expression analysis, DEcircRNA-DEmiRNA interaction MiRNA-mRNA pairs and circRNA-miRNA pairs were established in accordance with the findings of the differential expression analysis. The ceRNA (DEcircRNA-DEmiRNA-DEmRNA) regulatory network was constructed through integrating circRNA-miRNA pairs with miRNA-mRNA pairs, Subsequently, the regulatory network was visualized using Cytoscape 3.6.1. All DEmRNAs in the ceRNA regulation network underwent GO and KEGG pathway analysis with Metascape.

## RESULTS and DISCUSSION

### 1. Transcriptomic signatures

PDAC is considered to be of the most lethal diseases with high mortality and recurrence rates. The studies on the action mechanisms of circRNA are still in infancy stages. An integrative network-based analysis was performed to uncover the key circRNAs related to PDAC. We acquired publically available mRNA, miRNA, and circRNA microarray datasets of PDAC patients from the NCBI-GEO database to provide a definitive diagnosis of PDAC at an early stage. In this study, 248 DEmRNAs identified by analysing four set of mRNA expression profiles. 10 DEmiRNAs identified from three miRNA expression profiles and 110 DEcircRNAs identifies from two circRNA expression profiles as demonstrated in a Venn diagram (Fig. 1). After the GEO2R tool analysis, we established the cut-off point as an adjusted p-value<0.05 and defined the direction of gene expression using logFC<0 as down-regulated or logFC>0 as up-regulated. Following that, the mRNAs, miRNAs, and circRNAs were sorted according to the logFC absolute value (Fig. 2).

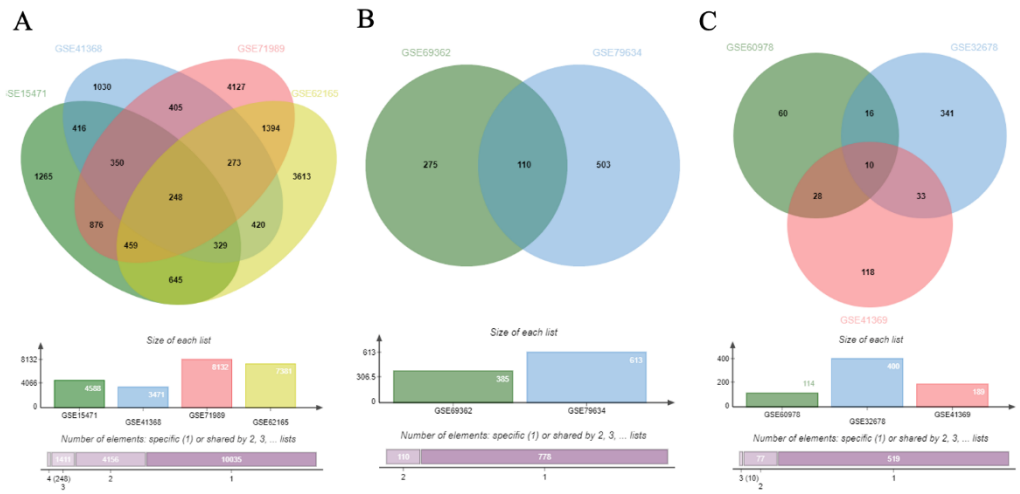



**Figure 1.** Identification of common DEmRNAs, DEmiRNAs, DEcircRNAs between the datasets. A) The mutual core 248 DEmRNAs identified between four mRNA datasets. B) The 110 common DEcircRNAs identified between two circRNA datasets. C) The 10 overlapping DEmiRNAs identified among three miRNA datasets.
*DEmRNAs, differentially expressed mRNAs; DEmiRNAs, differentially expressed miRNAs; DEcircRNAs, differentially expressed circRNAs.*

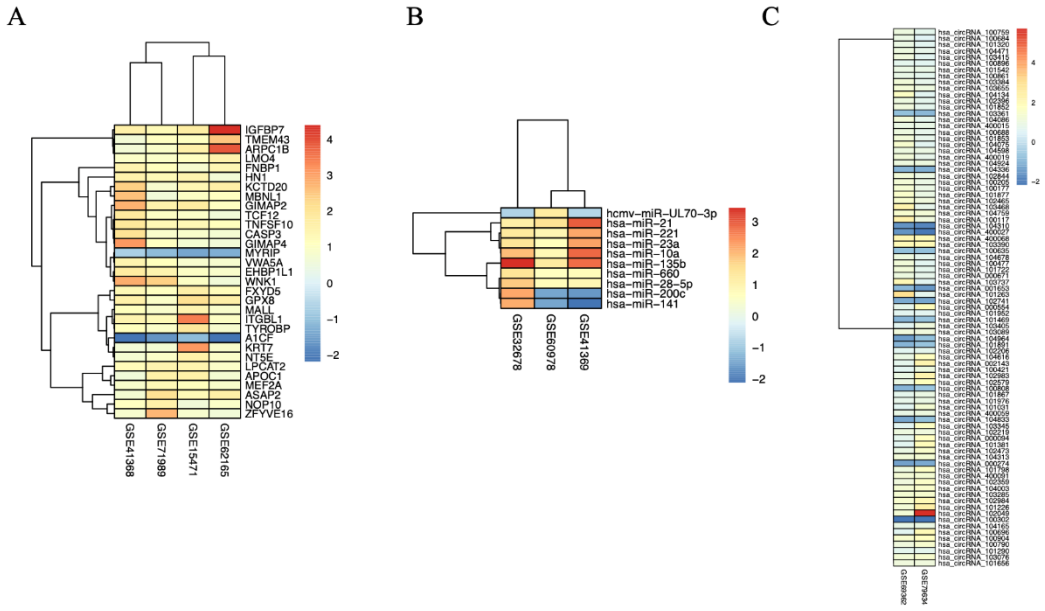
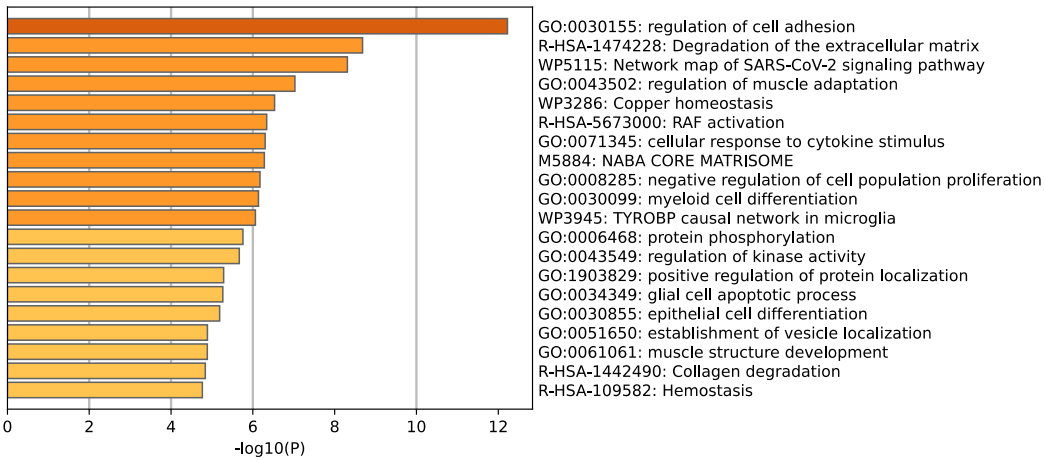



**Figure 2.** Heatmap analysis of DEmRNAs, DEmiRNAs, DEcircRNAs. A) Common upregulated and downregulated DEmRNAs, B) all common DEmiRNAs, C) common upregulated and downregulated DEcircRNAs. *DEmRNAs, differentially expressed mRNAs; DEmiRNAs, differentially expressed miRNAs; DEcircRNAs, differentially expressed circRNAs.*

## 2. Proteomic signatures

To identify the characteristic biological attributes and molecular functions of the DEmRNAs, the enrichment analysis was performed. The first 20 enriched annotations are shown in Fig. 3. GO term assessment illustrated that these genes, which were abundant in PDAC, are involved in regulation of cell adhesion, RAF activation. The PPI network of PDAC was constructed with 248 corresponding DEmRNAs. The network included 1700 nodes and 2136 edges (Fig. 4). Topological features were calculated for all nodes for identifying hub nodes in the networks. As a result, 15 major hub proteins were determined according to the degree and betweenness centrality (Fig. 5).



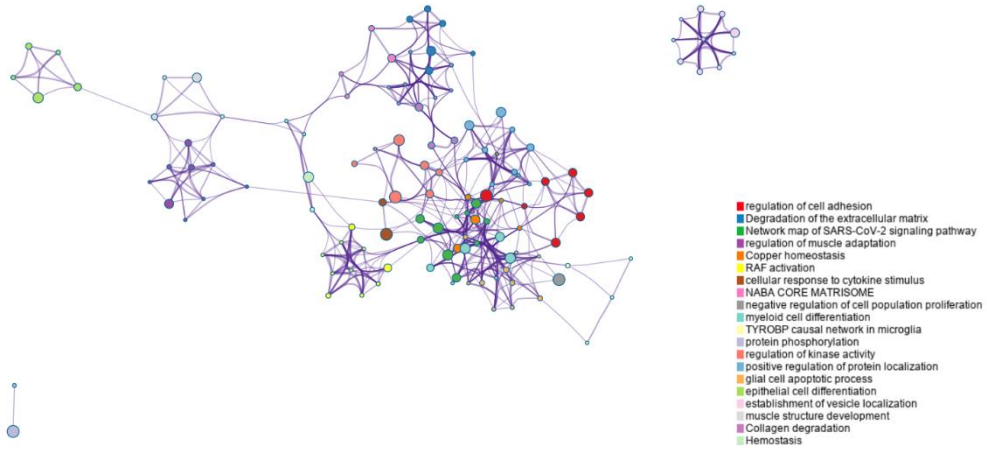



**Figure 3.** Enrichment results of common gene signatures.

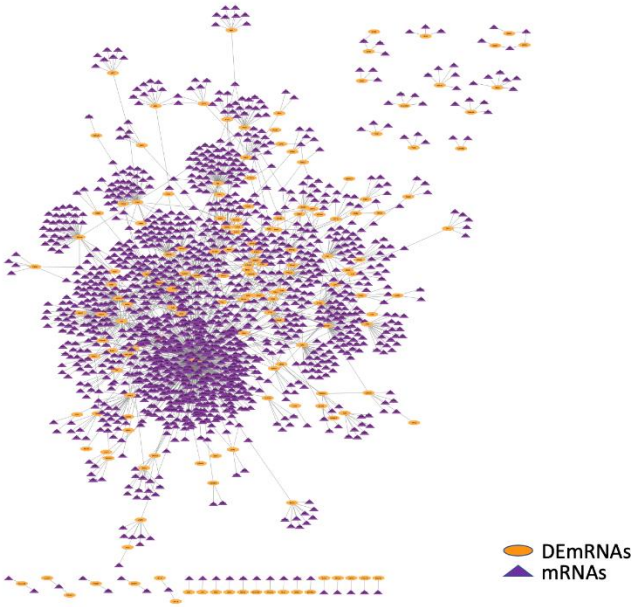



**Figure 4.** Protein-protein interaction map around common coding DEGs. 248 common proteins in PDAC samples are colored in orange while first neighbors are in purple.

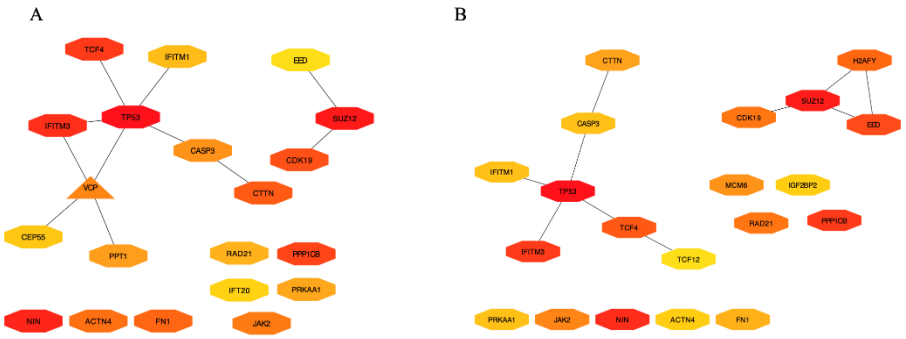



**Figure 5.** Hub proteins

Three major modules with high scores were identified from the PPI network using the MCODE plug-in (Fig. 6). It has been determined that 8 of the 20 hub DEGs (ACTN4, CDK19, EED, FN1, JAK2, IFITM1, IFITM3, TP53) in the PPI network were distributed in the three modules, strengthening the idea that these genes might play valuable roles in PDAC. One of the most frequently altered genes in cancer is TP53, which is mutated in 70% of pancreatic tumours (Maddalena et al., 2021). In a study, scientists were able to pinpoint elevated ACTN4 expression and gene amplification in PDAC and clarify their clinical and biological implications (Welsch et al., 2009). Thus, in addition to offering hub proteins as potential PDAC biomarkers, we also advise that a novel candidate for a module biomarker be found through the differential interactions of three modules.

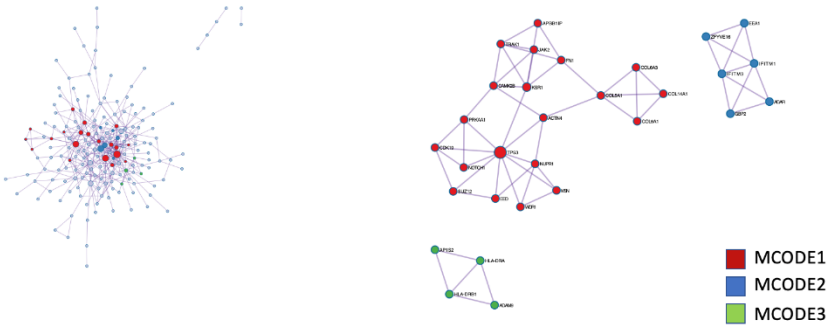



**Figure 6.** Three clusters extracted from PPI.

## CeRNA (DEcircRNA-DEmiRNA-DEmRNA) Regulatory Network

The ceRNA network possesses 56 circRNA-miRNA pairs and 41 miRNA-mRNA pairs, involving 46 circRNAs, 10 miRNAs, and 31 mRNAs (Fig. 7). GO enrichment analysis of 31 DEmRNA unveiled that top 15 clusters with their representative enriched terms. (Fig. 8.) These genes which were significantly enriched in focal adhesion, regulation of muscle tissue development and assembly of collagen fibrils and other multimeric structures.

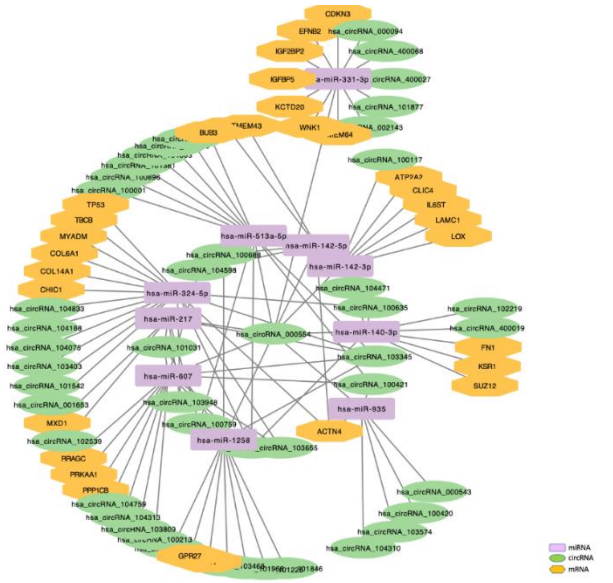



**Figure 7.** ceRNA network of circRNA-miRNA-mRNA in pancreatic ductal adenocarcinoma. The network composed of 46 circRNA nodes, 10 miRNA nodes, 31 mRNA nodes.

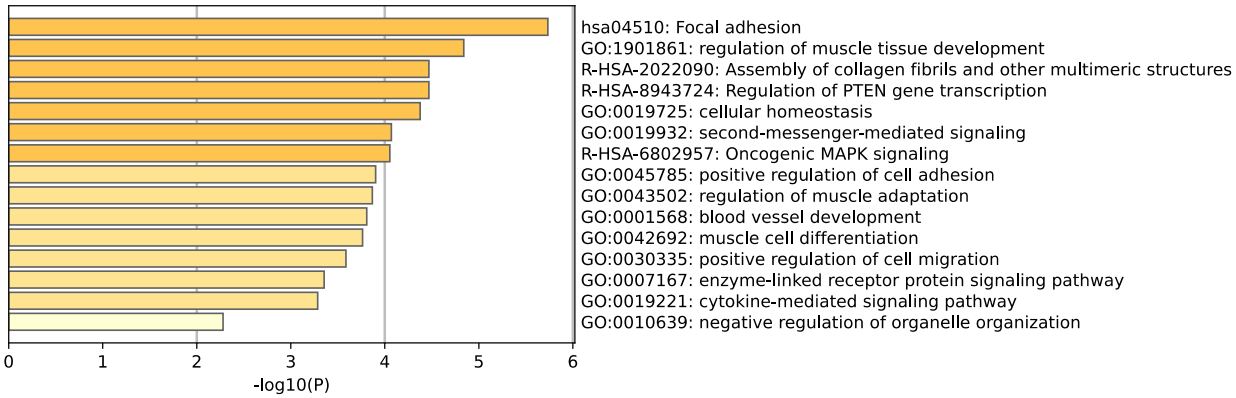



**Figure 8.** Enrichment analysis of 31 DEmRNAs in the ceRNA network.

Numerous studies have shown that PDAC exhibits dysregulated circRNA expression, which affects the pathogenesis and prognosis of the disease (Huang et al., 2018; Xu et al., 2019; Yang et al., 2017). As a result, circRNA is now thought to be a tumor-associated biomarker. In the current study, we ancticipated the association between the nine miRNAs and the one circRNAs that are part of the ceRNA network. Seven of these miRNAs have already been reported which are related to PDAC progression (Godfrey et al., 2018; Jiang et al., 2021; Wan et al., 2020; Wang et al., 2017; Zhan et al., 2020; Zhang et al., 2014; Zhu et al., 2020). A study indicated that MicroRNA-217 modulates pancreatic cancer progression via targeting *ATAD2* (Dutta et al., 2022).

## CONCLUSION

We used a network-based methodology to identify significant pathways and biomarkers for PDAC diagnosis, prognosis, and treatment. We successfully ascertained a circRNA- associated ceRNA network by utilizing bioinformatics analysis. The ceRNA network includes a number of key hub proteins (SUZ12, IGF2BP2, PPP1CB, FN1, ACTN4, TP53, and PRKAA1) that we identified through the interaction analysis. These hub genes which were significantly enriched in focal adhesion, regulation of muscle tissue development and assembly of collagen fibrils and other multimeric structures. Moreover, the results indicated that has_circRNA_000554 has the most miRNA interaction, further suggesting an important role in PDAC progression. Although our findings are based on only currently available tools and databases and need to establish through further *in vitro* analyses, the study opens new avenues into the molecular mechanism and identification of possible biomarkers for PDAC.

## ACKNOWLEDGEMENTS

"The scholarships under the program of TUBITAK-2210-C, and from the projects TUSEB 3629, and TUBITAK 121Y613 provided to Esra Yıldırım Sirkeci are greatly acknowledged."

## REFERENCES

Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashevsky, M., Marshall, K.A., Phillippy, K.H., Sherman, P.M., Holko, M., Yefanov, A., Lee, H., Zhang, N., Robertson, C.L., Serova, N., Davis, S., Soboleva, A., 2013. NCBI GEO: Archive for functional genomics data sets - Update. Nucleic Acids Res 41. https://doi.org/10.1093/nar/gks1193

Chin, C.H., Chen, S.H., Wu, H.H., Ho, C.W., Ko, M.T., Lin, C.Y., 2014. cytoHubba: Identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 8, 1–7. https://doi.org/10.1186/1752-0509-8-S4-S11

Du, W.W., Zhang, C., Yang, W., Yong, T., Awan, F.M., Yang, B.B., 2017. Identifying and characterizing circRNA-protein interaction. Theranostics. https://doi.org/10.7150/thno.21299

Dutta, M., Das, B., Mohapatra, D., Behera, P., Senapati, S., Roychowdhury, A., 2022. MicroRNA-217 modulates pancreatic cancer progression via targeting ATAD2. Life Sci 301. https://doi.org/10.1016/j.lfs.2022.120592

Godfrey, J.D., Morton, J.P., Wilczynska, A., Sansom, O.J., Bushell, M.D., 2018. MiR-142-3p is downregulated in aggressive p53 mutant mouse models of pancreatic ductal adenocarcinoma by hypermethylation of its locus article. Cell Death Dis 9. https://doi.org/10.1038/s41419-018-0628-4

Harris, M.A., Clark, J.I., 2006. The gene ontology (GO) project in 2006. Nucleic Acids Res 34. https://doi.org/10.1093/nar/gkj021

Huang, W.J., Wang, Y., Liu, S., Yang, J., Guo, S. xiang, Wang, L., Wang, H., Fan, Y.F., 2018. Silencing circular RNA hsa_circ_0000977 suppresses pancreatic ductal adenocarcinoma progression by stimulating miR-874-3p and inhibiting PLK1 expression. Cancer Lett. https://doi.org/10.1016/j.canlet.2018.02.014

Jiang, D., Yuan, X., Ni, J., Shen, L., Cai, M., Xu, L., 2021. Low Serum miR-607 Level as a Potential Diagnostic and Prognostic Biomarker in Patients of Pancreatic Ductal Adenocarcinoma: A Preliminary Study. Can J Gastroenterol Hepatol 2021. https://doi.org/10.1155/2021/8882129

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., Katayama, T., Kawashima, S., Okuda, S., Tokimatsu, T., Yamanishi, Y., 2008. KEGG for linking genomes to life and the environment. Nucleic Acids Res 36, 480–484. https://doi.org/10.1093/nar/gkm882

Maddalena, M., Mallel, G., Nataraj, N.B., Shreberk-Shaked, M., Hassin, O., Mukherjee, S., Arandkar, S., Rotkopf, R., Kapsack, A., Lambiase, G., Pellegrino, B., Ben-Isaac, E., Golani, O., Addadi, Y., Hajaj, E., Eilam, R., Straussman, R., Yarden, Y., Lotem, M., Oren, M., 2021. TP53 missense mutations in PDAC are associated with enhanced fibrosis and an immunosuppressive microenvironment. Proc Natl Acad Sci U S A 118. https://doi.org/10.1073/PNAS.2025631118

Maeda, S., Unno, M., Yu, J., 2019. Adjuvant and neoadjuvant therapy for pancreatic cancer. J Pancreatol. https://doi.org/10.1097/JP9.0000000000000028

Miller, K.D., Nogueira, L., Mariotto, A.B., Rowland, J.H., Yabroff, K.R., Alfano, C.M., Jemal, A., Kramer, J.L., Siegel, R.L., 2019. Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin 69. https://doi.org/10.3322/caac.21565

Qi, X., Zhang, D.H., Wu, N., Xiao, J.H., Wang, X., Ma, W., 2015. ceRNA in cancer: Possible functions and clinical implications. J Med Genet 52. https://doi.org/10.1136/jmedgenet-2015-103334

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., Smyth, G.K., 2015. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43. https://doi.org/10.1093/nar/gkv007

Salmena, L., Poliseno, L., Tay, Y., Kats, L., Pandolfi, P.P., 2011. A ceRNA hypothesis: The rosetta stone of a hidden RNA language? Cell. https://doi.org/10.1016/j.cell.2011.07.014

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: A software Environment for integrated models of biomolecular interaction networks. Genome Res. https://doi.org/10.1101/gr.1239303

Siegel, R.L., Miller, K.D., Jemal, A., 2019. Cancer statistics, 2019. CA Cancer J Clin 69, 7–34. https://doi.org/10.3322/caac.21551

Siegel, R.L., Miller, K.D., Jemal, A., 2015. Cancer statistics, 2015. CA Cancer J Clin 65. https://doi.org/10.3322/caac.21254

Smyth, G.K., Ritchie, M., Thorne, N., Wettenhall, J., Shi, W., Hu, Y., 2014. limma: Linear Models for Microarray Data User's Guide. Bioinformatics. May.

Wan, Y., Luo, H., Yang, M., Tian, X., Peng, B., Zhan, T., Chen, X., Ding, Y., He, J., Cheng, X., Huang, X., Zhang, Y., 2020. miR-324-5p Contributes to Cell Proliferation and Apoptosis in Pancreatic Cancer by Targeting KLF3. Mol Ther Oncolytics 18. https://doi.org/10.1016/j.omto.2020.07.011

Wang, C., Feng, Z., Jiang, K., Zuo, X., 2017. Upregulation of MicroRNA-935 promotes the malignant behaviors of pancreatic carcinoma PANC-1 cells via targeting inositol polyphosphate 4-phosphatase Type I gene (INPP4A). Oncol Res 25. https://doi.org/10.3727/096504016X14759554689565

Welsch, T., Keleg, S., Bergmann, F., Bauer, S., Hinz, U., Schmidt, J., 2009. Actinin-4 expression in primary and metastasized pancreatic ductal adenocarcinoma. Pancreas 38. https://doi.org/10.1097/MPA.0b013e3181b28d6f

Xu, Y., Yao, Y., Gao, P., Cui, Y., 2019. Upregulated circular RNA circ_0030235 predicts unfavorable prognosis in pancreatic ductal adenocarcinoma and facilitates cell progression by sponging miR-1253 and miR-1294. Biochem Biophys Res Commun 509. https://doi.org/10.1016/j.bbrc.2018.12.088

Yang, F., Liu, D.Y., Guo, J.T., Ge, N., Zhu, P., Liu, X., Wang, S., Wang, G.X., Sun, S.Y., 2017. Circular RNA circ-LDLRAD3 as a biomarker in diagnosis of pancreatic cancer. World J Gastroenterol 23. https://doi.org/10.3748/wjg.v23.i47.8345

Zhan, T., Chen, X., Tian, X., Han, Z., Liu, M., Zou, Y., Huang, S., Chen, A., Cheng, X., Deng, J., Tan, J., Huang, X., 2020. MiR-331-3p Links to Drug Resistance of Pancreatic Cancer Cells by Activating WNT/β-Catenin Signal via ST7L. Technol Cancer Res Treat 19. https://doi.org/10.1177/1533033820945801

Zhang, J., Zhao, C.Y., Zhang, S.H., Yu, D.H., Chen, Y., Liu, Q.H., Shi, M., Ni, C.R., Zhu, M.H., 2014. Upregulation of miR-194 contributes to tumor growth and progression in pancreatic ductal adenocarcinoma. Oncol Rep 31. https://doi.org/10.3892/or.2013.2960

Zhang, Q., Zeng, L., Chen, Y., Lian, G., Qian, C., Chen, S., Li, J., Huang, K., 2016. Pancreatic Cancer Epidemiology, Detection, and Management. Gastroenterol Res Pract. https://doi.org/10.1155/2016/8962321

Zhong, Y., Du, Y., Yang, X., Mo, Y., Fan, C., Xiong, F., Ren, D., Ye, X., Li, C., Wang, Y., Wei, F., Guo, C., Wu, X., Li, X., Li, Y., Li, G., Zeng, Z., Xiong, W., 2018. Circular RNAs function as ceRNAs to regulate and control human cancer progression. Mol Cancer. https://doi.org/10.1186/s12943-018-0827-8

Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A.H., Tanaseichuk, O., Benner, C., Chanda, S.K., 2019. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun 10, 1523. https://doi.org/10.1038/s41467-019-09234-6

Zhu, J., Zhou, L., Wei, B., Qian, Z., Wang, J., Hui, H., Sun, Y., 2020. MiR-142-5p inhibits pancreatic cancer cell migration and invasion by targeting PIK3CA. Mol Med Rep 22. https://doi.org/10.3892/mmr.2020.11251

## *ORAL PRESENTATION*

### Akciğer kanseri ve normal hücreleri arasında karnitin düzeyleri kıyaslaması

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**Özet**

Kanserle ilişkili ölümlerden en yaygın olanlardan biri akciğer kanseridir ve tüm kanser ölümlerinin yaklaşık üçte birini oluşturmaktadır. Karnitin, aktifleştirilmiş uzun zincirli yağ asitlerini hücre sitoplazmasından mitokondriye taşıyarak onların β-oksidasyonda önemli bir biyofaktördür ve enerji metabolizmasında önemli bir rol oynamaktadır. Yapılan çalışmalar, karnitinin mitokondriyi koruyarak karsinogenezi inhibe ettiğini göstermektedir. Bu çalışma ile akciğer kanseri hücreleri (A549) ve normal akciğer epitel hücrelerinin (BEAS-2B) karnitin düzeyleri kıyaslanarak karnitin metabolizması arasındaki farklar belirlenecektir. Yapılan çalışmada akciğer kanseri hücreleri (A459) ve normal insan akciğer epitel hücreleri (BEAS-2B) çoğaltılarak 24 saat inkübe edildi. Kazınarak toplanan hücre süspansiyonları her bir gutria kağıdına 50 µl olarak şekilde damlatılarak 2-3 saat 22-24 °C'de kurutuldu. Uygun prosedürde Sıvı Kromatografisi-Kütle Spektrofotometresi (LC-MS/MS) cihazı kullanılarak karnitin seviyeleri ölçülerek elde edilen veriler SPSS 22.0 ve GraphPad Prism 9 programları kullanılarak analiz edildi. Toplam 27 karnitin arasından A549 ve BEAS-2B gruplarında serbest karnitin (C0) (**p<0.01) ve Asetil L-karnitin (C2) (***p<0.001) düzeyleri kanserli hücrelerde normal ohücrelere göre anlamlı olarak arttığı bulundu. Diğer karnitin türevleri için istatistiksel olarak anlamlı bir fark tespit edilmedi (p>0.05). Serbest karnitin ve asetil L-karnitinin, yağ asitlerini β-oksidasyon için mitokondriyal matrise taşımaktır. Yağ asidi β-oksidasyonu, farklı koşullara bağlı olarak karsinomun ilerlemesini destekleyebilir veya engelleyebilir. HIF-1 aracılı yağ asidi oksidasyonunun baskılanması, kanser ilerlemesi için kritik öneme sahiptir. Bu çalışmada, CO ve C2 miktarları sağlıklı ve kanserli hücreler arasında kıyaslandığında C0 ve C2 miktarının kanserli hücrelerde sağlıklı hücrelere göre anlamlı düzeyde azaldığı bulunmuştur. Karnitin metabolizmasının karsinomun ilerlemesi ile negatif ilişkili olduğu düşünülmektedir.
**Anahtar Kelimeler:** A549, BEAS-2B, kanser, karnitin.

### Comparison of carnitine levels between lung cancer and normal cells

**Abstract**

One of the most common cancer deaths is cancer, accounting for about a third of all cancer deaths. Carnitine acts as an important biofactor in β-oxidation by transporting activated long-chain fatty acids from the cell cytoplasm to the mitochondria. Studies show that carnitine inhibits carcinogenesis by protecting mitochondria. In this study, the carnitine levels of lung cancer cells (A549) and normal lung epithelial cells (BEAS-2B) will be compared and the differences between carnitine metabolism will be determined. In the study, lung cancer cells and normal human lung epithelial cells were replicated and incubated for 24 hours. Cell suspensions collected by scraping were dropped to each gutria paper as 50 µl and dried at 22-24 °C for 2-3 hours. The data obtained by measuring carnitine levels using a High Performance Liquid Chromatography (LC-MS/MS) device in the appropriate procedure were analyzed using SPSS 22.0 and GraphPad Prism 9 programs. Among 27 carnitines, free carnitine (C0) (**p<0.01) and Acetyl L-carnitine (C2) (***p<0.001) levels were found to be significantly increased in cancer cells compared to normal cells in A549 and BEAS-2B groups. No statistically significant difference was found for other carnitine derivatives (p>0.05). Free carnitine and acetyl L-carnitine transport fatty acids to the mitochondrial matrix for β-oxidation. Fatty acid β-oxidation can promote or inhibit the progression of carcinoma depending on different conditions. Suppression of HIF-1-mediated fatty acid oxidation is critical for cancer progression. In this study, when the amounts of CO and C2 were compared between healthy and cancerous cells, it was found that the amount of C0 and C2 decreased

significantly in cancerous cells compared to healthy cells. Carnitine metabolism is thought to be negatively related to the progression of carcinoma.
**Keywords:** A549, BEAS-2B, cancer, carnitine.

## GİRİŞ

Akciğer kanseri, yılda tahmini 1,6 milyon ölümle dünyadaki en yaygın kanser çeşitlerindendir. Anormal enerji metabolizması, tümörlerin bir özelliğidir (Arbour ve ark. 2019). Tümör hücreleri, hücre biyosentezi ve hücre fonksiyonunun gereksinimlerini karşılamak için enerji metabolizmasını teşvik eder (Bose and Le, 2018, Zong 2016). Karnitin, aktifleştirilmiş uzun zincirli yağ asitlerini hücre sitoplazmasından mitokondriye taşıyarak onların β-oksidasyonda önemli bir biyofaktör olarak görev yapmaktadır. Bu kapsamda da enerji metabolizmasında önemli bir rol oynamaktadır. Yapılan çalışmalar, karnitinin mitokondriyi koruyarak karsinogenezi inhibe ettiğini göstermektedir. Yağ asitlerinin enerji olarak kullanılması, yağ asitlerinin aktivasyonu ve mitokondriye taşınmasında yer alan çeşitli enzimler ve taşıma proteinleri mevcuttur. Mitokondriyal yağ asidi oksidasyonunun kilit hız sınırlayıcı enzimleri karnitinler, mitokondriyal yağ asidi oksidasyonunu düzenler ve hem sağlıkta hem de kanser dahil hastalıkta çevreye uyumu kolaylaştırır (Schlaepfer ve Joshi, 2020). Yağ asidi metabolizmasının kanserdeki rolü son yıllarda artan bir ilgi görmüştür. Birçok çalışma, yağ asidi sentezi ve oksidasyonunda yer alan genlerin anormal ekspresyonunun, terapötik ilaç direnci, metastaz potansiyeli ve nüks dahil olmak üzere malign fenotiplerle korele olduğunu göstermiştir (Aiderus ve ark. 2018). Mitokondriyal yağ asidi β-oksidasyonu, yağ asitlerinin katabolizması için ana yoldur ve tüm vücut enerji homeostazını korumada önemli bir rol oynar. (Odle ve ark.,2014). Bu çalışma ile akciğer kanseri hücreleri (A549) ve normal akciğer epitel hücrelerinin (BEAS-2B) karnitin düzeyleri kıyaslanarak karnitin metabolizması arasındaki farklar belirlenmiştir. Bu sayede karnitin metabolizmasının bir biyobelirteç olarak kullanılabilirliği test edilmiştir.

## MATERYAL VE METOT

Hücre Kültürü ve Koşulları

Sterilizasyon

Hücreler çalışılmaya başlanmadan ve besiyeri hazırlanmadan önce laminar kabin sterilizasyonu için 30 dk UV ve %70'lik etil alkol kullanılmıştır. Steril edilen laminar kabinde kullanılacak malzemeler %70 alkolle silinerek içeri alınmıştır. Hücre kültürü çalışmaları için temin edilen sarf malzemeler 0,22 µm ve 0,45 µm por çapına sahip filtreler (Sartorius AG, Almanya) kullanılarak sıvı malzemelerin (Fetal Bovine Serum, Penicillin/Streptomycin ve L-Glutamine) (Sigma Aldrich, ABD) sterilizasyonu sağlanmıştır.

Besiyerinin Hazırlanması

BEAS-2B ve A549 hücre hatlarını geliştirmek için DMEM-F12/RPMI-1640 (500ml) besiyeri ortamları kullanılmıştır. Besiyeri ortamlarında 50 ml %10 FBS, 5 ml %1 Penicillin/Streptomycin, 5 ml %1 L-Glutamin kimyasalları kullanılmıştır.

Hücrelerin Çoğaltılması

Çalışmada ATCC'den temin edilip stoklanan BEAS-2B ve A549 hücre hatları kullanılmıştır. Sıvı azot tankından çıkarılan donmuş haldeki hücreler 37 °C'de çözülerek laminar kabin içerisine alınmıştır. Hücrelerin büyümesi için hazır hale getirilen DMEM-F12/RPMI 1640 (Sigma Aldrich, ABD) besiyerine hücreler aktarılarak 1200 rpm'de 5 dakika santrifüj edilip süpernatant uzaklaştırılmıştır. Pellet el yardımı ile hafifçe vurularak homojenize edilip 4 ml besiyerinde süspanse hale getirilmiştir. Süspansiyondan alınan hücreler (10µl) ve %5'lik Tripan-Blue (10µl) (Sigma Aldrich, ABD) karıştırılarak thoma lamında (Marienfeld, Almanya) hücre canlılığı değerlendirilerek 12 kuyucuklu hücre kültürü plakasına ekim yapılmıştır. Her bir kuyucuğa 1 ml DMEM-F12/RPMI 1640 besiyeri eklenip 37 °C' de %5 $CO_2$ ve %95 nemli ortam içeren ortamda inkübasyona bırakılmıştır. İnkübasyon sonrası hücreler scaper ile kazınarak bir ependorfa aktarılmıştır.

Hücre süspansiyonunda karnitin profilinin LC-MS/MS ile incelenmesi

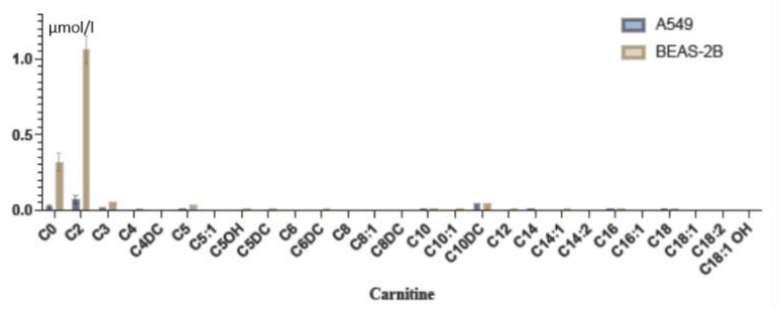
Ependorflara aktarılan hücre süspansiyonunun pipetlenerek homojen bir karışım olması sağlandıktan sonra yaklaşık 50 µl gutria kağıdına damlatılmış ve 2-3 saat 22-24 °C'de absorbe edilmesi sağlanmıştır. 200 µl NBS internal standart eklendikten sonra süpernatan yeni plate'e alınarak azot gazı altında 30 dk kurutulmuştur. 60 µl DRVT eklenerek 60 C de 15 dk inkübasyona bırakılarak türevlendirilmiştir. Daha sonra azot gazı altında 30 dk kurutulmuştur. 100 µl mobil faz (acetonitril: metanol: formicacid) solüsyonu eklenerek LC-MS-MS enjekte edilerek karnitin seviyelerinin ölçülmesi sağlanmıştır.

İstatistiksel Analizler

Deneyler sonrası elde edilen verilerin analizi için GraphPad prism 9 (GraphPad Software, Inc, San Diego, ABD) paket programı kullanılmıştır. Sürekli verilerin dağılımı ile normal dağılım arasında fark olup olmadığı Kolmogorov-smirnov ve Shapiro-Wilk testleriyle araştırılmıştır. Normal dağılan verilerin üç bağımsız grup kıyaslamalarında One-Way ANOVA testi kullanılırken, normal dağılmayan üç gruplu kıyaslamalarda Kruskal-Wallis H testi kullanılmıştır. $p<.05$ istatistiksel olarak anlamlı kabul edilmiştir.

## BULGULAR ve TARTIŞMA

Yapılan çalışmada akciğer kanseri hücreleri (A459) ve normal insan akciğer epitel hücreleri (BEAS-2B) Yüksek Performanslı Sıvı Kromatografisi (LC-MS/MS) cihazı kullanılarak karnitin seviyeleri ölçülerek elde edilen veriler GraphPad Prism 9 programları kullanılarak analiz edilmiştir. Toplam 27 karnitin arasından A549 ve BEAS-2B gruplarında serbest karnitin (C0) (**$p<0.01$) ve Asetil L-karnitin (C2) (***$p<0.001$) düzeyleri kanserli hücrelerde normal hücrelere göre anlamlı olarak arttığı bulundu. Diğer karnitin türevleri için istatistiksel olarak anlamlı bir fark tespit edilmedi ($p>0.05$). Serbest karnitin ve asetil L-karnitinin, yağ asitlerini β-oksidasyon için mitokondriyal matrise taşımaktır. Yağ asidi β-oksidasyonu, farklı koşullara bağlı olarak karsinomun ilerlemesini destekleyebilir veya engelleyebilir. HIF-1 aracılı yağ asidi oksidasyonunun baskılanması, kanser ilerlemesi için kritik öneme sahiptir (Wang ve ark. 2022). Bu çalışmada, CO ve C2 miktarları sağlıklı ve kanserli hücreler arasında kıyaslandığında C0 ve C2 miktarının kanserli hücrelerde sağlıklı hücrelere göre anlamlı düzeyde azaldığı bulunmuştur. Karnitin metabolizmasının karsinomun ilerlemesi ile negatif ilişkili olduğu düşünülmektedir.




**Şekil 1.** A-549 ve BEAS-2B hücre hatları karnitin metabolizması kıyaslaması.

## SONUÇ

Akciğer kanseri (A459) hücrelerinde karnitin metabolizmasının bir biyobelirteç olarak kullanılabileceği düşünülmektedir.

## KAYNAKLAR

Aiderus A., Black M.A., Dunbier A.K. Fatty acid oxidation is associated with proliferation and prognosis in breast and other cancers. BMC Kanser. 2018;18(1):805.

Arbour KC. Systemic therapy for locally advanced and metastatic non-small cell lung cancer: a review. *JAMA*. 2019;322:764-774.

Bose S, Le A. Glucose metabolism in cancer. *Adv Exp Med Biol*. 2018;1063:3-12.

Lane AN, Higashi RM, Fan TW. Metabolic reprogramming in tumors: Contributions of the tumor microenvironment. *Genes Dis*. 2019;7:185-198.

Schlaepfer R. I., Joshi M. Cpt1A-mediated Fat Oxidation, Mechanisms and therapeutic potential. Endocrinology. 2020 Feb 1;161(2):bqz046. doi: 10.1210/endocr/bqz046.

Odle J., Adams S.H., Vockley J. Carnitine. Adv Nutr. 2014;5(3):289-290.

Wang J., Zhou Y., Zhang D., Zhao W., Lu Y., Liu C., Lin W., Zhang Y., Chen K., Hui Wang H., Zhao L. CRIP1 supresses BBOX-1mediated carnitine metabolism to promote stemness in hepatocelular carcinoma. *The Embo Journal*. 41:e110218|2022.

Zong WX, Rabinowitz JD, White E. Mitochondria and cancer. *Mol Cell*. 2016;61:667-676.

## *ORAL PRESENTATION*

# Hidrojellerin biyofiziksel özellikleri

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## Özet

Hidrojeller, doku ortamlarını taklit eden su ile doldurulmuş üç boyutlu polimerik ağlardır. Hidrojeller suyu yapılarında tutabilme kabiliyetleriyle, yumuşak ve esnek yapıda olmalarıyla dokulara büyük benzerlik göstermektedirler. Herhangi bir toksik etkilerinin olmamasının yanısıra degrade olabilen, biyouyumlu yapıları sayesinde hidrojellerin biyomalzeme olarak kullanımı günden güne artmaktadır. Hücrelere madde taşınımı ve hasarlı dokuların rejenerasyonu hidrojellerin biyomalzeme olarak en yaygın kullanıldıkları alanlar arasındadır. Hidrojel ağlar, hücre işlevlerini sürdürmeye yardımcı olan adhezif ligandlar, büyüme faktörleri veya degrade olabilir bölgeler ile birlikte biyokimyasal işlevsellik kazanımı sağlamak amacıyla önemli ölçüde modifiye edilmektedir. Yakın zamanlı çalışmalarda hidrojellerin belirli biyofiziksel özelliklerinin hücre kaderini belirlemede kilit oyuncular oldukları öne sürülmüştür. Statik sertliğin (stiffness) ötesinde, hücrelerle etkileşimlerindeki zamana bağlı gerilim/gerinim (stress/strain) değişikliklerinin, hücre aracılı degradasyonun ve matris sentezinin de hücre durumunu ve doku onarım sürecini şekillendirdiği gösterilmiştir. Bu derlemede, doku rejenerasyonu için en uygun hidrojellerin tasarlanabilmesine ve geliştirmesine imkan verebilecek biyofiziksel özellikler incelenecektir.

**Anahtar Kelimeler:** Hidrojel, Biyofiziksel özellik, Doku rejenerasyonu

# Biophysical properties of hydrogels

## Abstract

Hydrogels are three-dimensional polymeric networks filled with water that mimic tissue environments. Hydrogels are very similar to tissues with their ability to hold water in their structures and to be soft and flexible. In addition to the absence of any toxic effects, the use of hydrogels as biomaterials is increasing day by day thanks to their degradable and biocompatible structures. Substance transport to cells and regeneration of damaged tissues are among the most common uses of hydrogels as biomaterials. Hydrogel networks are significantly modified to gain biochemical functionality with adhesive ligands, growth factors or degradable regions that help maintain cell functions. Recent studies have suggested that certain biophysical properties of hydrogels are key players in determining cell fate. Beyond static stiffness, time-dependent stress/strain changes in interactions with cells, cell-mediated degradation and matrix synthesis have also been shown to shape cell state and tissue repair process. In this review, the biophysical properties that may allow the design and development of the most suitable hydrogels for tissue regeneration will be examined.

**Keywords:** Hydrogel, Biophysical property, Tissue regeneration

## Giriş

Hidrojeller, suyla dolu üç boyutlu (3B) polimerik ağlardır. Su içeriği, polimer konsantrasyonuna bağlı olmakla beraber %90-99'a kadar ulaşabilmektedir. Bu yapı hidrojellerin yüksek hidrofilikliğini ve protein, hücre gibi biyolojik birimlere agrege olmadan güvenli bir şekilde uygulanabilmelerini sağlamaktadır. Hidrojellerin mekanik davranışı tipik olarak su ve polimer ağların akışkan ortamdaki hareketi sonucunda viskoelastiktir (Nam S., 2016).

Hidrojeller sentetik (ör. polietilen glikol (PEG), poliakrilamid (PAA), polidimetilsiloksan (PDMS)) ve doğal (ör. kollajen, jelatin, aljinat, hyaluronik asit (HA) ve kitosan) polimerler olarak karşımıza çıkabilmektedir (Li J, 2016). Hidrojeller, polimerlerin fiziksel veya kimyasal çapraz-bağlanma (cross-linking) organizasyonundan

oluşmaktadır. Fiziksel çapraz-bağlanma, polimer ağlar arasındaki zayıf etkileşimler yoluyla mümkündür; oysa kimyasal çapraz-bağlanma, polimer zincirler arasında güçlü bağlar oluşturur (Trappmann B, 2017). Fiziksel olarak çapraz bağlı hidrojeller mekanik etki altında kolayca deforme olabilirken, kimyasal olarak çapraz bağlı hidrojeller kalıcı deformasyona karşı direnç gösterir. Pek çok doğal polimer, pH veya sıcaklık değişimi ile fiziksel etkileşimlerle jelleşse de, kimyasal çapraz bağlama yöntemleri (örn. UV ile kürleme) sıklıkla kullanılmaktadır (Yue K, 2015).

Bir hidrojelin sertliği, hücre kaderini belirleyen önemli bir parametre olarak kabul edilmektedir. Engler ve ark. (2006) yılında yaptığı ve mezenkimal kök hücrelerin soy spesifikasyonunun PAA hidrojellerinin esneklik modülüsü tarafından belirlendiğini bildirilen preliminer çalışmalarının yanı sıra, birçok grup sertliğin pluripotent kök hücreler (Caiazzo M, 2016) nöral kök hücreler (Leipzig ND and Shoichet MS, 2009), hematopoietik kök hücreler (Choi JS, 2015) ve kanser hücreleri (Cavo M, 19) dahil olmak üzere çeşitli hücre tiplerinin yaşamında temel rol oynadığını göstermiştir.

Hidrojeller biyomedikal alanda kullanıldığından, biyolojik yapılarla etkileşimlerini iyileştirmek için çokça çaba sarf edilmiştir. Degrade olabilen hidrojeller, rejenere olan doku kendi yerini alacağından doku mühendisliğinde tercih edilmektedir. Bu nedenle, degradasyon hızının kontrolüne de odaklanılmıştır (Kloxin AM, 2009). Degradasyon hidrolitik veya enzimatik olarak gerçekleşebilmektedir. Ümit vadeden bir kontrollü degradasyon yaklaşımı, enzimatik olarak bölünebilir bölgelerin (örn. matriks metaloproteinaz bölünme bölgesi) polimer ağlar içine dahil edilmesidir (Khetan S, 2013). Degradasyon, nihai olarak, mekanik viskoelastisite de dahil olmak üzere hidrojel özelliklerinde zamanla değişikliklere yol açar.

**Hidrojellerin hücrelerle etkileşiminde rol oynayan biyofiziksel özellikler**

3B doku ortamlarını taklit etmenin doğası gereği, matriks-hücre etkileşimlerini yorumlamak için hidrojeller kapsamlı bir şekilde incelenmiştir (Dhaval ve ark. 2017). İlk çalışmalar, iki boyutlu (2B) hidrojel koşullarında hücre davranışlarını araştırmıştır (Engler ve ark. 2006). Sertlik, ligand yoğunluğu ve ağ gözenekliliği parametrelerinin uyarlanması nispeten kolaydır ve hücre davranışları için belirleyici faktörler olarak ele alınmaktadır. Kök hücrelerin soy spesifikasyonu üzerinde hangi parametrenin en etkili olduğu konusunda süregelen bir tartışma olmasına rağmen diğer parametrelerden bağımsız olarak sertliğin en kilit etken olduğu bulunmuştur (Wen ve ark. 2014).

Hidrojellerin statik sertlik değeri, 2015'te stres relaksasyonunun ortaya çıkmasına kadar hidrojellerde önemli bir parametre olmuştur (Chaudhuri ve ark. 2015). Hidrojeller fiziksel olarak (iyonik olarak) çapraz bağlandığında, sabit bir gerilim altında önemli derecede stres relaksasyonu (onlarca ila yüzlerce saniye içinde gerilimde %10'luk değişim) yaşadılar. Bu stres relaksasyonu davranışı, hücresel itme ve çekme kuvvetlerine hidrojel viskoelastik tepkilerini taklit edebilir ve bu nedenle hücrelerle etkileşimde dinamik olarak kabul edilir. İlginç bir şekilde, stres relaksasyonu olmayan kimyasal olarak (kovalent olarak) çapraz bağlı hidrojeller, hücrelerin çok sınırlı bir şekilde yayılmasına izin verdi. Stres relaksasyonu olan hidrojellerin sertliğinin zamanla azaldığı ve hücrelerin azalan sertliği tanıdığı göz önüne alındığında, stres relaksasyonu gösteren hidrojellerdeki bu tür bulgular beklentimizle bir şekilde çelişkilidir; PAA ve PEG gibi birçok hidrojelde kanıtlandığı gibi, hücrelerin daha sert jeller üzerinde daha iyi yayıldığı bilinmektedir (Engler ve ark. 2006; Rennerfeldt ve ark. 2013). Bununla birlikte, hücrelerin stres relaksasyonu olan hidrojellere yalnızca azalan sertlik bilgisini toplayarak yanıt verdiği görülmemektedir; bunun yerine, yapışkan ligandları kümeleyerek ve hücre iskeletlerini geliştirerek hidrojelleri şekillendirme ve yeniden modelleme yoluna gitmektedirler.

2B çalışmasının ardından, aynı grup, farklı çapraz bağlama yöntemleri aracılığıyla değişen stres relaksasyonu davranışlarla uyarlanmış 3B aljinat bazlı hidrojellerdeki mezenkimal kök hücre farklılaşmasını araştırmıştır (Chaudhuri ve ark. 2016). Hidrojeller daha hızlı stres relaksasyonu gösterdiğinde, osteojenik farklılaşma daha fazla uyarılmışken; adipojenik farklılaşma stres relaksasyonuna bağlı bulunmamıştır. Stres relaksasyonu gösteren hidrojeller, fokal adhezyonların hücresel olarak yeniden düzenlenmesine ve dolayısıyla osteojenik farklılaşmaya yardımcı olan şekillendirmeye ve yayılmaya olanak vermektedir. Bu çalışma, hidrojellerin stres relaksasyonunun uygun şekilde ayarlanmasının, kök hücreler ve kemik dokusu mühendisliği için osteojenik olarak uygun koşulları sağlamak için önemli bir strateji olduğunu vurgulamaktadır. Çalışmaların motive edici sonuçlarıyla, kondrosit davranışları üzerindeki stres relaksasyonu etkisi, 3B aljinat hidrojelleri kullanılarak daha fazla araştırılmıştır (Lee ve ark. 2017). Hızlı stres relaksasyonu olan hidrojellerdeki kondrositler, kıkırdak hücre dışı matrisini önemli ölçüde üretebilirken, yavaş stres relaksasyonu olan hidrojellerdekiler, elastisiyet nedeniyle sınırlı hacim genişlemesi gösterebilmiştir; çevreleyen matrisin uyguladığı stresler, kıkırdak dejenerasyonuna yol açmaktadır. Çalışma, kıkırdak parçalayıcı in vivo koşullardaki hücresel olguları

yorumlamak için yararlıdır ve hidrojellerin stres relaksasyonunun kıkırdak doku mühendisliği için önemli bir tasarım parametresi olabileceğini düşündürmektedir.

Stres relaksasyonu çalışmalarından bağımsız olarak, degradasyon konusu çok yakın zamanda ortaya çıkmıştır. Hidrojeller çoğunlukla zamanla hidrolitik ve/veya enzimatik olarak degrade olurlar, dolayısıyla degradasyon aracılı hücresel tepki hidrojel doku mühendislerinin ilgi alanına girer. Khetan ve ark. (2013), metakrilatlanmış hyaluronik asitten (MeHA) yapılmış hidrojeller tasarlayarak bu konuyu aydınlatmıştır. Hidrojeller, degradasyona bağlı mezenkimal kök hücre davranışlarını incelemek için degrade olmayan veya proteolitik olarak parçalanabilir nitelikte tasarlandı. İlginç bir şekilde, degrade olmayan hidrojellerdeki hücreler, çok çeşitli sertlik değerleri (4-95 kPa) için bile osteojenik bir farklılaşma gösteremedi; ancak, hidrojeller degrade olabilir özellikte olduğunda, hücreler ortam sertliğini hissedebilmenin yanı sıra osteojenik farklılaşmaya gidebilmiştir. Hücresel farklılaşma, degradasyon aracılı matris yeniden modellemesi ve etkileşimli hücresel yayılma ve mekanotransdüktör sinyalleme ile yakından ilişkilidir. Bu çalışma, matris degradasyonuyla sağlanan hücre kaynaklı matris yeniden organizasyonunun önemini bir kez daha vurgulamaktadır ve bunun, degradasyon sorununun henüz sayılmadığı stres relaksasyonu jellerle ortak temel moleküler mekanizmaları paylaştığı düşünülmektedir.

Derlememizde yer verilmiş anahtar bulgular birlikte ele alındığında, matriks aracılı hücresel olaylar, hücrelerin yeniden düzenlemeyi mümkün kılan matriksi tercih etmesi anlamında ortak bir noktaya işaret etmektedir ve bu, bir matriks degradasyonu veya bir ağ bağının çözülmesi (fiziksel çapraz bağlantılı hidrojeler) ile mümkündür. Hidrojeller yoluyla iletilen kök hücrelerin veya hidrojellerin etrafındaki dokuda yerleşik hücrelerin davranışlarına ilişkin gelecekteki anlayışımız bu nedenle bu noktada özel değerlendirmelere ihtiyaç duyabilir. Burada vurgulanan, hidrojellerin ortaya çıkan biyofiziksel yönü, hücre yüklü hidrojellerle ilgili in vitro ve in vivo olguların anlaşılmasına yardımcı olabilir ve doku onarımı ve rejenerasyonu için optimize edilmiş hidrojellerin daha fazla tasarımını ve geliştirilmesini motive edebilir.

**Sonuç**

Hidrojeller, 3B doku ortamlarını taklit eder. Bu nedenle, doku eşdeğeri matrikslerle hücre etkileşimlerini anlamak için bir çok çalışmada hidrojeller kullanılmıştır. Hidrojellerin çeşitli özelliklerinin hücre kaderini belirlemede önemli olduğu bulunmuş olsa da, hücrelerle dinamik etkileşimler yoluyla viskoelastik özelliklerdeki değişikliklerin, son zamanlarda hücre davranışlarını yöneten anahtar bir unsur olduğunu göstermiştir. Burada altı çizilen olgular dinamiktir ve matriksin hücrelerle karşılıklı etkileşimlerinden kaynaklanan farklı zaman dilimleri boyunca sürekli değişir. Hücre kaynaklı deformasyon, degradasyon ve yeni hücre dışı matriks oluşumu, hidrojel ile ilgili hücresel olaylara ilişkin anlayışımızı ve doku mühendisliği açısından hidrojellere yönelik gelecek tasarım stratejilerini yeniden inşa etmeye başlayacaktır.

## KAYNAKLAR

Baker BM, Trappmann B, Wang WY, et al. Cell-mediated fibre recruitment drives extracellular matrix mechanosensing in engineered fibrillar microenvironments. Nat Mater 2015; 14(12): 1262–1268.

Caiazzo M, Okawa Y, Ranga A, et al. Defined three-dimensional microenvironments boost induction of pluripotency. Nat Mater 2016; 15(3): 344–352.

Cavo M, Fato M, Peñuela L, et al. Microenvironment complexity and matrix stiffness regulate breast cancer cell activity in a 3D in vitro model. Sci Rep 2016; 6: 35367.

Chaudhuri O, Gu L, Darnell M, et al. Substrate stress relaxation regulates cell spreading. Nat Commun 2015; 6: 6364.

Chaudhuri O, Gu L, Klumpers D, et al. Hydrogels with tunable stress relaxation regulate stem cell fate and activity. Nat Mater 2016; 15(3): 326–334.

Choi JS, Mahadik BP and Harley BAC. Engineering the hematopoietic stem cell niche: frontiers in biomaterial science. Biotechnol J 2015; 10(10): 1529–1545.

Dhaval K and Rajesh V. Bi-functional oxidized dextran–based hydrogel inducing microtumors: an in vitro threedimensional lung tumor model for drug toxicity assays. J Tissue Eng 2017; 8: 2041731417718391.

Engler AJ, Sen S, Sweeney HL, et al. Matrix elasticity directs stem cell lineage specification. Cell 2006; 126(4): 677–689.

Guvendiren M and Burdick JA. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. Nat Commun 2012; 3: 792.

Khetan S, Guvendiren M, Legant WR, et al. Degradationmediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels. Nat Mater 2013; 12(5): 458–465.

Kloxin AM, Kasko AM, Salinas CN, et al. Photodegradable hydrogels for dynamic tuning of physical and chemical properties. Science 2009; 324(5923): 59–63.

Lee H-P, Gu L, Mooney DJ, et al. Mechanical confinement regulates cartilage matrix formation by chondrocytes. Nat Mater 2017; 16(12): 1243–1251.

Li J and Mooney DJ. Designing hydrogels for controlled drug delivery. Nat Rev Materials 2016; 1: 16071

Nam S, Hu KH, Butte MJ, et al. Strain-enhanced stress relaxation impacts nonlinear elasticity in collagen gels. Proc Nat Acad Sci 2016; 113: 5492–5497.

Rennerfeldt DA, Renth AN, Talata Z, et al. Tuning mechanical performance of poly(ethylene glycol) and agarose interpenetrating network hydrogels for cartilage tissue engineering. Biomaterials 2013; 34(33): 8241–8257.

Trappmann B, Baker BM, Polacheck WJ, et al. Matrix degradability controls multicellularity of 3D cell migration. Nat Commun 2017; 8(1): 371.

Trappmann B, Gautrot JE, Connelly JT, et al. Extracellularmatrix tethering regulates stem-cell fate. Nat Mater 2012; 11(7): 642–649.

Wen JH, Vincent LG, Fuhrmann A, et al. Interplay of matrix stiffness and protein tethering in stem cell differentiation. Nat Mater 2014; 13(10): 979–987. Connelly JT, Garcia AJ and Levenston ME.

within a hydrogel: novel strategy for human bone marrowderived stem cells neural/neuronal differentiation guidance and therapeutic secretome enhancement. Acta Biomater 2017; 49: 167–180.

Yue K, Trujillo-de Santiago G, Alvarez MM, et al. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. Biomaterials 2015; 73: 254–271.

## *ORAL PRESENTATION*

# Biological dressing of mesenchymal stem cells in 3D tissue culture secrete signaling molecules enhance wound healing in mice

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**Abstract**

Delayed wound healing significantly affect millions of people and impair their life quality and represent the most challenging disease in modern pathological medicine requiring modern effective therapies. Many studies collected secretions of mesenchymal stem cells (MSCs) in traditional cell culture and injected them around the wounds which is very invasive method. Therefore, this study has developed a biological dressing by growing MSCs on scaffold in 3-dimensional cell culture (3DCC) to be applied as biological dressing for wounds. Previously isolated and characterized MSCs were grown on Cytobuilder scaffold for 7 days to generate 3D tissue like structure of MSCs (MSC dressing). The developed MSC dressing was then applied for 15 days on induced wound in mice models and compared with control scaffold dressing containing culture media only. Results revealed that wounds covered with MSC dressing significantly healed faster than control wounds treated with culture media only. It could be concluded that MSC grown on Cytobuilder scaffold could be used as biological dressing to enhance wound healing.

**Keywords: Keywords:** Biological dressing, wound healing, 3D cell culture, MSC dressing.

## INTRODUCTION

Burns and chronic wounds damage the skin's outer layer, impairing other essential activities like regulating body temperature and preventing infection and evaporative losses. Burns and chronic wounds result in pain, contractures, scarring, and a decreased psychosocial well-being over time (Palackic et al., 2022). In order to improve the wound environment and patient outcomes, alternative wound substitutes have been used in large acute burns and scar resurfacing. Cell-based sheet replacements operate as biologically active dressings, releasing growth factors, cytokines, and extracellular matrix elements necessary for effective wound healing. They provide off-the-shelf temporary wound coverage (Moiemen, 2018). These coverages include biological dressings that do not integrate into the wound but nonetheless create a wound environment that promotes healing are temporary alternatives. Scaffolds are frequently used as permanent substitutes because they integrate into the wound and encourage host cells to move into them before eventually dissolving. Biological dressing are made from biological materials come from a natural source. This category includes dressings made from collagen sourced from bovine, porcine, or avian sources. These products are all intended to hasten the healing process. There are several different types of biological dressings, including gels, liquids, and semi-permeable sheets (Petrulyte, 2008). human mesenchymal stem cells (MSCs) are the pivotal player for coordinating the repair process by differentiation and secreting biologically active substances thereby recruiting other host cells. They are therefore involved in all phases of the healing process (Zahorec et al., 2015). For instance, MSCs modulate the immune response (Blaber et al., 2012) by enhancing the synthesis of anti-inflammatory cytokines including IL-10 and IL-4 (Aggarwal and Pittenger, 2005). Moreover, MSCs produce bioactive substances to act as inhibitors for fibrosis and apoptosis and to act as inducers for angiogenesis, mitosis and / or differentiation of progenitor cells, activating target cells or neighbouring cells to release biologically active substances (Caplan and Dennis, 2006). All in all, MSCs impact the whole phases of wound healing; inflammation, proliferation and tissue remodelling (Hocking and Gibran, 2012) including the cutaneous wound healing process (Rea et al., 2009). Many recent studies suggest that MSCs are the main candidates for cell mediated therapies and tissue regeneration (Sasaki et al., 2008) not only for their differentiation ability but also for their ability to produce active biomolecules (Caplan and Dennis, 2006). To avoid ethical issues related to applying animal material to human wounds, in this study, a cellular sheet of MSCs grown on polystyrene scaffold was applied as biological dressin to promote wound healing in mice for further appplications for human wound therapies.

## MATERIALS AND METHODS

### Cell Culture

All cell culture was performed in class II cabinets to reduce the risk of infection and contamination. Then, all the cells and 3D-BEM models were incubated at standard culture conditions (SCC) which were provided by humidified incubator adjusted to 37ºC, ≈20% $O_2$ and 5% $CO_2$. Unless otherwise stated, all culture conditions set out in this study for cell culture and cell experiments were SCC. MSCs were previously isolated form adipose tissue form donors undergone plastic surgery and grown in StemMACS MSC Expansion Media, human (UK) supplemented with foetal calf serum and L-Glutamine. Before use, it was supplemented with 5 ml of 100IU/ml penicillin - 100 µg/ml streptomycin (MSC Media) as described by Al-Shaibani (2022). To split cells, old media was aspirated, and cells were rinsed with phosphate buffered

saline (PBS), trypsinised with trypsin 1X and incubated for 10 minutes. A quantity of 10 ml of MSC Media was added to prepare the cell suspension and centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded, and cells were counted and a cell number of $5\times10^4$ was seeded into a T175 flask. A volume of 15-20 ml of fresh MSC Media were added to the flask and incubated under SCC. Trypan blue stain was used to assess viability of cells. A volume of 10 µl trypan blue was mixed with 10 µl of cell suspension. A quantity of 10 µl of this mixture was transferred to a Neubauer chamber and covered with a cover slip prior to counting under the microscope (Sandell and Sakai, 2008). (Equation 1) and (Equation 2) were used to calculate cell count and cell viability.

$$\textbf{Total Cell Count} = \textbf{No of cells in 25 small square} \times \textbf{Dilution Fcator} \times 10^4 \qquad \textbf{Equation 1}$$

$$\textbf{Cell Viability} = \frac{\textbf{No of viable cells in 25 small square}}{\textbf{Total cell count } (\textbf{Live} + \textbf{dead cells})} \times 100\ \% \qquad \textbf{Equation 2}$$

**Construction MSC dressing**

The basis of this model is the CytoBuilder scaffold developed by (Al-Shaibani 2020). This scaffold was made of polystyrene, which is widely used to synthesize plasticware for cell culture. Prior seeding cells, the CytoBuilder scaffold was activated with 70% ethanol for one minute, treated with MSC media for one minute, fixed in Petri dish (30×15 mm) and incubated under SCC until required. To establish MSC dressing, $3\times10^6$ cells were suspended in 200 µl MSC media, seeded onto the CytoBuilder scaffold and incubated at SCC for 90 minutes. Then, 20 ml of MSC media was added to the Petri dish and covered the entire scaffold, and the culture was incubated at SCC for 7 days, with regular feeding of the MSC dressing with MSC media every two days. Feeding the culture included withdrawal of old medium from the well using a micropipette and then 20 ml of fresh MSC medium was gently added on the inside wall of the petri dish without disturbing the cells on the CytoBuilder scaffold. Sections of MSC dressing were stained with haematoxylin-eosin (HE) to evaluate the formation of 3D structure of MSCs and to assess cell penetration through the CytoBuilder scaffold. Briefly, paraffin embedded sections of MSC dressing were always kept on ice during sectioning to ensure effective sectioning. The microtome was adjusted to 4 µm and sections were kept in a water bath adjusted at 40ºC for 2-3 minutes before transferring to slides. The slides were then heated in an oven at 60ºC overnight and the next day, dehydrated by washing for 5 minutes in each xylene, 100% ethanol, 95% ethanol, 70% ethanol and distilled water. stained with hematoxylin-eosin (HE) to evaluate the formation of 3D-tissue of MSC drssing. Sections were then washed four times in TBS for 2 minutes, covered with DPX, left in the dark at room temperature overnight, and analysed the next day using fluorescent microscope (Leica).

**Wound induction in mice and treatment with MSC dressing**

Four weeks age male albino mice (N=6) weighing 25–30 g was used in the study divided into two groups (test and control) of 3 animals each (n=3). To familiarize the animals with their new living conditions, they were brought to the experimental room one week before the trials and placed in cages with free access to water and regular pellet food in the form of dry pellets. The room temperature was set to 24°C with 12 hours light/dark intervals to manage daily physiological parameters. The experimental settings met the requirements of the Al-Nahrain University Ethics Committee in Baghdad, Iraq. Excisional wound was applied for assessing the wound healing activity. Before excision, the mice were given intraperitoneal injections of ketamine (60 mg/kg) and xylazine 288 (10 mg/kg). After shaving and sterilizing the area with 70% ethanol, a circular full thickness wound (1cm diameter) was punched on the back of each mouse. The animals were kept in separate enclosures. The treatment with MSC dressing commenced one day later than wound induction. Control group were treated with scaffold soaked with MSC media. All mice were exposed to UV-C 300 light for 30 min and immersed in 70% ethanol to be disinfected before treatment. MSC dressing was manually applied on the wound surface and covered with medical tape. Both the MSC dressing and the medical tape were being changed every 48 hours. Wound closure was observed by imaging wound surface at 5 days, 10 days, and 15 days post-wounding.

**Results**

MSC dressing generated in this study were prepared by seeding MSCs on the CytoBuilder scaffold for 7 days. As shown in figure (1a), the structure of MSC dressing is composed MSCs grown on the scaffold which was fixed by a plastic holder (orange part) and fed with MSC medium for 7 days. The scaffold now bearing MSCs was separated from the holder to get cellular layer of MSCs could be used as biological dressing for wounds. The MSC dressing upon staining with hematoxylin– eosin revealed that MSCs distributed within the scaffold and formed a tissue-like structure in which cells are distributed in a 3D environment that mimics an in vivo niche as illustrated in figure (1b). This MSC dressing was then applied on the top of the wounds induced in mice.

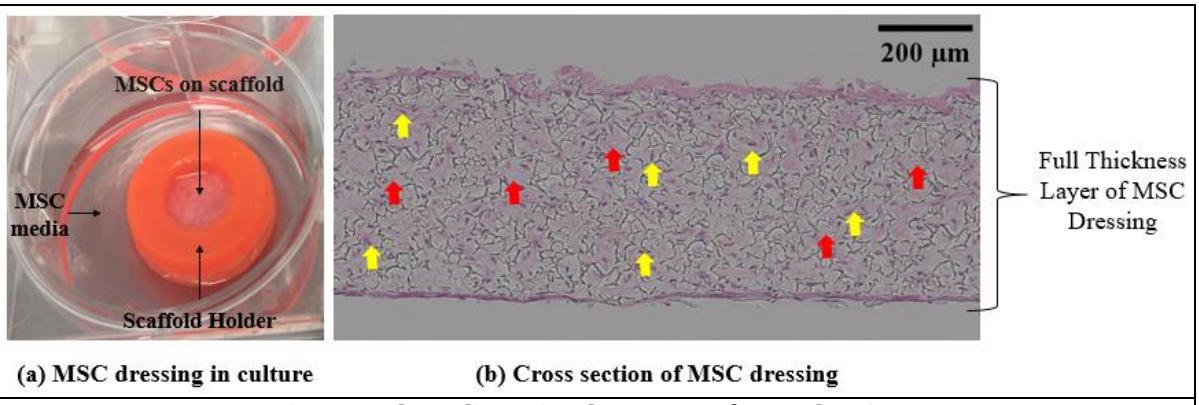


**Figure 1. Three-dimensional structure of MSC dressing.**

(a) MSCs grown on scaffold in tculture plate and fed with MSC medium for 7 days. (b) Hematoxylin–eosin (HE) staining shows 3D culture of MSCs at day 10 after seeding the MSCs over the CytoBuilder scaffold, revealing the formation of a cellular layer of MSCs that mimics an *in vivo* niche. Cells spread and deeply penetrated the scaffold and formed 3D tissue-like structure of MSC dressing. Yellow arraows are pointing out to MSCs, red arrows are pointing out to cytoplasm.

Representative images of wounds treated with MSC dressing and scaffold soaked with MSC media for control mice on days 5, 10 and 15 post-wounding are shown in figure (2), This also includes changes in wound areas. Throughout 15 days of the treatment period, none of the animals showed any postoperative adverse reaction such as bleeding of granulation tissue, fluid retention, infection or sepsis. All the animals stayed alive till the end of the experiment. Treated wounds showed closure with a small scar, smaller incision skin breaking stretch, and inflammation of edema disappeared. The present results revealed that the highest wound healing activity was observed in mice treated with MSC dressing on day 15 recorded a diameter of 0.2 cm for wounds treated with MSC dressing which is significantly smaller than wound size (0.9 cm) of control mice treated with MSC media.

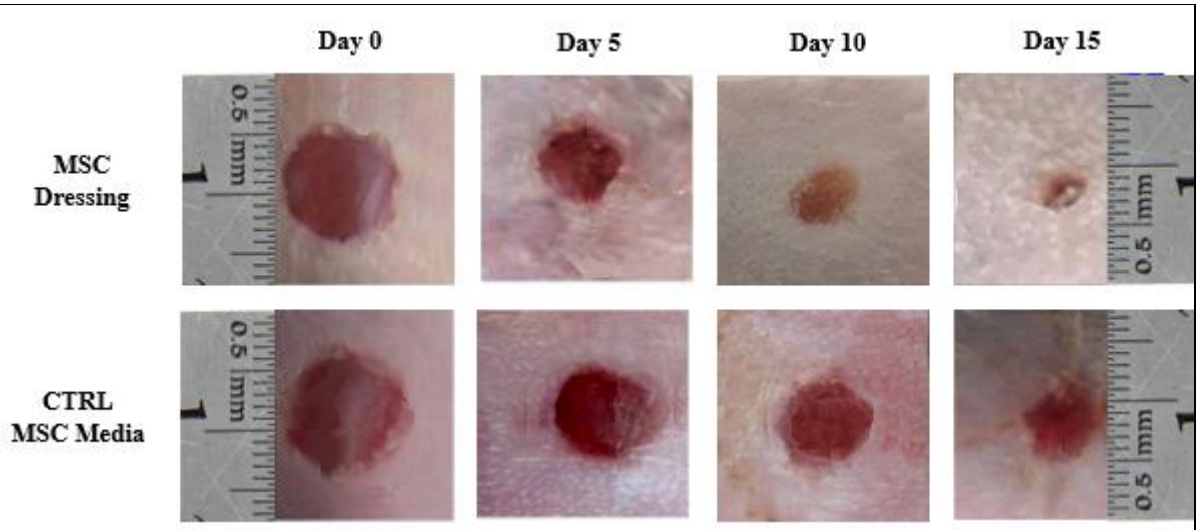


**Figure 2. Wound closure in mice treated with MSC dressing.**

Cross imaging of wound closure over 15 days. Representative images at each time point illustrate differences in wound closure rate and quality between wounds of mice covered with MSC dressing and wounds of control mice covered with scaffold soaked with MSC medum.

**Discussion**

It has been reported that human stem cells could be applied on and in mice models for therapeutic investigations (James *et al.*, 2006). Therefore, this study has investigated for the first time the use of biological dressing of mesenchymal stem cells (MSC dressing) for wound healing *in vivo* using mouse model. It has been previously reported that MSCs in 3D cell culture secrete many growth factors and cytokines including platelet derived growth factor (PDGF-AB), transforming growth factor-1 (TGF-1), hepatocyte growth factor (HGF), stromal derived factor-1 (SDF-1), interleukin 1 (IL-1), and interleukin 6 (IL-6) (Al-Shaibani, 2022). Therefore, MSCs has been used in this study to establish biological dressing in 3D cell culture and further applied on induced wounds in mice model. The healing process of wounds in mice treated with MSC dressing could be attributed to the fact that MSCs has formed 3D tissue like structure in 3D cell culture and secreted the above panel of growth factors and cytokines which are collectively or separately play vital roles in the healing process (Alfaro *et al.*, 2013). Availability of this proteomic panel in MSC-CM is sufficient to enhance cutaneous healing with full thickness recovery. These biomolecules work separately or synergistically to heal the damaged tissue. For example, KGF promotes keratinocyte migration and proliferation (Pastar et al., 2014), improving the healing rate, the

quality of epithelialisation, the density of blood vessels as well as it is playing a role in tissue remodelling (Maxson et al., 2012). PDGF participates in the inflammatory phase, and tissue remodelling and promotes keratinocyte proliferation (Safari et al., 2014). HGF enhances keratinocytes to migrate, proliferate and produce matrix metalloproteinase and stimulate new blood vessel formation. It has therefore been suggested to play a role in cutaneous wound repair (van de Kamp et al., 2013). TGF-β1 regulates the inflammatory phase (Barrientos et al., 2008b) by activating macrophages, and suppressing T-lymphocytes (Wang et al., 2012). TGF-β3 stimulates remodelling. It also supports wound repair and regeneration of hair follicles (Maxson et al., 2012). SDF-1 plays a role in regulating skin homeostasis and tissue remodelling (Werner, 2011). It also promotes wound closure by inducing cell migration. Many of which play an integral role in inflammation, proliferation, migration, differentiation, collagen regulation, angiogenesis and the remodelling phases of the healing process (Cowin et al., 2001). Collectively, these bioactive molecules as components of MSC secretion guide and judge the healing process in acute and chronic wounds by coordinating numerous cellular responses of different cell types resulting in the re-establishment of the damaged barrier (Barrientos et al., 2008).

## CONCLUSION

This study applied for the first time biological dressing from mesenchymal stem cells (MSC dressing) for wound healing *in vivo*. This method could be utilized for treatment of chronic wound by isolation of MSCs from patients and applied them as dressing tool. MSCs on the scaffold can keep grow and secrete growth factors and cytokines while it has been applied as wound dressing since MSCs on the scaffold can survive for 72 hours which allow continues exposure to MSC secretions.

## CONFLICT OF INTEREST

No conflict of interest

## REFERENCES

Aggarwal S, Pittenger MF 2005. 'Human mesenchymal stem cells modulate allogeneic immune cell responses', *Blood*, 105(4), pp. 1815-22. https://doi.org/10.1182/blood-2004-04-1559

Alfaro MP, Deskins DL, Wallus M, DasGupta J, Davidson JM, Nanney LB, Gannon M, Young PP 2013. 'A physiological role for connective tissue growth factor in early wound healing', *Laboratory Investigation*, 93(1), pp. 81-95. https://doi.10.1038/labinvest.2012.162

Al-Shaibani MBH 2022. Three-dimensional cell culture (3DCC) improves secretion of signaling molecules of mesenchymal stem cells (MSCs). *Biotechnology Letters*, 44, 143–155. https://doi.org/10.1007/s10529-021-03216-9

Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M 2008. 'Growth factors and cytokines in wound healing', *Wound Repair Regeneration*, 16(5), pp. 585-601. https://doi.org/10.1111/j.1524-475X.2008.00410.x

Blaber S, Webster R, Hill C, Breen E, Kuah D, Vesey G, Herbert, B 2012. 'Analysis of in vitro secretion profiles from adipose-derived cell populations', *Journal of Translational Medicine*, 10(1), p. 172. https://doi.org/10.1186/1479-5876-10-172

Caplan AI, Dennis, JE 2006. 'Mesenchymal stem cells as trophic mediators', *Journal of Cellular Biochemistry*, 98(5), pp. 1076-84. https://doi.org/10.1002/jcb.20886

Cowin AJ, Kallincos N, Hatzirodos N, Robertson JG, Pickering KJ, Couper J, Belford DA 2001. 'Hepatocyte growth factor and macrophage-stimulating protein are upregulated during excisional wound repair in rats', *Cell Tissue Research*, 306(2), pp. 239-50. https://doi.org/10.1007/s004410100443

Hocking AM 2012. 'Mesenchymal Stem Cell Therapy for Cutaneous Wounds', *Advanced Wound Care (New Rochelle)*, 1(4), pp. 166-171. https://doi.10.1089/wound.2011.0294

James D, Noggle SA, Swigut T, Brivanlou AH 2002. Contribution of human embryonic stem cells to mouse blastocysts, Developmental Biology,295 (1): 90-102. https://doi.org/10.1016/j.ydbio.2006.03.026

Maxson S, Lopez EA, Yoo D, Danilkovitch-Miagkova A, LeRoux MA 2012. 'Concise Review: Role of Mesenchymal Stem Cells in Wound Repair', *Stem Cells Translational Medicine*, 1(2), pp. 142-149. https://doi.org/10.5966/sctm.2011-0018

Moiemen NS 2018. Total Burn Care || The Role of Alternative Wound Substitutes in Major Burn Wounds and Burn Scar Resurfacing., 633–639.e1. https://doi.10.1016/B978-0-323-47661-4.00059-9

Palackic A, Duggan RP, Campbell MS, Walters E, Branski LK, El Ayadi A, Wolf SE 2022. The Role of Skin Substitutes in Acute Burn and Reconstructive Burn Surgery: An Updated Comprehensive Review, *Seminars in Plastic Surgery*; 36(01): 033-042. https://doi.10.1055/s-0042-1743455

Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, Patel SB, Khalid L, Isseroff RR, Tomic-Canic M 2014. 'Epithelialization in Wound Healing: A Comprehensive Review', *Advances in Wound Care*, 3(7), pp. 445-464. https://doi.org/10.1089/wound.2013.0473

Petrulyte S 2008. Advanced textile materials and biopolymers in wound management. *Danish medical bulletin*, 55(1):72-7. PMID: 18321446.

Rea S, Giles NL, Webb S, Adcroft KF, Evill LM, Strickland DH, Wood FM, Fear MW 2009. 'Bone marrow-derived cells in the healing burn wound--more than just inflammation', *Burns*, 35(3), pp. 356-64. https://doi.org/10.1016/j.burns.2008.07.011

Safari M, Ghahari L, Zoroufchi MD 2014. 'Effects of epidermal growth factor, platelet derived growth factor and growth hormone on cultured rat keratinocytes cells in vitro', *Pakistan Journal of Biological Sciences*, 17(7), pp. 931-6.

Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, Shimizu H 2008. 'Mesenchymal Stem Cells Are Recruited into Wounded Skin and Contribute to Wound Repair by Transdifferentiation into Multiple Skin Cell Type', *The Journal of Immunology*, 180(4), pp. 2581-2587. https://doi.org/10.4049/jimmunol.180.4.2581

van de Kamp J, Jahnen-Dechent W, Rath B, Knuechel R, Neuss S 2013. 'Hepatocyte growth factor-loaded biomaterials for mesenchymal stem cell recruitment', *Stem Cells International*, 2013, p. 892065.

Wang Z, Wang Y, Farhangfar F, Zimmer M, Zhang Y 2012. 'Enhanced Keratinocyte Proliferation and Migration in Co-culture with Fibroblasts', *PLOS ONE*, 7(7), p. e40951.

Werner S 2011. 'A Novel Enhancer of the Wound Healing Process: The Fibroblast Growth Factor-Binding Protein', *The American Journal of Pathology*, 179(5), pp. 2144-2147. https://doi.10.1016/j.ajpath.2011.09.001

Zahorec P, Koller J, Danisovic L, Bohac M 2015. 'Mesenchymal stem cells for chronic wounds therapy', *Cell Tissue Bank*, 16(1), pp. 19-26. https://doi.10.1007/s10561-014-9440-2

## *ORAL PRESENTATION*

# Biochemical pathway of Micro- plastic in seafood

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## Abstract

Microplastics (MPs), classified as all plastic particles smaller than 5 mm in size, are omnipresent in different environments from where they can be transferred among mediums, and from an aquatic environment to humans both by direct discharge and by the degradation of relatively larger plastic compounds. With changes in consumption tendencies of consumers and globalization, plastic, and therefore micro-plastic contamination rates, have increased over the recent years, globally. MPs are considered emerging pollutants of the aquatic environment due to their direct negative effects, such as facilitating the exposure of aquatic organisms to hazardous chemicals. The bioaccumulation and biomagnifications of MPs have become a threat to the seafood chain, resulting from the ingestion of these contaminants by aquatic organisms. While the determination of MPs in water bodies, aquatic organisms, and human health appears to be the most essential part of the risk, estimating the fate of these contaminants in the future should also have the same importance. While MPs contamination is often determined in water bodies, biota, and food chains partially, the whole assessment of MPs bioaccumulation and biodegradation chain from nature to consumer by seafood still remains well understood. In addition to risks of bioaccumulation of MPs resulting from aquatic water bodies on the raw material of seafood, the production line in terms of heat treatment and processing approaches may also impact the presence of MPs in the seafood. The packaging material that contacts directly to seafood products has also the potential to be a factor in the MP's bioaccumulation of the relevant products. In this study, the presence of micro plastic residue in the different commercial seafood product determined. The results could be useful for other commercial seafood products that have the risk of micro plastic contamination.

**Keywords:** micro plastic, biochemical degradation, seafood, food safety, public health, processing

## INTRODUCTION

Aquatic pollutions are serious emerging concern over the last years. Heavy metal, polycyclic carbon, polychlorinated biphenyls are known main pollutants found in the environment. Most of the aquatic pollutions reasoned by environmental pollutions, anthropogenic activities and climate change. Increasing of utilization of plastic material in the daily life, the exposure of micro plastic to the environment has risen gradually. Water bodies whether seas or fresh water can be considered one of the most vulnerable parts of nature due to higher contamination risk. The risk of micro plastic could be increased by the variation in the light and temperature of water. Sources of these aquatic pollutants most commonly resulted in agriculture and ordinary household products that are consequently discharged to water bodies (Badea and Niculescu,2022). Due to almost 80% of the world population has been suffered from water pollution-related issues, some monitoring process of these contaminants from nature to human health are needed.

Seafood has needed to be more attention to the aquatic pollutants due to the higher contamination, digestion and bioaccumulation rate of the relevant pollutants. Kwon et al.,(2020) highlighted that various types of micro plastics accumulated in different parts of fish such as gill, edible tissue and gut. Many studies have conducted on bioaccumulation of different mps on the several seafood from shrimp to crab and fish (Su et al., 2016;Li et al.,2019).Micro plastic bioaccumulation on seafood resulted by not only raw material but can be also during processing and storage. Packaging materials could be accelerating impact on the bioaccumulation of MPs on the seafood (Almeida et al.,2021).

Novel research indicated that bioaccumulation of micro plastic on seafood driven by food matrices; Diaz-Basantes et al.,(2022) indicated that different canned tuna sample show different micro plastic content as a result of canned tuna in brine solutions and oil. Silva et al.,(2022) reported that lipid level of fish impacted on

the micro plastic formation. While food matrices accepted as a factor that impact on the sensory quality of food products, it is actually resulted in different quality degradation, occurrence of various compounds at the different levels. Storage period also impacted on the bio formation of hazardous compound in the packaged seafood which is driven by the time of storage and the food material contacted package materials. Novakov et al.,(2017) detected heavy metal and polycyclic aromatic hydrocarbon at the different level in canned fish sample. The detection processes of micro plastic in the seafood has some advantages than other food products owing to the facilities of reaching muscle in the raw form, but the organic components of food matrices such as food additives, spices and vegetables can make difficult to reaching the micro plastic in processed seafood (Toussaint et al.,2019;Lee et al.,2022). Hydrogen peroxide and potassium hydroxide are mostly used for the extraction of micro plastic in muscle food. Following to extraction, detection and visualisation of micro plastic by different microscope and then characterization of the type of micro pollutants by different instrumental techniques (Sridhar et al.,2022).

While there is some research conducted on the micro plastic accumulation on the seafood impacted by tested species, packaged material and storage conditions, there is still need to more research on the investigations of biochemical pathway of micro plastic on seafood. This research aims to investigation of the presence of micro plastic in different kind of seafood.

## MATERIALS AND METHODS

Commercial seafood products; 2 different canned tuna,2 fillets vacuum packed sea bream were obtained from local supermarket located in Ankara, Turkey. All reagents used were in micro plastic extraction process of analytical grade and obtained from Sigma-Aldrich, Inc. and Merck. Can filling oil drained from canned tuna sample, then fish muscle dried for the frozen sea bream sample, the fish muscle remained at the room temperature until fish fillets reached to room temperature and moisture removed by sterile filtering paper. The micro plastic extraction process performed according to Diaz-Basantes et al.,(2022) methods; Drained canned tuna sample lyophilized at -80 ◦ C during 24 hours by a lyophilizator (Teknosem- TRS 2/2V). Then freeze dried fish sample suspended in distilled water (1:20,w/v). The 30% $H_2O_2$ was added to mixture at 1:10 rate to denaturation of protein found in the fish tissue. Following to three days of reaction, 10% potassium hydroxide (KOH) solution (Barboza et al., 2018). The mixture was incubated at 60 ◦C during 15 minutes for removing of the organic material (Karami et al., 2017).Following to incubation, all the heated mixture was filtered under vacuum through glass-microfiber filter membranes. Then, the filtrates were dried at the 40 ◦C during 24 h in glass petri dishes. Then dried filter membranes were visualised on a stereo microscope ZTX-20 morphologically depending on the colour or shape. Then micro plastic fragments characterized by Confocal-Raman NRS-4500 JASCO with different laser wavelengths from 532 to 785 nm (Xi et al., 2019).

## RESULTS and DISCUSSION

The Microplastic presence in the different seafood sample represented different packaging material and fish species are given in Table 1. The detected microplastics were Polyethylene (PE), Polyethylene Terephthalate (PET), Cellophane Polystyrene (PS) and Polyvinyl Chloride (PVC) in the tested sample.As shwon in figure 1, except Polyvinyl Chloride (PVC), other microplastic levels were found higher in the vacuum packaged sea bream than canned tuna samples regardless of brand.Due to the degradation of microplastic differ depending on processand storage conditions and packaging materials that the fish stored in (Smith et al., 2018;Alak et al.,2021).

**Table 1**. Micro plastic proportion (%) in the tested sample

| MPs proportion (%) | Polyethylene (PE) | Polyethylene Terephthalate (PET) | Cellophane Polystyrene (PS) | Polyvinyl Chloride (PVC) |
|---|---|---|---|---|
| **C1** | 25.4 | 26.7 | 12.1 | 31.4 |
| **C2** | 21.8 | 25.2 | 11.8 | 30.5 |
| **F1** | 38.3 | 39.9 | 24.1 | 28.7 |
| **F2** | 34.4 | 32.4 | 22.8 | 29.4 |

Groups: C1: Canned tuna from brand 1, C2: Canned tuna from brand 2; F1: Frozen vacuum packaged sea-bream fillet from brand 3; F2: Frozen vacuum packaged sea-bream fillet from brand 4.

As shown in figure 1, the micro plastic degradation was differed by the brand that the commercial seafood sample obtained which can be explained by the presence of micro plastic driven by processing line , used fish sample or packaging material even represent as same product.

## CONCLUSION

The results revealed that the biochemical pathway of micro plastic differed by several factors. In this research, samples from canned fish and vacuum packaged fish assessed as a representative sample from commercial seafood products. Further research would be conducted the proximal differences, the origin and aquaculture process of fish species and the whole process line factors on the bioaccumulation of micro plastic in seafood.

## REFERENCES

Alak G, Köktürk M, Atamanalp M (2021). Evaluation of different packaging methods and storage temperature on MPs abundance and fillet quality of rainbow trout. Journal of Hazardous Materials, 420: 126573.

Almeida C, Loubet P, da Costa T P, Quinteiro P, Laso, J, Baptista de Sousa, D., ... Marques, A. 2021. Packaging environmental impact on seafood supply chains: A review of life cycle assessment studies. Journal of Industrial Ecology.

Badea S L, Niculescu VC, 2022. Recent Progress in the Removal of Legacy and Emerging Organic Contaminants from Wastewater Using Metal–Organic Frameworks: An Overview on Adsorption and Catalysis Processes. Materials, 15(11), 3850.

Barboza L G A, Lopes, C, Oliveira P, Bessa F, Otero V, Henriques, B, Guilhermino, L. 2020. Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. Science of the Total Environment: 717, 134625.

Diaz-Basantes M F, Nacimba-Aguirre D. Conesa, JA. Fullana A 2022. Presence of microplastics in commercial canned tuna. Food Chemistry: 385, 132721.

Karami A. Golieskardi A. Choo C. K. Romano, N. Ho YB, Salamatinia B. 2017. A high-performance protocol for extraction of microplastics in fish. Science of the total environment, 578; 485-494.

Kwon JH, Kim JW, Pham T D, Tarafdar A. Hong S, Chun S H, Jung J. 2020. Microplastics in food: a review on analytical methods and challenges. *I*nternational journal of environmental research and public health, 17(18); 6710.

Lee SY. Woo S Y.Tian F Park J B, Choi K S, Chun H S2022. Simultaneous determination of okadaic acid, dinophysistoxin-1, dinophysistoxin-2, and dinophysistoxin-3 using liquid chromatography-tandem mass spectrometry in raw and cooked food matrices. Food Control, 139; 109068.

Li Q, Sun C,Wang Y Cai H, Li L, Li J, Shi H. 2019 Fusion of microplastics into the mussel byssus. Environ.Pollut., 252;420–426.

Novakov N J, Mihaljev Ž A, Kartalović BD, Blagojević B J, Petrović JM, Ćirković MA,Rogan DR, 2017. Heavy metals and PAHs in canned fish supplies on the Serbian market. *Food Additives &* Contaminants: Part B, 10(3); 208-215.

Silva DM, Almeida CMR, Guardiola F, Rodrigues SM, Ramos S.2022. Optimization of an Analytical Protocol for the Extraction of Microplastics from Seafood Samples with Different Levels of Fat. Molecules, 27; 5172.

Su L, Xue Y, Li L,Yang D,Kolandhasamy P, Li D, Shi H. 2016Microplastics in Taihu Lake, China. Environ.Pollut, 216, 711–719.

Sridhar A, Kannan D, Kapoor, A, Prabhakar S 2022. Extraction and detection methods of microplastics in food and marine systems: a critical review. Chemosphere, 286;131653.

Toussaint B, Raffael B, Angers-Loustau A, Gilliland D, Kestens V, Petrillo M, Van den Eede G. 2019. Review of micro-and nanoplastic contamination in the food chain. Food Additives & Contaminants: Part A, 36(5); 639-673.

Xi S C, Zhang X, Luan Z D, Du Z F, Li L F, Liang Z W, Lian C, Yan J. 2019. Micro-Raman study of thermal transformations of sulfi de and oxysalt minerals based on the heat induced by laser. Minerals , 9 (12): 751, https:// doi.org/10.3390/min9120751.

*ORAL PRESENTATION*

**Veteriner jinekolojide alfa-fetoproteinin kullanımı**

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**Özet**

Alfa-fetoprotein (AFP) fetüse özgü bir protein olarak ilk kez 1956 yılında tanımlanmıştır. Başlıca embriyonik kese ile fetal karaciğerden sentezlenen AFP'nin plasenta, fetal gastrointestinal sistem ve böbreklerden de salgılandığı bildirilmiştir. Kesin olarak günümüzde fizyolojik işlevleri netlik kazanmış değildir. Tümör belirteci olarak da kullanılan AFP, beşeri hekimlikte gebelik tarama testi parametresi olarak 1970'li yıllardan itibaren kullanılmaya başlanmıştır. İnsanlarda olduğu gibi evcil memeli hayvanlarda da, maternal kan ve fetal sıvılarda önemli bir belirteç olduğu yapılan çalışmalarla vurgulanmaktadır. Veteriner reprodüksiyonunda son yıllarda kısraklarda yapılan çalışmalar ile AFP' nin gebelik patolojilerindeki (embriyonik ölüm, abortus, plasentitis, ikiz gebelik) önemi ayrıntılı olarak incelenmeye alınmıştır. Tıpkı gebelik patolojisi gözlemlenen kadınlarda olduğu gibi, hayvanlarda (gerek deneysel gerekse spontan gelişen gebelik patolojilerinde) da AFP değerlerinde değişimler rapor edilmiştir.

**Anahtar Kelimeler:** Alfa-fetoprotein, Kısrak, Tümör, Gebelik Patolojileri, Plansenta

**Use of alpha-fetoprotein in veterinary gynecology**

**Abstract**

Alpha-fetoprotein (AFP) was first described in 1956 as a fetal-specific protein. It has been reported that AFP, which is mainly synthesized from the embryonic sac with fetal liver, is also secreted from the placenta, fetal gastrointestinal tract and kidneys. Precisely, its physiological functions are not clear today. AFP, which is also used as a tumor marker, has been actively used as a pregnancy screening test parameter in human medicine since the 1970s. It is emphasized by studies that it is an important marker in maternal blood and fetal fluids in domestic mammals as well as in humans. In veterinary reproduction, the importance of AFP in pregnancy pathologies (embryonic death, abortion, placentitis, twin pregnancy) has been examined in detail with studies conducted in mares in recent years. Just as in women with pregnancy pathology, changes in AFP values have been reported in animals (both experimental and spontaneous pregnancy pathologies).

**Keywords:** Alpha-fetoprotein, Mare, Tumor, Pathologies of Pregnancy, Plansenta

## GİRİŞ

İlk kez 1956 yılında Bergstrand ve Czar tarafından fetal bir protein olarak tanımlanan, insan alfa-fetoproteininin molekül ağırlığı ortalama 69.000 (65.000-72.000) dalton olarak (Milunsky, 1986) bildirilmiştir. Gitlin ve Boesman, Masopust ve ark., Zizkovsky ve Masopust, gibi bilim insanları tarafından ise, insanların dışında diğer memeli türlerinde de AFP'nin var olduğu ispat edilmiştir (Gitlin ve Boesman, 1967; Zizkovsky ve Masopust, 1974). Fetal serumun (pH 8,6)'da elektroforezinde, serum albümininden yavaşken alfa-1-globulininden daha hızlı göç ettiğinin saptanmış olması ve alfa globülinlerin bölgesinde bulunması sebebiyle bu fetal proteine ''alfa-fetoprotein-AFP'' denilmiştir (Bergstrand ve Czar, 1956; Masopust ve ark.1971). Fetal serum globülinlerinin % 90'ını oluşturan AFP, fetüse özgü olarak erken fetal hayatta dolaşımda en fazla bulunan proteindir (Milunsky ve Alpert, 1974).

Başlıca embriyonik kese ve fetal karaciğerden sentezlenen AFP, kopulasyondan 29 gün sonra fetal serumda belirlenecek düzeylere ulaşmaktadır. Fetal gastroisntestinal sistem, böbrekler ve plasentadan da AFP'nin sentezlendiği araştırmacılar tarafından bildirilmiştir (Milunsky ve Alpert, 1974).

Beşeri jinekolojide, gebeliğin ikinci trimester üçlü tarama testinde ölçümü yapılan maternal serum AFP ile gebeliklerin risk durumları ve fetal anomaliler hakkında bilgi sahibi olunabilmektedir. Başlıca fetal ve neoplastik oluşumlar sonucu gözlemlenen maternal serum AFP artışları, plasental veya maternal kaynaklı olarak da gözlemlenebilmektedir. Aynı zamanda karaciğer dokusu kaynaklı karsinomlarda, hepatitte ve sirozda karaciğer kaynaklı bir AFP artışı olduğu bildirilmiştir (Lamerz ve Fateh-Moghadam, 1975). İkiz/çoğul gebelikler, fetal tümörler, fetal anomaliler ve fetal ölümler sonucunda maternal kanda AFP artışı fetal kaynaklı olarak gözlemlenir. Ayrıca maternal AFP artışları, tümöral hastalıklar, endojen biyolojik değişiklikler, karaciğer metastazik tümörleri ve hepatit gibi durumlarda da gözlemlenebilmektedir. Plasenta defektleri, hemanjiom, anormal lokalizasyonlar (ektopik gebelikler) ve prematüriteden kaynaklı olarak maternal serumda AFP artışı gözlemlenebilmektedir (Yılmaz, 2005). McDowell ve ark. (1995) tarafından, atlarda AFP ağırlığının, 68,350 dalton olduğu bildirilmiştir. Sığırlarda gebelikte AFP varlığı Smith ve ark. (1979) tarafından fetal sıvılarda tespit edilirken, Butler ve ark. (1982) tarafından plasental dokularda tespit edilmiştir. Beriot ve ark. (2014) ise alageyik plasentasında AFP'yi izole etmişlerdir. Tavşanlarda gebelikte AFP piklerinin görüldüğü bildirilmiştir (Clarke, 1980).

Fetal karaciğerden AFP'nin dışında, alfa 1 antitripsin, prealbumin, albümin, alfa 2 makroglobulin, C1 esteraz inhibitör, hemopeksin, transferin ve betalipoprotein gibi birçok plazma proteinleri sentez edilmektedir. Fetal karaciğer gebeliğin ilerlemesiyle AFP'nin başlıca sentezlendiği yer olur (Gitlin ve Biasucci, 1969). Yapılan çalışmalar memelilerde amniyotik ve allantoik sıvılarda da gebelik boyunca bulunan AFP nin önemli bir protein olduğunu savunmaktadır (Okano ve ark. 1978; Smith ve ark. 1979; Luft ve ark. 1984; Deutsch, 1991).

Gebelik sırasında anne ve yavrunun hayatını tehlikeye sokabilecek anomaliler ve hastalıklar gibi istenmeyen olayların hepsi, gebelik patolojisi olarak tanımlanmaktadır. Gebeliğin herhangi bir zamanında ortaya çıkabilen bu patolojik durumlar, plasenta ile ilgili bozukluklar, yavru ile ilgili hastalıklar ve anne ile ilgili hastalıklardan kaynaklı olabilmektedir (Erk ve ark. 1972; Liepold ve Dennis, 1993; Güvenç ve Tekeli, 2015). Yapılan çalışmalar AFP' nin gebelik patolojilerinde tanı amacıyla kullanılabileceğini göstermektedir.

Özellikle ülke ekonomisine büyük katkı sağlayan sığır ve yarış atı yetiştiriciliğinde yavru kayıpları ile sonlanan gebelik patolojileri ekonomik kayıp olarak görülmektedir. Yapılan bu derleme makalesi ile AFP'nin veteriner reprodüksiyonundaki öneminin vurgulanması amaçlanmıştır.

## MATERYAL VE METOT

Gebelik patolojileri, hem yavrunun hem de annenin hayati tehlikesini barındıran istenmeyen kötü durumlardır. Beşeri hekimlikte prenatal tanı yöntemleri gelişmiştir. Özellikle kromozomal defektli, fetal anomalili gebeliklerin teşhisinde amniyosenteze ışık tutmak açısından, maternal kan tarama testleri aktif olarak kullanılmaktadır. Veteriner jinekolojisinde gebelik patolojilerinin tespitinde, laboratuvar testlerinin yanı sıra aktif olarak ultrasonografik muayeneler yapılmaktadır. Ultrasonografik muayenelerde gebelik teşhisi, yavru canlılığı, yavru sayısı, gebelik yaşı, plasentit, fetal acaibatlar gibi gebelik patolojileri gözle tespit edilirken kromozomal defektli yavruların tespiti yapılamamaktadır. Beşeri hekimlikteki gibi gebeliğin erken dönemlerinde yapılan maternal serum AFP ölçümleri ile gebeliklerin seyrinin değişebileceği, yavru kayıpları ve anne ölümlerinin önüne geçilebilineceği aşikârdır.

## BULGULAR ve TARTIŞMA

Beşeri hekimlikte yapılan çalışmalar ile gebelikte gözlemlenen neonatal enfeksiyonlarda,fetal gelişimde patolojik bozukluklarda, kongenital nefroz, ıntrauterin fetal ölüm, omfalosel, duodenal veya özofagus atrezisi, hidrosefali, spina bifida veya açık nöral tüp defektleri (anensefali, meningosel, miyelosel), down sendrom, plasenta patolojileri gibi durumlarda amniyotik sıvıda ve kanda AFP değerlerindeki değişimleri bizlere sunmaktadır (Seppala, 1975; Norgaard-Pedersen ve ark., 1976; Kupferminc ve ark., 1993).

Jilin ve ark.(2019) yapmış oldukları çalışmada, deneysel olarak gebe sıçanlara gebeliklerinin farklı günlerinde intraperitonal *Escherichia coli* enjekte edip, devamında ise normal salin çözeltisi ve lipopolisakkarit (LPS) ile tedavi seçeneklerinin maternal serumda AFP'ye etkisini incelemişler. Lipopolisakkarit ile tedavi edilen gebe

sıçanlarda, MSAFP seviyesinin yüksekliğinin sebebini, bozulmuş plasental bariyer nedeniyle, artan fetal-maternal AFP transferinin gerçekleşmesi sonucunda görüldüğünü bildirmişlerdir (Jilin ve ark., 2019).

Sığırlarda deneysel olarak *Aspergillus fumigatus*, *Sarcocystis cruzi*, *Campylobacter* ile enfekte edilen, plasentit olgularında, AFP seviyelerinde artış gözlendiği ve gebeliklerin fetal kayıplarla son bulduğu bildirilmiştir (Baetz ve ark., 1981). Plasentitisli kısraklarda AFP ölçümleri yapılmış ve maternal kanda, allantoik ve amnion sıvılarında arttığı rapor edilmiştir (Canisso ve ark. 2015). Vincze ve ark. (2015) gebe kısraklarda sorunlu gebeliklerde MSAFP düzeylerinin, sağlıklı gebelik sürdüren kısraklardakinden daha yüksek olduğun ayrıca kısrakların yaşı ile AFP arasında negatif bir korelasyon gözlemlediklerini bildirmişlerdir. Vincze ve ark. (2016) yapmış oldukları çalışmalarında kısraklarda ikiz gebeliklerde AFP oranının tekli gebeliklere göre yüksek olduğunu bildirmişlerdir.

## SONUÇ

Alfafetoprotein, kadın gebeliklerinin seyrinde prenatal tarama parametresi olarak kullanılmakta ve birçok fetal anomali ve gebelik patolojisinin teşhisinde hekimlere fayda sağlamaktadır. Alfafetoproteinin veteriner jinekolojisinde henüz insan jinekolojisindeki gibi önemi tam bilinmemektedir. Özellikle veteriner reprodüksiyonunda bu alanla ilgili birçok çalışmaya ihtiyaç duyulmaktadır. Yapılacak çalışmalarla beşeri hekimlikte olduğu gibi AFP'nin veteriner hekimlikteki önemi gün geçtikçe artacaktır. Yapılacak çalışmalarla, veteriner alanında gebelik patolojileri, yavru kayıpları ve damızlık hayvan kayıplarının önüne geçebilecek bir prenatal tarama parametresi olarak, AFP'nin klinik kullanımdaki öneminin anlaşılabileceği sonucuna varıldı.

## KAYNAKLAR

Baetz A.L., Crandell S.E., Schmer M.J., Barnett D., Bryner J.H., 1981. Plasma alphafetoprotein concentrations in pregnant cows exposed to Sarcocystis cruzi Campylobacter fetus, or Aspergillus fumigatus. Am. J. Vet. Res. 42: 2146–2148.

Beriot M., Tchimbou A.F., Barbato O., Beckers J.F., De Sousa N.M., 2014. Identification of pregnancy-associated glycoproteins and alpha-fetoprotein in fallow deer (Dama dama) placenta. Acta Vet. Scand. 13 (56): 4.

Bergstrand C. G. ve Czar B. 1956. Demonstration of a new protein fraction in serum from the human fetus. Scandinavian Journal Clinical Laboratory Investigation, 8 (2):174.

Butler J.E., Hamilton W.C., Sasser R.G., Ruder C.A., Hass G.M., Williams,R.J. 1982. Detection and partial characterization of two bovine pregnancy-specific protein. Biol. Reprod. 26: 925–933.

Canisso I. F., Ball B. A., Scoggin K. E., Squires E. L., Williams N. M., Troedsson M. H. 2015. Alpha-fetoprotein is present in the fetal fluids and is increase in plasma of mares with experimentally induced ascending placentitis. Animal Reproduction Science, 154: 48-55.

Clarke L.E., 1980. The distribution of rabbit alpha-foetoprotein (RAFP) and its molecular variants throughout gestation. J. Reprod. Immunol. 2:199–211.

Deutsch H. F. 1991. Chemistry and biology of alpha-fetoprotein. Advances in Cancer Research, 56: 253-312.

Erk H., Doğaneli M., Akkayan C. 1972. Gebelikte ve Doğumdan Sonra Memeler. Veteriner Doğum Bilgisi (Obstetrik) ve Jinekoloji. 2. Baskı, Ankara: Veteriner Fakültesi Yayınları; pp.189-192.

Gitlin D. ve Biasucci A. 1969. Development of gamma G., A, M, 1c, 1a, C1 esterase inhibitor, ceruloplasmine, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, alpha-1-antitrypsin, orosmucoid, beta-lipoprotein, alpha-2-75 macroglobulin and prealbumin in the human conseptus. The Journal of Clinical Investigation, 48(8): 1433-1446.

Gitlin D. ve Boesman M. 1967. Fetus specific serum proteins in several mammals and their relation to human alpha-protein. Comparative Biochemistry and Physiology, 21(2): 327-336.

Güvenç K. ve Tekeli T. 2015. Gebelik Patolojisi. İçinde Kaymaz M, Fındık M, Rişvanlı A, Köker A, (editörler). Kısraklarda Doğum ve Jinekoloji. Malatya: Medipress; pp. 115-142.

Kupferminc M.J., Tamura R.K., Wigton T.R., Glassenberg R., Socol M.L., 1993. Placenta accreta is associated with elevated maternal serum alpha-fetoprotein. Obstet. Gynecol. 82 (2): 266–269.

Lamerz R. ve Fateh-Moghadam A. 1975. [Carcinofetal antigens. I. alpha-fetoprotein (author's transl)]. Klinische Wochenschrift. 53(4):147-169.

Leipold H. W. ve Dennıs S. M. 1993. Congenital Defects ın Foals. In McKinnon, A. O., Voss, J. L. (edıtors). Equine Reproduction. Lea & Febiger, Philadelphia, London; Chapter 71: 604-613.

Luft A. J., Lai P. C., Roberston H. A., Saunders N. R., Lorscheider F. L. 1984. Distribution of alpha-fetoprotein in fetal plasma and in amniotic and allantoic fluids of the pig. Journal of eproduction and Fertility. 70(2):605-607.

Masopust J., Zizkovsky V. and Kithier K. 1971. Fetoproteins in different species of mammals. Protides of the Biological Fluids, 18: 63-74.

McDowell K. J., Adams M. H., Baker C. B. 1995. Molecular cloning and sequencing of equine AFP: synthesis of AFP by conceptus membranes and presence in fetal fluids. EMBL/GenBank/DDBJ databases. Erişim 23.09.2021, https://www.uniprot.org/uniprot/P49066/publications.

Milunsky A. 1986. The prenatal diagnosis of neural tube and other congenital defects. In: Milunsky A. (editor). Genetic Disorders and The Fetus. New York and London: Plenum Pres; 453-482.

Milunsky A. ve Alpert E. 1974. The value of alpha-fetoprotein in the prenatal diagnosis of neural tube defects. The Journal of Pediatrics, 84(6): 884-893.

Norgaard-Pedersen B., Jorgensen P.I., Trolle D. 1976. Alpha-Fetoprotein concentration in amniotic fluid during the last trimester in normal pregnancies and in pregnancies with severe fetal abnormalities. Acta. Ostet. Gynecol. Scand. 55: 59–62.

Seppala M. ve Rouslahti M. 1972. Radioimmunoassay of maternal serum alpha-fetoprotein during pregnancy and delivery. American Journal of Obstetrics and Gynecology, 112(2): 208-212.

Smith K. M., Lai P. C. W., Roberston H. A., Church R. B., Lorscheider F. L. 1979. Distribution of alpha-fetoprotein in fetal plasma, allantoic fluid, amniotic fluid and maternal plasma of cows. Journal of Reproduction and Fertility, 57(1): 235-238.

Okano A., Shioya Y., Obata T., Fukuhara R. 1978. Specific protein in bovine allantoic fluid and amniotic fluid. Japanese Journal of Zootechnical Science, 49(4): 276-282.

Vincze B., Caspardy A., Kulcsar M., Baska F., Balint A., Hegedüs G. T., Szenci O. 2015. Equine alpha-fetoprotein levels in Lipizzaner mares with normal pregnancies and with pregnancy loss. Theriogenology, 84(9): 1581-1586.

Vincze B., Gáspárdy A. ve Szenci O. 2016. Serum equine alpha-fetoprotein (AFP) levels as an indicator of twin pregnancy in the mare. (https://www.researchgate.net/profile/Boglarka-Vincze).

Yılmaz N. M. 2005. Gebelerde Üçlü Tarama Testlerinin Duyarlığının Değerlendirilmesi. Uzmanlık Tezi, İstanbul.

Zizkovsky V. ve Masopust J. 1974. The detection of specific fetal proteins in thirteen mammalian species. In Masseyeff R. (editor). Alpha-fetoprotein, INSERM, Nice: pp.125-137.

## ORAL PRESENTATION

# Sn-$Fe_3O_4$@s-rGO katalizörünün $H_2O_2$'nin elektrokimyasal tayininde performansının incelenmesi

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## Özet

Bu çalışmada hidrojen peroksitin elektrokimyasal tayini için öncelikle grafen SDBS ile fonksiyonelleştirilmiştir (s-rGO). Daha sonra sırası ile hidrotermal yöntemle $Fe_3O_4$ ve Sn grafen üzerine depolanmıştır (Sn-$Fe_3O_4$@s-rGO). Hazırlanan katalizörlerin karakterizasyonu alan emisyonlu taramalı elektron mikroskobu (FESEM), enerji dağılım X-ışını spektroskopisi (EDX), X-ışını kırınım spektroskopisi (XRD) ile incelenmiştir. Diğer taraftan katalizörlerin elektrokimyasal özellikleri, dönüşümlü voltametri (CV) ve impedans spektroskopisi ile incelenmiştir. Sn-$Fe_3O_4$@s-rGO 'nun $Fe_3O_4$@s-rGO ile kıyaslandığında düşük gözlenebilme sınırı (3µM) ile $H_2O_2$' ye karşı yüksek hassasiyet gösterdiği bulundu. Sn ile $Fe_3O_4$ arasındaki sinerjik etkinin, katalizörün $H_2O_2$ nin katakilitik indirgenmesindeki aktivitesinin arttırdığı düşünülmüştür.

**Anahtar Kelimeler:** Grafen, SDBS, $H_2O_2$ tayini, Sn-$Fe_3O_4$

# Investigation of the performance of Sn-$Fe_3O_4$@s-rGO catalyst for the electrochemical detection of $H_2O_2$

## Abstract

In this study, firstly, graphene was functionalized with SDBS for the electrochemical determination of hydrogen peroxide. Then, $Fe_3O_4$ and Sn were deposited on graphene via hydrothermal method, respectively (Sn-$Fe_3O_4$@s-rGO). Fabricated electrodes were characterized by Scanning Electron Microscopy, Energy Dispersive X-ray Spectrometry and X-ray Powder Diffraction. Besides, electrochemical properties of catalysts were investigated by cyclic voltammetry (CV) and impedance spectroscopy. It was found that Sn-$Fe_3O_4$@s-rGO showed high sensitivity toward $H_2O_2$ with low LOD value (3µM) compared to $Fe_3O_4$@s-rGO. It was concluded that the synergistic effect between Sn and $Fe_3O_4$ promotes the catalytic activity of catalysis for $H_2O_2$ reduction.

**Keywords:** Graphene, SDBS, determination of $H_2O_2$, Sn-$Fe_3O_4$

## GİRİŞ

Hidrojen peroksit, oldukça reaktif ve zararlı hidroksil radikallerinin oluşumu sırasında bir analit görevi görür. Öte yandan, hidrojen peroksit gıda endüstrisinde, biyoteknolojide, kâğıt imalatında ve kimya ve ilaç endüstrilerinde yaygın olarak kullanılmaktadır. $H_2O_2$ miktarının yüksek olması ciddi sağlık sorunlarına yol açtığı için düşük miktarda bile belirlenmesi önemlidir. Bugüne kadar birçok kalitatif $H_2O_2$ tayini kullanılmıştır. Bunların arasında elektrokimyasal teknik, kullanım kolaylığı, hızlı tepkisi, yüksek hassasiyeti ve seçiciliği nedeniyle büyük ilgi görmüştür (Tavakkoli ve ark., 2020). Elektrokimyasal yöntemde, üretilen elektrot $H_2O_2$'ye karşı yüksek tepki ve yüksek kararlılık ve seçicilik göstermelidir. Sensör uygulamalarında kullanılmak üzere hazırlanan elektrotların seçiciliği ve hassasiyeti kullanılan elektrotun iletkenliğine ve hedef moleküle olan ilgisine bağlı olarak değişmektedir (Liu ve ark., 2018).

Geçiş metal oksitlerinden $Fe_3O_4$ son yıllarda $H_2O_2$' in elektrokimyasal algılanmasında kimyasal kararlılığı, toksik ve kanserojen olmaması itibariyle yoğun çalışılmaktadır. Aynı zamanda yapılan çalışmalarda $Fe_3O_4$' ün $H_2O_2$' nin bozunmasında katalitik aktivite gösterdiği belirlenmiştir (Cao ve ark., 2014). Cao ve ark., literatüre ilk kez nano boyuttaki $Fe_3O_4$ eldesi için geliştirdikleri yöntem ile spinal yapıda ve 40-50 nm boyutta sentezledikleri $Fe_3O_4$ katalizörünü $H_2O_2$ nin indirgenmesi için incelemişler ve $H_2O_2$ tayininde sensör olarak

kullanılabileceğini göstermişlerdir. Salman ve ark., $Fe_3O_4$ ile doplanmış MOF sentezleyerek elde ettikleri MIL-101(Fe)@$Fe_3O_4$/NGC elektrodunun $H_2O_2$nin elektrokimyasal tayininde sensor olarak işlev yapabileceği araştırılmıştır. 1.76µM gibi düşük derişimindeki $H_2O_2$' yi bile algılayabildiği gözlenmiştir (Salman ve ark., 2020).

Grafenin yüksek iletkenlik, kararlılık ve geniş yüzey alanına sahip olması son derece ilgi çekmektedir. Bunun yanında grafenin SDBS, TRİTON-X gibi yüzey aktif maddeler ile fonsksiyonelleştirilmesi sonucu grafenin özellikleri iyileşmektedir (Agarwal ve ark., 2021). Dolayısıyla bu durum metallerin ve metal oksitlerin homojen olarak depolanmasında bir destek materyali olarak yaygın kullanılmasını da beraberinde getirmektedir (Rahmani ve ark., 2020). Literatürde $Fe_3O_4$' ün grafen tabakasına depolanması sonucu iletkenlik, kararlık, katalitik aktivite gibi özelliklerinin arttığı belirtilmiştir (Su ve ark., 2011).

Bu çalışmada $H_2O_2$ tayini dışındaki farklı reakisyonlarda $Fe_3O_4$ ile sinerjik etki gösterdiği belirlenen Sn, diğer çalışmamızda sentezlenen $Fe_3O_4$@s-rGO yüzeyine hidrotermal olarak depolanmıştır. Elde edilen Sn-$Fe_3O_4$@s-rGO' nin indirgenmesinde aktivitesi incelenmiş ve sensör olarak performansı incelenmiştir.

## MATERYAL VE METOT

### Materyal

Grafit tozu (325 mesh), demir (III) klorür hekzahidrat ($FeCl_3.6H_2O$), sodium asetat (NaAc), sodium dodekilbenzensulfanat (SDBS), % 80' lik hidrazin çözeltisi, Kalay (II)klorür dihidrat ($SnCl_2.2H_2O$), sodyum hidroksit, etanol, metanol, sülfürik asit, L-askorbik asit ve etilenglikol Sigma-Aldrich firmasından tedarik edilmiştir. Ayrıca Nafyon çözeltisi Ion Power'dan satın alınmıştır.

### s-rGO sentezi

Öncelikle grafen oksit(GO) Hummers metodu ile sentezlenmiştir. Daha sonra 150 mg GO 120 ml distile su içinde çözündüktekn sonra 140 mg SDBS eklenmiştir ve sonik banyoda 2 saat karıştırılmaya bırakılmıştır. Homojen bir çözelti eldesinden sonra %80' lik hidrazin çözeltisi eklenmiştir. Hazırlanan çözelti 24 saat manyetik karıştırıcıda karışmaya bırakılmıştır. .Elde edilen karışım $Fe_3O_4$@*s*-rGO sentezinde kullanılmıştır.

### $Fe_3O_4$@s-rGO sentezi

1.35g $FeCl_3.6H_2O$ and 3.60g NaAc 32 ml etilen glikol içerisinde çözülmüştür. sonra. Bu karışım önceden hazırlanan S-rGO çözeltisine eklenmiş and 15 dakiaka boyunca karıştırılmıştır. Daha sonra bu karışım Teflon hücreye alınarak 180 °C de 18 saat boyunca bekletilmiştir. Karışımın soğuması beklendikten sonra elde edilen katı partikül içeren çözelti sanrtfifujlenmiş ve bir gece 70 °C lik etüvde bekletilmiştir.

### Sn-$Fe_3O_4$@s-rGO sentezi

50 mg $Fe_3O_4$@s-rGO 60 mL etilen glikol içerisinde tamamen çözündükten sonra 85 mg $SnCl_2.2\ H_2O$ bu karışıma eklenir ve iyice karışması için bir saat ultrasonikte bekletilir. Daha sonra 0.1 g L-askorbik aist eklenerek çözelti karıştırılır. Karışım homojen hale geldiğinde Teflon hücreye alınarak 180 °C' de 2 saat boyunca bekletilmiştir. Karışımın soğuması beklendikten sonra katı partikül içeren çözelti sanrtifujlenmiş ve bir gece70 °C lik etüvde bekletilmiştir.

### Çalışma elektrodunun hazırlanması

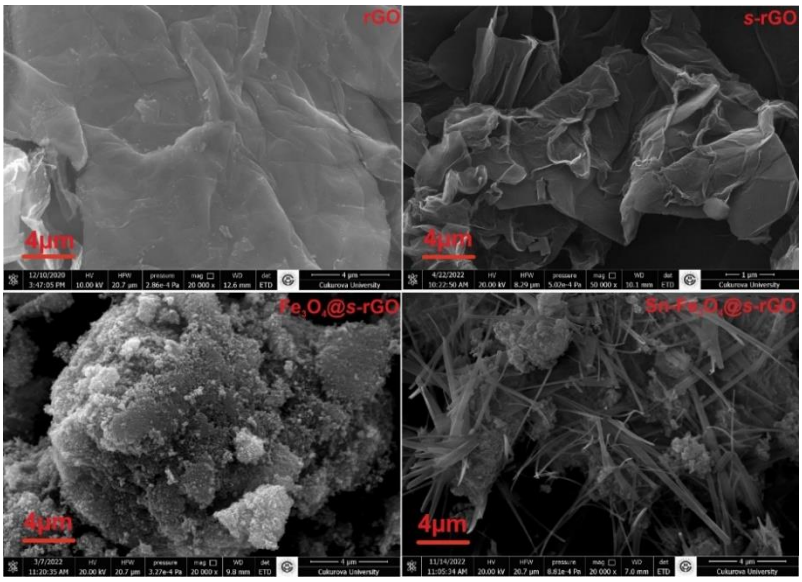
Öncelikle camsı karbon elektrot (GCE) alümina (50 nm) ile temizlendikten sonra alkol çözeltisinde 5 dakika bekletilerek katalizörün yüklenmesi için temiz bir yüzey elde edilmiştir. Sn-$Fe_3O_4$@s-rGO elektrodu için 5 mg Sn-$Fe_3O_4$@s-rGO 1:1 oranında karıştırılmış etanol-su ve 10 µL Nafion (Ion Power %15 alkol) karışımı homojen hale gelene kadar sonik banyoda bekletilmiş ve ardından mikropipetle 10 µL karışım camsı karbon elektrot yüzeyine damlatılarak kuruması için oda sıcaklığında bekletilmiştir. Kıyaslamak için $Fe_3O_4$@s-rGO elektrot aynı prosedür ile hazırlanmıştır.

### Elektrokimyasal Ölçümler

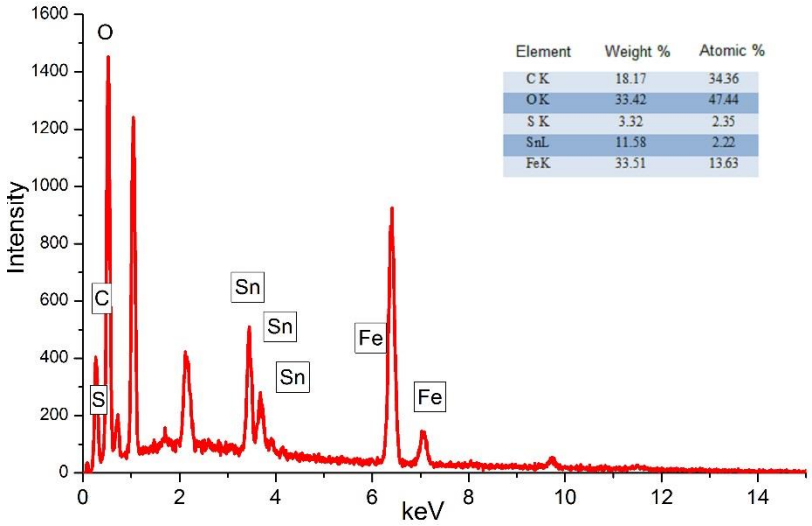
Elektrokimyasal ölçümlerinde çalışma elektrodu olarak GCE, karşı elektrot olarak Pt ve referans elektrot olarak Ag/AgCl (3,5M KCl) kullanılmıştır. Hazırlanan katalizörlerin elektrokatalitik aktivitesi dönüşümlü voltametri (CV), ve impedans spektroskopisi kullanılarak aydınlatılmaya çalışılmıştır. Ölçümler $N_2$ gazı geçirilmiş 0.1M PBS çözeltisi (pH=7) ile hazırlanan farklı derişimlerdeki hidrojen peroksit ortamında kaydedilmiştir.

## BULGULAR VE TARTIŞMA

rGO ve s-rGO, $Fe_3O_4$@s-rGO ve Sn-$Fe_3O_4$@s-rGO katalizörlerine ait SEM görüntüleri şekil 1. de verilmiştir. Grafenin SDBS ile fonksiyonelleştirilmesi sonucu grafen tabakaların katmanlarının açıldığı gözlenmiştir. Buda aktif yüzey alanının arttığını göstermektedir. Diğer taraftan $Fe_3O_4$ grafen tabakasına noktasal olarak depolanırken kalayın nano fibriller şekilde depolandığı gözlenmiştir. Sn-$Fe_3O_4$@s-rGO' e ait EDX spektrumu incelendiğinde katalizörde C, O, S, Sn ve Fe elementlerinin varlığı görülmektedir. S elementinin SDBS den ileri geldiğini, O miktarının yüksek çıkmasın da demirin oksitli halde bulunduğunu göstermektedir. Aynı zamanda EDX sonucuna göre (Şekil 2) demir miktarının % kütlesel olarak kalaydan yaklaşık üç kat daha fazla olduğu anlaşılmıştır.

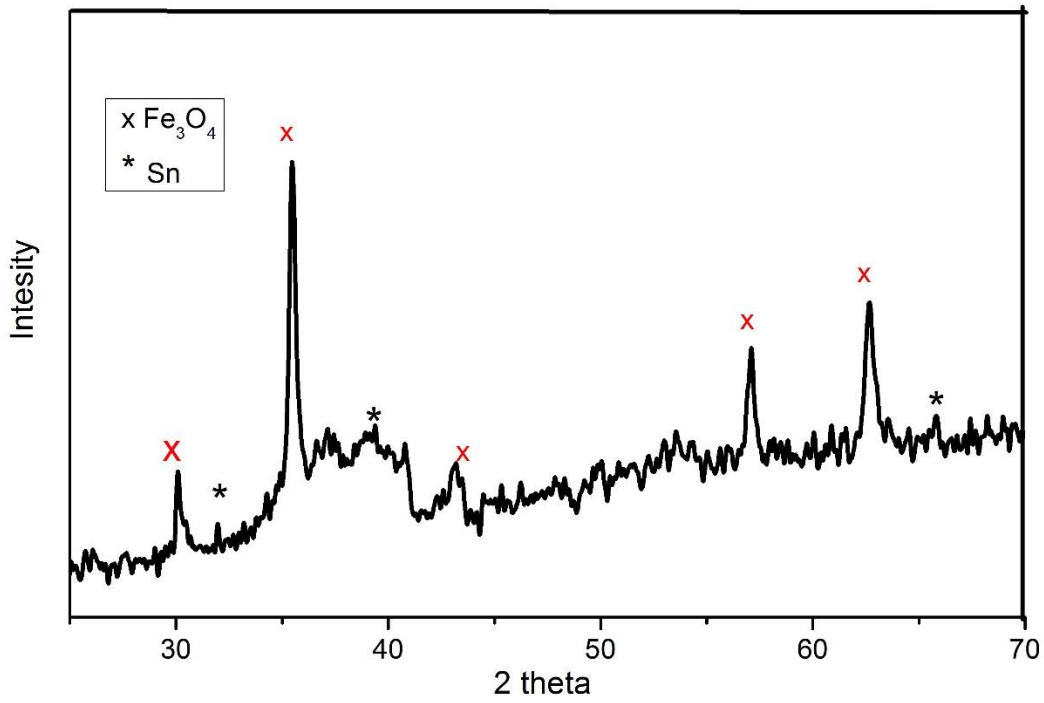



**Şekil 1.** rGO ve s-rGO, $Fe_3O_4$@s-rGO ve Sn-$Fe_3O_4$@s-rGO katalizörlerine ait SEM görüntüleri



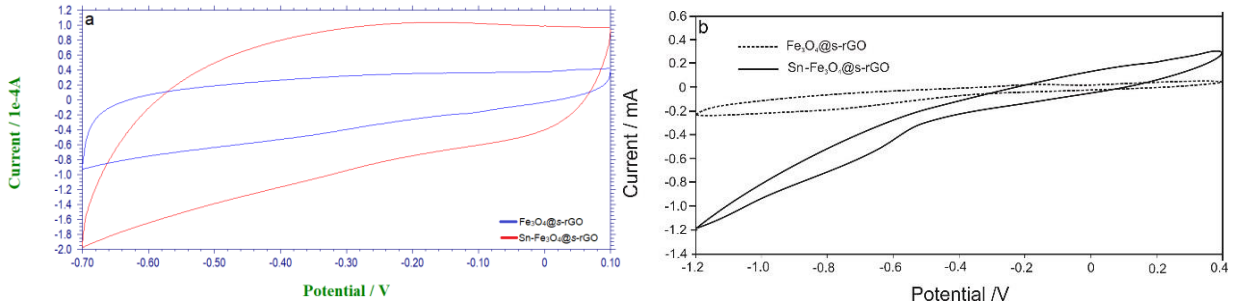

**Şekil 2.** Sn-$Fe_3O_4$@s-rGO' ye ait EDX spektrumu

Sn-$Fe_3O_4$@s-rGO' ya ait XRD spektrumu Şekil 3.' te verilmiştir. $Fe_3O_4$' e ait pik şiddetlerin kalaya ait pik şiddetlerinden daha kuvvetli olduğu görülmektedir. Bu durum $Fe_3O_4$' ün katalizör içerisinde kalaya nazaran daha fazla miktarda bulunduğunu göstermektedir. Spektrumda 2θ= 30.26 º, 35.2 º, 43.10, º57.11 º, 62.54 º de yer alan piklerin $Fe_3O_4$' e ait (2 2 0), (3 1 1), (4 0 0) (5 1 1), (4 4 0) kristal yapılarını simgelemektedir (Yetim ve ark., 2020). Diğer taraftan 2θ=31.9 º, 39.3º ve 65.7º de yer alan pikler Sn' ye ait piklerdir (JCPDS:4-673).



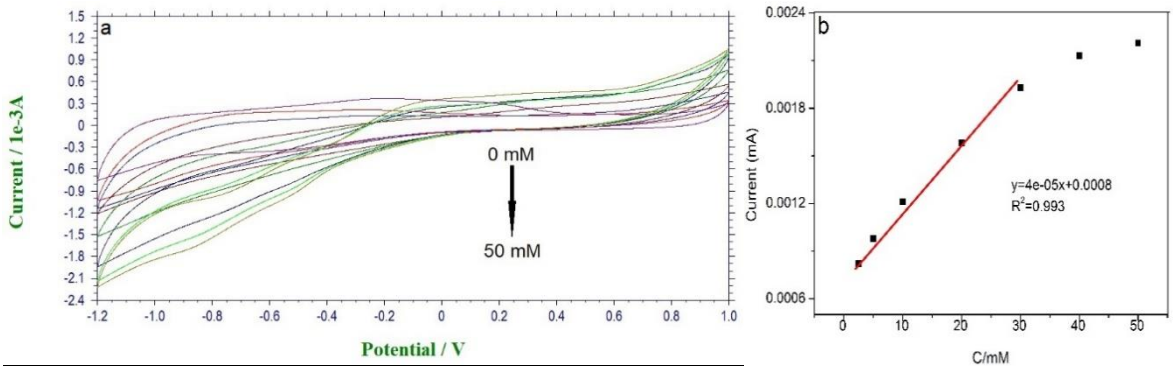

**Şekil 3.** Sn-$Fe_3O_4$@s-rGO ye ait XRD spektrumu

Şekil 4.' te $Fe_3O_4$@s-rGO ve Sn- $Fe_3O_4$@s-rGO' ya ait 0.1 M PBS ortamında alınmış CV' ler görülmektedir. Sn varlığıyla birlikte $Fe_3O_4$@s-rGO' ya ait akımın arrtığı gözlenmiştir. Bu durum Sn- $Fe_3O_4$@s-rGO'nın $Fe_3O_4$@s-rGO' ya kıyasla daha elektroaktif olduğunu göstermektedir.
Şekil 4.' te 20mM $H_2O_2$ ortamında alınmış dönüşümlü voltamları incelendiğinde katodik bölgede Sn-$Fe_3O_4$@s-rGO' ya ait akım artışının daha fazla olduğu gözlenmiştir. Bu durum Sn-$Fe_3O_4$@s-rGO' nun $H_2O_2$ indirgenmesinde diğer elektroda kıyasla daha fazla katalitik aktivite gösterdiği şeklinde yorumlanmıştır.



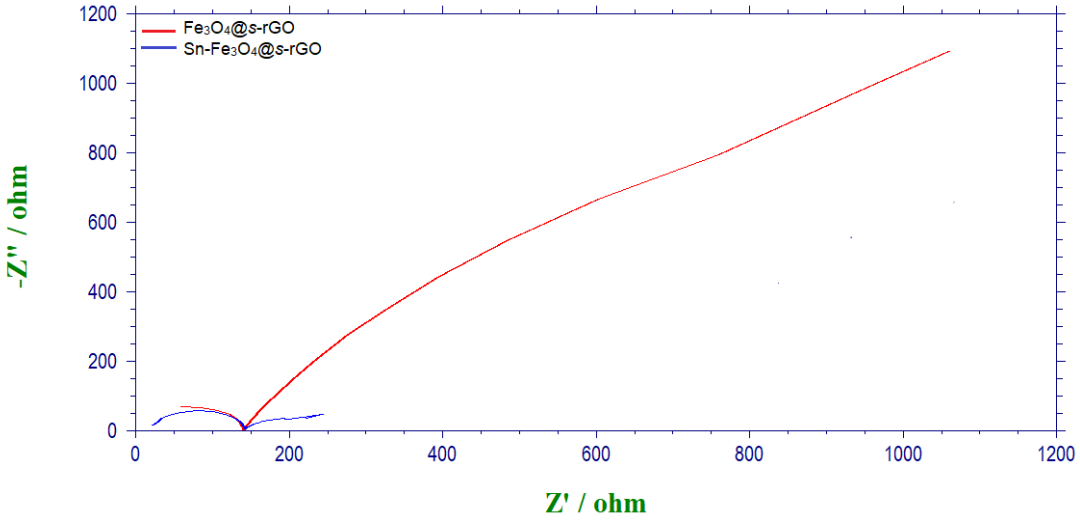

**Şekil 4. a.** Sn- $Fe_3O_4$@s-rGO ve $Fe_3O_4$@s-rGO ye ait 0.1 M PBS ortamında **b.** 20 mM $H_2O_2$ içeren 0.1 M PBS ortamında kaydedilmiş dönüşümlü voltamogramları

Sn-$Fe_3O_4$@s-rGO' un $H_2O_2$ tayininde sensor olarak kullanılabilme performansını incelemek üzere CV yönteminden faydalanılmıştır. Şekil 5.a.' da farklı derişimlerde $H_2O_2$ içeren 0.1 M PBS ortamında alınan CV' ler görülmektedir. Konsantrasyonun artması ile katodik akımında arttığı gözlenmiştir. Şekil 5b' te, bu derişimlere bağlı elde edilen akım değerleri grafiğe geçirildiğinde 0-30mM aralığında lineer bir artış olduğu görülmektedir. Bu lineer bölgeden limit dedeksiyon değeri (LOD) 3µM (S/N=3) olarak bulunmuştur.




**Şekil 5.a.** Sn-$Fe_3O_4$@s-rGO katalizörüne ait farklı $H_2O_2$ derişimleri ihtiva eden 0.1M PBS ortamında alınmış CV'ler **b.** derişime karşı kaydedilen akım değerlerinin değişiminin grafiksel gösterimi

Elektrokimyasal impedans spektroskopisi, bir reaskiyonu kinetiği hakkında bilgi vermektedir. Şekil 6'da Sn-$Fe_3O_4$@s-rGO ve $Fe_3O_4$@s-rGO' ye ait Nyquist eğrileri görülmektedir. Bahsedilen eğrileri yüksek frekans ve düşük frekans bölgesi olarak ikiye ayırmak ve incelemek mümkündür. Her iki elektrot için yüksek frekans bölgesinde tam kapanmamış bir lop ve düşük frekans bölgesinde birer kuyruk gözlenmektedir. Elde edilen eğriler Zview-2 yazılımı ile simule edilmiştir ve veriler Tablo 1'de gösterilmiştir. Her katalizör için R1 ve R2 olmak üzere iki direnç söz konusudur. R1 ile simgelenen direnç elektrodun iletkenliği ile ilgili olup, R2 direnci reaskiyon kinetiği ile ilgilidir (Çirmi ve ark, 2022). Sn ilavesi ile her iki direncinde azaldığı görülmektedir. Bu durum Sn ile $Fe_3O_4$ arasındaki sinerjik etkinin elektron haraketliliğini arttırarak katalitik aktiviteyi arttırdığı şeklinde yorumlanmıştır.




**Şekil 6.** Sn- $Fe_3O_4$@s-rGO ve $Fe_3O_4$@s-rGO' ye ait Nyquist eğrileri

**Tablo 1.** Sn-$Fe_3O_4$@s-rGO ve $Fe_3O_4$@s-rGO' ye ait Nyquist eğrilerinin simule edilmesi sonucu elde edilen veriler

| Electrode | Re | $R_1(\Omega)$ | $CPE_1$-T (µF) | $CPE_1$-P | $R_2$ (Ω) | $CPE_2$-T (mF) | $CPE_2$-P |
|---|---|---|---|---|---|---|---|
| $Fe_3O_4$@s-rGO | 1 | 138.1 | $1.34 \times 10^{-2}$ | 1.002 | 3495 | 0.64 | 0.759 |
| Sn-$Fe_3O_4$@s-rGO | 1 | 125.3 | $2.85 \times 10^{-1}$ | 0.93 | 146 | 6.3 | 0.58 |

## SONUÇLAR

Bu çalışmada, hidrotermal ve kimyasal yöntemleri içeren ve birkaç basamaktan oluşan sentez yöntemleriyle her bir katalizör örneği başarıyla sentezlendi. Karakterizasyon sonuçlarına göre, Sn ve $Fe_3O_4$ s-rGO' ya başarılı bir şekilde katkılandı. Hazırlanan katalizörlerin $H_2O_2$' ye duyarlılıkları araştırıldı ve en iyi sonucu veren katalizörün Sn ve $Fe_3O_4$ arasındaki sinerjisik etki sayesinde Sn-$Fe_3O_4$@s-rGO olduğu anlaşıldı.

Böylelikle; Sn-$Fe_3O_4$@s-rGO katalizörünün düşük algılama limiti (3µM), yüksek hassasiyet ve $H_2O_2$ 'ye karşı iyi bir elektrokimyasal tepki sağladığı gözlendi.

## KAYNAKLAR

Agarwal V, Fadil Y, Wan A, Maslekar N, Tran BN, Noor RAM 2021. Influence of anionic surfactants on the fundamental properties of polymer/reduced graphene oxide nanocomposite films. ACS Applied Materials & Interfaces, 13: 18338−18347.

Cao GS, Wang P, Li X, Wang Y, Wang G, Li J 2014. Hydrogen peroxide electrochemical sensor based on $Fe_3O_4$ nanoparticles. Micro & Nano Letters, 9: 16–18.

Çirmi D, Karatekin RS, Aydın R, Köleli F 2022. Hydrogen evolution and $CO_2$-reduction on a non-supported polypyrrole electrode. Synthetic Metals, 289: 117102.

Liu L, Zhou Y, Liu S, Xu M 2018. The applications of metal−organic frameworks in electrochemical sensors. ChemElectroChem, 5: 6–19.

Rahmani F, Nouranian S, Chiew YC 2020. 3D Graphene as an unconventional support material for ionic liquid membranes: computational insights into gas separations. Industrial & Engineering Chemistry Research, 59: 2203–2210.

Salman F, Zengin A, Kazici HÇ 2020. Synthesis and characterization of $Fe_3O_4$-supported metal–organic framework MIL-101(Fe) for a highly selective and sensitive hydrogen peroxide electrochemical sensor. Ionics, 26: 5221–5232.

Su J, Cao M, Ren L, Hu C 2011. $Fe_3O_4$_Graphene nanocomposites with ımproved lithium storage and magnetism properties. The Journal of Physical Chemistry C, 115: 14469–14477.

Tavakkoli H, Morteza A, Gholam AG, Ghodratollah A 2020. Electrochemical sensing of hydrogen peroxide using a glassy carbon electrode modified with multiwalled carbon nanotubes and zein nanoparticle composites: application to HepG2 cancer cell detection. Microchimica Acta, 108: 105.

Yetim NK, Baysak FK, Koç MM¸ Nartop D 2020. Characterization of magnetic $Fe_3O_4$@$SiO_2$ nanoparticles with fluorescent properties for potential multipurpose imaging and theranostic applications. Journal of Materials Science: Materials in Electronics, 31: 18278–18288.

## *ORAL PRESENTATION*

### Gestasyonel diyabetes mellitus hastalarında asprosin ve adiponektin hormon düzeyleri*

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*Bu çalışma Adıyaman Üniversitesi Bilimsel Araştırmalar Projeleri Birimi tarafından desteklenmiş bir yüksek lisans tezidir (TIPFYL/2019-0002).

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**Özet**

Gestasyonel diyabetus mellitus (GDM), gebelikte ortaya çıkan veya gebelikte tanısı konulan ve anne ve bebek sağlığı üzerinde olumsuz etkiler bırakabilen bir glukoz metabolizması bozukluğudur. Adipoz doku kaynaklı bir hormon olan adiponektin birçok farklı etkisinin yanında karbonhidrat ve lipid metabolizmasının düzenlenmesinde önemli rol oynar. Adipoz dokudan salgılanan asprosin karaciğerden glukoz salınımını artırıcı ve beta hücrelerinden insülin salgılanmasını olumsuz etkileyen bir hormondur. Bu çalışmada, gestasyonel diyabetus mellituslu hastalarda antidiyabetojenik etkili olarak değerlendirilen adinopektin ve diyabetojenik etkili asprosin seviyelerinin belirlenmesi ve hastaların klinik özellikleri ile karşılaştırılarak GDM patogenezindeki rollerinin belirlenmesi amaçlanmıştır.
Çalışmaya Adıyaman Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum polikliniğinde GDM tanısı almış 44 gebe ile 44 sağlıklı gebe olmak üzere toplam 88 birey dahil edildi. Serum adiponektin ve asprosin seviyelerinin belirlenmesi amacıyla hastaların serum örnekleri toplandı ve çalışma yapılana kadar -80 $^0$C de muhafaza edildi. Serum adiponektin ve asprosin konsantrasyonlarının ölçümü insana özgü kitler kullanılarak ELISA yöntemi ile yapıldı. Hasta ve sağlıklı gruptan alınan kan serumlarından adiponektin ve asprosin düzeyleri karşılaştırıldı. Ayrıca gruplar yaş, gebelik haftası, gravide, parite, abort ve vücut kitle indeksi kriterleri bakımından karşılaştırıldı.
Gestasyonel diyabeti olan hasta grubunda normal gebelere göre serum adiponektin düzeyi istatistiksel olarak anlamlı derecede düşük bulundu (1,58±0,43 mg/L ve 1,85±0,49 mg/L, p=0.0095). Serum asprosin düzeyleri GDM grubunda kontrol grubuna göre istatistiksel olarak anlamlı olmamakla birlikte yüksek bulundu (1,11±0,88 ng/L ve 0,76±0,75 ng/L, p=0.0933).
Sonuç olarak adiponektin düzeylerindeki düşüş ile birlikte asprosin düzeylerindeki yükseliş gestasyonel diyabetus mellitus gelişmesinde risk faktörleri olabileceği kanısına varıldı.
**Anahtar Kelimeler:** Gestasyonel diabetus mellutus, adiponektin, asprosin, gebelik.

### Adiponectin and asprosin levels in pregnant women with gestational diabetes mellitus

**Abstract**

Gestational diabetes mellitus (GDM) is defined as a type of diabetes that appears first in pregnancy in women that previously don't have the disease. An adipose tissue originated hormone adiponectin plays an important role in carbohydrate and lipid metabolism. Another adipose tissue hormone asprosin triggers glucose release from liver and suppresses insuline secretion from pancreatic beta cells. In the present study serum levels of adiponectin which has antidiabetogenic effect and asprosin which has diabetogenic effect was measured in 44 pregnant diagnosed with GDM and 44 pregnants without GDM. Study was conducted at the Department of Obstetrics and Gynecology of Adiyaman University Medical School. Serum adiponectin and asprosin concentration was measured using enzyme-linked immunosorbent assay (ELISA). Demographic parameters such as age, BMI, gravida, parity, abortus were also evaluated. Serum adiponectin levels were statistically significantly lower (1,58±0,43 mg/L vs 1,85±0,49 mg/L, p=0.0095). while serum asprosin levels were higher but not statistically significant (1,11±0,88 ng/L ve 0,76±0,75 ng/L, p=0.0933) in pregnants with GDM.

In conclusion, adiponectin levels were lower and asprosin levels were higher in pregnants with GDM and low levels of adiponectin and high levels of asprosin in pregnants may be evaluated as risk factors for GDM.

**Keywords:** Gestational diabetes mellitus, adiponectin, asprosin, pregnancy.

## GİRİŞ

Diabetes mellitus (DM), kalıtsal ve çevresel faktörlerin de rol aldığı ve kan glukoz seviyesinin aşırı yükselmesi ile kendini gösteren metabolik bir hastalıktır. Pankreas beta hücrelerinin insülin salgılama kapasitesinin düşmesi, insülinin etkinliğinin azalması veya her ikisinin birlikte bulunması diyabete neden olmaktadır. Tip 1 DM, Tip 2DM, spesifik diyabet tipleri ve gestasyonel diyabet gibi farklı tipleri mevcuttur (American Diabetes Association, 2014). Diyabet kalp hastalıkları, felç, körlük, kronik böbrek yetmezliği ve uzuv kaybı gibi ciddi komplikasypnlarına neden olan, dünyada yaklaşık her on bir kişiden birini etkileyen bir hastalıktır (Ugur, Yardim, 2018). Diyabet türlerinden biri olan Gestasyonel Diyabetes Mellitus (GDM), gebeliğin ikinci veya üçüncü trimesterinde teşhis edilebilen, gebelikten önce açıkça belirgin olmayan diyabet olarak tanımlanır (ADA 2014). Uluslararası Diyabet Federasyonunun (IDF) 2017 tahminlerine göre, GDM dünyadaki gebeliklerin yaklaşık % 14' ünü etkilemekte iken (Plows vd. 2018), 2021 yılında GDM prevalansının %14' ün üzerinde olduğu bildirilmiştir (H. Wang vd. 2022). GDM ilk olarak ileri gebelikte tespit edilen bozulmuş glukoz toleransı veya bozulmuş açlık glukozundan diyabetin açık göstergesi olan hiperglisemiye kadar olan geniş bir alanı kapsar (McIntyre vd. 2019).

GDM gebelik süresince hem anne hem bebek için ciddi komplikasyonlara neden olmaktadır. Ayrıca doğum sonrasındaki süreçte de hem anne hem çocuklar için diyebet, obezite ve kardiyovasküler hastalıklar bakımından yüksek risk oluşturmaktadır (Clausen vd. 2009) (Clausen vd. 2008) (Daly vd. 2018).

GDM genellikle doğumun ardından düzelirken, annede T2DM ve kardiyovasküler hastalık (CVD) riski ve çocukta da gelecekte obezite, CVD, T2DM ve / veya GDM gibi riskler de dahil olmak üzere uzun süreli sağlık sonuçları doğurabilmektedir. Bu, popülasyonun bütün olarak sağlığını etkileyen nesiller arası kısır bir obezite ve diyabet döngüsüne katkıda bulunur (Plows vd. 2018). GDM olan kadınlarda gebelikten 10-20 yıl sonra DM gelişme riski %35-60 oranında artmaktadır (Quintanilla Rodriguez ve Mahdy 2020). Ne yazık ki, şu anda GDM için yaşam tarzı müdahalesi (diyet ve egzersiz) ve zaman zaman insülin tedavisi dışında, yaygın olarak kabul edilmiş bir tedavi veya önleme stratejisi yoktur. Bu nedenle güvenli, etkili ve kullanımı kolay yeni tedaviler aranmaktadır. Bu tür tedavileri geliştirmek için GDM' nin patofizyolojisinin tam olarak anlaşılması gerekmektedir (Plows vd. 2018).

Epidemiyolojik çalışmalar çok sayıda faktörün GDM gelişimine katkıda bulunduğu bildirmektedir. Bu risk faktörlerinin arasında ileri gebelik yaşı, önceki gebeliklerde GDM varlığı, ailede tip 2 DM varlığı, artan vücut ağırlığı (vücut kitle indeksi 25'ten büyük), etnisite, fiziksel aktivite azalması, makrozomi (4000 gram veya daha fazla ağırlıkta) öyküsü, hipertansiyon, düşük HDL (35mg/dL den az), yüksek trigliserid düzeyi (250 mg/dL den fazla), polikistik over sendromu (PKOS), yüksek hemoglobin A1C (%5.7' den büyük) ve İnsülin direnci ile ilişkili diğer faktörler yer almaktadır (McIntyre vd. 2019).

Adipoz doku esas olarak fazla enerjinin depo edildiği bir doku olarak işlev yapmakla birlikte son yıllarda bir çok metabolik olayın düzenlenmesinde görev alan çok sayıda molekül sentezlediği bildirilmektedir. Adipoz doku kökenli proteinler olan adipokinlerin obezite ile insulin direnci, hipertansiyon, dizlipidemi gibi komplikasyonları arasında bağlantılarda rol alabileceği bildirilmektedir. (Fasshauer, Blüher, ve Stumvoll 2014). Adipokinler arasından leptin, adiponektin, asprosin, tümör nekrozis factor α (TNFα), adiposit yağ asidi bağlayıcı protein (AFABP),resistin, apelin (APLN), omentin vs. gibi çok sayıda molekülün gestasyonel diyebetes mellitus ile ilişkisi araştıtılmıştır (Fasshauer, Blüher, ve Stumvoll 2014) (Mohammadi ve Paknahad 2017) (Zhong vd. 2020).Adipokinler insülin salgısı, insülin duyarlılığı, insülin direnci, iştah, enerji tüketimi, inflamasyon, adipogenez gibi çok sayıda metabolik olaylarda etkilerini gösterirler. Bazı adipokinler doğrudan, bazıları ise dolaylı yollarla gebelikte glukoz homeostazını değiştirmek suretiyle gestasyonel diyabet gelişimine neden olabilirler. Doğrudan etkili olan mekanizmalar arasında pankreasın beta hücrelerinden insülin salgılanmasının modülasyonu ve özellikle karaciğer, kas, beyin gibi spesifik organlar olmak üzere bütün vücutta insülin duyarlılığının değiştirilmesi bulunur. Dolaylı mekanizmalar arasında ise inflamasyon, adipoz doku artışı ve vücutta yağların dağılımı gibi sonuçta glukoz metabolizmasını etkileyen mekanizmalar bulunmaktadır (Kralisch vd. 2007).

## Adiponektinin ve GDM

Adiponektin, enerji homeostazını, karbonhidrat ve lipid metabolizmasını düzenleyen büyük ölçüde adipositler tarafından salınan polipeptid bir hormondur (Mohammadi ve Paknahad 2017).
Adiponektin, özellikle glukoz ve lipid metabolizmasında etkin olan karaciğer, pankreas β hücreleri, böbreği, kas ve diğer birçok hücre tipine etki etmektedir. (26). Adiponektin, glukoz üretimine dahil olan genleri inhibe ederek hepatik glukoneogenezi güçlü bir şekilde baskılar. Adiponektin, temel metabolik dokulardaki yerel etkisi sayesinde insülin duyarlılığını artırır ve bu nedenle tüm vücut enerji homeostazını düzenler. Adiponektin, hücre ölümünü baskılar, iltihaplanmayı önleyerek ve hücre sağkalımını artırarak çeşitli hücrelerde bir dizi patolojik olaya karşı güçlü koruma sağlar (Z. V. Wang ve Scherer 2016).
Çalışmalar, obezite ve tip 2 diyabet gibi metabolik hastalıklar sırasında adiponektin konsantrasyonlarının azaldığını ve bunun gebelik komplikasyonları için bir biyobelirteç olarak kullanımını destekleyen yeni kanıtlar olduğunu bildirmiştir. Tehlikeli gebeliklerin sonucunu tahmin edebilecek maternal faktörlerin belirlenmesi, yüksek riskli gebeliklerin erken tanınmasına, anne ve çocukta yakın takip ve gebelik komplikasyonlarının önlenmesine olanak tanıyan değerli araçlar olarak kullanılabilir (Pheiffer vd. 2021).
Gestasyonel diyabetes mellitusta adiponektin düzeylerinin düşük olduğunu gösteren çok sayıda çalışma bulunmaktadır (Mohammadi ve Paknahad 2017). Adiponektin adenozin monofosfat ile aktive olan protein kinaz (AMPK) aktiviteisini artırmak suretiyle karaciğerde ve kasta glukonejenik enzimleri inhibe ederek kan glukozunu düşürücü ve yağ asidi oksidasyonun stimüle edici etkiler gösteren polipeptid yapılı bir hormondur (Yamauchi vd. 2002).
Düşük adiponectin düzeylerinin insulin direnci ve obezite ile ilişkisi olduğu rapor edilmiştir (Karpe 2013).

## Asprosin ve GDM

Asprosin, beyaz yağ dokusu tarafından salınan ve karaciğerden kan dolaşımına glikoz salınımını uyaran bir hormondur. Bu hormonun ana kaynağı beyaz adipoz dokudur. Asprosin, FBN1 geni (K, Ugur;M, Yardim; S 2018) tarafından kodlanan profibrilinin C-terminalinin bir bölünme ürünüdür. Romere ve arkadaşları, 2016 yılında asprosini yeni bir adipokin olarak tanıtan ilk kişilerdi (Hassanzadeh ve Nasri 2022). Beyaz adipoz doku tarafından salgılanan asprosin, açlık ile düzenlenir, nanomolar seviyelerde dolaşır ve karaciğerde G proteini-cAMP-PKA yolunu aktive ederek (karaciğeri glukoz salınımı için uyarır) kan dolaşımına hızlı glukoz salınımı sağlar (Romere vd. 2016). Kandaki glukoz miktarı düştüğünde asprosin seviyeleri artarken, glukoz miktarları arttığında asprosin miktarı da azalır (Zhang vd. 2017). İnsülin direncine sahip hayvanlarda ve insanlarda asprosin düzeylerinin arttığı bildirilmiştir (Romere vd. 2016).
Tip 2 DM ve Gestasyonel diyabetes mellitus hastalarında kan asprosin düzeylerini inceleyen çok sayıda çalışma mevcut olup büyük çoğunluğunda GDM'ta asprosin düzeylerinin yüksek olduğu bulunmuştur (Zhang vd. 2017) (Zhong vd. 2020) (Baykus vd. 2019).

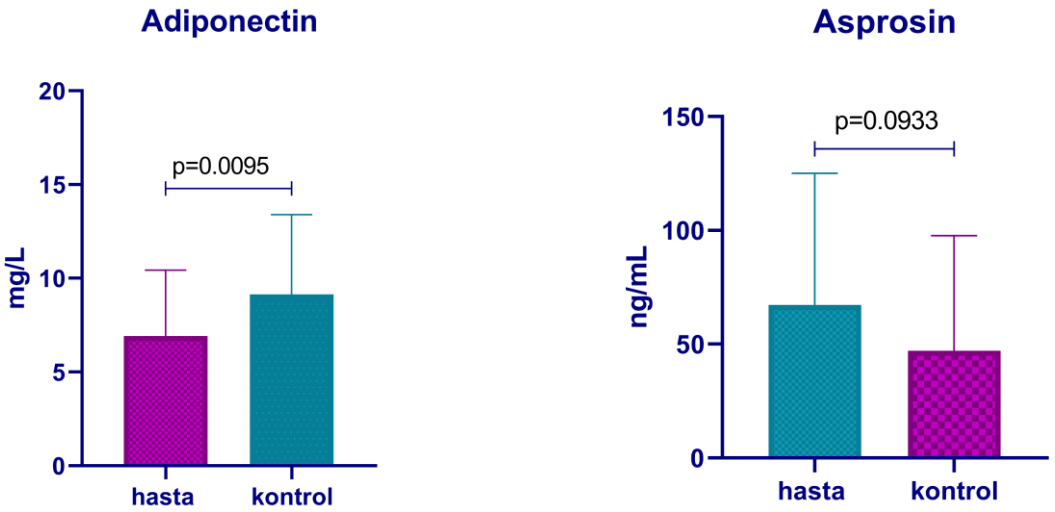
GDM gelişiminde insulin direncinin en önemli faktörlerden birisi olması ve adipoz dokudan salgılanan ve insulin direnci ve farklı diyabet tipleri ile ilişkileri olduğu yapılan çalışmalarla bildirilen adiponektin ile asprosin hormonlarının GDM' li hastalarda düzeylerinin ölçümü ve GDM ile ilişkilerinin değerlendirilmesi amaçlanmıştır.

## MATERYAL VE METOT

Bu çalışma Adıyaman Üniversitesi Tıp Fakültesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu'nun 22.10.2019 tarih ve 2019/7-11 sayılı Etik Kurul onayından sonra Ocak 2020 – Aralık 2020 tarihleri arasında Adıyaman Üniversitesi Eğitim ve Araştırma Hastanesi Kadın Hastalıkları ve Doğum Bölümüne başvuran gebelerde yapılmıştır. Çalışmada GDM tanısı almış (75 g glukozla OGTT ile tanı almış) 44 gebe ile GDM tanısı almamış 44 gebe olmak üzere toplam 88 kadından alınan kan numunesinde serum adiponektin ve serum asprosin hormon düzeyi ölçümü yapıldı. Kan örnekleri 1100Xg hızda 10 dakika santrifüj edilerek serumları ayrıldı ve çalışma yapılana kadar -80 $^{0}$C de muhafaza edildi. Serum asprosin ve adiponektin konsantrasyonlarının ölçümü insana özgü kitler kullanılarak ELISA yöntemi ile yapıldı. Kanı alınan sağlıklı ve hasta bireylerin yaş, VKİ, gravida, parite, abort gibi demografik kriterler de değerlendirmeye alınarak istatiksel analiz yapıldı. İstatistiksel analizler GraphPed V.8 programında yapıldı. Anlamlılık seviyesi en az $p<0,05$ olarak kabul edildi.

## BULGULAR

Adiponektin ve asprosin serum düzeylerinin ölçüldüğü bu çalışmamızda OGTT sonucuna göre GDM tanısı almış 27 ve 39. gebelik haftaları arasında taranan 44 GDM' li kadın (21-47 yaş) ve 44 GDM olamayan gebe (21-40 yaş) seçilmiştir. Vaka-kontrol çalışmamızda serum adiponektin ve asprosin konsantrasyonları ölçülmüş ve gruplar arasındaki konsantrasyonları karşılaştırılmıştır. Serum adiponektin konsantrasyonları GDM' li gebe bireylerde sağlıklı gebe bireylere göre önemli ölçüde daha düşük bulunmuştur (1,58±0,43 mg/L ve 1,85±0,49 mg/L, p=0.0095). Serum asprosin düzeyleri GDM grubunda kontrol grubuna göre istatistiksel olarak anlamlı olmamakla birlikte yüksek bulundu (1,11±0,88 ng/L ve 0,76±0,75 ng/L, p=0.0933). (Şekil 1).




Şekil 1: GDM' li ve sağlıklı gebelerde serum adipon

## TARTIŞMA

Bu çalışmada elde edilen veriler GDM'li gebelerde serum adiponektin düzeylerinin düştüğünü ve asprosin düzeylerinin arttığını göstermiştir. Adiponektinin hepatik glukoneogenezi güçlü bir şekilde baskıladığı bilinmektedir (25). Gestasyonel diyabetes mellitusta adiponektin düzeylerinin düşük olduğunu gösteren çalışmalar bulunmaktadır (Mohammadi ve Paknahad 2017). Adiponektin ile hiperglisemi arasındaki ilişkinin mekanizması tam olarak açıklanmamakla birlikte adiponektinin özellikle karbonhidrat ve lipid metabolizmasında görev alan doku ve hücrelerdeki reseptörlerine bağlanması ile AMPK yolağının aktive edildiği ve bunun sonucunda glukoneogenezin yavaşladığı ve yağ asidi oksidasyonunun arttığı bilinmektedir (Berg, Combs, ve Scherer 2002). GDM hastalarında adiponektin düzeylerinin düşmesi glukoneogenez üzerindeki baskılayıcı etkisinin azalması sonucu hiperglisemik bir durumun ortaya çıkmasına neden olabilir.

Asprosin karbonhidrat metabolizmasında etkileri olan bir hormon olup asprosin salgısı hepatik glukoz üretimini tetikleyerek normal kan glukoz düzeylerinin sürdürülmesine yardım eder (Romere vd. 2016). Tip 2 DM ve Gestasyonel diyabetes mellitus hastalarında kan asprosin düzeylerini inceleyen çalışmalarda Tip 2 DM ve GDM'ta asprosin düzeylerinin yüksek olduğu bulunmuştur (Zhang vd. 2019) (Zhong vd. 2020) (Baykus vd. 2019). Zhong ve ark., (2020) gebe kadınlarda GDM tanısından önce ve sonra asprosinin yükseldiği saptanmıştır. Bu sonuçlar, asprosinin, GDM tanısından önce bir durum olan insülin direncini indükleyerek GDM gelişiminde potansiyel bir rol oynayabileceğini göstermektedir. Artan asprosin miktarı GDM teşhisi konulmadan önce tespit edildiğinden, asprosinin GDM' nin bir belirleyicisi olarak hareket edebileceği bildirilmiştir.

## SONUÇ

Kırk dört GDM hastası ve 44 GDM olmayan gebede yapılan bu çalışmadan elde edilen bulgular GDM hastalarında hipoglisemik etkileri olan adiponektin düzeylerindeki düşüş ile hiperglisemik etkileri olan asprosin düzeylerindeki artışların bu iki hormonun glukoz metabolizması ile ilişkili fonksiyonları ele alındığında mevcut çalışmada mekanizmaları araştırılmamakla birlikte GDM gelişiminde risk faktörü olabileceği kanısına varılmıştır.

**TEŞEKKÜR**

Yazarlar bu çalışmada sağlanan finansal destek için Adıyaman Üniversitesi Bilimsel Araştırmalar Projeleri Birimi'ne teşekkür eder. (Yüksek Lisans Tezi Proje No:TIPFYL/2019-0002).

**KAYNAKLAR**

American Diabetes Association. 2014. "Diagnosis and Classification of Diabetes Mellitus". *Diabetes Care* 37 Suppl 1: S81-90.

American Diabetes Association, American Diabetes. 2018. "2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018." *Diabetes care* 41(Suppl 1): S13-27.

Baykus, Y. vd. 2019. "Asprosin in umbilical cord of newborns and maternal blood of gestational diabetes, preeclampsia, severe preeclampsia, intrauterine growth retardation and macrosemic fetus". *Peptides* 120. https://pubmed.ncbi.nlm.nih.gov/31400492/ (21 Aralık 2020).

Berg, Anders H., Terry P. Combs, ve Philipp E. Scherer. 2002. "ACRP30/Adiponectin: An Adipokine Regulating Glucose and Lipid Metabolism". *Trends in endocrinology and metabolism: TEM* 13(2): 84-89.

Clausen, Tine D. vd. 2008. "High Prevalence of Type 2 Diabetes and Pre-Diabetes in Adult Offspring of Women with Gestational Diabetes Mellitus or Type 1 Diabetes: The Role of Intrauterine Hyperglycemia". *Diabetes Care* 31(2): 340-46.

Clausen, Tine D. vd. 2009. "Overweight and the Metabolic Syndrome in Adult Offspring of Women with Diet-Treated Gestational Diabetes Mellitus or Type 1 Diabetes". *The Journal of Clinical Endocrinology and Metabolism* 94(7): 2464-70.

Daly, Barbara vd. 2018. "Increased Risk of Ischemic Heart Disease, Hypertension, and Type 2 Diabetes in Women with Previous Gestational Diabetes Mellitus, a Target Group in General Practice for Preventive Interventions: A Population-Based Cohort Study". *PLoS medicine* 15(1): e1002488.

Fasshauer, Mathias, Matthias Blüher, ve Michael Stumvoll. 2014. "Adipokines in Gestational Diabetes". *The Lancet. Diabetes & Endocrinology* 2(6): 488-99.

Hassanzadeh, Shakiba, ve Parto Nasri. 2022. "Asprosin; Effects and Associations". *Journal of Preventive Epidemiology* 7(1): e14-e14.

K, Ugur;M, Yardim; S, Aydin. 2018. "Is Asprosin, A Real Actor of Diabetes Etiopathology?" *Journal of Clinical and Molecular Endocrinology* 03(02).

Karpe, Fredrik. 2013. "Insulin Resistance by Adiponectin Deficiency: Is the Action in Skeletal Muscle?" *Diabetes* 62(3): 701-2.

Kralisch, S. vd. 2007. "Adipokines and Adipocyte Targets in the Future Management of Obesity and the Metabolic Syndrome". *Mini Reviews in Medicinal Chemistry* 7(1): 39-45.

McIntyre, H. David vd. 2019. "Gestational Diabetes Mellitus". *Nature Reviews. Disease Primers* 5(1): 47.

Mohammadi, Tanin, ve Zamzam Paknahad. 2017a. "Adiponectin Concentration in Gestational Diabetic Women: a Case-Control Study". *Clinical Nutrition Research* 6(4): 267.

Pheiffer, Carmen vd. 2021. "Adiponectin as a Potential Biomarker for Pregnancy Disorders". *International Journal of Molecular Sciences* 22(3): 1326.

Plows, Jasmine F vd. 2018. "The Pathophysiology of Gestational Diabetes Mellitus." *International journal of molecular sciences* 19(11).

Quintanilla Rodriguez, Bryan S., ve Heba Mahdy. 2020. "Gestational Diabetes. [Updated 2019 Dec 23]." İçinde *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*, StatPearls Publishing.

Romere, Chase vd. 2016. "Asprosin, a Fasting-Induced Glucogenic Protein Hormone." *Cell* 165(3): 566-79.

Wang, Hui vd. 2022. "IDF Diabetes Atlas: Estimation of Global and Regional Gestational Diabetes Mellitus Prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria". *Diabetes Research and Clinical Practice* 183: 109050.

Wang, Zhao V., ve Philipp E. Scherer. 2016. "Adiponectin, the past two decades". *Journal of Molecular Cell Biology* 8(2): 93-100.

Yamauchi, T. vd. 2002. "Adiponectin Stimulates Glucose Utilization and Fatty-Acid Oxidation by Activating AMP-Activated Protein Kinase". *Nature Medicine* 8(11): 1288-95.

Zhang, Lei vd. 2017. "Circulating asprosin concentrations are increased in type 2 diabetes mellitus and independently associated with fasting glucose and triglyceride". *Clinica Chimica Acta*.

Zhang, Lei vd. 2019. "Circulating Asprosin Concentrations Are Increased in Type 2 Diabetes Mellitus and Independently Associated with Fasting Glucose and Triglyceride". *Clinica Chimica Acta; International Journal of Clinical Chemistry* 489: 183-88.

Zhong, Lieqiang vd. 2020. "Continuous elevation of plasma asprosin in pregnant women complicated with gestational diabetes mellitus: A nested case-control study". *Placenta* 93(January): 17-22.

*ORAL PRESENTATION*

# Effects of Algal Diet on Sperm Quality

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## Abstract

All over the world, including developed countries, diseases related to eating habits have become a serious problem. Epidemiological and clinical studies show that nutrition is one of the main factors affecting susceptibility to lifestyle-related diseases. Recently, algae has emerged as a new food source with the potential for multi-purpose use in medicine. In addition, algae are a rich source of natural bioactive compounds with various biological activities. Considering the scientific researches, in order to protect the health and number of sperm; it is necessary to pay attention to exercising regularly, staying away from stress, avoiding alcohol and smoking, and eating a healthy and balanced diet. Individuals who cannot have children due to low sperm count can increase their sperm count by consuming the right foods. A healthy and balanced diet is very effective on reproductive health as it is in every aspect of health. In this study, the effects of algal diet on the male reproductive system and its relationship with infertility were reviewed.

**Keywords:** Male infertility, sperm quality, algal diet, reproductive health

## INTRODUCTION

We need over 40 essential nutrients (vitamins, essential amino acids, minerals and Omega fatty acids) to be a healthy individual. The only food source that can provide almost all of these forty essential nutrients is seafood. Seafood is a unique food that contributes to food security. Marine algae contain essential Omega-3 fatty acids and bioavailable micronutrients (vitamins A, B12 and D, as well as calcium, iron, iodine, zinc, selenium, phosphorus and zinc) (FAO, 2018).

Algae can be found in many areas of the earth. However, the main habitat of 70% is wet habitats. They can live by clinging to soil, trees and rocks in wetlands, thanks to stems or root-like structures with similar functions. They can form symbiotic relationships with other living species. They can live in glacial areas, at a temperature of 70°C or above, in a very salty environment, in waters with low light intensity and high pressure, where they can receive light for photosynthesis (Cirik and Cirik, 2011).

Microalgae have an important place in a healthy and balanced diet. Algae contain 55-60% protein, 16-50% carbohydrates, fats and various vitamins. They are especially important in terms of the vitamins they contain. β-carotene and astaxanthin, which are precursors of vitamin A, are found in algae. These organisms, which provide vitamin A support, contribute to the strengthening of the immune system. In addition, B group vitamins and vitamin E are also found in microalgae. Blue-green algae support the body's protection against radiation, thanks to the 31 chlorophyll they contain. Algae can have a positive effect by removing the radioactive particles that patients are exposed to during the cancer treatment process. However, in such cases, it is necessary to act in accordance with the doctor's advice. Blue-green algae are rapidly absorbed in the body and act as a detoxifier by balancing the intestinal system. It can be said that blue-green algae, which also helps to maintain the necessary mineral, vitamin and nutrient balance, have superior abilities in detoxification, which means purifying the body from harmful toxins with the chlorophyll it contains (Akyıl et al., 2016).

The inclusion of algae products in our diet is very advantageous in terms of development. For example, if we compare Spirulina with meat, egg and soybean according to their protein, carbohydrate and fat contents, the protein amount is 45-50% in eggs, 40-45% in meat, 35-40% in soybeans, while Spirulina has this value in %. It is 60-70 percent. Carbohydrates are 4-5% in eggs, 1-2% in meat, 30-35% in soybeans, 10-15% in Spirulina. Fat: 40-45% in eggs, 30-35% in meat, 15-20% in soybeans, only 6-7% in Spirulina. These types, which are very low in calories, also take their place in diet lists. They are both in proportion. A daily intake of only 10 grams of Spirulina, which is a protein store with a rate of 60-70%, also meets the entire daily iron requirement.

Due to these nutritious properties, algae are increasing their use in the list of alternative foods day by day (Arslaner and Türkoğlu, 2019).

Alginates are also used as the main drug substance. They are preferred as auxiliary materials because they have high fluidity. They are used as fillers in tablets and in the preparation of creams with high fat content. Alginates can be taken orally, as well as applied directly to the skin. It is the main ingredient of the bandage, which is frequently used in dermatology. At the same time, sodium alginate can be injected with the main active substances (insulin, antibiotics, hormones) that are kept constant at a certain fluidity and mixed with some vegetable oils and liquefied. Sodium alginate is also used in weight loss diets. This gel-like substance, which is low in calories, thickens stomach acid and creates a feeling of satiety. It can be used to lose weight by adding various flavors and calorie-free sweeteners or as a calorie-free, satiating food for diabetics (Williams and Laurens, 2010).

One of the important issues for male reproductive health is the amount of sperm. Insufficient sperm count can cause infertility in men. One out of every 6 couples of reproductive age is faced with the problem of infertility. In 1 or 2 of these 6 couples, the basis of infertility is low sperm count or quality. Men who are faced with this problem can increase the quality and quantity of sperm by taking some supportive measures in addition to medical treatment. Considering the scientific researches, in order to protect the health and quantity of sperm; it is necessary to pay attention to exercising regularly, staying away from stress, avoiding alcohol and smoking, and eating a healthy and balanced diet. Individuals who cannot have children due to low sperm count can increase their sperm count by consuming the right foods. A healthy and balanced diet is very effective on reproductive health as it is in every aspect of health. The decline in sperm counts in the past decades in Western countries has been a matter of debate for the reproductive health field and has become one of the focal points of ongoing studies. However, the potential reasons for this downward trend remain unclear. Increasing evidence indicates that diet affects semen quality. In fertility patients in the United States, especially saturated fat intake was found to be inversely related to sperm concentration and total sperm sperm count. In Spain, as in the USA, a decreasing trend was observed in semen quality during the same time period when the increase in caloric intake containing fat occurred. To further examine the effects of dietary fat intake on semen quality in healthy young adults, the relationship between trans fatty acid intake and sperm quality was studied in men from Southern Spain (Chavarro et al., 2014).

The steroid content in green algae can contribute to balanced hormonal regulation by forming hormones that play an important role in female fertility and regulate the function of the reproductive system in the best way. Therefore, algae can be used as an alternative treatment made from plants to overcome the toxic effects of free radicals that cause infertility in women as a result of disruption of the hormonal regulation of the reproductive system (Arini, 2021). However, the effect of Spirulina, a microalgae species, on reproductive functions is not well known. (Won et al. 2012; Murphy et al., 2017). Seaweeds are one of the largest reservoirs of nutrients with fewer side effects. For example, seaweeds such as Ulva lactuca, Laminaria japonica and Sargassum angustifolium have been reported to have the potential to improve male reproductive functions (Sobhani et al., 2015; Aly et al., 2013). It has been reported that fucoxanthin, found in the Sargassaceae family, reduces ROS such as hydroxyl radical, superoxide and hydrogen peroxide. It also increases the enzymatic antioxidant activity of superoxide dismutase (SOD). Fucoxanthin also inhibits some inflammatory mediators such as nuclear factor-kappa B (NF-κB), peroxisome proliferator-activated receptor-gamma (PPAR-γ) and activator protein 1 (AP-1) signaling pathways. Previous study reported the ameliorating effect of fucoxanthin in male reproductive dysfunction caused by streptozotocin-nicotinamide-induced diabetes in a rat model. They reported that it increased the expression of G protein-coupled receptor 54 (GPR54) and suppressor cytokine signaling 3 (SOCS-3) (Kong et al., 2019). Another study demonstrated the in vitro effect of fucoxanthin-rich extract from Sargassum glaucescens on cell viability and oxidative stress in RAW 264.7 macrophage cells and ameliorative effects on cisplatin-induced testicular damage in a hamster model. It has been reported that fucoxanthin prevents mitochondrial membrane loss in hamster sperm, reduces MDA levels in testicular tissue, sperm and plasma, and has ameliorative effects on testicular damage by reducing oxidative stress (Wang et al., 2020). It has been reported that the extraction of Chondrius crispus, which shows strong anti-inflammatory properties due to the presence of floridoside and isethionic acid, also has antioxidant potential. In an experimental rat model study, it was reported that Chondrius crispus extract injected into the testis showed a normal structure without causing any inflammation (Ibrahim et al., 2021).

## CONCLUSION

In experimental rat models, it has been shown that impaired antioxidant defense as a result of increased lipid peroxidation in male infertility induced by various agents causes ROS formation, which may be responsible for steroidogenesis and spermatogenesis. In fact, studies have reported that active polyphenols in algae and vitamins with antioxidant and anti-inflammatory potential can eliminate factors that cause infertility. As a result, it can regulate spermatogenesis by increasing testosterone production and increasing the secretory stimulation of accessory glands such as prostate. However, further studies are recommended to confirm the anti-inflammatory effect of algae on different experimental models.

## REFERENCES

Akyıl S, İlter I, Koç M, Kaymak-Ertekin F. Alglerden Elde Edilen Yüksek Değerlikli Bileşiklerin Biyoaktif/Biyolojik Uygulama Alanları. Akademik Gıda, 2016;14(4); 418-423.

Aly B. Okab, Emad M. Samara, Khalid A. Abdoun, Jan Rafay. Effects of dietary seaweed (Ulva lactuca) supplementation on the reproductive performance of buck and doe rabbits. Journal of Applied Animal Research, 2013;:3347-355.

Arini LA. IOP Conf. Ser.: Earth Environ. Sci. 2021;913:012085.

Arslaner A, Türkoğlu Z. Fonksiyonel Bir Gıda Katkısı Olarak Spirulina Platensis Spirulina Platensis As A Functional Food Additive. 1. Uluslararası Malatya Uygulamalı Bilimler Kongresi, 2019;308-313.

Chavarro JE, Mínguez-Alarcón L, Mendiola J, Cutillas-Tolín A, López-Espín JJ, Torres-Cantero AM. Trans fatty acid intake is inversely related to total sperm count in young healthy men. Hum Reprod. 2014;29(3):429-40.

Cirik Ş, Cirik S. Su bitkileri I-Deniz Bitkilerinin Biyolojisi, Ekolojisi ve Yetiştirme Teknikleri, Ege Üniversitesi Su Ürünleri Fakültesi Yayınları, 58:135-145. Content in eggs laid by hens fed with marine algae and sardine oil and stored at different times and temperatures. J. Appl. Phycol. 2011;24(3):593–599.

FAO, 2018. Dünyada Balıkçılık ve Su Ürünleri Yetiştiriciliğinin Durumu, Sürdürülebilir Kalkınma Amaçlarının Uygulanması.

Ibrahim NM, Ibrahim SR, Ashour OH, Abdel-Kader TG, Hassan MM, Ali RS. The effect of Red Seaweed (Chondrus crispus) on the fertility of male albino rats. Saudi J Biol Sci. 2021 Jul;28(7):3864-3869.

Kong ZL, Sudirman S, Hsu YC, Su CY, Kuo HP. Fucoxanthin-Rich Brown Algae Extract Improves Male Reproductive Function on Streptozotocin-Nicotinamide-Induced Diabetic Rat Model. Int J Mol Sci. 2019 Sep 11;20(18):4485.

Murphy EM, Stanton C, Brien CO', Murphy C, Holden S, Murphy RP, Varley P, Boland MP, Fair S. The effect of dietary supplementation of algae rich in docosahexaenoic acid on boar fertility. Theriogenology. 2017 Mar 1;90:78-87.

Sobhani A, Eftekhaari TE, Shahrzad ME, Natami M, Fallahi S. Antioxidant Effects of Brown Algae Sargassum on Sperm Parameters: CONSORT-Compliant Article. Medicine (Baltimore). 2015;94(52):e1938.

Wang PT, Sudirman S, Hsieh MC, Hu JY, Kong ZL. Oral supplementation of fucoxanthin-rich brown algae extract ameliorates cisplatin-induced testicular damage in hamsters. Biomed Pharmacother. 2020 May;125:109992.

Williams PJLB, Laurens LML. "Microalgae as biodiesel and biomass feedstocks: Review and analysis of the biochemistry, energetics and economics", Energy and Environmental Science, 2010;3(5):554-590.

Won HN, Il KK, Hae SA, Mi JK, Hee-Gyoo K, Jin HJ, Myung CG. Effect of Spirulina maxima on spermatogenesis and steroidogenesis in streptozotocininduced type I diabetic male rats. Food Chem. 2012;134:173-179.

## *ORAL PRESENTATION*

# Importance of Partial Hepatectomy in Liver Injuries

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## Abstract

It is one of the most important steps in modern surgery that liver regeneration becomes a common controllable procedure. For cancer treatment or transplantation, 60-70% of the liver volume can be safely removed to be used as a liver donor graft. Today, studies conducted with methods such as computed tomography, angiography and scintigraphy have shown that the liver reaches its former size in 3-6 months in adults and in less than 3 months in children after liver resection. In the presence of cirrhosis, this period increases up to 9-15 months. It has been reported that the human liver can tolerate even resections up to 80-85%. The determination of the mitotic index and more importantly the PCNA index has been used frequently in studies on liver regeneration and has played an important role in the interpretation of regeneration. Although liver cells regenerate slowly, it is a suitable organ for regeneration. Removal of a part of the liver by surgical means or its loss with the effect of toxic substances initiates regeneration. Cell proliferation begins in the periportal area and cell lines remodel towards the middle of the lobule. While cells continue to divide, they maintain their metabolic functions. In this study, the advantages and risks of partial hepatectomy in liver damage were reviewed.

**Keywords:** Liver, partial hepatectomy, regeneration, stem cell

## INTRODUCTION

Although liver cells regenerate slowly, it is a suitable organ for regeneration. Removal of a part of the liver by surgical means or its loss with the effect of toxic substances initiates regeneration. Cell proliferation begins in the periportal area and cell lines remodel towards the middle of the lobule. While cells continue to divide, they maintain their metabolic functions (1).

The first information about the regenerative capacity of the liver is found in Hesiod's Theogony. Prometheus, a Titan, is punished by Zeus for stealing fire and giving it to man and spoiling man. As punishment, he is chained to the highest peak of the Caucasus Mountains. Part of his liver is eaten by an eagle every day and is restored every night. However, the idea of liver regeneration was first introduced by Crueilhier in 1833 (2). Scientific studies in the 1900s by American scientists named Higgins and Anderson (3) removed the middle and left lobes with subtotal lobectomy (70-80%) under ether anesthesia in rats, and they observed that the liver weight loss of 75% was eliminated in a month. In the experimental animal resection model, the regeneration response is maximum when 2/3 of the liver is resected. When a smaller amount of parenchyma is removed, restoration progresses more slowly, DNA synthesis and mitotic activity are impaired in more than 2/3 resections, subtotal (90%) hepatectomy in rats leads to death without regeneration. Segmental or lobar resections are frequently performed in humans for tumor surgery or transplantation from living donors (4). However, after injuries, diseases (viral hepatitis, cirrhosis, and toxic events) that result in tissue loss in the liver, or after events such as surgical removal of a portion of the liver, a rapid compensatory growth is observed and this growth stops when the liver reaches adult sizes. The accepted general view for hepatic regeneration is a perfectly organized chain of events that continues until the liver reaches its optimum size, with the regulatory effect of the signals coming out of the organism. The very rare mitosis of the hepatocyte population in sections of a normal liver at any time is an expression of this stagnation (5,6).

The liver exhibits a distinctive feature from other organs with its regeneration capacity. In today's conditions, the liver is an organ that has important and vital functions that concern all systems of the human body, containing the mystery of many basic mechanisms such as biotransformation, regulation of metabolic functions, immunological events, which are still not fully and clearly deciphered. Regeneration is defined as the ability to regenerate tissue mass after loss or damage to a part of an organ. Regeneration is a complex process in which cytokines, hormones, transcription factors and oxidative stress products play a role. Studies

have shown that the liver has the ability to repair itself in case of significant tissue loss, thanks to intercellular interaction and a complex mediator network. Most of the studies on liver regeneration have studied pre-existing damaged liver in experimental animals, or liver damage has been created by using chemicals or by performing partial hepatectomy. Palmes and Spiegel described the model used in partial hepatectomy studies by specifying the ratios of each liver lobe to the total mass of all liver lobes in rats. According to the total liver mass, the right lobe contains 38%, the left lobe 30%, the tail lobe 8%, and the bipartite quadrate lobe contains 10%. It has been stated that the most appropriate model for partial hepatectomy studies in rats is resection of 68-70% of the liver (7,8).

Partial hepatectomy is one of the most frequently performed surgical procedures today and is an inevitable surgical treatment option in primary liver tumors, trauma, liver metastases of gastrointestinal tumors and liver transplantations. Liver resection has become safer, especially in the last 25 years, with the development of diagnostic methods, improvement and improvement in surgical techniques, and improvement in postoperative care. Postoperative mortality and morbidity in liver resection are directly dependent on preoperative liver functions and the functional and regenerative capacity of the liver remaining after postoperative resection. Regeneration of the liver tissue remaining with normal parenchyma is a very important support mechanism for the replacement of the functional hepatic mass in a short time after tissue injuries and hepatocellular necrosis. Active cell replication begins within 24 hours after partial hepatectomy, and this replication continues until the organ reaches its initial weight (9, 10).

We do not know the regulatory mechanisms in liver regeneration, the changes that occur and their relationships with each other. What we know for sure is that the liver knows when to start regeneration and when to stop. Loss of liver tissue initiates regeneration. The sensitive point that allows regeneration to stop is the relationship between body mass and liver mass. When the liver reaches a size that will meet the functional needs of the body and perform the metabolism, regeneration stops (11). Interestingly, when a larger liver tissue is transplanted than the recipient after transplantation, liver mass decreases until an optimal liver/body mass ratio is achieved.

Liver regeneration rates can be calculated by evaluating the liver mass taken in the injuries caused by partial hepatectomy and the masses of the whole liver taken after a certain period of time after hepatectomy. They reported that the remaining liver weight doubled 48 hours after 2/3 partial hepatectomy in rats and reached full weight within 7-10 days. In our study, it was found that regenerated liver weights approximately doubled in the partial hepatectomy groups 72 hours after partial hepatectomy (12). In some studies, liver wet weights and their 68% and 32% amounts were placed in Child's formula and used to determine the regeneration rate (12, 13). In our study, the relative liver weight defined by Fishback; Liver weight remaining after partial hepatectomy is subtracted from the liver weight at autopsy and calculated as the ratio of this value to the whole liver weight. The obtained value is multiplied by 100 to find the liver regeneration rate.

## CONCLUSION

It has been observed that hepatocyte proliferation is increased in cases where part of the liver lobes are surgically removed or hepatocytes are damaged by viruses or chemicals. However, studies have shown that after liver resection, regenerative activity is increased as a result of more stimulus. In addition, various drug applications are made to heal the medically damaged liver more quickly. However, as with many chemical drug treatments, side effects are inevitable in these studies. Therefore, determining the regeneration that occurs in the liver tissue after hepatectomy without any stimulus or chemical drug administration is an important issue and further studies are needed in this area.

## REFERNCES

1. Carnerio J, Kelley RO. Sindirim Kanalına Bağlı Bezler, Aytekin Y, Solakoglu S, Ahıshalı B(Ed).Temel Histoloji. 1. Baskı. İstanbul: Barış Kitabevi;1998.s.307-19.
2. Ethier C, Kestekian R, Christine B, Dube C, Havrankova J, Gascon-Barré M. Vitamin D depletion retards the normal regeneration process after partial hepatectomy in the rat. Endocrinology 1990;126(6): 2947-59
3. Bucher NLR, Larmer SR. Liver regeneration following partial hepatectomy; genes and metabolism. In: Strain AJ. Diehl AM. (Eds). Liver Growth and Repair. London: Chapman & Hall; 1998.s.3- 27.
4. Yang T, Sun YF, Zhang J, Lau WY, Lai EC, Lu JH, Shen F, Wu MC. Partial hepatectomy for ruptured hepatocellular carcinoma. Br J Surg. 2013 Jul;100(8):1071-9.
5. Naeem EM, Sajad D, Talaei-Khozani T, Khajeh S, Azarpira N, Alaei S, Tanideh N, Reza TM, Razban V. Decellularized liver transplant could be recellularized in rat partial hepatectomy model. J Biomed Mater Res A. 2019 Nov;107(11):2576-2588.
6. Francavilla A, Zeng Q, Polimeno L. Small-for-size liver tansplanted in to arger recipient: a model of hepatic regeneration. Hepatology 1994; 19: 210-16.

7. Palmes D, Spiegel HU. Animal models of liver regeneration, Biomaterials 2004;25(9):1601-11.
8. Court FG, Wemyss-Holden SA, Dennison AR, Maddern GJ. The mystery of liver regeneration. Br J Surg 2002; 89:1089-95.
9. Perek S, Kapan S, Ed: Değerli U,Bozfakıoğlu Y. Cerrahi Gastroenteroloji. 5. Basım. İstanbul: Nobel Tıp Kitabevleri 2000;194-208.
10. Meyers WC, Jones RS. Anatomy. In Meyers WC, Jones RS (eds) Textbook of liver and biliary surgery. Philadelphia: JB Lippincott Company; 1990.p.18–38.
11. Weinbren K, Hadjis NS. Compensatory hyperplasia of the liver. In "Surgery of the Liver and the Biliary Tract" Ed. LH Blumgart. Vol I. Edinburg: Churchill Livingstone inc; 1988.p.49.
12. Fan S-T, Lo C-M, Liu C-L, Lam C-M. Hepatectomy for hepatocellular carcinoma toward zero hospital deaths. Ann Surg 1999; 229: 322-30.
13. Chen G, Zhang J, Sun J, Wei S, Chen J, Ren H, Zhou S. Revisiting Partial Hepatectomy of Large Hepatocellular Carcinoma in Older Patients. Sci Rep. 2018 Sep 28;8(1):14505.

## *ORAL PRESENTATION*

# Nanoflower-Like Cu Doped ZnO Films Prepared by SILAR Method: Synthesis, Structural, Optical and Surface Properties

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## Abstract

ZnO thin films with nanoflower structures have recently received more attention owing to their applications in many fields such as electronics, magnetism and optics. In this study, we synthesized nanoflower-like ZnO films by doping Cu at different rates (1, 2 and 3 %) using the successive ionic layer adsorption and reaction (SILAR) method. The effect of Cu doping on the structural, optical and surface properties of the ZnO films was investigated using some characterization techniques such as FESEM (field emission scanning electron microscopy), EDS (Energy Dispersive Spectroscopy), Raman spectroscopy and UV-vis spectroscopy. The synthesized ZnO films were characterized morphologically by FESEM-Mapping techniques and results showed the nanoflower structures. In addition, ZnO films had higher stoichiometry with Cu addition in EDS results. Cu doping increased the transmittance of the ZnO films and caused the formation of double band gap structures in the band structure. The ZnO band gap value (~3.59 eV) was increased with Cu doping. In photoluminescence (PL) measurements, ZnO and Cu doped films clearly showed near band emission (~397 nm) and deep level emissions, and the defects that created these emissions were identified. Especially the surface and optical properties of ZnO films have been made more suitable with Cu (3%) doping for use in various application areas.

**Keywords:** Nanoflower, Cu doped ZnO films, Photoluminescence, RAMAN, FESEM/EDS

## INTRODUCTION

Recently, metal oxide films have found prominent physical properties and wide applications in electronic and optoelectronic-based semiconductor device technologies [1]. Especially Zinc oxide (ZnO) is used in optoelectronic systems owing to its large number of properties like wide optical band gap (3.37 eV) at room temperature, high electron mobility, high transparency and high chemical stability [1-3]. ZnO films crystallized in the quartzite structure are non-toxic, inexpensive, easily synthesized and abundant in nature semiconductor materials. Due to these properties, ZnO films are the subject of extensive research in many areas of technology such as photo diodes [4], biosensors [5], gas sensors [6], solar cells [7] and field effect transistors [8]. In order to change the some physical of ZnO films, applications are made with various additives such as Al, Cu [9], Ni [10], Sn [11], Ti [12]. In these applications, copper (Cu) element is especially preferred because of its low toxicity and abundance in nature [13-14]. In addition, parameters such as optical band gap and improving the physical performance of ZnO thin films can be controlled by varying the ratio of Cu doping [5]. Therefore, before applying ZnO thin films in many areas of technology, the effects of Cu doping on the physical properties such as structural, optical, morphological, electrical and magnetic should be investigated in detail [7]. It is used to control the structural, morphological and optical properties of metal oxide semiconductor films using the doping technique. The doped metal oxide produced by this technique is effective on many parameters (forbidden energy gap, surface porosity etc.) of semiconductor thin films. For this purpose, the effect of different doping elements and their concentrations on physical and chemical properties has become a focus for many researchers [1, 2].

To synthesize ZnO films, physical and chemical growth methods have been recently developed several techniques. These techniques can be listed such as chemical vapor deposition [15], chemical bath deposition [16], thermal evaporation [17], sputtering [18], sol-gel [19], ionic layer adsorption and reaction (SILAR) [20]

Among these techniques, the (SILAR) technique attracts attention due to its advantages such as easy use among semiconductor film growth methods, not requiring a vacuum environment, cheap and less time consuming [21].

Jellal et al. they determined the morphological properties of Cu-doped ZnO thin films using the SILAR method [22]. Its photo catalytic activity has been studied for the first time using X-ray photoemission spectroscopy (XPS) analysis. They emphasized that Cu doping with the SILAR method was especially effective on the morphological structures of ZnO thin films on the produced samples and that more research should be done on this subject. In a similar study, Devi et al [21] investigated the structural properties of Cu-doped ZnO thin films produced by the SILAR method. The detection performance in samples produced with ammonia vapor at room temperature was also investigated and it was emphasized that it was especially effective on the Quartzite geometry of ZnO thin films. In another study, Ghoset al. investigated the structural and optical properties of Cu-doped ZnO thin films produced by the SILAR method, depending on the temperature [23]. It has been seen that the ideal temperature for the samples to be used in technological applications of Cu-doped ZnO thin films produced by the SILAR method is in the range of 50-65 °C. Similarly, in this study, the SILAR method was used in the ideal temperature range. Abdel-Wahab examined the structural optical and photo-catalytic activity of Cu-doped ZnO thin films produced by the SILAR method [24]. In the study, the AFM method was used for the optical effects and structural properties of X-ray spectroscopy. As a result of the study, the effects of Cu doped especially on photo-catalytic were investigated and limited evaluations were made on their structural optical properties. It is a known fact that especially SEM/EDS mapping method is effective in structural investigations and this study needs to be examined with different structural and optical methods. Owing to high surface-to-volume ratio of doped ZnO films flower structures, they could be used in gas sensors to increase the sensitivity in gas detection measurements. Yong-Hui Zhang et al. revealed that the Al-doped ZnO nanoflower-like morphology with larger specific surface areas builds more active sites for high adsorption of toxic gas and multiple oxygen vacancies [25]. Devi et al., in his study indicated that Cu-doped ZnO samples, $Cu^{2+}$ ions participate in the reaction, crystal defects are formed that cause morphological nanoflower structure due to the formation of small particles [26]. Similarly, Dhaygude et al. observed that structures affecting nanoflower morphology were formed in Cu-doped ZnO thin films grown by the SILAR method, depending on the doping ratio [27]. They highlighted that this result provides an advantage in technological applications as it provides the expansion of the surface area of thin films. Studies show that doped ZnO thin films have superior electronic, magnetic, optical, chemical and catalytic properties depending on the surface morphology, surface components and atomic arrangement on the surface [28-29].

In this study, ZnO and Cu doped ZnO (CZO) films were produced on glass substrate using the SILAR method which is a cheap and easy technique and it can be used to produce gas sensors in the future. The main aim of this study is to determine the effects of Cu ratio on the structural, optical and morphological properties of ZnO and CZO thin film precipitate. In particular, we compared the variation of the band gap structure and forbidden band gap values, optical transmittance, deep and shallow defect situations and surface morphology as a function of Cu concentration in the produced films.

## MATERIAL METHOD

Glass was used as the substrate of the produced films. Glass substrates were cut 8 mm wide and 25 mm long. Afterwards, the glass substrates were thoroughly washed in soapy water to remove any possible dirt. After the washing process was completed, the substrates were ultrasonically cleaned in acetone for 10 minutes and ultrasonically cleaned in a one-to-one (1:1) ethanol water mixture for 10 minutes. The cleaned substrates were then dried in dry nitrogen gas. After these processes, the substrates became ready for sample augmentation with the SILAR method. Zinc chloride $ZnCl_2$ (99.9%), copper chloride $CuCl_2$ (99.9%) and ammonia $NH_3$ chemical sources as cationic solution were used for the growth of ZnO and Cu doped ZnO films. Ammonia ($NH_3$) (28%) was added into 0.1M $ZnCl_2$ in moles [Zn:$NH_3$=1:10] ratio and mixed, and the zinc-ammonia complex $[Zn(NH3)_4]^{2+}$ with a pH value of ~10 was obtained. The chemical reactions that take place during the preparation of the zinc-ammonia complex ($[Zn(NH_3)_4]^{2+}$) and the application of the SILAR method are given below.

$ZnCl_2 + 2NH_4OH \leftrightarrow Zn(OH)_2 + 2NH_4^+ + 2Cl^-$

$Zn(OH)_2 + 4NH_4^+ \leftrightarrow [Zn(NH_3)_4]^{2+} + 2H_2O + 2H^+$

$[Zn(NH_3)_4]^{2+} + 4H_2O \rightarrow Zn(OH)_2\,(s) + 4NH_4^+ + 2OH^-$

$Zn(OH)_2\,(s) \rightarrow ZnO(s) + H_2O$

The growth mechanism of ZnO films by the SILAR method is shown schematically in Fig. 1. After the necessary chemical solutions were prepared, the experimental SILAR cycle in Fig. 1 was applied to ensure the conversion of $Zn(OH)_2 \rightarrow ZnO$.

1. The glass substrate was soaked in the $[Zn(NH_3)_4]^{2+}$ solution for 20 seconds and an aqueous thin film layer containing the $[Zn(NH_3)_4]^{2+}$ complex was deposited onto the glass substrate surface.

2. The solution removed from the solution were kept in hot deionized (90 $^oC$) water for 7 seconds.

3. The solution were then removed from the water and air-dried for 60 seconds.

4. Finally, it was rinsed in distilled water at room temperature for 30 seconds.

5. Thus, a thin film ZnO layer was obtained on the glass substrate by completing a SILAR round.

This applied SILAR cycle was repeated until the ZnO films reached the desired thickness. The same cycle was applied to obtain Cu-doped ZnO films. By adding $CuCl_2$ chemical salt to the prepared starting solution, films with 1, 2, 3 % doped were produced. The films produced in the article are named ZnO for undoped ZnO, CZO1, CZO2 and CZO3 for 1, 2, and 3% Cu doped films, respectively.

The devices used in the structural, optical, elemental and surface characterization of ZnO and CZO films are listed below:

- Hitachi Su5000 Field Emission Scanning Electron Microscope (FE-SEM) for surface morphology.

- Oxford X-MaxN 80 model, EDX detector with 80 $mm^2$ detector area for elemental analysis.

- Laser Scanning Confocal Microscope System, Leica, TCS SP2

- The Perkin Elmer Lambda 900 UV/VIS/NIR spectrometer is a double-beam in the wavelength range from 200 to 1100 nm for transmittance measurements.

- Jobin Yuvon Farog-550 system with a 50 mW He-Cd laser ($\lambda$=325 nm) as an excitation light source fornPL measurements were performed at room temperature.

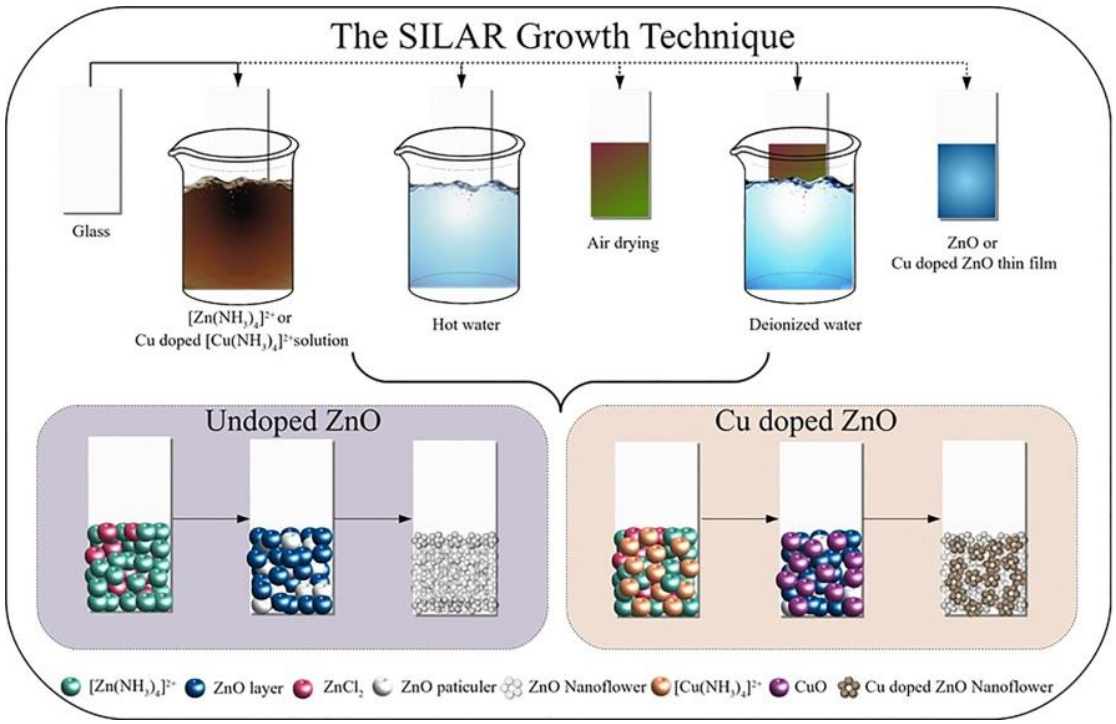


**Figure 1.** The schematic presentation SILAR growth technique [30]

## RESULTS AND DISCUSSIONS

Raman spectroscopy is a vibration spectroscopy technique. This technique examines the vibrational movements of the molecules and gives information about the vibration levels of the molecules in the structure and is used to determine the material structure and defects. Compared to techniques such as XRD and electron microscopy, Raman spectroscopy is a more sensitive, fast, practical and convenient technique for analysis in a narrow space. Structural analysis of ZnO and CZO films was performed by Raman spectroscopy and were analyzed the phonon properties, vacancies, and strain states using the obtained spectra. Raman spectra of the ZnO and CZO films are given in Fig. 1. According to group theory, the vibration modes of the hexagonal wurtzite ZnO structure are classified as follows [31]:

$$\Gamma\ (\text{Optical}) = A_1\ (LO) + A_1\ (TO) + 2B_1 + E_1(LO) + E_1\ (TO) + 2E_2$$

where $E_2$ is the non-polar phonon mode and it has two components low [$E_2$ (low)] and high-frequency [$E_2$ (high)]. $A_1$ and $E_1$ are polar modes and both $A_1$ and $E_1$ modes have longitudinal (LO) and transverse (TO) optical components. These components are called $A_1$ (TO), $A_1$ (LO), $E_1$ (TO), and $E_1$ (LO). Also, $B_1$ is inactive mode. In Fig. 1, the peaks of most dominant bands in the ZnO structure were detected as 213, 323, 332, 419, 427, 535 and 544 $cm^{-1}$. The peaks at 213, 323 and 332 $cm^{-1}$ show the $2E_2$ (low) and $E_2$ (low)-$E_2$ (high) components of the $E_2$ mode, respectivily [32]. These components are related to the Zn and oxygen sub-lattice vibrations. [33]. The peaks at 419 and 427 $cm^{-1}$ belong to the $E_2$ (high) phonon mode, which is caused by vibrations of oxygen atoms [34]. Also, the LO mode $A_1$ is symmetry at the peaks of 535 and 544 $cm^{-1}$ [35]. Raman vibration modes of these ZnO and CZO films show that the films are in hexagonal wurtzite structure. A clear change was observed in all peaks in the ZnO structure with Cu addition. As the Cu contribution increased, the peak intensity decreased and a slight shift in the peak positions was detected. The reason for this may be the increase of oxygen vacancies in the structure. The change in the $2E_2$ (low), $E_2$ (low)-$E_2$ (high) and $E_2$ (high) peaks can be caused by the energy changes in the Zn-O bonds by replacing the $Zn^{+2}$ ions of the Cu atoms entering the structure with doping. In addition, the change in $A_1$(LO) peaks may be the result of changing oxygen vacancies, interstitial zinc and other defect conditions in the structure with Cu doping [36].

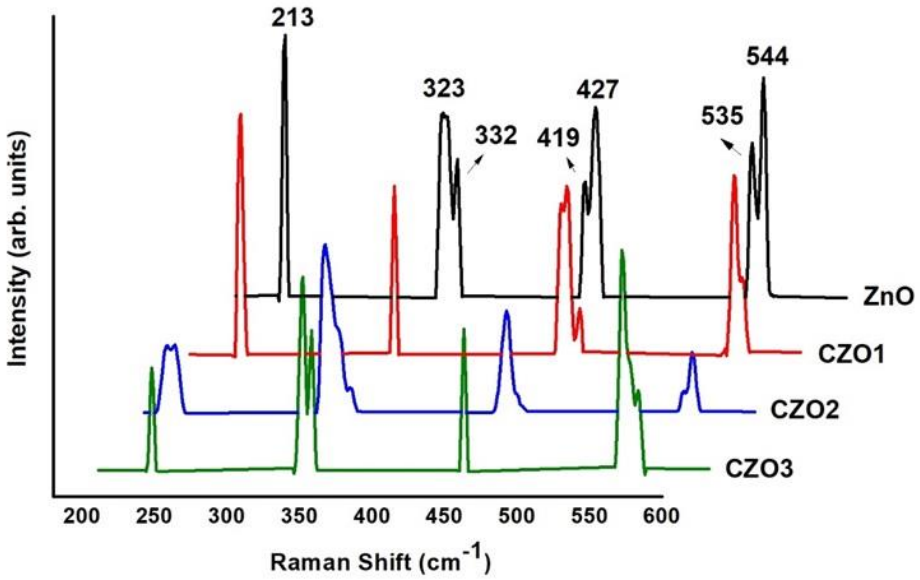


**Figure 2.** Raman spectra of ZnO and CZO films

In Fig. 2, transmittance spectra of ZnO and CZO films are given. The transmittance spectra of films were taken in the range of 300-1100 nm at room temperature. It is seen that all films are high transmittance in the visible region. It was determined that all films were high transmittance and the average transmittance values were 70% in the visible region. With low doping ratios (at. 1 and 2 % Cu), the transmittance value decreased and the band edge structure of ZnO was distorted. Especially in CZO1 films, this deterioration can be seen very clearly. This is due to the fact that the doping elements cannot enter the appropriate places in the structure and create defects in small additive ratios. As the Cu doping ratio increased, the transmittance values increased and the band edge structure improved, becoming sharper than the band edge of the ZnO films. This shows that CZO3 and CZO4 films have a smoother band edge than ZnO films. In addition, factors such as thickness, grain size, defect density, and roughness values of the films can cause changes in their optical properties [37].

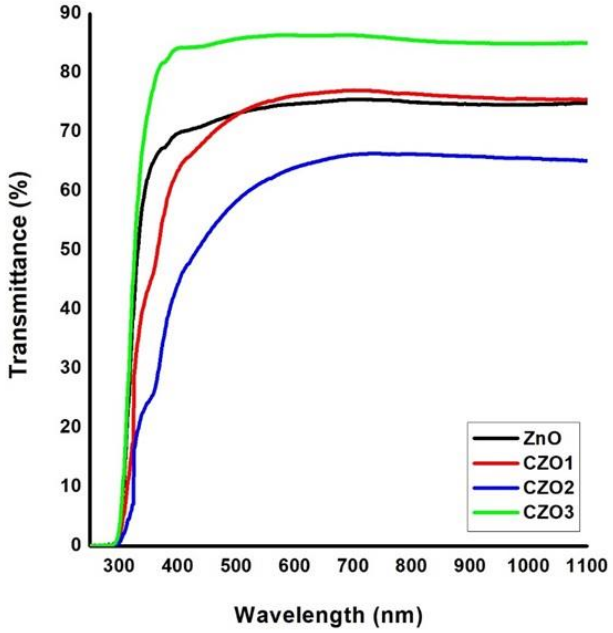


**Figure 3.** Transmittance spectra of ZnO and CZO films

Tauc relationship was used to calculate the optical band gap energy of ZnO and CZO films. This formula [38]:

$$\alpha h\nu = \alpha_0 (h\nu - E_g)^n \quad (1)$$

where is $\alpha_0$ is a constant, *hn* is a photon energy, n is an index factor and is used to determine the optical transition (n=1/2 allowed direct transition), α is an absorption coefficient. To determine the optical band gap of films, we plotted the $(ahn)^2$-*hn* and $E_g$ values were calculated by extrapolating the linear part to zero absorption coefficient. The graph between $(ahn)^2$ and *hn* is shown Fig. 3 and the calculated $E_g$ values are given in Table 1. The band gap energy of ZnO was determined approximately 3.52 eV and it was observed that the Cu doping changed the band structure of the films. The band edge shifted to red at low doping rates (at. 1 and 2 %) and to blue at higher doping rates (at. 3 and 4 %). In general, the ZnO band structure was preserved in all films, but double band gap energies were observed in CZO1 and CZO2 films. The band gap energy of ZnO films first decreased and then increased with Cu doped. Also, unlike others two different band energies were observed in CZO1 and CZO2 films. These energy values are named as $E_{g1}$ and $E_{g2}$ and are given in Table 2 and the schematic representation of the band diagram of ZnO and CZO films was shown in Fig. 4. While $E_{g1}$ band energies decreased in CZO1 and CZO2 films, this value increased in CZO3 and CZO4 films. This reduction in CZO1 and CZO2 films may occur due to band tailings caused by different Cu oxidation states in the ZnO structure [39]. Besides, the increase in the energies of CZO3 and CZO4 films is due to the increase of lattice distortion. This distortion causes changes of carries density and absorption band edges. The lowest levels in the conduction band are occupied by Cu a atom, which increases the $E_g$ energy. This situation is known as the Burstein-Moss (MS) effect in the literature, and the blue shift observed at the band edge is a result of this effect. [40]. Similar increase behavior (blueshift) in the band gap energy of Cu doped ZnO films has been reported in some studies in the literature [41, 42]. In addition, a second band energy $E_{g2}$ was calculated in the CZO1 and CZO2 films. This $E_{g2}$ band energy indicates the presence of one more band formation lower than the ZnO band energy in low Cu doped (1, 2 %) films (Fig. 4). This band energy can be thought of as a second-wide impurity band state resulting from the localized electrons of Cu impurities below the conduction band. This decrease in band energy was reported in different studies [43, 44].

Another reason for the change of the band edges of the films with Cu doping may be the lattice strain. Urbach energy ($E_u$) formula is used to examine the lattice strain and band edge distortions in the structure. This formula is as follows [45]:

$$\alpha = \alpha_0(h\nu/E_u) \qquad (2)$$

Using this formula, $E_u$ energies of the ZnO and CZO films were calculated and given in Table 1. Also Fig. 5 shows the graphs of the calculated $E_g$ and $E_u$ values. It was determined that the $E_u$ energies of the CZO1 and CZO2 films were increased compared to the ZnO film with the doping of Cu. In addition, $E_g$ values in these films decreased compared to the $E_g$ value of ZnO. This is an indication that lattice strain and band tails are higher in CZO1 and CZO2 films. Since Cu atoms could not be placed in suitable places in the lattice at low doping rates, the band structure was distorted and the strain in the lattice increased. In Fig 2, the change in the absorption edges of the CZO1 and CZO2 films is consistent with the change in the lattice strains. In the 3% Cu doped CZO3 film, the $E_u$ energy value was clearly decreased compared to the CZO1 and CZO2 films. Despite the decrease in $E_u$ value, $E_g$ value increased and reached the highest value compared to other films. This change in $E_u$ value indicates that the strains and band tails in the CZO3 film decrease.

**Table 1.** Band gap and Urbach energy values of ZnO and CZO films

| Films | $E_{g1}$ (eV) | $E_{g2}$ (eV) | $E_u$ (meV) |
|---|---|---|---|
| ZnO | 3.52 | - | 442.58 |
| CZO1 | 3.50 | 3.14 | 456.43 |
| CZO2 | 3.41 | 3.12 | 553.23 |
| CZO3 | 3.59 | - | 379.18 |

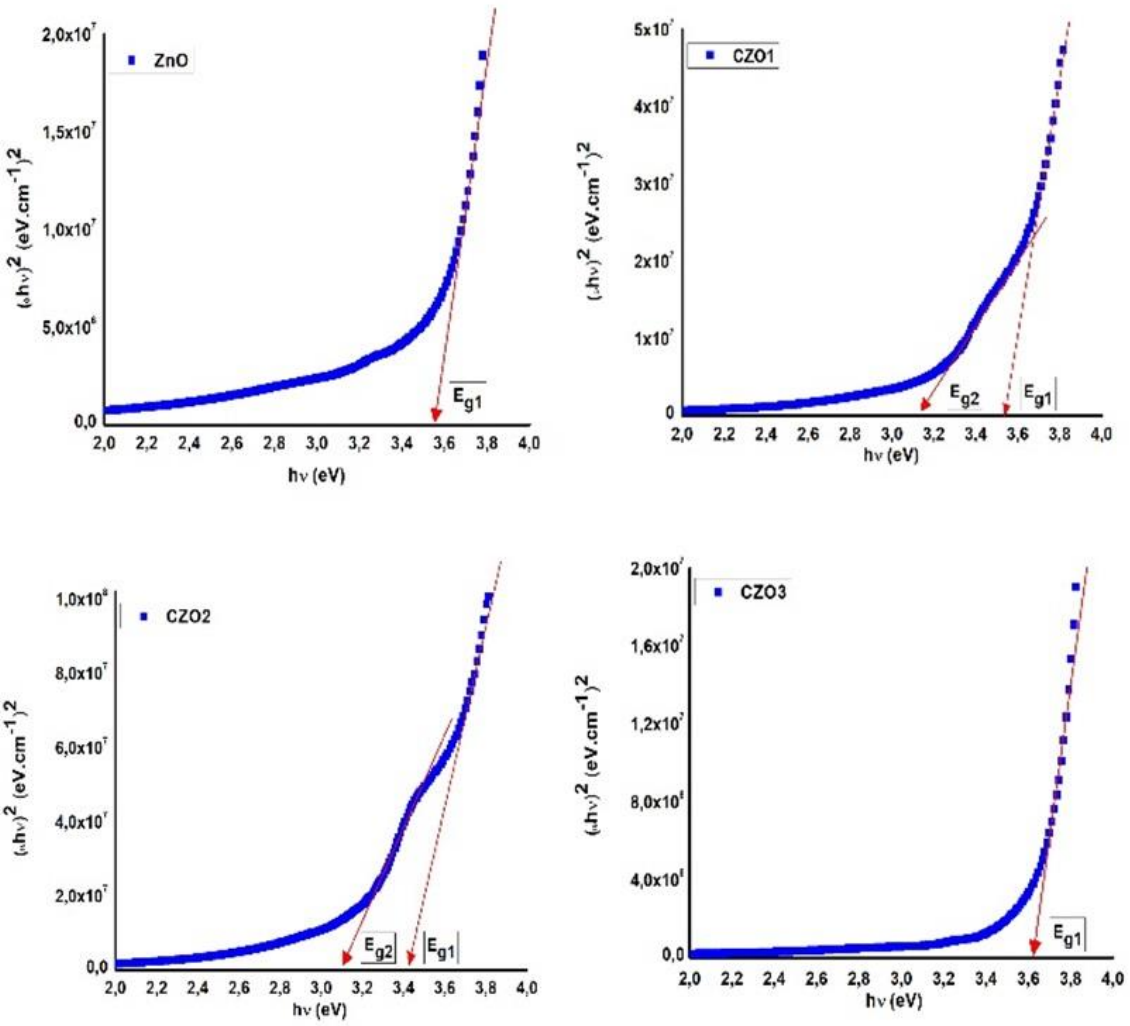


**Figure 4.** The plots of $(ahn)^2$ vs $(hn)$ for ZnO and CZO films

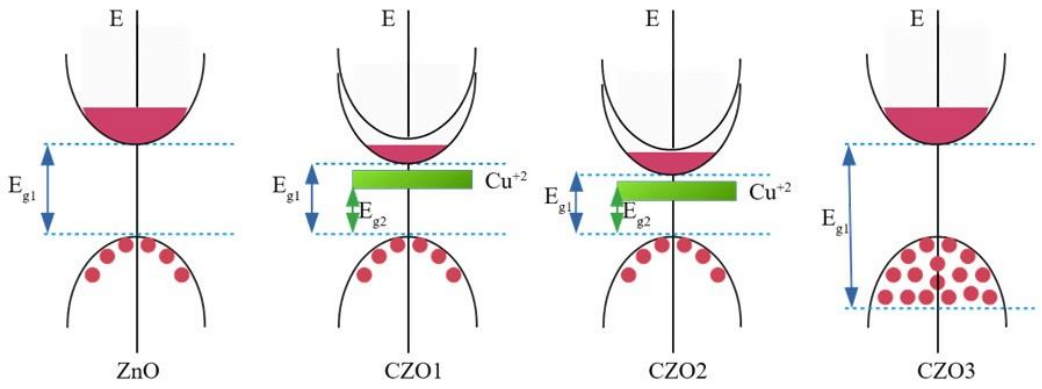


**Figure 5.** The schematic representation of the band diagram of ZnO and CZO films

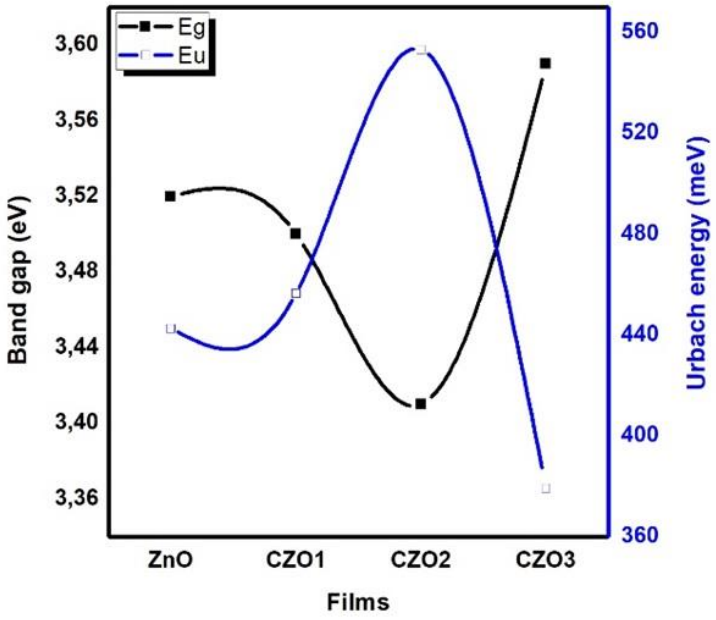


**Figure 6.** Band gap and Urbach energy versus of ZnO and CZO films

PL spectra of ZnO and CZO films are presented in Fig. 6 (a). In addition, the PL spectra of ZnO in Fig 6 (b) were analyzed by the Gaussian peak fit technique and narrow peaks were further deconvoluted. When Fig. 5 (a) is examined, it is seen that all films show similar photoluminescence properties. For ZnO and Cu doped ZnO, five peaks around 397 nm (3.12 eV), 445 nm (2.78 eV), 570 nm (2.17 eV), 660 nm (1.87 eV), and 735 nm (1.68 eV) were observed respectively. The peak at 397 nm in the PL spectra is called NBE emission. Other peaks (445, 570, 660, 735 nm) are indicative of deep level emissions (DLE) such as $Zn_i$, $O_i$, $V_{Zn}$, $V_O$ and O antisite ($O_{Zn}$) in the structure [46-48]. Also, the schematic representation of the transitions and defect states of the films are given in Fig. 7. From Fig. 7, the NBE emission (397 nm) of the films is clearly seen, and this emission is due to electron transitions passing from the lower levels of the conduction band (CB) to the upper levels of the valance band (VB) and it can also be caused by free or bound excitons at the band edge. The emission at 445 nm is called blue emission. This emission can be occurs in three ways. The first is the transition from the shallow donor levels of the interstitial zinc ($Zn_i$) statues to the VB band, the second is the transitions from the shallow donor levels of the O vacancies to the VB band and third is the transitions from interstitial zinc ($Zn_i$) statues to zinc vacancies ($V_{Zn}$). The emission at 570 nm is called green emission it related to oxygen vacancies ($V_o$) [49-54]. Also, it associated with the electron transitions from deep level donor of the ionized oxygen vacancies ($V_o$) to the VB and other vacancy defects [55]. Finally, the emissions at 660 and 735 nm are called orange and yellow emissions respectively and these emissions are due to the presence of high interstitial oxygen ($O_i$) in structure [56, 57]. Generally, these emissions are attributed to a transition from interstitial zinc ($Zn_i$) to oxygen vacancies ($O_i$) [58]. Elemental analysis results obtained for ZnO and CZO films also showed that there was an excess of oxygen in the structure [59].

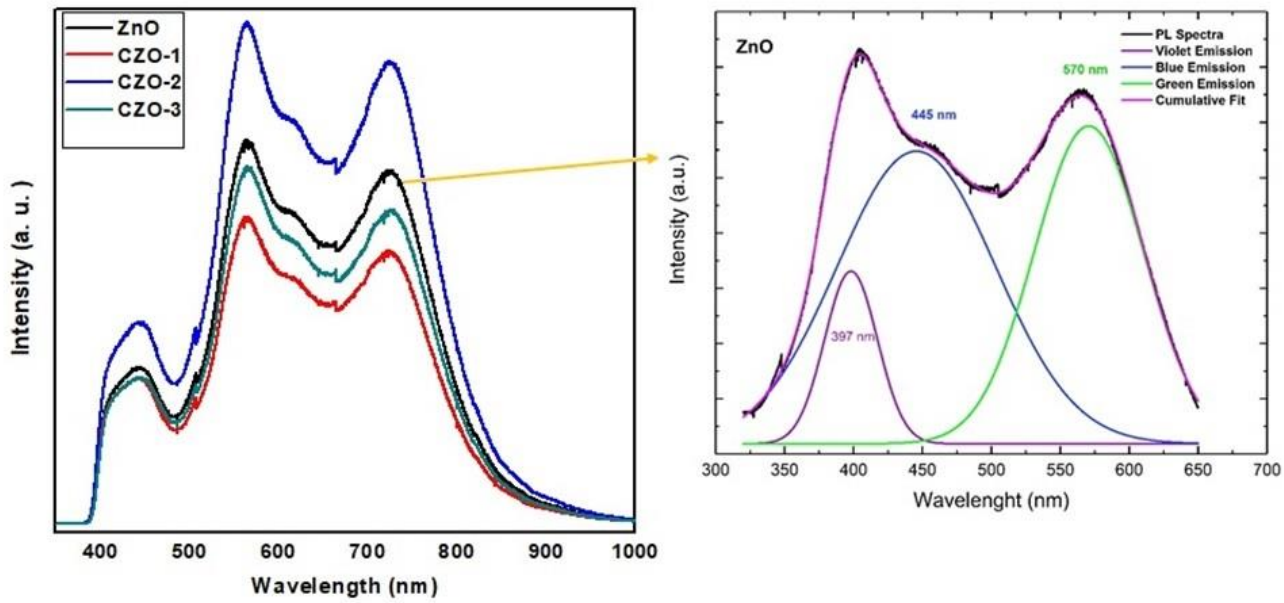


**Figure 7.** PL spectra of ZnO and CZO films

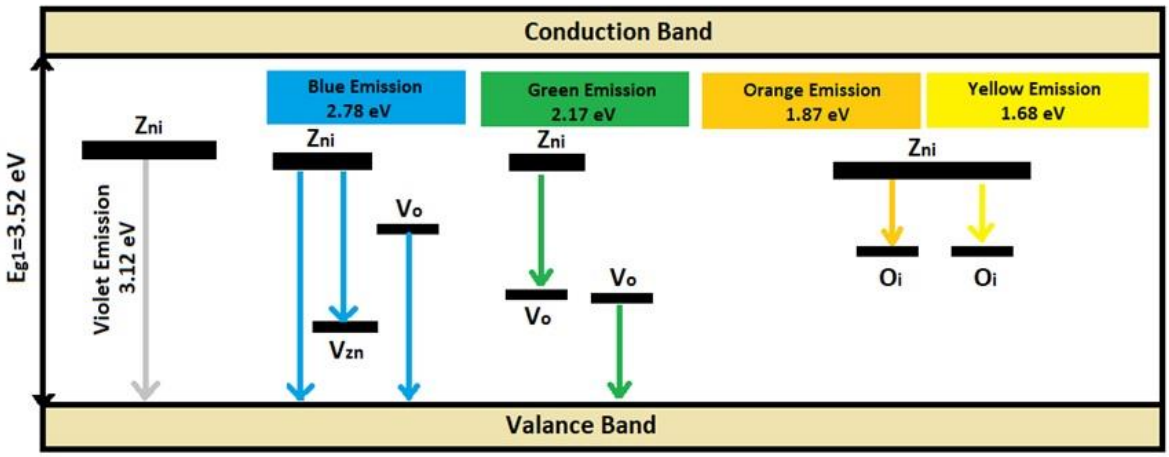


**Figure 8.** The schematic representation of the transitions and defect states of the ZnO and CZO films

The surface morphology was demonstrated by FESEM and the colorless and color FESEM images of ZnO and CZO films were shown in Fig. 9, respectively. When Fig. 8 was examined, ZnO films were densely formed on the surface and showed the presence of very small flower-like microstructures. It was observed that the surface formation of the films exhibited a more homogeneous dispersion with Cu doping. In addition, it has been determined that the atoms forming the films with Cu doping combine to form a nanoflower structure in an open way. It was determined that while the nanoflower structures were densely combined in low Cu doping, larger and more pronounced nanoflower structures were formed on the surface as the Cu doping ratio increased. In the CZO1 structure, smaller nanoflowers come together and the surface is closed. In CZO2 films, on the other hand, nanoflowers have increased in size and larger nanoflower structures are seen on the surface. Finally, the increase in the size of the nanoflowers clearly showed itself in the CZO3 film. Instead of a homogeneous surface distribution in CZO3 films, structures consisting of much larger nanoflowers were formed compared to CZO1 and CZO2 films.

In addition the elemental analysis of ZnO and CZO films was investigated using EDX. Fig. 9 shows elemental composition of Zn, Cu, O determined by EDX measurement. Besides, quantitative elemental analysis values of films are given in Table 2. When the elemental distribution results in Fig. 9 and Table 2 are examined, the presence of Zn and O, which are the primary elements for ZnO films, is seen in the structure. The ratio of Zn and O in the structure is not close to each other. This showed that the ZnO films were not in a stoichiometric structure. In addition, it is seen that the element Cu also enters the structure with the doping process in CZO films. When the atomic percentages of the elements in the structure were examined, as a result of doping, the amount of Zn and Cu increased while the O in the structure of the CZO1 and CZO2 films decreased. While the increase in Cu amount in CZO1 and CZO2 films increased the Zn/Cu and Zn/O ratios, the stoichiometry of the structure deteriorated. On the other hand, in CZO3 films, the amount of Cu in the structure increased and the ratio of Zn/Cu decreased. In addition, the amount of Zn and O in the structure of CZO3 films is the closest to each other compared to other films. For this reason, the Zn/O ratio in the structure of CZO3 films is the lowest and the stoichiometry of these films has clearly decreased. This is an indication that O accumulated at the interstate or grain boundaries in low-doped films enter the structure. Similar behaviors determined in the optical and structural properties of CZO3 films support this situation.

**Table 2.** The quantitative elemental analysis values of ZnO and CZO films

| Films | Atomic Weight (%) | | | Ratio | |
|---|---|---|---|---|---|
| | Zn | O | Cu | Zn/Cu | Zn/O |
| CZO | 64.4 | 35.6 | - | - | 1.80 |
| CZO1 | 71.6 | 25.2 | 2.1 | 34.09 | 2.84 |
| CZO2 | 77.8 | 20.4 | 1.8 | 43.22 | 3.81 |
| CZO3 | 46.1 | 48.4 | 5.5 | 8.38 | 0.95 |

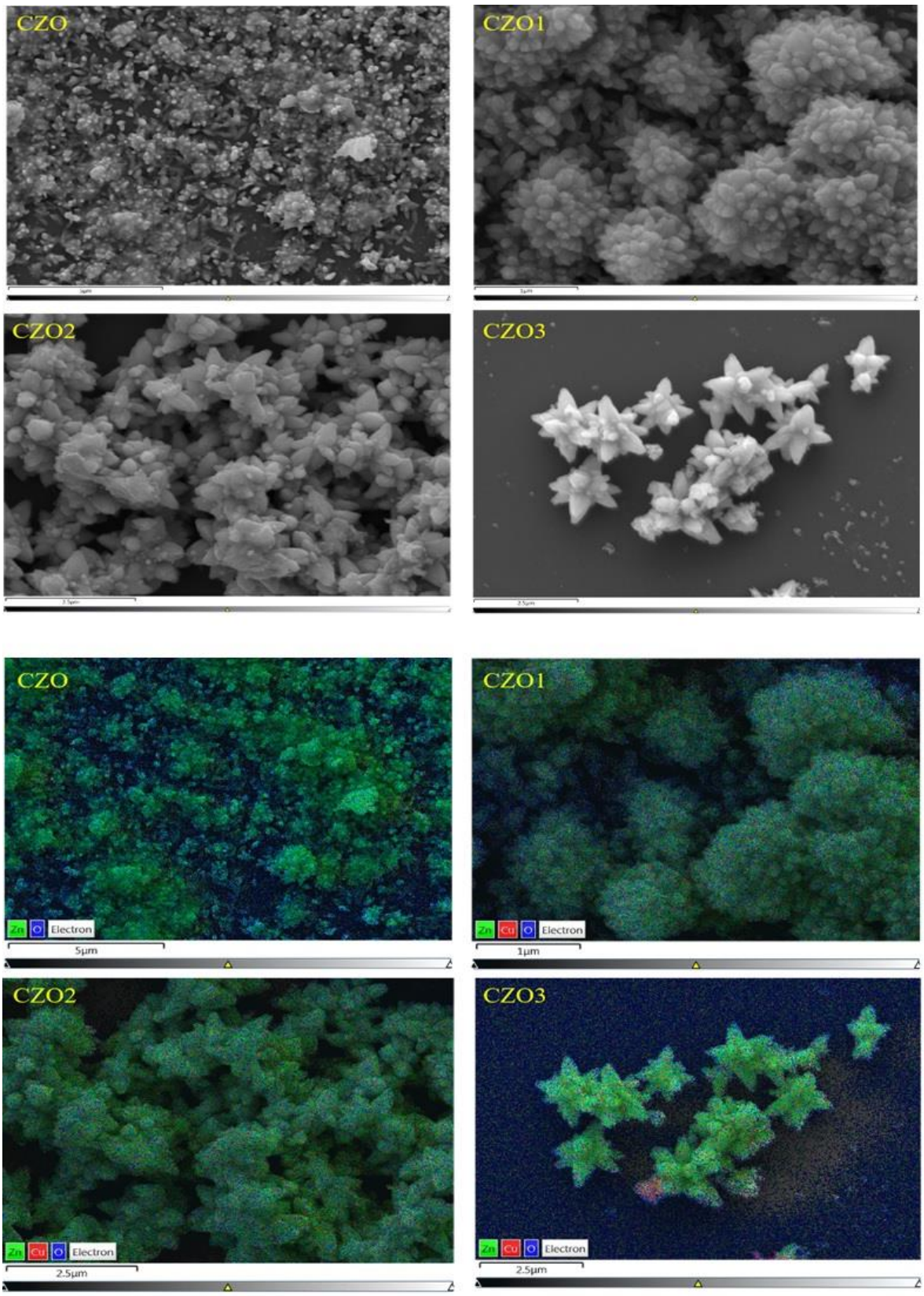


**Figure 9.** FESEM and SEM-Mapping analysis of ZnO and CZO films

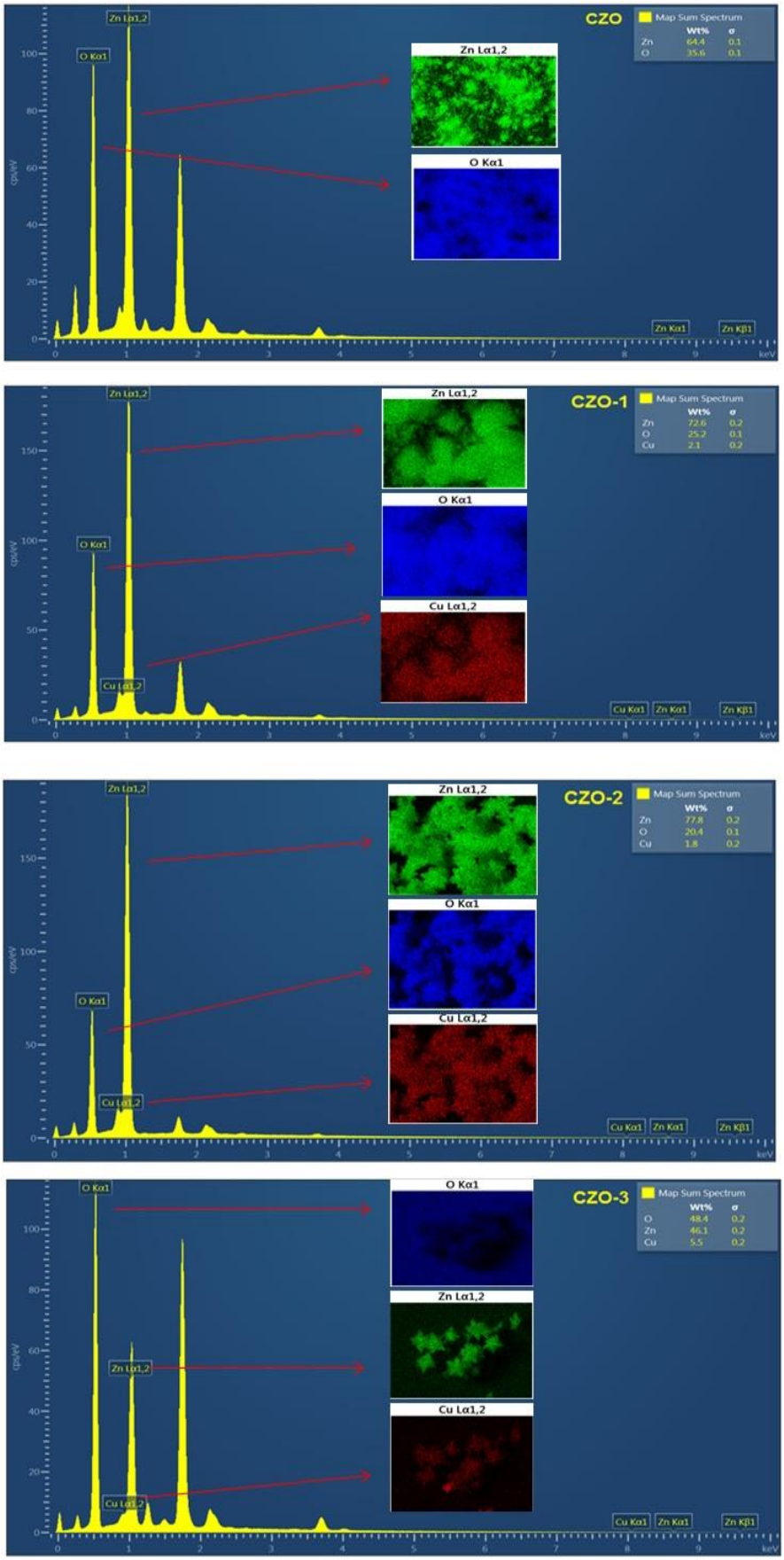


**Figure 10.** FESEM-EDS analysis of ZnO and CZO films

## CONCLUSIONS

In this study, ZnO and Cu doped ZnO films were produced using the SILAR method and the structural, optical and surface properties of the films were investigated. As a result of the examinations, the following results were obtained for the films we produced. Undoped and Cu doped ZnO films were all formed in the hexagonal wurtzite structure. The structure of low Cu doped ZnO films did not change, but defects occurred in the structure. The film with the best structural properties among the doping films was determined as CZO3 film. The transmittance value of ZnO films increased to approximately 90% with 3% Cu addition, while the transmittance value decreased at the small additive ratio. A $Cu^{+2}$ impurity band was formed in the band structure of the ZnO films with Cu doping of less than 3%. In the 3% Cu doped film, a band structure similar to ZnO was formed and the band gap of the films expanded. With Cu doping, ZnO films exhibited near band and deep level emissions at different wavelengths. Nanoflower type structures were formed in the surface morphology of ZnO films with Cu doping. According to the results obtained, it was seen that ZnO films could be improved and developed with 3% Cu doping with the SILAR method. It was determined that 3% Cu ratio was the most suitable additive ratio among the films produced for this method. In addition, it was determined that 3% Cu doped ZnO films have the potential to be used especially in gas sensor and LED applications.

## ACKNOWLEDGEMENTS

This work is supported by the Çankırı Karatekin University BAP under Project Number EYO080120B09. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## REFERENCES

[1] Kara I 2021. Investigation of structural, optical, and electrical properties of the (CuO/ZnO: Al/SnO2: F) heterostructure thin films using the spin coating method. Microscopy Research and Technique, 1-9.

[2] Sriram S, Lalithambika KC, Thayumanavan A 2017. Experimental and theoretical investigations of photocatalytic activity of Cu doped ZnO nanoparticles. Optik, 139:299-308.

[3] Shirazi M, Hosseinnejad MT, Zendehnam A, Ghoranneviss M, Etaati GRJ 2014. Synthesis and characterization of nanostructured ZnO multilayer grown by DC magnetron sputtering. Alloys Compdounds, 602:108-116.

[4] Zahedi F, Dariani RS, Rozati SM 2013. Ultraviolet photoresponse properties of ZnO:N/p-Si and ZnO/p-Si heterojunctions. Sens. Actuators A Phys, 199:123-128.

[5] Serin T, Atilgan A, Kara I, Yildiz A 2017. Electron transport in Al-Cu co-doped ZnO thin films. Journal of Applied Physics, 121(9):095303.

[6] Behera B, Chandra S 2016. An innovative gas sensor incorporating ZnO-CuO nanoflakes in planar MEMS technology. Sensors and Actuators B, 229:414-424.

[7] Yu X, Yu X, Zhang J, Hu Z, Zhao G, Zhao Y 2014. Effective light trapping enhanced near-UV/blue light absorption in inverted polymer solar cells via sol–gel textured Al-doped ZnO buffer layer. Sol. Energy Mater. Sol. Cells, 121:28–34.

[8] Fleischhaker F, Wloka V, Hennig I 2010. ZnO based field-effect transistors (FETs): solution-processable at low temperatures on flexible substrates. J. Mater. Chem, 20:6622.

[9] Dabir F, Esfahani H, Bakhtiargonbadi F, Khodadadi Z 2020. Study on microstructural and electro-optical properties of sol–gel derived pure and Al/Cu-doped ZnO thin films. Journal of Sol-Gel Science and Technology, 96(3):529-538.

[10] Ahmoum H, Li G, Belakry S, Boughrara M, Su'ait MS, Kerouad M, Wang Q 2021. Structural, morphological and transport properties of Ni doped ZnO thin films deposited by thermal co-evaporation method. Materials Science in Semiconductor Processing, 123:105530.

[11] Serrao FJ, Bappalige NN, Sandeep KM, Raghavendra S 2021. Dominance of c-axis orientation on the carrier transport properties of Sn doped ZnO thin films. Thin Solid Films, 722:138579.

[12] Ade R, Kumar SS, Valanarasu S, Kumar SS, Sasikumar S, Ganesh V, Yahia IS 2021. Improved opto-electronics properties of Ti-doped ZnO nanorods for photodetector applications. Ceramics International, 47 (17):24031-24038.

[13] Roguai S, Djelloul A 2020. A structural and optical properties of Cu-doped ZnO films prepared by spray pyrolysis. Applied Physics A, 126(2):1-8.

[14] Tsay CY, Fan KS, Lei CM 2012. Synthesis and characterization of sol–gel derived gallium-doped zinc oxide thin films. Journal of Alloys and Compounds, 512(1):216-222.

[15] Hu P, Han N, Zhang D, Ho JC, Chen Y 2012. Highly formaldehyde-sensitive, transition-metal doped ZnO nanorods prepared by plasma-enhanced chemical vapor deposition. Sensors and Actuators B: Chemical, 169:74-80.

[16] Rakhsha AH, Abdizadeh H, Pourshaban E, Golobostanfard MR, Mastelaro VR, Montazerian M 2019. Ag and Cu doped ZnO nanowires: a pH-Controlled synthesis via chemical bath deposition, Materialia, 5:100212.

[17] Zheng JH, Jiang Q, Lian JS 2011. Synthesis and optical properties of flower-like ZnO nanorods by thermal evaporation method. Applied Surface Science, 257 (11):5083-5087.

[18] Kumari N, Patel SR, Gohel JV 2018. Optical and structural properties of ZnO thin films prepared by spray pyrolysis for enhanced efficiency perovskite solar cell application. Optical and Quantum Electronics, 50(4):1-22.

[19] Dabir F, Esfahani H, Bakhtiargonbadi F, Khodadadi Z 2020. Study on microstructural and electro-optical properties of sol–gel derived pure and Al/Cu-doped ZnO thin films. Journal of Sol-Gel Science and Technology, 96(3):529-538.

[20] Gençyılmaz O, Taşköprü T 2017. The Production of ZnO and CuO Films by SILAR Method and Characterization for Use in Photovoltaic Solar Cells. Dokuz Eylul University-Faculty of Engineering Journal of Science and Engineering, 19 (56):468-484.

[21] Ge Z, Wang C, Chen T, Chen Z, Wang TL, Liu Guo J 2021. Preparation of Cu-doped ZnO nanoparticles via layered double hydroxide and application for dye-sensitized solar cells. Journal of Physics and Chemistry of Solids, 150:109833.

[22] Jellal I, Nouneh K, Toura H, Boutamart M, Briche S, Naja J, Touhami ME 2021. Enhanced photocatalytic activity of supported Cu-doped ZnO nanostructures prepared by SILAR method. Optical Materials, 111:110669.

[23] Ghos BC, Farhad SFU, Patwary MAM, Majumder S, Hossain MA, Tanvir NI, Guo Q 2021. Influence of substrate, process conditions, and post-annealing temperature on the properties of ZnO thin films grown by SILAR method. ACS, 6:2665−2674.

[24] Abdel-Wahab MS 2021. Substrate Temperature Impact on the Structural, Optical and Photo-Catalytic Activity of Sputtered Cu-Doped ZnO Thin Films. Journal of Electronic Materials, 1-9.

[25] Zhang YH, Li YL, Gong FL, Xie KF, Liu M 2019. H. L. Zhang, Al doped narcissus-like ZnO for enhanced $NO_2$ sensing performance: an experimental and DFT investigation. Sensor Actuators B, 305:127489.

[26] Devi KR, Selvan G, Prasad KH, Karunakaran M, Kasirajan K, Ganesh V, Alfaify S 2020. Effect of $Cu^{2+}$ doping on the structural, optical, and vapor-sensing properties of ZnO thin films prepared by SILAR method. Journal of Materials Science: Materials in Electronics, 31(19):16548-16560.

[27] Dhaygude HD, Shinde SK, Velhal NB, Takale MV, Fulari VJ 2016. Doping effect on SILAR synthesized crystalline nanostructured Cu-doped ZnO thin films grown on indium tin oxide (ITO) coated glass substrates and its characterization. Materials Research Express, 3(8):086402.

[28] Mehmood B, Khan MI, Iqbal M, Mahmood A, AlMasry W 2021. Structural and optical properties of Ti and Cu co-doped ZnO thin films for photovoltaic applications of dye sensitized solar cells. International Journal of Energy Research, 45(2):2445-2459.

[29] Boukhari A, Deghfel B, Mahroug A, Amari R, Selmi N, Kheawhom S, Mohamad A 2021. Thickness effect on the properties of Mn-doped ZnO thin films synthesis by sol-gel and comparison to first-principles calculations. Ceramics International, 47(12):17276-17285.

[30] Sadovnikov SI, Gusev AI 2017. Synthesis and characterization of novel stellate sea-urchin-like silver particles with extremely low density and superhydrophobicity. Journal of Materials Chemistry A, 5(38):20289-20297.

[31] Damen TC, Porto SPS, Tell B 1966. Raman Effect in Zinc Oxide. Review, 142:570.

[32] Song Y, Zhang S, Zhang C, Yang Y, Lv K 2019. Raman Spectra and Microstructure of Zinc Oxide irradiated with Swift Heavy Ion. Crystals, 9(8):395-403.

[33] Mingyuan Z, Zhiao Z, Min Z, Tariq M, Li Y, Li W, Jin H, Skotnicova K, Li Y 2017. Oxygen vacancy induced ferromagnetism in Cu-doped ZnO. Ceramic International, 43(3):3166-3170.

[34] Kuriakose S, Satpati B, Mohapatra S. 2015. Highly efficient photovoltaic degradation of organic dyes by Cu doped ZnO nanostructures. Phys. Chem. 17:25172-25181.

[35] Salem M, Massoudi I, Akir S, Litaiem Y, Gaidi M, Khirouni K 2017. Photoelectrochemical and opto-electronic properties tuning of ZnO films: Effect of Cu doping content. Journal of Alloys and Compounds, 722:313-320.

[36] Ajala F, Hamrouni A., Houas A, Lachheb H, Megna B, Palmisano L, Parrino F 2018. The influence of Al doping on the photocatalytic activity of nanostructured ZnO: The role of adsorbed water. Applied Surface Science, 445:376-382.

[37] Rai RC 2013. Analysis of the Urbach tails in absorption spectra of undoped ZnO nano films. Journal of Applied Physics, 113:153508.

[38] Tauc J 1974,. Amorphous and Liquid Semiconductor. New York: Plenum.

[39] Kim KJ, Parkın YR 2001. Large and abrupt optical band gap variation in In-doped ZnO. Applied Physics Letter, 78:475-479.

[40] Burstein E 1954. Anormalous optical absorption limit in InSb. Pyhsics Review, 93: 632.

[41] Gao D, Zhang Z, Fu J, Xu Y, Qi J, Xue D 2009. Room temperature ferromagnetism of pure ZnO nanoparticles. Journal Applied Physics, 105:113928.

[42] Sakai K, Kakeno T, Ikari T, Shirakata S, Sakemi T, Awai K, Yamamoto T 2006. Defect centers and optical absorption edge of degenerated semiconductor ZnO thin films grown by a reactive plasma deposition by means of piezoelectric photothermal spectroscopy. Journal Applied Physics, 99:043508.

[43] Muthukumaran S, Gopalakrishnan R 2012. Structural, FTIR and photoluminescence studies of Cu doped ZnO nanopowders by co-precipitation method. Optic Material, 34:1946-1953.

[44] Rahmati A, Sirgani AB, Molaei M, Karimipour M 2014. Cu-doped ZnO nanoparticles synthesized by simple co-precipitation route., The European Physical Journal Plus, 129(11):1-7.

[45] Urbach F 1953. The Long-Wavelength Edge of Photographic Sensitivity and of the Electronic Absorption of Solids. Physical Review, 92 (5).1324.

[46] Darma Y, Muhammady S, Hendri YN, Sustini E, Widita R, Takase K 2019. Tuning the point-defect evolution, optical transitions, and absorption edge of zinc oxide film by thermal exposure during molecular beam epitaxy growth. Material Science Semiconductors Process, 93:50-58.

[47] Jing L, Qu Y, Wang B, Li S, Jiang B, Yang L, Fu W, Fu H, Sun J 2006. Review of photoluminescence performance of nano-sized semiconductor materials and its relationships with photocatalytic activity. Sol. Energy Material Solar Cells, 90:1773–1787.

[48] Hou J, Huo L, He C, Yang C, Li Y 2006. Synthesis and absorption spectra of poly(3-(phenylenevinyl)thiophehe)s with conjucated side chains. Macromolecules, 39:594–603.

[49] Vanheusden K, Seager CH, Warren WL, Tallant DR, Voigt JA 1998. Correlation between photoluminescence and oxygen vacancies in ZnO phosphors. Applied Physics Letter, 68 (3):403.

[50] Vanheusden K, Warren WL, Seager CH, Tallant DR, Voigt JA, Gnade BE 1996, Mechanisms behind green photoluminescence in ZnO phosphor powders. Journal of Applied Physics, 79:79-83.

[51] Studenikin SA, Cocivera M 2022. Time-resolved luminescence and photoconductivity of polycrystalline ZnO films. Journal of Applied Physics, 91:5060.

[52] Look DC 2001. Recent advances in ZnO materials and devices. Material Science Enginerring B, 80:383-387.

[53] Zhao QX, Klason P, Willander M, Zhong HM, Lu W, Yang JH 2005. Deep-level emissions infuenced by O and Zn implantations in ZnO. Applied Physics Letter, 87:211912.

[54] Klason P, Borseth TM, Zhao QX, Svensson BG, Kuznetsov AY, Bergman PJ, Willander M 2008. Temperature dependence and decay times of zinc and oxygen vacancy related photoluminescence bands in zinc oxide. Solid State Communicaiton, 145:321.

[55] Becker J, Raghupathi KR, St Pierre J, Zhao D, Koodali RT 2011. Tuning of the crystallite and particle sizes of ZnO nanocrystalline materials in solvothermal synthesis and their photocatalytic activity for dye degradation. Journal of Physics Chemistry C, 115 (13):844–13850.

[56] Djurisic AB, Leung YH, Tam KH, Ding L, Ge WK, Chen HY, Gwo S 2006. Green, yellow, and orange defect emission from ZnO nanostructures: infuence of excitation wavelength. Applied Physics Letter, 88:103107.

[57] Studenikin SA, Golego N, Cocivera M 1998. Fabrication of green and orange photoluminescent, undoped ZnO flms using spray pyrolysis. Journal of Applied Physics, 84:2287.

[58] Cao B, Cai W, Zeng H 2006. Temperature-dependent shifts of three emission bands for ZnO nanoneedle arrays. Applied Physics Letter, 88:161101.

[59] Dellis S, Pliatsikas N, Kalfagiannis N, Lidor-Shalev O, Papaderakis A, Vourlias G, Sotiropoulos S, Koutsogeorgis DC, Mastai Y, Patsalas P 2018. Broadband luminescence in defect-engineered electrochemically produced porous Si/ZnO nanostructures. Scientific Reports, 6988(8):1-8.

## ORAL PRESENTATION

# Çanakkale Boğazı, Saroz Körfezi ve Güney Marmara Denizi Midye (*Mytilus galloprovincialis*) Örneklerinin Mikroorganizma Seviyelerinin Karşılaştırılması

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## Özet

Deniz suyu yıllık ortalama sıcaklıklarının küresel ısınma nedeniyle artış eğiliminde olması, deniz mikroflorasını kalitatif ve kantitatif açıdan doğrudan etkilemektedir. Genellikle, değişen su özelliklerine bağlı olarak sub-tropik denizlerde hakim mikroorganizmaların yaygınlaştığı görülmekte, bu da aynı zamanda denizlerimizdeki canlıların mikrofloralarında da değişime neden olmaktadır. Deniz canlıların birincil mikroflorasındaki değişim ise hem ekolojik hem de gıda güvenliği açısından önem taşımaktadır. Dolayısıyla bu çalışmada, Çanakkale ili ve çevresindeki kıyı bölgelerde mikroorganizmaların tür ve seviyelerindeki farklılıklar belirlenmeye çalışılmıştır. Bu amaçla Çanakkale Boğazı, Saroz Körfezi ve Güney Marmara Denizi'nde belirlenen istasyonlardan, Akdeniz midyesi (*Mytilus galloprovincialis*) örneklerinde, toplam heterotrofik mezofilik bakteri (THMB), *Vibrio* spp. ve *Enterobacteriaceae* ailesine ait mikroorganizmaların seviyesi belirlenmiştir. Elde edilen bulgularda, Saroz Körfezi örneklerinde *Vibrio* spp. ve Enterobacteriaceae üyesi mikroorganizmalara rastlanmazken, Güney Marmara Denizi'nden elde edilen midye örneklerinde *Vibrio* spp. (2,70 log kob/g), Çanakkale Boğazı'ndan temin edilen midye örneklerinde ise her iki mikroorganizma grubunun varlığı tespit edilmiştir. Çanakkale Boğazı'ndaki THMB sayısı, *Vibrio* spp. ve *Enterobacteriaceae* ailesine ait mikroorganizma sayıları sırasıyla 5,15 log kob/g, 4,67 log kob/g ve 3,11 log kob/g olarak belirlenmiştir. Elde edilen bulgulara göre Çanakkale Boğazı; Saroz Körfezi ve Güney Marmara Denizi'ndeki çalışma alanlarına göre daha fazla mikroorganizma içeriğine sahiptir. Boğazda tespit edilen yüksek mikrobiyal yük seviyesinin çevresel faktörler ile birlikte bölgeye özgü potansiyel kontaminasyonlardan kaynaklanabileceği düşünülmektedir. Sonuç olarak, mikroorganizma seviyelerinde bölgesel farklılıklara neden olan faktörlerin daha detaylı belirlenmesi gerektiği anlaşılmıştır. Ayrıca Çanakkale kıyı bölgelerinde mikroorganizma tür ve sayısında meydana gelen değişikliklerin canlı sağlığı ve gıda güvenliğine etkileri konusunda daha fazla çalışmaya ihtiyaç duyulacağı düşünülmektedir.

**Anahtar Kelimeler:** Çanakkale, Akdeniz midyesi, *Mytilus galloprovincialis, Vibrio, Enterobacteriaceae*

# Comparison microorganism levels of mussel (*Mytilus galloprovincialis*) samples in Canakkale Strait, Saros Gulf and Southern Marmara Sea

## Abstract

The increase in annual mean temperatures of sea water due to global warming directly affects the marine microflora both qualitatively and quantitatively. Generally, it is seen that dominant microorganisms become widespread in sub-tropical seas due to changing water properties, which also causes changes in the microflora of marine livings in Turkey seas. The change in the primary microflora of marine organisms is important both in terms of ecological and food safety. Therefore, in this study, it was tried to determine the differences in the types and levels of microorganisms in Canakkale province and its surrounding coastal regions. For this purpose, total heterotrophic mesophilic bacteria (THMB), *Vibrio* spp., and *Enterobacteriaceae* family the level of microorganisms belonging to were determined in Mediterranean mussels (*Mytilus galloprovincialis*). According to results, *Vibrio* spp. and microorganisms belonging to Enterobacteriaceae family were not

determined in the Saros Gulf samples. In the samples from the Southern Marmara Sea, *Vibrio* spp. were detected at 2.70 log cfu/g level, while no *Enterobacteriaceae* members were detected. In the samples from the station of Canakkale Strait, elevated levels of THMB, *Vibrio* spp., and *Enterobacteriaceae* were detected as 5.15 log cfu/g, 4.67 log cfu/g, and 3.11 log cfu/g, respectively. According to these findings, the Canakkale Strait samples have a higher microorganism content than the samples of both Saros Gulf and the Southern Marmara Sea. It is thought that the higher level of microbial load detected in the strait may be related to environmental factors and potential contaminations specific to the region. In conclusion, it is understood that the factors causing the regional differences in microbial levels should be determined in more detail. In addition, there will be a need for more studies on the effects of changes in microorganism species and number on the living health and food safety in the coastal areas of Canakkale.

**Keywords:** Canakkale, Mediterranean mussel, *Mytilus galloprovincialis*, *Vibrio* spp., *Enterobacteriaceae*

## GİRİŞ

Çanakkale, Ege ve Marmara Denizlerine olan kıyısı ve sahip olduğu boğaz ile coğrafik konum açısından önemli illerimizdendir. Çanakkale ilinde turistik ve ticari faaliyetler yaygın olarak gerçekleştirilmekte dolayısıyla bölgede kıyılar oldukça aktif kullanılmaktadır. Çanakkale kıyılarında ayrıca halkın büyük bir kesimi yetiştiricilik, profesyonel ve amatör avcılık ile uğraşmakta, su ürünlerinin üretimi ve tüketimi bölgede önem arz etmektedir (Çolakoğlu ve ark., 2019). Dolayısıyla kıyılarında yoğun insani aktivite olan bu bölgenin su ürünlerinde, canlı sağlığı ve gıda güvenliği konularının önemi her geçen gün artmaktadır.

Midyeler, besin olarak kullanılan önemli su ürünleridir. Ayrıca suyu süzerek beslenen bu canlılar su kalitesini belirlemek için iyi biyolojik indikatörler olup sudaki bir kontaminasyonun tespiti amacıyla kullanılabilmektedir (Çolakoğlu ve ark., 2020; Künili ve ark., 2021a; Künili ve ark., 2021b). Dolayısıyla, deniz kıyı alanlarında midyelerde aralıklı olarak mikroorganizmaların tür ve seviyelerinin belirlenmesi, o bölgede kontaminasyon olup olmadığının bir göstergesi olarak nitelendirilebilmektedir.

Bu çalışmada, deniz kıyıları aktif kullanılan ve ülkemizin en önemli çift kabuklu yumuşakça üretim alanlarının bulunduğu Çanakkale Boğazı midye örneklerinin mikrobiyolojik kaliteleri, Güney Marmara Denizi örnekleri ve boğaz bağlantısı olmayan Saroz Körfezi örnekleri ile kıyaslanmıştır. Bu bölgelerde belirlenen istasyonlardan temin edilen Akdeniz midyesi (*Mytilus galloprovincialis*) örneklerinde toplam heterotrofik mezofilik bakteri (THMB), *Vibrio* spp. ve *Entereobacteriaceae* ailesine ait mikroorganizmaların seviyesi belirlenmiştir. İstasyonlar karşılaştırılarak elde edilen bulgular, ekolojik ve gıda güvenliği açısından yorumlanmıştır.

## MATERYAL VE METOT

Araştırma materyali olarak Akdeniz midyesi (*Mytilus galloprovincialis*) kullanılmıştır. Materyaller Şekil-1'de koordinatları ile verilen A-Çanakkale Boğazı B- Saroz Körfezi ve C- Güney Marmara Denizi'nden serbest dalış yapılarak el ile toplanmıştır (Şekil 1). Her istasyondan farklı boylara sahip 50 adet midye toplanmış ve iki saat içerisinde önceden soğutulmuş izolasyonlu örnek taşıma çantaları ile Çanakkale Onsekiz Mart Üniversitesi, Deniz Bilimleri ve Teknolojisi Fakültesi Mikrobiyoloji Laboratuvarına getirilmiştir. Ölü veya kabuğu zarar gören midyeler ayırıldıktan sonra örnekler analizlere hazırlanmıştır.



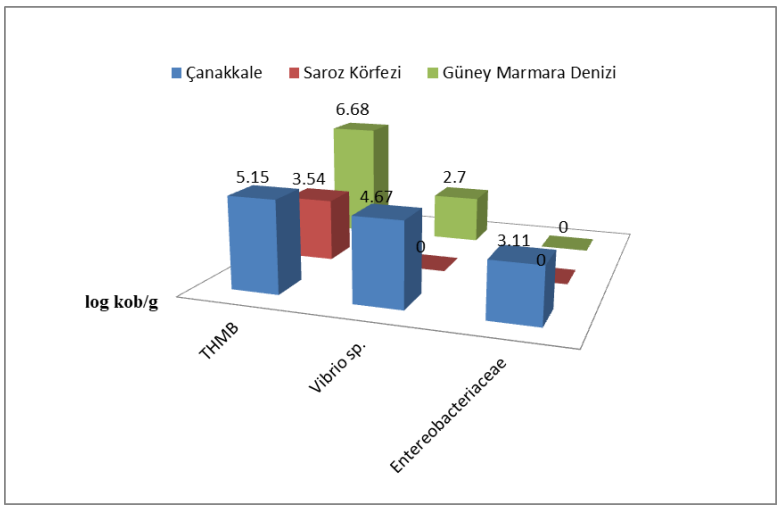

**Şekil 1**. Örnekleme istasyonları A- Çanakkale Boğazı (39° 46′ 57″ N; 26° 9′ 25″ E) B- Saroz Körfezi (40°33'194"N; 26°46'098"E) C- Edincik (40°22'116"N; 27°52'015"E)

## Mikrobiyolojik Analizler

Midyelerin kabukları kum, çamur ve makroorganizmalardan arındırıldıktan sonra, alkollü pamuk ile temizlenmiştir. Steril bıçak yardımıyla açılan canlı midyelerin içinde bulunan kabuklararası sıvı ve midye etlerinden toplam 10 g homojen örnek alınarak, 90 ml bakteriyolojik pepton (%0,1 Pepton + % 1NaCl) içerisine tartılmıştır. Tartım sonrasında örnekler Stomacher (Seward, 400) cihazında, 2 dk süre ile 2300 rpm'de homojenize edilmiştir. Homojenize edilen örneklerin yine peptonlu su kullanılarak $10^{-6}$'ya kadar desimal seyreltmeleri hazırlanmıştır. Yapılan her seyreltmeden yayma plak yöntemi ile Thio Citrate Bile Sucrose Agar (TCBS-Merck), Marine Agar (Difco) ve Endo Agar (Merck) besiyerlerine ekimleri yapılmıştır (FDA/BAM, 1998). Midye örneklerinden, ekim yapılan tüm petriler üreticilerin verdiği süre ve sıcaklıkta inkübasyona tabi tutulmuş, inkübasyon sonunda üreyen koloniler seçilerek biyokimyasal testler ile ön tanılama yapılmıştır.

## BULGULAR ve TARTIŞMA

Bu çalışmada aynı bölgede yer alan ve farklı mikrobiyal kirlenme seviyesine sahip olduğu düşünülen Çanakkale Boğazı, Saroz Körfezi ve Güney Marmara Denizinden seçilen istasyonlardan toplanan midye örneklerinin mikrobiyolojik yükleri belirlenmiştir (Şekil 2). Midyelerin THMB yükleri Güney Marmara Denizinde en yüksek seviyeye (6,68 log kob/g) ulaşmış, Çanakkale ve Saroz Körfezinde ise sırasıyla 5,15 ve 3,54 log kob/g olarak belirlenmiştir. Çift kabuklu yumuşakçaların gıda olarak tüketilebilmesi için toplam mezofilik aerobik bakteri (TAMB) limitinin $5x10^5$ kob/g olarak bildirildiği (ICMSF, 1986) standardı Güney Marmara Denizi midye örneklerinin aştığı tespit edilmiştir. Çanakkale Boğazı ve Saroz körfezi midye örnekleri ise mikrobiyal yük açısından standarda uygun olarak belirlenmiştir. Mikrobiyal yükteki değişimin ise bölgeler arası denizel faaliyetlerdeki farklılıktan kaynaklanabileceği düşünülmektedir. Çanakkale ili genelinde 11 farklı istasyondan alınan midye örneklerinin mikrobiyal yüklerinin belirlendiği bir çalışmada, tüm istasyonlardan alınan örneklerdeki toplam aerobik mezofilik bakteri sayısı 0,73 ila 3,56 log kob/g olarak belirlenirken (Tosun ve ark., 2021), Çanakkale boğazının iki yakasından örnekleme yapan bir başka çalışmada tüm istasyonlarda TAMB sayısı $5,4x10^5$-$1,6x10^6$ aralığında bulunmuştur (Şener ve ark., 2012). Çanakkale kıyılarından örneklenen çift kabuklu yumuşakça türlerinde (*Chamelea gallina*) ve su örneklerinde yapılan diğer bir çalışmada ise, TAMB sayılarının yaz aylarında $10^4$ kob/g değerine kadar çıktığı, kış aylarında ise $10^2$ kob/g değerine düştüğü ve bu aralıkta yıl boyu değiştiği bildirilmiştir (Çolakoğlu ve ark., 2010). Farklı çalışmalarda aynı bölgelerden toplanan midye örneklerinde farklı mikrobiyal yüke rastlanması mevsimsel değişimler, yıla bağlı olarak bölgedeki kirlilik farklarından kaynaklanabilmektedir.




**Şekil 2.** Çanakkale Boğazı, Saroz Körfezi ve Güney Marmara Denizi midye örneklerinin mikrobiyal yükleri (log kob/g)

*Vibrio* spp. su ürünleri açısından önemli bakteriler olup, *Vibrio* türlerini içeren su ürünleri ile temas veya bu ürünleri tüketim insan sağlığı açısından risk oluşturabilmektedir. Bu çalışmada seçilen istasyonlardan Saroz Körfezinden elde edilen midye örneklerinde *Vibrio* spp. türlerine rastlanmamıştır. Çanakkale Boğazı'nda ise en yüksek *Vibrio* spp. sayısı 4,67 log kob/g olarak tespit edilirken, Güney Marmara Denizi midye örneklerinde sayı 2,7 log kob/g olarak belirlenmiştir. Marmara Denizinden (Gelibolu Bölgesi) avlanan kum (*Venus gallina*) ve kara midyelerinin (*Mytilus galloprovincialis*) mikrobiyolojik kalitesinin belirlendiği bir çalışmada ise *Vibrio (V.) cholerea* ve *Vibrio parahaemolyticus* varlığını belirlemeye yönelik yapılan analizlerde bu mikroorganizmaların varlığı tespit edilememiştir (Yılmaz ve ark., 2005). Bandırma ve Balıkesir bölgesinde yapılan bir başka çalışmada da benzer şekilde kum midyesi örneklerinde *Vibrio* spp. varlığı bildirilmemiştir (İrkin ve ark., 2007). Diğer taraftan, *Vibrio* spp. ler arasında su ürünleri gıda güvenliği açısından en önemli suşlardan olan *V. parahaemolyticus*, Çanakkale bölgesi *C. gallina* örneklerinde, iki kez ve yalnızca yaz aylarında tespit edilmiştir (Çolakoğlu ve ark., 2010). Bu çalışmada ise Saroz körfezi örneklerinden *Vibrio* spp. tespit edilmemiştir, ancak örneklemeler sonbaharda yapılmış olmasına rağmen Güney Marmara ve Çanakkale Boğazı örneklerinde çok sayıda *Vibrio* spp. belirlenmiştir. Bu durumun genel olarak yıllık bazda deniz suyu sıcaklıklarının ortalamasındaki artışa bağlı olabileceği, Çanakkale civarındaki kıyı sularındaki doğal floraya *Vibrio* türlerinin dahil olabileceği ve bu bakteri türünün çift kabuklu yumuşakçalara bulaşması sonrasında kabukluların bünyesinden diğer mikroorganizmalara göre daha zor arındırması veya tamamen arındıramaması ile alakalı olabileceği düşünülmektedir (Çardak ve ark., 2013; Künili ve ark., 2013; Çolakoğlu ve ark., 2014).

Enterobacteriaceae sayımı, fekal patojen varlığı riskinin bir göstergesi olarak, fekal koliform, fekal Streptococcus ve *Escherichia coli* (*E. coli*) sayıları ile birlikte kullanılabilmektedir. Çanakkale Boğazı istasyonu midye örneklerinde Enterobacteriaceae sayımı 3,11 log kob/g belirlenirken, Saroz Körfezi ile Güney Marmara Denizi midye örneklerinde Enterobacteriaceae yüküne rastlanmamıştır. Çalışma sonucuna benzer şekilde Tosun ve ark. (2021) Çanakkale civarında yaptıkları çalışmada tüm örneklerde Enterobacteriaceae sayımı sonuçlarını 0-3,56 log kob/g olarak tespit etmişlerdir. Bununla birlikte Marmara Denizinde yapılan bir çalışmada koliform grubu bakteri ve *E. coli* sayıları sırasıyla 2,46 log kob/g ve 1,9 log kob/g olarak bildirilmiştir (Yılmaz ve ark., 2005). Şener ve ark. (2013) ise Çanakkale'nin iki yakasındaki istasyonlardan elde ettikleri kara midye örneklerinin koliform bakteri ve *E. coli* sayımlarını sırasıyla 5,0 – 5,1 log kob/g ve 5,4 – 6,1 log kob/g arasında bildirmişlerdir. Çanakkale kıyılarında yapılan çalışmalarda, Enterobacteriaceae familyasına ait olan koliform, fekal koliform ve *E.coli* mikroorganizmalarına ait seviyelerin akıntı olan yerlerden örneklenen çift kabuklu yumuşakça (*C. gallina*) türlerinde ve su örneklerinde 1100 EMS/100g'lara ulaştığı (Çolakoğlu ve ark., 2010); akıntının olmadığı ve muhtemel deşarjların olabileceği Çardak Lagünü gibi Çanakkale kıyı alanlarında ise daha yüksek seviyelere ulaşabildiği bildirilmiştir (Künili ve Ateş, 2021).

## SONUÇ

Sonuç olarak bu çalışma ile Çanakkale ve civarındaki kıyı bölgelerde deniz suyunun mikrobiyolojik kalitesinin değişken olduğu belirlenmiştir. Saroz Körfezi örneklerinde deniz trafiği ve kentleşmenin daha az olmasından dolayı, Çanakkale Boğazı ve Güney Marmara Denizi'ne göre çok daha az mikrobiyal yüke sahip olduğu tespit edilmiştir. Bu çalışma sonucunda elde edilen verilere göre mikroorganizma seviyesinin en yüksek olduğu

Çanakkale Boğazı'nda periyodik izleme çalışmalarının devam etmesi ekolojik, ekonomik ve gıda güvenliği açısından önem taşımaktadır.

## KAYNAKLAR

Çardak M, Çolakoğlu F, Künili İE, Çolakoğlu S 2013. Effect of depuration on *Manila clam, Ruditapes philippinarum,* contaminated with *Escherichia coli Salmonella typhimurium* and *Vibrio parahaemolyticus*. 40th International CIESM (Commission Internationale pour l'Exploration Scientifique de la mer Mediterranee) Congress,, Marsielle, Fransa, 28 Ekim - 01 Kasım 2013, 473-474.

Çolakoglu F, Ormancı HB, Künili İE, Colakoglu S 2010. Chemical and microbiological quality of the *Chamelea gallina* from the Southern Coast of the Marmara Sea in Turkey. Kafkas Universitesi Veterinerlik Fakültesi Dergisi, 16 (1): 153-158.

Çolakoglu F, Künili İE, Ormanci HB 2019. Fisheries food industry of Canakkale. Proceedings of The International Scientific and Practical Conference "Bulgaria of Regions", 2(1): 707-710.

Çolakoğlu S, Künili İE, Çolakoğlu F. 2020. Bioaccumulation monitoring of chemical contaminants in mussels *Mytilus galloprovincialis* from the southern coast of the Marmara Sea, Turkey. Turkish Journal of Veterinary & Animal Sciences, 44(2): 9.

Çolakolu F, Cardak M, Çolakoglu S, Künili, İE 2014. Depuration times of *Donax trunculus* and *Tapes decussatus*. Brazilian Journal of Microbiology, 45(3):1017-22.

FDA 1998. Bacteriological Analytical Manual, 8th ed. Revision A, Food and Drug Administration, AOAC International, Washington, DC.

ICMSF 1986. Microorganisms in foods. 2.sampling for microbiological analysis: principles and specific applications, 2nd ed. University of Toronto Press, Buffalo, NY.

İrkin R, Korukoğlu M, Tavşanlı H 2007. İhracata yönelik hazırlanan bazı deniz ürünlerinin mikrobiyal özellikleri. Türk Hijyen ve Deneysel Biyoloji Dergisi, 64 (1): 26-30.

Künili İE, Suat Ateş A 2021. Effects of seasonal changes and environmental factors on bioindicator bacteria levels in Çardak Lagoon, Çanakkale Strait, Turkey. Oceanological and Hydrobiological Studies, 50(3): 299-309.

Künili İE, Ertürk-Gürkan S, Aksu A, Turgay E, Çakir F, Gürkan M, Altinağaç U 2021b. Mass mortality in endangered fan mussels *Pinna nobilis* (Linnaeus 1758) caused by co-infection of *Haplosporidium pinnae* and multiple Vibrio infection in Çanakkale Strait, Turkey. Biomarkers, 26(5): 450-461.

Künili İE, Ormancı HB, Çolakoğlu F 2013. Akivades (*Tapes decussatus*) ve Kidonya (*Venus verrucosa*) Çift Kabuklu Yumuşakça Türlerinin Depurasyon Sürelerinin Belirlenmesi. 17. Ulusal Su Ürünleri Sempozyumu, İstanbul, Türkiye, 03-06 Eylül 2013, 196.

Künili İE, Çolakoğlu S, Çolakoğlu F 2021a. Levels of PAHs, PCBs, and toxic metals in *Ruditapes philippinarum* and *Donax trunculus* in Marmara Sea, Turkey. Journal of The Science of Food and Agriculture, 101: 1167-1173.

Şener A, Demir N, Çakıcı N, Kaya H, Bakar C 2012. Çanakkale Boğazı'ndan avlanan kara midyelerinin (*Mytilus galloprovincialis*) mikrobiyolojik incelemesi. Nobel Medicus, 26(9-2): 69-73.

Tosun M, Taylan G, Zorba NN 2021. Çanakkale bölgesinden alınan midye ve deniz suyu örneklerinin mikrobiyolojik kalitelerinin belirlenmesi. Gıda, 46(4): 949-958.

Yilmaz I, Bilgin B, Öktem B 2005. Occurrence of Vibrio and other pathogenic bacteria in *Mytilus galloprovincialis* and *Venus gallina* harvested from the Marmara Sea. Turkish Journal of Veterinary & Animal Sciences, 29(2): 409-415.

*ORAL PRESENTATION*

# Begonyada genetik çeşitliliğin morfolojik ve retrotranzpozon tabanlı iPBS markörleri ile belirlenmesi

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**Özet**

Begonya, tropikal ve subtropikal bölgelerde yaygın olarak yetiştirilen ekonomik önemi yüksek bir süs bitkisidir. Saksılı olarak iç mekân süs bitkisi olarak kullanımının yanında, bahçe bitkisi olarak da kullanılmaktadır. Ticari olarak yaklaşık 200 türünün kullanıldığı bilinen begonyalar yumrulu ve yumrulu olmayan çok sayıda değişik türü içermektedir. Bu çalışmada halk arasında salon bitkisi olarak ve bahçe bitkisi olarak yetiştiriciliği yapılan farklı türlere ait 20 begonya genotipi morfolojik ve moleküler özellikleri bakımından karakterize edilmiştir. Genotiplerin yaprak eni, yaprak boyu, yaprak alt rengi, yaprak üst rengi, apeks açısı, damarların dikkat çekiciliği, çiçek taç yaprak sayısı gibi morfolojik özellikleri belirlenmiş ve genotiplerin bitkisel özellikler bakımından oldukça farklı oldukları belirlenmiştir. Ek olarak, genetik çeşitlilik retrotranzpozon tabanlı bir moleküler markör sistemi olan iPBS (Inter-primer binding site) markörleri ile belirlenmiştir. Toplamda 10 adet iPBS primeriyle çalışılmış olup, 74 tane bant skorlanmış, bunlardan 71 tanesi polimorfiktir. Ortalama polimorfizm oranı %97 olarak saptanmıştır. Farklı türlere ait begonya genotipleri arasında önemli bir varyasyon olup, elde edilen dendogramda benzerlik katsayılarının 0,41 ile 0,84 arasında olduğu tespit edilmiştir. Bu çalışma da yaygın olarak yetiştiriciliği yapılan begonya genotiplerinin hem morfolojik özellikleri hem de genetik özellikleri bakılımdan oldukça geniş bir varyasyona sahip olduğu tespit edilmiş olup, gelecekte yapılacak ıslah çalışmalarına yol gösterici sonuçlar elde edilmiştir.
Anahtar Kelimeler: Begonya, *Begonia* L., iPBS

## Determination of genetic diversity in Begonia with morphological and retrotransposon-based iPBS markers

**Abstract**

Begonia is an ornamental plant of high economic importance that is widely grown in tropical and subtropical regions. It is used as a potted indoor ornamental plant as well as a garden plant. Begonias, of which about 200 species are known to be used commercially, contain a large number of tuberous and non-tuberous species. In this study, 20 begonia genotypes belonging to different species cultivated as indoor and garden plants were characterized in terms of morphological and molecular properties. Morphological properties of genotypes such as leaf width, leaf length, leaf under color, leaf top color, angle of apex, striking veins, number of flower petals were determined. Also, it was determined that genotypes were quite different in terms of vegetative characteristics. In addition, genetic diversity was determined with iPBS (Inter-primer binding site) markers, a retrotransposon-based molecular marker system. A total of 10 iPBS primers were studied and 74 bands were scored, 71 of which were polymorphic. The mean polymorphism rate was found to be 97%. There is a significant variation among begonia genotypes of different species, and the similarity coefficients in the obtained dendrogram were found to be between 0,41 and 0,84. In this study, it was determined that begonia genotypes, which are widely cultivated, have a wide variation in terms of both morphological characteristics and genetic characteristics, and guiding results were obtained for future breeding studies.
**Keywords:** Begonia, *Begonia* L., iPBS

## GİRİŞ

Begonya, tropikal ve subtropikal bölgelerde yaygın olarak yetiştirilen ekonomik önemi yüksek bir süs bitkisidir. Saksılı olarak iç mekân süs bitkisi olarak kullanımının yanında, bahçe bitkisi olarak da kullanılmaktadır (Hvoslef-Eide ve Munster, 2007). Çok çeşitli ortamlarda büyüyen 1.500'den fazla türle *Begonia* L. (*Begoniaceae*) yüzyıllardır yetiştiriciliği yapılan bir türdür (Neale ve ark., 2006; Brennan ve ark.,2012). Ticari olarak yaklaşık 200 türünün kullanıldığı bilinen begonyalar yumrulu ve yumrulu olmayan çok sayıda değişik türü içermektedir. Daha çok yağlı begonya, yumrulu begonya, çilli begonya olarak bilinen türler iç mekân süs bitkisi olarak kullanılmaktadır. Şeker begonya olarak bilinen türler ise genelde dış mekân süs bitkisi olarak bahçelerde kullanılmaktadır.

Begonyaların botanik isimleri:

- Yağlı begonya: *Begonia thurstonii*
- Söğüt begonya*: Begonia corallina*
- Çilli begonya: *Begonia corallina*
- Rex begonya: *Begonia rex*
- Yumrulu begonya*: Begonia tuberosa*
- Şeker begonya: *Begonia semperflorens* (Hvoslef-Eide ve Munster, 2007).

Begonyalar çok farklı bitkisel özelliklere sahip olan morfolojik olarak birbirinden oldukça farklı türleri içermektedir. Ancak ülkemizde begonyaların morfolojik özellikleri ile yapılmış çalışmalar oldukça kısıtlıdır. Moleküler ve genetik boyutuyla alakalı yapılmış çalışmaya rastlanmamıştır. Yoğun olarak çalışmaların gerçekleştiği morfolojik belirteçlerin aksine, moleküler belirteçler, DNA üzerindeki varyasyonu doğrudan ifade etmektedir. Moleküler belirteçler genom üzerinde bulunan ve bireyler arasındaki benzerlik ve farklıkların belirlenmesinde kullanılan DNA dizileridir (Yang ve ark., 2015). PCR temelli belirteç yöntemleri, özellikleri belirlenmek istenen tür ve çeşitlere uygun primerler kullanarak, primerlerin bağlanma bölgeleri arasında kalan DNA bölgesinin çoğaltılması esasına dayanmaktadır (Parveen ve ark., 2016).
iPBS yöntemi ise, retrotranspozon tabanlı ara primer bağlanma bölgesi olarak tanımlanan, genetik çeşitliliği belirlemede kullanılan önemli moleküler belirteç yöntemlerinden biridir. Bütün ökaryot genomlarında bulunan transpozon genlerinin kullanılmasına dayanan bir yöntemdir. Retrotranspozonlar, genom içerisinde dağınık halde bulunmaları, ortak yapısal özelliklere sahip dizilerinin olması, genom çapında düzenlemelere katılmaları gibi özellikleri yüzünden moleküler belirteç olarak kullanılmaya uygundurlar (Kalendar ve ark., 2011; (Kalendar ve Schulman, 2014).

Bu çalışmada ise çok fazla tür ve çeşiti barındıran Begonya genotiplerinin, morfolojik ve moleküler olarak genetik benzerlik ve farklılıklarının belirlenmesi amaçlanmıştır.

## MATERYAL VE METOT

### Bitkisel materyal

Bu çalışmada bitkisel materyal olarak, halk arasında en çok kullanılan türler toplanmıştır. Yumrulu begonya, yağlı begonya, çilli begonya, şeker begonya ve söğüt begonya türlerine ait 20 farklı begonya genotipi fidanlıklardan, evlerden ve ev bahçelerinden toplanmıştır. Saksılara alınarak sera ortamında yetiştirilen genotiplerde morfolojik özellikler belirlenmiştir. Ek olarak, genotiplerin genç yapraklarından alınan örnekler ile moleküler çalışmalar yürütülmüştür.

### Metot

### Morfolojik karakterizasyon

Yirmi genotip en, boy, yaprak alt rengi, yaprak üst rengi, çiçek taç yaprak sayısı, apeks açısı ve damarların dikkat çekiciliği gibi yedi morfolojik özellik bakımından UPOV kriterlerine göre değerlendirilmiştir.

### DNA izolasyonu ve PCR amplifikasyonu

Bitkilerin genç yapraklarından alınan örnekler CTAB yöntemi kullanılarak genomik DNA izole edilmiştir (Doyle, 1990). Bu yapraklardan izole edilen DNA'nın kalitesi ve miktarı agaroz jel elektroforezi ile belirlenmiştir. Genomik DNA, iPBS primerleri ile primer testine tabi tutulmuştur. Amplifikasyon gösteren, 10 iPBS primeri kullanılarak PCR amplifikasyonuna tabi tutulmuştur. PCR reaksiyonu, her bir örnek için 8,67 µL su ,1,5µL buffer,1,3 µL mg, 0,33µL dNTP,0,2 µL Taq DNA polimeraz, 2µL DNA, 2 µL IPBS primeri kullanılmıştır. PCR reaksiyonları, Bio-Rad C1000 termocycler cihazında gerçekleştirilmiştir. IPBS PCR amplifikasyonu için kullanılan döngü koşulları 94 °C'de 5 dakika, 94 °C'de 1 dakika 30 döngü, 55°C'de 1 dakika, 72°C'de 2 dakika, son uzatma adımı 72°C'de 5 dakika olmuştur. Amplifiye Edilen Ürünler 1X TBE (Tris-Borik asit-EDTA) tamponu kullanılarak 110 V'ta 3 saat boyunca %1.5'luk agaroz jel ile etidyum bromid yardımıyla boyanarak UV ışık altında görüntülendi.

### Veri Analizi

Moleküler analizler için, yalnızca net ve tekrarlanabilir DNA fragmanları, sırasıyla bir bandın varlığı ve yokluğu için 1-0 ikili veri matrisi olarak puanlandı. 20 begonya genotipi arasındaki kümeleme analizi, NTSYS-pc sürüm 2.1 (Sayısal Taksonomi Çok Değişkenli Analiz Sistemi, Exeter Yazılımı, Setauket, NY, ABD) kullanıldı. Her primer kombinasyonu için toplam fragman sayısı (TNF), polimorfik fragman sayısı (NPF) ve ortalama polimorfizm (MP) belirlenir. Polimorfizm oranları, $\frac{polimorfik\ bant\ sayısı}{toplam\ bant\ sayısı} * 100$ formülü kullanılarak hesaplanmıştır.

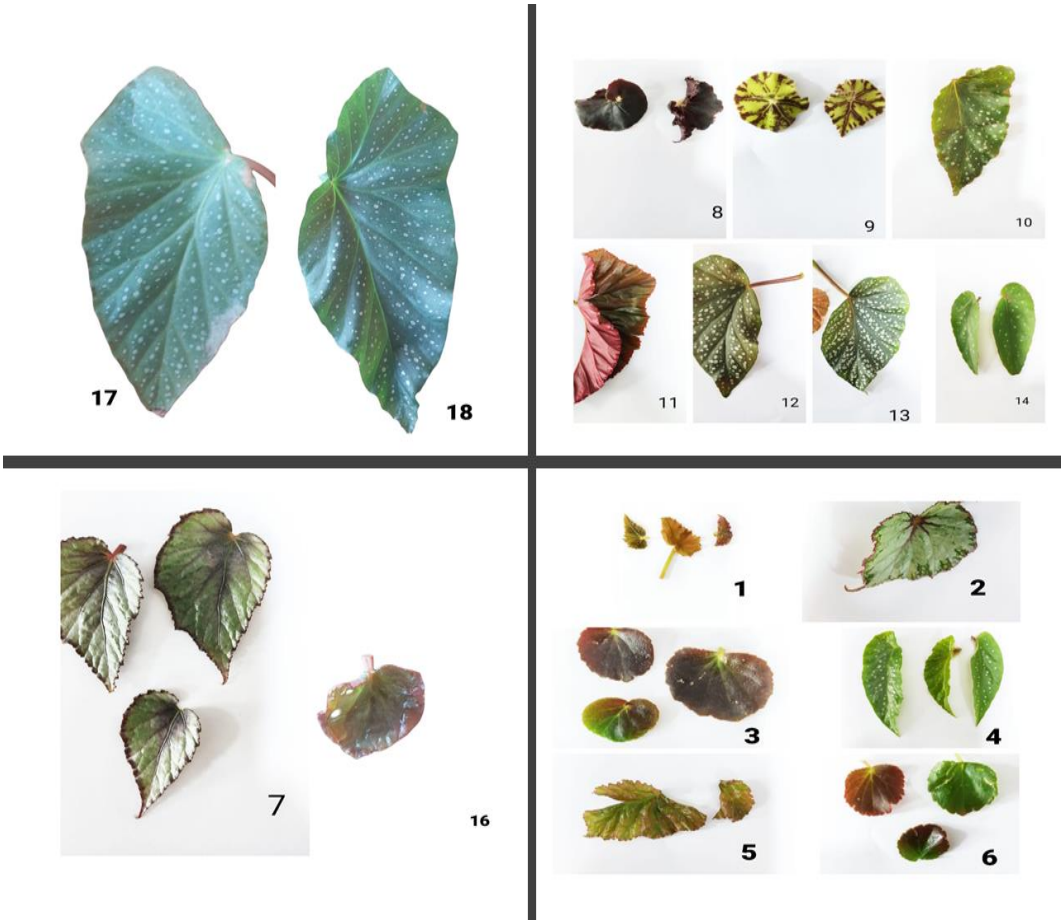
# BULGULAR

## Morfolojik Özelliklere Ait Bulgular

Farklı tür ve çeşitlere ait olan 20 farklı begonya genotipi, morfolojik ve moleküler özellikler açısından değerlendirilerek, genetik çeşitliliğin boyutları belirlenmeye çalışılmıştır. Morfolojik özellikler açısından 20 genotipten iki genotip yağlı begonya, altı genotip rex begonya, altı genotip çilli begonya, üç genotip şeker begonya, iki genotip söğüt begonya ve bir genotip yumrulu begonya grubuna aittir. Morfolojik özelliklerden, yaprak en ve boy değerleri ölçülmüş olup, yaprak eni bakımından en büyük genotipler yağlı begonyalar iken en küçük değere sahip genotipler söğüt ve şeker begonyalardır. Yaprak boy değerlerinde ise en küçük değere sahip genotipler şeker begonyalar iken en yüksek değere sahip genotipler çilli ve yağlı begonyalardır (Tablo1; Şekil 1).

**Tablo 2:** Begonya genotiplerinin yaprak özelliklerine ait bulgular

| Genotip no | Tip | Yaprak En | Yaprak Boy |
|---|---|---|---|
| **1** | Yumrulu | 3,3 | 2,0 |
| **2** | Rex | 4,0 | 4,0 |
| **3** | Şeker | 2,9 | 2,8 |
| **4** | Söğüt | 2,1e | 5,9 |
| **5** | Çilli | 4,4 | 5,4 |
| **6** | Şeker | 2,2 | 2,3 e |
| **7** | Rex | 3,1 | 5,0 |
| **8** | Rex-black membra | 4,0 | 2,6 |
| **9** | Rex | 3,3 | 2,0 |
| **10** | Çilli | 4,0 | 8,5 |
| **11** | Yağlı | 9,5a | 13,0b |
| **12** | Çilli | 8,5 | 17,0a |
| **13** | Çilli | 7,6 | 14,6a |
| **14** | Söğüt | 2,5e | 7,3 |
| **15** | Rex | 5,5 | 5,00 |
| **16** | Şeker | 2,5 | 2,7 |
| **17** | Çilli | 6,1 | 12,1 |
| **18** | Çilli | 7,4 | 14,0 |
| **19** | Rex | 3,2 | 4,6 |
| **20** | Yağlı | 9,6a | 12,7 |




**Şekil 1:** Begonya genotiplerinin yaprak morfolojilerine ait görsel

Morfolojik özelliklere ait parametreler UPOV kriterlerine göre belirlenmiştir. Bu bağlamda, yaprak alt rengi, yaprak üst rengi, apeks açısı, damarların dikkat çekiciliği, çiçek taç yaprak sayısı parametreleri ölçülmüştür. Yaprak alt rengi UPOV kriterlerine göre sadece yeşil, yeşil ve kırmızı, sadece kırmızı olarak değerlendirildi. Yaprak alt rengi yeşil ve kırmızı olan genotipler yumrulu, çilli ve rex begonyalardır. Sadece kırmızı olan genotipler ise rex, şeker ve yağlı begonyalardır. Sadece yeşil genotipler ise söğüt begonyalardır. Yaprak üst rengi UPOV kriterlerine göre açık yeşil, orta yeşil, koyu yeşil, kırmızımsı yeşil ve neredeyse siyah olarak değerlendirildi. Nerdeyse siyah olarak değerlendirilen genotipler black mamba ve rex begonya genotipleridir. Kırmızımsı yeşil olan genotipler şeker ve rex begonyalardır.

Damarların dikkat çekiciliği UPOV kriterlerine göre yok veya çok zayıf, zayıf, orta, güçlü ve çok güçlü olarak değerlendirildi. Damarların dikkat çekiciliği bakımından güçlü genotipler yağlı begonyalar ve rex begonyalardır. Söğüt ve black mamba genotipleri damar belirginlikleri, yok veya çok zayıftır. Yaprak apeks açısı değerleri UPOV kriterlerine göre çok küçük, küçük, orta, geniş, çok geniş olarak değerlendirildi. Çok geniş olan genotipler şeker begonyalar ve rex begonyalardır. Çiçek taçyaprak sayısı UPOV kriterlerine göre çok düşük, düşük, orta, yüksek ve çok yüksek olarak değerlendirildi. Genellikle begonyaların taç yaprak sayısı düşük ve çok düşük olarak değerlendirildi ama yumrulu begonya çiçek taç yaprak sayısı bakımından çok yüksek olarak değerlendirilmiştir.

**Tablo 3:** Begonya genotiplerinin bazı yaprak ve çiçek özelliklerine ait gözlemler

| Genotip no | Tip | Yaprak alt rengi | Yaprak Üst Rengi | Yaprak damar yoğunluğu | Yaprak Apeks açısı | Çiçek Taç Yaprak Sayısı |
|---|---|---|---|---|---|---|
| **1** | Yumrulu | Yeşil-kırmızı | Yeşil | Zayıf | Küçük | Çok Yüksek |
| **2** | Rex | Yeşil-kırmızı | Kırmızımsı yeşil | Güçlü | Küçük | Çok Düşük |
| **3** | Şeker | Kırmızı | Kırmızımsı yeşil | Zayıf | Çok geniş | Çok Düşük |
| **4** | Söğüt | Yeşil | Koyu yeşil | Çok zayıf | Çok küçük | Çok Düşük |
| **5** | Çilli | Yeşil-kırmızı | Kırmızımsı yeşil | Orta | Orta | Çok Düşük |
| **6** | Şeker | Kırmızı | Kırmızımsı yeşil | Zayıf | Çok geniş | Çok Düşük |
| **7** | Rex | Yeşil-kırmızı | Kırmızımsı yeşil | Güçlü | Çok küçük | Çok Düşük |
| **8** | Rex-black membra | Kırmızı | Neredeyse siyah | Yok | Çok geniş | Çok Düşük |
| **9** | Rex | Yeşil-kırmızı | Kırmızımsı yeşil | Güçlü | Çok geniş | Çok Düşük |
| **10** | Çilli | Yeşil-kırmızı | Koyu yeşil | Zayıf | Orta | Çok Düşük |
| **11** | Yağlı | Kırmızı | Kırmızımsı yeşil | Güçlü | Orta | Düşük |
| **12** | Çilli | Yeşil-kırmızı | Koyu yeşil | Orta | Orta | Çok Düşük |
| **13** | Çilli | Yeşil-kırmızı | Koyu yeşil | Orta | Geniş | Çok Düşük |
| **14** | Söğüt | Yeşil | Orta yeşil | Çok zayıf | Çok Küçük | Çok Düşük |
| **15** | Rex | Yeşil-kırmızı | Kırmızımsı yeşil | Zayıf | Geniş | Çok Düşük |
| **16** | Şeker | Yeşil-kırmızı | Kırmızımsı yeşil | Orta | Orta | Çok Düşük |
| **17** | Çilli | Yeşil-kırmızı | Orta yeşil | Orta | Orta | Çok Düşük |
| **18** | Çilli | Yeşil-kırmızı | Koyu yeşil | Orta | Orta | Çok Düşük |
| **19** | Rex | Kırmızı | Neredeyse siyah | Güçlü | Orta | Çok Düşük |
| **20** | Yağlı | Kırmızı | Koyu yeşil | Güçlü | Geniş | Düşük |

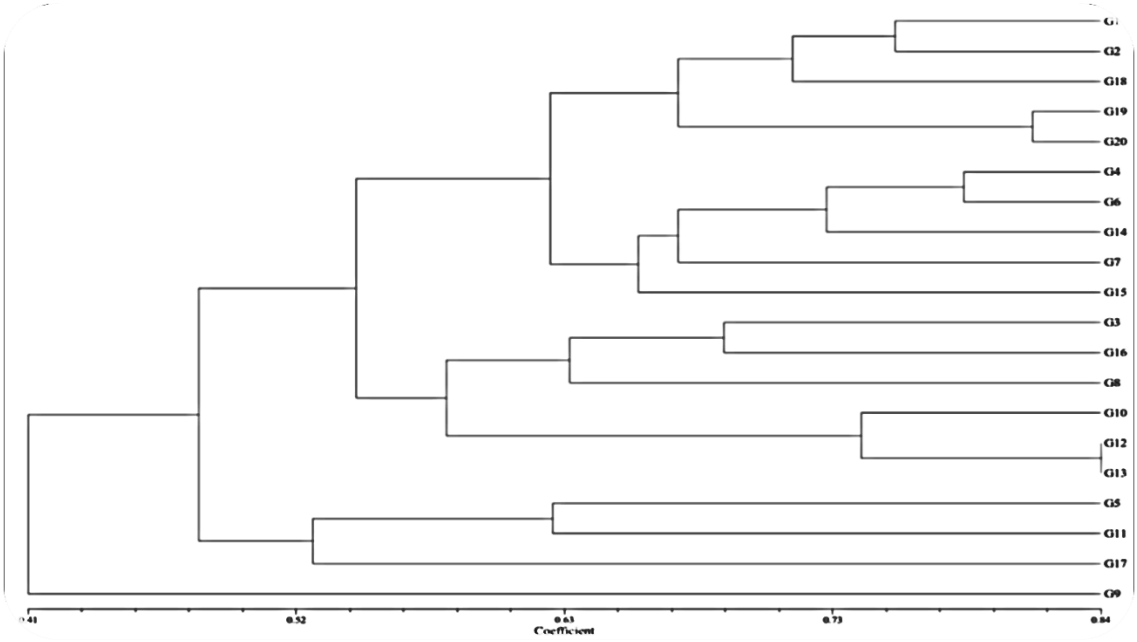
## Moleküler Özelliklere Ait Bulgular

Begonya genotiplerine ait genetik benzerlik ve farklılıklar iPBS moleküler markırları yardımıyla belirlenmiştir. Moleküler verilere dayalı olarak genotipler arasındaki genetik çeşitliliği tahmin etmek için UPGMA analizi ile dendogram oluşturulmuştur. Yirmi genotipin, iPBS belirteçleri ile ürettikleri skorlanabilir bantlardan, elde edilen veriler NTSYS programında analiz edilmiştir. Örneklerin moleküler analizleri için, değerlendirilen 52 primerden 10 iPBS primeri, 71'i (%97,2) olmak üzere 74 skorlanabilir bant üretmiştir. En yüksek bant veren primer, 14 skorlanabilir bant üreten iPBS-2085'tir. Primer başına ortalama 7,4 bant elde edilmiştir (Tablo 3).

**Tablo 4:** Begonya genotiplerinde iPBS primerleri ile elde edilen bat profilleri

| IPBS Primer | Sekans (5'>>3') | TNF* | NPF* | MP* (%) |
|---|---|---|---|---|
| **iPBS-2076** | ATCTGTCAGCCA | 5 | 5 | 100 |
| **iPBS-2077** | CTCACGATGCCA | 5 | 5 | 100 |
| **iPBS-2391** | ACCTAGCTCATGATCCC | 5 | 5 | 100 |
| **iPBS-2392** | ACCTAGCCTCGGATCC | 6 | 6 | 100 |
| **iPBS-2217** | TCATGGCTCATGATACCA | 7 | 7 | 100 |
| **iPBS-2221** | CGGTGACCTTTGATACCA | 8 | 8 | 100 |
| **iPBS-2231** | ACTTGCATCTCGATACCA | 7 | 6 | 86 |
| **iPBS-2084** | ATGCCGATACCA | 12 | 12 | 100 |
| **iPBS-2085** | GCAATGGAACCA | 14 | 12 | 86 |
| **iPBS-2403** | CATCGTAGGTGGGCGCCA | 5 | 5 | 100 |
| | **Toplam** | **74** | **71** | **97.2** |

DICE benzerlik matrisine dayalı UPGMA dendrogramına göre genotiplerin benzerlik katsayılar 0,41 ile 0,84 arasında değişmektedir. Genel olarak, genotipler, 0,41 benzerlik düzeyinde iki grupla sonuçlanmıştır. Begonya genotiplerinden, 9 numaralı genotip diğerlerinden ayrı olarak gruplanmıştır. Genotip 10, 12 ve 13 genotipi yüksek oranda benzer olup, 12 ve 13 %84 üzerinde genetik olarak benzer genotiplerdir. Genotip 19 ve genotip 20 ise aynı kolda konumlanmış benzerlik oranı yüksek genotiplerdendir. Genotip 4 ve genotip 14 söğüt begonya olup, aynı kol üzerinde konumlanmıştır (Şekil 2).




**Şekil 2:** Begonya genotiplerinde iPBS primerleri ile Dice benzerlik indeksine dayalı oluşturulan UPGMA dendogramı

Bu çalışma ile morfolojik olarak oldukça farklı olan begonyaların, genetik olarak da geniş bir varyasyona sahip olduğu tespit edilmiştir.

Bu çalışma, farkı morfolojik özelliklere sahip begonya genotipleri üzerinde yürütülmüştür. Daha önce begonyalar üzerinde yapılmış olan morfolojik ve genetik çalışmalar çok sınırlıdır. Bunun yanı sıra genetik çeşitliliğin belirlenmesi ve gen kaynaklarının korunması önemli çalışma konularındandır. Bu çalışmada 20 adet *Begonia* L. genotipinin süs bitkileri özellikleri ve genetik benzerlik düzeyleri incelenmiştir. Bu kadar az sayıda genotiple çalışılmış olmasına rağmen, hem morfolojik hem de moleküler olarak yüksek bir varyasyon olduğu tespit edimiştir. Genotipler için benzerlik katsayıları da 0,41 ile 0,84 arasında değişmektedir ve bu da ıslah programları için faydalı olabilir. Bu çalışma temel bir nitelikte olup, daha geniş bir populasyonla yapılabilecek olan ıslah çalışmaları için yol gösterici olacaktır.

## Kaynakça

Hvoslef-Eide, A. K., Munster, C. (2007). Begonia. In Flower Breeding and Genetics (pp. 241-275). Springer, Dordrecht.

Brennan, A. C., Bridgett, S., Shaukat Ali, M., Harrison, N., Matthews, A., Pellicer, J., Kidner, C. A. (2012). Genomic resources for evolutionary studies in the large, diverse, tropical genus, Begonia. *Tropical Plant Biology*, *5*(4), 261-276.

Neale, S., Goodall-Copestake, W., Kidner, C. A. (2006). The evolution of diversity in Begonia. Floriculture, ornamental and plant biotechnology, 4, 606-611.

Yang H.-B., Kang W.-H., Nahm S.-H., Kang B. C., 2015. Methods for Developing Molecular Markers.

Parveen S., Shahzad A., Yadav V., 2016. Molecular Markers and Their Application in Plant Biotechnology. In A. Shahzad, S. Sharma, S. A. Siddiqui (Eds.), Biotechnological strategies for the conservation of medicinal and ornamental climbers SE. 16, 389–413.

Kalendar R., Schulman A. H., 2014. Transposon-Based Tagging: IRAP, REMAP, and iPBS. Methods in Molecular Biology (Clifton, N.J.), 1115, 233–255.

Kalendar, R., Flavell, A.J., Ellis, T.H.N., Sjakste, T., Moisy, C., Schulman, A.H., 2011. Analysis of plant diversity with retrotransposon-based molecular markers. Heredity, 106, 520-530.

Doyle, J.J., 1990. Isolation of Plant DNA from fresh tissue. Focus. 12, 13-15.

Dice, L.R., 1945. Measures of the amount of ecologic association between species. Ecology, 26, 297-302.

## *ORAL PRESENTATION*

### *Cordyceps sinensis* mantarının antioksidan aktivitesi üzerine analitik çalışmalar

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**Özet**

Mantarlar, çok hücreli veya tek hücreli ökaryotik organizmaların bulunduğu alemin adıdır. Mantarlar klorofil benzeri bitkiler içermezler ve sporlarla çoğalırlar. Clavicipitaceae familyasına ait olan ve bilinen 750'den fazla türü bulunan Cordyceps mantarları, böcekler, eklembacaklılar ve diğer mantarlar üzerinde yaşayan bir endoparazittir. Cordyceps sinensis, Nepal, Çin ve Tibet'in yüksek rakımlı yaylalarında yetişen ve tıp bilimi için çok değerli bir mantardır. Bu mantar ishal, baş ağrısı, karaciğer ve böbrek rahatsızlıkları, yorgunluk, diyabet, romatizma, öksürük tedavisi gibi birçok alanda uzun yıllardır kullanılmaktadır. Bu kadar çok etki tek bir canlıda toplandığı için Çin kaynaklarında Cordyceps sinensis için "sihirli bir yaratık" tabiri kullanılmıştır. İçerdiği bileşenler insan vücudu üzerinde faydalı biyolojik ve farmakolojik etkilere sahiptir. Cordyceps sinensis son yıllarda tüm dünyada üne kavuşmuş ve popüler doğal ürünler arasında yerini almıştır. Bu çalışmada Cordyceps sinensis mantarının çeşitli etkileri nedeniyle bileşenleri ve antioksidan aktivite tayini ile ilgili literatür çalışmaları Springer, PubMed, Web of Science, Science Direct ve Scopus veri tabanları taranmış ve bu veri tabanlarından bilgiler elde edilerek çalışma gerçekleştirilmiştir. Bu veriler göz önüne alındığında tıbbi mantar olarak destekleyici tedavi için doktor kontrolünde kullanılması önerilebilir.

**Anahtar Kelimeler:** Cordyceps sinensis, Cordycepin, Antioksidan Aktivite Tayin Yöntemleri.

### Analytical Studies on the Antioxidant Activity of *Cordyceps Sinensis* Mushroom

**Abstract**

Fungi are the name of the realm in which multicellular or unicellular eukaryotic organisms are found. Fungi do not contain chlorophyll-like plants and reproduce by spores. Cordyceps fungi, belonging to the Clavicipitaceae family and with more than 750 known species, is an endoparasite that lives on insects, arthropods, and other fungi. Cordyceps sinensis is a mushroom that grows in the high plateaus of Nepal, China, and Tibet and is very valuable for medical science. This mushroom has been used for many years in many areas such as diarrhea, headache, liver and kidney ailments, fatigue, diabetes, rheumatism, and cough treatment. Because so many effects are concentrated in a single living thing, the term "magic creature" was used for Cordyceps sinensis in Chinese sources. The components it contains have beneficial biological and pharmacological effects on the human body. Cordyceps sinensis has become famous all over the world in recent years and has taken its place among popular natural products. In this study, Springer, PubMed, Web of Science, Science Direct, and Scopus databases were searched for literature studies related to the determination of its components and antioxidant activity due to various effects of Cordyceps sinensis mushroom, and the study was carried out by obtaining information from these databases. Considering these data, it can be recommended to be used as a medicinal mushroom for supportive treatment under the control of a doctor.

**Keywords:** Cordyceps sinensis, Cordycepin, Antioxidant Activity Determination Methods.

## GİRİŞ

Mantarlar, dünya üzerinde en yaygın dağılışa sahip organizmalar olup bu nedenle çevresel ve tıbbi açıdan büyük önem taşırlar. Bazı mantarlar karada ve suda tek başlarına yaşayabilirler, bazıları ise; hayvanlar ve bitkiler ile parazitik ya da simbiyotik ilişki kurarlar. Mantarlar, meyve ve sebzeler gibi çok çeşitli vitamin ve mineraller içerir. Yenilebilir mantarlar ve tıbbi mantarlar, besleyici, anti-enfektif, stres giderici ve antioksidan özellikleri nedeniyle gıda, ilaç ve kozmetik endüstrilerinde değerli kaynaklar olarak kullanılmaktadır. Doğada

bulunan ve yenebilen mantarlar yüzyıllardır çoğu Asya ülkesinde hem besin hem de ilaç olarak kullanılır. Mantarların tüketilmesinde rol oynayan özellikler kendine has kokusu ve tadı bulunması ayrıca içerdiği zengin karbonhidrat, diyet lif, mineral ve vitamin bunlara ilaveten yüksek oranda doymamış yağ asidi içermesidir. Yüksek protein ve düşük yağ oranı yenilebilen mantarların, özellikle spor yapan kişilerin diyette kullanması önemini daha da arttırmaktadır (Turp ve boylu 2018).

**Cordyceps Sinensis**

Gerçek mantarların en büyük alt bölümü olan Ascomycotina'nın bir üyesi olan yüzlerce Cordyceps türü, altı kıtada, çeşitli habitatlarda ve çeşitli gıda kaynaklarıyla tanımlanmıştır. Cordyceps türlerinin ilk yazılı kaydı MS 620 yılında, Tang Hanedanlığı döneminde (MS 618-MS 907) Çin'den gelir, mantarın yıllık varoluşu yazın hayvandan bitkiye, kışın ise yine bitkiden hayvana dönüşen bir yaratık gibi ima edilmiş ve bir zamanlar soyut olan alegorik anlatıya içerik getirmiştir. (Holliday ve Cleaver., 2008).



Şekil 1. Cordyceps sinensis

Cordyceps, Batı toplumuna ilk olarak 17. yüzyılda tanıtılmıştır. 1878'de, bir İtalyan bilim adamı olan Saccardo, Cordyceps adını Çin'de resmi olarak Cordyceps sinensis (Berk.) Sacc. olarak türetmiştir (Şekil 1) ve bu terminoloji günümüze kadar benimsenerek gelmiştir (Shashidhar ve ark. 2013). Cordyceps adı, Yunanca "kulüp" anlamına gelen "kordyle" ve "kafa" anlamına gelen Latince "-ceps" kökünden türetilmiştir. Tüm Cordyceps türleri benzer yaşam döngüsüne sahiptir ve konakçılarını istila etmek ve üzerlerinde büyümek için mekanizmalar geliştirmiştir. En büyük farkları büyüdükleri yer ve enfekte ettikleri konakçı böcektir. Cordyceps türleri, bir konakçıyla temasa geçene kadar toprakta uykuda kalır. Morfolojik çeşitliliklerinin yanı sıra, Cordyceps türleri böcekleri, eklembacaklıları ve diğer mantarları istila eder ve konağın bağışıklık sisteminden kaçarak, konağın yaşam döngüsünü hayatta kalma mekanizması ve üreme niyetiyle uyumlu hale getirir ve konak ile etkileşimleri, diğerleri arasında kordisepin, adenozin, guanozin, kordimin, γ-aminobutirik asit (GABA), ekzopolisakkaritler, kordisin A-E gibi farklı benzersiz ikincil metabolitler üretmelerini sağlar (Olatunji ve ark, 2018).Yaklaşık 20 Cordyceps türü Elapomyces cinsi üzerinde parazitlenirken, bazısı yine bir güve türü olan Hepialus armoricanus Oberthur'un (Lepidoptera) larvasının kafasında yaşar, Kalan türler Arachnida, Coleoptera, Hemiptera, Hymenoptera, Isoptera ve Lepidoptera sınıfına ait böcekler ve diğer eklembacaklılar üzerinde parazitlenir. Cinsteki türlerden birkaçı, geniş bir konakçı yelpazesini (birkaç familyadaki veya eklembacaklıların takımlarındaki türler) enfekte etme yeteneğine sahiptir. Bununla birlikte, çok sayıda Cordyceps türü, yalnızca tek bir konak türü veya birkaç yakından ilişkili türü enfekte etmekle sınırlıdır. (Shashidhar ve ark. 2013).

Cordyceps türleri Kuzey Amerika, Avrupa ve Asya'da, çoğunlukla Çin, Japonya, Nepal, Bhutan, Vietnam, Kore ve Tayland gibi ülkelerde yetişmektedir. Hindistan'da ise esas olarak Himalaya gibi yüksek rakımlı bölgelerde ve Avustralya'da bulunduğu bildirilmiştir. Metabolitlerinin bileşimi, yüksek rakımlarda (düşük sıcaklık, oksijen eksikliği ve UV radyasyonuna maruz kalma) karakteristik şiddetli koşulları tolere etmelerini sağlar. (Das ve ark, 2021; Maity, 2013; Chakraborty ve ark, 2014).

Geçmişi çok uzun yıllara dayanan Cordyceps sinensis'in özellikle eski Çin ve Tibet kaynaklarında adından oldukça bahsedilmiştir. Clavicipitaceae familyasına dahil olan ve bilinen 750'den fazla türü bulunan Cordyceps mantarları genelde böcek, eklem bacaklılar ve diğer mantarların üzerinde yaşayan bir endoparazittir. Çin kaynaklarında adından "Çin tırtıl mantarı" ya da orijinal ismiyle "DongChongXiaCao" türkçe karşılığı ise "yaz otu- kış solucanı" olarak bahsedilir. Bu drog geleneksel Çin tıbbında en önemli

drogların başında gelir. Başlangıçta Wu-Yilu tarafından Çin hanedanlığı boyunca "DongChongXiaCao" (materia medicanın yeni derlemesi)'ya kaydedilen bu drog, böbrek korunmasını ve akciğer gelişmesini sağlayarak Ying- Yang Double İnvigoration (ying-yangın çifte kuvvetlendirme, canlandırma prensibi) için önemlidir. Hindistan'da halk bu mantara "Yarsa gumba" demektedir, yarsa(kış) ve gumba (yaz) demektir. Yerel Nepalliler ise "Keera Jhar" (böcek otu) derler. (Winkler, 2009). Cordyceps'in medikal mantar olarak ilk defa Sheng Nung Bon Cas Chien (The Classic Herbal of the Divine Plowman) eserinde anonim bir yazar tarafından milattan önce 101 yılında bahsedilmiştir (Panda, 2010; Panda ve Swain, 2011; Önder ve ark, 2019).

Bu nadir ve ilginç şifalı mantarın yayılımı ise hava, yağmur ve böcekler aracılığıyla gerçekleşir. Tüm yaşam döngüsünde enfeksiyon, parazitlik ve saprofitizm olmak üzere üç aşamada bulunur. Cordyceps sinensis'in sporları rüzgar yardımıyla toprağın ve topraktaki bitkilerin üzerine saçılır, larvalarla temasta bulunurlar özellikle tırtıllar, otsu bitkilerin kökleriyle beslenmek için ortaya çıktıklarında temas sağlanır. Bu sayede tırtıllar mantar sporlarını ağız yolu ya da solunum gözenekleriyle vücutlarına alabilirler, vücuda giren sporlar ise filizlenerek yayılır. Cordyceps sinensis tırtıla saldırdığı vakit vücudunda bulunan boşluklara yayılarak boşlukları doldurur, dokuyu değiştirerek tırtılın ölmesine neden olur. Ölümü gerçekleşen tırtıl sarımsı kahverengi halde gözükür. 5-15 cm uzunluğunda koyu kahverengi siyah arası bir renk, silindirik kulüp şeklinde ana gövdesi ilkbahardan yazın başlarına kadar büyür ve tırtılın alnından çıkıntı yaparak dışarı çıkar. Dışarı çıkan mantar bulunduğu bölgenin yılın neredeyse 120 günü kar örtüsü ile örtülmesinden dolayı ancak hava sıcaklığı 0 °C üstünde kar ve buz örtüsünün kalkmasından sonra mantar toplayıcıları tarafından kolayca fark edilir çünkü mantarın yetiştiği bölgede odunsu ve büyük bitkiler yoktur. Bulunan mantarların altında genelde 16 cm uzunluğunda ve 2 cm çapında delikler bulunur bu delikler tırtılın bitki rizomunu yemek için toprağın altında oluşturduğu ve bu sırada mantarın saldırısına uğradığı deliklerdir bu da tırtıl ve mantarın ilişkisini gösteren ilk buluntudur. (Tuli ve ark, 2013; Das ve ark, 2021).

Cordyceps sinensis, 1964'ten beri Çin Farmakopesinde resmi bir ilaç olarak sınıflandırılmıştır (Pharmacopoeia Komitesi, Çin Sağlık Bakanlığı, 1964, 2005). Cordyceps sinensis, FDA değerlendirmeleri altında bir besin takviyesi olarak pazarlanmaktadır ve bu nedenle birçok ülkede kordiseps için büyük bir pazar talebi artmaktadır. Nesli Tükenmekte Olan Türlerin Uluslararası Ticaretine İlişkin Sözleşme (CITES) Çin Yönetim Otoritesi, bu mantarı resmi olarak nesli tükenmekte olan bir tür olarak sınıflandırmıştır (Shashidar ve ark).

Cordyceps sinensis ve diğer Cordycepsler, normalde yiyecek olarak kabul edilmemelerine rağmen, geniş bir yelpazede besleyici bileşikler içerir. Cordyceps, 18 temel aminoasidin tamamını içerir. Aminoasit içeriği, genellikle %5-10 aralığında oldukça yüksektir. Ayrıca E ve K vitaminleri ile B1, B2 ve B12 dahil olmak üzere suda çözünür B vitaminleri içermektedir. Heteropolisakkaritler, proteinler, steroller, birçok farklı nükleosit ve belki de içinde büyüdüğü zengin dağ toprağını yansıtan geniş bir mineral yelpazesi olan Zn, Mg K, Fe, Na, Ca, Cu, Mn, Pi, Al, Ni, Ti, Si, Ga, Sr, Se, Cr, V mineralleri de içermektedir (Holliday, 2017; Uhrinová ve Poľančíková, 2018).

Cordyceps cinsine ait mantarlar şu anda en pahalı mantarlar/mantarlar arasındadır. Geçmişte sadece Çin imparatorluk sarayının üyeleri veya soylular tarafından kullanılıyorlardı. Yararlı etkilerine olan ilginin artması, doğal Cordyceps'in toplanmasında önemli bir artışa ve sonuç olarak doğal pazar kıtlığına neden olmuştur. Bu, yapay kültürüne olan ilgiyi artırmış, yapay kültürde modern teknolojik ilerleme Cordyceps'in halka arzının artmasına ve aynı zamanda tıbbi etkinliğinin test edilmesine olanak sağlamıştır. Hem doğal hem de kültürlenmiş Cordyceps'in yüksek maliyeti, sonunda yüksek kaliteli ürünlerin kasıtlı olarak düşük dereceli, saf olmayan ürünlerle değiştirilmesine neden olmuştur.

Mevcut modern kültürleme yöntemleri, doğal mantarın büyüdüğü iklim koşullarına yaklaşabilir ve böylece yeterli miktarlarda kültürlenmiş Cordyceps üretebilir. Yeni prosedürler sayesinde, Cordyceps sinensis pirinç, darı, mısır ve diğerleri gibi besleyici ortamlardaki bitkilerde yetiştirilebilir. Kültürlenmiş Cordyceps'in kalitesi mannitol ve polisakkaritlerin içeriğine göre değerlendirilir: Polisakkaritlerin oranı %40'ı geçmeli ve mannitol içeriği en az %15'e ulaşmalıdır. (Uhrinová ve Poľančíková, 2018).

Cordyceps sinensis tırtıl ile mantarın oldukça nadir bir kombinasyonudur ve özellikle Hindistan'da 4500 metre rakımlı yerlerinde rastlanılmaktadır. Başlangıçta koyun ve keçilerini otlatan yerel çobanlar Cordyceps sinensisi yiyen hayvanlarının öbürlerine kıyasla daha iri ve güçlü olduğunu gözlemlemişlerdir. Daha sonra yerel halk, hayvanlarında süt üretimini ve üreme verimliliğini arttırmak için kullanmışlardır. Sonraları güneş ışığında

kuruttukları stroma (toprak üstü) kısmı toplanarak ilgili tıbbi özellikleri araştırmışlardır, mantarı tükettikten sonra kendilerini daha dinç ve canlı hissettiklerini ve afrodizyak etkilerinin olduğunu keşfetmişlerdir. Geleneksel şifacılar ve yerel halk bütün hastalıklar için tek başına ya da başka droglarla karıştırılarak kullanılmasını önermiştir çünkü enerji, iştah, dayanıklılık, libido, ve uyku düzenini geliştirdiğini savunmuşlardır. (Panda ve Swain, 2011).

Cordyceps'in bütün türlerinin farklı farklı yararlı özellikleri vardır. Anti-kanser, anti-proliferatif, anti-anjiyojenik, antimetastasis, apoptoz indüksiyonu, anti-inflamatuar, antioksidan, anti-fibrotik, anti-arterioskleroz, anti-hipertansif, anti-osteoporotik, anti-trombotik, anti-sıtma, anti-fungal, hipolipidemik, anti-diyabetik, hipoglisemik, anti-astmatik, anti-fertilite, anti-viral, yaşlanma karşıtı, steroidogenez, spermatojenik, böbrek fonksiyonlarını düzenleyici ve immünomodülatör etki dahil olmak üzere dikkate değer klinik sağlık etkilerine sahiptir (Liu ve ark. 2015; Chaturvedi ve ark, 2018; Das ve ark. 2022; Phull ve ark. 2022).

## MATERYAL VE METOD

Yaptığımız derleme çalışmasında; Cordyceps türleri, Cordyceps sinensis, Cordycepin, antioksidan özellikler, antioksidan aktivite tayin yöntemleri kelimeleri kullanılmıştır. İncelenen veri tabanları; US National Library of Medicine (PubMed), Web of Science, Scopus ve Google Scholar, Medical Literature Analysis and Retrieval System Online (MEDLINE) ve EMBASE, gibi akademik veri tabanlarındaki bulunan makaleler; deneysel verilerle oluşturulmuş ve sonuçlandırılmış, derleme olarak yapılmış ve hakemli dergilerde yayınlanmış çalışmalardan 2022 yılına kadar yapılmış çalışmalar dikkate alınarak derlemesi yapılmıştır.

## BULGULAR VE TARTIŞMA

### Cordyceps sinensis ve antioksidan özellikleri

Antioksidanlar, organizmalarda hücre hasarına neden olan serbest radikaller üreten oksidasyon reaksiyonlarını önleyebilen veya yavaşlatabilen bileşiklerdir. Oksitleyici türlerin artan oluşumu veya doğal antioksidan seviyelerinde önemli bir azalma ile ilişkili olan oksidatif stres, insanlarda farklı hastalıkların oluşumunda (hücresel nekroz, kardiyovasküler hastalık, kanser, nörolojik bozukluk, yaşlanma) rol oynar. Doğal kaynaklardan elde edilen, özellikle toksik olmayan antioksidan içeren bitkisel ürünler ve mantarlar, polifenolikler bakımından zengin olmaları nedeniyle oksidatif hasarı önlediği bilinmektedir.

Dong ve ark. 2008 yılında yapmış oldukları çalışmada, Çin'de doğal ve kültüre alınmış Cordyceps sinensis'in sıcak su ekstraktlarının antioksidan aktiviteleri in vitro olarak araştırılmış ve linoleik asit peroksidasyonunun inhibisyonu ile değerlendirilmiştir; Spektrofotometrik olarak DPPH, hidroksil ve süperoksit radikal anyonları üzerinde süpürme yetenekleri; ve demir iyonları üzerindeki indirgeme gücü (FRAP) ve şelatlama yetenekleri incelenmiştir.Linoleik asit peroksidasyonu kültür için: 0.15 ± 0.01, doğal için 0.14± 0.01 ($IC_{50}$ mg/ml); Süperoksit anyon radikalini kültür için: 1.00±0.08, doğal için 1.24±.0.05 ($IC_{50}$ mg/ml); ABTS yöntemi ile kültür için: 0.96±0.06, doğal için: 1.03±0.03 ($IC_{50}$ mg/ml); DPPH yöntemi ile kültür için: 0.93±0.01, doğal için: 1.23±0.01 ($IC_{50}$ mg/ml); FRAP yöntemine göre kültür için: 5.73 ± 0.14, doğal için:7.27± 0.08 ($IC_{50}$ mg/ml) hesaplanmıştır. Cordyceps sinensis'in doğal ve kültür misellerinin sıcak su ekstraktlarının antioksidan özellikleri incelendiğinde, güçlü antioksidan aktivitelere sahip olduğunu bulmuşlardır. Sıcak su ekstraksiyonunda, kültür miselinin, doğal miselden önemli ölçüde daha yüksek bir toplam verim ürettiğini, kültür misel ekstraktlarının polisakkarit içerikleri, doğal miselden daha yüksek, protein ve mannitol'ün ise doğal miselde kültürden daha yüksek olduğunu, antioksidan aktivite indirgeme gücü ve şelatlama kapasitesinin de kültürde doğala göre daha yüksek olarak tespit etmişlerdir. Cordyceps sinensis'in farmakolojik fonksiyonları, en azından kısmen oksidasyona karşı koruyucu etkilerinden kaynaklanmaktadır ve Cordyceps sinensis'in doğal kaynakları üzerindeki insan taleplerini azaltmak için antioksidan aktivite için mantarın kültürlenmiş misellerini kullanmanın umut verici bir yol olduğunu söylemişlerdir.

Uhrinova ve Polancikova, 2018, Hindistan ve Japonyadan'dan gelen Cordyceps sinensis mantar tozlarını metanol ve su ile ekstrakte etmişlerdir. Metanol ekstraktlarının su ekstraktlarından daha iyi stabilite gösterdiğini bulmuşlardır. Kültürü yapılmış farklı ortamlarda yetiştirilen iki Cordyceps sinensis örneğini iki farklı ekstraksiyon yöntemi ile hazırlamışlar ve antioksidan aktivitesini tayin etmişlerdir. Aralarındaki farkın mantarların üretiminde ortamdaki farklı bileşenlerin varlığından kaynaklanmış olabileceğini söylemişlerdir.

Qi-Lin Huang ve ark, 2013, Hong Kong'da patates üzerinden kültüre alınmış mantar örneklerinde antioksidan aktivite tayinlerinin; $ABTS^{\bullet+}$radikal süpürme aktivitelerini 107,1 mol Trolox/g, 276.8 mol Trolox/g, FRAP yöntemi ile de 104.3 mol $Fe^{2+}$/g, 285.9 mol $Fe^{2+}$/g olarak bulmuşlardır. Konsantrasyon artışı ile antioksidan aktivitenin arttığını tespit etmişlerdir.

Quang-Vinh ve ark, 2021, kültüre alınan Cordyceps sinensis'den elde edilen ekzopolisakkaritlerin $CCl_4$'ün neden olduğu karaciğer hasarı olan sıçanlar üzerindeki in vitro antioksidatif ve koruyucu etkilerini araştırmışlardır. Yetiştirilen sinensis'in sırasıyla 2.00 ve 3.78 mg/mL $IC_{50}$ değerleri ile orta düzeyde $ABTS^{\bullet+}$ ve hidroksil radikal süpürme yeteneklerine sahip olduğunu göstermiştir. Sonuç olarak ekzopolisakkarit (EPS) in serum AST ve ALT aktivitelerini azaltarak $CCl_4$'ün neden olduğu oksidatif etkiden sıçan karaciğerini koruyabileceğini, ayrıca histopatolojik karaciğer dokusundaki değişiklikler ile, EPS'nin karaciğerde $CCl_4$'ün neden olduğu hasarı önemli ölçüde azalttığını bulmuşlardır.

Singh ve ark, 2018, doğal ve kültür Cordyceps sinensis'in metanolik ekstraktlarının in vitro antioksidan potansiyelini araştırmışlardır. Örneklerin antioksidan kapasitesini DPPH, ABTS, serbest radikal ve hidroksil radikali süpürme aktivitesi ve demir iyonu şelatlama aktivitesi ile tayin etmişlerdir. İn vitro kültür Cordyceps sinensis'in metanolik özütü, doğal olandan daha fazla antioksidan potansiyel göstermiştir. Doğal ve kültür örneklerinin $IC_{50}$ değeri sırasıyla; DPPH'ye karşı 2,98 mg/ml ve 2,81 mg/ml, hidroksil radikaline karşı 3,28 mg/ml ve 2,95 mg/ml ve $ABTS^{\bullet+}$ serbest radikallerine karşı 4,94 mg/ml ve 4,65 mg/ml bulmuşlardır. Doğal ve kültür numuneleri, 1.6 mg/ml konsantrasyonda %81 ve %86 demir şelatlama aktivitesi göstermiştir. Buradan invitro kültürü doğal olana kıyasla iyi bir antioksidan kaynağı olarak kullanılabileceği sonucunu çıkarmışlardır.

Li ve ark, 2001, çeşitli doğal Cordyceps sinensis ve kültür Cordyceps sinensis misellerinin su ekstraktları, ksantin oksidaz, hemoliz indüksiyonu ve lipid peroksidasyon deneyi gibi üç farklı yöntem kullanarak antioksidan aktiviteleri için analiz etmişlerdir. Kültür Cordyceps misellerini, doğal Cordyceps ile karşılaştırıldığında eşit derecede güçlü antioksidan aktivitesi gösterdiklerini bulmuşlardır. Ayrıca, kültür Cordyceps miselinden kısmen saflaştırılmış polisakkarit fraksiyonlarında antioksidan aktivitelerinin 10 ila 30 kata çıktığını görmüşler, bunun da aktivitenin kısmen Cordyceps polisakkaritlerinden geldiğini düşündüklerini belirtmişlerdir.

Wang ve ark, 2017, Çin'de 3 farklı rakımdan temin ettikleri Cordyceps sinensis ve 1 adet kültür Cordyceps sinensis'in misellerini etanol ile ekstrakte etmişler ve kurutarak çeşitli işlemlerden sonra polisakkaritlerini elde etmişlerdir. Üç doğal Cordyceps sinensis ve kültür Cordyceps sinensis'ten ekstrakte edilen ham polisakkarit verimleri ağırlıkça sırasıyla; S1, S2, S3 ve S4 %9.43, %9.78, %8.94 ve %14.32 olmuş, proteinin Sevag yöntemiyle çıkarılmasından sonra ise verimler sırasıyla ağırlıkça %27.58, %31.57, %38.30 ve %49.36 olarak bulmuşlardır. S1, S2 ve S3'ün toplam fenolik içeriği sırasıyla 3.22±0.05, 5.16±0.02, 7.63±0.26 mg GAE/g ve S4, 7.05±0.46 mg GAE/g bulunmuştur. Kültüre alınmış S4 ün doğal olanlar ile anlamlı bir fark göstermediğini, doğal olanların arasındaki fenolik içeriğin farklılığının nem, rakım ve sıcaklık gibi farklı habitatlardan dolayı olabileceğini söylemişlerdir. Cordyceps sinensislere ait polisakkaritlerin in vitro antioksidan aktivitesini üç farklı yöntemle tayin etmişlerdir. Dört polisakkaritten kültür olanı, DPPH ve hidroksil radikali süpürme yeteneğine karşı biraz daha yüksek süpürme kapasitesi gösterirken(hidroksil radikallerinin yalnızca %40'ını süpürmüştür), doğal olan P1, muhtemelen içinde konjuge fenolik bileşikler ve amino asitler gibi faktörlerin bir kombinasyonu ile ilişkili olarak FRAP'a göre nispeten düşük indirgeme kabiliyetine sahip bulunmuştur Dört polisakkaritin FRAP değerleri, toplam fenolik içeriklerine göre sırasıyla 56.79, 53.74, 44.51 ve 30.19 değerleri ile S3>S4>S2>S1 şeklinde olup gözlenen antioksidan potansiyeli fenolik bileşenlerle ilişkili olduğunu söylemişlerdir.

## SONUÇ

Günümüzde, tıbbi tedaviye destek tedavi yaklaşımlarının, çeşitli tıbbi bitkiler, doğal maddeler ve fonksiyonel gıdalar alınması ile yapıldığı bilinmektedir. Geleneksel Çin ilaçlarından biri olan ve vücut sağlığının yenilenmesi için kullanılan Cordyceps sinensis tırtılın larvası üzerinde büyüyen kuru mantardır. Yapılan derleme çalışmasından bazı örneklerini sunduğumuz Cordyceps sinensis'in en önemli farmakolojik etkilerinden biri, antioksidasyon aktivitesidir. İncelenmiş olan Sonuçlara bakıldığında, Cordyceps sinensis'in içerdiği "Cordycepin" geniş spektrumlu biyolojik aktivitesi yüksek olan bir bileşik olup tıbbi değeri ve

nutrasötik potansiyeli nedeniyle kanser tedavisinde etkin olduğu ve tüm makalelerde Cordyceps sinensis mantarının güçlü bir antioksidasyon aktivitesine sahip olduğu görülmektedir. Bununla birlikte hem doğal hem de kültürlenmiş Cordyceps misellerinin, doğal Cordyceps ile karşılaştırıldığında genellikle eşit derecede güçlü antioksidasyon aktivitesine sahip olduğu da çalışmalardan tespit edilmiştir. Antioksidan özelliklerini ve sağlık üzerindeki olumlu etkileri incelenen makalelerde görülen Cordyceps sinensis'i tedavide kesinlikle doktor kontrolünde kullanılabileceğini söyleyebiliriz.

## KAYNAKLAR

Chakraborty S, Chowdhury S ve Nandi, G, 2014. Review on yarsagumba (Cordyceps sinensis)-an exotic medicinal mushroom. Int. J. Pharm. Phytochem. Res. 6, 339–346.

Chaturvedi VK, Agarwal S, Gupta KK 2018, Ramteke PW, and Sing MP, Medicinal mushroom: boon for therapeutic applications, Medicinal mushroom: boon for therapeutic applications, Biotech, 8(8): 334.

Das G ve ark, 2021, Cordyceps spp.: A Review on Its Immune-Stimulatory and Other Biological Cordyceps spp.: PotentialsFrontiers in Pharmacology. 11: 1-30.

Dong CH, Yao YJ, 2008, In vitro evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of Cordyceps sinensis, Lebensm Wiss Technol. 41(4): 669–677.

Holliday J, Cleaver M, 2008, Medicinal Value of the Caterpillar Fungi Species of the Genus Cordyceps (Fr.) Link (Ascomycetes). A Review. Int J Med Mushr.10.3:219–234.

Holliday J, 2017, *Cordyceps*: A Highly Coveted Medicinal Mushroom, Medicinal and Aromatic Plants of the World book series, 4:59–91.

Li SP, Li P, Dong TT ve Tsim KW, 2001, Anti-oxidation activity of different types of natural Cordyceps sinensis and cultured Cordyceps mycelia. Phytomedicine. 8: 207212.

Liu Y, Wang J, Wang W, Zhang H, Zhang XC, 2015, The Chemical Constituents and Pharmacological Actions of Cordyceps sinensis. Evidence-Based Complementary and Alternative Medicine. 1–12.

Maity D, 2013, A study on ethnomedicinal uses of yartshagumba, Cordyceps sinensis (berk.) sacc. (cordycipitaceae), by the trinal communities of North Sikkim and its conservation. Explore. Anim. Med. Res. 3, 95–101.

Olatunji OJ, Tang J, Tolac A, Auberond F, Oluwaniyie O, Ouyangb Z, 2018, The genus Cordyceps: An extensive review of its traditional uses, phytochemistry and pharmacology, Fitoterapia. 129:293–316.

Önder A, Çınar AS, Canatan M, 2019, Cordyceps Sinensis (Berk.) Sacc. and Its Pharmacological ImportanceJ Lit Pharm Sci. 8(2):85-96.

Panda AK, ve Swain KC, 2011, Traditional uses and medicinal potential of Cordyceps sinensis of Sikkim. Jayurveda ıntegr med. 2(1): 9–13.

Panda AK, 2010, Tracing historical perspective of Cordyceps sinensis- an aphrodisiac in sikkim Himalayas. Indian journal of history of science. 45(2):189-198.

Phull AR, Ahmed M, ve Park HJ, 2022, Cordyceps militaris as a Bio Functional Food Source: Pharmacological Potential, Anti-Inflammatory Actions and Related Molecular Mechanisms. Microorganisms 10, 405.

Qi-Lin Huang, Ka-Chai Siu, Wen-Qiang Wang, Yi-Ching Cheung, Jian-Yong Wu, Fractionation, characterization and antioxidant activity of exopolysaccharides from the fermentation broth of a Cordyceps sinensis fungus, Process Biochemistry 48 (2013) 380–386.

Quang-Vinh Nguyenhttps, ve ark, 2021, Antioxidant Activity and Hepatoprotective Effect of Exopolysaccharides From Cultivated Ophiocordyceps Sinensis Against CCl4-Induced Liver Damages, Naturel Product Communication, 16(2).

Shashidhar MG, Giridhar P, Sankar KU, Manohar B, 2013, Bioactive principles from Cordyceps sinensis: A potent food supplement - A review. J Funct Foods . 2013 Jul;5(3):1013-1030.

Singh S, Arif M, Ranjan S, Nasim M, 2018, Comparative in vitro antioxidant activity of Natural and Cultured Ophiocordyceps sinensis, Int J Adv Life Sci Res 1:3:30-39.

Tuli HS, Sandhu SS, Sharma AK, 2014, Pharmacological and therapeutic potential of Cordyceps with special reference to Cordycepin. Biotech 4:1–12.

Turp GY ve Boylu M, 2018, Tıbbi ve Yenilebilir Mantarlar & Et Ürünlerinde Kullanımı, YYU J AGR SCI, 28(1): 144-153.

Uhrinová, A, Poľančíková, N, 2018, Antioxidant Activity Of The Fungus Cordyceps sinensis grown on two different media, Folia Veterinaria. 62,3: 68-73.

Wang J, Nie S, Kan L, Chen H, Cui SW, Phillips AO, Phillips GO, ve Xie M, 2017, Comparison of structural features and antioxidant activity of polysaccharides from natural and cultured Cordyceps sinensisFood Sci. Biotechnol. 26(1): 55-62.

Winkler D, 2009, Caterpillar Fungus (Ophiocordyceps sinensis) Production and Sustainability on the Tibetan Plateau and in the Himalayas, Asian Medicine 5:291–316.

## *ORAL PRESENTATION*

# Treatment of food aroma and ingredients production wastewater by electrochemical processes

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**Abstract**
The world population is increasing rapidly, and accordingly, the need for food is increasing. The production of flavours and food additives, especially in ready-made food items, is constantly increasing. During these productions, very complex wastewater emerges. In this study, the treatability of the wastewater of a factory producing food additives and flavours by electrocoagulation (EC) and electrooxidation (EO) processes was investigated. The removal of COD and TOC from the EC process was found to be from 6000 to 3954 mg COD/L (34.1%) and from 2252 to 1770 mg TOC/L (21.4%) for aluminium iron (Al) anodes at operating conditions (initial pH = 3, current density = 60 A/m$^2$ and operating time = 2h), and from 6000 to 3152 mg COD/L (47.47%) and from 2252 to 1185 mg TOC/L (47.38%) for iron (Fe) anodes at operating conditions (initial pH of 6, the current density of 60 A/m$^2$ and operating time of 2h), respectively. With successive EC + EO processes, treatment effluent of COD and TOC concentrations at operating conditions (current density = 350 A/m$^2$ and 5 hours) were 45 mg/L and 10 mg/L, respectively. The total cost of the EC+EO process is calculated as approximately 79 US $/m$^3$.

**Keywords:** Food production wastewater, Treatment, Electrocoagulation, Electrooxidation.

## INTRODUCTION

The food and beverage industry is one of the major contributors to the growth of all economies (Temgire et al., 2021). The ready-made food industry is constantly evolving, offering us products with different flavours and tastes. Different flavours and additives are widely used in these foods. The food additives currently in use are added to food as emulsifiers, colourants, flavourings, nutrients, or preservatives. Preservatives inhibit the growth of yeasts, bacteria, and moulds in foods (Awuchi et al., 2020). From carbohydrates and proteins to extracts or flavours, the diverse food ingredients sector spans a wide range of products that are used to maintain or improve the safety, freshness, and nutritional value of our foods, as well as their taste, texture, or appearance. Food flavour is a very important part of the manufacturing of a lot of processed foods and ingredients. The presence of pollutants (like total suspended solids, colour, oils, and organic and inorganic compounds) in the wastewater produced from food production plants presented an issue, as there was no option to discharge this untreated wastewater into the sewer (Asgharnejad et al., 2021). This was a present major issue for many companies in the food and beverage sector as it is a specific pollutant that must often be removed for wastewater to be allowed into a public sewer stop also, pollutants can significantly increase the chemical oxygen demand (COD) biological oxygen demand (BOD) and total organic carbon (TOC) values of wastewater, which can again present an issue as many discharge licenses do not permit high BOD, COD and TOC values in wastewater being discharged into the sewer or into the environment.

There are numerous methods which have been applied to treat food industry wastewater such as physical-chemical processes (filtration, evaporation, centrifugation, gravity concentration, microfiltration, ultrafiltration, nanofiltration, dissolved air flotation, coagulation-flocculation, and electrocoagulation), aerobic and anaerobic oxidation, adsorption, advanced oxidation (Fenton/electro-Fenton, ozone, electrooxidation, photocatalytic oxidation, i.e.), etc. (Asgharnejad et al., 2021). However, effluents from the food processing industry are complex organic compounds and refractory organics containing high COD and TOC, which are difficult to remove, hence, requiring efficient treatment technologies (Xin and Rehmann, 2020). Also, some of these processes because of high operating costs and the generation of secondary pollutants have limited their applications. Thus, environmental discharge limits are not always reached.

New treatment processes are required to comply with legal environmental standards. Among them, the use of electrochemical processes such as electrocoagulation (EC) and electrooxidation (EO) could represent an interesting option as many chemical reactions occur simultaneously when they are applied. One such treatment technology is specialized EC, an electrochemical process often used in treatment to reduce many wastewater pollutants. EC wastewater treatment process has many benefits, but the four main points listed above encompass several of the primary benefits (Shahedi et al., 2020). EC has a lower lifecycle cost, is easy to operate, and is an environmentally friendly treatment system that has great potential for use in beverage & food wastewater treatment applications in the food industry (Khanitchaidecha et al., 2022; Barrera-Diaz et al., 2006). Electrooxidation or anodic oxidation (EO) is the most popular due to its simplicity. In this procedure, the pollutants are oxidized by direct electron transfer to the anode and/or mediated oxidation with heterogeneous $OH^{\cdot}$ formed from water discharge at the anode surface at a high current (Garcia-Segura et al., 2018). Since it can partly convert toxic and refractory compounds into simple matter or biodegradable substances without secondary pollution to the environment, it is considered an environmentally friendly technology.

This study, it is aimed to investigate the treatability of food aroma and ingredients production wastewater with important electrochemical wastewater treatment processes such as EC and EO. In fact, this wastewater was successfully treated using a sequential electrochemical process (EC + EO). The aim here is to provide pollutant treatment discharge values with electrochemical treatment processes of these wastewater.

## MATERIALS AND METHODS

*Wastewater characteristics used in this study*

The wastewater used in this study was obtained from a food aroma and ingredients production factory in Turkey. The food aroma and ingredients production wastewater were stored at 4 °C accordances with the standard methods (APHA, 2005). The initial values of the wastewater: pH of 8.83 and conductivity of 9.40 mS/cm, and COD, $BOD_5$ and TOC are 6000 mg/L, 3297 mg/L, and 2252 mg/L, respectively.

*Experimental setup and procedure*

The EC treatment studies were carried out in a batch mode using about 1000 mL plexiglass reactor (120 mm × 95 mm × 85 mm in dimension) using vertically positioned Fe and Al electrodes spaced by 15 mm and dipped in the wastewater solution. Rectangular plates of two anodes and two cathodes with dimensions of 50 mm × 75 mm × 20 mm made of iron (Fe: 99.5% purity) and aluminium (Al: 99.3% purity) plates were connected to a digital DC power supply (Agilent 6675A model; 120 V, 18 A) in monopolar parallel connection mode. The total effective electrode area was 225 $cm^2$. The phenol solution in the reactor was mixed at 300 rpm with a magnetic stirrer (Heidolp, 3600D model) to reduce the mass transport over the potential of the EC reactor. The constant current was adjusted according to a desirable value, and the experimental operation was started. To determine the phenol removal efficiencies, the samples were periodically taken from the reactor during the EC process and filtered through 0.45 μm membrane filters before analysis.

The EO treatment studies were performed in a 1000 mL Pyrex glass. 850 mL of electro-coagulated wastewater was placed in the Pyrex glass reactor for each run. One anode (BDD: Boron Doped Diamond) and one cathode (316 AISI stainless steel) in sizes of the rectangular plate (200 cm × 60 mm × 20 mm) were used in the reactor. The active surface area of the BDD anode was 144 $cm^2$. BDD electrodes used in the EO treatment study were supplied from DiaCCon GmbH Germany. BDD electrode was supplied from Baoji Changli Special Metal Co., Ltd. China. Both the anodes and cathodes were placed vertically parallel to each other. The inter-electrode gap was kept constant at 10 mm. Power was provided by a DC power supply (Agilent 6675A model) with current-voltage monitoring and a maximum output of 20 A-120 V. During each experimental run, 850 mL of wastewater was placed into the EO reactor. The reactor was equipped with a magnetic stirrer (Heidolp, 3600D model), and the stirring speed was adjusted at 300 rpm to keep the wastewater homogeny mixed. The current was held constant at 5 A (current density of 350 $A/m^2$) for the EO process. The samples taken at the different operating times from the EO batch reactor were filtered through a 0.45 μm membrane filter and subsequently analysed for COD and TOCs.

*Analytical methods*

All the chemical analyses were carried out by standard methods for the examination of water and wastewater (APHA, 2005). The COD value was determined by the dichromate open reflux method as per Standard Method 5220-B. The TOC levels were determined through the combustion of the samples at 680 °C using a non-dispersive IR source (Shimadzu, TOC-L model) by the non-purgeable organic carbon method. All the

experiments were repeated twice, for checking the reproducibility of the results. The maximum experimental error was below 2%, and the average values have been reported. Each measurement was made with three repetitions, and the average of 3 results was obtained. All experiments were performed at 25˚C. The pH and conductivity of solutions before and after the EC process were measured by a pH meter (Mettler Toledo, S220 Seven Compact) and a conductivity meter (Mettler Toledo, Seven Go Pro). The pH of the solutions was adjusted by adding either 0.10 N NaOH or 0.10 N HCl.

## RESULTS and DISCUSSION

### *EC treatment studies*

Since the COD concentration of the wastewater used in this study is very high, the pollution load of the wastewater should be reduced by first removing the COD concentration with the EC process. EC study is primarily focused on some crucial questions which must find suitable answers before processing the design step: What is the best sacrificial electrode material (Fe and Al) for a given food aroma and ingredients production wastewater? Is there any need for the adjustment of the initial pH of the wastewater before the EC stage? What are the optimum values of the operational variables such as current density, and operating time in the EC process?

In the EC process, the anodic reaction involves the dissolution of Al or Fe electrodes (Eqs. 1-3), and the cathodic reaction (Eqs. 2 and 5) involves the formation of hydrogen gas and hydroxide ions, then hydroxide ions formed at the cathode increase pH of the wastewater thereby inducing precipitation of metal ions as corresponding hydroxides and co-precipitation with iron hydroxides (Kobya et al., 2003; Mollah et al., 2001). The main anode and cathode reactions occurring at Al and Fe electrodes in the EC process are as follows:

Anode and cathodes reactions for Al electrodes:

Anode: $Al \rightarrow Al^{3+} + 3e^{-}$ (1)

Cathode: $3H_2O + 3e^{-} \rightarrow 3/2H_{2(g)} + 3OH^{-}$ (2)

Anode and cathodes reactions for Fe electrodes:

Anode: $Fe \rightarrow Fe^{2+} + 2e^{-}$ (3)

$Fe^{2+} \rightarrow Fe^{3+} + e^{-}$ (4)

Cathode: $2H_2O + 2e^{-} \rightarrow H_{2(g)} + 2OH^{-}$ (5)

In the case of Fe electrodes, dissolved iron (i.e., $Fe^{2+}$ or $Fe^{3+}$) hydrolyses by increasing pH to form precipitates as $Fe(OH)_{n(s)}$. The rate of the oxidation of $Fe^{2+}$ depends on the availability of dissolved oxygen (Kobya et al., 2003).

$$O_{2(g)} + 4Fe^{2+} + 2H_2O \rightarrow 4Fe^{3+} + 4OH^{-} \quad (6)$$

In the case of used aluminium electrodes in the EC process, $Al^{3+}$ and $OH^{-}$ ions generated by anode and cathode reactions (1) and (2) to form various monomeric and polymeric species, which transform finally into $Al(OH)_{3(s)}$ according to complex precipitation kinetics (Mollah et al., 2001). On the other hand, ferric ions ($Fe^{3+}$) generated by the electrochemical oxidation of Fe electrodes used EC process may form monomeric and polymeric species depending on the pH of the aqueous medium, which transform finally into $Fe(OH)_{3(s)}$ (Mollah et al., 2001). At pH greater than 10, monomeric anionic iron and aluminium species as $Al(OH)_4^-$ and $Fe(OH)_4^-$ prevail. Generally, $Al^{3+}$ or $Fe^{3+}$ ions released from the anode are gradually hydrolysed and formed $Al(OH)_{3(s)}$ and $Fe(OH)_{3(s)}$ if there are no other reactive species in the solution.

$$Al^{3+} + 3H_2O \rightarrow Al(OH)_{3(s)} + 3H^{+} \quad (7)$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_{3(s)} + 3H^{+} \quad (8)$$

Consequently, the removal mechanism of pollutants from wastewaters with both electrodes was related to forming of $Fe(OH)_{3(s)}$, $Al(OH)_{3(s)}$, monomeric and polymeric iron and aluminium species due to, coagulation, precipitation, co-precipitation, and electrooxidation (Mollah et al., 2001). Furthermore, freshly formed amorphous $Al(OH)_{3(s)}$ and $Fe(OH)_{3(s)}$ "sweep flocs" in the EC process have large surface areas which are beneficial for the rapid adsorption of soluble organic compounds and trapping of colloidal particles.

*Effect of initial pH on EC process using*

It has been established that the influence of pH is an important parameter influencing the performance of the electrochemical process (Daneshvar et al., 2006; Kobya et al., 2003). To examine its effect, the sample was adjusted to the desired pH for each experiment by using sodium hydroxide or sulfuric acid. It is known that the type of electrode and the reactions of the electrode during the EC process greatly affect the removal

efficiency of the process (Kobya et al., 2003). At a current density of 60 A/m$^2$, the COD removal from the food aroma and ingredients production wastewater as a function of operating time at various initial $pH_i$ values for Al and Fe electrodes were shown in Figure 1. COD and TOC removals at EC time of 2 h were 38.22% (3954 mg/L) and 32.24% (1526 mg/L) for $pH_i$ of 3, 34.75% (4176 mg/L) and 23.58% (1721 mg/L) for $pH_i$ 4, 32.97% (4290 mg/L) and 18.03% (1846 mg/L) for $pH_i$ 6, and 30.94% (4420 mg/L) and 26.21% (1662 mg/L) for $pH_i$ 8 for Al electrode, and 21.47% (5004 mg/L) and 31.39% (1445 mg/L) for pHi 3, 35.47% (4112 mg/L) and 16.96% (1870 mg/L) for $pH_i$ 4, 43.19% (3152 mg/L) and 47.47% (1185 mg/L) for $pH_i$ 6 and 32.52% (4300 mg/L) and 39.96% (1352 mg/L) for Fe electrode, respectively.

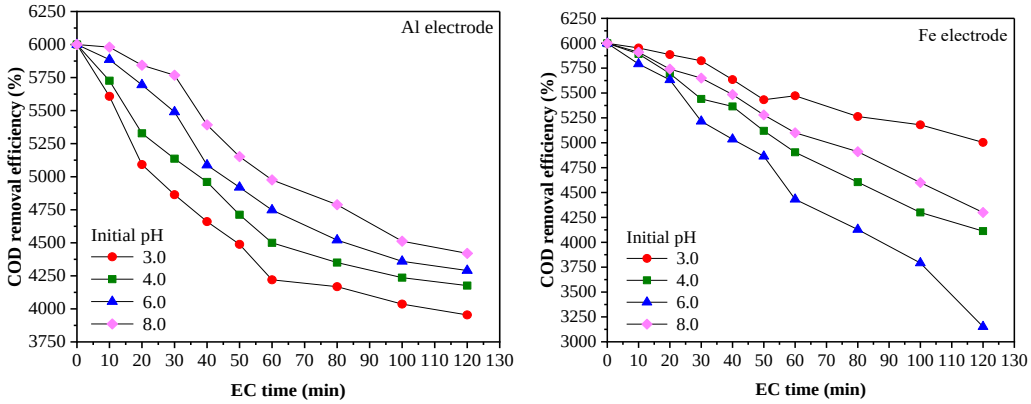


Figure 1. Effect of initial pH on COD removal by Al and Fe electrodes.

According to these results, it was seen that the COD and TOC removal efficiency decreased as pH increased for Al electrode. With Fe electrodes, the highest COD removal was seen at pH 6. The pH of the treated samples increased when the $pH_i$ was increased due to the excess of hydroxyl ions produced at the cathode according to Eqns. (2) and (5). On the other hand, final pH ($pH_f$) values after the EC process Al and Fe electrodes at EC time of 120 min were measured as 7.81 and 6.75 for a pHi of 3, 8.30 and 7.93 and for a pH of 4, 8.94 and 8.83 for a $pH_i$ of 6, and 9.43 and 9.79 for a $pH_i$ of 8. In the EC process using Fe and Al anodes, it can be concluded that at pH between 5 and 9, many iron and aluminium complexes (coagulants) are formed, and it is the optimum pH values ($pH_i$ 3 for Al and $pH_i$ 6 for Fe) for carrying out the EC. There was maximum COD and TOC removal efficiency at the initial $pH_i$ 3 for the Al electrode and at the initial $pH_i$ 6 for the Fe electrode. Since hydroxide ions, which were generated at the cathode, were neutralized by $H^+$ ions, a sufficient amount of iron and aluminium hydroxide complexes was formed at these pH values. At the pH > 9.5, COD and TOC removal efficiency decreased, because above this pH $Al(OH)_4^-$ and $Fe(OH)_4^-$ are the dominant species. $Al(OH)_4^-$ and $Fe(OH)_4^-$ are a dissolving species and it is unable to form flocs.

*Effect of current density on EC process*

To investigate the effect of current density on the COD and TOC removal efficiency, the EC process was carried out using various current densities at initial $pH_i$ of 6 for the Fe electrode and initial $pH_i$ of 3 for the Al electrode. Figure 2 shows the COD removal against the current density applied to the Al and Fe electrodes in the EC process. The COD and TOC removal efficiencies increased to 47.47% (3152 mg/L) and 46.74% (1185 mg/L) at 60 A/m$^2$ from 26% (4440 mg/L) and 19.27% (1818 mg/L) at 20 A/m$^2$ for Fe electrode and 34.10% (3954 mg/L) and 41.61% (1315 mg/L) at 60 A/m$^2$ from 23.47% (4592 mg/L) and 23.58% (1721 mg/L) at 20 A/m$^2$ for Al electrode, respectively. According to Faraday's law (Eq. (9)), when current density increases, the amount of ions ($Al^{3+}$, $Fe^{2+}$, and $Fe^{3+}$) produced on the electrodes increases.

$$C_{electrode} = \frac{i \times t_{EC} \times M_W}{z \times F \times v} \quad (9)$$

where $C_{electrode}$ (kg/m$^3$) is the iron and aluminium concentration electrochemically generated in the EC reactor, $t_{EC}$ is the time of EC (s), $M_{w,Fe}$ and aluminium are the molecular weight of the iron and aluminium electrodes ($M_{w,Fe}$ = 56 g/mol and $M_{w,Al}$ = 27 g/mol), z is the chemical equivalence of iron and aluminium ($z_{Fe}$ = 2 and $z_{Al}$ = 3), and $F$ is the Faraday constant (96,500 C/mol).

Therefore, there is an increase in floc production and hence an improvement in the COD and TOC removal efficiencies (Kobya et al., 2003). In other words, the higher current density will generate a significant amount of flocs, which in turn will trap the pollutant molecules and enhance the COD and TOC removal efficiencies. At EC time of 120 min, $C_{electrode}$ for Fe and Al electrodes were calculated as 1.104, 1.657, 2.761 and 3.308 kg/m$^3$ and 0.884, 1.324, 2.209 and 2.647 kg/m$^3$, respectively.

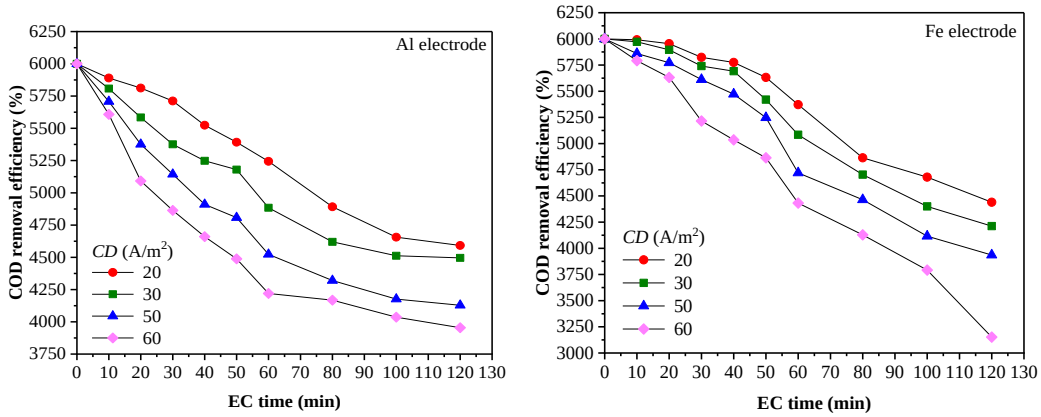


Figure 2. Effect of current density on COD and turbidity removal by Al and Fe electrodes

Another important issue for the EC process is the energy consumption of the process. The amount of energy consumed by the EC process for both electrodes, denoted by $C_{energy}$ and expressed in kWh/m$^3$ of wastewater, was calculated through Eq. (10) (Kobya et al., 2003):

$$C_{energy} = \frac{U \times i \times t_{EC}}{v} \quad (10)$$

where e U is cell voltage (V), i is applied current (A), $t_{EC}$ is the time of EC (h) and $v$ is the volume of wastewater in the EC reactor (m$^3$).

$C_{energy}$ at EC time of 120 min was calculated as 2.650, 4.929, 11.130 and 16.110 for Fe electrodes and 3.308 kWh/m$^3$ and 2.12, 4.77, 9.275 and 14.288 kWh/m$^3$ for Al electrodes. The operating costs involved in the EC treatment technology encompass electrical energy costs and costs of anode replacements. The operating cost ($OC$) in this study was calculated as US $ per m$^3$ of treated wastewater as illustrated in Eq. (11):

$$OC = \alpha C_{energ} + \beta C_{electrode} + \delta C_{chemical} \quad (11)$$

where $C_{energy}$ (kWh/m$^3$), $C_{electrode}$ (kg Fe or Al electrode/m$^3$), and $C_{chemical}$ (kg chemical/m$^3$) are energy, electrode, and chemical consumptions, respectively. The factors α, β, and δ for this study are quoted from the Turkish market as of August 2022 as follows: The factor "$\alpha$" indicates the unit electrical energy cost (0.12 $/kWh); "$\beta$" denotes the cost of the electrode, which is 0.952 $/kg for Fe and 1.78 $/kg for Al. The factor "$\delta$" denotes the costs of consumables like $H_2SO_4$ and NaOH, the unit costs were taken as 0.40 $/kg for $H_2SO_4$ and 1.01 $/kg for NaOH.

According to Eq. (11), the operating cost ($OC$, $/m$^3$) for a current density of 20, 30, 50 and 60 A/m$^2$ were calculated as 1.370, 2.169, 3.964 and 5.131 US $/m$^3$ for Fe electrode and 2.221, 3.521, 6.028 and 7.603 US $/m$^3$ for Al electrode, respectively. It is resulted that both the removal efficiencies (COD and TOC) and the operating costs, it was concluded that Fe electrodes are more effective than Al electrodes. Therefore, EO process studies following the treatment with the EC process were carried out with optimum Fe electrodes based on EC conditions

***EO treatment studies***

Food flavouring and additives production wastewater were treated with the EO process followed by the EC process using Fe electrodes. The removal efficiencies in the EC process were founded 47.47% (3152 mg COD/L) and 46.74% (1185 mg TOC/L) at optimum conditions (current density of 60 A/m$^2$, initial $pH_i$ of 6 and EC time of 120 min). EO studies were carried out with BDD anodes at 5 A (current density of 350 A/m$^2$) current conditions as stated in the material and method section of this study, and COD and TOC removal are shown in Figure 3. As can be seen from Figure 3, the COD and TOC removal efficiencies were found to be 98.57% (45 mg COD/L) and 99.16% (10 mg TOC/L) at EO time of 5 hours. According to these results, it has been observed that organic compounds containing COD and TOC concentrations in the wastewater are effectively removed by the EO process. The energy consumption of the EO process according to Eq. (9) is calculated as 615.3 kWh/m$^3$ and the operating cost is 73.8 US $/m$^3$. In this case, the total cost of the EC+EO process is calculated as approximately 79 US $/m$^3$.

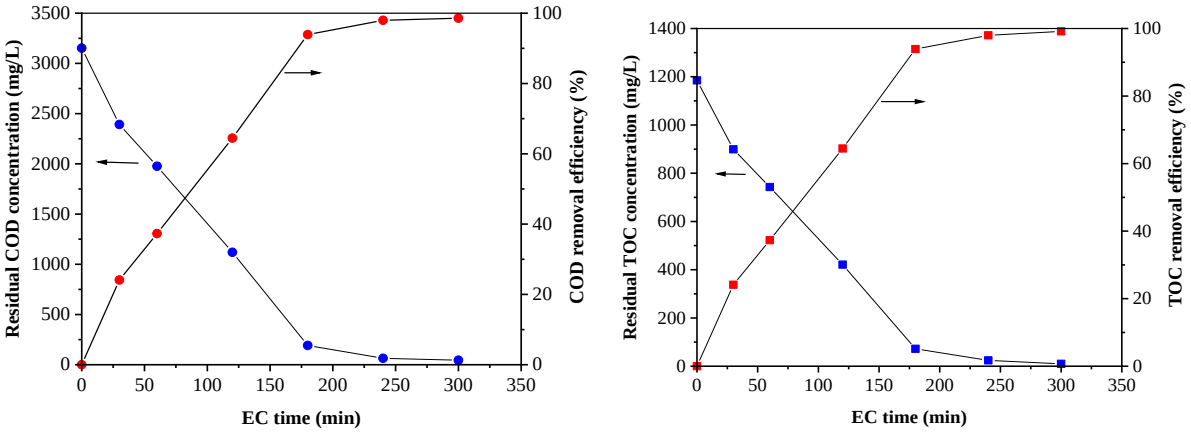


Figure 3. COD and TOC removals with EO process after EC process

The EO process is directly/indirectly utilizing oxidizing species such as hydroxyl radicals, active chlorine species, ozone, and hydrogen peroxide (Garcia-Segura, 2018). The BDD has been successfully used for EO to remove the organic pollutants from various wastewaters, since a large amount of hydroxyl radical (•OH) is formed by water oxidation on the electrode surface. The hydroxyl radical exhibits strong oxidant properties by which complex organic molecules are degraded to $CO_2$. At high potentials, a great number of organic pollutants (*R*) in wastewater by BDD are completely mineralized by the reaction with electrogenerated free •OH species (Panizza and Cerisola, 2005).

$$BDD + H_2O \rightarrow BDD(^{\bullet}OH) + H^+ + e^- \quad (12)$$

$$BDD(^{\bullet}OH) + R \rightarrow BDD + mineralization\ products + H^+ + e^- \quad (13)$$

$$BDD(^{\bullet}OH) + R \rightarrow BDD + mCO_2 + nH_2O \quad (14)$$

Moreover, BDD anodes have good chemical and electrochemical stability even in strong and aggressive media, with a long life and a wide potential window of water discharge. Therefore, the above-mentioned anode materials for treatment of the food flavouring and additives production wastewater, the BDD electrode has come forward for its robustness, versatility, high removal efficiency and little or no need for chemical addition.

COD removal in the food flavouring and additives production wastewater by the EC+EO process is given in Figure 4. As can be seen from this figure, it is seen that the incremental EC+EO process effectively removes the food flavouring and additives production wastewater and provides the desired treatment output values. However, the operating cost of the process is quite high.

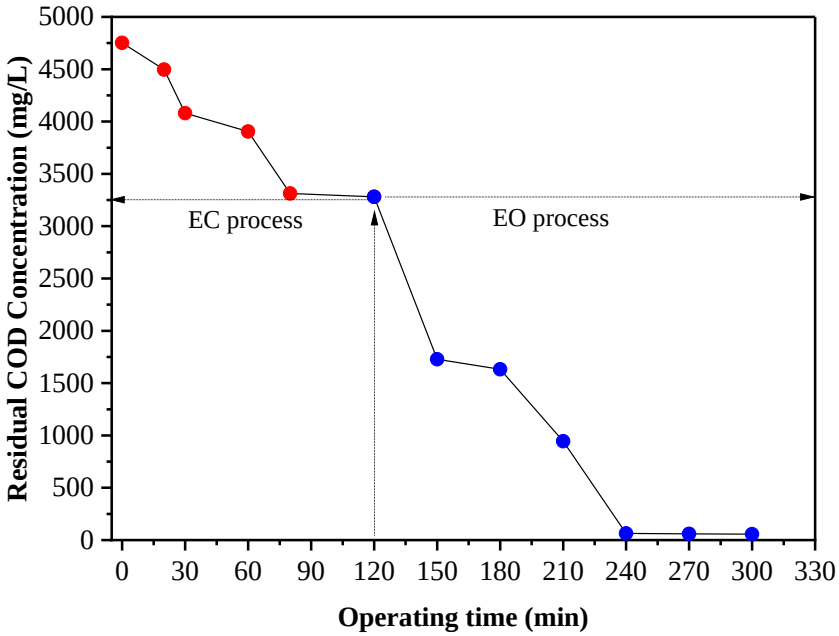


Figure 4. COD removal with successive EC + EO process.

## CONCLUSION

The food and beverage industry is one of the major contributors to the growth of all economies. During this industry, wastewater which contains high chemical oxygen demand (COD), biological oxygen demand (BOD) and total organic carbon (TOC) values have to be treated before being discharged into the sewer or into the environment. The initial pH, conductivity, COD, $BOD_5$ and TOC of wastewater are 8.83, 9.40 mS/cm, 6000 mg/L, 3297 mg/L, and 2252 mg/L, respectively. In this study, this wastewater was treated by a combination of electrocoagulation (EC) and electrooxidation (EO) processes. The EC treatment studies were carried out with Fe and Al electrodes. Otherwise, the EO treatment studies were performed with one anode (BDD: Boron Doped Diamond) and one cathode (316 AISI stainless steel). The removal efficiencies of COD and TOC were found as 34.1 % of COD and 21.4 % of TOC with Al at initial pH = 3, current density = 60 $A/m^2$ and operating time = 2h. On the other hand, the highest COD (47.47%) and TOC (47.38%) removal efficiencies by the EC process were obtained with Fe at initial pH of 6, a current density of 60 $A/m^2$ and an operating time of 2h. Then, the EC-treated wastewater was treated by EO (current density = 350 $A/m^2$ and 5 hours) and 45 mg/L of COD and 10 mg/L of TOC values were obtained as the final values of wastewater. The total cost of the EC+EO process is calculated as approximately 79 US $/$m^3$. The results showed that the combination of EC and EO is a very promising technology because of causes very low final pollutant content. However, its cost should be lowered.

## REFERENCES

AsgharnejadH, Nazloo EK, Larijani MM, Hajinajaf N, Rashidi H 2021. Comprehensive review of water management and wastewater treatment in food processing industries in the framework of water-food-environment nexus. Comprehensive Reviews in Food Science and Food Safety, May 2021: 1-37.

APHA 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

Awuchi CG, Twinomuhwezi H, Igwe VS, Amagwula IO 2020. Food additives and food preservatives for domestic and industrial food applications. Journal of Animal Health, 2: 1-16.

Barrera-Diaz C, Roa-Morales G, Avila-Cordoba L, Pavon-Silva T, Bilyeu B 2006. Electrochemical treatment applied to food-processing industrial wastewater. Industrial & Engineering Chemistry Research, 45: 34-38.

Daneshvar N, Oladegaragoze A, Djafarzadeh N, 2006. Decolorization of basic dye solutions by electrocoagulation: An investigation of the effect of operational parameters, Journal of Hazardous Materials, 129: 116–122.

Gameissa MW, Indrasti SNS 2012. Advanced treatment of food industry wastewater by electrocoagulation using stainless steel electrodes, E-jurnal Agro-Industri Indonesia,

Garcia-Segura S, Ocon JD, Chong MN, 2018. Electrochemical oxidation remediation of real wastewater effluents-A review. Process Safety and Environmental Protection, 113: 48-67.

Khanitchaidecha W, Ratananikom K, Yangklang B, Intanoo S, Sing-Aed K, Nakaruk A 2022. Application of electrocoagulation in street food wastewater. Water, 14: 655-663.

Kobya M, Can OT, Bayramoglu M 2003. Treatment of textile wastewaters by electrocoagulation using iron and aluminum electrodes. Journal of Hazardous Materials, 100: 163-178.

Mollah MYA, Schennach R, Parga JP, Cocke DL 2001. Electrocoagulation (EC)-science and applications. Journal of Hazardous Materials, 84: 29-41.

Panizza M, Cerisola G 2005. Application of diamond electrodes to electrochemical processes. Electrochimica Acta, 51: 191–199.

Shahedi A, Darban AK, Taghipour F, Jamshidi-Zanjani A 2020. A review on industrial wastewater treatment via electrocoagulation processes. Current Opinion in Electrochemistry, 22: 154-169.

Shrivastava V, Ali A, Marjub MM, Rene ER, Soto AMF, 2022. Wastewater in the food industry: Treatment technologies and reuse potential. Chemosphere, 293: 133553.

Temgire S, Borah A, Kumthekar S, Idate A 2021. Recent trends in ready to eat/cook food products: A review. The Pharma Innovation Journal, 10: 211-217.

Xin Z, Rehmann L 2020. Application of Advanced Oxidation Process in the Food Industry, Advanced Oxidation Processes, Edited by Ciro Bustillo-Lecompte, DOI: 10.5772/intechopen.92355.

## ORAL PRESENTATION

# Güney Marmara Bölgesi tarımsal hayvan barınaklarından gerçekleşen metan salınımı ve karbon ayak izinin tahmini

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## Özet

İklim değişikliği ve küresel ısınmaya sebep olan başlıca sera gazları, metan ve karbondioksittir. Tarımsal yapılar ve tarımsal inşaatlardaki eksiklik ve hatalar (özellikle gübre depolama yapılarındaki), atmosfere yayılan bu sera gazlarının miktarını artırmaktadır. Gübre yönetimi ve enterik fermantasyonla da doğrudan ilgili olan sera gazı emisyonunun, tarımsal kökenli kısmının tespit edilmesi gerekmektedir. Bu yüzden bitkisel üretime kıyasla, atmosferdeki sera gazı emisyonunu daha çok artıran hayvansal üretimin incelenmesi önem kazanmaktadır. Bu çalışmada; Bursa, Bilecik ve Eskişehir'deki 2004-2021 yılları arasındaki çiftlik hayvanı (büyükbaş ve küçükbaş) verileri kullanılarak, metan salınımı enterik fermantasyona göre, Tier 1 yöntemiyle hesaplanmıştır. Daha sonra bu sonuçlar karbon ayak izi sonuçlarına dönüştürülmüştür. Sonuçta, 2004-2021 yılları arasındaki periyot değerlendirildiğinde; büyükbaş hayvan kaynaklı karbon ayak izi ortalaması, Bilecik için 426 kg $CO_2$ e / gün (1 gündeki, kilogram cinsinden eşdeğer karbondioksit miktarı), Bursa için 2003 kg $CO_2$ e / gün ve Eskişehir için 1383 kg $CO_2$ e / gün olarak belirlenmiştir. 2004-2021 yılları arasındaki periyotta; küçükbaş hayvan kaynaklı karbon ayak izinin ortalama değerleri ise, Bilecik için 66 kg $CO_2$ e / gün, Bursa için 226 kg $CO_2$ e / gün ve Eskişehir için 389 kg $CO_2$ e / gün olarak tespit edilmiştir. Bu değerlerin azaltılıp, sera gazı emisyonlarının kontrol edilmesi için; özellikle gübre depolama yapılarının teknik özelliklerinin geliştirilmesi ve barınak içerisindeki havalandırma tesislerinin bilimsel olarak planlanması gerekmektedir.

**Anahtar Kelimeler:** Tarımsal yapılar, iklim değişikliği, karbon ayak izi, hayvan barınakları, Tier 1 yöntemi

# Methane emission from agricultural animal shelters in the Southern Marmara region and estimation of carbon footprint

## Abstract

The main greenhouse gases that cause climate change and global warming are methane and carbon dioxide. Deficiencies and errors in agricultural structures and agricultural constructions (especially in fertilizer storage structures) increase the amount of these greenhouse gases emitted into the atmosphere. The agricultural origin part of the greenhouse gas emission, which is directly related to manure management and enteric fermentation, needs to be determined. Therefore, it is important to examine animal production, which increases greenhouse gas emissions in the atmosphere more than plant production. In this study, methane emission was calculated by the Tier 1 method according to enteric fermentation, using livestock (cattle and small cattle) data between 2004 and 2021 in Bursa, Bilecik and Eskişehir. These results were then converted into carbon footprint results. As a result, when the period between 2004-2021 is evaluated, cattle carbon footprint average was determined as 426 kg for Bilecik $CO_2$ e / day (equivalent amount of carbon dioxide in kilograms in 1 day), was determined as 2003 kg $CO_2$ e / day for Bursa and was determined as 1383 kg $CO_2$ e / day for Eskişehir. In the period between 2004-2021, the average values of the carbon footprint originating from small cattle (sheep and goats) were determined as 66 kg $CO_2$ e / day for Bilecik, 226 kg $CO_2$ e / day for Bursa and 389 kg $CO_2$ e / day for Eskişehir. In order to reduce these values and control greenhouse gas emissions, it is necessary to develop the technical features of manure storage structures and scientifically plan the ventilation facilities in the shelter.

**Keywords:** Agricultural structures, climate change, carbon footprint, animal shelters, Tier 1 method

## GİRİŞ

Özellikle sanayi devriminden sonra, (deniz akıntısı, güneş ışınları ve volkanik faaliyetlerin sebep olduğu) doğal iklim değişikliğine ek olarak insani faktörler, iklimi düzensizleştirmiştir. Bunun sonucunda oluşan sıcak hava dalgaları, canlı hayatını tehlikeye atmaktadır.

Küresel ısınmanın % 14.5'u hayvansal üretim kaynaklı olup, bunun da %8'inin büyükbaş ve kümes hayvancılığından kaynaklandığı düşünülmektedir. Hayvancılığın iklime etkisi; gübre yönetimi ve enerji harcaması yönünden gerçekleşmektedir (Kılıç ve ark., 2018).

Karbondioksit, diazot oksit, metan, hidroflorür karbonlar, sülfürhegzaflorid v.b gazlar; atmosferde ısı tutulmasına sebep olduğundan, bunlara sera gazı denilmektedir. Temel olarak; $CO_2$'ye fosil yakıtlar, metana çeltik tarlası ile çöp alanları, diazot oksite suni gübreler, kloroflorakarbonlara sprey kutu içindeki aerosel sebep olmaktadır. Karbondioksit 200 yıla kadar, diazot oksit 114 yıla kadar ve metan ise 12 yıla kadar atmosferde kalabilmektedir. İnsan kaynaklı sera gazı emisyonlarına; fosil yakıt tüketimi, orman yangını, hayvancılık ve sentetik gübre kullanımı neden olmaktadır. İnsan kaynaklı metan; hayvancılık, çeltik üretimi, doğalgaz-kömür-petrol üretimi sebebiyle oluşmaktadır. Karbondioksit ise; canlıların solunumu ve organik madde çürümeleri sebebiyle atmosfere karışmaktadır. Diazot oksit ise, azotlu gübre kullanımı ve rasyondaki azot miktarıyla orantılı olarak atmosfere salınmaktadır (Koyuncu ve Akgün, 2018). İnsan kaynaklı sera gazı emisyonlarına; tarımsal emisyonun katkısı %18'dir. Bu tarımsal emisyon arasında ise; metan gazı emisyonunun oranı ise % 80'dir. Tarımsal metan emisyonunun % 7'si hayvan gübresi kaynaklı, %18'i çeltik üretimi kaynaklıdır. Tarımsal kaynaklı emisyonlar; Karbondioksit ($CO_2$), Diazot oksit ($N_2O$), Metan ($CH_4$) ve Amonyum ($NH_4$)'dur. $NH_4$'e çeltik tarlaları ve çiftlik hayvanı gübreleri sebep olmaktadır. Özellikle hayvancılık sektörü, başlıca sera gazı kaynağıdır (en az %35'lik bir oranla) (Bal, 2019; Kara ve ark., 2019).

Tarımsal sera gazı emisyonuna küçükbaş hayvancılığının katkısı, % 6.5'dur. Tarımsal sera gazı emisyonuna tavukçuluğun katkısı, % 8'dir. Tarımsal sera gazı emisyonuna büyükbaş hayvancılığının katkısı, % 65'dir (4.6 milyar ton$CO_2$ eşdeğerinde). Bunun %42'si et üretiminde, % 21'i ise süt üretiminde gerçekleşmektedir (Koyuncu ve Akgün, 2018).

Metan emisyonu hesabında kullanılacak, metan emisyon faktörü değeri, hayvan başına üretilen metan miktarı (kg)'nı ifade etmektedir. Bu değer; hayvanın ortalama enerji ihtiyacı için gerekli olan ortalama besin alımı ve kalitesine bağlıdır (Anonim, 2022).

Enterik fermantasyon; otçul hayvanların karbonhidrat emilimi sağlaması amacıyla, midesindeki mikroorganizmaların parçalama gerçekleştirdiği sindirim olayıdır. Bu olay sonucu, hayvan solunumuyla metan üretilmiş olur. Enterik fermantasyon kaynaklı metan üretimini azaltmak için, yemlere bitkisel yağ ve prebiyotik katılmalıdır. Geviş getiren hayvanların mide fermantasyonu sonucu oluşan hayvansal metan gazı emisyonu miktarlarını; yem kalitesi-içeriği, hayvanların yaş-cins ve cinsiyetleri etkilemektedir. Enterik fermantasyon kaynaklı metan emisyon faktörü belirlenirken, süt sığırları için 99 kg $CH_4$/baş.yıl ve koyun için 5 kg $CH_4$/baş.yıl olduğu kabul edilmektedir. (Ahmet, 2019; Bal, 2019).

Avrupa hayvancılığının sera gazı emisyonlarının % 36'sını enterik fermantasyon ve % 26'sını $N_2O$ oluşturmaktadır. $CO_2$ eşdeğeri cinsinden; 1 kg sığır eti 22.6 kg, 1 kg süt 1.3 kg ve 1 kg tavuk 1.6 kg emisyona sahiptir (Ahmet, 2019).

Hayvansal gübrelerin depolanmaları sırasında da metan açığa çıkmaktadır. Bu metan miktarı; gübre depolama biçimi-alanı ve gübre türüne göre değişmektedir. Hayvan gübresi kaynaklı metan gazının % 50'si domuz gübresi sebebiyledir. 1 yılda; sığır 4 ton, domuz 450 kg ve koyun 400 kg karbondioksit üretmektedir (Bal, 2019).

Karbon ayak izi; birim $CO_2$ cinsinden belirlenip, insan faliyeti sonucu üretilen sera gazını temsil etmek için kullanılmaktadır. Karbon ayak izinin hesabında; IPCC'nin ürettiği 3 adet Tier yaklaşımı kullanılabilmektedir. Bu yaklaşımlar sayesinde farklı sera gazlarının emisyonları hesaplanıp, bunların $CO_2$ eşdeğer miktarları belirlenebilmektedir (Kılıç ve ark., 2018).

IPCC; karbondioksit dışındaki sera gazlarını (metan ve diazot oksit gibi), enterik fermantasyon kaynaklı (metan) ve gübre yönetimi kaynaklı (metan ve diazot oksit gibi) olarak ikiye ayırmaktadır. IPCC'nin raporlarında Tier yöntemi eşitlikleriyle belirlenen bu sera gazı emisyonlarının, karbon ayak izi karşılıklarını belirlemek için, $CO_2$ eşdeğeri dönüşüm katsayıları ile çarpılması gerekmektedir (Ahmet, 2019).

Metanın, karbondioksit eşdeğeri ($CO_2$ e) 25 iken; Diazot oksitin 298'dir (Kılıç ve ark., 2018; Ahmet, 2019). Farklı çiftlik hayvanlarının atmosfere saldığı metan gazı miktarları ise Tablo 1'de gösterilmektedir.

**Tablo 1.** Çiftlik hayvanı çeşidine göre IPCC emisyon faktörleri (Kara ve ark., 2019)

| Çiftlik hayvanı çeşidi | Emisyon faktörü (kg metan/hayvan sayısı) |
|---|---|
| Sığır | 68 |
| Deve | 46 |
| At | 18 |
| Eşek | 10 |
| Koyun | 8 |
| Keçi | 5 |
| Manda | 4 |

Ahmet (2019) Bursa bölgesindeki 100 başlık süt sığırı, 180 başlık koyun ağılı, 12 000 başlık yumurta tavukçuluğu işletmelerinin karbon ayak izini belirlemiştir. Enterik fermantasyon ve gübre yönetimlerinden kaynaklanan metan gazı emisyonunu Tier 1 ve 2 yöntemlerine göre belirleyen araştırmacı; sonuçta kaliteli yem rasyonu kullanımını (hububat takviyeli) ve kompostlama ile gübre yönetimini önermiştir. Ayrıca; süt sığırı karbon ayak izinin, koyun karbon ayak izinden 10 kat fazla olduğu tespit edilmiştir. Sonuçta; Tier 1 yöntemiyle, süt sığırcılığında 1 L süt üretimi için 0.43 kg $CO_2$ e üretilmiştir. Bu miktarın %80'i enterik fermantasyon kaynaklı metandan, %20'si gübre yönetiminden oluşmaktadır. Tier 2 yöntemiyle ise, süt sığırcılığında 1 L süt üretimi için 1.1 kg $CO_2$ e tespit edilmiştir. Yani; karbon ayak izinin büyük bölümü enterik fermantasyon kaynaklıdır.

Barınaklardan gerçekleşen sera gazı emisyonları, enterik fermantasyona göre ve gübre yönetimine göre belirlenebilmektedir. Bu çalışmada; Bursa, Bilecik ve Eskişehir'deki 2004-2021 yılları arasındaki çiftlik hayvanı sayılarına TÜİK'ten ulaşılıp, metan salınımını enterik fermantasyona göre, Tier 1 yöntemiyle hesaplanmıştır.

## MATERYAL VE METOT

Çalışmada materyal olarak; Bursa, Bilecik ve Eskişehir'deki 2004-2021 yılları arasındaki çiftlik hayvanı sayıları kullanılmış ve metan salınımını enterik fermantasyona göre, Tier 1 yöntemiyle hesaplanmıştır. Bulunan değerler daha sonra eşdeğer $CO_2$ miktarına çevrilmiştir.

### MATERYAL

Güney Marmara bölgesindeki; Bursa, Bilecik ve Eskişehir İllerini kapsayan araştırma alanı, aynı zamanda iklimsel bir geçiş bölgesidir. Çalışma alanının; büyük metropol şehirlere yakınlığı sebebiyle, bu şehirlerin tarımsal ihtiyaçlarını karşılama özelliği bulunmaktadır (Şekil 1).




**Şekil 1.** Araştırma alanı

Çalışma alanındaki büyükbaş hayvan sayılarının; çoktan aza göre sıralanması Bursa, Eskişehir ve Bilecik şeklindedir (Tablo 2).

**Tablo 2.** Büyükbaş hayvan sayıları (TÜİK, 2022)

| Yıl | Bilecik | Bursa | Eskişehir |
|---|---|---|---|
| 2004 | 34335 | 124263 | 100298 |
| 2005 | 36392 | 142418 | 113227 |
| 2006 | 38730 | 132563 | 114083 |
| 2007 | 38783 | 148707 | 107887 |
| 2008 | 46887 | 147235 | 108634 |
| 2009 | 45983 | 149329 | 104308 |
| 2010 | 38695 | 160223 | 108173 |
| 2011 | 39591 | 172968 | 117018 |
| 2012 | 41153 | 189774 | 118881 |
| 2013 | 41535 | 199939 | 122661 |
| 2014 | 39637 | 193153 | 126778 |
| 2015 | 38741 | 197119 | 128466 |
| 2016 | 32161 | 186145 | 131459 |
| 2017 | 35775 | 201288 | 136779 |
| 2018 | 40685 | 236604 | 155273 |
| 2019 | 40953 | 242065 | 162855 |
| 2020 | 39018 | 235781 | 170073 |
| 2021 | 38557 | 265312 | 169938 |

Çalışma alanındaki küçükbaş hayvan sayıları ortalamalarının; çoktan aza göre sıralanması Eskişehir, Bursa ve Bilecik şeklindedir (Tablo 3).

**Tablo 3.** Küçükbaş hayvan sayıları (TÜİK, 2022)

| Yıl | Bilecik | Bursa | Eskişehir |
|---|---|---|---|
| 2004 | 74735 | 263769 | 482911 |
| 2005 | 80200 | 293599 | 555204 |
| 2006 | 74957 | 303794 | 556363 |
| 2007 | 73665 | 310692 | 526897 |
| 2008 | 83627 | 270227 | 493309 |
| 2009 | 81435 | 293814 | 508626 |
| 2010 | 90289 | 329837 | 496601 |
| 2011 | 107449 | 376531 | 581601 |
| 2012 | 117350 | 423968 | 603963 |
| 2013 | 120280 | 442013 | 675316 |
| 2014 | 136363 | 465345 | 704118 |
| 2015 | 117520 | 458823 | 808392 |
| 2016 | 126810 | 441859 | 765014 |
| 2017 | 140137 | 472467 | 733738 |
| 2018 | 155191 | 523198 | 800732 |
| 2019 | 187952 | 579525 | 1034359 |
| 2020 | 192814 | 587245 | 1184648 |
| 2021 | 202466 | 592090 | 1268047 |

## YÖNTEM

Çalışma alanındaki; büyükbaş ve küçükbaş hayvan barınaklarından gerçekleşen metan salınımı enterik fermantasyona göre, Tier 1 yöntemiyle hesaplanmıştır. Bulunan değerler daha sonra eşdeğer $CO_2$ miktarına çevrilmiştir.

Tier 1 yöntemi; yakılan yakıtın türü, miktarı ve emisyon faktörleriyle hesap yapmaktadır. Tier 2 yöntemi; Tier 1'deki parametrelere ek olarak, yakma teknolojisi bilgisine göre, ülke veya bölgeye has olarak özel emisyon faktörleriyle hesap yapmaktadır. Tier 3 yöntemi ise; Tier 2'deki parametrelere ek olarak, yakma tesisi ısıl gücüne ve beslenme tipine göre daha detaylı hesaplar yapmaktadır (Şenel ve Atabey, 2020).

Tier 1, 2 ve 3 yöntemlerinin denklemlerine, IPCC raporlarından ulaşılabilmektedir. IPCC'nin 2006 yılındaki kılavuzundaki çizelge ve eşitlikler kullanılarak, araştırılacak bölgenin ortalama sıcaklığına göre emisyon faktörü (EF) belirlenmektedir. Bu çizelgeler kullanılırken; Türkiye'nin gelişmekte olan ülkeler kategorisinde olduğu düşünülerek hesaplama yapılması gerekmektedir (Ahmet, 2019).

**Tier 1 Yöntemi** (Ahmet, 2019)**:**

Tier 1 yönteminde; gübre yönetimi kaynaklı ve enterik fermantasyon kaynaklı olarak $CH_4$ hesabı yapılabilmektedir.

$CH_4$ (gübre yönetimi kaynaklı)= $\sum \frac{EF*N}{10^6}$ (1)

Eşitlikteki;

$CH_4$: Metan emisyonu (Gigagram $CH_4$/yıl)

EF: Emisyon faktörü

N: Hayvan sayısı'dır.

$CH_4$ (enterik fermantasyon kaynaklı)= EF × $\frac{N}{10^6}$ (2)

Eşitlikteki;

$CH_4$: Metan emisyonu (Gigagram $CH_4$/yıl)

EF: Emisyon faktörü (kg $CH_4$/baş.yıl)

N: Hayvan sayısı'dır.

## BULGULAR

IPCC raporlarında belirtilen Tier 1 eşitliğine göre hesaplamalar yapılmıştır ve aşağıdaki çizelgelerdeki sonuçlar elde edilmiştir.

2004-2021 yılları arasındaki periyot değerlendirildiğinde; büyükbaş hayvan kaynaklı karbon ayak izi ortalaması, Bilecik için 426 kg $CO_2$ e / gün, Bursa için 2003 kg $CO_2$ e / gün ve Eskişehir için 1383 kg $CO_2$ e / gün olarak belirlenmiştir (Tablo 4).

**Tablo 4.** Güney Marmara'da 2004-2021 arasında büyükbaş hayvan barınaklarından gerçekleşen emisyon miktarları (kg $CO_2$ e / gün)

| | BİLECİK | BURSA | ESKİŞEHİR |
|---|---|---|---|
| 2004 | 372.26 | 1347.25 | 1087.42 |
| 2005 | 394.56 | 1544.08 | 1227.59 |
| 2006 | 419.91 | 1437.23 | 1236.88 |
| 2007 | 420.48 | 1612.26 | 1169.70 |
| 2008 | 508.34 | 1596.31 | 1177.80 |
| 2009 | 498.54 | 1619.01 | 1130.90 |
| 2010 | 419.53 | 1737.12 | 1172.80 |
| 2011 | 429.24 | 1875.30 | 1268.70 |
| 2012 | 446.18 | 2057.51 | 1288.89 |
| 2013 | 450.32 | 2167.72 | 1329.88 |
| 2014 | 429.74 | 2094.14 | 1374.51 |
| 2015 | 420.03 | 2137.14 | 1392.81 |
| 2016 | 348.69 | 2018.16 | 1425.26 |
| 2017 | 387.87 | 2182.34 | 1482.94 |
| 2018 | 441.10 | 2565.23 | 1683.45 |
| 2019 | 444.01 | 2624.44 | 1765.66 |
| 2020 | 423.03 | 2556.31 | 1843.91 |
| 2021 | 418.03 | 2876.48 | 1842.45 |
| TOPLAM | 7671.84 | 36048.05 | 24901.55 |
| ORTALAMA | 426.21 | 2002.67 | 1383.42 |

2004-2021 yılları arasındaki periyot değerlendirildiğinde ise; küçükbaş hayvan kaynaklı karbon ayak izinin ortalama değerleri, Bilecik için 66 kg $CO_2$ e / gün, Bursa için 226 kg $CO_2$ e / gün ve Eskişehir için 389 kg $CO_2$ e / gün olarak tespit edilmiştir (Tablo 5).

**Tablo 5.** Güney Marmara'da 2004-2021 arasında küçükbaş hayvan barınaklarından gerçekleşen emisyon miktarları (kg $CO_2$ e / gün)

| | BİLECİK | BURSA | ESKİŞEHİR |
|---|---|---|---|
| 2004 | 40.92 | 144.43 | 264.43 |
| 2005 | 43.92 | 160.77 | 304.01 |
| 2006 | 41.04 | 166.35 | 304.65 |
| 2007 | 40.34 | 170.13 | 288.51 |
| 2008 | 45.79 | 147.97 | 270.12 |
| 2009 | 44.59 | 160.88 | 278.51 |
| 2010 | 49.44 | 180.61 | 271.92 |
| 2011 | 58.84 | 206.18 | 318.47 |
| 2012 | 64.26 | 232.15 | 330.71 |
| 2013 | 65.86 | 242.03 | 369.78 |
| 2014 | 74.67 | 254.81 | 385.55 |
| 2015 | 64.35 | 251.24 | 442.65 |
| 2016 | 69.44 | 241.95 | 418.90 |
| 2017 | 76.73 | 258.71 | 401.77 |
| 2018 | 84.98 | 286.49 | 438.46 |
| 2019 | 102.92 | 317.33 | 566.38 |
| 2020 | 105.58 | 321.56 | 648.68 |
| 2021 | 110.86 | 324.21 | 694.34 |
| TOPLAM | 1184.53 | 4067.79 | 6997.86 |
| ORTALAMA | 65.81 | 225.99 | 388.77 |

## TARTIŞMA ve SONUÇ

Literatür ile kıyaslandığında, aynı bölgede benzer çalışma yapan Ahmet (2019) ve Kılıç ve ark. (2018)'nın bulgularına benzer sonuçlarla karşılaşılmıştır.

Tarımsal yapılar ve tarımsal inşaatlardaki eksiklik ve hatalar (özellikle gübre depolama yapılarındaki), atmosfere yayılan sera gazlarının miktarını artırmaktadır. Bu çalışma ile; Bursa, Bilecik ve Eskişehir'deki 2004-2021 yılları arasındaki çiftlik hayvanı (büyükbaş ve küçükbaş) verileri kullanılarak, metan salınımı enterik fermantasyona göre, Tier 1 yöntemiyle hesaplanmıştır. Daha sonra bu sonuçlar karbon ayak izi sonuçlarına dönüştürülmüştür. Farklı sera gazı emisyonlarının $CO_2$ eşdeğerlerinin hesaplanması, sonuçların kıyaslanmasına bir standart getirdiği için çok gereklidir. Sonuçta, 2004-2021 yılları arasındaki periyot değerlendirildiğinde; büyükbaş hayvan kaynaklı karbon ayak izi ortalaması, Bilecik için 426 kg $CO_2$ e / gün, Bursa için 2003 kg $CO_2$ e / gün ve Eskişehir için 1383 kg $CO_2$ e / gün olarak belirlenmiştir. 2004-2021 yılları arasındaki periyotta; küçükbaş hayvan kaynaklı karbon ayak izinin ortalama değerleri ise, Bilecik için 66 kg $CO_2$ e / gün, Bursa için 226 kg $CO_2$ e / gün ve Eskişehir için 389 kg $CO_2$ e / gün olarak tespit edilmiştir. Bu değerlerin azaltılıp, sera gazı emisyonlarının kontrol edilmesi için; özellikle gübre depolama yapılarının teknik özelliklerinin geliştirilmesi ve barınak içerisindeki havalandırma tesislerinin bilimsel olarak planlanması gerekmektedir. Ayrıca; Çiftlik hayvanlarından gerçekleşen metan emisyonunu azaltmak için; hayvanların genetik özellikleri iyileştirilmeli, bitkisel yağ barındıran kaliteli rasyon hazırlanmalı, az sera gazı emisyonuna sebep olan yem bitkileri üretilmelidir.

## KAYNAKLAR

Ahmet B 2019. Bursa bölgesinde faaliyet gösteren bazı hayvancılık işletmelerinin karbon ayak izinin belirlenmesi. Uludağ Üniversitesi Fen Bilimleri Enstitüsü, yüksek lisans tezi. Bursa.

Anonim 2022. $CH_4$ emissions from enteric fermentation. Erişim tarihi: 07.11.2022. Microsoft Word - 4.1_$CH_4$ _Enteric_Fermentation_20030113_rev3.doc (iges.or.jp)

Bal H 2019. Tarımdan kaynaklanan sera gazı emisyonlarının çevresel Kuznets eğrisi yaklaşımı çerçevesinde OECD ülkeleri için incelenmesi. Eskişehir Osmangazi üniversitesi sosyal bilimler ensititüsü, yüksek lisans tezi. Eskişehir.

Kara G, Yalınız İ, Sayar M 2019. Konya ili hayvansak gübre kaynaklı sera gazı emisyonları durumu. Ulusal çevre bilimleri araştırma dergisi, 2 (2): 57-60.

Kılıç İ, Yaylı B, Elekberov A 2018. Bursa bölgesinde faaliyet gösteren üç adet broyler işletmesinin karbon ayak izinin tahminlenmesi. Uluslararası tarım ve yaban hayatı dergisi, 4 (2): 224-230.

Koyuncu M, Akgün H 2018. Çiftlik hayvanları ve küresel iklim değişikliği arasındaki etkileşim. Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 32 (1): 151-164.

Şenel GU, Atabey T 2020. Diyarbakır ilinde farklı sektörlerden kaynaklanan karbondioksit salınımının hesaplanması. KSU mühendislik bilimleri dergisi, 23 (1): 37-47.

TÜİK (Türkiye İstatistik Kurumu) 2022. https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr Erişim tarihi: 07.11.2022.

## ORAL PRESENTATION

# Nöroblastomda Yeni Tedavi Adayı Olarak Kanser Kök Hücre Belirteçlerinin İn Silico Değerlendirilmesi

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**Özet**

**Giriş:** Nöroblastom çocukluk çağının en sık gözlenen solid tümörüdür. İleri evre ve relaps hastalarda tedavi modelleri hala istenen başarıya ulaşamamıştır. Bu nedenle nöroblastomda kök hücreleri hedef alacak bir tedavi geliştirmek için moleküler hedef arayışı vardır. Bu arayışta yapay zeka metodlarının kullanılmasını faydalı olabilir.

**Metod:** Nöroblastom kök hücreleri ve yapay zeka ile son 10 yıldır yapılan çalışmalar Pubmed, Google Scholar ve Pubmed Central veritabanlarından çekilmiştir. Bu çalışmalar daha sonra seçilerek nöroblastomda kök hücre belirteci olabilecek olası hedeflerden bir liste oluşturuldu.

**Bulgular:** Arama sonucunda 63 sonuç bulundu. Yayınların incelenmesi sonucu kök hücre hedefi olabilecek belirteçlerin manuel bir listesi oluşturulmuştur. Nöroblastom gelişiminde bilinen ALK, TERT, MYCN, PHOX2B, NOTCH genleri ve yolakları yanısıra LMO1, LIN28B, BARD1-Beta, BMP4 genleri moleküler patogenezde önemli ve hedeflenebilme açısından değerli bulunmuştur. Nöroblastom tedaviye direncinde kanser kök hücre açısından EGF-Cripto/FRL-1/Cryptic (EGF-CFC) ailesi önemli görünmektedir. Bu aile embriyonik gelişmede fonksiyonu tanımlanmıştır ve nöroblastom için kanser kök hücre markerı adayıdır. Bu aileden CFC1 Nöroblastom tümorosfer oluşumunda aktilin A ve SMAD ile etkileşmektedir.

**Sonuç:** Nöroblastomda kanser kök hücre markırı olarak önem kazanmış CFC1'in önemli bir kanser kök hücre hedefleyici ajan geliştirme aday molekülü olabileceği düşünülmektedir. Ayrıca BMI1 nöroblastomda önemli bir protein olarak tanımlanmaktadır. Kanser kök hücreye karşı tedavi molekülü geliştirmeye aday molekülü olabilir.

**Anahtar Kelimeler:** Nöroblastom, Kanser kök hücre, Yapay zeka, Hedeflenmiş tedavi

Bu çalışma Dokuz Eylül Üniversitesi Bilimsel Araştırma Koordinatörlüğü tarafından 2021.KB.MLT.003 numaralı proje ile desteklenmektedir.

## In Silico Evaluation of Cancer Stem Cell Markers as New Treatment Candidates in Neuroblastoma

**Abstract**

**Introduction:** Neuroblastoma is the most common solid tumor of childhood. Treatment models in advanced and relapsed patients still have not achieved the desired success. Therefore, there is a search for molecular targets to develop a therapy that will target stem cells in neuroblastoma. It may be useful to use artificial intelligence methods in this search.

**Method:** Studies with neuroblastoma stem cells and artificial intelligence for the last 10 years were drawn from Pubmed, Google Scholar and Pubmed Central databases. These studies were then selected to create a list of possible targets that could be a stem cell marker in neuroblastoma.

**Results:** 63 results were found as a result of the search. As a result of the review of the publications, a manual list of markers that could be stem cell targets was created. ALK, TERT, MYCN, PHOX2B, NOTCH genes and pathways known in the development of neuroblastoma, as well as LMO1, LIN28B, BARD1-Beta, BMP4 genes were found to be important in molecular pathogenesis and valuable in terms of targeting. EGF-Cripto/FRL-1/Cryptic (EGF-CFC) family seems to be important in terms of cancer stem cells in neuroblastoma treatment resistance. This family has been identified for its function in embryonic development and is a cancer stem cell marker candidate for neuroblastoma. CFC1 Neuroblastoma from this family interacts with actilin A and SMAD in tumorosphere formation.

**Conclusion:** CFC1, which has gained importance as a cancer stem cell marker in neuroblastoma, is thought to be an important cancer stem cell targeting agent development candidate molecule. In addition, BMI1 has been

identified as an important protein in neuroblastoma. It may be a candidate molecule to develop a treatment molecule against cancer stem cells.
**Keywords:** Neuroblastoma, Cancer stem cell, Artificial intelligence, Targeted therapy
This study is supported by the Scientific Research Coordinatorship of Dokuz Eylül University with the project numbered 2021.KB.MLT.003.

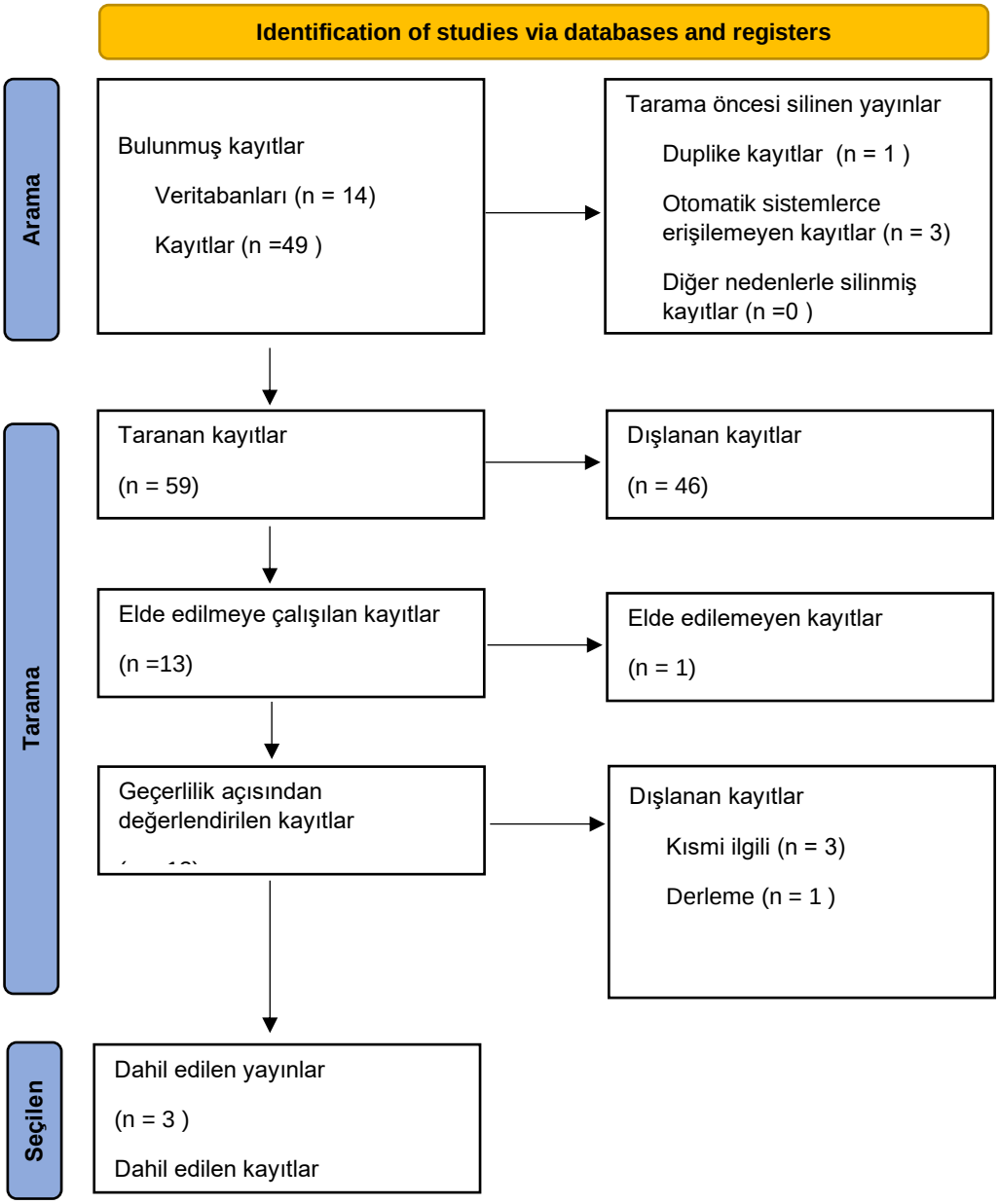
## GİRİŞ

Nöroblastom çocukluk çağının en sık gözlenen solid tümörüdür. Son on yılda tedavide önemli ilerlemeler sağlansada ileri evre ve relaps hastalarda tedavi modelleri hala istenen başarıya ulaşamamıştır. Bu tedavi başarısızlığının sebebinin nöroblastom kanser kök hücreleri olduğu düşünülmektedir. Bu nedenle nöroblastomda kök hücreleri hedef alacak bir tedavi geliştirmek için moleküler hedef arayışı vardır. Bu arayışta yapay zeka metodlarının kullanılmasını faydalı olabilir. Günümüzde artan çalışma sayısı ve veri miktarındaki üstel artışla başa çıkabilmek için yeni yapay zeka metodları geliştirilmekte ve kullanılan yapay zeka metodları sağlık alanına uyarlanmaktadır. Bu çalışmada nöroblastomda kök hücreleri yapay zeka ile inceleyen yayınları inceledik.

## MATERYAL VE METOT

Pubmed,PubmedCentral ve Google Scholar arama motoru olarak seçildi. Aramalar için Pubmed'de "(Stem cells OR regenerative medicine OR ("Stem Cells"[Mesh] OR "Stem Cell Research"[Mesh]) AND artificial intelligence OR machine learning OR knowledge engineering OR ("Artificial Intelligence/classification"[Mesh] OR "Artificial Intelligence/ethics"[Mesh] OR "Artificial Intelligence/instrumentation"[Mesh] OR "Artificial Intelligence/methods"[Mesh]) AND (Neuroblastoma)"terimi ; Pubmed Central'da " ((("stem cells"[MeSH Terms] OR ("stem"[All Fields] AND "cells"[All Fields]) OR "stem cells"[All Fields]) AND ("regenerative medicine"[MeSH Terms] OR ("regenerative"[All Fields] AND "medicine"[All Fields]) OR "regenerative medicine"[All Fields]) AND ("stem cells"[MeSH Terms] OR ("stem"[All Fields] AND "cells"[All Fields]) OR "stem cells"[All Fields])) AND ((("artificial intelligence"[MeSH Terms] OR ("artificial"[All Fields] AND "intelligence"[All Fields]) OR "artificial intelligence"[All Fields]) AND ("machine learning"[MeSH Terms] OR ("machine"[All Fields] AND "learning"[All Fields]) OR "machine learning"[All Fields]) AND ("knowledge"[MeSH Terms] OR "knowledge"[All Fields]) AND ("engineering"[MeSH Terms] OR "engineering"[All Fields])) OR ("Artificial Intelligence/classification"[Mesh] OR "Artificial Intelligence/ethics"[Mesh] OR "Artificial Intelligence/instrumentation"[Mesh] OR "Artificial Intelligence/methods"[Mesh]))) AND ("neuroblastoma"[MeSH Terms] OR "neuroblastoma"[All Fields])" terimi Google Scholar'da "artificial intelligence, machine learning (ML), deep neural network (DNN), induced pluripotent stem cells, stem cells, neuroblastoma" terimi kullanıldı. Arama sonucunda 63 sonuç bulundu

Tablo 1: PRISMA diagramı




## BULGULAR ve TARTIŞMA

Arama sonucunda 63 sonuç bulundu. Bu yayınların 3 tanesi anlamlı olarak değerlendirildi. Ayrıca 5 abstracttanda faydalanıldı. Bu yayınların ilki Jordan ve ark.'ın "Computational Algorithms for In Silico Profiling of Activating Mutations in Cancer" yayınıydı (Jordan ve ark., 2019). Bu yayında aktive edici mutasyonlar in silico metodlarla modellenmiştir. İkinci yayın "Tailoring Therapy for Children With Neuroblastoma on the Basis of Risk Group Classification: Past, Present, and Future" yayını olup nöroblastom sınıflamasına odaklanmaktadır (Liang ve ark., 2020). Son yayın ise "PD-L1, Inflammation, non-coding RNAs, and Neuroblastoma: Immuno-oncology Perspective" yayını olup nöroblastomda immun modülasyonun ilişkisine odaklanmıştır (Nallasamy ve ark., 2018). Yayınların yanında 5 abstractta çalışmaya alınmıştır.

Yayınların incelenmesi sonucu kök hücre hedefi olabilecek belirteçlerin manuel bir listesi oluşturulmuştur. Nöroblastom gelişiminde bilinen ALK, TERT, MYCN, PHOX2B, NOTCH genleri ve yolakları yanısıra LMO1, LIN28B, BARD1-Beta, BMP4 genleri moleküler patogenezde önemli ve hedeflenebilme açısından değerli bulunmuştur. LSAMP,OGR1, DAN ve P2X7 ön çalışmalarda gözlenen diğer

belirteçlerdir. PD-L1 önemli bir yolak olarak göze çarpmaktadır. Nöroblastom tedaviye direncinde kanser kök hücre açısından EGF-Cripto/FRL-1/Cryptic (EGF-CFC) ailesi önemli görünmektedir. Bu aile embriyonik gelişmede fonksiyonu tanımlanmıştır ve nöroblastom için kanser kök hücre markerı adayıdır. Bu aileden CFC1 Nöroblastom tümorosfer oluşumunda aktilin A ve SMAD ile etkileşmektedir.

## SONUÇ

Literatürdeki yapılan çalışmalar incelendiğinde nöroblastomda kanser kök hücre markırı olarak önem kazanmış CFC1'in önemli bir kanser kök hücre hedefleyici ajan geliştirme aday molekülü olabileceği düşünülmektedir. Ayrıca BMI1 nöroblastomda önemli bir protein olarak tanımlanmaktadır. Kanser kök hücreye karşı tedavi molekülü geliştirmeye aday molekülü olabilir. Ancak nöroblastomda kök hücrelerin in silico aranması açısından yapılan çalışma sayısı oldukça azdır. Bu alanda çalışma ihtiyacı vardır.

## TEŞEKKÜR

Bu çalışma Dokuz Eylül Üniversitesi Bilimsel Araştırma Koordinatörlüğü tarafından 2021.KB.MLT.003 numaralı proje ile desteklenmektedir.

## KAYNAKLAR

Jordan, E Joseph et al. "Computational algorithms for in silico profiling of activating mutations in cancer." *Cellular and molecular life sciences : CMLS* vol. 76,14 (2019): 2663-2679. doi:10.1007/s00018-019-03097-2

Liang, Wayne H et al. "Tailoring Therapy for Children With Neuroblastoma on the Basis of Risk Group Classification: Past, Present, and Future." JCO clinical cancer informatics vol. 4 (2020): 895-905. doi:10.1200/CCI.20.00074

Nallasamy, Palanisamy et al. "PD-L1, inflammation, non-coding RNAs, and neuroblastoma: Immuno-oncology perspective." Seminars in cancer biology vol. 52,Pt 2 (2018): 53-65. doi:10.1016/j.semcancer.2017.11.009

"Abstract." *Brain and Neuroscience Advances* vol. 3 2398212819855490. 23 May. 2019, doi:10.1177/2398212819855490

"Abstracts from the 53rd European Society of Human Genetics (ESHG) Conference: Interactive e-Posters." *European journal of human genetics : EJHG* vol. 28,Suppl 1 (2020): 141-797. doi:10.1038/s41431-020-00739-z

"Abstracts from Purines 2014, an International Conference on Nucleotides, Nucleosides and Nucleobases, held in Bonn, Germany, from July 23–27, 2014." Purinergic Signalling vol. 10,4 (2014): 657–854. doi:10.1007/s11302-014-9430-7

"Abstracts of the XIX International Congress of Neuropathology, Tokyo, Japan, September 23-27, 2018." Brain pathology (Zurich, Switzerland) vol. 29 Suppl 1,Suppl 1 (2019): 3-198. doi:10.1111/bpa.12675

"ACTRIMS-ECTRIMS MSBoston 2014: Poster Sessions 2." *Multiple sclerosis (Houndmills, Basingstoke, England)* vol. 20,1 Suppl (2014): 285-496. doi:10.1177/1352458514547846

## *ORAL PRESENTATION*

# Comparison of the content of heavy metals in *Sideritis reaseri* and *Salvia officinalis*

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***Abstract***

Heavy metals are becoming increasingly common in the environment and are harmful to plants, animals, and people's health. They are also known to cause human cancer, and even small quantities of exposure can have negative effects.
For therapeutic purposes, it is crucial to identify the presence of heavy metals in medicinal and herbal plants. This study is designed to evaluate five metals (Pb, Cd, Hg, Sn, and As) that were found in herbal tea that was being marketed in Albanian pharmacies on a qualitative and quantitative level. Following the initial processing of the samples utilizing microwave digestion, chemical treatment, and essential oil extraction using the traditional Clevenger equipment technique. The necessary results on these teas were obtained using the AOAC 2015, 6-19: 2019 method in conjunction with an analytical technique like ICP-MS. When comparing the levels of heavy metals in the leaves and the extracted oil of *Sideritis Reaseri*, it can be noticed that arsenic levels have decreased significantly from 0.125 mg/kg to < 0.001 mg/kg and tin levels have decreased significantly from 0.557 mg/kg to 0.004 mg/ kg. By comparing the presence of heavy metals in the leaves and the extracted oil *Salvia Officinalis*, a decrease in quantity is seen mainly for arsenic from 0.055 mg/kg to < 0.001 mg/kg, and a significant decrease is observed in lead from 0.242 mg/kg to 0.028 mg/kg. The levels of metals in these teas are differed. In particular, *Salvia Officinalis* had higher levels of As and Pb metals than *Sideritis reaseri*, which had higher levels of As and Sn metals. These contaminants have the potential to harm the environment irreparably even in low concentrations. More consideration must be given to the presence of these metals to protect the environment, the flora, and human health.

**Keywords***:* heavy metals, ICP-MS, HDC (hydro distillation), essential oil

## 1. INTRODUCTION

Heavy metals are becoming increasingly common in the environment and are harmful to plants, animals, and people's health. They are also known to cause human cancer, and even small quantities of exposure can have negative effects. For therapeutic purposes, it is crucial to identify the presence of heavy metals in medicinal and herbal plants. This study is designed to evaluate five metals (Pb, Cd, Hg, Sn, and As) that were found in herbal tea that was being marketed in Albanian pharmacies on a qualitative and quantitative level.

### 1.1. Characteristics of *Sideritis raeseri* tea – mountain tea

**Botanical Nomenclature:** Sideritis raeseri

**Common Name:** Ironwort, Mountain Tea, Shepherd's Tea

**Taxonomy- Family**: Lamiaceae

**Distinguishing Features of the Plant:** The perennial herbaceous *plant Sideritis raeseri* has opposite, oblong, fleshy leaves, spherical, brown fruits, and light-yellow flowers in inflorescences. It grows from 10 to 50 cm. Between July and August, the plants bloom and are harvested.

**Region Found:**Mediterranean regions, the Balkans, the Iberian Peninsula and Macaronesia, but can also be found in Central Europe and temperate Asia.

**Part of the Plant with Active Substances**: Leaves

**Active Substances:** Essential oil (which contains α-Pinene, β-pinene, limonene, sesquiterpenes), mucus, vitamin C, flavonoids, iridoids, coumarins, lignans, iron.

**Pharmacological Effects - Therapeutic Applications:** The plant is advised as an anti-inflammatory, antithrombotic, antihypertensive, antispasmodic, antibacterial, sedative, and antioxidant in the form of herbal tea prepared from the leaves. Additionally, it is used to prevent osteoporosis and anemia.

Numerous research are being done to see whether plant infusions could stop the growth of cancer cells. The herb is used in traditional medicine as a sedative, hypnotic, to warm up chilly patients, as well as to treat dyspepsia.

**Toxicity- Adverse Effects:** No side effects are reported. (Lambrou P. Spyrou, 1981)

### 1.2.Characteristics of *Salvia officinalis l* tea – Sage tea

**Botanical Nomenclature:** Salvia officinalis L.

**Common Name:** Garden Sage, Sage

**Taxonomy- Family:** Lamiaceae

**Distinguishing Features of the Plant:** Salvia officinalis is a perennial, evergreen shrub with gray-green, oblong, oval, and lanceolate leaves and yellow, blue, red, violet, or white flowers in cobs. It grows to a height of 50 to 80 cm (bunches). From June to July, the plants bloom, and from July to August, they are harvested.

**Region Found:** Although it only naturally occurs in Italy and the western portion of the Balkan Peninsula, it is widely farmed and has naturalized in many areas of southern and south central Europe.

**Part of the Plant with Active Substances:** Leaves

**Active Substances:** Tannins, bitter agents, terpenes, flavonoids, phenolic acid and essential oil, which contains 30% thujone, 15% cineole, camphor and beta-pinene.

**Pharmacological Effects - Therapeutic Applications:** Tonic, antidiarrheal, antibacterial, antiseptic, antispasmodic, and cardiac qualities are present in the herbal tea produced from the leaves. It is used to treat wounds, aphthous, pharyngitis, and gingivitis, and because of the estrogen-acting properties, it is also advised against amenorrhea, dysmenorrhea, and leucorrhea.

Additionally, the entire plant contains neurotronic characteristics and is used to treat mental health issues, melancholy, vertigo, and neuralgia as well as to enhance memory (sharpness). Finally, plant infusion is administered topically to the skin to treat muscle cramps, alopecia, lesions, infections, wounds, and acne. (Lewis, W.H. and Elvin-Lewis, 2003).

**Toxicity- Adverse Effects:** Plant's consumption must be avoided from hypertensive patient and from children, due to its stimulating action. It is not recommended during pregnancy. (Paul Schauenberg and Ferdinand Paris, 1981).

## 2. MATERIALS AND METHODS

### 2.1.Chemicals and apparatuses

All the following reagents used for microwave digestion and oil extraction: nitric acid, hydrogen peroxide, and hexane were from Merck. Digestion microwave (BERGHOF model Speedwave XPERT), Analytical balance (Ohaus Corporation, USA), ICP-MS (Agilent technologies model 7800). Clevenger equipment (Isolab Labogerate GmbH).

### 2.2.Preparation of the sample

The origin of *Salvia officinalis L.* used in this experimental work is from local Albanian herbs. The selection of the sample was random and was taken in the north of Albania in the area of Dibra. Part of the plant chosen to be analyzed were aerial parts (leaves).

Nitric acid is most frequently employed since it is the best oxidant for arsenic during microwave digestion; nevertheless, some substances are compatible with hydrochloric acid rather than nitric acid. The indirect

method involves first a closed vessel microwave digestion step, which is then followed by an analysis of the instrument. For this reason, we have used nitric acid for the digestion process by microwave.

### 2.2.1 Analysis of sample preparation by microwave digestion.

This analysis was based on the digestion of 0.5g of sample (plant leaves) after grinding and placing in a container to which 6 ml of concentrated $HNO_3$ and 1 ml of $H_2O_2$ (30%) were added. Then the container was placed in the microwave where the heating plan was carried out with three running steps.

**Table 1.** The microwave oven was at the programmed specific power and pressure.

| T [$^0$C] | p [bar] | Ramp (min) | Hold | P [%] |
|---|---|---|---|---|
| 120 | 40 | 3 | 4 | 40 |

The digested samples were transferred to PTFE tubes and then diluted to a final volume of 50 ml with $HNO_3$ 2% (Kilic et al. 2018).

**2.2.2. Oil extraction.** Clevenger apparatus was used to extract the oil from the leaves. Before extraction, the leaves of the *Salvia officinalis L*. plant were cleaned of various impurities such as soil, stones, or other foreign plants. From the plant taken for analysis, 50 g were weighed and then dried at 40$^0$C to constant weight. After drying the plant underwent a grinding process before coming into contact with steam. Steam distillation extraction was carried out in a Clevenger apparatus using a ratio of 10:1 (ml/g) water/dry herb, which means 50g of sample was placed in a 500ml volumetric flask and filled with water until in the sign.

After building the Clevenger apparatus equipped with a condenser, they were placed in the round flask, which would be heated in a heating bowl. The Clevenger system was connected to a continuous water source so that the cooling process is done as well as possible. The heat is controlled so that the oil with water vapor enters the graduated distillation tube and the excess water returns to the flask. The water-plant mixture was then subjected to distillation for an optimal number of hours which was determined to be 3 hours. In the first 30 minutes after the oil had begun to collect in the Clevenger column, about 1 mL of hexane was introduced into the condenser to prevent any polyphenols or other essential oil components from contacting the water.

The obtained essential oils were dried with anhydrous sodium sulfate and stored at 4 - 5$^0$C for further analysis.

## 2.3.Instrumentation

For the analysis, a conventional Agilent 7800 ICP-MS was employed. An Agilent SPS 4 autosampler was used for sampling. The typical sample introduction system was set up in the 7800 ICP-MS (Inductively coupled plasma mass spectrometry).

The optimization of the instrument is done every day according to the conditions recommended by the manufacturer.

### 2.3.1. Calibration and calibration verification

It is important specifying that:
For metals such as Hg and Sn, calibration curves of veranda 0 - 50 ppb have been built.
While for the other metals, a standard mix curve of 0-100 ppb was built.

## 3. RESULTS AND DISCUSSION

## 3.1.Data analytical methods results

Heavy metals of the sample 1 (*Sideritis raeseri*) and sample 2 (*Salvia officinalis L.)* were analyzed using the ICP-MS (151 Method). The percentage of oil after extraction was 0.05% for *Sideritis raeseri and 3.7% for Salvia officinalis L*.

**Table 2.** Detection of heavy metals in **herbal leaves** of *Sideritis raeseri* and *Salvia Officinalis.*

| Heavy Metals | *Sideritis raeseri* | *Salvisa Officinalis l.* | UdM |
|---|---|---|---|
| Arsenic | 0.125 | 0.055 | mg/kg |
| Cadmium | 0.024 | 0.005 | mg/kg |
| Mercury | 0.028 | 0.023 | mg/kg |
| Lead | 0.009 | 0.242 | mg/kg |
| Tin | 0.557 | 0.011 | mg/kg |

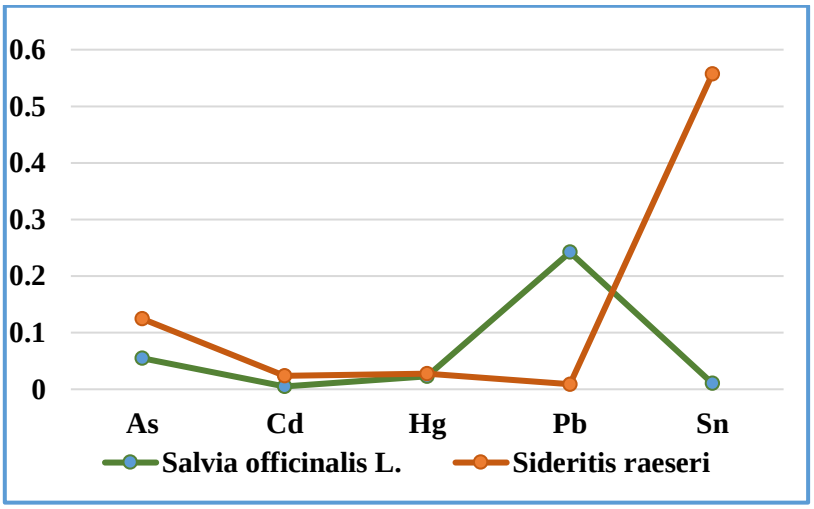


**Figure 1.** Comparison of heavy metals in the ***herbal leaves*** of *Sideritis raeseri and Salvia officinalis L.*

**Table 3.** Detection of heavy metals in **extracted oils** of Sideritis raeseri and Salvia Officinalis

| Heavy Metals | *Sideritis raeseri* | *Salvisa Officinalis l.* | UdM |
|---|---|---|---|
| Arsenic | < 0.001 | 0.001 | mg/kg |
| Cadmium | 0.001 | 0.002 | mg/kg |
| Mercury | 0.003 | 0.015 | mg/kg |
| Lead | 0.001 | 0.028 | mg/kg |
| Tin | 0.004 | 0.007 | mg/kg |

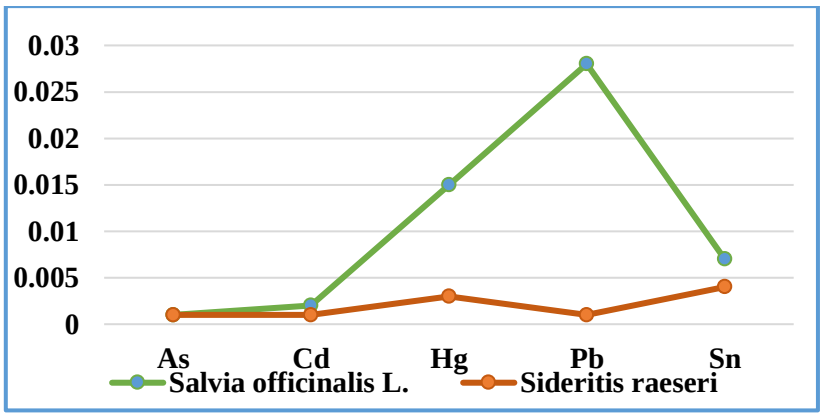


**Figure 2.** Comparison of heavy metals in the **extracted oil** of *Sideritis raeseri and Salvia officinalis L*

- When comparing the levels of heavy metals in the leaves and the extracted oil in Sample 1, it can be noticed that arsenic levels have decreased from 0.125 mg/kg to less than < 0.001 mg/kg and tin levels have decreased from 0.557 mg/kg to 0.004 mg/ kg. A decrease in the amount of other metals was also noticed.
- By comparing the presence of heavy metals in the leaves and the extracted oil in Sample 2, a decrease in quantity is seen mainly for arsenic from 0.055 mg/kg to les than < 0.001 mg/kg, and a significant decrease is observed in lead from 0.242 mg/kg to 0.028 mg/kg. A slight reduction in other metals is also observed.
- Another comparison was made by comparing the values obtained after the detection of heavy metals in the leaves and in the essential oil for both herbal teas. In the herbal leaves of *Sideritis raeseri* and *Salvia officinalis* L. It can be seen that the metal with the highest concentration in Salvia officinalis is Lead (0.242 mg/kg), while in *Sideritis raeseri* the metal with the highest concentration is tin with 0.557 mg/kg. In the extracted oil, we see that the highest concentration is lead with 0.028 mg/kg in the plant *Salvia officinalis L.*

## 4. CONCLUSION

- The physicochemical properties of soil including pH, temperature, redox potential, translocation exchange capacity and organic matter may influence the availability of metal to plants.
- Secondly, the phytological characteristics of medical plants themselves such as reduced biomass, root length and shoot length are common indicators of heavy metal toxicity.
- Furthermore, the interactions of soil-plant roots-microbes play vital roles in regulating heavy metal movement from the soil to edible plant parts. Certain plants are “hyper-accumulators” which grow on metalliferous soils and accumulate extraordinarily high levels of heavy metals without displaying phytotoxic effects.
- Thirdly, herbal plants could be contaminated during manufacturing and agronomic processes including growing, harvesting, transportation, processing and storage, due to pesticide formulations, chemical fertilizers and irrigation with poor-quality water (Oliveira et al., 2018).

## 5. REFERENCES

Barrachina A. C., Carbonell F. B., and Beneyto J. M. (1995) “Arsenic uptake, distribution, and accumulation in tomato plants: effect of arsenite on plant growth and yield,” Journal of Plant Nutrition, vol. 18, no. 6, pp. 1237– 1250.

Choong, G.; Liu, Y. (2014) Templeton, D.M. Interplay of calcium and cadmium in mediating cadmium toxicity. Chem. Biol. Interact. 211, 54–65.

Han FX, Su Y, Monts DL, Waggoner AC, Plodinec JM (2006) Binding distribution, and plant uptake of mercury in soil from Oak Ridge, Tennesse, USA. Sci Total Env 368:753–768.

Hohmann J., Zupko I. & Redei D. (1999). Planta Med.; 65, 576–578.

Hopenhayn, C.; Ferreccio, C.; Browning, S.R.; Huang, B.; Peralta, C.; Gibb, H.; Hertz-Picciotto, I. (2013) Arsenic exposure from drinking water and birth weight. Epidemiology 14, 593–602.

Israr M, Sahi S, Datta R, Sarkar D (2006) Bioaccumulation and physiological effects of mercury in Sesbania Drummond. Chemosphere 65:591–598.

Kilic S, Cam I ̇B, Tongur T, Kilic M (2018) Health risk assessment ofexposure to heavy metals and aflatoxins via dietary intake of dried red pepper from marketplaces in Antalya, Southern Turkey. J Food Sci 83(10):2675–2681

Lambrou P. Spyrou, (1981), Medicinal herbs and their therapeutic properties, Agro publishing house, Athens, Volume II, pages: 538-540

Lewis, W.H. and Elvin-Lewis, M.P.F. (2003). Medical Botany - Plants affecting human health. John Wiley & Sons, Inc., Hoboken, New Jersey. ISBN: 978- 0-471-62882-8

Mañay, N.; Cousillas, A.Z.; Alvarez, C.; Heller, T. (2008) Lead contamination in Uruguay: The "La Teja" neighborhood case. Rev. Environ. Contam. Toxicol. 195, 93–115.

Messer RL, Lockwood PE, Tseng WY, Edwards K, Shaw M, Caughman GB, Lewis JB, Wataha JC (2005) Mercury (II) alters the mitochondrial activity of monocytes at sublethal doses via oxidative stress mechanisms. J Biomed Mat Res B 75: 257–263

Morzck E Jr, Funiccllli NA (1982) Effect of lead and on germination of Spartina alterniflora Losiel seeds at various salinities. Environ Exp Bot 22:23–32.

Mukherji S, Maitra P (1976) Toxic effects of lead growth and metabolism of germinating rice (Oryza sativa L.) seeds mitosis of onion (Allium cepa) root tip cells. Ind J Exp Biol 14:519–521.

L.M. de Oliveira, D. Suchismita, et al. (2017), Arsenic uptake by lettuce from As-contaminated soil remediated with Pteris vittata and organic pp. 249-254.

Park, J.-D.; Zheng, W. (2012). Human exposure and health effects of inorganic and elemental mercury. J. Prev. Med. Public Health, 45, 344–352.

Paul Schauenberg, Ferdinand Paris (1981), Guide to medicinal plants, Publisher M. Giourdas, Athens, page 314.

Zhou ZS, Huang SQ, Guo K, Mehta SK, Zhang PC, Yang ZM (2007) Metabolic adaptations to mercury-induced oxidative stress in roots of Medicago sativa L. J Inorg Biochem 101:1–9.

https://www.unido.org/sites/default/files/200910/Extraction_technologies_for_medicinal_and_aromatic_plants_0.pdf

*ORAL PRESENTATION*

**Karaisalı yöresi kızılçam ağaç türüne ilişkin dip kütük çapı-göğüs çapı ilişkisi**

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**Özet**

Göğüs çapı, ağaçların kolayca ve doğru bir şekilde ölçülen bir özelliğidir. Ayrıca ağaçların birçok özelliği ile de yakın ilişki göstermektedir. Ormancılıkta envanter çalışmaları için oldukça önemli olan meşcere hacim tahmini için ormancılık literatüründe birçok yöntem bulunmasına karşın, pratik olmaları nedeniyle uygulamada genel olarak ağaç hacim tabloları tercih edilmektedir. Planlı silvikültürel uygulamalar veya kaçak kesimler sonucu ormandan uzaklaştırılan ağaçların hacimlerinin hesaplanabilmesi için, dip çap ile göğüs çapı arasındaki ilişkinin ortaya konulması gerekmektedir. Meşcere içerisinde bulunan ağaçlar yaklaşık olarak dip kütük yüksekliğinden kesildiğinden, usulsüz kesilen ağaçların hacim tahmini için ağaç hacim tablolarından faydalanılamamaktadır. Bu nedenle dip kütük çapının bağımsız bir değişken olarak yer aldığı göğüs çapını tahmin eden denklemlere ihtiyaç duyulmaktadır. Bu çalışmada, kızılçam meşcerelerinde göğüs çapı ile dip kütük çapı arasındaki ilişkiler belirlenmeye çalışılmıştır. Bu amaçla, Karaisalı yöresi kızılçam meşcerelerinden alınan örnek ağaçların göğüs çapları ve dip kütük çapları ölçülmüştür. Yapılan ölçümlere dayanarak, on adet regresyon modeli denenmiş ve bu modeller arasından çeşitli istatistiksel ölçütlere bağlı olarak en iyi sonucu veren denklem belirlenmeye çalışılmıştır.

**Anahtar Kelimeler:** Kızılçam, göğüs çapı, dip kütük çapı, Karaisalı

**The relationships between stump diameter-diameter at breast height for *Pinus brutia* Ten. in Karaisalı region**

**Abstract**

Diameter at breast height is an accurately and easily measured features of trees. It also shows a close relationship with many features of trees. Although there are many methods in forestry literature for stand volume estimation, which is very important for inventory studies in forestry, tree volume tables are generally preferred in practice due to their practicality. In order to calculate the volume of trees removed from the forest as a result of planned silvicultural practices or illegal cutting, the relationship between stump diameter and diameter at breast height should be revealed. Since the trees in the stand are cut approximately from stump diameter (30 cm above-ground), tree volume tables cannot be used for the volume estimation of felled trees. Therefore, there is a need for equations estimating diameter at breast height in which stump diameter is an independent variable. In this study, it were investigated the relationships between stump diameter and diameter at breast height for *Pinus brutia*. For that purpose, the diameter at breast height and stump diameter of the sample trees taken from *Pinus brutia* stands of Karaisalı region were measured. Based on the measurements made, ten regression models were evaluated and the most successful model, depending on various statistical criteria, was determined among these models.

**Keywords:** *Pinus brutia*, diameter at breast height, stump diameter, Karaisalı

## GİRİŞ

Orman kaynaklarından faydalanmada sürekliliğin sağlanması için ihtiyaç duyulan bilgileri elde etmenin temel yolu orman envanteridir (Köhl, 2004). Ormancılıkta bütün planlama işlemlerinin temelini yine orman envanteri oluşturmaktadır (Fırat, 1973). Bu temel bilgileri sağlayan orman envanterinin en önemli konularından birini ise meşcere hacmi oluşturmaktadır. Ormancılıkta meşcere hacim tahmini için birçok yöntem bulunmasına karşın, pratik olmaları nedeniyle uygulamada genel olarak ağaç hacim tabloları tercih edilmektedir. Ağaç hacim tabloları, kullandıkları bağımsız değişken sayısına bağlı olarak tek, çift ve çok girişli

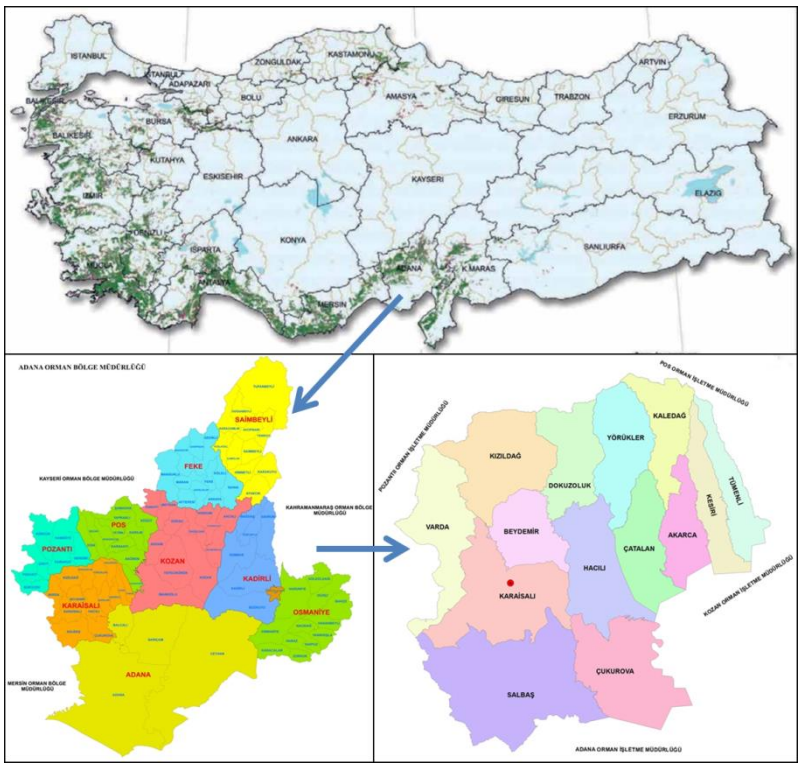
ağaç hacim tabloları olarak 3'e ayrılmaktadırlar. Bu tablolar dikili ağaçların sadece göğüs çapına ($d_{1.3}$) bağlı olarak tek girişli, göğüs çapı ile birlikte ağaç boyuna ($h$) bağlı olarak çift girişli ve ayrıca göğüs çapı ile ağaç boyuna ilaveten diğer bazı ağaç boyutlarının fonksiyonu olarak ise çok girişli ağaç hacim tabloları olarak isimlendirilirler (Kalıpsız, 1999). Tek girişli ağaç hacim tabloları, kolay ve doğru bir biçimde ölçülebilen bir parametre olan ağaçların göğüs çapının bir fonksiyonu olması bakımından uygulamada daha çok tercih edilmektedir (Vanclay, 1994; Kalıpsız, 1999). Buna karşın kayıt altına alınamayan kesimler sonucunda alandan uzaklaştırılan ağaçların göğüs çapı, ağaç boyu gibi birçok özelliği ortaya konulamamakta ve dolayısıyla hacimleri hesaplanamamaktadır. Bu ağaçların göğüs çaplarını kullanarak hacim hesaplamalarını mümkün kılmak amacıyla dip çap-göğüs çapı ilişkilerinin ortaya konulması çok büyük öneme sahip olmaktadır (Yavuz, 2000).

Çeşitli ağaç türleri için dip çap ile göğüs çapı ilişkilerinin ortaya konulması üzerine birçok ulusal ve uluslararası araştırma bulunmaktadır (Alemdag ve Honer, 1977; Bylin, 1982; Demaerschalk ve Omule, 1982; Wiant ve Williams, 1987; Omule ve Kozak, 1989; Kozak ve Omule, 1992; Johnson ve Weigel, 1997; Corral Rivas ve ark., 2007; Milios ve ark., 2016; Uğurlu ve Özer, 1977; Özer, 1981; Giray, 1982; Yavuz, 1996; 2000; Özçelik, 2005; Durkaya ve Durkaya, 2011; Şenyurt, 2012; Ercanlı ve ark., 2015; Sağlam ve ark., 2016; Sakıcı ve Özdemir, 2017; Özdemir ve ark., 2020; Durkaya ve Dağlı, 2021; Özçankaya ve Batur, 2022). Bu çalışmalardan Uğurlu ve Özer (1977) ve Özçelik (2005) tarafından yapılan çalışmalarda kızılçam için dip çap-göğüs çapı ilişkileri incelenmiştir. Kızılçam yaklaşık 5.2 milyon hektarlık yayılış alanıyla ibreli türler arasında ilk sırayı almaktadır (OGM, 2020). Oldukça geniş bir yayılış alanına sahip olan kızılçam meşcereleri Karaisalı yöresi için de yayılış bakımından öne çıkmaktadır.

Bu çalışmada, Karaisalı yöresi kızılçam ağaç türü için göğüs çapı ($d_{1.3}$) ile dip çap ($d_{0.3}$) arasındaki ilişkiler belirlenmeye çalışılmıştır. Bu amaçla, kızılçam meşcerelerinden seçilen örnek ağaçların göğüs çapları ile dip çapları ölçülmüştür. Yapılan ölçümlere bağlı olarak, on adet regresyon modeli denenmiş ve en iyi sonucu veren denklem belirlenmeye çalışılmıştır.

## MATERYAL VE METOT

Dip çap ile göğüs çapı ilişkilerinin belirlenmesi amacı ile Adana Orman Bölge Müdürlüğü'ne bağlı Karaisalı Orman İşletme Müdürlüğü bünyesinde bulunan Varda, Akarca, Yörükler ve Hacılı Orman İşletme Şeflikleri sınırları içerisinde bulunan kızılçam meşcerelerinde yapılan ölçümler sonucu elde edilen verilerden yararlanılmıştır (Şekil 1). Bu amaçla, bahsedilen şefliklerden sırasıyla 31, 21, 37, 32 adet olmak üzere toplam 121 örnek ağacın dip çap ve göğüs çapları ölçülmüştür. Ölçülen örnek ağaçların %85'ine (103 adet) ilişkin veriler modellerin geliştirilmesinde kullanılırken, %15'ine (18 adet) ilişkin veriler ise geliştirilen modellerin uygunluğunun test edilmesinde kullanılmıştır. Tablo 1'de model geliştirme ve kontrol verilerine ilişkin çeşitli istatistiksel değerler verilmiştir.

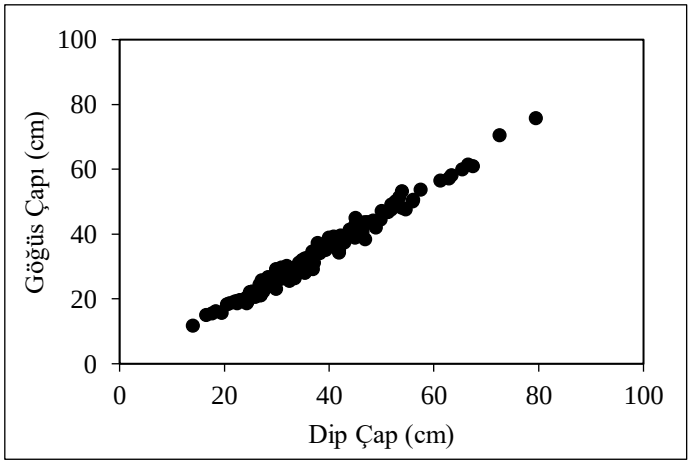



Şekil 1. Çalışma alanı

Tablo 1. Örnek ağaçlara ilişkin çeşitli istatistik bilgiler

| | Değişken | Örnek Sayısı ($n$, Adet) | Minimum (cm) | Maximum (cm) | Aritmetik Ortalama (cm) | Standart Sapma (cm) |
|---|---|---|---|---|---|---|
| Model | $d_{0.3}$ | 103 | 14,1 | 79,5 | 38,4 | 12,5 |
| | $d_{1.3}$ | | 11,6 | 75,6 | 34,3 | 12.4 |
| Kontrol | $d_{0.3}$ | 18 | 18,5 | 63,5 | 37,9 | 13,5 |
| | $d_{1.3}$ | | 16,0 | 58,0 | 33,5 | 12,5 |

Örnek ağaçlardan elde edilen dip çap ve göğüs çapı değerlerinin dağılımı Şekil 2'de verilmiştir. Bu dağılımının modellenmesi amacıyla aşağıda verilen eşitlikler denenmiştir.




Şekil 2. Örnek ağaçlara ilişkin dip çap-göğüs çapı dağılımı

$d_{1.3}=b_0+b_1\ d_{0.3}$ (Linear, 1)

$d_{1.3}=b_0+b_1\ln(d_{0.3})$ (Logarithmic, 2)

$d_{1.3}=b_0+b_1/\ d_{0.3}$ (Inverse, 3)

$d_{1.3}=b_0+b_1\ d_{0.3}+b_2\ d_{0.3}^2$ (Quadratic, 4)

$d_{1.3}=b_0+b_1\ d_{0.3}+b_2\ d_{0.3}^2+b_3\ d_{0.3}^3$ (Cubic, 5)

$d_{1.3}=b_0 b_1^{d0.3}$ (Compound, 6)

$d_{1.3}=b_0\ d_{0.3}^{b1}$ (Power, 7)

$d_{1.3}=e^{b0+b1/d0.3}$ (S, 8)

$d_{1.3}=e^{b0+b1d0.3}$ (Growth, 9)

$d_{1.3}=b_0 e^{b1d0.3}$ (Exponential, 10)

Bu eşitliklerde; $d_{0.3}$ Dip çapı (cm), $d_{1.3}$ Göğüs çapı (cm), $b_i$ Denklem parametrelerini ifade etmektedir.

Geliştirilen denklemler arasından en başarılı olan denklemin seçilmesi için Belirtme Katsayısı ($R^2$) ve Hata Kareler Ortalamasının Karekökü (*RMSE*) ölçütlerinden yararlanılmıştır. En yüksek Belirtme katsayısı ve en düşük hataya sahip olan regresyon modeli en başarılı model olarak seçilmiştir.

Belirtme Katsayısı:

$$R^2 = 1 - \frac{\sum(\hat{y}i - yi)^2}{\sum(yi - \bar{y})^2} \quad (11)$$

Hata Kareler Ortalamasının Karekökü:

$$RMSE = \sqrt{\frac{\sum(\hat{y}i - yi)^2}{n-p}} \quad (12)$$

Bu eşitliklerde; $y_i$ Ölçülen değeri, $\hat{y}_i$ Denklemden hesaplanan değeri, $p$ Denklemdeki parametre sayısını, $n$ Örnek sayısını ifade etmektedir.

103 örnek ağaçtan elde edilen veriler yardımıyla geliştirilen denklemlerin geçerlilikleri, kontrol için ayrılan veriler kullanılarak uygulanan Eşlendirilmiş Örnekler İçin t Testi yardımıyla kontrol edilmiştir. Çalışma kapsamında yapılan istatistiksel analizlerde SPSS 20.0 paket programından faydalanılmıştır.

## BULGULAR ve TARTIŞMA

Karaisalı yöresi kızılçam ağaç türünde dip çap ile göğüs çapı arasındaki ilişkileri ortaya koymak amacıyla geliştirilen modellere ilişkin parametre tahmin değerleri ile istatistiksel ölçütler Tablo 2'de verilmiştir. Tablo 2 incelendiğinde denklemlerin büyük çoğunluğunun başarılı denklemler olduğu ve bu denklemler arasında 1 nolu denklemin (Lineer model) dip çap-göğüs çapı ilişkisini ortaya koymada en başarılı denklem olduğu görülmektedir. Bu denklem, en yüksek belirtme katsayısına ($R^2$=0,981) ve oldukça düşük hataya (*RMSE*=1,716) sahip olması bakımından diğer denklemlere göre üstünlük sağlamaktadır.

Tablo 2. Dip çap-göğüs çapı modellerine ilişkin parametre ve istatistiksel ölçüt değerleri

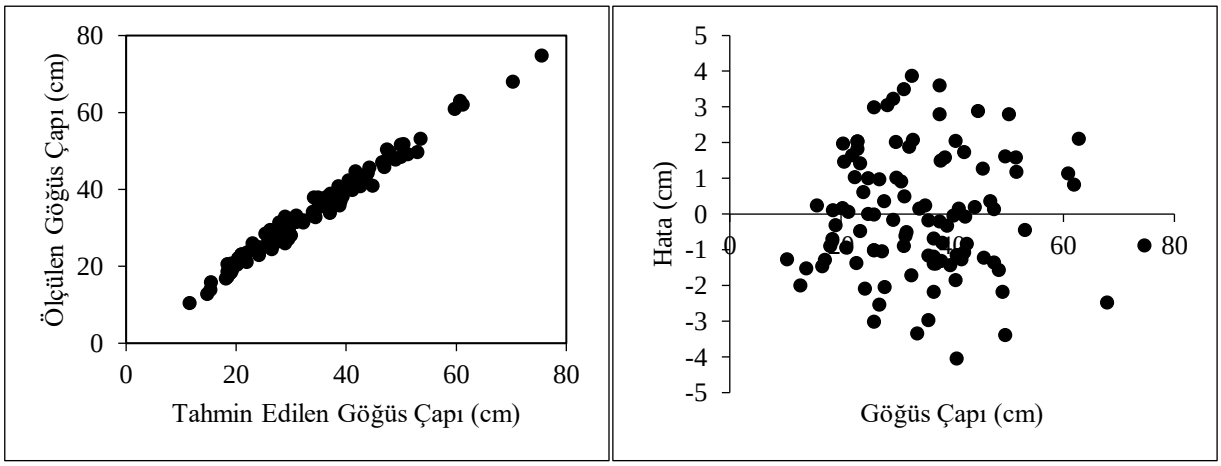
| Model | $b_0$ | $b_1$ | $b_2$ | $b_3$ | *F* | *p* | $R^2$ | *RMSE* |
|---|---|---|---|---|---|---|---|---|
| 1 | -3,555 | 0,984 | | | 5218,801 | 0,000 | 0,981 | 1,716 |
| 2 | -95,284 | 36,029 | | | 1288,941 | 0,000 | 0,927 | 3,358 |
| 3 | 65,327 | -1070,287 | | | 373,799 | 0,000 | 0,787 | 5,745 |
| 4 | -2,526 | 0,931 | 0,001 | | 2601,807 | 0,000 | 0,981 | 1,719 |
| 5 | 0,084 | 0,723 | 0,006 | -0,00004 | 1730,054 | 0,000 | 0,981 | 1,721 |
| 6 | 10,670 | 1,029 | | | 1466,693 | 0,000 | 0,936 | 4,443 |
| 7 | 0,607 | 1,103 | | | 4545,028 | 0,000 | 0,978 | 1,712 |
| 8 | 4,470 | -34,498 | | | 1164,895 | 0,000 | 0,920 | 3,809 |
| 9 | 2,367 | 0,029 | | | 1466,693 | 0,000 | 0,936 | 4,443 |
| 10 | 10,670 | 0,029 | | | 1466,693 | 0,000 | 0,936 | 4,443 |

Geliştirilen denklemlerin geçerlilikleri, 18 örnek ağaçtan elde edilen bağımsız bir veri grubu kullanılarak "Eşlendirilmiş örnekler için t Testi" yardımıyla test edilmiştir. Bu test ile örnek ağaçların göğüs çapı değerleri ile denklemler yardımıyla hesaplanan tahmini göğüs çapı değerleri karşılaştırılmış olup gerçek değerler ile tahmini değerler arasında istatistiksel olarak anlamlı fark bulunmayan denklemlerin kullanılabileceğine karar verilmiştir (Tablo 3). Tablo incelendiğinde diğer tüm denklemler gibi başarılı bulunan 1 nolu denklemin gerçek ve tahmini değerlerinin istatistiksel olarak farklılık göstermediği ($p$>0,05) ve bu denklemin göğüs çapı tahminlerinde kullanılabileceği görülmektedir.

Tablo 3. Denklemlere ilişkin t-testi sonuçları

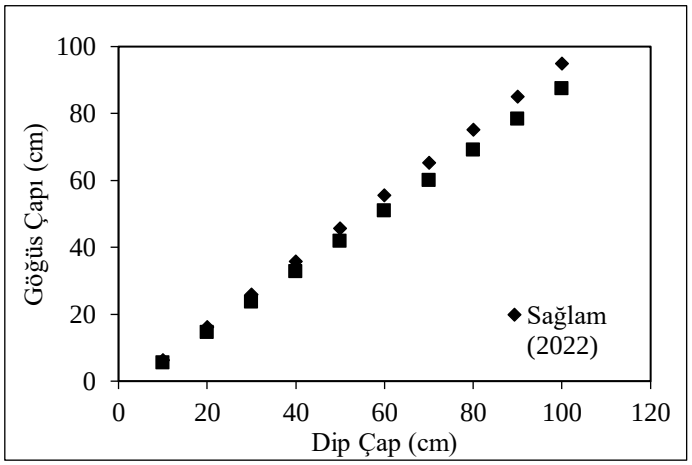
| Model | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| *t* | -0,707 | -0,254 | -0,290 | -0,690 | -0,686 | -0,822 | -0,608 | 0,267 | -0,822 | -0,822 |
| *p* | 0,489 | 0,803 | 0,775 | 0,499 | 0,502 | 0,422 | 0,551 | 0,793 | 0,422 | 0,422 |

Çalışma kapsamında alınan örnek ağaçların tümü için 1 nolu lineer denklem ile hesaplanan göğüs çapı tahminlerine ilişkin gerçek-tahmin grafiği ile hata dağılımları Şekil 3'te verilmiştir. Şekil incelendiğinde gerçek ve tahmin değerlerinin birbirine oldukça yakın oluğu ve hata dağılımlarının da rastgele bir dağılım gösterdiği söylenebilmektedir.




Şekil 3. Göğüs çapı değerlerine ilişkin hata dağılımı

Çalışma sonucunda geliştirilen 1 nolu lineer model ile Mut Orman İşletme Şefliği kızılçam meşcereleri için Özçelik (2005) tarafından geliştirilen lineer model karşılaştırılmıştır (Şekil 4). Şekil incelendiğinde her iki çalışma ile ortaya konan dip kütük çapı-göğüs çapı ilişkilerinin 40 cm dip kütük çapına kadar benzer olduğu görülürken, bu çaptan itibaren her iki çalışma ile yapılan sonuçların farklılık gösterdiği görülmüştür. Sağlam (2022) tarafından yapılan çalışma ile ortaya konan sonuçlar Özçelik (2005) tarafından ortaya konan sonuçlardan daha yüksek değerler almıştır.




Şekil 4. Özçelik (2005) ve Sağlam (2022) tarafından hesaplanan dip çap ($d_{0,3}$)-göğüs çapı ($d_{1,3}$) ilişkisi

## SONUÇ

Karaisalı yöresi kızılçam ağaç türüne ait dip çap ile göğüs çapı ilişkilerinin ortaya konulması amacıyla 10 adet regresyon denklemi denenmiş ve bu denklemler arasından istatistiksel ölçütlere göre en başarılı bulunan 1 nolu lineer denklem seçilmiştir. Bu denklem aşağıdaki şekilde ifade edilmektedir.

$d_{1.3} = -3{,}555 + 0{,}984\ d_{0.3}$

Uygulayıcıya kolaylık sağlaması açısından 1 nolu lineer denklem kullanılarak 10-80 cm dip çap değerlerine karşılık gelen göğüs çapı değerleri Tablo 3'te verilmiştir.

Söz konusu çalışmada Karaisalı yöresi kızılçam meşcereleri için dip çap ile göğüs çapı ilişkileri araştırılmış olup bu çalışmada elde edilen denklem yardımıyla herhangi bir sebeple meşcereden çıkarılan ağaçların göğüs çapı değerlerine ulaşmak mümkün olacaktır. Yine bu sayede göğüs çapının elde edilmesi ile tek girişli ağaç hacim denklemleri kullanılarak pratik bir şekilde hacim tahminlerinde bulunulabilecektir.

Diğer bütün meşcere özelliklerinde olduğu gibi dip çap-göğüs çapı ilişkileri de ağaç türü ve yetişme ortamına göre değişmektedir. Bu nedenle, söz konusu farklılıkların sonuçlara yansıtılabilmesi ve güvenilir sonuçların elde edilebilmesi için farklı ağaç türleri ve farklı yöreler için dip çap ile göğüs çapı ilişkileri ortaya konulmalıdır.

Tablo 3. Karaisalı yöresi kızılçam ağaç türü için dip çaplardan tahmin edilen göğüs çapı değerleri

| $d_{0.3}$ | $d_{1.3}$ | $d_{0.3}$ | $d_{1.3}$ | $d_{0.3}$ | $d_{1.3}$ | $d_{0.3}$ | $d_{1.3}$ | $d_{0.3}$ | $d_{1.3}$ | $d_{0.3}$ | $d_{1.3}$ | $d_{0.3}$ | $d_{1.3}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10** | 6,3 | **20** | 16,1 | **30** | 26,0 | **40** | 35,8 | **50** | 45,7 | **60** | 55,5 | **70** | 65,4 |
| **11** | 7,3 | **21** | 17,1 | **31** | 27,0 | **41** | 36,8 | **51** | 46,7 | **61** | 56,5 | **71** | 66,3 |
| **12** | 8,3 | **22** | 18,1 | **32** | 27,9 | **42** | 37,8 | **52** | 47,6 | **62** | 57,5 | **72** | 67,3 |
| **13** | 9,2 | **23** | 19,1 | **33** | 28,9 | **43** | 38,8 | **53** | 48,6 | **63** | 58,5 | **73** | 68,3 |
| **14** | 10,2 | **24** | 20,1 | **34** | 29,9 | **44** | 39,8 | **54** | 49,6 | **64** | 59,5 | **74** | 69,3 |
| **15** | 11,2 | **25** | 21,1 | **35** | 30,9 | **45** | 40,7 | **55** | 50,6 | **65** | 60,4 | **75** | 70,3 |
| **16** | 12,2 | **26** | 22,0 | **36** | 31,9 | **46** | 41,7 | **56** | 51,6 | **66** | 61,4 | **76** | 71,3 |
| **17** | 13,2 | **27** | 23,0 | **37** | 32,9 | **47** | 42,7 | **57** | 52,6 | **67** | 62,4 | **77** | 72,2 |
| **18** | 14,2 | **28** | 24,0 | **38** | 33,9 | **48** | 43,7 | **58** | 53,5 | **68** | 63,4 | **78** | 73,2 |
| **19** | 15,2 | **29** | 25,0 | **39** | 34,8 | **49** | 44,7 | **59** | 54,5 | **69** | 64,4 | **79** | 74,2 |

## KAYNAKLAR

Alemdağ İŞ, Honer TG 1977. Metric Relationships between breast-height and stump diameter for eleven tree species form Eastern and Central Canada, Canadian Forest Service, Forest Management Institute, Information Report, FMR-X-.

Anşin R, Özkan ZC 1993. Tohumlu bitkiler (*Spermatophyta*) odunsu taksonlar, KTÜ Basımevi, Genel Yayın No: 167, Fakülte Yayın No: 19, Trabzon, 512 s.

Bylin CV 1982. Estimating dbh from Stump Diameter for 15 Southern Species. USDA Forest Service Research Note SO286, Southern Forest Experiment Station, New Orleans.

Corral-Rivas JJ, Barrio-Anta M, Aguirre-Calderón OA, Diéguez-Aranda U 2007. Use of stump diameter to estimate diameter at breast height and tree volume for major pine species in El Salto, Durango (Mexico). Forestry 80(1): 29-40.

Demaerschalk JP, Omule SAY 1982. Estimating breast height diameter form stump measurements in British Columbia, Forest Chronicle, 58(3), 143-145.

Durkaya B, Dağlı C 2021. Kütük Çapı ve Kütük Yüksekliği ile Göğüs Çapı İlişkisi: Kastamonu- Göynük Örneği, Bartın Orman Fakültesi Dergisi, 23(3): 1004-1012.

Durkaya B, Durkaya A 2011. Zonguldak-Ulus Orman İşletme Müdürlüğü Göknar, Kayın ve Karaçam Ağaç Türleri için Kütük Çapı ve Boyu ile Göğüs Çapı İlişkisi. Kastamonu Üniversitesi Orman Fakültesi Dergisi, 11(1), 9-17.

Ercanli I, Gunlu A, Baskent EZ 2015. Mixed effect models for predicting breast height diameter from stump diameter of Oriental beech in Göldağ. Scientia Agricola, 72(3): 245-251.

Fırat F 1973. Dendrometri, Dördüncü Baskı, Kutulmuş Matbaası, İstanbul Üniversitesi Yayın No: 1800, Orman Fakültesi Yayın No: 193, İstanbul, 359 s.

Giray N 1982. Ağaçlarda kütük çapı, orta çap, göğüs çapı ilişkisi, O.A.E. Dergisi, Temmuz Sayısı, Cilt:28, 69-79.

Kalıpsız A 1999. Dendrometri, İstanbul Üniversitesi, Orman Fakültesi Yayını, No:3194/354, İstanbul.

Köhl M 2004. Forest ınventory and monitoring, Encyclopedia of Forest Science, Eds.: Burley, J., Evans, J., Youngquist, J.A., Elsevier, Spain, 403-409.

Kozak A, Omule SAY 1992. Estimating stump volume, stump ınside bark diameter and diameter at breast height form stump measurements, Forestry Chronicle, 68(5), 623-627.

Milios E, Kitikidou KG, Dalakouras V, Pipinis E 2016. Diameter at breast height estimated from stumps in Quercus frainetto in the region of Evros in Northeastern Greece. Cerne 22(3): 337-344.

Omule SAY, Kozak A 1989. Stump and breast height diameter tables for British Columbia Tree Species, FRDA Report 062, 67s.

OGM 2020. Türkiye orman varlığı. OGM Yayınları, Ankara.

Özçankaya N, Batur M 2022. İzmir Orman Bölge Müdürlüğü fıstık çamı (*Pinus pinea* L.) meşcerelerinde kütük çapı-göğüs çapı ilişkisinin modellenmesi. Ormancılık Araştırma Dergisi, 9(1) , 44-60.

Özçelik R 2005. Mut Orman İşletmesinde karaçam, sedir ve kızılçam ağaç türleri için dip çap-göğüs çapı ilişkileri. S.D.Ü. Fen Bilimleri Enstitüsü Dergisi, 9-3, 83-91.

Özdemir E, Şahin A, Özdemir GA 2020. Kuzey Marmara Yöresi sapsız meşe (*Quercus petraea* (Matt.) Liebl) meşcereleri için kütük çapı-göğüs çapı ilişkisinin belirlenmesi. Turkish Journal of Forestry 21(1): 25-34.

Özer E 1981. Sarıçamlarda kütük çapından yararlanarak göğüs çapının Bulunması, O.A.E. Dergi No: 53, Cilt:27, s.20-23, Ankara.

Sağlam F, Sakici OE, Seki M 2016. Stump diameter and diameter at breast height relationship for Chestnut (*Castanea sativa* Mill.) stands in Kastamonu coastal region. International Forestry Symposium (IFS 2016), 7-10 December, Kastamonu, Turkey, pp. 514-520.

Sakıcı OE, Özdemir G 2017. Stump diameter and diameter breast height relationships for oriental beech and Kazdağı fir at mixed stands in Karabük. Kastamonu Üniversitesi Orman Fakültesi Dergisi 17(2): 298-306.

Şenyurt M 2012. Batı Karadeniz Yöresi Sarıçam (*Pinus sylvestris* L.) Meşcerelerinde Kütük Çapı-Göğüs Çapı İlişkileri, Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi, 13(1),79-87.

Uğurlu S, Özer E 1977. Kızılçamlarda kütük çapından yararlanarak göğüs çapının bulunması, O:A.E. Dergisi, Cilt No: 23, Sayı:1, s.71-77, Ankara.

Vanclay JK 1994. Modelling forest growth and yield:Applications to mixed tropical forests. Wallingford CAB International.

Weigel DR, Johnson PS 1997. Estimating dbh of southern Indiana oaks from stump diameter. Tech. Brief TB-NC4 USDA Forest Service North Central Forest Experiment Station.

Wiant HUJr, Williams TB 1987. Lower bole diameter and volume of four appalachian hardwoods, Northern Journal of Applied Forestry, 4(212), 85-98.

Yavuz H 1996. Taşköprü Orman İşletmesinde sarıçam ve karaçam ağaç türlerimize ilişkin dip çap göğüs çapı-orta çap ilişkileri ile kabuk hacminin hesaplanması, K.T.Ü Orman Fakültesi Bahar Yarı Yılı Seminerleri, Seminer No:2, 67-75s.

Yavuz H 2000. Dişbudak ağaç türü için dip kütük çapı ile göğüs çapı arasındaki ilişkinin belirlenmesi, K.T.Ü Orman Fakültesi Güz Yarı Yılı Seminerleri, Seminer No:7, 10s.

## *ORAL PRESENTATION*

# The physical-chemical characteristics of the Albanian Capsicum annuum, a complete SC-$CO_2$ extract for the aromatic medicinal plant.

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## Abstract

Known by the plant's botanical name: Capsicum annuum, the chili pepper is considered an excellent source of antioxidants, including capsaicin, alkaloids, fatty acids, flavonoids, carbohydrates, carotene, essential oil, vitamins A, B1, B2, B3, B5, B6, B9, and C, fibers, folic acid, manganese, lutein, zeaxanthin, b-carotene, and lycopene. Capsaicin is used against atherosclerosis, diabetic vascular disease, stroke, hypertension, metabolic syndrome, etc.
After collecting the plant, its integrity and health are examined under a microscope, and the humidity is determined. Before the supercritical $CO_2$ extractions, the plant was under investigation for its physical properties. The extraction method used is supercritical $CO_2$ extraction, which requires a maximum humidity of 6% for production. The result provided by the GC-FID analysis of the essential oil extract of Capsicum annuum, with the SC-$CO_2$ has shown that the content of capsaicin is 36.82 % followed by nonivamide at 7.98%, which is used as a topical analgesic, as a flavoring ingredient, and exhibits anti-inflammatory properties in peripheral blood mononuclear cells and U937 macrophages. The GC-FID method was used for the identification of the components of the Capsicum annuum. The essential oil of Capsicum annuum, was under investigation for the presence of heavy metals, and ISO 11212 Spectrophotometry was used for the identification. Lead was the heavy metal that resulted in higher concentration in comparison with the other metals, but still, its presence was according to FAO / WHO levels for plants. All the analyses were performed according to the ISO standards.

**Keywords:** Capsicum annuum, Supercritical $CO_2$ extraction, GC -FID analysis,

## INTRODUCTION

The antioxidant effects of peppers, also known as capsicum, are well studied. Peppers come in a variety of colors (green, yellow, orange, and red), and it has been heavily studied for their positive effects in treating a variety of illnesses, including gastrointestinal problems and antipyretics, particularly in problems related to mental health. Along with substantial levels of beta-carotene (pro-vitamin A) and other active compounds, pepper also contains several bioactive substances. Red peppers had the highest concentration of beta-carotene and yellow peppers had the lowest when comparing pepper color and levels of the active substance. It has been discovered that the antioxidant activity in all peppers is similar. Pepper has been proven to stop the oxidation of important fatty acids when used in cooking. (Sun T et. al 2007). Capsaicin, alkaloids, fatty acids, flavonoids, carbohydrates, carotene, essential oils, vitamins A, B1, B2, B3, B5, B6, B9, and C, fiber, folic acid, manganese, lutein, zeaxanthin, b-carotene, and lycopene are just a few of the antioxidants that pepper is a great source of. The pharmacological effects of Capsicum annuum, sometimes known as chili paper, include anti-diarrheal, antibacterial, anti-rheumatic, and antispasmodic properties. Recent research has demonstrated that capsicum can stimulate the circulatory system, improve poor circulation, and treat excessive blood clotting. It is advised to take capsicum to avoid cardiovascular disorders because it can even lower high blood cholesterol levels. Of course, it has additional advantages beyond those mentioned; it can also be used to treat toothaches, nausea, drunkenness, malaria, and swallowing difficulties. Externally applied, it can help against osteoarthritis, and

rheumatoid arthritis (Gyula Mozsik,2018). Fresh pepper is one of the vegetables with such a higher vitamin C concentration (Vanderslice et al., 1990).

The major active substance of the Capsicum annuum is Capsaicin and Nonivamide. Capsaicinoids show antioxidant properties and potent antimutagenic and anticarcinogenic possessions. (Young – Joon Surh, 2002). Improved endothelial function, including eNOS activation/stimulation; induction of LXRalpha in foam cells, which promotes cholesterol export; Capsaicin is used to treat *atherosclerosis.*
Improved endothelial function, including eNOS activation/induction, in the treatment of *stroke and diabetic vascular disease*
Improved endothelium-dependent coronary artery vasodilation in angina
• *Hypertension:* reduced renal sodium retention and activation/induction of eNOS
• *Metabolic syndrome:* PPARgamma induction and decreased adipose inflammation
• *Cardiac hypertrophy* – PPARdelta induction
• Hepatocyte UCP2 induction in *fatty liver;* reduced adipose inflammation increased secretion of GLP-1.
Sympathetic stimulation of brown fat thermogenesis in *obesity*. Better ability to manage appetite thanks to improved adipocyte capacity for lipolysis and GLP-1, a vagal signal to appetite centers. Capsaicin exposure through the gastrointestinal tract lowers calorie and fat consumption.

Shengping Zhang MD, et. al. were able to look into the anticancer properties of capsaicin, according to a study that was published in 2019. They conclude that through stimulating capsaicin receptors or controlling other signaling pathways, capsaicin has potent anti-cancer effects on a variety of cancer types. Even the potential for clinical application in cancer therapy has been investigated, along with the impact of capsaicin on conventional chemotherapeutic medicines and radiotherapy in many cancer types. Capsaicin has broad clinical application prospects and may be used as a potential chemo-preventive or new auxiliary therapeutic drug for cancer, according to researchers who confirmed that when combined with conventional chemotherapy drugs or radiotherapy, it can improve patients' sensitivity to chemo-radiotherapy, reduce treatment dosages, and improve patient tolerance.

**Table 1.** Characteristics of Capsicum annuum.

| Name | Sweet peppers |
|---|---|
| **Scientific Name** | **Capsicum annuum** |
| **Native** | Central and South America |
| **Common/English Name** | **Capsicum Pepper,** Cayenne, Cayenne Pepper, Cherry Pepper, Chili, **Chilly Pepper,** Green Capsicum, **Paprika**, etc. |
| **Name in Other Languages** | **Albanian:** Specë djegës |
| **Plant Growth Habit** | Small, branched, mostly erect, annual or perennial herbaceous shrub |
| **Growing Climate** | Warm-season crop, which performs well under an extended frost-free season |
| **Origin of the plant:** | Gjinar, Elbasan. |
| **Cultivation** | collected by the harvesters from August to October |
| **Major Nutrition*** | Vitamin C (Ascorbic acid), Vitamin B6 (Pyridoxine)<br>Copper, Cu, Vitamin K (phylloquinone), Manganese, Mn<br>Vitamin B1 (Thiamin), Total dietary Fiber<br>Iron, Fe, Potassium, K, Carbohydrate |
| **Health Benefits** | It has anti-diarrheal actions. It can be used as an antibiotic, antirheumatic, antispasmodic, appetizing, antirheumatic, antiseptic, and sudorific. It has tonic and digestive actions.<br>- It can stimulate the cardiovascular system. It can improve poor circulation and reduces high blood cholesterol levels. It can prevent patients from cardiovascular (Kim C-S, 2007). disease and treats excessive blood clotting. |

The need for developing novel processing methods has increased because of the high cost of organic solvents, environmental considerations, and demands for ultrapure goods from, for example, the food and medical industries. In the manufacturing of pharmaceuticals, carbon dioxide is getting more attention. The "clean" synthesis of medicinal compounds utilizing carbon dioxide as a reaction medium holds tremendous potential for large-scale application in the pharmaceutical industry. These applications include particle creation with dense carbon dioxide. Since the end of the 1970s, supercritical fluid extraction has been used to separate natural products, but for a very long time, the applications were limited to a select few goods.

Supercritical extraction has the advantage of being able to extract much larger molecules like lipids (omega-3 and omega-6 fatty acids), chlorophyll, and waxes. Experiments and results have shown that these extracts are free of microorganisms, solvents, heavy metals, and preservatives. Since they are entire extractions of the plants, which comprise the essential oils as well as the fat- and water-soluble components, they are exceptionally concentrated. This means that while using $CO_2$ total extracts, we are utilizing both the plant's essential oil and other components that were also supercritically $CO_2$ extracted (T. A. Safapuri et.al 2019).
After the essential oil is obtained, it goes through different analyses, from the physical-chemical, even the microbial ones. GC – FID provided information regarding the composition of the chili pepper, after that using the UV-VIS Spectrophotometer we were able to determine the concentration of the heavy metals.

## MATERIALS AND METHODS

From August to October, harvesters in Gjinar Village, which is a component of the Administrative Unit of Gjinari, Elbasan Municipality, cultivate the plant. The fruit is the plant element that is extracted with supercritical $CO_2$. A regulated, dry, and ventilated environment is used for both plant selection and milling. The integrity and health of the plant are evaluated using a humidity monitor and a microscope. Regarding the current state of production: The plant particle size used is 0.3 mm by SCFE (supercritical fluid extraction) technology criteria with a maximum humidity of 6%. The extraction of solids from ground materials (or pellets, granulates) is frequently carried out in batch mode utilizing food carbon dioxide as a solvent. The apparatus comes with two separators where the $CO_2$ is removed from the extract before being recycled and two or more extractors that work above the $CO_2$'s critical pressure. The temperature and pressure of the carbon dioxide gas are increased until they reach the supercritical state as the first step in the supercritical $CO_2$ extraction procedure. At a temperature of 31.1 °C and a pressure of 1071 psi (72.87723 atm), carbon dioxide reaches a supercritical state. To do this, a heater and a high-pressure pump are employed. There is a lot of organic raw material in the extractors that the supercritical $CO_2$ passes through.

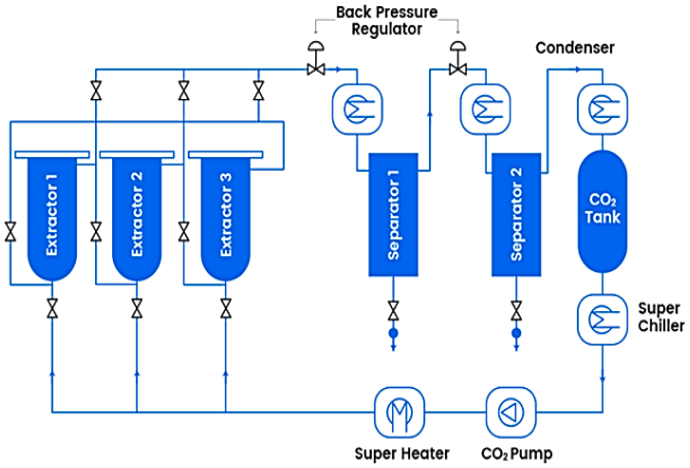


**Figure 1.** The Supercritical $CO_2$ extraction apparatus.

Depending on the elements present in the raw materials and the pressure-temperature conditions, the supercritical $CO_2$ removes the oils from the plant (*Capsicum annuum*) and passes through a series of pressure-regulating valves before entering the separators. The separator's two pressures are independently adjusted to separate the various extract components. The $CO_2$ is recycled by condensing and storing it as a liquid in the tank after the oil is removed and separated from the $CO_2$ that is released as gas. The oil is then collected in collection containers. The entire cycle is then repeated by utilizing this $CO_2$ throughout the batch. After obtaining the entire $CO_2$ extract, the extract is diluted with n-hexane in a ratio of 1:2 before being used for analysis.




**Figure 2.** The Supercritical $CO_2$ extraction apparatus while performing in the laboratory.

## RESULTS AND DISCUSSION

After $CO_2$ extraction, the main components were found using the GC-FID method performed by Essential's in-house laboratory. These data are shown in Table 2. The GC - FID method is faster, more sensitive, reproducible, and precise. (Branca M. Silva, 2019).

**Table 2**. GC- FID analysis of Capsicum annuum $CO_2$ Total Extract

| | Compound | % |
|---|---|---|
| *1* | heptadecane | 0.17 |
| *2* | octadecane | 0.12 |
| *3* | palmitic acid (hexadecanoic) | 0.22 |
| *4* | methyl 11-(3-pentyl-2-oxiranyl) undecanoate | 0.13 |
| *5* | 1-octadecanamine | 3.92 |
| *6* | 7,11-hexadecadienal | 0.2 |
| *7* | oxacyclohexadecan-2-one | 0.48 |
| *8* | 1-acetoxynonadecane | 0.88 |
| *9* | 9,12-octadecadien-1-ol | 0.2 |
| *10* | tetradecylcyclohexane | 0.32 |
| *11* | oleic acid | 0.1 |
| *12* | heneicosane | 0.7 |
| *13* | nonivamide | **7.98** |
| *14* | nordihydrocapsaicin | 0.65 |
| *15* | capsaicin | **36.82** |
| *16* | dihydrocapsaicin | **16.33** |
| *17* | N-vanillyldecanamide | 2.72 |
| *18* | homocapsaicin | 0.18 |
| *19* | docosane | 0.9 |
| *20* | homocapsaicin II | 1.12 |
| *21* | homodihydrocapsaicin | 3.49 |
| *22* | pentacosane | 1.4 |
| *23* | β-tocopherol | 0.1 |
| *24* | γ-tocopherol | 0.2 |
| *25* | hexatriacontane | 0.84 |
| *26* | vitamin E | 5.71 |
| *27* | ergost-5-en-3-ol | 0.39 |
| *28* | β-sitosterol | 0.6 |
| *29* | α-amyrin | 1.02 |
| *30* | β-amyrin | 1.5 |
| *31* | Lupeol | 0.12 |



**Figure 3.** Capsicum annum extract injection in GC – FID in the laboratory.

To employ the $CO_2$ extract for UV-VIS spectrophotometry analysis, the $CO_2$ extract must first be thoroughly extracted using $CO_2$ and then diluted with n-hexane in a ratio of 1: 2. The analyses were carried out using spectrophotometry by ISO-11212 Spectrophotometry.
The results regarding the presence of heavy metals are shown in the table below. These findings demonstrate that these metals' amounts for plants and vegetables are within WHO guidelines.

**Table 3.** The detection of heavy metals in Capsicum annuum with UV -VIS Spectrophotometer.

| Heavy Metals | Results | Specifications | Methods |
|---|---|---|---|
| Arsenic | 0.03 ppm | ≤1.5 ppm | **ISO-11212 Spectrophotometry** |
| Cadmium | 0.02 ppm | ≤1.0 ppm | |
| Mercury | < 0.05 ppm | ≤0.1 ppm | |
| Lead | 0.27 ppm | ≤5.0 ppm | |

The extract was obtained while it was still melting, applied without further processing, and then put in plates with the medium. The medium used for the identification of gram-positive bacteria was blood agar (Staphylococcus aureus), for gram-negative bacteria Endo Agar was used (i.e. Escherichia coli; Pseudomonas aeruginosa) and Sabouraud Dextrose Agar (SDA) for the identification of yeasts and molds. Since it contains waxes and esters, it is packaged at a temperature of 60 degrees Celsius so that the extract can be put into the container while it is still in a liquid state before freezing. The microbial analysis was performed according to the methods of ISO 21149, for the presence of Bacteria) and with ISO 16212 for Yeast and Mould.

0.1 gram of sample is mixed with 1 ml pure water, and centrifuged for 5 minutes / 3500 rpm at 20°C. Then 25 microliters are collected with a pipette and placed on the medium. These soils are then put in the thermostat for 48 hours at 36°C.

**Table 4.** The microbial analysis of Capsicum annuum.

| ***MICROBIAL ANALYSIS*** | **RESULTS** | **SPECIFICATIONS** | **METHOD** |
|---|---|---|---|
| ***Bacterial Count*** | | | |
| Staphylococcus aureus | CONFORMS | **< 1000 CFU/g** | **ISO 21149** |
| Escherichia coli | CONFORMS | **< 1000 CFU/g** | |
| Pseudomonas aeruginosa | CONFORMS | **< 1000 CFU/g** | |
| ***Yeast and Mould*** | | | |
| Candida Albicans | CONFORMS | **< 100 CFU/g** | **ISO 16212** |
| Aspergilius fumigatus | CONFORMS | **< 100 CFU/g** | |

## CONCLUSION

1. According to GC- FID analysis of Capsicum annuum $CO_2$ Total Extract, the main active ingredient of Capsicum annuum is capsaicin, which is present in 36.82% of the plant (together with its derivatives dihydrocapsaicin 16.33%) and nonivamide 7.98%. As a result of which chili pepper has a variety of beneficial effects.

2. By employing spectrophotometry, ISO-11212 Spectrophotometry was able to identify the presence of heavy metals. These results show that the levels of these metals in plants and vegetables are within WHO standards. Lead 0.27 ppm and mercury less than 0.05 ppm were the two most abundant heavy metals.

3. Microbial analyses are negative for the presence of various bacteria and yeast and molds. In some published regulations on standards and microbial analysis, the extracts obtained by extraction with carbon dioxide in the supercritical state are excluded from microbial analysis since the extract itself produced in this way does not allow microbial growth.

4. Supercritical $CO_2$ extraction has a variety of advantages, some of which we can list below:

    a) Compared to oil products made using the steam distillation process, CO2 extraction oil products are more pure and superior in quality.
    b) $CO_2$ extracts have an advantage over steam distillation in that heating does not destroy the elements in the material while the essential oil is being extracted. Supercritical extraction processes need temperatures between 88 and 100 degrees Fahrenheit to operate, whereas steam distillation processes need temperatures between 140 and 212 degrees Fahrenheit. Temperature manipulation during steam distillation can change the molecules of the plant material and essential oil. However, $CO_2$ extract is more chemically similar to the plant from which it is generated since it has a larger variety of the plant's elements.
    c) The $CO_2$ Extraction technique produces excellent lipids and aromatics (carrier oils). Many plant-based ingredients that are useful but difficult to extract using the cold pressing method are present in these products.
    d) The aroma of the actual herb, spice, or plant is present in $CO_2$ extracts. It is thicker than the same essential oils because of the variety of elements it includes. In products extracted by steam distillation, this amount is lower.

**REFERENCE**

Sun T, Xu Z, Wu CT, Janes M, Prinyawiwatkul W, No HK. Antioxidant activities of different colored sweet bell peppers (Capsicum annuum L.) J Food Sci. 2007;72: S98–102. [PubMed] [Google Scholar] [Ref list]

Gyula Mozsik, “Capsaicin and its human therapeutic development ISBN 978-1-78923-521-0, DOI 10.5772/INTECHOPEN.73124,2018.

Vanderslice JT, Higgs DJ, Hayes JM, Block G. Ascorbic acid and dehydroascorbic acid content of food-as-eaten. J Food Compos Anal. 1990; 3: 105–118. [Google Scholar] [Ref list]

Shengping Zhang MD, Dian Wang MD, Jingying Huang MD, Yueming Hu MD, Ph.D.‘ Application of capsaicin as a potential new therapeutic drug in human cancers’ 23 September 2019. https://doi.org/10.1111/jcpt.13039.

Kim C-S, Kawada T, Kim B-S, et al. Capsaicin exhibits anti-inflammatory properties by inhibiting IkB-a degradation in LPS- stimulated peritoneal macrophages. Cell Signal 2003; 15; 299 – 306.

T. A. Safapuri, F. A. Masoodi, S. A. Rather, S. M. Wani, and A. Gull, "Supercritical Fluid Extraction: A Review," J. Biol. Chem. Chron., vol. 5, no. 1, pp. 114-122, 2019.

Branca M Silva [1], Susana Casal, Paula B Andrade, Rosa M Seabra, M Beatriz Oliveira, Margarida A Ferreira ‘ Development and evaluation of a GC/FID method for the analysis of free amino acids in quince fruit and jam’ PMID: **14516081**, DOI: 10.2116/analsci.19.1285

## *ORAL PRESENTATION*

# The use of different analytical techniques for the identification of the presence of heavy metals in medicinal plants

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## Abstract

Sage is used for the treatment of gastrointestinal disorders, such as dyspepsia and ulcers, as well as heartburn, and upper respiratory complaints, including sore throat, and inflammations. Studies have shown the whole plant has neuro tonic properties and it is used against nervous disorders, depression, vertigo, and neuralgia and to improve memory (sharpness), in Alzheimer's patients. This work has as its main purpose to compare the two analytical techniques used ICP-PM and also UV -VIS Spectrophotometer, to identify the presence of heavy metals in the essential oils of Salvia Officinalis. We were able to investigate the presence of lead, arsenic, cadmium, and mercury in the essential oil of Salvia Officinalis obtained with the extraction using the Clevenger apparatus and then microwave disaggregation and the analysis performed with the ICP- MS. The second data were obtained using the supercritical $CO_2$ extraction. The results showed for the lead concentration present in the extracted oil of this plant, using two different techniques are different, lead is almost 0.22 ppm (mg/kg) compared to the 0.028 ppm result provided by ICP-MS. The concentration of the other metals found provided by the use of the SC- $CO_2$ with the ISO 11212 Spectrophotometer is almost 3 to 5 times higher compared to the results provided by the use of ICP-MS. Using supercritical $CO_2$ as a solvent produces exceptional quality in preserving sensitive components. These extracts are free of microorganisms, solvents, and preservatives.

**Keywords:** heavy metals, SC-$CO_2$, ICP-MS, UV -VIS Spectrophotometer,

## INTRODUCTION

The erosion of the bedrock, air pollution, and irrigation of the soil with contaminated water are among the so-called main factors that contribute to the contamination of groundwater, as Guerra F. also emphasizes in one of his scientific articles. As a result, the environment and the plants that will grow may both be contaminated with heavy metals (Guerra, F. et al. 2012). In this study, the four principal heavy metals—arsenic, cadmium, lead, and mercury—were identified in plants, specifically Salvia Officinalis. Usually, exposure to these metals occurs by ingestion (drinking or eating) or inhalation (breathing). Some effects of these metals on plant development and human health include the following: ***Mercury:*** $Hg^{+2}$ has recently been shown to accumulate abundantly in high and aquatic plants (Kamal M. et al. 2004; Israr M. et al. 2006). High levels of $Hg^{+2}$ are extremely phytotoxic to plant cells and can disrupt a plant's physiological processes (Zhou ZS. et al. 2007). **Lead** often interferes with essential enzymes, causing the plant to germinate and grow slowly (Mukherji S, and Maitra P. 1976). Lead is a heavy metal that also has negative effects on human health and should be avoided. Lead and ***cadmium*** both lower calcium levels, which causes osteomalacia and incorrect bone metabolism. One-third of the total amount of *Cd (II)* in the human body is stored in the liver and kidneys. Due to the hazards of cadmium, which can result in serious health issues even after brief contact, complete avoidance is suggested. The negative side effects of chronic exposure to cadmium ions include osteoporosis, kidney damage, bladder and prostate cancer, anemia, and insomnia. (Choong, G. et al, 2014;). ***Arsenic*** has a number of severe effects on plants, including decreased fruit yield, decreased leaf fresh weight, stunting, chlorosis, and wilting, as well as decreased seed germination, reduced seedling height, decreased leaf area, and decreased dry matter output (A. C. Barrachina], et al. 1995). In addition to lung and skin cancers, arsenic can also cause other types of cancer. The strongest associations between chronic arsenic exposure and cancer are

found in bladder, skin, and lung cancer. Malignancies of the kidney, liver (angiosarcoma), and other organs have only a weak association [IARC 2004; NRC 2000].
Linnaeus made the initial discovery of Salvia officinalis L. in 1753. In particular, Croatia, Bosnia and Herzegovina, Montenegro, and Albania are home to this plant, which is native to the Western Balkans. Studies have found that many problems with gastrointestinal disorders including dyspepsia and ulcers, as well as heartburn and upper respiratory problems such as sore throats and inflammation, can be alleviated by using sage (Sharifi-Rad, 2018). Which also has a large positive impact on age-related cognitive disorders (Adams, 2007). A study performed by Perry, E.K in 1999, found the importance of this plant for a very important and delicate group, Alzheimer's patients, the use of this tea-shaped plant helps the memories of the sufferers and promotes serenity. But not only in medicine, but even in the food sector, this plant for its gustatory, antioxidant, and antibacterial characteristics, specifically the leaves of the plants used in the food sector ( Eidi, 2005). In addition, the plant is used in the cosmetics and fragrance industry (Khedher, 2017). Essential oils and extracts contain hypotensive, depressive, and antispasmodic effects (Baricevic et al. 2001; Hohmann, 1999). In very small quantities, the plant's essential oil is used to cure rheumatism and clear mucus from the respiratory tract. It is also used to treat anxiety, depression, female sterility, issues during menopause, excessive lactation, nocturnal sweats, excessive salivation (as in Parkinson's disease), and excessive sweating (as in TB) (Plants for a Future 2012).

Thanks to its properties, Salvia Officinalis was gathered from two separate locations in Albania (the north and the center), exposed to various chemical analyses, and evaluated using various analytical techniques to determine the presence of heavy metals. Various values were found, highlighting the significance of each technique used. The main aim of this work is to analyze the concentrations of heavy metals, using two different extraction processes, traditional extraction using Clevenger, and then analyzing the extracted oil with ICP-MS. And in parallel use supercritical carbon dioxide extraction. Once the essential oil has been obtained, analyze it with UV-VIS spectrometry. After the use of the Clevenger apparatus extraction, we were able to determine if lead, arsenic, cadmium, and mercury were present in the essential oil of Salvia Officinalis, using ICP-MS analysis.

The second set of information was gathered using a supercritical $CO_2$ extraction. Supercritical carbon dioxide is reached at 1071 psi (72.87723 atm) and 31.1 °C. A molecule has both liquid and gas properties when it is in a supercritical state. This is important because $CO_2$ may behave as a liquid solvent and dissolve while still fitting into small areas like a gas. Supercritical $CO_2$ can attach to any molecule the producer selects since its physical qualities are flexible.

According to two independent methodologies, the lead concentration in the oil produced from this plant is about 0.22 ppm (mg/kg), and the concentration of the same metal in the Salvia Officinalif from the Dibras region is 0.028 ppm, value reported by ICP-MS.

The same comparison is made even for the other metals and the values obtained are very different for both procedures.

## MATERIALS AND METHODS

### 1. Oil extraction with Clevenger Apparatus

**Chemicals and apparatuses -** The reagents nitric acid, hydrogen peroxide, and hexane utilized for oil extraction were all produced by Merck, USA analytical balance, and ICP-MS (Agilent technologies model 7800). Clevenger apparatus (Isolab Labogerate GmbH).

**Preparation of the sample-** Salvia officinalis L., which was used in this experimental study, originated from native Albanian plants. The sample was chosen at random and was taken in the *Dibra region* of northern Albania. The aerial sections of the plant that was chosen for analysis (leaves).

**Extraction of oil. -** The oil from the leaves was extracted using the Clevenger apparatus. The Salvia officinalis L. plant's leaves were cleaned of numerous contaminants, including dirt, rocks, and other foreign plants, prior to extraction. 50 g of the plant sample that was removed for examination was weighed and dried at 400°C to a consistent weight. Before being exposed to steam, the plant underwent a grinding procedure after drying. Using a ratio of 10:1 (ml/g) water to dried herb, steam distillation extraction was performed in a Clevenger apparatus. To do this, 50g of sample was placed in a 500ml volumetric flask, and water was added until the sign appeared.

They were put in the round flask, which would be heated in a heating bowl, after constructing the Clevenger apparatus outfitted with a condenser. To ensure that the cooling process is completed as effectively as possible, the Clevenger system was connected to a constant water source. The heat is managed such that the excess water is returned to the flask and the oil with water vapor enters the graded distillation tube. After that, the water-plant mixture was exposed to distillation for the indicated optimal duration of three hours. About 1 mL of hexane was added to the condenser during the first 30 minutes after the oil started to gather in the Clevenger column in order to keep any polyphenols or other essential oil components from coming into touch with the water. After cooling, precisely weigh it, then make the following calculations:

$$\textbf{Oil \%} = \frac{\textbf{Weight of the oil x 100}}{\textbf{Weight of the material taken}} = \textbf{3.7 \%}$$

The generated essential oils were kept at 4 to 50 ºC for subsequent examination after being dried with anhydrous sodium sulfate.



**Figure 2:** Oil extraction with Clevenger equipment

## 2. Oil extraction with supercritical $CO_2$

Harvesters in **Gjinar Village,** which is a part of the Administrative Unit of Gjinari, Elbasan Municipality, collect the plant between July and September. Samples were analyzed at Esencial Laboratory, a laboratory located on Elbasan-Librazhd Road, Km. 2, Elbasan, Albania. Analysis of the essential oil was carried out in GC- FID test conducted by Esencial in-house laboratory, for the detection of the compound of the CO2 total extract of Salvia officinalis, instead for the identification of the heavy metals, ISO 11212 Spectrophotometry was used.

First, the process of choosing plants and milling is carried out in a controlled, dry, and ventilated area. Under a microscope and using a humidity gauge, the integrity and health of the plant are assessed. In relation to the production situation: According to SCFE (supercritical fluid extraction) technology specifications with a maximum humidity of 6%, the plant particle size employed is 0.3 mm.

On ground materials (or pellets, granulates), the extraction of solids is often done in batch mode using food carbon dioxide as a solvent. The apparatus comprises of two or more extractors that operate above the CO2's critical pressure and two separators where the $CO_2$ is separated from the extract before being recycled.

The initial step in the supercritical $CO_2$ extraction process is to raise the carbon dioxide gas's temperature and pressure until it reaches the supercritical state. At 1071 psi (72.87723 atm) pressure and 31.1°C temperature, carbon dioxide enters a supercritical state. A heater and a high-pressure pump are used to accomplish this. The extractors that the supercritical $CO_2$ goes through are loaded with organic raw material.

The supercritical $CO_2$ extracts the oils from the plant (Salvia Officinalis) and travels through a succession of pressure-regulating valves before entering the separators, depending on the elements present in the raw materials and the pressure-temperature parameters. To separate the various extract components, the separator's two pressures are individually controlled. Following the separation of the extracted oil from the $CO_2$ that leaves as gas, the oil is collected in collection containers, and the $CO_2$ is recycled by condensing and storing it as a

liquid in the tank. The entire cycle is then repeated for the duration of the batch using this $CO_2$. After the total extract of $CO_2$ was obtained, the extract is diluted with n-hexane in a ratio of 1:2 to proceed with the analysis.

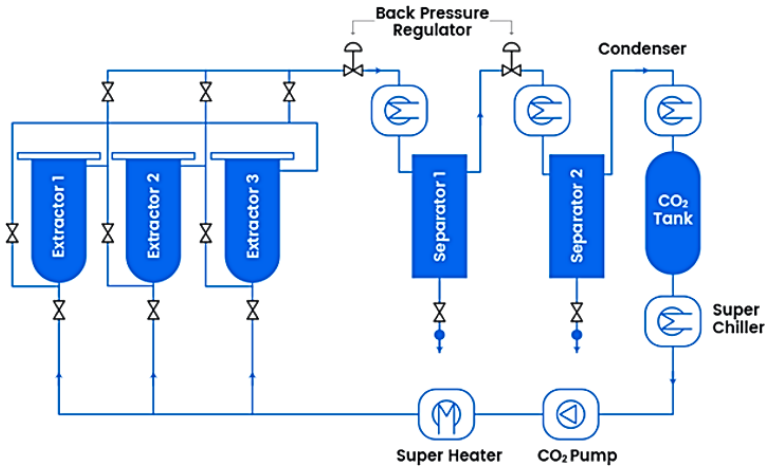


**Figure 3.** The Supercritical $CO_2$ extraction apparatus.



**Figure 4.** The Total extract of $CO_2$ was obtained.

### 3. Analysis with ICP-MS and UV-VIS Spectrophotometer.

Once the essential oils are obtained with 2 different extraction methods, we were able to analyze them using 2 different analytical techniques: ICP-MS and UV-VIS Spectrophotometer.

For the analysis, a conventional Agilent 7800 ICP-MS was employed. An Agilent SPS 4 autosampler was used for sampling. The typical sample introduction system was set up in the 7800 ICP-MS (Inductively coupled plasma mass spectrometry).
The optimization of the instrument is done every day according to the conditions recommended by the manufacturer.

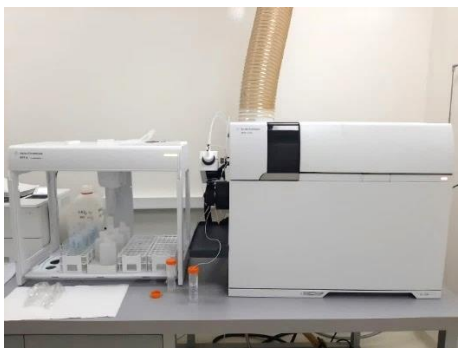

**Figure 4:** ICP-MS Agilent technologies model 7800

## RESULTS AND DISCUSSION

Validation tests for the created assay analytical method included those for specificity, linearity, the limit of detection and quantification, precision, and accuracy. ICP-MS was used to evaluate the heavy metal content of the herbal tea sample (Salvia officinalis L.), which was obtained from an herbalist in North Albania. After extraction, 3.7% of the oil remained.

**Table 2.** Detection of heavy metals in the extracted oil of *Salvia officinalis L.*

| Heavy Metals | Results | UdM | Methods |
|---|---|---|---|
| Arsenic | <0.001 | mg/kg | **Met. (151)** |
| Cadmium | 0.002 | mg/kg | |
| Mercury | 0.015 | mg/kg | |
| Lead | 0.028 | mg/kg | |

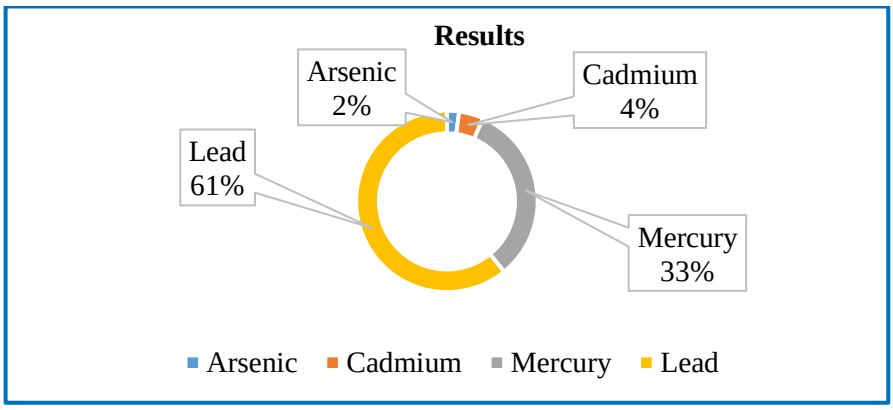


**Figure 5:** Percentage distribution of 4 heavy metals in Salvia officinalis L extracted oil.

The extract was diluted with n-hexane in a ratio of 1:2ml. The UV -VIS Spectrophotometer, was used to determine the presence of the heavy metals in the essential oil of Salvia Officinalis obtained with the SC-$CO_2$ extraction method. The percentage of the oil yield obtained is:

$$\textbf{Oil \%} = \frac{\textbf{Weight of the oil x 100}}{\textbf{Weight of the material taken}} = \textbf{3.5\%}$$

**Table 3.** The detection of heavy metals in Salvia Officinalis with UV -VIS Spectrophotometer.

| Heavy Metals | Results | Specifications | Methods |
|---|---|---|---|
| Arsenic | 0.03 ppm | ≤1.5 ppm | ISO 11212 Spectrofotometry |
| Cadmium | 0.12 ppm | ≤1.5 ppm | |
| Mercury | < 0.06 ppm | ≤0.1 ppm | |
| Lead | 0.22 ppm | ≤5.0 ppm | |

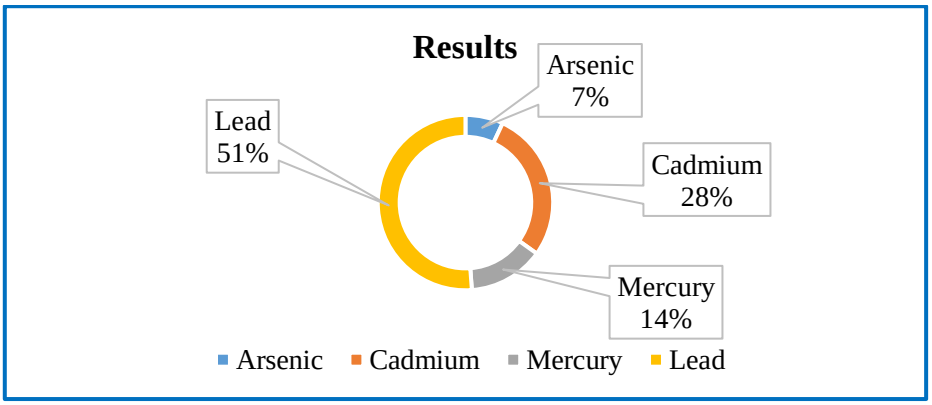


**Figure 6:** Percentage distribution of 4 heavy metals in Salvia officinalis L extracted oil.

## DISCUSSION

The following is the conclusion reached from the north of Albania utilizing the Clevenger apparatus and the ICP-MS analytical process: In 3.7% of the extracted essential oil, Lead was found in 0.028 ppm, followed by mercury at 0.015 ppm. The other two metals are arsenic and cadmium 0.001 ppm and 0.002 ppm respectively.

The essential oil of Gjinar Village, in the center of Albania, utilizing supercritical $CO_2$ extraction, the analysis for the determination of the heavy metal was performed with a UV-VIS spectrophotometer. : In 3.5 % extracted essential oil, lead was found at 0.22ppm, followed by cadmium at 0.12 ppm. The other two metals were found to be in a concentration less than 0.06 and arsenic 0.03 ppm.
What is important to underline is that: two different environments, the same plant, ( how do the environments influences the presence of the heavy metals? Two different extraction techniques were used, what is the importance of one and the other? And two different analytical techniques confirm the values obtained to confirm the regulations and limits provided by WHO.

For *Salvia Officinalis* of North Albania the two heavy metals found in abundance were lead and mercury, contrary to Salvia officinalis of Gjinar, where the two heavy metals in abundance were lead and cadmium. The Gjinar region maybe contains heavy metals in abundance even because that Elbasan is the Metallurgical Combine.

The primary source of air pollution, as well as surface pollution currently, is thought to be the metallurgical plant. However, additional research will be needed in the future to monitor the ecology and the levels of surface and subsurface waters and even other factors.

## CONCLUSION

For the purpose of analyzing trace metals, two different Salvia Officinalis, were cultivated in two different locations, north, and center of Albania.

- ***Salvia officinalis from Dibras Region:***

The *Clevenger apparatus* for the extraction of the essential oil and an *ICP-MS analytical* method is been used for the identification of heavy metals.
ICP-MS has several advantages, such as excellent detection limits (0.01 to 0.1 micrograms/L for several elements), easy specimen preparation, and the capacity to measure multiple elements at once.
In the extracted essential oil of Salvia Officinalis, and the analysis performed using ICP-MS, the two abundant metals present were lead 0.028 ppm followed by mercury 0.015 ppm. What is important are the concentrations of arsenic which are less than 0.001 ppm.

- ***Salvia officinalis from Gjinar***, after being obtained with the SC $CO_2$ extraction, were analyzed for the determination of the concentrations of heavy metals using the UV- VIS spectrophotometer.

The extract was then analyzed with the Spectrophotometer, and the values provided have shown that the two most abundant metals present are: lead and cadmium. Lead 0.22 ppm and cadmium 0.12 ppm.

The two analytical methods have shown that :

- ❑ The appearance of low heavy metal values is assumed to be largely influenced by the environment.

- Contrary to the plant that was planted in the Gjinar area, which was close to residential areas and increased the risk of contamination, in the Dibra's region, (second *Salvia Officinalis* analyzed) the environment where this plant was planted is quite clean because it is located far from housing.
- The SC - $CO_2$ extraction has proven to be a cleaning agent for the plant, though it should be noted that the concentrations of these metals are within the WHO's recommended limits.

**ACKNOWLEDGEMENTS**
Laboratory support acknowledgments are for the Essential® - Herbalist and Producer, Elbasan, Albania. (https://www.esencial.al/ ) which specialized in the production of 100% Natural Extracts from Medicinal Plants by applying Supercritical Fluid Technology (SCFE). Currently, Esencial® is the first and only company in Albania that applies SCFE Technology and produces 100% Natural $CO_2$ Total Extracts.

**REFERENCES**

Guerra, F.; Trevizam, A.R.; Muraoka, T.; Marcante, N.C.; Canniatti-Brazaca, S.G. (2012) Heavy metals in vegetables and potential risk for human health. Sci. Agric. 69, 54–60.

Kamal M, Ghalya AE, Mahmouda N, Cote R (2004) Phytoaccumu-lation of heavy metals by aquatic plants. Environ Intern 29:1029–1039.

Israr M, Sahi S, Datta R, Sarkar D (2006) Bioaccumulation and physiological effects of mercury in Sesbania Drummond. Chemosphere 65:591–598.

Zhou ZS, Huang SQ, Guo K, Mehta SK, Zhang PC, Yang ZM (2007) Metabolic adaptations to mercury-induced oxidative stress in roots of Medicago sativa L. J Inorg Biochem 101:1–9.

Mukherji S, Maitra P (1976) Toxic effects of lead growth and metabolism of germinating rice (Oryza sativa L.) seeds mitosis of onion (Allium cepa) root tip cells. Ind J Exp Biol 14:519–521.

Choong, G.; Liu, Y. (2014) Templeton, D.M. Interplay of calcium and cadmium in mediating cadmium toxicity. Chem. Biol. Interact. 211, 54–65.

Barrachina A. C, Carbonell F. B., and Beneyto J. M. (1995) "Arsenic uptake, distribution, and accumulation in tomato plants: effect of arsenite on plant growth and yield," Journal of Plant Nutrition, vol. 18, no. 6, pp. 1237– 1250.

Sharifi-Rad, M.; Ozcelik, B.; Altın, G.; Da¸skaya-Dikmen, C.; Martorell, M.; Ramírez-Alarcón, K.; Alarcón-Zapata, P.; Morais-Braga, M.F.B.; Carneiro, J.N.P.; Leal, A.L.A.B. Salvia spp. plants-from farm to food applications and phytopharmacotherapy. Trends Food Sci. Technol. 2018, 80, 242–263.

Adams, M.; Gmünder, F.; Hamburger, M. Plants traditionally used in age-related brain disorders—A survey of ethnobotanical literature. J. Ethnopharmacol. 2007, 113, 363–381.

Perry, E.K.; Pickering, A.T.; Wang, W.W.; Houghton, P.J.; Perry, N.S.L. Medicinal plants and Alzheimer's disease: From ethnobotany to phytotherapy. J. Pharm. Pharmacol. 1999, 51, 527–534.

Eidi, M.; Eidi, A.; Zamanizadeh, H. Effect of Salvia officinalis L. leaves on serum glucose and insulin in healthy and streptozotocininduced diabetic rats. J. Ethnopharmacol. 2005, 100, 310–313.

Khedher, M.R.B.; Khedher, S.B.; Chaieb, I.; Tounsi, S.; Hammami, M. Chemical composition and biological activities of Salvia officinalis essential oil from Tunisia. EXCLI J. 2017, 16, 160.

Baricevic D., Bartol T. & Kintzios S.E. (2000). (Ed.), Sagethe genus salvia, Harwood Academic Publishers, Amsterdam; 143–184.)

Hohmann J., Zupko I. & Redei D. (1999). Planta Med.; 65, 576–578.

## *ORAL PRESENTATION*

# Using surface-pnited carbon electrodes in ketoprofen separation by modifying

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## Abstract

The distinction of chiral compounds, which is one of the most popular topics of recent times, is actually of vital importance. This importance has caused the subject to be handled by many disciplines. Separation of enantiomers of chiral compounds was carried out using electrochemical methods within the scope of this study. Electrochemical sensor studies were carried out using carbon-based electrodes. As a result of the recent literature review, it has been determined that carbon material is frequently used in sensor studies. In this study, carbon material was synthesized as GO/β-CD derivative using chemical reduction method. SEM and XRD methods were used for the characterization of the synthesized catalyst. GO/SPCE and GO/β-CD/SPCE were produced by modifying the obtained carbon-based materials on the surface of surface-printed carbon electrodes. The activities of the electrochemical catalysts were characterized using the cyclic voltammetry (CV) method. Within the scope of this study, the enantioselectivity of ketoprofen, which is a chiral selective component, pain reliever and antipyretic drug substance, was determined by electrochemical methods.

**Keywords:** Carbon, Chirality, Drug, Racemic, Voltammetry

This work was financially supported by Van Yüzüncü Yıl University scientific research projects coordination unit of Turkey (BAP) project (project no: fba-2018-6596).

## INTRODUCTION

Recently, the use of pure drugs and nutritional additives used in agriculture has increased. The basis of these drugs and active substances is based on enantiomers. When the enantiomers are separated one by one, the results obtained from the use of the drugs also change. There are two different enantiomers in the structure of each drug. One of these enantiomers has a positive effect, while the other has a negative effect. Due to this difference in effect, it is important to make an enantiomeric distinction of drugs. Enantiomers can show many different effects in racemic drug mixtures. The use of two anantiomers together may cause different effects independently of each other. These different effects can create positive effects in the treatment phase [1].

The pharmaceutical industry is very interested in the production and sale of segregated chiral drugs. The various biological activities of enantiomers have increased the interest in optically active products. An optically active molecule has a symmetrical structure. Since optically active products are pure enantiomers, they are target specific and less harmful [2,3].

In other words, four different atoms or groups must be attached to a carbon atom in each molecule. For this purpose, investigation of the redox behavior of compounds by electrochemical techniques has the potential to provide valuable information about the biological redox reactions of these molecules. In racemic mixtures, enantiomers can act independently of each other. The combination of both enantiomers may be advantageous for therapy to achieve the desired activity, or the passive enantiomer may cause serious detrimental effects, while the two enantiomers together may have different therapeutic effects [4].

In this study, graphene oxide (GO/ β-CD supported catalysts were synthesized and enetiomeric separation of surface-printed carbon electrodes and ketophropene was made.

Electrochemical characterizations in the study were performed with cyclic voltammetry (CV). In addition, surface characterizations were also made. For these surface characterization processes, XRD and SEM

analyzes were carried out. With the electrochemical characterization, the chiral separation of the drugs on the market has been performed cheaper, more reliable and faster.

Within the scope of this thesis study, the chiral separation of our active ingredient, keteprofen, into the racemic form was made by electrochemical methods, and a contribution was made to the literature in this field.

## MATERIALS AND METHODS

In the experimental stage, firstly, carbon-derived electrocatalysts were prepared. Later, CD additions were made to these catalysts. Catalysts were prepared in line with the additions made at the determined rate. Conditioning of the SPC electrodes was carried out to perform the electrochemical characterization. Thus, the chiral separation was determined by the electrochemical method.

## RESULTS and DISCUSSION

For the surface printed electrode to be used in the study, conditioning was performed and activation was ensured. With this process, many beneficial results have been obtained by cleaning and activating the electrode surfaces. These results are increase in current, decrease in signal/noise ratio and decrease in irreversible reactions in electrochemical measurements.

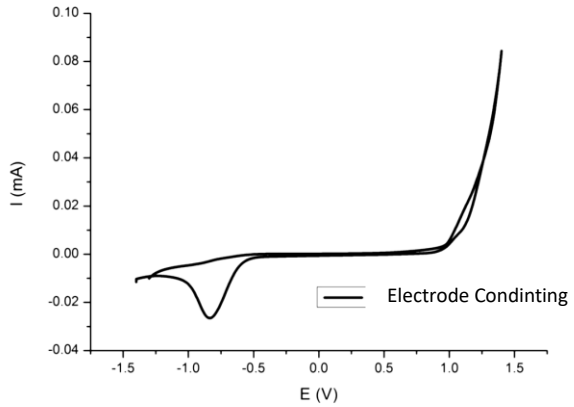


Figure 1. Electrode conditioning (measurement results in the range of -1.4 +1.4 V at 10 mV/s-1 in 0.1 M pH 8 $PO_4$ buffer).

After the conditioning process, the most suitable method for electrode activation was selected. Catalyst experiments were carried out using this method.

For the experimental procedure prepared within the scope of ensuring the enantiomeric separation of racemic drugs, the second selected model drug 0.1 mM (R, S) Ketoprofen was rotated in 0.1 M phosphate buffer solution (PBS) without a mediator, without a chiral selective group, with a GO/β-CD/NGCE electrode. voltammogram (CV) was obtained by the technique (Figure 2).

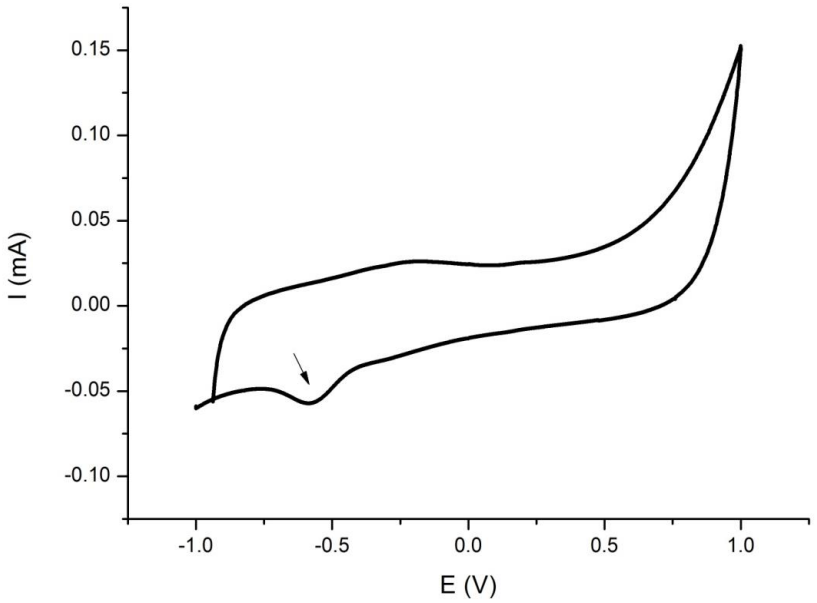


Figure 2. Cylic voltamogams of GO/NGCE in solution containing 0.1 mM (R, S) ketoprofen 0.1 M phosphate buffer solution (pH7)

As can be seen from the alternating voltammogram of the solution containing 0.1 mM (R, S) ketoprofen 0.1 M phosphate buffer solution (pH 7) with the GO/NGCE electrode, a significant reduction peak was observed around -0.5V with a current of about 70 µA. In the second stage, a series of experiments were carried out by attaching the chiral selective (α, β, γ –CD) group to the electrode.

The electrochemical characterization studies of the modified electrodes prepared within the scope of the thesis study, the 400 mg chiral selective (α, β, γ –CD) group used and the 0.1 mM (R, S) Ketoprofen model drug alternated in a mediator-free medium and in 0.1 M phosphate buffer solution (PBS). obtained by voltammetry (CV) technique (Figure 3).

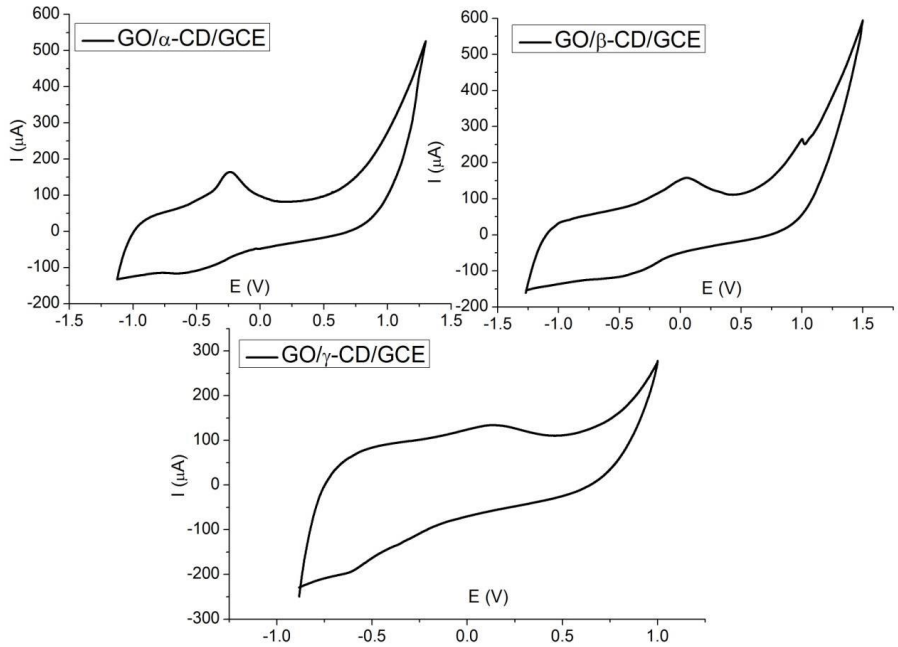


Figure 3. 0.1 mM (R, S) Ketoprofen 0.1 M phosphate buffer solution of GO/α-CD(400mg)/NGCE, GO/β-CD(400mg)//NGCE and GO/γ-CD(400mg)//NGCE ( Cylic voltamogams in solution containing pH 7.

As can be seen in Figure 3, two oxidation peaks around -0.1V (150 µA) and 1.1V (250 µA) were observed in the electrode modified with the chiral selective group (400mg)/β form. After the chiral selective effect was achieved with the GO/β-CD(400mg)/NGCE electrode, subsequent studies were continued with the (400mg)/β-CD chiral selective group.

How different ketoprofen concentrations affect chiral recognition was investigated using GO/ (400mg)β-CD/NGCE electrode with increasing drug concentrations added to the solution medium.

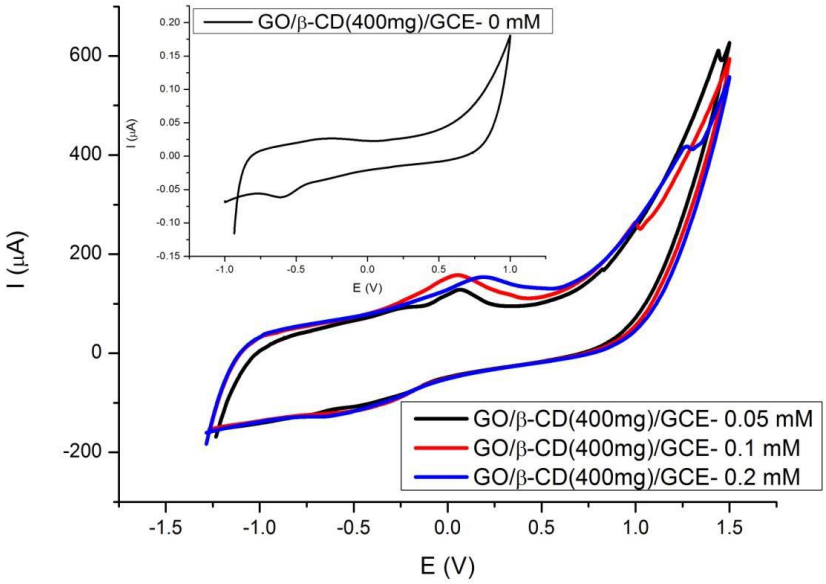


Figure 4. Cyclic voltamogams of ketoprofen (0-0.2 mM) of different concentrations (R, S) in solution containing 0.1 M phosphate buffer solution (pH 7) with a GO/ (400)β CD/NGCE electrode.

It has been studied to obtain more specific enantiomers by adding (R, S) Ketoprofen to the solution medium up to 0.2 mM for chiral recognition. As can be seen from Figure 4, with the addition of 0.05 mM (R, S) Ketoprofen, the GO/(400)β-CD/NGCE electrode provided chiral recognition and continued up to 0.2 mM.

(R, S) The effect of solution pH on the chiral selectivity of the Ketoprofen model drug was investigated. For this purpose, 0.1 M phosphate buffer solution was investigated at pH concentrations varying between 5-10 (Figure 5).

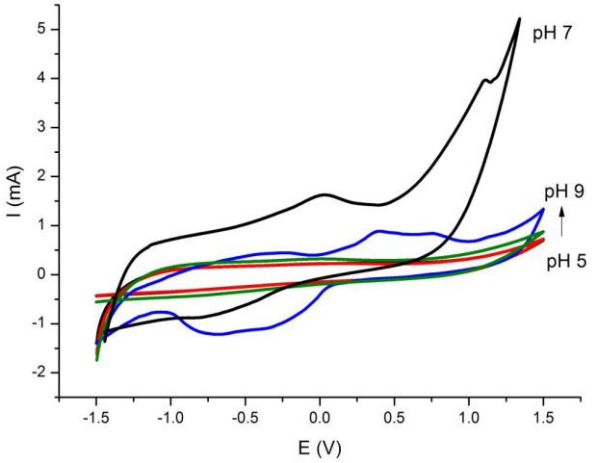


Figure 5. Cyclic voltamogams of ketoprofen (0.1 mM) at different pH concentrations (R, S) with GO/ (400)β-CD/NGCE electrode in solution containing 0.1 M phosphate buffer solution.

When the peaks obtained by adding 0.1 mM of (R, S) Ketoprofen to the solution medium were examined, it was determined that chiral recognition was achieved at pH 7. The pKa value of ketoprofen has been experimentally reported in the literature to be 4.39, 4.45 and 4.76 at 25◦C. In the same literature, it has been reported that the solubility of ketoprofen, which is a weak acid, increases with pH (Igor et al., 2012). Chiral recognition was achieved at a pH (7) greater than that of ketoprofen, with an average pKa of 4.7. This situation can be attributed to the effective solubility of ketoprofen in neutral medium (Pratap et al., 2016).

As a result of the electrochemical characterizations performed for the purpose of the study, it was observed that the chiral selectivity of Ketoprofen (0.1 mM) was achieved with the GO/β-CD/NGCE electrode (R, S). In the last stage of the study, voltammograms of (0.1 mM) S-Ketoprofen and (R, S) Ketoprofen were taken and interpreted in order to identify the enantiomers of R-Ketoprofen and S-Ketoprofen.

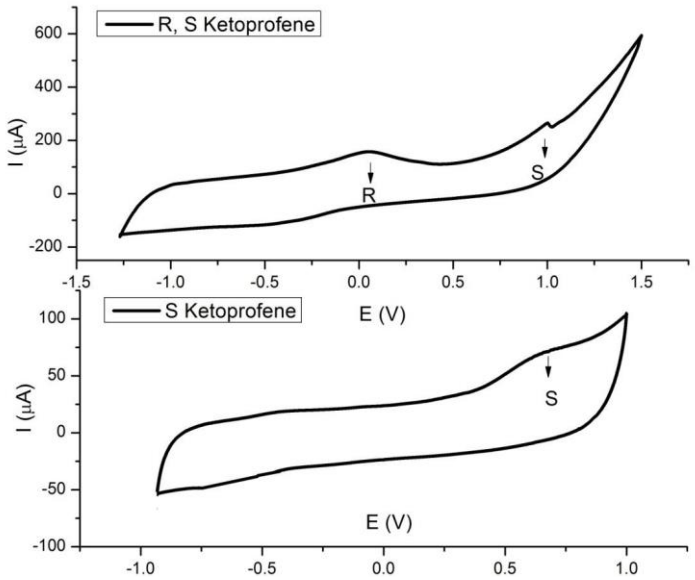


Figure 6. Cyclic voltamogams of (R, S) Ketoprofen (0.05 mM) and (S) Ketoprofen (0.1 mM) in solution with GO/ (400)β-CD/NGCE electrode and 0.1 M phosphate buffer solution.

When Figure 6 is examined, it was determined that Ketoprofen (0.05 mM) showing two oxidation peaks around 0.1 V and 1 V (R, S) and the oxidation peak (S) appearing around 1 V belonged to Ketoprofen, and chiral selectivity was successfully achieved.

## CONCLUSION

In this study, graphene oxide / β-cyclodextrin (GO/β-CD) supported catalysts were synthesized and characterized by the impregnation method, and electrochemical chiral separation of ketoprofen racemic drug was achieved with surface-modified surface-printed carbon electrodes.

Surface characterization of graphene oxide / β-cyclodextrin (GO/β-CD) supported catalyst was performed with XRD and SEM, and electrochemical characterization with CV methods.

First of all, GO catalysts with different β-CD concentrations (100, 200, 300, 400, 500 and 600 mg) were synthesized. These different concentrations were examined and the best amount was determined. After determining the best concentration of 400 mg for GO/β-CD, pH study was carried out.

Racemic separation of β-CD-supported synthesized carbon derivatives and ketoprofen drug was performed in different pH ranges (pH 5, 6, 7, 8, 9 and 10), and the most suitable range was 10 mV/s-1 in 0.1 M pH 7 phosphate buffer. It was determined to be in the range of – 1.5 V + 1.5 V. This showed that in a racemic drug, two different enantiomer structures differ according to their pH levels.

Activation experiments were carried out for the surface imprinted carbon electrode (SPCE) used in experimental studies and the most suitable method was selected for electrode activation. Activation studies were performed with a carbon supported (GO) catalyst and the appropriate activation method was carried out by applying potential for 300 s at +1.4V in 0.1 M pH 8 phosphate buffer. Literature studies on SPCE electrode have helped us to obtain important results such as cleaning and activation of the surfaces of the electrodes, increase in current in electrochemical measurements, decrease in signal/noise ratio and decrease in reversible reactions.

Support electrolyte study was carried out for the catalyst. Phosphate buffer and $H_2SO_4$ buffers were studied. It was concluded that phosphate buffer was the buffer medium with the best efficiency for 0.1 M and concentration studies were started using this buffer medium.

During the concentration study, the racemic concentration of the drug containing ketoprofen was studied between 0 – 0.3 mM. The best concentration ratio was determined for the active substance. For ketoprofen it is 0.2 mM. This showed that there are two different enantiomer structures in a racemic drug in our study. Then, the chiral separation processes of the drugs were studied with the same method.

The physical characterization of the carbon supported catalysts in which chiral separation was achieved in the experiments was carried out by XRD and SEM-EDX techniques. Since the GO/β-CD catalyst is the catalyst in which the chiral separation is realized experimentally, X-ray diffraction analyzes were carried out to determine the surface phases and crystal lattice structure. It has been observed that it is in the C (0 0 2) plane and has a value of $2\theta = 12.6°$. SEM (scanning electron microscope) images were taken to visually examine the surface morphology of the catalysts. For the GO/β-CD catalyst, it was observed that the SEM image of 400 mg β-CD chiral selective group had a high homogeneity and sheet-like structure.

With chiral and enantiomeric separation, the electrochemical behavior of some specific racemic drugs currently on the market was investigated quickly and reliably with a new electrode system. Our Ketoprofen drug active substance is an electroactive substance and it has not been distinguished as enantioselective before. In this thesis study, the electrochemical chiral separation of our active substance in racemic form has been realized and a contribution has been made to the literature.

## ACKNOWLEDGEMENTS

This work was financially supported by Van Yüzüncü Yıl University scientific research projects coordination unit of Turkey (BAP) project (project no: fba-2018-6596).

## REFERENCES

Minguillon C., 2012. Countercurrent Chromatography, Scope and Perspectives: Application to Chirotechnology,Chemical Engineering & Technology, 35.

Mustaranta, A., 1992. Use of lipases in the resolution of racemic ibuprofen, Appl. Microbiol Biotechnol, 38: 61-66.

Sheldon R. A., 1993. Chirotechnology. Marcel Dekker, Inc, 47-83, New.

Tüzün, C. 1996. Organik Kimya, 7. Baskı, Palme Yayın Dağıtım, Ankara.

## *ORAL PRESENTATION*

# Treatment of dye production wastewater by coagulation and flocculation processes

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**Abstract**

In this study, treatment studies were carried out using different coagulants and flocculants for the wastewater of the dye production industry which has very high COD (42301 mg/L), color, and TOC (16310 mg/L) values. The effects of pH and concentration of coagulants and flocculants used in the treatment of this wastewater were investigated. The wastewater was not filtered at the end of the treatment and the samples taken from the supernatants were analyzed.

The first coagulant used was commercial. This commercial coagulant effectiveness at 166-666 µL/L concentration was investigated in the presence of a commercial flocculant (0-500 µL/L) between pH 3.0 and 9.0. The results showed that 500 µL/L concentration of the coagulants at pH 5.0 provide optimum treatment efficiency in the presence of the 83.3 µL/L of flocculant.

The second coagulant chosen is $Fe_2(SO_4).18H_2O$. The efficacy of this coagulant (20-1000 mg/L) between pH 3.0 and 8.0 was tested in the presence of 0-333.3 µL/L flocculant. The optimum results were obtained at pH 3 with 667 mg/L of $Fe_2(SO_4).18H_2O$ and 333.3 µL/L of the flocculant. At these optimum conditions, a TOC and COD removal of more than 92% was achieved with both coagulants. However, the most striking result is that by only adjusting the pH to 3.0 (without adding coagulant and flocculant) a treatment efficiency of more than 69% in COD and TOC can be achieved.

**Keywords:** Dye production wastewater, Treatment, Coagulation, Flocculation.

## INTRODUCTION

Water pollution is one of the main causes of public health deterioration. A proper strategy has to be applied to diminish water pollution and optimize the use of recycled water. The dye production industry is one of the greatest substantial pollution sources. Moreover, it is very voracious to consume fresh water during its production processes[1]. Large amounts of dyes are annually produced and applied in many different industries, including the textile, cosmetic, paper, leather, pharmaceutical, and food industries[2]. The presence of even a trace concentration of dyes in the effluent is highly visible and undesirable[3]. The chemical reagents used are very diverse in chemical composition, ranging from inorganic and low molecular weight organic compounds to polymers. The dye molecules can be strongly fixed by forming covalent bonds that are caused by chemical reactions between substitutes on the dye molecules and the fibres[4]. Dyes in water are frequently toxic and have a significant influence on causing carcinogenicity to marine organisms and humans[5]. On the other side, marine life is significantly affected by the increase of organic and inorganic substances as well as suspended solids accompanying the discharged water. They can prevent natural light from penetrating the water which catalyzes the occurrence of the eutrophication process[5]. Dye effluent usually contains chemicals, including dye itself, which are toxic, carcinogenic, mutagenic, or teratogenic to various microbiological and fish species[6]. The concern arises, as many dyes are made from known carcinogens such as benzidine and other aromatic compounds[7]. The presence of dyes or their degradation products in water can also cause human health disorders such as nausea, hemorrhage, and ulceration of the skin and mucous membranes, and can cause severe damage to the kidney, reproductive system, liver, brain, and central nervous system[8]. Along with the recalcitrant nature

of dye wastewater, the frequent daily variability of characteristics of such wastewater adds to the difficulty of treatment[9].

The coagulation/flocculation process is a frequently used technique for numerous years as a main treatment or pretreatment stage in textile wastewater treatment plants. It has an excellent ability to discolor textile effluents and diminish a total load of suspended solids. The efficiency of this method depends on the characteristics of raw wastewater, especially pH, temperature, the type and dosage of the used coagulant, as well as the intensity and duration of mixing[10]. The addition of the flocculation process is highly recommended to improve coagulation process effluents by enlarging the size of flocs and, consequently causing rapid settling[11]. In general, aluminum salts and ferric ions are the principally used coagulants for wastewater treatment owing to their massive ability to attain particulate destabilization via charge neutralization for the negatively charged particles[12]. Several coagulants, such as $Fe_2(SO_4).xH_2O$, $AlCl_3$, $Al_2(SO_4)_3.18H_2O$ and $FeCl_3$ have been evaluated for wastewater treatment[10,11].However, there are many coagulants on the market, the content of which is not shared by the seller, especially in Turkey. This situation brings to mind the question of whether coagulants sold on the market and whose content is concealed and conventional coagulants cause different removal efficiencies.

This study aims (i) to determine the performance of commercial and conventional coagulants for dye production wastewater treatment plants, (ii) to optimize pH and coagulant dosage for each coagulant based on TOC removal efficiencies and reduce these pollutions load for the pre-treatment (before electrooxidation treatment process) of dye production wastewater (iii) to determine optimum flocculant concentration.

## 1. MATERIALS AND METHODS

*The coagulation-flocculation process*

Experiments were carried out using a jar test with a capacity of 300 mL. The effects of the coagulant dosage and initial pH were investigated on the residual concentrations of TOC. The initial pH of the samples was adjusted by adding 1 M $H_2SO_4$ or 1 M NaOH solutions. The coagulants used in experiments are commercial coagulant and ferric sulfate ($Fe_2(SO_4)_3.xH_2O$, Baker, > 99%). The sample pH was adjusted to the desired level, commercial coagulant was poured into the wastewater in liquid form and stirred at 150 rpm for 5 min. Ferric sulfate experiments were added in solid form. Slow mixing was conducted at 40 rpm for 10 min after the slow mixing process. Finally, mixing is terminated and precipitation is performed for 1 h. The samples were analyzed for the removal extent of TOC and COD.

*Analytical methods*

The concentration of total organic carbon (TOC) in water samples was measured using a TOC analyzer (Shimadzu, TOC-L model, Japan). COD was titrated with the appropriate Ferric Ammonium Sulphate Solution (FAS) until its color changed sharply from greenish blue to orange-brown after being heated at 150℃ for 2 h using a COD digester [13]. pH was measured using a pH meter (Mettler Toledo Seven Go). All the used chemicals were of analytical grade.

## 2. RESULTS and DISCUSSION

*Effect of pH on coagulation*

The pH of the solution in the coagulation process is the most crucial parameter. In the experiments, the pH varied from 3.0-9.0 at a constant dose for commercial coagulants, and pH 3.0-8.0 at a constant dose for Fe-based coagulants were added to the samples. **Fig. 1** presents the effect of pH values on the removal of TOC for dye production wastewater of the coagulation-flocculation process. As observed in these figures, low pH plays a significant role in the removal of TOC for commercial and Fe-based coagulants. The result of our experiments indicates that the optimum precipitation pH values are 5.0 and 3.0 for commercial and Fe-based coagulants, respectively. The efficiency was obtained at a pH of 5.0 as the maximum 86.6% for commercial coagulant. For Fe-based coagulant, organic matter was removed at pH 3, and TOC removal efficiency was 82.9%.

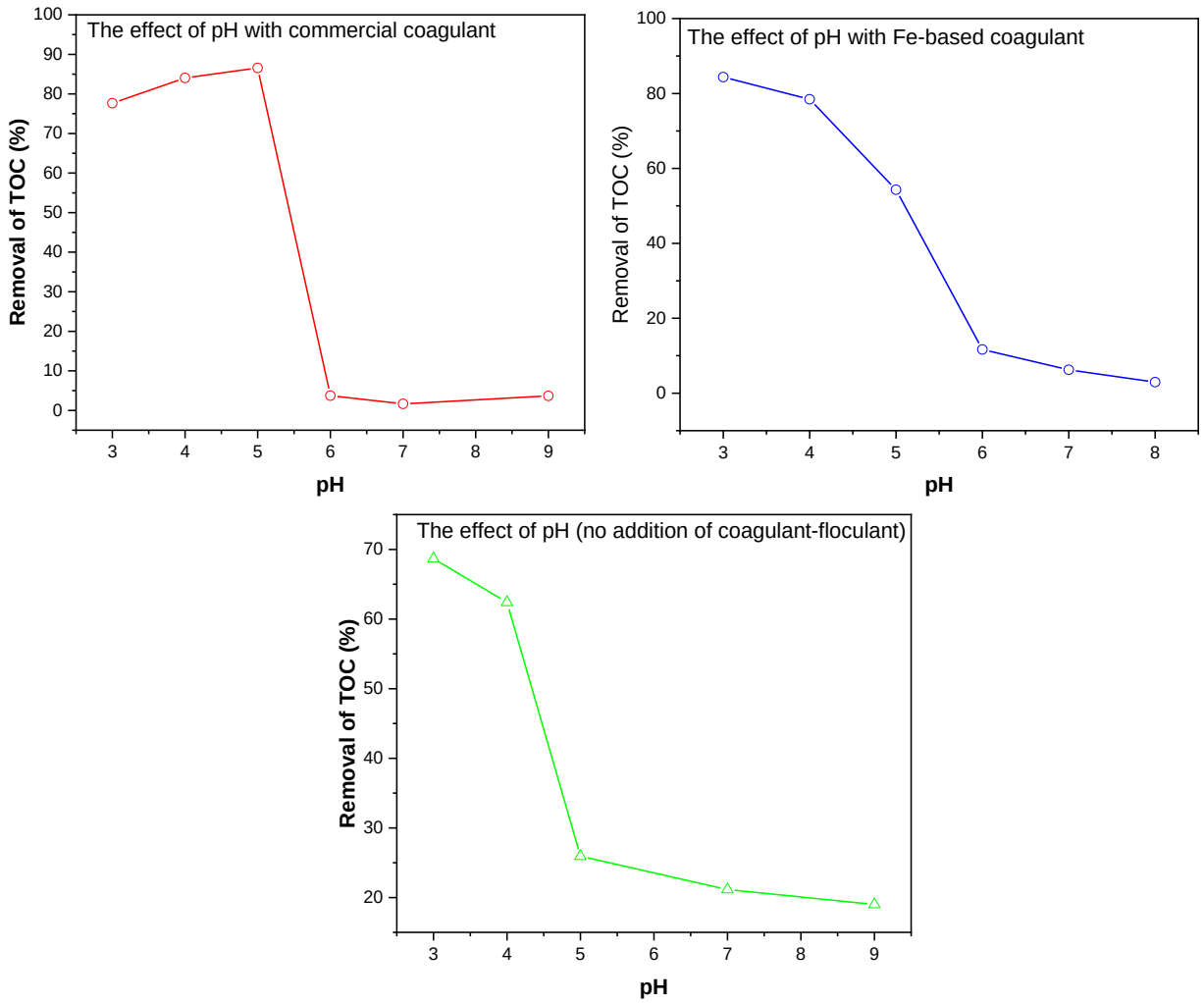


**Fig. 1.** Effect of pH for dye wastewater: TOC removals (a) 333 µL/L commercial coagulant + 166 µL/L flocculant and (b) 170 mg/L Fe-based coagulant + 166 µL/L flocculant (c) No addition of coagulant or flocculant.

In this study, in order to investigate the effectiveness of pH, the removal efficiency was investigated by changing the pH without adding any coagulant or flocculant. As can be seen in Figure 1.c, there is a serious removal in wastewater even when only the pH is changed. Serious precipitation was observed in the wastewater whose pH was adjusted to 3. As it is known, the net charge of wastewater is very important in the coagulation process. In this study, it is estimated that the isoelectric point of wastewater approaches zero at pH 3.0 level which causes high removal efficiencies.

*Effect of coagulant dosage*

Colloidal particles in water or wastewater are destabilized and easily settled by the coagulation-flocculation process[11]. Parameters such as coagulant type, concentration, type of pollutant, pH of the solution, and the concentration of used coagulant play an important role in this process.[14]

In our experiments, the effect of commercial and Fe-based coagulants were investigated on the removal efficiencies of organics from dye production wastewater. The dose of commercial coagulant varied between 166 and 666 µL/L. The dose of Fe-based coagulant varied between 20 and 1000 mg/L. The pH values were adjusted to 3.0 and 5.0 for commercial and iron-based coagulants, respectively.

**Fig. 2** shows the obtained removal efficiencies of organics from the experiments using the coagulants for dye production wastewater. According to the results given above, it is necessary to determine the optimum coagulant dosage and type, considering the treatment cost. High coagulant dosage means high cost and waste sludge. In experiments performed between 166 and 666 µL/L commercial coagulant doses, it was observed that the optimum removal efficiency was 500 µL/L. In iron-based coagulant experiments, the optimum removal efficiency was found to be 667 mg/L.

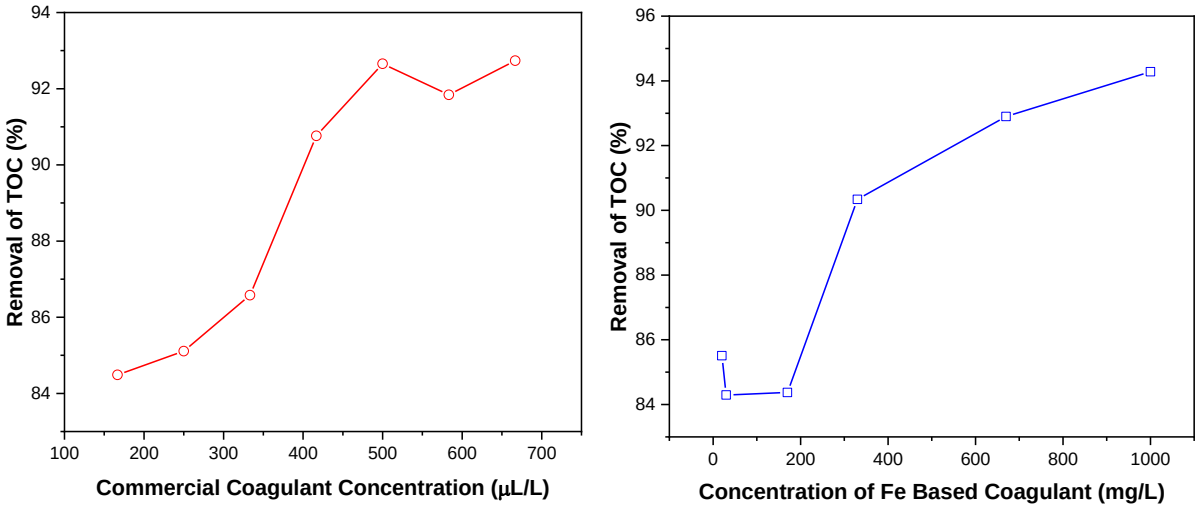


**Fig. 2.** Effect of coagulation dosage for dye production wastewater: TOC removals (pH 3.0 for Fe-base coagulants and pH 5 for commercial).

These results represent that the removal efficiency for dye production wastewater increase as the coagulant dosage increases. It is also observed that Fe-base coagulants are more effective in organics removal than commercial ones. The removal efficiencies of organics of the coagulants used are close to each other.

*Effect of flocculant on removal efficiencies*

The effect of flocculant concentration was observed in the optimum pH and coagulant dosages were determined in the last stage of this study. The results showed that less flocculant is needed when using commercial coagulant. In addition, it was observed that the efficiency decreased when a high flocculant concentration was used with commercial coagulant. The maximum removal efficiencies (> 94%) were obtained at 83.3 µL/L and 333.3 µL/L with commercial and Fe-based coagulants, respectively,

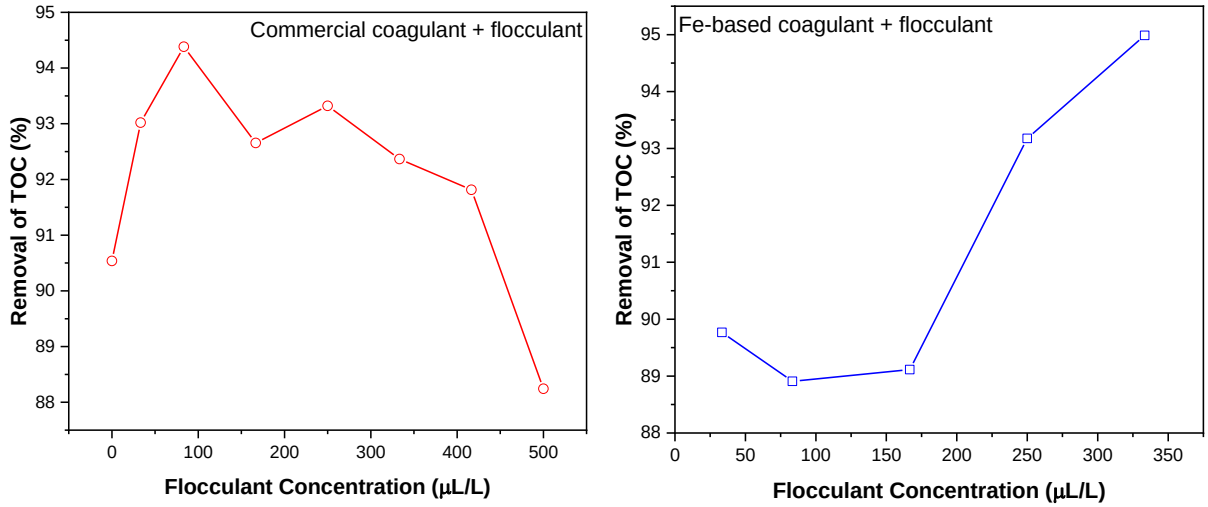


**Fig. 3.** Effect of flocculant dosage for dye production wastewater: TOC removals (pH 3.0 for Fe-base coagulants and pH 5 for commercial).

## CONCLUSION

In this study, removal efficiencies were measured with samples taken from the upper part of the wastewater without any filtering after treatment. Therefore, small fluctuations were observed in the study results. However, this situation presented important data in terms of predicting the results to be obtained in real practice.

The optimum coagulant dose and settling pH value for Al- and Fe-based coagulants in real dye production wastewater were investigated in this study. The following findings were obtained for optimum conditions.

- In dye production wastewater, the best precipitation results were obtained at pHs 5.0 and 3.0 for Al-based and Fe-based coagulants, respectively.
- Fe-based coagulants are more effective in TOC removal than Al-based coagulants.

Based on the findings, the coagulation process as a pretreatment can be more cost-effective for wastewater with a high pollutant concentration than electrochemical processes with very high electricity costs in the latter. Depending on the type of coagulant used, ions such as $SO_4^{2-}$ and $Cl^-$, which remain at high concentrations in the wastewater, are thought to probably help the electrochemical oxidation process by i) reducing energy consumption and ii) helping remove pollutants in terms of increasing the concentrations of $SO_4^{2-}$ and $Cl^-$ radicals.

## REFERENCES

1. Ghaly, A. E., R. Ananthashankar, M. Alhattab, and V. V. Ramakrishnan. 2014. “Production, Characterization and Treatment of Textile Effluents: A Critical Review.” J Chem Eng Process Technol 5 (1): 1–19.
2. Lourenco, N., Novais, J., biotechnology, H. P.-J. of & 2001, undefined. Effect of some operational parameters on textile dye biodegradation in a sequential batch reactor. *Elsevier*.
3. Hao, O. J., Kim, H. & Chiang, P. C. Decolorization of wastewater. *Crit Rev Environ Sci Technol* **30**, 449–505 (2000).
4. Carmen, Z. & Daniela, S. Textile organic dyes-characteristics, polluting effects and separation/elimination procedures from industrial effluents-a critical overview. (2012).
5. Lellis, B., Fávaro-Polonio, C., Innovation, J. P.-… R. and & 2019, undefined. Effects of textile dyes on health and the environment and bioremediation potential of living organisms. *Elsevier*.
6. Daneshvar, N., Ashassi-Sorkhabi, H., purification, A. T.-S. and & 2003, undefined. Decolorization of orange II by electrocoagulation method. *Elsevier*.
7. Robinson, T., McMullan, G., Marchant, R., technology, P. N.-B. & 2001, undefined. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Elsevier*.
8. Kadirvelu, K., Kavipriya, M., Karthika, C., … M. R.-B. & 2003, undefined. Utilization of various agricultural wastes for activated carbon preparation and application for the removal of dyes and metal ions from aqueous solutions. *Elsevier*.
9. Gürses, A., Yalçin, M., management, C. D.-W. & 2002, undefined. Electrocoagulation of some reactive dyes: a statistical investigation of some electrochemical variables. *Elsevier*.
10. Verma, A., Dash, R., management, P. B.-J. of environmental & 2012, undefined. A review on chemical coagulation/flocculation technologies for removal of colour from textile wastewaters. *Elsevier*.
11. Teh, C. Y., Budiman, P. M., Shak, K. P. Y. & Wu, T. Y. Recent Advancement of Coagulation-Flocculation and Its Application in Wastewater Treatment. *Ind Eng Chem Res* **55**, 4363–4389 (2016).
12. Ghafari, S., Aziz, H., Isa, M., hazardous, A. Z.-J. of & 2009, undefined. Application of response surface methodology (RSM) to optimize coagulation–flocculation treatment of leachate using poly-aluminum chloride (PAC) and alum. *Elsevier*.
13. Boyles, W. *Chemical Oxygen Demand*.
14. Xu, H. *et al.* Study on the effects of organic matter characteristics on the residual aluminum and flocs in coagulation processes. *Elsevier* **63**, 307–317 (2017).

## *ORAL PRESENTATION*

# Hayvan barınaklarının su kalitesi üzerine etkisi: Bursa, Bilecik ve Eskişehir örneği

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## Özet

Hayvansal üretim kaynaklı su kirliliğine sebep olan başlıca kirleticiler, toplam fosfor ve toplam azottur. Tarımsal gübre depolama yapılarındaki sorunlar sebebiyle, tarımsal hayvan barınakları yakınındaki yer üstü ve yer altı su kaynakları kirlenmektedir. Bu sebeple; Bursa, Bilecik ve Eskişehir'deki 2004-2021 yılları arasındaki büyükbaş ve küçükbaş hayvan verileri kullanılarak, toplam fosfor ve toplam azotun belirlenmesi bu çalışmada amaçlanmıştır. Sonuçta; 2004-2021 yılları arasındaki periyot için, büyükbaş hayvan kaynaklı toplam fosfor (TP) ve azot (TN) kirlilik yük ortalamaları sırasıyla, Bilecik için 35.87 ton/yıl (TP) ve 322.85 ton/yıl (TN), Bursa için 168.55 ton/yıl (TP) ve 1517 ton/yıl (TN), Eskişehir için 116.43 ton/yıl (TP) ve 1048 ton/yıl (TN) olarak belirlenmiştir. 2004-2021 yılları arasındaki periyotta, küçükbaş hayvan kaynaklı TP ve TN ortalamaları ise sırasıyla; Bilecik için 5.92 ton/yıl (TP) ve 124.36 ton/yıl (TN), Bursa için 20.34 ton/yıl (TP) ve 427 ton/yıl (TN), Eskişehir için 35 ton/yıl (TP) ve 735 ton/yıl (TN) olarak belirlenmiştir. Fosfor ve azotun optimum miktarları, bitki besin elementi olarak tarımsal ürünlere fayda sağlarken; bu elementlerin aşırı miktarlarının akarsulara ulaşması durumunda, önemli sorunlar yaşanabilmektedir. Bu sorunları tespit etmek için, su kalitesinin izlenmesi gerekmektedir. Ayrıca bu tehlikenin önlenmesinde; bilimsel tarımsal inşaat teknolojilerinden yararlanılarak, özellikle sıvı gübre depolama yapılarındaki sızıntıların engellenmesi gerekmektedir.

**Anahtar Kelimeler:** Tarımsal yapılar, çevre kirliliği, su kalitesi

# The effect of animal shelters on water quality: Bursa, Bilecik and Eskişehir example

## Abstract

The main pollutants causing water pollution originating from animal production are total phosphorus and total nitrogen. Due to the problems in agricultural fertilizer storage structures, surface and underground water resources near agricultural animal shelters are polluted. Therefore, the aim of this study is to determine the total phosphorus and total nitrogen by using the cattle and small cattle animal data between 2004 and 2021 in Bursa, Bilecik and Eskişehir. After all, for the period between 2004 and 2021, the total phosphorus (TP) and nitrogen (TN) pollution load averages from cattle were 35.87 tons/year (TP) and 322.85 tons/year (TN) for Bilecik, 168.55 tons/year (TP) and 1517 tons/year (TN) for Bursa, 116.43 tons/year (TP) and 1048 tons/year (TN) for Eskişehir, respectively. In the period between 2004 and 2021, the mean TP and TN originating from small cattle (sheep and goats) were determined as 5.92 tons/year (TP) and 124.36 tons/year (TN) for Bilecik, 20.34 tons/year (TP) and 427 tons/year (TN) for Bursa, 35 tons/year (TP) and 735 tons/year for Eskişehir (TN), respectively. Optimum amounts of phosphorus and nitrogen provide benefits to agricultural products as plant nutrients. If excessive amounts of these elements reach the rivers, significant problems can be experienced. To detect these problems, water quality needs to be monitored. In addition, in the prevention of this danger, it is necessary to prevent leakages, especially in liquid manure storage structures, by making use of scientific agricultural construction technologies.

**Keywords:** Agricultural structures, environmental pollution, water quality

## GİRİŞ

Şehirleşme, sanayileşme ve tarımsal üretimin artması (enerji, gübre, ilaç tüketimi v.b) sebebiyle, küresel ısınma artmaktadır. Bu da iklimin düzensiz değişmesine sebep olmaktadır. İnsan kaynaklı sera gazı emisyonlarının; % 49 enerji sektöründen, % 24'ü endüstriden, % 14'ü tarımdan ve % 13'ü ormansızlaşmadan kaynaklanmaktadır. Sera gazı konsantrasyonlarının artması ile su kirliliği arasında da ilişki bulunmaktadır. Atmosferde azot dioksit artışı dolaylı yoldan nitrit ve nitrat kirliliklerine de sebep olmaktadır (Koyuncu ve Akgün, 2018).

Evsel atıklar, noktasal kaynaklı kirliliğe örnek iken; tarımsal faaliyet, yayılı kirlilik kaynağına örnektir. Tarımda çok kullanılan N, P ve K içerikli gübreler; gereğinden çok uygulandığında özellikle yeraltı su kaynaklarını kirletmektedir. Özellikle yüksek nitratlı sular; hayvanlarda ishal, kusma ve ölüme sebep olabilmektedir (Hacısalihoğlu, 2022).

Tırınk (2021) Iğdır ilçelerindeki TP ve TN değerlerini belirlemiştir. Sonuçta; TP değeri 204 ton/yıl ve TN değeri 2510 ton/yıl olmuştur. Ayrıca; hayvanların kuru gübrelerinin; kapalı alanda biriktirilip, kurutup, kompost yapıp, biyogaz üreterek çevredeki su kalitesinin bozulmasının önleneceği bildirilmiştir.

Hacısalihoğlu (2022) Bursa ilçelerindeki hayvansal kaynaklı yayılı kirlilik yükü hesaplamıştır. Bunun için 2020 yılındaki büyükbaş, küçükbaş ve kümes hayvanı sayılarını kullanıp, yıllık yayılı toplam fosfor (TP) VE toplam azot (TN) değerlerini belirlemiştir. Sonuçta TP ve TN yükü en yüksek olan ilçeler; Yenişehir, Mustafakemalpaşa ve Karacabey olmuştur. Bursa genelinde, TP değeri 341.33 ton TP/yıl ve TN değeri 3242 ton TN/yıl olarak belirlenmiştir.

Bursa büyükbaş hayvancılığı kaynaklı toplam kirlilik yükünün; % 20.5'i Yenişehir'den, % 19'u Mustafakemalpaşa'dan ve %18.4'ü Karacabey'den kaynaklanmaktadır. Bu 3 ilçe Bursa'daki kirlilik yükünün yaklaşık % 58'ini temsil etmektedir (Hacısalihoğlu, 2022). Tırınk (2021) Iğdır'daki çiftlik hayvanlarının; yıllık toplam fosfor yükünü 203.5 ton/yıl, yıllık toplam azot yükü ise 2509.7 ton/yıl olarak belirlemiştir.

Sulardaki nitrit ve nitrat kirliliğinin kaynaklarından biri olan N_2 O (azot dioksit) konsantrasyonunun doğru tahminlenmesi oldukça önemlidir. Barınaklardan gerçekleşen $N_2O$ emisyonu, şu eşitlikle belirlenebilmektedir (Kılıç ve ark., 2018);

$$E = (C_e - C_i).Q \quad (1)$$

E: Barınaktan olan emisyon (g/h)

$C_e$: Kirletici gazın barınak içindeki yoğunluğu (g/$m^3$)

$C_i$: Kirletici gazın giriş yoğunluğu (g/$m^3$)

Q: Barınaktaki havalandırma miktarı ($m^3$/h)

Bu çalışmada; Bursa, Bilecik ve Eskişehir'deki 2004-2021 yılları arasındaki büyükbaş ve küçükbaş hayvan verileri kullanılarak, toplam fosfor ve toplam azotun belirlenmesi amaçlanmıştır. Bu sayede bölgenin kirlilik yükü belirlenmiş olacaktır.

## MATERYAL VE METOT

Çalışmada materyal olarak; Bursa, Bilecik ve Eskişehir'deki 2004-2021 yılları arasındaki çiftlik hayvanı sayıları kullanılıp, çeşitli eşitlikler yardımıyla toplam fosfor ve toplam azotun belirlenmiştir.

### MATERYAL

Araştırma alanı; Bursa, Bilecik ve Eskişehir İllerini kapsamaktadır. Çalışma alanındaki nüfus yoğunluğu sebebiyle, yapılan tarımsal faliyet büyük bir kirlilik yükü getirmektedir (Şekil 1).




**Şekil 1.** Araştırma alanı

Çalışma alanındaki büyükbaş hayvan sayılarının; çoktan aza göre sıralanması Bursa, Eskişehir ve Bilecik şeklindedir (Tablo 1).

**Tablo 1.** Büyükbaş hayvan sayıları (TÜİK, 2022)

| Yıl | Bilecik | Bursa | Eskişehir |
|---|---|---|---|
| 2004 | 34335 | 124263 | 100298 |
| 2005 | 36392 | 142418 | 113227 |
| 2006 | 38730 | 132563 | 114083 |
| 2007 | 38783 | 148707 | 107887 |
| 2008 | 46887 | 147235 | 108634 |
| 2009 | 45983 | 149329 | 104308 |
| 2010 | 38695 | 160223 | 108173 |
| 2011 | 39591 | 172968 | 117018 |
| 2012 | 41153 | 189774 | 118881 |
| 2013 | 41535 | 199939 | 122661 |
| 2014 | 39637 | 193153 | 126778 |
| 2015 | 38741 | 197119 | 128466 |
| 2016 | 32161 | 186145 | 131459 |
| 2017 | 35775 | 201288 | 136779 |
| 2018 | 40685 | 236604 | 155273 |
| 2019 | 40953 | 242065 | 162855 |
| 2020 | 39018 | 235781 | 170073 |
| 2021 | 38557 | 265312 | 169938 |

Çalışma alanındaki küçükbaş hayvan sayılarının; çoktan aza göre sıralanması Eskişehir, Bursa ve Bilecik şeklindedir (Tablo 2).

**Tablo 2.** Küçükbaş hayvan sayıları (TÜİK, 2022)

| Yıl | Bilecik | Bursa | Eskişehir |
|---|---|---|---|
| 2004 | 74735 | 263769 | 482911 |
| 2005 | 80200 | 293599 | 555204 |
| 2006 | 74957 | 303794 | 556363 |
| 2007 | 73665 | 310692 | 526897 |
| 2008 | 83627 | 270227 | 493309 |
| 2009 | 81435 | 293814 | 508626 |
| 2010 | 90289 | 329837 | 496601 |
| 2011 | 107449 | 376531 | 581601 |
| 2012 | 117350 | 423968 | 603963 |
| 2013 | 120280 | 442013 | 675316 |
| 2014 | 136363 | 465345 | 704118 |
| 2015 | 117520 | 458823 | 808392 |
| 2016 | 126810 | 441859 | 765014 |
| 2017 | 140137 | 472467 | 733738 |
| 2018 | 155191 | 523198 | 800732 |
| 2019 | 187952 | 579525 | 1034359 |
| 2020 | 192814 | 587245 | 1184648 |
| 2021 | 202466 | 592090 | 1268047 |

## YÖNTEM

Toplam P ve toplam N hesabı için gerekli çizelge ve eşitlikler aşağıda belirtilmiştir. Hayvansal kaynaklı kirlilik yük katsayısı Tablo 3'de gösterilmektedir.

**Tablo 3.** Hayvansal kaynaklı kirlilik yükü katsayısı (Hacısalihoğlu, 2022)

| Kirletici | Yük katsayısı | Büyükbaş | Küçükbaş |
|---|---|---|---|
| TP | $Q_{pyk}$ | 0.1 | 0.06 |
| | $Y_p$ | 5 | 5 |
| TN | $Q_{nyk}$ | 0.3 | 0.42 |
| | $Y_n$ | 15 | 15 |

Tablo 3'de kullanılan değişkenler şunlardır;

$Q_{pyk}$: Günlük fosfor yüküne bağlı kirletici yükü (kg/ton hayvan/gün)
$Y_p$: Fosfor kirliliğinin ortama ulaşma yüzdesi
$Q_{nyk}$: Günlük azot yüküne bağlı kirletici yükü (kg/ton hayvan/gün)
$Y_n$: Azot kirliliğinin ortama ulaşma yüzdesi

Bu değerler sayesinde, yıllık toplam kirlilik yükü belirlenebilmektedir (Hacısalihoğlu, 2022).

$Q_{TN}$: $Q_{nyk} * A_{CH} * Y_n *$ (365/1000) (1)

$Q_{TP}$: $Q_{pyk} * A_{CH} * Y_p *$ (365/1000) (2)

Eşitlikteki $A_{CH}$ değeri; büyükbaş hayvanlar için 500, küçükbaş hayvanlar için 45 kg olarak kabul edilmektedir. Eşitlik 1 ve 2 'deki değerler; ton/yıl cinsinden şu eşitlikler ile ifade edilebilmektedir (Hacısalihoğlu, 2022).

$Q'_{TP} = (Q_{TP} * N_{CH})/1000$ (3)

$Q'_{TN} = (Q_{TN} * N_{CH})/1000$ (4)

$Q'_{TP}$: Yıllık TP kirlilik yükü (ton/yıl)
$Q'_{TN}$: Yıllık TN kirlilik yükü (ton/yıl)
$N_{CH}$: Canlı hayvan sayısı'dır.

## BULGULAR

2004-2021 yılları arasındaki periyot için, büyükbaş hayvan kaynaklı toplam fosfor (TP) ve azot (TN) kirlilik yük ortalamaları sırasıyla, Bilecik için 35.87 ton/yıl (TP) ve 322.85 ton/yıl (TN), Bursa için 168.55 ton/yıl (TP) ve 1517 ton/yıl (TN), Eskişehir için 116.43 ton/yıl (TP) ve 1048 ton/yıl (TN) olarak belirlenmiştir (Tablo 4).

**Tablo 4.** 2004-2021 arası Bilecik-Bursa-Eskişehir'deki büyükbaş kaynaklı toplam P ve toplam N yükleri

| | Bilecik | | Bursa | | Eskişehir | |
|---|---|---|---|---|---|---|
| | Qtp (ton/yıl) | Qtn (ton/yıl) | Qtp (ton/yıl) | Qtn (ton/yıl) | Qtp (ton/yıl) | Qtn (ton/yıl) |
| 2004 | 31.33 | 281.98 | 113.39 | 1020.51 | 91.52 | 823.70 |
| 2005 | 33.21 | 298.87 | 129.96 | 1169.61 | 103.32 | 929.88 |
| 2006 | 35.34 | 318.07 | 120.96 | 1088.67 | 104.10 | 936.91 |
| 2007 | 35.39 | 318.51 | 135.70 | 1221.26 | 98.45 | 886.02 |
| 2008 | 42.78 | 385.06 | 134.35 | 1209.17 | 99.13 | 892.16 |
| 2009 | 41.96 | 377.64 | 136.26 | 1226.36 | 95.18 | 856.63 |
| 2010 | 35.31 | 317.78 | 146.20 | 1315.83 | 98.71 | 888.37 |
| 2011 | 36.13 | 325.14 | 157.83 | 1420.50 | 106.78 | 961.01 |
| 2012 | 37.55 | 337.97 | 173.17 | 1558.52 | 108.48 | 976.31 |
| 2013 | 37.90 | 341.11 | 182.44 | 1642.00 | 111.93 | 1007.35 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2014 | 36.17 | 325.52 | 176.25 | 1586.27 | 115.68 | 1041.16 |
| 2015 | 35.35 | 318.16 | 179.87 | 1618.84 | 117.23 | 1055.03 |
| 2016 | 29.35 | 264.12 | 169.86 | 1528.72 | 119.96 | 1079.61 |
| 2017 | 32.64 | 293.80 | 183.68 | 1653.08 | 124.81 | 1123.30 |
| 2018 | 37.13 | 334.13 | 215.90 | 1943.11 | 141.69 | 1275.18 |
| 2019 | 37.37 | 336.33 | 220.88 | 1987.96 | 148.61 | 1337.45 |
| 2020 | 35.60 | 320.44 | 215.15 | 1936.35 | 155.19 | 1396.72 |
| 2021 | 35.18 | 316.65 | 242.10 | 2178.87 | 155.07 | 1395.62 |
| Toplam | 645.70 | 5811.26 | 3033.96 | 27305.63 | 2095.82 | 18862.40 |
| Ortalama | 35.87 | 322.85 | 168.55 | 1516.98 | 116.43 | 1047.91 |

2004-2021 yılları arasındaki periyotta, küçükbaş hayvan kaynaklı TP ve TN ortalamaları ise sırasıyla; Bilecik için 5.92 ton/yıl (TP) ve 124.36 ton/yıl (TN), Bursa için 20.34 ton/yıl (TP) ve 427 ton/yıl (TN), Eskişehir için 35 ton/yıl (TP) ve 735 ton/yıl (TN) olarak belirlenmiştir (Tablo 4).

**Tablo 5.** 2004-2021 arası Bilecik-Bursa-Eskişehir'deki küçükbaş kaynaklı toplam P ve toplam N yükleri

| | Bilecik | | Bursa | | Eskişehir | |
|---|---|---|---|---|---|---|
| | Qtp (ton/yıl) | Qtn (ton/yıl) | Qtp (ton/yıl) | Qtn (ton/yıl) | Qtp (ton/yıl) | Qtn (ton/yıl) |
| 2004 | 3.68 | 77.33 | 13.00 | 272.94 | 23.80 | 499.70 |
| 2005 | 3.95 | 82.99 | 14.47 | 303.81 | 27.36 | 574.51 |
| 2006 | 3.69 | 77.56 | 14.97 | 314.36 | 27.41 | 575.71 |
| 2007 | 3.63 | 76.23 | 15.31 | 321.50 | 25.96 | 545.22 |
| 2008 | 4.12 | 86.54 | 13.32 | 279.62 | 24.31 | 510.46 |
| 2009 | 4.01 | 84.27 | 14.48 | 304.03 | 25.06 | 526.31 |
| 2010 | 4.45 | 93.43 | 16.25 | 341.31 | 24.47 | 513.87 |
| 2011 | 5.29 | 111.19 | 18.55 | 389.62 | 28.66 | 601.83 |
| 2012 | 5.78 | 121.43 | 20.89 | 438.71 | 29.76 | 624.97 |
| 2013 | 5.93 | 124.46 | 21.78 | 457.38 | 33.28 | 698.80 |
| 2014 | 6.72 | 141.11 | 22.93 | 481.53 | 34.70 | 728.60 |
| 2015 | 5.79 | 121.61 | 22.61 | 474.78 | 39.83 | 836.50 |
| 2016 | 6.25 | 131.22 | 21.77 | 457.22 | 37.70 | 791.62 |
| 2017 | 6.91 | 145.01 | 23.28 | 488.90 | 36.15 | 759.25 |
| 2018 | 7.65 | 160.59 | 25.78 | 541.39 | 39.46 | 828.58 |
| 2019 | 9.26 | 194.49 | 28.56 | 599.68 | 50.97 | 1070.33 |
| 2020 | 9.50 | 199.52 | 28.94 | 607.67 | 58.37 | 1225.84 |
| 2021 | 9.98 | 209.51 | 29.18 | 612.68 | 62.48 | 1312.14 |
| Toplam | 106.59 | 2238.47 | 366.05 | 7687.13 | 629.73 | 13224.26 |
| Ortalama | 5.92 | 124.36 | 20.34 | 427.06 | 34.98 | 734.68 |

## TARTIŞMA ve SONUÇ

Bursa, Bilecik ve Eskişehir'deki 2004-2021 yılları arasındaki büyükbaş ve küçükbaş hayvan verileri kullanılarak, toplam fosfor ve toplam azotun belirlenmesinin amaçlandığı bu çalışmadaki; Bursa'ya ait toplam P ve N kirletici yük değerleri, Hacısalihoğlu (2022)'nun Bursa'ya dair belirttiği sonuçlarla örtüşmektedir. Bu çalışmadaki; Bilecik'e ait büyükbaş kaynaklı ve Bursa'ya ait küçükbaş kaynaklı toplam P ve N kirletici yük değerleri belirlenmiştir. Bilecik'e dair belirlenen bu sonuçlar ise; Hacısalihoğlu (2022)'nun Muş'a dair belirttiği sonuçlarla (TP ve TN değerleri sırasıyla; 16.92 ton/yıl ve 543.4 ton/yıl'dır), benzerlik göstermektedir. Yani Bilecik ile Muş'un TP ve TN açısından kirlilik yüklerinin benzer olduğu söylenebilmektedir.

Fosfor ve azotun optimum miktarları, bitki besin elementi olarak tarımsal ürünlere fayda sağlarken; bu elementlerin aşırı miktarlarının akarsulara ulaşması durumunda, önemli sorunlar yaşanabilmektedir. Bu sorunları tespit etmek için, su kalitesinin izlenmesi gerekmektedir. Ayrıca bu tehlikenin önlenmesinde;

bilimsel tarımsal inşaat teknolojilerinden yararlanılarak, özellikle sıvı gübre depolama yapılarındaki sızıntıların engellenmesi gerekmektedir.

## KAYNAKLAR

Hacısalihoğlu S 2022. Hayvansal kaynaklı yayılı kirlilik yükleri hesabı, Bursa örneği. Uludağ üniversitesi mühendislik fakültesi dergisi, 27(1): 361-374

Kılıç İ, Yaylı B, Elekberov A 2018. Bursa bölgesinde faaliyet gösteren üç adet broyler işletmesinin karbon ayak izinin tahminlenmesi. Uluslararası tarım ve yaban hayatı dergisi, 4 (2): 224-230.

Koyuncu M, Akgün H 2018. Çiftlik hayvanları ve küresel iklim değişikliği arasındaki etkileşim. Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 32 (1): 151-164.

Tırınk S 2021. Iğdır ili ve ilçelerindeki hayvansal atıkların çevresel etkileri ve yayılı kirletici yükü hesabı. Black sea journal of engineering and science. 4 (2): 43-50.

TÜİK (Türkiye İstatistik Kurumu) 2022. https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr Erişim tarihi: 07.11.2022.

## *ORAL PRESENTATION*

# Turkish herbal remedies for snake bites and major compound analyses

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**Abstract**
The World Health Organization (WHO) defines a "medicinal plant" as any plant that has compounds that can be used to synthesize other medications, treat illnesses, or serve as precursors for one or more of the plant's organs. This concept makes a distinction between plants whose constituents and therapeutic capabilities have not yet undergone scientific study but are nevertheless recognized as being medicinal. Medicinal plants produce large number secondary metabolites that possess biological and pharmacological activities. These compounds are synthesized as a response to different stress of environmental factors. The main bioactive phytochemicals are terpenes, phenolics and alkaloids analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS). The biological and pharmacological effects of medicinal plants that have been investigated the most include their antibacterial, antioxidant, anticancer, anti-inflammatory, and hypoglycaemic properties. With its incredible plant diversity, Turkey has some of the richest flora in the entire globe. There are over 10,000 vascular plants in its flora, and about one-third of these (34.4%) are indigenous to the nation. The utilization of ethnobotanical data gathered from studies on therapeutic plants has recently drawn interest on a global scale. For this reason, a great deal has been written about medicinal plants in our nation and a ton of ethnobotanical research have recently been published. This study aims to provide information about how many taxa are used in traditional treatments against snake bites in Turkey and the compounds obtained from these species. Information about the scientific names, local names, families, parts used, and their usage in snake bites of these taxa are given. Also, major compounds and their analysis are given.

**Keywords:** Medicinal plants, Traditional treatment, Snake bite, Venom, Turkey

## INTRODUCTION

Many ailments (including diabetes, jaundice, shortness of breath, etc.) have been tested and researched to be treated with plants throughout the history of humanity. According to the World Health Organization (WHO), 80% of the world's population—roughly 4 billion people—first tried using herbal medicines to treat their health issues. Additionally, roughly 25% of prescription medications in wealthy nations contain active chemicals with a botanical origin (vinbilastine, reserpine, quinine, aspirin, etc.). The amount of use of these plants is growing daily, especially since the 1990s with the discovery of new applications for medicinal and aromatic herbs as well as the rise in demand for natural products (Çiğdem et al., 2019).

In the world, there are more than 3000 different kinds of snakes. A total of 375 of them, from five different genera, are toxic. The three regions with the highest rates of snakebite mortality and morbidity are South Asia, Southeast Asia, and Sub-Saharan Africa. According to findings from the World Health Organization, 35,000–50,000 people worldwide pass away annually from snakebites, predominantly in India. Varied forms of snake toxins have different effects and aggressive personalities (Chippaux, 1998; Yönetimi et al., 2014).

There are 40 species of snakes in Turkey. The most common venomous snakes are the Viperidae group snakes. Venomous snakes are divided into 5 main groups (Colubridae, Elapidae, Hydrophylidae, Viperidae, Atractaspididae). In the Cukurova region, there are generally *Vipera ammodytes* and *Vipera lebetina* venomous snakes. The venom of such snakes often has a hematotoxic, rarely neurotoxic effect. The toxic effect of the snake venom varies according to conditions such as the severity and amount of the venom, the sensitivity of the bitten person to the venom, the age of the snake, its physiological and pathological condition, etc. (Yönetimi et al., 2014).

In many parts of the world, especially in tropical and subtropical countries, snakebites pose a serious medical, social, and economic challenge. The majority of poisonous snakes in the world are found in developing nations, where access to treatment is poor. Around 2 million individuals are thought to be bitten each year, which can

result in up to 125,000 fatalities, 400,000 lasting impairments, and even amputation in the case of severe consequences. A complex blend of proteins and peptides, as well as at least 25 enzymes, make snake venom one of the most potent and "mystery" biological fluids in the Mammalia class. This makes it difficult to treat the effects of the venom on the body (Upasani et al., 2017).

However, only a few broad categories, such as systemic myolysis flaccid paralysis, coagulopathy and hemorrhage, cardiotoxicity, renal damage and failure, local tissue injury at the bite site, and other potential effects of snake envenoming on humans are of significant clinical importance. Secondary consequences, like potential morbidity and mortality, can occur occasionally (Upasani et al., 2017).

The first phase of management takes place in the field after a snake bite. To reduce the local and systemic symptoms of envenomation, immediate care is then provided in a hospital setting through triage. Although envenomation is thoroughly researched in terms of field treatment and management of acute symptoms, there is a dearth of literature covering the care of secondary sequelae in this particular patient population, leaving treatment recommendations uncertain (Russell et al., 2021).

Antibodies produced from the immunization of domestic animals with snake venom can negate its effects. A product called antisera, an antivenom, was created as a result. The high expense of serum therapy and the likelihood that victims will frequently be some distance from medical care when bitten are two key drawbacks. Therefore, the hunt for new venom inhibitors derived from natural materials is important given their potential to supplement serum therapy in primarily mitigating local effects of envenomation. With a variety of anti-venom actions, plant extracts make a great substitute (Okot et al., 2020). The results of ethnobotanical research utilizing standard medical procedures are recorded with the intention of assisting in the drug's creation. From one generation to the next, people have been passing down their understanding of how these medicinal or wild plants are used in traditional ways. After exploring ethnobotanical studies, taxa that are often used in Turkish traditional medicine for snake bites were also found in this study. The information on the families, scientific names, and sections of these taxa that are used to treat snake bites is intended to serve that purpose.

## MATERIALS AND METHODS

In this review, in addition to the analysis of ethnobotanical studies conducted in various parts of Turkey, postgraduate theses made by compiling local plants used in the treatment of snake bites at the National Higher Education Center were scanned.

## RESULTS AND DISCUSSION

A "medicinal plant," according to the World Health Organization (WHO), is any plant that contains substances that can be utilized to create additional medications, treat diseases, or act as precursors for one or more of the plant's organs. This idea distinguishes between plants and their constituents and therapeutic properties that have not yet undergone scientific investigation but are nevertheless acknowledged as being medicinal. Secondary metabolites that have biological and pharmacological effects are produced in enormous quantities by medicinal plants. As a reaction to various environmental stresses, these chemicals are created. Terpenes, phenolics, alkaloids, flavonoids, organic acids, essential oils, fixed oils, coumarins, saponins, tannins, carbohydrates, vitamins and minerals which are examined by Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry, are the primary bioactive phytochemicals (GC-MS)(Martinez et al., 2015; Sarı et al., 2020; WHO, 1977). These bioactive compounds obtained from traditionally used for treatment of snake bites via spectrophotometric analysis methods are shown in Table 1.

The most well studied biological and pharmacological effects of medicinal plants include their antibacterial, antioxidant, anticancer, anti-inflammatory, and hypoglycemic activities. Turkey has some of the richest flora on earth due to its remarkable plant diversity. Its flora includes approximately 10,000 vascular plants, of which about one-third (34.4%) are native to the country. Recently, there has been increased interest on a global scale in the use of ethnobotanical information discovered via research on medicinal plants. As a result, a ton of ethnobotanical research has just been published and a lot has been written about therapeutic plants in our country (Karahuseyin and Sari, 2019).

In this study, we compiled 21 plant species that were used in Turkish folk medicine to cure snake bites from ethnobotanical studies and theses published at the National Higher Education Center. Results are shown in Table 1. Most of the plant species are collected from nature and used externally.

Envenomation from snake bites is an underreported global health emergency. Since less severe snake bites are rarely seen by the healthcare system, the true incidence is probably underreported. Numerous studies back the

administration of adequate and early doses of antivenom to lessen the need for surgical intervention. Antivenom is, however, scarcely available in rural and resource-poor places(Russell et al., 2021).

In Turkey, the usage of medicinal plants for the treatment of snakebite is very common. On the other hand, there are no statistics on the envenomation and treatment of snakebite. Additionally, not many regions in Turkey have conducted ethnopharmacological studies of the herbs used to treat snakebites. This study set out to catalog the plant species utilized in Turkey to treat snakebite envenomation.

**Table 1**. Plants traditionally used in the treatment of snake bites in Turkey

| Botanical name | Family | Local name | Plant part used | Chemical content | Preparation, administration and use | Reference |
|---|---|---|---|---|---|---|
| *Allium sativum* L. | Liliaceae | Sarımsak | Bulb | Sucrose, glucose, fructose, vitamins A, B, C, saponin, allicin, alliin, allyl alliin, methyl alliin, allyl disulfurallylpropylsulfite | Ext. | ( Bulut and Tuzlaci, 2008; Tetik et al., 2013; Aktan, 2011; Uysal et al., 2009) |
| *Anchusa azurea* Mill. | Boraginaceae | Mor çiçek, Tatlı dil, Fısır, Sormuk | Leaves | Oleanazurosite and its derivatives, flavonoids, rosmarinic acid, lactic acid | Ext. | (Sargin et al., 2013; Tetik et al., 2013) |
| *Arctium platylepis* (Boiss. & Bal.) Sosn. ex Grossh. | Asteraceae | Devedabanı | - | Inulin, protein, essential oil, fixed oil, organic acid, carotenoids, flavonoids, tannin | Ext. | (Altundag and Ozturk, 2011) |
| *Borago officinalis* L. | Boraginaceae | Sığırdili, Zembilçiçeği, Hodan | Young Leaves | Gamalinolenic acid, linoleic acid, oleic acid, palmitic acid, stearic acid, eicosenoic acid, erucic acid | Ext. | (Yiğit, 2014) |
| *Bungea trifida* (Vahl) C.A. Meyer | Orobanchaceae | Üçkernek otu | Aerial Parts | - | Ext. | (Doğan, 2015) |
| *Calamintha nepeta* (L.) Savi | Lamiaceae | Yılan otu | Aerial Parts | Essential oils, triterpenes | Ext. | (Akgul et al., 2018) |
| *Calcitrapa iberica* (Trevir. ex Spreng.) Schur | Asteraceae | Kelembeşk | Young Leaves | Flavones, steroids, fatty acids, essential oils, sesquiterpene lactones, nitrogenous compounds, lignans | Int. | (Kaval et al., 2014) |
| *Carlina vulgaris* L. | Asteraceae | Deve kengeri | Aerial Parts | Triterpenes (lupeol and derivatives) | Ext. | (Doğan, 2015) |
| *Cedrus libani* A. Rich. | Pinaceae | Katran, Ardıç | Tar | Protoberberine type isoquinoline alkaloids, benzophenanthridine type isoquinoline alkaloids, protopine type isoquinoline alkaloids, caffeic acid derivatives | Ext. | (Yıldırım, 2015) |
| *Centaurea rigida* Banks. & Sol. | Asteraceae | Yenice, Tahliye | Leaves | Palmitic acid, oleic acid, linoleic acid | Dec., Ext. | (Akgul et al., 2018) |
| *Heliotropium europaeum* L. | Boraginaceae | Boz otu, Siğil otu, Pompul, Kircinnik otu | Latex<br>Leaves<br>Roots and Leaves | Pyrolizidine alkaloids and N-oxides, sterols, flavonoids, triterpenoids | Ext. | (Karagöz, 2013; Sargin et al., 2013) |
| *Juniperus oxycedrus* L. | Cupressaceae | Katran ardıcı | Tar | α-pinene, β-myrcene, α-phelandrene, limonene, epi-bicycloseschyphelandrene, δ-cadinene | Ext. | (Saday, 2009) |
| *Lycopsis pulla* L. | Boraginaceae | Güzrile | Leaves | Pyrolizidine alkaloids | Ext. | (Kaval et al., 2014) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Nerium oleander* L. | Apocynaceae | Sındılık, Zakkum, Acı Hayat | Stem Shell Latex | Cardiac steroids and glycosides | Ext. | (Güneş et al., 2018; Sargin et al., 2013) |
| *Olea europaea* L. var. *europaea* | Oleaceae | Zeytin, Sitin | Fruits | Tannin, essential oil, organic acid, fatty acids, phytosterol, flavonoids, organic acids, resin, vitamins (A and E), esters | Ext. | (Yıldırım, 2015) |
| *Peganum harmala* L. | Zygophyllaceae | Üzerik, Yüzerlik | Seeds | Alkaloids, quinazoline derivatives, anthraquinone | Ext. | (Metin, 2009) |
| *Rumex patientia* L. | Polygonaceae | Labada | Aerial Parts | Anthraquinone, naphthalene, tannin, napthoquinone | Ext. | (Bulut, 2006) |
| *Sambucus ebulus* L. | Caprifoliaceae | Şahmelik, Sultan otu | Leaves and Roots | Iridoids | Ext. | (Koçyiğit and Özhatay, 2006) |
| *Satureja cuneifolia* Ten. | Lamiaceae | Yayla kekiği, Kara kekik | Aerial Parts | Monoterpenes | Ext. | (Yıldırım, 2015) |
| *Thymbra spicata* L. | Lamiaceae | Zahter | Aerial Parts | Monoterpenes | Dec., Int. and Ext. | (Güzelşemme, 2014) |
| *Urtica dioica L.* | Urticaceae | Isırgan | Aerial Parts | Organic acids, salts (Ca, K, salicylic acid salts), biogenic amines, sitosterols, lectins, terpenes, essential oil, coumarins, vitamin C | Ext. | (Oral, 2007) |

Dec: Decoction, Ext: External, Int: Internal

## CONCLUSION

In conclusion, this list of medicinal plants becomes very important whenever there is knowledge loss. Studies on some plants' in vitro biological activity have been done in the past, but further studies are required to determine how well other species work. Future studies on these plants may result in the development of fresh drug candidates that are effective against multi-drug resistant strains.

## ACKNOWLEDGEMENTS

None.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

Akgul, A., Akgul, A., Senol, S. G., Yildirim, H., Secmen, O., Dogan, Y. (2018). An ethnobotanical study in Midyat (Turkey), a city on the silk road where cultures meet. Journal of Ethnobiology and Ethnomedicine 14:12.

Aktan, T. (2011). Yenişehir (Bursa) Köylerinin Etnobotanik Özellikleri. Celal Bayar Üniversitesi, Botanik Anabilim Dalı, Yüksek Lisans Tezi.

Altundag, E., Ozturk, M. (2011). Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. Procedia - Social and Behavioral Sciences, 19, 756–777. https://doi.org/10.1016/j.sbspro.2011.05.195

Bulut, G., Tuzlaci, E. (2008). Folk medicinal plants of Bayramiç (Çanakkale-Turkey). Journal of Pharmacy of Istanbul University, 40(1): 87–99.

Bulut, Y. (2006). Manavgat (Antalya) Yöresinin Faydalı Bitkileri. Süleyman Demirel University.

Chippaux, J. P. (1998). Snake-bites: appraisal of the global situation. Bull World Health Organ, 76(5): 515–524.

Çiğdem, F., Oruç, S., Ejder, İ., Deniz, E., Sadık, O., Oruç, H. (2019). Awareness of medical and aromatic plants in the western black sea region. International Journal of Agriculture, Environment and Food Sciences 3(1):1-4.

Doğan, A. (2015). Wild Edible Plants of Pertek (Tunceli-Turkey). Marmara Pharmaceutcal Journal 19(2):126-135.

Güneş, S., Savran, A., Paksoy, M. Y., Çakılcıoğlu, U. (2018). Survey of wild food plants for human consumption in Karaisalı (Adana-Turkey). Indian Journal of Traditional Knowledge 17(2):290-298.

Güzelşemme, M. (2014). Antakya'da Kullanılan Tıbbi Bitkiler ile Yabani Gıda Bitkileri. Mustafa Kemal Üniversitesi.

Karagöz, F. (2013). Suşehri (Sivas) Bölgesinin Etnobotanik Açıdan Değerlendirilmesi. Afyon Kocatepe University.

Karahuseyin, S., Sari, A. (2019). Plants used in traditional treatment against diarrhea in Turkey. Istanbul Journal of Pharmacy, 49(1): 33–44.

Kaval, I., Behçet, L., Cakilcioglu, U. (2014). Ethnobotanical study on medicinal plants in Geçitli and its surrounding (Hakkari-Turkey). Journal of Ethnopharmacology.

Koçyiğit, M., Özhatay, N. (2006). Wild Plants Used As Medicinal Purpose In Yalova (Northwest Turkey). Turkish Journal of Pharmaceutical Sciences 3(2):91-103.

Martinez, M., Poirrier, P., Chamy, R., Prüfer, D., Schulze-Gronover, C., Jorquera, L., Ruiz, G. (2015). Taraxacum officinale and related species - An ethnopharmacological review and its potential as a commercial medicinal plant. Journal of Ethnopharmacology, 169:244–262.

Metin, A. (2009). Mut Ve Çevresinde (Mersin) Yetişen Bitkilerin Etnobotanik Özellikleri. Selcuk University.

Okot, D. F., Anywar, G., Namukobe, J., Byamukama, R. (2020). Medicinal plants species used by herbalists in the treatment of snakebite envenomation in Uganda. Tropical Medicine and Health, 48(1):44-58.

Oral, D. (2007). Konya İlinde Kullanılan Halk İlaçları Üzerinde Etnobotanik Araştırmalar. Gazi University.

Russell, J. J., Schoenbrunner, A., Janis, J. E. (2021). Snake Bite Management: A Scoping Review of the Literature. Plastic and Reconstructive Surgery 9(4):e3506.

Saday, H. (2009). Güzeloluk Köyü Ve Çevresinin (Erdemli/Mersin) Etnobotanik Özellikleri. Selcuk University.

Sargin, S. A., Akçicek, E., Selvi, S. (2013). An ethnobotanical study of medicinal plants used by the local people of Alaşehir (Manisa) in Turkey. Journal of Ethnopharmacology 150(3):860-74.

Sarı, A., Özsoy, N., Karahüseyin, S. (2020). Taraxacum farinosum Hausskn. & Bornm. bitkisinin antioksidan aktivite yönünden incelenmesi* Investigation of antioxidant activity of the plant Taraxacum farinosum Hausskn. & Bornm. Journal of Advanced Research in Health Sciences, 3(1).

Tetik, F., Civelek, S., Cakilcioglu, U. (2013). Traditional uses of some medicinal plants in Malatya (Turkey). Journal of Ethnopharmacology.

Tuğba Aktan. (2011). Yenişehir (Bursa) Köylerinin etnobotanik özellikleri. Celal Bayar University.

Upasani, S. V., Beldar, V. G., Tatiya, A. U., Upasani, M. S., Surana, S. J., Patil, D. S. (2017). Ethnomedicinal plants used for snakebite in India: a brief overview. Integrative Medicine Research, 6(2): 114–130.

Uysal, İ., Onar, S., Karabacak, E., Çelik, S. (2009). Ethnobotanical aspects of Kapıdağ Peninsula (Turkey). Biological Diversity and Conservation, 3:15–22.

WHO. (1977). Resolution-Promotion and Development of Training and Research in Traditional Medicine.

Yiğit, S. (2014). Gaziantep İli Aktarlarinda Satilan Bitkiler Ve Etnobotanik Özellikleri. Gaziantep University.

Yıldırım, H. (2015). Alanya ve Gazipaşa (Antalya)'da Halk tarafından kullanılan bazı doğal bitkilerin etnobotanik özellikleri. Afyon Kocatepe University.

Yönetimi, Y. I., Kara, M. E., Oğuzhan Ay, M., Seğmen, S., Avcı, A., İçme, F. (2014). Arşiv Kaynak Tarama Dergisi Archives Medical Review Journal Management of Snake Bites Adana Numune Eğitim ve Araştırma Hastanesi Acil Tıp Kliniği, Adana, Turkey Atatürk Eğitim ve Araştırma Hastanesi Acil Tıp Kliniği, Ankara, Turkey 4 Çukurova Üniv. Tıp Fa. Arşiv Kaynak Tarama Dergisi, 23(2): 272–292.

## *ORAL PRESENTATION*

# Yeni periferal ve non-periferal $C_2$-simetrik diol substitue çinko ftalosiyaninler: sentez, karakterizasyon, agregasyon özellikleri ve katalitik uygulaması

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## Özet

Bu çalışmada, (1*R*,2*R*)-1,2-bis(2′-fenilfenil)etan-1,2-diol (**1**) bileşiğinin ftalosiyanin iskeletine periferal ve non-periferal konumlarından substitue edilmesi ile iki adet yeni tetrasubsitue çinko ftalosiyanin bileşiği (**4** ve **5**) sentezlenmiştir. Sentezlenen ftalosiyanin bileşiklerinin karakterizasyonu FT-IR, $^1$H NMR, UV-Vis ve MS analizleri ile gerçekleştirilmiştir. Yaygın olarak kullanılan organik çözücüler içerisinde ftalosiyanin bileşiklerinin çözünürlüklerinin yüksek olduğu gözlenmiştir. Yeni sentezlenen ftalosiyanin bileşiklerinin diklorometan, dietileter, kloroform, THF ve DMF çözücülerinin kullanılması ile $2x10^{-6}$ - $10x10^{-6}$ M derişim aralığında çözeltileri hazırlanmış ve ftalosiyanin bileşiklerinin bu çözücüler içindeki agregasyon davranışları UV-Vis spektrofotometresi ile incelenmiştir. Her iki ftalosiyanin bileşiğinin kullanılan çözücüler içinde agregatlaşmaya uğramadıkları belirlenmiştir. $2x10^{-6}$ - $10x10^{-6}$ M derişim aralığındaki çözeltilerinin detaylı UV-Vis ölçümleri ftalosiyanin bileşiklerinin Lambert-Beer kanununa uyduklarını göstermiştir. Buna ilave olarak, ftalonitril bileşikleri **2** ve **3** ile bunların çinko ftalosiyanin türevleri **4** ve **5**'in katalitik etkinlikleri aldehitlere enantiyoseçimli dietilçinko katılmasında test edilmiştir. 5 farklı aldehit ve iki farklı çözücü kullanılarak yapılan aldehitlere dietilçinko katılması denemelerinde enantiyoseçimlilik gözlenmemiş fakat belli oranlarda dönüşümler elde edilmiştir. En yüksek dönüşüm, **3** bileşiği varlığında toluen içerisinde gerçekleştirilen 2-metoksibenzaldehite dietilçinko katılması reaksiyonunda %34 olarak elde edilmiştir.

**Anahtar Kelimeler:** Ftalosiyanin, agregasyon, $C_2$-simetrik diol, kataliz, dietilçinko katılması

# Novel peripherally and non-peripherally $C_2$-symmetric diol substituted zinc phthalocyanines: synthesis, characterization, aggregation properties and cataytic application.

## Abstract

In this study, two new tetrasubstituted zinc phthalocyanine compounds (**4** and **5**) were synthesized by substituting the compound (1*R*,2*R*)-1,2-bis(2′-phenylphenyl)ethane-1,2-diol (**1**) to the peripheral and non-peripheral positions of the phthalocyanine skeleton. Characterization of synthesized phthalocyanine compounds was performed by FT-IR, $^1$H NMR, UV-Vis and MS analyzes. It has been observed that the solubility of phthalocyanine compounds is high in commonly used organic solvents. By using dichloromethane, diethylether, chloroform, THF and DMF solvents, solutions of newly synthesized phthalocyanine compounds were prepared in the concentration range of $2x10^{-6}$ and $10x10^{-6}$ M, and aggregation behavior of phthalocyanine compounds in these solvents was examined by UV-Vis spectrophotometer. It was determined that both phthalocyanine compounds did not undergo aggregation in the solvents used. Detailed UV-Vis measurements of solutions at concentrations in the range of $2x10^{-6}$ and $10x10^{-6}$ M showed that phthalocyanine compounds obey Lambert-Beer law. In addition, the catalytic activities of phthalonitrile compounds **2** and **3**, and their zinc phthalocyanine derivatives **4** and **5** were tested by enantioselective diethylzinc addition to aldehydes. Enantioselectivity was not observed in any of the diethylzinc addition to aldehydes experiments using 5 different aldehydes and two different solvents, but conversions were obtained at certain ratios. The highest conversion was obtained as 34% in the reaction of diethylzinc addition to 2-methoxybenzaldehyde in toluene in the presence of compound **3**.

**Keywords:** Phthalocyanine, aggregation, $C_2$-symmetrical diol, catalysis, diethylzinc addition

## GİRİŞ

Ftalosiyaninler yaklaşık 100 yıl önce şans eseri sentezlenmiş ve yapıları X-ray analizi ile 65-70 yıl önce doğrulanmıştır (Byrne vd., 1934; Dent ve Linstead, 1934). Ftalosiyaninlerin tarihi dikkat çekici spektral özellikleri ile başlar (Linstead, 1934). Ftalosiyaninler ve bunların metal kompleksleri, kimyasal ve termal kararlılıklarından dolayı başlangıçta sadece boyar madde olarak kullanılmıştır (Erk ve Hengelsberg, 2003; Topuz vd., 2013). Bu boyaların renklerinin derinliği ve saflığı, görünür bölgede 670 nm civarında çoğunlukla molar absorptivite katsayısı $10^5$ L $mol^{-1}$ $cm^{-1}$'i aşan değerlerdeki absorpsiyon bandından kaynaklanmaktadır (Stilmann ve Thomson, 1974). Görünür bölgede bu şekilde şiddetli bir absorpsiyon yapan bu bileşik sınıfına, aksiyal ligandların koordine olabilmesi ve farklı metallerin bağlanabilmesi kristal ve çözeltilerinde renklerinin çeşitlenmesine sebep olmaktadır. Bu durumun da spektroskopi ve sentez ile ilgili kimyagerlerin dikkatini çekmesi kaçınılmazdı. Linstead'ın ftalosiyaninler üzerine ilk makaleleri incelendiği zaman, komplekslerin renklerinin merkez kaviteye bağlanan metal ile nasıl değiştiği görülebilmektedir (Byrne vd., 1934; Dent ve Linstead, 1934). Son yıllarda substitue ve substitue olmayan ftalosiyanin türleri sanayinin birçok dalında ve özellikle tıpta çok değişik kullanım alanları bulmuştur (Rosenthal, 1987; Ben-Hur ve Chan, 2003; Makhseed vd., 2013). Ftalosiyanin türevlerinin optik ve elektrokimyasal aktiviteleri ticari uygulamalar sahasında büyük umutlar vaat etmektedir. Kimyasal sensör geliştirilmesi, nano yapılı materyal hazırlanması, metalli ftalosiyaninler ile değiştirilmiş elektrotların üretilmesi ve fotodinamik kanser tedavisi bu çok yönlü boyar maddelerin araştırma alanları içerisinde yer almaktadır (Leznoff ve Lever, 1998; McKeown, 1998). Yakın bir zamanda, titanyum oksit yüzeyine etkin bir tutunma sağlayabilen hacimli grupların ftalosiyanin iskeletinin periferal konumlarına bağlı olduğu bir çinko(II) ftalosiyanin bileşiği, güneş enerjisini elektrik enerjisine %17'lik bir verimle dönüştürebilmiştir (Cho vd., 2017).

Ftalosiyanin metal komplekslerinin teknolojik ve bilimsel alanlardaki uygulanabilme kabiliyetlerini kısıtlayan en önemli unsur, bu bileşiklerin yaygın olarak kullanılan organik çözücüler içerisindeki sınırlı çözünürlükleridir. Periferal ve non-periferal konumlarda çözünürlüğü artırıcı grupların olduğu ftalosiyanin bileşiklerinin tasarlanması ve sentezlenebilmesi, bu bileşiklerin teknolojik ve bilimsel alanlardaki kullanılabilme kabiliyetlerini artırabilmek için oldukça önemlidir. Bu yeni uygulamaların birçoğu ftalosiyanin halkasının üzerinde yapısal kontrolü sağlamaktan geçmektedir. Bu amaçla, ftalosiyanin molekülünün periferal ve periferal olmayan konumlarında çeşitli yapısal değişikliklerin yapıldığı çok sayıda farklı çalışmalar olanca hızıyla devam etmektedir (Bilgin ve Gök, 2002; Bilgin vd., 2003; Maksheed vd., 2013; Cho vd., 2017). Ftalosiyanin bileşiklerinin birçok uygulama alanında artan önemine rağmen, ftalosiyanin halkasına optikçe aktif bir bileşiğin bağlanması ancak 1993 yılında rapor edilmiştir (Kobayashi vd., 1993) ve o tarihten beri, optikçe aktif bileşikler içeren ftalosiyaninlerin sentezine yönelik çalışmaların sayısı oldukça sınırlı kalmıştır. Sınırlı yapılan bu çalışmalarda, ftalosiyanin iskeletine kiral binaftol bağlanması üzerine yoğunlaşmıştır ve kiral gruplar çoğunlukla ftalosiyanin halkasının β pozisyonunda bulunmaktadır. Ftalosiyaninler çok farklı kullanım alanlarına sahip olmalarına karşın günümüzde en çok çalışılan enantiyoseçimli sentez ve oksidasyon reaksiyonlarında henüz yer almamaktadırlar.

Bu çalışma kapsamında, bir kiral $C_2$-simetrik diolün ftalosiyanin halkasının periferal ve non-periferal konumlarına substitue olması ile tetrasubstitue metalloftallosiyaninlerin sentezi ve bu metalloftalosiyaninlerin aldehitlere enantiyoseçimli dietilçinko katılmasındaki katalitik etkinliklerinin incelenmesi yapılmıştır. Bu amaç için kiral $C_2$-simetrik diol içeren ftalonitril türevleri ve $Zn^{2+}$ metal merkezini içeren metalloftalosiyanin türevleri sentezlenerek karakterize edilmiştir. Daha sonra, kiral ftalonitril bileşikleri ve bunların tetrasubstitue çinko ftalosiyanin (ZnPc) türevlerinin aldehitlere enantiyoseçimli dietilçinko katılmasında katalitik etkinlikleri test edilmiştir. Enantiyomerik aşırılık değerleri ve dönüşüm oranları kiral kolonlu yüksek performanslı sıvı (HPLC) ve/veya gaz kromatografisi (GC) ile belirlenmiştir. Bütün bileşiklerin yapıları $^1H$, $^{13}C$ NMR, UV-Vis, FT-IR, polarimetre ve kütle ölçümleri ile karakterize edilmiştir.

## MATERYAL VE METOT

FTIR spektrumları KBr disk tekniği kullanılarak Perkin Elmer Spectrum 65 spektrometresinde kaydedilmiştir. $^1H$ ve $^{13}C$ NMR spektrumları, $CDCI_3$ ve THF-$d_8$ (%99.9) kullanılarak Varian Mercury 400 MHz spektrometresinde alınmıştır. Kütle spektrumları, Micromass Quatro LC/ULTIMA LC–MS/MS ve Bruker Daltonics MALDI-TOF spektrometresinde ölçülmüştür. UV-Vis spektrumları, oda sıcaklığında 1 cm yol uzunluğunda küvetler kullanılarak PG-T80+ spektrofotometre ile kaydedilmiştir. Optik çevirme açıları Rudolph Autopol-I serisi polarimetre ile ölçülmüştür. GC analizleri Shimadzu GC-2010 Plus kullanılarak gerçekleştirilmiştir. Erime noktaları bir elektrotermal cihazla ölçülmüş ve doğrulanmamıştır. Tüm çözücüler

Perrin ve Armarego'da verilen yöntemelere göre kurutulup ve saflaştırılmıştır (Perrin ve Armerago, 1989). Kolon kromatografisi silika jel (Merck, Kieselgel 60, 400-630 mesh) üzerinden gerçekleştirilmiştir.

**Çinko ftalosiyanin komplekslerinin sentezi için genel yöntem**

2(3),9(10),16(17),23(24)-tetrakis-{((1′*R*,2′*R*)-2-hidroksi-1′,2′-di(2′′-fenilfenil)etoksi)}ftalosiyaninato çinko bileşiği **4** ve 1(4),8(11),15(18),22(25)-tetrakis-{((1′*R*,2′*R*)-2-hidroksi-1′,2′-di(2′′-fenilfenil)etoksi)}ftalosiyaninato çinko ftalosiyanin bileşiği **5**'in sentezlerini gerçekleştirmek için, uygun ftalonitril bileşiğinin (**2** veya **3**) 1 ekivalenti ile 0,5 ekivalent çinko asetat bir Schlenk tüpü içerisine yerleştirildi. Daha sonra üzerine 2-2,5 mL n-pentanol ve 3-4 damla DBU ilave edilerek 140 °C'de 24 saat süreyle karıştırıldı. Süre sonunda, reaksiyon içeriği bir balon içerisine alınarak çözücüsü döner buharlaştırıcıda düşük basınç altında uzaklaştırıldı. Kalan ham ürün flash silikajel kolon kromatografisi ve/veya ince tabaka kromatografisi yöntemlerinin biri veya birkaçının kullanılması ile saflaştırıldı

**2(3),9(10),16(17),23(24)-Tetrakis-{((1′R,2′R)-2-hidroksi-1′,2′-di(2′′-fenilfenil)etoksi)}ftalosiyaninato çinko(II) (4) bileşiğinin sentezi**

4-((1′*R*,2′*R*)-2-hidroksi-1′,2′-di(2′′-fenilfenil)etoksi)ftalonitril (**2**) (0,2 g; 0,406 mmol) ve $Zn(CH_3CO_2)_2$ (0,037 g; 0,203 mmol); reaksiyon süresi: 24 saat; silikajel kolondan metanol çözücü sistemi kullanılarak flash kromatografi ile saflaştırıldı. Yeşil renkli katı elde edildi. Verim: 0.051 (%21). en: 197–198 °C; IR (KBr disk) $\nu_{max}/cm^{-1}$: 3548, 3449 (OH), 3058, 2925, 1704, 1609, 1481, 1393, 1336, 1269, 1224, 1094, 1049, 990, 746, 702. $^1$H NMR (400 MHz, $d_8$-THF, ppm) δ: 9.23-9.19 (m, 6H), 8.95-8.85 (m, 6H), 7.78-6.82 (m, 72H), 5.97-5.89 (m, 4H), 5.64-5.55 (m, 4H) ppm; UV–Vis (DMF): $\lambda_{max}$, nm (log ε): 354 (5.06), 616 (4.66), 684 (5.39); MS (MALDI- TOF) (m/z): $C_{136}H_{96}N_8O_8Zn$ için hesaplanan: 2032.6 $[M+H]^+$, bulunan: 2033.6 $[M+H]^+$

**1(4),8(11),15(18),22(25)-Tetrakis-{((1′R,2′R)-2-hidroksi-1′,2′-di(2′′-fenilfenil)etoksi}ftalosiyaninato çinko(II) (5) bileşiğinin sentezi**

3-((1′*R*,2′*R*)-2-hidroksi-1′,2′-di(2′′-fenilfenil)etoksi)ftalonitril (**3**) (0,2 g; 0,406 mmol) ve $Zn(CH_3CO_2)_2$ (0,037 g; 0,203 mmol); reaksiyon süresi: 24 saat; ilk silikajel kolondan sırasıyla hekzan/ dietil eter (8:2), hekzan/ dietil eter (6:4), hekzan/ dietil eter (4:6) ve kloroform çözücü sistemleri kullanılarak alınan ürün, ikinci silikajel kolondan önce metanol ve ardından kloroform çözücü sistemi ile saflaştırıldı. Yeşil renkli katı elde edildi. Verim: 0.021 (%9). E.n: 243–244 °C; IR (KBr disk) $\nu_{max}/cm^{-1}$: 3352 (OH), 3058, 2925, 2853, 1699, 1588, 1483, 1334, 1261, 1090, 1020, 945, 746, 701. $^1$H NMR (400 MHz, $d_8$-THF, ppm) δ: 9.40-8.99 (m, 12H), 8.05-7.15 (m, 72H), 6.14-5.89 (m, 8H) ppm; UV–Vis (DMF): $\lambda_{max}$, nm (log ε): 322 (4.78), 632 (4.67), 702 (5.39); MS (MALDI- TOF) (m/z): $C_{136}H_{96}N_8O_8Zn$ için hesaplanan: 2032.6 $[M+H]^+$, bulunan: 2035.5 $[M+3H]^+$

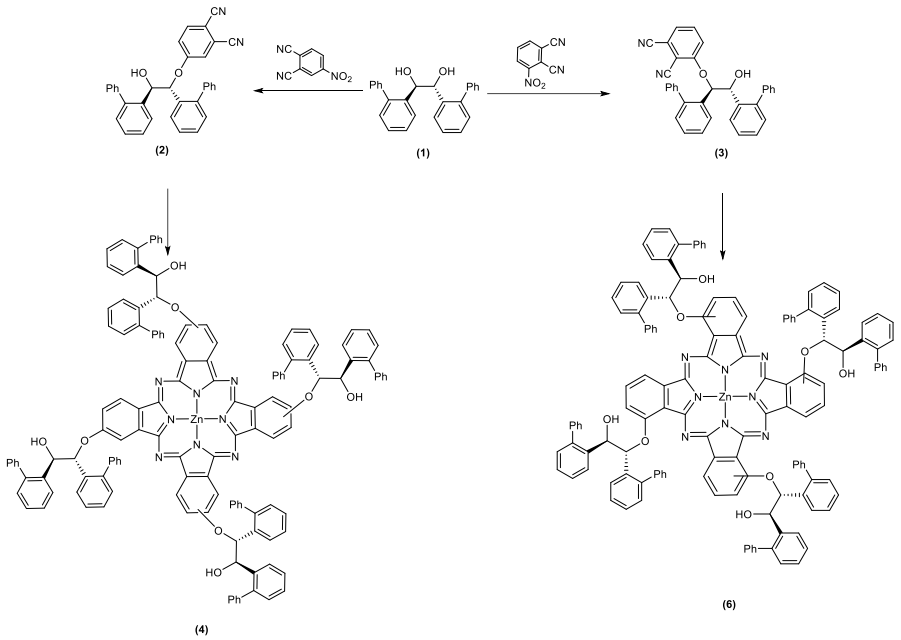
**Ftalonitril ve ftalosiyanin bileşiklerinin aldehitlere enantiyoseçimli dietilçinko katılmasındaki katalitik etkinliklerinin izlenmesi için genel yöntem**

Azot atmosferi altında bir schlenk tüpüne ligand (**2** ve **3** için %10 mol, **4** ve **5** için %2,5 mol) eklendi. Üzerine taze destillenmiş kuru çözücü (2,5 mL) eklendi ve oda sıcaklığında 5 dakika boyunca karıştırıldı. Karışan içeriğe, $Et_2Zn$ (2 mmol, 1 M hekzan çözeltisi) dikkat edilerek eklendi ve 20 dakika boyunca karışmaya bırakıldı. 20 dakika sonunda aromatik aldehit (1 mmol) şırınga yardımı ile çekilerek 10 dakika boyunca damla damla reaksiyon ortamına ilave edildi. Reaksiyon 24 saat boyunca karışmaya bırakıldı. Reaksiyon ortamı ince tabaka kromatografisi ile takip edildi. 24 saat sonunda reaksiyon ortamına doygun $NH_4Cl$ sulu çözeltisinden 1 mL ilave edildi. Bir süre karıştırıldıktan sonra distile su (25 mL) eklendi, içerik ayırma hunisine alındı. 15'er mL diklorometan ile 3 kez ekstrakte edildi. Organik fazlar birleştirildi ve $Na_2SO_4$ üzerinden kurutuldu. Kurutucuyu uzaklaştırmak için organik faz, por-4 krozeden süzüldü ve çözücüler düşük basınç altında kuruluğa kadar buharlaştırıldı. Ham ürün kolon kromatografisi ile saflaştırıldı. Elde edilen ürünlerin kiral GC analizi ile enantiyomerik aşırılıkları (% *ee*) belirlendi.

## BULGULAR ve TARTIŞMA

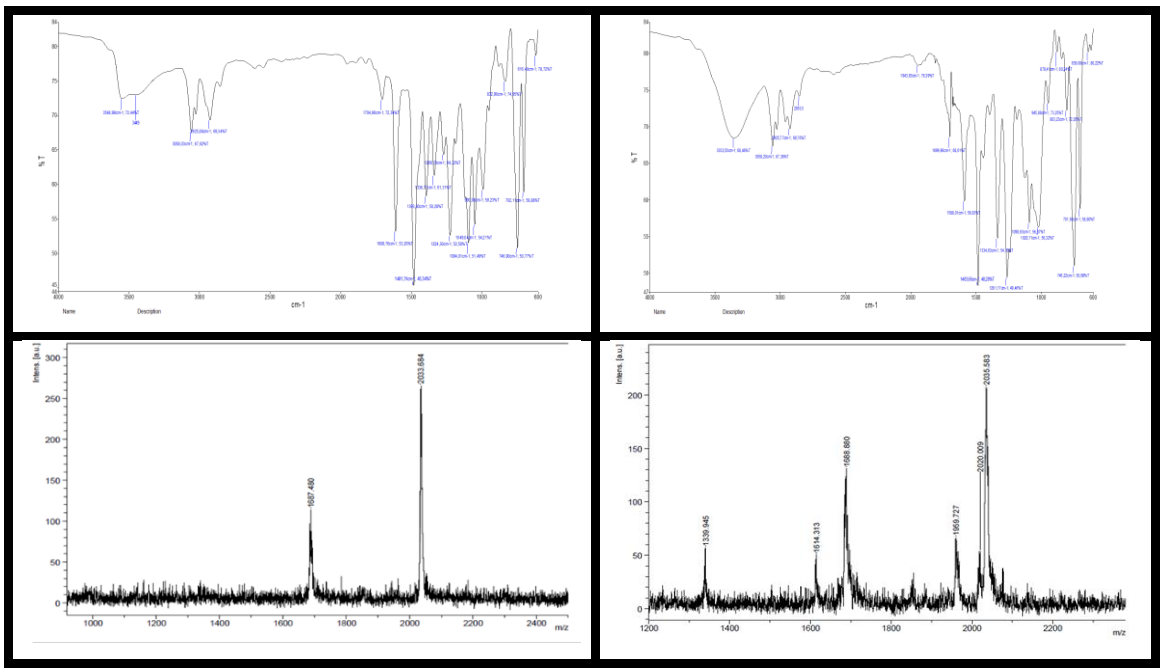
**Sentez ve karakterizasyon**

Periferal ve non-periferal $C_2$-simetrik diol tetrasubstitue çinko ftalosiyanin bileşikleri **4** ve **5**'in sentezlerine ait gösterim şekil 1'de verilmiştir. 3-Nitroftalonitril, 4-nitroftalonitril, (1*R*,2*R*)-1,2-bis(2′-fenilfenil)etan-1,2-diol **(1)**, 4-((1′R,2′R)-2-hidroksi-1′,2′-di(2′′-fenilfenil)etoksi)ftalonitril (**2**) ve 3-((1′R,2′R)-2-hidroksi-1′,2′-di(2′′-fenilfenil)etoksi)ftalonitril (**3**) bileşikleri literatürde kayıtlı yönetmelere göre hazırlanmıştır (George ve Snow, 1995; Young ve Onyebuagu, 1990; Gök vd., 2020).



**Şekil 1.** Çinko ftalosiyanin bileşikleri **4** ve **5**'in sentez yolu

Çinko ftalosiyanin bileşikleri **4** ve **5**, bir Schlenk tüpü içerisinde ilgili ftalonitril bileşiği (**2** veya **3**) ile susuz metal tuzu $Zn(CH_3CO_2)_2$'ın n-pentanol içindeki siklotetramerizasyonu reaksiyonu sonucu elde edilmişlerdir. Elde edilen ftalosiyanin bileşikleri silikajel kolon kromatografisi uygulanarak saflaştırılmıştır. Saflaştırma sonrası elde edilen bileşiklerin karakterizasyonları için FT-IR, UV-Vis, $^1$H NMR ve MS analizleri gerçekleştirildi. Periferal konumlardan substitue olan çinko ftalosiyanin bileşiği **4** ve non-periferal konumlardan substitue olan çinko ftalosiyanin bileşiği **5**'in alınan IR spektrumlarında dalga sayılarındaki küçük kaymalar dışında oldukça büyük benzerlikler gözlenmiştir (şekil 2). IR spektrumlarında C≡N grubuna ait 2230 cm$^{-1}$ civarlarındaki titreşiminin kaybolmuş olması ve 1610 cm$^{-1}$ civarlarında ftalosiyanin iskeletinde yer alan -C=N grubuna ait titreşim sinyallerinin ortaya çıkmış olması ftalonitril türevlerinin siklotetramerizasyonu sonucu ftalosiyanin iskeletlerinin oluştuğunun bir kanıtı olarak kabul edilebilir. Yine **4** ve **5** bileşiklerinin periferal ve non-periferal konumlarında bağlı substituentteki –OH grubuna ait sinyaller 3500-3400 cm$^{-1}$ civarlarında gözlenmiş olması önerilen yapılarla uygunluk göstermektedir.

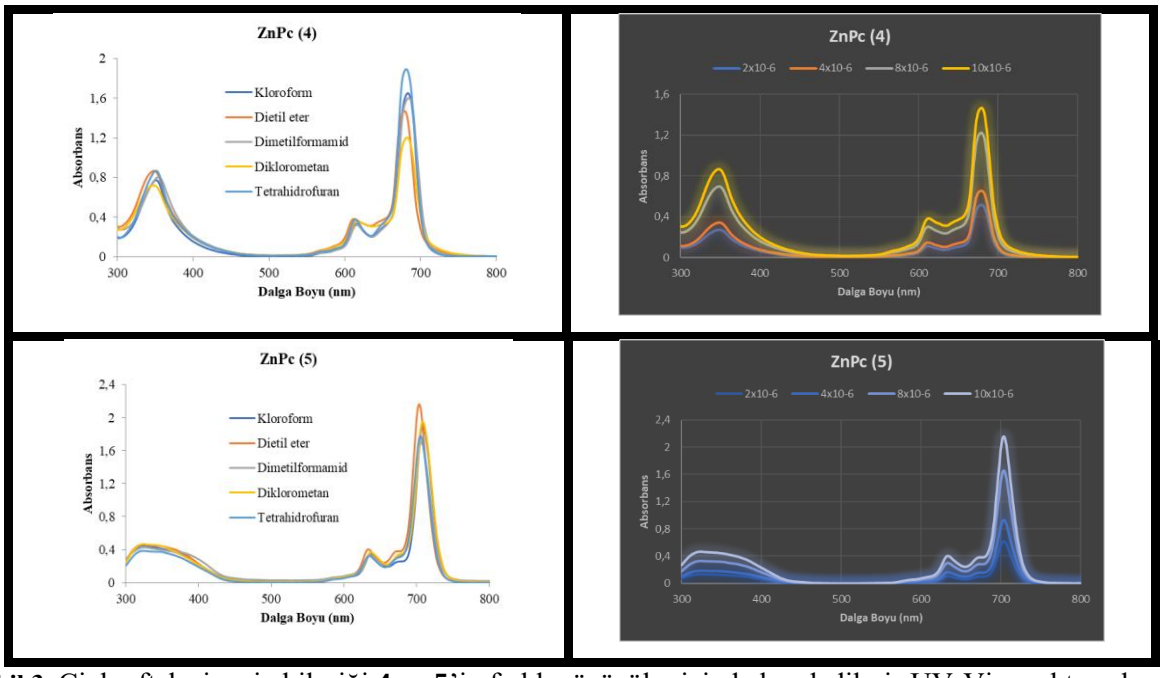



**Şekil 2.** Çinko ftalosiyanin bileşiği **4**'ün FT-IR spektrumu (sol üst) ve MS spektrumu (sol alt); çinko ftalosiyanin bileşiği **5**'in FT-IR spektrumu (sağ üst) ve MS spektrumu (sağ alt)

Sentezi gerçekleştirilen ftalosiyanin bileşikleri **4** ve **5**'in $^1$H NMR spektrumları $d_8$-THF'de kaydedilmiştir. Ftalosiyanin bileşikleri **4** ve **5**'in elde edilen spektrumlarında gözlenen sinyallerin kimyasal kayma değerlerindeki küçük kaymalar dışında başlangıç bileşikleri olan ftalonitril bileşiklerinin spektrumları ile büyük ölçüde benzerlik göstermektedir (Gök vd, 2020). Hem **4** hem de **5** bileşiğinin MALDI-TOF tekniği ile alınan kütle spektrumlarında bileşiklere ait moleküler iyon sinyallerinin (m/z), sırasıyla, 2033.5 $[M+H]^+$ ve 2035.5 $[M+3H]^+$ gözlenmesi önerilen yapılar ile uyum içindedir (şekil 2). FT-IR, $^1$H NMR ve MS analizlerinden elde edilen veriler, hedeflenen ftalosiyanin bileşikleri **4** ve **5**'in başarılı bir şekilde sentezlendiğini desteklemektedir.

**UV-Vis absorpsiyon spektroskopisi**

Sentezleri gerçekleştirilen ftalosiyanin bileşikleri **4** ve **5**'in, yaygın olarak kullanılan organik çözücüler olan diklorometan, kloroform, dietil eter, N,N-dimetilformamid ve tetrahidrofuran çözücüleri içinde çözünürlüklerinin iyi olduğu belirlenmiştir. Her bir ftalosiyanin bileşiğinin belirtilen bu beş farklı çözücü içerisinde çözündürülmesi ile belirli derişimlerde çözeltileri hazırlanmış ve bileşiklere ait ortaya çıkan absorbsiyon bantlarının yerleri belirlenmiştir. Metal ftalosiyaninlerde singlet Q bandı ve bunların metalsiz analoglarında yarılmış Q bandı gözlenmesi bu bileşikler için karakteristik bir özelliktir (Leznoff ve Lever, 1998). Substitue ve substitue olmayan metalli ftalosiyaninlerde singlet Q bandının gözlenmesi, bu bileşiklerin simetrisinin $D_{4h}$ olduğunun bir göstergesidir (Leznoff ve Lever, 1998). Ftalosiyanin bileşikleri **4** ve **5**, farklı çözücüler içinde alınan UV-Vis spektrumlarında şiddetli singlet Q bandının gözlenmesi bileşiklerin simetrisinin $D_{4h}$ olduğunu göstermektedir. $C_2$-simetrik diolün periferal konumlardan bağlanması ile elde edilen metal ftalosiyanin bileşiği **4** ile non-periferal konumlardan bağlanması ile elde edilen metal ftalosiyanin bileşiği **5**'in UV-Vis spektrumları incelendiğinde, non-periferal konumlardan susbtitue olmuş **5** bileşiğinin Q bandına ait absorpsiyon değerlerinin periferal analoğuna göre daha kırmızıya kaydığı gözlenmiştir (Şekil 3).




**Şekil 3.** Çinko ftalosiyanin bileşiği **4** ve **5**'in farklı çözücüler içinde kaydedilmiş UV-Vis spektrumları ile farklı derişimlerdeki dietileter çözeltilerinin kullanılması ile yapılan Lambert-Beer çalışması

α-Substitue çinko(II) ftalosiyanin bileşiğinin Q bandı absorpsiyonun β-substitue analoğunun Q bandı absorpsiyonuna göre kırmızıya kaydığının gözlenmesi literatür bilgisi ile uygunluk göstermektedir (Canlıca vd., 2011).

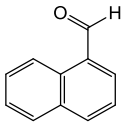
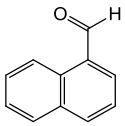
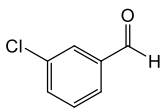
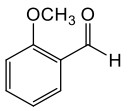
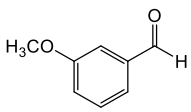
Ftalosiyanin bileşikleri **4** ve **5**'in agreagasyon davranışları diklorometan, kloroform, dietil eter, N,N-dimetilformamid ve tetrahidrofuran gibi çözücüler kullanılarak incelenmiştir. Farklı çözücülerde elde edilen UV-Vis spektrumları şekil-3'de verilmiştir. Ftalosiyanin bileşiklerinin farklı çözücüler içindeki Q bandın yerinin ve şiddetinin değişmiyor olması, bu bileşiklerin farklı çözücülerde agregasyona uğramadığını göstermektedir. Son olarak, bu bileşiklerin $2x10^{-6}$ ile $10x10^{-6}$ M derişimi aralığında dietileter çözücüsü içerisinde hazırlanan çözeltilerinin kaydedilen spektrumu incelendiğinde, artan derişim ile Q bandı absorpsiyonunun arttığı ve yüksek enerji tarafında herhangi bir yeni bandın oluşmadığı gözlenmiştir. Bu

bileşiklerin çalışılan derişim aralığında agregasyona uğramadıkları ve Lambert-Beer kanununa uydukları belirlenmiştir.

**Katalitik etkinlik çalışması**

Ftalonitril bileşikleri **2** ve **3** ile bu bileşiklerin çinko ftalosiyanin türevleri **4** ve **5**'in katalitik etkinlikleri aldehitlere enantiyoseçimli dietilçinko katılması reaksiyonunda test edilmiştir. Substrat olarak 5 farklı aldehit ve iki farklı çözücü ile denemeler gerçekleştirilmiştir. Bileşikler **2-5**'in varlığında 5 farklı aldehit ve iki farklı çözücü kullanılarak yapılan aldehitlere dietilçinko katılması denemelerinde enantiyoseçimlilik gözlenmemiş fakat belli oranlarda dönüşümler elde edilmiştir (Tablo 1). Çözücü değişiminin katalitik aktivite ve enantiyoseçimlilik üzerine kayda değer bir etkisi gözlenmemiştir. Her iki çözücüde elde edilen dönüşüm oranları birbirine oldukça yakındır. Ftalonitril bileşikleri **2-3**'ün varlığındaki denemelerde dönüşüm oranları %11-34 aralığında elde edilmiştir. En yüksek dönüşüm oranı, **3** bileşiği varlığında toluen içerisinde gerçekleştirilen 2-metoksibenzaldehite dietilçinko katılması reaksiyonunda %34 olarak elde edilmiştir. Ftalosiyanin bileşikleri **4** ve **5**'in katalitik etkinliklerini test etmek için çözücü olarak çözünürlüklerinin daha iyi olduğu diklorometan çözücü kullanılmıştır. Diklorometan içinde 3-metoksibenzaldehite dietilçinko katılması reaksiyonunda, **4** ve **5** katalizör olarak kullanıldığında elde edilen dönüşüm oranları %21 olarak kaydedilmiştir.

**Tablo 2.** Ftalonitril bileşikleri (**2, 3**)[a] ve ftalosiyanin bileşiklerinin (**4, 5**)[b] varlığında aldehitlere enantiyoseçimli dietilçinko katılması.

| Deneme | Aldehit | Ligand | Çözücü | Dönüşüm (%)[c] | ee (%)[d] |
|---|---|---|---|---|---|
| **1** |  | **2** | Diklorometan | 12 | ras |
| **2** | | **2** | Toluen | 13 | ras |
| **3** | | **3** | Diklorometan | 18 | ras |
| **4** | | **3** | Toluen | 12 | ras |
| **5** |  | **2** | Diklorometan | 16 | ras |
| **6** | | **2** | Toluen | 23 | ras |
| **7** | | **3** | Diklorometan | 22 | ras |
| **8** | | **3** | Toluen | 18 | ras |
| **9** |  | **2** | Diklorometan | 11 | ras |
| **10** | | **2** | Toluen | 14 | ras |
| **11** | | **3** | Diklorometan | 26 | ras |
| **12** | | **3** | Toluen | 15 | ras |
| **13** |  | **2** | Diklorometan | 17 | ras |
| **14** | | **2** | Toluen | 24 | ras |
| **15** | | **3** | Diklorometan | 31 | ras |
| **16** | | **3** | Toluen | 34 | ras |
| **17** |  | **2** | Diklorometan | 13 | ras |
| **18** | | **2** | Toluen | 16 | ras |
| **19** | | **3** | Diklorometan | 25 | ras |
| **20** | | **3** | Toluen | 28 | ras |
| **21** | | **4** | Diklorometan | 21 | ras |
| **22** | | **5** | Diklorometan | 21 | ras |

[a] Reaksiyon şartları: 1 ekivalent aldehit, 2 ekivalent $Et_2Zn$, 10 mol% ligand, oda sıcaklığı, 24 h. [b] 2.5 mol% ftalosiyanin. [c] Dönüşüm oranı. [d] HP-CHIRAL-20B kolon kullanılarak GC ile analiz edilmiştir.

## SONUÇ

Bu çalışma kapsamında literatürde kayıtlı olmayan bir adet periferal tetrasubtitue (**4**) ve bir adet non-periferal tetrasubstitue (**5**) çinko ftalosiyanin olmak üzere iki adet yeni ftalosiyanin bileşiği sentezlenmiştir. Bu çalışma ile periferal ve non-periferal konumlarda optikçe aktif $C_2$-simetrik kiral diollerin substitue olduğu ftalosiyanin komplekslerinin sentezlenmesi ile optikçe aktif ftalosiyaninlere yeni örneklerin katılması sağlanmıştır. Sentezlenen ftalosiyanin bileşiklerinin farklı organik çözücüler içerisinde iyi çözünebildikleri ve agregasyona uğramadığı belirlenmiştir. Ftalonitril bileşikleri ve bunların ftalosiyanin türevlerinin katalizör olarak katalitik etkinlikleri aldehitlere enantiyoseçimli dietilçinko katılması reaksiyonunda test edilmiştir. Yapılan denemelerde enantiyoseçimlilik elde edilememiş ancak düşük-orta oranlarda dönüşüm elde edilebilmiştir.

**TEŞEKKÜR**

Bu çalışma TÜBİTAK tarafından 1001-Bilimsel ve Teknolojik Araştırma Projelerini Destekleme Programı kapsamında desteklenmiştir (Proje No: MAG-115R015). Osmaniye Korkut Ata Üniversitesinden İlker Ümit Karayiğit ve Seda Kılıçarslan'a desteklerinden dolayı teşekkür ederiz.

**KAYNAKLAR**

Ben-Hur, E., Chan, W. S. 2003. *The Porphyrin Handbook: Applications of Phthalocyanines*. Kadish, K. M., Smith, K. M., Guilard, R. Elsevier.

Bilgin, A., Gök, Y. 2002. *Synthesis and Characterization of Novel Metal-Free Phthalocyanines Substituted with Four Diazadithiatetraoxa or Diazahexaoxamacrobicyclic Moieties.* Tetrahedron Letters, 43: 5343-5347.

Bilgin, A., Gök, Y., Ertem, B. 2003. *Synthesis and characterization of novel metal-free phthalocyanines containing four cylindrical or spherical macrotricyclic moieties*, Tetrahedron Letters, 44: 6937-6941.

Byrne, G.T., Linstead, R.P., Lowe, A.R. 1934. *Phthalocyanines. Part II. The Preparation of Phthalocyanine and Some Metallic Derivatives from o-Cyanobenzamide and Phthalimide.* Journal of Chemical Society, 1017.

Canlıca, M., Booysen, I.N., Nyokong, T. 2011. *Synthesis and electrochemical behavior of novel peripherally and non-peripherally substituted ball-type cobalt phthalocyanine complexes.* Polyhedron, 30: 522-528.

Cho, K. T., Trukhina, O., Roldán-Carmona, C., Ince, M., Gratia, P., Grancini, G., Gao, P., Marszalek, T., Pisula, W., Reddy, P. Y., Torres, T., Nazeeruddin, M. K. 2017. "*Molecularly Engineered Phthalocyanines as Hole-Transporting Materials in Perovskite Solar Cells Reaching Power Conversion Efficiency of 17.5%*. Advanced Energy Materials, 7: 1601733.

Dent, C. E., Linstead, R. P. 1934. *Phthalocyanines. Part IV. Copper Phthalocyanines*. Journal of Chemical Society, 1027.

Erk, P., Hengelsberg, H. 2003. *The Porphyrin Handbook: Applications of Phthalocyanines*. Kadish, K. M., Smith, K. M., Guilard, R. Elsevier.

George, R. D., Snow, A. W. 1995. *Synthesis of 3-nitrophthalonitrile and tetra-α-substituted phthalocyanines.* Journal of Heterocyclic Chemistry, 32: 495–498.

Gök, H. Z., Gök, Y., Yılmaz M. K. 2020. "*Oxidation of benzyl alcohol by novel peripherally and non-peripherally modular C2-symmetric diol substituted cobalt(II) phthalocyanines*". Applied Organometallic Chemistry, 1–14.

Kobayashi, N., Kobayashi, Y., Osa, T. 1993. *Optically active phthalocyanines and their circular dichroism.* Journal of American Chemical Society, 115: 10994-10995.

Leznoff, C.C., Lever, A. B. P. 1998. *Phthalocyanines: properties and applications.* vols.1-4. VCH, Weinheim.

Makhseed, S., Machacek, M., Alfadly, W., Tuhl, A., Vinodh, M., Simunek, T., Novakova, V., Kubat, P., Rudolfe, E., ve Zimcik, P. 2013. *Water-soluble non-aggregating zinc phthalocyanine and in vitro studies for photodynamic therapy.* Chemical Communication, 49: 11149-11151.

McKeown, N. B. 1998. *Phthalocyanine materials-synthesis, structure and function*. Cambridge University Press, Cambridge.

Perrin, DD, Armarego, WFL 1989. *Purification of Laboratory Chemicals, Second ed., Pergamon, Oxford, UK* 1989.

Rosenthal, I., Ben-Hur, E., ve Greenberg, S., Concepcion-Lam, A., Drew, D., Leznoff, C.C. 1987. *The effect of substituents on phthalocyanine photocytotoxicity*. Photochemistry and Photobiology, 46: 959-963.

Stilmann, M. J., ve Thomson, A. J. 1974. *Assigment of the Charge-transfer Bands in Some Metal Phthalocyanines. Evidence for the S=1 state of Iron(II) Phthalocyanine in Solution*. Journal of Chemical Society, Faraday Transaction II, 70: 790-804.

Topuz, B. B., Gündüz, G., Mavis, B., Çolak, Ü. 2013. *Synthesis and characterization of copper phthalocyanine and tetracarboxamide copper phthalocyanine deposited mica-titania pigments.* Dyes and Pigments, 96: 31-37.

Young, J. G., Onyebuagu, W. 1990. *Synthesis and Characterization of Di-disubstituted Phthalocyanines.* Journal of Organic Chemistry, 55: 2155-2159.

## *ORAL PRESENTATION*

# Kurkumin yüklü periyodik mezogözenekli organosilika nanotaşıyıcının sentezi ve ilaç salım özelliklerinin incelenmesi

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## Özet

Kurkumin doğal bir ürün olması ve gösterdiği anti-kanser özelliklerinden dolayı araştırmacılar tarafından son yıllarda yoğun bir şekilde çalışılmaktadır. Ancak, kurkuminin biyoyararlanımının ve kararlılığının oldukça zayıf olduğu belirlenmiştir. Bu durum kurkuminin kanser tedavisinde kullanımını oldukça sınırlandıran ciddi bir problem olarak karşımıza çıkmaktadır. Kurkuminin kullanımını kısıtlayan sorunların çözümü ve kurkuminin terapötik etkisini artırmak için, kontrollü/hedefe yönelik ilaç salımı çalışmalarında başarı gösteren mezogözenekli silika yapıların kullanılması bir çözüm olarak ortaya çıkmıştır. Bu çalışmada, kurkuminin kanser tedavisindeki çalışmalarda kullanımını kısıtlayan sorunların çözümü ve kurkuminin terapötik etkisini artırmak için periyodik mezogözenekli bir organosilika ilaç taşıyıcının (**PMO**) sentezi gerçekleştirilmiştir. Sentezlenen **PMO**'nun karakterizasyonu FT-IR, XRD, BET, TEM, SEM ve TGA analizleri ile yapılmıştır. İlaç taşıyıcı sistem **PMO**'ya kurkumin yüklemesi, kurkumin ve **PMO**'nun 24 saat karanlıkta karıştırılması ile gerçekleştirilmiştir. UV-Vis spektrofotometrede yapılan ölçümler sonrası, **PMO** için yükleme yüzdesi (%EE) ve yükleme kapasitesi (%LC) sırasıyla %13 ve %11,5 olarak hesaplanmıştır. Daha sonra, fizyolojik (pH =7,4) ve endosomal (pH = 5) pH'da zamana bağlı **Cur@PMO**'dan kurkumin salımı çalışılmıştır. 4 gün sonunda yapılan UV-Vis ölçümleri sonrası, **Cur@PMO**'dan kurkumin salım yüzdesi pH = 5'de %5 ve pH = 7,4'de %6 olarak bulunmuştur. Elde edilen düşük salım yüzdesi, **PMO** ile **PMO**'nun porları içine giren kurkumin arasında kuvvetli etkileşimin olduğuna işaret etmektedir. Bu kuvvetli etkileşim, ilaç taşıyıcı sistemi **PMO**'nun por kapatma ajanları olmaksızın taşıdığı kargonun %94'nü pH = 5 ve 7,4'de sızdırmadan taşıyabilmesine imkan vermiştir.

**Anahtar Kelimeler:** İlaç taşıyıcı sistem, periyodik mezogözenekli organosilika, ilaç salım

# Synthesis of curcumin loaded periodic mesoporous organosilica nanocarrier and investigation of drug release properties

## Abstract

Due to the fact that curcumin is a natural product and its anti-cancer properties, it has been studied extensively by researchers in recent years. However, the bioavailability and stability of curcumin were determined to be quite weak. This is a serious problem that limits the use of curcumin in cancer treatment. In order to solve the problems that limit the use of curcumin and to increase the therapeutic effect of curcumin, the use of mesoporous silica structures that have been successful in controlled/targeted drug release studies has emerged as a solution. In this study, a periodic mesoporous organosilica drug carrier (**PMO**) was synthesized in order to solve the problems that limit the use of curcumin in cancer treatment studies and to increase the therapeutic effect of curcumin. The characterization of the synthesized **PMO** was performed by FT-IR, XRD, BET, TEM, SEM and TGA analyses. Curcumin loading into the drug delivery system **PMO** was carried out by mixing curcumin and **PMO** in the dark for 24 hours. After the measurements in the UV-Vis spectrophotometer, encapsulation efficiency (%EE) and loading capacity (%LC) for **PMO** were calculated as 13% and 11.5%, respectively. Next, the time dependent release of curcumin from **Cur@PMO** at physiological (pH =7.4) and endosomal (pH = 5) pH was studied. After UV-Vis measurements at the end of 4 days, the percentage of curcumin release from **Cur@PMO** was calculated as 5% at pH = 5 and 6% at pH = 7.4. The low release percentage obtained indicates a strong interaction between **PMO** and curcumin in the pores of the **PMO**. This

strong interaction allowed the drug delivery system **PMO** to carry 94% of the cargo without leaking at pH = 5 and 7.4 without pore closing agents.

**Keywords:** Drug delivery system, periodic mesoporous organosilica, drug release

## GİRİŞ

Son on yıldır, yeni ve güçlü ilaç taşıyıcı sistemler üzerine yapılan çalışmaların sayısı oldukça hızlı bir şekilde artmaktadır. Özellikle ilaçların kontrollü salımı üzerine yeni yaklaşımlar ve metotlar geliştirilmektedir (Croissant, vd., 2015; Manzano & Vallet-regí, 2020; Vallet-Regí vd., 2018; Bagheri vd., 2018; Noureddine & Je, 2018; T. Sun vd., 2014). Nanoteknolojideki gelişmelerin tıp içinde kullanılmaya başlamasıyla nanotıp ortaya çıkmıştır. Nanotıp, ilaç taşınım sistemleri konusunda yapılan çalışmalar ile son yıllarda oldukça dikkat çeken bir alandır. Nanotıp sayesinde ilaç taşıyıcı sistemlerde yeni gelişmeler kaydedilmiştir. Bu taşıyıcı sistemler sayesinde, ilacın taşıyıcı sistem içerisine yerleştirilmesi ile aktif veya pasif yönlendirme şeklinde dağıtımı sağlanabilmektedir. Böylece, ilaçların yan etkisi en aza indirilmeye başlanmıştır. İdeal bir ilaç taşınım sisteminin temel amacı, ilacı tek bir dozdan sonra istenen terapötik aralıkta tutmak ve / veya ilacı belirli bir bölgeye hedeflerken aynı zamanda ilacın sistemik seviyelerini ayarlayabilmektir. İlaç taşınım sistemleri olarak günümüze kadar, lipozomlar, polimerler, polimerozomlar, silika nanotanecikler ve hidrojeller gibi farklı sistemler farklı formülasyonlar ile beraber kullanılmıştır. Bu sistemlerden bazıları Amerikan Gıda ve İlaç dairesi (FDA) tarafından da onaylanmıştır. Ancak bu taşıyıcı sistemlerin bazılarının uzun dönemli kullanımlarında ciddi sıkıntılar ortaya çıkabilmektedir. Örneğin, doza bağımlı toksisite, ilaç sızıntısı, kararlılık ve biyouyumluluk karşılaşılan sorunlardan bazılarıdır.

Mevcut tüm nanomalzemeler değerlendirildiğinde, mezogözenekli silika (Si-O-Si) yapılı nanotaşıyıcı (MSN) malzemeler yukarıda anılan diğer malzemeler arasından en umut vaat edici olanıdır. Bunun nedeni ise, bu malzemelerin farklı morfolojilere, farklı parçacık boyutuna, farklı por çapına ve farklı yüzey özelliklerine sahip olarak sentezlenebilmeleridir. MSN'ler içerdikleri kuvvetli Si-O bağlarından dolayı diğer nanotaşıyıcılara kıyasla bozunmaya ve mekaniksel baskıya karşı daha dayanıklıdırlar (Bharti vd., 2015). Ayrıca, FDA ve EFSA silikatların günlük 1500 mg'a kadar alınmasının insan sağlığına zararının olmadığını da bildirmiştir (Diab vd., 2017). Nanotaşıyıcılar içinde 2 nm ila 50 nm arasında değişen gözenek boyutuna sahip mezogözenekli silika nanoyapılar (MSN) ilaç dağıtımı ve biyomedikal uygulamalar için mükemmel adaylar olarak kabul edilmektedirler (Giri vd., 2007). İlaç taşıyıcı sistem olarak kullanılacak silika yapılı nanomalzemeler ile ilacın kimyasal kompozisyonunun uyumu oldukça önemlidir (Yang vd., 2019). Kullanılan terapötik ilaçlar, sudaki çözünürlüklerine bağlı olarak hidrofobik ve hidrofilik olmak üzere iki ana gruba ayrılır. Belirli bir ilaç türü için taşıyıcı olarak kullanılacak nanotaşıyıcıları seçerken veya tasarlarken, optimal bir kapsülleme verimliliği ve arzu edilen salım profili elde etmek için ilacın özelliklerini ve davranışlarını bilmek kritik önem taşır (Sun vd., 2014). Günümüzde kullanılan ilaçların çoğunluğu hidrofobiktir. Çünkü bu ilaçlar hücre içine varıncaya kadar geçtikleri sulu ortamlarda çözünmeden ilerleyerek hücre zarından geçebilme özelliğine sahiptir. Ancak suda çözünürlüklerinin az olmasından dolayı da ilaç uygulama konsantrasyonları çok düşük olabilmektedir. Uygulama konsantrasyonlarını artırabilmek için hidrofobik ilaçların nanotaşıyıcılar içinde taşınması durumunda sulu ortamdaki konsantrasyonları 1000 katına kadar artırılabileceği rapor edilmiştir (Huh vd., 2005). Klasik tedavide ve kanser tedavilerinde hidrofilik ilaçlar da oldukça sık kullanılmaktadır. Hidrofilik karaktere sahip yapıların hücre lipid zarından geçmesi oldukça zor olduğundan hücre içindeki biyobozunurlukları oldukça düşük olmaktadır. Ayrıca vücut içerisinde çok çabuk hidrolitik bozunmaya uğramaktadırlar. Bu ilaçların sistemik dolaşım sürelerini artırmak ve hücre zarından geçişini sağlamak için silika yapılı nanotaşıyıcılar oldukça etkili olmaktadır (Croissant vd., 2016). Hidrofilik ilaçların yüklenmesinde hidrofilik karakteri daha baskın olan mezogözenekli silika nanotanecikler (MSN) yapılar daha çok tercih edilirken, hidrofobik karakterdeki ilaçların taşınımı için hidrofilik/hidrofobik oranı ayarlanabilir olan mezogözenekli organosilika nanotanecikler (MON) ve periyodik mezogözenekli silika nanotanecikler (PMO) daha uygun bir alternatif olarak ortaya çıkmaktadır. MON veya PMO sentezi sırasında silika şebekeye katılacak organik yapının oranı değiştirilerek nanotaşıyıcının hidrofobik karakter yoğunluğu ilaca uygun olacak şekilde ayarlanabilmektedir (Croissant vd., 2016). Böylece, hidrofobik karakteri artırılan MON ve PMO ile hidrofobik ilaç arasında daha kuvvetli etkileşim olmakta ve ilaç sızması da engellenebilmektedir. Bu yaklaşıma uygun bir çalışma, 2016 yılında 1,4-bis(trietoksisilil)benzenin kullanılması ile bir PMO elde edilmiştir. Elde edilen bu PMO'ya hidrofilik doksorubisin hidroklorür (DOX) %56 ve kamptotesin (CPT) %64 oranında yüklenebilmiştir (Croissant vd., 2016).

Araştırmacıların son yıllarda üzerinde çokça çalıştığı doğal ürünlerden biri kurkumindir. Kurkumin (1,7-bis (4-hidroksi-3-metoksifenil)-1,6-heptadien-3,5-dion) zencefil ailesine ait çok yıllık bir bitki olan zerdeçalın (Curcuma longa) en aktif bileşenidir. Zerdeçal, Hindistan ve Çin başta olmak üzere Güney Asya ülkelerinde yaygın olarak medikal uygulamalar, gıda aroması ve kumaş boyama gibi çeşitli amaçlarla geleneksel olarak kullanılmaktadır (Hatcher vd., 2008). Kurkumin 2000 yılı aşkın bir süredir Çin ve Hindistan'da diyabet ve karaciğer fonksiyon bozuklukları gibi farklı hastalık gruplarının tedavisi için geleneksel tıpta yaygın olarak kullanılmıştır (Zhou vd., 2011). Bu nedenle, son yıllarda *Curcuma longa* ve onun ekstraktlarının biyolojik aktivitesi ve farmakolojik özelliklerini belirlemek üzere yapılan çalışmaların sayısı oldukça artmıştır. Kurkuminin kullanımını kısıtlayan sorunların çözümü ve kurkuminin terapötik etkisini artırmak için, kontrollü/hedefe yönelik ilaç salımı çalışmalarında başarı gösteren mezogözenekli silika yapıların kullanılması bir çözüm olarak ortaya çıkmıştır (Chen vd., 2018). Mezogözenekli silika yapılarda meydana gelen gelişmelere paralel olarak, kurkumin ile ilgili sorunların çözümüne yönelik çalışmalar da artmaya başlanmıştır. Son birkaç yıldır kurkuminin MSN'lere yüklenmesi ile terapötik etkilerinin incelenmesine yönelik çalışmaların sayısı artmıştır.

Yüksek ilaç yükleme kapasitesine sahip olduğu yapılan çalışma ile ortaya konan 1,4-bis(trietoksisilil)benzenden başlanarak sentezlenen **PMO** bu çalışma kapsamında tekrar sentezlenmiş ve bu ilaç taşıyıcı sisteme kurkumin yüklemesi yapılarak ilaç salım profili incelenmiştir.

## MATERYAL VE METOT

1,4-bis(trietoksisilil)benzenden başlanarak yapılan **PMO** sentezi literatürde kayıtlı yöntem takip edilerek gerçekleştirilmiştir (Croissant vd., 2016). Setiltrimetilamonyum bromür (CTAB), 1,4-bis(trietoksisilil)benzen ve diğer tüm reaktifler ticari tedarikçilerden (Merck veya Fluka) elde edildi. Deneyler sırasında kullanılan tüm malzemeler analitik saflıktaydı. Bu çalışmada kullanılan çözücülerin kurutulması ve saflaştırılması, Perrin ve Armarego'daki prosedür izlenerek gerçekleştirilmiştir (Perrin ve Armerago, 1989). Kızılötesi spektroskopi, Fourier dönüşümü kızılötesi spektroskopisi (FT-IR), KBr pelet tekniği kullanılarak Perkin Elmer spektrum 65 Fourier kızılötesi spektrometresi kullanılarak yapıldı. X-ışını kırınımı (XRD) ölçümü, 1°–10° ve 10°–80° saçılma açısı 2θ aralığında Cu-Kα radyasyonu (λ = 0.154056 nm) kullanılarak bir PANalytical Empyrean X-ışını kırınım ölçerde gerçekleştirildi. Örneklerin alan emisyon taramalı elektron mikroskobu (TEM) görüntülerini kaydetmek için JEOL JEM-1400 PLUS cihazı kullanıldı. Termogravimetrik analiz (TGA) ölçümleri, Seiko SII TG/DTA 7200 cihazında gerçekleştirilmiştir. TGA analizi sırasında $N_2$ gazının akış hızı 2 mL $dk^{-1}$ ve ısı hızı 10 °C $dk^{-1}$ idi. $N_2$ sorpsiyon izotermleri, Micrometrics Surface Area ve Porosity Tristar II analizöründe kaydedildi. Mezogözenekli malzemelerin yüzey alanını hesaplamak için Brunauer–Emmett–Teller (BET) yöntemi, gözenek boyutu dağılımını hesaplamak için Barrett, Joyner ve Halenda (BJH) yöntemi kullanıldı. İlaç yükleme ve salım deneylerindeki ölçümler için Optima 3000 marka UV-Vis spektrofotometre kullanıldı.

### Mezogözenekli organosilika nonataşıyıcının sentezi (PMO)

Kısaca, üç boyunlu bir balona CTAB (0.758 g, 2.08 mmol) ve ultra saf su (363 mL) konularak 40 °C sıcaklıkta berrak çözelti oluşuncaya kadar karıştırıldı. Üzerine 2 M NaOH çözeltisinden 2.7 mL eklenerek sıcaklık 80 °C'ye getirildi. Bu sıcaklıkta 1 saat karıştırıldı. Süre sonunda 1,4-bis(trietoksisilil)benzen (BTSB) (3,02 g, 7,7 mmol, 2.75 ml) şırınga ile tek seferde reaksiyon ortamında eklendi. Ekleme sonrası reaksiyon içeriği 80 °C de 2 saat daha karıştırıldı. Süre sonunda karıştırıcının sıcaklığı kapatılarak reaksiyon karıştırılmaya devam edildi. Reaksiyon sıcaklığının oda sıcaklığına gelmesi beklendi. Oda sıcaklığına soğuyan reaksiyon içeriği krozeden süzüldü. Krozede kalan katı kısım önce saf su ile sonra etil alkol ile yıkanarak vakum altında kurutuldu **(CTAB@PMO)**. Elde edilen **CTAB@PMO** yapısından CTAB yi uzaklaştırmak için, **CTAB@PMO** malzemesi $NH_4NO_3$'ın etanol içerisinde hazırlanan çözeltisi (6 g/L) ile 45 °C de 30 dakika süre ile 2 kez sonikasyon yapıldı. Sonikasyon sonrası süzülen katı 3 kez etil alkol, 1 kez su ve tekrar alkol ile yıkandı. Elde edilen katı kısım vakum altında kurutuldu.

### İlaç yükleme (Cur@PMO)

Kurkuminin **PMO**'ya yüklemesinin yapılabilmesi için, kurkuminin stok çözeltisinden (4 mg kurkumin/mL etil alkol) 25 mL alındı, içerisine 100 mg **PMO** ilave edilerek bir sonikatör yardımıyla disperse olması sağlandı. Dispersiyon sonrası elde edilen çözeltiler oda sıcaklığında ve karanlıkta 24 saat süresince karıştırıldı. Süre sonunda karışım santrifüj edilerek mezoyapılı **PMO** ilaç yükleme çözeltisinden ayrıldı. Kalan katı kısım 20 mL etil alkol ile yıkanıp, santrifüjlendi. Elde edilen katı kısımlar birleştirilerek vakum altında kurutuldu.

Yüklenen ilaç miktarı ve yükleme kapasitesi, yükleme ve yıkama çözeltilerinin absorbansının UV-Vis spektrofotometrede λ = 420 nm'de okunması sonrası Eşitlik 1 ve 2'nin kullanılması ile hesaplandı (Chen vd., 2018; Sun vd., 2019)

$$Yüklenen\ ilaç\ (\%) = \frac{yüklenen\ ilaç\ (mg)}{ilacın\ çözeltideki\ ilk\ miktarı\ (mg)} x100 \quad (1)$$

$$Yükleme\ kapasitesi\ (\%) = \frac{yüklenen\ ilaç\ (mg)}{yüklenen\ ilaç\ (mg) + PMO\ miktarı\ (mg)} x100 \quad (2)$$

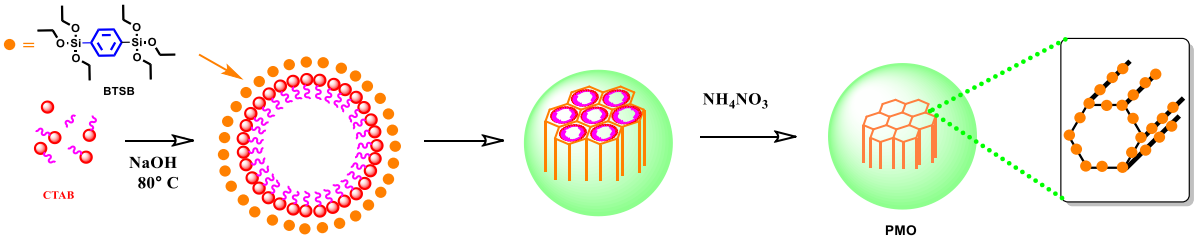
**İlaç salım (Cur@PMO)**

İlaç yüklenmiş olan **Cur@PMO** yapısından ilaç salım çalışmaları literatürde verilen yöntemlere göre yapılmıştır (Sun vd., 2014; Palanikumar vd., 2015). **Cur@PMO** (pH = 7,4 için 1,5 mg ve pH = 5 için 1,2 mg) yapıları %10 (v/v) tween-80 içeren 10 mL PBS (pH = 7.4 ve pH = 5) tamponunda disperse edilerek 37 °C'de ve 100 rpm'de karıştırılmıştır. Belirli aralıklarla ortamdan numune alınarak 12.000 rpm de 15 dakika santrifüjlendikten sonra süpernatant kısmının absorpsiyonu UV-Vis spektrofotometrede 420 nm'de okundu. Okuma sonrası süpernatant kısmı tekrar ilaç salım ortamına geri ilave edilerek karıştırılmaya devam edildi. Okunan absorpsiyon değeri standart çalışma grafiğine yerleştirilerek zaman bağlı salınan ilaç miktarı belirlenmiştir. Zaman bağlı olarak elde edilen veriler grafiğe geçirilerek mezoyapıların ilaç salım profilleri belirlenmiştir.

## BULGULAR ve TARTIŞMA

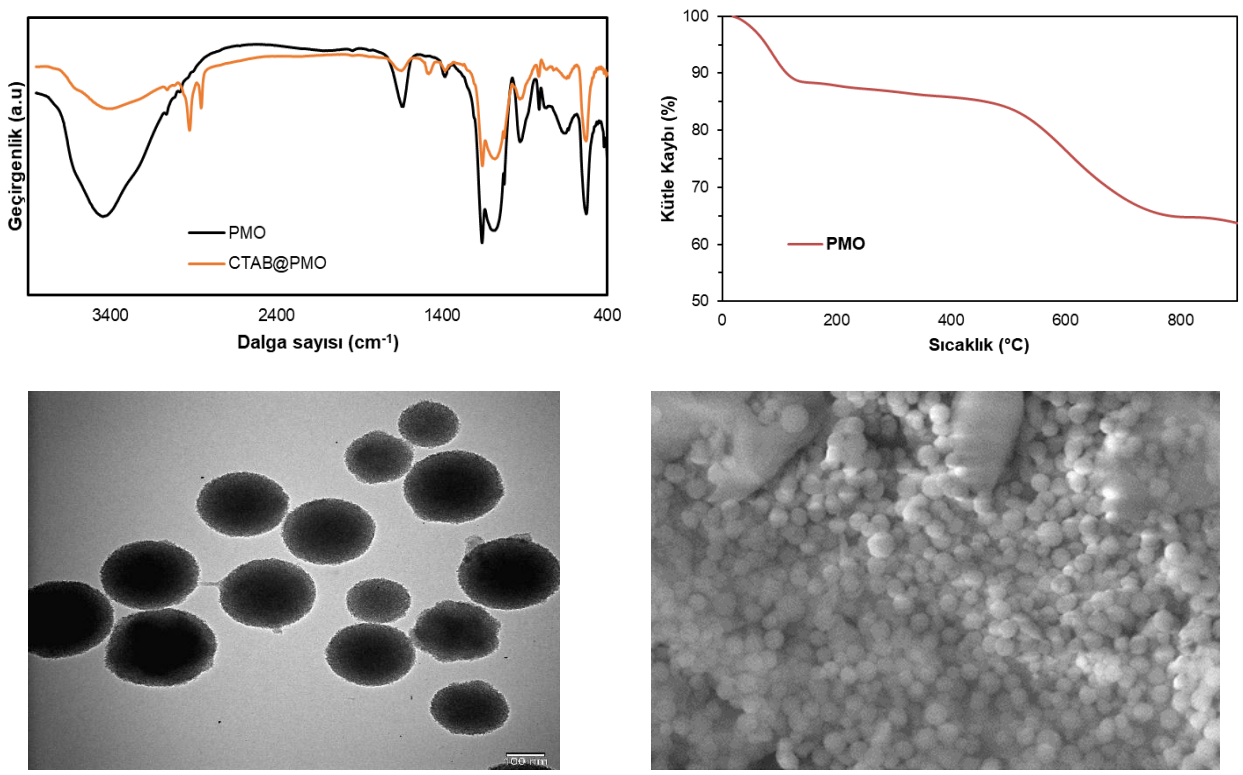
**İlaç taşıyıcı sistemin karakterizasyonu (PMO)**

İlaç taşıyıcı sistemin sentezi **(PMO)** literatürde kayıtlı yöntem takip edilerek gerçekleştirilmiştir (Croissant vd., 2016). Yönteme göre, bazik çözelti içinde şablon olarak triblok kopolimer setiltrimetilamonyum bromür (CTAB) varlığında 1,4-bis(trietoksisilil)benzen (BTSB)'in kondenzasyon reaksiyonu sonucunda periyodik mezogözenekli organosilika (**PMO**) malzemesi başarıyla sentezlenmiştir. Sentez yolu şekil 1'de gösterilmiştir.




**Şekil 1. PMO** ilaç taşıyıcı sistemin sentezi

Sentez sırasında kullanılan sürfaktan CTAB uzaklaştırılma dan önce alınan FT-IR spektrumu incelendiğinde, 2920 ve 2850 $cm^{-1}$'de gözlenen gerilim titreşimlerinin CTAB'nin hekzadesil grubunda yer alan $sp^3$ C'larına ait olduğu belirlenmiştir (Şekil 2). **CTAB@PMO** yapısının amonyum nitratın etil alkol çözeltisi ile sonikasyonu sonrası elde edilen son yapının alınan FT-IR spektrumunda 2920 ve 2850 $cm^{-1}$'deki gerilim titreşimlerinin gözlenmiyor olması CTAB'nin yapıdan başarılı bir şekilde uzaklaştırıldığını göstermektedir. Sentezi gerçekleştirilen **PMO**'ya ait FT-IR spektrumunda, aromatik halkada yer alan $sp^2$ =C-H gerilim titreşimleri 3061 ve 3010 $cm^{-1}$'de gözlenirken bunların bükülme titreşimleri 524 $cm^{-1}$'de gözlenmektedir. Siloksan ağında yer alan Si-O-Si grubuna ait titreşim sinyalleri 1143 ve 1074 $cm^{-1}$'de gözlenmiştir (Croissant, vd., 2016).

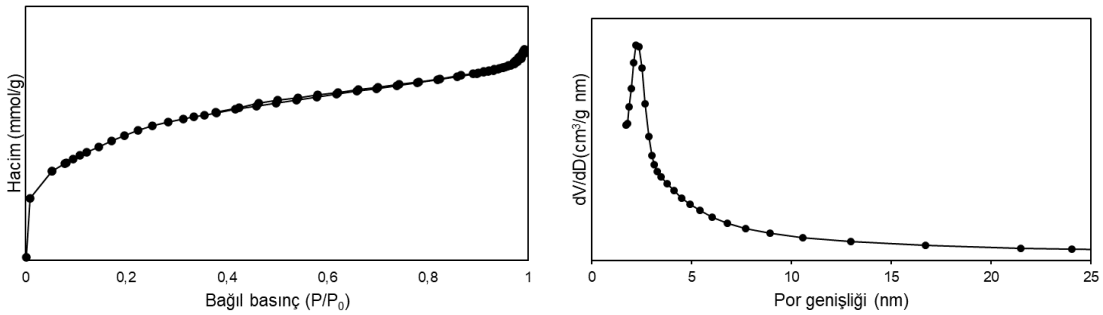



**Şekil 2.** CTAB@PMO ve PMO yapılarının FT-IR spektrumu (üstte solda), PMO'nun TGA eğrisi (üstte sağda), PMO'nun TEM mikrografiği (alt solda) ve SEM görüntüsü (alt sağda)

**PMO**'ya ait TGA eğrisi şekil 2'de gösterilmektedir. TGA eğrisinde 150 ºC'nin altında gözlenen ~%10'lık kütle kaybı, **PMO**'nun kanallarındaki porlar içerisine sıkışmış ve fiziksel olarak tutunmuş çözücülerden dolayıdır. 150 ºC ile 800 ºC arasında gözlenen ~%29'lık kütle kaybı, **PMO**'nun içermiş olduğu bozunabilir organik kısımlara karşılık gelmektedir.

**PMO** yapısına ait alınan TEM mikrografikleri şekil 2'de verilmiştir. TEM mikrografikleri, **PMO** yapısının düzenli yapıda olduğu ve yığıntı meydana getirmediğini göstermektedir. **PMO** yapısının gerçekleştirilen SEM analizi de bu durumu doğrulamaktadır (şekil 2).

**PMO** için adsorpsiyon-desorpsiyon izotermleri ve por büyüklüğü dağılım eğrileri şekil 3'de verilmiştir. Adsorpsiyon eğrisinden por büyüklüğü dağılımını hesaplamak için BJH (Barrett-Joyner-Halenda) metodu kullanılmıştır. Yapılan ölçümlerden **PMO** için elde edilen, Brunauer–Emmett–Teller (BET) yüzey alanı ($S_{BET}$) 1215 $m^2/g$, toplam por hacmi ($V_{total}$) 0,82 $cm^3/g$ ve ortalama por çapı ($D_{BJH}$) 3,4 nm olarak hesaplanmıştır.




**Şekil 3. PMO**'nun adsorpsiyon-desorpsiyon izotermi (solda) ve por genişliği dağılımı (sağda)

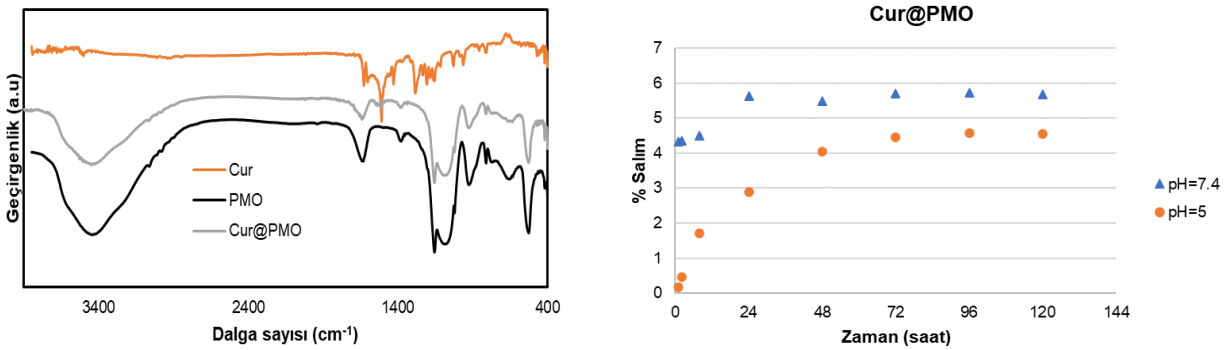
**İlaç yükleme ve salım çalışmaları**

**PMO**'ya doğal ürünlerden hidrofobik kurkumin yüklemesi çalışması yapılmıştır. Bunun için 4 mg kurkumin/mL etanol stok çözeltisi hazırlanarak içerisine **PMO** eklenip sonikatör yardımı ile disperse olması sağlandı. Dispersiyon sonrası oda sıcaklığında ve karanlıkta 24 saat karıştırıldı. Süre sonunda karışım santrifüj edilerek yükleme çözeltisi ile ilaç taşıyıcı sistem **(Cur@PMO)** birbirinden ayrıldı. Elde edilen **Cur@PMO** yapısı 2 kez etanol ile yıkanarak tekrar santrifüjlendi ve yıkama çözeltileri yükleme çözeltileri ile birleştirildi.

Yıkama ve yükleme çözeltileri içerisinde kalan kurkumin miktarını belirleyebilmek için UV-Vis spektrofotometrede 420 nm'da bu çözeltilerin absorbsiyon okumaları gerçekleştirildi. Yapılan ölçüm sonrası elde edilen değerler kullanılarak eşitlik 1 ve 2 yardımı ile **PMO**'nun ilaç yükleme yüzdesi (%EE) ve yükleme kapasitesi (%LC) hesaplandı (Chen vd., 2018; Sun vd., 2019). **PMO** için eşitlik 1 kullanılarak ilaç yükleme yüzdesi %13 ve eşitlik 2 yardımı ile yükleme kapasitesi de %11,5 olarak hesaplanmıştır.

Kurkuminin **PMO** ilaç taşıyıcı sistemine yüklendiği FT-IR analizi ile de doğrulanmaya çalışılmıştır. Bunun için, kurkumin, **PMO Cur@PMO** yapılarının FT-IR spektrumları alınarak üst üste çakıştırılmıştır. Elde edilen FT-IR spektrumları şekil 4'de gösterilmektedir.

Kaydedilen FT-IR spektrumları incelendiğinde, **PMO**'nun FT-IR spektrumundaki temel titreşimlerin (şekil 2) **Cur@PMO**'ya ait alınan FT-IR spektrumunda da net olarak ortaya çıktığı görülmüştür (şekil 4). Özellikle nanotaşıyıcının ağ örgüsünde yer alan Si-O grubuna ait gerilme titreşimleri 1090-1140 $cm^{-1}$ aralığında net olarak gözlenmektedir. Kurkuminin **PMO** yapısına yüklenmesi ile elde edilen **Cur@PMO**'nun FT-IR spektrumunda, 3400 $cm^{-1}$ üzerindeki bölgede ve 1800-2000 $cm^{-1}$ aralığındaki bölgede gözlenen zayıf şiddetli titreşimlerin **PMO**'nun FT-IR spektrumunda gözlenmemesi fakat saf kurkuminin FT-IR spektrumunda da gözleniyor olması kurkuminin **PMO** yapısına yüklendiğinin bir göstergesi olarak kabul edilebilir.




**Şekil 4.** Kurkumin, PMO ve Cur@PMO'ya ait FT-IR spektrumları (solda), İlaç yüklü Cur@PMO'nun pH= 5 ve pH =7,4'de UV-Vis spektrofotometre ile zamana karşı ilaç salımının izlenmesi (sağda)

**Cur@PMO**'nun ilaç salımına pH etkisini incelemek için, fizyolojik pH = 7.4 ve endosomal pH =5'de salım çalışması gerçekleştirilmiştir. Bunun için, 1,5 mg **Cur@PMO** tartılarak hacimce %10 tween-80 içeren 10 mL PBS (pH =7,4 ve 5) içerisinde disperse edilip 37 °C'de 100 rpm'de karıştırıldı. Belirli aralıklarla ortamdan numune alınarak 12.000 rpm'de 15 dakika santrifüjlendi ve süpernatant kısmının absorpsiyonu UV-Vis spektrofotometrede 420 nm'de dalga boyunda okundu. Okunan absorpsiyon değeri standart çalışma grafiğine yerleştirilmiş ve elde edilen yüzde salım oranları zamana karşı grafiğe geçirilmiştir. Elde edilen grafik şekil 4'de verilmiştir.

Salım grafiği incelendiğinde, **Cur@PMO** yapısı 5.gün sonunda taşıdığı kargonun pH = 7,4'de ~%6'sını ve pH = 5'de ~%5'ini salmıştır. İlk 24 saat içinde her iki pH değeri için de salım grafiğinde maksimum salım değerlerine ulaşılmış olup, ilk günü takip eden sonraki 4 gün salım değerlerinde bir artış kaydedilmemiştir. Yüklenebilen ilaç miktarının %5-6'nın salımının gerçekleşmiş olması, kurkumin ile **PMO** arasında kuvvetli moleküller arası etkileşimin olduğunu göstermektedir. Bu durum, por kapatma ajanları kullanmadan ilaç taşıma sistemi **PMO**'nun taşıdığı kargonun %94'nü pH = 5 ve 7,4'de sızdırmadan taşıyabildiğini göstermektedir.

Kanser tedavisinde, ilaç taşıyıcı sistemlerin taşıdıkları kargoyu hedefe kadar sızma yapmadan taşıması oldukça önemlidir. Kargonun erken bırakılması tedavinin gerçekleşmesine olanaksız kılmaktadır. Por kapatma ajanları kullanmadan **PMO**'nun taşıdığı kurkuminin ancak %6'sını 5 gün sonunda sızdırması oldukça önemli bir sonuç olarak değerlendirilebilir. İlaç sızmasının çok olması durumunda por kapatma ajanları ile bu sorun giderilebilmektedir. Ancak, por kapatma ajanlarının kullanılması sentez basamak sayısını dolayısı ile süresini ve nihayetinde maliyeti artırmaktadır. Bu yönler dikkate alındığında **PMO** ilaç taşıyıcı sistemi benzerlerine kıyasla daha ekonomik olarak değerlendirilebilir.

İlaç yüklü taşıyıcı sistemin taşıdığı kargoyu sızdırmadan taşıyabilmesi kadar taşıdığı kargoyu istenilen yerde ve istenilen zamanda bırakması da kanser tedavisinde istenilen başarının elde edilmesinde oldukça önemlidir. Bu çalışmada elde edilen kurkumin sızdırmayan **PMO** sisteminin, kurkumini istenildiği yerde ve zamanda salabilmesini sağlayacak şekilde değiştirilmesi için çalışmalarımız devam etmektedir.

## SONUÇ

Bu çalışma kapsamında, literatürde kayıtlı periyodik mezogözenekli organosilika nanomalzemeler sınıfında yer alan ilaç nanotaşıyıcı sistem **PMO** sentezlenmiş ve bu yapının ilaç yükleme ve salım çalışmaları gerçekleştirilmiştir. **PMO**'ya yüklenen kurkumin miktarı %13 olarak belirlenmiştir. Fizyolojik ve endosomal pH değerlerinde yapılan ilaç salım çalışmaları **PMO**'ya yüklenmiş olan kurkuminin ancak %6'sının salınabildiğini göstermiştir. İlaç salım yüzdesinin düşük çıkması, **PMO** ile kurkumin arasında kuvvetli moleküler etkileşim olduğunu göstermektedir. Bu durum **PMO**'nun, salım yapmasından ziyade sızdırmaz bir ilaç taşıyıcı sistem olmasına neden olmaktadır. Por kapatma ajanları kullanmadan **PMO**'nun taşıdığı kurkuminin 5 gün sonunda ancak %6'sını salması oldukça önemli bir sonuç olarak değerlendirilmektedir. İlaç yüklü taşıyıcı sistemin taşıdığı kargoyu sızdırmadan taşıyabilmesi kadar taşıdığı kargoyu istenilen yerde ve istenilen zamanda bırakması da kanser tedavisinde istenilen başarının elde edilmesinde oldukça önemlidir. Bu çalışmada elde edilen kurkumin sızdırmayan **PMO** sisteminin, kurkumini istenildiği yerde ve zamanda salabilmesini sağlayacak şekilde değiştirilmesi için çalışmalarımız devam etmektedir. Şu an devam eden çalışmalarımızda, kurkumin salımını istenilen zamanda başlatmak için **PMO** ilaç taşıyıcı sistemine pH ve enzim duyarlı tetiklerin yerleştirilmesi denenmektedir.

## TEŞEKKÜR

Bu çalışma Burdur Mehmet Akif Ersoy Üniversitesi Bilimsel Araştırma Projeleri Komisyonunca desteklenmiştir. Proje Numarası: 0828-YL-22.

## KAYNAKLAR

Bagheri E, Ansari L, Abnous K, Taghdisi SM, Charbgoo F, Ramezani M, Alibolandi M, 2018. *Silica based hybrid materials for drug delivery and bioimaging.* Journal of Controlled Release, 277, 57–76.

Bharti C, Gulati N, Nagaich U, Pal A, 2015. *Mesoporous silica nanoparticles in target drug delivery system: A review*. International Journal of Pharmaceutical Investigation, 5(3), 124.

Chen C, Sun W, Wang X, Wang Y, Wang P, 2018. *"Rational design of curcumin loaded multifunctional mesoporous silica nanoparticles to enhance the cytotoxicity for targeted and controlled drug release".* Materials Science and Engineering C, 85; 88–96.

Croissant J. G, Cattoën X, Wong Chi Man M, Durand JO, Khashab NM, 2015. *Syntheses and applications of periodic mesoporous organosilica nanoparticles.* In Nanoscale 7(48), 20318–20334.

Croissant JG, Fatieiev Y, Julfakyan K, Lu J, Emwas AH, Anjum DH, Omar H, Tamanoi F, Zink J. I, Khashab NM, 2016. *Biodegradable Oxamide-Phenylene-Based Mesoporous Organosilica Nanoparticles with Unprecedented Drug Payloads for Delivery in Cells.* Chemistry - A European Journal, *22*(42), 14806–14811.

Diab R, Canilho N, Pavel IA, Ha FB, Girardon M, Pasc A, 2017. *Silica-based systems for oral delivery of drugs , macromolecules and cells*. *249*,346–362.

Giri S, Trewyn BG, Lin VS, 2007. *"Mesoporous silica nanomaterial-based biotechnological and biomedical delivery systems".* 2, 99-111.

Hatcher H, Planalp R, Cho J, 2008. *"Curcumin: from ancient medicine to current clinical trials"*, Cellular and Molecular Life Sciences. 65:1631–1652.

He Y, Shao L, Usman I, Hu Y, Pan A, Liang S, Xu H, 2020. *A pH-responsive dissociable mesoporous silica-based nanoplatform enabling efficient dual-drug co-delivery and rapid clearance for cancer therapy.* Biomaterials Science.

Huh KM, Lee SC, Cho YW, Lee J, Jeong JH, Park, K, 2005. *Hydrotropic polymer micelle system for delivery of paclitaxel.* Journal of Controlled Release, *101*(1-3 SPEC. ISS.), 59–68.

Li QL, Xu SH, Zhou H, Wang X, Dong B, Gao H, Tang J,Yang YW, 2015. *PH and Glutathione Dual-Responsive Dynamic Cross-Linked Supramolecular Network on Mesoporous Silica Nanoparticles for Controlled Anticancer Drug Release.* ACS Applied Materials and Interfaces, 7(51), 28656–28664.

Lv Y, Li J, Chen H, Bai Y, Zhang L, 2017. *Glycyrrhetinic acid-functionalized mesoporous silica nanoparticles as hepatocellular carcinoma-targeted drug carrier.* International Journal of Nanomedicine, 12, 4361–4370.

Manzano M, Vallet-regí M, 2020. *Mesoporous Silica Nanoparticles for Drug Delivery. 1902634*, 3–5.

Noureddine A, Je C, 2018. *Pendant / bridged / mesoporous silsesquioxane nanoparticles : Versatile and biocompatible platforms for smart delivery of therapeutics*. 340,125–147.

Palanikumar L, Kim HY, Oh JY, Thomas AP, Choi ES, Jeena MT, Joo SH, Ryu J. H, 2015. *Noncovalent*

*surface locking of mesoporous silica nanoparticles for exceptionally high hydrophobic drug loading and enhanced colloidal stability.* Biomacromolecules, *16*(9), 2701–2714.

Pan J, Galactosylated PW, Liu W, Zhu Y, Wang F, Li X, Liu X, Pang J, Pan W, 2018. *Galactosylated chitosan-functionalized mesoporous silica nanoparticles for efficient colon cancer cell-targeted drug delivery*.

Sun T, Zhang YS, Pang B, Hyun D. C, Yang M, Xia Y, 2014. *Engineered nanoparticles for drug delivery in cancer therapy.* Angewandte Chemie - International Edition, *53*(46), 12320–12364.

Sun X, Wang N, Yang L. Y, Ouyang XK, Huang F, 2019. *Folic acid and pei modified mesoporous silica for targeted delivery of curcumin.* Pharmaceutics, *11*(9).

Yadav S, Deka SR, Tiwari K, Sharma AK, Kumar P, 2017. *Multi-Stimuli Responsive Self-Assembled Nanostructures Useful for Colon Drug Delivery*. *16*(8), 764–772.

Vallet-Regí M, Colilla M, Izquierdo-Barba I, Manzano M, 2018. *Mesoporous silica nanoparticles for drug delivery: Current insights.* Molecules, *23*(1), 1–19.

Yang B, Chen Y, Shi J, 2019. *Mesoporous silica/organosilica nanoparticles: Synthesis, biological effect and biomedical application.* In Materials Science and Engineering R: Reports, 137, 66–105.

Yu L, Chen Y, Lin H, Du W, Chen H, Shi J, 2018. *Ultrasmall mesoporous organosilica nanoparticles: Morphology modulations and redox-responsive biodegradability for tumor-specific drug delivery.* Biomaterials, *161*, 292–305.

Zhou H, Beevers CS, Huang S, 2011. *"The targets of curcumin"*, Current Drug Targets. 12(3):332-47.

## *ORAL PRESENTATION*

# Karsinogenezde yeni bir biyobelirteç: Kisspeptin

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**Özet**
Kanser hastalığı, dünyada en önemli ölüm nedenleri arasında yer almakta ve bu nedenle medikal araştırmaların ana alanlarından biri olarak kabul edilmektedir. Temel karsinogenez mekanizmaları olan kontrolsüz hücre büyümesi, invazyon ve metastaz ile ilgili bilinenler dışında kanser gelişimi ve evriminin çok aşamalı süreci hala tam olarak anlaşılamamıştır. Kisspeptin, başlangıçta melanom ve meme kanseri metastazını baskılama yeteneğine sahip bir insan metastaz baskılayıcı gen olarak tanımlanmıştır. Kisspeptin, KISS1 geni tarafından kodlanan ve G-proteinine bağlı reseptörü GPR54'ün aktivasyonu yoluyla farklı kanser hücrelerinin metastatik yeteneklerini baskıladığı bildirilen bir proteindir. Kisspeptin reseptörü olan GPR54 proteini; mesane, ovaryum, kolorektal, pankreas, hipofiz, prostat, karaciğer ve tiroid bezlerindeki metastatik kanserlerde anti karsinogenez olarak işlev görür. Bu proteinler, matris metalloproteinaz MMP-9 aktivitesinin baskılar, metastatik hücre göçü ve invazyonu inhibe eder. Kisspeptin ve reseptörü, kanser progresyonunun düzenlenmesinde önemli rol oynar. Pankreas ve ovaryum kanserlerinde tümör ilerlemesini baskılamak için Kisspeptin ve GPR54 ekspresyonunun başlangıçta artan bir ekspresyonu varken, hastalığın erken evrelerinde en yüksek ekspresyon seviyesine ulaşır ve yüksek gen ekspresyonu olan hastalarda daha uzun sağkalım ve daha iyi prognoz seyredilir. Kolorektal, mesane ve renal hücreli karsinomda, Kisspeptin ekspresyonu azalır ve bu azalış, daha düşük sağkalım oranları ve metastaz artışı ile ilişkilendirilmiştir. ERα'nın eksik olduğu triple negatif meme kanserinde, Kisspeptin ve GPR54 ekspresyonu yükselir ve bu yükseliş tümör büyümesini ve metastazı teşvik etmektedir ve bu kötü prognoz ile ilişkilidir. Sonuç olarak, Kisspeptin ve GPR54'ün kanser patogenezinde önemli rolleri olduğu ve bu proteinlerin yeni tedaviler geliştirilmesi ve/veya tedavide prognostik belirteçler olarak kullanılması için önemli potansiyele sahip olduğu düşünülmektedir.
**Anahtar Kelimeler:** Kisspeptin, GPR54, karsinogenez.

## GİRİŞ

1996 yılında, Danny Welch'in Pensilvanya/Hershey'deki grubu, habis melanom hücresine insan kromozom 6 eklenmesinin ardından bu melanom hücresinin metastaz yeteneğini kaybettiği gözlemlediler ve bu hücrelerden yeni bir cDNA izole ettiler (J. H. Lee et al., 1996). Bu cDNA'ya şehrin ünlü çikolata fabrikası olan 'Kisses Chocolate' 'dan gelen 'Kiss1'adını verdiler (J. H. Lee et al., 1996). Bu genin, C8161 melanom hücrelerine transfeksiyonun, metastazı baskıladığını gösterdiler. 1999 yılında farklı bir grup ise, galanin reseptörleriyle ilgili bir reseptör olan, aynı zamanda Kisspeptin proteinin reseptörü olan GPR54 (KISS1R)'ü keşfetti (D. K. Lee et al., 1999).
İnsanda Kiss1 geni, 1q32 kromozomunda bulunur. Sadece üçüncü ve dördüncü eksonları 145 amino asitlik peptite çevrilen dört ekzondan oluşur. 3. ve 4. ekzonun transkripsiyonu ile Kiss1 mRNA'sına çevrilip ardından Kiss1 mRNA'nın translasyonu ile beraber 145 amino asit uzunluğundaki Kisspeptin-145 öncü proteinine çevrilir. Bu öncü protein 3 proteolitik klivaja uğrayarak 4 aktif peptid oluşturur. Bu aktif Kisspeptin peptitleri; 54 amino asit uzunluğu 5,9 kDa ağırlığındaki Kisspeptin-54, 14 amino asit uzunluğu 1,7 kDa ağırlığındaki Kisspeptin-14, 13 aminoasit uzunluğu 1,6 kDa ağırlığındaki Kisspeptin-13 ve 10 amino asit uzunluğu 1,3 kDa ağırlığındaki Kisspeptin-10'dur (Hu et al., 2019).
G-proteinine bağlı reseptör GPR54'ün en bilinen görevi; memelilerde fertilitenin başlatılması ve sürdürülmesindek, önemli rolüdür. GPR54 geninde mutasyonlara sahip olan insanlar ve fareler, cinsel maturasyonun başlamaması ve düşük seviyelerde gonadotropik hormonlar ile karakterize edilen hipogonadotropik hipogonadizme sahiptir (Colledge, 2008).

**Kisspeptin/GPR54 Sinyalizasyonu**

Kiss1 geni tarafından kodlanan Kisspeptin proteini G-protein-bağlı reseptör olan GPR54'e baplanır. GPR54, G protein alt birimi $G_{q/11}\alpha$ ile birleştirilmiş bir GPCR'dir (Kotani et al., 2001; Muir et al., 2001). Ligand (Kisspeptin) bağlanması fosfolipaz C 'yi (PLC) aktive ederek fosfatidil inositol bisfosfatın (PIP2) hidrolizine ve diasilgliserol (DAG) oluşumuna yol açar. DAG daha sonra protein kinaz C'yi aktive eder. Mitojenle aktive olan protein kinazlar (MAPK'ler) fosforile edilir ve böylece β-arrestin ve inositol-1,4,5-trifosfat (IP3) aktive edilir. Sonuç olarak, hücre içi kalsiyum salınır ve nöronu depolarize duruma geçirir (Liu, Lee, & Herbison, 2008).
PLC'den bağımsız olan düzeltici potasyum kanalları ve seçici olmayan katyon kanallarının açılması hücre içi kalsiyum seviyesini artırabilir. Kisspeptin nöronları, gonadotropin salgılayan hormon (GnRH) eksprese eden nöronlarla sinapslar oluşturur (Pampillo et al., 2009). Kisspeptin nöronlarının depolarizasyonu, GnRH nöronlarının depolarizasyonuna ve ardından lüteinize edici hormon ve folikül uyarıcı hormon salınımının modülasyonuna yol açar.

**Kisspeptin ve Kanser**

2020'de dünya çapında yaklaşık 19,3 milyon yeni kanser vakası ve yaklaşık 10,0 milyon kansere bağlı ölüm olduğu tahmin edilmektedir. Yaşam boyu (75 yaşından önce) kansere yakalanma riski yaklaşık %20 ve kanserden ölme riski %10 civarındadır (Ferlay et al., 2021). 2020'de tahmin edilen ortalama 2,26 milyon yeni vakayla, kadın meme kanseri şu anda dünya çapında en sık teşhis edilen kanser türüdür ve onu yakından takip eden ortalama 2,21 milyon ile akciğer kanseridir. Kanserden ölümün en yaygın nedeni ortalama 1,80 milyon ölüm ile akciğer kanseridir, ardından 0,83 milyon ile karaciğer kanseri ve 0,77 milyon ile mide kanseri izlemektedir (Ferlay et al., 2021).
Dünya genelinde erkeklerde en sık ortaya çıkan kanser akciğer kanseri olmakla birlikte, ardından prostat ve melanom olmayan deri kanseri gelmektedir. Mortalite oranlarında ise başta akciğer kanser ardından karaciğer kanseri ve mide kanseri gelmektedir. Kadınlarda ise dünya genelinde insidansı ve mortalitesi en yüksek kanser meme kanseri olmakla birlikte ardından akciğer ve rahim ağzı kanseri gelmektedir (Ferlay et al., 2021).
Karsinogenezdeki süreci yönlendiren ve sürecin ilerlemesini etkileyen değişikliklerin tanımlanması, dizileme ve veri analizi gibi son zamanlarda geliştirilen teknolojilerle belirlenebilir. Ortalama 3-6 mutasyon sayısının yetişkin tümörlerinde karsinogenezi teşvik ettiği düşünülse de, gen aktivitesini değiştirdiği tahmin edilen mutasyonların (non-synonymous mutations) toplam sayısı çoğu katı tümörde 40 ila 100 arasında değişir ve mikrosatellit kararsızlığı olan bazı tümörlerde (akciğer ve kolorektal kanserler gibi ) yüzlerceye kadar çıkabilir (Zaimy et al., 2017).
Kanser, hücrelerin kontrolden çıkmış bölünmesi ve öngörülen tedavi/tedavilere dirençli birçok türün oluşmasıyla karakterize edilen yıkıcı bir durumdur (Balon, Sheriff, Jackow, & Laczmanski, 2022). Kanserin 6 ayırt edici özelliği vardır (Hanahan & Weinberg, 2011) ve kanserli hücre oluşumuna neden olan somatik mutasyonlar kademeli olarak birikir ve bir kanser tipine, bireysel bir hastaya ve hatta tek bir tümör lezyonuna özgü bir mutasyon karakteristiği yaratır (Balon et al., 2022). Yeni çıkan bi method olan CRISPR/Cas9 temelli araçlarla ilgili gen bölgelerinin düzenlenmesi oluşmuş olan mutasyonların kesin ve kalıcı olarak hedeflenmesini sağlar. Bununla birlikte, kanser için güvenli ve etkili gen düzenleme terapilerinin geliştirilmesi, normal hücreleri korumak ve diğer mutasyonları ortaya çıkarmaktan kaçınmak için dikkatli bir tasarıma dayanır (Balon et al., 2022).
Son yıllarda, onkoloji araştırmacıları, çalışmalarını metastaz baskılayıcı genlerin tanımlanmasına odaklamıştır. 2008 yılına kadar gen transferi, gen ekspresyonu indüksiyonu, bir genetik ürünün eksojen uygulaması, metastaz baskılayıcıların hedeflenmesi ve sinyal iletimini içeren kansere özgü terapötik stratejiler için yararlı tümör süpressör genleri sağlayan yaklaşık 30 metastaz baskılama geni izole edilmiştir (Stafford, Vaidya, & Welch, 2008). Fakat 2017 yılı itibariyle 55 onkogen ve 70 tümör süppressör gen tanımlanmıştır (Zaimy et al., 2017). Kanser hücrelerini diğer hücrelerden ayıran 6 temel özellik vardır. Bunlar; proliferatif sinyalleme, ölüme direnç, büyüme baskılayıcılardan kaçma, replikatif ölümsüzlük, anjiyogenezi indükleme, invazyon ve metastazdır (Hanahan & Weinberg, 2011).

**Kanser Proliferasyonu ve Metastazında Kisspeptin**

Kisspeptin/GPR54 sisteminin aktivasyonunun, kanser hücresi biyolojisi üzerinde motilitenin baskılanması, kültür stratchının onrımı, proliferasyon, metastaz ve invazyonu üzerinde etkili olduğu gösterilmiştir (Ji, Ye, Mason, & Jiang, 2013). Kisspeptinin antimetastatik olduğu bilinse de, bunu hangi

mekanizma ile yaptığı spesifik olarak belirsizdir. Ancak Kisspeptinin çeşitli hücre içi sinyal yolaklarını tetikleyebileceği düşünülmektedir (Ciaramella, Della Corte, Ciardiello, & Morgillo, 2018).

GPR54, PLC sinyal yolunun aktivasyonunu ve ardından $Ca^{2+}$ alımını belirleyen, Gq/11 ile ilişkili G-protein-bağlı reseptördür. GPR54'ün Kisspeptin tarafından uyarılması ayrıca fokal adezyon kinazın (FAK) fosforilasyonuna yol açar. Aşırı fokal adezyonların ifadesi stres fiberlerinin oluşumuna yol açar, böylece Kisspeptinin kemotaksiyi inhibe etmiş olur. Ayrıca, GPR54 aktivasyonu, metastazın baskılanmasına katkıda bulunan kalsinörin aktivitesini azaltır (Ciaramella et al., 2018).

**Kisspeptin ve MMP-9**

Bugüne kadar, Kisspeptinlerin metastaz baskılayıcı aktivitesi, melanom, tiroid, over, mesane, mide, özafagus, pankreas, akciğer ve hipofiz kanserlerindeki önemli rolü keşfedilmiştir (Cvetkovic, Babwah, & Bhattacharya, 2013). Bu kanserlerin birçoğu için, Kisspeptin/KISS1R'ye bağlı metastatik baskılamanın altında yatan birleşik bir ortaya çıkan mekanizma, matris metalloproteinaz (MMP)-9 aktivitesinin baskılanması ve ardından kanser hücresi göçü ve istilasının inhibisyonudur (Makri, Pissimissis, Lembessis, Polychronakos, & Koutsilieris, 2008). Kisspeptinler, insan kanserlerinde p38 MAP kinazını aktive ederek MMP-9 ifadesini bastırır (K. H. Lee & Kim, 2009). MMP-9 proteinin embriyonik gelişim, üreme sistemi, anjiyogenez, kemik gelişimi, yara iyileşmesi, hücre göçü, öğrenme ve hafıza, metastazda önemli rolleri vardır (N. Zhu, Zhao, Song, Ding, & Ni, 2022).

**Kisspeptin ve Warburg Etkisi**

Hücre metabolizması, tümör hücrelerinin çoğalması, hayatta kalması ve metastazı ile yakından ilişkilidir. Tümör hücreleri, glukoz alımını arttırarak Warburg etkisi gösterirler ve ATP için oksidatif fosforilasyon (OXPHOS) yerine glikolizi tercih ederler (Hanahan & Weinberg, 2011). Aerobik glikoliz genellikle tümör metabolizmasında kritik bir özellik olarak kabul edilir. Özellikle Kisspeptin, hücre dışı ortamın asitlenmesini, glikoz alımını ve laktat sekresyonunu azaltmak da dahil olmak üzere, tümör hücrelerinde Warburg etkisinin tersine çevrilmesinde yer alır (Lu, Tan, & Cai, 2015).

**Çeşitli Kanser Türlerinde Kisspeptin**

Kisspeptin proteini, melanoma, meme kanseri, kolorektal kanser, NSCLC, gastrik tümörler, tiroid kanseri, mesane kanseri ve diğer birkaç tip kanserde metastaz baskılayıcı olarak görev alırken; baş ve boyun skuamoz hücre karsinomasında ve üçlü negatif meme kanserine sahip hastalarında iyileşmenin sağlanmasında başlıca sınırlayıcı faktör olan ilaç direç sisteminde bulunur (Fratangelo, Carriero, & Motti, 2018). GPR54 aktivitesinin, ERα-negatif meme hücre hatlarında ve TNBC (üçlü negatif meme kanseri) hücrelerinde ilaç direncini artırmak için gerekli olduğunu gösteren çalışmalar mevcuttur. GPR54 antagonisti hücrenin doksorubisine duyarlılığını geri kazandırır. Bu bulgular, GPR54 proteinini TNBC'den etkilenen hastalarda ilaç duyarlılığını eski haline getirmek için olası yeni bir terapötik hedef olabileceğini düşündürmektedir (Blake et al., 2017).

Kolorektal kanser, çok sık görülen ve ölümcül kanser türlerinden biridir. Kolerektal kanserin önemli bir komplikasyonu, karaciğer metastazı yapmasıdır. Kolerektal kanserdeki metastazlar, MMP-9 ifadeleri ile ilişkilidir. Kolorektal karaciğer metastazlarında Kisspeptin ve GPR54 ekspresyonunun analizi, hastaların prognozu ile bir korelasyonun varlığını göstermiştir. Düşük Kisspeptin seviyelerine sahip kolekrektal kanser dokuları, yüksek seviyelerde MMP-9 ifade eder ve uzak bölgelere daha sık metastaz yapar (Fratangelo et al., 2018; C. Zhu et al., 2015). Karşıt olarak; Kisspeptin ve reseptörünün meme kanserinde hem normal hücrelere göre daha fazla ekspresyonunu gösteren hem de daha az eksprese edildiğini gösteren çalışmalar mevcuttur (J. H. Lee & Welch, 1997; Martin, Watkins, & Jiang, 2005; Papaoiconomou et al., 2014).

**SONUÇ**

Kisspeptin/GPR54 sistemi pek çok kanser türünde MMP-9'un aktivitesinin baskılanmasında, Warburg etkisinin geri çevrilmesinde ve baskılanmasında görev almaktadır. Tüm bunların yanı sıra Kisspeptin proteini bir çok kanser türünde, metastas, proliferasyon, invazyon, hayatta kalım, büyüme, farklılaşma gibi süreçlerde de önemli rol oynamaktadır. Kisspeptin/GPR54 sisteminin kanser hücrelerinin fizyolojisindeki önemli etkileri, bu proteinin gelecekteki kanserin tanı aşamasında marker olarak kullanılabilmesini ve kanserli hastalarda terapötik tedavilerde kullanılma potansiyeli öngörülmektedir. Tüm bunları yanı sıra Kisspeptin ve reseptörü GPR54'ün etki mekanizmasını tam olarak anlayabilmemiz kanser hücresinin metabolizmasını ve karsinogenez sürecini daha iyi anlamamıza yardımcı olacaktır.

**KAYNAKLAR**

Balon, K., Sheriff, A., Jackow, J., & Laczmanski, L. (2022). Targeting Cancer with CRISPR/Cas9-Based Therapy. *Int J Mol Sci, 23*(1). doi:10.3390/ijms23010573

Blake, A., Dragan, M., Tirona, R. G., Hardy, D. B., Brackstone, M., Tuck, A. B., . . . Bhattacharya, M. (2017). G protein-coupled KISS1 receptor is overexpressed in triple negative breast cancer and promotes drug resistance. *Sci Rep, 7*, 46525. doi:10.1038/srep46525

Ciaramella, V., Della Corte, C. M., Ciardiello, F., & Morgillo, F. (2018). Kisspeptin and Cancer: Molecular Interaction, Biological Functions, and Future Perspectives. *Front Endocrinol (Lausanne), 9*, 115. doi:10.3389/fendo.2018.00115

Colledge, W. H. (2008). GPR54 and kisspeptins. *Results Probl Cell Differ, 46*, 117-143. doi:10.1007/400_2007_050

Cvetkovic, D., Babwah, A. V., & Bhattacharya, M. (2013). Kisspeptin/KISS1R System in Breast Cancer. *J Cancer, 4*(8), 653-661. doi:10.7150/jca.7626

Ferlay, J., Colombet, M., Soerjomataram, I., Parkin, D. M., Pineros, M., Znaor, A., & Bray, F. (2021). Cancer statistics for the year 2020: An overview. *Int J Cancer*. doi:10.1002/ijc.33588

Fratangelo, F., Carriero, M. V., & Motti, M. L. (2018). Controversial Role of Kisspeptins/KiSS-1R Signaling System in Tumor Development. *Front Endocrinol (Lausanne), 9*, 192. doi:10.3389/fendo.2018.00192

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell, 144*(5), 646-674. doi:10.1016/j.cell.2011.02.013

Hu, K. L., Chang, H. M., Zhao, H. C., Yu, Y., Li, R., & Qiao, J. (2019). Potential roles for the kisspeptin/kisspeptin receptor system in implantation and placentation. *Hum Reprod Update, 25*(3), 326-343. doi:10.1093/humupd/dmy046

Ji, K., Ye, L., Mason, M. D., & Jiang, W. G. (2013). The Kiss-1/Kiss-1R complex as a negative regulator of cell motility and cancer metastasis (Review). *Int J Mol Med, 32*(4), 747-754. doi:10.3892/ijmm.2013.1472

Kotani, M., Detheux, M., Vandenbogaerde, A., Communi, D., Vanderwinden, J. M., Le Poul, E., . . . Parmentier, M. (2001). The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem, 276*(37), 34631-34636. doi:10.1074/jbc.M104847200

Lee, D. K., Nguyen, T., O'Neill, G. P., Cheng, R., Liu, Y., Howard, A. D., . . . O'Dowd, B. F. (1999). Discovery of a receptor related to the galanin receptors. *FEBS Lett, 446*(1), 103-107. doi:10.1016/s0014-5793(99)00009-5

Lee, J. H., Miele, M. E., Hicks, D. J., Phillips, K. K., Trent, J. M., Weissman, B. E., & Welch, D. R. (1996). KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *J Natl Cancer Inst, 88*(23), 1731-1737. doi:10.1093/jnci/88.23.1731

Lee, J. H., & Welch, D. R. (1997). Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1. *Cancer Res, 57*(12), 2384-2387.

Lee, K. H., & Kim, J. R. (2009). Kiss-1 suppresses MMP-9 expression by activating p38 MAP kinase in human stomach cancer. *Oncol Res, 18*(2-3), 107-116. doi:10.3727/096504009789954591

Liu, X., Lee, K., & Herbison, A. E. (2008). Kisspeptin excites gonadotropin-releasing hormone neurons through a phospholipase C/calcium-dependent pathway regulating multiple ion channels. *Endocrinology, 149*(9), 4605-4614. doi:10.1210/en.2008-0321

Lu, J., Tan, M., & Cai, Q. (2015). The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Lett, 356*(2 Pt A), 156-164. doi:10.1016/j.canlet.2014.04.001

Makri, A., Pissimissis, N., Lembessis, P., Polychronakos, C., & Koutsilieris, M. (2008). The kisspeptin (KiSS-1)/GPR54 system in cancer biology. *Cancer Treat Rev, 34*(8), 682-692. doi:10.1016/j.ctrv.2008.05.007

Martin, T. A., Watkins, G., & Jiang, W. G. (2005). KiSS-1 expression in human breast cancer. *Clin Exp Metastasis, 22*(6), 503-511. doi:10.1007/s10585-005-4180-0

Muir, A. I., Chamberlain, L., Elshourbagy, N. A., Michalovich, D., Moore, D. J., Calamari, A., . . . Harrison, D. C. (2001). AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J Biol Chem, 276*(31), 28969-28975. doi:10.1074/jbc.M102743200

Pampillo, M., Camuso, N., Taylor, J. E., Szereszewski, J. M., Ahow, M. R., Zajac, M., . . . Babwah, A. V. (2009). Regulation of GPR54 signaling by GRK2 and {beta}-arrestin. *Mol Endocrinol, 23*(12), 2060-2074. doi:10.1210/me.2009-0013

Papaoiconomou, E., Lymperi, M., Petraki, C., Philippou, A., Msaouel, P., Michalopoulou, F., . . . Koutsilieris, M. (2014). Kiss-1/GPR54 protein expression in breast cancer. *Anticancer Res, 34*(3), 1401-1407.

Stafford, L. J., Vaidya, K. S., & Welch, D. R. (2008). Metastasis suppressors genes in cancer. *Int J Biochem Cell Biol, 40*(5), 874-891. doi:10.1016/j.biocel.2007.12.016

Zaimy, M. A., Saffarzadeh, N., Mohammadi, A., Pourghadamyari, H., Izadi, P., Sarli, A., . . . Tavakkoly-Bazzaz, J. (2017). New methods in the diagnosis of cancer and gene therapy of cancer based on nanoparticles. *Cancer Gene Ther, 24*(6), 233-243. doi:10.1038/cgt.2017.16

Zhu, C., Takasu, C., Morine, Y., Bando, Y., Ikemoto, T., Saito, Y., . . . Shimada, M. (2015). KISS1 Associates with Better Outcome via Inhibiting Matrix Metalloproteinase-9 in Colorectal Liver Metastasis. *Ann Surg Oncol, 22 Suppl 3*, S1516-1523. doi:10.1245/s10434-015-4891-7

Zhu, N., Zhao, M., Song, Y., Ding, L., & Ni, Y. (2022). The KiSS-1/GPR54 system: Essential roles in physiological homeostasis and cancer biology. *Genes Dis, 9*(1), 28-40. doi:10.1016/j.gendis.2020.07.008

# 2. POSTER PRESENTATIONS
# 2.1. ABSTRACTS

## *POSTER PRESENTATION*

# Composition analysis of pumpkin seed oil extracts and investigation of their antimicrobial, antioxidant and photoluminescence properties

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**Abstract**

Pumpkin seeds have been used for curing and protecting against various diseases. They are an excellent source of bioactive compounds and have been shown to possess a variety of biological activities, such as, antidiabetic, antifungal, antibacterial, anti-inflammation activities and antioxidant effects [1]. Pumpkin seed oil is an edible oil and has been shown to have nutraceutical value, providing advantageous health benefits. The literature has reported pumpkin seed oil as consisting of bioactive compounds, mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) which can act as natural antioxidants [2].
This study was designed to isolate pumpkin seed oil of different pumpkin varieties using the Soxhlet and sonication extraction techniques, with ethanol as the extraction solvent. These extracts were then utilized to evaluate their antioxidant and antibacterial activities. The antioxidant properties were assessed by the *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenger technique and antibacterial activities by the disc diffusion method. All the pumpkin seed oil sources showed remarkable antioxidant activities. Additionally, spectroscopical analyses of the pumpkin seed oils were investigated to evaluate their photoluminescence properties. Pumpkin seed oil can further supplement usage in the food and pharmaceutical industries.

**Keywords:** Pumpkin seed oil, Antimicrobial activity, Antioxident activity.

**References:**
[1] Dar, A.H., Sofi, S.A., Rafiq, S. Pumpkin the functional and therapeutic ingredient: A review. International Journal of Food Science and Nutrition, **2017**, 2(6), 165-170.
[2] Kulaitienė, J., Černiauskienė, J., Jarienė, E., Danilčenko, H., Levickienė, D. Antioxidant activity and other quality parameters of cold pressing pumpkin seed oil. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, **2018**, 46(1), 161-166

## *POSTER PRESENTATION*

# Influence of electrical conductivity, parity and stage of lactation on milk yield and biological value in Simmental cows

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**Abstract**

The current research aimed to assess the effects of electrical conductivity, parity and stage of lactation on both milk yield and quality. Study was performed between January and September 2021, on 358 Simmental cows (94 primiparous and 258 multiparous cows). A significant influence of electrical conductivity ($p \leq 0.01$) was recorded for both primiparous (10.15±0.09 mS/cm) and multiparous (8.79±0.15 mS/cm). The milk electrical conductivity decreased according to days in milk, being higher in the first interval (10.12±0.16 mS/cm for 0-30 days) compared to second interval (10.03±0.08 mS/cm for 31 to 60 days $p \leq 0.001$) and third interval (9.24±0.21 mS/cm for 61-90 days, $p \leq 0.001$). The multifactorial regression model employed, highlights significant influence of month of calving ($p \leq 0.001$) and days in milk ($p \leq 0.01$) on the electrical conductivity, while parity had no influence ($p > 0.05$). Medium correlations were calculated between electrical conductivity and chemical components (-0.11 for fat, -0.08 for protein), also for milk yield (-0.12). No significant correlations were obtained according to lactose content (-0.013). Dynamics of milk related traits proved to be significantly influenced by month of calving ($p \leq 0.001$), stage of lactation ($p \leq 0.01$) and cows' parity ($p \leq 0.01$). No significant influence of electrical conductivity on milk related traits was recorded ($p > 0.05$).

**Keywords**: electrical conductivity, milk related traits, Simmental

## *POSTER PRESENTATION*

# $Cu^{2+}$ İyonunun Sulu Ortamda Seçici ve Hassas Tayini İçin Naftakinon Tabanlı Yeni Bir Kimyasal Probun Geliştirilmesi

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**Özet**

Bakır iyonu tirosinaz, süperoksit dismutaz, sitokrom oksidaz, dopamin b-hidroksilaz, seruloplazmin, lizil oksidaz gibi çeşitli enzimler için katalitik kofaktör olmak gibi çeşitli temel fizyolojik olaylarda önemli rol oynamaktadır. Fizyolojik öneminin yanı sıra antimikrobiyal aktiviteye de sahip olan $Cu^{2+}$ iyonunun içme suyunun arıtımında ve taşınmasında da yararlı olduğu bilinmektedir. Ayrıca $Cu^{2+}$, $Fe^{3+}$ ve $Zn^{2+}$'dan sonra insan vücudunda en çok bulunan üçüncü metal iyonudur. Biyolojik öneminin yanı sıra alaşım yapımında, gübrelerde, çeşitli makine parçalarında, pil yapımında, elektrik tellerinin içeriğinde bulunma gibi çeşitli alanlarda da önemli yere sahip olan $Cu^{2+}$ iyonu normal koşullar altında yetişkin bir insan vücudunda tipik olarak 70-80 mg kadar bulunmaktadır. İnsan vücudu tarafından aşırı miktarda alındığında toksik etkisinin yanında karaciğer ve böbrek hasarına yol açabilmekte ve Alzheimer, Parkinson, Wilson hastalığı, Menkes, amyotrofik yanal skleroz ve prion hastalıkları gibi nörodejeneratif hastalıklarla ilişkili bozukluklara neden olabilmektedir. Dünya Sağlık Örgütü (WHO) ve Amerika Birleşik Devletleri Çevre Koruma Ajansı (EPA) tarafından içme sularında bulunabilecek maksimum $Cu^{2+}$ seviyesi sırasıyla 2.0 mg $L^{-1}$ ve 1.3 mg $L^{-1}$ olarak belirlenmiştir. Bu nedenlerden dolayı, $Cu^{2+}$ iyonu seviyesinin çeşitli ortamlarda insan sağlığı ve çevresel etkiler açısından takip edilebilmesi ve tayini büyük önem arz etmektedir.

Bu çalışmada, 8-aminokinolin modifiye naftakinon temelli ve $Cu^{2+}$ iyonuna karşı seçici yeni bir prob geliştirildi. Sentezlenen molekül NMR, MALDI-TOF kütle spektroskopisi, IR spektroskopisi gibi çeşitli spektroskopik metotlarla karakterize edilmesinin yanı sıra sentezlenen molekülün yapısı X-ışını kırınım analizi (XRD) ile de aydınlatıldı. Sentezlenen probun $Cu^{2+}$ iyonuna karşı seçiciliği $Li^{+}$, $Na^{+}$, $K^{+}$, $Cs^{+}$, $Ag^{+}$, $Be^{2+}$, $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$, $Cd^{2+}$, $Al^{3+}$, $Cr^{3+}$, $Mn^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $F^{-}$, $Cl^{-}$, $Br^{-}$, $I^{-}$, $NO_3^{-}$, $NO_2^{-}$, $HSO_3^{-}$, $HCO_3^{-}$, $CO_3^{2-}$, $PO_4^{3-}$, $ClO_4^{-}$, $S_2O_3^{2-}$ gibi yarışmacı iyonlar varlığında kanıtlandı.

**Anahtar Kelimeler:** Naftakinon, 8-aminokinolin, Bakır, Kemosensör, X-ışını

## *POSTER PRESENTATION*

# Composition analysis of garlic extract and evaluation of the antimicrobial, antioxidant, DNA protective and photoluminescence properties

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**Abstract**

Medicinal plants continue to be valuable therapeutic agents. Garlic (*Allium sativum L.*) has been documented as bearing medicinal and spicy qualities by many cultures around the world. It is one of the most traditional plants used for bacterial related diseases and in flavourings, foodstuffs and condiments. The literature has demonstrated its use in treating tumor and cardiovascular diseases and also to combat ageing [1]. Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) and ajoene as well as some thiosulfinates and a wide range of other organosulphurate compounds are the main and most important principal bioactive compounds present in garlic extracts, having very effective antifungal, antibacterial, antiviral and antiparasitic properties [2]. However the bioactive component ajoene is the most attractive compound due to it having a greater number of *in vitro* and *in vivo* biological activities, where antithrombotic, anti-tumoral, antifungal, and antiparasitic effects have been detected [2].
This study dealt with the extraction of ajoene from raw garlic, and its potential use to treat resistant bacterial infections. Ajoene was extracted from raw garlic by reflux using ethanol as the extraction solvent. Extraction was completed using ethyl acetate, where the combined organic extracts were washed with water and brine and then concentrated by evaporation *in vacuo*. The antioxidant and antibacterial activities of the garlic extract were assessed. The antioxidant properties were examined *in vitro* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenger assay and antibacterial activities by the disc diffusion method. The garlic extract showed remarkable antioxidant activity. Further, spectroscopical analyses of the garlic extract were conducted to evaluate the photoluminescence properties. Potential DNA protective activities were also investigated.

**Keywords:** Garlic extract, Ajeone, Antioxident, DNA protective activitiy, Photoluminescence

**References:**
[1] Lawal, A., Dangoggo, S.M., Umar, K.J. Phytochemical and antibacterial screening of garlic (*Allium sativum*). Katsina Journal of Pure & Applied Sciences, **2010**, 2(2):101-104.
[2] Ledezma, E., Apitz-Castro, R. Ajoene the main active compound of garlic (*Allium sativum*): A new antifungal agent. Revista Iberoamericana de Micología, **2006**, 23(2):75-80.

## *POSTER PRESENTATION*

# Synthesis and characterization of SAPO 34 supported catalysts containing Pd, $ZrO_2$ and $CeO_2$ for total oxidation of methane

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**Abstract**

Methane is an alternative energy source to fossil fuels, which can be found in large quantities in natural gas reserves and can be produced from biologically purified sources through anaerobic digestion. Alternative energy sources are constantly being researched due to the recent emission of gasoline containing pollutants. In this study, the synthesis and characterization of SAPO34 zeolite type supported catalyst containing Pd, $CeO_2$, $ZrO_2$ for the total oxidation of methane was carried out. Palladium metal is used for its high activity for the total oxidation of methane. Because it is expensive, it is added to the catalyst structure in low amounts. While $ZrO_2$ component was added to the structure for high temperature resistance of the catalyst, $CeO_2$ booster component was added to increase oxygen mobility. Literature studies have shown that acidic zeolite type is more effective in total oxidation of methane. Therefore, SAPO34 zeolite type was synthesized by hydrothermal synthesis method. Other components were added to the porous structure of the zeolite support by the surfactant assisted impregnation method. The produced Pd/SAPO34, Pd-$CeO_2$/SAPO34, Pd-$ZrO_2$/SAPO34 and Pd-$CeO_2$-$ZrO_2$/SAPO34 catalysts were characterized by X-Ray diffraction pattern (XRD) and $N_2$ physisorption analyses. SAPO34 zeolite support was also analyzed by Fourier transform infrared spectrometry (FTIR). As a result of the comparison with the studies in the literature, XRD and FTIR analyzes showed that the SAPO34 zeolite supplement was synthesized successfully. XRD analysis results showed that the desired crystal phase structure could be achieved. In general, the diffraction peaks originating from PdO, $CeO_2$, $ZrO_2$ crystal phases and SAPO34 zeolite support were obtained in the structure of the catalysts. This result shows that the targeted crystal phase structure has been achieved. Surface areas, pore volumes and average pore diameters of catalysts and support were determined by $N_2$ physisorption analysis. The SAPO34 zeolite support has a surface area of 71 $m^2/g$; A surface area of 40 $m^2/g$ was obtained in the Pd-SAPO34 catalyst due to the blocking of the small pores with the introduction of PdO into the structure. The surface area increased significantly with the addition of $CeO_2$ and $ZrO_2$ to the structure. Surface areas of 106 $m^2/g$, 192 $m^2/g$, 143 $m^2/g$ were obtained for Pd-CeO2/SAPO34, Pd-ZrO2/SAPO34 and Pd-CeO2-ZrO2/SAPO34 catalysts, respectively. The results show that the $ZrO_2$ component has a much more positive effect on the surface area. It was determined that the catalyst structure was mesoporous.

**Keywords:** SAPO34, Zeolite, Catalysts, Characterization.

## *POSTER PRESENTATION*

# Investigation and comparison of CO methanation activities of nickel-doped zeolite supported catalysts in both conventional and microchannel reactors

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**Abstract**

In recent years the great interest exists on the systems which contain microchannel reactor. The interest on the microchannel is increasing because of the advantage of the microchannel reactor which are the high surface area to volume ratio, high heat/mass transport. In this study, SAPO34 and HZSM5 supported catalysts containing 15% Ni for CO methanation were synthesized by both classical impregnation method and surfactant assisted impregnation method. In order to determine the effect of the solvent used in the impregnation process, four different solvents were used during the preparation (water, acetone, 1-propanol, ethyl alcohol). Their catalytic activities were tested for CO methanation in both the conventional reactor and the microchannel reactor. In both reactors, the reactor temperatures were changed from 125 °C to 375 °C. The reaction feed gas mixture was adjusted to be 1% CO, 50% $H_2$ and the remainder He. The microchannel stainless steel reactor is 100x90x15 mm in size and contains 48 channels with a channel depth of 0.17 mm, a channel width of 0.50 mm and a channel length of 30 cm. Among the HZMS5 supported catalysts, the best activity results were obtained from the CO methanation studies performed with the conventional vertical reactor, as well as from the catalysts prepared using 1-propanol as a solvent in both methods. With the HZSM5 catalysts prepared with 1-propanol, 100% conversion was obtained at 375 °C in the classical reactor, while 100% CO conversion was obtained from 325 °C in the microchannel reactor. Positive developments were also obtained as a result of the activity experiments carried out in the microchannel reactor with SAPO34 zeolite supported catalysts. It was determined that the CO conversion increased to approximately 25%. Again, among the SAPO34 supported catalysts, the catalysts prepared using 1-prppanol showed the best catalytic activities.

**Keywords:** Carbon monoxide, methanation, zeolite support, microchannel reactor.

## *POSTER PRESENTATION*

# Evaluation of the effects of heavy metals "mercury and lead" on neutrophil degranulation

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**Abstract**

Among the environmental pollutants known for their toxicity and their propagation throughout the world, heavy metals are certainly of primary concern. Since humans are exposed to these toxic metals, in particular lead and mercury, their effects on the immune system require evaluation. The organism defends itself against these aggressions thanks to its immune system composed of several molecules and cells, in particular the neutrophils which constitute one of the pivots of the innate immunity and a powerful defense system of the Man against the xenobiotics. The present work was undertaken in order to understand the effect of Lead and Mercury, on the effector functions and properties of the neutrophil respectively degranulation and adhesion. In order to answer this objective, an evaluation test of the effect of these two metals was performed on neutrophils isolated from healthy donors, and stimulated in vitro with different concentrations (10, 25, 50, 80, 100µg/ml) of Lead and Mercury. Non adherent cells were counted on MALASSEZ cells, and degranulation was quantified by measuring myeloperoxidase, a reference enzyme for this function, by spectrophotometry. The results showed that depending on the concentration of the two metals, the adhesion and degranulation of the neutrophil are modified, with a concentration of 25µg/ml of Mercury and Lead, more than 80% and 100% of cells are adhered, respectively. For the same concentration, Mercury induces degranulation equal to 50% of that obtained with fMLP, which is used as a positive control. Therefore, the results allowed to conclude that Lead and Mercury have an effect on the effector functions and properties of neutrophils.

**Keywords:** neutrophils, lead, mercury, degranulation, myeloperoxidase.

## *POSTER PRESENTATION*

# Biological applications and spectroscopic investigations , intramolecular charge transfer,nonlinear optical and density functional studies of borazine derivatives

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**Abstract**

In this work, a comprehensive investigation of the borazine derivatives, is carried out using density functional theory at three functionals, vis. B3LYP, CAM-B3LYP and LanL2DZ with 6-311++G(d,p) basis set to determine their linear and non-linear optical properties.The results of the optimized molecular structure are presented and compared with the available X-ray data of very similar molecules. A complete analysis of the observed spectra of the FT-IR,UV–Vis absorption and fluorescence emission spectral measurements have been given with TD-DFT.Charge distributions from Mulliken population, NBA and MEP are correlated and gap energy are calculated. The low energy gap value indicates the possibility of intramolecular charge transfer.The optical properties are determined and show that they have extremely high nonlinear optical properties.The frontier molecular orbital calculations clearly show the inverse relationship of HOMO–LUMO gap with the first-order hyperpolarizability, confirming that the borazine derivatives can be used as attractive future NLO materials.

**Keywords**: NLO,TD-DFT,DFT, borazine derivatives, HOMO–LUMO

## *POSTER PRESENTATION*

# Protective effect of caffeic acid against hemolysis induced by heavy metals "mercury and lead"

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**Abstract**

Mercury (Hg) and lead (Pb) are environmental pollutants affecting human health and ecosystems. To assess the health effects of Hg and Pb, human erythrocytes from healthy subjects were exposed to Hg and Pb (10, 25, 50 and 100 (µg/ml) alone and in the presence of caffeic acid. For the hemolysis test, erythrocytes and test products were incubated with 0.9 % NaCl. After a centrifugation step, the amount of hemoglobin released into the medium was measured in the supernatant by spectrophotometry at 405 nm. The percentage of red blood cell protection is then quantified relative to total lysis samples with 0.2 % NaCl. The study showed that mercury alone at 100 µg/ml induced more than 70 % of erythrocytes hemolysis versus 15 % hemolysis for lead alone at the same concentration. The rate of protection of caffeic acid against mercury-induced hemolysis is dose-dependent. With 100 µg/ml caffeic acid the protection rate is about 80 %. The results presented confirm the harmful effects of heavy metals, particularly Hg, on the physiology of erythrocytes. The caffeic acid present in the plant kingdom with multiple biological effects has a protective effect on the membranes of erythrocytes.

**Keywords:** Erythrocytes, hemolysis, mercury, lead, caffeic acid.

## *POSTER PRESENTATION*

# Plant extracts and oxidative brain injury in bile duct ligation induced biliary cirrhosis

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**Abstract**

Cirrhosis is a globally important cause of morbidity and mortality, affecting not only the liver but also many organs such as the brain. The aim of the present study was to evaluate the possible protective effects of bitter melon (BM), chard and parsley extracts on brain injury in rats with bile duct ligation (BDL) induced biliary cirrhosis. In this study, female Sprague Dawley rats were divided into five groups: Control group (C): Sham-operated, 0.9% physiological saline was given; BDL group: The common bile duct was separated and ligated by double ligatures with suture silk; BDL+BM group: BM extract (50 mg/kg) administered BDL animals; BDL+chard group: Chard extract (2 g/kg) administered BDL animals; BDL+parsley: Parsley extract (2 g/kg) administered BDL animals for 28 days. At the end of the study, all the animals were sacrificed and brain tissues were taken and homogenized in physiological saline to make 10% (w/v) homogenates. Acetylcholinesterase (AChE), xanthine oxidase (XO), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH) and carbonic anhydrase (CA) activities were determined in brain homogenates. According to the results, AChE, XO and LDH activities were found to increase, while G6PDH and CA activities were decreased in the BDL group as compared to the control group. Administration of the plant extracts reversed these effects probably by their unique antioxidant property. In this study, it was observed that the plant extracts prevented the damage caused BDL induced biliary cirrhosis in rats' brains.

**Keywords:** Brain, enzymes activities, bitter melon, chard, parsley, bile duct ligation.

## *POSTER PRESENTATION*

# Understanding the thermochemistry of the reaction of alkanethiol with O($^3$P). A DFT study

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**Abstract**

The reactions of O($^3$P) with alkanethiols are well-known to be relevent to some important industrial, environmental, and biochemical problems.
The obtained products of the reactions of alkanethiols with O($^3$P) are consistent with a mechanism involving two major steps [1]. Firstly, the addition of oxygen to sulfur takes place to form an oxygen atom-sulfide complex [2, 3]. Secondly, the energy-rich adduct passes through a fragmentation process giving rise to many products (Figure 1.) in a very similar manner as it is discussed when treating the olefin reactions [4, 5].
The calculated thermodynamics quantities are in good agreement with experimental results.

**Keywords**: Multichannel reactions, O($^3$P), alkanethiol, O-addition, DFT calculations.




**Figure 1**. General mechanism of the reaction of O($^3$P) with alkanethiols.

**References :**

[1] B. Dill, H. Heydtmann, Infrared Chemiluminescent Reactions of Halogen Atoms with Hydrogen Sulfide and Methanethiol. Chemical Physics, 35 (1978), 161-176.

[2] S.W. Benson, Thermochemistry and Kinetics of Sulfur-Containing Molecules and Radicals, Chemical Reviews, 78 (1978), 23-35.

[3] M. Tranquille, P. Labarbe, M. Fouassier and M.T. Forel, Détermination des Champs de Forces de Valence et des Modes Normaux de Vibration des Molécules $(CH_3)_2S$ et $(CH_3)_2SO$, Journal of Molecular Structure, 8 (1971), 273-291.

[4] T. Oguchi, A. Ishizaki, Y. Kakuta, H. Matsui, *Mechanism of the Reactions of Butenes with O($^3$P): The Yields of $CH_3$ and $C_2H_5$*, Journal of Physical Chemmistry A, 108 (2004), 1409-1416.

[5] R. P. Wayne, Chemistry of Atmospheres, Oxford Science Publication Inc., Oxford, 1991.

## *POSTER PRESENTATION*

# Viscoelastic and Thermal Properties of 3,4-Dihydro-2H-pyran Containing Copolymer/Clay Nanoarchitectures

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**Abstract**

Organically modified layered-silicates or nanoclays have become an attractive class of hybrid materials due to their prospective use in a great variety of applications from industry to health [1]. For design, synthesis and characterization for potential biomedical nanocomposites, antitumor-active copolymer; poly(3,4-Dihydro-2H-pyran-*alt*-maleic anhydride);[Poly(DHP-*alt*-MA)] were used to prepare nanocomposites [2]. The functional copolymers, having a combination of rigid/flexible linkages and an ability of complex-formation with interlayered surface of organo-silicate, and their nanocomposites have been synthesized by interlamellar complex-radical copolymerization of monomer complexes of MA and DHP with three alkyl ammonium cations with alkyl chain length from a range of C14 to C18.

Comparative analysis of structure–composition–property relationship of the poly(DHP-*alt*-MA) and its organically modified bentonite nanocomposites with varying modification of quaternary ammonium cations exhibited significant increase of the thermal stability of nanocomposites by analyzed by *Differential Scanning calorimetry* (*DSC*), Differential Thermal Analysis (DTA) and Thermogravimetric Analysis (TGA). All the nanocomposites exhibited improved thermal stability compared to the copolymer. Viscoelastic properties were analyzed by Dynamic mechanical analyzer (DMA). The results obtained from study suggest that these nanocomposites with anhydride functional groups prepared by *in situ* interlamellar copolymerization may be promising as novel potential antitumor materials.

**Keywords:** Poly(DHP-*alt*-MA), Copolymer, Nanoarchitecture, Viscoelastic properties, DMA

**References**:

[1] H. Kaplan Can "Charge transfer complex formation in in-situ maleic anhydride and N-vinyl caprolactam copolymer and copolymer/organo-montmorillonite nanoarchitectures". Journal of Macromolecular Science, Part A: Pure and Applied Chemistry,53(1):26–33,2016.

[2] Ö. Şahin and H. Kaplan Can "Design, Synthesis and Characterization of 3,4-Dihydro-2H-pyran Containing Copolymer/Clay Nanocomposites", Journal of Macromolecular Science, Part A: Pure and Applied Chemistry, 52:6, 465-475, 2015.

## *POSTER PRESENTATION*

# The Role of Apocynin Against Radiation and Chloroquine-Induced Testicular Damage

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**Abstract**

Gamma-radiation (RAD) causes changes in chemical balance of cells and impairs the reproductive potential. Although chloroquine (CQ) is a drug used in treatment of malaria, chronic inflammatory diseases and of coronavirus (COVID-19). It is known that apocynin (APO) strongly inhibits NADPH oxidases, and with this effect, it is a very important antioxidant member to reduce oxidative stress. This study, it was aimed to evaluate the effects of RAD and CQ on testicular tissue and to examine the effects of APO, which has antioxidant properties as a protective factor to reduce or prevent these effects, on male fertility by biochemical and histological methods. In our study, male rats were divided into 8 groups; (1)Control, (2)CQ, (3)APO, (4)RAD, (5)CQ+APO, (6)CQ+RAD, (7)APO+RAD and (8)CQ+APO+RAD. At the end of the experiment, testicular tissues were taken and used for biochemical and histological experiments. Reduced glutathione (GSH), lipid peroxidation (LPO), total antioxidant status (TAS), total oxidant status (TOS), reactive oxygen species (ROS), oxidative stress index (OSI), nitric oxide (NO) levels and myeloperoxidase (MPO), lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH) activities were determined. In CQ, RAD and CQ+RAD groups, LPO, TAS, TOS, ROS, OSI, NO levels and MPO, LDH, SDH activities were found to be increased while GSH levels decreased. Administration of APO reversed these changes. Tubules were categorized as normal, regressive, degenerative or atrophic as histological. Regressive and degenerative seminiferous tubule structures and partial separations reflecting damage to the spermatogenic series were observed in the testicular tissues of the RAD, CQ and CQ+RAD groups. Moreover, intercellular space enlargements were noted. It was observed that healthy seminiferous tubule appearances were common in the damaged groups treated with APO, similar to the control group. Results showed that APO had a protective effect on the damage caused by CQ, RAD and CQ+RAD over biochemical and histological parameters.

**Keywords:** Radiation, Chloroquine, Apocynin, Testis Tissue.

## *POSTER PRESENTATION*

# Integrated transcriptomic and metabolomic analysis reveals the potential mechanism of seed germination of *Pistacia chinensis*

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**Abstract**

Seed germination, an important stage in the life style of seed plants, is regulated by complex mechanisms. As an important oil, ornamental and afforestation tree species, there is an urgent need to determine the mechanism for the erratic germination of Chinese pistache (*Pistacia chinensis*) seeds. Transcriptomic sequencing and widely targeted metabolomics were used to detect the molecular mechanism of *P. chinensis* at different stages of seed germination. A total of 1648 differentially expressed genes (DEGs) and 121 differentially accumulated metabolites (DAMs) were identified during germination in this study. The KEGG enrichment analysis of DEGs and DAMs revealed significant differences in "Flavonoid biosynthesis", "Phenylpropanoid biosynthesis" and "Plant hormone signal transduction". During germination of *P. chinensis* seeds, the flavonoids were accumulated significantly, which was attributed to the upregulation of biosynthetic genes of flavonoid pathway, such as *PAL*, *CHS*, *CHI*, *F3H* and *FLS*. The relative content of ABA and JA reduced significantly, and Abscisic Acid Insensitive 5 (ABI5), as a core regulator of the modulation of seed germination, was downregulated dramatically. By WGCNA and K-means analysis, several transcription factors, *MYB1*, *MYB2*, *MYB4* and *MYC*, were indentified and may be strongly correlated with ABI5 signalling and flavonoid biosynthesis. The interaction among plant hormone, flavonoid and transcription factors may play a critical role in the germination of *P. chinensis* seeds. This study enhanced the understanding of the overall mechanism in seed germination and provided the basis for the molecular mechanism of *Pistacia* species.

**Keywords:** *Pistacia chinensis*, Seed germination, Plant hormones, Transcriptome, Flavonoid

## *POSTER PRESENTATION*

# Relationship between diet quality and type 1 diabetes in children

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**Abstract**

The present study aimed to assess dietary diversity and variety and their relationship with micronutrient adequacy in children and adolescents with type 1 diabetes (T1D). A cross-sectional study was carried among 240 children and adolescents with T1D. Weight and height were measured and BMI was calculated. Dietary intake data were obtained from two 24-h recalls. A Dietary diversity (DDS) and dietary variety scores (DVS) and mean adequacy ratio (MAR) and nutritional adequacy ratios (NARs) were calculated and compared according to socio-demographic/anthropometric categories. 52.1% of the patients were female. The mean age of the patients was 8.49 ± 4.1 years. The mean BMI was 19.44 ± 5.24 kg/m2; the mean DDS was 4.62±1.20 and the mean MAR was 0.66 ± 0.11. Older children living in rural areas have a low DDS/DVS. Parental education and income level are associated with DDS/DVS. General and central obesity were significantly elevated in children with high DDS. In addition, a high intake of vegetables, eggs, fiber and micronutrients (Magnesium, Calcium, Potassium, Zinc, Phosphorus and Vit B1) is associated with a high DDS; however, high DVS is associated with high consumption of dairy products, carbohydrates and low intake of protein and fat. There are also positive correlations between DDS/DVS and NARs for various nutrients. The quality of the respondent's diets is moderately diversified. DDS or DVS can be used as indicators of micronutrient adequacy in Moroccan T1D children. Nutritional education needs to be strengthened to improve dietary diversity in children, especially in rural areas.

**Keywords:** Diet quality, nutritional adequacy, T1D.

## *POSTER PRESENTATION*

# Assessment of nutrition education and dietary intake in children with type 1 diabetes

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**Abstract**

Type 1 diabetes (T1D) is a common disease in children and adolescents, characterized by a high prevalence of patients with poor glycemic control (GlyC). Well-conducted nutrition education (NE) is one of the key elements for the treatment of T1D. The objective is to evaluate the impact of two methods of NE on anthropometric parameters, GlyC, lipid profile and dietary intake. The study included a sample of 110 children and adolescents, aged 15 years or younger with T1D. A structured questionnaire was used to collect information on socio-demographic status. Weight and height were measured and Waist-to-Height ratio (WHtR) and body mass index (BMI) were calculated. $HbA_1c$ was determined. Food intake was assessed using two 24-hour recalls. Participants were divided into two groups: a collective group (CG=54) that received a collective NE and another group that received an individual NE (IG=56). A significant reduction of the studied parameters was observed after three and six months in two groups. However, the intergroup difference was significant for WHtR and $HbA_1c$ only. After 6 months, a significant increase in HDL, calorie, carbohydrate, MUFA, and fiber intakes and a significant reduction in TC, LDL, lipids, and SFA intakes were observed in both groups. In the IG, the contribution to carbohydrate intake was 50.61%, that of protein was about 15.50%, and that of fat intake was about 34%, which is in line with recommendations. Individual NE has proven to be the most effective method in improving $HbA_1c$ and dietary intake in poor GlyC T1D patients.

**Keywords:** nutrition education, type 1 diabetes, dietary intake.

## *POSTER PRESENTATION*

# Allelokimyasal içeren bazı bitkilerin Umbelliferae tohumlarının canlılık parametreleri üzerine etkisi

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## ÖZET

Allelopati, organizmalar tarafından salgılanan birtakım kimyasallar ile oluşan etkileşim, bu biyokimyasallar ise allelokimyasallardır. Bunlar diğer bitkilerin büyüme ve canlılığını etkileyebilmektedir. Allelopatiyle ilişkili kimyasalların çoğu primer metabolik yolun yan ürünü olarak üretilen sekonder metabolitlerdir. Bu metabolitler asetat veya şikimik asit yoluyla karbonhidrat, yağ ve amino asitlerin metabolizmasıyla sentezlenmektedir. Allelokimyasallar kök, yaprak, gövde, çiçek, tohum gibi bitki aksamının tümünde bulunmaktadır. Çalışmada; dereotu (*Anethum graveolens*), rezene (*Foeniculum vulgare*), zakkum (*Nerium oleander*) ve kimyon (*Cuminum cyminum*) gibi allelokimyasal içeren bitkilerin yaprak ekstraktları ile ikişer çeşit maydanoz (*Petroselinum crispum*) ve havuç (*Daucus carota*) türlerinin tohumlarına yapılan uygulamaların tohum canlılık ve güç oranlarına olan etkileri belirlenmiştir. %5-10'luk hazırlanan ekstraktlarda tohumlar oda sıcaklığında 12 saat süre ile tutulmuştur. Ardından çimlendirme (24 C'de, 14 gün), çıkış (25 C'de 21 gün), kontrollü bozulma (%18 nemde, 45 $^0$C'de 24 saat) ve hızlı yaşlandırma (42 $^0$C'de 48 saat) testleri kurulmuştur. Denemede normal-anormal çimlenme oranı (%) ve ortalama çimlenme- çıkış zamanı (gün) belirlenmiştir. Tür olarak havuç en yüksek canlılığı vermiştir. Nantes çeşidi havuç tohumlarında ise kontrol grubu çimlenme performansının yüksekliği tüm önuygulamalar sonucununda da devam ederek, allelopatik etki belirlenmemiştir. Bununla beraber normal çimlenme oranı yükselmiştir. Uygulanan güç testleri karşılaştırıldığında hızlı yaşlandırma testleri sonucu canlılık önemli düzeyde kaybolmuştur. Çeşitler karşılaştırıldığında uygulanan yaprak ekstratları farklılık göstermekle beraber, tohum gücü çok düşük olan kontrol grubu maydanoz partilerinde Italian Giant çeşidi tohumları olumlu derece etkilenmiştir.

**Anahtar kelimeler:** Allelokimyasal, Umbelliferae, Bitki yaprak ekstrakları, Priming

## *POSTER PRESENTATION*

# D-amino acid transaminase from *Aminobacterium colombiense* as a potential biocatalyst for stereoselective amination of α-keto acids.

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**Abstract**

D-amino acid transaminases (DAATs) are pyridoxal-5'-phosphate-(PLP)-dependent enzymes that catalyze the reversible and stereoselective transfer of an amino group from D-amino acid to a prochiral α-keto acid, forming a new D-amino acid and α-keto acid. D-amino acids play an important role in physiological processes of living organisms as neurotransmitters, immunity regulators, and as part of antibacterial and anticancer compounds [1]. DAATs are promising biocatalysts for the synthesis of optically pure D-amino acids because chiral purity of chemical products is one of the important requirements in fine organic synthesis.
We discovered a sequence encoding transaminase in a genome of the mesophilic bacteria *Aminobacterium colombiense* (Amico). The transaminase was expressed in *E. coli* cells, purified and investigated. We found that Amico was active with various D-amino acids with D-glutamate being the best substrate. Amico was found to catalyze transamination in a temperature range 30-60 °C, at pH 8-9. We investigated properties of Amico as a potential biocatalyst for amination of α-keto acids. We studied specificity of Amico to various keto acids, stabilization of holo form and apo form of Amico as well as the effect of pH and temperature on the Amico activity and stability. We studied the PLP leakage and approaches to stabilize the holo form. A one-pot-three-enzyme system to produce D-amino acids was developed. Product yields and enantiomeric excess (e.e.) in the reactions were determined. Optimization of one-pot-three-enzyme system conditions resulted in the production of 215 mM of norvaline and 300 mM of 2-aminobutyrate with e.e. more than 99%. We found that substitution K237A led to a shift of the pH-optimum of Amico activity to acidic pH 5-6 while maintaining the level of activity.

1. Bastings JJAJ at all. D-amino Acids in Health and Disease: A Focus on Cancer. Nutrients. 2019 Sep 12;11(9):2205. doi: 10.3390/nu11092205.

**Keywords:** D-amino acids, stereoselective amination, transamineses, biocatalysts, biotechnology

**Acknowledgements:** The study is supported by the Russian Science Foundation project 19-14-00164

## *POSTER PRESENTATION*

# Computational Study of Electronic Properties and Reactivity of Rome TTF Rerivatives

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**Abstract**

This work includes a theoretical study by molecular modeling via the Gaussian program by the density functional theory (DFT/B3LYP) with 6-31G (d,p) as base set, of certain derivatives of TTF, and this in order to determine the parameters geometric and structural: geometric optimization, molecular electrostatic potential (MEP), energy parameters and descriptors of global and local reactivity such as: electronegativity (χ), electrophile (ω), difficulty (η ) and overall softness (S). The Fukui index was used to determine the reactivity of each atom to help predict reactive sites on the molecule for nucleophilic, electrophilic, and radical attacks.

**Keywords**: Tétrathiafulvalene, density functional theory (DFT), reactivity descriptors.

## *POSTER PRESENTATION*

# Antimicrobial activity screening of *Acorus calamus*

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**Abstract**

Like many animals, human beings have been using plants to combat diseases and infections since they first appeared on the scene of history. On the other hand, thousands of antibiotics have been synthesized and used since Alexander Flameng's discovery of penicillin in 1928. Antibiotics, which are an effective weapon in this war against bacteria, are rapidly losing their effectiveness today. Bacteria, on the other hand, cause more and more deaths every day, thanks to the resistance and resistance mechanisms they have acquired. The *Staphylococcus aureus* bacteria, known simply as the "hospital virus", is responsible for the death of 10% to 30% of patients receiving treatment. This number corresponds to more than 100,000 people per year. Due to the increasing resistance, new antibiotics are sought today. In this search, studies are carried out on plants and continue to increase. *Acorus calamus* plant, known to have been used for its healing effects long before antibiotics, is a monocotyledonous perennial wetland plant. Ease of digestion, diuretic and sedative effects are some of its known benefits. In this study, antimicrobial effects of *Acorus calamus* were observed as a result of disc diffusion test performed on ethanol extract. It has been shown to be effective even in small doses, especially its effects on *S.aureus* strains are promising.

**Keywords:** Antibiotic, Antimicrobial, Disk diffusion, *Acorus calamus.*

## *POSTER PRESENTATION*

# Antimicrobial activity screening of *Daucus carota* ssp. *sativus* var. *atrorubens* Alef.

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**Abstract**

Plants have been used in many areas from health to industry throughout human history and continue to be used today. *Daucus carota* ssp. *sativus* var. *atrorubens* Alef. which is rich in vitamins and minerals and is also known colloquially as the black carrot. It originated in Türkiye, the Middle, and the Far East and has been cultivated for about 3000 years. This valuable plant is used commercially in areas such as turnip making, food coloring production, etc. In addition, studies with plants that act against pathogenic bacteria, which are one of the important problems of today, have an important place in the research community. According to the World Health Organization (WHO) data, more than 17 million people die every year in the world due to infectious diseases. In this study, this disc diffusion test was performed on 29 strains, *Daucus carota* ssp. *sativus* var. *atrorubens* Alef.'s 30 µl, 70 µl, and 150 µl ethanol extract was studied by impregnating the discs. In the results of the disc diffusion test, black carrots were found to have formed various zones on pathogenic bacteria and showed antimicrobial activity. While 8-8-10 mm zones were observed in *Staphylococcus aureus*, which causes hospital-acquired infections, 9-11-16 mm zones were observed in *Enterococcus faecium*, which causes meningitis and endocarditis in newborns, and 9-9-9 mm zones were observed in *Streptococcus pneumonia*, which causes diseases such as meningitis, pneumonia, middle ear inflammation, blood inflammation. This plant, which acts on pathogenic bacteria that threaten human health and cause great problems, is promising in the development of new antimicrobial agents against infectious diseases.

**Keywords:** Antimicrobial, Disc diffusion, *Daucus carota* ssp. *sativus* var. *atrorubens* Alef.

## *POSTER PRESENTATION*

# Design of maleimide based fluorine containing polymers

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**Abstract**

Maleimide and its N-substituted derivatives are 1,2-disubstituted ethylenes. Their radical polymerization and copolymerization behaviours and thermal properties of the polymers have been extensively studied by many workers [1]. It is well known that polymaleimide is a potential heat and chemical resistant material so maleimide is widely used as a comonomer for modified polymeric systems.
Taking advantage of the low surface energy of fluoropolymers makes them useful in a number of applications. Fluoropolymers impart resistance to oils, water, and soils and therefore are used prevalently in the textile and carpet industries. Other common uses include nonstick cookware, car finishes, cosmetics, and pharmaceutical applications [2, 3].
Since we aim to use the many unique advantages provided by the fluorine group a new type of perfluorinated maleimide monomer containing aromatic group has been synthesized and this monomer has been polymerized to form copolymers that can contain more fluorine groups instead of fluorines present only at the chain ends. The synthesized monomer was characterized by $^{1}$H-NMR, $^{19}$F-NMR and FT-IR spectroscopic methods.
After the synthesis and characterization of the new monomer its copolymerization with several different monomers such as styrene and methylmethacrylate were realized. Styrene was carried out in the presence of azobisisobutyronitrile as initiator using free radical polymerization method. The molecular weight and polydispersity of the synthesized polymers were measured by gel permeation chromatography.
The resulting fluorine-containing polymers were characterized by Fourier transform infrared spectroscopy and $^{1}$H-NMR. Its thermal properties were investigated by differential scanning calorimetry and thermogravimetric analysis methods. The copolymers showed a single glass transition temperature. Melting endotherms were not observed in differential scanning calorimetry traces suggesting that the copolymers are amorphous. To examine the wetting behavior of obtained polymers thin films of these polymers were prepared by spin coating method and the surface contact angle values were measured.

**Keywords:** Maleimide copolymers, floropolymers, hyrophobic surfaces

**References:**

[1] F. Morel, C. Decker, S. Jonsson, S.C. Clark, C.E. Hoyle, “Kinetic study of the photo-induced copolymerization of N- substituted maleimides with electron donor monomers”, Polymer, vol. 40, pp. 2447-2454, 1999.
[2] R. E. Banks, B. E. Smart, J. C. Tatlow, Organofluorine Chemistry Principles and Commercial Applications. New York, NY: Springer, 1994.
[3] L. N. Pierandrea, “Phase Separation Properties of Fluorocarbons, Hydrocarbons and Their Copolymers”, Advs. Coll. Int. Sci., vol.56, pp. 245-287, 1995.

## *POSTER PRESENTATION*

# Avocado seed extractions and evaluation of their antimicrobial, antioxidant, and photoluminescence properties

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**Abstract**

Scientific studies on seeds and shells of various plants continue to be carried out for health and cosmetic purposes. Plant extracts can provide many useful vitamins and minerals beneficial for human health and the pharmaceutical and cosmetic industries. The pulp of avocados is generally used in various industries, while the skin and seeds are usually discarded as waste. The literature shows such residues as being rich in polyphenols, having antioxidant and antimicrobial activities [1]. In addition to the polyphenols, other useful components of the avocado residues have also been identified and include (+)-catechin and (−)-epicatechin, which too have revealed antioxidant and antimicrobial activities [2].
In this study avocados (*Persea americana*) were purchased from a local store and their seeds were subsequently separated from their edible parts. The avocado seeds were the sundried and ground into a powder. This powder was then subjected to extractions using the Soxhlet and sonication extraction techniques, with ethanol as the extraction solvent. These extracts were then utilized to evaluate their antioxidant and antibacterial activities. The antioxidant properties were assessed by the *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenger technique and antibacterial activities by the disc diffusion method. The obtained avocado seed oils were also used for soap preparations of differing aroma and colours for further socio-economic benefit and contribution in the soap and cosmetic fields. Additionally, spectroscopic analyses of the avocado seed oils were investigated for their photoluminescence properties.

**Keywords:** Avocado seed extracts, Antioxidant, Antibacterial activitiy, Photoluminescence

**References:**
[1] Rodríguez-Carpena, J.G., Morcuende, D., Estévez, M. Avocado by-products as inhibitors of color deterioration and lipid and protein oxidation in raw porcine patties subjected to chilled storage. Meat Science, **2011**, 89, 166-173.
[2] Soong, Y.-Y., Barlow, P.J. Antioxidant activity and phenolic content of selected fruit seeds. Food Chemistry, **2004**, 88, 411-417.

## *POSTER PRESENTATION*

# Syntheses, electrochemistry and spectroelectrochemistry of non-peripheral tetra methoxylated pyrazoline bearing CoII, CuII and MnIIICl phthalocyanines

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**Abstract**

Metallated phthalocyanines (MPcs) represent an important class of macrocyclic compounds that have been used in modern applications such as, cancer, liquid crystalline, electrocatalytic, optical and electrochemical, photodynamic antimicrobial activity, nonlinear optical, photodynamic therapy. Solubility properties of phthalocyanines can be increased by binding bulky groups in the appropriate position in addition, phthalocyanines with redox properties that are rich with suitable metal ions can be designed and their use in this field can be increased. The most important aim of this study is to design phthalocyanine compounds that can show good solubility and electrochemical properties. The redox properties of phthalocyanine can be tailored by changing the metal center and substituent to design the required physicochemical properties. MPcs containing redox active metal centers are very important in terms of their superior electroactive nature. Especially, MPcs containing $Fe^{2+}$, $Co^{2+}$ and $Mn^{3+}$ center cations have promising electrochemical properties due to their oxidation state range from $M^{+}$ to $M^{4+}$. In this study, we wanted to investigate both electrochemistry and spectroelectrochemistry properties of phthalocyanines (M: $Co^{II}$ (***Pc*-Co**), $Cu^{II}$ (***Pc*-Cu**), $Mn^{III}Cl$ (***Pc*-Mn**)) containing methoxylated pyrazoline group and redox active/inactive metal center ions ($Cu^{2+}$, $Co^{2+}$, and $Mn^{3+}$). In this study, we wanted to investigate the contribution of the methoxy group to the electrochemical properties of phthalocyanine compounds. ***Pc*-Co** and ***Pc*-Mn** compounds have developed electrochemical properties by metal-based redox processes in various electrochemical application areas. Considerable color changes observed during the redox reactions indicated the solvotochromic properties of the phthalocyanines [1].

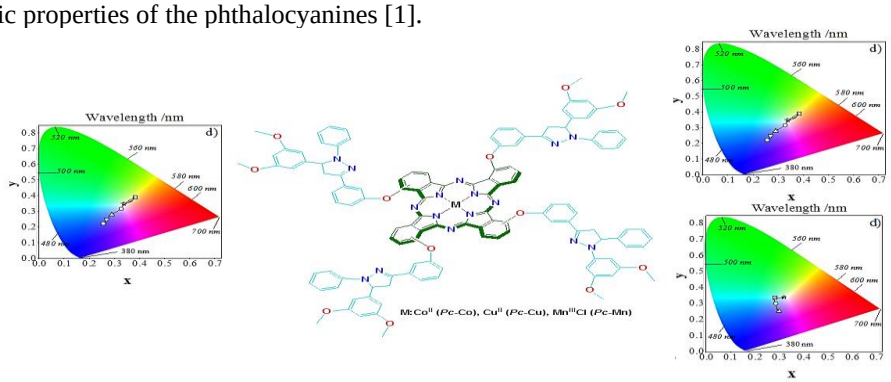


**Figure**: Non-peripheral tetra methoxylated pyrazoline bearing $Co^{II}$, $Cu^{II}$ and $Mn^{III}Cl$ phthalocyanines

**Keywords**: Electrochemistry, Phthalocyanine, Spectroelectrochemistry

**Acknowledgements**: *This work was supported by Office of Scientific Research Projects of Karadeniz Technical University. Project number:* FDK-2021-9351

**Reference:**

[1] H. Yalazan, H. Kantekin, Ö. Budak, A. Koca, Journal of Organometallic Chemistry 973–974 (2022) 122405.

## *POSTER PRESENTATION*

# İzmir/Kaynaklar florasına katkı

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**Özet**

Ülkemiz içinde bulunduğu Holarktis; Avrupa-Sibirya, Turan-Önasya ve Akdeniz olmak üzere üç flora bölgesine sahiptir. Eski çağlardan bu yana yapılan geziler ya da yapılan araştırmalar sonucunda Türkiye'nin doğal yayışlı bitki zenginliği her zaman göze çarpmaktadır. Bu zenginlikte Türkiye'de görülen iklim çeşitleri, toprak yapısı, yükselti ve üç floristik bölgenin görülmesi etkili olmaktadır. Bu çalışmada İzmir'e bağlı Buca ilçesinin Zafer Mahallesi'ne ait Kaynaklar Semti'nden toplanan doğal yayılışlı bitkilerin araştırması yapılmıştır. Yapılan flora çalışması sonucunda Amaryllidaceae (1), Apiaceae (8), Asparagaceae (2), Asteraceae (11), Boraginaceae (3), Brassicaceae (2), Campanulaceae (1), Caryophyllaceae (2), Cistaceae (1), Crassulaceae (2), Dioscareaceae (1), Fabaceae (12), Geraniaceae (5), Lamiaceae (3), Orchidaceae (2), Orobanchaceae (1), Papaveraceae (1), Plantaginaceae (3), Poaceae (10), Primulaceae (1), Rosaceae (1), Rubiaceae (4) ve Scrophulariaceae (1) olmak üzere 79 farklı tür tespit edilmiştir. Tespit edilen bu türlerin çoğunluğu Kaynaklar Semti'nde bulunan Gürlek Şelalesi boyunca toplanmıştır.

**Anahtar Kelimeler:** Bitki, Doğal Yayılış, Flora, Tür

## *POSTER PRESENTATION*

# Evaluation of photocatalytic properties of substituted cobalt phthalocyanines on benzyl alcohol photoxidation

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**Abstract**

In recent years, the emission of greenhouse gases, global warming and increasing industrialization have been a source of great concern for the sustainable environment as well as human life.Considering sustainable life, water is one of the most important natural resources for the survival of all living things.Photocatalysis is a green technology that has received a lot of attention by researchers because it is used to mineralize pollutants in water and convert them into non-hazardous compounds [1]. Metallated phthalocyanine (MPcs) compounds are important photocatalysts. MPcs are conjugated organic molecules with 18-π electron structure and often play a key role in electrochromic devices, semiconductors and catalysis fields due to their outstanding structural and photoelectric properties. Because these compounds have intense absorption in the blue-green light region, they can efficiently harvest energy from visible light, which is widely used in photocatalysis [2]. An effective way to increase the efficiency of phthalocyanines in the photocatalytic field is molecular design. In this study, it is aimed to design a cheap and low-cost ideal photocatalyst containing different pyridine or quinoline groups with the same central atom in the phthalocyanine compound. Photocatalytic activities of DMSO soluble cobalt phthalocyanines were searched to catalyze the oxidation of benzyl alcohol with low toxicity and rescue studies of photocatalysts were carried out. **This study was supported by The Research Fund of Karadeniz Technical University (Project no: 9129), Trabzon, Turkey.**

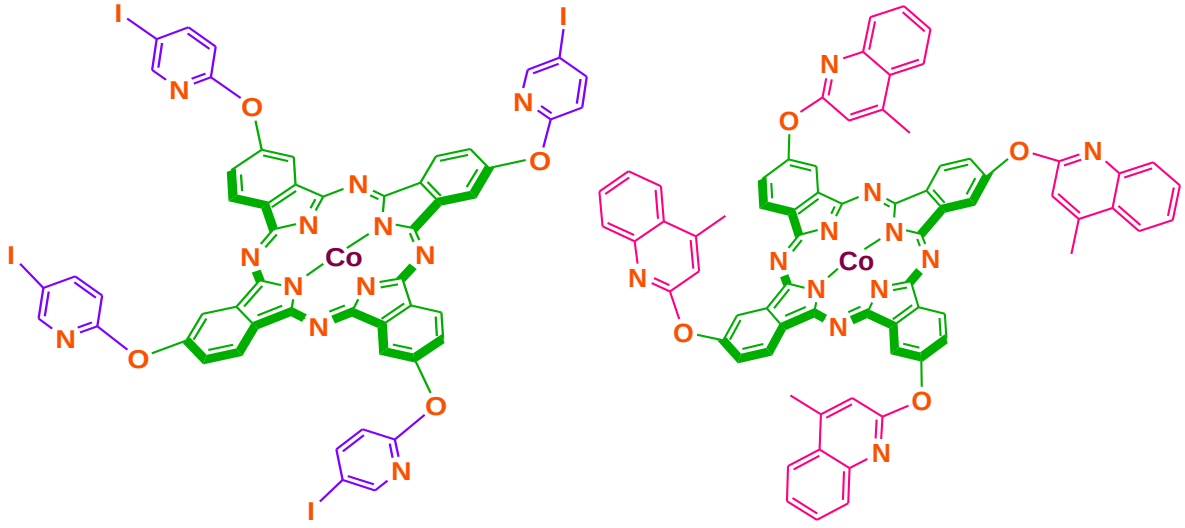


**Figure**: Peripheral pyridine (a) or quinoline (b) substituted cobalt phthalocyanines

**Keywords**: Phthalocyanine, Photocatalytic, Photoxidation.

**References:**

[1] I. Arora, H. Chawla, A. Chandra, S. Sagadevan, S. Garg, Inorganic Chemistry Communications 143 (2022)109700.
[2] S. Wu, Y. Li, X. Cui, Q. Duan, Microporous and Mesoporous Materials 338 (2022)111993.

## *POSTER PRESENTATION*

# Discovery of Estrogen Receptor Alpha (ER-α) Inhibitors by *In Silico*

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**Abstract**

Breast cancer stands out among all cancer types because it covers 15% of all cancer-related deaths and is diagnosed frequently. Breast cancer occurs when exposed to many endogenous and exogenous factors, and this type of cancer has a hereditary feature for the human body. Estrogen receptors are a nuclear hormone receptor that plays a role in the development and control of the female reproductive system, also it considered as a good potential target in the control and treatment of breast cancer cases. ER-α is a group of estrogen receptors and has been proven to be the main factor in almost 65% of breast cancer cases. There is a need to develop new small molecules that modulate ER-α due to the resistance and side effects that develop against current breast cancer treatment [1]. To meet this need, in this study, a virtual screening was performed to determine the most affinity for the ER-α receptor among 900 compounds with a benzothiophene skeleton. In this screening, ER-α crystal structure in complex with 4-hydroxytamoxifen ligand (PDB code: 3ERT) was preferred as receptor protein and the complex structure was prepared using Schrödinger Protein Preparation Wizard [2]. The ligand in the prepared structure was determined as the binding site. In addition, the library with the benzothiophene skeleton (900 compounds) was created and their 3D structures were prepared with the LigPrep module. Next, virtual screening was performed using Glide docking modules. Three compounds with the lowest Glide XP score were identified as hit inhibitors as a result of the virtual screening. In conclusion, these three hit compounds with benzothiophene skeleton could be new and effective anticancer drugs for breast cancer.

**Keywords:** ER-α, Virtual Screening, Benzothiophene

**Acknowledgement:** This work was funded by Ataturk University the Coordination Unit of Scientific Research Projects (Project number FLP-2021-9543).

**References:**

[1] Kumar, N., Gulati, H. K., Sharma, A., Heer, S., Jassal, A. K., Arora, L., ... & Bedi, P. M. S. (2021). Most recent strategies targeting estrogen receptor alpha for the treatment of breast cancer. Molecular Diversity, 25(1), 603-624.
[2] Shiau, A. K., Barstad, D., Loria, P. M., Cheng, L., Kushner, P. J., Agard, D. A., & Greene, G. L. (1998). The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell, 95(7), 927-937.

## *POSTER PRESENTATION*

# Effect of Shoc2 Elimination in Urinary Bladder Function

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**Abstract**

The RAS-RAF-MEK-ERK signalling pathway plays an important role throughout mammalian development, from embryogenesis to tissue-specific cellular homeostasis and its aberrant activation is a major driver of human cancer. Mouse models have highly improved our current understanding of the RAS-ERK pathway in physiological and pathological contexts.
RAF activation is a complex process that involves multiple regulatory steps in addition to RAS binding. Key among them is the dephosphorylation of a conserved inhibitory site (S259-RAF1, S365-BRAF) by a phosphatase complex comprised of SHOC2, MRAS and PP1 (SHOC2 complex).
In order to study the role of Shoc2 in tissue homeostasis we have generated a mouse model of conditional Shoc2 inactivation. Shoc2 knock-out (KO) mice are embryonic lethal indicating Shoc2 function is required during mouse development. To study Shoc2 function in adult tissue homeostasis, Shoc2 KO mice were crossed with animals carrying an inducible ubiquitously expressed CreERT2 recombinase (R26-CreERT2). Treatment with tamoxifen leads to efficient recombination (>80%) in all tissues examined except brain.
In this study, we discovered a sexually dimorphic role of Shoc2 in urinary function, with male, but not female KO mice developing severely distended bladders full of urine. No significant histopathological change such as epithelial hyperplasia or tumour formation were detected in bladder and urethra of Shoc2 KO mice. In addition, no renal damage is obvious in mice with enlarged bladders. Also, the prostate glands of Shoc2 KO mice were of normal size and appearance without any obvious histological abnormalities. Furthermore, affected mice appeared able to urinate normally and water consumption was unaltered. As a result, urethral or prostate obstruction appear unlikely to be responsible for enlarged bladders of Shoc2 KO mice.

**Keywords:** Shoc2, Transgenic Mouse Models, Urinary Bladder

## *POSTER PRESENTATION*

# Physiological Role of Shoc2 in Skin Homeostasis

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**Abstract**

The mammalian skin is the largest organ of the body and serves a main protective barrier between the body and the external environment against mechanical pressure, physical damage, pathogen infection, water loss, and it has immunological functions that help the maintenance of body homeostasis. The EGFR/RAS/RAF/ERK pathway has a well-described role in the skin and hair follicle development. Its function in the skin and its appendages, including hair follicles, is necessary for proper development and tissue homeostasis, and its deregulation rapidly results in defects in cellular proliferation and differentiation.
Dephosphorylation of a conserved inhibitory site on RAF (S259-RAF1, S365-BRAF) by a phosphatase complex comprised of SHOC2, MRAS and PP1 (SHOC2 complex) is one of the key steps in the process of RAF activation.
In order to study the role of SHOC2 in vivo/in tissue homeostasis we have generated 2 mouse models of conditional SHOC2 inactivation. A knockout model (KO) was generated by flanking exon 4 with loxP sites. A Knock-in (KI) model was generated using a minigene strategy, where wild-type SHOC2 is expressed in a cDNA configuration under its endogenous promoter and replaced after Cre-mediated recombination by a mutant SHOC2 D175N allele that is defective for interaction with MRAS and PP1.
The results show that systemic inactivation of SHOC2 in adult mice by using KO and KI models, results in skin dermatitis as well as a range of histopathological alterations that include epidermal hyperproliferation with hyperkeratosis, an increased number of hair follicles that fail to enter catagen and remain in aberrant anagen. The KO and KI mice skin also developed an extensive dermal and epidermal inflammatory infiltrate containing a variety of immune cells. With all of these alterations, Shoc2 KO/KI mice are sharing pathological features associated with human chronic inflammatory skin syndromes such as Atopic Dermatitis and Psoriasis.

**Keywords:** Shoc2, Skin Homeostasis, Mouse Models

## *POSTER PRESENTATION*

# Molecular docking studies of pheromone-lıke compounds within the binding site of a lepidopteran odorant binding protein

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**Abstract**

Lepidopterans are considered quarantine pests in the US, the primary destination market, so exported flowers are rigorously controlled due to the ability of the pest to establish itself in places with a favorable temperature for its development, particularly the Heliothis genus. One of the strategies for its control is using sexual pheromones, where the sexual behavior of the species is used to capture males and monitor the pest in the field. At the physiological level, the female compounds are detected by the trichoid sensillae present in the male antennae, where pheromone-binding proteins (PBPs) initiate signal transduction, triggering an intraspecific orientation and courtship response. Using *in-silico* methods that simulate the protein-ligand interaction allows evaluating of a wide variety of compounds and determining which of these may be potential pheromones. For this reason, the objective of this study was to determine the interaction of different type-I pheromonal compounds (n > 60) reported for lepidopterans with odorant-binding protein (OBP). A homological model of the OBP was constructed, adapted from the crystal structure reported for *Heliothis virescens*, using Yasara Structure software. Subsequently, using the algorithms incorporated in the Molegro and Vina packages, a Docking analysis was performed between the constructed receptor and the type-I pheromonal compounds to determine the interaction. This analysis indicated that aldehydes and acetates having C16-C18 carbon chains were the compounds with the best docking scores. Residual interaction analysis informed the binding mode through Pi-sigma contact between aromatic residues and the hydrophobic ending of the aliphatic chain of test compounds. An *in vitro* interaction assay is in the design phase for the respective validation of these results. This study is derived from the IMP-CIAS-3399 project, financed by the UMNG Vice-Rector for Research, validity 2021.

**Keywords:** Molecular docking, pheromones, lepidoptera, noctuidae

## *POSTER PRESENTATION*

# Molecular networking-based analysis of extracts from sex glands of female *Copitarsia* spp. (Lepidoptera: Noctuidae) obtained by different methods

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**Abstract**

*Copitarsia* spp. is a phytophagous moth of several important plants, distributed in towns of the Bogotá highlands, with cut flowers and cruciferous as its main host plants. Moths of the genus Copitarsia are considered quarantine pests. They are subject to rigorous controlling practices to avoid the damage they cause and their entry since they can establish themselves in areas with favorable temperatures for their development. Hence, its management in cultivated areas is critical, and sex pheromones as the ethological approach can be one of the strategies used to capture and control males. However, the information about Copitarsia pheromones is insufficient. For this reason, this work aimed to compare various sampling methods to identify those compounds accumulated in the sex glands of the Copitarsia females. The methods consisted of direct extractions and captures of accumulated compounds. The resulting extracts and captured compounds were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Different chromatographic profiles were obtained from the test methods, obtaining more compounds through direct extraction in cold maceration due to other glandular fatty compounds. The molecular networking-based analysis led to individualizing the particular contributions of each method and indicated that each method could provide different information about the chemical composition of Copitarsia females. However, three compounds were observed in all profiles, mainly associated with type I infochemicals, which could be related to the sexual pheromone of *Copitarsia* spp. Behavioral and bioactivity studies are in progress to discriminate the joint action of these pheromonal candidates. This study is derived from the IMP-CIAS-3399 project, financed by the UMNG Vice-Rector for Research, validity 2021.

**Keywords:** Molecular network, Copitarsia, lepidoptera, noctuidae.

## *POSTER PRESENTATION*

# Synthesis of Quinaldinium-Appended Calix[4]arene Derivative for Metal Ion Sensing in Fluorescence Sensor Studies

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**Abstract**

Calixarenes, as third generation supramolecular hosts, can be readily functionalized at their lower and upper rims [1]. Furthermore, the fact of their selective ion/molecule binding capacity by incorporating suitable functional groups highly increases their importance. Thus, different derivatives of calixarenes have been widely used as model structures, catalysts and analytical sensors in biomimetic studies [2]. For example, some metal ions are added to drinking water in very small amounts for vital activities. However, these metal ions can have harmful effects on people mentally and physically, especially for children. Therefore, it has become important to develop new methods to determine the amounts of such metals that can be found in our environment. In this work, a new calix[4]arene compound bearing quinaldinium groups at its upper rim was synthesized to be evaluated in fluorescence sensor studies for sensing some metal cations ($Na^+$, $K^+$, $Li^+$, $Mg^{2+}$, $Ni^{2+}$, $Ba^{2+}$, $Ca^{2+}$, $Cu^{2+}$, $Ca^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Ag^+$ etc.) and anions ($F^-$, $Cl^-$, $Br^-$, $H_2PO_4^-$, $NO_3^-$, $I^-$, $HSO_4^-$ etc.). The structure of the synthesized calix[4]arene derivative was characterized by spectroscopic techniques (FTIR, $^1$H-NMR , $^{13}$C-NMR).

**Keywords:** Calix[4]arene, Quinaldinium, Fluorescent calixarenes

**References:**
**[1]** Gutsche, C.D., et al., *Tetrahedron*, 1986, 42:6, 1633–1640.
**[2]** Yilmaz, M.. et. al *Calixarenes in Organo and Biomimetic Catalysis; Calixarenes and Beyond*; Springer International Publishing, 2016, 719–742.
**[3]** Kumar R., et al., *Chem. Rev*., 2019, 119:16, 9657–9721.

## *POSTER PRESENTATION*

# Determination of $Hg^{2+}$ with a new type of rhodamine-cyanine compound that absorbs and emits in the near IR region

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**Abstract**

Mercury is a metal that is used in industrial settings such as power plants, cement kilns, and gold mining, as well as in everyday life in dentistry and in devices such as thermometers and barometers. Mercury, which pollutes the environment even at low concentrations, can pose a major threat to human life and disrupt the ecological balance. When mercury enters the organs through the skin or the food chain, it accumulates in the human body and causes diseases such as Minamata disease, neurological disorders, cognitive deficits, movement disorders, and cardiac arrhythmias [1-4]. Therefore, the highest mercury concentration in drinking water is limited to a maximum of 2 ppb (10 nM) according to the regulations of US Environmental Protection Agency [5]. For this reason, it is necessary to promote a selective and sensitive method for continuous monitoring of mercury, which seriously threatens human and environmental health, in the environment and biological samples.

Methods such as high-performance liquid chromatography [6], atomic absorption spectrometry [7], inductively coupled plasma mass spectrometry [8], and electrochemical detection [9] have been reported in the literature for the determination of mercury in the environment. Since these methods have disadvantages; such as the need for a large amount of samples, expensive equipment, well-trained experts, and long and complicated procedures, it is important to develop analytical methods based on fluorometric and colorimetric methods [10-13].

In this study, a new biocompatible rhodamine -cyanine type compound CSME; was synthesized, characterized, and utilized as a probe for $Hg^{2+}$ cations. Colorimetric and fluorometric analytical methods against $Hg^{2+}$ ions were developed using selective and sensitive probe CSME which responses responds quickly with a wide linear range in the medium of acetonitrile:water (v/v, 9/1, pH 7.4 HEPES). In addition, the CSME probe was used as a bioimaging agent in 3T3 cells, and $Hg^{2+}$ ion presence was visualized using a fluorescence microscope. Furthermore, CSME can be used for instantly detection of $Hg^{2+}$ using a cell phone application.

**Keywords**: rhodamine, cyanine, sensor, smart phone, colorimetric, fluorometric

This project is supported by The Scientific and Technological Research Council of Türkiye (TUBITAK) with the project number of 219Z085.

**References:**

[1] Tchounwou, P. B., Ayensu, W. K., Ninashvili, N., Sutton, D. 2003. "Environmental exposure to mercury and its toxico pathologic implications for public health", Environ. Toxicol., 18, 149–175.
[2] Natale, F. D., Lancia, A., Molino, A., Natale, M. D., Karatza, D., Musmarra, D. 2006. "Capture of mercury ions by natural and industrial materials", J. Hazard. Mater.,132, 220-225.
[3] Grandjean, P., Weihe, P., White, R. F., Debes, F. 1998. "Cognitive performance of children prenatally exposed to Safe levels of methylmercury", Environ. Res., 77, 165–172.
[4] Harada, M. 1995. "Minamata disease: methylmercury poisoning in Japan caused by environmental pollution", Crit. Rev. Toxicol., 25, 1–24.
[5] Mercury Update: Impact on Fish Advisories (EPA Fact Sheet EPA-823-F-01-011), Office of Water, EPA, Washington DC, 2001.
[6] Leermakers, M., Baeyens, W., Quevauviller, P., Horvat, M. 2005. "Mercury in environmental samples: speciation, artifacts and validation", TrAC Trends Anal. Chem., 24, 383–393.
[7] Gao, Y., Galan, S. D., Brauwere, A. D., Baeyens, W., Leermakers, M. 2010. "Mercury speciation in hair by headspace injection–gas chromatography–atomic fluorescence spectrometry (methylmercury) and combustion-atomic absorption spectrometry (total Hg)", Talanta, 82, 1919–1923.
[8] Moreno, F., Garcia-Barrera, T., Gomez-Ariza, J. L. 2010. "Simultaneous analysis of mercury and selenium species including chiral forms of seleno methionine in human urine and serum by HPLC column-switching coupled to ICP-MS", Analyst, 135, 2700–2705.
[9] Fu, X. C., Chen, X., Guo, Z., Xie, C. G., Kong, L. T., Liu, J. H., Huang, X. J. 2011. "Stripping voltammetric detection of mercury (II) based on a surface ion imprinting strategy in electropolymerized microporous poly(2-mercaptobenzothiazole) films modified glassy carbon electrode", Anal. Chim. Acta., 685, 21–28.
[10] Mako, T. L., Racicot, J. M., Levine, M. 2019. "Supramolecular Luminescent Sensors", Chem. Rev., 119 (1), 322–477.
[11] Li, Z., Askim, J. R., Suslick, K. S. 2019. "The Optoelectronic Nose: Colorimetric and Fluorometric Sensor Arrays", Chem. Rev., 119 (1), 231–292.
[12] You, L., Zha, D., Anslyn, E. V. 2015. "Recent Advances in Supramolecular Analytical Chemistry Using Optical Sensing", Chem. Rev., 115 (15), 7840–7892.
[13] Wu, J., Kwon, B., Liu, W., Anslyn, E. V., Wang, P., Kim, J. S. 2015. "Chromogenic/Fluorogenic Ensemble Chemosensing Systems", Chem. Rev., 115 (15), 7893–7943.

## *POSTER PRESENTATION*

# *In vitro* antidiabetic and anti-inflammatory activities of *Centaurea urvillei* subsp. *armata* Wagenitz and *Centaurea virgata* Lam.

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**Abstract**

This study was designed for the first time to investigate the anti-diabetic and anti-inflammatory activities of ethanol extracts obtained from all parts of *Centaurea urvillei* subsp. *armata* (CUE) and aerial part of *Centaurea virgata* (CVE). *In vitro* anti-inflammatory activity of extracts was carried out by 5-lipoxygenase inhibition method while antidiabetic activity was assessed by α-amylase and α-glucosidase inhibition methods. CUE and CVE with $IC_{50}$ values of 0,14 and 0,21 mg/mL exhibited a good inhibitory effect against 5-lipoxygenase enzyme compared to standard indomethacin (0,04 mg/mL), respectively. CUE with an $IC_{50}$ value of 0,96 mg/mL and CVE with an $IC_{50}$ value of 0,41 mg/mL showed weak and moderate activity against α-amylase enzyme, while CUE with an $IC_{50}$ value of 1,50 mg/mL and CVE with an $IC_{50}$ value of 1,04 mg/mL exhibited poor activity against α-glucosidase enzyme, respectively. These results indicate that CUE and CVE possess anti-inflammatory activity. However, further studies, such as *in vivo* studies, are needed to fully reveal the anti-inflammatory properties of extracts.

**Keywords:** *Centaurea urvillei* subsp. *armata*, *Centaurea virgata*, antidiabetic activity, anti-inflammatory activity

## *POSTER PRESENTATION*

# Determination of antibacterial effect of curcumin on *Staphylococcus aureus*

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**Abstract**

Staphylococcal food poisoning is one of the most common foodborne diseases worldwide that causes acute food poisoning in humans as a result of consumption of enterotoxin-contaminated food. There is a need to develop new agents for the prevention of foodborne *S.aureus* infections. Curcumin is a yellow-colored, powerful antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral agent obtained from the turmeric (*Curcuma longa*) plant. In this study, it was aimed to investigate the antibacterial effect of curcumin on *S. aureus*. The in vitro (in broth) and in vivo (in food) antibacterial activity of curcumin on *S.aureus* was determined. Chicken samples were experimentally contaminated with *S.aureus* at the level of $2.0x10^{8'}$cfu/ml. Then, it was kept in 1, 2 and 3 % curcumin solution for 15 minutes. After the application, chicken samples were stored at +4 ºC. The number of *S.aueus* in chicken samples at 0, 2, 4 and 6 days of storage was determined according to the method reported by EN ISO 6888-1-2:1999. The macrodilution method was used to determine the in vitro Minimum Inhibitory Concentration (MIC) value of curcumin for *S.aueus*. In the result of study, it was found that the minimum inhibitory concentration (MIC) of curcumin was 125 µg/ml for *S. aureus*. In the experimentally contaminated chicken samples, the number of *S.aureus* decreased by 2.43, 2.70 and 3.49 log at the end of the sixth day as a result of the application of 1, 2 and 3 % curcumin, respectively. Although 3% curcumin showed a significant decrease in bacterial count, its sensory acceptability decreased. This research was supported by Ondokuz Mayıs University, Scientific Research Projects, Project no: PYO.VET.1904.21.019.

**Keywords:** Curcumin, *S.aureus*, chicken, antibacterial activity.

## *POSTER PRESENTATION*

# Extraction and characterization by GC-MS of the essential oil of *Thymus vulgaris* , evaluation of its antibacterial and antioxidant activity, and complexation by beta-cyclodextrin

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**Abstract**

*Thymus vulgaris* is a plant known and used empirically by our ancestors in traditional medicine, such as whooping cough, bronchitis, pleurisy, and other lung diseases such as emphysema.Our objective was to extract and charctrize thymus vulgaris essential oils and to study its antibacterial and antioxidant activity Then enhance its solubility in water by formulating inclusion complex with amphiphilic molecule. In this work, the extraction of *Thymus vulgaris* essential oils was carried out by Clevenger extractor. A part of this oil was characterized by GC-MS using an apolar column, and the other part was used for the evaluation of the antibacterial and antioxidant activities The essential oil of *Thymus vulgaris* showed a remarkable antibacterial effect against Gram( +) and Gram (–) bacterial strains. For the antioxidant activity, the IC50 (concentration that inhibits 50% of DPPH free radicals) was 36.55462 ul/ml.
To improve its solubility, it was complexed with an amphiphilic molecule widely used in the cosmetic and pharmaceutical field, beta-cyclodextrin,.The improvement of the bioavailability of this complex has allowed its application against several diseases such as parasites and pathogenic bacteria that affect animals such as broilers and laying hens.

**Keywords:** *Thymus vulgaris* , essntial oils , antibacterial activity , antioxidant activity, b-cyclodextrin

## *POSTER PRESENTATION*

# Novel Bioactive Compound from Gypsogenin Aglycone

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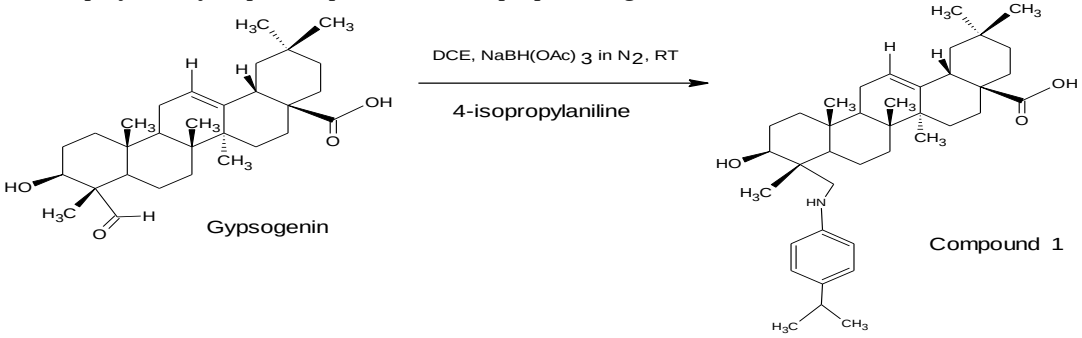
**Abstract**

Gypsogenin compound, a natural saponin derived from *Gypsophila* plant species, is obtained from the plant roots. Gypsogenin compound in pentacyclic triterpene structure has important biological activities. At the same time, semi-synthesis gypsogenin compounds, combined with different substituted derivatives, have higher activity than gypsogenin aglycon such as anticancer, antimicrobial, antioxidant, antithyrosine kinase activity. So, we aimed to synthesize new bioactive gypsogenin derivative. The starting material gypsogenin aglycone was combined with 4-isopropylaniline by using sodium triacetoxyborohydride in DCE at room temperature. The purification of reaction mixture was carried out by using chromatographic methods (with column chromatography). The synthesized compound (**Compound 1**)was determined by IR, UV, $^{1}$H NMR, APT, and LCMS analysis. The biological activities of new semi-synthesized compound was employed very important pharmaceutical properties against HeLa, MCF-7, Jurkat, and K562.




**Keywords:** Gypsogenin; Amine; Semi-synthesis; Cytotoxic.

**References:**

[1] Öztürk S., Karayildirim T., Capaci-Karagoz A., Alankus-Caliskan O., Ozmen A., Poyrazoglu-Coban, European Journal of Medicinal Chemistry, 82, 565-573, 2014.
[2] R. Gevrenova, T. Stancheva, Y. Voynikov, D. Laurain-Mattar, M. Henry, Enzyme and Microbial Technology, 47, 97-104, 2010.
[3] Emirdag-Ozturk S .; Babahan I .; Ozmen A., Bıoorganıc Chemıstry, 15-23, 2014.
[4] J.-G. Luo, L. Ma, L.-Y. Kong, Bioorganic & Medicinal Chemistry, 16, 2912-2920, 2008.

## *POSTER PRESENTATION*

# Overview of Palbociclib, the next-generation neoadjuvant chemotherapy agent

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**Abstract**

Cancer is a serious disease characterized by the uncontrolled proliferation of cells and the formation of metastases as a result of the accumulation of DNA damage. While chemotherapy, radiotherapy, and surgical procedures are among the main cancer treatment methods, immunotherapies, PARP inhibitors, and anti-androgen treatments are also promising. In recent years, neoadjuvant chemotherapy has been reported as a new strategy in some cancer types, especially in locally advanced breast, gastroesophageal, and rectal cancers. While this therapy has advantages such as timely treatment of lymph node/distant micrometastases, increased resection margin, and evaluation of chemosensitivity after treatment, it also carries risks of delayed surgical treatment, progression of the disease, intestinal obstruction, and chemotoxicity. In neoadjuvant chemotherapy, drugs such as Palbociclib providing selective cyclin-dependent 4/6 kinase inhibition (CDK4/6i) have been developed and approved. In the G1-S transitional phase, Palbociclib provides cell cycle control by blocking the progression of tumor cells from G1 to the S phase. In several studies, it has been reported that Palbociclib interrupt the growth, migration, and invasion ability of OSCC cells, increased cellular senescence and apoptosis in a significant manner. Furthermore, a promising therapeutic strategy can be obtained in combination with endocrine therapies in metastatic cancers. Studies show that the combination of Palbociclib with other anticancer drugs can increase drug activities and endocrine therapy resistance, making it the best oral CDKi in its class. However, CDK4/6 inhibitor therapy not only affects tumor cells but also some other normal cell types within the tumor microenvironment, potentially damaging or enhancing tumor responses to therapy. Furthermore, drugs after discharging into the environment may induce some genotoxic potentials on the organisms in water, soils, and other non-targeted species. Therefore, more genotoxicity studies are needed on drugs used for cancer for a safer life.

**Keywords:**Neoadjuvant Chemotherapy, Palbociclib.

## *POSTER PRESENTATION*

# Monkeypox cases in Bulgaria - differential laboratory diagnosis

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**Abstract**

Human monkeypox virus (MPXV) is a doublestranded DNA virus of the Orthopoxvirus genus of the Poxviridae family. Since May 2022, an outbreak of MPXV has been registered in non-endemic countries, and by September 16, 2022, the total number of reported cases in the world was 61 282.
The study aimed to optimize the protocols for MPXV detection, included differential diagnostic aproach to laboratory study of all possible cases of MPXV in Bulgaria. By September, 2022, the total 25 patients (total 81 patient`s clinical materials) with possible MPXV infections were laboratory tested in Bulgaria. Six of them were proven for MPXV. The infected were men who have sex with men between 29 and 44 years of age.The tested clinical materials were: samples of vesicle contents, pustules, nasal swabs, urine and ejaculate. Molecular genetic methods to detection of G2R (TNF receptor gene) region of the MPXV genome were used. MPXV were detected in 6 vesicle contents, 4 pustules, 6 nasal swabs, 2 urine and 2 ejaculates, taken in the first days of onset of clinical symptoms. In three of negative for MPXV patients were proven HSV 2 infection and in four enteroviruses.
The unusually rapid human-to-human transmission of the virus and an exponential increase in the number of infected since May 2022, classify MPXV as a pathogen of "global health concern". The laboratory and epidemiological surveillance of cases is critical to contain the outbreak
Acknowledgments: The study was supported by the European Regional Development Fund through Operational Program Science and Education for Smart Growth 2014–2020, Grant BG05M2OP001-1.002-0001-C04.

**Keywords:** monkeypox, enteroviruses, herpes simplex virus 1 and 2, PCR

## *POSTER PRESENTATION*

# Mumps virus - reemerging disease

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**Abstract**

Mumps is a vaccine-preventable disease caused by an RNA virus (MuV) of the family Paramyxoviridae. Despite vaccination strategies, recent outbreaks in European contries, United States, and Canada highlight the need for rapid diagnosis. Serological methods have limited (folse positive and negative) utility for the diagnosis of mumps in partially immunized individuals. Detection of mumps virus RNA by reverse transcriptase PCR (RT-PCR) is the most sensitive method for laboratory confirmation.
The study aimed to detection of MuV circulated in Bulgaria by RT-PCR assay.
Material and Methods: The total 54 oral fuides from patients with possible MuV infection were tested. Molecular methods with a target amplifying the highly variable small hydrophobic (SH) gene of the mumps virus genome by an one-step RT-PCR were used.
Results: In 49 (91%) of tested patients data for previous MuV immunization were shown. Patients ranged in age from 4 to 32 years. In 28 (52%) positive MuV SH signals were found. The resulting parotitis symptoms were quickly resolved and the outcome of the disease was good.
In summary, the RT-PCR assay is sensitive and specific. In addition, it is significantly less expensive and labor-intensive, making it ideal for mumps diagnosis. Since mumps outbreaks have been documented worldwide, the one-step RT-PCR validated can be used for monitoring of infection.
Acknowledgments: The study was supported by the European Regional Development Fund through Operational Program Science and Education for Smart Growth 2014–2020, Grant BG05M2OP001-1.002-0001-C04.

**Keywords:** mumps virus, RT-PCR, RNA

## *POSTER PRESENTATION*

# Biyoloji Eğitimi Sırasında, Bağımsız Etkinliğin Temeli Olarak Öğrencilerde Yaratıcılığın Oluşumu

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**Özet**

Ortaokullarda öğretmenin çalışmalarında ana yerlerden birini yaratıcı düşüncenin gelişimi tutar. Bu nedenle yaratıcı düşüncenin gelişimi öğrencinin erken yaşlardan itibaren başlamalıdır. Çünkü erken yaşlardan itibaren geliştirilen yaratıcı düşünme, yeni bilimsel keşiflere yol açan ve giderek gelişen düşünmedir. Ders sırasında anlatılan ders , tabloların doldurulması, şemalar, nesnelerin resimlerinin çizilmesi, yazılı cevaplar vb. dahil olmak üzere çeşitli şekillerde öğrencilere anlatılabilir. Bu, öğretmenin ders sırasında öğrencilerin etkinliklerini izlemesine ve ayrıca kapsadıkları konuya nasıl hakim olduklarını görmesine olanak tanır. Öğrencilerde bağımsız aktivitenin gelişimi, yaratıcı yeteneğin gelişimini doğrudan etkiler. Öğretmen öncelikle konuya uygun öğrenme teknolojilerini, amaçlarını, yöntemlerini ve araçlarını doğru seçmeli ve sonunda öğrencileri bu doğrultuda yaratıcı etkinliklere yönlendirmelidir. Bu amaçla, öğrencilerin yaratıcı yeteneklerinin ve aktif öğrenme faaliyetlerinin oluşum koşullarının geliştirilmesine yönelik modern pedagojik teknolojilerin analizi yapılmıştır. Öğrencilere uygun teknolojilerin seçimi, adaptif sistemin amacına göre yapılır. Bağımsız etkinlik oluşturmaları için öğrencilere aşağıdaki yaratıcı görevler verilebilir:

- Bitki ve hayvan hücrelerinin yapısını karşılaştırın, karşılaştırma için belirteçleri adlandırın, uygun sonuçlar verin.
- Prokaryotların yapı ve yaşam aktivitelerinin özelliklerine göre bakteri kavramını belirleyin. Konsept iki bölümden oluşmalıdır. İlk olarak, canlı organizmaların genel grubuna ait olduğu gösterilmeli ve ikinci olarak bu grubu diğer gruplardan ayıran temel özellikler gösterilmelidir.
- Yaz aylarında, su kitlelerinde tek hücreli yosunların aşırı çoğalması görülür. Bu süreci oluşturan faktörler nelerdir? Havza için sonuçları ne olabilir?

Söylenen tüm fikirleri özetlemek gerekirse, öğrencilerde yaratıcı düşünme oluşturulurken aynı zamanda öğrencilerde bağımsız etkinlik oluşturmanın da mümkün olduğu son olarak not edilebilir. Bu, önce öğretmen tarafından verilen görev bazında yapılır ve daha sonra öğrencilerin görev etrafında aktif bir şekilde çalışmasıyla devam eder.

**Anahtar kelimeler:** biyoloji, yaratıcı düşünce, öğrenci, öğretmen.

## *POSTER PRESENTATION*

# Synthesis and characterization of mesoporous iron phosphate catalyst for the propargylamine synthesis

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**Abstract**

Iron phosphate catalysts have attracted considerable attention of researchers because of their electrochemical properties, compositional and good activity. This material is known as an electrode and a catalyst for oxidation reaction [1]. The method of the preparation of the $FePO_4$ catalyst is the key of their structure and activity. The use of our catalyst stays limited In organic synthesis comparing with others heterogeneous one especially in the C-C coupling reaction for the propargylamines synthesis which are important intermediates to synthesize drugs and heterocyclic molecules [2].
In the present study, the mesoporous iron phosphate catalyst is prepared by hydrothermal rout using urea. The catalyst was characterized by XRD, FTIR, MEB-EDS, UV-Vis, Raman spectroscopy, and MET. The catalyst showed a high activity in the propargylamines synthesis via the A3 coupling of aldehyde, amine and alkyne.

**Keywords:** $FePO_4$, Heteregeneous catalyst, C-C coupling, propargylamine.

**References:**
[1] Y. Xu, Y. Lu, P. Yin, L. Yan, Z. Yang and R. Yang, *journal of materials processing technology* **2008**, *204*, 513-519.
[2] M. Bensaad, A. Berrichi, R. Bachir and S. Bedrane, *Catalysis Letters* **2021**, *151*, 1068-1079.

## *POSTER PRESENTATION*

# Can the standard culture medium for breeding the fruitfly, *Drosophila melanogaster*, be improved?

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**Abstract**

The fruit fly, *Drosophila melanogaster* is one of the most widely used model organism in research on genetics and genome evolution. Mass culture of *D. melanogaster* is important to produce sufficient fruit flies for research purposes. Various culture media have been formulated using simple and economical methods to produce the fruit fly. The objectives of this study were to (a) optimize different fruit-based culture media for the hatching and growth of *D. melanogaster*, and (b) determine the effect of different culture media for the collection and hatching of wild-caught *Drosophila* spp. In this study, ten different culture media were formulated to culture inbred *D. melanogaster* and for use as attractant for wild-caught *Drosophila* spp. Banana medium was used as the positive control medium and plain agar was used as the negative control medium. For inbred *D. melanogaster*, the number of pupal cases and hatched flies were calculated for two generations while only the number of pupal cases was calculated for wild-caught *Drosophila* species. The results were analyzed by one-way ANOVA, Tukey's HSD multiple range test and paired sample t-test. Ten different culture media were tested along with banana medium (positive control) and plain agar (negative control). One-way ANOVA showed that there were significant differences ($p \leq 0.05$) in the numbers of inbred offspring and the numbers of wild-caught *Drosophila* species among different culture media. For inbred *D. melanogaster*, the banana and egg medium produced the highest number of offspring for both generations. From the Tukey's HSD test, the number of first-generation pupal cases observed in this medium was significantly higher than for all the other culture media. Conversely, media containing pineapple, starfruit, guava and M3 (with oatmeal) failed to breed *D. melanogaster* in this study. Parental flies cultured in these media failed to survive and did not produce any offspring. The medium containing a mixture of banana, apple, grapes and potato managed to attract and propagate the highest number of wild-caught *Drosophila* compared to other media. In conclusion, this study has contributed to the information on the effect of different culture media for breeding inbred *D. melanogaster* and also for the collection and culture of wild-caught *Drosophila*.

**Keywords:** *Drosophila melanogaster*, inbred, wild-type, wild-caught, culture media, fruits

## *POSTER PRESENTATION*

# Freshwater fish diversity and distribution in ex-tin mining ponds of Kampar, Perak, Malaysia

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**Abstract**

Tin mining industry was once a major contributor to the Malaysian economy from 1820-1992. This transformed areas like Kampar, which is rich in tin resource, into a busy tin-mining town. When the mining industry ended, the mining sites were left deserted, thus forming freshwater ponds over time. These freshwater ponds were able to sustain a variety of organism such as fishes and shrimps, but their diversity still remained largely unknown. Hence, with the aim to study the fish diversity in these ponds, the rehabilitated mining ponds surrounding the Universiti Tunku Abdul Rahman, Kampar campus in Perak were sampled over a period of four months. Samples were collected at four different sites to study the differences of the fish diversity and abundance at different habitat. Water samples were collected in order to study the water quality of disused tin mining ponds, including the level of dissolved oxygen, nitrate, nitrite, ammonia, turbidity, suspended solids, pH, water hardness, temperature and salinity. A total of 3022 fishes which belong to 12 species and 8 families were sampled. *Gambusia holbrooki* was the most abundant (35.24%), followed by *Cichlasoma urophthalmus* (29.09%), *Parambassis ranga* (8.88%), *Stigmatogobius poecilosoma* (6.59%), *Plecostomus* spp. (6.3%). The remaining species contributed the remaining 13.9% of the total catch. Fish diversity in the ponds was found to be closely related to human activities. Introduced species such as *G. holbrooki* and *C. urophthalmus* dominated the fish diversity in the ponds as they were more competitive and reproductive than native species. At site C, the number of species and individuals was the highest, resulting in the highest mean of Shannon-Weaver's Diversity Index (0.536). However, site D contained the highest mean of Simpson's Diversity Index (0.657) and Pielou's Evenness Index (0.84) due to a more uniformly distributed data. The water quality of sampling sites was found to be under Class IIA based on the Interim National Water Quality Standards for Malaysia.

**Keywords:** Tin mining ponds, teleosts, biodiversity, water quality, Kampar

## *POSTER PRESENTATION*

# Photocatalytic degradation of pollutant using biosynthesis photocatalyst assisted UV-visible

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**Abstract**

Photocatalytic degradation using a variety of semiconductor-based materials has recently emerged as an efficient method for environmental purification. Among these materials $TiO_2$ has gained an attention due to its thermal stability, high electron mobility and cost efficiency [1]. Biosynthesis of titanium dioxide nanoparticles ($TiO_2$NPs) by plant extracts is an alternative process to physical and chemical methods that leads to ecofriendly products due to the use of non-toxic solvents, and does not require high temperatures and pressure [2].
The aim of this work is the preparation of titanium dioxide nanoparticles ($TiO_2$NPs) using *Eucalyptus* leaf extract and their characterization by photocatalytic activity. This extract is used as a reducing agent for titanium metal ions in the aqueous medium.
Photochemical oxidation experiments were carried out under UV (365nm) in order to evaluate and compare the performance of the processes.
The results revealed that the biosynthesis material, thermally calcined in air at 500°C for two hours, exhibited enhanced rate of photocatalytic degradation of toxic dye (BM). Furthermore, a comparative study with commercial $TiO_2$ was carried out for the oxidation of MB under UV (365nm). The results showed that the $k_{app}$ for the both photocatalysts (biosynthesis material and commercial $TiO_2$) is almost identical, which confirms the significant performance of the biosynthesis material.
The biosynthesis of the photocatalyst nanoparticles presents promising performances in the field of water purification which require additional studies.

**Keywords:** Eucalyptus leaf, $TiO_2$, biosynthesis, UV (365nm), water purification, photocatalytic activity.

## *POSTER PRESENTATION*

# Molecular assessment of taxonomic status of pine vole species *Microtus subterraneus* and *Microtus majori* distributed in southeastern Europe and Turkey

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**Abstract**

*Microtus subterraneus* and *Microtus majori* are the pine voles that spread throughout northern parts of Turkey. Intra- and interspecific phylogenetic relationships and the taxonomical status of pine vole populations have been controversial for years, and cryptic species are thought to exist especially in *M. subterraneus*' distribution area. To clarify the taxonomical status of Turkish populations of these species, mitochondrial (Cytochrome-b and Cytochrome oxidase-I) and nuclear (Interphotoreseptor retinoid binding protein) gene markers were analysed adding GenBank data from Europe and Caucasia. Considering obtained data based on mean genetic distance, genetic diversity and fixation index values, Bayesian trees and Median-joining networks, it was proved that *M. subterraneus* and *M. majori* are valid species. It was also suggested that although Anatolian, Thracian (Turkish Thrace and Greece) and European populations of *M. subterraneus* and Anatolian and Caucasian populations of *M. majori* have been in the process of divergency since the Pleistocene Glacial Periods, these intrapopulations don't appear to be different species. Besides, considering the high intraspecific variation in *M. subterraneus*, it is still likely that new species in its' distribution area could be identified by the aid of future studies.

**Keywords:** *Microtus subterraneus*, *Microtus majori*, pine vole, Anatolia, mtDNA, nucDNA

## *POSTER PRESENTATION*

# Relationship between the evolutions of blood biomarkers of stress in sea bass *Dicentrarchus labrax* under rearing conditions in M'diq Bay - the Moroccan Mediterranean

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**Abstract**

The aim of this study was to determine the presence of statistically significant correlations between the evolutions of blood indicators of stress in the European sea bass Dicentrarchus labrax. This study was conducted on 48 fish of the bass *Dicentrarchus labrax*. The blood biomarkers studied were cortisol, glucose, lactate and total cholesterol. The correlation test was performed by IBM SPSS Statistic (Version-25) with a significance risk $\alpha = 0.05$. Our results showed the presence of a positive, statistically significant correlation between the evolution of plasma cortisol levels and blood glucose and lactate levels, and between the evolution of glucose and lactate levels. It could be concluded that the increase in the level of the stress hormone "cortisol" activates the secretion of the energy metabolites "glucose and lactate" in the bloodstream of the bar wolf to cope with the stress events.

**Keywords:** farmed sea bass, M'diq bay, stress biomarkers, correlation.

## *POSTER PRESENTATION*

# Tick fauna of cattle in northwestern part of Serbia

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**Abstract**

Ticks are obligate haematophagous ectoparasites which have multiple adverse effects on the host organism. A particular problem is that they spread diseases to humans, domestic and wild animals,which can be reservoirs, vectors and/or transient hosts for the tick-borne pathogens. Ticks are carriers of babesiosis, theileriosis and anaplasmosis in cattle and are of exceptional epidemiological importance. For these reasons, we started systematic research on ticks in cattle in Serbia and here we present the results for northeastern part of Serbia (Mačva). The present study was conducted in 35 cattle herd in the period of March to October 2018, during the grazing season was to established tick fauna in that arae. Ticks were collected from cattle and from pastures where they were grazed. The tick species and sex/gender were identified by morphometric characteristics.Relative abundance analysis revealed that the *I. ricinus* was absolutely dominant species found in 44.22%, followed by *Rhipicephalus bursa* (22.01%). *Haemaphysalis punctata* (18.22%), Boophilus annulatus (12,22%), *Dermacentor marginatus* (9.72%) and *Rhipicephalus sanguineus* (4.22%) Out of the total number of ticks collected, 53.65% were females and 46.35% were males. The sex ratio showed a higher number of females in five species (*Ixodes ricinus, Haemaphysalis punctata*, *Boophilus annulatus*, *Rhipicephalus sanguineus* and *Dermacentor marginatus)*, while higher number of males were detected in *Rhipicephalus bursa.* The population dynamics of recorded tick species showed two annual maxima, in spring (April-May) and in autumn (September-October). Two species *D.marginatus* and *H.punctata* occurred population maximum in April. May was the month of the population peak for *I.ricinus* and it was noted that this species started to decrease in abundance in June. *R. sanguineus* and *R.bursa* reached their maxima decreasing gradually until August, and disappearing completely in Septembar and October. The autumn population peak in Septembar and in October occurred for the *I.ricinus, D.marginatus, Boophilus annulatus* and *H.punctata.*

**Keywords:** cattle, ticks, Northwestern Serbia

## *POSTER PRESENTATION*

# The effect of extract *Astragalus armatus* on liver structure in mice induced by hyperhomocysteinemia.

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**Abstract**

Hyperhomocysteinemia (HHcy) defined by the increase in the homocysteine (Hcy) level in the plasma (1), is related with hepatic lesions and abnormal lipid metabolism(2).
Our research aims to determine the protective effect of the extract on HHcy, which has risk factor of liver diseases, we are looking for whether HHcy leads to hyperlipidemia and endogenous oxidative stress, alterations on liver. And the treatment with the extract of medicinal plant *Astragalus armatus* could correct these alterations induced by high dose of L- methionine.
We want to find out whether the HHcy leads to endogenous oxidative stress, the histopathological changes in the liver, and the extent to which it is reduced by the extract.
The plasma HHCY concentrations were elevated after the following oral administration of L-methionine in high dose to mice, These were associated with appearance of pathological alterations in the liver tissues. While the treatment with *Astragalus armatus* extract is effect in protecting liver tissues in mice fed a diet rich in L-methionine.

**Keywords**: Hyperhomocysteinemia, *Astragalus armatus*, cardiovascular diseases.

**References:**

**1** - Zhang, X., Li, Y., Yang, X., Wang, K., Ni, J., Qu, X. (2005). Inhibitory effect of Epimedium extract on S-adenosyl-l-homocysteine hydrolase and biomethylation. Life Sciences. 78(2), 180-186.
**2**- Latour A, Salameh S, Carbonne C, Daubigney F, Paul JL, Kergoat M, Autier V, Delabar JM, Geest BD, Janel N. 2015. Corrective effects of hepatotoxicity by hepatic Dyrk1a gene delivery in mice with intermediate hyperhomocysteinemia. Molecular Genetics and Metabolism Reports. 2, 51–60.

## *POSTER PRESENTATION*

# Is beta-adrenoceptor antagonist nadolol cytotoxic to HT-29 human colorectal adenocarcinoma cells?

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**Abstract**

The beta adrenergic (β-AR) signaling pathway drives the pathogenesis of the benign vascular tumor infantile hemangioma and several malignant tumor types (1). Pharmacological inhibition of the β-AR receptors with beta blockers has shown clinical efficacy against infantile hemangiomas and angiosarcomas (1,2). Nadolol is a non-cardioselective β-AR antagonist used for the treatment of various disease including hypertension (3). However, it is not known whether nadolol has antiproliferative or cytotoxic properties. In this work, we aimed to investigate whether nadolol has anti-proliferative effects against HT-29 human colorectal adenocarcinoma cells in vitro. HT-29 cells were seeded at a density of 2.5 x $10^4$ cells per ml into 96-well microplates and incubated for 24 h to allow sufficient cell adhesion, then treated with various concentrations of nadolol (0.01-1000 µM) for 24, 48, and 72 hours. 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide (MTT) colorimetric assay was performed to ascertain cell proliferation and cytotoxic activity. MTT assay clearly showed that the HT-29 cell proliferation was not decreased as the concentration of nadolol increases. After treatment with 1000 µM of nadolol for 24, 48, and 72 h, proliferation of HT-29 cells was 104.4%, 95.3%, and 102.9%, respectively. These experimental results indicated that nadolol did not show any cytotoxic effect at concentration up to 1000 µM. Taken together, our data showed that nadolol has no cytotoxic or cytostatic activity against HT-29 human colorectal adenocarcinoma cells in vitro. However, the effects of nadolol on human colorectal cancer cells and other cancer types should be investigated.

**Keywords:** Beta-adrenoceptor antagonist, colorectal cancer, nadolol

**References:**

1- Nagaraja, A. S., Sadaoui, N. C., Lutgendorf, S. K., Ramondetta, L. M., Sood, A. K. (2013). β-blockers: a new role in cancer chemotherapy? *Expert Opinion on Investigational Drugs*, *22*(11), 1359-1363.

2- Yap, A., Lopez-Olivo, M. A., Dubowitz, J., Pratt, G., Hiller, J., Gottumukkala, V., Sloan, E., Riedel, B., Schier, R. (2018). Effect of beta-blockers on cancer recurrence and survival: a meta-analysis of epidemiological and perioperative studies. *British Journal of Anaesthesia*, 121(1), 45-57.

3- Frishman, W. H. (1981). Nadolol: a new β-adrenoceptor antagonist. *New England Journal of Medicine*, 305(12), 678-682.

## *POSTER PRESENTATION*

# Effects of sulfisomidine, a sulfonamide derivative, on HT-29 human colorectal cancer cell line in vitro

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**Abstract**

Sulfisomidine is a sulfonamide analogue and an antibacterial agent (1). However, it is not known whether sulfisomidine has antiproliferative or cytotoxic activities. In the present study, we aimed to investigate whether sulfisomidine has anti-proliferative effects against the proliferation of HT-29 human colorectal cancer cells in vitro. HT-29 cells were seeded at a density of 2.5 x $10^4$ cells per ml into 96-well microplates and incubated for 24 h to allow sufficient cell adhesion, then treated with various concentrations of sulfisomidine (0.01-1000 µM) for 24, 48, and 72 hours. 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide (MTT) colorimetric assay was performed to ascertain cell proliferation and cytotoxic activity. MTT cell proliferation assay clearly showed that the HT-29 cell proliferation was not decreased as the concentration of sulfisomidine increases. After treatment of HT-29 cells with 1000 µM of sulfisomidine for 24, 48, and 72 h, proliferation of cells was 92.3%, 111.2%, and 97.5%, respectively. These results indicated that sulfisomidine did not show any cytotoxic effect at concentration up to 1000 µM. In summary, these data showed that sulfisomidine has no cytotoxic activity against HT-29 human colorectal cancer cells in vitro. However, the effects of sulfisomidine on human colorectal cancer cells and other various types of cancer should be investigated in vitro and in vivo studies.

**Keywords:** Colorectal cancer, HT-29 cell line, sulfisomidine

**References:**

1-Fox, C. L., Modak, S., Stanford, J. W., Fox, P. L. (1979). Metal sulfonamides as antibacterial agents in topical therapy. *Scandinavian Journal of Plastic and Reconstructive Surgery*, *13*(1), 89-94.

## *POSTER PRESENTATION*

# Lateritik Nikel Cevherinin Yüksek Basınçlı Asit Liçi Sonrası Proses Atığından Liç Yöntemi ile Nikel ve Kobaltın Geri Kazanımı

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**Özet:**

Dünya metal nikel rezervinin toplamı 78 milyon ton, görünür ve muhtemel rezerv toplamı ise 130 milyon tondur. Bu rezervlerin yaklaşık %40'ı sülfürlü yataklarda, %60'ı lateritik yataklarda bulunmaktadır. Ülkemizde Manisa-Gördes-Çaldağ, Uşak-Banaz, Bolu-Mudurnu, Eskişehir-Mihalıççık başta olmak üzere yaklaşık 40 milyon tonluk lateritik tipte nikel rezervi bulunmaktadır. Lateritik cevherlerden metal kazanımları hidrometalurjik ve pirometalurjik yöntemler ile yapılmaktadır. Atmosferik liç ve yüksek basınçlı asit liçi bu yöntemlerden en sık kullanılanlarıdır. Ülkemizdeki tek nikel işletmeciliği olan Manisa'nın Gördes ilçesinde bulunan Meta Nikel Kobalt Madencilik Sanayi ve Ticaret A.Ş.'ye ait tesiste yüksek basınçlı asit liçi yöntemi uygulanmaktadır. Lateritik cevherin 245-250 ºC sıcaklık ve 44-46 bar basınç altında liçlenmesi ve sonrasında uygulanan saflaştırma işlemleri ile elde edilen karışık hidroksit çökeleği ara ürün öncesi basamaklarından, ters akımlı dekantasyon tikiner basamağının alt akım bölgesindeki çamur atık olarak depolanmaktadır. Atıkta bulunan değerli metallerin geri kazanılması, çözeltiye alınarak faydalı hale dönüştürülmesi ekonomik açıdan oldukça önem taşımaktadır. Özellikle emisyon değeri düşük, çevreci elektrikli araçların artması, pil teknolojilerinin gelişmesi ile nikel-kobalt ihtiyacı artmakta ve metal iyonlarının geri kazanım yollarına olan ilgi artmaktadır. Dolayısıyla bu çalışmada Meta Nikel Kobalt Madencilik Sanayi ve Ticaret A.Ş.'nin prosesinden sağlanan atıktan nikel ve kobaltın geri kazanımı amaçlanmış ve bu amaç doğrultusunda liç yöntemi çalışılarak farklı parametreler incelenmiştir. Çalışmada öncelikle atık, saf su ile pulp haline getirilmiştir. Sonrasında sülfürik asit kullanılarak atmosferik koşullarda pH, sıcaklık, zaman, % katı miktarı ve süre gibi parametrelerin etkisi incelenmiştir. En uygun koşullara $H_2SO_4$ ile pH=0.5, 90ºC sıcaklık, %30 (w/w) katı oranı ve 2 saat liç süresinde %44.1 nikel ve % 74.6 kobalt verimi ile ulaşılmıştır. Katı ve sıvı faz analizleri ICP-OES cihazı ile yapılmıştır.

**Anahtar Kelimeler:** Nikel, kobalt, lateritik cevher, atık, liç.

## *POSTER PRESENTATION*

# Pektinaz Enziminin Farklı Polimerik Destekler Üzerine Klik Reaksiyonu ile İmmobilizasyonu ve Karakterizasyonu

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**Özet**

Meyve suyu endüstrisinde aydınlatma, kalınlık ve bulanıklık azaltma gibi işlemlerde kullanılan pektinazlar, poligalakturonik asidin a-1,4-glikosidik bağının hidrolizini katalize eder. Pektinaz gibi ticari olarak kullanılan enzimler yüksek aktiviteye sahip olmalarına rağmen, saflaştırılmaları maliyetli, endüstriyel uygulamalarda tekrar kullanılabilirliklerinin olmaması ve zaman içeresinde aktivitelerini kaybetmeleri, depolama sürelerinin kısıtlı olması gibi nedenlerle sürekli kullanımları mümkün değildir. Enzimlerin kullanılabilirliğinin artırılması amacıyla bir desteğe tutturulmaları yaygın olarak kullanılmaktadır.
Bu çalışmada, asetilen fonksiyonilitesine sahip pektinaz enzimi azid fonksiyonilitesine sahip PVC, PVC-PEG, PVC-Kitosan ve PVC-PEG-Kitosan desteklere klik reaksiyonu ile immobilize edildi. Polimerik desteküzerindeki fonksiyonel gruplar immobilizasyon öncesi ve sonrası ATR-FTIR ile karakterize edildi. Serbest ve immobilize enzim üzerine sıcaklık, pH etkileri incelendi, kinetik parametreleri (Vmax ve Km) hesaplandı, immobilize enzimin tekrar kullanılabilirliği, serbest ve immobilize enzimin stabil olduğu zaman aralığı (depolama süresi) tayin edildi. Ayrıca immobilize enzimin taze sıkılmış elma suyunda bulanıklığa etkisi türbidimetrik olarak incelendi. Serbest enzim Ph 6.5'ta maksimum aktivite gösterirken farklı desteklere immobilize edilmiş enzimin pH 7.5'ta maksimum aktivite gösterdiği Ayrıca pH değişikliklerine karşı immobilize enzimin dirençli olduğu ve serbest enzime göre daha yüksek aktivite gösterdiği tayin edildi. Tüm desteklerde immobilize enzimin sıcaklık değişikliklerine karşı daha iyi direnç gösterdiği bulundu. 60 gün sonunda serbest enzim ilk aktivitesinin %40'ını koruken bu değer immobilize enzimlerde yaklaşık %70 olarak tayin edildi. 12 tekrar kullanımdan sonra immobilize enzimlerin 62-76 aralığında değişen yüzdelerde aktivite gösterdikleri bulundu.

**Anahtar Kelimeler:** İmmobilizasyon,klik,pektinaz

## *POSTER PRESENTATION*

# Genotoxicity and mutagenicity studies using *Saccharomyces cerevisiae* strain RS112 on three natural peptide inhibitors to allosterically target SHP-2 in cancer

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**Abstract**

Genotoxicity damages the genetic information of cells and causes mutations that can lead to the development of cancer [1]. Genotoxicity assays are commonly used to identify compounds with genotoxic activity early in the new drug development process [2]. Regulatory agencies such as the Food and Drug Administration (FDA) recommend the use of these assays prior to conducting clinical trials and approving new drugs [3]. Allosteric SHP-2 inhibitors offer great opportunities for drug development due to their potent cytotoxic and anticancer effects [4, 5]. In this study, we aimed to investigate the *in vitro* genotoxic/mutagenic effects of three novel allosteric SHP-2 peptide inhibitors of natural origin with potential anticancer activities (NSC524592, NSC523374 and NSC374121), previously designed by our team using different virtual screening methods [6]. For this purpose, the deletion (DEL) assay was used and the mutagenic/genotoxic effect of the test compounds on the mutant strain *Saccharomyces cerevisiae* RS112 was examined. Eight non-cytotoxic doses ranging from 0.015 to 245.76 µM were tested for each compound and ethyl methanesulfonate (EMS) (100 µg/mL) was used as a positive control. Our results have revealed for the first time that these compounds do not have genotoxic/mutagenic effects through DEL and gene conversion mechanisms. In summary, they can be considered genotoxically safe up to 245.76 µM and could be included in further research in the field of anticancer drug development.

**Keywords:** Allosteric SHP-2 Inhibitors, Natural Peptides, Genotoxicity, Mutagenicity, Yeast DEL Assay

**Acknowledgement:** This work was financially supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) [120Z828] and Atatürk University Scientific Research Projects Coordination Unit [FHD-2022-10235]. Mine İsaoğlu was supported by the Council of Higher Education (YÖK) 100/2000 and TÜBİTAK-BİDEB 2211/A National PhD Scholarship Programs.

**References:**

1. Ren, N., Atyah, M., Chen, W-Y., Zhou, C-H. (2017). The various aspects of genetic and epigenetic toxicology: testing methods and clinical applications. Journal of Translational Medicine, 15(1), 110.
2. Custer, L. L., Sweder, K. S. (2008). The Role of Genetic Toxicology in Drug Discovery and Optimization. Current Drug Metabolism, 9(9), 978-985.
3. Barabadi, H., Najafi, M., Samadian, H., Azarnezhad, A., Vahidi, H., Mahjoub, M. A., Koohiyan, M., Ahmadi, A. (2019). A Systematic Review of the Genotoxicity and Antigenotoxicity of Biologically Synthesized Metallic Nanomaterials: Are Green Nanoparticles Safe Enough for Clinical Marketing?. Medicina (Kaunas), 55(8), 439.
4. Yuan, X., Bu, H., Zhou, J., Yang, C-Y., Zhang, H. (2020). Recent Advances of SHP2 Inhibitors in Cancer Therapy: Current Development and Clinical Application. Journal of Medicinal Chemistry, 63(20), 11368-11396.
5. Wu, J., Zhang, H., Zhao, G., Wang, R. (2020). Allosteric Inhibitors of SHP2: An Updated Patent Review (2015-2020). Current Medicinal Chemistry, 27, 1-17.
6. İsaoğlu, M. (2022). Hedefe Dayalı Sanal Tarama Yöntemleri İle Yeni Allosterik SHP-2 İnhibitör Adaylarının Keşfi ve Biyolojik Etkinliklerinin Araştırılması. Doktora Tezi, Fen Bilimleri Enstitüsü, Erzurum.

## *POSTER PRESENTATION*

# Nephrogenic adenoma

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**Abstract**

Nephrogenic adenomas are uncommon benign lesions of the urothelium, which were initially thought to be a metaplastic process of the urothelium in response to injury or chronic inflammation. It is now considered as a proliferation of implanted renal tubular cells and can occur in patient of all ages. This benign lesion can both clinically and histologically mimic malignancy and presents a potential diagnostic pitfall. Therefore, we present our experience and review the current aspects with regards to the diagnosis and clinical management of nephrogenic adenomas.
We identified 19 cases of nephrogenic adenoma. Lesions were equal to males and females with an average age at diagnosis of 50.1 years (range 11-79). Bladder was the most common site of occurrence (68%), and most patients (47%) were urinary tract irritation and presented with hematuria (26%). Fourteenth (74%) patients were managed with endoscopic resection of their tumors. In particular, one patient underwent radical surgery. Pathological cells express PAX8, CK7, P504s and 34βE12, but do not express GATA3, P63, CK20, NKX3.1 and PSA, Ki67.
Nephrogenic adenomas are benign lesions that can bring discomfort due to the urinary tract irritation and hematuria in addition, these tumors can clinically and histologically mimic malignant tumors and be a diagnostic trap. Urologists and pathologists should have an increased index of suspicion in patients with predisposing factors for nephrogenic adenoma. Immunohistochemistry and morphology can be helpful in distinguishing this entity from malignant processes. In the future, using next-generation sequencing technology may find the pathogenesis and treatment strategies of nephrogenic adenoma.

**Keywords:** nephrogenic adenoma, pathology, treatment

## *POSTER PRESENTATION*

# Morphological analysis of drought stress effect in *Solanum lycopersicum* plant

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**Abstract**

As in many countries of the world, tomato is one of the most important plants for production, consumtion and export of agricultural products in Turkey. According to studies conducted in recent years, Turkey is among the top four in the world in terms of tomato production. Unfortunately, adverse climatic conditions all over the world negatively affect the growth and development of plants, and together with rapidly increasing human-induced damage, plant biodiversity is gradually decreasing and even facing the danger of extinction. Recently, drought stress, causes economic distress by damaging many crops such as tomato. Within this study, the seeds of the "pink sandik tomato", a variety of tomato plant grown and exported in Turkey, were transferred to the in vitro environment free from microorganisms by surface sterilization. Then, germinated seeds were reached to sufficient numbers by in vitro clonal propagation. The shoot tips obtained from the in vitro grown micro-shoots were transferred to MS medium supplemented with 0%, 2.5%, 5%, 10%, 20% or 30% Polyethylene glycol (PEG) for drought stress application. Plant materials were incubated at 27±2 °C, 16/8 hour photoperiod and 50 µmol-1 m-2 s-1 white daylight fluorescently illuminated ambient conditions. After one month, plant materials growing under drought stress conditions were analyzed morphologically. When the groups formed with different concentrations of PEG for drought stress were examined, it was observed that the control group developed better than the other groups and plant loss was very low. Depending on PEG treatment at increasing concentrations, it was observed that there was an increase in yellowing, shedding and plant loss in the leaves of the plant. In addition, it was observed that almost all of the plants were transferred to the medium containing 30% PEG lost their viabilities.

**Keywords:** Abiotic stress, morphologic differentiation, PEG, tissue culture

## *POSTER PRESENTATION*

# Atık sulardan bisfenol A'nın aktif karbona adsorpsiyonu

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**Özet**

Son zamanlarda içme suyunda saptanan endokrin bozucu bileşikler (EDC'ler) ciddi bir sorun olarak ortaya çıkmıştır. Özellikle artan nüfus ve gelişen sanayideki yığın kimyasallarının üretiminden kaynaklı olarak bu bileşikler çevreye deşarj olmaktadır. EDC'lerden biri olan bisfenol A (BPA), plastik endüstrisinde polikarbonat ve epoksi reçinenin üretimi için yaygın olarak bir monomer olarak kullanılır. Fenolik polikarbonat plastikleri, epoksi reçineleri ve polivinilklorürün endüstri ve hane halklarında sıkça kullanılmasından dolayı nehirlerde, denizlerde ve topraklarda BPA birikimi gözlenmektedir. Su ortamlarına salınan başlıca BPA kaynakları su arıtma tesisleri ve endüstriyel atık sulardır. EDC'ler; estron, 17p-strodial ve estriol gibi doğal östrojenlerin yanı sıra etinilestrodial gibi yapay olarak sentezlenmiş estrojenler ve BPA, nonilfenol gibi diğer endüstriyel bileşikler dahil olmak üzere ve bir dizi diğer fenolik bileşikler bir veya daha fazla fenolik grup içeren organik kimyasallardır. Yapıları ve fizikokimyasal özellikleri nedeniyle bu moleküller endokrin sistemlerini bozabilir ve sudaki yaşam ve insan sağlığı üzerinde olumsuz etkilere neden olabilir. Doğal hormonların aktivitesini bloke edebilir veya taklit edebilir. Böylelikle üreme sistemlerine müdahale edebilir, doğurganlığı azaltır ve meme, yumurtalık ve testis kanserlerinin olasılığını artırabilir.
Çalışmada atık suda bulunan endokrin bozucu bileşik BPA'nın suda aktif karbona (20-40 mesh) adsorpsiyonu ceket tipi ısı değiştirici özelliğine sahip 2 L hacimli amber camdan imal edilmiş mekanik karıştırmalı ve sıcaklık kontrollü bir sistemde gerçekleştirilmiştir. Tüm deneylerde belli başlangıç derişimine sahip 1 L BPA çözeltisi kullanılmıştır. Sulu çözeltisinden BPA'nın aktif karbona adsorpsiyon sürecinde; kalma süresi (t=~24 h), başlangıç BPA derişimi ($C_0$=10-100 ppm), adsorban miktarı (m=1-3 g), karıştırma hızı (N=100-300 rpm), ortam sıcaklığı (T=10-60 $^oC$) e pH'ı (2-13) ve bunun yanı sıra iyon türleri (NaCl, KCl, $MgCl_2$, $Na_2CO_3$) ve derişim etkisi gibi temel adsorpsiyon parametrelerinin adsorpsiyon verimine etkisi incelenmiştir. Ayırma süreci için en uygun koşul; t=6 h, $C_0$=80 ppm, T=25 $^oC$, $m_{AC}$=2 g, N=200 rpm, ve doğal pH olarak saptanmıştır. 0.5 M NaCl derişiminin kısmen adsorpsiyon verimini arttırdığı belirlenmiştir.

**Anahtar Kelimeler:** Endokrin bozucu bileşik, Bisfenol A, Aktif karbon, Adsorpsiyon

## *POSTER PRESENTATION*

# Laktoz İntoleransının Azaltılması için Rekombinant Olarak β-Galaktosidaz Enziminin Üretilmesi

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**Özet**

Laktoz, sütte bulunan ana karbohidrattır ve memelilerde emzirme döneminde ana enerji kaynaklarından biridir. Glukoz ve galaktozdan oluşan laktozun bağırsaklarda az sindirilmesi ya da sindirilmemesi sonucu oluşan rahatsızlık, laktoz intoleransı olarak adlandırılmaktadır. Laktoz intoleransı olan bireylerin tolere edebildikleri kadar laktoz almaları önerilmektedir. Çünkü, laktozun kaynakları olan süt ve süt ürünleri kalsiyum, fosfor, çinko ve B grubu vitamin kaynağı açısından oldukça zengindir. Süt ve süt ürünlerinin tamamen beslenme düzeninden çıkarılması bazı hastalıklara sebep olmaktadır. β-galaktosidaz enzimi ilaç endüstrisindeki popülerliğinin yanı sıra gıda sektöründe de kullanımı oldukça yaygındır. Terapötik alanda ve gıda alanında ihtiyacın artması ile enzime olan talep her geçen gün artmaktadır. Bu noktada biyoteknoloji, rekombinant teknolojiyi kullanarak enzim üretimini ve verimini artırmaya yardımcı olur. Bu çalışmada rekombinant β-galaktosidaz enziminin üretilmesi hedeflenmiştir. Yüksek verimde üretim kapasitesine sahip pET28a plazmidi seçilmiştir. UniProt veri tabanında P16278 erişim koduna sahip β-galaktosidaz sekansının TOP10 E.coli bakterisine göre kodon optimizasyonu yapılmıştır. Enzim geni tasarlanarak hizmet alımıyla ürettirilmiştir. Sipariş edilen gen sekansı pUC plazmidi içinde gönderilmiştir. Bu plazmid Üretim TOP10 E.coli bakterisine ısı şoku ile transforme edilmiştir. Kanamisin içeren sekans LB/kanx içeren agarda büyütülerek tek koloni seçimi yapılmıştır. Plazmit izolasyonu için Thermo GeneJET Plasmid Miniprep Kit kullanılmaktadır. Plazmit nanodrop içinde ölçülür ve agaroz jel içinde UV ışığı altında görselleştirilir. pET28a ve elde edilen gen sekansı ligasyon yöntemi ile birleştirilerek TOP10 E.coli bakterisine ısı şoku ile transforme edilmiştir. Gen doğruluğu agaroz jel işe kontrol edilmiş olup üretime geçilmiştir. SDS-PAGE yapılarak protein varlığı kanıtlanmıştır. Bu aşamadan sonra, besiyeri optimizasyonu yapılarak maksimum verim sağlayacak kompozisyon belirlenmiştir. Optimize edilen besiyeri ile üretim yapılarak hücreler sonikasyon yöntemi ile parçalanmıştır. Saflaştırma işlemi Hi Trap IMAC kolonu ile tek adımda gerçekleştirilmiştir. Saflaştırma sonunda proteinin moleküler ağırlığı belirlenmiştir. Moleküler ağırlığı aynı zamanda QTOF ile doğrulanmıştır. Aktivite ve stabilite deneyleri ile enzimin uniti belirlenmiş aynı zamanda da sıcaklık ve pH değişikliğinin etkileri incelenmiştir.

**Anahtar Kelimeler:** Rekombinant enzim üretimi, laktoz intoleransı, β-galaktosidaz enzimi

## *POSTER PRESENTATION*

# Yaban mersini tohumu yağının süperkrtik akışkan ekstraksiyonu

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**Özet**

Dünyada son yıllarda fenolik bileşikler ve antosiyanin içerikleri nedeniyle Yaban Mersin'i (*Vaccinium* spp.) gibi meyvelerin üretim ve tüketiminde büyük oranda artışlar meydana gelmiştir. Yaban Mersin'i meyvesi; meyve suyu işleme sektörünün yanı sıra reçel ve jöle gibi çeşitli gıda üretim proseslerinde özel ilgi görmektedir. Bu süreçlerde üretimden kaynaklanan posa ve tohum gibi atıklarda değerli biyokimyasal bileşiklerin ekonomiye yeniden kazandırılması önemlidir. Kütlece %24-30 arasında yağ içeriğine sahip olan Yaban Mersin'i tohumunun yağı; güçlü bir antioksidan kaynağı olup özellikle cilt hasarına ve gözle görülür yaşlanma belirtilerine neden olan çevredeki serbest radikalleri nötralize edebilme potansiyeline sahiptir. Ayrıca yüksek Omega 9, beta-karoten formunda bir A vitamini ve sterol konsantrasyonuna sahiptir. Bu olumlu özellikleri sergileyen tohumun yağı bu nedenle fonksiyonel gıdalarda, farmasötiklerde ve kozmetiklerde bir bileşen olarak kullanılabilir. Günümüzde değerli ürünlerin iyi üretim uygulamaları ile geri kazanımları için süperkritik $CO_2$ ile ekstraksiyon uygulamaları popüler hale gelmiştir. Süperkritik akışkan ekstraksiyonu gözenekli katı materyallerde ya da sıvı çözeltilerdeki istenilen ürün/ürünlerin süperkritik koşullardaki çözücüyle denge çözünürlüğü farkına dayanarak ayrılması ve bu ayırma işlemi sonunda ekstrakte edilmiş ürün/ürünleri içeren süperkritik koşullardaki çözeltinin atmosferik koşullara ya da belirli basınç koşullarına indirgenmesiyle süperkritik akışkan bileşimindeki çözünen ürün/ürünlerin ayrılması işlemi olarak tanımlanabilir. Bu çalışmada, yaban mersini tohumu yağının süperkritik akışkan ekstraksiyon sürecinde ayırma verimine etki eden temel proses parametrelerinin etkisi süperkritik çözücü fazına göre sürekli katı faza göre kesikli olarak işletilen; sıcaklık, basınç ve akış hızı kontrollü ISCO Marka SFX 220 model SFE sisteminde araştırılmıştır. Meyvenin tohumları sırasıyla; yıkma, kurutma ve öğütme işlemlerinin ardından hem nem içeriği hem de partikül boyutu ekstraksiyon işlemine hazır hale getirilmiştir. Hekzan ile gerçekleştirilen Soxhlet ekstraksiyon sürecinde 24 h sonunda tohumlardaki yağ içeriği kütlece %26 olarak saptanmıştır. Süperkritik $CO_2$ ile gerçekleştirilen ekstraksiyon sürecinde bu yağın %90 geri kazanım verimine en uygun ayırma koşulu olarak saptanan; 350 bar, 50 $^oC$ ve 2 mL/min koşullarında 3 h süre sonunda ulaşılmıştır.

**Anahtar Kelimeler:** Yaban mersini tohumu yağı, Süperkritik $CO_2$, Süperkritik akışkan ekstraksiyonu.

## *POSTER PRESENTATION*

# Synthesis and investigation of anti-cancer activity of novel substituted alkylsulfonyl 1*H*-benzo[*d*]imidazole derivatives in MCF-7 breast cancer cells

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**Abstract**

This study aimed to synthesize and evaluate a series of novel alkylsulfonyl 1H-benzo[d]imidazole derivatives in continuation of our previous work on benzimidazole scaffold. Several new compounds (23-36) were synthesized and evaluated for their cytotoxic and anti-cancer activities over MCF-7 breast cancer cell line. Based on the in vitro antiproliferative activity of the derivatives, compounds 23 (2-(4-Chlorophenyl)-1-cyclohexyl-5-(methylsulfonyl)-1H-benzo[d]imidazole) and 27 (1-(3,4-Difluorobenzyl)-2-(3,5-difluorophenyl)-5-(methylsulfonyl)-1H-benzo[d]imidazole) were found to be the two most cytotoxic against MCF-7 cells. The effects of the compounds on gene expression profiles were also elucidated. Forty five genes which are related to microtubule organisation, tumor suppression, apoptosis, cell cycle and proliferation were selected. Vincristine, a renowned tubulin polymerase inhibitor was also applied to MCF-7 cells and this group was used as a positive control in order to investigate the anti-cancer effect of our newly synthesized benzimidazole compounds. Among the studied genes, there is high level of expression of ABL-1 in compounds 23 and 27-treated MCF-7 cells indicating more inhibitory effect compared to standard chemotherapeutic agent, vincristine. Similarly, the expression of BCL-2 is significantly downregulated in cells following treatment with the compounds by 128 and 256 folds respectively. Furthermore, there is overexpression of p53 consequent to treatment with the compounds. On the other hand, BRCA1 and BRIP1 overexpressions were only observed in compound 23 treated cells indicating that the compound may exert its cytotoxic effect through BRCA1/BRIP1 gene. Overall, our compounds exert cytotoxic activity as multi-target gene inhibitors, especially as BCL-2 inhibitors. In addition, molecular docking analysis suggests the presence of halogens and sulfonyl groups in these compounds played crucial roles in their H-bond interactions with DNA bases leading to good docking profile.

**Keywords:** Benzimidazole, alkylsulfonyl, cytotoxic activity, MCF-7, molecular docking, BCL-2 genes.

## *POSTER PRESENTATION*

# Evaluation of a pesticide-enriched potato diet (Metribuzin and Mancozeb) on biochemical parameters and kidney histology in the wistar rat

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**Abstract**

Pesticides have made tremendous progress in controlling food resources and improving public health (especially in the fight against insects, vectors of diseases), and the flip side of the coin has emerged rapidly. Resistance phenomena in insects and reproductive disorders in birds have shown dramatically the limits and dangers of these substances to the environment, to ecosystems but also to human beings.
The aim of our work is to show the effect of the consumption of potatoes contaminated by two types of pesticides, Metribuzin herbicides and Mancozeb fungicide in western Algeria on the biochemical parameters and kidney histology in the wistar rat.
The experimental protocol includes different rat groups, control rats receive a potato diet that is uncontaminated by pesticides, and other groups of rats that receive a pesticide-contaminated potato diet, Metribuzin in two different doses (D1 1.3 mg / kg / day and D2 13 mg / kg / day) and Mancozeb (D1 5 mg / kg / day and D2 50 mg / kg / day) for three months. biochemical parameters measured are Glucose, urea, creatinine and clearance, kidney is removed for histological analysis.
The results obtained show a significant hyperglycaemia, increased blood urea, Creatinine and clearance of Mancozeb and Metribuzin compared to control rats. Histological analysis of kidney shows an inflammatory infiltrate and architectural disorganization of kidney cells in rats consume both types of pesticides.
Conclusion Mancoezb and Metribuzin have an effect on the biochemical parameters and disorganization of kidney architecture in the rat.

**Keywords**: Metribuzin, Mancozeb, kidney, wistar rat.

## *POSTER PRESENTATION*

# Biochemical Profile In People With Hepatic Insufficiency

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**Abstract**

Liver failure is the failure of liver function that can be acute or chronic. liver failure means a loss of function of hepatocyte cells and is influenced by several factors such as
toxins, infections and autoimmune. the aim of this work is to determine some biochemical parameters in people with hepatic insufficiency in the mostaganem region.
for this study we chose men and women with hepatic insufficiency and healthy people belonging to the age group (30-40 years). as assayed biochemical parameters; (alat, asat,
alkaline phosphatase, albumin, gamma glutamyltransferase and total and direct bilirubin) and hematological parameters (white blood cells, red blood cells, platelets and prothrombin level) within the biochemical laboratory of the university hospital center (chu) of kharouba in mostaganem.
the results obtained show an increase in the level of transaminases (alat and asat), alkaline phosphatase, gamma glutamyltransferase and direct bilirubin, a decrease in the level of white blood cells and the level of prothrombin.
to live with this disease, it is recommended to follow a diet low in protein in addition to monitoring liver function if necessary to avoid bleeding while avoiding the use of drugs and alcohol.

**Keywords:** Hepatic impairment, biochemical parameters, adults

## *POSTER PRESENTATION*

# A novel Na-nanoparticles containing solid catalyst for biodiesel production

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**Abstract**

Na-nanoparticles containing solid catalyst (Na-Nps) derived from the leach solution of *Alyssum murale* was developed for the production of biodiesel from waste frying sunflower seed oil (WFSO). In the material synthesis method, the metallic ions in *A. murale* were taken into an acidic solution and then these ions were reduced via $NaBH_4$ as a reductant agent. The developed method is independent of synthetic metal salts and harmful products do not occur at the end of the synthesis process. The characterization studies of Na-Nps were carried out via TEM, SEM, EDX, XRD, FT-IR analyses. TEM and SEM analyses showed that the synthesized particles were in nano-size and heterogeneous spherical-like structure. According to EDX analysis, Na-Nps contained 56.75% O, 39.43% Na, 1.32% Mg, 0.38% K, 0.7% Ca, and 1.42% Ni elements in weight. XRD and FT-IR analyses included the peaks originating from both sodium element and plant content. Na-Nps were applied for biodiesel production from the transesterification of waste frying sunflower seed oil and the transformations from WFSO to biodiesel were confirmed via $^1$H NMR. According to $^1$H NMR spectra, the peak in the 3.5-4.0 ppm range indicated the presence of fatty acid methyl esters (FAME), ie biodiesel, while the peak around 5.5 ppm showed the presence of glycerol. On the other hand, the peak assigned to the methyl group was seen in the 3-3.5 ppm range while the peak belonging to the 5-carbon ring (triazole) structure occurred at around 7.5 ppm. A maximum biodiesel peak intensity in $^1$H NMR was achieved using the methanol:oil volume ratio of 2:5, Na-Nps:oil ratio of 1:1000 (w (g) : v (mL)), the reaction time of 30 min, and the reaction temperature of 65°C.

**Keywords:** *Alyssum murale*, solid catalyst, biodiesel production, waste frying sunflower seed oil.

## *POSTER PRESENTATION*

# Investigation of the anti-cancer effect of *Lamium purpureum* on prostate cancer cell lines

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**Abstract**

Cancer is the most common and deadly disease in the world after cardiovascular diseases. Human prostate cancer ranks second in incidence worldwide after lung cancer in men. Anticancer, anti-inflammatory, antioxidant, antimicrobial, antiviral and antiobesity effects of some plants are known. The genus Lamium is a herbaceous species in the Lamiaceae family. Antioxidant, antibacterial, antimicrobial, anti-inflammatory, cytotoxicity and antityrosinase activities of the genus Lamium have been shown before. In this study, the anticancer effect of Lamium purpureum, a species belonging to the genus Lamium, was studied in LNCaP androgen-dependent human prostate cancer cell lines. Extracts derived from n-hexane (SSH), aqueous ethanol (SSSE), chloroform (SSK), ethyl acetate (SSEA), maceration ethanol (SSME) prepared from Lamium purpureum plant were used. The cytotoxic effect of the extracts was examined with the CCK-8 kit after 24 and 48 hours of incubation. IC50 values were calculated using the results obtained. Accordingly, the IC50 values for SSH, SSSE, SSK, SSEA, SSME in the LNCaP cell line were 20.33, 86.66, 78.07, 70.41, 27.18 µg/ml for 24 hours; 19.93, 48.81, 35.05, 56.92, 15.35 µg/ml for 48 hours, respectively. The JC-1 mitochondrial membrane potential test was used to determine possible depolarization in the mitochondrial membrane potential. As a result of the 48-hour study with SSH and SSK, no depolarization tendency was observed in the mitochondrial membrane potential. These preliminary results suggest that the extracts suppress cell proliferation by showing cytotoxic effects, but may not stimulate the intrinsic apoptotic pathway. Further studies will be carried out to support the results.
This study was supported by Marmara University Scientifical Research Projects Commission with project number TYL-2022-10417.

**Keywords:** Lamium purpureum, LNCaP, prostate cancer, apoptosis, anticancer.

## *POSTER PRESENTATION*

# Physical Properties of the excellent Second Harmonic Generation material $LiMg(IO_3)_3$

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**Abstract**

The excellent second harmonic generation (SHG) material, $LiMg(IO_3)_3$ has been elaborated in 2019. It exhibits an extremely strong SHG effect of roughly 24 x KH2PO4 (KDP) under 1064 nm laser more than $Zn(IO_3)_2$. In this work, we carry out the DFT calculations as implemented in Cambridge Sequential Total Energy Package CASTEP module of material studio. We employed the pseudo-potential plane wave method approach on new $LiMg(IO_3)_3$ theoretical compound to investigate its structural, electronic, mechanical and optical properties. The electronic structure show that $LiMg(IO_3)_3$ has an indirect band gap of 2.789 eV. The imaginary part of the dielectric functions was calculated as well as the refractive index. Elastic properties and mechanical stability were investigated. The present optical material Lithium magnesium iodate could be considered as a promising material for optoelectronics applications too.

**Keywords:** Lithium Magnesium iodate, DFT, Electronic structure, Band gap, Refractive index.

## *POSTER PRESENTATION*

# 4-Aminoantipiren temelli farklı Schiff bazlarının Glukoz biyosensörü olarak incelenmesi

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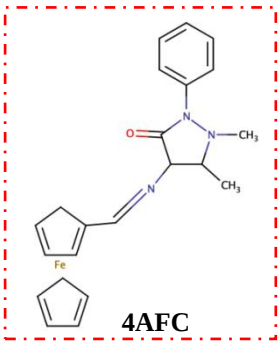
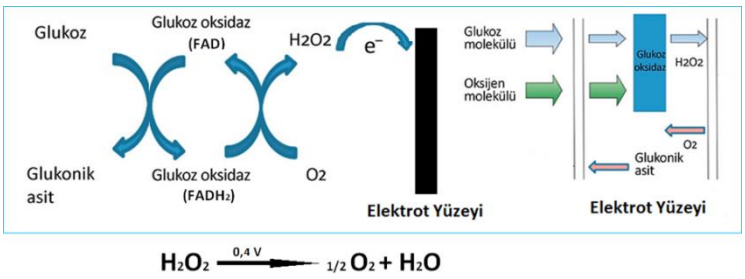
**Özet**

Glukoz tayini; gıda işlemleri, klinik, biyolojik ve kimyasal örneklerde oldukça önemlidir. Örneğin obezite günümüzde birçok ülkede en önemli sağlık sorunu olarak yer almaktadır. Obeziteye bağlı olarak şeker hastalarının sayısında da artış söz konusudur. Kan glukoz değerinin hızlı, düşük maliyetli ve pratik bir şekilde tayin edilmesi önem arz etmektedir. Şeker hastalığının izlenmesi ve tıbbi teşhisi amacıyla glukoz tayinleri yaygın olarak kullanılmaktadır. Deri altı dokularında glukoz derişimini sürekli bir değerlendirmeye tabi tutabilmek ve kandaki glukoz seviyesini belirleyebilmek amacıyla duyarlı, seçici, güvenilir ve düşük maliyetli glukoz sensörü üretimi giderek artmaktadır [1-2].

Bu çalışmada, enzimatik tepkimeler sonucunda sıklıkla meydana gelen hidrojen peroksit'e ($H_2O_2$) duyarlı modifiye karbon pasta elektrot (MCPE) hazırlandı. Enzim biyosensörleri de enzimatik olarak üretilen $H_2O_2$'in oksidasyonu ile hazırlanabilirler. Bu nedenle de hazırlanan elektrodun $H_2O_2$'e duyarlı olması biyosensörlerin tasarlanmasında oldukça önemlidir [1]. Bu amaçla, 5 farklı madde (N-ferroseniliden-4-aminoantipiren, Salisiliden-4-aminoantipiren, 5-floro-3-metilsalisiliden-4-aminoantipiren, 5-floro-3-kloro-4-aminoantipiren, 5-florosalisiliden-4-aminoantipiren) karbon pasta elektrodu modifiye etmek için kullanıldı ve duyarlılık-cevap akımları incelendikten sonra en yüksek cevap akımı gözlenen madde karbon pasta elektrodu modifiye etmek için kullanıldı. Glukoz miktarının belirlenmesi için ise, modifiye edilmiş karbon pasta elektrot ile glukoz oksidaz (GOD) içeren bir enzimatik biyosensör sistemi hazırlandı. Bu modifiye elektrota enzim immobilizasyonu yapılarak yeni bir biyosensör tasarlandı. Hazırlanan biyosensör ile glukoz tayini; enzimatik reaksiyon sonucu açığa çıkan $H_2O_2$'in anodik akımlarının ölçülmesi esasına dayanılarak yapıldı.




(N-ferroseniliden-4-aminoantipiren)




$$H_2O_2 \xrightarrow{0,4\ V} 1/2\ O_2 + H_2O$$

Tasarlanan biyosensörün amperometrik cevabına; karbon pasta elektrodu modifiye etmek için kullanılan 4AFC kodlu Schiff bazı (bileşiğin yapısal karakterizasyonları spektroskopik yöntemler kullanılarak yapılmıştır) miktarının, çalışma potansiyeli değerinin ve substrat derişiminin etkisi araştırıldı. Amperometrik i-t yöntemi ile tayin için optimum çalışma koşulları; *0,4 V* çalışma potansiyeli, modifiye etmek için *2,50 mg* 4AFC miktarı olarak belirlendi. Biyosensörün doğrusal çalışma aralığı, alt tayin sınırı, $Km_{(göz)}$ ve $Imax_{(göz)}$ değerleri tespit edildi.

Biyosensörün performansını etkileyen faktörler olan tekrarlanabilirlik, raf ömrü, girişim etkisi parametreleri incelendi.

**Anahtar Kelimeler:** Glukoz, biyosensör, karbon pasta elektrot, Schiff bazı, ferrosen, amperometrik

**Kaynaklar:**

**1.** Arslan, F., Ustabaş, S., & Arslan, H. (2011). An amperometric biosensor for glucose determination prepared from glucose oxidase immobilized in polyaniline-polyvinylsulfonate film. *Sensors*, *11*(8), 8152-8163.

**2.** Fitzpatrick, D., (2015). Glucose Biosensors, Implantable Electronic Medical Devices, s.37-51 doi: 10.1016/B978-0-12-416556-4.00004-8.

## *POSTER PRESENTATION*

# In vitro antioxidant activity of leaves and stems from an Oudneya species

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**Abstract**

The aim of this study was to evaluate the antioxidant activity of three extracts; Chloroform (CHCL3), Ethyle acetate (AcOET) and (n-BuOH ) obtained by using maceration apparatus, of the leaves and stems of Oudneya medicinal plant used in traditional pharmacopoeia of Algeria. The determination of total phenolic content by the Folin-Ciocalteu method produced values of: 98.6, 137.16 and 143.26 µg gallic acid equivalent/mg (GAE/mg) respectively, while the evaluation of total flavonoids content using Aluminum chloride (AlCl3) method showed that this plant contain 15.62, 21.96 and 27.63 µg quercetin equivalent/mg (QE/mg) for CHCL3, AcOEt and n-BuOH, respectively.
In vitro antioxidant methods such as DPPH was used to determinate the antiradical activity of leaves and stems of the plant extracts, The three extracts showed a very interesting DPPH radical scavenging with low values of IC50 (12.05, 14.91 and 24.57 µg/ml for n-BuOH, AcOEt and CHCL3, respectively) of leaves extracts.

**Keywords:** phytochemical, polyphenols, flavonoids, antioxidant activity, DPPH.

## *POSTER PRESENTATION*

# Development of UV light emitting naphthol-based fuel marker

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**Abstract**

Legal violations in fuels include the dilution of high-tax fuels with low-tax fuels, adulteration of quality fuels with poor-quality fuels, and smuggling of illegal fuel. These legal violations do not only cause loss of income for the states, and poor performance of fuels but also cause serious damage to the environment due to the formation of harmful gases released through fuel emissions. As a solution to prevent all these problems, fuel markers are added to fuels. Fuel markers are used to track fuels, and their sources, customize fuels, monitor their quality, classify fuels, and identify the types of fuels available in the market. The suitability of a fuel marker is determined by examining the following characteristics. Such as solubility in fuel, no color in fuel, inertness, and detectability by photophysical techniques quickly.
For these reasons, we synthesized a naphthol-derived compound containing a long-chain alkyl group that enables dissolution in the fuel. We investigated the solubility of this compound in the fuel, its marking properties, and photophysical properties in the fuel.

**Keywords:** Naphthol, Long-chain alkyl, Fuel marker.

## *POSTER PRESENTATION*

# The synthesis and the photophysical properties of 2,4,6-substituted pyrylium salts

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**Abstract**

2,4,6-Aryl pyrylium salts (Katritzky salts) are stable dyes having high absorption coefficients and fluorescence quantum yields in the visible region. These properties let them be used in various studies as photocatalysts, light emitters, sensors, and sensitizers. Since the main purpose of these studies was the application of the dye, the information about the effect of substitutions on the photophysical properties of the dye has remained scattered. In our work, we aimed to investigate these effects by synthesizing pyrylium compounds with different substituents at 2,4 and 6 positions. The UV-VIS absorption and photoluminescence properties of the synthesized dyes were investigated in different solvents. The obtained results indicated that the photophysical properties of pyrylium salts can be easily tuned by making small modifications on the substituents.

**Keywords:** Pyrylium, Uv-vis absorption, Photoluminescence.

## *POSTER PRESENTATION*

# The removal potential of two aquatic macrophyte species (*Pistia stratiotes* L. and *Egeria densa* Planch.) for copper, chromium and vanadium contaminated waters

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**Abstract**

Aquatic habitats are the final sink for several pollutants including heavy metals released into environment. Therefore, they pose a a serious threat to aquatic life. They are persistent to degradation and have a tendency to accumulate in tissues, thus may get involved in the food chains. Thus, rehabilitation of contaminated water resources and treatment of wastewaters are vital from an environmental standpoint. Phytoremediation is an ecofriendly and cost-effective technique used to remove heavy metals from polluted water bodies. Although, several aquatic macrophytes have been proposed for removal of several pollutants; there are still some gaps on the optimization of removal rates. In this study, we aimed to investigate the removal potential of *Pistia stratiotes* and *Egeria densa* for copper, chromium and vanadium. Plants were exposed to 3 different concentrations of each element (for copper: 0.025, 0.1 and 0.4 ppm, for chromium: 2.5, 5 and 10 ppm, for vanadium: 0.2, 0.6 and 1.8 ppm) in %25 Hoagland solutions. The concentration of heavy metals in the water was measured with the ICP-MS on 1, 4 and 7$^{th}$ days. Removal percentages were calculated and compared. The maximum removal rate for copper was found as 96.7% and 83% for *P. stratiotes* and *E. densa*, respectively. Removal rates for chromium (6.5 to 28.1%) and vanadium (13.7 to 22,1%) were lower for both plants indicating their limited ability to remove these metals from aqueous media.

**Keywords:** *Pistia stratiotes*, *Egeria densa*, heavy metal, pollution, phytoremediation.

**Acknowledgements:** A part of this study is supported by the Ankara University Coordinatorship of Scientific Research Projects (Project no: 18B0430006, Investigation of two different macrophyte species (*Pistia stratiotes* and *Egeria densa*) for their removal potential of mercury, copper and vanadium under laboratory conditons).

## *POSTER PRESENTATION*

# Detection of Microsomal Prostaglandin E Syntase-1 (mPGES-1) Inhibitors by Structural Based Virtual Screening and Investigation as In Vitro

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**Abstract**

Microsomal prostaglandin E synthase-1 (mPGES-1) is an inducible prostaglandin E synthase after exposure to pro-inflammatory stimuli. The mPGES-1 enzyme represents a new target for the therapeutic treatment of acute and chronic inflammatory disorders and cancer. New mPGES-1 inhibitors should be determined for the purpose of drug design and discovery aiming to develop new generation anti-inflammatory drugs.[1] For this purpose, compounds containing indole scaffolds (30000) were downloaded from the Zinc-15 database, their 2D structures were converted to 3D and their different conformations were obtained. The interactions between mPGES-1 and compounds with an indol scaffold were determined by structure-based virtual scanning. These interactions were examined and ZINC000002639093 (compound 8) and ZINC000003323374 (compound 9) targeting the active site of mPGES-1 were selected as potential target inhibitors. Molecular dynamics simulations of the reference and the two best compounds were performed to analyze the dynamics between protein-ligand complexes. The antiproliferative effects of two selected target compounds in lung cancer cells and their anti-inflammatory effects were determined by measuring the PGE2 levels formed in these cells. In vitro experiments found that two compounds targeting the active site of mPGES-1 could be potential novel inhibitors.

**Keywords:** mPGES-1, Virtual Screening, Molecular Simulation, Anticancer, Anti-enflammation.

**Acknowledgment:** This work was funded by Scientific Research Projects (BAP) (Project no: FYL-2021-9085).

**References:**
[1] Salem, M., Khormali, A., Arshadi, A. K., Webb, J., & Yuan, J. S., 2020. Transcreen: transfer learning on graph-based anti-cancer virtual screening model. Big Data and Cognitive Computing, 4(3), 16.

## *POSTER PRESENTATION*

# *In Vitro* Evaluation of Novel Hsp90 Inhibitors Determined with *In Silico* Drug Repurposing Approach

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**Abstract**

Hsp90 is an evolutionarily conserved molecular chaperone that modulates many cellular processes in eukaryotes through signaling pathways regulated by Hsp90-related proteins such as proliferation, cell cycle, and gene expression. Thanks to control of the Hsp90 protein, cellular functions such as cell proliferation and apoptosis are stabilized [1,2]. In addition, Hsp90 inhibitors have a wide therapeutic window for cancer therapy, since the level of Hsp90 between cancer cells and normal cells is distinctive. Therefore, targeting Hsp90 today is a promising strategy for developing a new anticancer drug [3]. For this purpose, the interactions of all drugs (FDA approved, experimental, research) with Hsp90 protein in the Drugbank database were determined using structure-based virtual screening. In this study, all molecules (~10767) in the Drugbank database were downloaded and examined for interactions with the Hsp90 protein and potential hit inhibitors were identified. DB00157, DB00581, DB00256, DB0786, DB00577, DB00415 targeting the active site of Hsp90 were identified by virtual screening and molecular dynamics simulations were performed. After MD simulation (200 ns), the effects of NADH, Lactulose and Ampicillin (DB00157, DB00581 and DB00415) drugs on human breast cancer cell lines (MCF-7 and MCF10) were determined as *in vitro*. $IC_{50}$ values of 17-AAG, Ampicillin, Lactulose and NADH applied (48 hours) on MCF-7 and MCF10A cell lines were determined experimentally. It was determined that three of these six target drugs (Lactulose, NADH and Ampicillin) determined by *in silico* modeling according to their $IC_{50}$ values, acted against MCF-7 and MCF10A human breast cancer cell lines at moderate micromolar concentrations. The antiproliferative effects of Lactulose, Ampicillin, NADH and reference 17-AAG drugs were evaluated in MCF-7 breast cancer and MCF10A cells after prolonged incubation with increasing compound concentrations. These results accelerated their identification as new Hsp90 inhibitors that could be used in cancer therapy. Considering the ligand RMSD values obtained as a result of molecular dynamic simulation studies of these target compounds, new Hsp90 target compounds can be designed by removing the groups with high fluctuations on the ligand.

**Keywords:** Hsp90, Virtual Screening, Molecular Simulation, Anticancer.

**Acknowledgment:** This work was financed by the Scientific and Technological Research Council of Türkiye (TUBITAK) (Project number: 221Z272).

**References:**

[1] Whitesell, L., Mimnaugh, E. G., De Costa, B., Myers, C. E., & Neckers, L. M. (1994). Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proceedings of the National Academy of Sciences, 91(18), 8324-8328.
[2] Taipale, M., Jarosz, D. F., & Lindquist, S. (2010). HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nature reviews Molecular cell biology, 11(7), 515-528.
[3] Park, H. K., Yoon, N. G., Lee, J. E., Hu, S., Yoon, S., Kim, S. Y., ... & Kang, B. H. (2020). Unleashing the full potential of Hsp90 inhibitors as cancer therapeutics through simultaneous inactivation of Hsp90, Grp94, and TRAP1. Experimental & molecular medicine, 52(1), 79-91.

## *POSTER PRESENTATION*

# Charachterization of resistance profile of Methicillin-resistant *Staphylococcus aureus* isolated from Algerian hospitals

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**Abstract**

The spread of a nosocomial outbreak often originates from cross-contamination and the most common means of pathogen transference occurs between the hands of healthcare professionals, hospital equipment and patients. However, drug-resistant bacteria cause many of these nosocomial infections, with an increasing predominance of Methicillin-resistant *Staphylococcus aureus* (MRSA). It is a major public health concern because of their resistance to all other penicillin and cephalosporin, and is responsible for both hospital and community associated infections worldwide. The study included MRSA isolated from patients hospitalized for at least 48 hours (urine, blood, pus…) and from environmental samples (surfaces and adjacent equipment) in Algerian hospital. The isolates were identified by microbiological methods (Api Staph system) and confirmed by MALDI-TOF MS. Antibiotic susceptibility of the isolates was performed according to the antibiotic susceptibility standard disc diffusion method on Mueller-Hinton agar. MICs of antibiotics, including, oxacillin and vancomycin, were determined using Vitek 2 compact. MRSA and vancomycin-resistant *S. aureus* (VRSA) phenotypic mechanisms were revelled using Vitek 2 compact too. A total of 25 *S. aureus* isolated from clinical and surfaces specimens were screened, all strains presented a MRSA profile with oxacillin MICs ≥ 4 µg/ml, confirmed by Vitek 2 compact. Fourth of them were resistant to vancomycin (VRSA) with MICs ≥16 µg/ml and 02 were intermediary to vancomycin (VISA) with MIC=4 µg/ml. It remains very important to confirm these phenotypic results by genotypic characterization as soon as possible.

**Keywords:** MRSA, VRSA, VISA, nosocomial, infections.

## *POSTER PRESENTATION*

# Determination of antibacterial effect of curcumin on *Salmonella Typhimurium* in chicken meat

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**Abstract**

Salmonellosis is one of the most important foodborne diseases transmitted by contaminated poultry products and is widespread worldwide. Salmonella serotypes are important zoonotic pathogens that cause a wide range of diseases such as enteric fever, gastroenteritis and bacteremia in humans. Although a wide variety of chemical agents are used in the prevention of foodborne *Salmonella* spp. infections, consumers prefer additives that are natural, do not harm human health and do not impair the properties of food. Curcumin is a yellow colored, hydrophobic polyphenol, natural component obtained from the rhizome of the *Curcuma longa* plant known as turmeric. Curcumin has antimicrobial, antioxidant, anti-inflammatory, appetite enhancer, immunomodulatory and gastroprotective effects on human health. In this study, it was aimed to determine the antibacterial effect of curcumin on *S.Typhimurium* in chicken meat in vivo and in vitro. In the first step, chicken samples were experimentally contaminated with *S.*Typhimurium at a level of 2.8x107'cfu/ml. Then, they were kept in 1, 2 and 3 % curcumin solution for 15 minutes. At the end of the treatment, chicken samples were stored at +4 ºC. On days 0, 2, 4 and 6 of storage, the number of *S.*Typhimurium in chicken samples was determined according to the method reported by EN ISO 6579-1:2017. At the end of the study, the number of *S.Typhimurium* in chicken samples experimentally contaminated with *S.Typhimurium* decreased by 2.37, 2.71 and 2.84 log levels at the end of the 6th day as a result of 1, 2 and 3% curcumin treatment. Macrodilution method was used to determine the Minimum Inhibitory Concentration (MIC) value of curcumin on *S.* Typhimurium (CLSI, 2018). The organoleptically acceptable dose of curcumin in poultry meat was determined to be 2%. 1% curcumin caused a bland taste, flavor and odor in chicken, while 3% curcumin caused a dominant yellow color, taste and odor. This research was supported by Ondokuz Mayıs University, Scientific Research Projects, Project number PYO.VET.1908.22.001.

**Keywords:** Curcumin, *S.Typhimurium*, chicken, antibacterial activity.

## *POSTER PRESENTATION*

# Development of New Generation Fluorescent In Situ Hybridization Probes by PCR

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**Abstract**

Leukemia is the sixth most common cause of cancer deaths worldwide. There are several types of leukemia, such as CML (Chronic Myeloid Leukemia), AML (Acute Myeloid Leukemia) and MDS (Myelodysplastic Syndromes). Fluorescent In Situ Hybridization (FISH) is the most common technique used in leukemia diagnosis. CML patients usually carry a translocation that results in fusion of ABL in 9q34 and BCR in 22q11. In AML, there occurs a fusion of PML in 15q24 and RARA in 17q21. Lastly, MDS patients carry an EGR1 deletion in 5q. In this study, we aim to produce FISH probes with PCR method to detect common translocations and deletions in CML, AML and MDS. For CML, primer sets were designed and synthesized in order to produce 10 amplicons of 1 kb size in BCR gene and 10 amplicons of 1 kb size in ABL gene. Similarly, primer sets were designed and synthesized to produce 10 amplicons of 1 kb size in PML and RARA genes for AML and in EGR1 gene for MDS. PCR amplifications with these primers were optimized in terms of PCR conditions such as annealing temperature and a total of 50 amplicons were produced by PCR in high amounts and purified. In order to label PCR products, florescent dUTP molecules with two different chemistries were used in PCR reactions. Different concentrations of florescent dUTP and different Taq polymerases were tested for labelling, however, a very low yield of florescent dUTP incorporation into PCR products was obtained. Therefore, labelling studies with alternative approaches such as chemical or enzymatic labeling are still ongoing to produce PCR FISH probes with post PCR florescent labeling.

**Keywords:** Fluorescent In Situ Hybridization (FISH), Leukemia, Probes, PCR, Florescent Labeling.

## *POSTER PRESENTATION*

# Evaluation of The Methods Used in The Production of 100 bp DNA Ladders

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**Abstract**

Determination of nucleic acid size is a basic need in molecular biology. DNA ladders used for this purpose contain a mixtureof double-stranded DNA products with known sizes, increasing in size at regular intervals. During electrophoresis, these DNA fragments separate according to their mobility in the electric field and they form bands on the gel. The resulting bands are used as references to estimate the size of DNA fragments of unknown size. Three most common methods used in the preparation of DNA ladders include ligation, amplification by PCR and restriction enzyme digestion. Ligation method is a very time-consuming and costly method. Restriction digestion enzymes utilizes bacteriophage and plasmid DNA and is good for the production of large size DNA fragments. The target DNA region can be amplified in a very short time and obtained in high amounts by PCR method. This method is advantageous for the production of smaller size DNA fragments. In the PCR method, a target sequence is cloned into a plasmid. Primer sets are designed and optimized to produce fragments with different sizes in the ladder. Amplification is performed in multiplex PCR approach, however this step needs careful optimization of several parameters including primer sequences, annealing temperature and primer concentration to prevent primer dimer formation and secondary structures. In addition, touch-down PCR may be used to minimize non-specific primer binding during PCR amplification. In this analysis, we focus on cost-effective and fast PCR-based production methods of 100 bp DNA ladders.

**Keywords:** DNA Ladder, Production Methods, Polymerase Chain Reaction (PCR), Multiplex PCR.

## *POSTER PRESENTATION*

# Development of In-house Viral Nucleic Acid Extraction Media for COVID-19 Testing by RT-qPCR

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**Abstract**

Quantitative Reverse Transcription PCR (RT-qPCR) tests are widely used for COVID-19 diagnosis. Samples obtained from COVID-19 patients must be transported and preserved until PCR tests are performed. Various viral transport media (VTM) have been used to transfer and preserve nasopharyngeal swab samples collected from patients for PCR tests during the COVID-19 pandemic. Some are just for the preservation of viral material during transport to the test center, some contain agents for quick extraction of viral nucleic acids. However, there is not enough and clear data regarding recipes of media used for nucleic acid extraction. In this study, we produced different extraction media by combining various reagents including Tris-Cl, Tris-EDTA, Triton-X and guanidinium isothiocyanate (GITC). A total of 20 different media combinations were prepared and tested. First, we tested the RT-qPCR amplification profile of extraction media using RNA obtained from COVID-19 patients to observe if they interfere with PCR. We observed that GITC at 4M inhibits PCR amplification. It was also demonstrated that Triton-X in extraction media does not interfere with RT-qPCR amplification. Next, extraction media were tested in COVID-19 RT-qPCR tests using swabs obtained from 5 COVID-19 patients, directly immersed into extraction media. It was shown that Triton-X in may be used for transportation and preservation of COVID-19 patient samples for RT-qPCR test. At the same time, stability tests were performed to investigate sample stability in these media. Patient samples could be stored at 4 ºC for 24 hours without interfering with RT-qPCR results of COVID-19 patients. In conclusion, multiple alternatives for nucleic acid extraction media that can be prepared in-house were tested and shown to efficiently extract and preserve COVID-19 patients' samples for RT-qPCR testing.

**Keywords:** COVID-19, Viral Transport Medium (VTM), Nucleic Acid Extraction, RT-qPCR Test

## *POSTER PRESENTATION*

# Slight increase in reactive oxygen species production reduces the cancer stem cell population of human pancreatic adenocarcinoma cells

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**Abstract**

Cancer stem cells (CSCs) as tumor initiating cells are responsible for cancer reoccurrence and drug resistance and differentiate into heterogeneous lineages of cancer cells. Information on the importance of the slight increase in Reactive Oxygen Species (ROS) levels induced by low hydrogen peroxide ($H_2O_2$) in cancer stem cell biology is limited. We aimed to investigate the effects of ROS increase on pancreatic cancer stem cell population and properties in this study. PANC-1 cells were treated with different concentrations of $H_2O_2$ and the increase in ROS levels was investigated. Following treatment with $H_2O_2$, the cancer stem cell population was analyzed by aldehyde dehydrogenase-positive ($ALDH^+$) cell population. Besides, the changes in cancer stem cell properties were determined by transwell migration, invasion assay, and colony formation assay. According to our results, administration of 0.05 mM and 0.1 mM $H_2O_2$ to PANC-1 cells for 24 hours slightly increased ROS levels and decreased the cancer stem cell population. The decrease in cancer stem cell population was significant at 0.1 mM $H_2O_2$ treated PANC-1 cells. Consistent with a decrease in the stem cell population, the invasive and motility abilities were reduced and the number of colonies in soft agar was less when compared to control groups. According to our results, slightly increased level of ROS suppresses the population of cancer stem cells (CSC) and aggressiveness of pancreas adenocarcinoma cells.

**Keywords:** Cancer stem cell, pancreatic adenocarcinoma, reactive oxygen species, tumor aggressiveness.

## *POSTER PRESENTATION*

# Slight increase in ROS levels increases global DNA methylation and changes the methylation status of genes related to WNT and Shh pathways in cancer stem cells of human pancreatic adenocarcinoma

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**Abstract**

During carcinogenesis, epigenetic alterations contribute to malignant properties that leads to inappropriate silencing or activation of cancer-associated genes. There are limited number of studies investigating the relationship of a slight increase in the level of ROS production in cancer stem cells with the regulation of DNA methylation. We aimed to investigate whether a slight increase in ROS levels changes methylation status at CpG sites in the promoters of more than 200 genes related to stemness, metabolism, signaling pathways, epithelial-mesenchymal transition, drug transporters, antioxidant enzymes, and glycosyltransferases. PANC-1 cells were treated with 0.1 mM $H_2O_2$ for 24h and cancer stem cells were sorted by their ALDEFLOUR positivity. After DNA isolation, methylated DNA was immunoprecipitated and labeled. Methylated promoters were detected by microarray and analyzed bioinformatically. Besides any changes in global DNA methylation were also researched following $H_2O_2$ treatment. The promoters of genes related to Wnt and Sonic hedgehog (Shh) pathways were differently methylated in cancer stem cells exposed to 0.1 mM $H_2O_2$. The levels of 5-mC increased in the experimental group compared to the control. These results suggest that slight increase in ROS level may lead to epigenetic changes via DNA methylation in pancreatic cancer stem cells and effect the properties of stemness.

**Keywords:** Cancer stem cell, reactive oxygen species, stemness, MeDIP array, global DNA methylation.

## *POSTER PRESENTATION*

# Effect of indolin-2-on-based derivatives on viability and apoptosis of breast cancer cells

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**Abstract**

Recent analyses indicate that breast cancer has become one of the most common malignancies worldwide. Despite various types of treatment strategies used for the disease, it is still a need to seek alternatives. It is known that indolin-2-on derivatives have a wide variety of biological effects including antimicrobial, anti-malarial and anti-cancer activities. In this study, antiproliferative and apoptotic effects of originally synthesized different indolin-2-on derivatives were investigated in two breast cancer cells and a non-cancerous breast cell line. To expose the anti-carcinogenic effect of derivatives on cell proliferation and cell death, MTT and two different fluorescence staining (AO/EB and PI/DAPI) methodologies were performed, respectively. For all compounds, $IC_{50}$ values were calculated and analysed by Two-way ANOVA, and then SI values were calculated based on the $IC_{50}$ values. Our results indicated that all four indolin-2-one compounds reduced cancer cell viability and induced dose-dependent apoptosis. However, compounds did not show slight anti-proliferative and apoptotic effects on non-cancerous cell lines till high concentrations, which may show that derivatives are more selective to cancer cells rather than non-cancerous cells.

**Keywords:** Breast cancer, indolin-2-one, anti-proliferative, apoptosis

## *POSTER PRESENTATION*

# Synthesis and Determination of Biological Activity of Sugar Based Coumarin Derivatives

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**Abstract**

Coumarin derivatives have been widely used in a derivative that has survived from the past. It has been observed to have antitumor, antituberculous, antioxidant, anti-inflammatory, antiviral and antimicrobial uses [1]. Some coumarin derivatives exhibited anti-HIV activity as well as inhibition of HIV replication and anti-tumor activities [2]. Coumarin derivatives are used in demand as pests, as potential protectors of the agricultural sector [3]. Coumarin derivatives include them as suitable candidates for bioimaging and intracellular fluorescence applications [4]. Apart from these, the list of various coumarin derivatives is included as enzymatic inhibitory teams in degenerative like Alzheimer's and their diversity in ongoing processes [5]. The main purpose of this study is to combine chiral coumarin derivatives, which are known to be effective in biological applications, especially in anticancer activity studies, with sugars, which are widely used for biomedical purposes, especially as theranostic agents, thus obtaining new multifunctional structures and efficacy tests in in vitro cell culture. In this study, chiral chiral sugar-based coumarin syntheses, their characterization and biological applications are carried out starting from per acetyl sugars within the scope of the project. The structure and purity of sugar-based chiral coumarin derivatives synthesized were verified by $^{1}H$ and $^{13}C$ NMR, IR, UV, elemental analysis, melting point and optical rotation angle analysis methods. In addition, the anticancer activities of synthesized sugar-based chiral coumarin derivatives will be examined using the widely used breast cancer cell line MCF-7 cell proliferation method.

**Keywords:** Coumarin, Schiff Base, Theranostic Agents, Anticancer Activity, Breast Cancer.

**References:**

1. Jain, P. K.; Joshi, H. , **2012** *J. Appl. Pharm. Sci*. 2(6), 236-240.
2. Zhou, P., Takaishi, Y., Duan, H., Chen, B., Honda, G., Itoh, M., Takeda, Y., Kodzhimatov, O. K., Lee, K. H., **2000** *Phytochemistry. 53*, 689-697.
3. Brooker, N., Windorski, J., Bluml, E., **2008** *Commun. Agric. Appl. Biol. Sci., 73*, 81-89.
4. Zhao, L., Zhang, J., Liu, T., Mou, H., Wei, C., Hu, D., Song, B., **2019** *J. Agric. Food Chem., 68*, 975-981.
5. Sun, Q., Peng, D. Y., Yang, S. G., Zhu, X. L., Yang, W. C., Yang, G. F., **2014** *Bioorg. Med. Chem. Lett., 22*, 4784-4791.

## *POSTER PRESENTATION*

# Development of a Multiplex Real Time PCR (qPCR) Kit for GMO Screening

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**Abstract**

Genetically modified organism (GMO) is a term used for organisms produced using genetic engineering methods. Legislations in most countries impose various restrictions on the use of Genetically Modified (GM) products and require indicative labeling of foods containing GM products. According to the Turkish Biosafety Legislation, only a limited number of modified genes are allowed in animal feeds, while the presence of GM products in human food products is completely prohibited. Therefore, both the presence and quantity of GM products in food and feed products must be monitored with high precision. The presence of GM products can be identified by detecting various biomolecules and, DNA is the most effective choice because of its stability, abundance, and easy amplification. When it comes to DNA-mediated GMO detection, real-time polymerase chain reaction (qPCR) is the most widely used method because of its sensitivity and robustness. With GMO screening kits in Turkey market, 3-4 basic transgene regions can be screened. Other genes can be examined separately by qPCR. In this study, specific primer-probes were designed for 14 different transgene regionsto be studied in multiplex manner. These regions consist of 4 terminator/promoter (tE9, T-NOS, PFMV, CaMV-P35S), 4 element specific gene (CP4-EPSPS, Cry1Ab/Ac, bar, pat), and 6 plant specific gene regions for maize and soy (DP-305423-1, DAS-68416-4, DAS-40278-9, DAS-81419-2, VCO-Ø1981-5, DP-2Ø2216-6). With the simultaneous screening of these 14 transgene regions, a highly sensitive monitoring system will be developed for routine analysis. In this context, single qPCR reactions were performed using the above mentioned primer-probe sets. In these qPCR reactions, DNA isolated from Certified reference material (CRM), each containing a different target gene region, were used. 8 primer-probe sets designed for transgene regions were tested and successfully confirmed by qPCR. Following the completion of qPCR studies of the remaining 6 transgene regions, multiplex qPCR studies will be started.

**Keywords:** Genetically modified organism (GMO), Soybean, Maize, Transgene Regions, qPCR, Multiplex PCR.

## *POSTER PRESENTATION*

# Development of a New Generation Quantitative Multiplex PCR Kit for Detection of Adulteration in Meat Products

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**Abstract**

Meat is one of the basic foods that has a very important place in human nutrition. Today, mostly due to the increase in food prices, imitation and adulteration in food has increased considerably. In this respect, determination of meat types used in a meat product is very important in terms of protecting consumers and preventing food adulteration. Quantitative PCR (qPCR) is one of the laboratory methods that give high precision results with high sensitivity. A significant majority of qPCR kits for detecting meat species available on the market can detect only one or two species simultaneously. This brings extra costs and leads to a loss of time as well. The aim of this study is to produce a multiplex meat species-specific qPCR kit that can detect horse, donkey, bovine, pork species in one reaction tube using species-specific probes. Initially, the primers to be used in this study were designed from conserved gene sequences common to the species. Taqman probes were designed to distinguish between horse-donkey, bovine and pork species. DNA was isolated from the species to be detected. After singleplex qPCR studies performed for each primer-probe set, multiplex qPCR studies were performed for simultaneous detection of species. In multiplex reactions, DNA from each target species has been successfully amplified. No peaks were observed in no-template negative control reactions, indicating that the kit is free of false positive results. In addition, species-specific probes did not amplify other species meaning that there is no cross-species amplification. In conclusion, a multiplex kit was produced that simultaneously detects four different species with high sensitivity. This kit can be used to increase food safety and prevent adulteration in meat products and saves time and cost.

**Keywords**: Adultery, qPCR, Meat Species, Multiplex.

## *POSTER PRESENTATION*

# Cloning, Expression, and Production of Wild Type and Mutant Versions of HIV Reverse Transcriptase

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**Abstract**

Reverse transcriptase (RT) is the essential enzyme that generates a complementary strand of DNA (cDNA) from RNA. Retrovirus RTs are one of the most commonly used enzymes in molecular biology and used in the diagnosis of COVID-19 disease. Among all known RTs, the reverse transcriptase from human immunodeficiency virus (HIV) is one of the most studied and well-known. Although HIV-RT has higher activity than other MLV and AMV RTs at temperatures above 50ºC, HIV-RT has relatively low fidelity. In the literature, it has been reported that some mutations in the HIV-RT confer increased fidelity. In this study, the RT encoding genes p66 and p51 of HIV were amplified by PCR, and the PCR products were cloned into *the pJET1.2 cloning* vector. Nucleotide sequence of plasmids carrying HIV-RT genes, were verified by the next-generation sequencing (NGS). To express the heterodimer proteins produced by p66 and p51 genes in the different hosts, the genes were cloned into the pET-28a(+) expression vector. Protein expression was confirmed by SDS-PAGE and coomassie brilliant blue staining. Amino acids in ß3-ß4 hairpin loops of the enzyme are important for the fidelity of HIV-RT. By examining this region K65R, V75I, D76V, and R78A mutations were inserted by site-directed mutagenesis techniques at cloned p66 gene on pET-28a(+). To test the enzyme activity, lysates of expressed protein subunits p66 and p51 were used as RT enzyme in cDNA reaction and qPCR for COVID-19 samples and, also in cDNA reaction and conventional PCR for ß-actin. In these reactions, combined lysates of p66 and p51 showed a good performance, which is comparable to the commercial enzyme. Single point mutations on p66 gene were by confirmed by NGS and, expression of mutant versions, activity assays and comparisons with wild type are still ongoing.

**Keywords:** Reverse transcriptase (RT), Cloning, Expression, Mutant Enzyme, RT Activity Test.

## *POSTER PRESENTATION*

# Cloning, Expression, Production and Activity Test of Taq Polymerase

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**Abstract**

Polymerase chain reaction (PCR) is a basic and primary technique used in clinical, forensic and food laboratories. This technique is a key tool for forensic DNA analysis, food safety, clinical diagnosis, and safety decision-making in bioterrorism. In addition, it is used in the diagnosis of infectious diseases such as tuberculosis, AIDS, COVID-19, to characterize the infection and to treat it appropriately. DNA polymerase is the enzymeused in PCR. DNA polymerase makes it possible to detect and analyze the target DNA sequence by producing copies of specific DNA and amplifying traces of target DNA sequence billions of times this way. Although various DNA polymerases are available for various PCR techniques, Taq polymerase obtained from *Thermus aquaticus* bacteria is widely used and, it has a high resistance to denaturing conditions such as high temperature required during PCR. The importance of Taq polymerase has been further realizedduring the COVID-19 pandemic and ithas become a great need for COVID-19 detection kits produced across the country. In this product-oriented study, it was aimed to produce Taq DNA polymerase in our laboratory and to make it more stable and effective by optimizing it with different buffers. To achieve this, the Taq DNA polymerase gene, which is a GC-rich region, was amplified by PCR using specific primers and was first cloned into a cloning vector and, then into pET28a(+) expression vector. Cloned sequence was confirmed by next generation sequencing. Taq polymerase was expressed in E. coli BL21(DE3) cells and partially purified. Partially purified enzyme was tested in PCR and it was observed that our enzyme works as efficient as commercial Taq polymerases. In the continuation of the study, this enzyme will be purified to high purity level and optimized with different buffers. Thus, more efficient, domestic and long-lasting enzyme production will be achieved.

**Keywords:** Taq polymerase, Cloning, Expression, Purification, PCR Activity Test, Domestic Product.

## *POSTER PRESENTATION*

# Paracetamol Containing Oral Jelly Formulations for Pediatric Usage

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**Abstract**

Paracetamol (PARA), N-acetyl-p-aminophenol, which is also called as acetaminophen has been using widely since 1950s. It is using as analgesic at fever and pain treatment. Although PARA has a bitter taste, it is known with its well-tolerated profile at pediatric usage. Oral jelly formulations have a gummy-like look, taste, and texture. Due to these advantages, oral jellies can be administered more easily than other oral formulations to pediatric patients. The aim of this study was to prepare PARA containing oral jelly formulations. Pectin based PARA oral jelly formulations were prepared with carrageenan and/or alginate. Any possible interactions between PARA and polymers were analyzed with FT-IR and DSC studies. Hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience of the oral jelly formulations were measured with a texture profile analyzer. The release profiles were evaluated with in-vitro dissolution studies. No interactions were observed between PARA and polymers according to DSC and FTIR studies. The formulations, prepared as PK7 (5% pectin LM-HC 25 AS – 3% alginate), PK9 (5% pectin type LM-104 AS-FS – 3% carrageenan), PK13 (5% pectin (citrus) type USP/100 – 3% carrageenan), PK14 (5% pectin LM-HC 25 AS – 3% carrageenan), PK29 (%5 pectin type LM-104 AS-FS – 1.5% carrageenan – 1.5% alginate), and PK30 (5% pectin amid Cu 020 – 1.5% carrageenan – 1.5% alginate), were successfully removed from the molds. The formulations were found to be suitable for oral applications according to texture profile analyzes. All of oral jelly formulations completely dissolved in 15 mins. It was concluded that oral vegan paracetamol jellies could prevent bitter taste in pediatric usage.

**Keywords:** Oral jelly formulations, Paracetamol, Pediatric dosage forms.

## *POSTER PRESENTATION*

# An overview of heterogenous photocatalysis applications for the water treatment

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**Abstract**

The rapid development of industrialization and population growth has resulted in the depletion of water resources as a result of increased demand, as well as water contamination as a result of the overwhelming discharge of micropollutants into the natural water cycle. Therefore, it is necessary to adopt various strategies to reduce the risk of water scarcity.
Photocatalytic processes in heterogeneous phases are the most promising and are currently attracting the scientific community's attention. Indeed, these processes are simple to use, clean, and inexpensive; they are based on the absorption of solar energy (a natural resource available to everyone) by a semiconductor, which causes the transfer of an electron from its valence band to its conduction band, resulting in redox reactions that degrade the adsorbed pollutants. Titanium dioxide is commonly used as a semiconductor because it is stable and non-toxic. Nonetheless, its photocatalytic activity is limited by the fact that it can only be activated by ultraviolet radiation, which accounts for only 5% of the energy emitted by the sun. Furthermore, the discovery and sharing of useful knowledge regarding photocatalytic technology as a potential solution to water scarcity and wastewater treatment are among the primary conclusions of this scientific overview.

**Keywords:** Photocatalysis, Wastewater Treatment, Environment, Photocatalysts.

## *POSTER PRESENTATION*

# Antioxidant potential and gastroprotective effects of aqueous extract from Algerian *Thymelaea hirsuta* L. areal parts

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**Abstract**

*Thymelaea hirsuta*, a plant belonging to Thymeleaceae family. It has been used in folk medicine for its biological properties. This research focused primarily on the antiulcer properties of aqueous decoction extract (ADE) from *Thymelaea hirsuta* areal parts on gastric ulceration induced by ethanol in rats, mucosal damage was compared with that given with ranitidine. Total polyphenol and flavonoid content were evaluated and *in vitro* antioxidant activity of ADE were investigated with ABTS scavenging and reducing power assays. ADE showed a large amount of total polyphenols and a low content of flavonoids with 114.0±1.35 mgGAE/g and 16.80±1.03 mgQE/g DW of extract, respectively. In addition, ADE showed a high ability to scavenge ABTS radical with IC50 at 52.93±0.9 µg/mL and a high redox potential with a concentration at A0.5 =124.5±1.79 µg/ml. The antiulcer effect of *Thymelaea hirsuta* aqueous decoction extract was evaluated by the 100% ethanol-induced ulcer test. The results obtained revealed that the aqueous decocted extract with 125, 250 and 500 mg/Kg exerted a dose dependent effect of protecting the stomach at 52.35±3.51%, 69.57±2.16 and 83.01±1.92, respectively.

**Keywords**: antioxidant, antiulcer, Thymelaea hirsuta, polyphenols, flavonoids.

## *POSTER PRESENTATION*

# Investigation of the removal of Reactive Red 195 dye from aqueous solutions by using capia pepper seeds

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**Abstract**

Water is the essential need for the life cycle of all living things. Therefore, the existence and sustainability of water resources is the most important factor. The rapid increase in human population and the effects of industrialization are the main reasons for the rapid decrease in clean water resources day by day. At the beginning of the chemicals mixed into the wastewater; heavy metals, dyestuffs, toxic organic compounds, dissolved inorganic solids, etc. can be pollutants that threaten our lives. There are many physical and chemical methods applied to make the aquatic environments polluted by wastes from various sources reusable. Among these methods, adsorption is the one of the most preferred methods used to remove waste materials from water due to the simplicity, cheapness and easy-to-use method.
The selection of appropriate adsorbents is one of the most effective parameter in adsorption studies. In this respect, the adsorbent should be highly efficient, economically cheap and easily accessible. Therefore, in the present study, it was aimed to investigate the potential use of capia pepper seeds, which is considered as food waste, for the removal of Reactive 195 dye from aqueous solutions. The effect of several parameters such as pH, temperature and adsorbent amount were studied by using Box Behnken design and adsorption mechanisms were evaluated. Moreover, according to the data obtained from the adsorption experiments, the most suitable adsorption isotherm and kinetic models were examined. Based on the results, capia pepper seed has found as an effective and eco-friendly alternative for the cleanup of dye-polluted aqueous media as a new natural sorbent with desirable absorption capacity.

**Keywords:** Adsorption, Reactive Red 195, Capia Pepper Seeds, Optimization.

## *POSTER PRESENTATION*

# Effect of temperatures on performance of sorbent for desulphurization of diesel

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**Abstract**

The increasing demand for renewable energy sources in recent years has increased the importance of fuel cells. Since the hydrogen to be used in fuel cells is not found in pure form in nature, it must be obtained by converting another natural fuel. The hydrogen required for use in fuel cell systems can be obtained from liquid hydrocarbons. However, in the case of using liquid hydrocarbons containing sulfur compound impurities, such as diesel, it is advantageous to remove these impurities before the reforming unit, which is the step of obtaining a hydrogen-rich gas mixture. Sulfur compounds poison the catalysts of the reformer unit in fuel cell systems and shorten their lifetime. Additionally, it causes poisoning of the materials used in the anodes and deterioration of their structure. The study aims to remove sulfur compound impurities in diesel for systems using diesel as a hydrogen source. For this purpose, the adsorptive desulphurization process was chosen within the scope of the study. So far, a commercially available nickel-based sorbent has been studied. In the tests, three different temperature values were tried, namely ambient temperature, 125 °C, and 270 °C. Due to the adsorption study in the liquid phase, the reactor was kept in the pressure range of 10-12 bar so that the diesel would not evaporate at the temperature values of 125 °C and 270 °C. As the operating temperature increases, the adsorptive desulphurization kinetics improve. In addition, polyaromatic hydrocarbons attached to the sorbent surface are separated, allowing more sulfur compounds to adhere to these areas. However, as the contact time between diesel and sorbent increases, the adsorption process results improve. Within the scope of the study, the sorbent capacity value of 0.283 mg S/g was reached under the conditions of 270 °C temperature and 1.08 hours of contact time.

**Keywords:** Desulfurization, Adsorption, Sorbent, Diesel.

## *POSTER PRESENTATION*

# Characterization of Oil from Fruit Seeds for Potential Cosmetic Application

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**Abstract**

Oil extracted from plant seeds have various applications in cosmetic products such as face cream, soap and shampoo and are becoming increasingly popular. This is due to their favourable fatty acid composition and high amount of bioactive compounds such as squalene, sterols, vitamin E and total phenolic content which has been known to be vital ingredients in skin care products. In this research, oil was extracted from two fruit seeds namely Phyllanthus acidus locally known as cermai or Malay gooseberry and Cucumis melo L also known as honeydew. The oil content of Malay gooseberry seeds and honeydew seeds were 2.5% and 14.8% respectively. Malay gooseberry seeds oil is a viscous oil consisting predominantly of 45.89% C18 unsaturated fatty and 24.64 % C16 and C18 saturated fatty acid. The major unsaturated fatty acid being linolenic (18:3) with 23.63% . Hence, Malay gooseberry seeds oil can be considered as a high ω−3 fatty acid oil. Honeydew seeds oil on the other hand, is composed of 85.22% unsaturated fatty acid where 67% being linoleic acid (ω−6 unsaturated fatty acid). The oil from Malay gooseberry seeds contains 1.514 mg/g of squalene, 38.293 mg/g of β-sitosterol, 0.311 mg/g vitamin E and 105.85mg/g total phenolic content, while honey dew oil contains 0.699 mg/g of squalene, 2.951 mg/g of β-sitosterol, 0.379 mg/g vitamin E and 3.03 mg/g total phenolic content. The oil from Malay gooseberry seeds has the highest total phenolic and β-sitosterol content compared to some seeds oils currently used in cosmetic such as rosehip oil, olive oil and pomegranate oil and comparable amount of squalene and vitamin E making it possible to be utilized as cosmetic ingredients.

**Keywords:** seeds oil, squalene, sterol.

## *POSTER PRESENTATION*

# Synthesis and characterization of magnetic graphene oxide

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**Abstract**

In recent years, magnetic nanoparticles have been widely used in various applications such as magnetic drug delivery, hyperthermia, and separation methods due to their good magnetic properties, high surface area, excellent biocompatibility, and low cost. In this study, magnetic properties were imparted to the material by forming $Fe_3O_4$ magnetic nanoparticles on the surface of graphene oxide. Magnetic iron oxides were preferred as magnetic nanoparticles due to their superparamagnetic properties, low toxicity, and excellent biocompatibility in physiological environments. Graphene oxide was synthesized by the modified Hummers method within the scope of the study. Magnetic graphene oxide (MNGO) composite material was prepared by co-precipitation method with a weight ratio of magnetite ($Fe_3O_4$) to GO of 4:1. XRD, VSM, BET, SEM analyses were performed to determine the characteristic properties of the material prepared with a $Fe^{2+}/Fe^{3+}$ ratio of 3/2. While the surface area of the synthesized graphene oxide was initially 39.4 $m^2/g$, it was observed that it increase to 132 $m^2/g$ after $Fe^{2+}/Fe^{3+}$ ion loading at a ratio of 3/2. The crystallinity of the MNGO composite material was investigated by the X-ray diffraction (XRD) method and characteristic peaks of $Fe_3O_4$ nanoparticles were obtained while graphene oxide peaks were also observed. The crystallite size of magnetic graphene oxide was obtained as 10 nm. Thus, it was determined that the material has superparamagnetic properties. Vibrating Sample Magnetometer (VSM) measurements showed that the MNGO composite material has magnetic properties.

**Keywords:** Magnetic nanoparticles, Graphene oxide, magnetite, -$Fe^{+2}$, $Fe^{+3}$

**Acknowledgments:**Gazi University Research Fund (FGA-2022-7426) grants are acknowledged.

## *POSTER PRESENTATION*

# *Veronica cinerea* less ve *Helleborus vesicarius* aucher ex boiss endemik bitkilerinin metanol ekstratlarının ve gümüş nanopartiküllerinin biyofilm tabaka üzerine olan etkilerinin karşılaştırılması

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**Özet**

Salgın hastalıkların bazı faktörleri içerisinde yer alan biyofilm oluşumu, bakteri aracılığı ile insan sağlığını tehdit etmektedir. Biyofilm tabakası bakterilerin oluşturduğu su bazlı polimerik matriks yapısının bazı yüzeylere tutunması sonucu oluşan hücre topluluklarıdır. Bu bağlamda bilim insanları, biyofilm tabakası oluşumunu engelleyici, ucuz ve atık miktarının az olduğu çevre dostu alternatif yollar aramaktadırlar. Literatür araştırması neticesinde *Veronica cinerea* Less ve *Helleborus vesicarius* Aucher Ex Boiss endemik bitki türlerinde, yeşil sentez yöntemi ile ilgili bir çalışmaya rastlanmamıstır. Çalışmamızda, *Veronica cinerea* ve *Helleborus vesicarius* endemiklerinin methanol ile elde edilen ekstraktlarının ve AgNP (gümüş nanopartikül)'lerinin; *Enterobacter aerogenes (*ATCC 13048)*, Klebsiella pneumoniae, Enterococcus durons, Salmonella typhimurium, Stophylococcus aureus (*ATCC 25923), *Bacillus suptilis* (DSMZ 1971) bakteri suşları üzerindeki antibiyofilm etkileri karşılaştırmalı olarak incelenmiştir. Elde edilen çalışmalar sonucunda gümüş nanopartikül çözeltilerinin, biyofilm inhibe edici yüzde değerlerinin daha anlamlı olduğu tespit edilmiştir. *Veronica cinerea* bitkisinin metanol ile elde edilen ekstraktlarında *Enterococcus durons* bakteri suşu üzerinde 50 mg/mL konsantrasyonda %39, gümüş nanopartiküllerinde ise %57 oranında en yüksek biyofilm inhibisyonunu göstermiştir. Aynı konsantrasyonda metanol ile elde edilen ekstraktlarda en düşük antibiyofilm etki ise *Klebsiella pneumoniae* bakteri türü üzerinde %9, gümüş nanopartiküllerinde ise %16 olarak gözlenmiştir. *Helleborus vesicarius* bitkisinin metanol ile elde edilen ekstraktlarında *Stophylococcus aureus* (ATCC 25923) bakteri suşu üzerinde 50 mg/mL konsantrasyonda %43, gümüş nanopartiküllerinde ise %48 oranında en yüksek antibiyofilm etki göstermiştir. Aynı konsantrasyonda metanol ile elde edilen ekstraktlarında en düşük antibiyofilm etki ise *Bacillus suptilis* (DSMZ 1971) türünde %0, gümüş nanopartiküllerinde ise %0 olarak gözlenmiştir.

**Anahtar Kelimeler:** Veronica cinerea, Helleborus vesicarius, Antibiyofilm, Gümüş Nanopartikül, Yeşil Sentez.

## *POSTER PRESENTATION*

# Risk assessment of fluorosis in the Algerian sub-Saharan region

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**Abstract :**

Fluoride is a widely used element and essential for the different function of the human body, with a daily dose that should not exceed 0,05 mg/L according to WHO. It is mainly used for the prevention of dental caries, at high dose, it constitutes a considerable health risk. our study aims to assess the risk of fluorosis by impregnating water, dates and tea infusions; as the main sources of fluorine exposure in the sub-Saharan endemic zone in Algeria, El Oued, Biskra and the region of Constantine as a control region. based on the results obtained, the fluorine levels in the waters exceed the WHO recommended standards of 0.8 mg/L only for the Biskra region with an average of 1.08 mg/L in the El Oued region, the average is 0.43 ppm because of the use of the albian nappe for irrigation in drinking water; high levels are also recorded in tea infusions (1.62-2.03 ppm) and dates (Daglet Noor) 35.7 mg/Kg . The prolonged ingestion of this hyper-fluorinated water, as well as the foods made by the cultures watered by it, the tea infusions and dates are the main cause of dental and bone fluorosis in the region of Biskra and El Oued; this is also confirmed by the high levels of fluorine stored in the toothing by signaling generation less than 16 years.as a primary means of preventing tooth decay, confirmation of the compliance of fluorine intakes by toothpaste pastes is also performed.

**Keywords:** fluorine, water, food, Algerian sub-Saharan region.

## *POSTER PRESENTATION*

# Ömerler, Yeniköseler ve Sazılar(Polatlı-Ankara) ve Çevresinin Lokal Endemik Bitkileri

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**Özet**

Ankara'nın Polatlı ilçesinin Ömerler, Yeniköseler ve Sazılar köylerinde 2019-2022 yılları arasında yüksek lisans tez çalışması kapsamında yapılmış olan arazi çalışmalarında endemik taksonlar tespit edilmiştir, bunlardan 7 tanesi lokal endemiktir. Bu taksonlar; *Acantholimon gemicianum* Kaptaner İğci, Körüklü & Aytaç, *Cephalaria aytachii* Göktürk & Sümbül, *Hedysarum duruae* Yıld., *Hedysarum hasanyildirimii* Yıld., *Salvia aytachii* Vural & Adıgüzel, *Aethionema dumanii* Vural & Adıgüzel, *Alyssum niveum* T.R.Dudley taksonlardır. Habitat tipi ve çevresel faktörler sebebi ile baskı altında bulunan türlere dair koruma önerileri ve tehlike kategorileri çalışma içerisinde belirtilmiştir.

**Anahtar Kelimeler:** Endemik, Jips, IUCN, Polatlı.

## *POSTER PRESENTATION*

# Synthesis and characterization of mesoporous carbon containing magnetic nanoparticles

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**Abstract**

Magnetic nanoparticles are composed of metal and metal oxides with sizes between 0.1-100 nm and also show magnetic properties. Due to their easy control, biocompatibility, superior physical and chemical properties, the use of these particles in various fields such as industrial, environmental, analytical and biomedical applications are remarkable. Having a high surface area/volume ratio enables high adsorption capacity. This adsorption capacity, gives the opportunity to be used in separation processes. Due to these properties, studies on magnetic nanoparticle synthesis and characterization have been carried out in recent years. In this study, synthesis and characterization studies of mesoporous carbon composite material which containing magnetic nanoparticles were carried out. First of all, SBA-15 was synthesized to use as a template, then mesoporous carbon was obtained by hard template method. Iron was added to the synthesized mesoporous carbon, using the hydrothermal synthesis method, with a magnetic nanoparticle/mesoporous carbon ratio of 4/1. In the composite material, the $Fe^{+2}/Fe^{+3}$ ratio was studied to be 1/1. XRD, $N_2$ adsorption desorption, and VSM analyzes were used for the characterization of the obtained materials. The BET surface areas of the mesoporous carbon and mesoporous carbon containing magnetic nanoparticle samples were found to be 850 $m^2/g$ and 252 $m^2/g$, respectively. The pore diameters calculated by the BJH method were 3.00 nm and 3.46 nm, respectively. As a result of the XRD analysis of mesoporous carbon, characteristic peaks of CMK-3 were obtained. The characteristic peaks of $Fe_3O_4$ nanoparticles were observed in XRD analysis of mesoporous carbon containing magnetic nanoparticles. Result of VSM analysis showed that magnetic nanoparticles containing mesoporous carbon composite material displays magnetic properties. The crystallite size, calculated by Scherrer's law, was 17.7 nm, indicating that the particles exhibited superparamagnetic properties.

**Keywords:** Mesoporous carbon, magnetic nanoparticle, $Fe_3O_4$ nanoparticles

**Acknowledgments:**Gazi University Research Fund (FGA-2022-7426) grants are acknowledged.

## *POSTER PRESENTATION*

# Grain size, pHpzc and adsorption capacity of barium onto vermiculite

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**Abstract**

The use of barium in industry (plastics, rubbers, paints, carpets, ceramics, sealants) and in agriculture (fertilizers and pesticides) can lead to the contamination of underground surface waters by leaching. Understanding the mechanisms of barium adsorption on vermiculite could lead to the improvement of its maximum adsorption capacity and therefore its elimination from the environment. The purpose of this work is to contribute to the understanding of the barium adsorption mechanism on vermiculite by determining the effect of the zero charge point pHpzc on the increase in its adsorption capacity.
The grinding of the vermiculite leads to an increase in the pHpzc accompanied by an improvement in the adsorption. The study at free pH of the effect of the grain size of vermiculite on the isotherms of adsorption of barium at 25°C showed that the maximum capacity of adsorption increases with the reduction in the grain size. This study was carried out for the following grain sizes: d1>1000 µm, 100 µm<d2<500 µm and 25 µm<d3<80 µm. The maximum adsorption capacity is 24.8 $mg.g^{-1}$ for a grain size d1, 28.09 $mg.g^{-1}$ for a grain size d2 and 29.61 $mg.g^{-1}$ for a grain size d3. The determination of the pHpzc for the previous grain size showed that their decrease led to an increase in the pHpzc. The pHpzc for fractions d1, d2, d3 is 2.49, 2.65 and 3.27 respectively.

**Keywords:** adsorption, barium, vermicumite, pHpzc.

## *POSTER PRESENTATION*

# Quantitative Structure Property Relationship study to predict soil sorption partition coefficient

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**Abstract**

The Koc partition coefficient is an important parameter for determining the distribution of cantaminants in the environment. The experimental value of koc is difficult to measure, that's why it is important to establish models to predict this parameter.
In the literature, several studies have been conducted to predict its value for certain categories of molecules.
In this work, we have undertaken QSPR study to relate Koc to molecular structure. The study was established on a database of 643 molecules. Among the methods used for the establishment of the QSPR model, the multilayer neural networks gave excellent results in comparison with those of the literature.

**Keywords:** QSPR- Neural Network RNN- Koc - Linear regression.

## *POSTER PRESENTATION*

# Synthesis and characterization of SAPO-34 catalyst for light olefin synthesis from methanol

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**Abstract**

Olefins, commonly known as alkenes, are unsaturated organic species containing C=C bond and have a carbon content of 2-4, are important industrial monomers. Light olefins; ethylene ($C_2H_4$) and propylene ($C_3H_6$) are short-chain valuable liquid hydrocarbons which are used in large scale plastics production. Olefins are traditionally produced via petroleum cracking. However, due to fossil fuel supplies and environmental issues, production is shifting to environmentally friendly and renewable sources. In olefin production, methanol, which can be obtained from renewable resources, can replace petroleum. SAPO-34 molecular sieve catalyst is one of the most widely used commercial catalysts in MTO production and it has good conversion and selectivity. However, the catalyst has transfer limitations when it is deactivated by the catalytic coke formation. Synthesis method development studies that seek to improve SAPO-34 optimize catalyst performance by reducing coke formation via structure manipulation.

In this study, SAPO-34 synthesis was carried out via hydrothermal method using DEA template. The structure of the synthesized catalyst significantly affects SAPO-34 catalyst's performance. Therefore, the characterization of the SAPO-34 catalyst is of great importance. The MTO reaction requires mild acidity, high pore volume, and the SAPO-34 molecular sieve structure to be cubic and crystallised. All these properties are analysed by various characterization methods.

The catalysts synthesized in this study were characterized by FT/IR, XRD and SEM analyses. SAPO-34 catalyst is a cubic Chabazite material containing silica, alumina and phosphate in its structure. In the FT/IR analysis, it was seen that the molecular structure of the SAPO-34 catalyst was properly formed. The strong peak at $2\theta$=9.58, minor peaks at 13, 20.8, 16.2, 17.8, 25 and 26.2, 30.9-31.7 double peak in XRD analyses show that the SAPO-34 catalyst is of the Chabazite (CHA) crystal structure. With the SEM analysis, cubic Chabazite structures of around 100 μm were visually observed.

**Keywords:** MTO, SAPO-34, hydrothermal synthesis.

## *POSTER PRESENTATION*

# Modified glassy carbon paste electrodes in voltammetric studies of hydroquinone, catechol, vanillin and melatonin solutions

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**Abstract**

Hydroquinone, catechol, vanillin, and melatonin are very interesting (bio)active compounds that are important for many biological and industrial processes. In the present work, voltammetric procedures for their determination employing carbon paste electrodes manufactured using natural glassy carbon and polydimethylsiloxane ($GC_n$/PDMS) were developed. Two types of electrodes were examined: (i) unmodified and (ii) modified by addition of $Y_2O_3$, $Yb_2O_3$, $La_2O_3$ or mixture of $Y_2O_3$ and $Yb_2O_3$. All tested electrodes provided measurable voltammetric signals of catechol and hydroquinone when acetate (pH 4.66) or phosphate buffer (7.36) were used. In most of the experiments, the sensitivity of the modified electrodes was higher than that offered by the unmodified paste electrode. The modification of carbon paste was also beneficial for the separation of catechol and hydroquinone signals recorded by cyclic voltammetry. In this respect, the $Y_2O_3$, $Yb_2O_3$ modified electrodes turned out to be the most advantageous. The favourable peak separation, greater than 0.14 V[1], allowed the simultaneous determination of catechol and hydroquinone. The type of buffer did not significantly affect peak separation, but phosphate buffer provided better developed voltammograms with well-defined signals of both analytes. The phosphate buffer also provided better analytical signals for vanillin compared to the acetate buffer. Modification of the carbon paste did not cause any significant changes when the determination of vanillin was considered. On the contrary, in the case of the melatonin solution in phosphate buffer, a small but noticeable 40% increase in melatonin peak current was observed when the electrode modified with $Y_2O_3$, $Yb_2O_3$ was applied. When the results of the conducted research are summarized, it can be stated that the $GC_n$/PDMS-based carbon paste electrodes, both unmodified and modified, allow the recording of well-shaped voltammograms originating from the oxidation of phenol derivatives, but their application to recording of electrooxidation signals of other compounds is less beneficial.

**Keywords:** Hydroquinone, catechol, vanillin, melatonin, voltammetry, carbon paste electrodes

The research was carried out through participation in the Ceepus programme (M-CZ-0212-16-2223-M-161191 - Education of Modern Analytical and Bioanalytical Methods)

1.Fan, J., et al., *Simultaneous detection of hydroquinone and catechol with decreasing pH at a bare glassy carbon electrode surface.* Analytical Methods, 2019. **11**(5): p. 604-609.

## *POSTER PRESENTATION*

# Akgöl Su Kalitesinin Organik Parametreleri Değerlerine Göre Su Kalite İndeksinin Değerlendirilip Karşılaştırılması

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**Özet**

Su, insanlığın temel ihtiyaç kaynaklarından birisi olmuş ve insanlık tarihi boyunca da toplumlara yön vermiştir. Su kaynaklarına yakın toplumlar oluşturulurken günümüzde bu yapı değişse de suyun önemini değişmiş değildir. Buna karşın su tükenebilir bir kaynaktır ve doğru kullanılması insanlığın geleceği adına büyük önem taşımaktadır. Çalışma kapsamında önemli su kaynaklarından birisi olan Sakarya Akgöl'ün su kalitesinin incelenmesi amaçlanmaktadır. Bu doğrultuda Eylül 2021 – Ocak 2022 dönemi arasında 5 ay boyunca 2 farklı noktadan ayda 2 kez su örnekleri alınarak 10 gözlemden oluşan bir örneklem geliştirilmiştir. Bu örneklem özelinde de 22 farklı parametre üzerinden su kalitesinin değerlendirilmesine çalışılmıştır. Yapılan analizler doğrultusunda pH değerinin ortalama olarak 7.9 seviyesinde olduğu belirlenmiştir ki bu da su için ideal pH aralığında kabul edilmektedir. Kimyasal oksijen ihtiyacı düzeyi 2.sınıf kalitede yer alırken, çözünmüş oksijen düzeyi 1.sınıf kalitede, toplam fosfor düzeyi 3.sınıf kalitede, biyolojik oksijen ihtiyacı 3.sınıf kalitede, toplam organik karbon düzeyi 4.sınıf kalitede, yağ ve gres düzeyi 1.sınıf kalitede, fenolik maddeler düzeyi 1.sınıf kalitede, mineral yağlar ve türevleri düzeyi 4.sınıf kalitede, sülfat düzeyi 4.sınıf kalitede, klorür iyonu düzeyi 2.sınıf kalitede, florür düzeyi 2.sınıf kalitede, nitrit azotu düzeyi 1.sınıf kalitede ve son olarak nitrat azotu da 1.sınıf kalitede belirlenmiştir. Elde edilen bu veriler ışığında Sakarya Akgöl'deki su kalitesine dair kabul edilebilir seviyede olduğu çıkarımında bulunmak mümkündür.

**Anahtar Kelimeler:** Su, kalite, Akgöl, indeks.

## *POSTER PRESENTATION*

# RNA-seq based detection and prioritization of aberrant splicing events in rare diseases: A benchmark study for recently developed tools

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**Abstract**

80% of rare diseases have genetic origins and the average time to get an accurate diagnosis for a rare disease is 6-8 years. Although genome sequencing is the most successful approach in rare disease diagnosis, the success rate is between 35%-50%. Recent studies have shown that RNA Sequencing (RNA-Seq) provides additional information about causative genes in rare diseases, increasing the diagnostic rates up to 70%. RNA sequencing data provides information about genomic variants, expression levels of genes as well as alternative splicing events. Additionally, it was shown that 62% of all disease-causing single nucleotide variants can affect RNA splicing events. Therefore, several tools have been developed in the recent years to identify causative genes based on aberrant splicing. Here, we set up a benchmark study to evaluate recently developed RNA-Seq bioinformatics tools; - FRASER, dasper and SCISSOR - for the detection of aberrant splicing events to discover disease causing genes. The RNA-Seq samples were obtained from a public dataset, where patients with Cystic Fibrosis disorder were analyzed in response to a drug treatment, and drug-responder patients and drug-nonresponder patients are also known. As a result, although dasper and SCISSOR could detect outlier splicing events in differentially expressed genes, they were not successful in recognizing aberrant splicing between responders and non-responders. Moreover, albeit requiring additional annotation steps, FRASER provided a more data type-sensitive and -specific analysis than the others.

**Keywords:** rare disease, RNA-Seq, aberrant alternative splicing.

## *POSTER PRESENTATION*

# Zinc-catalyzed synthesis of salicylate esters by transesterification of methyl salicylate

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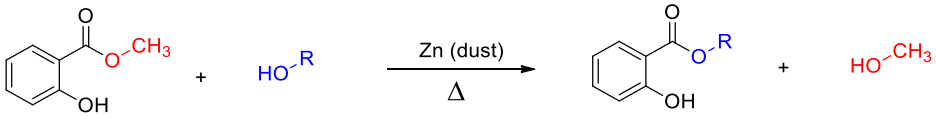
**Abstract**

The salicylate esters are of great importance because of their usage as food preservatives, drugs, plasticizers, solvents, perfume and cosmetics.[1] Traditionally, they have been obtained by esterification of salicylic acid with an alcohol in the presence of inorganic acid catalysts.[2] However, this method offers some disadvantages such as unwanted side products, difficulty in purification step, longer reaction times, elevated temperatures and thermodynamic reversibility. In order to synthesize the salicylate esters in an efficient manner, many considerable synthetic efforts have been performed involving acid or base catalysed transesterification of alkyl salicylate with alcohol.[3,4] Depending on the nature of catalyst, the transesterification is carried out at lower temperatures and shorter reaction time than those used in esterification. Considering the industrial scale synthesis of salicylate esters, the utilization of catalytic system in transesterification is desired to be readily available, reusable and stable. On the basis of above consideration, in this study, the transesterification of methyl salicylate with 10 different alcohol have been accomplished by the use of Zn catalyst. The using Zn dust provided shorter reaction time by giving moderate to excellent yields. The structural elucidation of the products was done by the means of GC-MS data obtained from the Wiley GC/MS Library and in-house "Parkim Fragrance House GC/MS Library".

**Keywords:** Salicylate esters, transesterfication, methyl salicylate, inorganic catalyst




**R**: Isoamyl, cis-3-hexenyl, benzyl, citronellyl, cyclohexyl, isobuthyl, phenethyl, propyl, bornyl, carvacryl

**Scheme 1**: Zn-catalyzed transesterification of methyl salicylate with various alcohols

**References:**

1- Lapczynski, A., McGinty, D., Jones, L., Bhatia, S., Letizia, C. S., & Api, A. M. (2007). Fragrance material review on ethyl salicylate. Food and chemical toxicology, 45(1), S397-S401.
2- Tanaka, S., Nakashima, T., Satou, N., Oono, H., Kon, Y., Tamura, M., & Sato, K. (2019). Epoxide as precatalyst for metal-free catalytic transesterification. Tetrahedron Letters, 60(30), 2009-2013.
3- Tao, D. J., Wu, J., Wang, Z. Z., Lu, Z. H., Yang, Z., & Chen, X. S. (2014). SO 3 H-functionalized Brønsted acidic ionic liquids as efficient catalysts for the synthesis of isoamyl salicylate. Rsc Advances, 4(1), 1-7.
4- Shamshuddin, S. M., & Nagaraju, N. (2007). Liquid phase transesterification of methyl salicylate and phenol over solid acids: kinetic studies. Journal of Molecular Catalysis A: Chemical, 273(1-2), 55-63.

## *POSTER PRESENTATION*

# Phenolic compounds and biological activity of Algerian Fabaceae plant

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**Abstract:**

This work involved the quantification of phenol and flavonoid content in methanol and n-butanol extracts, and the evaluation of the antioxidant activities of the extracts. Secondary metabolites were obtained by maceration with water/methanol (80:20;V:V).After liquid-liquid extraction with increasing polarity of solvent, the obtained extracts (*n*-BuOH, $CHCl_3$) were subjected to phytochemical analysis and biological activity. The evaluation of antioxidant activity was assessed by DPPH assay. The quantification of polyphenols and flavonoid was performed using The Folin-Ciocalteu reagent and the aluminum trichloride method. The results showed that *n*-BuOH extract had the highest content of total polyphenols and flavonoids ,which was 96.03±6.36mg GAE/g.
The DPPH test used to determine antioxidant activity showed that *n*-BuOH extract had the highest antioxidant activity with IC50= 0.038±0.002 mg/ml. The results of phytochemical studies and quantitative tests , as well as the antioxidant activity , explain the abundance of this plant and its use as a medicinal plant.

**Keywords:** phenolic content, flavonoid content, Folin-Ciocalteu , antioxidant activity.

## *POSTER PRESENTATION*

# Extraction methods and evaluation of the antioxidant activity of a medicinal plant from *Ferula* genus

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**Abstract**

Extraction is a very important step in the separation and recovery of target compounds. It is affected by several factors, including the method used and the presence of interfering substances. The purpose of this study was to compare three extraction methods by evaluating the total flavonoid content and antioxidant activity of the aerial parts of *Ferula*.
Extraction rates were determined by three extraction methods, namely maceration, infusion, and decoction, and antioxidant activity was quantified using the method of FRAP Reducing Power.
Impregnation had the highest extraction rate of 15.815%. Apart from the antioxidant activity, the extract obtained by infusion exhibited the most important antioxidant activity, IC50=152.01±1.74 µg/ml, irrespective of the plants examined. This study demonstrated that maceration extraction was the optimal extraction and maceration method to obtain the highest antioxidant capacity of the studied extracts.

**Keywords:** extraction methods,antioxidant activity,FRAP.

## *POSTER PRESENTATION*

# Computational Study Of Structural Parameters And Chemical Reactivity Of a TTF Derivative With Optical Interst

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**Abstract**

The present work includes a computational study of a TTF derivative of optical interest, by molecular modeling via the Gaussian9 program. The Density Functional Theory (DFT/B3LYP) method with the basis of 6-31G (d,p) was used to identify the electronic parameters and descriptors of the global reactivity as well as the properties of nonlinear optics (NLO). The results obtained allowed us to predict the reactive sites on the molecule for nucleophilic, electrophilic and radical attacks. The possibility of using this organic material in the field of nonlinear optics has also been verified.

**Keywords:** Tetrathiafulvalene, Density Functional Theory (DFT), Reactivity Descriptors, Nonlinear Optics (NLO).

## *POSTER PRESENTATION*

# The chemical profile of the *Salvia officınalis* growing wild in Kosova

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**Abstract**

This study presents the chemical profile of the essential oil extracted from *Salvia officinalis* growing wild in Kosova. The *S. officinalis* plant is a member of Lamiaceae family and is native to the Balkan region as well as in Kosovo areas. *S. officinalis* are reach on chemical compounds such as α- and β-Thujone, Cineol, Borneol, Cornsole, Caffeic and Nicotinic acids, flavones etc. Essential oils of *S. officinalis* samples were analysed from different areas of Kosova (in different altitude and in different soil chemical composition). The plant *S. officinalis* were collected in the end of June, 2022. The air dried *S. officinalis* was extracted by Clevenger apparatus within 4 hours to obtain essential oil. The chemical composition of the essential oils was analysed using GC/FID technique, and VF-1ms capillary column (30 m x 0.33 mm x 0.25 um) were used for separation of compounds. α- and β-Thujone were identified as main constituents for the all analysed essential oils of *S. officinalis* samples from Kosova areas. Oxygenated monoterpenes (Camphor, Cineol and Borneol) were found in higher percentage for all samples. As conclusion, the changes observed in the chemical profile of the essential oil of *S. officinalis* from the areas of Kosova are mainly related to the altitude, humidity, air temperature, hours of sunshine and soil geology of the areas where wild *S. officinalis* grew.

**Keywords:** *Salvia officinalis*, Essential oils, α- and β-Thujone, GC/FID.

## *POSTER PRESENTATION*

# Use of avocado oil and vacuum packaging in poultry meats

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**Abstract**

The place of foods of animal origin in human nutrition is very important. The popularity of poultry meat in animal production in the world is increasing day by day. The role of poultry meat, which is rich in protein, iron, zinc, folic acid, vitamin B and vitamin A, is very important in compensate the animal protein requirements. Although it is known that the nutritional value of meat is high, this also shows that meat is susceptible to physical, chemical and microbiological changes. In line with the increasing demands of consumers and developments in packaging technology, food manufacturers focus on innovations in the packaging system to ensure the highest possible product quality. One of the important parameters affecting the quality of poultry meat is the use of appropriate packaging systems. Vacuum packaging is one of the most widely used systems in the packaging of fresh meat in the meat industry. Vacuum packaging is a packaging method used to preserve the quality of fresh meat and extend its shelf life. At the same time, the importance of many different applications is gradually increasing in order to improve the textural properties of fresh meat. Avocado is a fruit native to the Americas and is a plant that grows in temperate and subtropical climates. There is increasing interest in the use of avocado oil in a nutrition and wide variety of industries such as food and cosmetics. Biological active substances such as tocopherols, tocotrienols, phytosterols and carotenoids in its structure and monounsaturated fatty acids have made avocado oil the focus of research. Avocado oil also has good durability at high temperatures, increasing its usability in technological applications. In this review, it is aimed to give general information about the effect of vacuum packaging and avocado oil in poultry meat on some properties of meat.

**Keywords:** Poultry meat, Vacuum packaging, Avocado oil, Meat quality.

## *POSTER PRESENTATION*

# Active and intelligent packaging systems in meats

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**Abstract**

Containers, covers or wraps made of special materials such as metal, glass, paper, plastic, which protect the product from external influences and facilitate its marketing and consumption are called packaging. Packaging has three basic functions; I- To protect the product inside, II- To provide ease of loading, unloading, stocking and use, III- To promote the product and to encourage the consumer to buy. Traditional packaging systems are not sufficient to satisfy the increasing demands of the consumer and have some limitations, especially in terms of extending shelf life and ensuring food safety. Therefore, active and intelligent packaging systems have been developed to satisfy increasing consumer demands and industrial production trends. Increasing health awareness and increasing food safety concerns have led to more consumer acceptance of active and intelligent food packaging.
Active packaging systems include scavengers such as oxygen, carbon dioxide, ethylene, moisture and diffusers such as carbon dioxide, ethanol, antioxidants and antimicrobials. Oxygen scavengers are the most widely used among active packaging systems. Oxygen scavengers are used to protect meat products containing red and white meat from rancidity and discoloration. Films containing antimicrobial substances are used as packaging materials, especially in antimicrobial systems used in meat products.
Intelligent packages are packaging systems that provide information to producers and consumers in terms of food safety and quality and provide them with early warnings about product quality. The working principles of intelligent packaging depend on temperature-time measurement, enzymatic quality changes and microbiological activity measurement. Packaging indicators are indicators that inform the consumer about the presence or absence of a substance, the reaction between two or more substances. The aim of this review is to give general information about active and intelligent packaging systems used in meat and meat products.

**Keywords:** Active packaging, Intelligent packaging, Meat, Meat quality.

## *POSTER PRESENTATION*

# Çanakkale İli ve Çevresinde Satışa Sunulan İşlenmiş Su Ürünlerinden İzole Edilen *Staphylococcus* spp. türlerinin antibiyotik dirençliliklerinin belirlenmesi

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**Özet**

Çanakkale ili su ürünleri üretimi ve tüketimi açısından Türkiye'nin en önemli illerindendir. Su ürünleri tüketiminin nüfusta yaygın olması hem ilde, hem de yakın bölgelerde su ürünlerine olan talebi artırmaktadır. Bu talep karşısında ise yoğun taze tüketimin yanında, ülkemizin farklı bölgelerinde üretilen çoğu işlenmiş su ürünlerine de perakende satış noktalarında ulaşmak mümkündür. Bu çalışmada, Çanakkale ili ve çevresinde yer alan güney Marmara illerindeki perakende satış noktalarından, farklı şekillerde işlenerek dondurulmuş veya raflarda yer alacak şekilde paketlenmiş ürünlerden satın alınarak *Staphylococcus* spp. izolasyonu yapılmış, izole edilen türler tanımlandıktan sonra beta-laktamların da içinde olduğu farklı antibiyotiklere karşı gösterdikleri dirençlilik seviyeleri belirlenmiştir. Ülkemizdeki kullanımları da göz önünde bulundurularak dirençlilik testlerinde ofloksasin (OFX-5), amoksisilin + klavulanik asit (AMC-30), vankomisin (VA-5 ve VA-30), ampisilin (AMP-10), tetrasilin (TE-30), kanamisin (K-5), aztreonam (ATM-30), sefuroksim (CXM-5 ve CXM-30), seftazidim (CAZ-30), sefotaksim (CTX-30) ve gentamisin (CN-120) antibiyotikleri kullanılmıştır. *S. aureus* haricinde izole edilen *Staphylococcus spp*. türleri *Staphylococcus equorum, Staphylococcus sciuri, Staphylococcus xylosus, Staphylococcus lentus* ve *Staphylococcus haemolyticus* olarak belirlenmiştir. Dirençliliğin en fazla olduğu antibiyotikler aztreonam, sefuroksim ve ofloksasin olarak belirlenmiştir.
Sonuç olarak, farklı türlerde *Staphylococcus* spp.'nin işlenmiş su ürünlerinde bulunabileceği ve bu türlerin yaygın kullanıma sahip olan önemli antibiyotiklere karşı dirençli olabilecekleri tespit edilmiştir. Gıdaların mikrobiyal ekolojisinde, hem hijyenik, hem de teknolojik açıdan önem taşıyan mikroorganizmaların izolasyonları ve benzer antibiyotiklere karşı dirençlilik seviyelerinin belirlenmesine yönelik çalışmaların durum takibi açısından gerekli olabileceği öngörülmektedir.

**Anahtar Kelimeler:** *S. equorum*, *S. sciuri*, *S. xylosus*, antibiyotik, işlenmiş su ürünleri.

## *POSTER PRESENTATION*

# Identification of disinfection by-products (trihalomethane) in drinking and utility waters

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**Abstract**

In the disinfection process, substances with complex structures formed by the reaction of organic substances in water with chlorine are called disinfection by-products or trihalomethanes. It is extremely important to detect trihalomethanes, which are Chloroform, Dichlorobromomethane, Chlorodibromomethane, Bromoform etc. species and affect human health toxicologically. Considering human exposure to THMs more broadly, people use chlorine-disinfected tap water not only for their drinking water use, but also for cooking, hand-washing, bathing, showering, swimming, laundry, cleaning, etc. It is known that they are more exposed to THMs by using It is expected that trihalomethane levels will be high, especially in swimming pool waters where high amounts of chlorination are made. As a matter of fact, according to the study conducted in the swimming pool, trihalomethane was found at an average of 150 µg/l.

**Keywords:** trihalomethane, drinking and utility water.

## *POSTER PRESENTATION*

# Highly sensitive ascorbic acid electrochemical sensor based on reduced graphene oxide and application of real samples

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**Abstract**

In this study, a graphene/Au modified glassy carbon (GC) electrode was developed to detect ascorbic acid (AA) in human blood and drug samples using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The structural properties and morphological properties of the obtained modified electrodes were investigated using different techniques. This newly designed graphene oxide/Gold-based electrochemical sensor can measure AA content in phosphate buffer solution (PBS) with a wide range of concentration. The obtained ascorbic acid sensor gave very high acceptability results for the determination of AA in human blood serum and vitamin C tablets.

**Keywords:** Ascorbic acid, graphene oxide, gold nanoparticle, voltammetry

# 2.2. FULL-TEXT PAPER

## *POSTER PRESENTATION*

# Evaluation of Pentosidine Level in Human Dentin Samples for Chronological Age Estimation

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## Abstract

Age estimation is an important procedure in the human identification. Teeth are retained longer than soft tissue after death. Aging is a natural process accumulating advanced glycation end products, especially pentosidine. This molecule has been studied in age estimation but there are a few data supporting for the Thai population. The purpose of this study was to determine the relationship of pentosidine level composed in the second molar and chronological age in the Northern Thai population. Powder of 35 non-carious dentine samples (male = 25, female = 10) were prepared and examined by high-performance liquid chromatography – fluorescence. The concentration of pentosidine contained in the second molar dentine found 59.50 ng/mL. Mean value of pentosidine concentrations in the female samples (64.90 ng/mL) were higher than in the males samples (57.33 ng/mL). The pentosidine concentration related with chronological age which a correlation coefficient (r) showed 0.67. Bias, inaccuracy, and standard error value revealed 0, 10.1, and 2.1, respectively. Tooth position did not effect to the pentosidine concentration. Both age and gender might be further investigated from the only pentosidine concentration.

**Keywords:** : Age estimation, Pentosidine, Second molar teeth

## INTRODUCTION

Person identification is one of the most important processes in forensic science. Age estimation methods are an important tool for giving accurate information to age identity. There are many techniques have been applied for age assessment including, examining the cranial sutures, epiphysial plates, pubic symphysis, parts of cartilage that connect between the sternum and ribs, the lateral portion of the hipbone (auricular surface of the ilium), vertebrae and teeth [1]. These methods are only precise in adolescent but highly deviate in adults. It also requires an expert forensic anthrophologist and the samples must be completed [2].

Currently, post-translational protein modification, aspartic acid racemization and advanced glycation end products (AGEs) have been applied for age estimation. AGEs, heterogeneous compounds, are produced from reducing sugars binding with proteins, lipids or nucleic acids. Pentosidine, a type of AGEs, is permanent chemical and can accumulates through a person's lifetime.

The analysis of pentosidine is an interesting method for age estimation. It is more rapidly, and accurate analytical procedure than the other methods [3]. Therefore, detection of pentosidine may provide a new method for age estimates. The aim of this study was to study a relationship and estimate chronological age by pentosidine concentrations contained in the second molar.

## MATERIALS AND METHODS

*chemical*

Standard material of pentosidine was purchased from Cayman (Michigan USA). Ethylenediaminetetraacetic acid disodium salt dihydrate ($Na_2EDTA$) and Heptafluorobutyric acid (HFBA) were obtained from Sigma-Aldrich (St.Louis, USA). All reagents were analytical grade, excepted methanol was HPLC grade.

*Preparation of dentine samples*

The second molar teeth were obtained from Department of Forensic Medicine, Faculty of Medicine,

Chiang Mai University. This study was approved by the Research Ethics Committee of Chiang Mai University's Faculty of Medicine (FOR2565-09049). The teeth were washed with distilled water and dried at room temperature. Then, the samples were stored at -20 °C before use. The information including age, gender, and tooth position were recorded. Dentine sample was prepared according to the method described by Greis F et al. [3]. The outer layer of cementum and enamel was screwed in with a high-speed diamond bar after this phase was completed using a low-speed teeth saw.

*Preparation of pentosidine from dentine samples*

Dentine powder (50 mg) was added to a 2 mL microcentrifuge tube and demineralized with 2 mL $Na_2EDTA$ (0.5 M, pH 7.4 adjusted with 2 M NaOH). The sample was vigorously shaken for 20 min and centrifuged at 13,000 rpm for 5 min. The residue was washed 3 times with distilled water to remove free EDTA, Ca and Mg-EDTA complexes [4]. Hydrochloric acid (6 M; 500 µL) was added and then hydrolyzed the sample at 100 °C for 20 h. The hydrolyzed sample was dried under nitrogen. Dried samples were redissolved with 0.1 M HCl before analysis with high performance liquid chromatography system (HPLC).

*Pentosidine analysis*

Samples were injected into a HPLC-fluorrescence system (HPLC 1100 Series, Agilent, CA). An analytic column is a Purospher® STAR RP-18 endcapped (0.5 µ x150 mm) column. The mobile phase consisted of (A) 0.1% HFBA and (B) Acetonitrile. A linear gradient program at 0 minutes (A100: B0), 20 minutes (A0: B100), and 9 minutes (A100: B0) at a flow rate of 1 mL/min. The injection volume was 10 µL. The fluorescence detector (FLD) was set at 385 and 335 for emission and excitation wavelength [3].

*The self-consistency test*

The self-consistency test was used to evaluate the age prediction models' efficacy. The following equation was used to compute bias, inaccuracy, and the standard error value.

- Bias = $\Sigma$ (predicted age – chronological age)/n
- Inaccuracy = $\Sigma$|predicted age – chronological age|/n
- Standard error = $\{[\Sigma(\text{predicted age} - \text{chronological age})^2]^{1/2}\}/n$

*The statistical significance.*

The analysis was performed by IBM SPSS Statistics 22 program. Linear regression was performed the relationship between the pentosidine level and chronological age. Correlation between pentosidine level with gender and location was performed by Pearson correlation.

## RESULTS AND DISCUSSION

The non-caries samples were included in this study and obtained from 25 males (71%) and 10 females (29%). The average age of both males and females presented 35 years. They consisted of 22 (63%) and 13 (37%) for upper and lower second molar teeth respectively. The details of the example are shown in Table 1.

**Table 1.** Demographic data of the dentine samples

| Age range (years) | Number of teeth (persons) | Gender | | Tooth positions | |
|---|---|---|---|---|---|
| | | Male | Female | Upper | Lower |
| <30 | 8 | 7 | 1 | 4 | 4 |
| 31 – 50 | 16 | 13 | 3 | 10 | 6 |
| >51 | 11 | 5 | 6 | 6 | 5 |

*Effect of gender and location of dentine on pentosidine level*

The average pentosidine concentrations contained in the sample presented 59.50 ng/mL. Effect of gender ($p$ = 0.06) and tooth position ($p$ = 0.81) did not affect on pentosidine concentrations. Pentosidine concentration contained in females samples showed 64.90 ng/mL which was higher than male's (57.33 ng/ml). The apparent relationship of menopause with pentosidine accumulation, the gender difference could be related to changing estrogen levels. Estrogen regulates insulin-dependent glucose transport [5-6] and it acts as an antioxidant [7]. It is possible that the higher pentosidine level in the female is due to the loss of estrogen during menopause. Oxidative stress might be one of the causative factors for osteoporosis through inducing a high content of pentosidine in bone in postmenopausal diabetic women.

*A correlation of pentosidine concentration and chronological age*

A correlation between the concentration of pentosidine and chronological age was estimated by using linear regression. The correlation was described by the equation one and the results is shown in Figure 1.

Calculated age (years) = (pentosidine concentration (ng/mL) - 17.405)/1.045 (eq1)

The correlation coefficient (r) between chronological age and pentosidine concentrations was 0.67. The value of bias, inaccuracy, and standard error were 0, 10.1, and 2.1 years, respectively.

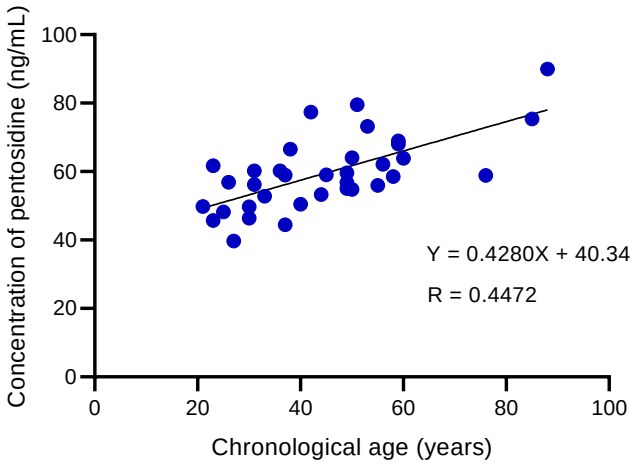


**Figure 1** A linear relationship between chronological age and pentosidine concentrations in dentine samples.

The pentosidine concentration contained in the dentine samples were subdivided into three groups including less than 30, between 31 to 50, and higher than 51 years. The results are shown in table 2.

**Table 2**. Pentosidine concentrations contained in dentine samples.

| Age range (years) | Mean concentrations of pentosidine (ng/mL) | | | |
|---|---|---|---|---|
| | Gender | | Tooth position | |
| | Female | Male | Upper | Lower |
| <30 | 50.33 | 45.69 | 53.51 | 45.99 |
| 31 – 50 | 58.29 | 57.26 | 57.07 | 59.82 |
| >51 | 71.91 | 64.61 | 80.78 | 65.05 |

There was no statistically significant difference in the three age groups in the dentine samples ($p$ = 0.52). The tooth position and gender did not affect on pentosidine, as shown in Table 3. The results presented that the pentosidine concentrations depend on age. In humans, age-dependent AGEs relate to increase in the natural aging process and accumulation of AGEs due to the progressive creation of AGEs or pentosidine [8]. Extracellular collagen is an AGEs target because the collagen containing lysine, hydroxylysine, and arginine residues. As a result, the accumulation of AGEs in living organisms is regarded a biomarker of aging processes [9-10]. A number of pathologies have been identified, including diabetes, heart disease, degenerative brain diseases, and cancer [11-15]. In addition, the grilled and fried foods have influence to the pentosidine accumulation in the body and affects the amount of pentosidine [16-17]. Food consumption might an important factor that disturb to the age estimation using pentosidine.

**Table 3** comparison of the mean pentosidine concentrations in dentine samples.

| Age range (years) | Mean concentrations of pentosidine (ng/mL) |
|---|---|
| <30 | 49.75 ± 6.36 |
| 31 – 50 | 58.10 ± 7.13 |
| >51 | 68.60 ± 9.83 |

## CONCLUSION

Pentosidine level corelated with chronological age. The equation between pentosidien and the equation of age estimation showed 2 years for a standard error. The position of teeth and the gender were not affect the concentration of pentosidine. Therefore, pentosidine level can be applied for age estimation model in future. Influences of underlying disease and gender should be studied in further.

## REFERENCES

1. Seubwai, w., & Khunkitti, W., 2557. Application of Molecular Biology Techniques on Forensic Sciences. Srinagarind Med J, 29 (3): 321-326.
2. Alkass, K., et al., 2010. Age estimation in forensic sciences: application of combined aspartic acid racemization and radiocarbon analysis. Mol Cell Proteomics, 9(5): 1022-1030.
3. Greis, F., et al., 2017. Analysis of advanced glycation end products (AGEs) in dentine: useful for age estimation? Int J Legal Med, 132(3): 799-805.
4. Ritz-Timme, S., et al., 1993. Postmortem estimation of age at death based on aspartic acid racemization in dentin: its applicability for root dentin. Int J Leg Med, 105(1): 289-293.
5. Godsland, I.F., 2005. Oestrogens and insulin secretion. Diabetologia, 48(1): 2213–2220.
6. Margolis, K.L., et al., 2004. Effect of oestrogen plusprogestin on the incidence of diabetes in postmenopausal women: results from the Women's Health Initiative Hormone Trial. Diabetologia, 47(1):1175–1187.
7. Miquel, J., et al., 2006. Menopause: a review on the role of oxygen stress and favorable effects of dietary antioxidants. Arch. Gerontol. Geriatr, 42(1): 289–306.
8. Simm, A., 2013. Protein glycation during aging and in cardiovascular disease. J Proteomics, 92(1): 248-59.
9. Valenzuela, A., et al. 2018. Differences in non-enzymatic glycation products in human dentine and clavicle: changes with aging. Int J Legal Med, 132(6): 1749-1758.
10. Kida, Y., et al., 2019. Non-invasive skin autofluorescence, blood and urine assays of the advanced glycation end product (AGE) pentosidine as an indirect indicator of AGE content in human bone. BMC Musculoskelet Disord, 20(1): 627.
11. Ohtani, S., & Yamamoto, T., 2005. Strategy for the estimation of chronological age using the aspartic acid racemization method with special reference to coefficient of correlation between DL ratios and ages. J Forensic Sci, 50(5).
12. Ohtani, S., et al., 2003. Differences in the D/L aspartic acid ratios in dentin among different types of teeth from the same individual and estimated age. Int J Legal Med, 117(3): 149-152.
13. Peng, X., et al., 2011. Naturally occurring inhibitors against the formation of advanced glycation end-products. Food Funct, 2(6): 289-301.
14. Franco, A., et al., 2019. Case-specific characteristics of pink teeth in dental autopsies - A systematic review. J Forensic Leg Med, 68(1): 101869.

15. Ahmed, N., 2005. Advanced glycation endproducts-role in pathology of diabetic complications. Diabetes Res Clin Pract, 67(1): 3-21.

16. Stirban, A., et al., 2014. Vascular effects of advanced glycation endproducts: Clinical effects and molecular mechanisms. Mol Metab, 3(2): 94-108.

17. Ramalho, J.S., et al., 1996. Role of Glycation in Human Lens Protein Structure Change. EUR J Ophthalmol, 6(2): 155–161.

## *POSTER PRESENTATION*

# Determination of Biomolecules Contained in Human Dentine Samples by Proton Nuclear Magnetic Resonance Spectroscopy ($^1$H-NMR)

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## Abstract

The tooth is the hardest organ in the human body and the most remained tissue after death. The tooth layer covered by the enamel and dentine layer. Dentine contains many chemical constituents which is one of popular sample for forensic science study but less data to support. The purpose of this study was to identify biomolecules contained in dentine by $^1$H-NMR technique. Dentine sample was prepared from second molar. The dentine powder was dissolved in 0.6 M hydrochloric acid and hydrolyzed under 100°C for 24 hr. The biomolecules contained in the solution were determined by $^1$H-NMR spectroscopy. Sixty three biomolecules were found in dentine including amino acid, sugar, lipid, organic acid, amine compounds and theirs metabolites. D-and L-aspartic acid, pentosidine, carnitine and *N*6-carboxymethylsine can be applied for chronological age estimation. Pyroglutamic acid, malic acid, L-leucine can be estimated for gender. Moreover, Hydroxyl indole acetic acid and ethyl glucuronide which are metabolites of ethanol were established. These biomolecule and $^1$H-NMR techniques can be applied for identification of cause of death, poisonous and diseases in forensic science further.

**Keywords:** Human dentine, $^1$H-NMR, Forensic science, Biomolecule

## INTRODUCTION

Human dentine is a white layer and contained with solid substances as the main tooth. The dentine structure is similar to the bone which will be used to identify the person. Forensic investigation has been present that tooth has specific and individual characteristics in each person. Chemical contained in dentine have been applied for forensic investigation including in mass disasters, accidents and criminal investigations.

Teeth are the hardest organ in the human body and difficult to decompose from charred burning and bury a cropse. Chemical components in dentine both organic and inorganic compounds were illuciated. Enamel contains inorganic mattix (96%, w/w), the protein (90% amelogenin), collagen, lipids and water (4%, w/w). Dentine is the next part of the tooth enamel. The composition of dentine is similarly to the bone combinding with70% (w/w) of inorganic matters, 20% of organic matters and 10% of water. Chemical compounds have been investigated and utilized for many forensic science study.

In Forensic odontology, especially aspartic acid racemization has been applied for age and gender estimation from dentine samples. The chromatography tehcniques have been identified for D and L-aspartic acid. Nuclear magnetic resonance spectroscopy (NMR) was first discovered in the 1940. The NMR uses the magnetic properties of assured atomic nuclei and is widely being used in physic and chemistry. At a present, NMR technique is identified for metabolomics in human samples including blood, serum, plasma, saliva, urine and feces. The results have been used for prediction and diagnosis of non-communicable diseases( NCDs).

The purpose of this study was to identify biomolecules contained in dentin by $^1$H-NMR technique. The solution was determined by 1H-NMR spectroscopy at a frequency of 500 MHz. The chemical composition and the 1H-NMR technique can be applied in forensic science by gender and age determination. Moreover, cause of death ,diseases and toxic substances will be identified and linked to specific chemical contained in dentine samples.

## MATERIALS AND METHODS

### 2.1 Chemicals

Hydrochloric acid (HCl) was obtained from Merck (Darmstadt, Germany). Deuterium oxide ($D_2O$), 3-(trimethylsilyl) propanoic acid sodium salt (TSP) were obtained from Sigma-Aldrich (Ontario, Canada).

### 2.2 Dentine sample collection

Forty five samples of non-carious permanent second molar (30 males and 15 females) age ranged 21 to 85 years old. The teeth samples were obtained from Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, Thailand. This research was approved by the Ethics Committee of Faculty of Medicine, Chiang Mai University (FOR-2565-09049). The teeth samples were cleaned with 0.9% normal saline solution and distilled water to eliminate blood remaining according the method of Manuela *et al*. The dried samples were stored in plastic bag at -20 °C until use.

### 2.3 Dentine preparation

Dentine samples were isolated by transverse section (1 mm) method using a low-speed cross-sectional saw and the enamel surface was subsequently removed by a high speed diamond bur. Both preparing steps were under cooling water condition to avoid the heat.

### 2.4 $^1$H-NMR spectroscopy

Dentine samples were firstly pulverized by a freezing mill (SPEX CertiPrep - 6750 Freezer/Mill) for 15 min. Fifty grams of the dentine powder was digested with 50 mg of 0.6 M HCl at 100 °C for 24 hrs. The digested solution was lyophilized. 0.1 M of TSP in $D_2O$ was added into the sample tube and then the mixed solution was transferred into NMR tube. Record the proton NMR spectrum was acquired at 27 ◦C on a Bruker AVANCE 500 MHz (Bruker, Bremen, Germany) with Carr-Purcell-Meiboom-Gill (CPMG, -RD-90◦-(t-180◦-t) n-acquire) using water-suppression pre-saturation pulse sequence. A 90◦ pulse with a 16 number of signal averaging (NSA) was applied. The baseline and phase correction were carefully adjusted by Bruker TopSpin versions 4.0.7 software.

### 2.5 Peak Assignment and Untargeted Metabolite Identification

Identification of each biomolecule was determined by using The Human Metabolome database (HMDB) and previous published paper [David et al., 2013]. Acquisition of peak, *J* couplings joint value were analyzed by using Bruker TopSpin versions 4.0.7 software.

**Table 1.** Chemical Shifts and Multiplicities of chemical compounds contained in human dentine samples.

| Position | Chemical compounds | $^1$H-NMR Human dentine | HMDB ID |
|---|---|---|---|
| 1 | L-alpha-aminobutyric acid | 0.96 (t; *J* = 6.256) | HMDB 0000452 |
| 2 | Isobutyric acid | 1.05 (d; *J* = 5.3) | HMDB 0001873 |
| 3 | EtG | 1.17 (t; *J* = 7.0) | HMDB 0010325 |
| 4 | L-Allothreonine | 1.32 (d; *J* = 7.8) | HMDB 0004041 |
| 5 | L-lysine | 1.47 (s) | HMDB 0000182 |
| 6 | Citrulline | 1.54 (d; *J* =2.6) | HMDB 0001257 |
| 7 | L-leucine | 1.70 (m) | HMDB 0000687 |
| 8 | Cadaverine | 1.71 (t; *J* = 7.2) | HMDB 0002322 |
| 9 | N6-Carboxymethyllysine | 1.75 (d; *J* = 6.6) | HMDB 0240347 |
| Position | Chemical compounds | Human dentine | HMDB ID |
| 10 | Ornithine | 1.81 (s) | HMDB 0000214 |
| 11 | Thymine | 1.86 (s) | HMDB 0000262 |
| 12 | 2-Hydroxy-3-methylbutyric acid | 2.01 (m) | HMDB 0000407 |
| 13 | Isovaleric acid | 2.04 (d; *J* = 7.3) | HMDB 0000718 |

| | | | |
|---|---|---|---|
| 14 | L-Glutamic acid | 2.06 (m) | HMDB 0000148 |
| 15 | L-Homoserine | 2.15 (t; *J* = 7.0) | HMDB 0000719 |
| 16 | L-Acetycarnitine | 2.18 (s) | HMDB 0000201 |
| 17 | L-Valine | 2.26 (m) | HMDB 0000883 |
| 18 | Gamma-carboxyglutamic acid | 2.29 (s) | HMDB 0041900 |
| 19 | Malic acid | 2.34 (d; *J* = 7.0) | HMDB 0000156 |
| 20 | Pyruvic acid | 2.36 (s) | HMDB 0000243 |
| 21 | Succinic acid | 2.39 (s) | HMDB 0000254 |
| 22 | L-glutamine | 2.41 (d; *J* = 7.0) | HMDB 0000641 |
| 23 | Pyroglutamic acid | 2.43 (d; *J* = 2.2) | HMDB 0000267 |
| 24 | Dimethylamine | 2.50 (s) | HMDB 0000087 |
| 25 | Citric acid | 2.52 (d; *J* = 2.1) | HMDB 0000094 |
| 26 | L-Methionine | 2.63 (t; *J* = 3.9) | HMDB 0000696 |
| 27 | Sarcosine | 2.72 (s) | HMDB 0000271 |
| 28 | L-Aspartic acid | 2.80 (d; *J* = 6.2) | HMDB 0000191 |
| 29 | N-methyl-D-aspartic acid | 2.82 (d; *J* = 5.5) | HMDB 0002393 |
| 30 | D-Aspartic acid | 2.84 (d; *J* = 5.5) | HMDB 0006483 |
| 31 | L-Asparagine | 2.88 (d; *J* = 5.9) | HMDB 0000168 |
| 32 | Creatine | 3.02 (s) | HMDB 0000064 |
| 33 | L-arginine | 3.19 (t; *J* = 6.5) | HMDB 0000517 |
| 34 | Glycerophosphocholine | 3.23 (s) | HMDB 0000086 |
| 35 | L-Carnitine | 3.22 (s) | HMDB 0000062 |
| 36 | L-Phenylalanine | 3.25 (d; *J* = 6.8) | HMDB 0000159 |
| 37 | Taurine | 3.39 (d; *J* = 12.7) | HMDB 0000251 |
| 38 | Glycerol | 3.42 (d; *J* = 8.7) | HMDB 0000131 |
| 39 | Choline | 3.51 (d; *J* = 3.8) | HMDB 0000097 |
| 40 | L-Glycine | 3.56 (s) | HMDB 0000123 |
| 41 | L-Proline | 3.60 (d; *J* = 3.8) | HMDB 0000162 |
| 42 | L-Isoleucine | 3.66 (d; *J* = 3.1) | HMDB 0000172 |
| Position | Chemical compounds | Human dentine | HMDB ID |
| 43 | Acetylglycine | 3.73 (s) | HMDB 0000532 |
| 44 | L-Alanine | 3.77 (m) | HMDB 0000161 |
| 45 | Hydroxylysine | 3.88 (s) | HMDB 0000450 |
| 46 | L-Serine | 3.99 (m) | HMDB 0000187 |
| 47 | Fructosamine | 4.00 (d; *J* = 3.2) | HMDB 0002030 |
| 48 | O-Acetylserine | 4.07 (d; *J* = 4.8) | HMDB 0003011 |
| 49 | Creatinine | 4.09 (d; *J* =5.1) | HMDB 0000562 |
| 50 | L-Lactic acid | 4.11 (dt; *J* = 12.3,6.0) | HMDB 0000190 |

| | | | |
|---|---|---|---|
| 51 | Myo-inositol | 4.12 (d; *J* = 13.0) | HMDB 0000211 |
| 52 | Heparan sulfate | 4.19 (d; *J* = 3.5) | HMDB 0000693 |
| 53 | Pyrraline | 6.33 (s) | HMDB 0033143 |
| 54 | L-Histidine | 7.09 (s) | HMDB 0000177 |
| 55 | L-Tyrosine | 7.19 (d; *J* = 9.7) | HMDB 0000158 |
| 56 | L-Tryptophan | 7.33 (d; *J* = 6.9) | HMDB 0000929 |
| 57 | 5-HIAA | 7.37 (d; *J* = 14.9) | HMDB 0000763 |
| 58 | 5-HTOL | 7.43 (d; *J* = 7.3) | HMDB 0001855 |
| 59 | Pentosidine | 7.44 (d; *J* = 4.6) | HMDB 0003933 |
| 60 | Guanine | 7.60 (s) | HMDB 0000132 |
| 61 | Adenine | 8.01 (s) | HMDB 0000034 |
| 62 | Formic acid | 8.46 (s) | HMDB 0000142 |
| 63 | Cytosine | 8.81 (d; *J* = 0.9) | HMDB 0000630 |

s, singlet; d, doublet; dd, doublet of doublets; t, triplet; dt, doublet of triplets; q, quartet; dq, doublet of quartets; m, multiplet.

**RESULTS and DISCUSSION**

In this study, dentine samples received from donor who drink alcohol and then the sample were hydrolyzed with hydrochloric acid to obtain free molecules. The sixty three biomolecules were found in dentine sample and the results are shown in Table 1. The NMR chromatograms are presented in Figures 1 and 2. Biomolecules composed of α and β form of amino acids, sugars, lipids, organic acids, amine compounds and ethanol metabolites. Enantiomer and derivatives of 36 amino acids were found including L-glutamine, L-glycine, L-histidine. Organic acid (citric acid, lactic acid, succinic acid and pyrulic acid, malic acid, 2-hydroxy-3-methylbutyric acid were identified. Dimethylamine, isobutyric acid, isovalerric acid are grouped in lipid acid. Pentosidine, *N*6-carboxymethyllysine, fructosamine, pyrraline, haparan sulfate, myo- inositol are classified as sugar and its derivatives. Ethanol and its metabolites including 5-hydroxy indole acetic acid (5-HIAA), 5-hydroxytryptophol (5-HTOL), ethyl glucuroide (EtG) were presented. Thymine, adenine, guanine and cytosine which are nucleic acid were determined. Cadaverine (amine compounds) was displayed.

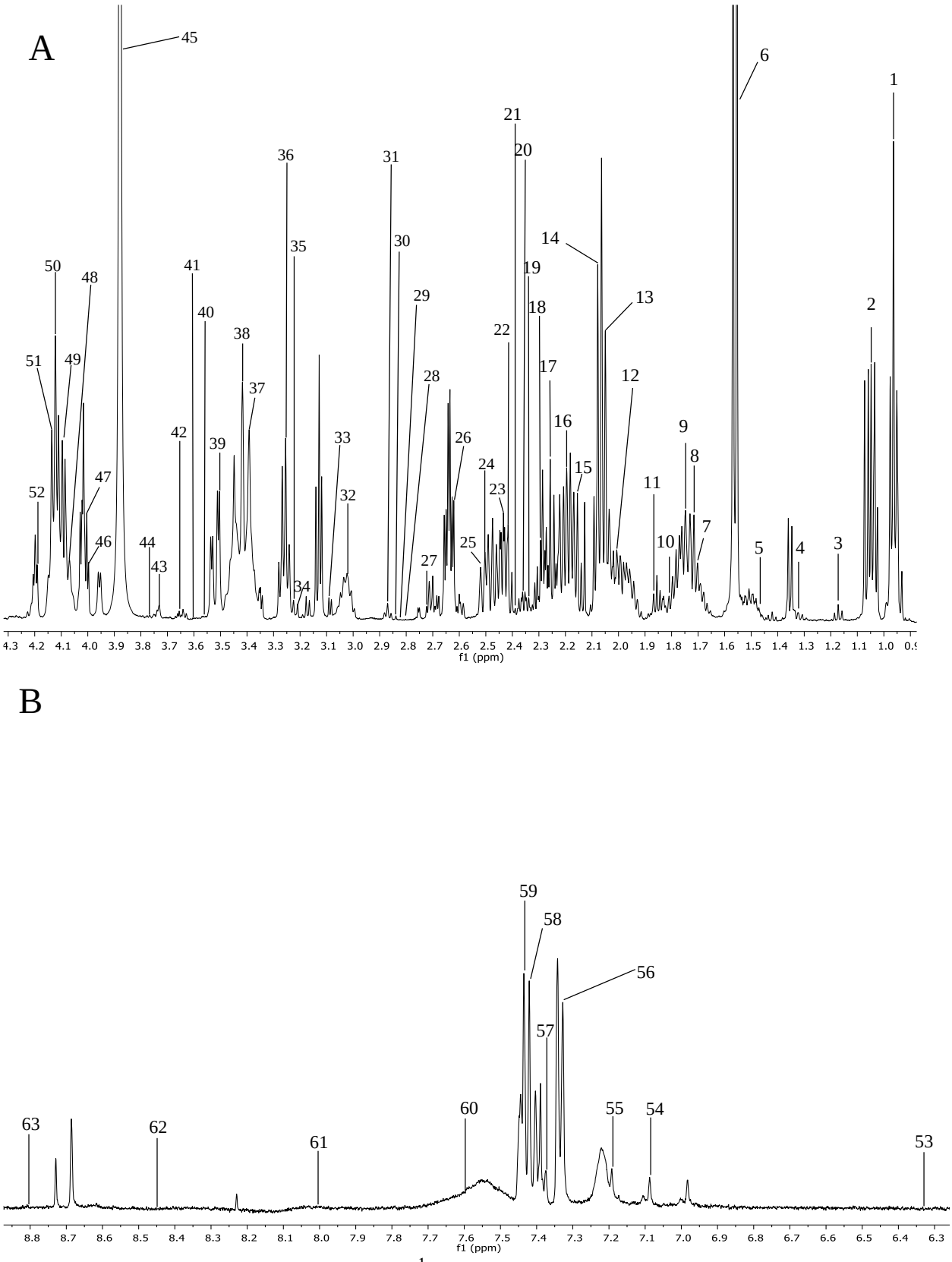


**Fig.1** Chromatograms of typical 500 MHz of $^{1}$H-NMR spectra of hydrolyzed dentine at 0.7-4.3 and 7.05-7.80 ppm regions are presented in Figure A and B, respectively. The main signals have been assigned in the apectra. 1-L-Alpha-aminobutyric acid, 2-Isobutyric acid, 3-Ethyl glucuroide (ETG), 4-L-Allothreonine, 5-L-Lysine, 6-Citrulline, 7-L-Leucine, 8-Cadaverine, 9-N6-Carboxymethyllysine, 10-Ornithine, 11-Thymine, 12-2-Hydroxy-3-methylbutyric acid, 13-Isovaleric acid, 14-L-Glutamic acid, 15-L-Homoserine, 16-L-Acetycarnitine, 17-L-Valine, 18-Gamma-carboxyglutamic acid, 19-Malic acid, 20-Pyruvic acid,21-Succinic acid, 22-L-Glutamine, 23-Pyroglutamic acid, 24-Dimethylamine, 25-Citric acid, 26-L-methionine, 27-Sarcosine, 28-L-aspartic acid, 29-N-methyl-D-aspartic acid, 30-D-aspartic acid, 31-L-asparagine, 32-Creatine,

33-L-arginine, 34-Glycerophosphocholine, 35-L-Carnitine, 36-L-phenylalanine, 37-Taurine, 38-Glycerol, 39-Choline, 40-L-Glycine, 41-L-proline, 42-L-Isoleucine, 43-Acetylglycine, 44-L-Alanine , 45-Hydroxylysine, 46-L-Serine, 47-Fructosamine, 48-O-acetylserine, 49-Creatinine, 50-L-Lactic acid, 51-Myo-inositol, 52-Heparan sulfate, 53-Pyrraline, 54-L-Histidine, 55-L-Tyrosine, 56-L-Tryptophan, 57- 5-Hydroxy indole acetic acid (5-HIAA), 58-5-Hydroxytryptophol (5-HTOL), 59-Pentosidine, 60-Guanine, 61-Adenine, 62-Formic acid, 63-Cytosine.

NMR technique has been used for structural identification, define mechanisms and link molecules in chemistry. This technique provides an information on the chemical structure of various substances and can provide details about specific chemical bonding. The NMR is a potential technique to assist in biochemical processes studies better than other imaging techniques. The $^{1}$H-NMR technique is a popular method for screening untargeted metabolomes in various biological samples including blood, urine, saliva and feces.

This first study in Thailand for analysis of untargeted metabolite containing in dentine was performed by $^{1}$H-NMR. Specific central peaks were identified and matched for each chemical substance.

In forensic science, Application of $^{1}$H-NMR, these metabolites can be used for studying potentially lethal diseases or to predict cause of death.and can be used in forensic science Predict gender and age from teeth found in anonymous corpses. or death from alcohol can be known as it is detected. 5-HIAA, 5-HTOL metabolites. even death from an unknown poison can use NMR to identify the type of chemical that which can be used in forensic medicine Due to modern advances in science, it is difficult to determine the cause of death.

Dentistry uses NMR to treat and study the use of biomaterial in tooth restoration. These biomaterials must be studied for their biocompatibility and do not pose a hazard when used. As a result, NMR spectroscopy provides an in-depth understanding of chemical reactions and chemical compounds. in terms of the composition of the biological substance and the potential effects when using Extensive research on gas ionomer cement (GIC), resin composites, dental bone cements, and periodontal membranes materials has been conducted using the NMR spectroscopy. Prosser et al used NMR spectroscopy and reported the role of tartaric acid in the setting reaction of GIC, was “The fluid cement pastes have shown that tartaric acid reacts more readily than the polyacid with the glass, and hence suppresses the premature gelation of the cement.”

## CONCLUSION

From the experiment, $^{1}$H-NMR spectroscopy technique can identify 63 biomolecules in dentine samples. The chemical compounds and their metabolites were classified as amino acids, sugars, lipids, organic acids, amine compounds. For application, D and L-aspartic acids, pentosidine, carnitine and N6-carboxymethylsine might be used for age estimating. Pyroglutamic acid, malic acid, L-leucine for gender investigation. Furthermore, hydroxyl indoleacetic acid and ethyl glucuronide, which are metabolite of ethanol can be interogated alcohol consumption. Another biomolecules will be identified and applied for in forensic cases in further.

## ACKNOWLEDGEMENTS

We greatful thanks Dr. Rattakorn Pernsuwan for collcecting the teeth samples.

## REFERENCES

Almhojd US, Lingstrom P, Nilsson A, Noren JG, Siljestrom S, Ostlund A, et al. Molecular Insights into Covalently Stained Carious Dentine Using Solid-State NMR and ToF-SIMS. Caries Res. 2017;51(3):255-63.

Andreev AS, Livadaris V. Characterization of Catalytic Materials through a Facile Approach to Probe OH Groups by Solid-State NMR. The Journal of Physical Chemistry C. 2017;121(26):14108-19.

Berglund B, Vaughan RW. Correlations between proton chemical shift tensors, deuterium quadrupole couplings, and bond distances for hydrogen bonds in solids. The Journal of Chemical Physics. 1980;73(5):2037-43.

Chan W, Banks R, Lynch E, Grootveld M. High-Resolution <sup>1</sup>H NMR Investigations of the Oxidative Consumption of Salivary Biomolecules by a Tooth-Whitening Formulation: Relevance to Safety Issues. Advances in Chemical Engineering and Science. 2012;02(01):62-73.

Coppel Y, Prigent Y, Gregoire G. Characterization of hydrogenated dentin components by advanced (1)H solid-state NMR experiments. Acta Biomater. 2021;120:156-66.

de Falco B, Incerti G, Pepe R, Amato M, Lanzotti V. Metabolomic Fingerprinting of Romaneschi Globe Artichokes by NMR Spectroscopy and Multivariate Data Analysis. Phytochem Anal. 2016;27(5):304-14.

Diez C, Rojo MÁ, Martín-Gil J, Martín-Ramos P, Garrosa M, Córdoba-Diaz D. Infrared Spectroscopic Analysis of the Inorganic Components from Teeth Exposed to Psychotherapeutic Drugs. Minerals. 2021;12(1).

Gardner A, Parkes HG, Carpenter GH, So PW. Developing and Standardizing a Protocol for Quantitative Proton Nuclear Magnetic Resonance ((1)H NMR) Spectroscopy of Saliva. J Proteome Res. 2018;17(4):1521-31.

Grootveld M, Page G, Bhogadia M, Edgar M. Updates and Original Case Studies Focused on the NMR-Linked Metabolomics Analysis of Human Oral Fluids Part I: Emerging Platforms and Perspectives. Applied Sciences. 2022;12(3).

Kim H-Y, Nam S-H, Han M-S. Hard Tissue Analysis of NMR after Fluoride Administration. Journal of Magnetics. 2016;21(4):599-602.

Letieri AdS, Freitas-Fernandes LB, Albarello LL, Fontes GP, de Souza IPR, Valente AP, et al. Analysis of Salivary Metabolites by Nuclear Magnetic Resonance Before and After Oral Mucosa Cleaning of Infants in the Pre-dental Period. Frontiers in Dental Medicine. 2021;2.

Nagana Gowda GA, Gowda YN, Raftery D. Expanding the limits of human blood metabolite quantitation using NMR spectroscopy. Anal Chem. 2015;87(1):706-15.

Ni Q, Chen S. Assessment of structural changes of human teeth by low-field nuclear magnetic resonance (NMR). Meas Sci Technol. 2010;21(1):15803.

Ni Q, Nicolella DP. The characterization of human cortical bone microdamage by nuclear magnetic resonance. Meas Sci Technol. 2005;16(3):659-68.

Pickard CJ, Mauri F. All-electron magnetic response with pseudopotentials: NMR chemical shifts. Physical Review B. 2001;63(24).

Qingwen N.; Shuo C. Assessment of structural changes of human teeth by low-field nuclear magnetic resonance (NMR). Molecular Biology. 2010 (27):1-15.

Tsai YL, Kao MW, Huang SJ, Lee YL, Lin CP, Chan JCC. Characterization of Phosphorus Species in Human Dentin by Solid-State NMR. Molecules. 2020;25(1).

Turunen S, Puurunen J, Auriola S, Kullaa AM, Karkkainen O, Lohi H, et al. Metabolome of canine and human saliva: a non-targeted metabolomics study. Metabolomics. 2020;16(9):90.

Verma M, Verma N, Sharma R, Sharma A. Dental age estimation methods in adult dentitions: An overview. J Forensic Dent Sci. 2019;11(2):57-63.

Vyalikh A, Mai R, Scheler U. OH(-) deficiency in dental enamel, crown and root dentine as studied by (1)H CRAMPS. Biomed Mater Eng. 2013;23(6):507-12.

Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, et al. HMDB 3.0--The Human Metabolome Database in 2013. Nucleic Acids Res. 2013;41(Database issue):D801-7.

Zhou J, Ning K, Yang Y, Zou L, Xue J, Kong X, et al. 1H-NMR -based metabolic analysis on biocompatibility of dental biomaterials. Process Biochem. 2022;114:163-73.

Zia K, Siddiqui T, Ali S, Farooq I, Zafar MS, Khurshid Z. Nuclear Magnetic Resonance Spectroscopy for Medical and Dental Applications: A Comprehensive Review. Eur J Dent. 2019;13(1):124-8.

## *POSTER PRESENTATION*

# DPPH radical scavenging activity and anticancer potential of collagen extracted from buffalo

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**Abstract**

This study aimed to evaluate the DPPH radical scavenging activity and cytotoxicity potentials of collagen extracted from buffalo. The result of antioxidant (DPPH) indicated that collagen possessed the ability to scavenge free in a concentrations dependant manner. They were (67.86±3.65, 54.44± 2.05, 47.92 ±0.53, 44.29±1.80 and 18.02±5.74%) for (200,100,50,25 and 12.5 mg\ml) respectively. the results of cytotoxicity assay matches the antioxidant results, the cell viability decrease by concentrations increasing to be 40.20± 3.06, 48.64± 1.48, 61.57± 1.33, 71.14± 0.82 and 83.21± 1.10% for400,200,100,50 and 25 µg/ml respectively withen IC50 of 98.54 µg/ml. A little effect on viability of normal cells were observed after collagen treatment (72.33±2.41, 76.27±5.12, 83.17±1.31, 94.56±1.51 and 94.13±0.99 %) for 400,200,100,50 and 25 µg/ml respectively withen IC50 of 122 µg/ml.

Key words: collagen, DPPH, antioxidant, anticancer.

## Introduction

Collagen is the very important dominant protein in connective tissue, and it is found in various forms in tissues of all species of multicellular organisms; it exercises various functions, depending on its location .(1)It can be extracted from various animal species and it is generally derived from slaughter by-products. The main sources of collagen are the skin, tendons, cartilage , bovine and bones. Some studies have addressed ways of obtaining collagen from different animal sources, such as fish and birds. ( 2)

There is growing interest in the processes used to extract collagen and its derivatives due to the growing tendency to use this protein in place of synthetic agents in various industrial processes, and also to provide a greater appreciation of the by-products of animal slaughter (3). Collagen is one of the most useful biomaterials because it has a wide range of industrial applications (4). Hence, the use of natural antioxidants is replacing the use of these synthetic antioxidant ingredients due to their safety, nutritional, and therapeutic values.

In recent years, the use of antioxidants as a functional ingredient in the diet of people increased significantly. A popular ingredient considered to be an antioxidant is collagen in its hydrolyzed form.

The use of natural peptides like collagen hydrolysates has been widely utilized due to their excellent biocompatibility, easy biodegradability, and weak antigenicity (5). Some protein hydrolysates from casein, wheat gluten, lactalbumin, and soya sources have been studied due to their potential anti-aging properties and fibroblast renewal in monolayered cell culture (6). However, less is known about the benefits of HC on the skin and its properties as an anti-aging, UV protector, enhancer of fibroblast production and extracellular matrix (ECM), moisturizer, and nutraceutical.

Guided tissue regeneration (GTR) is a procedure used to control early periodontal surgical wound healing dynamics to promote periodontal regeneration (7). In addition, it has been applied to bone and preimplant defects, and to bone augmentation procedures prior to implant placement (8). The principle of GTR is to use a membrane as a mechanical barrier to protect the blood clot, create space and facilitate the repopulation of the defect with cells with regenerative potential (9). Several materials have been used as barriers, namely polytetrafluoroethylene (PTFE), collagen, polyglycolic acid and copolymers (10).

Consequently, a number of bioabsorbable barrier materials have been clinically used in GTR procedures (11). These barriers are currently produced by collagen reconstitution using cross-linking techniques (8) to reduce the fast resorption and the lack of stability in the surgical area. However, the techniques used to multiply the links between collagen molecules, increasing the stiffness and slowing down the resorption of the collagen barrier, may increase its cytotoxicity.

## Methods

### Extraction of collagen from buffalo

The collagens were extracted by the method described by (12) with a slight modification. All the extraction procedures were performed at 4 °C with the continuous stirring. To remove non-collagenous proteins, the meat was mixed with 0.1 N NaOH at a sample/alkali solution ratio of 1:10 (w/v) for 6 h. The alkali solution was changed every 2 h. Then, the protein free skin were washed with cold water until neutral or faintly basic pH of wash water were obtained. The protein free skins were defatted with 10% butyl alcohol with a solid/solvent ratio of 1:10 (w/v) for 18 h and the solvent was changed every 6 h. Defatted meat were washed with cold water, followed by soaking in 0.5 M acetic acid with a solid/solvent ratio of 1:30 (w/v) for 24 h. The mixture was filtered. The residue was re-extracted under the same conditions. Both filtrates were combined. The collagen was precipitated by adding NaCl to a final concentration of 2.6 M in the presence of 0.05 M tris(hydroxymethyl)aminomethane, pH 7.0. The resultant precipitate was collected by centrifuging at 4000 rpm for 15 min. The obtained pellet was dissolved in 0.5 M acetic acid, dialysed against 0.1 M acetic acid and distilled water, respectively, and then freeze-dried. The natural triple helix and fibrillary structure of the collagen were preserved during the extraction process.

### DPPH antioxidant activity of collagen

The antioxidant activity of collagen and standard (vitamin C) were assessed on the basis of the radical scavenging effect of the stable DPPH free radical, and the method of (13) was followed. An aliquot of 0.1 ml of collagen or standard (200, 100, 50, 25 and 12.5 mg\ml) was added to 3.9 ml of DPPH solution in a test tube. After incubation at 37°C for 30 minutes, the absorbance was determined at 517nm using spectrophotometer. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}}\right) \times 100$$

### Cytotoxic Effect of collagen on MCF-7 breast cancer cell line using MTT assay

After cell line maintenance according to(14), and reach final cell concentration that was accomplished by counting the cells using the haemocytometer and applying the formula:

Total Cell Count/ml: cell count × dilution factor (or sample volume) $\times 10^4$.

Different concentrations were used in this test (12.5, 25, 50, 100, 200 and 400 µg/mL) of collagen to perform MTT assay by using ready to use kit which composed of MTT solution 1ml ×10 vials and solubilization solution 50 ml× 2 bottle. Briefly, tumor cells ($1x10^4$ –$1x10^6$ cells/ml) were grown in 96 flat well micro-titer plates, in a last volume of 200 µL complete culture medium per each well and sterilized parafilm covered the microplate and incubated at 37 °C, 5 % $CO_2$ for 24 hours. Followed by removing media and serial concentrations of collagen (12.5, 25, 50, 100, 200 and 400µg/mL) were added to the wells. Plates were incubated at 37 °C, 5 % $CO_2$ for (24 hours), then , 10 µl of the MTT solution was added to each well and further incubated at 37 °C, 5 % $CO_2$ for 4 hours. The media were cautiously removed and 100 µL of solubilization solution was added per each well for 5 min. finally, by using an ELISA reader for measurement at 575 nm wave length. The data of optical density was subjected to statistical analysis in order to evaluate the concentration of compounds required to cause 50 % reduction in cell viability for each cell line.

## Results and discussion

### Antioxidant activity of collagen by DPPH radical scavenging activity

Collagen was effective in DPPH radical scavenging activity at all concentrations tested (12.5, 25,50, 100 and 200 mg/ml). The concentrations 100 and 200 mg/ml of collagen shared an approximated radical scavenging activity (54.44 ±2.05 and 67.86 ± 3.65 %, respectively). Vitamin C also showed variations between all concentrations ranged from (22.90± 1.83 to 83.37± 1.85) for 12.5 and 200 mg\ml respectively (table 1, figure 1).

**Table 1: DPPH radical scavenging activity of *collagen* and vitamin C**

| Concentration (Mg\ml) | DPPH Radical Scavenging Activity (mean± S.D.; %) | |
|---|---|---|
| | Collagen | Vitamin C |
| 200 | 67.86±3.65 [a] | 83.37±1.850[a] |
| 100 | 54.44± 2.05[b] | 74.07±1.00[b] |
| 50 | 47.92 ±0.53[c] | 57.60±2.20[c] |
| 25 | 44.29±1.80[d] | 39.00±1.73[d] |
| 12.5 | 18.02±5.74[e] | 22.90±1.83[e] |

Different letters: Significant difference ($P \leq 0.05$) between means of columns.

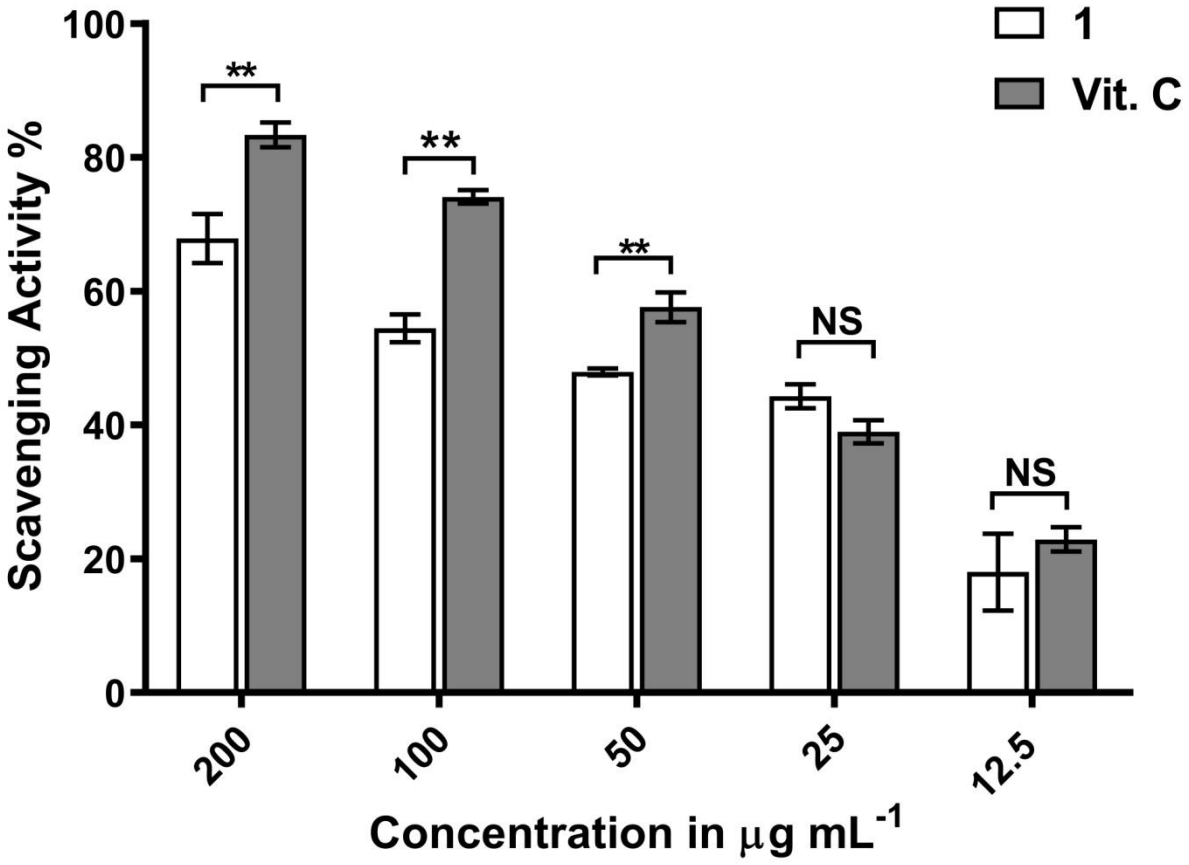


**Figure 1: DPPH radical scavenging activity of *collagen* and vitamin C,[1: represented collagen).**

**Cytotoxic activity of collagen on MCF-7 cancer cell line**

The test of 3-(dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) was accomplished to conclude the cytotoxic effect of collagen on breast cancer cell line (MCF-7) in order to calculate the cell viability and inhibition rate on the tumor cell line by using different concentrations of collagen. The percentage viability of treated cells was calculated in a comparison with normal cell line WPL-68. The cytotoxic effect of green synthetic NPs in concentration ranged from 25-400 µg/ml on MCF-7 cells (Table 2) presented a decrease in cell viability in a dose-dependent pattern. The cell viability is reduced by increasing the concentration of collagen. The decreasing in MCF-7 cell viability (%) was noted by 400µg/ml (40.20±3.06 %) while the highest MCF-7 cell viability at 25 µg/ml reached (83.21±1.10 %). Collagen exhibited significantly the most potent cytotoxic activity with IC50 value of 98.54 µg/ml. however an IC50 of 122 µg/ml was obtained from the effect of collagen on WRI-68 normal cell line (Figure 2).

**Table (2): Cytotoxicity effect of collagen on MCF-7 and WRI-68 cells after 24 hours incubation at 37ºC**

| Concentrations µg/ml | MCF-7 | WRL-68 |
|---|---|---|
| | Viable cell counts (Mean± S.D.) | Viable cell counts (Mean± S.D.) |
| 400 | 40.20± 3.06 | 72.33±2.41 |
| 200 | 48.64± 1.48 | 76.27±5.12 |
| 100 | 61.57± 1.33 | 83.17±1.31 |
| 50 | 71.14± 0.82 | 94.56±1.51 |
| 25 | 83.21± 1.10 | 94.13±0.99 |

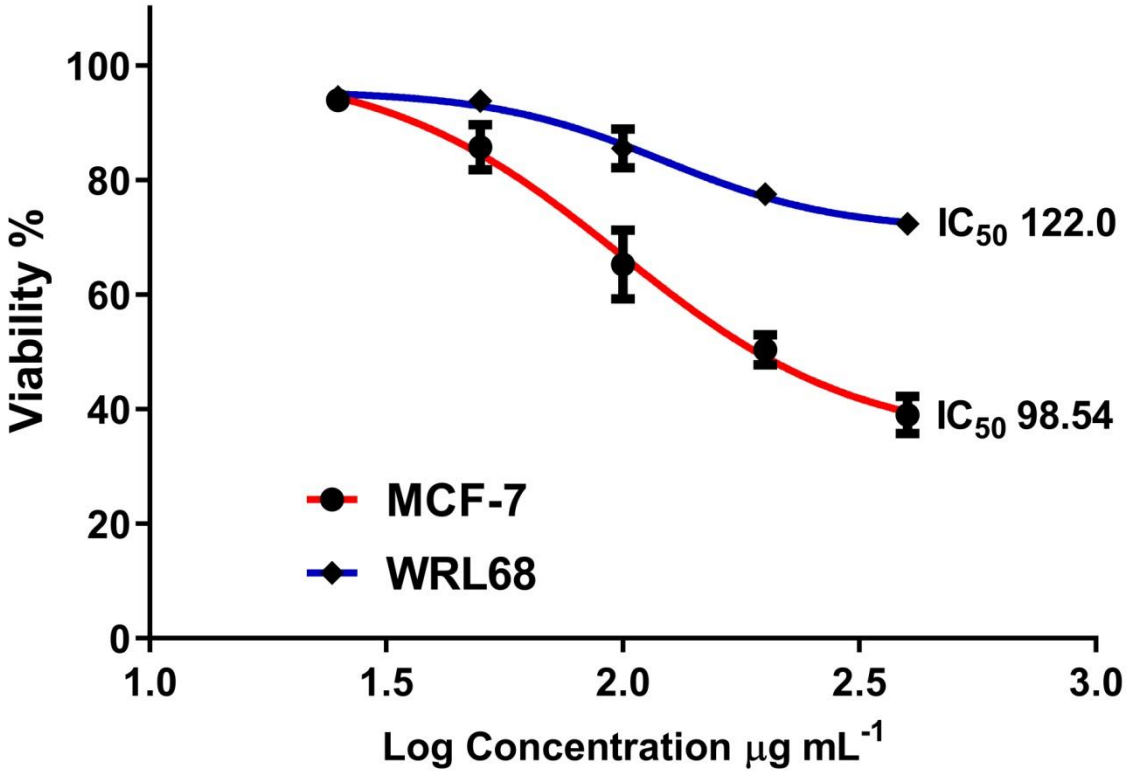


**Figure (2): Cytotoxicity effect of collagen on MCF-7 and WRI-68 cells after 24 hours incubation at 37ºC**

Discussion.

Collagen is abundant inside the individual body and extracellular matrix network of molecules that holds together the body's tissues. the protein in the human being body and three quarters of the dry weight of skin consist of collagen. performs a key function in the natural development of wound healing reducing inflammation attracting cells that promote wound healing (15).Collagen antioxidant activity influenced by the composition, structure and hydrophobicity of amino acids, and the weight of collagen molecules (16). Three of the dominant amino acids in these collagen were non-polar amino acids, so collagen tends to be hydrophobic. Antioxidant activity that can inhibit the formation of certain radical chains .Collagen is a protein arranged of three polypeptide chains together with a triple-helix structure and has three intermittent residues in the form of Gly-X-Y. Glycine is the major amino acid set up in collagen, while point X is for proline as well as Y is for hydroxyproline (17). The structure and position of amino acids in peptides perform an essential function in defining the antioxidant activity of collagen. The inhibition of chain reactions for the free radicals produced by the high content of proline and glycine in the sequence of collagen amino acids which act as donors to protons against free radicals (18). Additional probability is the presence of -OH and -NH2 groups in collagen peptides that are efficient of binding with free radicals (19). Amino acid collagen of skin parang-parang dominated by glycine, proline, glutamic acid, alanine, and arginine. Total Hydrophobic Amino Acid (THAA) has a high-level of solubility in fat and increases antioxidant activity. C- or N- peptide endings in hydrophobic amino acids can act together along with lipid molecules and contribute protons to free radicals.

Furthermore, indicated the relationship between antioxidants and molecular weights, which is peptides that get antioxidant action commonly consist of 2-20 amino acids with molecular weights less than 3000 Da (20).

## References

1. Shimokomaki M, Olivo R, Terra N.N. and Franco B.D.G.M. 2006. Atualidades em ciência e tecnologia de carnes. São Paulo: Varela. 236p.
2. Kaewdang, O., Benjakul, S., Kaewmanee, T. and Kishimura, H. 2014. Characteristics of collagens from the swim bladders of yellowfin tuna (Thunnus albacares). Food Chemistry 155: 264-270.
3. Karim, A.A. and Bhat, R. 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids 23(3): 563– 576.
4. Lafarga, T. and Hayes, M. 2014. Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. Meat Science 98(2): 227-239.
5. Liu, D.; Nikoo, M.; Boran, G.; Zhou, P.; Regenstein, J.M. Collagen and gelatin. Annu. Rev. Food Sci. Technol. 2015, 6, 527–557. [CrossRef].
6. Frei, V.; Perrier, E.; Orly, I.; Huc, A.; Augustin, C.; Damour, O. Activation of fibroblast metabolism in a dermal and skin equivalent model: A screening test for activity of peptides. Int. J. Cosmet. Sci. 1998, 20, 159–173. [CrossRef] [PubMed].
7. Bornstein MM, Heynen G, Bosshardt DD, Buser D. Effect of two bioabsorbable barrier membranes on bone regeneration of standardized defects in calvarial bone: a comparative histomorphometric study in pigs. J Periodontol 2009;80:1289- 1299.
8. Lee CK, Koo KT, Kim TI, Seol YJ, Lee YM, Rhyu IC, et al.. Biological effects of a porcine-derived collagen membrane on intrabony defects. J Periodontal Implant Sci 2010;40:232-238.
9. Patino MG, Neiders ME, Andreana S, Noble B, Cohen RE. Cellular inflammatory response to porcine collagen membranes. J Periodontal Res 2003;38:458-464.
10. Behring J, Junker R, Walboomers XF, Chessnut B, Jansen JA. Toward guided tissue and bone regeneration: morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. Odontology 2008;96:1-11.
11. Patino MG, Neiders ME, Andreana S, Noble B, Cohen RE. Collagen as an implantable material in medicine and dentistry. J Oral Implantol 2002;28:220-225.
12. Kukhareva, L.; Shamolina, I.; Polevaia, E. Tsitologiia 2009, 52, 597-602.
13. Sanja, S.D., Sheth, N.R., Patel, N.K., Dhaval, P. and Biraju, P. (2009). Characterization and evaluation of antioxidant activity of portulaca oleracea. *Int. J. Pharm. Pharm. Sci*., 1: 74-84.
14. Freshney, R. I., 2015. *Culture of animal cells: a manual of basic technique and specialized applications*. John Wiley & Sons.
15. Ahmad M, Benjakul S and Nalinanon S. 2010. Compositional and physicochemical characteristics of acid solubilized collagen extracted from the skin of unicorn leatherjacket (Aluterus monoceros). Food Hydrocolloids. 24: 588-594.
16. Mayasari D. 2016. Ekstraksi kolagen optimum dari kulit ikan tuna (Thunnus sp.) sebagai antioksidan [skripsi]. Bogor (ID): Institut Pertanian Bogor.
17. Fawzya YN, Chasanah E, Poernomo A, Khirzin MH. 2016. Isolasi dan karakterisasi parsial kolagen dari teripang gamma (Sticopus variegatus). JPB Kelautan dan Perikanan. 11(1): 91- 100.
18. Wu R, Chen L, Liu D, Huang J, Zhang J, Xiao X, Lei M, Chen Y and He H. 2017. Preparation of antioxidant peptides from salmon byproducts with bacterial extracellular protease. Marine Drugs. 15(4): 1-19
19. Zhu X, Zhou X, Yi J, Tong J, Wu H and Fan L. 2014. Preparation and biological activity of quaternized carboxymethyl chitosan conjugated with collagen peptide. Int J Biol Macromol. 70: 300-305.
20. Mendis E, Rajapakse N, Byun HG and Kim SK. 2005. Investigation of jumbo squid (Dosidicus gigas) skin gelatin peptides for their in vitro antioxidant effects. Life Sci. 17: 2166 – 2178.

## *POSTER PRESENTATION*

# Comparison of polyphenolic components in olive oil extracted with the continuous system and ultrasound.

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**Abstract**

Virgin olive oil is extracted from olive fruit (Olea europaea L.) using physical or mechanical processes and it can be used without further treatments. The extraction of olive oil includes several stages that affect oil quality and the optimal yield of its extraction. Therefore, finding innovative techniques such as ultrasonic extraction in order to eliminate oil residues in pomace has become very significant nowadays. This study aims to better understand the mechanism of ultrasound and determine the optimal conditions for extra virgin olive oil extraction for Kalinjot and Korroneik cultivars ,through the use of ultrasound technique without affecting oil quality, compared to the continuous extraction three-phase system. For the realization of this study, two different olive cultivars were used, "Kalinjot" and "Korroneik". The analyses carried out are physic-chemical analysis, total polyphenols, orthodiphenols, etc. The results of this study shows that ultrasound treatment leads to a significant improvement of olive oil extraction and industrial yield. This treatment has shown to reduce the time of oil extraction by halves of the time compared to the continuous system.

**Keywords:** Olive oil, ultrasound, continuous extraction system, polyphenols.

## 1.INTRODUCTION

The olive tree is a subtropical perennial tree cultivated mainly in temperate and subtropical zones between latitudes 30 and 45º north and south, under different climatic and soil conditions. The olive tree needs mild winters (minimum 4 ºC) and hot, dry summers (maximum 40 ºC) with an average annual temperature of 15-20 ºC (Kabourakis, 2000).

In regular climatic conditions, the process of growing and ripening olive fruits takes about 5 months. Fresh and ripe olive fruits are mainly composed of water, oil, sugars, proteins and cellulose (Kiritsakis, 1998). Other important components are pectin; phenolic compounds; organic acids, such as citric, oxalic, malonic, fumaric, tartaric, acetic and triterpenic acids; tannins; and inorganic salts (Fedeli dhe Jacini, 1971).

Virgin Olive Oil means the oily liquid obtained from the olive fruit harvested at optimal maturity, derived from a healthy fruit, bypassing any mechanical, physical, and above all thermal treatment or manipulation from which it can alienate nature chemical composition of its components (Codex Alimentarius, 2014).

The chemical content of olive oil is different and depends on many different factors such as varietal, agronomic, technological and degree of maturity. (Gharbi et al., 2015). The extraction of olive oil is carried out after the fruits are harvested, cleaned and selected, and go through several stages that can be briefly summarized as follows: Grinding the fruits aims to destroy the structure of the pulp by starting the separation of the fat from the vacuoles with the parts other pulp. The homogenization of the paste is carried out immediately after grinding the fruit, and its purpose is to release and combine the oil droplets extracted from the fruit tissues. And the last stage is the separation of the solid-liquid phase.

The extraction and homogenization of olive paste can be done with different technologies, but in this study, ultrasound technology was applied, which helps to break up the paste formed by the grinding process, thus releasing the oil and the monorital compounds found in olive fruit. Intensification of the ultrasonic process results in higher yield of extra virgin olive oil, higher nutritional quality and shorter homogenization (Jimenez et al., 2006). Ultrasound increases the content of antioxidants and polyphenols in extra virgin olive oil.

The aim of this study is to determine the effects of ultrasonic extraction parameters, including time and temperature of the paste on the yield and quality characteristics of olive oil from two olive cultivars.

## 2.MATERIALS AND METHODS

*Study Plan*

For the realization of this study, 2 different olive cultivars were used, namely: 'Kalinjot' and 'Korroneik'. A laboratory mill, equipped with a mixer, a centrifuge, was used to extract the oil from the olive fruits. After the washing process, the olives were pressed and the olive pastes were sonicated for about 15 minutes and then malaxed for 45 minutes at 27 ºC. The ultrasound system used in this study was an ultrasound bath (150 kW and ultrasonic frequency 37±2 KHz; tank volume 4 L). Each olive paste sample was centrifuged for 1 minute and the oil was collected in dark colored 500 ml bottles. No auxiliary or pre-heating processes were applied during extraction and all experiments were performed in triplicate. While oil extraction with the three-phase system was carried out at the "3 Miqte" Ndroq oil factory. The oil obtained was filtered to remove impurities, coded and stored in a refrigerator at a temperature of -15ºC.

### 2.1. Analytical determination.

To determine the quality of olive oil (values of free acidity and specific absorbance at 232 and 270 nm (K232 and K270) and the peroxide index) were used according to the official methods described in European Regulation EC 2568/91 and amendments (CCE, 1991).

### [1] 2.2. Total polyphenols content.

Polyphenols content was analysed as described by (Vázquez-Roncero, 1973) using the Folin–Ciocalteau reagent and absorbance measurement at 726 nm, the results were expressed as mg/kg of gallic acid.

### 2.3. Determination of the content of orthodiphenols

The determination of the content of orthodiphenols was carried out using the method described by Vázquez-Roncero et al. (1973). From the methanolic extract used for the determination of total polyphenols, 10 mL is taken, of which 5 mL is used for the white test by adding 1 mL of 50% ethanol. The sample is prepared using the other 5 mL of the extract to which 1 mL of 5% sodium molybdate in 50% ethanol is added. The reaction time is 15 min. The quantification of orthodiphenols is performed by calculating the calibration curve, the results are expressed in mg/kg of caffeic acid. The results are measured using a spectrophotometer at a wavelength of 370 nm.

### 2.4. Statistical analysis

For each sample three replications were used. Medium and SD was performed using Statistix 9.0

## RESULTS AND DISCUSSIONS

*Analytical Determination*

The amount of free acids in olive oil is a very important indicator, as it determines the quality of the olive oil (Uceda et al., 2001). For the category of extra virgin and virgin olive oil, the free acid values must not be more than 1 – 0.8 % oleic acid (CEE, 2015). Table 1 shows the values of total free acidity of olive oil extracted from the two varieties taken in this study.

**Table 1.** Olive oil quality parameters of cultivars obtained in this study.

| Varieties | Total acidity (% oleic acid) | $K_{232}$ | $K_{270}$ | Peroxide index (meq $O_2$/kg oil) |
|---|---|---|---|---|
| Koroneik | 0.46 ± 0.05 | 0.82 ±0.00 | 0.06 ±0.00 | 1.33 ±0.00 |
| Kalinjot | 0.20 ± 0.00 | 0.82 ± 0.00 | 0.06 ± 0.00 | 1.00 ± 0.33 |
| Kalinjot ultrasound | 0.60 ± 0.00 | 0.58 ± 0.01 | 0.04 ± 0.01 | 1.79 ± 0.05 |
| Koroneik ultrasound | 0.60 ± 0.00 | 0.61 ± 0.00 | 0.04 ± 0.00 | 1.27 ± 0.07 |
| Mean | 0.4667 | 0.7117 | 0.0509 | 1.3492 |
| SD | 0.1723 | 0.1175 | 0.0109 | 0.3316 |
| Variance | 0.0297 | 0.0138 | 0.00019 | 0.1100 |
| C.V. | 36.927 | 16.516 | 21.440 | 24.582 |
| Minimum | 0.2000 | 0.5700 | 0.0300 | 0.67 |
| Maximum | 0.6000 | 0.8300 | 0.0610 | 1.8400 |

As can be seen from Table 1, the olive oils extracted from the two cultivars belong to the category of extra virgin olive oil. Regardless of the cultivar, the oil extracted with the ultrasound system presents slightly higher free acidity values than in the traditional system.

UV spectrophotometric values K232 and K270 are very important indicators regarding the quality of olive oil, the state of storage and the technological changes that have occurred during its extraction. Absorption at these wavelengths is attributed to the formation of conjugated dienes and trienes. Table 1 shows the values of K232 and K270 of olive oil of the cultivars 'Kalinjot' and 'Koroneik' extracted with two different systems. As can be seen from the table, the values of K232 and K270 for the extracted oils are within the established limits, as well as for total free acidity, these oils are classified in the category of extra virgin oil.

The peroxide index is a parameter that determines the initial oxidation state of olive oil, expressed in milliequivalents of active oxygen per kilogram of fat. Peroxides or initial oxidation, comes as a result of incorrect oil extraction practices, or the oil is not protected and stored from light and heat and, as a consequence, the higher the peroxide value, the lower the antioxidant capacity of the oil.

As can be seen from Table 1, the average values of the peroxide index of the olive oil obtained from the two cultivars studied, extracted with two different methods, range from 1.00 - 1.79 meq O2/kg. These values are within the established limits and classify these oils as extra virgin olive oil.

*Total polyphenols*

Most of the antioxidant compounds found in olive oil are phenolic in nature. These components have two functions, the first is to protect the oil from auto-oxidation and the second is to protect human health (Beltrán et al., 2005). During the ripening process of the fruits there is a decrease in polyphenols in the oil, this tendency varies according to the variety and the area of cultivation (Gutiérrez, et al., 1999, Beltrán, 2000).
Figure 1 shows the values of total polyphenols of olive oil extracted from the studied varieties in two extraction systems.

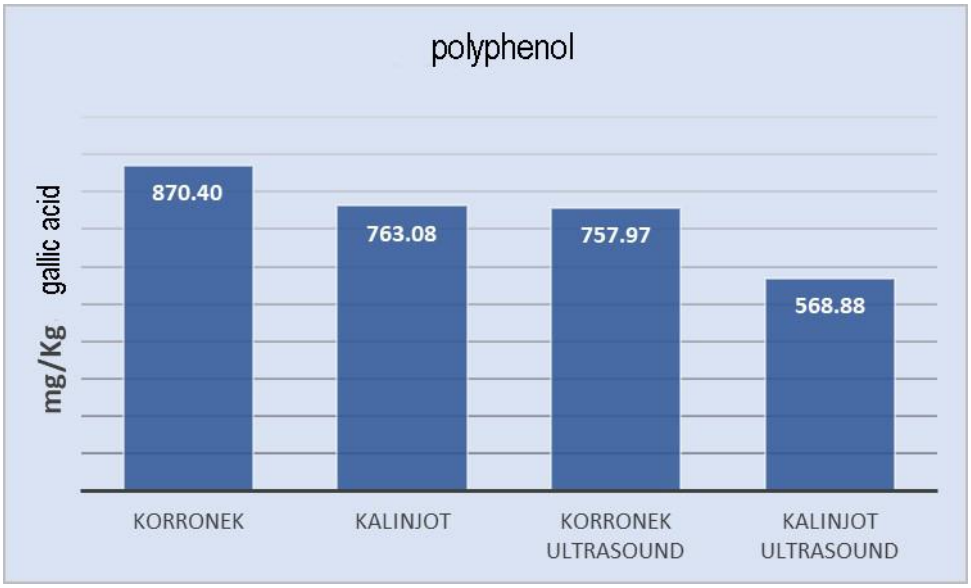


**Figure 1.** Total polyphenol content of olive oil extracted from the cultivars taken in the study.

Olive oil extracted from the variety obtained in the study presented a higher content in total polyphenols ranging from 870.4 – 568.88 mg/kg of gallic acid. Referring to Cimato *et al.*, (1990), Tsimidou *et al.*, (1998) and Beltrán *et al.*, (2000) they claim that the differences that exist on the levels of phenolic compounds of the same variety are due to agronomic, technical and genetic factors (Yousfi *et al.*, 2006). Supported by these studies, we affirm that the differences in the content of total polyphenols for the olive oil of the varieties studied may be the result of the genetic component and the technological process of extraction. From these results, it can be observed that the oil extracted from the Koroneik cultivar without ultrasound treatment presented higher values than extraction with ultrasound. The total phenolic content decreased when the paste was sonicated. This observation can be explained by the presence of oxygen, which acts as a cofactor in many enzymatic reactions and as a promoter of non-enzymatic oxidations.

*Orthodiphenol content*

Regarding orthodiphenols, in our study the olive oil extracted with two systems shows a heterogeneous distribution of values, however the average concentration of ortho-diphenols in the oils extracted with ultrasound treatment was higher than the oils of the continuous system. The average values of this parameter are presented in Figure 2. In this group of samples, the highest values were observed in Koroneik treated with ultrasound 44.56 mg/kg caffeic acid.

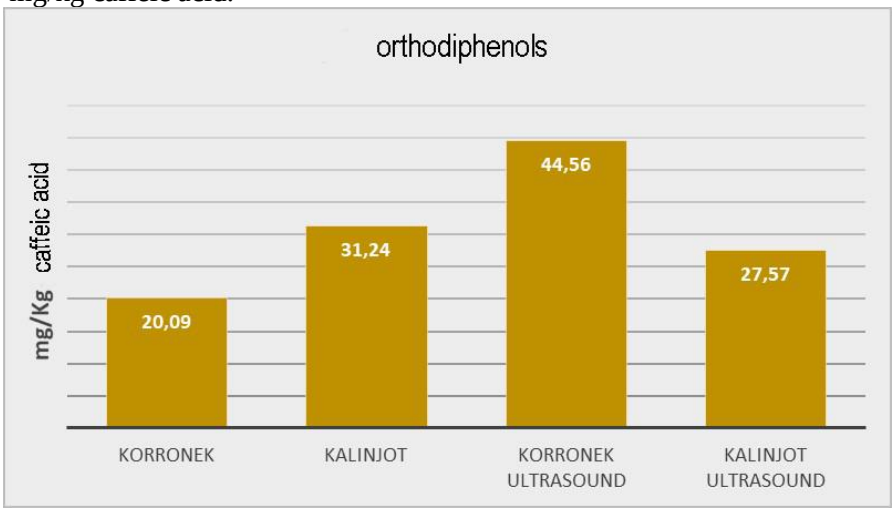


**Figure 2.** The content of orthodiphenols of olive oil extracted from the cultivars taken in the study.

Thus, we can say that the high range observed in the average concentration of this chemical parameter of each of the olive oil samples is due to many factors mentioned previously such as cultivar, stage of fruit maturation, geographical origin and practices agronomic. The high values of the phenolic content and, consequently, in the treated olive oils can be explained by the influence of this treatment on the oil extraction process.

## CONCLUSIONS

Ultrasound is known to have a significant effect on the speed of various processes in the food industry. The olive oil industry can benefit from ultrasound systems in the oil extraction process. In fact, ultrasound reduces the duration of homogenization while increasing the quality of the product. From the obtained results we can say that the ultrasound treatment is accompanied by obtaining an extra virgin oil. In addition, ultrasound technology ensures improvements in the extraction process and a high content of small compounds in the oils. Based on these results, we can say that ultrasound treatment appears to be more efficient compared to continuous system technology, since ultrasound treatment leads to a significant improvement in olive oil extraction and industrial yield. However, this study is preliminary as it will be followed by other ongoing studies to see the impact of other extraction conditions of higher quality olive oil.

## REFERENCES

AOAC .Official "Method of Analysis of AOAC International". 2000 Edn 17, Vol. 2, Washington: Association of Analytical Chemists.

Beltrán G. Beltrán, Jiménez A. Jiménez, Aguilera M. P. Aguilera, Uceda M., 2000. Análisis mediante HPLC de la fracción fenólica del aceite de oliva virgen de la variedad, Arbequina'. Relación con la medida del amargor K225 y la estabilidad. Grasas Aceites, 51, 320– 325.

Beltrán G. Beltrán, M Aguilera. P. Aguilera, Del Rio C. Del Rio, Sanchez S. Sanchez, Martinez L. Martinez, 2005. Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. Food Chem., 89, 207–215.

Cimato, A., 1990. La qualité de l'huile d'olive et les facteurs agronomiques. Olivae, 31, 20-31.

CODEX, 2009. Codex Alimentarius. Norme codex pour les huiles d'olive et les huiles de grignons d'olive, CODEX STAN 33-1981, 9 p. (Adoptée: 1981; révisions: 1989, 2003; amendée: 2009).

European Commission: Regulation no. EEC/2568/91, 1991. The characteristics of olive and olive pomace oils on their analytical methods. Off J Eur Communities. 1991, L 248, 1–82.

European Community (EC). 2014, Commission implementing Regulation (EU) No 836/2014 f 31 July 2014 amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council

Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. In: Official Journal of the European Communities. 2008R0889 - EN-01.01.2015- 010.003- 1. EC, Brussels, Belgium.

Fedeli, E., & Jacini, G., 1971. Lipid composition of vegetable oils. Advances in Lipid Research 9, 335–382.

Gharbi, I., Issaoui, M., Mehri, S., Cheraief, I., Sifi, S. and Hammami, M., 2015. Agronomic and Technological Factors Affecting Tunisian Olive Oil Quality. Agricultural Sciences, 6, 513-526. doi: 10.4236/as.2015.65051.

Gutierrez, F., Arnaud, T., & Albi, M.A., 1999. Influence of ecological cultivation on virgin olive oil quality. Journal of the American Oil Chemists' Society 76, 617–621.

Jimenez, A., Beltran, G., Uceda, M., & Aguilera, M. P., 2006. Empleo de ultrasonidos de potencia en el proceso de elaboracion del aceite de oliva virgen. Resultados a nivel de planta de laboratorio. Grasas y Aceites, 57(3), 253e259.

Kabourakis, E., 2000. Learning processes in designing and disseminating ecological olive production systems in Crete. In: Knowing and learning for change in agriculture: case studies from industrialised countries, ed. M. Cerf, D. Gibbon, B. Hubert, J. Jiggins, M. Paine, J. Proost, & N. Rolling. INRA Editions, Paris, 97–111.

Kiritsakis A.K., (1998.a) Olive oil: from the tree to the table, 2nd ed. Food and Nutrition Press, Trumbull, CT.

Uceda, M., Hermoso, M., 2001. La calidad del aceite de oliva. In: Barranco, D.,Fernandez-Escobar, R., Rallo, L., (Eds), El Cultivo del Olivo. Mundi Prensa, Madrid, Spain, pp. 589-614.

Vazquez-Roncero A. Vazquez-Roncero, Janer del Valle C. Janer del Valle, Janer del Valle M. L., Janer del Valle 1973.: Determinación de polifenoles totales en el aceite de oliva. Grasas Aceites, 24, 350–357.

Yousfi, K., Cert, R.M., García, J.M., 2006. Changes in quality and phenolic compounds of virgin olive oils during objectively described fruit maturation. Eur. Food Res. Tech. 223, 117 – 124.

## *POSTER PRESENTATION*

# Detection of Asia-I genetic lineage of *Canine distemper virus* (CDV) in domestic dogs in Turkiye

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## Abstract

*Canine distemper virus* (CDV) causes canine distemper disease, characterized by cutaneous, respiratory, digestive, and central nervous system findings, with a high morbidity and mortality rate in domestic and wild carnivores. The virus has a cross-species transmission and is isolated also from wild cat populations as well as dogs. The most susceptible age group to infection is 0-6 months old. For this reason, uptaking maternal antibodies and making vaccination programs according to maternal antibodies uptaking status are significant for struggling with this disease in puppies. The increase in the incidence of CDV infection in vaccinated animals in recent years reveals that the efficacy of the vaccine strains in use should be reconsidered. The high mutation frequency in RNA viruses causes changes in the level of amino acids in the Hemagglutinin gene region, which stimulates the formation of neutralizing antibodies of the virus, and strains with different antigenic properties occur. The use of one type of strain in vaccines may result in variable levels of neutralizing antibody formation or the absence of antibodies in susceptible animals. In order to create effective immunity in animals, the viruses circulating in the field should be characterized and compared with the strains in the vaccine contents. In this study, phylogenetic analysis was performed based on the hemagglutinin gene region using the nucleotide sequences of samples obtained from vaccinated and unvaccinated infected dogs, CDV isolates from different genetic lineages and vaccine strains. It has determined that the samples used in this study are similar to the Asia-I genetic lineage common in China and Thailand and different from the vaccine strain, unlike the Northern European and Arctic-like lineages circulating in Turkiye.

**Key Words:** canine distemper disease, phylogeny, hemagglutinin, mutation

## INTRODUCTION

Canine Distemper is a viral disease with high morbidity and mortality that affects domestic carnivores as well as wild carnivores such as coyotes, foxes, wolves, raccoons, weasels, sea lions and mink (Çalışkan, 2007; Oğuzoğlu et al., 2018). Detection of *Canine distemper virus* (CDV) in mountain lion and leopard populations from the *Felidae* family in wildlife studies shows the cross-species transmission characteristic of the virus and this situation endangers the existence of threatened with extinction isolated populations (Deem et al., 2000).

Maternal antibody uptake and vaccination are significant in preventing this infection. Due to the endotheliochorial placenta structure in carnivores, the transmission of immunoglobulins from mother to offspring is limited, so passive immunity is provided by colostrum (Chastant and Mila, 2019). The disease progresses severely in puppies which not receive sufficient colostrum. Vaccination programs should be planned to take into account the maternal antibody uptake status of puppies.

The causative agent, which is characterized by skin, respiratory, digestive, and central nervous system findings, is in the *Morbillivirus* genus of the *Paramyxoviridae* family (ICTV,2022; Börkü et al., 2012). The 15.690 base pair long single-stranded viral RNA genome encodes six important proteins: Nucleocapsid protein (N), Phosphoprotein (P), Matrix protein (M), Fusion protein (F), Hemagglutinin protein (H), and Polymerase

protein (L) (Abraham and Banerjee, 1976). The H protein enables CDV to attach to cellular receptors and stimulates the formation of neutralizing antibodies in the host (Örvell et al., 1990). As a result of mutations in the H gene region, 21 genetic lineages were formed (Echeverry-Bonilla et al., 2022). Despite being monotype serologically, there are significant differences between the vaccine strain and field strains due to genotype diversity. The use of a single strain in vaccines results in variable levels of neutralizing antibodies in susceptible animals. Therefore, the characterization of field strains and comparison with vaccine strains are important in the fight against this infection.

In this study, diagnostic materials taken from two dogs by veterinarians, one vaccinated against CDV and the other unvaccinated, were sent to the laboratory for routine diagnosis, and CDV nucleic acid positivity was detected in these individuals. This research is aimed at the molecular characterization of the obtained positives.

## MATERIALS AND METHODS

Nasal swab samples taken by the veterinarian from two dogs (Herkul and Bal) brought to the private clinic with symptoms of dehydration, anorexia, nasal and eye discharge were sent to the laboratory for routine diagnosis and were subjected to Phenol-Chloroform-Isoamyl alcohol extraction (Chomczynski and Sacchi, 1987). The viral RNA was dissolved 30 µl nuclease-free water and stored at -80°C for use in RT-PCR.

Reverse transcription was performed to obtain complementary DNA (cDNA) from viral RNA (Shimizu et al., 1994). RevertAid Reverse Transcription Kit was used for this process (Thermo Fisher Scientific). The PCR was conducted in a 30-µl reaction mixture containing 3 µl of cDNA template, 10 pmol HF1 primer (5'-TGTGTGTAGAAGAGAGCACTGT-3'),10 pmol HR1 primer (5'-AGTTTTTTTATAATGCTAGAGATGGT) (Lan et al., 2005) and 1U of *Taq* polymerase (Thermo Fisher Scientific). The cycling conditions were 95°C for 3 min, 95°C for 1 min, 58°C for 1 min, 72°C for 2 min; and a final extension is at 72°C for 10 min. The 1045 base pair length positive PCR products were visualized by 1% agarose gel electrophoresis (Figure 1).

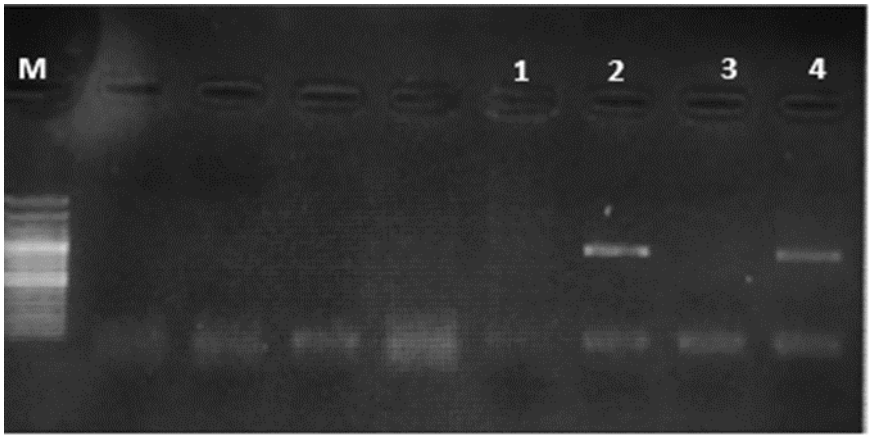


**Figure 1.** Agarose gel image of amplicons obtained with primer pairs of Hemagglutinin gene region of the samples used in the study.

M: GeneRuler 100 bp plus DNA ladder (Thermo Fisher Scientific)

2 and 4: Positive PCR products

After sequencing the positive PCR amplicons, validation with GenBank BLAST was performed. In order to compare the sequence results, alignment was performed with BioEdit (v.7.0) using references from sequences belonging to lineages in different geographies. The phylogenetic tree of the aligned sequences was created with MEGA 6.0 software using the Neighbor-Joining method.

## RESULTS AND DISCUSSION

In previous studies in Turkiye, it was determined that circulating viruses were similar to Northern European and Arctic-like lineages (Özkul et al., 2004; Koç et al., 2021). The field viruses in this study were found close to the Asia-I genetic line circulating in China and Thailand. The vaccine strains used worldwide are in the America-I lineage (Figure 2).

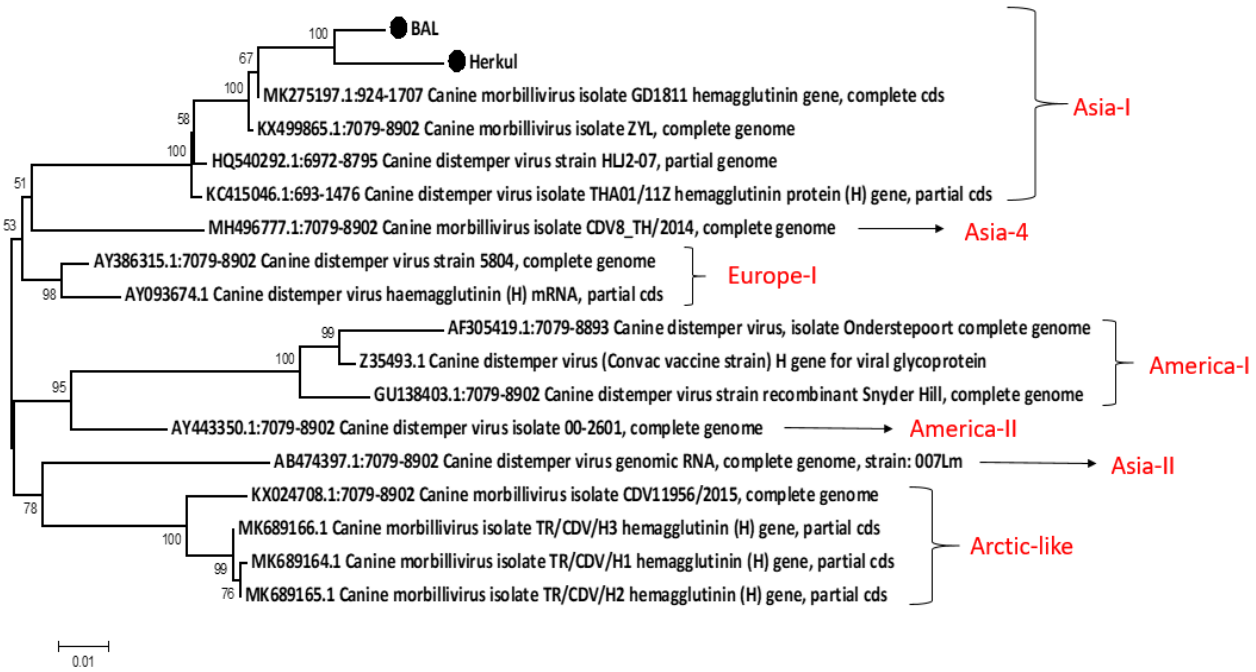


**Figure 2.** Based on the nucleotide sequences of the hemagglutinin gene region; phylogenetic relationship between viruses used in the study, vaccine strains and other CDV lineages. Bal and Herkul marked with ● are samples used in this study.

Hemagglutinin protein of CDV is considered to be the most appropriate viral protein for the evaluation of amino acid changes and antigenic differences, as it is an important target of neutralizing antibodies. Due to the nature of RNA viruses, the high mutation ability causes the formation of new wild-type strains, making it difficult to fight infection. In addition, because mutations cause antigenic differences, the success of vaccines prepared using only a single genetic lineage is limited. Considering that vaccinated animals may be susceptible to infection like unvaccinated individuals, it is thought that characterizing the viruses circulating in the country for an effective control and incorporating existing strains into vaccine formulations will increase the success of the vaccine.

The fact that vaccinated animals may also be susceptible to infection may be explained by the fact that the current vaccine strains are antigenically different from viruses circulating in the field. In support of the data obtained in this study, Harder et al. (1996) found that the titers of neutralizing antibodies developed against the field strain in blood serum samples of CDV-infected animals were higher than the titers developed against the vaccine strain. Iwatsuki et al. (1997), on the other hand, stated that the changes in the epitope regions of the H protein exposed to continuous immunological pressure are related to the differences in the neutralization efficiency.

## CONCLUSIONS AND RECOMMENDATIONS

In this study, the CDV strains belonging to the Asia-I lineage were detected, unlike the Arctic-Like and Northern European strains detected in Turkiye in previous years. Although lineage differences in CDV vaccines do not affect cross immunity, significant differences may occur in the neutralization kinetics of antibodies against variable epitopes (Diallo, 1990) In this context, it was concluded that the CDV strains of America-1 origin included in the vaccines may have some inadequacies in terms of providing full protection against infection. According to the results of this study:

- It is recommended to include strains belonging to Arctic-like and Asia-I lineages in vaccine compositions.

- It is important for protection to vaccinate with booster vaccines containing strains of other lineages.

- For effective prevention, mothers must be vaccinated before pregnancy and puppies must uptake maternal antibodies with colostrum. It has importance for veterinary clinicians to consider this issue when the vaccination programs are made.

- Periodic control of CDV field strains, investigation of novel and different lineages circulating in the field with molecular characterization studies are necessary for evaluation of vaccine effectiveness. In this context, there is a need for studies to investigate antigenic differences by molecular methods.

## REFERENCES

Abraham G, Banerjee AK 1976. Sequential transcription of the genes of vesicular stomatitis virus. Proc Natl Acad Sci U S A.73(5):1504-8.

Borku MK, Haydardedeoglu AE, Colakoglu EC, Alihosseini H, Baydın A, Oguzoglu TC 2012. Canine Distemper Virus Infection in A Terier Dog with Pyotraumatic Dermatitis. Kafkas Univ Vet Fak Derg, 18 (6): 1083-1084.

Chastant S, Mila H 2019. Passive immune transfer in puppies. Anim Reprod Sci. 207:162-170.

Chomczynski P, Sacchi N 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 162(1):156-9.

Çalışkan E 2007. Canine distemper virus isolation, characterization of H protein coding region and seroepidemiology. Doctoral thesis. Ankara University.

Deem SL, Spelman LH, Yates RA, Montali RJ 2000. Canine distemper in terrestrial carnivores: a review. J Zoo Wildl Med. 31(4):441-51.

Diallo A 1990. Morbillivirus group: genome organisation and proteins. Vet Microbiol. 23(1-4):155-63.

Echeverry-Bonilla DF, Buriticá-Gaviria EF, Orjuela-Acosta D, Chinchilla-Cardenas DJ, Ruiz-Saenz J 2022. The First Report and Phylogenetic Analysis of Canine Distemper Virus in Cerdocyon thous from Colombia. Viruses. 1;14(9):1947

Harder TC, Kenter M, Vos H, Siebelink K, Huisman W, van Amerongen G, Orvell C, Barrett T, Appel MJ, Osterhaus AD 1996. Canine distemper virus from diseased large felids: biological properties and phylogenetic relationships. J Gen Virol. 77 (Pt 3):397-405.

ICTV 2022. International Committee on Taxonomy of Viruses. Available at: https://ictv.global/taxonomy/ [29.10.2022]

Iwatsuki K, Miyashita N, Yoshida E, Gemma T, Shin YS, Mori T, Hirayama N, Kai C, Mikami T 1997. Molecular and phylogenetic analyses of the haemagglutinin (H) proteins of field isolates of canine distemper virus from naturally infected dogs. J Gen Virol. 78 (Pt 2):373-80.

Koç BT, Akkutay-Yoldar Z, Oğuzoğlu TÇ 2021. New members to Arctic-like lineage of canine distemper virus from Turkey. Comp Immunol Microbiol Infect Dis. 78:101678.

Lan NT, Yamaguchi R, Furuya Y, Inomata A, Ngamkala S, Naganobu K, Kai K, Mochizuki M, Kobayashi Y, Uchida K, Tateyama S 2005. Pathogenesis and phylogenetic analyses of canine distemper virus strain 007Lm, a new isolate in dogs. Vet Microbiol. 31;110(3-4):197-207.

Oğuzoğlu TÇ, Yücel-Tenekeci G, Çalışkan M, Bozkurt MF, De Massis F 2018. Detection and characterization of Distemper virus in a mink (Neovison vison) in Turkey. Vet Ital. 31;54(1):79-85.

Örvell C. Moller-Blinxenkrone M. Svanson V 1990. Immunological relationships between phocid and canine distemper virus studies with monoclonal antibodies. J. Gen.Virol.71: 2085-2092.

Özkul A, Sancak AA, Güngör E, Burgu I 2004. Determination and phylogenetic analysis of canine distemper virus in dogs with nervous symptoms in Turkey. Acta Vet Hung. 52(1):125-132.

Shimizu H, Shimizu C, Burns JC 1994. Detection of novel RNA viruses: morbilliviruses as a model system. Mol Cell Probes. 8(3):209-14.

## *POSTER PRESENTATION*

# Age estimation from frontal bones by using pentosidine concentrations in Northern Thailand

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## Abstract

Age estimation is an important procedure for the personal identification in forensic science. Pentosidine is a stable advanced glycation end products from heat and humidity. It is strongly associated with age and can be used as a diagnostic biomolecule for age estimation but there are less data to provide in Thailand. The aim of this study was to investigate age-related pentosidine contained in frontal bone. Quantitative and correlation analysis of pentosidine were investigated from thirty- eight individuals which known chronological age of the frontal bone samples. The samples were subdivided into three groups including aged less than 40, between 41 and 60, and higher than 60 years. The mean value of the pentosidine concentration contained in the frontal bone was 119.8 ng/mL. The pentosidine levels in the bones of female presented higher than male but not significantly different. The pentosidine concentration in the frontal bone related with chronological age ($r = 0.468$ , $p = 0.008$). The accurate age range presented between 41 and 60 years old. The mean standard error of using pentosidine to estimate age from the frontal bone was 2.7 years.
The pentosidine contained in the frontal bone is suitable biomolecule for age estimation. Influences of nationality and congenital disease should be studied in further.

**Keywords:** Age estimation, Pentosidine, Frontal bone

## INTRODUCTION

Personal identification is an important step in forensic science. The process can assess physical appearance, clothing, organs and bones. Bone is the most stable structure. Now, gender, ethnicity and chronological age can be investigated from bone. The common methods available for age estimation are based on physiological changes that occur in dental and skeletal structures with aging [1]. However, these methods are only accurate in adolescents. But, there is a high discrepancy among middle-aged and elderly people due to limitation of morphological information. The integrity and completeness of the bone sample are important citerias for bone identification method. It is challenging to evaluate the new method for age estimation. A small amount bone should be uses and accurate to identification.

Advanced glycation end products (AGEs) generated by amino acids, lipids, nucleic acids and reducing sugars [2-5]. Pentosidine is a subtype of AGEs and can be found in the human body. In addition, AGEs is an important biomaker for the aging process and is permanent accumulation throughout life. It is found in the organs containing with collagen deposits such as dentine [6] crystalline lens [7] articular cartilage [8] rib cartilage. intervertebral disc [9] skin [10] and bone [11]. The relationship between age and bone pentosidine content has been found [11,13]. The amount of pentosidine is differ in each bone such as dentine and clavical bone samples [12,13].

The aim of this study was to explore the correlation between chronological age and pentosidine content in frontal bone for evaluting a new age estimation method in forensic science. Morever, the effect of age and gender might be studied to pentosidine level.

## MATERIALS AND METHODS

*Chemicals and Reagents*

Standard substance of pentosidine was purchased from Cayman (Michigan, USA). . Heptafluorobutyric acid (HFBA) and ethylenediaminetetraacetic acid disodium salt dihydrate ($Na_2EDTA$) were purchased from Sigma-Aldrich (St.Louis, USA). All chemical and reagent were analytical grade..

*Sample collection and preparation*

Thirty eight indivadual frontal bones that age range from 16 to 87 years old were obtained from Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University. This study was approved by the Research Ethics Committee of Chiang Mai University's Faculty of Medicine (FOR-2565-09049). Bone samples were washed out three times in an ultrasonicator with water and ethanol. The samples were dried at ambient temperature, pulverized in a freezing mill, and stored at -20°C before use, respectively. [16]

*Pentosidine Analysis*

Pentosidine concentration was determined according to the method described by Greis F et al. [13] with minor modifications. Bone powder (50 mg) was demineralized with 2 mL $Na_2EDTA$ (0.5 M, pH 7.4 adjusted with 2 M NaOH). The sample was vigorously shaken for 20 min and centrifuged at 13,000 rpm for 5 min. The residues were washed 3 times with distilled water to remove $Na_2EDTA$ from the sample. 500 µL of HCl 6 M was added into the residues and hydrolyzed at 100 °C for 20 h. After that, the hydrolyzed sample was dried under nitrogen gas. Dried samples were diluted with 0.1 M HCl and filtered through a 0.22 µm syringe filter. Finally, 10 uL of samples were injected into the High Performance Liquid Chromatography-FLD system (HPLC 1100 Series, Agilent, CA). Purospher® STAR RP-18 endcapped (0.5 µm x150 mm) analytic column was separated. The mobile phase contained (A) 0.1% HFBA and (B) Acetonitrile with a flow rate of 1 mL/min. A step gradient was used in the following sequence: 0 minutes (A100: B0), 20 minutes (A0: B100), and 9 minutes (A100: B0). Excitation 335 nm and Emission 385 nm were set for fluorescence detector [12].

*The self-consistency test*

The self-consistency test was used to evaluate the age prediction models' efficacy. Bias, inaccuracy and standard error were used to calculate value following equation;

$$\text{Standard error} = \{[\Sigma(\text{predicting age} - \text{chronological age})^2]^{1/2}\}/n$$

*Statistical analysis*

The data of pentosidine concentration frontal bone were expressed as the mean and standard deviation. The paired T-Test were used for examining differential and gender-based intracranial pentosidine concentration. Then IBM SPSS Statistics Base version 22.0 was used to do a linear regression and linear correlation study on the pentosidine quantity correlation with age. Pearson correlation was used to examine statistically significant correlations. This was taken into account at a 95% confidence level ($p<0.05$).

## RESULTS and DISCUSSION

*General information of samples*

The samples were collected from 38 people which composed of 20 males (53%) and 18 female (47 %). The samples were subdivided into three groups according to age ranged: less than 40 (n = 11), between 41 to 60 (n = 15), and higher than 60 years (n = 12).

*Analysis of pentosidine concentration*

The concentration of pentosidine in the bone samples was investigated with High Performance Liquid Chromatography – Fluorescence detector. The results found that the frontal bones had pentosidine concentrations in the range of 83.6 - 156.4 ng/mL. The mean value of the pentosidine level contained in the frontal bone was 119.8 ng/mL. The mean of pentosidine concentration in females was 125.3±18.2 ng/mL and in males was 112.2±19.2 ng/mL. The concentration of pentosidine contained in the frontal bone depended on age.

**Table 1.** Pentosidine level in the Frontal bones

| Age group (years) | Mean pentosidine level (ng/mL) |
|---|---|
| ≤40 | 108.5±19.0[a] |
| 41 – 60 | 121.9±17.8[a] |
| > 60 | 131.6±17.2[b] |

small letters significantly difference from the other groups ($p$<0.05).

The concentrations of pentosidine in each age groups and gender were compared and data are shown Table 1. Pentosidine concentrations depended on age. In humans, age-dependent AGEs relate to increase in the natural aging process and accumulation of AGEs due to the progressive creation of AGEs or pentosidine. Extracellular proteins, collagen, are target of the AGEs molecules because they are long-lived proteins containing with lysine, hydroxylysine, and arginine residues that can combine with reducing sugar. Extracellular glucose promotes the buildup of AGE-damaged proteins. [13-15] As a result, the accumulation of AGEs in living organisms is regarded a biomarker of aging processes. [17-19] The concentration of pentosidine had relationship between the chronological age of the frontal bone samples (r = 0.468, $p$<0.05).

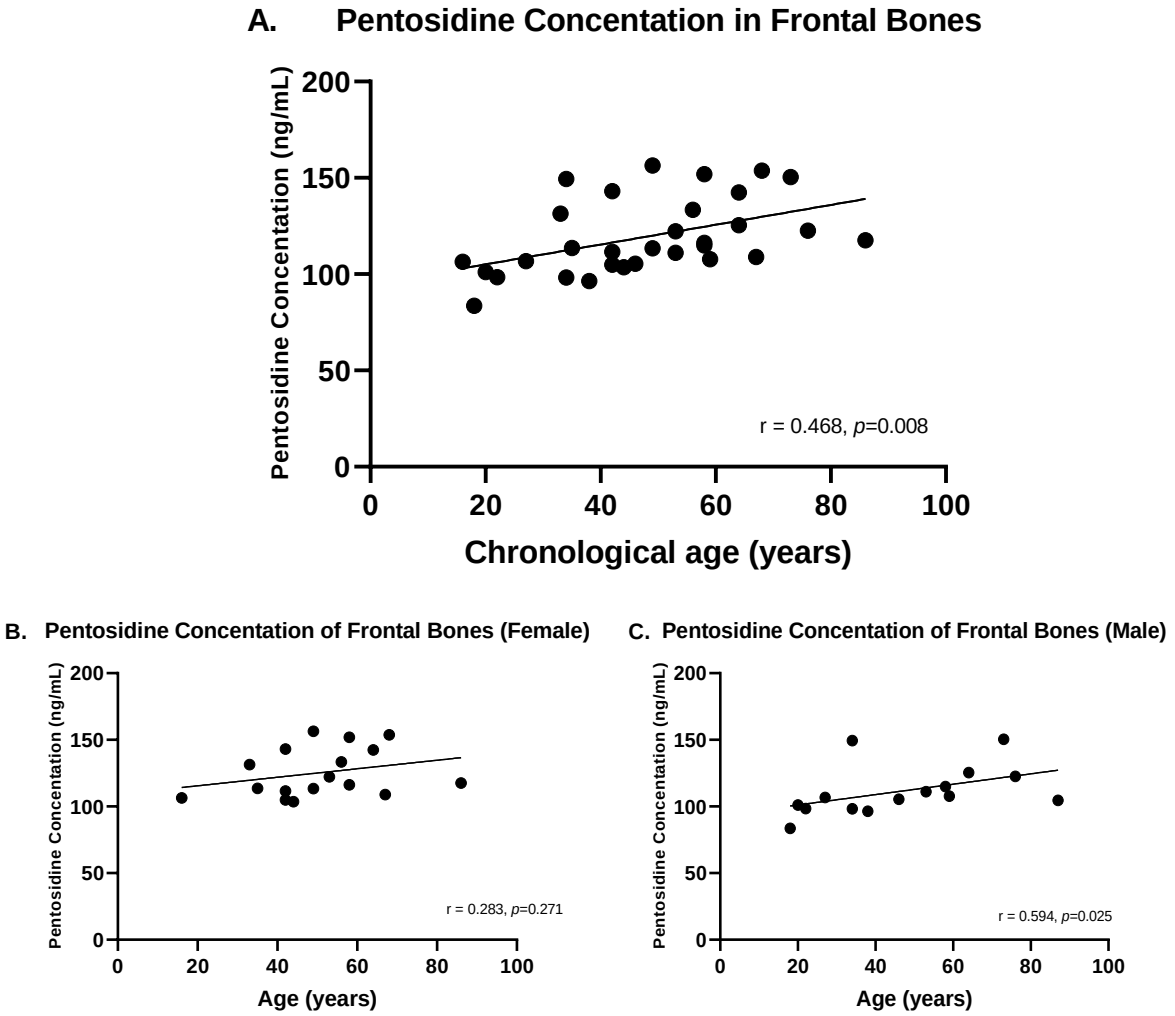


Figure 1. The relationship between the amount of pentosidine in the frontal bone (r = 0.468 , $p$ = 0.008) and obtaining age estimation equations from linear regression analysis obtained from frontal bone is Estimate age (years) = 27.652 + (0.189[pentosidine concentration in frontal bone]). The relationship between pentosidine concentrations and age in the frontal bone (ng/mL) and the chronological age (year) of frontal bone samples. The correlation between pentosidine concentrations in the frontal bone was compared with chronological age

(years) in females with chronological age (r = 0.283, $p$ = 0.217) and chronological age (years) in males (r = 0.594, $p$ = 0.025).

In Table 1, the amount of pentosidine in each gender is usually different, but not statistically significant ($p$ = 0.086). The pentosidine in the frontal bones in age range 41 to 60 years presented higher than the other groups. Individual's lifestyle, food consumption, especially grilled and fried foods can increase the level of AGEs about 10 to 100 times. AGEs are abundant in foods high in fat and protein, [19]. The concentration of pentosidine was analyzed to determine the relationship with the age of the frontal bone samples separated by gender as shown in Figure 1.

*Age estimation from linear regression analysis*

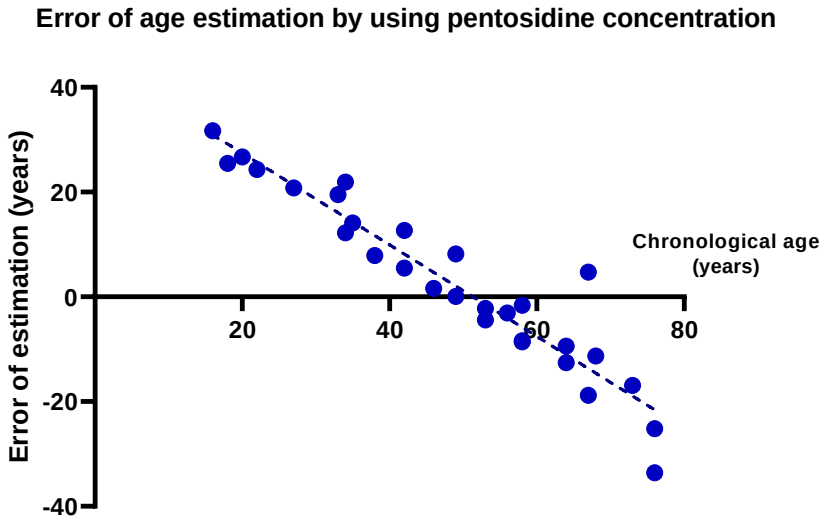


**Figure 2.** Error of age estimation by using pentosidine concentration.

The estimated age and the chronological age differed and the results are presented in Figure 2. The error value was range 1 to 39 years. The estimated age was similar value to the chronological age in age range 41 to 60 years. However, in age range less than 40 and higher than 60 were different the value. In elder age, Aging, and non-communicable diseases (Diabetes mellitus, coronary artery disease) can affected to pentosidine level. Changing of lifestyle especially food consumption about grilled and fried foods might increase the level of pentosidine in adolescents.

*Calculation of the performance of the age prediction model*

The efficiency of the age prediction model was evaluated using the calculations using a self-consistency test, which included bias, errors, and standard error. In the frontal bone, there is a standard discrepancy of age prediction models. The standard error of frontal bone was 3 years.

## CONCLUSION

The pentosidine level contained in frontal bone moderately related to chronological age. It may be used as a new diagnostic tool for age estimation in human remains which the mean standard error was 3 years. The pentosidine level in the frontal bone of female presented higher than male. It might be applied to categorize gender in further.

**REFERENCES**

[1] Wunchana S, Wirut K. Application of Molecular Biology Techniques on Forensic Sciences. Srinagarind Med J 2014 ;29(3):321-6.

[2] Garay-Sevilla M.E. et al. Nutritional Modulation of Advanced glycation end products. J Nutr Health Aging 2016:263-276.

[3] Vlassara H., Palace M.R. Diabetes and advanced glycation endproducts. J Intern Med 2002;251:87-101.

[4] Pattawadee S. Project to investigate the pharmacological activity of rhododendron honey. [Research report]. Burapha University 2017.

[5] Peng X et al. Naturally occurring inhibitors against the formation of advanced glycation end-products: Review. Food & Function 2011;(2).

[6] Miura J et al. Accumulation of advanced glycation end-products in human dentine. Arch Oral Biol 2014;59(2):119–24.

[7] Ramalho J et al. Role of glycation in human lens protein structure change. Eur J Ophthalmol 1995;6(2):155–61.

[8] Verzijl N et al. Age-related accumulation of Maillard reaction products in human articular cartilage collagen. Biochem J 2000;350(2):381–87.

[9] Pilin A et al. Changes in colour of different human tissues as a marker of age. Int J Legal Med 2007;121(2):158–62.

[10] Dyer DG et al. Accumulation of Maillard reaction products in skin collagen in diabetes and aging. J Clin Investig 1993;91(6):2463.

[11] Masaki N et al. Pentosidine and carboxymethyl-lysine associate differently with prevalent osteoporotic vertebral fracture and various bone markers. Sci Rep 2020;10:22090.

[12] Aurora V et al. Differences in non-enzymatic glycation products in human dentine and clavicle: changes with aging. Int J Legal Med 2018;132:1749–58.

[13] Greis F et al. Analysis of advanced glycation end products (AGEs) in dentine: useful for age estimation?. Int J Legal Med 2018;132(3):799-805.

[14] Ahmed N. Advanced glycation endproducts--role in pathology of diabetic complications. *Diabetes Res. Clin. Pract* 2005;67(1):3-21.

[15] Nina SM et al. Molecular clocks in ancient proteins: Do they reflect the age at death even after millennia?. Int J Legal Med 2021;135:1225–33.

[16] Tawachai M et al. Age estimation using aspartic amino acid racemization from a femur. Aust J Forensic Sci 2019;51(4):417–425.

[17] Jacobson J et al. Biomarkers of aging in Drosophila. Aging Cell 2010; 9: 466-77.

[18] Unoki H et al. Advanced glycation end products attenuate cellular insulin sensitivity by increasing the generation of intracellular reactive oxygen species in adipocytes. Diabetes Res Clin Pract 2007;76:236-44.

[19] Uribarri J et al. Advanced Glycation End Products in Foods and a Practical Guide to Their Reduction in the Diet. J Am Diet Assoc 2010;110:911-6.

## *POSTER PRESENTATION*

# Hepatoprotective Activity of *Ammi majus* on CCL4 Induced Albino Mice

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**Abstract**

This study was amid to evaluate the possible protective effects of the water and alcoholic extract of *Ammi majus* seeds against liver damage induced in mice by CCl4. The plant was collected from Baghdad markets, water and alcoholic extract was prepared and used at one concentration for injecting six groups of mouse, the first one is control negative that are mice was not injected, the second was positive control which is the mice was injected with CCl4, the third group had mice that injected with water extract, the fourth group included mice that injected with CCl4 and water extract, the fifth group had mice that injected with alcoholic extract , the last group includes mice injected with CCl4 and water extract. Injected intraperitoneally (0.1 ml) for seven days as single dose\day, Finally mice were sacrificed at day 8 for laboratory assessments. Blood and liver tissue were collecting , blood used for administration of liver enzyme (GOT, GPT, ALP) and liver tissue used for assessment of the hepatoprotective effect of *Ammi majus* extracts.

The results show that for the liver enzymes, the GPT activity was 199±2.1 Unit/ L in control negative (Untreated mice); the GPT value decrease to 76±5.2 Unit/L in positive control suggesting that there was positive damage upon its treatment. Interestingly, it got normal value of GPT which was 142±2.5 Unit/L after its treatment with 100 mg/Kg water extract of *Ammi majus* extract and about same value 145±2.5 Unit/L when injected with water extract and CCL4, also it got decrease value 154±2.5 when injected the alcoholic extract of *Ammi majus* and highly increased value 203±2.5 Unit/L with injection of alcoholic extract and CCl4. While GOT activity is checked in order to see the hepatoprotective role of *Ammi majus* plant extract, the GOT value of CCL4 injected mice had shown high level 67.1±1.2 Unit/L compared with those untreated mice was 33±4.5 Unit/L, while upon the treatment with *Ammi majus* water extract, it got a in GPT about normal value which was 40.3±3 Unit/L 100 mg/Kg, and highly decrease value 16±2.5 Unit/L when injected water extract of *Ammi majus* and CCL4, also it got increase value 50±2.5 when injected the alcoholic extract of *Ammi majus* and few decrease value 28±2.5 Unit/L with injection of alcoholic extract and CCl4. Also, ALP activity was the mice that were injected with CCL4 also produced high levels of ALP 132.3±2.5 Unit/L compared with those untreated mice that was 93.6±4 Unit/L. But, upon the treatment with *Ammi majus* water extract, it got a in ALP about normal value which was 90.3±3 Unit/L with a dose of 100 mg/Kg, and increase value 102±2.5 Unit/L when injected with water extract of *Ammi majus* and CCL4, also it got decrease value 61±2.5 when injected the alcoholic extract *Ammi majus* and highly decrease value 43±2.5 Unit/L with injection of alcoholic extract and CCl4.

The Histopathological Evaluation of liver and further assessment of the hepatoprotective effect of *Ammi majus* water and alcoholic extracts, the results show that, the section of normal liver structure, which consists of central vein, surrounded by hepatocyte cells without any changes, while section of liver tissue in mouse treated with CCl4 as a control positive showing congestion, degenerative and necrosis of paranchymal tissue cells; with mild cells inflammation. In addition, section of a liver tissue in mouse treated with water extract of *Ammi majus*, section of liver showing normal histological structure and section of a liver tissue in mouse treated with water extract of *Ammi majus* and CCl4, Section showing look like normal histological structure appearance but with mild dilatation of sinusoid and no fatty changes seen. On the contrary, section of a liver tissue in mouse treated with alcoholic extract, section of liver showing congestion evident depletion of glycoprotein granules inside the hepatocyte cells and Section of a liver tissue in mouse treated with Section of a liver tissue in mouse treated with alcoholic extract of *Ammi majus* and CCl4, section showing congestion ,sinusoidal dilation, with few hepatic cells showing fatty changes.

From the result above it was concluded that the water extract give a good protection activity for liver enzyme and histology while the alcoholic extract was toxic, harmful, toxic and not produce productive activity for liver enzyme and histology.

## Introduction

Coumarins and flavonoids are both secondary metabolic heterocyclic organic phytochemicals, which are very important phytochemicals for the formulation of drugs due to their medicinal values. Recently, the literature showed that both the phytochemicals have been extensively used as synthetic antioxidants to foodstuffs. There is some extensive curiosity in protective medicine and in food manufacturing in the expansion of natural antioxidants found from plant sources, especially herbal plants (Djilas *et al*., 2003).

The liver is a key organ in regulating the homeostasis of the body by carrying various essential functions.Many chemicals that are inhaled or swallowed can damage the liver among these, are drugs, industrials and pollutants. The severity of liver injury may vary from nonspecific structural and functional change to acute liver failure or chronic injury (Recknagel *et al.,* 1989, Weber *et al*., 2003).

*Ammi majus* is considered as a wild vegetable *plant* which is used traditionally to treat different ailments. The roots, leaves, stems, fruits and their paste of the selected plant are equally used traditionally to treat particular diseases (Satrani *et al*., 2004 , Abdul-Jalil *et al*., 2010).

*A. majus* is considered as a wild vegetable plant which is used traditionally to treat different ailments. The roots, leaves, stems, fruits and their paste of the selected plant are equally used traditionally to treat particular diseases (Satrani *et al*., 2004 , Abdul-Jalil *et al*., 2010).

Since ancient times, scientists are working continuously on the various parts of the selected plant species related to phytochemicals and pharmacological activities for medicinal uses (Rose, 1992)] Based on pharmacological activities, the researchers are working continuously on different parts as well as the different polarities of extracts of the selected plant and isolate and characterized some pharmacologically active phytochemicals which can be used as remedy to treat diseases (Satrani *et al* ., 2004).

our group is trying to work on that selected plant species to isolate the bioactive phytochemicals that can be used to treat different diseases.

## 2. Methods

Plant Collection

The seeds of Plant were collected from the local market in Baghdad in December , 2020. The plant was previously identified by Iraqi center of herbs then it homogenized to fine powder .

Plant extraction(water and Alcoholic extract)

For water extract the powder seeds of plant (10 g) were added to 100 ml of distilled water in cleaned flask, put in shaker incubator for 24 h at 37C. then the mixture was filtered by gauze in to glass tube , centrifuged at 500 rpm for 10 min, the upper phase was filtered by filter paper then distribute it in sterile petry dishes and put in oven at 60 C for about 5 day utile obtained dry scaled of the extract (chanda and Parekh, 2007).Then put the extract in sterile sealed tube and keeping in freezing at -20°C until used. For alcoholic extract the seeds of plant (20 g) were extracted with 250 ml of methanol (70%) by soxhlet extraction apparatus for 6 hours at 55°C (10). After that the extract filtered and evaporated by using rotary evaporator to dry extract and stored at 4°Cuntil used (Harborn *el al*., 1975).

Plant Tested Doses

The selection of these doses which used in this research were (10%) of LD50 (20 mg /kg) which determined in according to EFSA journal (2008).

Experimental animals

Twenty four adult males' albino mice were used. Their weights at the beginning of experiment were (22- 28g). These mice which were obtained from General Company of veterinary medicine, maintained in well-conditioned room, on special pellets regime and water. The animals were separated into six groups as below :

-Group 1: mice were administrated with D.W (negative control negative group 4 animals).

-Group 2: mice were administrated with water extract 20 mg/kg of R.communis (4 animals).

-Group3: mice were administrated water extract 20 mg/Kg and ccl4 2o mg/kg (4 animals).

-Group4: mice were administrated with alcoholic extract 20 mg/kg of extract (4 animals).

- Group5 : mice were administrated with 0.1 ml of alcoholic extract 20 mg/kg and CCL4 20 mg/kg (4 animals).

- Group 6: mice were administrated with CCL4 20 mg/ml as control positive (4 animals).

In order to repair these doses, the water plant extract dissolved in distilled water directly, while alcoholic plant extract dissolved in a few drops of DMSO and then completed by distilled water to reach the required doses. In addition the CCL4 dissolved in virgin olive oil. Injected intraperitoneally (0.1 ml) for seven days as single dose\day, Finally mice were sacrificed at day 8 for laboratory assessments.

Determinations of Hepatoprotecive effects

For Hepatoprotective determinations, the parameters of assessment were ALT, AST and ALP enzymes in serum, The activity of enzyme of ALT was determined in mouse serum according to (Reitman and Frankel, 1957), as in GOT determination using a commercial kit (Randox Company). The enzyme activity of Aspartate Amino-Transferees (GPT) was calculated in mouse serum according to evaluation method of (Gometi *et al*., 2014). For this purpose, a commercial kit (Randox Company) was used.

The ALP enzyme was measured in mouse serum using a specific kit manufactured by Bio Merieux Company and the greatest traditional way used is that of (Camargo and Martinez, 2007)

Histopathological Study

Samples were obtained & cut into small pieces (2×2×2 mm.) then pre-fixed in 2.5% gluteraldehyde diluted in phosphate buffer PH (7.4). After that specimen were rinsed in the same buffer for several times and left in PBS for 12 hrs, and the procedure of (Dogan and Celik,2012) was followed to prepare histopathological sections.

**Results and Discussion**

**3.1. Effect of water extract and alcoholic extract on liver enzymes (GOT, GPT, Alkaline Phosphatase )**
Here, we would like to refer that we injected the mice with CCL4 before their treatment with our plant extract in order to produce certain damage in liver function.

**3.1.1 GPT activity**

The results had shown that the GPT activity was 199±2.1 Unit/ L in control negative (Untreated mice) while, upon its injection with CCl4 as a positive control; the GPT value decrease to 76±5.2 Unit/L, suggesting that there was positive damage upon its treatment. Interestingly, it got normal value of GPT which was 142±2.5 Unit/L after its treatment with 100 mg/Kg of water extract of *Ammi majus* extract and about same value 145±2.5 Unit/L when injected with water extract and CCL4, also it got decrease value 154±2.5 when injected the alcoholic extract *Ammi majus* and highly increased value 203±2.5 Unit/L with injection of alcoholic extract and CCl4. As shown in Table (3-1).

**Table (3-1) shown the values of GPT level of liver in control negative (untreated), control positive (CCL4), water extract *Ammi majus* and water extract with CCL2, and alcoholic extract of *Ammi majus* and alcoholic extract with CCl4.**

| Liver enzyme | Negative control Unit/L | Positive control CCl4 Unit/L | Water extract of *Ammi majus* Unit/L | Water extract of *Ammi majus* and CCl4 Unit/L | Alcoholic extract of *Ammi majus* Unit/L | Alcoholic extract of *Ammi majus*and CCl4 Unit/L |
|---|---|---|---|---|---|---|
| GPT | 199±2.1 | 76±5.2 | 142±2.5 | 145±2.5 | 154±2.5 | 203±2.5 |

It was found that from the result that the water extract, water extract with CCl4 and alcoholic extract are given a good protection effect for GPT enzyme level while the alcoholic extract with CCl4 is not give protection effect for enzyme level.

### 3.1.2. GOT activity

GOT activity is another parameter checked in order to see the hepato-protective role of *Ammi majus* plant extract. In table (3-2), the GOT value of CCL4 injected mice had shown high level 67.1±1.2 Unit/L compared with those untreated mice was 33±4.5 Unit/L, while upon the treatment with *Ammi majus* water extract, it got a in GPT about normal value which was 40.3±3 Unit/L 100 mg/Kg, and highly decrease value 16±2.5 Unit/L when injected with water extract of *Ammi majus* and CCL4, also it got increase value 50±2.5 when injected the alcoholic extract *Ammi majus* and few decrease value 28±2.5 Unit/L with injection of alcoholic extract and CCl4.

**Table (3-2) shown the values of GOT level of liver in control negative(untreated), control positive (CCL4), water extract *Ammi majus* and water extract with CCL2, and alcoholic extract of *Ammi majus* and alcoholic extract with CCl4.**

| Liver enzyme | Negative control Unit/L | Positive control CCl4 Unit/L | Water extract of *Ammi majus* Unit/L | Water extract of *Ammi majus* and CCl4 Unit/L | Alcoholic extract of *Ammi majus* Unit/L | Alcoholic extract of *Ammi majusand* CCl4 Unit/L |
|---|---|---|---|---|---|---|
| GOT | 33±4.5 | 67.1±1.2 | 40.3±3 | 16±2.5 | 50±2.5 | 28±2.5 |

From the result above it was found that no good effect for protection level of GOT enzyme with water extract while alcoholic extract have good effect that's mean the alcoholic extract helped in reregulation of liver activity upon the reduction of GOT in CCL4 mice.

### 3.1.3. ALP activity

The mice that were injected with CCL4 also produced high levels of ALP 132.3±2.5 Unit/L compared with those untreated mice that was 93.6±4 Unit/L. But, upon the treatment with *Ammi majus* water extract, it got a in ALP about normal value which was 90.3±3 Unit/L with a dose of 100 mg/Kg, and increase value 102±2.5 Unit/L when injected with water extract of *Ammi majus* and CCL4, also it got decrease value 61±2.5 when injected the alcoholic extract *Ammi majus* and highly decrease value 43±2.5 Unit/L with injection of alcoholic extract and CCl4.

**Table (3-3) shown the values of ALP level of liver in control negative(untreated), control positive (CCL4), water extract *Ammi majus* and water extract with CCL2, and alcoholic extract of *Ammi majus* and alcoholic extract with CCl4.**

| Liver enzyme | Negative control Unit/L | Positive control CCl4 Unit/L | Water extract of *Ammi majus* Unit/L | Water extract of *Ammi majus* and CCl4 Unit/L | Alcoholic extract of *Ammi majus* Unit/L | Alcoholic extract of *Ammi majus and* CCl4 Unit/L |
|---|---|---|---|---|---|---|
| ALP | 93.6±4 | 132.3±2.5 | 90.3±3 | 102±2.5 | 61±2.5 | 43±2.5 |

From the result it was shown that water extract and water extract with CCl4 provide a protection effect for ALP level while there is decrease in this enzyme when treated with alcoholic extract and alcoholic extract and CCl4.

From the results mentioned above which explain the important role of *Ammi majus* plant extract (water and alcohol)in controlling the regulation of liver function that were represented by GPT, Got and ALP enzymes, we should look for the mechanism or any explanation behind that positive effect. To answer this question, we treated the mice with both of our plant extract and CCL4 together in order to check if this reduction in enzymatic activity that came from plant treatment is due to its ability to interact with the CCL4 or not. Regarding GPT activity, the dual treatment with CCL4 and water *Ammi majus* plant extract had shown positive increase in its values which were 142±2.5 and 203±2.5 Unit/L compared with the group which was treated with CCL4 alone. while this result was confirmed by GOT values that produce decrease in its activity upon dual treatment which were 16±2.5 and 28±2.5Unit/L compared with CCL4 treatment alone. Again, ALP activity was down regulated significantly 102±2.5and 43±2.5Unit/ L if we compare it with single treatment of CCL4. So, we can conclude that the dual treatment of mice with CCL4 and *Ammi majus* produced a fundamental increase in GPT and reduction in Got & ALP activity.

Liver is the major site of detoxification and the primary target of drug exposure in the body. High levels of drugs cause various hepatic disorders by producing pro-oxidants/reactive oxygen species (ROS), which are able to induce cellular damage in a variety of ways by affecting the cellular biomolecules, such as lipids, DNA and proteins (Ziech *et al*., 2010). The hepatotoxicity induced by $CCl_4$ is mainly due to its metabolite $CCl_3$, which is a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids. In the presence of oxygen, lipid peroxides are produced, leading to liver damage, which is characterized by fatty liver, cirrhosis and necrosis (Zeashan *et al*., 2008).

In addition, in $CCl_4$ induced hepatotoxicity, the extent of hepatic damage is assessed by the increased level of cytoplasmatic enzymes (ALT, AST and ALP), which leads to leakage of large quantities of the enzymes into the blood circulation and could be regarded as an index of the liver parenchymal cells damage (Shankar *et al*., 2008). Hepatocellular necrosis and liver injury leads to elevation of these serum marker enzymes, which are released from the liver into blood (Rezende *et al*., 2014). The present study revealed a significant increase in the activities of GPT, and decrease in GOT and ALP upon exposure to $CCl_4$, indicating considerable hepatocellular injury. Clinically, the general strategy for prevention and treatment of the $CCl_4$-induced hepatotoxicity includes reducing the production of reactive metabolites (Wong *et al*., 2012), increasing evidence indicates that oxidative stress causes organ injury and carcinogenesis (Kucharská *et al*., 2004, Cheng *et al*., 2013).

Treatment of rats with different doses of *A. majus* seeds' extract could cause hepatoprotective effects against CC14-induced liver damage, in a dose-dependent fashion(Mutlag *et al*., 2011).

### 3.2. Histopathological Evaluation of Liver

For a further assessment of the hepatoprotective effect of *Ammi majus* water and alcoholic extracts, the liver of the six groups in the present experiment was examined blindly by the histpathologist Professor Dr. Salim R. Hamoudi (Department of Pathology, College of Medicine, University of Baghdad). The results of examination are presented for each treated group of mice under represented pictures (Figures 3-1, 3-2, 3-3, 3-4, 3-5 and 3-6).

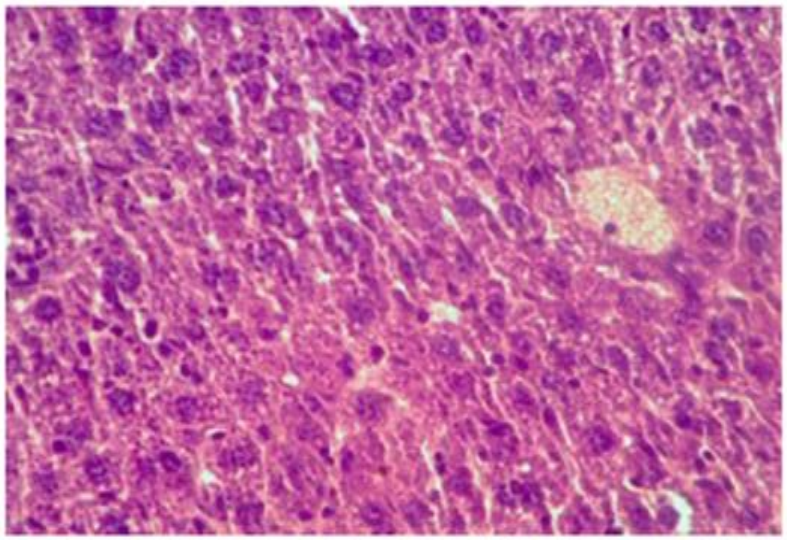

**Figure 3-1: Section of a liver tissue in mouse as a negative control, section of normal liver structure, which consists of central vein, surrounded by hepatocyte cells (400X; H and E).**

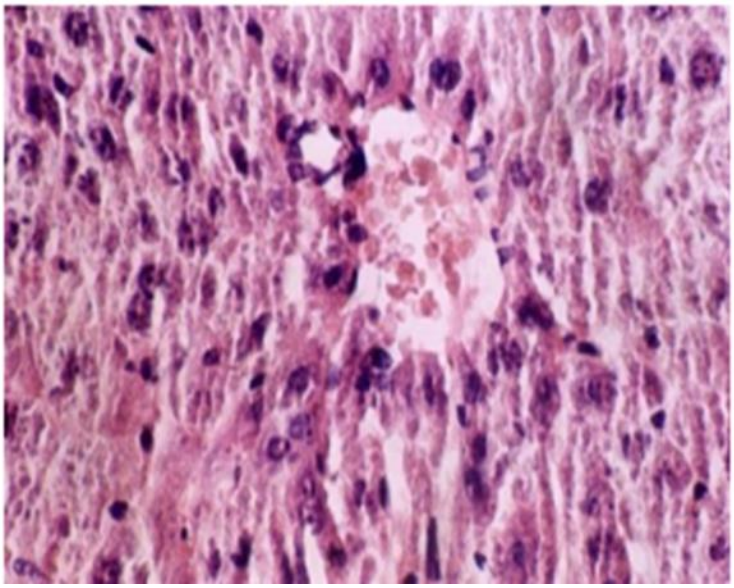

**Figure 3-2: section of liver tissue in mouse treated with CCl4 showing congestion, degenerative and necrosis of paranchymal tissue cells; with mild cells inflammation as a control positive (H&E, x400).**

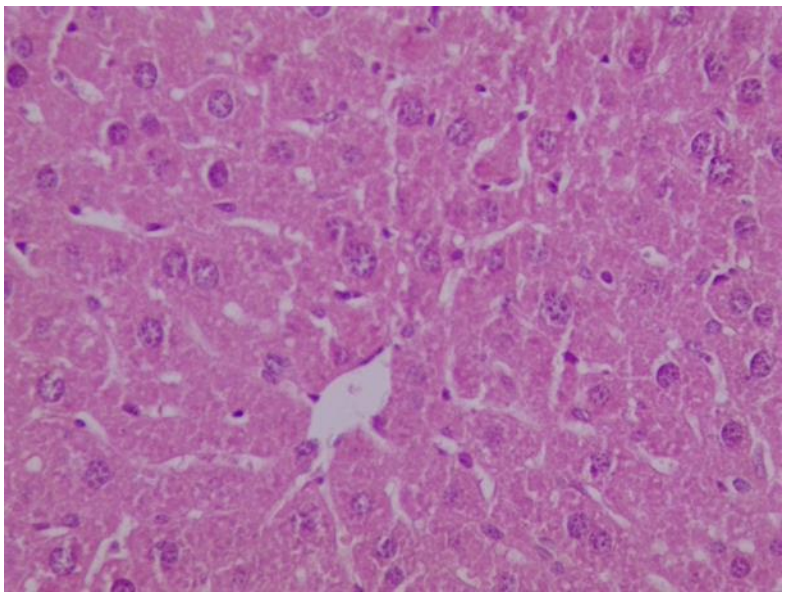

**Figure 3-3: Section of a liver tissue in mouse treated with water extract of *Ammi majus*, Section of liver showing normal histological structure (200X; H and E).**

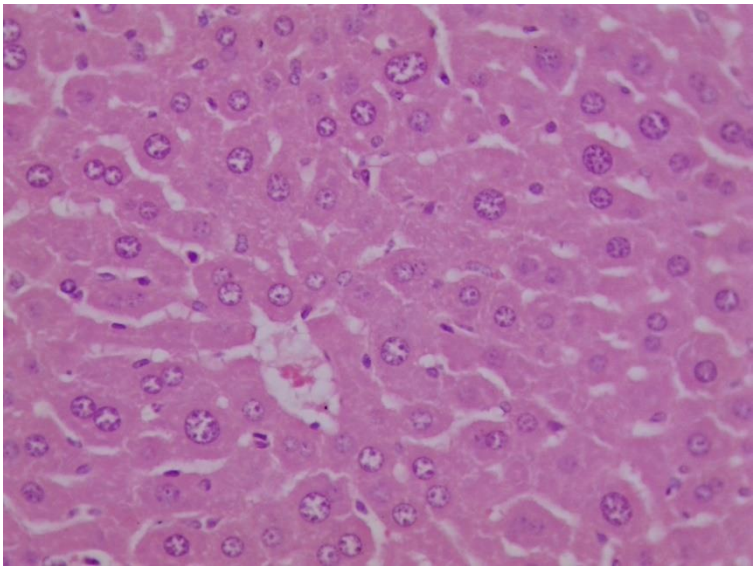

**Figure 3-4: Section of a liver tissue in mouse treated with water extract of *Ammi majus* and CCl4, Section showing look like normal histological structure appearance but with mild dilatation of sinusoid and no fatty changes seen (200X; H and E).**

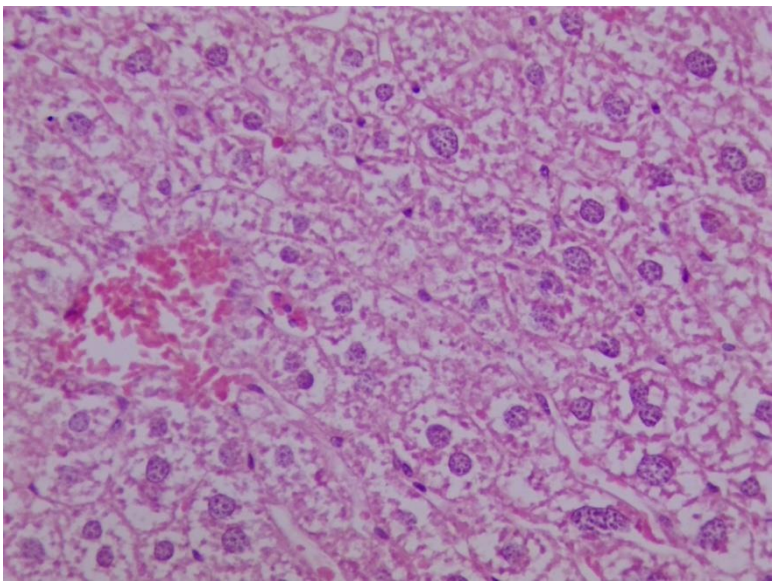

**Figure 3-5: Section of a liver tissue in mouse treated with alcoholic extract, section of liver showing congestion evident depletion of glycoprotein granules inside the hepatocyte cells (200X; H and E).**

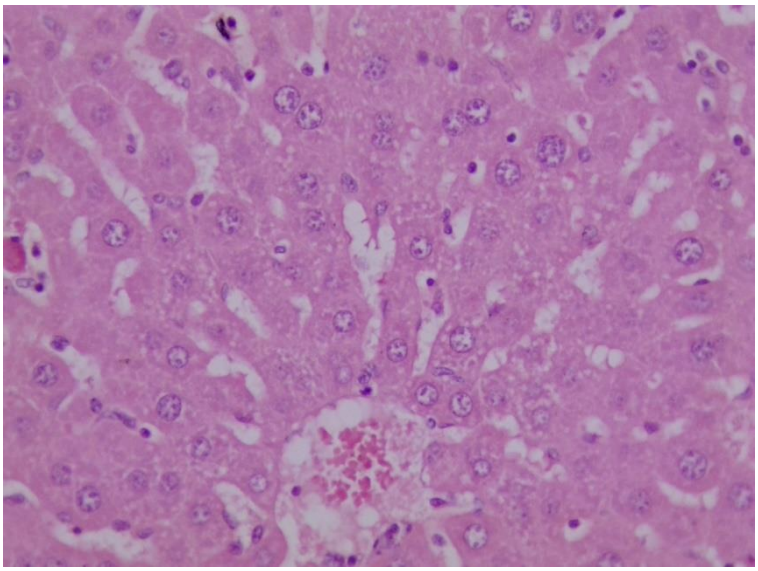

**Figure 3-6: Section of a liver tissue in mouse treated with Section of a liver tissue in mouse treated with alcoholic extract of *Ammi majus* and CCl4, Section showing congestion ,sinusoidal dilation, with few hepatic cells showing fatty changes (200X; H and E).**

Histopathological examination of liver confirmed the results of tissue homogenate and serum markers, and also indicated that $CCl_4$ induced liver injury was less severe in the water extract of *Ammi majus* treated groups. The present results show that water extract of *Ammi majus* reduces the extent of hepatic injury caused by $CCl_4$ in mice. This suggests the beneficial effect of *water extract of Ammi majus* in modulating the hepatotoxic effects of $CCl_4$ in treated mice; an observation that is favored by Abdel-Salam *et al*. (2012).

The histopathological examinations of liver sections in $CCl_4$-treated mice revealed a regeneration of hepatic cells after treatments with the water plant extract especially at a dose of 100 mg/kg; therefore it is possible to suggest that the plant extract being able to condition the hepatic cells to a state of accelerated regeneration (Aydin *et al*., 2014).

*Ammi majus* contain Linear furancoumarins (xanthotoxin , bergabten , imperatorin and isioimpiellin) which inhibt human liver CYP450 simple coumarins induce number of enzymes like aldehyde reductase , glutathione Stransferase(GST), and NAD(P)H quinone oxidoredctase in the liver that are possible for detoxification of alfatoxin B1 and also contain flavoniods (quercetin and keampferol) which has antioxidant and anti-tumor activity (lin *et al*., 2006 and Donnini *et al*., 2006).

The antioxidant properties of *Ammi majus* extract may be attributed to the presence of quercetine (Muragundla and Kanwaljit, 2004). It has been shown that chronic administration of an oral dose of quercetine (10mg/kg/day) for 5 weeks reduce blood pressure, increase glutathione activity and reduce both plasma and hepatic malondialdehyde (MDA) levels ( Duarte *et al*., 2001).

## Conclusion and Recommendation

### Conclusion

1- CCl4 had a toxic and harmful effect at liver enzyme and histology.

2- Water extract have benefit effect and productive activity for liver enzyme and histology.

3- Alcoholic extract have toxic effect and not give productive activity.

### Recommendation

1- Investigated on active compound that are found in *Ammi majus* water extract and study.

2. Study effect of water extract of *Ammi majus* with different concentration and know toxic dose for it.

3. Study effect of water extract of *Ammi majus* on another organ.

4- Investigated on active compound found in alcoholic extract and why it was toxic.

## ***References***

A Nostro, MP Germanò, VD'angelo, et al. (2000) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol 30: 379-384.

Abdul-Jalil TZ, Saour KY, Nasser AA (2010) Phytochemical study of some flavonoids present in the fruits of two Ammi L. species wildly grown in Iraq. Iraqi J Pharm Sci 19: 48-57.

Abdul-Jalil TZ, Saour KY, Nasser AA (2010) Phytochemical study of some flavonoids present in the fruits of two Ammi L. species wildl18. Duarte J, Torres AI, Zarzuelo A (2000) Cardiovascular effects of visnagin on rats, Planta Med 66: 35-39.y grown in Iraq. Iraqi J Pharm Sci 19: 48-57.

Abdul-Jalil TZ, Saour KY, Nasser AA (2010) Phytochemical study of some flavonoids present in the fruits of two Ammi L. species wildly grown in Iraq. Iraqi J Pharm Sci 19: 48-57.

Abu Mustafa EA, Fayez MBE (1961) Natural coumarins I. Marmesin and marmesinin further products from the fruits of Ammi majus L. J Org Chem 26: 161-166

Ammor K, Bousta D, Jennan S, et al. (2017) Total polyphenol content and antioxidant power of Ammi visnaga from Morocco. Der Pharma Chemica 9: 73-78.

Aourabi S, Driouch M, Sfaira M, et al. (2019) Influence of phenolic compounds on antioxidant and anticorrosion activities of Ammi visnaga extracts obtained ultrasonically in three solvent systems. Int J Electrochem Sci 14: 6376-6393.

Bencheraiet R, H Kherrab, A Kabouche, et al. (2011) Flavonols and antioxidant activity of Ammi visnaga L. (Apiaceae). Rec Nat Prod 5, 52 55.

Boll M, Weber L W D, Becker E and Stampfl A; Mechanism of carbon tetrachloride induced toxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. Z Naturforsch. 2001; 56:649-659.

Camargo, M. M. P. and Martinez, C. B. R. (2007).Histopathology of gills, kidney and liver of a Neotropical

fish caged in an urban stream .Neotrop Ichthyol., 5:327-336.
Canadanovic-Brunet JM, Djilas SM, Cetkovic GS, et al. (200) Free radical scavenging activity of wormwood (Artemisia absim thium L.) extracts. J Sci Food Agric 85: 265-272.
Dogan, A. and Celik, I. (2012). Hepatoprotective and antioxidant activities of grapeseeds against ethanol-induced oxidative stress in rats. Br J Nutr., 107: 45-51.
Donnini , S. , Finetii, F, Morbidelli , L.L. , Cheneir , V., Barron , D. , Williamson, G. , Waltenberger, J. and Ziche. M. (2006). Divergent effect of quercetin conjugate on angiogenesis: British Journal of Nutrition . 95:1016-1023.
Donnini S, Finetii F, Morbidelli L L, Cheyneir V, Barron D, Williamson G, Waltenberger J and Ziche M; Divergent effect of quercetine conjugate on angiogenesis. British Journal of Nutrition. 2006; 95:1016-1023.
Duarte J, Ocete M A, Vizcaino F P, Zarzuelo A and Tamargo J. (2001). Effect of chronic querectin treatment on hepatic oxidative status of spontaneously hypertensive rats. Molecular and cellular Biochemistry journal. 221:155-160.
Duarte J, Torres AI, Zarzuelo A (2000) Cardiovascular effects of visnagin on rats, Planta Med 66: 40-41.
El Gamal M H A, Shalaby N M M, Duddeck H and Hiegemann M; Coumarins and coumarin glycoside from the fruit of Ammi majus L. Phytochemistry 1993;
El Mofty AM (1948) A preliminary clinical report on the treatment of leucodermia with Ammi majus Linn. J Egypt Med Assoc
El-Mofty AM (1952) Further study on treatment of leucodermia with Ammi majus Linn. J Egypt Med Assoc 35: 1-19.31: 651-665.
Farnsworth N R; Present and future of pharmacognosy. ibid. 1979; 43:239-243.
Fua, W., Chena, J., Caia, Y., Leia, Y., Chenb, L., Peic, L., Zhoua, D., Lianga, X. and Ruana, J. (2010). Antioxidant, free radical scavenging, antiinflammatory and hepatoprotective potential of the extract from
function of Flavonoids. Academic press, New York, P: 970.
Gometi, S.A., Ogugua, V. N., Odo, C. E. and Joshua, P. E. (2014). Effects of some anti-diabetic plants on the hepatic marker enzymes of diabetic rats. Afr J Biotechnol.,13: 905-909.
Hakim RE (1969) Rediscovery of a treatment for vitiligo. Clio Medica 4: 277-289.
Harborn, J. B.; Mabray, T. J. and Mabray, H. (1975). Physiology and
Hussain I, Saleem S, Khan H, et al. (2015) Screening of Ammi Majus and Convolvulus Arvensis for antioxidant activities. MDSRC- 2015 Proceedings, Wah/Pakistan.
Khalfallah A, Labed A, Semra Z, et al. (2011) Antibacterial activity and chemical composition of the essential oil of Ammi visnaga L. (Apiaceae) from constantine, Algria. Int J Med Arom Plants 1: 302-305.
Lai PK, Roy J (2004) Antimicrobial and chemopreventive properties of herbs and spices. Curr Med Chem 11: 1451-1460.
Lin, J., Zhan, S.M. , Wu, K. , Willett , W. C , Fuchs, C.S. , and Giovannucci , E. (2006). Flavoniod intake and Colorectal Cancer in Men and Women :American journal of Epidemiology . 164:644-651.
Marimuthu P, Wu CL, Chang HT, et al. (2008) Antioxidant activity of the ethanolic extract from the bark of Chamaecyparis obtusevar. formosana. J Sci Food Agric 88: 1400-1405.
Milic BLJ, Djilas SM, Canadanović-Brunet JM, et al. (2000) Polyphenols in plants. Faculty of Technology, University of Novi Sad,Novi Sad, 277-309
Mothana RA, Abdo SA, Hasson S, et al. (2010) Antimicrobial, antioxidant and cytotoxic activities and phytochemical screening of Some Yemeni medical plants avid based complement alternat MED 7;323-330
Mounting: Mounted the slide was with a Canada balsam and covered with a cover slip. Then, the slide was examined microscopically to inspect the histopathological change function of Flavonoids. Academic press, New York, P: 970.
Muragundla A and Kanwaljit C. (2004). Quercetine antioxidant bioflavonoid, attenuataes diabetic nephropathy in rats; Clinical and Experimental pharmacology and physiology. 31:244-248.
Muthukumran P, Begumand VH, Kalaiarasan P (2011) Anti-aiabetic activity of Ammi majus (L) leaf extracts. Inter J Pharm Tech Res 3: 136-139.
Mutlag SH, Ismael DK, Al-Shawi NN. Study the possible hepatoprotective effect of different doses of Ammi majus seeds'extract against CCl4 induced liver damage in rats. Pharm Glob (IJCP) 2011; 9: 1-5.
Recknagel R O, Glende E A, Dolak J A, Waller R L; Mechanism of Carbon-tetrachloride Toxicity. Pharmacology Therapeutics. 1989; 43:139-154.
Rehab MSH, Hossain MA (2016) Evaluation of antimicrobial and cytotoxic activities of polar solvents extracts

of leaves of Ammimajus used by the Omanis. Pacific Science Review A: Natural Science and Engineering 18: 62-65.

Rehab MSH, Hossain MA (2016) Evaluation of antioxidant, anti-microbial and cytotoxic activities of seed crude extracts of Ammimajus grown in Oman. Egyptian Journal of Basic and Applied Sciences 3: 329-334.

Reitman, S. and Frankel, S.( 1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 28:56-63.

Rose J, Hulburd J (1992) The aromatherapy book applications and inhalations. North Atlantic Books, Berkeley, California, 94712.

Rose J, Hulburd J (1992) The aromatherapy book applications and inhalations. North Atlantic Books, Berkeley, California, 94712.

Ruqaia M. Al-Ezzy, ZainabYaseen Mohammedand Farah T.O. AlJumaili (2016). Hematological toxic effect and the frequency of micronucleus formation of cyproheptadinediffrent doses on albino male mice blood pictures. IRAQ JOURNAL OF HEMATOLOGY,5 (2): 154-164.

Satrani B, Farah A, Fechtal M, et al. (2004) Chemical composition and antimicrobial and antifungal activities of the essential oil of Ammi visnaga (L.) Lam. Acta Bot Gal 151: 65-71.

Satrani B, Farah A, Fechtal M, et al. (2004) Chemical composition and antimicrobial and antifungal activities of the essential oil of Ammi visnaga (L.) Lam. Acta Bot Gal 151: 65-71.

Sonnenberg H, Kaloga M, Eisenbach N, et al. (1995) Isolation and characterization of an angular-type dihydropyranocoumaring-25. Al-Snafi A (2015) Chemical constituents and pharmacological activities of Ammi majus and Ammi visnaga. A review. Inter J Pharm Ind Res 3: 257-265

Vanachayangkul P, Byer K, Khan S, et al. (2010) An aqueous extract of Ammi visnaga fruits and its constituents khellin and visage prevent cell damage caused by oxalate in renal epithelial cells. Phytomedicine 17:653-658.

Weber L W, Boll M, Stampfl A; Hepatotoxicty and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Critical Reviews in toxicology. 2003; 33(2):105-36.

Zgorka G, Dragan T, Glowniak K, et al. (1998) Determination of furanochromones and pyranocoumarins in drugs and Ammi visnaga fruits by combined solid-phase extraction high-performance liquid chromatography and thin-layer chromatography-high performance liquid chromatography. J Chromatogr A797: 305-309. Ghareeb AM, Zedan TH, Gharb LA (2011) Antibacterial and an-tifungal activities of Ammi visnaga extracts against pathogenic microorganisms. Iraqi J Sci 52: 30-36.

## *POSTER PRESENTATION*

# Quantitative phytochemical analysis of *Inula viscosa* extracts from Izmir, Türkiye

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## Abstract

*Inula viscosa* (L.) is one of the most used medicinal plants in countries of the Mediterranean region. *I.viscosa* has been used for many years in folk medicine for its anti-inflammatory, antipyretic, antiseptic, and antiphlogistic activities, and for different diseases. This study aimed to assay phytochemical analysis and antioxidant activity from different parts and different methods of *Inula viscosa* extract. After 300 kg of plants collected in September 2020 were separated as leaves, flowers, stems, and aerial parts. These different parts were extracted by maceration method, using different solvents (50% ethanol, 70% ethanol, methanol, water) and their % yield was calculated. Thus, it was decided to use the aerial parts of extract obtained by maceration with 70% ethanol solvent for this study. After that, total phenolic compound, total flavonoid compound ratios, and antioxidant activity were evaluated. *Inula viscosa* extract showed antioxidant activity and had a strong correlation with total flavonoid content. Our future goals are to conduct in vitro, in-vivo, and ex-vivo experiments of *Inula viscosa*, to elucidate its molecular mechanisms of action and to investigate its effects on different diseases. Thanks to the increase in studies to be carried out on such medicinal plants, which are frequently used among the public, it is thought that the side effects encountered because of misuse can be minimized by preparing reliable, dose-specific, modern phytotherapeutic preparations. This project has the code 2020.KB.MLT.005 and was supported by the Dokuz Eylül University,Department of Scientific Research Project.

**Keywords:** Inula *viscosa*, Extraction, Antioxidant, Phenolic Compound, Flavonoid Content, Medicinal plants

## INTRODUCTION

Inula, from Compositae (Asteraceae), is a large genus with more than one hundred species. *Inula viscosa* (L.) is one of the most used medicinal plants in countries of the Mediterranean region. *I. viscosa* is a strong-smelling plant. Woody at the base, *I. viscosa* presents at the top of the stem numerous yellow flowered heads. It is represented by 27 species with 7 endemic species in Türkiye flora. *I.viscosa* has been used for many years in folk medicine for its anti-inflammatory, antipyretic and antiseptic activities, and for treating different disease. Inula species revealed a large spectrum of biological and pharmaceutical activities. Pharmacologicaly active compounds of *I. viscosa* are phenolic acids, terpenes (mono-, sesqui-, di-and triterpenes), flavonoids and glycolipids (Gökbulut, 2016).

Maceration, which is one of the most commonly used extraction methods in Inula viscosa, is one of the methods used in the extraction of medicinal plants. In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a certain period until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing (Handa vd., 2008). The obtained contents can be analyzed by different methods.

Several analytical methodologies including modern chromatographic techniques are being used for the chemical standardization of marker compounds or active constituents in herbal preparations (Bansal ve Bansal, 2011).

Antioxidant compounds play an important role for the prevention of damage caused by free radicals. For this purpose, antioxidant compounds of natural and synthetic origin are used. Various in vitro test systems are used for the discovery of compounds of natural origin and synthetic origin with antioxidant effects. One of these test systems is the DPPH radical scavenging method. The DPPH method is one of the most widely used

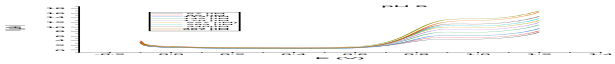
spectrophotometric methods for measuring antioxidant activity. This test is considered a valid, inexpensive, fast, accurate, easy and economical method to evaluate the activity of antioxidants, since the radical compound is stable and does not need to be produced (Ilg, 2016; Seyhan, 2019). Free radical scavenging activities of solvent extracts included in Inula viscosa studies can be determined by DPPH studies (Ouariachi ve Bouyanzer, 2015).
Several reports have revealed that the majority of the antioxidant activity in *Inula viscosa* and many other medical plants may be from biochemicals such as flavonoids, isoflavones, flavones, anthocyanins, catechins and other phenolics (Parthasarathi ve Park, 2015). Therefore, the total phenol contents and total flavonoid compositions of medicinal plants including *Inula viscosa* are analyzed.

## MATERIALS AND METHODS

### Plant Materials and Extraction

In September 2020, 300 kg of Inula viscosa material (whole plant, leaves, stems, flowers, and roots) were collected from Izmir and air-dried in a dark place at ambient temperature for 15 days. After the impurities were separated, the plant was cut into small pieces and dried in the shade at room temperature. The dried plant material was ground in the mill. It was then pulverized and stored at -20$^{o}$C.

5 g of dry powdered plant material sample was extracted using 150 ml of different solvents (50% ethanol, 70% ethanol, methanol, water). Extraction was carried out at 40-50 $^{o}$C for 6 hours with maceration and mixing. The softened extracts were filtered and the solvents were evaporated to dryness at 50-55$^{o}$C under reduced pressure, the concentrated extract solution was poured into petri dishes and incubated at room temperature for 24 hours. A yield percentage of plant extract product was calculated by the following formula (1).



(1)

### Total Phenolic Substance Analysis by Folin-Ciocalteu Method

2.8 mL of deionized water and 2 mL of 2% sodium carbonate were added to the samples at a concentration of 10 mg/mL and kept for 1 minute. Then, 0.1mL 50% Folin-Ciocalteu reagent was added to the mixture and at 25C and kept in the dark for 30 minutes. After that, the absorbance of the mixture was measured at 750 nm. The total amount of phenolic content of the extracts was calculated according to the measurement curve drawn with gallic acid and the regression equation y = 0.0728x + 0.1785.

### Determination of Antioxidant Activity by DPPH Radical Scavenging Method

A solution of 10 mg/mL concentration was setting from the extract prepared with 70% ethanol. Then, 200 µL of the prepared solution was completed to 1 mL with methanol. A final concentration of 400 µg/mL was prepared by adding 4 mL of DPPH's stock solution (4mg DPPH/100 mL methanol) onto 1 mL of extract. The blank sample without extract was prepared with a mixture of 1mL of methanol and 4 mL of DPPH stock solution. Solution was kept in no-light condition for 30 minutes; the absorbance of the extracts was measured against methanol at 517 nm wavelength in a spectrophotometer. Percent inhibition values were calculated by using the absorbance of the extracts at 517 nm against the absorbance value of the blank sample. At last, using the standard curve prepared with α-tocopherol, the antioxidant activity values of the samples equivalent to α-tocopherol were calculated. The % inhibition is calculated by the following formula (2).

**% DPPH inhibition= [(A-B/A)] x 100** (2)

(A= absorbance of the blank, B= absorbance of the extract at 517 nm)

**Total Flavonoid Substance Analysis by Aluminium Chloride Method**

0.5 mL of the sample was taken at 10 mg/ml concentration. Then, 1.5 mL volume of 96% ethanol, 0.1mL of 10% ethanol aluminium chloride solution and 2.8mL of deionized water were added to the sample. Sample was kept in the dark at room temperature for 40 minutes. Then, the absorbances were measured by using spectrophotometer at a wavelength of 415 nm. The determination of total flavonoids was calculated according to the standard measurement curve drawn with quercetin and the calculated regression equation y= 0.0274x-0.021, and the values were found as total flavonoid values equivalent to quercetin (QE).

**RESULTS and DISCUSSION**

As shown in **Table 1.**, the most extractive solvent was 70% ethanol. The highest extraction yield of *I. viscosa* aerial part was the extract from 70% ethanol with %36.1.

The total phenolic compound was determined in comparison with standard gallic acid. As shown in **Table2.**, the most extractible solvent of phenols was ethanol and the highest amount of total phenolics found in I. viscosa aerial part was the extract from 70% ethanol with 18,8%.

Antioxidant activity was determined by DPPH (1, 1-diphenyl-2-picryl hydrazil) radical scavenging method for 70% ethanol extracts. Antioxidant activity of 70% ethanol extract I. viscosa was determined from the reduction of aerial parts. Absorption of DPPH radicals at 517nm. Effective concentrations with 50% radical inhibition activity ($EC_{50}$) were 11.37µg/ml and the scavenging effect (%) on DPPH was 59.50 ± 0.120.

Total flavonoid content determination was made by the Aluminum Chloride method for 70% ethanol extract. Total flavonoid content was found to be 34.74µgQE/ml

As reported, phenolic compounds were attributed to the overall antioxidant activities and they have reserved considered attention because of their physiological function, including cardio protective action and hepatoprotective activity which was mainly attributed to the antioxidant potential that might occur by reduction of lipid peroxidation and cellular damage(Babu vd., 2013; Zhou ve Yu, 2006). Chahmi et al. suggested that the phenolic contents they found were responsible for the free radical scavenging activity of *I. viscosa* (Chahmi vd., 2015).

In our study, similarly with Chahmi et al. *Inula viscosa* extract showed antioxidant activity as expected in the literature and had a strong correlation with total flavonoid content.

**Table 1**. Maceration % Yield

| **Parts of I. viscosa** | **Maceration % Yield** | | | | |
|---|---|---|---|---|---|
| | **Methanol** | **50% Ethanol** | **70% Ethanol** | **96% Ethanol** | **Water** |
| **Leaves** | 26,4 | 25,8 | 31,5 | 25,1 | 19,7 |
| **Flowers** | 18,3 | 13,2 | 19,0 | 17,4 | 12,8 |
| **Stems** | 21,5 | 20,4 | 22,3 | 19,2 | 13,3 |
| **Aerial Parts** | 32,9 | 27,6 | 36,1 | 29,1 | 21,0 |

**Table 2.** Total Phenolic Compound (%)

| Parts of I. viscosa | Total Phenolic Compound (%) | | | | |
|---|---|---|---|---|---|
| | **Methanol** | **50% Ethanol** | **70% Ethanol** | **96% Ethanol** | **Water** |
| **Leaves** | 15,3 | 16,4 | 17,5 | 13,2 | 8,9 |
| **Flowers** | 4,6 | 3,5 | 5,2 | 3,1 | 1,2 |
| **Stems** | 6,7 | 4,8 | 6,5 | 4,0 | 0,8 |
| **Aerial Parts** | 13,6 | 11,5 | 18,8 | 13,0 | 7,5 |

## CONCLUSION

*Inula viscosa* extract showed antioxidant activity and had a strong correlation with total flavonoid content.

Our future goals are to conduct in vitro, in-vivo, and ex-vivo experiments of *Inula viscosa*, to elucidate its molecular mechanisms of action and to investigate its effects on different diseases.

Thanks to the increase in studies to be carried out on such medicinal plants, which are frequently used among the public, it is thought that the side effects encountered because of misuse can be minimized by preparing reliable, dose-specific, modern phytotherapeutic preparations.

## ACKNOWLEDGEMENTS

This project has the code 2020.KB.MLT.005 and was supported by the Dokuz Eylül University,Department of Scientific Research Project.

## REFERENCES

Babu, D., Gurumurthy, P., Borra, S.K., Cherian, K.M., 2013. Antioxidant and free radical scavenging activity of triphala determined by using different in vitro models 7, 2898–2905. https://doi.org/10.5897/JMPR2013

Bansal, Y., Bansal, G., 2011. Analytical methods for standardization of Aegle marmelos : A review. JournL Pharm. Educ. Res. 2, 2–9.

Chahmi, N., Anissi, J., Jennan, S., Farah, A., Sendide, K., El Hassouni, M., 2015. Antioxidant activities and total phenol content of Inula viscosa extracts selected from three regions of Morocco. Asian Pac. J. Trop. Biomed. 5, 228–233. https://doi.org/10.1016/S2221-1691(15)30010-1

Gökbulut, A., 2016. Determination of Hispidulin in the flowers of Inula viscosa ( L .) Aiton Using HPLC and HPTLC Methods. Turk. J. Pharm. Sci. 13, 159–166.

Handa, S., Khanuja, S., Longo, G., Rakesh, D., 2008. Extraction Technologies for Medicinal and Aromatic Plants.

Ilg, S., 2016. Original article Antioxidant Properties and Phenolic Composition of Salvia. Turk J Pharm Sci 13, 201–212.

Ouariachi, E. El, Bouyanzer, A., 2015. Antioxidant activity of solvent extracts of Inula viscosa from Morocco. Arab. J. Chem. ….

Parthasarathi, S., Park, Y.K., 2015. Determination of total phenolics, flavonoid contents and antioxidant activity of different mBHT fractions: A polyherbal medicine. Pak. J. Pharm. Sci. 28, 2161–2166.

Seyhan, S.A., 2019. DPPH•Antioksidan Analizinin Yeniden Değerlendirilmesi. Batman Univ. J. Life Sci. 9, 125–135.

Zhou, K., Yu, L., 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT - Food Sci. Technol. 39, 1155–1162. https://doi.org/https://doi.org/10.1016/j.lwt.2005.07.015

## *POSTER PRESENTATION*

# Electrochemical sensor based on graphene oxide modified glassy carbon electrode for azithromycin determination

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**Abstract**

A voltammetric nanosensor using a glassy carbon electrode coated with a graphene oxide (GO/GCE) has been constructed for a sensitive determination of azithromycin (AZI). The electrochemical behaviour of modified electrode GO/GCE was tested using cyclic and differential pulse voltammetry (CV/DPV) in PBS solution pH 8.5. The nanomodified sensor exhibits an enhanced effectiveness for the oxidation of azithromycin and produce an anodic peak at about 0.8 V. The sensitivity, selectivity, limit of detection, linear range and repeatability of the proposed method were also evaluated. The analytical performance of the GO/GCE sensor under optimal condition assessed through differential pulse voltammetry proved to be in the linear range of 4.4 µM to 396 µM with the detection of limit 3.8 µM and correlation coefficient 0.969 for Azithromycin. The sensor is reproducible with the relative standard deviation for four repetitive measurements in 57 µM Azithromycin were found to be 2.7% respectively. The proposed method was successfully applied in the pharmaceutical dosage forms and results indicate that the nanomodified electrode (GO/GCE) could be a reliable sensor for azithromycin determination.

**Keywords:** Azythromycine, Graphene oxide, Nanosensor, Diferential pulse voltammetry, Glassy carbon electrode

## INTRODUCTION

Graphene has grabbed exceptional research importance owing to their high electronic conductivity, thermal stability, high mechanical strength, processability, distinguished biocompatibility, large unique surface area, possibility for surface functionalisation, minimal cost and ease of preparation (Pumera 2011), (Geim and Novoselov, 2007), (Huang et al., 2011). GO contains several oxygen functional groups which decorated all over the graphitic structure and introduced $sp^3$ -hybridised carbon within the sp2 -hybridised carbon network of graphite (Ambrosi et al., 2014). Waterloving nature of GO is rendered by the oxygen containing functional groups of GO. Hence, GO shows good dispersibility in water and some polar solvents. The structural defects arising from covalent oxygen functionalities mostly epoxy and hydroxyl groups makes it very fascinating for electrochemical studies and executions (Leila Shahriary, 2014).
Azithromycin (AZI), as a novel macrolide antibiotic, has a broad-spectrum antibacterial activity. It mainly inhibits the process of bacterial transpeptidation to inhibit the synthesis of proteins in bacteria, thereby acting as an antibacterial agent. It has the characteristics of long half-life and high tissue drug concentration. It can be used clinically to treat various infections such as urinary system infection and respiratory system infection. However, when the accumulation of AZI in the body exceeds a certain amount, it will bring a series of side effects such as cardiovascular death, gastrointestinal and nervous system damage, and anaphylactic shock (Patil et al., 2019) Abdel-Hamid and Newair (2015). Excessive use of antibiotics leads to the resistance of bacteria strains to them, allergic reactions, liver damage, yellow teeth, and digestive disorders. It also results in the transfer of genes resistant to antibiotics and can potentially cause cancers in those who consume animal source foods contaminated with antibiotics (Han et al., 2015). So far, several analytical methods have been reported for determination of antibiotics and pharmaceuticals, such as spectrophotometry (Chavada et al., 2017), HPLC (Ibrahim et al., 2017), atomic-spectroscopic (Viñaset et al., 2014), electrochemiluminescence (Hu et al., 2018), and electrochemistry (Benvidi et al., 2018; Wang et al., 2017). These methods are time-consuming, sample preparation is expensive, and large amounts of specimens are needed. Among different sensing methods, electrochemical technique has received considerable attention in analytical and bioanalytical chemistry. This is due to its being fast, simple, and cost-effective as well as its high selectivity and

reproducibility (Wang et al., 2017; Aghili et al., 2017), Broli et al., (2020), (2021) (2022). Cui et al. fabricated a graphene/gold nanocomposite modified glassy carbon electrode (GCE) for the sensitive quantification of EPN in the presence of vitamin C (ascorbic acid (AA) (Cui and Zhang, 2012).Tezerjani et al. developed hydroquinone derivatives of graphene oxide nanocomposite sensor for EPN, from the mixture of acetaminophen (AC) and dopamine (DP) (Tezerjani et al., 2017). These examples clearly illustrate that the graphene based materials contribute a good platform for electrochemical analysis of diffrent drugs. The propose of the present study is to develop a simple and sensitive method for the determination of azithromycin based on the high conductivity effect of carbon nanomaterials (graphen oxide) on the surface of glassy carbon electrode using voltammetric technique; CV and DPV. Under optimal condition the analytical performance of nanocomposite sensors toward azithromycin were evaluated. Obtained result showed that GO/GCE sensor exhibits high sensitivity, good reproducibility and could be a good alternative for quantitative detection of azithromycin in real sample.

## MATERIALS AND METHODS

*Reagents and Chemicals*

Reagents are of analytical grade and are used without the purification procedure. All solutions were prepared using double distilled water. Antibiotic Azithromycin (AZT) (with a purity of 90%). Graphene oxide nanomaterial GO was purchase by *PubChem* company supplier. The paraffin oil (Olio di Vaselina) used was from Zeta farmaceutici company and the graphite powder synthetic with particles size 90–71 μm was from Alfa Aesar (99.9%). Dipotassium phosphate, and dihydrogen phosphate were purchased from Merck (99% Merck). Acetate buffer solution 0.1 M (pH=5) was prepared using right quantities of $CH_3COOH/CH_3COONa$ and dissolved in aqueous solution. Phosphate Buffer Solution 0.1 M (pH =7) prepared by mixing suitable amounts of 0.1 M $K_2HPO_4$ x $3H_2O$ and $KH_2PO_4$, KCl. PBS, 0.1 M (pH=8.5) was prepared using aqueous solutions of 0.1 M $Na_2HPO_4$ and adjusted with 0.1 M NaOH to the desired pH value. Stock solutions of Azithromycin with the concentration of 13.00 mM was prepared by dissolving suitable amounts of solid standards in distillate water; was stored in the refrigerator at 4–6 °C when not in use. Working solutions of lower concentrations were freshly prepared on the day of experiment

*Apparatus*

The voltammetric measurements were performed with the Palm Sens 4 electrochemical analyzer using the three system electrode, whereas the working electrode was used the glassy carbon electrode modified with grapheme oxide. As reference electrodes were used Ag + / AgCl / Cl- and platinum wire as auxiliary electrodes. For the determination of AZI was used electrochemical technique differential pulse voltammetry (DPV). The voltammograms were recorded from -0.2 to 1.2 V (vs. SCE) in PBS buffer pH 8.5. Experimental condition: pulse amplitude 50 mV and scan rate 50 mV/s and step potential 5 mV. In the electrochemical cell containing 25 ml of PBS solution 0.1 M, the background current is recorded after a rest period of 10 sec, and then voltammograms are recorded after each addition of the standard solution of azithromycin. The same procedure was used for all experimental measurement and was repeated three times. The measurements were performed at room temperature.

### Pretreatment and Preparation of working electrodes (GO/GCE)

Firstly the surface of glassy carbon electrode was polished starting with aluminum oxide powder of larger sizes and continuing after the other with smaller sizes (1.0 μm, 0.5 μm, 0.05 mμ), on a polishing cloth until a mirror-like finish was obtained. The electrode was then thoroughly rinsed with double-distilled water and cleaned in an ultrasonic bath for 15 s. To prepare the modified glassy carbon electrode, first was prepared the alcoholic modifying suppsesion solution: GO-Nafion: In 10 mg of GO add 10 ml of distilled water and then place it in ultrasonic for 30 min. Then it was taken 50 μL of the prepared solution and was added 10 μL Nafion 2.5%, also it was placed in ultrasonic for 30 min. From the prepared mixture was taken 20 μL volume and was dripped on the surface of the glassy carbon electrode. The modified electrode was keept for 24 hours in an alcoholic environment. The electrode was then cycled between –0.2 and +1.2 V at a scan rate of 50 mV/ s until a stable current–voltage profile was obtained.

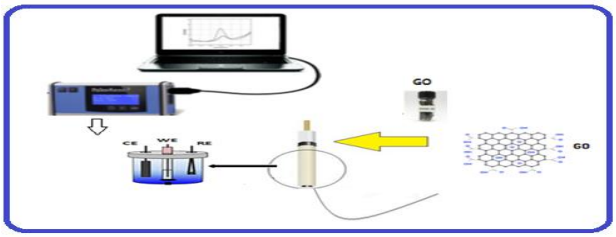


**Figure 1.** Schematic Preparation of GO/GCE Sensor and measurements

### Pharmaceutical preparations

Azithromycin tablets were purchased in local casual pharmacy. The declared amounts of azithromycin are 250 mg per capsule. The contents of ten capsules were crushed in a mortar pestle. An accurately weighted portion of the powder, equivalent to 250 mg of azithromycin, was transferred into a 50 ml volumetric flask. About 25 ml of methanol (99.8%) was added to dissolve the active material. For analys an aliquot of the clear supernatant was transferred to an electrochemical cell and a series of dilutions was made with PBS buffer pH 8.5 as a supporting electrolyte and analyzed with the DPV technique Azithromycin content was determined using the method of standard additions and a calibration curve

## RESULTS AND DISCUSSION

### Electrochemical behavior of modified nanocomposite sensor GO/GCE

The aim of this study was to improve the analytical performance of a glassy carbon electrode using differential pulse voltammetry for determination of azithromycin. In order to improve the electrochemical response of GCE toword the drug, the surface of electrode was modified (by coating) with graphene oxide (carbon nanostructed material).

The effect of nanomodifiers at GCE/GO response was estimated by comparing the electroanalytical signal of modified sensor in 1 µM Fe (II) solution, with the bare electrode GCE, by using CV and DPV at potential -0.2 to 1.0 V vs Ag/AgCl, in acetate buffer solution 0.1 M pH 5.

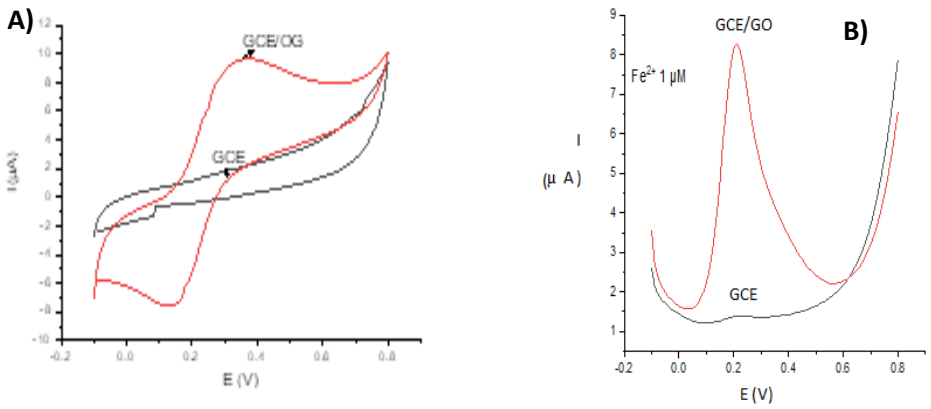


**Figure 2.** The cyclic voltammograms (A) and (B) differential pulse voltammograms obtained with GCE and GCE/GO, at 1 µM Fe (II), in acetate buffer (pH 5), scan rate 50 mV/s

In cyclic voltammograms CV obtained with the modified electrode, a pair of well-defined, quasi-reversible redox peaks at –0.2 V and 0.4 V was observed. The reversibility and the height of this pair of redox peaks were related to the modification of GCE electrode with GO, which is known for high conductivity property (Pumera, 2011; Huang et al., 2011). The same behavior of modified sensor GO/GCE was obtained with differential pulse voltammograms, the oxidation peak of Fe (II) was appear at potential 0.3 V and a higher current intensity was obtained compared with the bare one GCE.

The electrochemical behavior of modified sensor GO/GCE was evaluated toward azithromycin using

voltammetric techniques: CV, DPV and SWV (square wave voltammetry) in PBS 0.1M solution pH=8.5. The registered voltamograms with DPV, CV and SWV are shown in following figure.

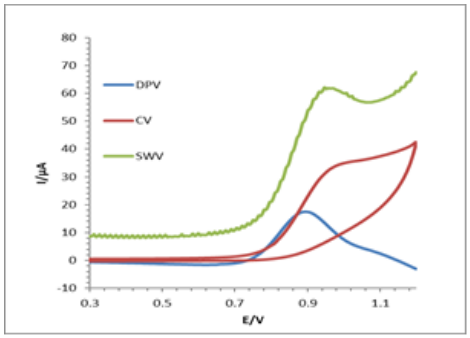


**Figure 3.** DPV, CV and SWV voltamogramms obtained with GO/GCE in PBS buffer pH 8.5, at concentration of azithromycin 99 µM.

As can be observed in fig 3 the oxidation peak of azithromycin using three technique : DPV, SWV and CV was apear at potencial 0.8 V. In cyclic voltamogram, an anodic peak owing to the oxidation of tertiary amino group on the desosamine sugar residue was observed.( Patil et al., 2019) In a reverse scan, no cathodic peak was noticed, which indicates that the azithromycin oxidation is irreversible. Regarding signal of AZI obtained with SWV and DPV technique the more sensitiv signal, with well defined oxidation peak and with a low backgraund was obtained with DPV technique. This technique was used in futher experiments.

**Effect of pH, scan rate on peak parameters of AZI**

The pH of supporting electrolite has a great impact on the electrochemical reaction of AZI at electrode surface interface. The influence of the pH on the voltammetric response was tested between pH 6 and 8.5. From a previous study, it was found that the value of the peak current without adsorption increased in the pH range of (6 – 8), and then decreased up to alkaline pH conditions (Abdel-Hamid and Newair, 2015). In figure 4 are shown DPV voltammograms at different concentration of AZI registred in PBS solution pH=6 and pH 8.5.

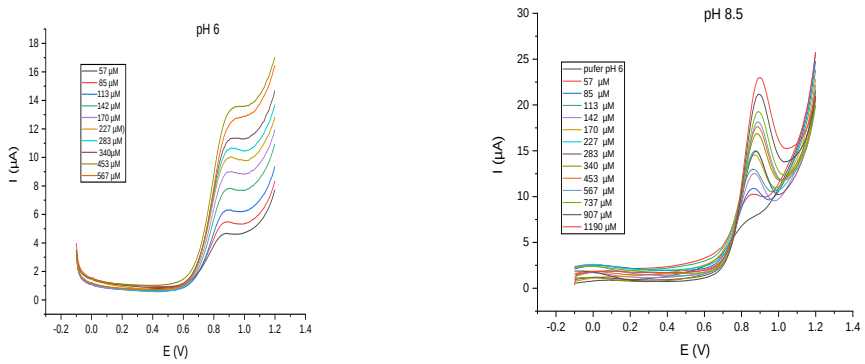


**Figure 4.** The DP voltammograms obtained with GO/GCE at concentration of AZI from 57µM to 567 µM, in PBS (pH 6 and 8.5), scan rate 50 mV/s.

The best analytical performace of GO/GCE toword AZI could be attained in a PBS 0.1M solution at pH 8.5. This pH value was selected for the other measurmets. In order to find the optimal conditions for the quantification of azithromycin, the effect of instrumental parameters on the response of the GO/GCE using differential pulse technique were studied. The best peak definition was found when using a 30 mV pulse amplitude, a 50 mV/s scan rate and a 30 ms pulse width.

***Calibration plots***

Under the optimum analytical conditions, the performance of modified sensor GO/GCE using differential pulse peak current at different concentrations of azithromycin was evaluated. In table 1 are summarizes the characteristics of the calibration plots.

**Table 1** Analytical parameters of the calibration plots for the determination of azithromycin using the proposed method

| Parameters | GO/GCE | GCE |
|---|---|---|
| Measurements (n) | 3 | 3 |
| Standard Deviation Sb (µM) | 0.05 | 0.1 |
| Relative standard deviation (%) | 2.7 | 4.2 |
| Sensitivity (µA/µM) | 0.04 | 0.02 |
| Correlation coefficient (R2) | 0.969 | 0.970 |
| Linearity range (µM) | 4.4-396 | 11.3- 113 |
| Limit of detection (µM) | 3.8 | 30 |

The variation of current intensity with AZI concentration was studied, and the results are shown in Figure 5. It can be seen from Figure 5(A) that the current intensity increases with the increase of the AZI concentration. The linear regression analysis of the AZI concentration by the current intensity shows that the AZI concentration is within the range of 4.4 -396 µM. It shows a good linear relationship with current intensity (Figure 5(B). The linear corresponding equation is y =0.0392x + 4.15 (R2 = 0:969), and the limit of detection (LOD) was calculated about 3.3 µM (S/N = 3).

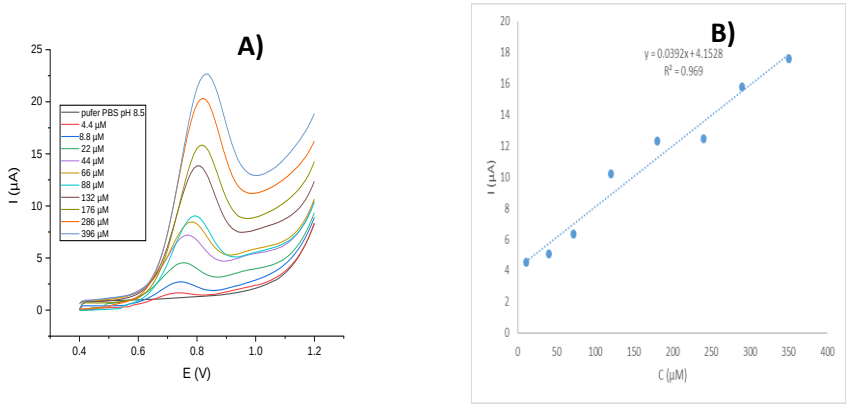


**Figure 5. (A)** Differential pulse voltammograms for modified sensor recorded in different concentration of AZI, in PBS buffer pH 8.5, amplitude 30 mV and step potential 5 mV, scan rate 50 mV/s **(B)** Corresponding calibration curves (n=3);

**Determination of azithromycin in pharmaceutical samples**

The determination of the azithromycin content in pharmaceutical formulations was performed with GO/GCE sensor using the differential pulse voltammetry. The content of azithromycin in the tablets was determined by the standard addition method and resulted to be 253 ± 40 mg per tablet, indicating adequate precision and accuracy of the proposed method. In table 2 are shown the recovery result for required amount of AZI spiked in tablet sample.

**Table 2.** Results of recoveries of real samples spiked with different concentrations of AZI

| Sensor | Spiked AZI (µM) | Detected (µM) | Recovery (%) |
|---|---|---|---|
| GO/GCE | 22 | 19.8 ±0.6 | 90 |
| | 44 | 48 ±0.8 | 109 |

The percentage recovery values obtained using GO/GCE was in the acceptable range, indicating that the method established in this paper can be used to determine the AZI in the pharmaceutical sample.

## CONCLUSIONS

In the present study, we designed a modified glassy carbon nanosensor (coated) with a graphen oxide (GO/GCE) for a sensitive detection of azithromycin by using cyclic CV and differential pulse voltammetry DPV. Immobilization of a nanomodifier on the surface of glassy carbon electrode improve the analytical performance of modified GO/GCE sensors. The enhanced of sensitivity of GO/GCE toward azithromycin is attributed to improved modified sensor conductivity and increased the electron transfer of the reaction. The optimal experimental and working parameters for voltammetric determination of azithromycin with GO/GCE sensor were obtained with DPV technique, in the PBS buffer solution pH 8.5, with scan rate 50 mV/s . The modified sensor (GO/GCE) was used to determine azithromycin in pharmaceuticals tablets. The proposed method is an attractive method due to the low cost of the instrumentation and the short time required for the analysis.

## REFERENCES

Abdel-Hamid R and Newair EF 2015. Voltammetric determination of ferulic acid using polypyrrole-multiwalled carbon nanotubes modified electrode with sample application. Nanomaterials, 5(4), pp. 1704–1715.

Ambrosi A, Chua CK, Bonanni A, and Pumera M 2014. Electrochemistry of Graphene and Related Materials. Chem. Rev, 114:14, pp. 7150–7188.

Benvidi A, Yazdanparast S, Rezaeinasab M, Tezerjani MD, Abbasi S 2018. Designing and fabrication of a novel sensitive electrochemical aptasensor based on poly (Lglutamic acid)/MWCNTs modified glassy carbon electrode for determination of tetracycline. J. Electroanal. Chem. 808 311–320.

Broli N, Vallja L, Vasjari M, Shehu A and Duka S 2021. Electrochemical Behaviour of Gold Thin Film Glassy Carbon Electrode for Determination of Chromium (VI). Asian Journal of Chemistry; ISSN: 0970-7077, Vol. 33: 5 , pp.1001-1005. https://doi.org/10.14233/ajchem.2021.23121

Broli N, Vasjari M, Vallja L, Shehu A, Duka S, Cenolli S 2022. A carbon paste modified with banana tissue for determination of atenolol in pharmaceutical tablets. Journal of Hygienic Engineering and Design, ISBN/ISSN: 1585-8489, 37, pp. 149-154.

Broli, N, Vasjari M, Vallja L, Duka S, Shehu A and Cenolli S 2021.Electrochemical Determination Of Atenolol And Propranolol Using A Carbon Paste Sensor Modified With Natural Ilmenite. Open Chemistry, ISSN: 2391-5420, vol. 19, no. 1, pp. 875-883; https://doi.org/10.1515/chem-2021-0071

Chavada VD, Bhatt NM, Sanyal M, Shrivastav PS 2017. Surface plasmon resonance based selective and sensitive colorimetric determination of azithromycin using unmodified silver nanoparticles in pharmaceuticals and human plasma, Spectrochim. Acta A Mol. Biomol. Spectrosc. 170: 97–103.

Cui F and Zhang X 2012. Electrochemical sensor for epinephrine based on a glassy carbon electrode modified with graphene/gold nanocomposites J. Electroanal. Chem., 669: pp. 35-41

Geim AK, Novoselov KS 2007. The rise of grapheme. Nat. Mater. 6: pp. 183–191.

Han R, Zheng N, Yu Z, Wang J, Xu X, Qu X, et al 2015. Simultaneous determination of 38 veterinary antibiotic residues in raw milk by UPLC–MS/MS. Food Chem. 181: 119–126.

Hu L, Zhou T, Feng J, Jin H, Tao Y, Luo D, et al 2018. A rapid and sensitive molecularly imprinted electrochemiluminescence sensor for azithromycin determination in biological samples. J. Electroanal. Chem. 8131–8

Huang X, Yin Z, Wu S, Qi X, He Q, Zhang Q, Yan Q, Boey F, and Zhang H 2011. Graphene-Based Materials: Synthesis, Characterization, Properties, and Applications. Small, 7, 1876.

Ibrahim FA, Wahba MEK, Galal GM 2017. Two spectrophotometric methods for the determination of azithromycin and roxithromycin in pharmaceutical preparations. Eur. J. Chem. 8 (3): 203–210

Jafari S, Dehghani M, Nasirizadeh N, Akrami HR 2017. Voltammetric determination of basic red 13 during its sonoelectrocatalytic degradation. Microchim. Acta 184 (11): 4459–4468.

Leila Shahriary AA 2014. Graphene Oxide Synthesized by using Modified Hummers Approach

Int. J. Renew. Energy Environ. Eng, 02: 01, pp. 58-63

Patil SD, Dugaje T, and Kshirsagar SJ 2019. Development and validation of UV spectrophotometric method for estimation of cyproheptadine hydrochloride. Asian Journal of Research in Chemistry, 12 (2), pp. 112–115.

Pumera M 2011. Graphene in biosensing, Material today. 14: (7-8), pp. 308-315.

Tezerjani MD, Benvidi A, Dehghani Firouzabadi A, Mazloum-Ardakani M, Akbari A, 2017. Epinephrine electrochemical sensor based on a carbon paste electrode modified with hydroquinone derivative and graphene oxide nano-sheets: Simultaneous determination of epinephrine, acetaminophen and dopamineMeas. J. Int. Meas. Confed., 101: 183-189

Viñas P, Campillo N, López-García I, Hernández-Córdoba M 2014. Dispersive liquid–liquid microextraction in food analysis. A critical review, Anal. Bioanal. Chem. 406 (8): 2067–2099.

Wang H, Yao S, Liu Y, Wei S, Su J, Hu G 2017. Molecularly imprinted electrochemical sensor based on au nanoparticles in carboxylated multi-walled carbon nanotubes for sensitive determination of olaquindox in food and feedstuffs, Biosens. Bioelectron. 87: 417–421.

## *POSTER PRESENTATION*

# Comparison Of The Physico-chemical Parameters Of Pasteurized And Raw (Unpackaged) Cow's Milk

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## Abstract

Milk is a highly nutritious food, and it is a source of necessary macro and micro-nutrients for the growth, development and maintenance of human health. In Albania, non-packaged (without thermal treatment) milk continues to be an alternative for its sale and remains preferred by a large part of consumers. The aim of this study is to compare of the quality of pasteurized and raw milk, sold in Tirana, based on physico-chemical parameters. So, our study is focused on observing the difference between these analysed parameters. Physico-chemical parameters determined by Lactoscan are: density, fat, protein, lactose, added water, solid non fat etc., pH and acidity were also determined for each samples. Based on the results of physico-chemical parameters, we can conclude that pasteurized milk is more appropriate for consumption as compared to the lower quality raw milk. The problematic parameter of unpackaged milk remains the added water. Since milk s a great importance in human diet, particularly to infants and children, its quality and safety become a major concern for consumer health.

**Keywords**: physico-chemical parameters, milk, Tirana.

## INTRODUCTION

Milk used as food material has a great importance for the nutrition of all people from young to old. Additionally, milk is the main source of protein, carbohydrate, fat, and mineral matter necessary for healthy growth and is the sole source of nutrition for feeding newborn organisms. (Maijala, 2000)

Raw milk may be contaminated with microorganisms from the animal, cowshed, humans, milking machines, air, and tools and equipment used, and these microorganisms may rapidly proliferate if milk is not stored under appropriate conditions. (Özdemir 2020) Moreover, the insufficient cold capacity and long storage times can also increase the bacterial count owing to the bacterial growth during milk storage. (Elmoslemany et al. 2009)

During the storage time (especially in the room temperature), the physico-chemical parameters of raw milk change by microorganismis activity. (Deeth and Fitz-Gerald, 2006). Pasteurization aims to reduce the number of viable pathogens so they are unlikely to cause disease (Hassan, 2009).

Milk is also an important vehicle for transmission of pathogenic microorganisms to human beings unless it is produced and handled under good hygienic conditions. Thus, hygienic production of milk has to get due attention in order to provide more and better quality milk for the general public (Dehinenet et al. 2013).

Raw milk sold by the villagers stays for a long time without refrigeration facilities and is stored only at room temperature. The purpose of this study is to compare the quality of milk, based on the physicochemical properties of pasteurized milk produced by Albanian company's and fresh milk sold by villager. The fresh (raw) milk is still an alternative and remains preferred by a large proportion of consumers, due to its lower price. The results were also compared with milk composition reported by the FAO (Food and Agriculture Organization) standards for nutritional quality of milk.

## MATERIALS AND METHODS

### Sample collection

Two types of milk samples, pasteurized milk and fresh (raw) milk were purchased in Tirana in May 2022. Experiments were performed using five locally produced pasteurized milk samples and five samples of unpacked and non-heat-treated that sold by the villagers. First, all the samples were analyzed with milk analyzer and are tested for the presence of alkaline phosphatase. Then the untreated samples were pasteurized in laboratory conditions and analyzed again with the milk analyzer and tested for the presence of alkaline

phosphatase. The characteristics of various milk samples were determined shortly after they were brought to the laboratory.

**Physicochemical Properties of Milk**

Physicochemical parameters were determined with Lactoscan milk analyzer. This apparatus measures some characteristics of milk such as: density, fat, solid non-fat (SNF), lactose, protein and added water. The milk analyzer is simple to use, fast and low cost, it also requires a small sample quantity.

The temperature and pH value of the milk samples was determined by portable pH meter (Testo 205, Germany) (AOAC, 2005). The pH meter was firstly calibrated using buffers of pH 7.0 and 4.0 each time before the pH of milk sample was measured.

Titratable acidity

Titratable acidity of the milk samples was determined according to the method of the Association of Official Analytical Chemists (no. 947.05; AOAC, 1990). Nine ml of milk sample was pipetted into an erlenmeyer flask and 3 to 5 drops of 1 % phenolphthalein indicator was added to it. The milk sample was then titrated with 0.1N NaOH solution until a faint pink color persisted. The titratable acidity, expressed as % lactic acid, was finally calculated using the following formula.

$$\text{Titratable acidity (\%)} = \frac{\text{0.1 N NaOH (ml) x 0.9}}{\text{Weight of milk sample}}$$

**Determination of alkaline phosphatase in cow's milk by rapid test**

For a quick check of correct pasteurization of milk is a valuable auxiliary a rapid test as Phosphatesmo MI (Macherey-Nagel, Germany). This test has a limit of sensitivity: 0.5 % raw milk in pasteurized milk. This test was performed on all samples with pasteurized milk according to the procedure: 1. Dip test strip briefly into the milk. 2. Shake off excess liquid. 3. In order to prevent the test pad of the test strip from drying out, place the test strip in the enclosed PE bag. Use a new bag for each test to avoid cross-over contamination. 4. Incubate the test strip at 36 °C for 1 h.

In the presence of alkaline phosphatase, the test field turns yellow. For control measurement use raw milk and heated milk. A yellow coloration indicates that raw milk is present, or the milk was not sufficiently heated. No coloration indicates that the pasteurization was correctly completed.

**RESULTS and DISCUSSION**

Some factors such as density, fat, total protein, total solid non fat, lactose, added water, ph and acidity are important parameters in studying the physicochemical compositions and nutritional aspects of milk. The data presented are the average values of analyzed parameters.

The physicochemical parameters of five pasteurized milk samples are shown in Figure 1. We can show that the sample 4 has the lowest values of SNF, density, protein, lactose, because it has the highest value of the added water parameter of about 6%. While the sample 5 has highest values for these parameters, and the added water 0.0 %. The pH and acidity are respectively 6.6 and 0.13-0.14 , for all the samples of pasteurized milk.

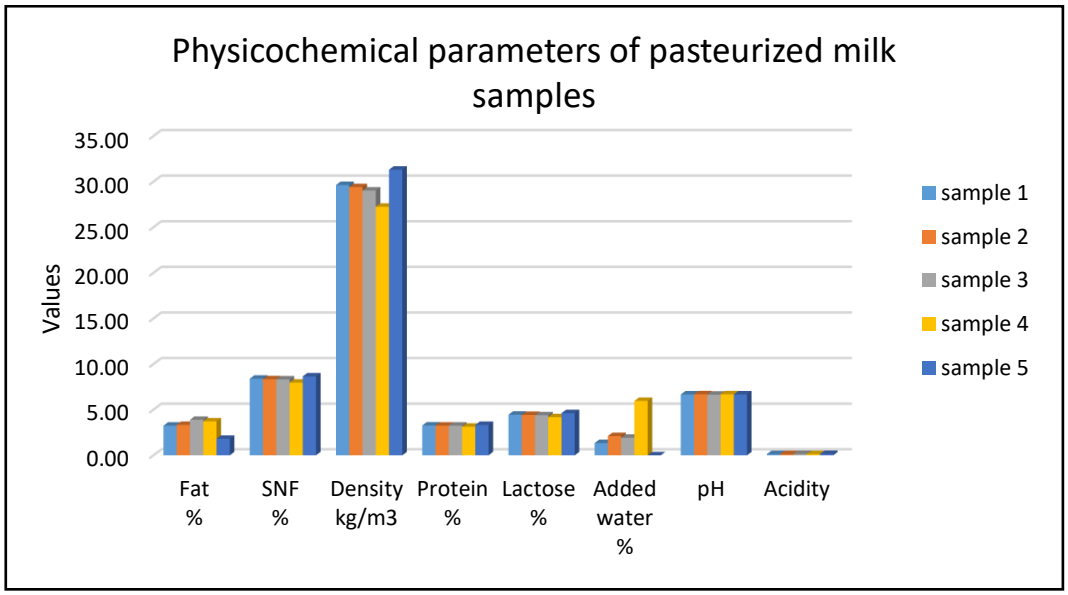


**Figure 1.** Physicochemical properties of pasteurized milk samples

The physicochemical parameters of five raw milk samples are shown in Figure 2. The samples 2 and 5 have the lowest values of SNF, density, protein, lactose, because it have the highest value of the added water parameter of about 25% and 12 % respectively. While the sample 4 has highest values for these parameters, and the added water 0.0 %. The pH and acidity are respectively 6.5-6.7 and 0.13-0.18, for all the samples of raw milk. We can show that four from five samples have high value of added water to 5-25 %.

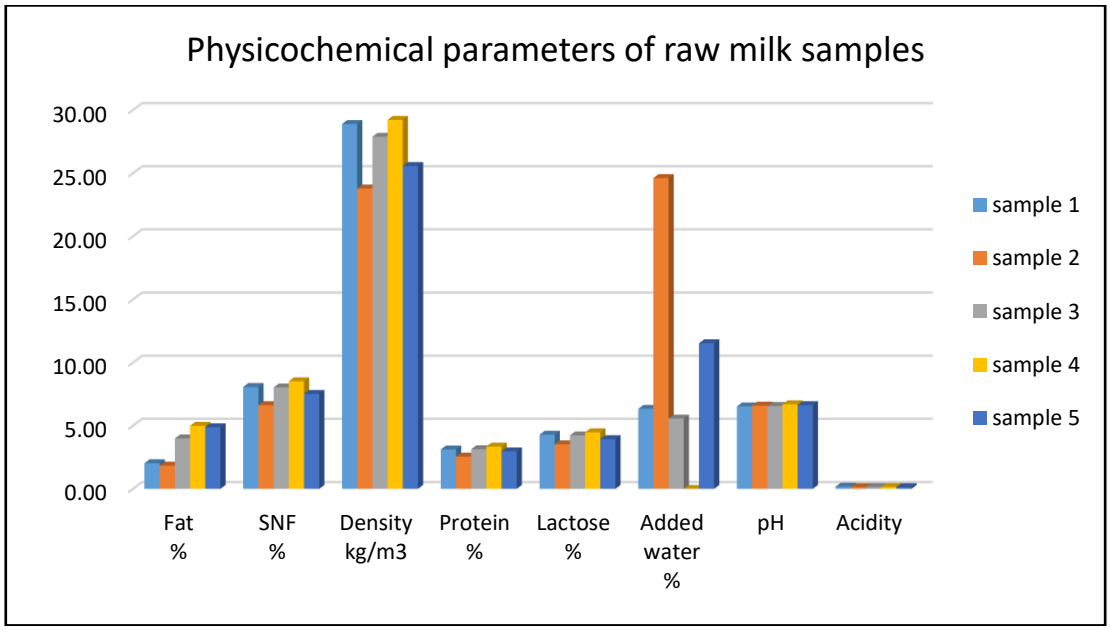


**Figure 2.** Physicochemical properties of raw milk samples

Raw milk samples were boiled for 5 minutes, after the analysis of physicochemical parameters. Also, the physicochemical parameters were measured again in the milk samples after boiling, and the results are show in the Figure 3.

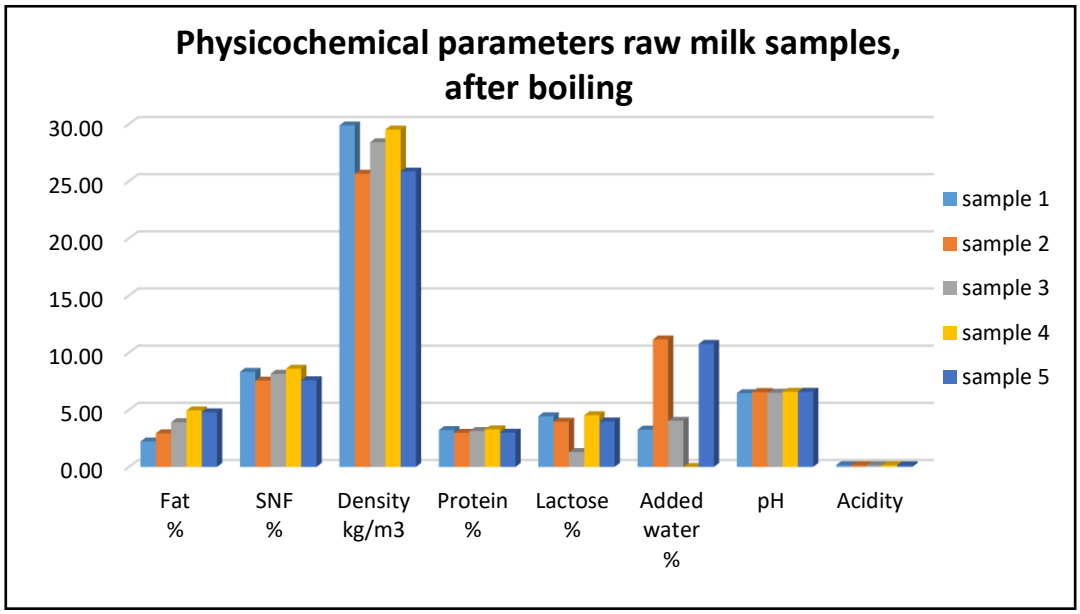


**Figure 3.** Physicochemical properties of raw milk samples after boiling

From the results we see that we have a decrease in the values of added water (in the sample 1 and 2, it is halved) and normally an increase in density values and other parameters. The pH remains between 6.5-6.6, while the acidity is slightly decreases a little to 0.14-0.15, for raw milk samples after boiling. The smallest changes in parameters after boiling are represented by sample 4, which had no added water.

For the physicochemical parameters that we have analyzed from the milk samples, FAO has set certain ranges, which a cow's milk must have to be of quality for the consumer, present in Table 1.

**Table 1.** Values of physico-chemical parameters that quality cow's milk must have

| **Parameters** | **Fat %** | **SNF %** | **Density kg/m$^3$** | **Protein %** | **Lactose %** | **Water %** | **pH** | **Acidity %** |
|---|---|---|---|---|---|---|---|---|
| **Values** | 3.1 - 3.3 | 8.4 - 10.1 | 27 - 33 | 3.2 - 3.4 | 4.5 - 5.1 | 87.3 -88.1 | 6.6 - 6.7 | 0.12 - 0.19 |

Comparing the values obtained from the analysis of pasteurized milk samples with those of the standards set by FAO, we see that sample 4 has the largest deviation of all the pasteurized samples, this is because it has the highest percentage of water added. So, sample 4 is not quality milk.

Also, comparing the values obtained from the analysis of raw milk samples after boiling, with those of the standards set by FAO, we see that samples 2 and 5 have the largest deviation of all samples, this is because it has the highest percentage of water added. Samples 1 and 2 are very close to the lower limits of a quality milk. So, only sample 4 is a quality milk.

The alkaline phosphatase test was performed on pasteurized milk samples. After incubation, test strips were observed and the color intensity was compared with the initial color of the strips before incubation. From the results shown in the Figure 4, none of the pasteurized milk samples changed in color. But all the raw milk samples are underwent a change in color, as also shown in Figure 5. This indicates that the villagers did not heat treat the milk samples before selling them.

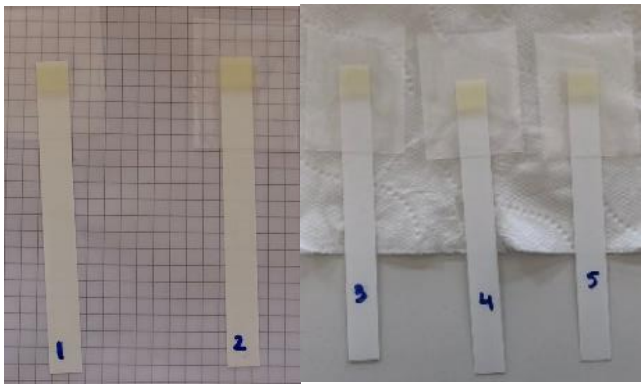


**Figure 4**. Result of phosphatase test in pasteurized milk

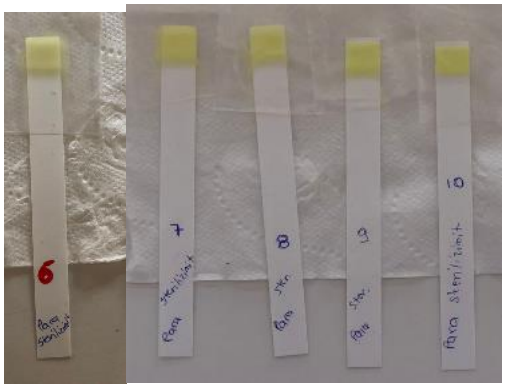


**Figure 5**. Result of phosphatase test in raw milk

This test was also performed on raw milk samples after boiling and showed that the heat treatment time was sufficient to reduce the presence of alkaline phosphatase.

## CONCLUSION

- Based on results of physicochemical properties, we conclude that the pasteurized milk is of better quality than raw milk that sold by villagers.
- In 80% of the pasteurized milk sold for consumption in the Albanian market, the physico-chemical parameters are generally stable referring to the international standard of FAO. But only 60% of raw milk samples approached the FAO standard
- The most problematic parameter in the raw milk remains the added water
- In all pasteurized milk samples, the pasteurization was done properly, since the presence of alkaline phosphatase was not found.
- The presence of alkaline phosphatase was found In all samples of raw milk, so the villagers did not heat it before selling it to increase shelf life.
- These findings may be helpful for the Albanian consumer (to help them choose milk quality) and government stakeholders (to monitor the quality of the milk and to prevent villagers selling raw milk direct to the consumer).

## REFERENCES

AOAC (2005). “Official methods of analysis”. The association of official analytical chemists. 16th edition. 481.North Fredrick Avenue Gaithersburg, Maryland, USA,

AOAC (1990). “Official Methods of Analysis”. Association of Official Analytical Chemists Inc., Virginia, USA.

Deeth HC, Fitz-Gerald CH, (2006) Lipolytic enzymes and hydrolytic rancidity. In: Advanced Dairy Chemistry –2 Lipids, 3rd edn (eds P.F. Fox and P. McSweeney), pp. 481–556.

Dehinenet, G, Mekonnen H, Ashenafi M and Emmanuelle G, 2013. Determinants of raw milk quality under a smallholder production system in selected areas of Amhara and Oromia National Regional States, Ethiopia, Agric. Biol. J. N. Am., 4:84-90

Elmoslemany AM, Keefe GP, Dohoo IR, Jayarao BM, 2009. Risk factoris for bacteriological quality of bulk tank milk in Prince Edward Island dairy herds. Part 1: Overall risk factors. Journal of Dairy Science, 92: 2634-2643.

FAO 2013. Milk and dairy products in human nutrition, Food And Agriculture Organization Of The United Nations, Chapter 3. Available at: https://www.fao.org/3/i3396e/i3396e.pdf

Hassan A, Amjad I, Mahmood S, 2009. Microbiological and physicochemical analysis of different UHT milk available in a local market. Asian Journal of Food & Agro-Industry, 2 : 434-447.

Maijala K, 2000. Cow milk and human development and wellbeing. Livestock Production Science, 65: 1-18.

Özdemir D, Tahmas Kahyaoglu D, 2020. Identification of microbiological, physical, and chemical quality of milk from milk collection centers in Kastamonu Province. Turkish Journal of Veterinary and Animal Sciences, 44: 118-130.

***POSTER PRESENTATION***

# Energy produced by photovoltaic panels and their chemical composition

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## Abstract

A solar cell, also known as a photovoltaic cell, is a miracle of nature and engineering that transforms incident photon energy into electrical energy. A frame module, commonly referred to as a solar panel, can be attached to a different cell unit. The increased use of photovoltaic panels in our nation is a result of the rising demand for solar energy generated by them. The monthly consumption and photovoltaic panel efficiency for a family living in the city of Elbasan were measured for the study. The type of panel used is monocrystalline on grid 11.9 kWp on a surface of 65 $m^2$, with till 17grade direction southwest. The time period studied includes May to October 2022. From the data obtained, it was observed that the highest production was in July with 1857.61 kWh, followed by 1707.30 kWh in June, 1688.83 kWh in May, and 1606.33 kWh in August. We have a decrease in production in September with 1370.12 kWh and in October with 1189.59 kWh. In these 6 months, the day with the highest production was 18.06.2022 with 67.23 kWh and the day with the least production was 11.10.2022 with 08.13 kWh. In this study, a comparison was also made regarding the energy produced and consumed. It is noted that the production of these panels varies from month to month and this depends on many factors such as the weather, the angle formed by the light with the Solar panel, the positioning of the panels, and the structure of the panels. The information provided in this 'Photovoltaic Report' is very concise and the aim is to provide a rough overview of electrochemical performance, production technology, monthly savings, and environmental impact.

**Keywords:** Panel, photovoltaic, production, consumption, chemical composition.

## 1. INTRODUCTION

In photovoltaic, or solar, cells, the conversion of light to energy is based on the photovoltaic (PV) effect. The PV effect can be summarized as follows: Light, which is pure energy, enters a photovoltaic cell and gives some electrons—negatively charged atomic particles—enough energy to set them free. These electrons are affected by a built-in potential barrier in the cell, which creates a voltage (referred to as photovoltage) that can be utilized to move a current through a circuit.

### 1.1. Electron configuration of Silicon

Chemical element silicon has the chemical symbol Si and atomic number 14. It is a tetravalent metalloid and semiconductor that has a blue-grey shiny appearance and is a hard, brittle crystalline solid. It belongs to group 14 of the periodic table, which is above carbon and below germanium, tin, lead, and flerovium.

It doesn't react all that much. Therefore, the Silicon electron configuration will be $1s^2 2s^2 2p^6 3s^2 3p^2$. The Si-Si bond distance in the crystalline solid is 235 pm, and it crystallizes like a diamond. A denser deformed form may be created under extremely high pressure, but the Si-Si bond spacing essentially stays the same. The four ionization energies added together are very high. Therefore, it is not advantageous to lose four electrons in order to achieve a noble gas structure. The production of four covalent bonds with sp3 hybridization is the most common method used to achieve it.

### 1.2. The Barrier's Function

The barrier in a PV cell is created by opposing electric charges that are aligned with one another on either side of a separating line. This hypothetical barrier divides the electrons and holes produced by light into two distinct groups, sending more electrons to one side of the cell and more holes to the other. The electrons and holes are

less likely to reunite and lose their electrical energy as a result of being divided. The voltage differential created by this charge separation can be used to create an electric current in an external circuit.

**Forming the Barrier**

It has an additional, unbonded electron when an impurity atom, like phosphorus, which has five valence electrons, is replaced into a silicon crystal. A potential barrier can be created in a solar cell in a number of ways. One is to gently change the crystal's structure so that it changes on either side of the dividing line.

**The Negative-Carrier (Donor) Dopant.** The four valence electrons in silicon, as previously mentioned, are all typically a part of bonds inside silicon crystals. Imagine placing a phosphorus atom with five valence electrons in place of a silicon atom to introduce an impurity into a crystal of otherwise pure silicon.

The impurity atom, which would provide an electron for each of silicon's four bonds, would occupy the same space in the crystal as a regular silicon atom. The phosphorus atom, however, had an additional valence electron, thus there would be one electron that was free of a bond. The additional electron in the impurity atom is relatively free in comparison to a bound electron. Even though it would leave behind a positively charged impurity atom, the crystal has enough thermal energy at ambient temperature to shake this electron away.

Dopants, or impurities introduced in this method, are those that have one additional valence electron, like phosphorus injected into a silicon crystal, and donors, or impurities that give the crystal an electron, are those that don't. Because it has free negative charges, this form of donor-doped crystal is referred to as n-type (Meinel, Aden B. et al. 1976).

**The Positive-Carrier (Acceptor) Dopant.** Impurity atoms with one fewer valence electron than silicon can be substituted into the silicon crystal to create an appropriately changed material. The initial silicon atom would be replaced by an impurity atom (such as boron) with three valence electrons, but there would be a hole in one of its links to the silicon. As we've just seen, holes have about the same mobility as conduction-band electrons. This causes the numerous holes in a silicon crystal doped with a lot of these boron atoms to behave as free positive charges travelling across the crystal lattice.

Because its holes absorb electrons from the surrounding silicon crystal (either bound valence electrons or conduction-band electrons), a three-valence electron impurity in silicon is known as an acceptor. A silicon material that has been acceptor-doped is referred to as p-type because it has free positive charges (Johnston, W.D., Jr. 1980).

**The junction**: The position of a potential barrier necessary for the functioning of a solar cell is established by a line separating n-type from p-type silicon.

Additionally, holes on the adjacent p-type side can move into the n-type material when valence band electrons on the n-type side jump into them. This quick charge transference process sends a large number of electrons to the p-type side and holes to the n-type side along the dividing line (junction). This immediately results in a charge imbalance, with more positive charges (ions) along the n-type side of the interface and more negative charges (extra electrons) along the p-type side.

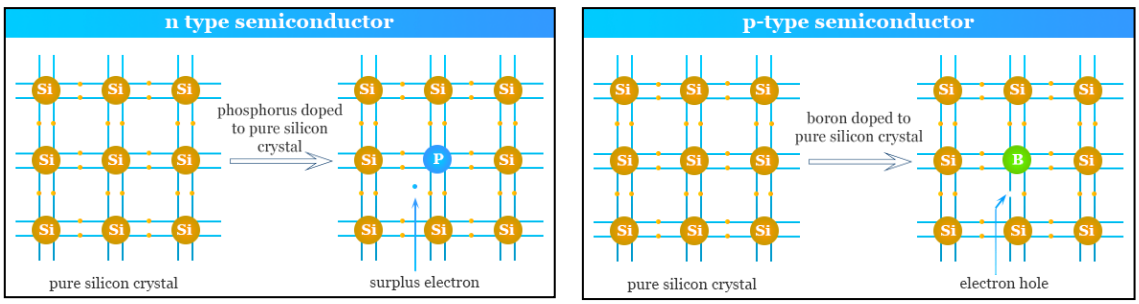



**Figure 1.** (a) When an impurity atom such as phosphorus with five valence electrons is substituted into a silicon crystal, it has an extra, unbonded electron. (b) A three-valence-electron impurity (such as boron) in a silicon crystal is normally bonded, except one of the bonds is missing an electron.

**Electronic current**

Since this lessens the light-induced charge imbalance in the cell, if we link the n-type side of the cell to the p-type side using an external electric circuit, current flows through the circuit. The electrons flow into the p-type side, where they recombine with holes close to the electrode, after performing meaningful work on the load (such as heating the filament of the light bulb to incandescence) through the electrode on the n-type side. The light energy that the electrons first absorbed is depleted as they power the external circuit. As a result, an equilibrium is preserved: the incident light continuously generates more electron-hole pairs, leading to an increase in the charge imbalance; the charge imbalance is then corrected by the current, which expends energy while moving the charge imbalance. Current generated by a cell is nearly proportional to the amount of light that strikes it (Chalmers, Bruce. 1976).

The greatest energy that may be collected from an electron in an external circuit is equal to the energy required to bring an electron to the conduction band. This energy is the same energy that light initially imparts to the electron. All the prerequisites for current to flow have been seen: incident light to release the charge carriers, a barrier to accelerate the carriers over the junction and maintain them at opposing ends of the cell, and a charge imbalance to move the current (charged carriers) along a circuit. (Photovoltaics Report, 2016)

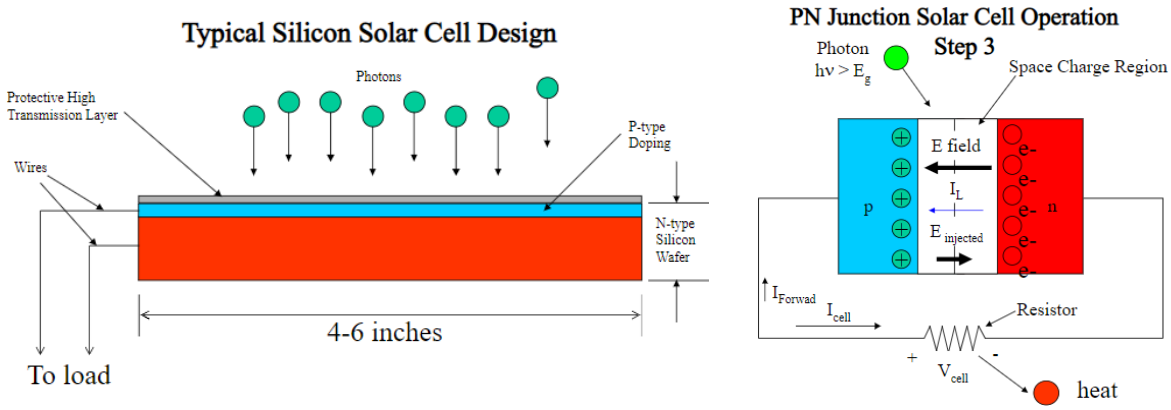



**Figure 2**. During junction formation, electrons move from the n-type silicon into the p-type, while holes move in the opposite direction. Movement of electrons into the p-type silicon and holes into the n-type silicon builds up a fixed potential barrier at the junction opposing the further movement of free carriers and creating a state of equilibrium

**Solar cells made of monocrystalline silicon**

In comparison to polycrystalline silicon's blue color specks, monocrystalline solar panels have a more aesthetically pleasing appearance because of their uniform appearance. Monocrystalline solar cells are produced using the Czochralski technique. They are extracted from premium cylindrical silicon ingots that have been accurately sliced on all four sides, yielding a highly respectable efficiency of 20–21%. The monocrystalline silicon solar panels use less area and can generate two to three times as much power as thin film technology. These solar panels typically last for 25 years. Shade, dirt, and snow commonly have a substantial negative impact on solar panel performance and can cause the circuit to malfunction. In general, monocrystalline solar panels perform worse as the temperature rises and are more effective in hotter climates.

**Solar cells made of polycrystalline silicon**

After being melted and placed into a square mold, raw silicon is then carved into the desired shape. With no rounded corners, polycrystalline solar cells appear to be exactly rectangular. Polycrystalline silicon is straightforward and less expensive. When compared to the production of monocrystalline semiconductors, silicon wastage is quite low**. (** Mekhilef, S et al. 2012)

## 2. MATERIALS AND METHODS

### *2.1. Equipment*

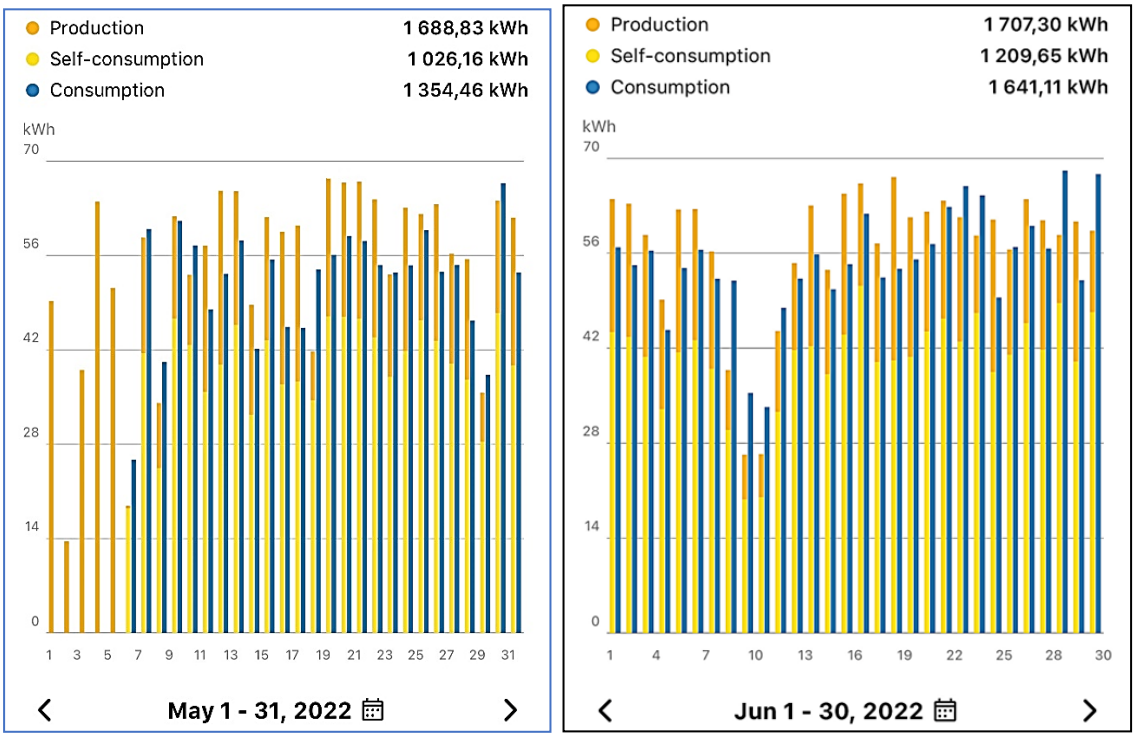
Monocrystalline photovoltaic panels 340W, Fronius inverter 12.5 kw (SYMO 10.0-3-M-OS) on grid, Fronius Smart meter.

### *2.2. Method used for detection of energy consumption and energy production:*

PV system monitoring with Fronius solar.web. Fronius Solar.web is not just a professional monitoring tool that provides installers with real-time system data at all times, but it also offers comprehensive analysis functions that enable individual consultation and ongoing optimization of the PV system.

## 3. RESULTS

The monthly consumption and photovoltaic panel efficiency for a family living in the city of Elbasan were measured for the study. The type of panel used is monocrystalline on grid 11.9 kWp on a surface of 65 $m^2$, with till 17grade direction southwest. The time period studied includes May to October 2022.



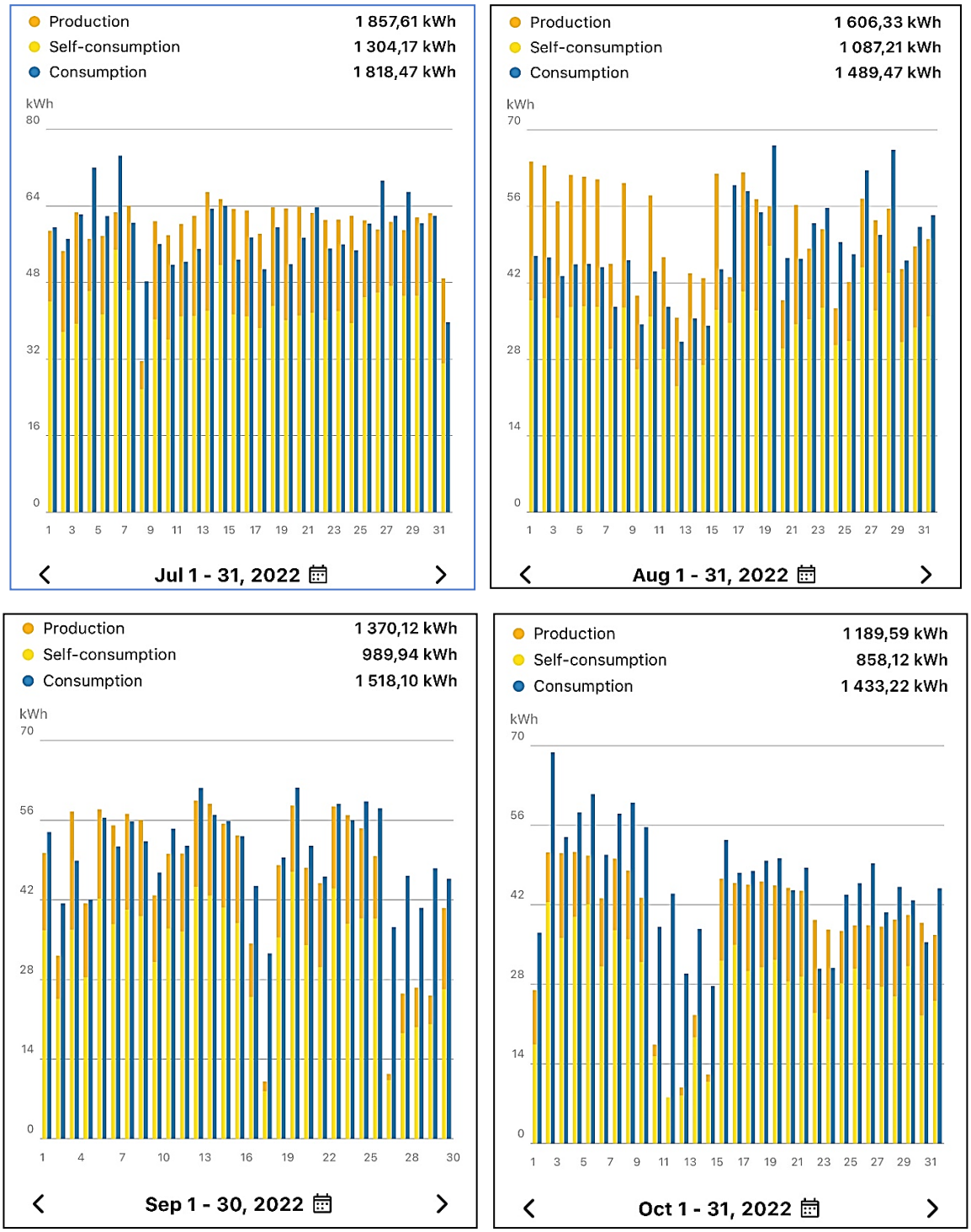



**Figure 3**. Distribution in kWh of energy production, self-consumption and consumption from May to October.

From the distribution chart for the month of May, it can be seen that the device for detecting the energy consumed by the subject was installed after May 7.

- **Result for May 2022:** The day with the highest production 67.03 kWh belongs to the date 19.05.2022. The day with the lowest production 13.62 kWh belongs to the date 02.05.2022.
- **Result for June 2022:** The day with the highest production 67.23 kWh belongs to the date 18.06.2022. The day with the lowest production 26.29 kWh belongs to the date 09.06.2022.
- **Result for July 2022:** The day with the highest production 66.94 kWh belongs to the date 13.07.2022. The day with the lowest production 31.57 kWh belongs to the date 08.07.2022.

- **Result for August 2022:** The day with the highest production 64.21 kWh belongs to the date 01.08.2022. The day with the lowest production 35.64 kWh belongs to the date 12.08.2022.
- **Result for September 2022:** The day with the highest production 59.46 kWh belongs to the date 12.09.2022. The day with the lowest production 10.09 kWh belongs to the date 17.09.2022.
- **Result for October 2022:** The day with the highest production 51.31 kWh belongs to the date 04.10.2022. The day with the lowest production 8.13 kWh belongs to the date 11.10.2022.

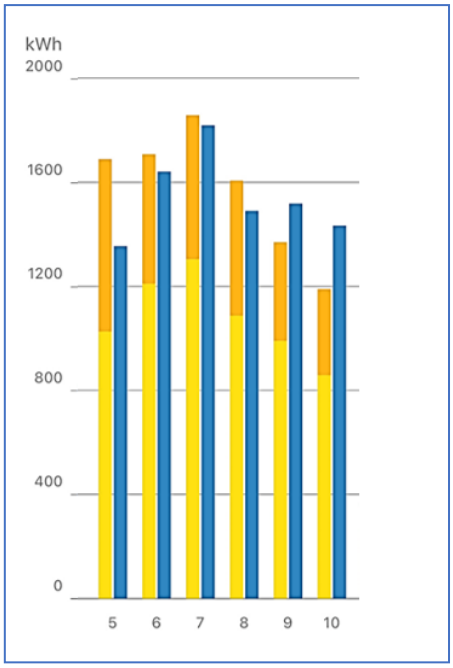



**Figure 4.** A total distribution in kWh of energy production, self-consumption and consumption from May to October.

From the data obtained, it was observed that the highest production was in July with 1857.61 kWh, followed by 1707.30 kWh in June, 1688.83 kWh in May, and 1606.33 kWh in August. We have a decrease in production in September with 1370.12 kWh and in October with 1189.59 kWh. In total for 6 month production was 9419.78 kWh.

## 4. CONCLUSION

In this study, a comparison was also made regarding the energy produced and consumed. It is noted that the production of these panels varies from month to month and this depends on many factors such as the weather, the angle formed by the light with the Solar panel, the positioning of the panels, and the structure of the panels. The information provided in this 'Photovoltaic Report' is very concise and the aim is to provide a rough overview of electrochemical performance, production technology, monthly savings, and environmental impact.

## 5. ACKNOWLEDGEMENTS

This study has Panele Besi as a financial support.

## 6. REFERENCES

Backus, Charles E. (ed.}. 1976. Solar Cells. New York, NY: IEEE Press.

Chalmers, Bruce. 1976. "The Photovoltaic Generation of Electricity." Scientific American. Vol. 235 (No.4): pp.34-43.

Johnston, W.D., Jr. 1980. Solar Voltaic Cells. New York, NY: Marcel Dekker, Inc.; pp. 19-51.

Meinel, Aden B.; Meine], Marjori P. 1976. Applied Solar Energy. Reading, MA: Addison-Wesley Publishing Co.; pp. 526-544.

Mekhilef, S., Saidur, R. and Kamalisarvestani, M., 2012 Renewable and Sustainable Energy Reviews,. 16(5): p. 2920-2925.

Noll, Edward M. 1975. Wind/Solar Energy. Indianapolis, IN: Howard W. Sams and Co., Inc.; pp. 7-44.

Pulfrey, David L. 1978. Photovoltaic Power Generation. New York, NY: Van Nostrand Reinhold Co.; pp. 66-113.

Tauc, Jan. 1972. Photo and Thermoelectric Effects in Semiconductors. New York, NY: Pergamon Press; pp.90-129.

Photovoltaics Report, 2016.

https://www.priyamstudycentre.com/2021/02/silicon.html

## *POSTER PRESENTATION*

# Neuroblastoma-targeted anti cancer drug delivery

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**Abstract**

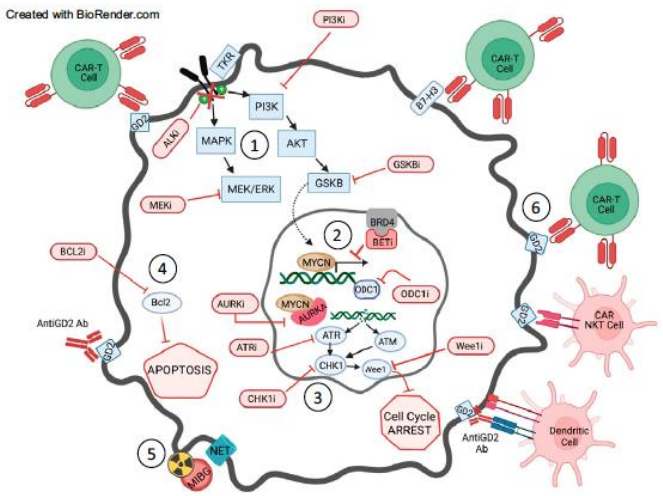
Everolimus is mTOR inhibitor and Tozasertib is an AURORA inhibitor. Both of these proteins plays an important role in stabilizing MYCN protein. MYCN is an important prognostic marker of neuroblastoma. Ganglioside2 (GD2) is a protein that overexpressed in tumor tissue in primary NB. Therefore, anti-GD2 mAb agents such as DTX-B appear to be an effective molecule for immunotherapy. Drug delivery systems that directly target tumor cells is an effective method for presenting drug combinations together.
The aim of this study is to determine the methodology for loading dual chemotherapeutic agents to PEG-b-PLGA nanoparticle and targeting the agents with mAb and to design a targeted nanoparticle loaded with dual drugs. The designed nanoparticle will be used as a therapeutic agent in NB for future studies.
20 mg of PEG-b-PLGA was used to form nanoparticle. $IC_{50}$ doses of therapeutics were added to the nanostructure with 1:3 rate. FTIR,size,loading capacity(LC) and zeta potential were measured. mAb was attached to target the formed nanoparticle. 500 µg of DTX-B was loaded with EDC/NHS synthesis. FTIR,zeta potential and size were measured. LC was determined by BCA assay.
The amount of Everolimus and Tozasertib loaded into the nanoparticle was 24% and 43%, respectively. The zeta potential is -27.6 mV, PDI is 0.371, and the size is 164.1 nm. FTIR result peaked at 1752.58 $cm^{-1.}$ The loading efficiency of mAb is 52%. The zeta potential is -41.1 mV. PDI is 0.401 and the size is 170 nm. In FTIR, it peaked at 1753.13 $cm^{-1.}$
EVER-TOZA@PEG-b-PLGA/DTX-B nanoparticle protects the dual drug combination in its structure. Nanostructure has a high loading capacity. NP is at the desired size. In future studies, the therapeutic potential of the formed nanoparticle will be examined *in vitro* and *in vivo* for the treatment of NB.

**Keywords:** Neuroblastoma, Nanoparticle, Drug Delivery

## INTRODUCTION

Neuroblastoma (NB) is the most common extracranial solid tumour of childhood, with 90% of cases occurring in those <5 years of age, and at an average of 2 years of age at diagnosis. Primary neuroblastomas arise from cells of the sympathetic nervous system in the adrenal medulla or along the sympathetic chain. NBs are remarkably heterogeneous, with distinct yet variable biological and clinical characteristics, including localized or widely disseminated disease that spontaneously resolves, low- to- intermediate- risk tumours that can be surgically resected with or without the need for postoperative chemotherapy, and high- risk disease, which is often lethal. Nonetheless, 5- year survival durations in patients with high- risk disease have increased from <20% to >50% over the past few decades owing to progressive improvements in the efficacy of multimodal therapies tested in clinical trials (Qiu and Matthay 2022).

MYCN status has been known be one of the most powerful biological indicators for NB, that is associated with aggressive illness and poor prognosis for a subpopulation of individuals with amplified MYCN. The assessment of tumor molecular profile has become increasingly significant to establish the overall prognosis. Down-regulators of MYCN expression, including retinoid derivatives, Histone deacetylase (HDAC) inhibitors, and PI3K/AKT/mTOR pathway inhibitors, vary according to the mechanism of action and efficacy. The coding genes of critical cell cycle regulators, especially in the mitosis phase, Aurora A (AURKA) and Aurora B (AURKB), are distributed differently in the cell, and their protein products perform different roles. AURKA mRNA expression stabilizes MYCN through a direct protein-protein interaction, which makes it less proteasome degradable. AURKB has been identified as a direct transcriptional target of MYCN (Salemi et al. 2022).




**Fiugre 1.** Molecular targets of therapeutics in NB (DuBois, Macy, and Henderson 2022).

All neuroblastoma cells express glycolipid antigens known as gangliosides. Gangliosides are sialic acid-containing glycosphingolipids that can be classified into four series: 0-, a-, b-, and c-series. Gangliosides are mainly engaged in signal transduction, cell adhesion, and recognition. Normal tissues usually express a-series gangliosides. NB expresses b-series gangliosides rather than a-series, as aberrant glycosylation is a hallmark of malignant cellular transformation. This includes disialoganglioside (GD2) and hematoside (GD3) (Chan and Chan 2022). Circulating GD2 levels in the blood isn't high enough to interfere with binding to anti-GD2 specific mAbs. These properties make it an attractive target for immunotherapy. Because of the relative specificity for NB, GD2 has been utilized as a vehicle to deliver drug directly to the tumor, where it is endocytosed and selectively released into the tumor cell for killing. Targeting GD2 antigen with immune effectors, including mAbs has the potential to achieve substantial clinical benefits in NB therapy (Sait and Modak 2017).

Drug delivery systems have long been studied as a way of delivering cytotoxic chemotherapy directly to a tumor or other site of action, while potentially reducing systemic side effects (Aktaş et al. 2021). Nanocarriers can be functionalized at their surface with mAbs in order to deliver drugs selectively towards tumor tissues (targeted nanomedicines) or with hydrophilic molecules (i.e. poly(ethylene glycol) (PEG)) to increase their blood circulation time in the body (Chen, Trout, and Towbin 2018). Several experiments have been performed by linking anti-GD2 antibodies or their fractions to NPs. Recently, SN-38 was loaded into anti-GD2 poly(lactic-co-glycolic) acid (PLGA)- PEG NPs (Monterrubio et al. 2017).

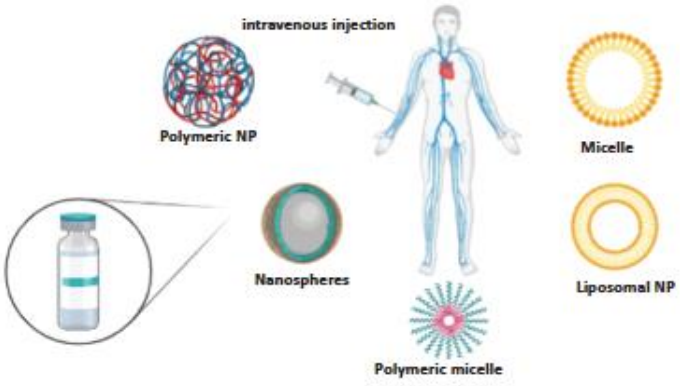



**Figure 2.** The most commonly used NPs in cancer immunotherapy and intravenous administration (Aktaş et al. 2021).

## MATERIALS AND METHODS

The chemical formation method was used to form the nanostructure. Briefly, 20 mg of PEG-b-PLGA (Mn 5000, Mn 15000, lactide:glycolide (50:50) was used to form NP. $IC_{50}$ doses of therapeutics were added to the nanostructure with 1:3 rate. 20 mg of NP was measured and dissolved in 1 mL of acetonitrile. Then, Everolimus and Tozasertib were added to the mixing falcon at the determined doses. It was stirred for 4-6 hours with the help of a magnetic stirrer at 500 rpm. At the end of the 6th hour, the NP was washed with PBS and centrifuged at 10,000 rpm for 10 min. The resulting pellet was left to dry for two days at -20 degrees. Size studies were performed by separating the supernatant. FTIR, size, loading capacity (LC) and zeta potential were measured. mAb was attached to target the formed nanoparticle. To attached the NP with mAb, 500 µg of DTX-B was loaded with EDC/NHS synthesis. FTIR,zeta potential and size were measured. LC was determined by BCA assay.

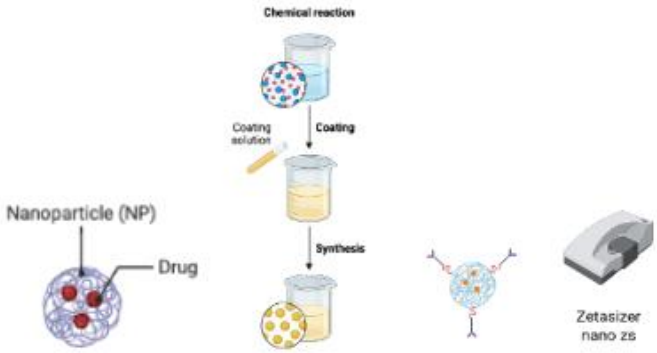



**Figure 3.** Methodology was explained briefly.

## RESULTS and DISCUSSION

The amount of Everolimus and Tozasertib loaded into the nanoparticle was 24% and 43%, respectively. The zeta potential is -27.6 mV, PDI is 0.371, and the size is 164.1 nm. FTIR result peaked at 1752.58 cm-$^{1.}$ The loading efficiency of mAb is 52%. The zeta potential is -41.1 mV. PDI is 0.401 and the size is 170 nm. In FTIR, it peaked at 1753.13 cm$^{-1.}$

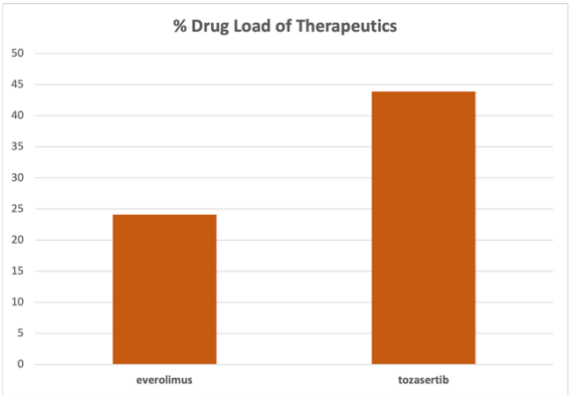



**Figure 4.** Drug loading capacity was measured by using spectro.

The zeta potential of EVER-TOZA@PEG-b-PLGA is -27.6 mV, PDI is 0.371, and the size is 164.1 nm. FTIR result peaked at 1752.58 cm-1. The loading efficiency of DTX-B is 52%, detected by BCA assay. The zeta potential is -43.2 mV. PDI is 0.401 and the size is 170 nm. FTIR of EVER-TOZA@PEG-b-PLGA/DTX-B , it peaked at 1753.13 cm-1.

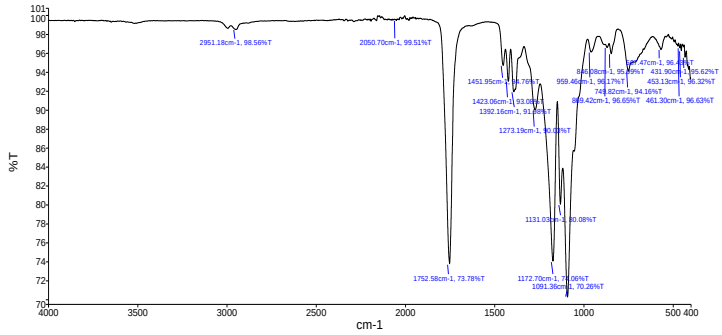



**Figure 5.** FTIR spectrum of EVER-TOZA@PEG-b-PLGA .

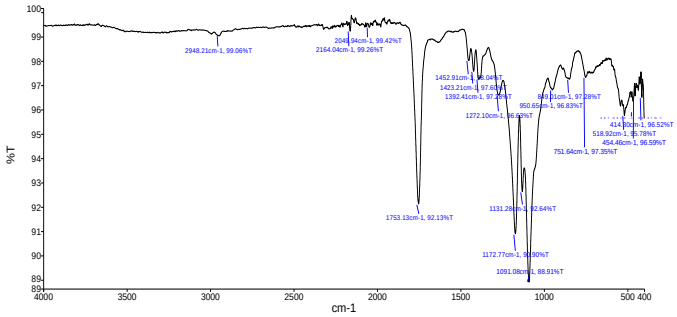



**Figure 6.** FTIR spectrum of EVER-TOZA@PEG-b-PLGA/DTX-B .

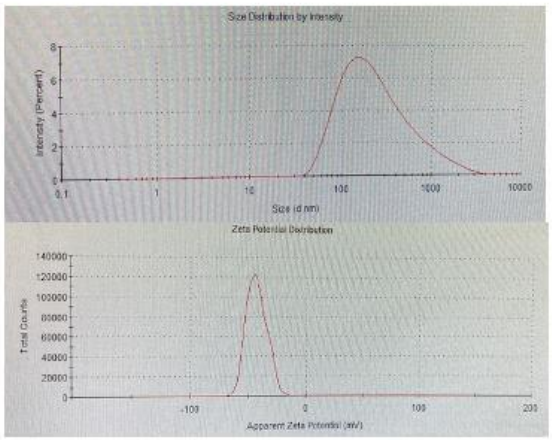



**Figure 7.** Size and zeta potential of EVER-TOZA@PEG-b-PLGA/DTX-B

Tozasertib is an AURORA Kinase inhibitor and plays an important role in stabilizing MYCN protein. Everolimus is responsible for the suppression of the mTOR protein in the PI3K/AKT/mTOR pathway, and the mTOR protein is associated with MYCN. Both proteins, mTOR and Aurora proteins act on MYCN protein in the signaling cascade. With the mTOR and Aurora proteins being genetically defected, these proteins are far from being able to control MYCN and cause an increase in expression of MYCN. This results in a poor prognosis in NB patients. Tozasertib and Everolimus have been separately loaded and studied in nanoparticle systems but have not been combined together before. These data, which we present as an original aspect of our study, will contribute to the literature. DTX-B is an anti-GD2 agent and an antibody produced against GD2, an antigen often expressed in NB. Targeting the nanodrug to NB tissue with DTX-B increases the specificity of the drug against tumor. Conventional chemotherapeutics can cause undesirable side effects after they enter the bloodstream. In addition, the bioavailability of the given treatments by the body is also limited to a certain extent. Targeting nano-drugs and drugs by encapsulation aims to prevent undesirable systematic

effects. The designed nanodrugs reveal less side-effect profiles than conventional chemotherapeutics in phase studies.

In this study, we describe our preliminary data. Everolimus and Tozasertib drugs which are used in our study have not been used as dual combination before, and in this respect, our study has an original quality. The loading efficiencies of drugs loaded into nanoparticles are compatible with the literature (Kirchner, Meier-wiedenbach, and Manns 2004), (Duong Le et al. 2016). DTX-B showed loading efficiency in accordance with the literature (Tivnan et al. 2017). Nanoparticle formulation for this study was based on the protocol by Cheng et al. which used a nanoprecipitation method for dual drugs encapsulation and formulation of PLGA nanoparticles (Cheng et al. 2007).

## CONCLUSION

EVER-TOZA@PEG-b-PLGA/DTX-B nanoparticle form with the dual drug combination in its structure has been formed. Nanostructure has a high loading efficiency. NP is at the desired size. In future studies, the therapeutic potential of the formed nanoparticle will be examined in vitro and in vivo for the treatment of NB.

## ACKNOWLEDGEMENTS

This project was supported by the Turkish Pediatric Oncology Group.

## REFERENCES

Aktaş, Safiye, Özde Elif Gökbayrak, Aylin Erol, and Hatice Nur Olgun. 2021. "Neuroblastoma-Targeted Anticancer Drug Delivery." *Journal of Dr. Behcet Uz Children s Hospital* 11(3): 219–25.

Chan, Godfrey Chi Fung, and Carol Matias Chan. 2022. "Anti-GD2 Directed Immunotherapy for High-Risk and Metastatic Neuroblastoma." *Biomolecules* 12(3): 1–17.

Chen, Alan M, Andrew T Trout, and Alexander J Towbin. 2018. "A Review of Neuroblastoma Image-Defined Risk Factors on Magnetic Resonance Imaging." *Pediatric radiology* 48(9): 1337–47.

Cheng, Jianjun, Benjamin Teply, Ines Sherifi, and Josephine Sung. 2007. "Formulation of Functionalized PLGA-PEG Nanoparticles for In Vivo Targeted Drug Delivery." *Biomaterials.* 28(5): 869–76.

DuBois, Steven G., Margaret E. Macy, and Tara O. Henderson. 2022. "High-Risk and Relapsed Neuroblastoma: Toward More Cures and Better Outcomes." *American Society of Clinical Oncology Educational Book* (42): 768–80.

Duong Le, Thi Thuy et al. 2016. "In Vitro Evaluation of Aurora Kinase Inhibitor -VX680-in Formulation of PLA-TPGS Nanoparticles." *Advances in Natural Sciences: Nanoscience and Nanotechnology* 7(2).

Kirchner, Gabriele I, Ivo Meier-wiedenbach, and Michael P Manns. 2004. "Of Everolimus." *Clinical Pharmacokinetics* 43(2): 83–95.

Monterrubio, Carles et al. 2017. "Targeted Drug Distribution in Tumor Extracellular Fluid of GD2-Expressing Neuroblastoma Patient-Derived Xenografts Using SN-38-Loaded Nanoparticles Conjugated to the Monoclonal Antibody 3F8." *Journal of controlled release : official journal of the Controlled Release Society* 255: 108–19.

Qiu, Bo, and Katherine K. Matthay. 2022. "Advancing Therapy for Neuroblastoma." *Nature Reviews Clinical Oncology* 19(8): 515–33.

Sait, Sameer, and Shakeel Modak. 2017. "Anti-GD2 Immunotherapy for Neuroblastoma." *Expert Review of Anticancer Therapy* 17(10): 889–904.

Salemi, Fateme et al. 2022. "Neuroblastoma: Essential Genetic Pathways and Current Therapeutic Options." *European Journal of Pharmacology* 926(February): 175030. https://doi.org/10.1016/j.ejphar.2022.175030.

Tivnan, Amanda et al. 2017. "Anti-GD2-Ch14.18/CHO Coated Nanoparticles Mediate Glioblastoma (GBM)-Specific Delivery of the Aromatase Inhibitor, Letrozole, Reducing Proliferation, Migration and Chemoresistance in Patientderived GBM Tumor Cells." *Oncotarget* 8(10): 16605–20.

## *POSTER PRESENTATION*

# Evaluation Of Nitrite And Nitrate Present In Salami Consumed In Tirana

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## Abstract

Salami are made with meat (traditionally pork), are blended with fat, and then mixed with herbs and seasonings, such as salt, garlic, vinegar etc. But usually as food additive are used and nitrates or nitrites. For positive effects that give as: a better flavor, taste, and aroma; preserve the red-pink color of the meat; and prevent the risk of bacterial contamination of the cured meat, especially from *Clostridium botulinum*. They also have a negative effect because they contribute to the formation of a group of compounds known as nitrosamines, some of which are carcinogenic.
The purpose of this study is to determine the concentrations of nitrites and nitrates in salami traded in Tirana, produced by Albanian and imported companies. Also, for the determination and physicochemical properties such as water activity, humidity, and pH.
Based on the results, none of the analyzed salami samples were above the EU maximum limit for nitrites and nitrates of 100 mg/kg and 250 mg/kg respectively.
Therefore, the assessment of nitrate and nitrite levels in salami is important to assess their safety for consumers.

**Keywords:** nitrite, nitrate, salami, Tirana.

## INTRODUCTION

Meat is processed by methods such as: smoking, curing, or adding salt or preservatives, in a way to extend the shelf life or change the taste. Processed meat includes bacon, sausages, hot dogs, salami, corned beef, beef jerky and ham as well as canned meat and meat-based sauces. (Lonergan et al., 2019)

The salts of nitrite and nitrate are commonly used for curing meat and other perishable produce. They are added to food to preserve it and also help hinder the growth of harmful microorganisms, in particular *Clostridium botulinum*, the bacterium responsible for life-threatening botulism. Nitrites, together with nitrates, are also added to meat to keep it red and give flavour. (EFSA, 2017)

Despite the advantages of nitrite application in the meat industry (pinkish-red color, and antioxidant and antimicrobial properties), it is harmful to human health. The negative effects of nitrite as a meat additive were first recorded in the early 1950s and 1960s when *N*-nitroso compounds (NOCs) were initially discovered. (Bedale et al., 2019) Nitrate per se is relatively non-toxic but approximately 9% of all ingested nitrate is converted in saliva and in the gastrointestinal tract to the more toxic nitrite. In the stomach, nitrite reacts with amines and amides, to form a group of molecules known as nitrosamines (Sindelar and Milkowski, 2012) that have been classified by International Agency for Research on Cancer as probable carcinogens belonging to Group 2A (IARC, 2010). Nitrate and its derivate nitrite, is also associated to a disease affecting the oxygen transport in blood (methaemoglobinaemia), of which babies are most susceptible (Walker 1990).

In this context, the use of sodium and potassium nitrates and nitrites is regulated as food additives in the European Union (EU) according to the Regulation (EC) No 1333/2008.

The aim of this study is to determine the concentrations of nitrites and nitrates in salami traded in Tirana, produced by Albanian and imported companies, during may 2022.

## MATERIALS AND METHODS

The study was based on 6 samples of salami obtained from Albanian companies and 6 samples of imported salami, collected in some of the biggest markets in Tirana. The samples were analysed to determine the content of nitrites and nitrates by the spectrophotometric method.

Nitrite by the spectrophotometric method is determined by diazotizing with sulphanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured at 540 nm. The calibration curves for nitrite and nitrate were initially prepared. The linearity of the calibration curves were good (R=0.99) in the concentration range 0.2-1.2 mg/l. Nitrate was determined after reduction of nitrate to nitrite with zinc powder. The nitrite present in the sample is determined by analyzing without the reduction step. The nitrate is calculated as the difference between the total nitrite content after reduction and the initial nitrite concentration. Physicochemical parameters as water activity and pH were determined with correspondent apparatus, and moisture content was determined via a thermogravimetric method, i.e., by weight loss after drying.

**RESULTS and DISCUSSION**

In the Table 1 are given data for the physicochemical parameter as: water activity (Aw), pH and moisture for each samples analyzed. The data are the average values of analyzed parameters.

Based on these results, we can see that the water activity for the salami produced by the Albanian factory is generally 0.91, except for one sample that are 0.75. Aw for the imported salami is from 0.80-0.93.

The pH values were 5.28-6.47 for salami products in Albania and 4.62-6.45 for imported salami, we see that the pH range is generally from 5.3 to 6.4 for both types of local and imported salami product, only one sample of imported salami has a lower pH value of 4.62.

It is noted that in 66.7 % of the analyzed samples the moisture content is from 61.67-73.25 %, while only one sample from local products has the lowest moisture of 21.18 % because it was dry salami, while three samples from imported salami have moisture from 31.55-41.73 % these are semi-dry salami.

From data we see that have a relationship between water activity and moisture content.

**Table 1.** Physicochemical properties of analysed salami

| | $a_w$ | | pH | | Moisture % | |
|---|---|---|---|---|---|---|
| **No. sample** | **Albanian producer** | **Imported** | **Albanian producer** | **Imported** | **Albanian producer** | **Imported** |
| 1 | 0.91 | 0.92 | 5.90 | 6.29 | 61.67 | 65.55 |
| 2 | 0.91 | 0.93 | 6.24 | 6.45 | 63.73 | 73.25 |
| 3 | 0.75 | 0.93 | 5.30 | 6.36 | 21.18 | 69.14 |
| 4 | 0.91 | 0.84 | 5.28 | 5.35 | 62.45 | 33.15 |
| 5 | 0.91 | 0.87 | 5.98 | 4.62 | 61.90 | 41.73 |
| 6 | 0.92 | 0.80 | 6.47 | 5.74 | 70.30 | 31.55 |

Nitrite and nitrate concentrations in the salami were determined by the spectrophotometric method, based on the reduction of nitrate to nitrite with zinc powder. The nitrite present in the sample is determined by analysing without the reduction step. The nitrate is calculated as the difference between the total nitrite content after reduction and the initial nitrite concentration.

Calibration curves were first constructed for nitrite ($NO_2^-$) and nitrate ($NO_3^-$). The concentration of nitrite and nitrate was then calculated for each sample and shown in Table 2.

**Table 2.** The concentration of nitrites and nitrates in the analysed salami from the Albanian producer and imported

| | Albanian producer | | Imported | |
|---|---|---|---|---|
| **No. sample** | **Conc. $NO_2^-$ mg/kg** | **Conc. $NO_3^-$ mg/kg** | **Conc. $NO_2^-$ mg/kg** | **Conc. $NO_3^-$ mg/kg** |
| 1 | 29.03 | 35.23 | 49.00 | 146.51 |
| 2 | 46.47 | 1.82 | 52.33 | 2.24 |
| 3 | 92.32 | 108.06 | 57.90 | 236.77 |
| 4 | 46.41 | 118.70 | 11.56 | 1.66 |
| 5 | 46.51 | 91.71 | 2.89 | 44.93 |
| 6 | 46.45 | 144.21 | 11.55 | 65.66 |

Referring to the data, it is noted that the levels of nitrites and nitrates in the samples produced in Albania were from 29.03-92.32 mg/kg and 1.82-144.21 mg/kg respectively. Also, the concentration of nitrite and nitrate in imported samples were from 2.89-57.90 mg/kg and 1.66-236.77 mg/kg respectively.

From the figure 1, it can be seen that the locally produced samples present higher nitrite concentration values than imported samples.

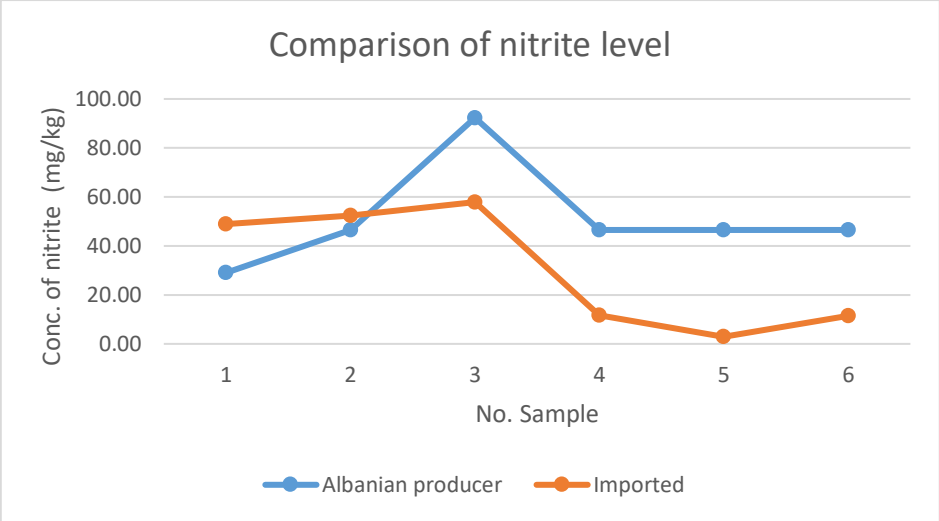



**Figure 1.** Comparison of nitrite level in analysed samples

Regarding the concentration of nitrates in figure 2, there is no significant difference between locally produced and imported salami, but the two samples with the highest value are the import samples.

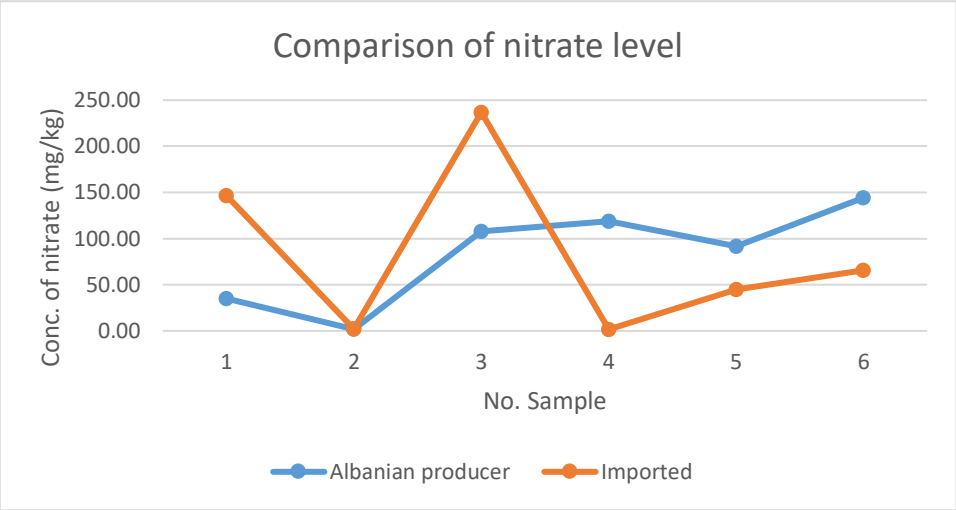



**Figure 2.** Comparison of nitrate level in analysed samples

The results obtained showed that in 75 % of the analysed samples the nitrate concentrations were higher than the nitrite concentration. In three samples (25 %), it happens that the concentration of nitrite is higher than nitrate, and they are those samples in which there is almost no nitrate content.

In all the analysed samples the nitrite and nitrate content were below the maximum admitted concentrations established by European Commission Regulations in meat products, for nitrites and nitrates of 100 mg/kg and 250 mg/kg respectively.

## CONCLUSION

Based on results of physicochemical properties, we conclude that there are no significant differences between salami products in Albania and imported ones. But the nitrite content is lower in salami imported than these products in Albania.

Nitrite levels in analyzed salami are lower than nitrate levels. In all analyzed samples, the concentration of nitrates and nitrites detected in the meat products (salami) were lower than the maximum concentrations allowed by the Regulation of Food Additives, but in 8.3 % of samples the content of nitrites and nitrates was very near to the maximum limit.

In conclusion the concentrations of nitrates and nitrites in the end products should be limited and monitored, in order to cannot cause a risk to human health.

## REFERENCES

Bedale W, Sindelar JJ, Milkowski AL, 2016. Dietary nitrate and nitrite: Benefits, risks, and evolving perceptions. Meat Science,120: 85–92.

EFSA 2017. Nitrites and nitrates added to food, EFSA explains risk assessment. Available at: https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/nitrates-nitrites-170614.pdf

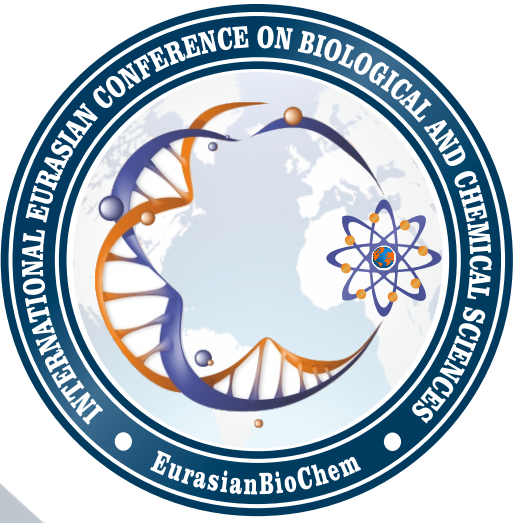
European Regulation, 2008. Regulation of the European Parliament and of the Council of 16 December 2008 on food additives, 1333/2008/CE. In: Official Journal, L 354/16, 31/12/2008.

International Agency For Research On Cancer World Health Organization, 2010. Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 94. Lyon (France).

Lonergan SM, Topel DG, Marple DN, 2019. The Science of Animal Growth and Meat Technology (Second Edition) Chapter 13. Academic Press pp. 205-228.

Sindelar JJ, Milkowski AL, 2012. Human safety controversies surrounding nitrate and nitrite in the diet. Nato Sci S A Lif Sci, 26: 259-66.

Walker R, 1990. Nitrates, nitrites and N-nitrosocompounds: a review of the occurrence in food and diet and the toxicological implications. Food Additive Contamination, 7:717–768.

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