

7th INTERNATIONAL EURASIAN CONFERENCE ON

BIOLOGICAL AND CHEMICAL SCIENCES

02 - 04 October 2024
Ankara / Turkey

(EurasianBioChem2024)

www.eurasianbiochem.org

PROCEEDING BOOK

(Abstracts and Full-Texts)

EurasianBioChem

ISBN : 978-605-72134-4-0

7th International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2024)

October 02-04, 2024
Ankara, Turkey
www.EurasianBioChem.org

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Editor

Prof. Dr. Muhittin DOĞAN

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ISBN: 978-605-72134-4-0

Publication Date: October 2024

Organized by

Avrasya Araştırma Geliştirme Bilim ve Teknoloji Merkezi

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* This list is arranged in alphabetical order.

FOREWORD

7th International Eurasian Conference on Biological and Chemical Sciences (*EurasianBioChem 2024*) was successfully held on October 02-04, 2024 with participation from many countries.

The objective of *EurasianBioChem 2024* 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 2024* has made important contributions to you at large and hope to see you at the *8th International Eurasian Conference on Biological and Chemical Sciences* (*EurasianBioChem 2025*).

Prof. Dr. Muhittin DOĞAN

Chairman of *EurasianBioChem 2024*

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1. ORAL PRESENTATIONS

1.1. ABSTRACTS

ORAL PRESENTATION

Biological properties of transgenic hairy roots of *Plectranthus venteri* van Jaarsv. & L. Hankey

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Abstract

Plectranthus venteri van Jaarsv. & L. Hankey is a plant species belonging to the family Lamiaceae. Discovered in 1997, the endemic plant is mainly found in the Sekukuniland region of South Africa. Among the main secondary metabolites of *Plectranthus venteri* are acetophenones and terpenoids [1, 2]. One of the key elements of biotechnology is to interfere with metabolic pathways to increase the production of valuable secondary metabolites, for example by introducing a 1-deoxy-D-xylulose-5-phosphate reductase (DXR) gene construct. This enzyme is required for terpenoid biosynthesis in some organisms, as it is an essential enzyme in the MEP pathway for the production of isoprenoid precursors. Obtaining highly productive transgenic roots from *Plectranthus venteri* could lead to an increase in plant material with a higher content of selected metabolites in the short term. Our work resulted in obtaining transgenic roots of *Plectranthus venteri* with overexpression of the DXR gene by *Rhizobium rhizogenes*-mediated transformation, as well as estimating the content of secondary metabolites and checking their biological properties in an *in vitro* model. Obtaining highly productive plant *in vitro* cultures may in the near future be a major source of biologically active compounds with very broad applications.

Keywords: *: Plectranthus venteri*, DXR gene, transgenic hairy roots, *in vitro* cultures, *Rhizobium rhizogenes*

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ORAL PRESENTATION

Assessment of physical-chemical properties, chemical alteration and origin of the geothermal springs in Llixha, Elbasan

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Abstract

The thermal springs of Llixha, Elbasan, emerge in the central Albania and rank among the springs with the highest temperature in the Albanides region. This study aims to analyze their chemical composition, examine the physicochemical indicators of the springs, and establish correlations with the springs in Bënja and Sarandaporos (South Albania). The physical-chemical properties of the Llixha springs are closely linked to the geological setting and the infiltration regime of surface waters. The springs are located at the contact zone between Upper Oligocene and Aquitanian deposits, occurring at the lithological contact with olistoliths, conglomerates, and sandstone horizons, and in areas characterized by longitudinal and transverse tectonic faults. The springs in Llixha are divided into two groups: the first comprising springs in the northern sector, and the second those in the Hidraj area of the southern sector. The total mineralization reaches up to 7,381 mg/l, with hardness values ranging between 23.1 mg/l and 152.2 mg/l. The thermal waters from Llixha, Elbasan, belong to the Cl-Mg-Na-Ca group, suggesting an intermediary origin due to the interaction of interstitial fluids with evaporitic rocks, along with the mixing of regional hydrothermal and infiltration fluids. These waters are chemically altered and influenced by infiltration fluids, with a high hydrogen sulfide (H_2S) content. This gas in the Llixha geothermal spring results from the thermal reduction of sulfur compounds, while in the Hidraj area, it is probably produced by thermochemical sulfate reduction. Given these characteristics, is strongly recommended further investigation into the concentration of hydrogen sulfide in the atmosphere, along with the hydrocarbon gases associated with these geothermal springs. Additionally, the discharge of these thermo-mineral waters into the river system should be preceded by treatment in purification facilities.

Keywords: Geothermal spring, Chemical alteration, Mineralization, Interstitial fluid interaction, H_2S, Llixha Elbasan.

ORAL PRESENTATION

Synthesis, characterization, antimicrobial and molecular docking studies of thiazolidin-4-one derivatives fused with indole and pyrimidine moieties

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Abstract

A series of fifteen 2-substituted-3-(4-(pyridin-2-yl)-6-(1H-pyrrolo[2,3-b]pyridin-4-yl)pyrimidin-2-yl)thiazolidin-4-one, was prepared and characterized using analytical techniques like FT-IR, NMR (^{1}H & ^{13}C), and mass spectrophotometry. On screening, the compounds for antibacterial and antifungal potential, the findings portrayed to be the potent inhibitor of bacterial and fungal strains. The cytotoxicity of the prepared molecules was checked against HepG2 cells to calculate the percent viable cells, which was observed to be in the range of 70-81 % up to 100 µM. The molecular docking assay was performed to assess the binding modes, the extent of the H-bond, and binding affinities against the receptor glucosamine-6-phosphatase (GlcN-6P) and lanosterol 14-alpha-demethylase. The findings portrayed that TYR 312, SER316, GLU396, LEU448, SER303, GLY398, and SER 401 were the only amino acids of GlcN-6P that formed the H-bond with the prepared molecules. Similarly, the residues GLC5, GLC6, GLC7, SER60, TYR63, THR104, ASP229, LEU230, SER249, SER251, THR 485 were the only amino acids of lanosterol 14-alpha-demethylase) that formed H-bond with the prepared molecules. The binding affinities for both receptors were found to be in the range of -8.5 to -6.3 kcal/mole (GlcN-6P), and -8.4 to -6.8 kcal/mole (lanosterol 14-alpha-demethylase).

Keywords: Thiazolidinone, pyrimidine, indole, antimicrobial, MTT assay, molecular docking

Acknowledgment
The author, Dr. Mohammad Arshad, is highly thankful to Dr. Hamza M. Maghraby (The Dean) and Dr. Talal Maghrabi (The Vice Dean), Al-Fayha College, Al-Jubail, Saudi Arabia for providing the necessary support to accomplish this study.

ORAL PRESENTATION

Immunoassays of alkyl- and bisphenols as representatives of hydrophobic low molecular weight compounds

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Abstract

Alkyl- and bisphenols are low molecular weight hydrophobic compounds used in industry as plasticizers to form film coatings, packaging, various resins and adhesives. They migrate into the surrounding space – food products and drinking water – creating risks to human health due to the their estrogen-like effects. The difficulties of their immunoanalysis are associated with poor solubility in aqueous media, micelle formation that lead to «escape» from the reaction medium. The report presents the results of experimental work aimed at obtaining hapten-protein conjugates in a suitable solvent medium, effectively recognizing polyclonal antibodies, using solubilization methods and conducting immunoassays in non-polar organic solvent media. The given approaches provide various options for immunoassays of nonylphenol, bisphenol A in water and food matrices using poly-monoclonal antibodies and aptamers. Replacing the dimethylformamide solvent with dimethylsulfoxide allowed to obtain hapten-protein conjugates, as well as polyclonal antibodies for determining bisphenol A. The detection limit of bisphenol A was 8.3 ng/mL, the working range was 18.5 – 290.3 ng/mL for the selected antiserum. It was shown that replacing colorimetric detection with chemiluminescent detection in enzyme immunoassay of nonylphenol allowed reducing the detection limit from 55 to 9 ng/ml, and expanding the working range to 28-1800 ng/ml. Preliminary concentration of the sample containing nonylphenol with magnetic immunosorbents allowed to achieve the detection limit of 3.8 ng/mL. In addition to the enzyme immunoassay protocols, a comparison was made of lateral flow immunoassay (LFIA) schemes for determining nonylphenol with detection limits of 0.4 and 1.1 μg/mL. Multiplex LFIA was developed for the determination of endocrine disruptors dimethyl phthalate and bisphenol A with detection limits of 2.22 and 0.67 ng/mL, respectively. The developed analytical systems allowed to detect analytes in real matrices with high recoveries.

Keywords: immunoassay, hydrophobicity, bisphenol A, nonylphenol.

This work was financially supported by the Russian Science Foundation (grant 22-13-00293).

ORAL PRESENTATION

Toxicology of Glue Thistle "*Atractylis gummifera.* L*"* Analytical Study and Toxicokinetics of the Active Ingredient "Atractyloside

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Abstract

Glue Thistle or *Atractylis gummifera* L., is used in traditional medicine for its healing properties in local application (for syphilis, boils, abscesses) and in the form of tea as a diuretic, purgative, antipyretic, abortifacient, and emetic. Its acute toxicity is mainly characterized by hepatic necrosis and renal failure. Our objective is to assess, through in vivo tests, the toxic power of atractyloside, the active ingredient of glue Thistle. This toxic power is evaluated in vivo by estimating the LD50 in mice and conducting a kinetic study after administering a sub-toxic dose in rats. We also studied the molecule's binding percentage and assessed the likelihood of toxicity related to an intermediate metabolite. The kinetic study of atractyloside in Wistar rats revealed nonlinear kinetics, with a maximum concentration of 68.8 µg/mL and a half-life of 90 minutes, with approximately 65% of the fraction being free. Using the Karber/Behrens and Miller and Tainter methods, the LD50 was estimated at 115+22.16 mg/kg. The phenobarbital induction test showed a significant decrease in maximum concentration and AUC, suggesting that the toxicity of atractyloside is related to the parent molecule and/or its metabolites. Understanding the in vivo biology of the molecule could help mitigate the severity of acute toxicity associated with the Glue thistle and similar plants, given the absence of specific treatment and the symptomatic nature of current therapeutic approaches.

Keywords: Glue Thistle, atractyloside, in vivo, toxicokinetics, enzyme induction, Algeria.

ORAL PRESENTATION

Microbiological Diagnostics of the Facial Skin Among Beauty Salons' Clients Depending on the Age

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Abstract

Introduction: Human's skin is inhabited by numerous microorganisms that make up for their peculiar microbiome. Many factors cause the modification of the skin's microbiome, often leading to dysbiosis, the consequence of which might be inflammation around the skin, as well as chronic dermatosis for example: *acne vulgaris* or *seborrheic dermatitis*. Aim of the study: The aim of the study was the microbiological diagnosis of the facial skin 30 beauty salon's clients divided among groups: 16 - 20 years old, 21 - 29, 30 - 39, 40 - 49, 50 - 59 and 60 - 70 years old. Methods: The facial skin samples were taken through the Rodact Contact Test plates for the overall number of bacteria and the overall number of yeast and mold. Then, all colonies were characterized and dyed through the Gram method. Simultaneously, a subjective evaluation of participant's skin condition was made. Results: Results showed that age lead to the alterations of facial skin microbiome. The coccus and rod-shaped bacteria were isolated in all testes groups but in different amount. In addition, work conditions of the participants play a role in determining of skin microbiota. Conclusions: The conducted research allowed to indicate the differences in morphology, as well as the amount of microorganisms depending on the age. It can be assumed that the isolated bacteria from the skin are above all *Staphylococcus epidermids* and *Cutibacterium acnes.*

Keywords: microbiome skin, *Staphylococcus epidermidis, Cutibacterium acnes.*

ORAL PRESENTATION

Can food cause acne? Correlations between diet and skin

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Abstract
Acne vulgaris is classified as a disease of civilization, which also includes insulin resistance, obesity, type II diabetes, metabolic syndrome and cancer. It affects about 83-95% of young people between the ages of 16 and 19, as well as adults over the age of 30 (late acne). Most people undergo mild acne, with moderate lesions. 15% are said to have severe forms of the disease. Regardless of the severity of acne, the presence of acne lesions affects the psyche of those who deal with it. Increasing attention in the etiology of acne is being paid to aspects related to diet and the role of insulin-like growth factor IGF-1. Its elevated levels have been detected in adolescents with acne vulgaris and in women with late-onset acne. People with Laron-type dwarfism, which is characterized by IGF-1 deficiency, do not suffer from Acne vulgaris. The research of Otto Schaefer, who spent 30 years looking at Inuit populations adopting the eating habits of Western civilizations, is well-known. Initially, the lesions typical of acne did not occur in them, but over time the incidence of the disease was similar to Western countries. Currently, there are more and more reports in the literature on how to support the therapy of acne vulgaris with a properly adjusted diet. Attention is paid not only to the external use of cosmetic preparations, but also to the patient's diet, lifestyle and ability to cope with stress.

Keywords: acne, diet, eating habits, IGF-1, skin

ORAL PRESENTATION

Utilization and reimbursement trends of oral anticoagulant drugs in Albania

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Abstract

Nowadays, research studies suggest that direct oral anticoagulants (DOACs) compared to vitamin K antagonists (VKAs) are more convenient because they do not require routine blood monitoring, have fewer food and drug interactions, an improved safety profile and increase anticoagulation rates in people with atrial fibrillation. Since 2016, different DOACs acting as factor Xa inhibitors have been introduced into the Reimbursed Drug List in Albania as alternatives to the previously dominant VKAs. The study aimed to evaluate and compare the utilization and reimbursement trends of oral anticoagulant drugs in Albanian pharmaceutical market. A retrospective comparative study was used to analyze trends of utilization and reimbursement costs of DOACs and VKAs in Albania from 2014 to 2023, by using Anatomical Therapeutic Chemical/Defined Daily Dose (ATC/DDD) methodology recommended by World Health Organization. The data on oral anticoagulant drugs were collected from the Mandatory Health Care Insurance Fund database, while relevant population data were gathered from the Institute of Statistics' database. Drug utilization was quantified as DDD per 1,000 inhabitants per day (DID), providing a standardized measure of consumption. The total use of oral anticoagulant drugs for the selected period, 2014 to 2023, almost was quadrupled. VKAs had the highest utilization in 2016, while DOACs increased their use till 2023, approximately six times higher than in 2016. The utilization ratio of VKAs to DOACs was reversed from 75/25 in 2016 to 28/72 in 2023, resulting in a significant reimbursement cost increase for DOACs, accounting 98% of the total oral anticoagulant drugs reimbursement. The most used and expensive DOAC was rivaroxaban. To conclude, the utilization and reimbursement trend of DOACs in Albania was in accordance with the international recommendations. With the inclusion of generic DOACs in the Reimbursed Drug List 2024, the financial impact of these drugs on the healthcare budget may be more limited.

Keywords: Oral anticoagulants, Direct oral anticoagulants, Vitamin K antagonists, Utilization, Reimbursement trend

ORAL PRESENTATION

Epidemiological, clinical and biochemical profile of metabolic syndrome in patients from west Algeria

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Abstract

Introduction Metabolic Syndrome (MetS) is not defined as a disease and therefore there is no universal definition for it, but it is characterized by abdominal obesity, high blood pressure, low HDL-cholesterol blood level, high triglycerides and insulin resistance. Our objective was to evaluate the epidemiological, clinical and biochemical profile of metabolic syndrome in west Algerian patients. Patients and methods 52 cases with MetS with an average age of 62.5 years (M/F sex ratio: 1.47) were screened in different laboratories over a period of 2 months. The metabolic syndrome was retained according to the NCEP-ATP III criteria. Results The body mass index showed that 51.92% of our cases are obese, of which 34.62 % presented moderate obesity with a male predominance. 50 % of patients had an increased waist size compared to 50% who were normal. The distribution of patients according to comorbidities reported the following percentages: diabetes estimated at 100% of which 88.37% are type 2 diabetics, arterial hypertension at 61.54%, cardiovascular disease at 23.08% and finally a digestive disease estimated at 23.08%. On the biochemical level, fasting hyperglycemia was noted in 86.54% of cases. The lipid abnormalities observed were as such: hypertriglyceridemia (82.69%), hypercholesterolemia (21.15%), low HDL-cholesterol level (55.77%) and high LDL-cholesterol level (9. 62%). Hyperuricaemia and hypercreatinaemia were observed in 5.77% and 13.46% respectively. Liver function tests showed elevated TGO and TGP levels in 3.85% of patients. Conclusion Our results demonstrated that fasting hyperglycaemia and hypertriglyceridaemia seem to be the two most significant criteria of the metabolic syndrome.

Keywords: Metabolic syndrome; Biochemical profile; Diabetes; Obesity.

ORAL PRESENTATION

Evaluation of the effectiveness of cryolipolysis treatment in reducing excessive body fat

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Abstract

Cryolipolysis is a non-invasive method that freezes fat cells, leading to their natural elimination by the body. The purpose of this study was to test the effectiveness of cryolipolysis treatment in reducing body fat and to determine the extent of this reduction. The study was conducted on a group of 10 respondents between the ages of 20 and 50, including 2 people with normal BMI, 5 overweight people and 3 obese people (including one with grade II obesity). Respondents underwent cryolipolysis using the Body Bell CRYO ANGEL 360 SMALL, and changes in body fat, abdominal circumference and BMI were monitored before and after the procedure. Measurements were taken using a Seca mBCA 515 body composition analyzer and a tape measure. The results of the study showed that 8 of 10 respondents showed a reduction in body fat, confirming the effectiveness of the treatment. Among 6 of 10 people, abdominal circumference decreased, indicating a local reduction in body fat. In addition, BMI was reduced in 7 of 10 people, indicating the overall effect of the treatment on body weight. The treatment had no side effects, confirming its safety.
The cryolipolysis procedure has proven effective in reducing body fat, both locally and overall. It is safe, non-invasive and well tolerated by patients. The results suggest that cryolipolysis may be an effective tool in the fight against excessive body fat. Further studies on a larger group of respondents are recommended to confirm the results and better understand the long-term effects of the procedure.

Keywords: cryolipolysis, overweight, obesity, bmi

ORAL PRESENTATION

In vitro screening for acetylcholinesterase inhibitory activity in vacuum liquid chromatography fractions of an Algerian endemic plant *Genista* sp. Roots

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Abstract

Currently, products derived from nature play an important role as a potential source of medicine. Many pharmaceutical agents have been discovered by screening natural products from plants, ethnopharmacological data can guide the search for medicinal plants with potential bioactivity.The genus Genista is an important genus of the Fabaceae (Leguminosae) family with approximately 100 species distributed mainly in the Mediterranean region and in Western Asia. This genus is represented in Algeria by 23 species, eleven of which being endemic to Algeria. With its cosmopolitan distribution, has attracted the human interest since ancient times, as it is used in folk medicine. Many Genista species show interesting biological properties such as hypoglycemic, anti-inflammatory, antiulcer, and cytotoxic activity against different human cancer cell lines. In a continuation of our work on the chemical constituents of Genista species growing in Algeria, the roots of Genista Sp. were investigated. Alzheimer's disease, a progressive neurodegenerative disease, is characterized by hypofunction of acetylcholine (ACh) neurotransmitter in the distinct region of brain. Acetylcholinesterase (AChE) is an enzyme that metabolizes the ACh at synaptic cleft resulting in Alzheimer's disease. The present work summarizes and discusses acetylcholinesterase inhibitory activity aiming to highlight the recent advances in current knowledge on the roots of Genista species as a new source of bioactive compounds. The crude extract of this plant's roots was obtained after macerating 700 grams of dried roots in a hydroethanolic mixture, followed by filtration and evaporation, fractionation of the crude extract was accomplished through VLC (vacuum liquid chromatography), the elution is initially carried out by a 70/30 distilled water/methanol system, then the polarity is increased by methanol as follows 60/40, 40/60, 20/80, 100% with 3 repetitions for each system. 14 VLC fractions were tested against Acetylcholinesterase (AChE), Galanthamin was used as standard. Based on our results, we determined that fractions 8, 9, 10, and 11 showed higher inhibition percentage (g/ml) values ranging from 93,50 g/ml to 95.24 g/mL against AChE respectively compared to standard compounds. This study indicated that the roots of Genista species have a wide scope and possess therapeutic potential to efficiently manage neurological disorders associated with AChE dysregulation.

Keywords: *Genista* sp. Roots, Fabaceae, Enzyme inhibition, AChE, Alzheimer, Vacuum Liquid Chromatography**.**

ORAL PRESENTATION

Total phenolic and total flavonoid content, acetylcholinesterase inhibitory activity of an Algerian endemic plant *Chrysanthemum* sp.

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Abstract

Asteraceae is a large family of medicinal plants, containing over 13000 species most of which have been used as traditional medicines. Many genera of this family such as Chrysanthemum have attracted the attention of various authors for their phytochemical and biological properties, particularly antioxidant, antimicrobial and cytotoxic activity against different human cancer cell lines. Alzheimer's disease, a progressive neurodegenerative disease, is characterized by hypofunction of acetylcholine (ACh) neurotransmitter in the distinct region of brain. Acetylcholinesterase (AChE) is an enzyme that metabolizes the ACh at synaptic cleft resulting in Alzheimer's disease. The present work summarizes and discusses acetylcholinesterase inhibitory activity aiming to highlight the recent advances in current knowledge on the aerial parts of Chrysanthemum species as a new source of bioactive compounds. The plant was collected from Ghardaïa located in the south of Algeria. The crude extract CEC of this plant was obtained by maceration in a hydroethanolic mixture for 48h and then filtrated. After evaporation, the residue was dissolved in water. The resulting solution was extracted successively with petroleum ether, chloroform, ethyl acetate and finally n-BuOH. The evaporation of solvents in vacuum led to the respective dried extracts PEC, CEC, EEC, BEC. 5 extracts were tested against Acetylcholinesterase (AChE), Galanthamin was used as standard, determination of total flavonoid content (TFC) was carried out using UV-Vis spectrophotometry method with AlNO3 reagent at 415 nm. TFC results are expressed in quercetin equivalent (QE) and determination of total phenol content (TPC) was carried out using UV-Vis spectrophotometry method with Folin-Ciocalteu reagent at 760 nm. TPC results are expressed in pyrocatechol equivalent (PE). The TPC results are ranging from 17.45±1.27 to 135.25±5.09 µg PEs/mg extract and the TFC results are between 17.85±0.38 and 135.2±1.23 µg QEs/mg extract. We determined that only PEC and CEC showed inhibition percentage (g/ml) values 54.02±0.97g/ml and 18.62±0.75 g/mL against AChE respectively compared to standard compounds. Based on the research results, we conclude that the extracts contain active compounds of flavonoids and phenols through UV-Vis spectrophotometry test and that the aerial part of Chrysanthemum species don't possess therapeutic potential to efficiently manage neurological disorders associated with AChE dysregulation.

Keywords: *Chrysanthemum sp*., Enzyme inhibition, AChE, Total phenolic, Total flavonoids.

ORAL PRESENTATION

Eco-innovative Laser-Printed Paper ELISA Assay for Noninvasive and Colorimetric AFP Detection in Saliva

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Abstract

Alpha-fetoprotein (AFP) is a well-established biomarker for liver cancer diagnosis, but it is also crucial for detecting congenital anomalies such as Down syndrome and spinal canal defects during pregnancy. Despite the importance of AFP screening, conventional immunological assays like ELISA dominate the market, often generating significant plastic waste and requiring complex procedures. To address these challenges, this study proposes a novel, eco-friendly paper-based ELISA (pELISA) for point-of-care AFP detection in saliva. The innovative platform utilizes laser-printed hydrophobic barriers on filter paper, which is a sustainable alternative to traditional inkjet and wax printing methods. This approach integrates hydroxyapatite for antibody immobilization and silver-enhanced gold nanoparticles for colorimetric detection, offering a wide detection range (1.0–800 ng/mL) and a low limit of detection (1.0 ng/mL). The LP-pELISA demonstrates high selectivity, repeatability, and stability, performing effectively in spiked human saliva samples. Achieving a high AGREE score, the LP-pELISA aligns with green analytical chemistry principles, presenting a reliable, low-waste, and cost-effective diagnostic tool. This method represents a significant advancement in noninvasive diagnostics, bridging the gap between sustainability and high-performance biomarker detection.

Keywords: Paper-based ELISA, Alpha-fetoprotein, Congenital anomalies, Saliva diagnostics, Green analytical chemistry.

ORAL PRESENTATION

Microbial Contamination of Cosmetics Testers

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Abstract

Introduction: Health and hygiene awareness demands that cosmetic manufacturers provide not only aesthetically pleasing packaging but also ensure that their products are safe and do not pose health risks to consumers. Cosmetics testers, frequently touched by human hands and skin face, as well as oral mucosa become source of different microorganisms including pathogens.
Aim of the study: The aim of this study was to assess the microbiological purity of color cosmetics testers.
Methods: Short microbial diagnostic were done. Samples were taken from 5 cosmetics testers and 5 cosmetics using by individual students. Baird-Parker agar, McConkey agar, Agar N, Sabourauda agar with dextrose and agar for the overall number of bacteria were used. Colonies were characterized and dyed using Gram method.
Results: Growth of bacteria, yeast and molds were not observed for all testers cosmetics. For cosmetics using by individual students staphylococci, yeast and molds were observed.
Conclusions: The amount of cosmetics testers users as well as environmental conditions of store them not influenced on microbial contamination. Interestingly cosmetics used by individual users were contaminated by staphylococci, yeast and molds.

Keywords: cosmetics testers, contamination

ORAL PRESENTATION

The Gut Microbiota And Diabetes

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Abstract

The discovery of the role of the gut microbiota in the mechanisms of type 1 diabetes has recently opened new perspectives. The gut microbiota consists of billions of non-pathogenic bacteria, viruses and fungi. It is located in the small intestine and colon. However, what is the role of the gut microbiota? Diet plays an important role in the composition of the gut microbiota. Indeed, from the consumed foods, intestinal bacteria produce organic compounds, metabolites, which can affect health. Furthermore, type 1 diabetic patients also exhibit multiple impairments in the intestine, with low grade local inflammation, a weakening of the mucosal integrity and a disruption of the immune system and gut microbiota. Recent studies have shown that diabetes affects the intestines. Indeed, poorly managed diabetes can promote the dominance of pathogenic bacteria. This can lead to alterations in the intestinal mucosa. Which represents a barrier between bacteria and the intestine, potentially increasing the risk on infections and inflammation. To understand the mechanisms responsible for these alterations, researchers used a mouse model and showed a loss of production by mucosal immune cells of cytokine that are involved in strengthening the intestinal barrier. The production of these cytokines stimulated by some bacteria in the microbiota, such as segmented filamentous bacteria, which are found at very low levels in the gut microbiota of animals that will develop diabetes. These animals thus exhibit a loss mucus production, compromised integrity of digestive mucosa and increased passage of microbiota compounds and bacteria into the bloodstream towards the liver. These results highlight the importance of intestinal alterations in the pathophysiology of type 1 diabetes, as well as the therapeutic potential associated with their treatment. In conclusion, balanced diabetes is influenced by a balanced diet, consuming plenty of fibers, which promotes the development of intestinal probiotics

Keywords: Probiotc, Microbiota, Diabete, Immune system, Gut.

ORAL PRESENTATION

Aqueous environment and the role of ALD-Al_2O_3 coating on $MnCl_2$(TPA)-PDMS luminescent films

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Abstract

A novel eco-friendly luminescent $MnCl_2$(TPA) organic-inorganic complex was synthesized in powder form and incorporated as an emissive additive in an in-situ formed PDMS composite. Photoluminescence measurements were conducted to determine emission intensity, while luminescence decay and lifetime analysis provided insights into the stability and dynamics of excited states. Raman spectroscopy and FTIR were utilized to study the structural properties of the $MnCl_2$(TPA) powder. Morphological and structural characteristics of both the powder and the PDMS composite were examined using optical microscopy. The luminescent behavior of the $MnCl_2$(TPA)-PDMS composite was further evaluated in aqueous environments. Atomic layer deposition (ALD) of Al_2O_3 on the PDMS surface was tested for its protective effects, showing enhanced stability and preservation of photoluminescent properties in water, reducing degradation. This comprehensive study highlights the optical performance and structural resilience of $MnCl_2$(TPA)-embedded PDMS films, with and without ALD-Al_2O_3 coatings, in water-based environments.

Keywords: $MnCl_2$(TPA), PDMS polymer, photoluminescence, ALD-Al_2O_3 coating, water stability.

Acknowledgement
We acknowledge the support for this work from the Scientific and Technological Research Council of Türkiye (TÜBİTAK) through the 2219 International Postdoctoral Research Fellowship Program (Grant# 1059B192202512), QLEDCures LLC, DARPA Award No. 140D0422C0020, and the Florida High Tech Corridor Council (FHTCC) matching grant.

ORAL PRESENTATION

Investigating Hepatoprotective Potential of Mesenchymal Stem Cells Against Acetaminophen-Induced Damage

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Abstract

The liver is a crucial organ involved in metabolism, playing a significant role in maintaining homeostasis, synthesizing essential components, storing nutrients, and facilitating detoxification. Mesenchymal stem cell (MSC) therapy is considered a promising strategy for treating liver damage in the context of regenerative medicine. In liver tissues, MSCs can migrate to the site of injury, differentiate into hepatocytes, mitigate inflammation, reduce fibrosis, and demonstrate antioxidant activity. The purpose of this study is to explore the effects of Wharton's jelly-derived mesenchymal stem cells on acetaminophen (APAP)-induced hepatotoxicity. For this purpose, an initial optimization study was performed to establish the appropriate mesenchymal stem cell ratios. Afterward, liver cells were cultured in an *in vitro* microenvironment model to replicate liver physiology. Subsequently, a triple cell culture model was developed, and the effects of various APAP concentrations were evaluated through the MTT cytotoxicity assay. After 24 hours of drug exposure, the IC50 value was calculated, and live/dead analysis was conducted using the optimal drug concentration. According to the MTT assay results, APAP exhibited a dose- and time-dependent cytotoxic effect. Moreover, it was found that different APAP concentrations had distinct effects on MSCs. The live/dead analysis, which also examined morphological changes, indicated about %45-50 reduction in cell viability compared to the control group, along with diverse morphological alterations depending on the MSC ratios. In conclusion, the data suggest that MSCs may exert a protective effect in *in vitro* hepatotoxicity models. Further research, incorporating functional molecular assays and characterization studies, is required to enhance these findings.

Keywords: Hepatotoxicity, APAP-induced liver injury, Wharton's jelly-derived mesenchymal stem cells, MTT, Live/Dead.

Acknowledgment
This study was supported by Turkish Scientific and Technological Council (TUBİTAK 1004- Regenerative and Restorative Medicine Research and Applications) under the grant numbers of 20AG003, 20AG031 and 122C158 (TUBİTAK 2218- National Postdoctoral Research Fellowship Program). This work also has been supported by Eskisehir Osmangazi University Scientific Research Projects Coordination Unit under grant number of TYL-2024-3141 and TOA-2022-2307.

ORAL PRESENTATION

Investigation of mesenchymal stem cell ratios on liver regeneration

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Abstract

Drug-induced liver injury (DILI) has the potential to exacerbate pre-existing hepatic conditions and accelerate the progression of liver disease. Hepatotoxicity disrupts liver function, leading to tissue damage, and poses a significant risk, particularly with chemotherapy drugs like cyclophosphamide. The present study aims to investigate the effects of cyclophosphamide drug-induced hepatotoxicity on mesenchymal stem cells (MSCs) and assess cell viability in a 2D static culture system. Mesenchymal stem cells (MSCs) have garnered significant attention in recent years for their potential therapeutic applications in liver diseases, particularly in the context of hepatotoxicity. Therefore, liver cells were first cultured in conditions that mimic liver microphysiology and exposed to varying concentrations of cyclophosphamide. Additionally, an optimization study was conducted to determine the appropriate MSCs concentration to evaluate their regenerative potential. Different concentrations of MSCs were co-cultured with liver cells. After 24 hours of drug exposure, the toxic doses of cyclophosphamide were identified using the WST-8 cytotoxicity assay. Following the determination of the optimal MSC ratio and drug concentration, live/dead staining was performed using fluorescence microscopy to observe changes in cell viability and morphology. The results showed a dose- and time-dependent increase in cytotoxic effects, while different concentrations of MSCs exhibited a protective effect. In conclusion, the development of this study will help the establishment of a hepatotoxicity model, as well as the application of stem cell therapy from a regenerative medicine perspective.

Keywords: Cyclophosphamide, DILI, Mesenchymal stem cells, Co-culture, Live/Dead Analysis

Acknowledgment
This study was supported by Turkish Scientific and Technological Council (TUBİTAK 1004- Regenerative and Restorative Medicine Research and Applications) under the grant numbers of 20AG003, 20AG031 and 122C158 (TUBİTAK 2218- National Postdoctoral Research Fellowship Program). This work also has been supported by Eskisehir Osmangazi University Scientific Research Projects Coordination Unit under grant number of TYL-2024-3145 and TOA-2022-2307.

ORAL PRESENTATION

Investigation of Antiviral Activity of *Vitis vinifera* Against SARS-CoV-2

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Abstract

In case of pandemic, the major challenge to overcome in antiviral drug development is to discover potential unknown virus-host interaction, reproducibility of clinical data on drug-related efficacy and safety. These needs addressed the potential use of common herbs and spices as antiviral agents and immunity boosters. The aim of this research is to determine the antiviral activity of the *Vitis vinifera* against the SARS-CoV-2 and to contribute its use as an alternative therapeutic agent. MTT cell viability assay, Hematoxylin & Eosin staining, Annexin V/PI/Hoechst staining, and DCFDA assay were carried out to investigate *V. vinifera* extract' effects on the cell viability, cell morphology, apoptosis, and oxidative stress by using HEK293T, Vero E6, Caco-2, and Calu-3 cell lines, respectively. Subsequently, high throughput screening of antiviral activity of *V. vinifera* extract was analyzed targeting inhibition of RBD-ACE2 binding with ELISA and inhibition of main protease (Mpro) activities with FRET test. Additionally, SARS-CoV-2 host cell entry inhibition potential of *V. vinifera* extract was investigated by performing pseudovirus S1 neutralization assay using HEK293T cell line. The results of in vitro assays for *V. vinifera* extracts were demonstrated significant antioxidant activity and antiviral potential preventing 60% of SARS-CoV-2 infection on in vitro model. *V. vinifera* is a very powerful promising therapeutic agent in case of viral infection.

Keywords: SARS-CoV-2, *Vitis vinifera*, Antiviral activity, Antioxidant effect, MTT assay, DCFDA assay

ORAL PRESENTATION

Determination of antimicrobial, anti-biofilm and anti-quorum sensing properties of *Myosotis alpestris* and *Cardamine bulbifera* plant extracts

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Abstract

The genus *Myosotis* is one of the largest genera in the family *Boraginaceae*. The *Boraginaceae* family includes plants of significant importance in pharmacology and cosmetics. Compounds isolated from these plants are known to exhibit antimicrobial, antitumor, antiviral, anti-inflammatory, cardiotonic, contraceptive, and antiplatelet activities. *Cardamine L.* is one of the largest genera in the family *Brassicaceae*. Plants from the *Brassicaceae* family have antioxidant, anticarcinogenic, and cardiovascular protective activities. It has been reported that some *Cardamine* species contain compounds in both their aerial parts and roots that are used in folk medicine to treat colds, respiratory conditions, gastrointestinal issues, and urogenital infections. This research aims to determine the antimicrobial, antibiofilm, and anti-quorum sensing activities of extracts from *Myosotis alpestris* and *Cardamine bulbifera*. In the antibiofilm assay *C. bulbifera* exhibited biofilm inhibitory activity at concentrations of 20 mg/ml and 12.5 mg/ml against the *Pseudomonas aeruginosa* strain; however, biofilm inhibition was not detected at the remaining concentrations examined. *M. alpestris* exhibited no inhibitory effects at concentrations of 40 mg/ml, 25 mg/ml, or 12.5 mg/ml. For the *P. aeruginosa* PA01 strain, *C. bulbifera* demonstrated biofilm inhibition at concentrations of 20 mg/ml and 12.5 mg/ml, yet no inhibition was evident at the other concentration. *M. alpestris* did not show any effect at concentrations of 40 mg/ml, 25 mg/ml, or 12.5 mg/ml. For the *P. aeruginosa* PA01 strain, *C. bulbifera* exhibited biofilm inhibition at concentrations of 20 mg/ml and 12.5 mg/ml, but no inhibition was observed at the other dose. *M. alpestris* exhibited biofilm inhibition at concentrations of 40 mg/ml and 25 mg/ml, but no effect was observed at the 12.5 mg/ml dose. In the anti-quorum sensing experiment conducted with *Chromobacterium violaceum*, neither plant extract demonstrated anti-quorum sensing properties for any of the bacterial strains tested.

Keywords: *Myosotis alpestris*, *Cardamine bulbifera*, antimicrobial activity, anti-biofilm, anti-quorum sensing.

Acknowledgments: This research was conducted with the support of TUBİTAK 2209-A Research Project Support Programme for Undergraduate Students.

ORAL PRESENTATION

Investigation of Loading Packages and Door Opening on Energy Consumption of Bottle Coolers

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Abstract

Bottle coolers are one of the commonly used commercial coolers. They are frequently preferred in restaurants, cafeterias, and supermarkets to keep beverages at optimal temperatures. Various features related to the system, such as dimensions, capacity, noise level, energy efficiency, *etc*., are taken into consideration during purchasing them. The energy consumption of these systems is also directly linked to user behaviour, such as the total number of bottles loaded into the cabinet and the frequency of opening doors, *etc*. In this study, a laboratory-scale cooling system was tested with different total quantities of water bottles (0-4000 mL) inside a cabinet at temperatures ranging from 0-7 ℃ to simulate a bottle cooler [1,2]. It was revealed that the compressor off-time is highly dependent on the number of water bottles loaded into the cabinet. The compressor running time was determined to be 20.0% without any water bottles. This value decreased to 18.8% with a low product load (800 mL); however, the running time increased with the number of loaded water bottles. The compressor running time was 23.1% with a total water bottle volume of 2000 mL, while this value was found to be 30.0% with a total water bottle volume of 4000 mL. Moreover, the effect of opening the cabinet door at certain intervals on the system performance was examined with a total water bottle volume of 4000 mL. The door opening every 10 minutes at a 180° angle for 30 seconds resulted in an increase in compressor running time up to 33.3%. In sum, both optimizing the loading amount of bottles and decreasing the frequency of door opening are of great importance in reducing system energy consumption in bottle coolers.

Keywords: Bottle coolers, energy consumption, door opening, load packages

Acknowledgements

This research was supported financially by the Scientific and Technological Research Council of Turkey (TUBITAK), 2209-A - University Student Research Project Support Program (Project no:1919B012328680).

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ORAL PRESENTATION

Synthesis, characterization, and antituberculosis activity of novel 5-(trifluoromethoxy)-1H-2-indolinones bearing naphthalene ring

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Abstract

Tuberculosis (TB) is an airborne disease caused by the bacterium *Mycobacterium tuberculosis* (Mtb), affecting various tissues, primarily the lungs (Jain et al., 2024). Though TB, which can be seen in all countries and age groups, is treatable and preventable, it is the second most infectious killer disease globally behind COVID-19 (above AIDS). According to World Health Organization (WHO) estimates, 10.6 million people contracted tuberculosis in 2022 and a total of 1.3 million people died (WHO, 2023). The organization recommends a 6-month treatment consisting of four first-line drugs (isoniazid=INH, rifampicin, ethambutol, and pyrazinamide) for drug-susceptible TB patients. Resistance to both INH and rifampicin in multidrug-resistant (MDR-TB) and rifampicin-resistant (RR-TB) strains threatens public health (Wulandari et al., 2024). In a study on isoniazid hybrid compounds indicated that the compound which was substituted with bromine at position 5- of indole ring in INH-indole derivatives had same antituberculosis activity as INH, while the compounds derived by condensation from position 2- or 3- of indole ring provided equally higher activity than INH (Oliveira et al., 2017; Wahan et al., 2024). Several compounds with IC_{50} values ranging from 31.25 to 62.5 µg/ml were identified when the antituberculosis activity of 5-fluoro-1*H*-indole- 2,3-dione compounds with thiosemicarbazone structure was tested against rifampicin (IC_{50}: 25 µg/ml) in MTB H37Rv cell lines (Sevinçli et al., 2020). In this study, two series of novel 1-[(naphthalen-1/2-yl)methyl] substituted 5-(trifluoromethoxy)-1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazone) compounds (**5a-c** and **6a-c**) were synthesized by condensation of 1-[(naphthalen-1/2-yl)methyl]-5-(trifluoromethoxy)-1*H*-indole-2,3-dione with 4- (substituted phenyl)thiosemicarbazides in ethanol medium (Figure 1). The structures of these compounds **5a-c** and **6a-c** have been established using analytical and spectroscopic findings. The antituberculosis activities of compounds were assessed using MTB H37Rv cell group in comparison to rifampicin as standart. The screening results showed that the compounds' IC_{50} value ranged from 62.5 to 125 µg/ml based on the inhibitory profile. The IC_{50} value of compounds (**5a** and **6a**) that were 4-methylphenyl substituted (as R group) in both series was 62.5 µg/ml.

Keywords: indole, naphthalene, thiosemicarbazone, synthesis, antituberculosis activity.

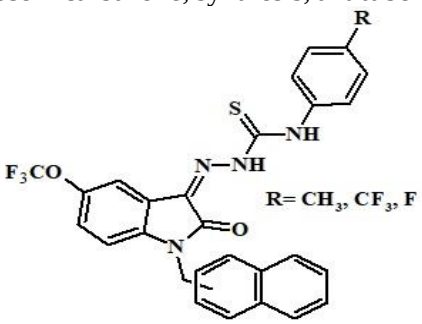

Figure 1. General structure of 1-[(naphthalen-1/2-yl)methyl]-5-(trifluoromethoxy)-1*H*-indole-2,3-dione 3-(4-phenylthiosemicarbazones) (**5a-c** and **6a-c**)

Acknowledgements

This study was supported The Scientific and Technological Research Council of Turkey (TUBITAK) (grant number: 1003-215S011).

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ORAL PRESENTATION

Photocatalytic Hydrogen Evolution from ZnO-loaded Carbon Nanotube

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Abstract

Graphene-based materials attract important interest due to their enhanced electron transfer efficiency in photocatalytic hydrogen evolution reactions. In this study, 1D graphene-based nanomaterial combined with ZnO and produced heterostructured CNT/ZnO nanocomposite catalyst enhance the hydrogen evolution. Herein, co-catalysts (MoS_x and Pt) were photodeposited onto CNT/ZnO nanocomposite catalyst in water, resulting in the formation of CNT/ZnO/MoS_x and CNT/ZnO/Pt through the reduction of $(NH_4)_2MoS_4$ and $H_2PtCl_6.6H_2O$, respectively. The photodeposition of co-catalysts on the CNT/ZnO nanocomposite provided enhanced catalytic efficiency and stability due to increased active surface area and enhanced electron transfer capabilities.

Keywords: photodeposition, photocatalytic activity, hydrogen, graphene-based nanomaterials

Acknowledgements
The authors would like to thank the Turkish Academy of Science (TUBA). This study is prepared from a section of Ph.D. thesis by Münevver Tuna Genç, which is also supported by Selçuk University (SUBAP-Grant no: 23211019 and SUBAP-Grant no: 23401013), Türkiye Council of Higher Education YOK-100/2000 scholarship, TUBITAK 1002-B (Grant number:124Z584) and TUBITAK 2211-C Domestic Priority Fields Doctoral Scholarship Program.

ORAL PRESENTATION

Development of new dihydropyrazole derivatives as potent anticancer agents against NSCLC and breast carcinoma

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Abstract

Lung cancer is the most prevalent cause of cancer-related death worldwide and non-small-cell lung cancer (NSCLC) accounts for nearly 85% of all lung cancer cases. Besides, breast cancer is the most prevalent and second deadly cancer type in women. Although many anticancer drugs have been developed for the treatment for both cancers, the clinical effectiveness of drug therapy is often limited by drug resistance and severe toxic side effects.

On the purpose of reaching selective and potent anticancer agents, in this research, new dihydropyrazole derivatives (**BH1-14**) were synthesized via the reaction of proper 2-bromo-1-arylethanone with 3-(3,4-dimethylphenyl)-5-(2-(4-chlorophenyl)pyrimidin-5-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (**B8**) and 3-(benzo[*d*][1,3]dioxol-5-yl)-5-(2-(4-chlorophenyl)pyrimidin-5-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (**B10**). On the other hand, **B8** and **B10** were obtained through the reaction of thiosemicarbazide wih (*E*)-3-(2-(4-chlorophenyl)pyrimidin-5-yl)-1-(3,4-dimethylphenyl)prop-2-en-1-one (**B3**) and (*E*)-3-(2-(4-chlorophenyl)pyrimidin-5-yl)-1-(benzo[*d*][1,3]dioxol-5-yl)- prop-2-en-1-one (**B5**), respectively. All these new compounds (**B3, B5, B8, B10** and **BH1-14**) were evaluated for their cytotoxic effects against human A549 NSCLC and MCF-7 breast carcinoma cell lines. According to the results, **B3** revealed the most promising anti-NSCLC effects in this series with an IC_{50} value of 3.91±1.22 μM compared to lapatinib (IC_{50}= 17.41±4.36 μM). The second most significant anti-NSCLC result was achieved by **B5** with an IC_{50} value of 16.82±4.18 μM. On the other hand, the anti-breast cancer effects of **B3** and **B5** were found to be lower compared to the anti-NSCLC effects (IC_{50}= 42.99±8.15 μM; 29.08±4.53 μM, respectively). The cytotoxic effects of **B3** and **B5** were also determined between Jurkat human leukemic T-cell line and peripheral blood mononuclear cell (PBMC) line. **B3** exhibited notable tumor selectivity between these cell lines with a Selectivity Index (SI) value of 57.25. Overall, **B3** stands out as a promising anticancer agent for future mechanistic studies.

Keywords: NSCLC, breast cancer, dihydropyrazole, chalcone, pyrimidine, drug development

Acknowledgement: This study was supported by Scientific and Technological Research Council of Turkey (TUBITAK) under the Grant Number 122Z775. The authors thank to TUBITAK for their supports.

ORAL PRESENTATION

Synthesis of New Bodipy Compounds and Investigation of Their Photophysical Properties

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Abstract

BODIPY compounds are used in important applications such as sensors, light harvesting agents, solar cells, energy transfer systems, and photodynamic therapy [1]. For biological applications, the absorption and emission wavelength of the dye is desired to be in the near infrared (NIR) region (650-900 nm) [2]. However, the absorption and emission wavelength of isolated BODIPY compound is in the range of 480-540 nm [3]. Therefore, extending the absorption and emission wavelengths of BODIPYs to the NIR region is of great interest to researchers. In this study, two novel BODIPY compounds were synthesized via Knoevenagel condensation reaction. In the designed molecules, biphenyl-4-carboxaldehyde was preferred to enhance the conjugation of the BODIPY core. These compounds are thought to be potential building blocks in materials to be developed for applications requiring NIR radiation.

Keywords: BODIPY, biphenyl-4-carboxaldehyde, near infrared, knoevenagel condensation

Acknowledgements

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) 2209/A program within the scope of the University Students Research Projects Support in the 2023/2.

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ORAL PRESENTATION

Risk Factors in Canine Idiopathic Gastroenteritis Diseases

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Abstract

Gastroenteritis diseases are one of the most important issues in the field of health from past to present and the fact that the clinical findings caused by lesions occurring in the gastrointestinal system are specific for some etiological agents is important for accurate diagnosis and treatment methods. However, the etiopathogenesis of some gastroenteritis diseases has not been fully elucidated and the broad spectrum drugs or corticosteroid therapy used in the treatment methods applied in this regard may not have a positive effect on the course of the disease. Idiopathic gastroenteritis is one of these diseases and many studies have been conducted in humans. Nevertheless, there are some deficiencies in animal studies. In this study, necropsies were performed on 20 dogs that came to Ankara University, Faculty of Veterinary Medicine, diagnosed with IBD and breed, age and sex data were collected. The aim of this study was to raise awareness of veterinarians by sharing current data on IBD disease in dogs in our country.

Keywords: IBD, Breed, Age, Dog

This study was financially supported by TUBITAK BIDEP 2209-A (Number:1919B01221449).

ORAL PRESENTATION

Antioxidant activity and total phenolic content of hawthorn vinegar produced in Yozgat region

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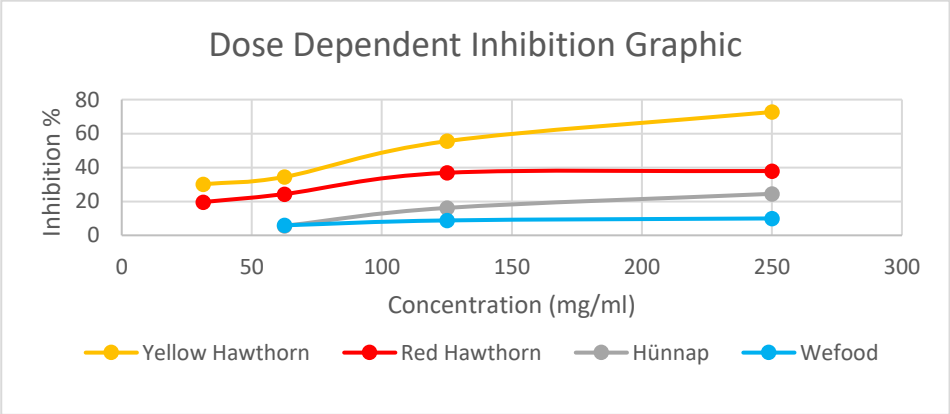
Abstract

The hawthorn plant which grows naturally in many regions of Turkey and has traditional use as folk remedy. Various researchers have conducted studies on the chemical content and activities of hawthorn products available in the market. In this study we aimed prepare vinegar of *Crataegus meyeri* Pojark collected from Yozgat region by using traditional methods, and determine whether it has any superiority in terms of antioxidant activity and phenolic compound content compared to organic hawthorn vinegars available in the market. The antioxidant activity was determined using the DPPH method, and the total phenolic content was determined using the Folin-Ciocalteu reagent. As a result, it was determined that the vinegar prepared from yellow hawthorn had highest antioxidant activity among samples (Figure 1). The equation related to the concentration of gallic acid is given as (y = 13260x + 136.33), with an (R^2) value of 0.9979. The sample with the highest total phenol content based on gallic acid is red hawthorn vinegar. It has been concluded that hawthorn vinegar prepared by traditional methods will be more beneficial than hawthorn vinegar sold as organic in the market in terms of antioxidant activity.

Figure 1. Dose dependent DPPH radical inhibiton of vinegar samples.



Keywords: Antioxidant activity, Hawthorn vinegar, Total phenolic content.

This study was supported by a grant of TUBITAK (1919B012302087).

ORAL PRESENTATION

Electrochemically modification of glassy carbon electrode with Fast Dark Blue R Salt and determination of Pb (II) ion

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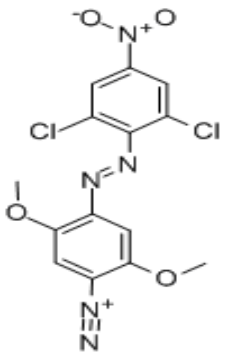
Abstract

Modifications of glassy carbon electrodes by various methods have been widely used in recent years. Electrochemical modification studies are encountered in the literature, especially using aril diazonium reduction method. Chemically modified electrodes (CMEs) have been very actively investigated in electrochemistry and many researches about the preparation, characterization, and electrochemical behaviors of CMEs have been performed for a long time. In this study, Fast dark blue R (FDBR) salt was modified by covalent bonding to the glassy carbon electrode surface. The modification process was done with the cyclic voltammetry (CV) technique. In electrochemical characterization, CV voltammograms of the solution prepared using ferrosen and ferrisiyanür were examined. Determination of heavy metal was applied to the GC electrode modified with FDBR material.

Keywords: Chemically modified electrode; electrochemical oxidation; Fast dark blue R salt; Voltammetry.



Scheme. Molecular structure of FDBR

This Study was Supported by Amasya University FMB-BAP Project No. 22-0571.

ORAL PRESENTATION

Predicting botanical composition using geostatistical methods in grassland

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Abstract

Grasslands play a crucial role in supplying livestock with forage resources, especially during the summer, while also preserving ecological balance. To ensure the continuity of this ecological balance, botanical composition needs to be accurately determined. For this reason, it is crucial to accurately predict botanical composition exchange in grasslands. This study aimed to predict botanical composition at un-sampled points using geostatistical methods, and to evaluate the modeling performance. Botanical composition was determined by line interception method at 150 random sampling points. Spatial variability of botanical composition was analyzed by semivariogram. The variability in decreaser plant percentage was the lowest (CV = 26.88%), while the increaser plant percentage showed a much higher variability (CV = 291.72%) compared to other plant characteristics. According to the results of geostatistical analysis, the spatial variations of decreaser, increaser, and invader plants were strongly dependent, while *Poaceae, Fabaceae*, and other families were moderately dependent. The shortest range value was recorded for *Fabaceae* plants (%) at 42 m, while the longest range value was for plants from other families (%) at 6330 m. The results showed that vegetation can be successfully modeled in semiarid grassland with uncontrolled sheep grazing. The spatial distribution maps helped pinpoint and define regions within the study area that should be left ungrazed, lack palatable vegetation, and are occupied by proliferating invasive plant species. More research is needed to predict changes in vegetation across different climatic conditions and larger areas, to develop effective grazing and management plans in grasslands.

Keywords: Spatial variability, kriging, spatial distribution, grassland, botanical composition

Acknowledgments
The authors would like to thank Scientific Research Projects Coordinator of Cankiri Karatekin University for providing financially for the study. This research was funded by Scientific Research Project Unit of Cankiri Karatekin University (BAP), Cankırı, Turkey under project no. BAP-OF210621B18.

ORAL PRESENTATION

Antimicrobial Activity of Waste Walnut (*Juglans regia* L.) Inner Shell Based Silver Nanoparticles

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Abstract

Increasing infections and antibiotic resistance developed by microorganisms in recent years have negatively affected sustainable health conditions. Therefore, it is important to develop products with antimicrobial activity. Silver nanoparticles are a broad-spectrum antimicrobial agent with antibacterial, antifungal, and antiviral properties. However, silver nanoparticles obtained using chemical techniques cause the formation of components that are harmful to the environment and human health during and after synthesis. For this reason, silver nanoparticles are obtained using green synthesis techniques that are environmentally friendly, economical, and not harmful to human health. In this study, the waste walnut inner membrane was used in the green synthesis of silver nanoparticles. The obtained nanoparticles were characterized and then the antimicrobial activity of silver nanoparticles was determined using the disk diffusion method. It was observed that the obtained nanoparticles formed zones of different diameters on microorganisms. This result shows that silver nanoparticles obtained by the green synthesis technique can be used as antimicrobial agents.

Keywords: green synthesis, silver, antimicrobial, *E.coli*, waste management

*This study is a part of Merve Can's Master's Thesis. It was supported by Bilecik Şeyh Edebali University (BAP) with project number of 2022-02.BŞEÜ.01-03.

ORAL PRESENTATION

Green-synthesized silver nanoparticles from *Alcea rosea* and *Calendula officinalis* flower extracts: nanosupports for lysozyme immobilization

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Abstract

Nanotechnology includes the design, production, processing and use of nanoparticles which possess at least one dimension of 1-100 nanometers [1]. They can be produced in different morphologies by different methods from components such as carbon, metal, metal oxides or organic precursors [2]. Although they can be produced by conventional physical and chemical methods, toxic chemicals threat the environment and restrict biotechnological applications of nanoparticles. Biogenic or green synthesis is a bottom-up approach that uses bacteria, fungi, actinomycetes, yeasts, algae, and plants and has advantages over classical methods such as nature-friendliness, low energy requirement, low cost, sustainability, and single-step synthesis [3]. Among the organisms used for green synthesis, plants appear to be the best candidates. Herein, silver nanoparticles (AgNPs) were fabricated using aqueous extracts of *Alcea rosea* and *Calendula officinalis*. Flowers of the plants were extracted with ultrapure water at 65°C and the filtrate was mixed with $AgNO_3$ solution to generate AgNPs. Optimum conditions were determined as 3.0 mM and 5.0 mM $AgNO_3$, 1:10 and 1:20 metal ion:extract volume, 120 min and 60°C for *A. rosea* and *C. officinalis,* respectively. AgNPs formation was followed by UV-Vis spectrophotometry and a SPR peak at ~450 nm verified the synthesis of AgNPs. Characterization of nanoparticles was carried out using FTIR, TEM and EDS. Antioxidant activities of AgNPs and extracts were determined as 75% and 91% for *A. rosea*, and 94% for both extract and AgNPs of *C. officinalis* by DPPH method. Lysozyme was immobilized covalently onto AgNPs via EDC/NHS chemistry and 72 µg protein/mg AgNPs was loaded. Enzyme activity measurements showed that immobilized lysozyme worked as effectively as free enzyme for lysis of *Micrococcus lysodeikticus* cells. It can be concluded that green-synthesized nanoparticles are potential support material candidates for immobilization of different enzymes and immobilized enzymes may be utilized for various applications.

Keywords: green synthesis, nanoparticle, extract, *Alcea rosea, Calendula officinalis.*

Acknowledgments: This study covers a part of Fatma OLĞAÇ's master's thesis. The authors are thankful to Aydın Adnan Menderes University Graduate School of Natural and Applied Sciences for their contributions and financial support provided by Scientific Research Project Unit (BAP) of Aydın Adnan Menderes University (Project No: FEN-24007).

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ORAL PRESENTATION

Design, synthesis and biological investigation of the novel 4-oxo-4*H*-chromene-(2/3)-carboxamide derivatives as an anti-Alzheimer agents

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and a major public health concern. Key pathophysiological features of AD include acetylcholine deficiency, neuroinflammation, oxidative stress, metal ion imbalance, and protein dysregulation, such as amyloid-β aggregation and tau hyperphosphorylation. Although the precise mechanisms are still under investigation, it is clear that a multifactorial interplay of genetic, environmental, and lifestyle factors contributes to AD development. Current treatments focus on symptom management, primarily using cholinesterase inhibitors and NMDA receptor antagonists, but these do not address the underlying disease processes and offer limited efficacy [1]. Therefore, there is a critical need for innovative, disease-modifying therapies to halt or slow AD progression. Recently, drug development has shifted toward multitarget-directed ligands (MTDL) rather than the traditional 'one-target, one-drug' approach [2]. In this study, we aimed to develop novel cholinesterase (ChE) and monoamine oxidase (MAO) inhibitors with additional antioxidant and metal-chelating properties using the MTDL strategy. Building on our previous efforts to develop anti-AD heterocyclic agents, in the current study we focused on chromone, a versatile heterocycle recognized as a privileged scaffold for drug design. The chromone ring selected in the design is a structure that potentially inhibits cholinesterase by interacting with the peripheral anionic site and has also been identified as a pharmacophore for MAO inhibitory activity [3, 4]. The chromone core was coupled with tertiary nitrogen-bearing structures known to interact with cholinesterase's catalytic site via a carboxamido ethylene bridge [5]. As a result, we synthesized twenty novel 4-oxo-4*H*-chromene-(2/3)-carboxamide derivatives (Figure 1) and evaluated their biological activities, including inhibitory effects on acetylcholinesterase, butyrylcholinesterase, MAO-A, and MAO-B, as well as their antioxidant and metal-chelating properties.

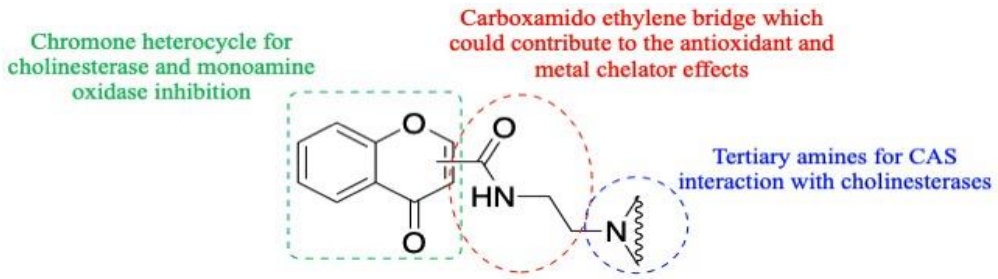

Figure 1. Design strategy and general structures of the final compounds.

Keywords: Alzheimer, chromone, cholinesterase, monoamine oxidase, antioxidant, metal chelator.

Acknowledgements: This study was supported by the Gazi University Scientific Research Projects Unit (Gazi BAP) under grant number TGA-2021_6910.

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ORAL PRESENTATION

Regulation of Oxidative Stress by *Capparis spinosa* and Glutamine Combination in Wound Healing

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Abstract

Oxidative stress is critical in delaying wound healing by exacerbating cellular damage and inflammation. *Capparis spinosa* is known for its antioxidant properties, while glutamine is an essential amino acid supporting tissue repair and modulating oxidative stress. We aimed to determine the effects of *Capparis spinosa*, a natural phenolic compound, and glutamine on oxidative events occurring in the wound healing process in excisional wounds created in hyperglycemic experimental animals. 42 adult male Wistar-albino rats with streptozotocin-induced (STZ) diabetes were divided into seven groups: control, untreated, treated with 0.2 g/kg glutamine, treated with 20 mg/kg *C.spinosa* by topical and oral gavage, and treated with a combination of 0.2 g/kg glutamine + 20 mg/kg by topical and oral gavage. Animals were sacrificed on day 7 of wound healing and levels of malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NOx) were measured spectrophotometrically in the wound tissues. As a result, C .*spinosa* and glutamine applications in rats decreased the MDA levels of the wound tissue compared to the untreated group ($p < 0.05$) The GSH levels were significantly elevated in all treatment groups compared to the untreated control group ($p<0.05$). The NOx levels of all treated groups were statistically decreased compared to the untreated group ($p<0.05$). The combination of *Capparis spinosa* and glutamine effectively reduces oxidative stress and enhances wound healing in a diabetic wound model. This suggests that using both agents together or separately may be a promising strategy to improve wound repair and reduce oxidative damage.

Keywords: Wound healing, Diabetes, Antioxidant, Glutamine, *Capparis spinosa*, Oxidative stress

Acknowledgement: This research was supported by Gazi University Scientific Research Projects (BAP), Ankara, Turkey (Project Number: FYL-2023-8513)

ORAL PRESENTATION

Determination of cadmium levels in seedlings developed by germination from the seeds of some wheat and barley varieties treated with different cadmium molarities

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Abstract

In this study, three durum wheat [*Triticum durum* L.], three bread wheat (*Triticum aestivum* L.), three barleys (*Hordeum vulgare* L.) varieties and three species of wheat landraces [*Triticum spelta* L., *Triticum dicoccum* L. and *Triticum monococcum* L.] were used. The experiments were designed according to a randomized plot design with three replications. Cadmium chloride ($CdCl_2$) solutions (1, 2 and 3 mM) and distilled water were applied to experimental groups and control group respectively, and germination was proceeded under optimized conditions. The results indicated that root and shoot parts of seedlings were separated from each other in each group and were dried at 45 ° C for 5 days. The dried samples were hydrolyzed by nitric acid-hydrogen peroxide [1:1] and analyzed by atomic absorption spectroscopy to measure the amount of cadmium stored in the roots and shoots. The lowest amounts of cadmium for each application were detected as 2.52 ppm, 9.2 ppm and 18.91 ppm in the roots of Kızıltan-91 and Ovidio varieties, and 0.61 ppm, 1.01 ppm, 2.56 ppm in the shoots of Çeşit-1252 and İnce-04 varieties respectively. The highest amounts of cadmium for each application were observed for both roots (31.07, 61.01 and 109.81 ppm) and shoots (4.29, 8.55 and 32.75 ppm) of Golia. In conclusion, heavy metal accumulation in wheat landraces and barley varieties were determined in similar levels, while bread wheat varieties other than Golia and durum wheat varieties were determined in similar levels. Heavy metal accumulation was higher in roots compared to shoots of seedlings. This might be due to the limited transfer of cadmium to the shoots by accumulating most of the cadmium in the root cells in vacuoles or by forming complexes with phytochelatins. It was recommended that further advanced research methods and more variety samples could produce more efficient results in investigating of cadmium heavy metal stress on germination physiology in cold climate cereals.

Keywords: Atomic Absorption Spectroscopy, Barley, Cadmium, Wheat, Wheat landraces

Acknowledgement: This study is a part of Funda Küyük's PhD thesis project, which was supported by Scientific Research Projects Coordinator (BAP) of Hitit University with the project numbers (FEF19001.20.006 and FEF19004.23.001).

ORAL PRESENTATION

Investigation of the potential protective and therapeutic efficacy of two different antioxidant combinations in d-galactose induced aging model

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Abstract

Aging, a natural part of life, is a process that begins at fertilization in the womb and ends with death. The aging process, which underlies many chronic diseases through deterioration of physiological functions and metabolic processes, is associated with increased oxidative stress. Antioxidant treatment strategies are gaining attention for their potential to mitigate and prevent the degenerative effects caused by oxidative stress. The aim of this study was to investigate the potential protective and therapeutic effects of two different antioxidant combinations, squalene (SQ) and saponin (SP), in a D-galactose (D-Gal)-induced aging model. A total of 48 male Sprague-Dawley rats (200-250 g) were used in the study. The animals were randomly divided into three groups (6 rats per group): Control group, D-Gal group, and D-Gal+SQ+SP combination group. The antioxidant treatment groups were administered SQ (2.66 ml/kg/day) and SP (100 mg/kg/day) orally. Following the treatment, brain tissue samples were collected from the sacrificed rats, and the levels of Sirtuin 1 (SIRT1), Forkhead box O3a (FOXO3a), and Paraoxonase 1 (PON1) in the tissues were measured using the ELISA method. Levels of SIRT1, FOXO3a, and PON1 in brain tissue were found to be decreased in the D-Gal group ($p<0.05$). In contrast, the combined antioxidant treatments resulted in a statistically significant increase in SIRT1, FOXO3a, and PON1 levels ($p<0.05$). The SQ and SP combinations demonstrated protective and therapeutic effects on aging-related oxidative stress biomarkers.

Keywords: Aging, Squalene, Saponin, Sirtuin1, FOXO3a, Paraoxonase1.

Acknowledgements: This study was supported by the Gazi University Scientific Research Coordination Unit (BAP) under project code FYL-2023-8665.

ORAL PRESENTATION

Incidence of subclinical hypocalcaemia or hypoglycaemia in Holstein dairy farms in Muğla Milas region (Yatağan, Çine-Aydın)

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Abstract

The development of postpartum secondary metabolic disorders due to subclinical hypocalcemia and the induction of subclinical ketosis induced by SCH have only recently been recognized globally. The incidence of subclinical form of hypocalcemia, a metabolic disorder that, due to genetic predisposition, occurs especially often in dairy breeds in the third and higher lactation and causes economic losses by triggering many secondary metabolic diseases, was studied for the first time in our country. Our study was carried out in a minimum of 12 farms in the Muğla Milas region (Yatağan, Çine-Aydın) that are involved in commercial fattening and dairy cattle breeding and have a herd monitoring system. Of these farms, 155 cows were randomly selected, with approximately 10-15 Holstein breed dairy cows that have recently given birth among them. SCH or hypocalcemia was identified with high accuracy and early diagnosis by evaluating the iCa (ionized calcium) value that was rapidly and highly reliably obtained using Edan İ15 VET blood gas device with fresh whole blood taken from newly delivered Holstein dairy cattle in the first 72 hours, which is a critical period after birth. According to the iCa <1.0 mmol /L value, 32 out of 97 cows fed anyoic were evaluated as SCH and it was determined that 33% SCH prevalence was observed. When the iCa reference value of the same cows was taken as <1.05 mmol /L, 50 out of 97 cows were determined to be SCH, with a prevalence of 51.5% SCH. In farms where nonanionic feeding was practiced, 26 out of 58 cows with iCa <1.0 mmol /L had SCH and the prevalence rate of SCH was 44.8%. When the iCa reference value for the same cows was taken as <1.05 mmol /L, 35 out of 58 cows had SCH and the prevalence rate of SCH was 60.4%. Statistical data showed that when the reference value for SCH was taken as <1.0 mmol /L, the odds value for nonanionic/anionic comparison was 1.65, and when the reference value for SCH was taken as <1.05 mmol /L, the odds value for nonanionic/anionic comparison was 1.43. In farms with non-anionic feeding, SCH prevalence was observed in 26 out of 58 cows (44.8%) according to iCa <1.0 mmol/L, whereas with an iCa reference value for the same cows <1.05 mmol/L SCH Prevalence was observed in 35 of 58 cows (60.4%) SCH was <1.0 mmol/L, the odds ratio for non-anionic versus anionic was 1.65, and when the reference value for SCH was <1.05 mmol/L , the odds ratio for non-anionic versus anionic was 1.43 It was once again established, based on all the data collected, that anionic feeding combined with a suitable ration and preventive measures (prenatal D-Vit, etc.) in the near phase are critical and essential for SCH prevention or reduction. Our study was the first and only one to use iCa, or ionized calcium, to reliably and quickly diagnose and detect SCH, allowing for the prompt initiation of treatment or the taking of necessary precautions.

Keywords: Subclinical hypocalcemia, Holstein, Metabolic and reproductive diseases, Urine pH, Blood Gas

This study was supported by Tübitak within the scope of 2209-A student projects and Muğla Sıtkı Koçman University BAP unit Lökap projects (Project No: 22/138/03/3/5)

ORAL PRESENTATION

The Use of Bio-oil Obtained by Rice Husk Pyrolysis in Rubber Paste Mixture

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Abstract

Rubber, one of the most important materials of the polymer industry, is used in many areas, such as the production of spare parts in the automotive industry, automobile tires, and conveyor belts. In the production phase, mixtures consisting of rubber, other raw materials and additives are prepared, which can be adjusted and vulcanized according to the desired properties. Some raw materials, from some types of rubber used in the mixture to resins and fillers, are petroleum derivatives. One of the renewable alternatives to fossil resources is biomass. Biomass is subjected to various thermochemical processes to produce solid (char), liquid (bio-oil) and gaseous products. Solid products can be used as fillers in carbon black, activated carbon and various products, while liquid products can be used as alternative fuel for fuel oil and as binders in various products. Gas products can be separated and used in industry. In this study, the use of the bio-oil obtained as a result of the pyrolysis of rice husk instead of process facilitating oil (aromatic oil) and binder resin in rubber dough mixtures used in conveyor belt production was investigated. For this purpose, bio-oil was substituted for resin and aromatic oil at 25%, 50%, 75% and 100%. Rheometer tests were carried out to examine rubber compound mixtures' cooking times and vulcanization behaviors. Afterward, the adhesion strengths of the prepared tape samples between rubber-cloth (rubber-tissue) and cloth-cloth (tissue-tissue) were tested and compared. In addition, a SEM analysis was carried out to understand the surface morphology of the rubber samples prepared with different recipes and to observe how bio-oil use in the blends affects the appearance and distribution of the surface. As a result of the studies, it was determined that the biofuel obtained from rice husk pyrolysis can be used in rubber products.

Keywords: Rice Husk, Pyrolysis, Bio-oil, Rubber, Conveyor Belt

Reference

Gürer, Hüseyincan. 2021. The Use of Bio-oil Obtained by Rice Husk Pyrolysis in Rubber Paste Mixture. Afyon Kocatepe University, Graduate School of Natural and Applied Sciences, MSc Thesis. Afyon.

Acknowledgements

This study was supported by Afyon Kocatepe University BAPK unit with project number 20.FEN.BİL.44.

ORAL PRESENTATION

The investigation of association between schizophrenia and haplotypes of NRG1 rs3802160 and rs10503929 variants in a Turkish case-control group

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Abstract

The first component of neuregulin signaling pathway (NSP) is *Neuregulin-1* protein *which* is encoded by NRG1 gene. NRG1 gene is the earliest gene whose genetic association was shown with schizophrenia. A number of SNPs located in the NRG1 gene have been reported to be associated with schizophrenia in several populations. The SNPs called rs3802160 and rs10503929 were also reported to be associated with the disease in some populations. In the present study, we determined the genotypes of those two SNPs and analyzed their single SNP and haplotype associations in a case-control group (96 schizophrenia patients and 100 controls) collected from Malatya-Turkey. A real-time PCR based genotyping method was used for allelic determination. The haplotype estimation and case-control association analyses were done using Haploview Software. None of those SNPs showed any association with schizophrenia. Also, we have found 4 different haplotypes constructed by rs3802160 and rs10503929. Nonetheless, we did not find any significant differences between the case and control groups for the distribution of these haplotypes. Indicating the absence of sufficient evidence to support the association between these haplotypes with schizophrenia in our sample. The reason for this may be the sample size, or the limited geographical region we have collected our sample. Screening the SNP in larger groups of family trios would help to find a potential association.

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

Acknowledgement: This study has been supported by Inonu University BAP project Number: FCD-2021-2552 to M.M.S.

ORAL PRESENTATION

Investigation of Hepatotoxic Effects of Ayurvedic Herbal Products by *In Vitro* Test

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Abstract

Ayurveda is a traditional medical system that originated in the Indian subcontinent. Complementary and alternative medicine involves the unregulated use of three Ayurvedic herbal preparations from the *Asteraceae* family: *Atractylodes lancea, Stevia rebaudiana,* and *Senecio vulgaris*. Despite the widespread use of plants containing diterpene glycosides and pyrrolizidine alkaloids in Ayurveda, their hepatotoxic effects and mechanisms in humans are not fully understood. The aim of this study is to investigate the *in vitro* hepatotoxicity of selected diterpene glycosides and pyrrolizidine alkaloids on the human hepatocellular carcinoma (HepG2) cell line using MTT and LDH assays. In this study, diterpene glycosides (atractyloside and stevioside) and pyrrolizidine alkaloids (senecionine and senecionine N-oxide) were tested at five different concentrations. The MTT assay was employed to examine the cytotoxic effects of various Ayurvedic plants and to assess their hepatotoxicity. In addition to evaluating mitochondrial pathways, the cytotoxic effects of various plant extracts on HepG2 cells were also assessed by measuring lactate dehydrogenase (LDH) release, which evaluates membrane permeability. LDH release indicates the leakage of cytoplasmic LDH enzyme due to membrane damage. Our results demonstrate that atractyloside A, senecionine, senecionine N-oxide, and stevioside disrupt membrane integrity and induce changes in metabolic activity in HepG2 cells. Based on *in vitro* test results, the cytotoxic effects of plant standards were ranked as follows: atractyloside A>stevioside>senecionine>senecionine N-oxide. Our study concluded that atractyloside A had the lowest IC50 value compared to the other standards, indicating the highest cytotoxicity. These findings suggest that atractyloside poses a higher risk of cellular toxicity compared to the other compounds, causing irreversible damage to cell membranes and mitochondrial activity. The data obtained from this study clearly indicate that glycoside standards are more toxic than pyrrolizidine alkaloid standards. In the risk assessment of xenobiotics, understanding the mechanisms of toxicity is crucial for predicting potential risks.

Keywords: Ayurvedic, Asteraceae, Hepatotoxic, MTT, LDH

Acknowledgements: This study was supported by a research project of the Ankara University BAP Projects (Project No: TDK-2023-2853).

ORAL PRESENTATION

Development of A New Method For The Electrochemical Determination of Some Pesticides In Anilino Pyrimidine Group

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Abstract

In this study, pencil graphite electrodes (PGE) were modified with l-cysteine and silver nanoparticles (Ag-NPs) and then pyrimethanil fungicide in anilino pyramidine group was determined by square wave voltammetric (SWV) technique. Firstly, the PGE working electrode, which was immersed in the 0.10 M H_2SO_4 solution in the 10.0 mL voltammetric cell, was activated by cyclic voltammetry (CV) (at a scan rate of 100 mV/s) by performing 5 cycles between −0.65 V and +1.10 V. Then, cyclic voltammetry (CV) scans were performed on PGE immersed in $1.0x10^{-3}$ M l-cysteine in 0.1 M phosphate buffer (pH 6) with a scan rate of 100 mV/s for 5 cycles between -0.50 V and +1.80 V, and in this way the graphite pencil electrode was coated with cysteine (L-cysteine PGE). Then, the L-cysteine/PGE electrode washed with distilled water was immersed in voltametric cell containing $1.0x10^{-4}$ M $AgNO_3$ prepared in 0.10 M KNO_3 and then modification was performed by cyclic voltammetry (CV) within the potential range of +0.30 V and -1.40 V, at a scanning speed of 50 mV/s with a single cycle and the AgNP/L-cysteine/PGE electrode was prepared. In order to compare the electrode performances, cyclic voltammograms (CV) were recorded in $5.0x10^{-3}$ M $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$ for activated in H_2SO_4 (PGE), L-cysteine-activated (L-cysteine/PGE) and L-cysteine/$AgNO_3$-activated(AgNP/L-cysteine/PGE) electrodes (Figure 1). For the validation of the method, pyrimethanil and cyprodinil determinations were performed with a proper accuracy, precision, sensitivities and interference effects were also investigated.

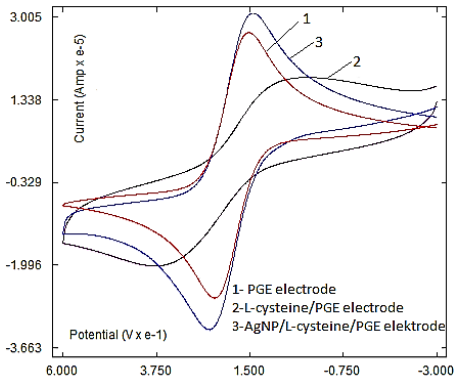

Figure 1. Cyclic voltammograms (CV) for $5.0x10^{-3}$ M $K_3[Fe(CN)_6]$ /$K_4[Fe(CN)_6]$ on PGE, L-cysteine/PGE and AgNP/L-cysteine/PGE electrodes.

Keywords: Anilino pyrimidines; Modified electrodes; Determination.

Acknowledgment: This master's thesis study was supported by Gazi University BAP (Project Code: FYL-2023-8759)

ORAL PRESENTATION

The effect of K_{ATP} channel mediators on insulin secretion in INS1 cell-created pseudoislets

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Abstract

This study investigates how the KATP channel activator diazoxide and inhibitor tolbutamide affect insulin secretion in the ins1 cell line. KATP channels represent a significant mechanism regulated by glucose, free fatty acids, and other metabolic signals. This mechanism plays a critical role in the homeostatic control of insulin and glucagon secretion, with central importance in the pathophysiology of metabolic diseases such as diabetes. However, the effect of diazoxide and tolbutamide, commonly used KATP channel activator and inhibitor, on the ins1 cell line has not been fully elucidated. The study employs a static approach using the incubation method. Pseudoislets were formed using ins1 cells under conditions of 1 mM glucose (G1), 10 mM glucose (G10), 20 mM glucose (G20), 20 mM glucose + 250 μM diazoxide (G20 Dz(250 μM)), and 20 mM glucose + 250 μM tolbutamide (G20 Tb(100 μM)), incubated for 1 hour. Supernatants collected after incubation were analyzed for insulin secretion using the radioimmunoassay method. The results show that insulin secretion in pseudoislets formed with ins1 cells increases parallel to increasing glucose concentration, while a significant decrease is observed in the group treated with 250 μM diazoxide ($p < 0.05$). Moreover, a significant increase in insulin secretion is detected in the group treated with 100 μM tolbutamide ($p < 0.05$). In conclusion, static insulin secretion in pseudoislets formed with ins1 cells significantly changes with diazoxide and tolbutamide treatments. While insulin secretion decreases in response to diazoxide, an increase is observed in response to tolbutamide.

Keywords: Diazoxide, tolbutamide, insulin, ins1 cells, pseudoislet.

ORAL PRESENTATION

Relationships Between Oxidative Stress in Healthy Bees Collected from Antalya Province and Antioxidant Levels of Their Pollen and Propolis Samples

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Abstract

In our country, as in the world, the production of propolis and pollen, which are beekeeping products, and their contribution to the economy are gradually increasing. Propolis and Pollen are considered very valuable bee products for the health sector in terms of their bioactivity and the antioxidant levels they contain. In this study, bee thorax samples and pollen and propolis samples produced in these colonies were collected from 20 different bee colonies in the Antalya Province Region. The exposure of the bees to oxidative stress was evaluated by measuring MDA and catalase levels in the thorax samples of the bees. Total phenol (TP), total flavonoid (TF), ferric reducing antioxidant assay (FRAP) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid assay (ABTS) levels of pollen and propolis samples were measured and Antioxidant content levels were evaluated. Additionally, it was evaluated whether there was a correlation between pollen and propolis samples in terms of antioxidant content levels. A positive correlation was found between MDA and Catalase levels determined in bee thorax samples. A negative correlation was found between TF levels determined in propolis samples and MDA and Catalase levels determined in bee thorax samples. A negative correlation was found between TF and TP levels determined in pollen samples and MDA and Catalase levels determined in bee thorax samples. As a result, it was concluded that measuring MDA and catalase levels in bee thorax samples can be used effectively to evaluate the oxidative stress status of bee colonies, measuring total phenol and total flavonoid levels in propolis samples can be used effectively to evaluate the antioxidant capacity of propolis, and measuring total phenol levels in pollen samples can be used effectively to evaluate the antioxidant capacity of pollen.

Keywords: Antalya Province Region, Antioxidant, Bee thorax, Oxidative stress, Pollen, Propolis.

ORAL PRESENTATION

The effect of convective heat transfer coefficient on a 26650 cylindrical lithium-ion battery thermal behavior: A computational study

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Abstract

Lithium-ion batteries are of great importance for electric vehicles owing to their high energy density, power density, and long cycle life. However, the thermal behavior of lithium-ion batteries restricts their use in electric vehicles. Therefore, investigation of the thermal behavior of lithium-ion batteries is necessary to achieve robust battery thermal management systems. In this study, the thermal behavior of a 26650 cylindrical lithium-ion battery was numerically examined to determine the effect of the convective heat transfer coefficient (CHTC) on maximum battery temperature, minimum battery temperature, and temperature homogeneity. The thermal behavior of the lithium-ion battery discharged at 3C-rate and 300 K free stream temperature was obtained by applying the NTGK model. The highest maximum and minimum battery temperatures were 342.651 K and 340.172 K respectively when the CHTC was 10 W/m K. The lowest maximum and minimum battery temperatures were 324.602 K and 319.739 K respectively when the CHTC was 40 W/m K. As expected, both maximum and minimum battery temperatures decreased with increasing the CHTC. Similarly, the temperature homogeneity decreased with increasing the CHTC. A linear change was observed for the maximum battery temperature while an exponential change was determined for the minimum battery temperature. Applying the CHTC at 40 W/m K was recommended for a lower maximum battery temperature and to achieve temperature homogeneity in limit values.

Keywords: lithium-ion battery, thermal behavior, maximum temperature, convective heat transfer coefficient.

ORAL PRESENTATION

Zingiber officinale extracts and 6-gingerol inhibit HDAC 1 activity

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Abstract

Enzymes known as histone deacetylases have been linked to a number of illnesses, including cancer, neurodegeneration, and inflammation. HDACS were discovered to be overexpressed in numerous human malignancies, whereas HDAC 1 activity was primarily observed in pancreatic, prostate, colorectal, gastric, and hepatocellular cancers. While the potential of ginger extracts of varied polarity was not comparatively shown and the exact HDAC type remained unclear, some compounds from the rhizomes of ginger, such as 6-gingerol and 6-shogaol, have been found with HDAC inhibitory activity. In vitro HDAC 1 inhibitory activity was particularly assessed in this work using ginger (Zingiber officinale Roscoe) extracts prepared to span a wide polarity range with hexane, dichloromethane, 2-methyltetrahydrofuran, ethanol, 70% ethanol and water. UPLC-DAD and ESI-MS/MS were used to analyze 6-gingerol content, while spectrophotometry was used to quantify the total phenol and flavonoid amounts. Hexane and dichloromethane yielded the highest 6-gingerol levels of 131 mg/g and 79 mg/g extract, respectively, among the crude extracts. The extracts of ethanol (122 mg gallic acid equivalent/g extract) and hexane (126 mg gallic acid equivalent/g extract) produced the greatest total phenol content, whereas ethanol extract (74 mg quercetin equivalent/g extract) was determined with the highest total flavonoid content. There was no direct correlation found between the total phenol and flavonoid contens and the ginger extracts' ability to inhibit HDAC 1 activity. The extracts with the greatest 6-gingerol content, as dichloromethane (IC50: 332 µg/ml) and hexane, exhibited highest HDAC 1 inhibitory activity. 6-gingerol's individual IC50 value was found to be 7 µg/ml. Future research might assess the 6-gingerol rich extracts for potential application in products targeting disorders connected to HDAC 1.

Keywords: Zingiber, Ginger, 6-gingerol, HDAC 1, LC-MS

ORAL PRESENTATION

Waste Utilization for Biopolymer Production with Bacterial Cellulose Producers

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Abstract

Plant cellulose is a naturally abundant biopolymer. However, the presence of contaminants such as pectin, hemicellulose, and phytate requires the implementation of various physical, mechanical, and chemical purification processes to obtain pure cellulose. Therefore, in recent years, scientists have begun exploring alternative sources for obtaining pure cellulose from microbial sources. Accordingly, microbial cellulose, which can be obtained pure from culture media, is more durable and cost-effective compared to plant cellulose, thus garnering significant attention. Additionally, unique properties as moldability, three-dimensional structure, porosity, flexibility, mechanical strength, large surface area, biocompatibility, and high water-holding capacity, allow its use in various industrial fields such as biomedical applications, agriculture, food, cosmetics, paper industry, and environmental remediation. This research aims to produce microbial cellulose as an alternative to plant cellulose by using cellulose producers isolated from home-made wine and vinegar. To determine the impact of incorporating industrial waste into the production process on cellulose production, the amount of cellulose produced in media containing whey, molasses, and olive black water were examined using the isolated cellulose producers. Consequently, the effects of these media on microbial growth and cellulose yield were also evaluated. In addition to the standard Hestrin-Schramm medium, the use of industrial waste aims to reduce the cost of cellulose production and contribute to environmental protection by utilizing industrial waste. The physicochemical properties of the cellulose obtained from this study was analyzed using Scanning Electron Microscopy (SEM), Fourier-Transform Infrared Spectroscopy (FTIR), and Thermogravimetric Analysis (TGA). Thus, this study will yield renewable and natural microbial cellulose, obtained purely from the production medium without the need for chemical purification processes, by utilizing industrial waste.

Keywords: Bacterial Cellulose, Waste utilization, Characterization

ORAL PRESENTATION

Biosynthesis of *Pepino* Fruit-Cu (II) and Co (II) Hybrid Nanoflowers: Cytotoxic Activity Assay Process

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Abstract

In the study, the synthesis of organic-inorganic hybrid nanoflowers, which have a flower-like structure and are called nanoflowers, was carried out. For this purpose, hybrid nanoflower synthesis was carried out using *pepino* fruit as the organic part and Cu (II), Co (II) metal ions as the inorganic part. Various analyzes were carried out to characterize the synthesized nanoflowers (such as SEM, EDX, FTIR, XRD). In addition, the cytotoxic activities of hybrid nanoflowers were tested on the MCF7 (breast cancer) cell line. It was concluded that there was a good increase in cytotoxic activity when *pepino* was converted into hybrid nanoflower form. When metal ions were compared, it was observed that the cytotoxic activities of hybrid nanoflowers synthesized with Cu metal ion were better than those of hybrid nanoflowers synthesized with Zn metal ion.

Keywords: Nanoflower, *Pepino*, Cytotoxic

ORAL PRESENTATION

Development of hydrogel-based smart label systems containing anthocyanin derivatives from various natural sources for the colorimetric determination of food freshness

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Abstract

Current standards for monitoring food freshness often rely on estimates based on potential storage conditions. Colorimetric food freshness indicators can differentiate between fresh, sub-fresh, and spoiled food without opening the package. One proposed method for food freshness indicators is the use of smart labels with pH indicators. These labels change color in response to pH changes in the food, caused by the release of gases such as ammonia (NH_3), carbon dioxide (CO_2), trimethylamine (TMA), dimethylamine (DMA), and others during microbial growth and food spoilage. This study aims to develop a hydrogel support material from biocompatible and environmentally friendly Pullulan (Pull) and Poly(vinyl alcohol) (PVA), and incorporate anthocyanin from three different sources (purple cabbage, tradescantia flower, and blueberry) as a pH indicator into these hydrogels to create smart label systems for effectively monitoring food freshness. In this study, the PVA/Pull hydrogel was synthesized using the freeze-thaw method, an inexpensive and clean physical cross-linking technique that does not involve toxic cross-linkers. Structural analysis of the hydrogels was performed using FTIR, swelling values were measured gravimetrically, and mechanical strength was tested with a Zwick Z010 universal mechanical tester. Hydrogels were prepared with varying concentrations and molecular weights of PVA, with and without Pull addition, which significantly affected their elasticity and homogeneity. The optimal PVA/Pull ratio was identified and selected as the support material for the smart labels. Anthocyanins were extracted from purple cabbage, tradescantia flower, and blueberry, and their color changes at different pH levels were analyzed using UV/Vis spectroscopy. These anthocyanins were loaded into the optimal hydrogels, and a rapid color change (within 20 seconds) was observed when the anthocyanin-loaded hydrogel was exposed to ammonia gas, a primary indicator of food spoilage. The synthesis of hydrogels using the PVA/Pull combination is a novel and scarcely explored topic, with no existing studies on pH indicators, food freshness indicators, or smart labels derived from this combination.

Keywords: Hydrogel, Poly(vinyl alcohol), Pullulan, Anthocyanins, Food freshness indicators

ORAL PRESENTATION

Comparative Yeast Beta Glucan Analysis by Using Different Carbohydrate Analysis Methods

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Abstract

Betaglucans are polysaccharides derived from oats, barley, yeast and fungi, consisting of D-glucose monomers linked together by β-glycosidic bonds and vary in molecular mass, solubility, viscosity and three-dimensional structure. Commercially available betaglucan products are generally offered as immune system supplements, health products and functional foods. Yeasts can be used as ideal raw materials for betaglucan production because they are cheaply available and their cell walls can have approximately 50-55% betaglucan content. In addition, studies show that yeast-derived betaglucans are the most effective immune system protective agent. In this study, analyses were carried out on yeast-derived beta glucan tablets available in the market as dietary supplements. The aim of this study was to establish a correlation between antron analysis, which is a total carbohydrate analysis, and DNS analysis, which is a reducing sugar analysis, and to analyse the beta glucan content without the need for enzymatic analysis which is an expensive and time consuming method. Thus, beta glucan analysis may be performed with accuracy by more fast, efficient and economical way. In the experiments, beta glucan samples was hydrolysed with H2SO4 firstly followed by antron, DNS and enzymatic analysis. By using three analysis method same procedure was repeated for glucose in order to draw a calibration graph. As a result of the enzymatic analysis, the beta glucan content of commercial sample was found to be %35.51 . According to the antron analysis, the total carbon content of it was 0.18 mg/mL and according to the DNS analysis, the reducing sugar content was obtained as 0.125 mg/mL. When the results obtained were examined, it was proved that beta glucan analysis can be performed without the need for enzymatic analysis thanks to the correlation between antron and DNS analyses.

Keywords: Yeast betaglucan, antron analysis, DNS analysis, enzimatic analysis.

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ORAL PRESENTATION

Production of resin-based dental composites from MSN-Ciplofloxain conjugates with antimicrobial properties

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Abstract

In this research, porous silica nanoparticles (MSN) were synthesized and conjugated with the antibiotic Ciprofloxacin using NHS/EDC linkers. These MSN-Ciprofloxacin bioconjugates form the inorganic component of the dental composite. The dental composite is composed of 60% resin and 40% inorganic material, with varying mass ratios of MSN-Ciprofloxacin at 0%, 10%, 20%, 30%, and 40%. The morphology of the synthesized MSN-Ciprofloxacin and dental composites was characterized using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDS), Thermogravimetric Analysis (TGA), and Differential Scanning Calorimetry (DSC). Additionally, the chemical resistance (Fenton reaction) and water solubility-absorption (at 37°C) of the dental composites were evaluated in accordance with ISO standards. To assess the antimicrobial effects of the materials, minimum inhibitory concentration (MIC) tests were conducted with gram-negative bacteria Escherichia coli (ATCC® PTA-5976TM) and gram-positive bacteria Staphylococcus aureus (ATCC® 25923™). The results demonstrated that the chemical stability, water absorption, and dissolution of the dental composites met ISO requirements and exhibited antimicrobial properties.

Keywords: Ciprofloxacin, MSN, dental composite

ORAL PRESENTATION

***Kluyveromyces marxianus* M59 suşundan izole edilen ekzopolisakkaritin *in vitro* insan lenfositlerinde genotoksik ve antigenotoksik etkisinin kromozomal aberasyon testi ile değerlendirilmesi**

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Özet

Günümüzde sağlıklı beslenmeye ilginin artmasıyla birlikte fonksiyonel gıdaların dolayısıyla temel beslenmenin ötesinde sağlık için yararı olan gıdalar ve gıda bileşenlerinin önemi artmıştır. Bu bağlamda mayalar, gıda endüstrisinde yaygın olarak kullanılmakla beraber beslenmede potansiyel kaynak olarak değerlendirilmektedir. Bu çalışmaya konu olan *Kluyveromyces marxianus* türü maya geleneksel fermente süt ürünleri, gıda katkı maddeleri, hurma vb. gıdalarda bulunması nedeniyle önemli bir mayadır. Birçok çalışmada mayaların antikanser ve antineoplastik özellikte olduğu vurgulanmaktadır. Ekzopolisakkaritler (EPS'ler) hücre dışına salgılanan polisakkarit yapıdaki biyopolimerlerdir. Günümüzde EPS'lerin kullanımı gitgide yaygınlaşmakta olup ilaç, gıda ve kozmetik sanayinde kullanılmaktadır. Bu çalışmada kromozomal aberasyon (KA) testi ile *Kluyveromyces marxianus* M59 suşundan izole edilen EPS'nin (EPS_{M59}) potansiyel genotoksisitesi ve mitomisin-C kaynaklı kromozom hasarına karşı antigenotoksik etkisinin incelenmesi amaçlanmıştır. Bir erkek ve bir kadın olmak üzere iki sağlıklı donörden alınan kan ile *in vitro insan lenfositlerinde* 48 saatlik kültür boyunca EPS_{M59}'un tek başına beş farklı konsantrasyonu (640; 320; 160; 80; 40 µg/mL) ve eş zamanlı olarak tüm konsantrasyonlarla birlikte mitomisin-C (0,20 µg/mL) ile 37 ˚C'de muamele edilmiştir. Her muamele için bir negatif (distile su) ve bir pozitif (mitomisin-C) kontrol grubu bulundurulmuştur. Çalışmada tüm konsantrasyonlarda negatif kontrole kıyasla anormal hücre ve hücre başına düşen anormallik (KA/Hücre) frekansında anlamlı bir değişiklik olmamıştır. Bu bağlamda EPS_{M59}'un genotoksik potansiyeli olmadığı sonucuna varılmıştır. Antigenotoksisite çalışmasında pozitif kontrole (mitomisin-C) kıyasla EPS_{M59}+mitomisin-C muamelesinde tüm konsantrasyonlarda anormal hücre ve KA/hücre frekansında anlamlı bir düşüş meydana gelmiştir. Sonuç olarak bu çalışmada EPS_{M59}'un tüm konsantrasyonlarında herhangi genotoksik etki görülmemiştir. Aynı zamanda EPS_{M59}+mitomisin-C'nin tüm konsantrasyonlarında antigenotoksik yani kromozomal aberasyonda iyileştirici/koruyucu etkisinin olduğu gözlenmiştir. Bu etkilerin diğer genotoksisite testleriyle de değerlendirilmesi gerekmektedir.

Anahtar Kelimeler: *Kluyveromyces marxianus*, Ekzopolisakkarit, Kromozomal Aberasyon, Genotoksisite, Antigenotoksisite

Teşekkür
Bu çalışma Gazi Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından FYL-2024-9366 numaralı proje kapsamında desteklenmiştir.

ORAL PRESENTATION

Protective Effect of Melatonin on Subacute Toxicity Caused by Abamectin in Rat Brain Tissue

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Abstract

Pesticides essential for good agriculture applications, but pesticides consist of dangerous-chemicals for organisms. Abamectin, an organophosphate pesticide, is frequently used in the cultivation of cotton, citrus, fruits and vegetables. Abamectin readily crosses the blood-brain barrier in mammals. Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone produced mainly by the pineal gland. This is regulate our circadian rhythm and also protect cellular damage. Melatonin has been widely used as a preservative in toxicological studies in recent years. In this study, the effect of abamectin on antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST)] and malondialdehyde (MDA) levels in rat brain tissue and the protective role of melatonin were investigated. 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. For this purpose, Wistar Albino rats were divided into 4 groups. 1st group: control group, 2nd group: melatonin (10 mg/kg) treated group, 3rd group: abamectin (0.5 mg/kg) treated group, 4th group: abamectin plus melatonin treated group. The substances were given to the rats by gavage once daily for 28 days. At the end of the experiment, no statistically significant difference was observed between the control and melatonin treated group in the investigated parameters. When the control group was compared with the abamectin-treated group, a statistically significant increase in MDA levels was observed, while a decrease in antioxidant enzyme activities SOD, CAT, GPx and GST enzyme activities was observed compared to the control group rats. In the study, it was observed that abamectin caused oxidative stress in rat brain tissue and melatonin treatment reduced abamectin-induced toxicity.

Keywords: Abamectin, Melatonin, Brain, Antioxidants, Oxidative stress

ORAL PRESENTATION

pH-Responsive Caffeic Acid Phenethyl Ester Release from Polyazomethine-Poly(ethylene glycol) Copolymer Micelles

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Abstract

Biodegradable polymers are advantageous as biomaterials because they break down and are naturally removed from the body once they complete their function, allowing to be temporarily used biomedical applications. Poly(lactide-co-glicolide), polycaprolactone, polyanhydrides are the mostly known biodegradable polymers used in many applications. However, utilization of polyazomethines in such applications is very limited, although they have pH sensitive biodegradability feature[1]. In this study, a new block copolymer of polyazomethine-poly(ethylene glycol) (PAM-PEG) was produced for the delivery of hydrophobic cargo molecules. Caffeic acid phenethyl ester (CAPE) was used as the model drug, which has anticancer, immunomodulatory, anti-inflammatory, and antioxidant properties[2]. In the study, PAM-PEG block copolymers were produced and characterized with NMR and FTIR spectroscopies. Then, the CAPE-loaded PAM-PEG micelles were prepared with dialysis technique. Analysis using scanning electron microscopy (SEM) showed that the micelles are spherical with an average diameter of 135.5 nm. The release profile of CAPE from these micelles was evaluated under different pH conditions: a physiological pH of 7.2 and a lysosomal pH of 5.0. The study revealed that CAPE release was pH-dependent, with only 2.14% released at pH 7.2, while a much higher release of 38.8% was observed at pH 5.0 after 24 hours. Furthermore, in vitro tests on human-derived fibroblast cells indicated that the CAPE-loaded micelles exhibited minimal toxicity after 48 hours, in contrast to both free CAPE and unloaded micelles. These promising results reveal the potential of polyazomethine-based micelles as efficient vehicles for the targeted delivery of therapeutic agents, offering a viable approach to improving drug delivery systems.

Keywords: drug delivery, micelle, pH-sensitive, polyazomethine

Acknowledgment: We would like to acknowledge the financial support provided to Nermin Saliha Topuzogullari through the 100/2000 YÖK Ph.D. Scholarship Program from Council of Higher Education Republic of Türkiye (YÖK).

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ORAL PRESENTATION

İletken nanofiber matrikslerde kullanılmak üzere yeşil sentezle elde edilen indirgenmiş grafen oksitin sentezi ve karakterizasyonu

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Özet

Kalp ve kas doku mühendisliği çalışmaları dikkate alındığında iletken nanofiber matrikslerin üretilmesi rejeneratif tıp alanında tedavi seçeneklerine yeni bir boyut kazandırmıştır. İletken nanofiber matriks çalışmalarında önemli bir malzeme türü ise grafen türevlerinden olan indirgenmiş grafen oksittir. Elektriği iletme özelliğinin yanı sıra ayarlanabilir hidrofilik/hidrofobik yüzey özellikleri sayesinde hücre davranışlarını yönlendirebilmekte ve yapısında bulundurduğu fonksiyonel gruplar sayesinde zarar gören dokularda hücre transplantasyonu sonrası ortamdaki reaktif oksijen türlerini sönümleyerek hücre sağkalımını arttırabilmektedir. Bununla birlikte, indirgenmiş grafen oksit, grafen oksitin hidrazin, borhidrid gibi kimyasal indirgeyici ajanlar kullanılmasıyla elde edilmektedir. Tüm bu ajanlar hücre çalışmalarına uygun olmamakla beraber hem çevre hem de insan sağlığı açısından son derece toksiktirler. Bu nedenle, bu çalışma da grafen oksitin indirgenmesinde ülkemizde yetişen latince adı *Momordica Charantia* olan kudret narı bitkisi rGO'nun yeşil sentezinde ilk defa kullanılmıştır. Temmuz ayında hasat edilen bitkinin hem etanol hem de distile su ile ekstraktı elde edilmiş ve antioksidan kapasitesi ve toplam fenolik içerik analizleri yapılmıştır. Daha sonra bitki ekstraktları kullanılarak indirgenmiş grafen oksitler sentezlenmiş ve indirgeme işlemi UV-Görünür, Raman, FT-IR spektroskopileri ile ve SEM-EDS ile karakterize edilmiştir. Elde edilen indirgenmiş grafen oksitlerin biyolojik uygulamalarda kullanılabilirliğini göstermek adına L929 fare fibroblast hücre hattı üzerinde sitotoksisite çalışmaları da yapılmıştır.

Anahtar Kelimeler: Nanofiber matriks, yeşil sentez, kudret narı, indirgenmiş grafen oksit

Teşekkür: Bu çalışma Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) tarafından desteklenmiştir (Proje No: 222M052).

ORAL PRESENTATION

Synthesis of Potentially Biological Active Coumarin-fused Compounds

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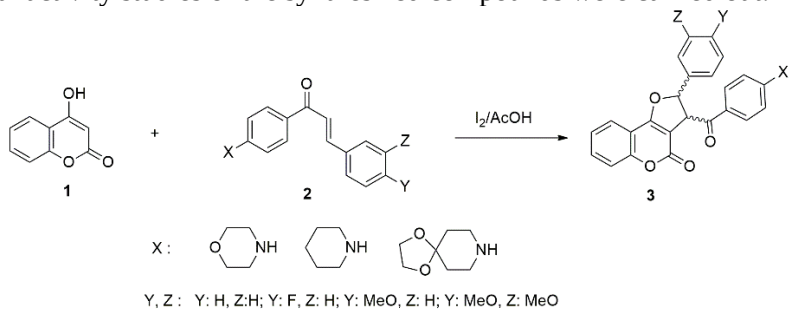
Abstract

'Coumarin' compounds, an important member of the heterocycle class, have played a key role in the synthesis of many different compounds that have been determined to show pharmaceutical and biological activity over the years, as their core structure is derivatized with different substituents and they are extremely prone to cyclization reactions. Studies in the cardiovascular field where it is used as an anticoagulant in the treatment of cardiovascular diseases are its most common known applications [1]. In recent years, in addition to their use in medicine due to their important biological and pharmacological activities such as anticancer, antiviral, antibacterial, cytotoxic and anti-inflammatory, it has also been determined that they are effective in current fields such as optical materials and chemosensor production due to their fluorescence properties [2]. For this purpose, interest in the synthesis of hybrid molecules bearing the coumarin core structure and especially adjacent heterocycle derivatives is increasing day by day and new methods are being developed [3]. In this study, firstly, the synthesis of chalcones (**2**) (α,β-unsaturated aromatic ketone derivative) from the condensation reaction of morpholine, piperidine, and 1,4-dioxa-8-azaspiro[4.5]decane [DASD] substituted acetophenone derivatives with various benzaldehyde compounds. has been carried out. Later, the cyclization reactions of the 4-hydroxycoumarin (**1**) compound with all the synthesized chalcones in the presence of iodine catalysts were studied, and new coumarin-fused compounds (**3**) were obtained (**Scheme 1.**). The structures of all synthesized new compounds were identified by elemental analysis, mass spectrometry (ESI-MS), FTIR, one-dimensional {^{1}H and ^{13}C-NMR} and two-dimensional {COSY, HSQC, and HMBC} advanced NMR techniques. Biological activity studies of the synthesized compounds were carried out.



Scheme 1. Synthesis of new coumarin-fused compounds.

Keywords: Coumarin, chalcone, heterocyclic amin, iodine, biological activity.

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ORAL PRESENTATION

Biosynthesis of CeO_2 NPs using *Persea americana* extract and evaluation of their antioxidant activity

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Abstract

This study presents a green and efficient method for synthesizing cerium oxide nanoparticles with regard to their antioxidant activity. This research emphasizes plant-based resources, particularly derived from waste, to enhance their sustainability and accessibility for functions in biosynthesized CeO_2 NPs. The CeO_2 NPs were synthesized using *Persea americana* exocarp extract and characterized by dynamic light scattering (DLS), zeta potential (ZP), transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared (FTIR). In addition, the antioxidant activities of the CeO_2 NPs were evaluated by the scavenging capacity for 2,2-diphenyl-1-picrylhydrazyl free radicals. The TEM, DLS, and ZP characterization demonstrated that the prepared nanoparticles had a spherical shape, average size of 30 nm, and zeta potential of −32.5 mV. The compounds in the *Persea americana* exocarp extract are responsible for reducing metal ions. According to FTIR and ZP data, the capping particles on the surface of CeO_2 NPs are primarily made up of negatively charged groups, which contribute to the considerable stability. Furthermore, EM and DLS analysis showed that the obtained particle sizes resulted in a nanoscale range with agglomeration. Moreover, the results of the nanoparticle based antioxidant strategy indicated that the CeO_2 NPs have a comparably good scavenging capability, inhibiting 42.7% of DPPH. The obtained results show sustainable, cost effective, facile synthesis CeO_2 NPs with considerably good radical scavenging activity.

Keywords: Green synthesis, Nanotechnology, Antioxidant, Radical scavenging

Acknowledgments: C.E. gratefully acknowledges the Scientific and Technological Research Council of Turkey (TÜBİTAK) for supporting through the TÜBİTAK 2211-A National PhD Fellowship Program.

ORAL PRESENTATION

Investigating the potential of biodegradable nanofiber membrane for effective dye removal

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Abstract

Water pollution is one of the most serious global challenges threatening biodiversity, mainly because of the discharge of industrial and household wastes into water resources. Industrial wastes, notably effluent from the textile industry, electroplating, and the chemical industry, are of great concern due to their toxicity and potential consequences on the environment. Textile wastes, majorly synthetic dyes, cause an imbalance in the aquatic ecosystem, impede photosynthesis, and raise the chemical oxygen demand. The current study investigates the use of biodegradable nanofiber membranes made of silk fibroin for the removal of dyes from wastewater, taking Methylene Blue as a model contaminant. In this work, the efficiency of the silk nanofiber membrane for charge selective adsorption-desorption of MB dye was tested and taken advantage of by adjusting with the variation in different pH through the zwitterionic nature of the silk protein. The natural degradation of nanofiber membranes in soil and salty water environments was further tested to indicate their eco-friendly nature. The results indicated that the silk fibroin nanofiber membrane efficiently removed the MB dye and provided a sustainable alternative solution. Besides, natural degradation provided an alternative way to avoid micro/nanoplastics pollution because of membrane aging and material qualities in the conventional membrane filtering system. This environmentally friendly approach might incredibly enhance wastewater treatment procedures to reduce water pollution.

Keywords: Water pollution, Nanofiber membrane, Silk fibroin, Soil degradation

Acknowledgments: C.E. gratefully acknowledges the Scientific and Technological Research Council of Turkey (TÜBİTAK) for supporting through the TÜBİTAK 2211-A National PhD Fellowship Program.

ORAL PRESENTATION

DNA barcoding of *Aethionema grandiflorum* Boiss. & Hohen var. *sintenisii* (Hausskn. & Bornm.) Govaerts and determining the phenolic content of its methanol extract

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Abstract

Aethionema grandiflorum var. *sintenisii* is an endemic taxon predominantly distributed in the north part of the Black Sea Region of Turkiye. In this study, air dried plant samples were grounded and macerated with methanol . The extract was filtered and evaporated using rotary evaporator. The Nexera-i LC-2040C 3D Plus HPLC system was used. A DAD was used as the detector in the analysis process, and scanning was performed at 254 nm. Phenylhexyl 4.6 × 150 mm, 3 µm (UP) reversed phase filler column was used for separation of the phenolic components. The pump flow program with gradient elution was applied with 0.1% formic acid/deionized water (solvent A) and acetonitrile (solvent B) as the mobile phase. Throughout the analysis, the flow rate of the mobile phase was adjusted to 1 ml/minute. In total, 10 µl of sample and 10 µl of standard were injected into the device. The column temperature was set as 30°C. Identification and quantitative analysis of 15 phenolic compounds were done by comparison with standards. ITS region was amplified by using universal ITS4 and ITS 5m primers by PCR and sequenced commercially. Blast analysis was performed on NCBI to confirm. The sequence was registered to genbank with the accession numer of PP999647. This result is the first record of *A. grandiflorum* var. *sintenisii* ITS sequence. According to our results; The predominant components of the methanol extract have been found to be quercetin (31.9 mg/g extract), rutin (23.6 mg/g extract) and ferulic acid (21.7 mg/g extract). Blast analysis showed that this DNA sequence is identical with a percent identity of 97.91% *A. grandiflorum* (AM905710.1). This results comfirmed that the plant material in interest is *A. grandiflorum* var. *sintenisii* and its methanol extract should be used for further investigations due to its phenolics.

Keywords: *Aethionema*, *Aethionema grandiflorum* var. *sintenisii*, DNA barcoding, Phenolic content

ORAL PRESENTATION

Removal of Malachite Green From Dye Wastewater Using Cranberry Kernel (*Cornus mas* L.) Adsorbent

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Abstract

Untreated textile wastewater is highly toxic because it contains many metal complex dyes. Therefore, wastewater discharged from various textile and dyeing industries are hazardous colored wastes. Adsorption techniques are a simple, fast and cost-effective method used in the treatment of wastewater containing dyes. The use of adsorption in the textile industry has many advantages, including its high efficiency, low cost and environmentally friendly nature. In this study, it was aimed to examine the equilibrium and kinetic data in malachite green adsorption onto cranberry kernels (CK). XRD, SEM and FTIR analyzes were carried out to determine the surface properties of CK. The effects of the concentration, pH, amount of adsorbent and temperature of malachite green dye in textile wastewater on the adsorption efficiency were examined. We successfully used the adsorption method in malachite green removal. CK have effective surfaces for use in adsorption in textile wastewater. During the determination of the surface properties of CK, it was found that it had a mesoporous structure from the SEM image and that it showed an amorphous feature from the XRD diffractogram. The optimal conditions of adsorption were contact time of 75 min, amount of adsorbent 0.05g, pH 5, Malachite green concentration of 10 mg/L, and temperature at 296 K. The maximum adsorption capacity of malachite green adsorption with this agricultural waste was found to be 138.12 $mg.g^{-1}$. The adsorption isotherms of the malachite green onto CK matched well with the Freundlich model at 296 K. Thermodynamic studies revealed that the adsorption processes of malachite green are exothermic and non-spontaneous. These results confirm that CK is promising as an efficient, low-cost, and stable adsorbent.

Keywords: Adsorption, Biomass, Malachite Green, Wastewater, Cranberry Kernel (*Cornus mas* L.)

ORAL PRESENTATION

A Tri Co-Culture Model in Human Feto-Placental Steroidogenesis and the Effects of Vortioxetine

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Abstract

The placenta is a dynamic organ that is constantly changing throughout pregnancy. It is critical for hormone production and fetal development during pregnancy. This study aims to investigate the structural intricacies of the placental barrier using a novel in vitro model consisting of three different cell lines: BeWo (human placental choriocarcinoma cells), HUVEC (human umbilical vein endothelial cells) and H295R (human adrenocortical epithelial carcinoma cells). BeWo cells have the ability to convert androgens to estrogens in the system, while H295R cells have the enzymatic capacity of the fetal adrenal gland to produce steroids. HUVEC cells play a critical role in the formation and maintenance of placental blood vessels. These cells, grown in a Transwell® system, were exposed to vortioxetine, the active ingredient in the antidepressant Brintellix. Vortioxetine, a selective serotonin reuptake inhibitor (SSRI), modulates serotonergic processes. Due to a lack of information on the use of this drug during pregnancy, further research is needed to elucidate its pregnancy toxicity, particularly in studies using human cell lines. This study investigated the effects of vortioxetine on cell viability, placental barrier integrity, hormone production and gene expression profiles. In addition, it is believed that the optimised tri co-culture model will provide a fundamental framework for evaluating the safety and effects of various substances during pregnancy. In conclusion, vortioxetine affects cell viability, androgen-estrogen interactions and placental hormone production, providing important information for assessing the potential risks of SSRI-type antidepressants to pregnancy and fetal development.

Keywords: BeWo, H295R, HUVEC, Vortioxetine, Steroidogenesis

ORAL PRESENTATION

Kuarsetin ve D-alaninin Ga(III) ve In(III) komplekslerinin sentezi ve karakterizasyonu, DPPH radikal süpürme aktivitelerinin incelenmesi

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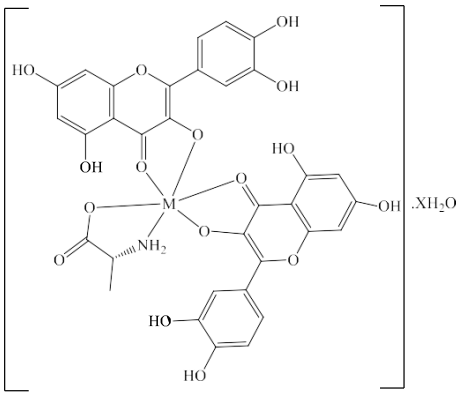
Özet

Flavonoidler bitki kökenli polifenol bileşikleridir. Yapılarında bulunan 2-fenil-benzo-α-piron halkasına bağlı hidroksil grupları ile ilişkili olarak antosiyanin, flavonol, flavon, kateşin, flavanon ve izoflavonoid olarak farklı isimler alırlar [1]. Flavonoidler bitkilerde antioksidan ve enzim inhibitörü gibi önemli özelliklere sahiptir. Sahip oldukları karbonil ve hidroksil gruplarının varlığı nedeniyle nötr veya anyonik formlarında metal iyonları ile kompleks oluşturabilir [2]. Bir flavonoid olan kuarsetin, tümör oluşumunu inhibe etmek ya da tersine çevirmek için modülatör olarak sinyal iletim yolları üzerinde etki eden bir kanser koruyucusu olarak kabul edilmektedir. Bu aktivitesi ile kuarsetin apoptoz, hücre göçü, farklılaşma ve çoğalma, oksidatif denge, inflamasyon ve yara iyileşmesi gibi birçok süreçte etki göstermektedir [3]. Amino asitler proteinlerin temel yapı taşlarıdır. Amino asitler kokusuz, renksiz, suda çözünen, erime noktaları yüksek, polar yapılı ve katı maddelerdir. Peptitler yani amino asit zincirleri birçok farklı işlev görür. Amino asitlerin yapısal pek çok özelliği ve koordinasyon bileşiklerinde ligand olarak kullanılabileceği fikrini akıllara getirmiştir [4]. Bu çalışmamızda ligant olarak kuarsetin ve D-alanin kullanılarak, yeni galyum(III) ve indiyum(III) kompleksleri (Şekil 1) sentezlendi. Komplekslerin yapıları FT-IR, NMR ve UV-GB spektroskopisi, element analizi, manyetik duyarlılık ile iletkenlik ölçümleri ile karakterize edildi. Bu komplekslerin antioksidan özellikleri 2,2-difenil-1-pikrilhidrazil (DPPH) radikal süpürme yöntemi ile DMSO ortamında ve UV-GB spektroskopisi ile çalışıldı.



Kompleks	Metal	H_2O
K-1	Ga(III)	2
K-2	In(III)	-

Şekil 1. Sentezlenen yeni Ga(III) ve In(III) komplekslerinin olası açık yapıları.

Anahtar Kelimeler: flavonoid, kuarsetin, krizin, antioksidan, kompleks, radikal süpürme.

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ORAL PRESENTATION

Production and Characterization of 3D PEG/PCL Nerve Conduit Containing CoO Nanoparticles Integrated *H. perforatum* Oil

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Abstract

Peripheral nerve diseases are a common clinical problem worldwide. Peripheral nerve injuries occur in 360,000 people each year in the United States and more than 300,000 people annually in Europe. Autologous nerve grafts are generally used in the treatment of peripheral nerve injuries. The use of autologous nerve grafts causes morbidities such as loss of sensation and scarring in the donor area. Since this procedure is a surgical intervention, it is not only a difficult technique but also a limited resource. In order to overcome such problems, synthetic biomaterials have begun to be used in recent years. Synthetic nerve conduits are classified in two different ways, depending on the properties of the materials used: non-degradable (non-absorbable) and degradable (absorbable). Silicone synthetic nerve conduits are widely used polymers, but since it is a non-absorbable polymer, it has some disadvantages such as causing inflammation in tissues. In order to overcome such disadvantages, it is aimed to produce ideal absorbable synthetic nerve conduits. For this purpose, the design, production and characterization of nerve channels in 3 dimensions were carried out. The structure of synthetic nerve conduits includes PEG (Polyethylene glycol), PCL (Polycaprolactone) polymers, *H. perforatum* oil and CoO nanoparticles. The production of three-dimensional nerve conduits was carried out using the electrospinning technique. While producing the nerve conduits, *H. perforatum* oil was integrated in PEG, and CoO nanoparticles were integrated in PCL, and co-spinning was performed and three-dimensional nerve conduits were generated. Some characteristics of the 3D nerve conduits produced within the scope of the study such as, chemical structure (FTIR), surface morphology (SEM), wettability (% swelling ratios), hydrophilic/hydrophobic properties (contact angle measurements), time-dependent mass loss, release profile of *H. perforatum* oil and CoO nanoparticles from the nerve conduits were determined by performing various in-vitro characterization studies and nerve conduits performance was evaluated.

Keywords: Nerve Conduits, Polyethylene glycol (PEG), Polycaprolactone (PCL), *H. perforatum* oil, CoO nanoparticles, Electrospinning

ORAL PRESENTATION

Production and Characterization of 3D PEG/PCL Nerve Conduit Containing Ni_2O_3 Nanoparticles Integrated Jojoba Oil

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Abstract

Peripheral nerve injurys are a serious disease that significantly affects the quality of life of patients and often causes lifelong disability, causing a huge socioeconomic problem. Different surgical procedures are available today, including direct suturing, autograft, and synthetic conduits. In spaces that require tension-free nerve sutures smaller than 5 mm, the direct suture method known as end-to-end suture is used. In gap larger than 5 mm, the use of three-dimensional nerve conduits produced from biodegradable synthetic polymers has begun to be studied in recent years. Since the need for a second surgery due to the non-absorbable nature of silicone-based synthetic nerve conduits negatively affects patients both financially and psychologically, the production of synthetic nerve channels with biodegradable materials is aimed. An ideal nerve channel is mechanically durable, elastic, functional, biocompatible, does not require a secondary operation, has an absorbable structure, degradation products are not toxic, tumorigenic, apoptotic and necrotic on the cells forming the nerve and surrounding tissue, and supports the healing process of damaged nerve channels. It should be conductive, support neural signaling by passing electric current through it, support biocompatibility, have a neural tube lumen that does not narrow when swollen by taking in water, have structural and functional biomimetic properties, and be easy and economical to implant. While producing the nerve conduits, Jojoba oil was integrated in PEG, and Ni_2O_3 nanoparticles were integrated in PCL, and co-spinning was performed and three-dimensional nerve conduits were generated. Some characteristics of the 3D nerve conduits produced within the scope of the study such as, chemical structure (FTIR), surface morphology (SEM), wettability (% swelling ratios), hydrophilic/hydrophobic properties (contact angle measurements), time-dependent mass loss, release profile of Jojoba oil and Ni_2O_3 nanoparticles from the nerve conduits were determined by performing various in-vitro characterization studies and nerve conduits performance was evaluated.

Keywords: Nerve Conduits, Polyethylene glycol (PEG), Polycaprolactone (PCL), Jojoba Oil, Ni_2O_3 nanoparticles Electrospinning

ORAL PRESENTATION

Clustering XRF Data for Nickel Laterite Using K-Means Algorithm

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Abstract

This study focused on the analysis of the chemical composition of nickel laterite mineral samples using X-ray Fluorescence (XRF) by clustering the dataset through K-Means algorithm and visualizing the cluster by the Principal Component Analysis (PCA) method. The analysis of 14 chemical elements in nickel laterite performed data exploration in the data set. Descriptive statistics and heatmap of the result indicated the potential of the laterite mineral as a viable source of nickel. The determination of the number of clusters in the dataset was done by the Elbow method and Silhouette Score method with; respectively, 3 and 2 clusters with optimal performance. The K-Means clustering categorized the samples into three clusters: Centroid 0 showed clusters with highly sulfur, iron, phosphorus, aluminum, and chromium contents; Centroid 1 showed clusters with very high magnesium, silicon, and calcium contents; Centroid 2 showed clusters with high nickel, cobalt, and manganese contents. 3D PCA visualization of the K-Means model was used to understand the data structure of the samples by reducing the dimension of the dataset, thus enabling the identification of clusters with different elemental characteristics. Clusters with high nickel and cobalt content were identified as potential candidates for exploration and mining, while clusters with high manganese and sulfur content required specific processing strategies.

Keywords: XRF dataset, Machine Learning, K-Means, Nickel Laterite.
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ORAL PRESENTATION

Preparation of isoniazid and NSAIDs co-loaded PLGA microparticles and *in-vitro* evaluation

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Abstract

The objective of this work was to formulate a biocompatible PLGA microparticle co-loaded isoniazid (ISN) and NSAID [1]. Flurbiporfen (FB) was chosen as the NSAID. There is a gap in the literature regarding cytotoxic effects on concomitant administration of isoniazid and NSAID co-loaded microparticles. In this study, PLGA was synthesized primarily from lactide and glycolide by polycondensation and ring-opening polymerization [2]. Then, PLGA microparticles were prepared using a w/o/w double emulsion, solvent evaporation technique. The chemical and thermal property–structure relationships of PLGA microparticles were identified. Scanning electron microscopy (SEM) were showed that the presence of drugs in the PLGA microparticles created porous features and indentations on the surface of the microstructures. The cell culture studies were performed according to the procedure reported in the mouse fibroblast (L929 cells) cell line [4]. The samples (raw MPs, ISN, ISN+FB, ISN MPs, ISN+FB MPs, control (Dulbecco's modified eagle's medium supplemented with 10% fetal bovine serum, 50 U/mL penicillin and 50 µg/mL streptomycin supplemented with 10% fetal bovine serum, 50 U/mL penicillin and 50 µg/mL streptomycin) were applied to the cells at appropriate concentrations for 24h. The results of cell culture studies were analyzed by two-way ANOVA test using the GraphPad Prism 8 statistical program. Cell culture studies have shown that, in general, the prepared samples do not have a significant toxic effect on cells. It was observed that the biocompatibility of drugs was enhanced by PLGA microparticles.

Keywords: isoniazid, flurbiprofen, PLGA, microparticle, cell culture.

Acknowledgements We would like to thank Prof. Dr. Öztekin ALGÜL for the isoniazid drug supply.

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ORAL PRESENTATION

Effect of Different Azinphos Ethyl and Azinphos Methyl Concentrations on *Scenedesmus obliquus* Growth in Culture Conditions

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Abstract

Azinphos methyl (S - 3,4 - dihydro - 4 - oxo - 1,2,3 - benzotriazine - 3 - ylmethyl O,O - dimethylphosphorodithioate) and azinphos ethyl (S - 3,4 - dihydro - 4 - oxo -1,2,3 - benzotriazin - 3 - ylmethyl O, O - diethylphosphorodithioate) are pesticides containing two phosphorus. These pollutants are widely used as agricultural insecticides and acaricides, which are used as broad-spectrum pesticides. It is thought that these pesticides are washed away from the soil by rain, flood and snow water and pollute river, lake and sea waters. The aim of the research is to determine the effect of different azinphos ethyl and azinphos methyl concentrations on the growth of green algae to be isolated from streams under culture conditions. Thus, the effects of pesticides on aquatic ecosystems will be investigated. In this study, *Scenedesmus obliquus* cultures were prepared in BG-11 medium for use in laboratory experiments. The growth of the species in cultures containing azinphos methyl (1 mg/l, 1.1 mg/l, 1.2 mg/l, 1.4 mg/l) and azinphos ethyl (0,1 mg/l, 0,5 mg/l, 1 mg/l, 1,5 mg/l, 2 mg/l) was compared with the species grown in cultures without these pesticides. This research attempted to determine the change in algal growth in cultures depending on the increase in the concentration of these pesticides. In addition, pH measurements were made and chlorophyll-*a* values were calculated in the control group and in the cultures to which azinphos ethyl and azinphos methyl were added during the study. The number of cells showed different changes over time depending on the pesticide concentration. As the dose increased, a decrease in the number of organisms was observed, especially from the 24th hour. For azinphos-ethyl, the highest cell count was determined as 2.34x10^{6} cells/ml at the end of the 48th hour in the control group, and the lowest cell count was determined as 1.0065x10^{6} cells/ml at the end of the 96th hour at a dose of 1 mg/L. In azinphos-methyl, the highest cell count was 2.48 x 10^{6} mg/l at a dose of 1.1 mg/l at the end of the 72nd hour, and the lowest cell count was 1.655x10^{6} cells/ml at the end of the 96th hour at a dose of 1.4 mg/L has been determined. pH and chlorophyll-*a* values did not fluctuate much for either pesticide. As a result, it was determined that the doses studied for both pesticides negatively affected algae growth, although not too much.

Keywords: algae, pesticide, azinphos ethyl, azinphos methyl.

ORAL PRESENTATION

Epilithic Algal Flora of Bulancak Stream

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Abstract

As the primary producer in the food chain, algae have a very important place in determining the biodiversity of aquatic ecosystems. The epilithic algal flora of Bulancak Stream (Giresun) was investigated at 3 stations between February 2018 and January 2019. A total of 73 taxa belonging to Bacillariophyta, Chlorophyta, Cyanophyta, Euglenophyta, Chrysophyta and Cryptophyta divisions were identified. 57.53% of the algal flora consists of members of the Bacillariophyta group. This is followed by Chlorophyta and Cyanophyta divisions which constitute 28.76% and 5.47%, respectively. *Amphora ovalis*, *Cocconeis pediculus*, *Cymbella affinis*, *Nitzschia palea* and *Ulnaria ulna* were determined as common species in Bacillariophyta which is the dominant divisio. Other algal groups did not contribute significantly to biomass. Algae showed similar seasonal variation in all stations; an increase in the total number of organisms was observed in July, September and March. As a result of the measurements made throughout the year, water temperature was determined as 6.13 - 19.4°C, dissolved oxygen 8.1 - 9.2 mg/L, pH 6.95-7.84, electrical conductivity 342.28 - 496 µS/cm.

Keywords: stream, algae, diatom, Bacillariophyta.

ORAL PRESENTATION

Circadian rhythm and chronopharmacology in veterinary medicine

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Abstract

In animals, many biological, physiological, hormonal and behavioral conditions occur in accordance with specific rhythms. Various physiological elements such as the sleep-wake cycle, body temperature, blood pressure regulation, heart rate, melatonin synthesis, cortisol and neurotransmitter release have circadian rhythms. If drugs are administered in the morning or evening, before or after feeding, there is a delay in absorption in the circadian rhythm and differences in the animal's response to the drug due to food-drug interactions. Chronopharmacology refers to the relationship of the timing of drug administration to the circadian rhythm and how it affects the circadian rhythm.Chronopharmacology has critical roles such as reducing drug dosage, increasing the therapeutic effectiveness of the drug, shortening the treatment period, minimizing side effects, slowing down the rate of progression of the disease, and preventing drug toxicity, especially in case of damage to body systems.Chronobiology is successfully transferred to veterinary medicine, it will be possible to apply effective drug treatment at the right time through the relationship between chronopharmacology and circadian rhythm in animals.

Keywords: Chronopharmacology, chronokinetics, circadian rhythm, chronotherapeutics

ORAL PRESENTATION

Apoptotic, Stemness and Anti-Proliferative Effects of A Novel Indole Derivative in NSCLC

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Abstract

Lung cancer (LC) is associated with the highest mortality rate among diagnosed cancers. Non-Small Cell Lung Cancer (NSCLC) is responsible for 85% of all lung cancers. In addition to conventional treatment methods, targeted therapies aim to protect healthy cells and tissues and kill cancerous cells and tissues by triggering various pathways. Indoles and their derivatives are both naturally occurring and synthetically available substances containing indole skeleton and are known to be anticancer agents including apoptosis induction, anti-proliferation and suppression of protein kinase activity. In this study, we investigated the apoptotic, stemness and anti-proliferative effects of Substance 10, an indole derivative synthesized at Ankara University, on healthy lung cell line BEAS-2B and cancerous lung cell lines p53 wild-type A549, p53 mutant-type H1299 in vitro. In terms of anti-proliferative effect, MTT assay showed that Substance 10 showed a higher cytotoxic effect on cancer cell lines than healthy BEAS-2B and suppressed Ki-67 gene expression in a statistically significant manner. CD24 gene expression level, which was examined in terms of stemness, was statistically significantly suppressed in cancerous cell lines after 24 hours of application, while it was significantly increased in H1299 at 48 hours of application. This result was interpreted as a correlation of high expression of CD24 in p53 mutant-type cells with increased stemness and treatment resistance. In terms of apoptosis induction, BCL-2 expression was significantly decreased in A549 at 24 hours, while BAX expression was significantly increased in H1299 at 48 hours. The study is the first study to investigate the efficacy of indole derivative Substance 10, which has not been applied on LC before, on LC, and as a result of the experiments, it was observed for the first time that it decreased cell viability, induced apoptosis and decreased proliferation rate and contributed to the literature in these respects.

Keywords: apoptosis, indole derivative, lung cancer, NSCLC, stemness

ORAL PRESENTATION

Synthesis, Structural Characterization and Investigation of Antiproliferative Activities of New Drug Candidate Molecules

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Abstract

Cancer is one of the deadliest diseases that affects millions of people worldwide, and today there are many different treatment methods depending on the type and stage of cancer [1]. Chemotherapy is one of the methods used in the treatment process of cancer patients, and it is known that these drugs have side effects such as hair loss, diarrhea and loss of appetite during the treatment process. Considering the side effects and toxicity of existing drugs, it is important to conduct studies on the discovery of new drugs with minimum side effects and maximum therapeutic potential. So examination of the chemical basis of the pharmacological properties and mechanism of action of inorganic and organometallic compounds is great importance for drug research [2]. Research on metal-based chemotherapeutics has gained momentum after the groundbreaking discovery of cisplatin and related complexes as potential anticancer agents. Schiff bases, which have many biological and pharmacological activities, are widely used in this field due to their anticancer activities. Schiff base transition metal complexes with anticancer activity are promising molecules for cancer chemotherapy, and therefore their synthesis and activity studies have recently gained great importance in the field of medicine and pharmacy [3]. In this study, an imine compound was synthesized and then this compound was used as a ligand for the preparation of metal complexes. Elemental analysis and some spectroscopic techniques such as FT-IR, ^{1}H-NMR, ^{13}C-NMR, XRD, SEM and UV-Vis were used at structural characterization [4]. The antiproliferative activities of these compounds were investigated in breast cancer MCF-7 and MDA-MB-453 cell lines [5]. It was found that the complexes exhibited greater activity compared to the ligand in both cell lines. The Ni complex also had a greater effect with a lower IC_{50} value compared to positive carboplatin in both cancer cell lines.

Keywords: Antiproliferative Activity, Cancer, Imine Compound, Ligand, Metal Complex

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ORAL PRESENTATION

Effect of Topiramate on Mouse Detrusor Contractility

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Abstract

Topiramate (TPM) is a sulfonamide derivative approved for the treatment of epilepsy and migraine. TPM has a broad spectrum of activity due to its multiple mechanisms of action including the inhibition of voltage-dependent sodium and calcium channels, the augmantation of GABAergic transmission and the suppression of glutamergic transmission. Recently, TPM has been reported to cause bladder dysfunction such as urinary incontinence and urinary retention, but its mechanism has not been elucidated. This study aims to determine the effect of TPM on isolated mouse detrusor contractility by using isometric tension testing. The contractile responses of the detrusor strips to KCl (80 mM), carbachol (CCh, 10^{-8}-10^{-4} M), or $CaCl_2$ (10^{-6}-10^{-1} M) were measured in the presence of TPM or vehicle (dimethyl sulfoxide). The relaxant effect of TPM on KCl-precontracted detrusor strips were also evaluated. TPM ($3x10^{-3}$ and 10^{-2} M) reduced KCl-induced contractions (Emax=33.10±3.67% and 28.62±1.23%, respectively). TPM at 10^{-2} M inhibited the contractile responses induced by CCh and $CaCl_2$ (Emax=44.94±6.21% and 73.84±6.02%, respectively). Moreover, TPM (10^{-2} M) produced a significant relaxation in the detrusor strips precontracted with KCl (Emax=78.78%±3.20%). To our results, TPM inhibited contractions and caused relaxation in the detrusor strips concentration-dependently. The present study is the first report demonstrating the inhibitory effect of TPM on detrusor smooth muscle contractility.

Keywords: Carbachol, Contraction, Detrusor, Isolated organ bath, Relaxation, Topiramate

This study was supported by a grant from the Scientific and Technological Research Council of Türkiye (TÜBİTAK-2209/A, grant no. 1919B012306351).

ORAL PRESENTATION

Evaluation of the Oxidative and Apoptotic Effects of Epigallocatechin Gallate and Cetuximab in Lung Cancer Cells

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Abstract

Lung cancer, which is the type of cancer with the highest mortality and incidence worldwide, ranks first among the causes of cancer death in men and second in women. Anticancer drugs also target healthy cells, causing serious complications and life-threatening side effects. Today, many natural products obtained from plants with anticancer properties are purified or chemically synthesized and used for therapeutic purposes. Although cetuximab, a commonly used chemotherapeutic agent targeted to epidermal growth factor receptor (EGFR), provides successful results in many types of cancer when used alone or in combination with different drugs, it is known to cause serious side effects. For this reason, the therapeutic effectiveness of cetuximab remains limited. In cancer therapy, the use of anticancer drugs in combination with natural products has become a very important strategy in strengthening the effects of these drugs and minimizing their side effects on healthy cells by reducing drug doses. Combined treatment studies in which cetuximab, a targeted chemotherapeutic agent, is applied together with various substances obtained from plants to increase its effectiveness may be a promising treatment strategy in this respect. Green tea catechins, especially epigallocatechin-3-gallate (EGCG) have been associated with cancer prevention and treatment. In our study, cytotoxic, oxidative and apoptotic effects of cetuximab alone and together with EGCG on A-549 cells (NSCLC) were investigated. In order to determine $<IC_{50}$ values of cetuximab and EGCG, 3-(4,5-Dimethyl2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) assay were performed on the cell lines. After A-549 cells treated with cetuximab and EGCG together, the activity of GSH-Px was found to be approximately 1.5 times greater than in cetuximab alone treated cells. Thus, these data suggest that cetuximab and cetuximab combined with EGCG induced oxidative stress producing reactive oxygen species in the cells. Caspase-3 activity increased approximately 1.6-fold compared to the control when cetuximab coadministered with EGCG in A-549 cells.

Keywords: Cetuximab, Combined therapy, Epigallocatechin-3-gallate, Oxidative effect, Apoptotic effect.

ORAL PRESENTATION

Green synthesis of olive leaves-based silver nanoparticles

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Abstract

Nanoparticles show properties considered to be much different and superior to bulk structured materials. These characteristics make nanoparticles useful in various industries, including pharmaceuticals, electronics, textiles, chemistry, medicine, and defense [1, 2]. Nanoparticles could be synthesized using different techniques such as chemical, physical, and biological methods. Chemical methods are harmful to human health and the environment. For this reason, biological synthesis which is also called green synthesis has been popular in recent years [3]. In this technique, plants, fungi, and bacteria could be used as electron precursors. Olives are produced significantly in Türkiye and their leaves are natural waste. They are rich in bio compounds such as phenolics, flavonoids, etc. These compounds could reduce silver ions to metallic silver. In this study, waste olive leaves were used in the green synthesis of silver nanoparticles. The obtained nanoparticles (OL-AgNPs) were characterized by using a UV-Vis spectrophotometer and Fourier Transform Infrared Spectrometer (FTIR). It was determined that the obtained nanoparticles gave maximum absorbance at 390 nm. There were differences between olive leaf extract and OL-AgNPs functional group bands in FTIR spectrums. The obtained results show that olive leaves had the potential as a precursor in the biological synthesis of silver nanoparticles.

Keywords: green synthesis, olive leave, sustainability, waste

Acknowledgement We are grateful to Gazi University Scientific Research Projects Coordination Unit (Project number: FDK-2022-7739) for providing financial support.

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ORAL PRESENTATION

Transient expression of the brucella OMP25 protein in tobacco plants by using the agrobacterium system

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Abstract

Brucellosis is a significant zoonotic disease caused by Brucella species, prevalent in developing countries. It leads to economic and health issues in livestock, including reproductive failures, reduced milk production, and infertility. The disease is widespread in the Mediterranean, South and Central America, Africa, Asia, the Arabian Peninsula, the Indian subcontinent, and the Middle East. Due to its rapid transmission and the challenges in controlling it, combating brucellosis is crucial for the socio-economic development of rural livestock breeders.Vaccination is the most cost-effective strategy for controlling brucellosis. However, current live attenuated vaccines have drawbacks, such as causing pathogenicity in humans and adverse effects in animals, including abortions and sterility. They also complicate disease management, as serological tests cannot distinguish between infected and vaccinated animals. These issues drive the need for safer vaccine alternatives.One promising solution is edible vaccines, which offer advantages like eliminating the need for purifying antigens, reducing production costs, and avoiding the need for sterile injection conditions and specialized storage equipment. This study aims to develop an edible vaccine by expressing the OMP25 **(**25 kDa outer-membrane immunogenic protein**)** protein of Brucella abortus in plants. The gene for OMP25 will be transferred into Agrobacterium tumefaciens, which will then transfer it to tobacco plants. Successful expression of OMP25 in these plants could provide a safer, more cost-effective vaccine, improving livestock health and productivity in affected regions. In this study, the target gene was transferred to Agrobacterium using the Gateway system, gene transfer to plant was performed through Agrobacterium tumefaciens and the agroinfiltration method on 50 leaves. The results were confirmed using a GFP imaging device.

Keywords: Brucellosis, OMP25, Gateway system, Agrobacterium

ORAL PRESENTATION

Tenebrio molitor (Linnaeus, 1758) (Coleoptera: Tenebrionidae) as an edible insect

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Abstract

The rapid growth of the human population in the second half of the 21st century may cause food shortages, especially animal proteins. Considering recent changes in population, sustainability of food sources, and environmental issues, insects are of great interest as novel sources of human nutrition. The recognition of insects as novel foods in Europe has paved the way for further research on the nutritional value of insects and allowed their incorporation into foodstuffs. The mealworm *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae), an edible insect, is a holometabolic insect that probably originated in the Mediterranean and has now spread worldwide due to colonization and trade. *T. molitor* is considered a pest affecting stored grains. However, studies on their use for food and feed purposes and waste management have accelerated in recent years. Microbiology analysis showed that *Escherichia coli* and *Salmonella* spp. were not detected in larvae, adults or even feces. *T. molitor*, one of the most remarkable insect species in the search for alternative and sustainable food sources, provides environmental and economic advantages due to its significant protein, lipid, vitamin, and mineral contents. *T. molitor* larvae are a more valuable food source for humans than traditional meat foods. Fresh larvae contain more total protein and total fat than conventional meats such as eggs, chicken, pork, beef, and fish. The protein found in *T. molitor* is also rich in amino acids such as isoleucine, leucine, and lysine, which meet the nutritional value recommended by the Food and Agriculture Organization. Additionally, *T. molitor* larvae show a good composition of vitamins A, C, D, E, K, and B. These results further support the possibility of introducing mealworms into human food consumption. In recent years, many farms and food companies worldwide have started to use this insect.

Keywords: *Tenebrio molitor*, Edible insect, Food.

ORAL PRESENTATION

An insect with versatile use potential: *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae: Hermetiinae)

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Abstract

The black soldier fly (BSF) *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae: Hermetiinae) is a unique insect with a wide range of uses, from waste treatment to use as feed and fertilizer, from biodiesel to chitin production. Having a short life cycle and rapid growth, BSF can be grown and harvested without special facilities. Adults consume nothing but water, do not approach humans, do not bite or sting, and do not vector or spread any specific diseases. The black soldier fly larvae (BSFL) feed voraciously on substrates as diverse as decomposing fruits and vegetables, animal and human feces, municipal organic waste, distillers grains, and even crop straw. They reduce the initial weight of organic waste by approximately 50% in less time than traditional composting. Therefore, BSF can be used as a sustainable alternative to treat organic waste. Apart from this, BSF has the potential to be used in many different areas. BSF, an edible insect, can provide a sustainable source of protein to feed a growing global population. Besides, the residue from the BSFL bioconversion process can be used as fertilizer. Since the larvae contain high amounts of crude protein and fat, they are used as animal feed. These insects can replace fishmeal, which is used as animal protein feed in aquaculture and animal husbandry. BSFL is also used in poultry feed as a partial replacement for corn or soy-based feeds. Another interesting area of use is biodiesel production. BSF oil is high in medium-chain saturated fatty acids and low in polyunsaturated fatty acids, providing biodiesel with low viscosity and good oxidative stability. Biodiesel obtained from BSF has good combustion and fuel efficiency and reduced environmental emissions. Additionally, BSF can also be used as a potential source of chitin and chitosan and can serve as a potential source of these biopolymers.

Keywords: Black soldier fly, Waste treatment, Fertilizer, Feed.

ORAL PRESENTATION

Molecular investigation of BVDV and BPI-3 infections in a cattle farm using one-step RT-PCR in Ankara province

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Abstract

Bovine Viral Diarrhea Virus (BVDV) and Bovine Parainfluenza Virus Type 3 (BPI-3) are significant diseases in cattle that have a substantial impact on the health and production of the animals. BVDV is notorious for inducing a variety of symptoms, ranging from simple respiratory problems to severe immunosuppression and gastrointestinal abnormalities. BPI-3 predominantly impacts the respiratory system, resulting in ailments like pneumonia and making animals more susceptible to subsequent bacterial infections. The aim was to gather current data on the prevalence of BPI-3 and BVDV. This investigation involved the collection of nasal swab samples from 75 cattle of different ages on a farm in Ankara. Some of the animals on the farm exhibited respiratory distress, and there were cases of calf deaths associated with bacterial infections. The phenol-chloroform extraction was applied to extract the RNAs from the processed samples. Subsequently, the presence of BVDV and BPI-3 viruses was examined using the one-step RT-PCR technique. There was no data indicating that bacterial infections made the cattle more susceptible to viral infections. All the samples were found to be negative for both BVDV and BPI-3. Additionally, appropriate bands located between the non-specific bands were sequenced, however they yielded negative results. The positive and negative controls yielded precise outcomes as anticipated. Both cytopathogenic (cp) and non-cytopathogenic (ncp) biotypes of BVDV were also demonstrated individually for BVDV positive controls. These results show that BVDV and BPI-3 infections were not present in the studied cattle farm and that bacterial infections that was detected as E. coli did not predispose the animals to these viral infections. According to preliminary results, the herd either has a low viral prevalence or is virus-free. Furthermore, these findings might suggest that stringent biosecurity protocols, good herd management techniques, restricted animal transportation, and efficient vaccination programs are productive.

Keywords: Ankara, BVDV, BPI-3, cattle, Türkiye.

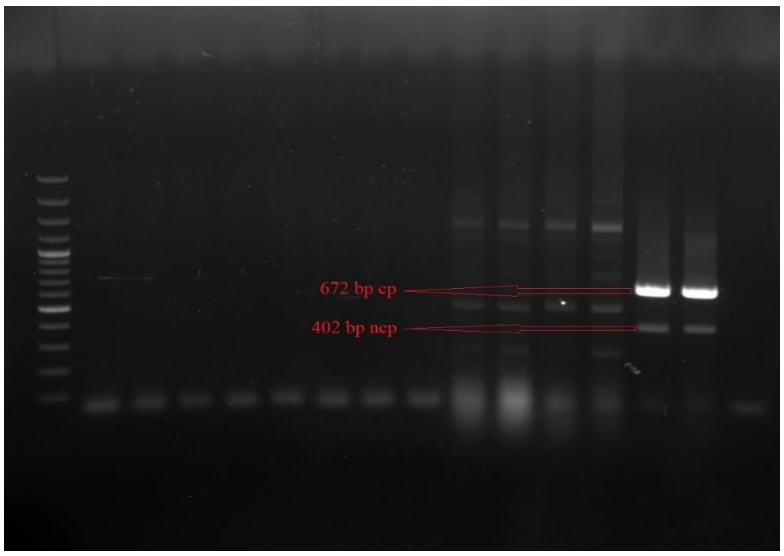

Figure 1: BVDV amplicon agarose gel electrophoresis image for both cytopathogenic and non-cytopathogenic biotype

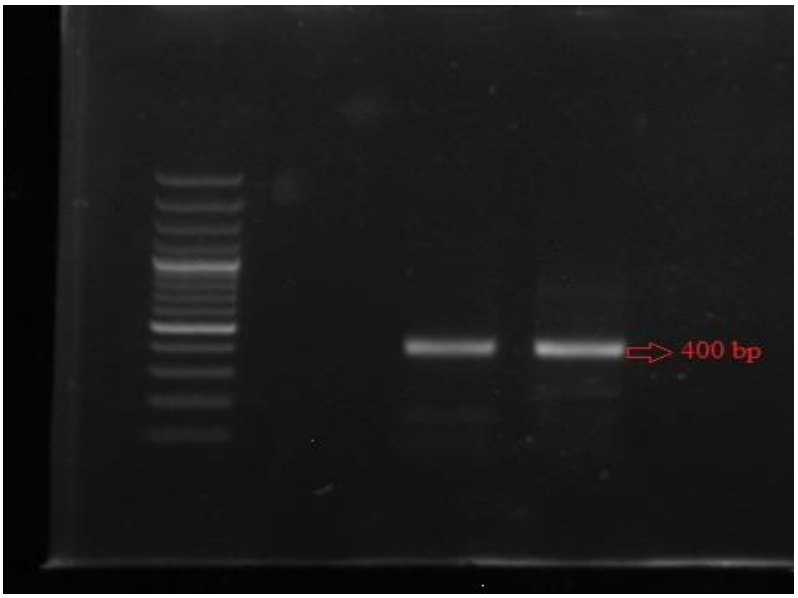

Figure 2: BPI-3 amplicon agarose gel electrophoresis image

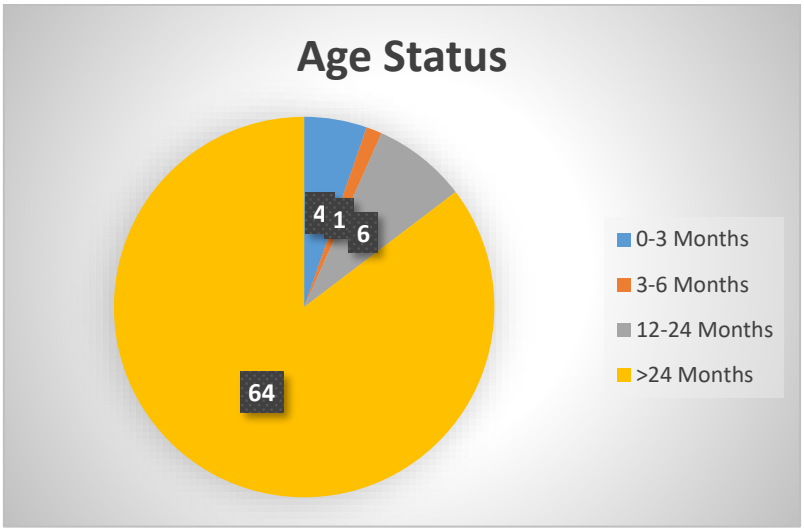

Figure 3: Age status of sample group

ORAL PRESENTATION

Electrochemical biosensor designing for investigation of the interaction between DNA and Quercetin

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Abstract

It is known that the bioactive flavonoid, quercetin, has anticancer, antiviral, antioxidant, and anti-inflammatory effects. Flavonoids, which are secondary metabolites of plants, are named according to the differences in their phenylbenzopyran structures. Quercetin is classified as flavonol due to its structure. Antioxidant action mechanisms; Flavonoids, defined as neutralizing free radicals, are lipophilic antioxidants. In addition to their antioxidant properties, they have antitumoral, antiviral, antiallergic, vasodilatation, and coronary disease protective effects. Since they can bind to free radicals, proteins, and enzyme structures, they are thought to have a wide range of action mechanisms. Various chromatographic, spectrophotometric, and enzymatic methods are used for flavonoid determination in the literature review. However, no study using electrochemical DNA biosensors has been encountered so far. The most important advantage of biosensors over other methods is that they allow economical, practical, and specific measurements for the substances to be determined. This study aimed to develop electrochemical biosensors for the determination of the effects of quercetin flavonol, which has antioxidant and analgesic activity, on the biosensing layer, DNA.

Keywords: DNA, biosensors, quercetin

ORAL PRESENTATION

Kendi Kendini Temizleyen, Çevre Dostu, Nefes Alabilen, Antimikrobiyal Özellikli Yeni Bir Maske Üretimi

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Özet

Covid-19 pandemisinin etkisiyle maske ihtiyacının artması, ucuz, pratik, kendini temizleyen ve antimikrobiyal özelliklere sahip bir maskeye olan gereksinimi ortaya çıkardı. Literatür taramamız, bu niteliklere sahip bir maskenin mevcut olmadığını ve hidrotermal sentez yöntemi ile üretimine dair bilgi olmadığını gösterdi. Bu çalışmada, kenevir tozuna magnezyum ve çinko eklenerek hidrotermal sentez yöntemi ile Mg ve Zn nanopartiküller (NP) üretildi. Sentezlenen NP'lerin PVDF polimeri ile karıştırılmasıyla elektro-eğirme yöntemi ile Mg ve Zn nanofiber yapılar elde edildi. Elde edilen nanofiber yapılar SEM ve EDX kullanılarak karakterize edildi. Bu çalışma, özgün nitelikte, nefes alabilen, çevre dostu ve kendini temizleyebilen bir maske üretim yöntemi sunmaktadır. Bu yöntem sunulurken iki basamaklı yöntem protokolü uygulandı. Birinci basamakta, TiO_2 altlık üzerine Zn ve Mg nanopartiküller kenevir tozu katkılanarak hidrotermal sentez yöntemi ile üretildi. İkinci basamakta, PVDF polimeri ile nanopartiküller karıştırılarak elektro-eğirme işlemine tabi tutuldu ve Mg ve Zn nanofiber yapılar elde edildi.

Anahtar Kelimeler: Hidrotermal sentez yöntemi, kenevir tozu, elektro-eğirme yöntemi, kendi kendini temizleme

ORAL PRESENTATION

Bazı bitkilerden yeşil kimya ile sentezlenecek bazı yeni nanopartiküllerinin yara tedavisi kullanımının araştırılması

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Özet

Çeşitli bitkilerden elde edilen birçok nanopartikül bulunmaktadır. Elde edilen nanopartiküllerin sağlık alanında ve yara tedavisinde birçok kullanımı literatürde yer almaktadır. Havacıva bitkisi geleneksel olarak bir gıda maddesi ve önleyici ve iyileştirici bir ilaç olarak da kullanılır. İkinci derece yanıklara balmumu, zeytinyağı ve *A. tinctoria* (L.) Tausch karışımı uygulandığında bu durum epitelizasyonu hızlandırmış, pansuman değişiklikleri sırasında yaşanan ağrıyı azaltmış ve hastaların hastanede kalış sürelerini kısaltmıştır. Havacıva bitkisi sedef hastalığı da dahil olmak üzere dermatit tedavisinde etkili olduğu belirlenmiştir. Bu çalışmada yer alan çinkooksit nanopartikülü bir metalik nanopartikül olup ana maddesi olan çinko, antibakteriyel, antikanser, antiinflamatuar, yara iyileştirme, antifungal ve antimikrobiyal madde olarak yıllardan beri çeşitli alanlarda kullanılmaktadır. Bu çalışmada özellikle üzerinde çalıştığımız havacıva bitkisi farklı alanlarda kullanıldığı tespit edilse de nanopartikül sentezlenerek yara iyileşmesi üzerinde denemeler yapılmadığı görülmüştür. Araştırmalarımız sonucu literatürde bu alanda bir boşluk olduğu anlaşılmıştır. Bu anlamda bu çalışmayla birlikte havacıva bitkisinden elde edilen nanopartikülün yara tedavisi üzerine etkisinin araştırılmaktadır. Bu projede havacıva bitkisinden elde edilen ekstrakt ile çinko elementi hidrotermal sentez yöntemi ile katkıladıktan sonra elde edilen nanopartiküllerin SEM görüntüleri alınmıştır. Diğer karekterizasyon ile ilgili çalışmalar devam etmektedir.

Anahtar Kelimeler: Havacıva, hidrotermal, çinko, yara tedavisi

ORAL PRESENTATION

Elucidating the impact of TACC3 levels on colorectal cancer tumorigenesis

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Abstract

Colorectal cancer (CRC) ranks as the third most common cancer as incidence and second most lethal cancer. It usually begins in the colon or rectal epithelium and can progress into invasive and metastatic profile through the accumulation of epigenetic abnormalities or mutations. The majority of CRC patients have competent DNA mismatch repair (pMMR) status and microsatellite stability (MSS) in which mechanisms function correctly. However, microsatellite instability (MSI) is one of the molecular hallmarks of CRC detected in about 15% of the cases and mainly caused by the DNA-mismatch-repair system deficiency. Transforming Acidic Coiled-Coil Containing Protein 3 (TACC3) is one of the highly deregulated protein playing critical roles in mitotic spindle formation and centrosome localization during cell division. Previous research show that TACC3 is upregulated in different cancer tissues compared to normal counterparts, while others indicate that it also acts as a tumor suppressor by reducing invasiveness and cell proliferation. Therefore, we first aimed to investigate TACC3's role in CRC with a particular focus on MSI and MSS profiles of CRC patients. We analyzed different CRC patient datasets including The Cancer Genome Atlas (TCGA) data and independent high throughput datasets from GEO database to investigate the effects of TACC3 levels on disease subtype, progression, aggressiveness, and survival. We demonstrated that TACC3 was significantly upregulated in MSI/dMMR patient tumor samples compared to MSS/pMMR profiles. Similar profile was also observed in CRC cell lines as MSI cells expressed higher TACC3. Importantly, we also showed that higher TACC3 levels were associated with better overall survival, disease-free survival, and relapse-free survival in MSS/MSI-L (low) patients. Altogether, our results indicate that TACC3 upregulation is possibly behaving a protective role in MSI patients compared to MSS patients, which still needs further analysis investigating the distinct functional roles of TACC3 as oncogene or tumor suppressor in CRC.

Keywords: Colorectal cancer, TACC3, microsatellite instability

ORAL PRESENTATION

Herbal medicine: Antibacterial and anti-biofilm activity of tea tree oil

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Abstract

In the last two decades, infections caused by multidrug resistant bacteria became one of the greatest global health threats. Antibiotic resistance challenge led the researchers to develop the effective antibacterial agents by investigating the bioactivity of natural compounds such as plant-derived components. Thus, this study aimed to determine the antibacterial and anti-virulence activity of tea tree oil (TTO) against *Pseudomonas aeruginosa* (*P. aeruginosa*), which causes various infections via its planktonic cells or in the biofilm formation phase. With this aim, minimum inhibitory concentration (MIC) of TTO against *P. aeruginosa* strain PAO-1 was detected by broth micro dilution assay, then the effectiveness of sub-MIC of TTO on virulence factors of *P. aeruginosa* strain PAO-1 (biofilm formation, protease activity, swarming, swimming and twitching) were investigated. Crystal violet method was used to examine the biofilm inhibition capability of TTO. According to the obtained data, it can be stated that, TTO showed inhibitory effect on the swarming, swimming and twitching ability of *P. aeruginosa* strain PAO-1 at 0.5xMIC concentration. Additionally, reduced protease activity and 80% biofilm inhibition were determined at this low concentration of TTO. This study showed the efficacy of TTO to attenuate the virulence of *P. aeruginosa.* Hence, it was revealed that TTO is a promising replacement for the treatment of biofilm associated infections with its inhibitory effect on biofilm formation.

Keywords: Tea tree oil, *P. aeruginosa*, antimicrobial, anti-biofilm, anti-virulance.

ORAL PRESENTATION

Advancements in Plant-Based Protein Extraction Using Deep Eutectic Solvents

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Abstract

In recent years, plant-based proteins have gained popularity due to the increasing global population and the negative environmental impact of animal-based proteins. Plant-based food waste presents a sustainable and cost-effective alternative. Traditional methods of protein extraction often have low recovery rates and require high solvent consumption. In contrast, deep eutectic solvents (DES) are low-cost, offer improved solubility and biodegradability, and are safe and easy to synthesize. Recent studies have utilized various fruits, grains, legumes, and some of their seeds and shells as sources of plant-based protein. Choline chloride is commonly used as a hydrogen bond acceptor (HBA), while glycerol, urea, and glucose are frequently chosen as hydrogen bond donors (HBD) in DES. DES extractions have been compared with alkaline and pressurized liquid extraction (PLE) methods. In protein extraction from sea buckthorn seeds, the choline chloride-urea DES demonstrated higher amino acid content and better *in vitro* digestibility compared to alkaline methods. Another study found that choline chloride-urea extracted protein from canola with a lower efficiency than alkaline methods (68.34%) but higher efficiency compared to other DES (52.89%). Sequential application of PLE and DES extraction in pomegranate seeds resulted in the recovery of 23.8 g of protein per 100 g of pomegranate seeds, accounting for 94% of the protein present in the seeds. Protein extraction from sesame using choline chloride-ethylene glycol achieved a purity of 93.3%. Overall, DES offers a promising approach for protein extraction, providing high protein yield, recovery, and purity.

Keywords: deep eutectic solvents, plant-based proteins, protein extraction

ORAL PRESENTATION

Synthesis, Characterization and Photophysical Properties of a Novel Fluorene Derivative BODIPY Dye

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Abstract

Cancer, a global health problem, has been one of the leading causes of death worldwide in recent years, and the primary prevention of cancer is an area of great interest at scientific, economic and political levels [1,2]. It is known that chemotherapy, radiotherapy and surgical intervention are effective treatment methods, especially in early diagnosis, and alternative treatment methods are also being developed and used in addition to these treatment methods. Photodynamic therapy (PDT) is an alternative, non-surgical method for cancer treatment [3]. Today, the PDT method attracts great attention as an alternative method to chemotherapy, radiotherapy and surgical methods used for cancer treatment due to its low side effects, the fact that cells do not develop resistance to the drug and that the drug can be administered to the patient in low doses. The basic principle of the PDT method is the production of singlet oxygen ($^1O_2$) as a result of the interaction of a light-sensitive molecule with light and the production of toxic $^1O_2$ killing the cancer cell. The substances that undertake this task are called photosensitizer (PS) [4]. Fluorescent dyes attract great attention from researchers in the ever-expanding multidisciplinary scientific world. Recent developments in the field of personal diagnosis and organic electroluminescent devices are rapidly increasing the interest in the development of new generation dyes. BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes are good PS candidates due to their properties such as high fluorescence quantum yields, narrow emission bands, thermal and photostability, good solubility in different solvents and easy functionalization from various positions [5]. Within the scope of this study, the design, synthesis, structure elucidation and photophysical properties of a fluorene derivative new BODIPY dye (1) that may be PS candidate in cancer treatment with PDT are aimed to be examined (Figure 1).

Keywords: BODIPY, Fluorene, Photophysical property.

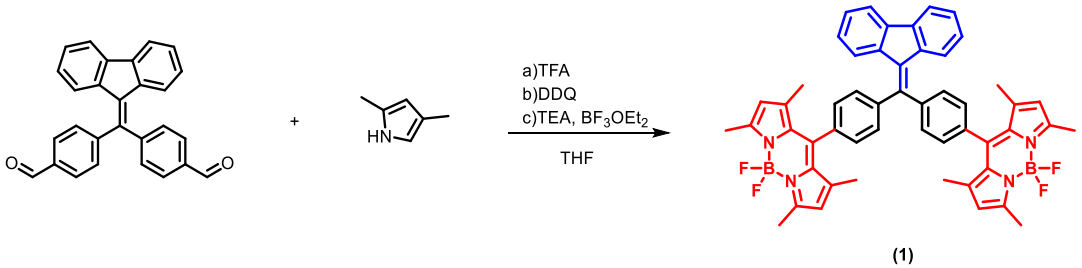

Figure 1. Synthesis pathway of fluorene based BODIPY dye (1).

Acknowledgement: We would like to thank Kafkas University Scientific Research Projects Coordinatorship (Project No: 2024-FM-20) for financial support.

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ORAL PRESENTATION

The effect of thermal production temperature of Zn/L-tryptophan molecular organic framework's on morphologic and crystallographic structures

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Abstract

This study investigates the impact of thermal production temperature on the morphology and crystallographic structure of Zn/L-tryptophan molecular organic frameworks (MOFs). Various temperatures ranging at 20°C, 30°C, and 50°C were employed during the synthesis process. Zn/L-tryptophan ratio was 1:1 (mol:mol). The resulting frameworks were characterized using techniques such as scanning electron microscopy (SEM) and X-ray diffraction (XRD) to analyse their morphological features and crystalline properties. Results indicate that variations in production temperature significantly influence the size, shape, and arrangement of the crystalline structures. Higher temperatures tend to enhance crystallinity, while lower temperatures favour the formation of unique morphologies. This research provides insights into optimizing the synthesis conditions for Zn/L-tryptophan MOFs, potentially enhancing their application in fields such as catalysis and drug delivery.

Keywords: L-tryptophan, MOFs, Crystallinity, Morphology.

ORAL PRESENTATION

Comparative heavy metal analysis of tar phases isolated from a heated tobacco product and 1R6F research cigarette

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Abstract

Cigarette smoking is among the leading preventable causes of mortality and poses a significant socioeconomic challenge in our nation due to its association with chronic illnesses. Furthermore, the tar collected in cigarette filters, which are often disposed of in urban environments, can flow into rivers, and ultimately reach the ocean, raising new concerns for environmental health. This study aimed to analyze the accumulated heavy metal profiles of manually isolated tar phases from a 1R6F research cigarette (TarC) and heated tobacco product (TarH) comparatively. TarC and TarH were isolated according to a slight modification of our previous method (Reis et al., 2024). Tar collected from each product on Whatmann filter papers was digested with ultrapure HNO_3 (67-69%) + H_2O_2 (30%) for 2 h at 80 ºC and analyzed via ICP-MS after diluting the sample solutions (1:10) with a diluent containing 2% HNO_3. Sample blanks were prepared with original paper filters. The content of the analyzed elements (^{7}Li, ^{65}Cu, ^{66}Zn, ^{75}As, ^{85}Rb, ^{111}Cd, ^{202}Hg, ^{205}Tl, ^{208}Pb) was expressed as µg/g tar. Moreover, cell morphology exposed to the dimethylsulfoxide extracts of TarH and TarC filter papers up to 72 hours was imaged via microscopic examination at 10x magnification rate. Our results revealed a notable difference in heavy metal concentrations and variations in cell morphology when exposed to these two products. TarH samples had a lower heavy metal content, with values below the detection limit, while TarC samples contained detectable levels of Cd, Tl, Pb, Li, Zn, and Rb, leading to a notable decrease in cell viability compared to both control and TarH groups. These findings indicate that heated tobacco products could potentially lead to lower heavy metal exposure compared to traditional cigarettes. Nonetheless, further investigation is essential to clarify the potential presence of other harmful substances in their tar. Future studies should also examine the environmental consequences of cigarette butt disposal, particularly focusing on the leaching of heavy metals.

Keywords: Environmental pollution, cigarette tar, heated tobacco products, heavy metal.

Acknowledgments
This project is supported by TUSEB 2023-A3-01 grant (#33176).

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ORAL PRESENTATION

Syntheses and spectroscopic properties of novel *spiro-bino-spiro-* phosphazene derivatives

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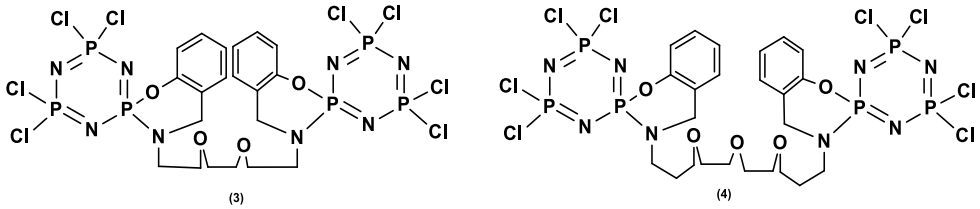
Abstract

Hexachlorocyclotriphosphazene (HCCP, $N_3P_3Cl_6$, trimer), has six very reactive P-Cl bonds, and it is a well-known starting compound for preparation of countless trimeric and polymeric organophosphazene derivatives [1-2]. These compounds can be easily functionalized as they can easily undergo nucleophilic substitution reactions [3]. The product range in the field of phosphazene chemistry is quite wide. Over the years, cyclotriphosphazene and its derivatives have garnered outstanding attention due to their exceptional physical and chemical properties, such as important thermal/ radiation stability, chain flexibility, flame retardancy, biocompatibility, and biodegradability [4]. In this study, Schiff bases obtained by reacting salicylaldehyde with 1,8-diamino-3,6-dioxaoctane and 1,13-diamino-4,7,10 trioxatridecane (2:1) were reduced with $NaBH_4$ and N_2O_2 donor atom tetradentate compounds (L1 and L2) were obtained. As a result of the condensation reactions of the synthesized N_2O_2 donor atom tetradentate ligands with potassium salt, trimer ($N_3P_3Cl_6$) (2:1), in the presence of Et_3N in dry THF medium, partially substituted *spiro-bino-spiro-* derivatives (3 and 4) (Schema 1.) were synthesized and pure by column chromatography. The structures and spectroscopic properties of the synthesized compounds (3 and 4) were determined using elemental analysis, mass spectrometry (ESI-MS), FTIR, ^1H, ^{13}C and ^{31}P NMR techniques.

Keywords: Schiff base, Spectroscopy, *Spiro-bino-spiro*-phosphazene, Tetradentate ligands.



Schema 1. *Synthesized spiro-bino-spiro-cyclotriphosphazenes*

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ORAL PRESENTATION

Antimicrobial and DNA Cleavage Activities of Tetraaminomono(4-fluorobenzyl)spiro(N/O) cyclotriphosphazenes

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Abstract

Hexachlorocyclotriphosphazene, (HCCP; $N_3P_3Cl_6$) has served as an important starting compound for numerous cyclotriphosphazene derivatives. Its six-membered ring offers a stable platform for multifunctional molecular arrangements [1]. The HCCP presents an almost planar structure, with six paired P-Cl bonds stationed on either side of the ring [2]. These P-Cl bonds are justly weak, inducing their susceptibility to nucleophilic reagents, which accounts for the extensive abundance of HCCP compounds [3]. There are studies in the literature investigating the antibacterial, antifungal, cytotoxic, antioxidant activity and DNA cleavage of HCCP derivatives [4-5]. Tetrachloro-4-fluorobenzyl-spiro(N/O)cyclotriphosphazene was synthesized from the substitution reaction of HCCP with sodium 3-(4-fluorobenzylamino)-1-propanoxide. Reactions of tetrachloro-spiro with excess 1-(2-aminoethyl)-piperidine, 4-(2-aminoethyl)-morpholine, 1-(2-hydroxyethyl)piperidine and 4-(2-aminoethyl)morpholine gave the fully substituted cyclotriphosphazene derivatives [6]. Moreover, antibacterial and antifungal activities of the fully substituted 4-fluorobenzylspiro(N/O)cyclotriphosphazenes (2a–2d) were investigated against G(+) and G(-) bacteria and fungi. The DNA cleavage activities of these compounds on plasmid DNA were evaluated using agarose gel electrophoresis.

Keywords: Antimicrobial activity, DNA cleavage, Tetraaminomono(4-fluorobenzyl)spiro(N/O)cyclotriphosphazenes.

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ORAL PRESENTATION

Optimizing a green chromatographic method for the determination of sweeteners in beverages: A Box-Behnken approach

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Abstract

The term "green analytical chemistry" draws on the green chemistry movement of the 1990s and is focused on reducing hazardous substances associated with chemical analysis processes. Recently, these approaches have grown under increasing interest as the demand for green and sustainable analytical techniques, particularly in chromatography, becomes more critical. In this study, a green chromatographic method employing ethanol in the mobile phase was developed and validated for the determination of three sweeteners, namely acesulfame K (ACE-K), saccharin (SAC), and aspartame (ASP), in beverages. Box-Behnken design with desirability function was employed as a chemometric tool to optimize selected chromatographic parameters: ethanol ratio at the beginning of gradient (2-25%), ethanol ratio at the end of gradient (40-80%), and gradient time (12-24 min). Under the optimized conditions, baseline separation of sweeteners was achieved with a total analysis time of 15 min. The method was linear for all analytes ($r \geq 0.9999$). Accuracies of intra- and inter-day experiments at low-, middle-, and high-quality control levels ranged from 97.7-101.7% with acceptable relative standard deviation values ($\leq 3.6\%$). The limits of detection were estimated as 0.42, 0.12, and 0.38 µg/mL for ACE-K, SAC, and ASP, respectively. The applicability of the method was demonstrated by analyzing ten beverages. Mean recoveries obtained for beverage samples ranged from 99.04% to 102.0% for all analytes. The findings suggest that ethanol-based chromatographic methods could be more widely used in the beverage sector.

Keywords: Design of experiments, HPLC, Beverage analysis, Sweeteners.

ORAL PRESENTATION

Evaluated of possible effects of acute exercise on spexin and orexin levels in football players

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Abstract

Exercise has many positive effects, such as reducing the risk of cardiovascular disease, lowering blood cholesterol and glucose levels. Spexin and orexin, which are peptide biomarkers, have the physiological functions such as obesity, energy homeostasis, insulin resistance, cardiovascular diseases, and type 2 diabetes. Exercise is also believed to have an impact on these biomarkers. In this study, we aimed to investigate the effects of acute exercise on spexin and orexin in football players. We compared licensed amateur football players who regularly exercise with a control group that does not exercise regularly. Additionally, we determined the effects of acute and chronic exercise on these parameters by comparing spexin and orexin levels before and after 45 minutes of acute exercise in amateur football players who regularly exercise. 24 healthy male individuals who were licensed amateur football players at Gaziantep University Faculty of Sports Sciences were included in this study. Blood samples were taken from participants twice: before exercise and after 45 minutes of exercise. The control group included 24 male volunteers over the age of 18 who did not regularly engage in sports. Blood samples from the control group were took after 8 hours of fasting. Demographic data of the all participants were recorded. Serum spexin and orexin levels in were measured with ELISA. The results of the study showed that the serum spexin and orexin levels of football players were significantly higher after exercise compared to before exercise ($p<0.05$). When the pre-exercise serum spexin and orexin levels of football players were compared with those of the control group, it was found that the serum spexin levels were significantly higher in football players ($p<0.05$), while there was no significant difference in serum orexin levels between the two groups ($p>0.05$). In conclusion, acute exercise was observed to increase both orexin and spexin levels.

Key Words: Acute exercise, exercise, spexin, orexin

ORAL PRESENTATION

Opopanax hispidus Ekstrelerinin Fenolik İçeriği ile Biyolojik Aktivitelerinin Belirlenmesi

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Özet

Opopanax üyelerinden daha geniş bir coğrafi dağılıma sahip olan *Opopanax hispidus* Batı Avrupa'dan Balkanlar'a ve Asya'nın batı bölgelerine kadar dağılım göstermektedir. Yaygın olarak havuç veya maydanoz ailesi olarak bilinen Apiaceae veya Umbelliferae, yaklaşık 300 cins ve 3000'den fazla tür içerir (Downie ve ark. 2000). Bu büyük aile, zengin ikincil metabolit kaynağı ve yüksek ekonomik ve tıbbi değere sahip çok sayıda cinsi bünyesinde barındırması nedeniyle büyük ilgi görmüştür (Olle & Bender 2010). Apiaceae familyasının bir cinsi olan *Opopanax* W. Koch'un 4 türü vardır: *Opopanax hispidus* (Friv.) Gris., *Opopanax chironium* (L.) W. Koch, *Opopanax persicus* Boiss. ve *Opopanax siifolius* (Boiss. & Heldr.) Menemen. *Opopanax* türleri tıbbi etkileri nedeniyle geleneksel olarak hem Türkiye'de hem de dünyada kullanılmaktadır (Sahranavard vd. 2014). Türkiye'de *O. hispidus*'un "Mayasil otu" olarak bilinen bir bitki grubu içerisinde yer aldığı ve hemoroid tedavisinde kullanıldığı rapor edilmiştir (Sevgi ve Kızılarslan 2013). 10-11. yüzyıllara ait "Dynameron" yazısında *O. hispidus*'un panzehir olarak kullanıldığı bildirilmektedir (Valiakos vd. 2015). Çalışmamızda Muğla'dan toplanan *Opopanax hispidus* topraküstü kısımlarının hegzan, kloroform ve metanol ekstreleri elde edildi. *Opopanax hispidus* topraküstü kısımlarından elde edilen metanol ekstresinin fenolik bileşenleri 41 standart fenolik bileşik ile tek kromatogramda kıyaslanarak HPLC-DAD cihazı yardımıyla belirlendi. Opopanax hispidus'dan alınacak olan farklı özellikteki hegzan, kloroform ve metanol ekstrelerinin 4 farklı metot ile (DPPH, ABTS, CUPRAC ve *β*- Karoten linoleik asit) antioksidan aktiviteleri karşılaştırıldı. Aynı zamanda elde edilen ekstrelerin asetilkolinesteraz ve bütirilkolinesteraz, α-amilaz, α-glukosidaz enzim inhibisyonları Elisa reader cihazı yardımıyla belirlendi. Yapılan bu çalışma ile, Türkiye'nin Ege bölgesinde sebze olarak tüketilen yabani yenilebilir O*popanax hispidus*'dan elde edilen tüm ekstrelerin fenolik içeriği, antioksidan aktivite ve yanısıra antikolinesteraz, α-Amilaz, α-Glukosidaz enzim inhibisyon kapasiteleri hesaplanarak elde edilen fenolik içerik sonuçlarına bakıldığında metanol ekstresinde; 4-hidroksi benzaldehid, hesperedin, oleuropein, hesperetin, protokateşik asit, teofilin, 4-hidroksibenzoik asit, metil-1,4-benzokinon, vanilik asit, kafeik asit, *p*-kumarik asit, ferulik asit, *trans* sinnamik asit, 1,4-diklorobenzen, krisin fenolik asitleri tespit edildi. Ayrıca metanol ekstresinde diğer ekstrelerle kıyaslandığında çok iyi α-Glukosidaz enzim inhibisyon aktivite tespit edildi. Elde edilen ekstrelerin ilave çalışmalarla ileri kimyası ve *in vivo* aktiviteleri araştırılarak söz konusu olan hastalıkların terapötiklerinin geliştirilmesi mümkün olabilecektir.

Anahtar kelimeler: *Opopanax hispidus*, Fenolik içerik, Antioksidan aktivite, Antikolinesteraz Enzim İnhibisyonu, α-Amilaz ve α-Glukosidaz Enzim İnhibisyonu

Teşekkür: Bu çalışma 2023/2 yılında Üniversite Öğrencileri Araştırma Projelerini Destekleme Programı kapsamında 1919B012332563 numaralı proje ile TÜBİTAK 2209-A tarafından desteklenmiştir. Laboratuvar çalışmaları Muğla Sıtkı Koçman Üniversitesi Fen Fakültesi'nde gerçekleştirilmiştir. Desteklerinden dolayı Prof. Dr. Sayın Mehmet Öztürk'e teşekkür ederim.

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ORAL PRESENTATION

Kenevir Kökünün Farklı Yöntemlerle Ekstraksiyonu ve Antioksidan Özelliğinin Belirlenmesi

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Özet

Kenevir bitkisi çoklu kullanımlarıyla bilinir: yaprakları, çiçekleri, tohumları ve sapları kısacası bitkinin tümü gıda, yakıt, lif, ilaç vb. uygulamalar için kullanılmıştır. Günümüzde her ne kadar kenevirin en çok kullanılan kısımları yaprakları, çiçekleri, tohumları ve sapları olarak bilinse de çok eski tarihlerde kenevirin kökü de geleneksel tıpta sıklıkla kullanılmıştır. Literatürde kenevir kökünün tıbbi kullanımına ilişkin ilk kanıtlar Romalı tarihçi Plinius'un yazdığı "Natural Histories" adlı kitapta kenevir kökünün suda kaynatılmasının eklemlerdeki kasılmaları gevşettiğini, gut ve benzeri hastalıkları iyileştirdiğinden bahsedilmiştir. Çeşitli hekimler ve bitki uzmanları ateş, iltihaplanma, gut, artrit ve eklem ağrısının yanı sıra cilt yanıkları ve tümörleri tedavi etmek için kenevir kökünü önermişlerdir. Kenevir kökleri; friedelin, friedelan-3-one, epifriedelinol, B-sitosterol, etil linoleat, karvone, p-kumarik asit, metil hekzadekanoat, oleamid, 4-hidroksi-3-öetoksibenzaldehit, feruloitriamin, B-Amrin, fukasterol olmak üzere birçok farklı yapıda aktif bileşik içerir. Bu çalışmamızda "Endüstriyel Kenevir Kök" özütü çıkartılması için Soxhlet Ektraksiyonu, Ses Dalgaları-Destekli Sıvı Ekstraksiyonu ve Süperkritik CO_2 Ekstraksiyon yöntemi olmak üzere üç ayrı ekstraksiyon yöntemi kullanılmıştır. Bu yöntemlerle elde edilen üç kenevir kök özütünün antioksidan özelliği belirlenmiştir. Antioksidan özellik belirlemede "Radikal Söndürücü Yöntemi" ve "Demir (III) İyonu İndirgeyici Antioksidan Gücü (FRAP) Yöntemi" kullanılanılmıştır.

Anahtar Kelimeler: Endüstriyel Kenevir Kökü, Ekstraksiyon, Antioksidan aktivite

Bu çalışma TÜBİTAK 1002- Hızlı Destek Programı kapsamında (**Proje No:123Z841**) desteklenmiştir.

ORAL PRESENTATION

Green Synthesis of paper-based flexible SERS substrate for biomedical applications

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Abstract

Over the last few decades, the green or biological synthesis of nanoparticles has gained significant recognition across the globe, largely due to its cost-effectiveness, low toxicity, and environmentally friendly characteristics. One promising application of this approach is the production of paper-based silver nanostructures, which can be employed as effective substrates for Surface-Enhanced Raman Scattering (SERS). These substrates offer a high signal-to-noise ratio and remarkable uniformity, making them valuable tools in various analytical applications. In this study, a paper-based, flexible silver nanostructure surface was successfully fabricated using a single-step, in-situ, and green approach. The *Satureja hortensis L.* plant extract served as both the reducing and capping agent, enabling the formation of silver nanoparticles directly on the paper substrate. The results demonstrated that the silver nanoparticles formed on the SERS substrate were uniformly distributed. To evaluate the SERS activity of the fabricated substrate, rhodamine 6G, a commonly used dye in SERS studies, was applied. The substrate exhibited excellent repeatability and reproducibility, with variation in measurements being less than 10%. This level of consistency highlights the substrate's potential for providing not only significant signal enhancement but also uniform spectra across different measurements. The high performance and reliability of the fabricated SERS substrate suggest that it holds great promise for a wide range of applications, particularly in the biomedical field. Potential uses include the identification of pathogens and the detection of diseases, where accurate and sensitive detection methods are critical. As such, this paper-based SERS substrate could play a pivotal role in advancing diagnostic techniques and improving healthcare outcomes.

Keywords: Green synthesis; SERS; detection

This work was supported by Erciyes University Scientific Research Projects Coordination Unit under the project number FHD-2023-13096.

ORAL PRESENTATION

BSA-coated magnetic carbon nanotubes for mitoxantrone delivery: Drug loading/release studies and cytotoxicity evaluation

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Abstract

Magnetic nanocarriers may be more effective for cancer treatment under a magnetic field compared to non-magnetic nanomaterials due to enhanced tumor-targeting ability and release in the tumor site. CNTs are very encouraging delivery carriers for anticancer drug delivery systems and they can combine with magnetic nanoparticles. Non-functional CNTs are initially hydrophobic and toxic. Proteins are an important group of organic structures used to improve the biocompatibility of nanocarriers. Bovine serum albumin (BSA) is the most abundant protein in the plasma and has attracted attention because of its solubility in water, biodegradability, and nontoxicity. In this study, BSA-coated magnetic single-walled carbon nanotube (mCNT-BSA) was synthesized using covalent functionalization. To prove the covalent attachment of BSA to mCNT, a control experiment was also conducted in which mCNT was modified with BSA using a non-covalent method. The success of the functionalization process was demonstrated through various analysis techniques such as Raman spectroscopy, vibrating sample magnetometer (VSM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Mitoxantrone (MTO) was chosen as model drug and loading/release profiles from BSA-coated nanocarriers were evaluated. A magnetic drug delivery carrier based on mCNT-BSA nanoparticles was developed with demonstrated advantages such as high drug release and magnetic targeting. The cytotoxicity effects of the nanocarriers were examined on HEK293T, and then MTO-loaded nanocarriers were studied on MDA-MB-231 cell lines using an MTT assay. Modification with biocompatible BSA decreased the toxicity of mCNTs. Furthermore, cell viability (%) is significantly reduced with MTO-loaded mCNT and mCNT-BSA, and it is higher than that of free MTO. Considering drug release and cytotoxicity assay results, MTO-loaded mCNT-BSA nanoparticles have great potential for cancer treatment.

Keywords: Bovine serum albumin, Carbon nanotube, Drug delivery, Magnetic nanocarrier, Mitoxantrone

Acknowledgements: This work was supported by Istanbul Technical University Scientific Research Project Foundation (MDK-2020-42751).

ORAL PRESENTATION

The role of computational methods in the drug discovery and development process: Opportunities and challenges

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Abstract

Drug discovery and development is an expensive and time consuming process. Computer aided drug design (CADD) plays a substantial role in expediting the drug development process, reducing the cost, and effort. CADD is mainly employed in the drug discovery step of the process. Specifically, CADD is used in discovering the hit compound, hit to lead progression, and lead optimization. For this purpose, drug target identification, virtual screening, drug repurposing, and *in silico* pharmacokinetic investigations are widely utilized by the academia and pharmaceutical industry. The role of CADD in the drug discovery and development has been increasing in recent years. This is attributed to the increase in the available data, data processing capacity, development of new methods that predict from potency to solubility, and involvement of state of the arts like artificial intelligence.

CADD methods have been employed in the development and repurposing of drugs available in the pharmaceutical market. Especially, these methods play an immense role in conditions that needs urgent response. The role of the CADD in pandemic emergency cases was proven in the fight against COVID-19 recently. There are also some drugs used in the treatment of AIDS that are developed through the involvement of CADD. Although CADD has played a tremendous role in the drug discovery and development process, there are also bottlenecks to be surmounted. The accuracy quest, scarcity of specialists that know both CADD and the state of the art, quality of the available data, overdependence on CADD, computational cost and availability, and the flexibility representation issues are some to mention. Despite the existing challenges, the applications of CADD in the drug discovery process is increasing. The relevance of the CADD methods in shaping the future of drug discovery is anticipated to increase as the limitations are resolved.

Keywords: CADD, drug development, drug discovery, drug repurposing

ORAL PRESENTATION

Molecular detection of *Toxocara canis* in a golden jackal (*Canis aureus*) in Bingol Province, Türkiye

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Abstract

Golden jackals have a wide distribution spanning almost two-thirds of Türkiye, encompassing an area of approximately 289,350 km2 along river lines. It is believed to have a significant function in the spread of parasites to the environment and in serving as a direct or indirect means of infection for other hosts. The objective of this study was to conduct molecular characterisation of Toxocara sp. isolated from a deceased golden jackal discovered in the rural area of Bingöl province, Türkiye. The golden jackal was taken to Bingöl University, Faculty of Veterinary Medicine for a postmortem examination. Upon inspecting the intestines, only the Toxocara sp. isolate was found. The acquired isolate was visually evaluated using a stereomicroscope, and genomic DNA (gDNA) was extracted for confirmation using sequence analysis. Following the separation of genomic DNA, the target region was amplified using the polymerase chain reaction (PCR) technique. The amplification was carried out using JB3-JB4.5 primers, which specifically amplify a 446 bp fragment of the mitochondrial cytochrome oxidase subunit 1 (mt-CO1) gene region. The PCR results of the amplified mt-CO1 were subjected to one-way DNA sequence analysis. The BLAST search results have identified the sequence of the isolate as Toxocara canis. Phylogenetic studies were conducted using mt-CO1 gene segments from several hosts recorded as T. canis in the Genbank database. This study represents the initial molecular characterisation of Toxocara canis in golden jackals in Turkey.

Keywords: Golden jackal, Toxocara canis, PCR, Türkiye

ORAL PRESENTATION

Identification of a novel hsa_circ_HIPK3/miR-375 regulatory network function in prostate cancer

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Abstract

Aim: Prostate cancer (PCa) is one of the most common malignant tumors for men, and its early symptoms are not obvious. Therefore, it is of great significance to study the mechanism of PCa occurrence and development for the early diagnosis, treatment and improvement of patients' prognosis. Noncoding RNAs, especially circular RNA (cirRNA) and microRNA (miRNA), have been shown to play a unique role in tumor development. To investigate the role of the hsa_circ_0000284 (circHIPK3)/miR-375/SLC16A2 axis in PCa by using bioinformatics method.
Methods: The cicRNAs data of prostate were obtained from the CircR2Disease database.
Circular RNA Interactome was used to predict the potential circRNA/miRNA interaction. miRNA expression profiles were provided from the GSE21036 database on the Gene Expression Omnibus (GEO) and miRNET database. GEO2R was used to determine the differences between PCa and control samples. The interaction between miR-375 and SLC16A2 were predicted by miRDB database. The expression change and overall survival analysis of the SLC16A2 gene were analyzed by Gene Expression Profiling Interactive Analysis (GEPIA) and UALCAN databese. Protein-protein and gene-gene interaction networks were determined using STRING and GeneMANIA, respectively.
Results: Hsa_circ_0000284 was down regulated in PCa. While miR-375 was identified as the target of hsa_circ_0000284, the target of miR-375 was found to be SLC16A2. Downregulation of hsa_circ_0000284 may contribute to the anti-cancer functions of miR-375 on PCa tumorigenesis, or upregulation of miR-375 may remove the anti-cancer effects of SLC16A2 on PCa tumorigenesis. Down expression of SLC16A2 predicts an unfavorable prognosis in PCa.
Conclusion: The overexpressing hsa_circ_0000284 and/or blocking miR-375 could become a new target for PCa treatment, and demonstrate that miR-375 may be a potential biomarker for PCa. Further studies with these findings may potentially help in the prognosis and treatment of PCa.

Keywords: Prostate cancer, Hsa_circ_0000284, miR-375, **SLC16A2**

ORAL PRESENTATION

Extraction of some origanum species with new deep eutectic solvents and comparison of their antimicrobial activities

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Abstract

In this study, the synthesis of deep eutectic solvents, which is one of the important topics of green chemistry, compared to general solvents were evaluated. Mixtures of chemicals separated as Hydrogen Bond Donor and Hydrogen Bond Acceptor in amounts ranging from 1:1 to 1:10 in 10mmol and multiples were prepared and heated to 60 ℃ with a microwave oven and then left to cool at room temperature. The antimicrobial activities of the extracts obtained with deep eutectic solvents were investigated and compared with organic solvents. The characterization properties of the synthesized deep eutectic solvents were elucidated by infrared and differential scanning calorimetric analyses. The plant species used in this study were selected as Origanum micranthum and Origanum minutiflorum from the Labiatae (Lamiaceae) family. Studies were carried out on 1 pathogenic fungus and 4 bacteria for antimicrobial activities. The inhibition zone diameters of the samples applied with Agar Well Diffusion Test were measured with a ruler and recorded in mm. It was determined that *Candida albicans, Staphylococcus aureus, Bacillus cereus, Staphylococcus epidermis* fungi and bacteria had various degrees of antimicrobial activity.

Keywords: Antimicrobial Activity, Deep Eutectic Solvents, Differential Scanning Calorimetric Analysis, Origanum Species.

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ORAL PRESENTATION

The effect of *Lactobacillus sakei* and *Staphylococcus carnosus* bacteria on the antioxidant activity capacity in fish sauce production

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Abstract

Fish sauce is a high-quality functional product produced through fermentation techniques. The structural properties of this amber-colored, flavorful liquid are shaped by the microorganisms and enzyme activities present in the environment. In this study, fish sauce was produced using anchovy (*Engraulis encrasicolus*) with traditional methods and starter cultures of *Lactobacillus sakei* and *Staphylococcus carnosus* (BFL-F06, Germany). The use of starters represents a production method that promises both a lower salt content and improved flavor development compared to traditional production. In the study, 20% salt was used in traditional production and 3% in starter-added production, and the resulting product groups were also evaluated in terms of quality characteristics. In the sauce groups, the crude protein content was determined as 10.72-12.43% and the crude fat content as 3.21-2.17%, respectively. Sensory evaluation revealed that the starter culture group had a sour taste, while the traditional sauce group had a salty and fishy taste. In terms of DPPH scavenging activity, the traditional production method resulted in sauces with stronger antioxidant activity.

Keywords: *Engraulis encrasicolus*, fish sauce, *Lactobacillus sakei*, *Staphylococcus carnosus*, quality, antioxidant activity

Acknowledgement
We would like to thank TÜBİTAK for their support of this study. This study was supported by project number 1919B012222851 within the scope of the 2nd term call of 2209-A 2022.

ORAL PRESENTATION

Apis mellifera L. Türü Arı Zehirinin Enzimatik Aktivitelerinin Araştırılması

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Özet

Her ülkenin kendi fitocoğrafik koşullarına uyum sağlayan saf bal arısı ırkları ve yerel ekotiplere sahip olduğu bilinmektedir. Ülkemiz sahip olduğu ekolojik zenginlik ile 5 saf bal arısı ırkına (Anadolu arısı, Kafkas arısı, Karniyol arısı, İran arısı ve Suriye arısı) ve onlarca ekotipe (Gökçeada arısı, Muğla arısı, Yığılca arısı, Efe arısı vb.) ev sahipliği yapmaktadır. Farklı bal arısı ırk ve ekotiplerin farklı fizyolojik, morfolojik ve davranışsal özelliklere sahip olduğu bilinmektedir. Arı zehiri, bal arılarının zehir bezlerinde ürettikleri; enzimler, düşük moleküllü bileşikler ve peptidlerin karışımından oluşan biyoaktif kompleks bir karışımdır. Arı zehirinin fosfolipaz A2 , fosfolipaz B, hiyaluronidaz, fosfataz ve a-glukosidaz aktiviteli peptidler içerdiği bildirilmiştir. Ayrıca arı zehirinde sadece bal arısı zehirinde bulunan melittin ile birlikte apamin, mast hücre degranüle edici peptid, adolapin, tertiapin, secapin, melittin F ve kardiyopep gibi bileşenlerin olduğuda raporlanmıştır. Bu çalışmada Ankara, İzmir ve Mersin olmak üzere üç farklı bölgeden elektriksel stimulasyon yöntemi ile toplanan Apis mellifera L. ham zehirinin bileşenleri kromatografik ve elektroforetik yöntemler ile ayrıştırılmış ve karışımların peptid profilleri ortaya çıkarılmıştır. Ham zehirin proteolitik aktivitesi jelatin ve kazein substratları varlığında zimogram çalışması ile belirlenmiştir. Ayrıca ham zehirin hyaluronidaz ve fosfolipaz aktiviteleri araştırılmıştır. Ham zehrin Yüksek Performans Sıvı Kromotografisinden alınan sonuçlarına göre Ankara zehri, en az 30, İzmir zehri en az 52, Mersin zehri ise, en az 40 farklı fraksiyondan oluşmaktadır. Elektroforetik profili hem Tris-Glisin hemde Tris-Tricine SDS-PAGE ile, her üç lokalite de benzer bandlar göstermiştir. Her üç lokalitede de 37-52 kDa arasında zimogram sonucunda pH 7,4 ‘de beyaz band gözlemlenerek, kazeinolitik aktivite belirlenmiştir. Ayrıca İzmir lokalitesinde diğerlerinden farklı olarak 30-37 kDa arasında da kazeinolitik aktivite gözlenmiştir. Jelatinolitik etki ise yalnız İzmir zehrinde 95-130 kDa ve yaklaşık 270 kDa bölgesinde beyaz band gözlemlenmiştir. Fosfolipaz testinde 16 saat inkübasyon sonucunda her üç zehir örneğinde de 1 cm zon çapı ile oluşan PLA2 aktivitesi görülmüştür. Ayrıca 6,5-16 kDa bandları arasında görülen beyaz band ile hyaluronidaz aktivite gösterilmiştir. Çalışma sonuçları ile bal arısı zehirlerinde varlığı gösterilen proteolitik, lipolitik ve hyaluronidaz etkili peptidlerin arı zehirlerinin tıbbi ve ticari kullanımının potansiyelini artırabileceğini düşünmekteyiz. Her lokalitede bulunan aynı tür arı ırklarında dahi intraspesifik varyasyonlar ile yapısal farklılıklar gösterme potansiyeli içeren zehirlerin, enzimatik etkili bileşenlerinin zehir aktivasyon mekanizmasını anlamak içinde yararlı araçlar olacağı ön görülmektedir.

Anahtar kelimeler: *Apis mellifera*, Bal arısı zehiri, Proteolitik, Fosfolipaz, Hyaluronidaz

ORAL PRESENTATION

Sector-based evaluation of aquaculture wastes

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Abstract

Aquaculture wastes are wastes generated during seafood processing and usually include parts such as fish head, spine, skin, scales, scales, liver, intestines. These wastes can both cause environmental problems and have a significant economic value. Recycling and sectoral utilization of aquaculture waste ensures sustainable management of these wastes and provides a source of raw materials for various sectors. Recycling of aquaculture waste facilitates waste management and creates economic value at the same time. The utilization of these wastes is of great importance in terms of reducing environmental impacts and sustainable resource management. Industries such as food, agriculture, cosmetics and biotechnology can create added value by making extensive use of products derived from these wastes.

Keywords: Recycling, Aquaculture, Waste, Seafood

ORAL PRESENTATION

Electrostatic Separation of bran from Fine Bulgur by Using Teflon and PVC Surfaces

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Abstract

In packaged bulgur products, adherence of the bran to the inner surface creates a visual imperfection that negatively affect the perception of the consumer and compromise the quality standards for bulgur. The objective of this study is to separate the bran from the bulgur by creating an electrostatic field with and without the application of electricity. In order to facilitate the electrostatic separation, Teflon and PVC surfaces with varying properties were employed. Additionally, Teflon and PVC surfaces were configured as flat and folded, respectively, to examine the extent of bran separation. The width of the tunnel within the system was designed to be 4, 5 and 6 cm, while the length was set at 20, 40 and 60 cm. Additionally, the angle of the system was configured to be 30, 35 and 40 degrees. The most effective separation was observed in different configurations, depending on whether the system was Teflon or PVC and whether it was folded or flat. As a result, a fine bulgur mixture containing 5 g of bran per 1000 g of bulgur was passed through the system at a flow rate of 0.89 g/s, resulting in 60 % reduction of bran in bulgur. More effective results were obtained on the Teflon surface than on the PVC surface.

Keywords: Bulgur, Bran, Electrostatic separation, Electric field, Teflon, PVC

ORAL PRESENTATION

pH- Duyarlı Kırılabilir Kovalent Bağlarla İşlevselleştirilebilir Yeni Tip Polimerlerin Hazırlanması

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Özet

Bu çalışmada, alkol grubu içeren hedef moleküller ile silil eter bağları oluşturacak şekilde yan dallarında fonksiyonel gruplar içeren polimerler hazırlanmıştır. Bu amaç için silil eter klorür grubu içeren yeni tip monomerler sentezlenmiş ve bu monomerlerin hidrofilik monomerler ile RAFT (Tersinir Katılma-Ayrışma Zincir Transferi) polimerizasyonları gerçekleştirilmiştir. Elde edilen fonksiyonel polimerlere alkol grubu içeren model moleküller asidik ortamda hidroliz olabilir silil eter gruplarıyla bağlanmıştır. Kullanılan monomerler silisyum atomu üzerindeki alkil grupları değiştirilerek elde edilen üç farklı yapıda seçilmiştir. Silil eter grubu bazik ortamda kararlı ama asidik ortamda hidroliz olabilen bir yapıdır. Silisyum atomu üzerinde bulunan küçük alkil grupları silil eterlerin asidik ortamda daha kolay hidroliz olmasını sağlarken sterik olarak daha hacimli sübstitiye gruplar silil eter hidrolizinin daha yavaş olmasını sağlamaktadır. Bu farklılıktan yararlanılarak, alkol grubu içeren moleküllerle işlevselleştirilmiş polimerlerin asidik şartlarda silil eter grubunun kırılması ile bağlı molekülleri salması amaçlanmıştır. Farklı oranlarda reaktif monomer içeren polimerlerin hazırlanması ile polimerik platformlara istenen seviyede hedef moleküllerin bağlanabilmesi mümkün olmuştur. Hazırlanan yeni tip reaktif polimerlere farklı yapıda alkol grubu içeren model moleküller bağlanmış ve farklı pH şartlarında salımları araştırılmıştır.

Anahtar Kelimeler: Polimerik malzemeler, pH- duyarlı polimerler, reaktif polimerler

Bu projedeki çalışmalar TÜBİTAK tarafından 122Z201 nolu proje kapsamında desteklenmiştir.

ORAL PRESENTATION

İklim değişikliğinin deniz balıkları üretim sektörüne etkileri

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Özet

Türkiye su ürünleri yetiştiricilik sektörü, belirlenen stratejik hedefler doğrultusunda her geçen yıl büyümektedir. Ülkemizde 2023 yılında gerçekleşen toplam su ürünleri yetiştiriciliğinin, %72,1'ini (399 bin 529 ton) deniz balıkları yetiştiriciliği oluşturmaktadır. Deniz balıkları üretiminin büyük çoğunluğu denizlerde ağ kafeslerde yapılmakla birlikte, bir kısmı da karasal alanlarda toprak havuzlarda yapılmaktadır. Su kaynakları üzerindeki etkisi nedeniyle, iklimsel özellikler balık üretim sürecini doğrudan etkilemektedir. İklim değişikliği, insan faaliyetleri sonucunda meydana gelen, yerkürenin ortalama yüzey sıcaklıklarındaki artışı ve iklimde oluşan değişiklikleri ifade eder. Su sıcaklığı; balıkların yemlenme miktarı, yem alımı, yem dönüşüm oranı, balıkların büyümesi, üremesi, solunum ihtiyacı gibi unsurlar üzerinde oldukça etkilidir. Yapılan meteorolojik izleme sonuçlarına göre tüm denizlerimizde, su sıcaklık değerlerinin son 30 yılda 1-2 ˚C arttığını göstermektedir. Bunun yanında, yıl içerisinde görülen maksimum su sıcaklığı değerlerinin de artış gösterdiği görülmektedir. Deniz suyu sıcaklığında meydana gelen bu artış, özellikle son yıllarda ağ kafeslerde balık üretim miktarı artan Akdeniz'de yaz aylarında olumsuzluklara neden olmaktadır. Bu sıcaklık artışının devam etmesi Ege Denizi'nde de yaşanacak olumsuzlukları artıracaktır. Balıkların yem almaması, büyümenin azalması ve hatta durması, yem dönüşüm oranlarının artması, üretim sürecinin uzaması, suyun oksijen tutma miktarının azalması, zararlı alg çoğalmaları, balık hastalıklarının artması ve balıkların hastalıklara karşı direncinin azalması, yaşanan olumsuzlukların bazılarıdır. Toprak havuzlarda yapılan üretimde de atmosferik sıcaklığın artmasına bağlı olarak meydana gelen su sıcaklığı değişimlerinden kaynaklı sorunlar yaşanmaktadır. İklim değişikliğinin sonucu olarak meydana gelen doğa olayları da üretim sistemlerine zarar verebilmekte ve üretim süreçlerini olumsuz etkilemektedir. Bunun yanı sıra balıkların beslenmesinde kullanılan yem ham maddelerinin sürdürülebilir temini ile ilgili de riskler bulunmaktadır. İklim değişikliğinin olumsuz etkilerinin önceden tahmin edilmesi ve neden olabileceği zararların önlenmesi veya azaltılması için uygun önlemlerin alınması, sektörün sürdürülebilir gelişimi açısından önem arz etmektedir.

Anahtar Kelimeler: Su ürünleri yetiştiriciliği, Balık üretimi, Küresel ısınma, Ağ kafes balıkçılığı, Toprak havuz balıkçılığı, Deniz suyu sıcaklığı

ORAL PRESENTATION

Evaluation of the secretome effects of mesenchymal stem cells cultured in 3D cadherin-based hydrogel on endothelial cells

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Abstract

The use of mesenchymal stem cells(MSCs) in clinical applications is increasing due to their regenerative properties. It is known that cadherin-based biomaterials enhance the adhesion of MSCs, improve their paracrine effects, induce proliferation, increase anti-apoptotic effects, and reduce fibrosis. N-cadherin biomimetic hydrogel may regulate the effects of MSCs on endothelial cells by altering the secretion of specific growth factors, but this effect has not yet been clearly defined. The aim of this study is to investigate the effects of conditioned medium obtained from a 3D N-cadherin mimetic environment on the viability and migration of endothelial cells. The observed effects are also compared with 2D classical culture and 3D spheroid culture. In this study, the purity and characterization of the synthesized peptides were demonstrated by HPLC and Mass Spectrometry analyses. The experimental groups were designed as 2D classical culture, 3D spheroid culture, 3D N-cadherin mimetic hydrogel (at a concentration of 1 mM), and 3D hydrogel containing scram sequence peptides. To evaluate paracrine effects, the impact of conditioned media obtained from 48-hour culture on the proliferation and migration of Human Umbilical Vein Endothelial Cells (HUVECs) was investigated using MTT viability analysis, calcein staining, and wound healing methods. We reported that MSCs cultured in N-cadherin hydrogel secreted more PDGF-BB, FGF-2, and VEGF-A growth factors compared to the 2D classical culture media, as demonstrated by the ELISA method. Conditioned media obtained from the N-cadherin hydrogel were demonsrated to increase the viability of endothelial cells at 1:2 and 1:5 dilutions and to have a higher migration potential compared to other groups. N-cadherin biomimetic peptide hydrogel may be considered a more effective culturing strategy than the 2D classical culture to enhance the paracrine effects of MSCs and their effects on endothelial cells.

Keywords: Mesenchymal stem cells, Regenerative medicine, N-Cadherin, Peptide hydrogels, biomaterial

ORAL PRESENTATION

Effects of UC-MSCs derived exosomes on apoptosis and cell cycle mechanisms of pancreatic ductal adenocarcinoma cells

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Abstract

Mesenchymal stem cells(MSCs) secrete exosomes that influence important mechanisms of cancer cells, such as proliferation, apoptosis, metastasis, angiogenesis, chemoresistance, and epithelial-mesenchymal transition. The effects of MSC derived exosomes on tumor cells and their potential for modification and use in cancer therapy are being investigated. In this study, the aim was to clarify the effects of Umbilical Cord(UC) derived MSCs on the apoptosis and cell cycle mechanisms of pancreatic adenocarcinoma cells. In this study, after particle size, number, and marker analyses were performed, the UC-MSCs exosomes were commercially obtained and treated to Panc-1 cells at 4000, 8000, and 12000 exosomes per cell for 24 hours. Apoptosis analysis was performed using Annexin V staining, and cell cycle analysis was performed using Propidium Iodide staining, both analyzed by flow cytometry. In the cell cycle analysis, the percentage of cells in the S phase was found to be 31.87% in the non-treated group, 29.98% in the 1:4000 group, 28.4% in the 1:8000 group, and 27.68% in the 1:12000 group. In the apoptosis analysis, the percentage of total apoptotic cells was 12.07% in the non-treated group, 13.04% in the 1:4000 group, 13.24% in the 1:8000 group, and 13.15% in the 1:12000 group. Our preliminary findings suggest that UC-MSC derived exosomes do not induce proliferation of Panc-1 cells. We also demonstrated that they do not significantly affect apoptosis of Panc-1 cells. Further explanation of the effects of UC-MSC derived exosomes on other antitumor molecular mechanisms will be an important step in the development of potential therapeutic approaches.

Keywords: Mesenchymal stem cells, PDAC, exosomes, pancreatic cancer, cancer therapy

ORAL PRESENTATION

Knowledge, Opinion, and Attitude Towards Apitherapy: A Comparative Study of Healthcare Professionals

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Abstract

Apitherapy is a traditional and complementary medicine approach in which bees and bee products are used to support the treatment of various diseases (1). Upon reviewing the literature, it is evident that there are a limited number of studies evaluating the knowledge, opinions, and attitudes of health professionals about apitherapy practice (2,3). This study aimed to determine the knowledge, opinions, and attitudes of healthcare professionals about apitherapy. The descriptive survey was conducted using an online survey technique on healthcare professionals throughout Türkiye. The study included 703 questionnaires suitable for data quality. Statistical analysis was performed using the SPSS 23.0 package program. To identify the variables that have the most effect on apitherapy knowledge, a binary logistic regression analysis was done using the backward LR elimination method. The statistical significance level is regarded as $p < 0.05$. It was determined that the study population consisted of 304 (43.2%) pharmacists, 123 (17.5%) physicians, 122 (17.4%) nurses, and 154 (21.9%) other healthcare professionals. Of the respondents, 55.9% stated that they knew the concept of apitherapy. The analysis of logistic regression demonstrated that educational attainment ($p = 0.023$), profession ($p = 0.000$), work experience ($p = 0.017$), and the presence of chronic disease ($p = 0.012$) significantly had an effect on apitherapy knowledge. The best-known apitherapeutics were honey (98.4%), propolis (96.4%), and royal jelly (92.7%), while the less well-known were api-air (8.4%), apilarnil (5.1%), and apis totale (podmore) (3.1%). Apitherapy, according to 87.5% of participants, should only be practiced by a physician. 51.6% of healthcare professionals mentioned they wanted to learn more about apitherapy or execute studies concerning the topic in their careers. The study's findings revealed that healthcare professionals have a low level of knowledge about apitherapy. There should be more approaches that improve healthcare professionals' knowledge and awareness of apitherapy practices.

Keywords: apitherapy, bee product, healthcare professionals.

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ORAL PRESENTATION

Yenilenebilir tartarik asit türevi tartarimidlerin poliüretan sentezinde monomer bileşen olarak değerlendirilmesi

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Özet

Petrol başta olmak üzere fosil kaynaklarının giderek azalması ve artan sera gazı emisyonları, ana bileşenleri petrokimya ürünlerine bağlı polimerlerin eldesinde alternatif kaynakların kullanımının araştırılmasını zorunlu hale getirmektedir. Poliüretanlar (PU) dünya çapında kullanımda olan en önemli ticari polimerler arasındadır. PU bazlı malzemeler, yapı kimyasalları, taşımacılık ve mobilya sektörlerindeki kullanımları başta olmak üzere emtia plastiklerinin imalatında, ileri uygulamalarda iyon seçici elektrot membranların, membran sensörlerinin ve biyomedikal cihazların üretiminde geniş bir uygulama alanına sahiptir. PU polimerler temel olarak izosiyanat ve alkollerin polimerizasyonu ile elde edilmektedir. Sürdürülebilirliğin artırılması açısından bu polimerlerin eldesinde yenilenebilir alkollerin monomer olarak kullanılması önemli bir husus olarak karşımıza çıkmaktadır. Bu projede doğal kaynaklardan elde edilebilen ve yenilenebilir bir organik bileşen olan tartarik asitten yola çıkılarak elde edilecek diol yapıdaki tartarimid monomerlerinin poliüretan sentezinde kullanımının araştırılması amaçlanmaktadır.

Anahtar Kelimeler: Poliüretanlar, yenilenebilir ürünler, sürdürülebilir üretim, doğal kaynaklı monomerler

ORAL PRESENTATION

Antioxidant Effects of Oak (*Quercus* sp.) Acorn and Leaf Extracts on Shelf Life of Rainbow Trout (*Oncorhynchus mykiss*) Fillets

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Abstract

The preservation of fish products is crucial for ensuring food safety and quality, particularly with rising concerns over synthetic preservatives and their residues. This study explores the potential use of oak (*Quercus* sp.) acorn and leaf extracts as natural antioxidants to extend the shelf life and maintain the nutritional quality of rainbow trout (*Oncorhynchus mykiss*) fillets. Oak extracts were prepared with different solvents (water, ethanol, and methanol) and applied to five treatment groups along with one control group. The treated fillets were stored under refrigeration, and malondialdehyde (MDA) analyses were conducted on days 0, 5, 10, and 15 to assess lipid oxidation. Results indicate that the application of oak acorn and leaf extracts significantly reduces MDA levels, indicating a decrease in lipid oxidation and preservation of fish quality. The results demonstrate that the application of oak acorn and leaf extracts significantly reduced MDA levels, suggesting a decrease of lipid oxidation and an improvement in the preservation of fish quality. Notably, the most effective results were obtained from oak acorn extracts (both ethanol and methanol), followed by leaf extracts (both ethanol and methanol) over the 15-day period. Conversely, leaf water extracts performed well in the early days (0-5 days), but MDA values increased after 5 days. These findings underscore the potential of oak extracts as natural antioxidants for enhancing the shelf life of fish products. Given the potential health and environmental risks associated with synthetic antioxidants, the use of natural alternatives like oak extracts offers a safer and more sustainable approach. Further research is recommended to explore the efficacy of oak extracts at different concentrations and their potential applicability in the food industry.

Keywords: Oak acorn extracts, Oak leaf extracts, Food preservation, Rainbow trout, Natural antioxidants

ORAL PRESENTATION

Synthesis, Spectroscopic Characterization and ADME studies of Novel *N*-Acylhydrazones of 2-methoxy-5-nitrophenol

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Abstract

Hydrazones, used as intermediates in the synthesis of bioactive heterocyclic compounds, have attracted considerable interest among researchers due to their importance in various biological and clinical applications. The activity of hydrazones is related to the active pharmacophore group (-NH-N=C-). In medicinal chemistry studies, hydrazone derivatives have been extensively investigated due to their prominent functional group with respect to activity. Compounds of this structure have shown anticonvulsant, antidepressant, antimalarial, antiviral, vasodilatory, antimicrobial, and antitumor effects. These encouraging findings have maintained the significance of hydrazone synthesis. In recent years, anticancer screening of new hydrazone-containing compounds has gained importance. Based on this information, a series of new hydrazide-hydrazones were synthesized from 2-methoxy-5-nitrophenol (Figure 1). The purity of these synthesized compounds was confirmed through elemental analysis, while their structures were elucidated using IR, ^{1}H-NMR and ^{13}C-NMR analyses. Today, chemoinformatic softwares give us important information about the molecular structure of the synthesized compounds, their possible effects on metabolism and their drug-likeliness properties without conducting experimental studies. We utilized SwissADME for in silico ADME studies, which calculated the physicochemical, pharmacokinetic, lipophilicity, and water solubility properties of our compounds. Today, chemoinformatic softwares give us important information about the molecular structure of the synthesized compounds, their possible effects on metabolism and their drug-likeliness properties without conducting experimental studies. Additionally, the compounds are being evaluated for anticancer activity against various cancer cell lines using the MTT assay.

Keywords: Hydrazone, Phenol, Characterization, ADME

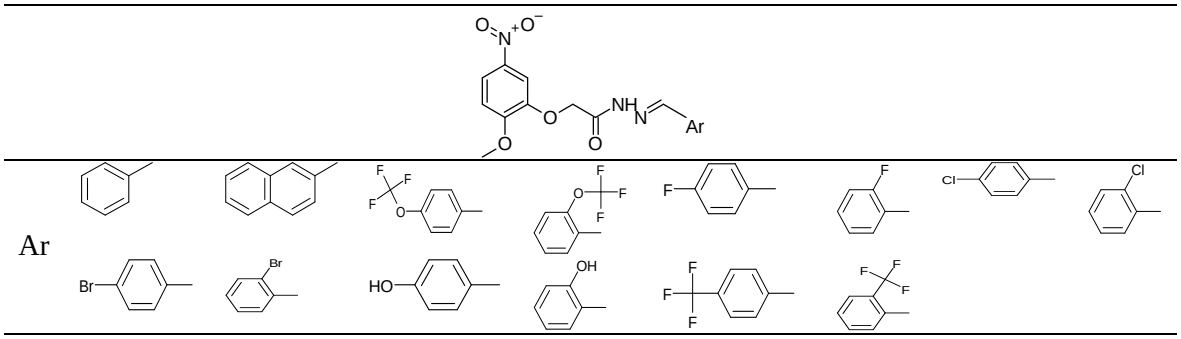

Figure 1. Chemical structures of the synthesized compounds

Acknowledgment: This work has been supported by Marmara University Scientific Research Projects Coordination Unit under grant number TYL-2024-11224.

ORAL PRESENTATION

Fluorescence, singlet oxygen generation, and photodegradation studies of pyrazoline substituted Zn-phthalocyanine-based photosensitizers

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Abstract

In recent years, cancer has become a worldwide problem that has put pressure on the healthcare system. Photodynamic therapy (PDT) is one of the most promising approaches for treating cancer. Phthalocyanines have been widely used as photosensitizers for therapeutic applications, particularly in photodynamic therapy (PDT). This work aims to investigate the photophysicochemical characteristics of peripherally tetra substituted and non-peripherally tetra substituted Zn-phthalocyanines that have been recently synthesized and described using pyrazoline substitution. The impact of substituting 3-(5-(4-(dimethylamino)phenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole-3-yl)phenol from the peripheral and non-peripheral positions on solubility and aggregation behaviors was examined. The potential use of the pyrazoline substituted Zn-phthalocyanines as a photosensitizers in photodynamic therapy was investigated by examining their the fluorescence, singlet oxygen generation, and photodegradation studies. Non-peripheral pyrazoline substituted Zn-phthalocyanine (HY-ZnPc^{np}) may be a potential photosensitizer for PDT, owing to an examination of the results [1].

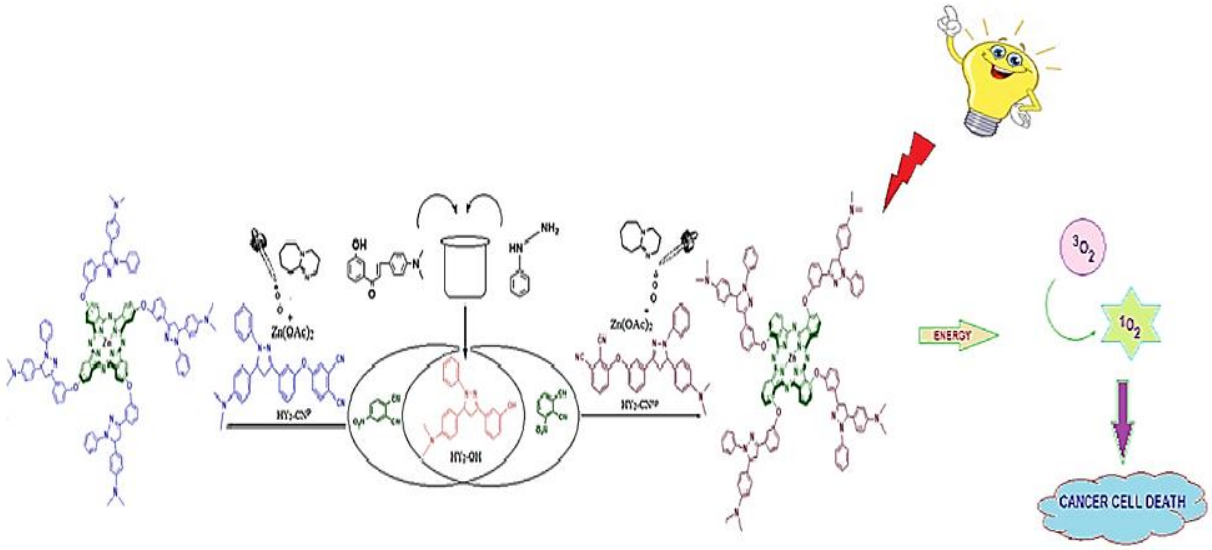

Figure 1. The pyrazoline substituted Zn-phthalocyanine-based photosensitizers

Keywords: Fluorescence, Phthalocyanine, Photodynamic therapy, Pyrazoline, Singlet oxygen

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

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ORAL PRESENTATION

Relationship between estrogen with progesterone and ovarian irisin levels during the sexual cycle in domestic cats: A correlation analysis

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Abstract

This study aimed to determine the relationships between estrogen, progesterone levels, and ovarian irisin levels in cats during the sexual cycle. For this purpose, 30 female cats were used in sexual period. After blood and ovarian samples were taken from the animals, estrogen and progesterone levels were measured in the blood samples, and irisin levels were measured in the tissues. The relationship between ovarian irisin levels (31.292±18.325) ng/ml and estrogen (31.793±11.871) pg/ml was measured by Pearson correlation (r (28) = .188, p= 0.319). Similarly, the relationship between ovarian irisin levels (31.292±18.325) ng/ml and progesterone (5.281±7.790) ng/ml was measured by Spearman's rho (r (28) = .039, p= 0.838). In the light of the data obtained, no significant relationship was found between these variables. As a result, it is thought that fluctuating increases in reproductive hormones during the sexual cycle may cause this result. As a result, further research may uncover the relationships between reproductive hormones and local hormones in cats, potentially benefiting feline reproductive activities.

Keywords: Ovarian irisin, estrogen, progesterone, domestic cats, correlation

ORAL PRESENTATION

Determination of d-SPE clean-up performance of doped graphene for neonicotinoid pesticides

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Abstract

Pesticides are used for protect to agricultural products from some harmful organisms such as insects, fungicides and herbicides. The control of foods before consumption became an obligation, due to the noncontrolled and excessively usage of pesticides to the products. The pesticide residue analysis in foods is challenging matter owing to interference factors of complex matrixes. The naturally pigments such as chlorophyll and carotenoid are one of the interference factors, that decrease the sensitivity and reliability of analysis, to be overcome in green, leafy vegetables. Carbonous materials are preferred for removal of chlorophylls and carotenoids from extract. In this study, d-SPE clean-up step of QuEChERS method was modified by using iron-nitrogen doped graphene (Fe-N-GN). Arugula was preferred as a matrix that was rich about chlorophyll and carotenoids. Acetamiprid, imidacloprid and thiamethoxam were studied as representative neonicotinoid pesticides. In the scope of validation linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability and reproducibility parameters were studied via LC-MS/MS. As a result of validation study the mean recoveries of acetamiprid, imidacloprid and thiamethoxam were calculated 97,77%, 98,02% and 90,92%, respectively. The LOQ and relative standard deviations (RSD) for repeatability and reproducibility of all three pesticides were calculated <0.01 mg kg^{-1} and <20%, respectively.

Keywords: graphene, QuEChERS, pesticide, LC-MS/MS

ORAL PRESENTATION

Metformin as a Neuroprotective Agent: Inhibition of GSK3β and Attenuation of LPS-Induced Oxidative Stress in SH-SY5Y Cells

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Abstract

Neuroinflammation and oxidative stress play a crucial role in the progression of neurodegenerative disorders that are commonly studied and often modeled using lipopolysaccharide (LPS). This work aims to investigate the neuroprotective effects of metformin in the SH-SY5Y neuroblastoma cell line when exposed to LPS. The experimental design comprised untreated control cells, cells treated with LPS (1 µg/mL), cells treated with metformin, and cells co-treated with both LPS and metformin. The ideal concentration of metformin (5 mM) was established based on initial cell viability tests. A variety of oxidative stress indicators were evaluated, encompassing reactive oxygen species (ROS), protein oxidation, lipid peroxidation, glutathione peroxidase (GPx) activity, glutathione (GSH) levels, and the overall total antioxidant capacity. The findings of this study indicate that the administration of LPS resulted in notable oxidative stress, marked by elevated levels of ROS generation, intensified oxidation of proteins and lipids, and decreased activity of GPx and GSH. Metformin administration, alone or in conjunction with LPS, successfully alleviated these oxidative effects. Metformin specifically decreased ROS levels and significantly enhanced the overall antioxidant capacity, contributing to a more balanced redox state within the cells. Moreover, we analyzed the function of glycogen synthase kinase 3 beta (GSK3β) in regulating the inflammatory reaction. Exposure to LPS resulted in a significant rise in GSK3β activity, linked to increased levels of pro-inflammatory signaling molecules, including IL-1β, IL-6, and TNF-α. Efficacious inhibition of GSK3β activity by metformin led to a substantial decrease in these pro-inflammatory markers. These results indicate that metformin reduces oxidative stress and blocks important inflammatory pathways, therefore reducing neuroinflammation and providing possible therapeutic advantages for neurodegenerative disorders.

Keywords: Metformin, LPS, SH-SY5Y, GSK3, Oxidative stress, Interleukin

ORAL PRESENTATION

Cell Culture Techniques in Veterinary Medicine: New Horizons in Treatment and Research

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Abstract

Cell culture techniques in veterinary medicine are crucial for understanding diseases at the molecular level, developing new drugs, and researching innovative treatment methods. Cell culture involves the growth and proliferation of cells isolated from animal tissues in a laboratory environment. This technique provides veterinary researchers with a powerful tool to better understand disease mechanisms and develop new therapeutic strategies. The use of cell culture has become widespread in studies on cancers, infectious diseases, and genetic disorders commonly encountered in pets such as dogs and cats. In cancer research, culturing cancer cell lines derived from animals in the laboratory allows for the testing of new chemotherapy drugs and a deeper understanding of tumor biology. For example, cell culture is being used to investigate how individual tumors, such as the commonly seen mammary tumors in dogs, respond to treatment. This opens the door to personalized treatment approaches in veterinary medicine. Cell culture techniques also play an essential role in studying infectious diseases such as viral infections. In vaccine development for veterinary medicine, growing viruses and bacteria in cell cultures contributes to the formulation of effective vaccines. Additionally, the use of stem cell cultures is gaining increasing interest for tissue engineering and regenerative medicine applications in animals. This presentation will explore the latest applications of cell culture techniques in veterinary medicine and how these methods can revolutionize the diagnosis and treatment of diseases. Cell culture stands as a critical component in veterinary research and clinical practice, shedding light on future treatment strategies.

Keywords: veterinary medicine, cell culture, domestic animals

ORAL PRESENTATION

Current Genetic Diagnostic Tests in Veterinary Medicine: Detection and Management of Hereditary Diseases

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Abstract

Genetic diagnostic tests in veterinary medicine are becoming increasingly important tools for the early diagnosis and management of hereditary diseases. Recent advancements in genome sequencing technologies have enabled a better understanding of the genetic foundations of common hereditary diseases in pets, particularly in dogs and cats. Specific genetic mutations have been identified as the root cause of many diseases in these animals. These tests allow for the early detection of genetic disease carriers, the regulation of breeding programs, and the development of appropriate treatment strategies. Current genetic tests offer innovative methods for confirming diseases at the molecular level. Techniques such as single nucleotide polymorphism (SNP) analysis and whole-genome sequencing provide valuable insights into the diagnosis of genetic diseases and the overall genetic health of animals. For example, the genetic basis of conditions such as degenerative myelopathy, retinal degeneration, hypothyroidism, and certain heart diseases in dogs can be easily identified using these tests. One significant advantage of genetic testing is that it not only helps veterinarians treat existing diseases but also offers the potential to prevent such diseases in future generations. Genetic diagnostic tests aid in breeding programs by selecting individuals that do not carry hereditary diseases, thus contributing to the emergence of genetically healthier offspring. This presentation will explore the applications of current genetic diagnostic tests in veterinary medicine and how they can revolutionize the management of hereditary diseases. Genetic tests hold great potential for animal health and pave the way for more personalized approaches in veterinary diagnostics and treatment.

Keywords: veterinary medicine, genetic, treatment

ORAL PRESENTATION

CYP19 gene polymorphism in some native Turkish cattle breeds

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Abstract

In the present study, PvuII polymorphism in the promoter region CYP19 (cytochrome P450 aromatase) gene was investigated in some native Turkish cattle breeds. The study was carried out using PCR-RFLP in 151 animals including Eastern Anatolian Red, Turkish Grey and Turkish Holstein breeds. While 3 genotypes (AA, AG, GG) were determined in Turkish Grey, two genotypes (AA and AG) were determined in all other breeds. The A allele frequency (0.8229-0.9419) was detected at a considerably higher frequency than the G allele when all breeds were considered. The highest A allele frequency was determined to be 0.9419 in the Eastern Anatolian Red breed. All populations were found to be in Hardy-Weinberg equilibrium for this locus.

Keywords: CYP19 gene, polymorphism, cattle, PCR-RFLP

ORAL PRESENTATION

Immobilization of laccase onto $NiFe_2O_4$@poly(GMA) microparticles: application for dye removal

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Abstract

Enzymes are biological catalysts that accelerate chemical reactions by lowering the activation energy. They have a variety of uses in industries such as chemistry, pharmaceuticals, food, cosmetics, paper and detergents. Their activities, purities, thermal, storage and operational stabilities must be as high as possible, therefore, techniques called "immobilization" have been developed to ensure the reuse of these biomolecules which can be achieved by one or more of the methods of adsorption, entrapment, cross-linking, encapsulation and covalent binding [1,2]. Laccase is one of the extensively researched enzymes to be used in the immobilized form for its promising uses such as lignin degradation, pulp bleaching, dye decolorization, toxic substance degradation, biosensors, and biofuel cells [3]. In this study, laccase immobilization onto $NiFe_2O_4$@poly(GMA) microparticles was achieved. $NiFe_2O_4$ nanoparticles were synthesized by co-precipitation and loaded into poly(GMA) microparticles. Microparticles were characterized and visualized by FTIR, SEM and EDX. SEM images revealed the spherical particles with average size of 1µm. $NiFe_2O_4$@poly(GMA) microparticles were then modified with 3-mercaptopropionic acid to be functionalized with carboxylic acid moiety and laccase enzyme was covalently immobilized onto microparticles through EDC/NHS. The amount of immobilized laccase was calculated to be 173 µg/mg. The activities of free and immobilized laccase were measured. Optimum pH was determined as 3.0 and optimum temperature was 65°C. Laccase immobilized microparticles were investigated for Congo Red (CR) and Reactive Green 5 (RG5) dye removal from aqueous solutions. Decolorization efficiencies of 91% and 84% were obtained for CR and RG5 (initial concentration of 0.01 mg/mL, 25°C, pH 3.0), respectively. It was observed that immobilized laccase was stable up to five re-uses. The results of this study are expected to offer novel, efficient, low-cost and reusable treatment for dyestuff wastewater.

Keywords: $NiFe_2O_4$, glycidyl methacrylate, laccase, immobilization, dye removal, microparticle.

Acknowledgments: This study covers a part of Ezgi DEREBAŞI's master's thesis. The authors are thankful to Aydın Adnan Menderes University Graduate School of Natural and Applied Sciences for their contributions.

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ORAL PRESENTATION

***CLPB* is overexpressed in breast cancer and its expression correlated with unfavorable survival values**

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Abstract

CLPB gene, also known as *SKD3*, encodes a mitochondrial chaperone protein. This protein is important for the protection of mitochondrial cristae structure and it is overexpressed in acute myeloid leukemia (AML) cases. CLPB's contribution to drug resistance and anti-apoptotic properties have been demonstrated in leukemia cells in vitro and in vivo. Breast cancer is the most common type of cancer in women. Chemotherapy resistance is a common problem in breast cancer treatment and it is important to identify new target genes for adjuvant treatments that reduce this resistance. By employing Bc-GenExMiner and UALCAN in silico tools that analyze DNA microarray and RNA-seq datas, we investigated *CLPB* expression and its effect on breast cancer patient survival. The expression level of *CLPB* gene is significantly higher in breast cancer patients (n=1097) than in normals (n=114) (P<1e-12). Similarly, all breast cancer subtypes express *CLPB* gene significantly higher compared to the normal samples. High expression of *CLPB* negatively affects the overall survival (OS), disease-free survival (DFS) and distant-metastasis-free survival (DMFS) values of breast cancer patients. According to the results of our anaylsis by employing the UALCAN in-silico tool, the promoter region of the *CLPB* gene is hypomethylated in both normal and tumor samples. However, the methylation level is significantly lower in HER2+ and triple negative subtypes than that of normal samples (P<0.05). Based on our Q-RT-PCR results, *CLPB* gene is differentially expressed in breast cancer cell lines representing different subtypes. By examining the promoter methylation levels in breast cancer cell lines, we continue to investigate the effect of promoter methylation on *CLPB* expression. According to our results, *CLPB* gene is overexpressed in breast cancer samples and negatively affects patient survival. Promoter hypomethylation could be one of the factors regulating the *CLPB* expression. We continue to investigate the role of *CLPB* gene in breast carcinogenesis and its effect on chemotherapy-induced cell death by reducing *CLPB* expression in breast cancer cell lines with siRNAs.

Keywords: *CLPB*, breast cancer, survival, methylation, expression

ORAL PRESENTATION

Evaluation of Hydrogen Peroxide-Induced Oxidative Stress in SH-SY5Y and HMC-3 Cells by MTT and MTS assay

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Abstract

MTT and MTS tests are the most widely used tests for measuring cell viability in molecular and cellular biology. The principles of both tests show some similarities. Tetrazolium salts are reduced to formazan depending on the mitochondrial NADH activity of the cells. The MTT test produces insoluble formazan, whereas the MTS test produces water-soluble formazan, and the absorbance values are measured at wavelengths of 570 nm and 490 nm. In addition, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is used in the MTT test and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) is used in the MTS test. Here we investigated the viability of the neuroblastoma cell line SH-SY5Y and glial HMC-3 cells under 0.1 M H_2O_2 condition for 0, 1, 2 and 4 hours using both tests. Both cell lines were sensitive to oxidative stress. Our results showed that there was no significant change between MTT and MTS results in SH-SY5Y and HMC-3 cell lines. HMC-3 cells were found to be more sensitive to H_2O_2-induced oxidative stress than SH-SY5Y cells. It was observed that the death rate of HMC-3 cells was higher than that of SH-SY5Ycells depending on the time of exposure. It was observed viability of both cell lines decreased with increasing time compared to the control ($p<0.05$). Our results of SH-SY5Y and HMC-3 neuronal cell lines induced with H_2O_2 showed that there was no significant change between MTT and MTS results ($p>0.05$), and HMC-3 cells were more sensitive compared to SH-SY5Ycells. In this study, the test duration is a limiting factor.

Keywords: Cytotoxicity, microglia, oxidative stress

ORAL PRESENTATION

The New Method Development for The Determination of Favipiravir in Saliva By HPLC

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Abstract

Viral infections affect the patient's life both acutely and chronically, and in acute cases, the patient's quality of life is often permanently reduced. Favipiravir (FA) was first approved against RNA viruses in Japan and was also used in the Sars-CoV-19 pandemic. FA targets RNA polymerase enzymes as a mechanism. In addition to influenza, it is also used to treat diseases caused by other RNA viruses such as Ebola, Arenavirus, Bunyavirus, Filovirus, West Nile Virus and Yellow Fever. Considering the ease of obtaining it from the patient, using saliva instead of plasma or serum to monitor changes in active substance concentrations may be a new and easy option for drug level monitoring. Favipiravir dosing regimen varies in different viral infections. Doses administered to achieve drug efficacy can be at very high concentrations. The aim of the study is finding a new, easy method that allow the separation and determination of FA in artificial saliva. Artificial saliva was used to prepare solutions of FA at the desired concentration values. FA extractions were performed by liquid-liquid extraction procedure. FA separation was performed by XBridge, C_{18} (100 x 4.6 mm, particle size 5 µm) analytical column and acetonitrile: phosphate buffer (50:50, v/v, pH=3.5, 20 mM) containing mobile phase at 1.2 mL/min. Detection of FA was performed by DAD detector at 320 nm. The calibration curves were linear over the concentration ranges of 0.5-3.0 µg/mL. The recovery value was found 97.508 % (n=6) for 2.0 µg/mL. This method gives easy separation techniques without any interference peaks and good resolution. Consequently, this study describes a simple, sensitive, and practical HPLC-DAD method which permits determination of FA in saliva.

Keywords: Favipiravir, HPLC-DAD, Artificial Saliva

ORAL PRESENTATION

The Ameliorative Role of Berberine and Resveratrol Against the Cardiotoxic Oxidative Damage of Imidacloprid

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Abstract

Pesticides are chemical substances used to reduce the destructive effects of life forms such as insects, rodents that live on or around the human and animal body. Imidacloprid (IMI), a neonicotinoid insecticide, is one of the most widely used insecticides. Berberine (BBR), has a wide range of biological activities, including antioxidant properties. Resveratrol is a natural antioxidant substance with polyphenol structure. In this study, we investigated the protective effects of berberine and resveratrol on the toxicity caused by imidacloprid in rat heart tissue. This experimental study was carried out with the approval of the Local Ethics Committee. In the study, 42 rats were divided into 7 groups like, control group, Resveratrol (RES), Berberine (BBR), Imidacloprid (IMI), Imidacloprid and Resveratrol, Imidacloprid and Berberine, Imidacloprid+Resveratrol and Berberine treated groups. All groups were administered by oral gavage once daily for 28 days. At the end of the experimental period, antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx)] and malondialdehyde (MDA) levels were evaluated in heart tissues from rats. After the fourth week, malondialdehyde (MDA) levels and antioxidant enzyme activities (SOD, CAT, GPx and GST) in rat heart tissues were investigated spectrophotometrically by various methods. No significant difference was observed between control and other treated groups. After treatment, imidacloprid increased MDA levels, while decreased SOD, CAT, GPx and GST enzyme activities were observed compared to control rats. In the study, it was observed that imidacloprid caused oxidative stress in rat heart tissue, and berberine and resveratrol reduced imidacloprid-induced toxicity.

Keywords: Pesticide, Heart, Berberine, Resveratrol, Toxicity

ORAL PRESENTATION

Synthesis of Steroid-Derived Drug Active Ingredients for the Treatment of Alzheimer's Disease

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Abstract

Alzheimer's disease is a serious neurodegenerative disorder, known as the most common form of dementia, with no current curative treatment and resulting in mortality in its advanced stages. With the global increase in average life expectancy, a significant rise in the prevalence of this disease is anticipated, posing a critical global health issue (Sang et al., 2019). This study focuses on the synthesis of steroid-derived drug active ingredients potentially usable for the treatment of Alzheimer's disease and their inhibitory effects on the enzyme Acetylcholinesterase (AChE). Steroids are known as biomolecules that play a key role in various biochemical processes in human physiology, as well as in many other organisms (Yang et al., 2020). Due to their low toxicity profile and minimal side effects, these compounds are considered an attractive option as therapeutic agents (Zhang et al., 2019). In this study, a series of pharmacological derivatives have been developed, based primarily on Dehydroepiandrosterone (DHEA), a steroid known for its neuroprotective properties. The synthesis processes of these derivatives have been discussed in detail, and comprehensive evaluations have been made regarding their biological activities. The findings indicate that steroid-derived drugs could potentially offer various benefits in the treatment of Alzheimer's disease, including improving patients' quality of life, preserving cognitive functions, and inhibiting specific enzymes.

Keywords: Alzheimer's disease, Acetylcholinesterase inhibition, Dehydroepiandrosterone, Steroid.

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ORAL PRESENTATION

The assessment of potential toxic effects of the novel fungicide penthiopyrad

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Abstract

Penthiopyrad, a chiral succinate dehydrogenase inhibitor (SDHI), is known as a novel fungicide. SDHI fungicides inhibit fungal respiration by explicitly blocking the ubiquinone binding sites in mitochondrial complex II. Due to this unique molecular mechanism of action, they exhibit high activity against diseases that are resistant to major fungicides on the market. Therefore, penthiopyrad has noteworthy activity against various plant diseases including rust, gray mold, apple scrab, wheat disease etc. Although reports indicate that penthiopyrad may result to liver, kidney toxicity and reproduction/developmental effects for humans, the toxic effects of penthiopyrad have not yet been assessed for reproductivity. Therefore, the present study focused on evaluating the potential toxicity of the fungicide penthiopyrad on the male reproductive system, given the high occupational exposure. For this purpose, the Leydig cell line (TM3) was used to assess potential toxicity of fungicide penthiopyrad *in vitro* conditions. Firstly, cytotoxicity was determined and then ROS production, apoptosis and DNA damage was investigated. The cytotoxic activity of penthiopyrad was determined by the MTT assay for 24 h and the IC_{50} was calculated as 58 µM in TM3 cells. According to IC_{50} value, the exposure doses were determined as 0-25 µM for the experiments. H_2DCF-DA, a molecular probe, was used to measure ROS production by flow cytometry. Apoptosis was evaluated using the FITC Annexin V Apoptosis Detection Kit with PI, and DNA damage was assessed with Comet assay. While the levels of ROS changed with exposure concentration (**p<0,01, ****p<0,0001), there was no significant difference in apoptosis between the exposure doses. The comet assay results showed a significantly higher average 7-fold increase in tail intensity at exposure doses compared to the negative control (p<0.0001). The data obtained in this study are expected to form the basis for further investigations to understand the mechanisms responsible for the toxicity of penthiopyrad in TM3 cells.

Keywords: penthiopyrad, male reproductivity, cytotoxicity

ORAL PRESENTATION

Spiro-bino-spiro (sbs)-bis(tetraalkilamino)siklotrifosfazenlerin sentezi ve biyoaktivite çalışmaları

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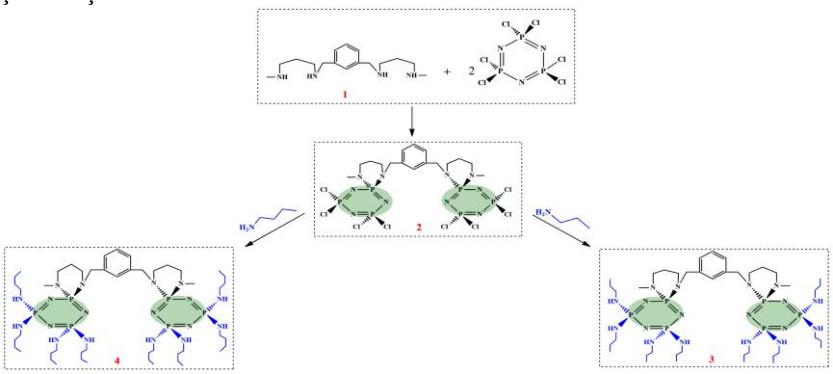
Özet

Klorosiklofosfazenler, P-Cl bağının yüksek derecede reaktifliğe sahip olması sayesinde farklı nükleofiller ile sübstitüsyon reaksiyonları verebilmektedir. Nükleofilik sübstitüsyon reaksiyonları neticesinde elde edilen ürünler, kararlı yapıları, özgün fiziksel, kimyasal özellikleri ve kullanım alanları nedeniyle büyük ilgi görmektedir [1, 2]. Organosiklo-, dendrimerik- ve polimerik fosfazen bileşiklerini sentezlemek üzere başlangıç bileşiği olarak yaygın bir şekilde kullanılan klorosiklotrifosfazenlerden birisi, hekzaklorosiklotrifosfazendir [$N_3P_3Cl_6$ (HCCP), trimer]. Trimerik fosfazen bileşiğinin tetra-fonksiyonel ligantlar ile tepkimeleri sonucunda spiro-bino-spiro (sbs) ve spiro-ansa-spiro (sas) fosfazenler oluşmaktadır [3, 4]. Bu çalışmada, trimerin tetra-fonksiyonel bir ligant olan benziltetraamin ile reaksiyonları incelenmiştir. Çalışmaların ilk aşamasında, ligant olarak kullanılmak üzere benziltetraamin bileşiği (1) sentezlenmiştir. Ardından bu tetraamin bileşiğinin, trimerik fosfazen ile regioselektif olarak etkileşmesinden (spiro-bino-spiro)fosfazen (2) ve oluşan bu bileşiğin n-propilamin ve n-butilamin ile tepkimelerinden spiro-bino-spiro (sbs)-bis(tetraalkilamino)siklotrifosfazenler (3, 4) hazırlanmıştır. Bu orijinal bileşiklerin; spektroskopik özellikleri ve biyolojik aktiviteleri araştırılmıştır. Yapısal karakterizasyon çalışmaları, tek boyutlu NMR teknikleri kullanılarak gerçekleştirilmiştir. Biyolojik aktivite çalışmaları kapsamında, bileşiklerin antibakteriyel ve antifungal aktiviteleri standart antibiyotiklerin aktiviteleri ile karşılaştırmalı olarak değerlendirilmiştir. Bununla birlikte, bileşiklerin pBR322 plazmid DNA ile etkileşimleri araştırılmıştır.



Anahtar kelimeler: Trimer, (spiro-bino-spiro)fosfazen, spektroskopi, biyolojik aktivite.

Bu çalışma "19B0430005" numaralı BAP Projesi ile desteklenmiştir.

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ORAL PRESENTATION

Computational Molecular Docking Analysis of Inhibitory Effects of Quercetin and Myricetin on Human Acetylcholinesterase (AChE)

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Abstract

The aim of this study is to analyze the inhibitory effect of phenolic compounds by computational docking studies. For this, flavanol compounds quercetin and myricetin were used as ligands for molecular interaction. The crystallographic structure of the molecular target Human acetylcholinesterase (AChE) was obtained from the PDB database (PDB ID: 4EY6). Human acetylcholinesterase (AChE) is a significant target for therapeutic drugs. By re-docking for the drug galantamine in complex with the Crystal Structure of Recombinant Human Acetylcholinesterase, it was taken as a standard for comparative analysis. Computational docking analysis was performed using the AutoDock program based on scoring functions. Acetylcholinesterase is one of the important enzymes in the central and peripheral nervous systems. Phenolic compounds are potent inhibitors and are very suitable probes for investigating the chemical reaction mechanism of inhibition. Studies have shown us many biological functions of phenolic compounds. (Cong et al., 2017). Understanding the chemistry of the enzyme is important for preventing and for designing new drugs for cholinergic-related diseases such as Alzheimer's disease. Quercetin and myricetin showed optimum binding affinity with a molecular target (Human acetylcholinesterase (AChE)) with binding energy of -8.20 kcal/mol and -8.19 kcal/mol, respectively, compared to the standard (-8.04 kcal/mol). These results indicated that flovob glycosides (quercetin and myricetin) could be one of the potential ligands that can be used in the treatment of Alzheimer's disease.

Keywords: AChE, Alzheimer, Phenolic compounds, Molecular Docking.

ORAL PRESENTATION

Evaluation of protective effects of bromelain against high sugar-induced neuroinflammation in cell culture

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Abstract

Hyperglycemia and hypoglycemia, or fluctuations in blood sugar levels, have been linked to microglial activation in the brain, a phenomenon involving the immune cells of the central nervous system. It has been demonstrated that this activation results in an increased release of pro-inflammatory mediators. This results in the initiation of an inflammatory cascade within the brain. Bromelain is a mixture of protein-digesting enzymes that occur naturally in the stem, fruit, and juice of pineapple. The anti-inflammatory effects of bromelain have been demonstrated in numerous studies. The objective of this study was to investigate whether bromelain is effective in reducing hyperglycaemia-induced neuroinflammation in the SH-SY5Y neuroblastoma cell line. Glucose monophosphate was employed to simulate the effects of hyperglycaemia in cells. In order to ascertain the optimal doses for the subsequent study, an MTT test was conducted on both the glucose monophosphate and the bromelain. The IC_{50} dose for glucose monophosphate (350 mM) and the highest dose of bromelain that did not induce cytotoxicity or cell proliferation (75 µg/ml) were selected. Subsequently, the levels of C-reactive protein (CRP) and interleukin-18 (IL-18) were quantified in the study groups. Furthermore, histological examinations of the study groups were conducted using a light microscope. It was observed that administration of a high level of sugar resulted in a significant increase in both CRP levels (18.65%) and IL-18 levels (57.4%). The administration of bromelain was observed to result in a statistically significant reduction in both CRP and IL-18 levels (13.34% and 33.96%, respectively) in the high sugar group. The histological analysis demonstrated that exposure to elevated levels of sugar resulted in neurodegeneration, characterised by a reduction in the number of neuronal cells, a shortening of neurite length, and cellular senescence. The administration of bromelain has been demonstrated to mitigate these effects. These findings suggest that bromelain may possess efficacy in reducing hyperglycaemia-induced neuroinflammation. Given that neuroinflammation is a key driver of numerous neurodegenerative disorders, further investigation into the potential therapeutic role of bromelain is warranted.

Keywords: Neuroinflammation, bromelain, SH-SY5Y neuroblastoma cells, hyperglycemia, cytokines

ORAL PRESENTATION

Recombinant Production and Purification of the HECW2 C-lob for NMR-Based Structural Studies

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Abstract

Ubiquitin is a regulatory protein consisting of 76 amino acids, which marks proteins in post-translational modifications to initiate the degradation process. The ubiquitination mechanism, which works with three different enzyme complexes (E1, E2, E3) to activate ubiquitin, is a post-translational modification whose primary purpose is to lead proteins to degradation in 26S proteasomes. E3 enzymes, which play the final role in the ubiquitination process, are divided into two categories based on their structure: RING and HECT. The HECT domain, short for "Homologous to the E6-AP Carboxyl Terminus," is found in ubiquitin ligases and is further subdivided into heterogeneous members, including HERC, NEDD4, and 13 other families. HECW2, a HECT E3 ligase from the NEDD4 family, plays a crucial role in cell division, neurodegenerative developments, and nervous system development, and has been observed to be involved in epilepsy, developmental regression, Hirschsprung's disease, and several other conditions. In this study, the target protein was produced and purified using different fusion tags such as SUMO and GB1. It was determined that the SUMO protein caused the target protein to form misfolded soluble aggregates, while using the GB1 fusion protein, the target protein was found to be highly soluble and correctly folded, as confirmed by NMR spectroscopy.

Keywords: NMR Spectroscopy, BioNMR, HECW2, ubiquitination

ORAL PRESENTATION

Knowledge and Awareness of Pharmacy Students Regarding Personal Protective Equipment and Chemical Substances

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Abstract

Students studying in the field of health are exposed to various risks during laboratory practices. Since there are many chemical substances used in the processes in the laboratories of the pharmacy education, there is a risk caused by chemicals. It is important that students need to learn basic laboratory safety rules before taking laboratory sessions during their studies. They also have to knowledge how to work safely with chemicals. Laboratories are classified as very hazardous/dangerous workplaces among occupational health and safety risk factors. Due to high risks which may cause accidents during pharmacy studies in the laboratories, it is important to evaluate the knowledge of students about health and safety. This study focusses on the knowledge and awareness of students about personal protective equipment and on the processes in laboratory sessions which include chemical substances. The necessary legal permission was obtained for the survey, and it was conducted for all laboratories for the students in different years of their pharmacy studies using a survey which has been applied for health workers and medical laboratory students. A total of 98 pharmacy undergraduate students participated in our study. 69.4% of the participants were female. The mean age of the participating students was 23.05 ± 2.649 years. According to the answers given by the students, the top three personal protective equipment used in the laboratory were gloves with 78.6 percent, shoes with 76.5 percent, and long pants with 65.3 percent. The data obtained showed that the students' level of knowledge about occupational risks was quite high. In addition, the results showed that it is important to organize information and training programs for students at regular intervals. This study makes it possible to design to reduce the dangers that should be conducted in the laboratories through applying preventive and corrective activities specified in relevant regulations worldwide.

Keywords: Pharmacy, students, health and safety, laboratory, occupational health

ORAL PRESENTATION

A Comparative Review of Molecular and Biochemical Methods Used in the Diagnosis of Feline Infectious Peritonitis

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Abstract

Feline Infectious Peritonitis (FIP) is a fatal viral disease caused by feline coronavirus (FCoV) with a high mortality rate. This makes early and accurate diagnosis of the disease crucial for ensuring the effectiveness of treatment and to protect individual animal health. The diagnostic methods for FIP are categorized into two main groups: molecular genetic analyses and biochemical methods. These methods show significant differences in diagnostic performance. Molecular methods have the capacity to directly detect the genetic material of the FCoV virus and provide high specificity and sensitivity in the detection of pathogenic mutations with techniques such as Polymerase Chain Reaction (PCR) and Real Time PCR (RT-PCR). These methods are considered among the most reliable diagnostic tools for the confirmation of FIP. However, despite the possibility of specific diagnosis, these methods have limitations such as high cost and the need for advanced technology. On the other hand, biochemical methods work on parameters that reflect the metabolic and immunologic changes that occur during the progression of the disease. Parameters such as the globulin/albumin ratio, liver and kidney function tests are especially important for evaluating the clinical course of FIP. Increased globulin levels and decreased albumin levels are noteworthy biochemical findings. In addition, the Rivalta test is frequently used to evaluate the accumulated effusion in the “wet” form of FIP. Although biochemical methods are rapid and economical, they are supportive rather than providing a specific diagnosis. Therefore, synergistic use of molecular and biochemical methods is inevitable for optimal diagnostic accuracy and comprehensive evaluation. This review aims to compare the diagnostic performance of molecular and biochemical methods used in diagnosis of FIP and to discuss the role and impact of both methods in clinical practice.

Keywords: biochemical, FIP, methods, molecular

ORAL PRESENTATION

Investigation of phytochemicals as potential therapeutics for Multiple Sclerosis by computational approaches

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Abstract

Multiple sclerosis (MS) is an autoimmune disorder characterized by myelin degeneration in the central nervous system, leading to various neurological symptoms. Currently, there are approximately 23 FDA-approved disease-modifying therapies (DMTs) for MS, including interferon beta-1a, glatiramer acetate, dimethyl fumarate, ocrelizumab, and natalizumab. However, these treatments can cause side effects such as flu-like symptoms, fatigue, and increased infection risk, prompting interest in alternative therapies and phytochemicals that may offer additional benefits with fewer adverse effects. Recent research highlights cannabinoids from Cannabis sativa, particularly cannabidiol (CBD) and tetrahydrocannabinol (THC), for their efficacy in modulating immune responses and reducing neuroinflammation. Additionally, curcumin provides anti-inflammatory and antioxidant effects, while epigallocatechin-3-gallate (EGCG) offers myelin protection and neural regeneration. Resveratrol modulates inflammatory pathways, and black garlic extract shows significant antioxidant effects in MS models. Hypericum perforatum (St. John's wort) also demonstrates potential in reducing immune infiltration in MS. In our in-silico study, we identified 1,780 phytochemicals associated with MS, among which caffeic acid, quercetin, curcumin, apigenin, and ascorbic acid ranked highest for activity, utilizing Dr. Duke's phytochemical database. Together, these phytochemicals represent a promising area for adjunctive MS therapies and warrant further research for their safety and efficacy. We also examined the ChEMBL database for small molecules at Phase 3 clinical trials, applying the Rule of Five, and their target proteins. We identified Laquinimod as a small molecule as potentially associated with MS. Furthermore, we identified 20 proteins related to Laquinimod that may serve as viable drug targets. Ongoing computational research aims to investigate phytochemicals from Dr. Duke phytochemical databases and literature as potential drugs against our candidate protein targets driven from ChEMBL database, enhancing therapeutic strategies against MS.

Keywords: Multiple Sclerosis, Phytochemicals, Therapeutics, Computational Biology, Databases

ORAL PRESENTATION

Investigation of the effects of boric acid on blood pressure in rats

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Abstract

Boric acid, or orthoboric acid, is a compound of boron, oxygen and hydrogen with the formula B(OH)3. Boric acid is used as an additive in pharmaceutical products, supplements, cosmetics, lotions, soaps, mouthwashes and eyewashes. Boric acid is known to have important effects on many biological and physiological stages such as the cardiovascular, endocrine, and immune systems. Wistar-albino male rats (180-200 gr) were used as experimental animals in this study. They were divided into 4 groups as control and three different dose groups (10 mg/kg, 100 mg/kg and 1000 mg/kg). A total of 16 rats were used, with 4 rats (8-10 weeks old) in each group (n=4). Boric acid application groups were given a single dose of 10 mg/kg, 100 mg/kg, and 1000 mg/kg orally daily. Tail blood pressure and pulse data were measured before and 90 minutes after boric acid application for 5 days. The change in pulse rate at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg of boric acid was not statistically significant ($p>0.05$). However, the decrease in blood pressure was only significant on the 5th day at a dose of 1000 mg/kg boric acid compared to the control group ($p<0.05$). In conclusion, although regular oral use of boric acid does not significantly change the pulse rate, it may cause a significant decrease in blood pressure, in a dose-dependent manner.

Keywords: Boric acid, blood pressure, pulse rate

ORAL PRESENTATION

Current Approaches to Carbapenem Resistance and Novel Treatment Strategies

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Abstract

Carbapenems are considered the last line of defense among broad-spectrum beta-lactam antibiotics and play a crucial role in the treatment of severe bacterial infections. However, the rise in carbapenem resistance has become a significant public health concern, threatening the efficacy of these drugs. This paper addresses the fundamental mechanisms of carbapenem resistance and current treatment approaches. The primary causes of carbapenem resistance include the production of bacterial carbapenemases, loss of outer membrane porins, and overexpression of efflux pumps. Carbapenemases produced by pathogens such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* have significantly contributed to the spread of this resistance. This situation has heightened the need for more effective and alternative treatment options in clinical practice. Recent years have seen the development of novel treatment strategies to combat carbapenem resistance. These strategies include new beta-lactam/beta-lactamase inhibitor combinations, compound antibiotics, and alternative classes of antibiotics. Clinical studies on these treatment methods have shown promising results in overcoming resistance and controlling infections. The aim of this paper is to summarize the current status and molecular mechanisms of carbapenem resistance and evaluate the potential of new treatment approaches. Additionally, it provides insights into how these strategies can be integrated into clinical practice and outlines future research directions.

Keywords: Carbapenems, antibiotic resistance, carbapenemases, treatment strategies, beta-lactam/beta-lactamase inhibitor combinations.

ORAL PRESENTATION

Effect of heat and ultrasound on quality parameters of tomato juice

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Abstract

Tomato juice is a valuable source of nutrients, particularly vitamin C and lycopene, which are important for human health due to their antioxidant properties. This study aimed to investigate the effects of ultrasound and thermal treatments on the quality parameters of tomato juice derived from both peeled and unpeeled tomatoes. The quality parameters assessed included vitamin C, lycopene, Brix, acidity, and color, all of which are critical indicators of the nutritional and sensory qualities of tomato juice. Ultrasound was applied at varying frequencies (4, 8, and 12 mHz) for different durations (5, 10, and 15 minutes), while thermal treatment was performed at temperatures of 45, 55, and 65 degrees Celsius for the same time intervals. The results revealed that both treatments led to a decrease in vitamin C and lycopene content, with more pronounced effects observed at higher intensities and temperatures. Vitamin C is particularly sensitive to heat, as it is a water-soluble and unstable compound that can degrade quickly at high temperatures. This degradation is often accelerated during thermal processing, leading to a significant reduction in vitamin C content. Lycopene, on the other hand, is a fat-soluble antioxidant, and while it is relatively more stable than vitamin C, it can still degrade under prolonged exposure to heat and physical agitation, such as ultrasound. The mechanical forces generated by ultrasound can break down cellular structures, potentially leading to the degradation of lycopene. Overall, the observed decrease in both vitamin C and lycopene during heat and ultrasound treatments is consistent with what is generally expected from the processing of plant-based foods, where these sensitive compounds can be lost due to exposure to high temperatures and physical stress. However, no significant changes were detected in Brix, acidity, or color values.

Keywords: tomato, tomato juice, ultrasound, heat, vitamin c, lycopene.

ORAL PRESENTATION

Use of ultrasound on carrot juice production and its effects

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Abstract

Carrot is a biennial herbaceous crop plant in the Apiaceae family, grown for its cone-shaped fleshy root, scientific name Daucus carota. Among the foods consumed, the vegetable with the highest carotene content is carrot. Carrot juice is obtained from the root of the carrot vegetable, which is widely grown in many parts of the world. Carrot juice has a particularly high content of β-carotene, a source of vitamin A, but it is also high in B complex vitamins like folate, and many minerals including calcium, copper, magnesium, potassium, phosphorus, and iron. Ultrasound is one of the nonthermal methods used in the food industry to develop various effective and reliable food processing applications. It can be applied to different food systems (solid,liquid and gas) for different purposes. This study aimed to understand the effect of ultrasound on quality parameters of carrot juice like brix, color, vitamin C, beta carotene and acidity. Ultrasound treatment was applied to carrot juice at 4 mHz, 8 mHz and 12 mHz for 5 minutes, 10 minutes and 15 minutes. The values of quality parameters such as brix, acidity, color, beta carotene and vitamin C were measured in carrot juices that were subjected to ultrasound treatment one by one at different times and at different frequencies. In this application, quality parameters increased and decreased depending on time and ultrasound frequencies. It was observed that, the amount of acidity increased with increasing frequency and increasing time. The amount of vitamin C decreased with increasing ultrasound frequency and time. Beta carotene and brix values increased proportionally with the increase in ultrasound frequencies and time.

Keywords: carrot juice, quality parameters, ultrasound treatment.

ORAL PRESENTATION

Monkeypox Şüpheli Vakada Tüm Genom Dizileme Yöntemi ile Human Herpesvirus 3 Tespiti

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Özet

Maymun çiçeği (Monkeypox/MPX), Poxviridae ailesindeki Orthopoxvirus cinsinin bir üyesi olan maymun çiçeği virüsünün (MPXV) neden olduğu zoonotik bir hastalıktır. Türkiye'de ilk maymun çiçeği virüsü vakası 30 Haziran 2022 tarihinde tespit edilmiştir. Maymun çiçeği, suçiçeği, kızamık gibi diğer döküntülü hastalıklarla benzerlik gösterdiğinden klinik tanıda yanlış teşhis edilebilmektedir. En yaygın yanlış tanı taksonomik olarak Human herpes virüs 3 olarak bilinen varicella zoster virüsünün (VZV) neden olduğu suçiçeği hastalığıdır. Bu çalışmada, laboratuvarımıza MPX şüpheli gelen ancak PCR ve seroloji testleri negatif çıkan örneklerde shotgun genom dizileme yöntemi kullanılarak kesin tanıyı koymak amaçlanmıştır. MPX şüpheli, aynı kişiye ait orofaringeal,lezyon eksüda sürüntüsü ve serum materyallerinden DNA ekstraksiyonu sonrasında iki farklı kit (Bio-Speedy Monkeypox Virus qPCR Kit ve RIVM primerleri) ile MPXV qPCR çalışması yapılmış ve sonuçları negatif çıkmıştır. Ekstraksiyon ürünleri birleştirilerek içerisinde RNA da olacağı düşünülerek sekans öncesi rtPCR (Reverse transcriptase Polimerase Chain Reaction) yapılarak cDNA elde edilmiştir. Ardından Oxford Nanopore Ligation Sequencing Kit (SQK-LSK109) – Native Barcoding Expansion Kit 1-12 (EXP-NBD104) kiti kullanılarak Oxford Nanopore GridION cihazında shotgun dizileme yapılmıştır. Elde edilen FastQ dosyaları çeşitli bioinformatik araçlar (Flye, Blast+, Minimap2, Geneious Prime) kullanılarak analiz edilmiştir. Shotgun dizileme sonucunda elde edilen datalar, MPXV referans genomu ile haritalama işlemine tabii tutulmuş ve bu işlem sonucunda hiçbir okumanın Maymun Çiçeği genomuna ait olmadığı görülmüştür. Örneğin okumaları, virüs veri tabanında tarandığında, 177 adet okumanın Human herpesvirus 3 (Varicella Zoster Virus / VZV) genomuna ait olduğu bulunmuştur. Elde edilen ortalama okuma derinliği 2,1X, referans genom ile sekansların bazlarının ikili benzerlik oranı %89,1 ve referans genomu yatay olarak kapsama oranı %88,1'dir. Klinik tanının ve mevcut laboratuvar tanı metodlarının yetersiz kaldığı durumlarda shotgun dizileme yöntemi bütün dünyada yaygın olarak kullanılmaktadır. Bu çalışmada Maymun çiçeği virüsü klinik tanısı ile laboratuvarımıza gelen ve MPXV qPCR negatif çıkan numunede shotgun dizileme yöntemi kullanılarak Human Herpes 3 virüsü tespit edilmiştir.

Anahtar kelimeler: Maymun çiçeği virüsü, Human Herpes 3 virüsü, Shotgun Genom Dizileme.

ORAL PRESENTATION

Glass Transition Dynamics of Lignin in Tetrahydrofuran - Water

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Abstract

Lignin, one of the main components of lignocellulosic plant cell wall, is the most abundant non-carbohydrate polymer. Lignin removal is key for the deconstruction of biomass during biofuel production. Polar aprotic solvents have become a subject of significant research interest due to their impact on biomass fraction. The driving force behind this research is the limited information about the dynamics of lignin polymers in polar aprotic solvents. Here, we performed all-atom MD simulations for lignin polymers with the same chemical compositions, but different linkage and sub-unit sequences, to understand how tetrahydrofuran (THF) affects their dynamics. Lignin polymers were obtained using four distinct models: two having different syringyl (S) / guaiacyl (G) subunit sequences linked by βO4, and the other two having only G-subunit linked with different linkage sequences of ββ, β5, αO4, βO4 and 4O5. This work demonstrates that the glass transition temperature (T_g), at which lignin polymers cross over from a glassy state to a rubbery/ more viscous state, shifted to a lower temperature (decreased by approx. 60 K) when THF was added as co-solvent. Moreover, in THF : water, the glass transition dynamics is found to depend on both the linkage and sub-unit sequences.

Keywords: Lignin, Glass Transition Temperature, Dynamics, Molecular Dynamics Simulations.

ORAL PRESENTATION

Enalapril quantum dots

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Abstract

Hypertension is one of the most common chronic diseases and is a global public health problem. Hypertension can cause problems such as stroke, heart disease, kidney disease and premature death, and also causes a significant economic burden. Enalapril (EN) is an antihypertensive drug used to treat hypertension, and is an angiotensin converting enzyme inhibitor (ACE) that is poorly soluble and has limited oral bioavailability. Carbon quantum dots (CQDs) are fluorescent nanomaterials with particle sizes smaller than 10 nm that have been discovered in recent years and are known for their biocompatibility, unique optical properties, easy synthesis, low toxicity, and high stability in aqueous solutions. Due to their optical properties, CQDs provide high fluorescence stability and have adjustable excitation and emission wavelengths, making them a significant contribution to fluorescent nanomaterials. Oral administration is a popular route preferred today due to its advantages such as non-invasiveness, high patient compliance and ease of drug administration. However, low solubility and low dissolution rate in gastrointestinal fluids are limiting factors for *in vivo* bioavailability of oral formulations. Therefore, it is important to improve the dissolution of low-solubility drugs as well as their bioavailability. ENCQDs were shown to be successfully prepared by a simple and single-step method. The particle sizes of the prepared ENCQDs were found to be 9.15 ± 0.122 nm, their polydispersity index was 16.7 ± 0.035 and their zeta potential was -10.8 ± 0.012 mV. Cytotoxicity of formulation was evaluated wit MTT assay on H9c2 cardiomyolastic cell line. The formulation affected cell viability in a dose-dependent manner. IC50 was detected to be 3.36% after 24 h exposure. These results indicate that they show less cytotoxicity than solution and are ready for other future purposes such as biological imaging.

Keywords: hypertension, carbon quantum dots, cytotoxicity

ORAL PRESENTATION

The anticytotoxic and antioxidant effects of sorafenib and osage orange extract combination in A549 cell lines

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Abstract

Lung cancer is the leading cause of cancer-related deaths worldwide. Developing new therapeutic strategies that are effective, safe, and specific for lung cancer cells is a priority in cancer research. This study investigates the anticytotoxic and antioxidant effects of a combination of sorafenib and *Maclura pomifera* extract, known as Osage orange, in A549 human lung cancer cell lines. Sorafenib, a multikinase inhibitor, has shown promising results in the treatment of various types of cancer. However, its therapeutic potential remains limited due to side effects and resistance developed by cancer cells. Natural extracts, especially those obtained from plants such as Osage orange, show significant antioxidant properties and may improve the efficacy and reduce the toxicity of conventional chemotherapy drugs. A549 human lung cancer cell lines were treated with various concentrations of sorafenib, Osage orange extract, and their combination. Cell viability was evaluated by MTT assay, while the levels of total oxidant status (TOS) and total antioxidant status (TAS) were measured. The combination of sorafenib and Osage orange extract demonstrated a significant decrease in cell viability compared to sorafenib treatment alone. In addition, the combination treatment significantly reduced total oxidant levels, indicating increased antioxidant activity. The findings of this study demonstrate that the combination of sorafenib and Osage orange extract exhibits potent anticytotoxic and antioxidant effects in A549 human lung cancer cell lines. This combination therapy has the potential to be a more effective and safer alternative in the treatment of lung cancer, but further *in vivo* studies and clinical trials are required.

Keywords: Lung cancer, Sorafenib, *Maclura pomifera*, Anticytotoxic, Antioxidant, A549 cell lines

ORAL PRESENTATION

Application of Sous Vide Technology in Seafood Processing

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Abstract

Sous vide is an advanced thermal processing method that plays a significant role among minimal processing techniques and has gained increasing attention in recent years. It involves cooking vacuum-sealed food in a water bath with controlled circulation at low temperatures, followed by rapid cooling. The key differences between sous vide and traditional methods are the use of heat-resistant, food-grade vacuum bags and precise temperature control. As a type of pasteurization, sous vide ensures uniform heat distribution, enhancing food safety. Combined with cold storage, this method significantly extends the shelf life of food. Food prepared using sous vide can be cooked with or without spices and reheated for serving in a hot water bath or microwave. Seafood, while nutritionally valuable, is highly prone to microbiological, chemical, and physical spoilage due to rapid microbial growth and enzymatic activity. Sous vide technology helps preserve the nutritional value, texture, and sensory qualities of seafood while improving microbiological safety. It also minimizes oxidative spoilage, maintaining product quality. In vacuum-sealed seafood, protein denaturation occurs more slowly, leading to better water retention and a softer texture. Additionally, sous vide extends shelf life, reduces spoilage, and helps minimize food waste. Sous vide technology not only improves the safety and quality of seafood, but it also offers consistency in cooking, making it ideal for both home kitchens and large-scale food production. The precise temperature control reduces the risk of overcooking, ensuring optimal texture and flavor. This technique has been increasingly adopted in the seafood industry due to its ability to maintain freshness and enhance the sensory attributes of various seafood products. This review aims to provide an overview of the use of sous vide technology in the seafood sector, based on national and international articles and theses.

Keywords: Sous vide, Seafood, Minimal processing, Food safety, Shelf life

ORAL PRESENTATION

Morphological, structural and photoluminescence properties of boron nitride film coated on glass substrate using different nitrogen precursor

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Abstract

Boron nitride (BN) is a material that is very similar to graphene in terms of its structure and applications. When produced as a film, they exhibit unique physical and chemical properties. This study aims to form BN films on glass surfaces at low temperatures by hydrothermal synthesis method using different nitrogen precursor. BN films were created using boric acid as the boron source and urea and melamine as the nitrogen source. The effects of different nitrogen precursor on the structural, optical and photoluminescence (PL) properties of the film were examined. Films obtained using different nitrogen sources generally showed similar morphology, while their photoluminescence properties changed. BN formation on the surface was confirmed by XPS, FT-IR and SEM analyses. Boron nitride nanolayers with a diameter of 200-300 nm grew vertically on the glass surface. The different nitrogen sources play an important role in the formation of various defects in the boron nitride lattice. The emergence of weak and deep level emission in the PL spectra for the film obtained by longer acid etching indicates that the boron nitride film deposited under these conditions has a good crystalline structure and contributes positively to its optical properties. This result is also parallel with the AFM histograms. The films obtained on the surface etched for 150 seconds using melamine have fewer defects. BN coating significantly improved the optical properties of the glass surfaces and completely absorbed the UV region while allowing a certain amount of transmittance in the visible region. It is envisaged that the films have potential especially in UV protective coating and various sensor applications.

Keywords: Boron nitride, nitrogen precursor, photoluminescence, coating, hydrothermal method

This study was funded by Bilecik Şeyh Edebali University Scientific Research Projects Unit with the project number of 2023-01.BŞEÜ.03-07.

ORAL PRESENTATION

Investigation of the effect of low pH on miR-191 in bovine milk

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Abstract

Recent evidence indicates microRNAs (miRNAs) in plant- or animal-based foods can enter the human circulation and could have a potential nutritional impact. However, our understanding of how food processing and digestion conditions might impact the stability and function of miRNAs is limited. The miR-191, which has a similar sequence in cows and humans, plays a role in various biological pathways associated with human health issues. Our previous study has shown that miR-191 abundantly exists in consumed cow milk; here, we aimed to investigate the resistance of miR-191 to a low pH environment similar to the acidic conditions of the human stomach. Pasteurized milk products were treated with hydrochloric acid (HCL) until they reached a pH of 2, simulating the acidity of the human stomach. Total RNA was isolated at 1-hour intervals after the milk reached pH 2, and the levels of miR-191 were analyzed using the RT-qPCR method. The results were compared with an untreated milk sample with a pH of 7. The study found that miR-191 could be detected up to the 2nd hour, but by the 3rd hour, there was no detectable amount of bovine miR-191 (bta-miR-191) using the RT-qPCR method. After normalization, there was no statistically significant difference in the ΔCT values of the acid-treated samples after 2 hours ($p>0.05$). These findings suggest that miR-191 may resist the acidic environment of the gastrointestinal system when consumed in milk. However, further extensive studies are needed to understand miRNA degradation in low pH environments better and its potential impact on human health.

Keywords: microRNA, miRNA, bovine milk, miR-191, low pH

ORAL PRESENTATION

A spectroscopic study on the ROS scavenging potential of *Rumex patientia* L. by the Phosphomolybdenum method

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Abstract

Oxygen has two unpaired electrons and tends to form oxygen-centered free radicals known as ROS (hydroxyl radicals, superoxide anion radicals). Antioxidants are compounds that protect the human body from the harmful effects of ROS. Antioxidants can be of natural or synthetic origin. In recent years, the interest of researching the antioxidant activities of plants has increased significantly. One of these plants is *R. patientia* L. It.is a plant of Polygonaceae family and the leaves of this plant are consumed as green vegetables. In this study, ROS scavenging (total antioxidant) potential of *R. patientia* L. were determined by the Phosphomolybdenum method described by Prieto et al. For this method, water and methanol extracts of dry and fresh plant leaves were prepared. 0.4 mL aliquot of the plant extract was mixed with 3.6 mL of the reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate) and incubated in a water bath at 90 ◦C for 90 min. The samples were cooled to room temperature and the absorbance was measured at 695 nm against a blank. Each experiment was repeated 3 times. The results are provided as mean values in ascorbic acid equivalents per gram of plant extract (AAE/g). Water extract of fresh plant had the highest antioxidant activity of 24.55 mg of AAE/g, and methanolic extract had 24.11 mg of AAE/g. Additionally, the highest antioxidant capacity of dry plant leaves methanol and water extracts were determined as 20.73 mg/g and 20.59 mg/g, respectively. The results of present study confirm that the *R. patientia* L. extracts have shown antioxidant activity by the Phosphomolybdenum method. In conclusion, the leaves of this plant could be used *in vitro* pharmacology studies as a natural antioxidant instead of synthetic antioxidants.

Keywords: Antioxidant, Phosphomolybdenum method, *Rumex patientia*, ROS.

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ORAL PRESENTATION

Chronoimpedimetric biosensor for detection of toxoplasmosis

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Abstract

Toxoplasma is a parasite that infects cats. The contamination occurs by the oocytes from cat feces. Therefore, controlling supplies and nutrients in a home environment is important. Infections form different forms located in tissues, and tachyzoite forms are found in body fluids, leading to chronic infections. This chronic form is the source of the antibodies for this disease. Therefore, in this study, a non-faradaic biosensor for the detection of toxoplasma gondii antibodies was developed. A gold ultra-micro electrode was used as a transducer and modified by a self-assembly monolayer of amino thiophenol (ATP) and carboxy fullerene layer to increase sensitivity and anti-IgG for toxoplasma immobilized on those layers. Carboxy fullerenes were activated in NHS/ EDC (40mM/10mM) and dropped on the ATP layer. Subsequently, the anti-IgG layer was immobilized on the already activated fullerene surface. All modifications were followed by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) in ferri /ferro redox probes. Chronoimpedance parameters were set as 1000Hz 50mV DC and 5mV AC. The detection was performed in 25 seconds by chronoimpedimetric detection and the biosensor is capable of detecting anti-IgG between 0.001U/mL to 1UmL with a regression coefficient of 0.9992. The biosensor showed a good and linear detection of anti-toxoplasma IgG as a biomarker of toxoplasmosis.

Keywords: Biosensor, Toxoplasma, Chronoimpedance

ORAL PRESENTATION

Development and characterization of collagen/keratin/alginate hydrogel

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Abstract

Hydrogels are commonly used as cell-culture scaffolds to mimic the extracellular matrix due to their biocompatibility, three-dimensional structure, water-holding capacity, and degradability. This study aimed to produce collagen/keratin/alginate hydrogels and evaluate their physical, chemical, and biological properties. Keratin isolated from human hair and collagen isolated from bovine Achilles tendon were used in hydrogel groups. The protein structures and stability of keratin and collagen were confirmed with FTIR and SDS-PAGE analysis. Hydrogels were prepared by mixing 1% (w/v) alginate, keratin, and collagen solutions, then cross-linked with either 0.10 M or 1.0 M $CaCl_2$ at 4°C for 30 min, followed by 0.0214 M EDC/0.065 M NHS crosslinking at 4°C overnight. Two hydrogel groups, cross-linked with different $CaCl_2$ molarities, were compared for morphology, chemical properties, swelling behavior, *in vitro* degradation profile, and biocompatibility. SEM results show that both groups had porous structures, and characteristic FTIR peaks of the components were observed in all hydrogel groups. The group crosslinked with 0.1 M $CaCl_2$ reached a swelling ratio of 686±44% after 2 hours, while the group crosslinked with 1 M $CaCl_2$ showed a swelling ratio of 918±35%. In both groups, the percent swelling ratios remained constant in PBS solution throughout the 3 days, with averages of 709±42% and 915±54% for the group crosslinked with 0.1 M and 1.0 M $CaCl_2$, respectively. As a result of the 15-day *in vitro* degradation study, it was observed that the structure of the hydrogels was preserved more as the crosslinker concentration increased. *In vitro* cell viability of mouse fibroblast L929 cells on hydrogels was assessed at 1, 3, and 14 days using resazurin assay. Cell viability decreased as crosslinker concentration increased, but both groups showed adequate viability properties.

Keywords: Collagen, keratin, alginate, hydrogel

ORAL PRESENTATION

Virological Investigation of Bovine Adenovirus (BAdV) Infections in Dairy and Beef Cattle Farms Using Semi-Nested PCR

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Abstract

Bovine Adenovirus (BAdV) causes significant disease in cattle, affecting various systems and leading to a range of symptoms, including diarrhea, reduced appetite, abdominal distension, coughing, serous nasal discharge, dyspnea, and tachypnea. These clinical signs are more common and severe in calves. In this study, BAdV was investigated in both symptomatic and asymptomatic animals from two different cattle farms using semi-nested PCR. From the first farm, nasal swab samples were collected from 101 animals and rectal swab samples from 100 animals. Additionally, from the second farm, 4 nasal and 4 rectal swab samples were collected. In the second farm, four animals showed signs of respiratory distress and varying degrees of diarrhea, ranging from mild to severe. The nasal and rectal swabs were processed to ensure efficient viral DNA extraction. Total viral genetic material was extracted using the guanidine isothiocyanate extraction technique as outlined by Sambrook and Russell (2001). Semi-nested PCR, employing broad-spectrum primer sets, was utilized to detect the presence of BAdV in these samples. Both positive and negative control samples were included to ensure the reliability and accuracy of the PCR results, confirming that the technique was functioning correctly throughout the experiment. All samples tested negative for BAdV, indicating that BAdV infection was not present in the studied animals. These results are consistent with previous studies conducted in Turkey, where only seropositivity was detected, and virologic detection was not possible in most cases. A limited number of positive results were observed in symptomatic animals in earlier studies. These findings highlight the need for further epidemiological and virological studies to fully understand the prevalence and impact of BAdV in cattle populations.

Keywords: BAdV, cattle, diarrhea, respiratoy system, semi-nested PCR.

ORAL PRESENTATION

Keten tohumu gamı ile soğan kabuğu ekstraktı katkılı yenilebilir film üretimi

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Özet

Bu çalışmada, keten tohumu gamından üretilmiş yenilebilir filmlerin farklı soğan kabuğu ekstraktı konsantrasyonları ilave edilerek soğan kabuğu ekstraktının yenilebilir film üzerine fonksiyonel, mekanik, fiziksel özellikleri incelenmiştir. Çalışmada yapılan ön denemeler sonucunda yenilebilir film özelliklerine uyan 4 parametre belirlenmiş olup bunlar F0,F1,F5,F10 numaralı filmlerdir (F0:soğan kabuğu ekstraktsız yenilebilir film,F1:%1 soğan kabuğu ekstraktı içeren yenilebilir film,F5:%5 soğan kabuğu ekstraktı içeren yenilebilir film, F10: %10 soğan kabuğu ekstraktı içeren yenilebilir film). Soğan kabuğu ekstraktının yenilebilir filmin özelliklerine etkilerini incelemek için FTIR, SEM, antioksidan, antimikrobiyal, germe-çekme, suda çözünürlük, kalınlık, renk, ışık geçirgenliği, su kontakt açısı, toplam fenolik madde analizleri yapılmıştır. Soğan kabuğu ekstraktı katkısı ilavesiyle birlikte filmlerde kalınlık artışı gözlemlenmiş olup katkısız filmle kıyasla ışık geçirgenliğinde bir azalma görülmüştür. Germe-çekme testi bulgularında filmin istenilen esneklikte olduğu görülmekte olup bu durum soğan kabuğu ekstraktında bulunan biyoaktif bileşenlerin kaplama materyali ile etkileşime girerek mevcut kaplamanın yapısal özelliklerini ve esnekliğini olumlu yönde etkilediği görülmüştür. Yenilebilir filmlerin toplam fenolik madde analizinde ise soğan kabuğu ekstraktının miktarı arttıkça toplam fenolik madde miktarında artış görülmüş olup yenilebilir filmlerin buna bağlı olarak antioksidan ve antimikrobiyal özelliklerinde olumlu yönde bir etki ettiği bulgusuna ulaşılmıştır. Aynı zamanda soğan kabuğu ekstraktı katkılı filmlerin ilavesiyle yenilebilir filmlerin yüzey homojenliğine ve pürüzsüzlüğüne etkisi SEM görüntülerinde incelenmiştir. Araştırmada elde edilen bulgulara sonucunda soğan kabuğu ekstraktı ilaveli yenilebilir filmlerin ilavesiz filme kıyasla önemli fonksiyonel, mekanik, fiziksel etkileri görülmekte olup elde edilen yenilebilir filmlerin gıda endüstrisinde aktif ambalaj olarak kullanılabilme potansiyelinin olduğunu göstermektedir.

Anahtar kelimeler: Keten tohumu gamı, soğan kabuğu ekstraktı, yenilebilir film, sürdürülebilirlik

Teşekkür: Bu çalışma Türkiye Bilimsel ve Teknik Arsştırm Kurumu (TÜBİTAK) tarafındaan desteklenmiştir (TÜBİTAK 2209A, Proje no.: 1919B012305732)

ORAL PRESENTATION

Combination therapy of hesperidin and gemcitabine exerts anti-inflammatory effects in human pancreatic cancer cells

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Abstract

Pancreatic cancer is the fourth most common cause of cancer-related mortality in the world, after lung cancer. Gemcitabine is commonly prescribed in all stages of PDAC despite its suboptimal clinical efficacy. However, underlying molecular mechanisms that inhibit the cellular uptake and activation of gemcitabine lead to the development of chemoresistance, limiting its efficacy. Therefore, new combinations are needed to increase the activity and prevent resistance. Hesperidin is a flavanone glycoside with various pharmacological effects, including anti-inflammatory, analgesic, anti-fungal, anti-viral and anti-cancer effects. This study aimed to investigate the anti-inflammatory activity of a hesperidin and gemcitabine combination on Mia-Paca2 and BxPC-3 pancreatic cancer cell lines. For this purpose, the cytotoxic effects of hesperidin and gemcitabine on cell lines were evaluated by MTT assay for 48 and 72 hours, respectively. Analyses of IL-6, IL-17A, IL-1Beta, IL-22, IFN-γ and TNF-α were determined by ELİSA methods. The expression of these inflammation-related genes was analyzed using qRT-PCR. Statistical analysis was performed using ANOVA and Bonferroni post hoc test. For MiaPaCa-2 and BxPC-3 cells, Gemcitabine IC_{50} values were found to be 0.83 and 1.03 µM, hesperidin IC_{50} values were found to be 228.8 and 240,5 µM, respectively. Our results showed that gemcitabine, hesperidin and gemcitabine plus hesperidin significantly decreased cell viability, protein levels and gene expression of inflammatory parameters (IL-6, IL-17A, IL-1Beta, IL-22, IFN-γ and TNF-α) in MiaPaCa-2 and BxPC-3 cells compared to the control group ($P<0.05$). Interestingly, hesperidin significantly increased IL-1B, IL-6 and IL-17A protein levels in the BxPC-3 cell line ($P<0.05$). Taken together, hesperidin co-administered with gemcitabine decreased cell viability and increased the anti-inflammatory activity of the drug. Further in vivo and clinical studies are needed to elucidate the underlying mechanism of hesperidin used in combination with anti-cancer drugs.

Keywords: Gemcitabine, Hesperidin, Inflammation, Pancreatic Cancer.

ORAL PRESENTATION

Potansiyel Antikanser Özelliğine Sahip Yeni Azo Bileşiğinin Sentezi, Karakterizasyonu ve *İn Siliko* Yöntemlerle Antikanser Etkinliğinin Araştırılması

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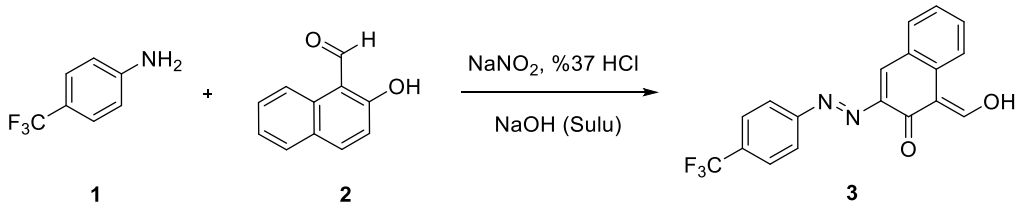
Özet

Kanser, kontrolsüz bir şekilde bölünebilen hücrelerin neden olduğu ve vücudun diğer dokularına yayılma potansiyeline sahip bir hastalık türüdür. Ölüm sıralamasında kalp hastalıklarından sonra ikinci sırada yer almaktadır [1] ve istatistik verilerine göre her 6 ölümün birinden sorumludur [2]. Kanserin tedavisinde kullanılan yöntemlerin istenilmeyen yan etkileri, hastalığın tedaviside kullanılabilecek yeni ilaçların erişebilirliğinin düşük olması, sayılarının yetersizliği ve yüksek maliyetleri, bu alanda potansiyel uygulamalara sahip yeni bileşenlerin tasarımı ve sentezi ile ilgili araştırmaların artmasına neden olmuştur. Ayrıca, bu bileşenlerin antikanser özelliklerinin bilgisayar destekli ilaç tasarımı yöntemleri ile değerlendirilmesine yönelik çalışmalar önem kazanmıştır [3]. Bu sebeple çalışma kapsamında ilk olarak; antikanser özellik gösterme potansiyeline sahip yeni azo bileşiğinin sentezi (Şekil 1), yapısal karakterizasyonu gerçekleştirilmiştir. Sonrasında ise; sentezlenen bileşiğin *in silico* yöntemlerle ilaç olabilme potansiyeli araştırılmıştır. Moleküle ait ADME ve toksisite (ADMEt) parametreleri web sunucuları kullanılarak hesaplanmıştır. Dört farklı Kanser proteini (PDB ID: 2XIR, 1M17, 1E8X, 1MP8) ile olan etkileşimleri ise moleküler kenetlenme yöntemi ile incelenmiştir.



Şekil 1. Yeni azo bileşiğine (**3**) ait sentez şeması

Anahtar Kelimeler: Azo Bileşikleri, Antikanser, ADMEt, Moleküler Kenetlenme.

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ORAL PRESENTATION

Heterosiklik Piridazino-karbazol-on Türevi: Sentezi, Karakterizasyonu, Antikanser Aktivitesinin *İn siliko* ve *İn Vitro* Yöntemler ile Araştırılması

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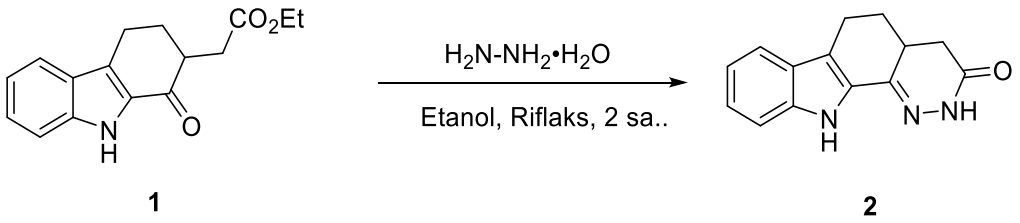
Özet

Heterosiklik bileşikler, yapılarında en az bir tane azot, oksijen veya kükürt gibi heteroatom içeren yapılardır. Bu yapılar ilaç keşfinde önemli bir yere sahiptirler [1]. Özellikle yapısında azot atomu bulunduran heterosiklik bileşikler farmasötik kimya açısından da önem taşımaktadır ve 2010 yılından sonra hedefe yönelik kanser tedavilerinde kullanılan mevcut bazı kanser ilaçlarına ait molekül yapıları incelendiğinde büyük bir çoğunluğunun yapısında azot heteroatomu içerdiği görülmektedir. Alkaloidler, bitkilerden izole edilen, yapısında azot heteroatomu içeren ve önemli farmakolojik aktifliğe sahip doğal bileşiklerdir. Alkaloidler, tarih boyunca ilaç kimyasında önemli yere sahip bileşikler olarak yerini korumaktadırlar [2]. Yapısında heterosiklik azot atomu bulunduran karbazol alkaloidlerinin antikanser aktiviteleri de dahil olmak üzere çeşitli farmakolojik aktivitelere sahip oldukları bilinmektedir [3]. Bu sebeple yapısında karbazol iskeleti içereren yeni türevlerin sentezi ve farmakolojik aktivitelerinin incelenmesi üzerine yapılan çalışmalar devam etmektedir [4]. Çalışma kapsamında yapısında heterosiklik karbazol ve piridazin halkası içeren yeni piridazinokarbazal-on türevi bileşik (2) sentezlenmiştir. Sentezlenen bileşiğin yapısı spektroskopik yöntemlerle (^{1}H-NMR, ^{13}C-APT NMR, FTIR, Mass) karakterize edilmiştir. Bileşiğe ait ADMEt parametrelerinin belirlenmesinde önemli rol oynayan Fizikokimyasal, Lipofilisite, Çözünürlük, Farmakokinetik, İlaçbenzerlik ve Toksisite özellikleri web sunucuları kullanılarak hesaplanmıştır. Sentezlenen bileşiğin üç farklı kanser hücresine (DLD-1, MDA-MB-231, HepG2) karşı sitotoksik aktivitesi araştırılmış ve bu kanser hatları ile ilişkili kanser proteinleri ile etkileşimleri *in siliko* yöntem kullanılarak incelenmiştir.



Şekil 1. Piridazino-karbazol-on bileşiğine ait sentez şeması

Anahtar Kelimeler: Heterosiklik bileşikler, Alkaloid, Karbazol, Antikanser, *in vitro*, *in siliko*

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ORAL PRESENTATION

Synthesis and structure elucidation of some new substituted 1,3,4-oxadiazol-2-amine derivatives

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Abstract

Throughout history, various methods have been utilized for diagnosing, treating, and preventing diseases. Today, synthetic molecules are of primary interest. Pharmaceutical chemists engage in the research and development of new drug molecules by designing pharmacophore structures based on literature and synthesizing them according to planned protocols. In drug discovery, heterocyclic structures such as pyridine, oxadiazole, and triazole are often favored due to their ability to interact with target cells and tissues, including enzymes and receptors. This study focuses on synthesizing new 1,3,4-oxadiazole compounds from methyl 6-chloropyridine-3-carboxylate. Because, 1,3,4-oxadiazoles have numerous biological and pharmaceutical properties. Many drug structures bearing the 1,3,4-oxadiazole ring are known, such as zibotentan (anticancer), furamizole (antibacterial), tiodazosin (anti-hypertensive) and raltegravir (anti-retroviral). The synthesis process involved three main steps: hydrazide synthesis, semicarbazide synthesis, and 1,3,4-oxadiazole synthesis. Initially, a hydrazide compound was synthesized from the ester structure, which was then reacted with various isocyanates to produce semicarbazide. These semicarbazide compounds were subsequently reacted with phosphorus(V) oxychloride to form 1,3,4-oxadiazol derivatives. A total of twelve semicarbazide and twelve oxadiazole compounds were synthesized in this study. The structures of all compounds were verified by some spectroscopic methods such as IR, ^{1}H-NMR, ^{13}C-NMR and MS. In addition, their physicochemical, pharmacokinetic and drug-likeness properties were assessed by the SwissAdme web tool.

Keywords: Oxadiazole, semicarbazide, synthesis, pyridine.

ORAL PRESENTATION

Design, synthesis and cytotoxic activity of novel triazolopyrimidinone derivatives for immune-mediated diseases

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Abstract

Immune-mediated inflammatory diseases (IMID) are a large group of illnesses (rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, lupus). These diseases are characterised by systemic inflammation and multiorgan involvement [1]. The JAK/STAT signaling pathway has been associated with the development of various inflammatory conditions, such as psoriasis and rheumatoid arthritis. There are several FDA licensed JAK inhibitors for the treatment of inflammatory and autoimmune diseases [2]. Tofacitinib (Xeljanz®) and Baricitinib (OLUMIANT®) are the first orally available JAK inhibitors to be approved in treating rheumatoid arthiritis and other autoimmune diseases. We rationally designed and synthesized JAK inhibitor compounds with appropriate reactions according to the reports. JAK enzyme inhibiton levels of these compounds were reported. Between the compounds, 6 of them are selected according to the enzyme inhibition results and their cytotoxicity rates were examined on THP-1 human leukemia monocytic cell line and L929 mouse fibroblast-like cell lines. Cytotoxicity levels have been found to be within a reliable range. We also calculated compunds' physicochemical properties, lipophilicity, water solubility and evaluate druglikeness using SwissADME. Following these promising results with the novel JAK enzyme inhibitors, further studies are ongoing to explore the potential of these compounds for the treatment of immune-mediated diseases.

Keywords: JAK/STAT, TYK2, triazolopyrimidinone, autoimmune, inflammatory diseases

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ORAL PRESENTATION

Importance of SOX proteins in the cell

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Abstract

The development of multicellular organisms and the maintenance of homeostasis in adults, as well as the complex control of basic cellular processes such as cell differentiation, proliferation and death, are provided by transcriptional factors. Transcription factors determine the fate of cells within tissues, organs and organisms, controlling many physiological processes, especially development. One of the transcription factors that play a role in the control of embryonic development processes in these processes is SOX proteins. SOX proteins are members of a 20-member transcription factor known as the high mobility group (HMG-box) associated with the sex-determining region Y (SRY) and divided into groups from A to H. It has been stated that the functions of the members in different groups of SOX proteins in different developmental processes are generally not the same. Thus, it has been stated that these proteins, in addition to their roles being adaptable to developmental conditions and cellular contexts, can perform synergistic or antagonistic functions in some cases. According to their groups, SOX proteins play critical roles in the development of many cells, tissues and organs, especially in the development of some stem cells and the nervous system (SOX1/2/3), in the proliferation and differentiation of testicular cells and chondrogenesis (SOX9), in endothelial cells and vascular development (SOX7/17/18). In conclusion, SOX proteins are a family of transcription factors (TFs) that play critical roles in various biological processes, including the regulation of development in a wide variety of cell and tissue types.

Keywords: Cell differentiation, SOX proteins, Transcription factors

ORAL PRESENTATION

Investigation of the Inhibitory Effect of Bryophyte Metabolites with Anti-tumor Properties on BRAF V600E Mutation by *In silico* Methods

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Abstract

The BRAF V600E mutation is a common genetic alteration found in various cancer types, particularly melanoma, thyroid cancer, colorectal cancer, and lung cancer. This mutation leads to the constitutive activation of the BRAF protein, resulting in excessive stimulation of the MAPK/ERK signaling pathway, which promotes cell proliferation and survival. Consequently, cells grow and multiply uncontrollably, contributing to tumor formation. Clinically, targeted therapies have been developed for patients with the BRAF V600E mutation, and BRAF inhibitors have emerged as significant treatment options for cancers harboring this mutation. However, the drugs used in cancer treatment are associated with a range of side effects. Considering these adverse effects, it is well recognized that medicinal plants exhibit fewer side effects and possess high therapeutic efficacy. Bryophytes (mosses, liverworts, and hornworts) are a primitive group of plants. Various secondary metabolites found in these plants, particularly phenolic compounds and terpenoids, have been shown to inhibit the growth of cancer cells and induce apoptosis. Furthermore, the low toxicity profile of bryophytes makes them attractive candidates as potential natural anti-tumor agents. This study aims to investigate bryophyte metabolites as potential alternatives to the inhibitors currently used for the treatment of cancers with the BRAF V600E mutation.

Keywords: Bioinformatics, Molecular Docking, Drug Discovery, Bryophyte, BRAF V600E

ORAL PRESENTATION

Determination of tin(IV) in wastewater using a smartphone-based digital image colorimetric method

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Abstract

In our study, a rapid, easy-to-use, low-cost method was developed a smartphone-based digital image colorimetric method for determination of Sn(IV). To observe the color change in the presence of tin, silver nanoparticles were first synthesized using the green synthesis method. Clove extract was used as bioreducant in our green synthesis method. All parameters that may affect our method were optimized before starting the analysis. Under optimum conditions, when the tin(IV) was added to the medium containing silver nanoparticles, a color change was observed. The intensity of the color change showed a linear relationship with increasing tin concentration. The color change in the sample medium was monitored with the application called Color Detector, which can be loaded onto smartphones. In order to monitor the color change in the most appropriate way in our analyses, the distance of the sample placed in the digital imaging box to the camera lens, the location of the point where the color change is monitored, and the size of the point where the color change is monitored have been optimized. The analytical performance of the method we developed was determined by taking measurements with samples containing increasing concentrations of tin under optimum conditions. After determining the analytical performance of our method, a recovery study was applied to demonstrate its applicability in a real sample environment. In the recovery studies, wastewater was used as the real sample medium.

Keywords: Tin, Silver nanoparticle, Digital imaging, Smartphone.

ORAL PRESENTATION

In vitro Cytotoxic Effects of Some Food Additives on L929 Cells

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Abstract

Food additives are substances of natural or synthetic origin that are not in the main composition of the food and are added to the food during its production, processing, packaging and/or storage. These substances are used to add sensory values such as taste, aroma, odor, or texture and nutritional values such as vitamins, minerals and salts to the food, as well as to extend the shelf life of the food and prevent spoilage. However, studies have revealed that dose-dependent and long-term exposure to synthetic food additives can lead to different health problems such as obesity, cancer, asthma and attention deficit. Therefore, the presence of food additives in foods causes concerns and studies on toxicity in terms of risk assessment are increasing. In this study, the cytotoxic effects of potassium benzoate and potassium sorbate used as food preservatives and mono sodium glutamate used as flavor enhancer on L929 (fibroblast cell line) were investigated by using the WST-1 assay. The results showed that potassium benzoate, potassium sorbate and mono sodium glutamate didn't show any significant effect on cell viability at applied doses. In conclusion, since these food additives may have dose-dependent toxicological effects, they should be screened for genotoxicity, mutagenicity and hepatotoxicity by further different methods for their safety use.

Keywords: mono sodium glutamate, potassium benzoate, potassium sorbate, cytotoxicity

ORAL PRESENTATION

Bodipy – ferrocene dyad system and its spectroscopic investigation

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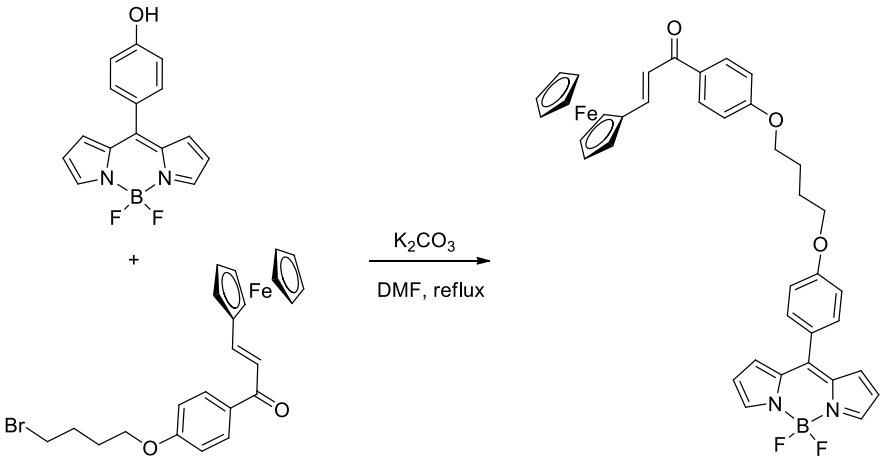
Abstract

Conjugated heterocyclic organic structures are a versatile class of compounds used frequently in ion receptor or sensor applications due to their photophysical properties. Bodipy structures obtained from pyrrole compounds that exhibit high fluorescence properties are frequently used in today's chemistry. When gathered with another chromophoric structure, Bodipys can display fluorometric responses by means of emission intensity. Ferrocene and its derivatives are also known for their electron donating ability and have been extensively used in electrochemistry studies. In line with these information, in the presented study, the synthesis of new dichromophoric dyad system of Bodipy and ferrocene derivative that are linked by an alkyl chain have been realized and the investigation of its photophysical properties were screened by means of absorption and emission spectroscopy techniques.



Keywords: Bodipy, ferrocene, dyad, spectroscopic analysis

ORAL PRESENTATION

In vitro Antibacterial Activity of Two Different Plant (*Mentha pulegium* and *Stachys iberica*) Methanolic Extracts on Some Fish Pathogenic Bacteria

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Abstract

Novel disease outbreaks and increased use of antibiotics have led to the rise of antibiotic-resistant bacterial strains. As the effectiveness of commonly used antibiotics diminishes, natural medicinal herbs have emerged as potential pharmaceutical resources for combating microbial antibiotic resistance. This study aimed to evaluate the in vitro antibacterial potential of methanolic extracts from *Mentha pulegium* and *Stachys iberica* against several fish pathogenic bacteria, including *Aeromonas hydrophila*, *Aeromonas sobria*, *Aeromonas veronii*, *Yersinia ruckeri*, *Edwardsiella tarda*, *Enterococcus faecalis*, *Vibrio anguillarum*, and *Plesiomonas shigelloides*. The sensitivity of the tested strains to the plant methanolic extracts were assessed using the disc diffusion assay. The results indicated that the methanolic extracts from both plants were bactericidal for all test bacteria except *P. shigelloides*. Notably, the methanolic extract of *M. pulegium* exhibited the highest zone of inhibition against *E. faecalis* (11.33 mm). Further research on phytochemicals and the isolation of specific bioactive components is needed to gain detailed insights into the antibacterial properties of these medicinal plants. Additionally, to evaluate the efficacy of the extracts and their potential impact on fish, further studies are required.

Keywords: Antibacterial Activity, Plant Methanolic Extracts, Fish Pathogenic Bacteria

ORAL PRESENTATION

Investigation of anticancer activity of naphthoquinone compound in mouse breast cancer model

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Abstract

Compounds containing the naphthoquinone scaffold have a critical place in drug research studies, and many drug molecules contain the naphthoquinone scaffold in their molecular structures [1-3]. In this study, the anticancer effects of a naphthoquinone derivative compound at various doses were investigated in 4T1 cell line-induced mouse breast cancer model. We observed that the compound reduced tumor volume by approximately 59% in the administered mice compared to the cancer group. As a result of the study, we determined that serum and tissue caspase-3, 6, and 8 levels increased in the compound applied group compared to the cancer group. Additionally, we found that tissue and serum HSP90, topoisomerase alpha and beta levels were decreased compared to the cancer group. As a result, we think that the compound shows anti-cancer activity by inducing apoptosis in cancer cells via the caspase pathway and inhibiting molecules that play a role in cell proliferation such as hsp90 and topoisomerase. (The protocol was approved by the Ethic committee on animal research of Mersin University: Ethics committee number: 2021/23)

Keywords: Naphthoquinone, Breast cancer, *In vivo* anticancer activity.

Acknowledgments: We are thankful to Mersin University (project grant 2021-1-AP5-4364) for financial support.

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ORAL PRESENTATION

Chemosensor properties of tetrakis-1,2,3-triazole derivatives

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Abstract

Anions have a critical place in health and the environment, and their determination is of critical importance [1]. Since the 1,2,3-triazole structure is present in the structures of many compounds that exhibit chemosensory properties [2], in this study, new tetrakis-1,2,3-triazole derivatives were synthesized and their chemosensory properties against various anions were determined by UV-vis spectrophotometric titrations. It was determined that the compounds showed chemosensory properties against fluoride and hydroxide ions. The sensor mechanisms of the compounds were elucidated by ^{1}H NMR titrations.

Keywords: Anion sensor, Chemosensor, Spectrophotometric titration, Tetrakis-1,2,3-triazole.

Acknowledgments: We are thankful to Mersin University (project grant 2023-2-TP2-4933) for financial support.

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ORAL PRESENTATION

Characterization, Synthesis, and Biological Activities of Silver Nanoparticles Produced via Green Synthesis Method Using *Verbascum uschakense* Aqueous Extract

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Abstract

Silver nanoparticles were obtained by green synthesis using the aboveground parts of *Verbascum uschakense* plant, one of the endemic *Verbascum* species growing in Turkey. First, the nanoparticles were characterized and their antimicrobial and antioxidant activities were investigated. In the literature, it was determined that *Verbascum* genus plants contain secondary metabolites such as iridoid glycosides, oleanane type triterpenic saponosides, phenylpropanoid and phenylethanoloid glycosides, flavonoids, steroids and alkaloids. Considering that they contain different active ingredient groups and that each ingredient group will have different activities, Verbascum species stand out as potential medicinal plants. The characterization of silver nanoparticles (VAgNP) obtained by green synthesis at 60 C˚ from *Verbascum uschakense* species was carried out by various spectroscopic and microscopic techniques (UV-visible (UV-vis) absorption spectroscopy, X-ray Diffraction Analysis (XRD), High Resolution Transmission Electron Microscopy (HR-TEM) and Scanning Electron Microscopy Energy Dispersive X-Ray Spectroscopy (SEM-EDS). According to UV-Vis spectroscopy data, it was determined that VAgNP showed a plasmon resonance peak at 432 nm. According to TEM and SEM analysis results, VAgNPs were found to be spherical nanoparticles with a size of 4-14 nm. When the XRD results were evaluated, the reflections of the VAgNP spherical structures in the (111), (200), (220) and (311) planes were respectively 38.21°, 44.46°, 64.59° and 77.48° and it was determined that these values belong to the cubic crystal structure. While DPPH free radical scavenging was determined as 86.93% in *Verbascum uschakense* extract at a concentration of 1 mg mL-1, it was determined as 60.04% for VAgNP. For ABTS radical scavenging, VAgNP inhibition values at a concentration of 1 mg/ml were determined to be equivalent to Trolox. The antimicrobial effect of VAgNP was investigated by line method and it was determined that they had activity against various microorganisms (*S. aureus*, *S. mutans*, *E.coli*, *P. aeruginosa*, *L. monocytogenes*, *Candida* sp).

Keywords: Green synthesis, Silver nanoparticle, Antioxidant capacity, Antimicrobial activity

ORAL PRESENTATION

Rat Spermi Sulandırıcısına İlave Edilen L-Glutathione ve Alpha-Tocopherol'ün Donmuş Çözdürülmüş Sperm Kalitesi Üzerine Etkileri

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Özet

Sunulan çalışmada, Rat sperminin dondurulabilirliği üzerine, %8 laktoz, %23 yumurta sarısı ve %10'luk tris solüsyonu içeren sperm kriyoprezervasyon medyumuna ilave edilen farklı dozlardaki L-Glutathione (2.5 mM, 5 mM ve 10 mM) ve Alpha-Tocopherol (0.1 mM, 0.2 mM ve 0.5 mM)'ün etkileri araştırıldı. Araştırmada Rat sperminin dondurulmasında antioksidan ilave edilmeyen karışım kontrol grubu olarak tutuldu. Araştırmada 12-16 haftalık yaşta, 7 farklı çalışma grubunda toplam 56 adet Wistar-Albino ırkı erkek rat kullanıldı (n=8). Ratların servikal dislokasyon yöntemiyle sakrifiye edilmesiyle kaudalarından elde edilen spermler kontrol ve çalışma grubu medyumları içerisinde sulandırıldı. Kryopreserve edilen sperm payetleri 37ºC'lik su banyosunda 30 saniye süreyle çözdürüldü. Dondurulup çözdürülen payetlerde motilite, ölü spermatozoon oranı, anormal akrozom oranı, plazma membran bütünlüğü, HTF (Human Tubal Fluid) içerisinde 90 dakikalık motilite dayanıklılık ve hücre apoptozu yönlerinden analizler yapıldı. Dondurma çözdürme sonrası %12.50±1.36'lik kontrol grubu ile karşılaştırıldığında en yüksek motilite %23.89±1.82 ile 0.1 mM Alpha-Tocopherol ve %28.00±2.71 ile 2.5 mM L-Glutathione gruplarında tespit edildi *($p<0.01$)*. Ölü spermatozoon ve anormal akrozom oranları bakımından araştırma grupları ile kontrol grubu arasında önemli bir fark bulunmadı *($p>0.01$)*. Sağlam plazma membran oranları %25.78±2.39'luk 0.1 mM Alpha-Tocopherol ve %29.40±1.12'lik 2.5 mM L-Glutathione gruplarında, %20.39±2.00'lik kontrol grubundan daha yüksek bulundu ($p<0.01$). Dondurma çözdürme sonrası, 90. dakikaya kadar en iyi motilite dayanıklılık (longevity) süresi, kontrol grubu ile karşılaştırıldığında 0.1 mM Alpha-Tocopherol ve 2.5 mM L-Glutathione gruplarında belirlendi *($p<0.01$)*. Çözüm sonrası, kontrol grubu ile karşılaştırıldığında en düşük apoptotik hücre oranı 0.1 mM Alpha-Tocopherol ve 2.5 mM L-Glutathione gruplarında tespit edildi *($p<0.01$)*.
Sonuç olarak, Rat spermi kriyoprezervasyon medyumuna 0.1 mM Alpha-Tocopherol veya 2.5 mM L-Glutathione ilavesinin dondurup çözdürme sonrası motilite, fonksiyonel plazma membran bütünlüğü, motilite dayanıklılığını artırdığı ve apoptotik hücre oranını düşürdüğü ve böylece Rat spermi dondurma medyumunda başarılı bir şekilde kullanılabileceği belirlendi.

Anahtar Kelimeler: Rat Spermi, Kriyoprezervasyon, Antioksidan, Alpha-Tocopherol, L-Glutathione, Apoptozis.

ORAL PRESENTATION

Assessment of the Nanofiber Hydrogels Cytotoxicity Fabricated via Electrospinning Method

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Abstract

The term ‘’hydrogel’’ is used to describe three-dimensional structures composed of polymers with hydrophilic functional groups. These structures can be either naturally occurring or intentionally synthesized. Hydrogels display distinctive characteristics in aqueous media, including high water content, insolubility, and swelling [1]. Hydrogels are ideal synthetic materials favoured in several biomimetic and biomedical applications due to their resemblance to the extracellular matrix, exceptional biological performance, and intrinsic capacity to interact with cells [2]. However, hydrogels' mechanical characteristics are occasionally suboptimal, particularly when they swell in water or absorb wound exudate [3]. Consequently, their frequent breakdown impedes their applications as wound dressing materials. Researchers propose combining hydrogel with a nanofiber electrospun mat to enhance existing hydrogels' mechanical and degrading characteristics.[4]. The electrospinning method is preferred choice among these several approaches due to its traditional and controllable nanofiber production technology, which is capable of mimicking the microstructure of the extracellular matrix (ECM) [5]. Chitosan is a material with a wide range applications in biological contexts, due to a number of favourable properties including antibacterial activity, biocompatibility, non-toxicity, and biodegradability [6]. In the present study, PEO/Chitosan-based cross-linked nanofibers (nanofiber hydrogels) containing different amounts of chitosan were prepared by electrospinning, and their cytotoxic properties were investigated. According to the results, it was determined that the prepared nanofiber hydrogels did not contain toxic properties for biological applications.

Keywords: Hydrogel, Nanofibers, Electrospinning, Cytotoxicty, Chitosan

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ORAL PRESENTATION

Morphometric evaluation of female genital organs in roe deer (*Capreolus capreolus*)

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Abstract

The roe deer (*Capreolus capreolus*) inhabits a large area that includes all of Europe except Northern Europe, as well as Turkey, Syria, Iraq, the Caucasus, and the northern part of Iran. In Turkey, its habitat is primarily in the Black Sea and Marmara regions. The roe deer is an endangered species due to hunting and poaching, and its long gestation period (290 days), typically producing one or two offspring, along with a high potential for miscarriage under stress, makes understanding the anatomy of the female genital system crucial for the species' survival. The lack of studies on the anatomy of the female genital system in roe deer makes the morphometric data obtained from this study significant as the first of its kind. As part of the study, female reproductive organs from roe deer, with an average weight ranging between 20-25 kg, brought to our faculty clinics and nature conservation and national parks directorates due to reasons such as traffic accidents, were used. After the dissection of the female genital organs from six roe deer, the morphometric measurements of each organ were taken and evaluated. After dissection of the female genital organs of the roe deer, the lengths of parts of the uterus, except for the uterine horns (cornua uteri), were measured. As a result of these measurements, the craniocaudal length of the right ovary was found to be 12.98±3.92 mm, and its lateromedial length was 7.73±1.15 mm; the craniocaudal length of the left ovary was 12.85±2.74 mm, and its lateromedial length was 8.18±0.66 mm. The length of the uterine tube (tuba uterina) was measured as 82.56±18.24 mm, while the length of the uterine body (corpus uteri) was 55.88±29.74 mm, and the length of the cervix uteri was 40.73±22.03 mm. The lengths of the vagina and vestibulum vagina were determined to be 46.92±10.92 mm and 38.64±9.24 mm, respectively. The diameter of the caruncles, which belong to the uterus, was 35.53±19.67 mm, and the number of these structures was found to be an average of 8.2±1.09. All roe deer, except one, were at different stages of pregnancy. Therefore, the lengths of the uterine horns (cornua uteri) varied depending on the stage of pregnancy. In the non-pregnant roe deer, the right cornu uteri was 67.31 mm, and the left cornu uteri was 70.08 mm. The lengths of the uterine horns (cornua uteri) and the length of the fetuses from the cranium to the anus were measured according to the stages of pregnancy and are shown in Table 1. Based on these measurements, it was determined that the length of the uterine horns increased proportionally with the stage of pregnancy. In conclusion, this study has presented the results of morphometric data of the female genital organs of the roe deer, which has an important place in wildlife. It is thought that these findings will contribute to the anatomy literature.

Key words: Anatomy, female genitalia, roe deer, morphometric

Table 1. Uterine cornu and offspring lengths (mm) of roe deer with different pregnancy periods

Sample No	Right uterine cornu length (mm)	Left uterine cornu length (mm)	Hatchling length in right cornu (mm)	Hatchling length in left cornu (mm)
1	67,31	70,08	—	—
2	153	138,01	82,19	76,42
3	142	100	18,57	—
4	155,43	162,95	165,52	168,59
5	337,45	312,58	359,43	316,83
6	319,43	328,29	202,8	212,42

ORAL PRESENTATION

Diclofenac Sodium Loading into Poly(NVI- *co*-PEGMA) Cryogels, Release Studies, Kinetic Parameters and Anti-Inflammatory Activity

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Abstract

Cryocopolymers of N-vinyl imidazole (NVI) and poly(ethylene glycol) methacrylate (PEGMA) were synthesized under cryogenic conditions, and their anti-fouling properties have been previously reported. The anti-inflammatory drug diclofenac sodium (DS) was loaded into these gels, and both the drug loading efficiency and capacity were evaluated. Drug release studies were conducted in three different pH environments (pH 2.0, 5.0, and 7.4), demonstrating a pH-dependent sustained release of DS. While limited release was observed at stomach pH (2.0), a significant increase in release occurred within 15 hours at pH 5.0 and 7.4. Further investigation of DS release from copolymer cryogels using various kinetic models (zero-order, first-order, Higuchi, and Korsmeyer-Peppas) revealed that over the linear release period of 1–6 hours, the release followed a zero-order kinetic model in P(NVI-*co*-PEGMA) (9), whereas it conformed to the Korsmeyer-Peppas model in sample P(NVI-*co*-PEGMA) (6). The anti-inflammatory activity of the released DS was tested via inhibition of Bovine Serum Albumin Denaturation. The amount of DS released from the copolymer cryogels was sufficient to demonstrate effective anti-inflammatory action. In conclusion, the P(NVI-*co*-PEGMA) cryogel drug delivery system shows great potential as a targeted carrier for the delivery of diclofenac sodium to the colon, offering promising applications in site-specific drug release.

Keywords: Cryogel, diclofenac sodium, anti-inflammatory activity, release kinetics, colon targeted delivery.

ORAL PRESENTATION

Evaluation of 2-hydroxyethyl methacrylate and N-(2-hydroxyethyl) acrylamide based cryogels as potential quercetin release matrices

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Abstract

Copolymer samples of poly(2-hydroxyethyl methacrylate–*co*-N-hydroxyethyl acrylamide), P(HEMA-*co*-HEAA), as well as homopolymers of 2-hydroxyethyl methacrylate (HEMA) and N-hydroxyethyl acrylamide (HEAA) were previously produced through free radical polymerization using the APS/TEMED redox initiator system under cryogenic conditions. The samples' drug loading efficiency (DLE) and loading capacity (DLC) values were calculated by varying the initial drug concentrations to determine the optimal drug loading conditions. Fourier-transform infrared spectroscopy (FTIR) spectra of the drug-loaded samples indicate the successful drug-loading process. Cumulative quercetin release percentages reveal sigmoidal release curves of PHEMA (DLC%, 1.97 % ± 0.02), P(HEMA-*co*-HEAA) (3) (F_{HEAA}= 0.26) (DLC%, 1.86 % ± 0.03), P(HEMA-*co*-HEAA) (7) (F_{HEAA}= 0.80) (DLC%, 1.85 % ± 0.03), and PHEAA (DLC%, 1.34 % ± 0.05), at pH=7.4, 37 °C and shaken at a speed of 130 rpm. Release kinetic studies show that quercetin release from the cryosamples follows the zero-order release model. Quercetin release percentages from PHEMA, PHEAA, and their copolymers show limited quercetin release under stomach-mimicking conditions at pH=2.0 indicating that these cryogels are promising matrices for successfully delivering quercetin to the small intestine, where its primary absorption occurs. In addition, the antioxidant activity of the quercetin concentrations released from the samples was evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

Keywords: Drug release matrices, 2-hydroxyethyl methacrylate, N-hydroxyethyl acrylamide, quercetin

ORAL PRESENTATION

Investigation of Coexistence of Renal Artery and Vein Variations in Adults Using CT

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Abstract

Variations may occur in renal vessels due to various reasons during the embryological process. Renal artery and renal vein variations, which are important in transplantation surgery, have been examined separately in many studies. Our study aimed to evaluate the association of renal artery and vein variations. The images of 200 individuals (103 males, 97 females) aged 18-93 who underwent contrast-enhanced abdominal CT at Tokat Gaziosmanpaşa University Hospital were retrospectively examined. The vertebral level where the right and left renal arteries branch off from the abdominal aorta was determined as L1 in 46.2% of the patients, L2 in 28.5%, and between L1 and L2 in 22.6%. Our results were consistent with the literature. Unlike other studies, in our findings, retroaortic left renal vein was seen in 8.3% of those with double right renal artery. Double right renal vein was observed in 20% of those with double left renal artery. Arterial variations were more common in the right kidney, and venous variations were more common in the left kidney. We believe that we have contributed to retroperitoneal surgery by evaluating the association of renal artery and vein variations with our study.

Keywords: Computed tomography, Renal artery, Renal vein, Coexistence of variations

ORAL PRESENTATION

Determination of antibacterial activity of resveratrol on some pathogenic bacteria

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Abstract

This study aims to investigate the antibacterial effect of resveratrol, a natural polyphenol, on some pathogenic bacteria. Resveratrol is a compound found in plants such as red grapes and is known for its antioxidant, anti-inflammatory and antimicrobial properties. Due to these properties, it is considered as a potential agent in the prevention and treatment of bacterial infections. In this context, it was aimed to determine the antibacterial effects and effective dosage of resveratrol on *Salmonella typhimurium, Staphylococcus epidermidis, Pseudomanas aeruginosa, Yersinia pseudotuberculosis, Proteus vulgaris, Enterococcus faecalis, Enterobacter cloaceae, Escherichia coli*, and *Listeria monocytogenes*. Antibacterial activity studies were performed by agar well diffusion method. As a result of antibacterial activity studies, inhibition zones with a diameter of 14mm for *Staphylococcus epidermidis*, 15mm for *Enterococcus faecalis*, 15 mm for *Enterobacter cloaceae* and 16mm for *Listeria monocytogenes* were measured at 5 mg\ml dosage of resveratrol. However, resveratrol was found to have a bacteriostatic effect against *Salmonella typhimurium, Pseudomanas aeruginosa, Yersinia pseudotuberculosis, Proteus vulgaris* and *Escherichia coli*. According to these results, it can be said that resveratrol may be effective against some pathogenic bacteria and may contribute to the development of new strategies for its use as an antibiotic adjuvant, although its use in terms of both food safety and public health is foreseen.

Keywords: Resveratrol, pathogenic bacteria, inhibition, antibacterial effect

ORAL PRESENTATION

Electroanalytical Determination of Phenothiazine Derivative with Nanowire Structured Electrodes

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Abstract

The purpose of this study is using an electroanalytical method to detect promethazine which is a phenothiazine derivative compound, in the urine sample. To determine promethazine, we preferred a pencil graphite electrode (PGE) that has not been used in the literature before. We preferred polypyrrole, a conductive polymer often used to modify electrodes. The electrodes were prepared by electropolymerization of pyrrole using cyclic voltammetry (CV). Determination of promethazine was performed by differential pulse voltammetry (DPV). The preparation of working nanowire electrodes was accomplished using two different procedures. In the first procedure, 15 mL of distilled water was used to dissolve 0.1 M pyrrole, 0.1 M $LiClO_4$, and 0.1 M Na_2CO_3 to produce electrolyte solutions. To eliminate CO_3^{2-} ions, the obtained electrode was first thoroughly cleaned with pure water before being conditioned in 10% $HClO_4$ solution for 24 hours [1]. In the second procedure, 0.1 M Na_2HPO_4 was used instead of 0.1 M Na_2CO_3 [2]. Platinum wire, Ag/AgCl reference electrode, and PGE were immersed in the solution. The electrode was obtained by CV method. The optimum solution pH was determined as 4. Surface characterization and elemental analysis of the electrodes were carried out by SEM and SEM-EDS. From the SEM images, desired nanowire structure, which provides a larger surface area, was obtained. To assess selectivity, a non-nanowire polypyrrole electrode was prepared for comparison. The working range of the nanowire electrodes was determined as 0.05-0.3 mM with DPV. The LOD values for electrodes from the first and second procedures were 0.015 mM and 0.012 mM, respectively. Promethazine analysis was performed in the urine sample by the standard addition method. According to the analysis results with the prepared nanowire electrodes, the recoveries for 0.1 mM promethazine were 101.5% and 97.9%, respectively, while the recoveries for 0.2 mM promethazine were calculated as 100.5% and 98.95%, respectively.

Keywords: Pencil graphite electrode, Nanowire structured electrode, Promethazine, Voltammetry

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ORAL PRESENTATION

Antibacterial Activities of *Cymbopogon winterianus* Jowitt and Carvacrol Combination

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Abstract

The continuous increase of antibiotic resistance and the lag in development of novel antibiotics are two important challenges in treatment of infectious diseases. Essential oils (EOs) and their combinations are promising alternatives for treatment of bacterial infections. *Cymbopogon winterianus Jowitt* EO, reported by the EMA to improve sleep and relieve anxiety, has sedative properties that help with sleep disorders, according to the German Commission E monograph. The aim of the study was to investigate antibacterial activities of the *C.winterianus* and carvacrol combination. Commercial C.winterianus EO and carvacrol were tested for antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Klebsiella pneumoniae* using the broth microdilution method. Ciprofloxacin served as the standard antimicrobial agent. A microdilution checkerboard assay was used to evaluate the combined effects of C.winterianus and carvacrol. *C.winterianus* had moderate effect against both Gram negative and Gram positive bacteria. Additive effects were observed in combinations of essential oil with carvacrol on all bacteria tested. It has also been observed that the antibacterial effect of the essential oil increases 2-16 times in combination with carvacrol.

Keywords: Synergy, combination, essential oil, Cymbopogon, carvacrol, antibacterial

ORAL PRESENTATION

Quantitative analysis of orange II dye by smartphone-assisted colorimetric method

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Abstract

Dyes have an important place as environmental pollutants, especially in industrial wastes and water resources. Orange II (OII) is resistant to almost all acids, bases, light, and oxygen, and it is incapable of decomposing in treatment facilities for wastewater [1]. Furthermore, long-term exposure to OII can impair reproduction, leading to infertility and birth abnormalities [2]. Therefore, it is important to detect OII dye. In this study, a smartphone-based colorimetric method was developed for the quantitative analysis of OII dye in water. For this purpose, the RGB values of the colors of the sample solution were obtained with the help of software. The results obtained with this method were validated with the spectrophotometric method at the 95% confidence level. The obtained data show that smartphone-based colorimetric analysis provides sufficient accuracy and sensitivity in the quantitative determination of OII dye. This smartphone-based methodology has the potential to provide a practical alternative in areas such as environmental monitoring and waste management by providing low-cost, portable, and rapid analyses. This study demonstrates that smartphone-based colorimetric analyses offer an effective alternative to traditional spectrophotometric methods and can be used in environmental analyses.

Keywords: Orange II, Smartphone colorimetry, Quantitative analysis, Environmental monitoring.

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ORAL PRESENTATION

Protective Effects Of Coenzyme Q10 Against Methotrexate-Induced Ovarian Damage: A Biochemical, Flow Cytometry, And Histopathological Study

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Abstract

Objective: The present study aimed to investigate the protective effects of coenzyme Q10 (CoQ10) on methotrexate (MTX)-induced ovarian damage. Methods: Female twenty-four wistar albino rats (10-12 weeks old) were divided into four groups. First group was accepted as control and 1 ml corn oil was applied by oral gavage (p.o.). In the second group, only 20 mg/kg MTX intraperitoneal (i.p.) was applied one time on the first day of the experiment. In the third group 20 mg/kg MTX i.p. was applied on the first day of the experiment and 100 mg/kg CoQ10 in 1 ml corn oil was applied p.o. for seven days. In the fourth group, 100 mg/kg CoQ10 in 1ml corn oil was applied by p.o. for seven days. At the end of the seventh days, animals were euthanized under anesthesia. Blood samples were taken by intracardiac and both side ovarium tissues were removed. The left and right ovaries were put in to neutral formalin. The left ovaries were used for apoptosis analysis by flow cytometry and right ovaries were used for the histopathological analysis and follicle counting. After tissue processing, right ovaries were embedded into paraffin. Follicle counting and morphometric analysis were performed on the hematoxylin &eosin stained sections. The left ovaries were used for analysis of apoptosis by flow cytometry. Biochemical analysis of serum anti-mullerian hormone (AMH) was performed from blood samples. Results: According to results of follicle counting and morphometic analysis; there was no statistically significant difference in the follicle numbers and ovarium diameter between all groups. Apoptosis increased significantly in the MTX group and it decreased significantly in the MTX+CoQ10 group. AMH level was significantly increased in the only CoQ10 treatment group and decreased in the MTX group. Conclusion: As a result, CoQ10 may reduce MTX-induced apoptosis and have positive effects on AMH.

Keywords: Apoptosis, Coenzyme Q10, Flow cytometry, Methotrexate, Ovarium

ORAL PRESENTATION

The Regulatory Impact of Thymoquinone on Mitophagy in Cancerous and Healthy Cells

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Abstract

Mitochondrial function and dynamics play a critical role in the progression of various pathological conditions, including metabolic disorders, neurodegenerative diseases (such as Alzheimer's, Parkinson's, and Huntington's), and cancer. These dynamics are regulated through key processes, including mitochondrial fusion, fission, and mitophagy. In this study, the effects of thymoquinone, the primary bioactive compound in *Nigella sativa* extracts and essential oil, were investigated with a focus on mitophagy in HepG2 (liver cancer) and HDF (human dermal fibroblast) cells. Thymoquinone is known for its antioxidant, anti-inflammatory, and hepatoprotective properties, and has been proposed as a complementary agent in cancer therapies. MTT assays were conducted to assess thymoquinone's cytotoxic effects, and IC_{50} values were determined. Additionally, cell proliferation was analyzed using colony formation assays. The primary objective was to evaluate whether thymoquinone activates mitophagy through the PINK1-Parkin pathway and its relationship with mitophagy inhibition via the fission mechanism. The involvement of DRP1 protein in fission was specifically examined. Both Real-time PCR and Western blot analyses were performed to investigate these processes. The findings demonstrated that thymoquinone activates mitophagy and contributes to apoptosis through the release of cytochrome c, with its effects on fission being mediated by DRP1. Overall, this study highlights the potential role of thymoquinone in modulating mitophagy in both cancerous and healthy cells, suggesting its beneficial use as an adjunct in therapeutic strategies.

Keywords: Thymoquinone, Mitophagy, Mitochondrial Dynamics, Cancer Cells, Healthy Cells, PINK1-Parkin Pathway

ORAL PRESENTATION

Bazı *Ornithogalum* türlerinin antioksidan ve antidiyabetik etki potansiyellerinin araştırılması

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Özet

Ornithogalum L. cinsi Asparagaceae familyasına ait olup dünyada yaklaşık 150 türü bulunmaktadır. *Ornithogalum* türleri sebze, süs bitkisi ve tıbbi bitki olmak üzere çeşitli amaçlarla kullanılmaktadır (1). Bu çalışma üç farklı *Ornithogalum* türünün (*Ornithogalum armeniacum* Baker, *O. neurostegium* Boiss. & Blanche, *O. sphaerocarpum* A.Kern.) toprak üstü ve soğan kısımlarından hazırlanan %80 etanol ekstrelerinin antioksidan ve antidiyabetik aktivitelerinin incelenmesi amacıyla yapılmıştır. Ekstrelerin antioksidan aktivitelerinin araştırılmasında demir indirgeyici güç, fosfomolibden analizi ile toplam antioksidan kapasite ve ABTS radikal süpürücü etki testleri kullanılmıştır. Antidiyabetik etki potansiyelinin araştırılmasında ise *α*-glukozidaz enzim inhibisyon yöntemi kullanılmıştır. *O. sphaerocarpum* toprak üstü ekstresinin diğer ekstrelere kıyasla en etkili ABTS radikal süpürme aktivitesine (%99.82 ± 0.18) ve total antioksidan kapasitesine (93.34 ± 3.14 mg askorbik asit eşdeğeri/g ekstre) sahip olduğu bulunmuştur. Ekstrelerin indirgeme güç sonuçları incelendiğinde en yüksek absorbans değeri (0.736 ± 0.02) *O. sphaerocarpum* toprak üstü ekstresinde (2 mg/mL) bulunurken, en düşük absorbans değeri (0.058 ± 0.01) *O. neurostegium* toprak üstü ekstresinde (0.5 mg/mL) bulunmuştur. *O. armeniacum* toprak üstü ekstresi 2 mg/mL konsantrasyonda *α*-glukozidaz enzimini %80.11 ± 0.50 oranında inhibe etmiştir (IC_{50}: 74.63 ± 0.76 µg/mL). Literatürde yer alan bilgiler doğrultusunda bu çalışma ile *O. armeniacum, O. sphaerocarpum, O. neurostegium* türlerinin antioksidan ve *α*-glukozidaz enzim inhitör aktivitesi ilk kez araştırılmıştır. *O. sphaerocarpum* toprak üstü ekstresinin antioksidan etki yönünden, *O. armeniacum* toprak üstü ekstresinin ise antidiyabetik (*α*-glukozidaz enzim inhibitör aktivite) etki yönünden potansiyel bir kaynak olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Antidiyabetik, Antioksidan, *Ornithogalum armeniacum*, *Ornithogalum neurostegium*, *Ornithogalum sphaerocarpum*

Kaynaklar

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ORAL PRESENTATION

Investigation of the Effects of Lycorine and Galanthamine Extracted from *Galanthus elwesii* on Viral and Parasitic Targets: An *In-Silico* Analysis and DFT Study

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Abstract

In this study, Density Functional Theory (DFT), ADME property analysis, and molecular docking simulations were employed to evaluate the electronic structure, antiviral potential, and antiparasitic effects of lycorine and galanthamine, two alkaloids extracted from *Galanthus elwesii*. DFT calculations revealed that lycorine has a lower e_gap compared to galanthamine, suggesting higher reactivity and lower stability, enhancing its potential as a drug candidate. Pharmacokinetic profiling indicated that galanthamine (TPSA: 41.93 Å², logP: 0.797) has a lower total polar surface area (TPSA) and higher lipophilicity (logP) compared to lycorine (TPSA: 62.16 Å², logP: -0.268), indicating that galanthamine may possess superior absorption and permeability characteristics. ADME analysis also identified galanthamine as having a lower AMES toxicity score, implying reduced mutagenic risk. A total of nine target proteins, representing viral and parasitic diseases Zika virus, malaria, leishmaniasis, and dengue, were chosen for molecular docking. Molecular docking studies demonstrated that lycorine exhibited superior binding interactions (-8.76 kcal/mol), particularly against Leishmania, and displayed stronger binding affinity across all selected target proteins. Despite galanthamine's lower toxicity profile, lycorine's enhanced reactivity and stronger binding properties suggest its higher efficacy as a therapeutic candidate based on DFT and molecular docking results, while galanthamine shows potential based on its favourable ADME profile.

Keywords: Lycorine, Galanthamine, ADMET, Antiviral and Rapasitic, Molecular Docking

ORAL PRESENTATION

Sarı kantaron atıklarından üretilen katı biyoyakıtların Coats-Redfern yöntemi ile yanma kinetiklerinin belirlenmesi

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Özet

Sarı kantaron, özellikle içerdiği biyoaktif maddeler sayesinde gıda, ek gıda, ilaç ve kozmetik alanında oldukça yoğun kullanılan ticari bir bitkidir. Bu bitkinin biyoaktifçe zengin yağı alındıktan sonra geriye kalan kısmı çoğunlukla faydası olmayan bir atık olarak değerlendirilmektedir. Bu posa, uygun şekilde işlenmediği takdirde çevresel atıklara dönüşmekte ve ekonomik bir değer taşımamaktadır. Bunun önlenmesi için bu atıklar çeşitli termal yöntemler kullanılarak değerli biyoyakıtlara dönüştürülmekte ve fosil yakıtlara alternatif olarak kullanılabilmektedir. Bu çalışma, sarı kantaron atıklardan elde edilen katı biyoyakıtların yanma davranışlarının ve kinetiklerinin Coats-Redfern yöntemi kullanılarak incelenmesini amaçlamaktadır. Bu kapsamda ham sarı kantaron atıkları ve bunlardan 400 °C sıcaklık ve 20 °C/dk ısıtma hızı koşullarında azot atmosferinde üretilen biyocharlar bir termogravimetrik analiz cihazında yakılmıştır ve yanma aktivasyon enerjisi değerleri farklı model denklemleri kullanılarak Coats-Redfern yöntemi ile belirlenmiştir. Elde edilen sonuçlara göre kuru sarı kantaron örneklerinin yanması iki aşamada gerçekleşirken, biyocharların yanması tek aşamada gerçekleşmiştir. Biyokütlenin yanma aktivasyon enerjisi değerleri birinci aşamada 45 - 116 kJ/mol arasında değişmişken, ikinci aşamada ise 45 - 313 kJ/mol arasında değişmiştir. Biyocharın yanma aktivasyon enerjisi ise 61 - 208 kJ/mol arasında değişmiştir. Elde edilen R^2 değerlerine göre biyokütle (birinci ve ikinci aşama) ve biyochar örneklerinin yanma mekanizmaları için en uygun modeller sırasıyla R3 (53.85 kJ/mol), F1 (93 kJ/mol) ve F2 (140.63 kJ/mol) olarak belirlenmiştir. Elde edilen sonuçlara göre sarı kantaron atıklarının biyocharlara oranla daha kolay yandığı, ancak enerji verimlerinin daha düşük olduğu anlaşılmıştır. Bu biyocharlar yanma sistemlerinde kömüre alternatif olarak veya kömürle karıştırılarak kullanılabilir ancak yanma verimi ve kalitesinin arttırılması için tüm mekanizmaların belirlenmesi ve optiizasyon çalışmalarının yapılması gerekmektedir.

Anahtar kelimeler: Biyokütle, Sarı Kantaron atığı, Biyochar, Termogravimetrik Analiz, Coats-Redfern Yöntemi

ORAL PRESENTATION

Investigation of Waste Frying Oil to Biofuel Using Advanced Thermal Pyrolysis Techniques

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Abstract

Waste oils originating from food consumption is hazardous to human health and the environment, as they contain degraded additives and undesirable components. In Turkey, restaurants, fast food chains and other commercial enterprises produce approximately 300,000 tons of waste frying oil (WFO) annually as a result of the use of vegetable oils. Due to its low cost and easy availability, it can be considered as a promising alternative for biofuel production. There are various methods such as thermal pyrolysis, transesterification and hydrothermal cracking to convert WFO into a useful fuel. In this study, experiments were conducted under different conditions using the thermal pyrolysis method, and products with diesel-like properties were targeted as a result of fast pyrolysis. A new experimental setup was designed for fast pyrolysis. In this setup, the reactor is heated to a predetermined temperature, the sample to be pyrolyzed is sprayed into the system with the help of a pressurized pump, and the product fed into the system in the form of small particles is pyrolyzed instantly. Before starting the experiment, the reactor was purged with nitrogen gas to remove oxygen and again, 5 ml/m nitrogen was fed into the system during the reaction period. The system temperatures were set to 500, 550, and 600 °C. WFO was injected into the system for 0.1 s and then waited for 5 s for the system to become stable. Each injection delivered about 0.35 ml of WFO, with the system automatically controlled by a programmable logic controller (PLC). Transformation temperatures were monitored using thermocouples placed at three points in the reactor. FT-IR analyses revealed structural transformations in the synthesized samples due to the hydrothermal reaction. At 1745 cm^{-1}, C=O represents the ester stretching and a decrease in these bonds is observed.

Keywords: Waste frying oil, Fast pyrolysis, FT-IR

ORAL PRESENTATION

Mechanisms of pexidartinib-induced hepatotoxicity

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Abstract

Pexidartinib is a kinase inhibitor that was approved by the Food and Drug Administration (FDA) in 2019 for the treatment of tenosynovial giant cell tumors, a highly aggressive type of neoplasm. Pexidartinib selectively inhibits colony-stimulating factor 1 (CSF1), which is overexpressed by tenosynovial giant cell tumors. Pexidartinib has a black box warning for hepatotoxicity and FDA warns the patients about concomitant administration with other substances that especially have hepatotoxic potential. However, the mechanism of pexidartinib-induced is not yet known. In the present study, it was aimed to investigate the molecular mechanisms of pexidartinib-induced hepatotoxicity using HepG2 cell line. The cells were exposed to 0-25 μM pexidartinib for 24 h. Cell viability was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Oxidative stress was measured by determination of glutathione and malondialdehyde levels using ELISA kits, and reactive oxygen species (ROS) production with a fluorometric kit. Additionally, changes in mitochondrial ROS, mitochondrial membrane potential, and mitochondrial mass were evaluated fluorometrically using specific fluorescence dyes. According to the results, Pexidartinib decreased cell viability by nearly 30% at the highest concentration. Pexidartinib induced oxidative stress and increased mitochondrial mass and mitochondrial ROS production in HepG2 cell line at high concentrations. Besides, it caused fluctuations in mitochondrial membrane potential after 24 h exposure. This preliminary study suggests that oxidative stress and changes in mitochondrial function may play a role in pexidartinib-induced hepatotoxicity. However, further detailed studies should be performed to confirm our findings. Understanding underlying mechanisms of pexidartinib-induced hepatotoxicity may contribute to developing new treatment strategies to prevent liver damage.

Keywords: pexidartinib, hepatotoxicity, oxidative stress, mitochondrial toxicity,

ORAL PRESENTATION

Novel retinoid-benzimidazole derivatives: Synthesis, anticancer activity, molecular docking and *in silico* ADME studies

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Abstract
Cancer remains a significant public health concern due to its devastating impact on human health. Hence, the search for novel compounds that could mitigate this challenge continue to garner considerable interests in cancer research. Retinoids are novel family of compounds structurally related to vitamin A or retinol with remarkable anticancer properties. In this study, we aimed to synthesize and evaluate the cytotoxic activity of novel retinoid benzimidazole derivatives against human breast adenocarcinoma cell line (MCF-7). The synthesis follows a biphasic multi-step reaction in which the final compounds bear tetrahydronaphthalene moiety or retinoidal head fused to the C2 position of N^1-substituted alkylsulfonyl benzimidazole derivatives. The synthesized compounds (AZ1 – AZ14) were characterized by mass spectrometry, ^{1}H NMR and ^{13}C NMR analyses. Three of the compounds tested (AZ1, AZ8, AZ14) via MTT assay demonstrated the most potent activity against MCF-7 cells with respect to the reference drug all-*trans* retinoic acid (ATRA) and were thus selected for further cytotoxic evaluation by using xCELLigence Real-Time Cell Analysis. Molecular docking simulation using RAR ligand-binding domain in complex with ATRA showed these potent derivatives bind to the RAR's active site as ATRA with slightly higher docking scores. Further *in silico* ADME studies indicate these compounds exhibit favourable physicochemical, pharmacokinetic and drug-like properties that may render them promising candidate as anti-cancer compounds.

Keywords: Retinoid**,** benzimidazole, ATRA, RAR, MCF-7

ORAL PRESENTATION

Şalgam Suyu ve Üretimi

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Özet

Fermentasyon, uzun yıllardır yiyecekleri muhafaza etme amacı ile kullanılan yöntemlerden birisidir. Fermentasyon sonucu oluşan laktik asit aracılığı ile gıdanın asidik özellikleri artmakta; gıdanın, kokusu ve aroması değişmekte ve meyve ve sebzeler dayanıklı hale gelmektedir. Günümüzde tüketilmekte olan gıdaların 1/3'ünü fermente gıdalar oluşturmakta ve turşular, yoğurt, kefir, bazı alkollü içkiler, zeytin, tarhana ve şalgam gibi ürünler önemli fermente gıdalar arasında sayılmaktadır. Ülkemize özgü bir içecek olan şalgam suyu geleneksel olarak bulgur unu, ekşi hamur, içme suyu ve yemeklik tuzun karıştırılması ile laktik asit fermentasyonuna tabii tutulması ile üretilmektedir. Elde edilen bu özüt, mor havuç ve isteğe bağlı olarak acı toz biber ilavesi ile tekrar fermentasyona tabii tutulduktan sonra ısıl işlem yardımı ile dayanıklı hale getirilmektedir. Şalgam, içeriğindeki laktik asit sayesinde kolay sindirilir, iştah açıcıdır ve mineral açısından zengindir. En fazla Adana ve yakın çevrelerinde tüketilirken, şalgam suyunun şişelenmesi ile birlikte ülke genelinde tüketimi artmaktadır. Ayrıca son yıllarda İstanbul, Ankara gibi büyük şehirlerde de üretimi gerçekleştirilmektedir. Şalgam suyu üretiminde kullanılan siyah renkli havuç, antosiyanin bakımından zengindir ve şalgam suyunun kendine özgü rengi siyah havuçtan gelen renk pigmentlerinden kaynaklanmaktadır. Şalgam suyunun farklı yöntemlerle üretimi mevcuttur ancak endüstriyel olarak genellikle geleneksel yöntem ile üretim ve doğrudan yöntem tercih edilmektedir. Bazı kaynaklarda starter kültür ilavesi ile şalgam suyu üretimi üçüncü bir yöntem olarak da ele alınmaktadır. Üretim yöntemleri de dahil olmak üzere hammadde çeşitliliği dolayısıyla duyusal tercihlere göre üretim gerçekleştirilmektedir.

Anahtar kelimeler: Şalgam suyu, fermantasyon, laktik asit bakterileri

ORAL PRESENTATION

Determination of antioxidant activity, total phenolic and flavonoid contents of *Glycyrrhiza glabra* L. varieties and investigation of anticancer effect in breast cancer cells

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Abstract

Cancer is one of the leading global health problems with a high mortality rate. Some plants with their biologically active contents can increase cytotoxicity on cancer cells. This study aims to determine the anticancer effect,antioxidant activity and total phenolic(TPC), flavonoid(TFC) contents of ethanol extracts of *Glycyrrhiza glabra* L. varieties. For this purpose;three root from two different varieties of G*lycyrrhiza glabra* L.-*Glycyrrhiza glabra* var.*glabra*(GB), *Glycyrrhiza glabra* var.*glandulifera*(GF1) and *Glycyrrhiza glabra* var.*glandulifera*(GF2)- were freshly supplied.80% ethanol extracts of GB, GF1 and GF2 were prepared.Then TPC,TFC were determined. The antioxidant activities were evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging capacity tests. The effect of 80% ethanol extracts of GB,GF1,GF2 on cell viability in breast cancer cells was determined by MTT and MCF-7 cell line was used. In addition, NIH-3T3 cell line was used to determine the effect on healthy cells. The TPC was calculated as 100.60±5.06, 127.90±0.30, 69.01±0.30 mg GAE /g extract for GB,GF1,GF2,respectively.TFC was calculated as 80.07±0.15, 25.35±0.0, 16.58±0.31 mg KE/g extract for GB,GF1,GF2, respectively.IC50 values of GB,GF1,GF2 were determined as 371.00±0.14 and 74.22±0.42; 87.47±1.27 and 19.89±0.45; 119.10±0.35 and 62.98±1.33 for DPPH and ABTS assay, respectively.GB,GF1,GF2 were applied to the cells at seven different concentrations as 400- 0 µg/mL for 24 and 48 hours. As a result, it was observed that all of GB,GF1,GF2 inhibited cell viability in the MCF-7 cell line for 24 and 48 hours. When the effect of GF1, which has more effective anticancer activity than the other two types, on cell viability in the NIH-3T3 cell line was applied, it was observed that it did not significantly reduce cell viability in healthy cells. The obtained results, when supported by further studies, will contribute to the creation of new treatment strategies that can be used in cancer treatment of *Glycyrrhiza Glabra* L plant roots.

Keywords: MCF-7, *Glycyrrhiza glabra* L, Breast cancer, Antioxidant activity, Anticancer, Total phenolic content

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ORAL PRESENTATION

Characterization of VTE5 Gene in Bread Wheat (*Triticum aestivum* L.) and Its Partial Sequencing

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Abstract

Wheat is one of the most important crop plants consumed by people. Wheatgrass is rich for vitamin E and C, some minerals, phenolic compounds and bioactive compounds. Phytol kinase encoded by the VTE5 gene has an important role for tocopherol synthesis. The aim of this study was to explore the VTE5 gene and its sequencing in bread wheat (*Triticum aestivum* L.). Seeds of the Tosunbey cultivar were surface sterilized by sodium hypochlorite (3%) for 5 minutes and rinsed with sterile distilled water several times. Also, seeds were washed with ethanol (80%) for one minute and rinsed with sterile distilled water. Seeds were sown using Hoagland's solution. The VTE5 gene (NCBI accession number: DQ163025.1) was searched in the Ensembl Plants database (https://plants.ensembl.org/index.html) to find VTE5 homoeologous. The CTAB method was used to isolate DNA from wheat seedlings. Gene specific primers were designed, and PCR was performed. Sequencing of PCR products was performed by using ABI 3500 XL. Vector NTI program and NCBI BLAST tools were used to analyze sequenced results. DQ163025.1 mRNA sequence was 100%, 96.7% and 97.9% similar to chromosomes 2D, 2A and 2B, respectively. The VTE5 gene has six exons and five introns. In the light of sequencing results, a 405 bp fragment of the gene was amplified from PCR products. Fragment included the last part of exon five and complete of exon six. As a result, a good target for gene expression and functional genomic studies for the VTE5 gene was determined.

Keywords: VTE5 gene, Bread wheat, Sequencing, Phytol kinase.

Acknowledgements: This study was a part of the M.K. Master of Science Thesis. This study has been supported by Van Yuzuncu Yil University Scientific Research Projects Coordination Unit under grant number FYL-2020-8844.

ORAL PRESENTATION

Drug candidate molecule vulpinic acid targets apoptosis signaling pathway in oral squamous cell carcinoma

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Abstract

Oral cancers are the sixth most common type of cancer worldwide and 90% of all oral cancers are oral squamous cell carcinomas (OSCC). Although survival rates have increased in recent years, there is still a need to identify drug candidate molecules for use in the treatment and prevention of OSCC. The aim of this study is to reveal the therapeutic effect potential of vulpinic acid (VA) on OSCC. In order to ascertain the potential of VA to exert an antiproliferative effect, wound healing, colony formation and gene expression profiles, a series of experiments was conducted within the scope of the study. The study demonstrated that VA reduced cell viability in OSC19 cancer cells in a dose and time dependent manner without exerting a significant cytotoxic effect on normal epithelial cells by xCELLigence analysis. It was determined that the invasive potential of OSCC cells was suppressed by applying VA. In colony formation assay, a statistically significant decrease was observed in the number of colonies after VA application compared to the control. According to the qRT-PCR analyses, significant increase in the expression levels of the *BID* (2.48) gene was determined after VA treated. An increase in gene expression levels of *CASP4* and *CASP7* was determined, which may indicate that apoptosis has been induced. The gene expression of the *BIRC5* gene has shown downregulated, which may indicate increased in apoptotic signaling. The relationship between *BID, BIRC5, CASP4* and *CASP7* genes were also evaluated and it was deduced that the intrinsic apoptosis pathway, which was characterized by mitochondrial damage and caspase activation, was strongly activated and programmed cell death was induced in VA treated OSC19 cells. This finding highlights the potential of VA as a drug candidate molecule that can be used in the development of new therapeutic strategies for the treatment of OSCC.

Keywords: apoptosis, oral squamous cell carcinoma, therapeutic activity, vulpinic acid

ORAL PRESENTATION

Synthesis, Characterization and BSA Interactions of a New Copper(II) Complex Containing Azo Type Ligand

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Abstract

In this study, a novel copper(II) complex featuring an azo type ligand bearing sulfanilamid and 1,3-dimethyl barbituric acid was synthesized and characterized. The structure of the ligand was confirmed using FTIR and ^{1}H NMR, while the structure of copper(II) complex was elucidated through various analytical techniques including melting point determination, FTIR spectroscopy, UV-Vis. spectroscopy, elemental analysis, magnetic moment measurements and MALDI-TOF-MS. The FTIR spectrum revealed characteristic stretching bands that confirmed the N2O2 type of coordination of the ligand to the copper(II) center. Elemental analysis was consistent with the proposed molecular formula, while mass spectrometry provided 2:1 (ligand to metal ratio) coordination and the molecular weight of the complex. The binding affinity of the synthesized copper complex towards bovine serum albumin (BSA) was investigated to explore its potential as a bioactive compound. UV-Vis spectroscopy and fluorescence quenching methods were employed to study the interaction between the copper complex and BSA. The results indicated strong binding affinity (K_{bin}=5.13x10^{4}), suggesting the potential of this complex for therapeutic applications, particularly in drug delivery systems. Fluorescence quenching constant (K_{sv}=2.17x10^{4}) and binding parameters (n, dG) were calculated, demonstrating a static quenching mechanism. Given the unique structural features of the copper(II) complex, which includes a combination of sulfanilamid and barbituric acid derivatives, further studies are warranted to explore its biological activities and potential applications in medicinal chemistry. The strong interaction with BSA highlights the complex's potential as a carrier molecule for pharmaceutical agents.

Keywords: metal complexes, azo type ligands, copper(II) complexes, BSA interactions.

ORAL PRESENTATION

RP-HPLC Method Development for Determination of New Butenafine and Curcumin Combination

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Abstract

The combination of butenafine, an antifungal agent used to treat skin infections, and curcumin, a natural anti-inflammatory compound, holds promise for enhancing therapeutic efficacy in various dermatological disorders. Butenafine exerts its antifungal effect by inhibiting fungal growth, while curcumin potentiates its activity through synergistic mechanisms, including modulation of inflammatory pathways and antioxidant properties that enhance the overall therapeutic response. The purpose of this work was to develop a dependable HPLC method for assessing the combination of butenafine and curcumin active medicinal components.
A UV-Vis detector and a C18 column (150 mm × 4.6 mm, 5 µm particle size) were used in a high-performance liquid chromatography (HPLC) system. Methanol and water were combined in an isocratic elution method to form the mobile phase. The wavelength used for detection was 254 nm, and the flow rate was fixed at 1.0 mL/min. Curcumin and butenafine were shown to have retention times of 1.8 and 10.6 minutes, respectively, according to our research. The HPLC method demonstrated excellent linearity for butenafine and curcumin over the concentration ranges of 1.25-15 µg/mL with correlation coefficients (R^2) of 0.997.
The devised HPLC method is a dependable instrument for simultaneously quantifying butenafine and curcumin in a combination formulation. This method's accuracy, precision, and specificity make it appropriate for routine quality control analysis, assuring consistent and dependable supply of these active components. This synergistic interaction between butenafine and curcumin could lead to more effective treatments and broaden the scope of therapeutic applications in dermatology.

Keywords: butenafine, curcumin, HPLC, development

ORAL PRESENTATION

Simultaneous Analysis of Major Compounds in Aronia-Containing Food Supplements by HPLC

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Abstract

Aronia melanocarpa fruit is a valuable component of a healthy diet due to its rich antioxidant activity and high content of bioactive compounds. Recently, many food products and food supplements have emerged due to the increasing interest in aronia. In this study, a liquid chromatography method for the simultenous quantification of the anthocyanins cyanidin-3-galactoside, cyanidin-3-arabinoside and cyanidin-3-glucoside in aronia was developed and validated according to ICH Q2 (R1) guidelines. In this HPLC system, the simultenous analysis of the three active substances was performed on a Phenomenex, Gemini C18, (5µm, 150 x 4.6mm) column at 30°C. As mobile phase, 1% glacial acetic acid:ACN:phosphoric acid (510:485:5) (h:h:h) and 0.5% phosphoric acid solutions were used and a gradient system was applied at a flow rate of 0.8 mL/min. Cyanidin-3-galactoside and cyanidin-3-arabinoside were found linearly in the concentration ranges 2.5-12.5 µg/mL and cyanidin-3-glucoside 0.25-4.0 µg/mL ($r>0.99$). The developed and validated method was successfully applied to the analysis of samples containing aronia.

Keywords: Cyanidin-3-galactoside, Cyanidin-3-glucoside, Cyanidin-3-arabinoside, Aronia, HPLC

ORAL PRESENTATION

Antimicrobial, Antibiofilm, and Anticancer Potential of L-Tyrosine Amino Acid

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Abstract

Amino acids play a crucial role in numerous biological processes, including protein synthesis, enzyme and hormone production, immune system modulation, neurotransmitter synthesis, energy production, and tissue repair. They also exhibit protective effects against infections and tumour cells due to their antimicrobial, antioxidant, anticancer and immunomodulatory properties. Therefore, in this study, we aimed to determine the antimicrobial, antibiofilm, and anticancer effect of L-Tyrosine amino acid that is a precursor to several important neurotransmitters, including dopamine. The antimicrobial activities and biofilm inhibitions against *Candida tropicalis* T26, *Proteus mirabilis* U15, and *Staphylococcus epidermidis* W17 clinical strains were evaluated using the Broth Dilution and Crystal Violet Binding assays respectively. Meanwhile, the cytotoxicity of L-Tyrosine on HeLa and MDA-MB-231 cell viabilities was determined through the MTT assay. According to our results, L-Tyrosine did not cause any antimicrobial effect against any of the tested strains with a Minimum Inhibition Concentration (MIC) and Minimum Microbicidal Concentration (MMC) values > 200 mg/mL. However, 0.5MIC of L-Tyrosine caused inhibitions against biofilm formation of all the tested clinical isolates, with the highest inhibition observed against *S. epidermidis* W17 (89.75 %). Cell viability results showed that in both cancer cell types, viability at 24 hours decreased significantly starting from the lowest concentration of 0.01 mg/mL. As the concentration increased, the number of dead cells significantly increased compared to the control ($p<0.05$). Additionally, a statistically significant decrease in cell viability was observed with increasing concentrations in the 48-hour data compared to the control ($p<0.05$). Moreover, compared to the MDA_MB_231 cell line, cell viability was more effectively suppressed in HeLa cells. The findings of this study will contribute to the development of anti-biofilm and anti-cancer strategies focused on L-Tyrosine synthesizable molecules. Nonetheless, it would be beneficial to conduct comprehensive experimental and clinical trials as well.

Keywords: Antibiofilm effect, Anticancer activity, *Candida tropicalis*, Cytotoxicity, *Proteus mirabilis*, *Staphylococcus epidermidis*

ORAL PRESENTATION

Synthesis, Characterization and Determination of Biological Activities of Sugar Based Coumarin Metal Complexes

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Abstract

Carbohydrates are the most abundant biomolecules in nature because they are the primary source of energy for living organisms. They are also essential for numerous biological recognition processes. Coumarin derivatives have strong pharmacological activity, low toxicity and side effects, less drug resistance, high bioavailability, wide spectrum, better curative and the like many coumarin compounds with effects are being actively studied as medical candidates for drugs [1]. Coumarins and their schiff bases have many biological activities, such as antiviral, antimicrobial, antidiabetic, anticancer, antioxidant, anticoagulant, enzyme activity and anti-inflammatory [2]. Furthermore, copper complexes of coumarin Schiff-base have been shown to have antifungal, antibacterial, antioxidant, antiproliferative, and antiviral properties [3]. For this importance, we decided to investigate the biological activity of a series of coumarin Schiff base ligands and their copper complexes containing different sugar substituents. Primarly, the syntehsis of the sugar based coumarin Schiff base ligands and their metal complexes from D-Arabinose, D-Galactose, and D-Glucose were done. All compounds are purified by column chromatography and then the pure compounds were characterized by ^{1}H and ^{13}C NMR, FT-IR, mass analysis, and melting point determination methods. Finally, the biological activities of the synthesized compounds were determined by antimicrobial activity tests.

Keywords: Coumarin, Schiff Base, Copper Complex, Antimicrobial Activity

Acknowledgment: We would like to thank the Faculty of Science, Ege University (FLP-2021-23487) for financial support of this work.

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ORAL PRESENTATION

Determination of the Phenolic and Flavonoid Contents in Lyophilized Ethanolic Extracts of Marshmallow Plant (*Althaea officinalis* L.)

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Abstract

The marshmallow plant, which is a medicinal plant, (*Althaea officinalis* L.) belongs to the Malvaceae family. The aim of this study was to investigate the total phenolic and flavonoid contents in marshmallow flower (MF) extracts prepared in different alcohol concentrations (MF+EtOH-80%, MF+EtOH-90% and MF+EtOH-100%). For this purpose, commercially available marshmallow flowers were purchased from local market. Flowers were ground using a blender. 30g of flower powder was put into a 300 mL-alcohol solution at different concentrations, and mixed. Solutions were filtrated with the aid of a vacuum pump system. Alcohol was evaporated using rotavapor equipment. Frozen samples were freeze dried to obtain lyophilized extracts. Extract yield was calculated for each sample. Total phenolic content was calculated as mg gallic acid equivalent/g extract. Total flavonoid content was calculated as mg quercetin equivalent/g extract. According to extract yield results, the highest yield was determined in the MF+EtOH-80% as 18.3%. Yield was decreased depending on increasing alcohol concentration, 11.5% and 7.4% in MF+EtOH-90% and MF+EtOH-80%, respectively. MF+EtOH-80% had a significantly higher total phenolic content than EtOH-100%. MF+EtOH-90% had the significantly higher total flavonoid content than MF+EtOH-80% and MF+EtOH-100%. As a conclusion, ethanol concentration used in extract preparation affects extract yield, total phenolic and flavonoid contents.

Keywords: Marshmallow plant, Total phenolic, Total flavonoid, Ethanol.

ORAL PRESENTATION

Investigation of adsorptive properties of chitosan-alginate biocomposite for effective removal of erythrosine B dye

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Abstract

Biocomposite materials are effective and efficient adsorbents in the treatment of dye-contaminated wastewater. In this study, the adsorption properties of chitosan (Ch)-alginate (A) biocomposite for Erythrosine B dye were investigated. FT-IR, SEM-EDX, and point of zero charge analyses support that the adsorption process can occur via hydrogen bonds and electrostatic interactions in Ch-A biocomposite-containing functional groups. The maximum adsorption capacity of Ch-A biocomposite was 319 mg g^{-1} and the adsorption energy value showed the physical nature of the adsorption process. Adsorption thermodynamic studies showed that the adsorption process was endothermic and spontaneous. When all adsorption studies were evaluated together, Ch-A biocomposite is an alternative, effective, efficient, and promising adsorbent for the removal of erythrosine B from wastewater.

Keywords: Chitosan, alginate, biocomposite, Erythrosine B, removal, wastewater treatment

Acknowledgements

The present study was supported by the Sivas Cumhuriyet University Projects Commission (Project number: SBF-2023-101).

ORAL PRESENTATION

Design and biological assessment of novel platinum(II) complexes with benzimidazole ligands against cancer cells

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Abstract

Platinum-based drugs, such as cisplatin, carboplatin, and oxaliplatin, have been widely used in anticancer treatments for many years. However, their effectiveness is limited by significant side effects, the development of drug resistance, and reduced efficacy against certain cancer types. This study aimed to develop new platinum(II) complexes (PtL_2Cl_2) (Fig 1) containing (*S*)-1-(1*H*-benzimidazol-2-yl)ethyl acetate, (*R*)-1-(1*H*-benzimidazol-2-yl)ethyl acetate, and (*RS*)-1-(1*H*-benzimidazol-2-yl)ethyl acetate, and evaluate their cytotoxic effects on human hepatocellular carcinoma (HepG2), human colorectal adenocarcinoma (LoVo), and human healthy fibroblast (WS-1) cells in vitro. Initially, 2-substituted benzimidazole ligands were synthesized, followed by the creation of novel PtL_2Cl_2 complexes through their reaction with K_2PtCl_4. The structures of these new platinum complexes were confirmed using high-resolution mass spectrometry (HRMS), elemental analysis, infrared (IR) spectroscopy, and nuclear magnetic resonance (NMR). The in vitro cytotoxicity was assessed using the ATP assay. Among the tested compounds, the platinum complex **MG1**, which has the S isomer as a ligand, showed the highest effectiveness against the cancer cell lines, and all complexes demonstrated selective cytotoxicity towards cancer cells.

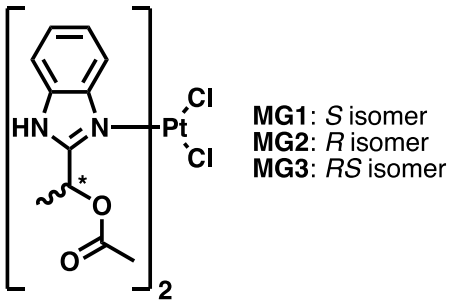

Fig 1. The structure of novel platinum complexes

Keywords: Platinum complexes, Benzimidazole, LoVo, HepG2, WS-1

ORAL PRESENTATION

Use of Ulvan as coating material in soft-gel production

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Abstract

In soft-gel (soft-gel, soft capsule) products, polysaccharide-based alginate, starch, carrageenan, pectin, and gums are employed as coating materials, while protein-based whey and gelatin are utilized as coating materials. In this study, the potential of ulvan polysaccharide as an alternative to the traditionally used gelatin and/or modified starch was investigated. Ulvan is a sulfated polysaccharide derived from green macroalgae that has the potential to be utilized as a stabilizing and emulsifying agent in food and pharmaceutical applications due to its amphiphilic character. These characteristics render it biocompatible and biodegradable. The extant research indicates that ulva can serve as an alternative to other natural algal polysaccharides and synthetic chemicals. Given its higher density than Arabic gum and its structural similarity to alginate, ulvan emerges as a novel coating material. It was thus demonstrated that a polysaccharide derived from entirely sustainable sources could be employed as an alternative to gelatin or as a supportive outer coating material for the soft gel capsule structure, commonly referred to as a "soft-gel" for encapsulation. In the initial phase of the investigation, the Ulvan polysaccharide was successfully extracted from the Ulva sp. macrolags gathered from the Marmara Sea. To characterize the extracted crude ulvan polysaccharide, an FTIR analysis was conducted. The results of the analysis indicated that the extracted polysaccharide exhibited significant structural similarities to commercially available ulvan. The polysaccharide was supported with different alginate, ulvan, and protein isolate in order to investigate the possibility of its use without the need for an additional purification process. The outer phase was used for "oil encapsulation." The soft gels were prepared using the emulsification method and subsequently subjected to vacuum drying and storage in airtight packages at 4°C. The characterization results indicated that the samples prepared with an inner phase:outer phase ratio of 1:3.5 and an ulvan:pea protein:alginate ratio of 1:2:4 exhibited stronger characteristic bonds and binding. These ratios may be applicable for further ulvan polysaccharide applications and analyses.

Keywords: Soft-gel, Soft Capsule, *Ulva lactuca*, Ulvan, Encapsulation

ORAL PRESENTATION

Antioxidant Activity of The Various Extracts From *Maesa kamerunensis* Mez.

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Abstract

The Cameroonian medicinal plant, *Maesa kamerunensis* (Myrsinaceae) is used in the treatment of several illness such as typhoid and the leaves are consumed as tea. In this study, the antioxidant properties of (hexane, ethyl acetate, methanol, n-butanol and water) extracts of *Maesa kamerunensis* were determined. ABTS·+ scavenging, *β*-carotene-linoleic acid, Cupric-reducing antioxidant capacity (CUPRAC), DPPH·scavenging, and metal chelating assays were performed to evaluate antioxidant activities of the extracts. The spectrophotometric methods were used to determine the total phenolic, flavonoid, ascorbic acid, b-carotene, and lycopene contents. Among the extracts tested, the crude extract of stem' showed the highest antioxidant activity in CUPRAC ($A_{0.50}$: 9.34±0.71 µg/mL), ABTS·+ (IC_{50}: 3.27±0.25 µg/mL), DPPH (4.09±0.45 µg/mL), *β*-carotene linoleic acid (IC_{50}: 6.18±0.25µg/mL) assays. These results show that *M. kamerunensis* could be used of antioxidants in food, pharmaceutical, and cosmetic industries.

Keywords: *Maesa kamerunensis*, antioxidant, DPPH, ABTS

ORAL PRESENTATION

The Effects of Plastic Pollution on Algae

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Abstract

Plastics are the most adaptable material in the world because they are so cheap to make and can be molded into a huge range of sizes, shapes, and densities. It is well known that tons of plastic enter the environment each year and do harm, despite its many advantages. High levels of microplastics are found in freshwater systems, marine ecosystems, and sediments due to inadequate waste management, ongoing accumulation, and poor biodegradation brought on by the breakdown of bigger plastic trash. Less than 5 mm in size, microplastics are extremely important due to the possible damage they could do to aquatic food webs. It is well recognized that ingesting microplastics can have detrimental consequences on a variety of trophic levels of creatures, including fish, crabs, mammals, and zooplankton. These drawbacks include stunted growth, decreased fertility, changed oxygen uptake, shorter life span, restricted ability to absorb nutrients, and elevated antioxidant activity with associated enzymes. Microplastics also have an impact on phytoplankton. Additionally, fundamental consumers like zooplankton, tiny fish, and crustaceans receive their nourishment from phytoplankton. These foods are subsequently ingested by higher-level consumers. Thus, higher trophic level animals may suffer harm as a result of the harmful impacts of contaminants on algae. Both macro- and microalgae are impacted by microplastics. The aim of this study was to investigate the interaction of microplastics with microalgae and macroalgae and thus their effects on living organisms in aquatic ecosystems.

Keywords: Algae, Aquatic ecosystems, Macroalgae, Microalgae, Phytoplankton, Plastic

ORAL PRESENTATION

Design and synthesis of two novel hydrazone derivatives as potent tubulin inhibitors

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Abstract

Cancer is the most common cause of death in the world after cardiovascular diseases. Various enzyme inhibitors, such as tubulin inhibitors, can be used for cancer chemotherapy. Microtubules are critical structures for eukaryotic cells. Since they play a role in cell division, they stand out as important targets in anticancer therapy. Rapid and uncontrolled proliferation in cancer cells makes these cells more sensitive than healthy cells. The colchicine binding site is one of the most studied binding sites in this enzyme. This inhibitor type usually carries trimethoxyphenyl moiety to interact with Cys241 in the enzyme. On the other hand, hydrazones are of interest functional groups with various activities, including anticancer. Based on the literature, two novel hydrazone derivatives carrying trimethoxyphenyl moiety were designed: *N*'-[(3-substitued-4-hydroxy-5-methoxyphenyl)methylidene]-3,4,5-trimethoxybenzohydrazide; TR-MP, and TR-FP. Molecular docking studies revealed that both *E* and *Z* isomers of the compounds have binding scores ranging from -6.6 to -7.5 kcal/mol (coligand: -6.8 kcal/mol). The compounds occupy the cavity in the colchicine binding site with various hydrogen binds: GLN245, LEU246, ARG221, CYS239, and THR351. Then, the compounds were synthesized, and their structure was characterized *via* ^{1}H and ^{13}C NMR spectroscopy. In the second part of the study, the compounds *in vitro* were tested on the human endometrial adenocarcinoma cell line (HEC-1B).

Keywords: Anticancer, hydrazone, tubulin inhibitör

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ORAL PRESENTATION

Fresh and Dry Weight of *Ceratophyllum demersum* L. Under Different Light Conditions with Arsenic and Mercury Exposure

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Abstract

This study investigates how different light conditions affect the fresh and dry weights of *Ceratophyllum demersum* L. plants exposed to arsenic (As) and mercury (Hg). The light conditions tested were white LED, red LED, blue LED, and white fluorescent light.
Fresh Weight of Arsenic-Treated Plants: Fresh weight decreased with higher As concentrations and longer exposure durations. The control group exhibited the highest fresh weights on the 21st day, while the lowest weights were observed under 1 mg/L As on the same day. White LED light enhanced fresh weights at lower As concentrations (0.01 and 0.1 mg/L) on days 1 and 7 compared to the control, but significant reductions occurred at higher concentrations on days 14 and 21. Similar patterns were observed under red and blue LED lights. The lowest fresh weights across all conditions were found under white fluorescent light on the 21st day, with white LED, blue LED, and red LED following.
Dry Weight of Arsenic-Treated Plants: The control plants, which were not treated with As, had the highest dry weights. High As concentrations and extended exposure reduced dry weights, with the lowest values recorded on the 21st day at 1 mg/L As across all light conditions.
Fresh Weight of Mercury-Treated Plants: Fresh weight significantly decreased with increasing Hg concentrations ($p<0.05$). The highest fresh weights were recorded in the control group, while the lowest were found on the 21st day with 0.2 mg/L Hg exposure. Lower Hg concentrations led to some increases in fresh weight compared to the control, whereas higher Hg exposure resulted in reductions. The lowest fresh weights were observed under white fluorescent light, with blue LED, white LED, and red LED following on the 21st day.
Dry Weight of Mercury-Treated Plants: Control plants had the highest dry weights. Hg exposure led to reductions in dry weights, which varied with concentration and duration. The lowest dry weights were recorded under white fluorescent light, followed by white LED, blue LED, and red LED on the 21st day.
This study underscores the influence of light conditions and heavy metal exposure on the growth of *C. demersum*, highlighting their importance in phytoremediation strategies.

Keywords: Dry weight, Fresh weight, Heavy metal exposure, Plant growth

Acknowledgment

This study was supported by the Scientific and Technological Research Council of Turkey - TUBITAK [Project number: 218Z144]. We gratefully acknowledge the support of the Scientific and Technological Research Council of Turkey (TUBITAK).

ORAL PRESENTATION

Üreazın TiO_2 Nanopartiküllere İmmobilizasyonu ve Tekrar Kullanım Performansının Araştırılması

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Abstract

Üreaz (EC 3.5.1.5) bir molekül üre hidrolizi ile bir molekül karbondioksit, iki molekül amonyağın açığa çıkmasını katalizleyen ve hidrolazlar sınıfında yer alan bir enzimdir. İmmobilize üreaz; ürenin kandan uzaklaştırılmasında ya da hemodiyaliz işleminde, sentetik diyalizat çözeltisinin rejenere edilmesinde; kanda, idrarda ve atık sularda bulunan ürenin belirlenmesi amacıyla biyosensörlerde; gıda endüstrisinde içeceklerden ürenin uzaklaştırılmasında ve gübre atık sularında bulunan ürenin dönüştürülmesinde potansiyel kullanım alanına sahiptir (Alptekin, 2022). Son on yılda giyilebilir yapay böbrek cihazlarının kullanımına yönelik çalışmalar kronik böbrek yetmezliği olan hastaların hareketliliğini sağlamak ve sürekli homeostaz iyileştirmesi için düşük akışlı renal replasman tedavisi sağlamak amacıyla ivme kazanmıştır (Bazaev ve ark. 2016). Sürekli rejenerasyon için sorbent bazlı diyalizat rejenerasyon sisteminin "giyilebilir" ağırlıkta olması gerekir. Diyalizat ve sorbent içeren ekipmanı yaklaşık 5 kg olup bunun sorbent kısmının 65 gramı Al_2O_3 üzerinde üreazın immobilize edildiği immobilize üreaz preparatı, 600 gramı zirkonyum fosfat, 51 gramı zirkonyum oksit, ve 153 gramı aktif karbondur (Gelder ve ark. 2020).Giyilebilir teknolojilerin gelişiminde hafif, biyouyumlu ve yüksek aktivite ve kararlılığa sahip immobilize üreaz preparatlarının elde edilmesi amacıyla bu alanda oldukça fazla araştırma yapılmaktadır. Bu çalışmada TiO_2 nanopartiküller polietilenimin ile modifiye edilerek ve iyonik adsorpsiyon yöntemi ile üreaz (Jack bean kaynaklı) immobilize edilmiştir. İmmobilize üreaz için optimum pH 7,0, optimum sıcaklık 60° C olarak belirlenmiştir. Kinetik çalışmalar sonucunda immobilize üreaz için K_m değeri 9,9 mM, V_{max} değeri ise 15,5 IU/mg protein olarak hesaplanmıştır. İmmobilize üreazın 50, 60 ve 70 °C deki termal kararlılıkları incelendiğinde 8 saat sonunda başlangıç aktivitelerinin sırasıyla %91, %79 ve %74'ü kalmıştır. İmmobilize üreazın tekrar kullanım kararlılığı ile ilgili kesikli reaktör sisteminde yapılan çalışmalarda 50 mg ve 100 mg immobilize üreaz kullanıldığında 5 kullanım sonunda hala aktivitelerini %90'dan fazla koruduğu belirlenmiştir. Sonuç olarak, TiO_2 üzerine immobilize edilen üreaz yüksek termal kararlılık, tekrar kullanım performansı ve aktivite açısından değerlendirildiğinde diyalizat rejenerasyonunda giyilebilir teknolojilerde kullanım potansiyeli göstermektedir.

Keywords: üreaz, immobilizasyon, polietilenimin, reaktör

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ORAL PRESENTATION

The effect of boron level on some biochemical and haematological parameters in sheep

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Abstract

Boron is one of the most important underground resources, with Turkey holding around 73.5 per cent of the world's reserves. It has many applications, especially in industry. In recent years, it has attracted attention especially in the field of health. The mechanism of boron's action on living organisms is not yet clearly understood. However, since it has a high coefficient of action, the boundary between deficiency and toxicity is quite thin. Therefore, in this study, the effects of boron levels in living organisms and some biochemical and haematological parameters depending on the distance of boron exposure were investigated. For this purpose, blood samples were taken from 10 sheep in Bigadiç district, which is close to the boron mine site, and 10 sheep in Balıkesir city centre, which is far from the mine site. Boron levels were determined by ICP-OES, some biochemical parameters and haemogram values were determined by autoanalyser. It was observed that the serum boron levels of sheep at close distance to the boron mine site were statistically higher than those at far distance. This suggests that the distance of exposure to boron may be effective in serum concentration. Significant differences were also found in urea, BUN and creatine levels. No significant difference was observed between WBC, LYM, MID, GRAN, LYM%, MID%, PLR, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, PDW-CV, PDW-SD, PCT, P-LCC, P-LCR among haemogram parameters, whereas statistically significant difference was determined between GRAN%, NLR, RDW-CV, PLT and MPV. This shows that boron exposure causes changes in both its concentration in blood and some biochemical and haematological parameters. The results determined have the characteristics of a suitable infrastructure for more comprehensive projects. In order to understand the changes that boron may cause in haematological and biochemical parameters in more detail, studies should be carried out in different animal species.

Keywords: Boron, Hemogram, ICP-OES, Sheep.

ORAL PRESENTATION

Antioxidant activity potential of green natural deep eutectic solvent extraction of *Salvia officinalis* plant

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Abstract

Salvia officinalis has been known to have strong antioxidant activity for many years. The scientific community has increased its efforts to obtain antioxidant agents from natural sources in recent years [1]. Moreover, with the inclusion of green solvents in the studies, there is a growing interest in this field in the literature. This study aimed to investigate the antioxidant activity of dried *Salvia officinalis* plant leaf extracts by comparing the effect of natural deep eutectic solvents (NADES) [2], a class of green solvents, and conventional solvents. For this reason, extracts were prepared by ultrasound-assisted extraction [3] using both NADES and conventional solvents (water and methanol) and then antioxidant determination using DPPH (2,2-diphenyl-1 picryl-hydrazyl-hydrazyl-hydrate) analysis was carried out by UV-Vis spectrophotometer. In these experiments, the ability to scavenge DPPH radical was measured by the color change of the solution. The results showed that NADES has great potential as an agent with strong antioxidant activity. The highest values of antioxidant activity were detected in the samples obtained by extraction using the NADES combination of choline chloride with oxalic acid (1/1). Based on the results obtained, it was determined that the NADES extract of the *Salvia officinalis* plant obtained with the combination of choline chloride with oxalic acid (1/1) showed lower IC_{50} values than other NADES extracts.

Keywords: Natural deep eutectic solvents (NADES), Green extraction, DPPH, Antioxidant activity, *Salvia officinalis*, Ultrasound-assisted extraction

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ORAL PRESENTATION

Development of a green synthesis-assisted microextraction method for the determination of Allura Red dye in children's play materials

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Abstract

Color is of great importance in the industry. Color is of great importance as it has a wide area in every field of industry as it directly affects the choice of consumers and the product's commercial success. Among these, 10,000 different dyes and pigments are produced worldwide every year. These dyes are chemically, photolytically, and biologically highly stable and quite permanent. Since synthetic colorants are associated with undesirable and harmful effects, most of them are banned worldwide. They are not allowed in foods at any level. However, they are used in different industrial and scientific applications due to their low cost, color, and visual appearance due to excellent stability [1-3].
In this study, determination of Allura Red dye in different play material samples produced for children using deep eutectic solvents, deep eutectic solvents were used in different molar ratios (1:1, 1:2, 1:3, 1:4, 1:5) prepared with tetrabutyl ammonium bromide and decanoic acid as green solvents. Parameters such as pH, DES volume and type, THF volume, ultrasonic bath and centrifugation time, sample volume, and matrix effects that affect the recovery of the method were investigated. The most optimum parameters for the recovery of Allura Red dye with deep eutectic solvent were determined. The developed method was applied to red play dough, red finger paint, red face paint, and red crayon samples and the values were found to be satisfactory.

Keywords: Allura Red, Deep eutectic solvent, Liquid-liquid microextraction, Children's play materials

Acknowledgment: This research was supported by the Düzce University Scientific Research Projects Fund (Project No: 2023.05.03.1420) for contributing to the financial portion of the project.

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ORAL PRESENTATION

COVID-19'a Karşı Gelişen Doğal Antikor Yanıtın Pnömoni ve Hastalık Şiddetiyle İlişkisi

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Özet

Amaç: Bu çalışma semptomatik COVID-19 enfeksiyonu sonrası oluşan doğal IgG antikor yanıtının; hastaların demografik özellikleri, hastalık ve pnömoni şiddeti ve yoğun bakım ihtiyacı ile ilişkisini irdelemektedir.
Method: Çalışmaya COVID-19 RT-PCR testi pozitif, aşılanmamış, semptomatik ve hospitalize 125 hasta dahil edilmiştir. Hastaların demografik bilgileri, WHO hastalık şiddeti değerleri ve pnömoni şiddeti değerleri kaydedilmiştir. RT-PCR pozitifliğini takiben 18. günde alınan serum örneklerinden hastalık sonrası oluşan doğal IgG antikor tayini ELFA yöntemine dayalı VIDAS Anti-SARS CoV-2 IgG kitleriyle VIDAS analizöründe çalışılmıştır. İstatistiksel analizler IBM SPSS 25.0 paket programı ile yapılmış, anlamlılık düzeyi <0,05 olarak dikkate alınmıştır.
Bulgular: Çalışmaya dahil edilen hastaların %52,8'i kadın %47,2'si erkektir. Tüm hastaların %53,6'sı 65 yaş üstünde, kalanlar 65 yaş ve altındadır. Hastaların %51,2'sinin tedavisi YBÜ'de, geri kalan hastaların tedavisi ise serviste sürdürülmüştür. Hastaların oluşturdukları IgG antikor titresiyle yaşları arasındaki ilişkide 65 yaş üzeri hastaların IgG antikor titresi diğer hastalara oranla anlamlı derecede yüksektir. Benzer şekilde cinsiyet-IgG ilişkisinde erkek hastalar kadın hastalara göre daha yüksek düzeyde antikor oluşturmuştur (sırasıyla z=-6,995;p<0.001, z=-1,941;p<0,052). Hastaların YBÜ ihtiyacı ile oluşturdukları antikor titreleri arasında anlamlı bir ilişki bulunmamaktadır. Hastaların pnömoni şiddeti ve WHO hastalık şiddeti kriterleri arttıkça oluşturdukları IgG miktarları da artmaktadır (sırasıyla z=-2,741;p<0,06, z=-4,587;p<0,001).
Sonuç: COVID-19 sonrası antikor yanıtı, reenfeksiyon, hastaneye yatış ve mortalite açısından değerlidir. Hastalarımızın tamamının aşılanmamış olması, tespit edilen antikorların hastalığın oluşturduğu doğal IgG antikorları olması ve antikor oluşumuna rağmen pnömoni ve hastalık şiddetinin yüksek seyretmesi hastalığın akciğer filtrasyonunun yüksek olduğunu düşündürmekte aynı zamanda hastalığa karşı oluşan doğal antikor yanıtın koruyuculuğunun düşük olduğunu göstermektedir. COVID-19'da hastalık ve pnömoni şiddeti arttıkça oluşturulan antikor titreleri de artmaktadır. Fakat bu antikorların nötralize edici fonksiyonları ne derece yansıttıkları konusu hala tartışmalıdır. Enfeksiyon hastalıklarına karşı gelişen immün yanıt hayati önem taşımaktadır. Dahil ettiğimiz hastaların tamamının aşılanmamış ve semptomatik olduğu göz önüne alındığında; hastalığa karşı gelişen immün yanıtın nötralize edici etkisinin tam olarak anlaşılması için benzer çalışmaların aşılanmış bireyleri de kapsayacak şekilde yapılması önerilmektedir.

Anahtar kelimeler: Enfeksiyon hastalıkları, COVID-19, Nötralizan Antikor, Hastalık Şiddeti

ORAL PRESENTATION

Synthesis and Characterization of Chitosan/Ulvan Hydrogel Wound Dressings Containing Various Phytochemical Additives

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Abstract

Damage and deterioration of the skin are defined as wounds, and especially chronic wounds seriously affect the quality of life of patients. World Health Organization (WHO), states that an estimated 180,000 people die each year from wounds caused by burns. For this reason, it claims that the wounds that occur are a global public health problem. One way to prevent wound-related deaths is to treat wounds correctly. Traditional wound care materials can be time-consuming and uncomfortable in a busy pace of life, as they need to be changed frequently. For this reason, intensive research is being carried out on modern alternatives such as hydrogel wound dressings today. In this context, in this study, the incorporation of common phytochemicals (such as aloe vera and collagen) used in wound treatment into the hydrogel structure was investigated. For this purpose, hybrid hydrogel wound dressings were synthesized with various phytochemicals (aloe vera and collagen) using chitosan and ulvan polysaccharides for the first time in the literature. The experimental plan for the synthesis of hydrogels was obtained using the Box-Bhenken Design using the Dizayn Expert 11 program. Moisture, water absorption capacity and antibacterial efficacy analyzes were performed for characterization of the obtained hydrogels. As a result of the experiments, hybrid hydrogels were successfully synthesized and it was determined that the antibacterial properties decreased as the amount of ulvan in the structure of the hydrogels increased. The highest antibacterial properties were achieved in aloe vera-doped hybrid hydrogels. Moreover, it has been determined that aleovera doped hydrogels have a higher moisture content. In addition, in the water absorption experiments, the hydrogel with the highest water absorption capacity was achieved as a result of using 5% ulvan polysaccharide, 90% chitosan and 5% phytochemicals. The water absorption value of this hydrogel was determined as 270.57%.

Keywords: Hydrogel, Wound Healing, Ulvan, Chitosan

ORAL PRESENTATION

Nutritional Composition and Antioxidant Potential of Radish Microgreens

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Abstract

Radish microgreens are young plant sprouts that contain more concentrated nutrients and biologically active compounds compared to mature vegetables. These microgreens are particularly notable for their antioxidant properties and high nutritional content. Typically low in calories, radish microgreens offer a dense source of nutrients that can be incorporated into a healthy diet. This study examines the nutritional content and antioxidant properties of radish microgreens. Radish microgreens are rich in vitamins, minerals, and phytochemicals. They are particularly high in vitamins C, K, E, and folate, which support the immune system and contribute to cell regeneration. Additionally, they contain important minerals such as potassium, calcium, magnesium, and iron, which promote bone health and help maintain electrolyte balance. Radish microgreens are also high in fiber, which has a positive effect on the digestive system. Radish microgreens are known for their potent antioxidant properties. These antioxidants protect cells against free radicals and reduce the negative effects of oxidative stress on the body. Radish microgreens are particularly rich in antioxidants such as anthocyanins, flavonoids, and phenolic compounds. These compounds help reduce inflammation in the body, aiding in the prevention of chronic diseases, especially degenerative conditions like heart disease and cancer. Studies have shown that regular consumption of radish microgreens is effective in preventing cell damage caused by oxidative stress. In addition to antioxidants, radish microgreens contain phytochemicals like glucosinolates, which contribute to their anticancer potential. Glucosinolates are particularly noted for their ability to reduce the risk of colon, breast, and prostate cancers. In conclusion, radish microgreens stand out among functional foods due to their high nutritional value, strong antioxidant properties, and protective effects on health.

Keywords: Antioxidant Potential, Broccoli, Healthy nutrition, Microgreens, Radish

ORAL PRESENTATION

Evaluating the Efficacy of Cinnamon in Managing Type II Diabetes

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Abstract

The potential role of cinnamon in managing Type II diabetes has garnered increasing scientific interest in recent years. Type II diabetes is a widespread metabolic disease characterized by chronically high blood sugar levels and insulin resistance. Management of this disease is typically achieved through lifestyle changes, dietary adjustments, and medication. However, it is suggested that some herbal and natural components can also aid in Type II diabetes management, and cinnamon is one of them. Cinnamon is found in two main types: *Cinnamomum verum* J. Presl (true cinnamon) and *Cinnamomum cassia* (Chinese cinnamon). Research on the effectiveness of cinnamon in managing Type II diabetes has generally focused on Cinnamomum cassia. The potential benefits of cinnamon in managing Type II diabetes are attributed to its active components, such as cinnamaldehyde, cinnamic acid, and cinnamate. These components have been reported to exhibit blood sugar-regulating effects in various studies. In some clinical studies, cinnamon supplements have been observed to lower blood sugar levels and reduce insulin resistance in individuals with Type II diabetes. Additionally, cinnamon consumption has been reported to decrease total cholesterol, LDL cholesterol, and triglyceride levels. These results indicate that cinnamon could provide positive effects on both glycemic control and cardiovascular risk factors in individuals with Type II diabetes. However, not all studies have yielded consistent results. Some research has reported that the effects of cinnamon on blood sugar levels are minimal or not statistically significant. These conflicting results may stem from methodological differences in studies, variations between cinnamon types, and the doses used. In conclusion, cinnamon could be a potentially beneficial herbal component in managing Type II diabetes. To better understand the role of cinnamon in Type II diabetes management, larger-scale, long-term, and standardized clinical studies are needed.

Keywords: Blood Sugar Levels, Glycemic Control, Phytotherapy, Supplementation, Therapeutic Potential

ORAL PRESENTATION

Botanical Nutraceuticals and Their Anti-inflammatory Properties

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Abstract

Botanical nutraceuticals are natural plant-derived compounds with health-promoting properties, particularly noted for their anti-inflammatory effects. Inflammation is the body's defense mechanism against infections and injuries, but when it becomes chronic, it can lead to the development of various diseases. Therefore, controlling inflammation through natural means has become an important area of research in modern medicine. Botanical nutraceuticals offer a promising solution in this regard. The anti-inflammatory effects of botanical nutraceuticals are linked to the bioactive compounds they contain. Plants like turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), and green tea (*Camellia sinensis*) are rich in phytochemicals that regulate inflammatory processes. The active ingredient in turmeric, curcumin, suppresses the production of key enzymes involved in inflammation, such as COX-2 and TNF-α. These properties make turmeric a popular nutraceutical in the management of chronic inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease. Similarly, the gingerols and shogaols in ginger reduce the production of inflammatory cytokines, preventing inflammation. Green tea contains polyphenols such as epigallocatechin gallate (EGCG), which also possess potent anti-inflammatory properties. EGCG inhibits the NF-κB pathway, a key player in triggering the inflammatory response, thereby reducing cellular inflammation. Regular consumption of green tea may contribute to the prevention of chronic inflammation, potentially reducing the risk of diseases such as cancer and heart disease. In conclusion, botanical nutraceuticals play a significant role in maintaining health through their anti-inflammatory effects. These natural compounds hold great potential as complementary therapies in managing diseases associated with chronic inflammation.

Keywords: anti-inflammatory, botanical nutraceuticals, chronic inflammation, phytochemicals

ORAL PRESENTATION

Nutritional Value and Health Benefits of Broccoli Microgreens: A Functional Food Perspective

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Abstract

Broccoli microgreens have gained popularity in recent years among health-conscious consumers due to their dense nutrient content. Compared to mature broccoli, these young plant sprouts contain higher levels of phytochemicals and antioxidants, making them rich in various bioactive compounds that support a healthy lifestyle. This study examines the nutritional content and health benefits of broccoli microgreens, which are particularly high in glucosinolates, flavonoids, and vitamins, with sulforaphane being one of the most notable compounds. Broccoli microgreens are rich in essential micronutrients such as vitamins C, E, and K, as well as folate, iron, and calcium. They are also high in fiber and contain a modest amount of protein, contributing to digestive health. Sulforaphane, the most well-known active compound in broccoli microgreens, has been shown to have cancer-fighting properties and the ability to activate detoxification enzymes in cells. Additionally, its antioxidant properties provide protection against free radicals in the body. The health-promoting effects of broccoli microgreens can be attributed to their rich antioxidant content and phytochemicals, offering a wide range of benefits. Sulforaphane's anticancer properties have been linked to reducing the risk of breast, prostate, and colon cancers. Research indicates that broccoli microgreens may enhance immune function, support cardiovascular health, and provide anti-inflammatory effects. These benefits play a crucial role in preventing and managing chronic diseases. Furthermore, broccoli microgreens are low in calories, making them an ideal component of a healthy diet and for weight management. The fiber content supports digestion and gut health, while glucosinolates are known to benefit liver health and detoxification processes. In conclusion, broccoli microgreens stand out among functional foods for their high nutritional value and potential health benefits.

Keywords: Broccoli, Healthy nutrition, Microgreens, Natural nutrition, Phytochemicals

ORAL PRESENTATION

Evaluation of Measurement Metrics of Bioinformatic Algorithms for Determination of Intron Retention

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Abstract

Intron retention (IR), one of the alternative types of splicing, occurs when an intron is inserted into the pre-mRNA and remains in the final mRNA. It has been reported that IR is much more frequent in mammals than previously thought and has been determined to affect the transcripts of most genes. Misregulation of alternative splicing is also associated with cancers and abnormal expression or mutations in splicing factors contribute to tumor formation. Because these events are difficult to measure reliably, they constitute a class of alternative splicing that is often neglected. This study aims to examine the IRFinder, IRFinder-S, iREAD, and Whippet algorithms, which are widely used in determining intron retention in RNA-seq data, and to determine the operational differences between the algorithms. As a result of the evaluation, it was observed that IRFinder and IRFinder-S algorithms use IRratio as the metric of intron retention, iREAD uses a measurement metric based on Entropy score, and the Whippet algorithm determines the retained introns according to Percent Splice In (PSI) values. In differential gene expression measurements, IRFinder, IRFinder-s algorithms used DEseq2, IREAD used edgeR and whippet used Delta PSI values. In addition, IRFinder-S was found to be effective in long read sequences.

Keywords: Intron Retention, IRFinder, IRFinder-S, IREAD, Whippet

This study was supported by Erciyes University, Scientific Research Projects Coordination Office with the project coded TYL-2022-12231.

ORAL PRESENTATION

Investigation of the binding interraction of synthetic retinoid-benzimidazole derivatives towards RAR and RXR receptor targets: A molecular docking approach

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Abstract

In this study, we aimed to investigate the molecular binding interraction of new synthetic retinoid benzimidazole derivatives towards two molecular targets of retinoid compounds – retinoic acid receptor (RAR) and retinoid X receptor (RXR). Crystallographic data of the proteins for RAR (PDB ID: 6EU9) and RXR_{β} (PDB ID: 1H9U) were obtained from Protein Data Bank. Molecular docking of synthesized retinoid benzimidazole derivatives towards these molecular targets was performed using Cresset Flare program. All-*trans* retinoic acid and 9-*cis* retinoic acid were used as standard drugs. The molecular docking analysis revealed that compound **AZ3** exhibited the highest binding affinity (-6.815 kcal/mol) when complexed with RAR, in comparison to the ATRA reference (- 4.404 kcal/mol) and formed bonding interraction with Ser448, Arg494, Asp313 residues. In the case of the RXR target, compound **AZ10** (-14.47 kcal/mol) demonstrated the highest docking energy score relative to 9-cis RA (- 7.201 kcal/mol) and formed bonding interraction with Ala343, Lys511, Arg373, Asp344, Trp376, Leu380 and Phe384. Notably, all the derivatives exhibited higher docking scores compared to their corresponding references. Moreover, they all bound to the proteins' active sites in a manner similar to the standard drugs and demonstrated the potential to compete for the protein's binding site with standard drugs. Docking simulation with RXR resulted in higher binding energies compared to the RAR protein, indicating stronger and more stable protein-ligand complex interactions. The binding of the synthesized derivatives to retinoid receptors suggest they may have anticancer activity.

Keywords: Molecular docking, retinoid-benzimidazole, RAR, RXR, all-*trans* retinoic acid, 9-*cis* retinoic acid

ORAL PRESENTATION

Investigation of the effect of CRISPR-Cas system on GFP gene using artificial intelligence

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Abstract

The viral epidemics that are currently occurring and are likely to happen in the future have some negative effects on humans. The CRISPR-Cas13d system enables the production of prototype targets for antiviral drugs without harming human health using biotechnological methods. In this study, when lentivirus was transferred to mesenchymal stem cells (MSCs) expressing green fluorescent protein (GFP) as a model, the onset of GFP expression and the efficiency of transduction were tested. Then, another MSC line carrying the CRISPR-Cas13d system will be created using the same method, and exosome isolation will be performed. Exosomes will be applied to GFP+ MSCs and the suppression of GFP expression will be analyzed. Determining the effectiveness of CRISPR-Cas13d in silencing GFP requires a large number of analyses that cannot be completed with human power. With the use of an artificial intelligence system, important data will be obtained with sensitivity far beyond human power and at a low cost. The study was supported by TÜSEB (#38898), Health Institutes of Türkiye.

Keywords: viruses, CRISPR-Cas systems, mesenchymal stromal cells, supervised machine learning

ORAL PRESENTATION

Melamine exposure from the weaning period causes gonadotoxicity

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Abstract

Objective: The present study aimed to investigate the gonadotoxic effects of melamine exposure from the weaning period on ovarium tissue. Melamine caused food scandal years ago due to illegaly usage. It used for providing false high protein result in milk, dairy products and formula. Methods: Eighteen female wistar albino rats were divided into three groups. Group 1; this group was accepted as control and 0.1 ml saline was applied by gavage (p.o.) for 3 weeks. Group 2; 50 mg/kg melamine in 0.1 ml saline was applied by p.o for 3 weeks. Group 3; 75 mg/kg melamine in 0.1 ml saline was applied by p.o for 3 weeks. At the end of the experiment, all animal were euthanised under anesthesia. The intracardiac blood samples were taken and both side ovaries were removed. Both ovaries were pun into neutral formalin. The left ovaries were used for analysis of apoptosis by flow cytometry. The right ovaries were used for histopathological and morphometric analysis. Blood samples were used for analysis of plasma AMH (anti-Mullerian hormone) level. Results: There was no statistically significant difference in the number of the primordial, primary, secondary, antral and graaf follicle and ovarian diameter between the control and melamine-treated groups ($p > 0.05$). A significant decrease in the corpus luteum and a significant increase in the number of atretic follicles were found($p < 0.05$). AMH plasma level significantly decreased in the 75 mg/kg group compare to control group ($p < 0.05$). Apoptosis (Annexin V) increased in the 50 and 75mg/kg melamine groups compare to control group ($p < 0.05$). Conclusion: Melamine exposure from the weaning period may cause gonadotoxicity in parallel with dose increase.

Keywords: Anti-Mullerian hormone, Apoptosis, Flow cytometry, Melamine, Ovarian follicle, Weaning period, Rat

ORAL PRESENTATION

Ekstrüzyon İşlemindeki Ürün Kalitesini Etkileyen Değişkenler

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Özet

Ekstrüzyon prosesi, nihai ürünün kalite özelliklerine göre değişen içeriğe (nişasta, protein, nem ve diğer ingredientlerin oranı) sahip bir hamur karışımının, yüksek sıcaklık ve basınç koşulları altında tek veya çift vidalı yoğurucuların bulunduğu bölmelerde yoğurulduktan sonra hamurun, namlunun ucundaki oldukça dar bir alandan geçirilmeye zorlanmasıyla pişmiş üründe ani sıcaklık ve basınç değişimi sonucunda ekzotermik genleşme sağlayan kompleks bir teknoloji olarak tanımlanabilmektedir. Ekstrüzyon teknolojisi, sıcaklık ve basınç ile nişastanın jelatinize, proteinlerin denatüre, lipitlerin modifiye ve enzimlerin inaktive olduğu modern bir gıda işleme teknolojisi olup; tek bir proseste karıştırma, pişirme, soğutma, taşıma, kesme, hava muhafaza ederek şişme, şekil verme ve kısmen kurutma gibi işlemlerin yapıldığı çok yönlü bir prosestir. Ekstrüzyon işleminde kullanılan karışımların özellikleri ve ekstrüzyon işleminin çalışma parametreleri, ekstrüder ile üretilen ürünün renk, tat ve aroma gibi özelliklerini ve besinsel özelliklerini etkilemektedir. Ekstrüzyon işleminde kullanılan karışımın nem içeriği en önemli değişkenler arasında yer almaktadır. İşlem sırasında nem doğrudan karışıma tavlama yöntemiyle ilave edilebilmekte ya da ekstrüde işlemi sırasında su veya buhar olarak sisteme eklenebilmektedir. İşleme sırasında karışımın nem içeriği, ekstrüzyon işleminin verimliliğini (sıcaklık, basınç, kesme kuvveti vb.) ve sertliğini etkileyebilmektedir. Nem, nişasta jelatinizasyon oranını, ayrışmasını ve dekstrinizasyonunu değiştirerek ürünün genleşmesini, yoğunluğunu, gözenek yapısını, kırılganlığını ve sertliğini etkilemektedir. Uygulanan mekanik enerjiye bağlı olarak proteinlerin, nişastaların ve liflerin su tutma kapasitesi, çözünürlüğü, viskozitesi ve boyutu değişim göstermektedir. Öte yandan, bu değişiklikler elde edilen ürünlerin tekstürel özelliklerini belirlemektedir. Ekstrüzyon işlemindeki ana değişiklik, nişastadaki kristal formun jelatinizasyon yoluyla parçalanması ve muhafaza koşullarına ve zamana bağlı olarak tekrar kristal yapıya dönüşmesidir. Ayrıca, ekstrüzyon işlemi yoğunluğuna göre nişastanın sindirilebilirliğini etkilemekte ve amiloz-lipit kompleksine bağlı olarak da nişastanın dirençli nişasta içeriğinde bir artış sağlanabilmektedir. Bu bildiride ekstrüzyon işlem parametrelerinin gıda ürününde meydana getirdiği fizikokimyasal değişikliklerden anlatılacaktır.

Anahtar Kelimeler: Ekstrüzyon, fizikokimyasal değişimler, nişasta, protein

ORAL PRESENTATION

Bioethanol Production from Alkaline Pretreated *Microcystis aeruginosa*

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Abstract

The increasing demand for sustainable energy sources has intensified research on bioethanol production from non-food biomass. *Microcystis aeruginosa*, a common cyanobacterium, presents a promising feedstock due to its high carbohydrate content and rapid growth rate. This study investigates the efficacy of alkaline pretreatment in fermentation of *Microcystis aeruginosa* biomass for bioethanol production. In this study, *Microcystis aeruginosa* were cultivated and subjected to alkaline pretreatment using sodium hydroxide (NaOH) at varying concentrations (0.75%, 1.0%, and 1.5% w/v) and reaction times (15, 30 and 60 min) at 100°C, and finally fermented with using *Saccharomyces cerevisiae* under anaerobic conditions. Bioethanol yield was quantified using gas chromatography. Highest bioethanol yield was obtained as 12.44% under condition of 0.75 w/v NaOH, pre-treatment temperature of 100°C, and 15 minutes of pre-treatment time, respectively. This research highlights the viability of *Microcystis aeruginosa* as a sustainable feedstock for bioethanol production. Alkaline pretreatment significantly enhances the fermentation process, making it an alternative approach for biofuel production.

Keywords: Bioethanol, *Microcystis aeruginosa*, fermentation

ORAL PRESENTATION

A morphological and molecular study on *Mentha pulegium* L.

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Abstract

Mentha pulegium commonly (European) pennyroyal, or pennyrile, also called mosquito plant, and pudding grass, is a species of flowering plant in the mint family, Lamiaceae, native to Europe, North Africa, and the Middle East. Crushed pennyroyal leaves emit a very strong fragrance similar to spearmint. Pennyroyal is a traditional folk remedy, emmenagogue, abortifacient, and culinary herb, but is toxic to the liver and has caused some deaths. This study aimed to reveal both the morphological and molecular differences and similarities of the *M. pulegium* species, which particularly shows morphological variation in flower color during field studies. Individuals of *M. pulegium* with different colors were collected from the Karameşe forests of Ardanuç district in Artvin province (Türkiye) and studied both morphologically and molecularly. In the morphological character analyses, measurements of leaves, verticillate, calyx, corolla, trichomes, and pedicels were conducted. Molecularly, chloroplast DNA matK gene regions were studied, and the sequence analyses were arranged and subjected to phylogenetic analyses. As a result of the analyses, in both samples, it was observed that stems were erect and prostrate, strongly branched, and measured 15-30 (50) cm in length. It was observed that the calyx teeth, calyx length, and calyx width of the purple-colored *Mentha* samples showed morphological differences. The purple *M. pulegium*, the calyx measured. 20-23 x 6-8 mm, the short calyx teeth were 3-7.9 x 2-3.7 mm, and the long calyx teeth were 7-9.3 x 1.2-1.9 mm. In contrast, in the white *M. pulegium*, the calyx measured 16.5-19.4 x 7.8-9.3 mm, the short calyx teeth were 4.3-5.8 x 2-3 mm, and the long calyx teeth were 6.4-7.6 x 1-1.5 mm. The flower pedicel was 1.3-1.4 mm in the white-flowered species and 1.2-1.9 mm in the purple species. As a result of molecular analyses, a single nucleotide difference was observed in the comparison of the matK gene region of the two species with morphological differences. In the phylogenetic tree constructed with both internal and GenBank sequences, the species showed 99% similarity. Although they appeared morphologically different, it was determined that they shared almost identical gene regions at the molecular level. Due to the medicinal and aromatic properties of this species and its use by the public, confirming the species identification is of significant importance.

Keywords: Artvin, *Mentha pulegium*, morphology, matK, Türkiye

ORAL PRESENTATION

Synthesis and anti-biofilm activity studies on novel sulfonamide carrying benzamide derivatives

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Abstract

Pseudomonas aeruginosa (P. aeruginosa) is a multi-drug resistant opportunistic pathogen which is responsible for numerous hospital-acquired infections. It needs host colonization and enhances its resistance by forming biofilms. Biofilm associated *P. aeruginosa* enfections could be treated with difficulty because of their strong resistance to well known antibiotics [1-3]. Sulfonamides are an important class of antimicrobial compounds. They have come forward with the invention of Prontosil's chemotherapeutic property by Domagk in 1930s. Since then various sulfonamide conjugates have been synthesized and used in therapy. Currently, sulfonamides are still popular, still used in theraphy and a great number of sulfonamide structured compounds are in clinical trial for various medical conditions [4]. Benzamides are important pharmacophoric structures that display a wide range of biological activities such as antimicrobial, antiviral and antiinflammatory [5]. In the light of aforementioned evidence we have synthesized ten novel sulfonamide carrying benzamide compounds and evaluated their anti-biofilm potential on biofilm formation of *P. aeruginosa* PAO1.

Keywords: Benzamide, sulfonamide, anti-biofim activity.

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ORAL PRESENTATION

Salmonella enteritidis UDP-N-asetilglukozamin 1-karboksiviniltransferaz (MurA)'ının farklı homoloji modellerinin karşılaştırılması

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Özet

Salmonella enteritidis, insanlarda mide-bağırsak yolu enfeksiyonuna neden olarak ateş, karın krampları, kusma, ishal ve ölüme yol açabilen en önemli *Salmonella enterica* serovarlarından biridir. *S.* enteritidis, bazı β-laktamları da içeren çeşitli antimikrobiyal ilaçlara karşı direnç ggeliştirmiştir. Bu durum, *S.* enteritidis enfeksiyonlarına karşı yeni ilaçların keşfedilmesi ihtiyacını ortaya çıkarmaktadır. İlaç hedefi belirlenmesinde peptidoglikan sentezinde yer alan UDP-N-asetilglukozamin 1-karboksiviniltransferaz (MurA) (EC 2.5.1.7), bakteriyel hücre büyümesinin inhibisyonu için önemli bir enzimdir. *S.* enteritidis MurA enziminin literatürde tanımlanmış bir 3-boyutlu yapısı bulunmamaktadır. Bu nedenle yapılan çalışmada ilaç hedefi olarak bu enzim homoloji modellenmiştir. *S.* enteritidis MurA'nın amino asit dizisi (Erişim numarası: AM933172.1) NCBI veritabanından alınmıştır (https://www.ncbi.nlm.nih.gov). İlk olarak SWISS-MODEL (https://swissmodel.expasy.org/) web yazılımı kullanılarak 3 boyutlu yapısı bilinen bir protein kalıbı (PDB ID: 1EJC) belirlenmiş ve *S.* enteritidis MurA'nın homoloji modeli oluşturulmuştur. SWISS-MODEL kullanılarak belirlenen protein kalıbı (PDB ID: 1EJC) aynı zamanda MODELLER 9.15 programında protein modeli oluşturmak için kullanılmıştır. Oluşturulan 50 model içerisinden en düşük DOPE skoruna sahip model seçilmiştir. Son olarak bir yapay zeka sistemi olan AlphaFold (https://alphafold.ebi.ac.uk/) üzerinden *S.* enteritidis MurA'nın modeli elde edilmiştir. Elde edilen üç modelin validasyonu için PROCHECK, ERRAT (https://saves.mbi.ucla.edu/) ve ProSA programları kullanılmıştır. PROCHECK programı kullanılarak elde edilen Ramachandran plot grafiklerine göre enzimin SWISS-MODEL, MODELLER ve AlphaFold modelleri için sırasıyla tüm amino asit kalıntılarının %94,7'si (342); %95,3 'ü (344); %94,7'si (342) en çok tercih edilen bölgede yer almaktadır. ERRAT programı sonuçlarına göre modellerin kalite faktörleri de aynı sırayla 94.321; 93.674; 93.6548 olarak bulunmuştur. ProSA programı sonuçlarına göre ise Z-Score'ları sırasıyla -10,21; -10,38; -10,05 olarak bulunmuştur ve tüm modeller X-ray kristallografisi ile 3 boyutlu yapısı çözümlenmiş proteinler arasında yer almaktadır. Tüm validasyon sonuçları değerlendirildiğinde elde edilen üç *S.* enteritidis MurA modelinin de skorlarının birbirine yakın olduğu, iyi modellendiği ve ileriki çalışmalar için uygulanabilir olduğu tespit edilmiştir.

Anahtar kelimeler: *S. enteritidis*, İlaç hedefi, MurA, Protein modelleme

ORAL PRESENTATION

Synthesis and Characterization of Fe_3O_4@VT@AZI Drug Delivery System

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Abstract

Antibiotic resistance is one of the significant issues, being the third leading cause of death worldwide after ischaemic heart disease and stroke. Nano based drug delivery systems (NDDSs) can be efficient tool to diminish the resistance to antibiotics and prolong the lifetime of current antibiotics and they can provide targeting drug delivery in comparison with traditional antibiotics (1). Magnetic nanoparticles (MNPs) have unique properties such as being able to transmit cargo by magnetising in a magnetic field and ultimately target the tissue, as well as antibacterial properties (2). Azithromycin (AZI) is a macrolide antibiotic that is effective against positive and especially gram-negative bacteria. It is used to treat pneumonia, chronic obstructive pulmonary disease and cystic fibrosis. Besides, it has a low oral bioavailability of 37% due to poor permeability (3). In this study, we aimed to enhance AZI permeability and develop sustained drug release. For this purpose, iron oxide (Fe_3O_4) magnetic nanoparticles were synthesised and the MNPs were loaded with drugs after being modified with valonia tannin (VT). In conclusion, drug loaded MNPs were confirmed by FT-IR, XRD and characterised in terms of Zeta potential, *in vitro* drug release and SEM.

Keywords: Iron oxide magnetic nanoparticles, azithromycin, antibiotic resistance, drug release.

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ORAL PRESENTATION

The effects of curcumin and vitamin D on the Apelin/APJ System

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Abstract

Apelinergic system is constituted from apelin receptor, apelin and elabela. The main effects of apelinergic system are cardiac inotropic signalling, vasodilation, body fluid homeostatis, increasing insulin response, alleviation of oxidative stress and immune response modulation. Curcumin is a polyphenol and it is derived from Curcuma Longa L. It serves as a potent exogenous non-enzymatic antioxidant, via scavenging free radicals, reducing reactive oxygen species production. Vitamin D is a fat-soluble vitamin and it has been known that decreases chronic inflammation and oxidative stress. To our knowledge, the effects of the combined administration of curcumin and vitamin D on the apelinergic system have not yet been studied. In our study, we aimed to evaluate the effects of vitamin D and curcumin on apelinergic system. Forty-two Wistar rats were divided into six groups. Group I as control received physiological saline solution, group II received 200 mg/kg/d curcumin, group III received 0.4 µg/kg/d vitamin D_3, group IV received 200 mg/kg/d curcumin and 0.4 µg/kg vitamin D_3, group V received 400 mg/kg/day curcumin, group VI received 400 mg/kg/day curcumin and 0.4 µg/kg vitamin D_3. Serum apelin, elabela and nitric oxide (NO) levels were measured by ELISA. No statistically significant difference was found between the groups in serum apelin, elabela and NO levels. Our results suggest that the administration of curcumin and vitamin D does not have an adverse effect on the apelinergic systems of healthy rats. However, further research is needed to evaluate the potential effects of curcumin and vitamin D on the apelinergic system.

Keywords: Curcumin, vitamin D, apelin, elabela, nitric oxide.

ORAL PRESENTATION

Synthesis of phosphonate-substituted calixphyrin

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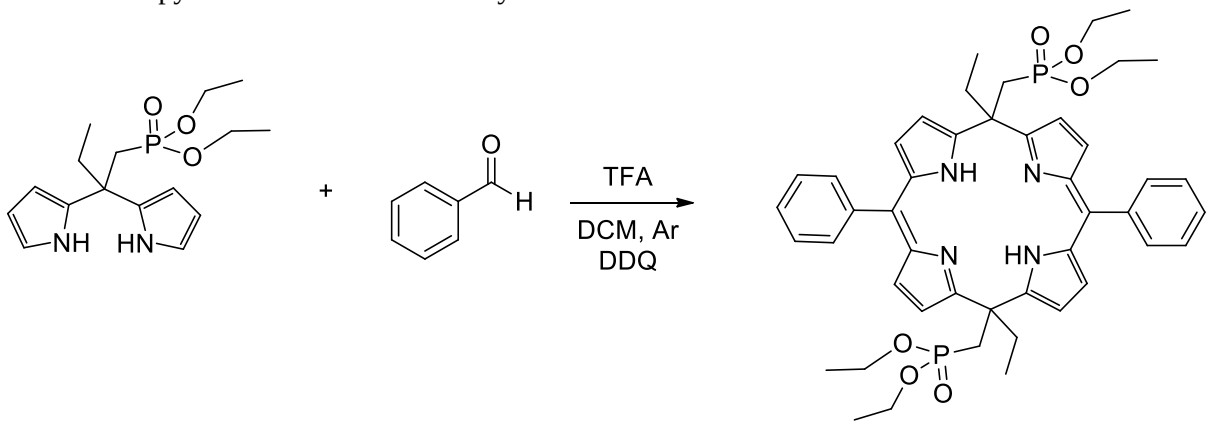
Abstract

Calixphyrins are a class of molecules at the interface between porphyrins and calixpyrroles. Calixphyrins are characterized as alternating sp^2- and sp^3-hybridized *meso*-carbon bridges, that leads to these compounds unique chemical properties. Because calixphyrins show new features, they are important in coordination chemistry, as catalysts and as sensors for ions and in supramolecular chemistry. Dipyrromethanes are important building blocks in organic synthesis for the preparation of porphyrins and porphyrin analogues. On the other hand, phosphonate group including compounds continues to attract considerable interest as they have important biological activities and synthetic aspects. Considering the important features mentioned above, in this study, the synthesis of phosphonate-substituted calixphyrin was successfully achieved by using phosphonate-substituted dipyrromethane and benzaldehyde.



Keywords: calixphyrins, dipyrromethanes, phosphonates.

ORAL PRESENTATION

Traditional plants used for medicinal-aromatic purposes in north villages of Ardanuç, Artvin (Türkiye)

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Abstract

A variety of traditional medicinal plants has been widely used by different indigenous people in Türkiye. This study, which was conducted between 2021 and 2023 to record the traditional medicinal plant usage culture of the inhabitants of North Villages of Ardanuç (Bağlıca, Çakıllar, Hisarlı, Kutlu, Cevizlik, Aşağıırmaklar, Yolağzı, Beratlı, Yaylacık, Hamurlu, Boyalı, Kaşıkçı, Konaklı, Karlı, Bulanık) was the first to be conducted in the district. face-to-face interviews with individuals with advanced ethnobotanical knowledge were conducted in the villages (15) of the district, and the medicinal plants which were used were gathered and identified. Besides descriptive statistics, the data were analyzed using some ethnobotanical analysis tools like preference ranking, paired comparison, direct matrix ranking, informant consensus factor, and fidelity level index. The information gathered as a result of the interviews with participants was recorded for this purpose. A total of 79 species of medicinal plants were identified and collected with 62 genera and 35 families were used to treat 44 different diseases. The most used medicinal plants were from the family "Rosaceae" (17 taxa) followed by "Lamiaceae" (8 taxa), and "Moraceae (4 taxa)". The usage value (UV) was calculated for each species. Out of these,56 species (70,88%) were used against human ailments. The most frequently used plant part was fruit (35,02%), followed by leaves (21.26%), and the condition of preparation was fresh plant materials (74.03%). The most widely used method of preparation was uncooked (23.79%) followed by cooked (22.94%). The most common route of administration was oral (74.03%) followed by dermal (25.97%). Furthermore, diseases for which medicinal plants were used were classified using The International Classification of Primary Care (ICPC), accepted by the WHO, and the Informant Consensus Factor (FIC) values for each disease category were calculated. Diseases with high FIC values are digestive problems (27 taxa). This is followed by the respiratory and skin disease categories (11 taxa for each). There was a high preference for vinegar form of *Malus sylvestris* was the most preferred species for the treatment of febrile illness. Anthropogenic factors are the major threats to medicinal plants. In general, the study area is rich in medicinal plants that have a significant role in the management of various human and livestock diseases.

Keywords: Artvin, Ardanuç, Medicinal-Aromatic Plants, Traditional, Türkiye

Acknowledgement: This study was produced from the project numbered 2021-TAB-01 titled 'Identification of Potential Areas for Medicinal and Aromatic Plants in Artvin (Inventory), Measurement of Public Awareness, Procurement of Live Material and Seeds' supported by the Presidency of the Republic of Turkey Strategy and Budget Directorate within the scope of the project themed "Regional Development Oriented Mission Differentiation and Specialization of Universities". We would like to thank the Presidency of Strategy and Budget of the Republic of Turkey for their support.

ORAL PRESENTATION

Carbonic anhydrase inhibitory activity of N-[(3-(4-substituelphenyl)-1,2,4-oxadiazol-5-yl)methyl]ethanamine compounds

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Abstract

Carbonic anhydrases (CA), which are commonly found in many tissues, are enzymes mainly responsible for catalyzing the conversion between CO2 and HCO3-. CA enzymes have been reported to be abnormally activated in the body in many pathological conditions. The use of CA enzyme inhibitors in the clinic for the treatment of various diseases such as glaucoma, cancer and obesity is a known pharmacological approach. Therefore, selective inhibition of CA enzymes has been the focus of many in vitro and in vivo studies. In the light of this information, in this study, molecular docking studies were performed with the compounds (N-[(3-(4-(furan-2-yl/1H-pyrrol-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)methyl]ethanamine) and hCAI and hCAII enzymes obtained by replacing the 3-thienyl ring in compound N-Methyl-N-[(3-(4-(thiophen-3-yl)phenyl)-1,2,4-oxadiazol-5-yl)methyl] ethanamine in our previous studies with furan and pyrrole rings, which are bioisosteres. The human carbonic anhydrase I (hCA I) enzyme's was imported from the protein data bank (PDB ID: '2FOY') (https://www.rcsb.org/). The human carbonic anhydrase II (hCAII) enzymes was imported from the protein data bank (PDB ID: '2ILI' (https://www.rcsb.org/). The enzymes were prepared in pdbqt file format using AutoDockTools 1.5.6 program. Ligands were drawn with the Chem3D 19.0 program, minimized, saved in pdb format. And converted to pdbqt file format with AutoDockTools 1.5.6 program. The molecular docking process was carried out with the latest AutoDock Vina program. The results were displayed in 2D and 3D with the Discovery Studio Client program. The binding energies of compound N-[(3-(4-(furan-2-yl)phenyl)-1,2,4-oxadiazol-5-yl)methyl]ethanamine are -6.4 kcal/mol for the hCAI enzyme and -6.8 kcal/mol for the hCAII enzyme. The binding energy of compound N-[(3-(4-(1H-pyrrol-1-yl)phenyl)-1,2,4-oxadiazol-5-yl)methyl]ethanamine is -6.9 kcal/mol for the hCAI enzyme and -7.0 kcal/mol for the hCAII enzyme. As a result of the molecular docking study, these compounds are thought to have potential inhibitory effects for hCAI and hCAII enzymes.

Keywords: 1,2,4-oxadiazole, carbonic anhydrase, molecular docking

ORAL PRESENTATION

Green Synthesis of Gold Nanoparticles with *Sorbus aucuparia* L. fruit Extract

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Abstract

This work describes a particular method for producing environmentally friendly gold nanoparticles (AuNPs) by extracting from *Sorbus aucuparia* L. fruit and to analyze the resulting particles. *Sorbus aucuparia* L. fruits underwent a thorough cleaning process, including multiple washes with deionized water and was dried under appropriate conditions. Subsequently, 100 g of dried fruits of *Sorbus aucuparia* L. were boiling for 60 minutes in 500 mL of deionized water. The produced aqueous solution (0.1–0.6 mL) of was mixed with different concentrations (0.05-1 mM) of $HAuCl_4.3H_2O$ solution in a microwave device. Au nanoparticles were produced after the combination was subjected to microwaves at different power (180-360-600W) for one to fifteen minutes. Zeta potential and UV-visible spectroscopy were used to characterization of the produced AuNPs (1,2).

Keywords*:* AuNP, *Sorbus aucuparia* L., UV–Vis spectroscopy.

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ORAL PRESENTATION

Effect of telomerase inhibitor BIBR1532 on Wnt signaling pathway in breast cancer and breast cancer stem cells

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Abstract

Breast cancer is a leading disease in cancer-related deaths in women. It is one of the most common types of cancer in Turkey as it is in the whole world. Breast cancer and breast cancer stem cells are associated with tumor growth, multidrug resistance, metastasis, and relapse of cells, and targeting these cells in the treatment process is thought to be effective. Cancer stem cells are a subpopulation of cells within a tumor that create resistance to metastasis and therapy and are the cells responsible for tumor initiation. These cells, like stem cells, have self-renewal, proliferation, and differentiation properties. The Wnt Signaling Pathway is tightly linked to cancer and is associated with reduced survival in breast cancer patients, and this signaling pathway controls the properties of cancer stem cells. However, BIBR1532 is a telomerase inhibitor and is widely used in cancer research to inhibit active telomerase in tumors. It is a preferred telomerase inhibitor because it has few side effects in normal tissues. In our study, it is aimed to investigate the effect of telomerase inhibitor BIBR1532 on Wnt signaling pathway in breast cancer and breast cancer stem cells.
First, the cytotoxicity of the active substance BIBR1532, which was applied to MCF-7 and BCSCs (breast cancer stem cells), which we used in this study, was determined. The IC50 doses of the cells whose apoptotic effects were investigated later, were found to be 34.59 μM in MCF-7 cells and 29.91 μM in BCSC line at 48th hour. Considering the apoptosis results, it was observed that apoptosis was severely induced in MCF-7 and BCSC cell lines. Gene expression changes in genes in the Wnt signaling pathway were determined by RT-qPCR. When the gene expression changes of the genes in the Wnt pathway in our study were examined, it was seen that some of the genes that we know to be oncogenes were down-regulated, and some tumor-suppressing genes were up-regulated. Gene expression results obtained from genes that have not been studied much in breast cancer studies will also contribute to the literature in this sense.

Keywords: Breast Cancer; Breast Cancer Stem Cells; Wnt Signaling Pathway; BIBR1532; Telomerase inhibitör

ORAL PRESENTATION

Immunohistochemically iNOS Expression in the Cerebellum of Dogs Infected with Canine Distemper Virus and its Relationship with Cell Death

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Abstract

Canine distemper is a highly contagious and fatal disease of wild and domestic carnivores. Canine distemper virus causes multifocal demyelination in the central nervous system in infected dogs. Clinical findings such as apathy, behavioral disorders, muscle atrophy, hyperesthesia, ataxia and seizures occur in infected animals. Histopathological findings of the disease include demyelination in the substantia alba of the cerebellum, gemistocytic astrocytes, and intranuclear and occasionally cytoplasmic inclusions in glial cells. In this study, iNOS expression in the cerebellum of distemper disease in dogs and its relationship with apoptosis were investigated. For the study, 22 tissue blocks diagnosed with distemper obtained from the pathology department archive were used. Eight tissues that died from other diseases and whose cerebellum was not affected were used as controls. All 5-micron sections taken on the slide in the microtome were histopathologically stained with hematoxylin-eosin, distemper, iNOS antibody and TUNEL. Histopathologic and immunohistochemically, all tissues were diagnosed with distemper. At the end of the study, it was seen that iNOS expressions and TUNEL positive staining in distemper tissues were significantly increased compared to controls. These results suggested that iNOS may be one of the factors that can trigger apoptosis in the neuropathology of distemper disease. More detailed studies are needed regarding the neuropathology of the disease.

Keywords: Distemper, Immunohistochemistry, iNOS, TUNEL.

ORAL PRESENTATION

Investigation of CB_1R Mechanism of Migraine-inducing Nitroglycerin by *In Silico* Molecular Docking Analysis

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Abstract

Nitroglycerin (NTG), one of the nitrovasodilators, is known to trigger migraine attacks in the clinic and is also used in pre-clinical studies to create a migraine model in animals. In recent studies, cannabinoid type-1 receptor (CB1R) activation has been shown as a therapeutic target in migraine treatment. However, the binding affinity and binding modes of CB1R with the migraine trigger NTG remain unclear as a scientific gap. The aim of this study was to predict the binding energy and binding sites of NTG and CB1R.

Molecular docking studies were performed with AutoDock 4.0 to predict the possible binding sites of NTG on the crystal structure of the CB1R (PDB ID: 6WSK). Before docking analysis, the parameters of the receptor and ligand (NTG) molecules were prepared with AutoDockTools (ADT). After 100 independent docking runs for the NTG molecule, all possible binding modes were clustered by the program and ranked for the selected NTG pose according to the binding free energy kcal/mol of the conformation with the best docking pose with the lowest binding free energy. The AutoDock 4.0-mediated best docking pose between ligand and receptor was visualized using BIOVIA Discovery Studio Visualizer 2016.

NTG binds to CB1R with a Gibss free binding energy of -10.09 kcal/mol, which is stronger than the threshold binding energy (-6.00 kcal/mol). The NTG ligand binds to the CB1R protein with 8 different chemical bonds and interacts with 8 different amino acids.

The results of the study show that NTG binds strongly to the CB1R protein. This preliminary study may guide more comprehensive studies in the future.

Keywords: Nitroglycerin; CB1R; Molecular Docking

ORAL PRESENTATION

iNOS Expression in Intestinal Inflammation Caused by Paratuberculosis Disease in Goats

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Abstract

In this study, paratuberculosis disease, which causes loss of productivity in goats in our country and around Adana and is mostly latent, was investigated pathologically. The material of the study consisted of 25 goat intestinal tissues and mesenteric lymph nodes from districts and villages around Adana that died with suspicion of paratuberculosis and were necropsied and subsequently diagnosed with paratuberculosis using the Ziehl-Nelson staining method. Intestinal tissues from 8 animals that died from diseases that did not affect the digestive system were also used as controls. The collected intestinal tissues were kept in a 10% formaldehyde solution for at least 48 hours and then washed in tap water overnight. Afterwards, they were passed through graded alcohols and made transparent in xylene, then embedded in paraffin and blocked. Sections were cut onto 5 µ slides from the blocked tissues. Sections were stained with hematoxylin-eosin, Ziehl-Nelson and immunohistochemically with iNOS antibody. After diagnosis of paratuberculosis was made with histopathological findings and Ziehl-Nelson staining, iNOS antibody staining results were evaluated. As a result, it was determined that iNOS expression in paratuberculosis tissues was significantly increased compared to control animals. With these results, it was thought that the prevalence of paratuberculosis in goats in the region was high and iNOS expression may play an active role in the pathogenesis of intestinal inflammation in the disease. However, more extensive research is needed on the subject.

Keywords: Goat, Paratuberculosis, iNOS

ORAL PRESENTATION

Anti-cancer effect potential of evernic acid on ovarian cancer

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Abstract

Ovarian cancer, which is caused by the uncontrolled proliferation of cells on the surface of the ovarium or in the fallop tubes, is the eighth most common cancer in women worldwide. Ovarian cancer, which can usually be detected in advanced stages, has high mortality rates. Despite the advances in diagnostic and therapeutic processes, it is still an important need to identify innovative drug candidate molecules with lower toxicity and low side effects, with anti-cancer effects, and to develop alternative treatment methods. The aim of this study was to reveal the potential therapeutic effect of evernic acid (EA), a lichen secondary metabolite with important bioactive properties, on ovarian cancer cells. The antiproliferative effect of EA on different ovarian cancer cell lines (A2780, SKOV-3, and OVCAR-3) and the normal ovarian cell line OSE was determined by the xCELLigence RTCA system. Afterwards, various experiments, including colony formation, wound healing, cell cycle arrest, and apoptosis rate determination, were performed to reveal the therapeutic effect of EA on cells. The results showed that EA decreased cell viability in ovarian cancer cells, especially OVCAR-3 cells, in a dose- and time-dependent manner without showing significant cytotoxic effects on normal cells. It was determined that the invasion and colony-forming abilities of EA-treated ovarian cancer cells have decreased compared to the control. Moreover, it was found by flow cytometry that EA caused cell cycle arrest in the G2/M phase in ovarian cells and significantly increased the rate of early and late apoptosis. The findings have demonstrated that EA has a significant anti-proliferative effect on ovarian cancer cells with low cytotoxic effects in normal cells, providing the potential of EA as a novel and alternative drug candidate molecule for the treatment of ovarian cancer.

Keywords: evernic acid, ovarian cancer, apoptosis

ORAL PRESENTATION

The new drug candidate molecule evernic acid shows therapeutic activity through the apoptosis pathway by regulating gene and protein expression levels in ovarian cancer cells

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Abstract

Ovarian cancer has the highest mortality rate among female gynecological cancers. Because current treatments have serious side effects and patients develop resistance to treatment, treatment processes are difficult, resulting in a decrease in survival rates. Therefore, there is a need to search for innovative drug candidate molecules with high therapeutic efficacy and low side effects for the treatment of ovarian cancer. The aim of this study was to reveal the association of the therapeutic efficacy of evernic acid (EA), a lichen secondary metabolite with proven anti-proliferative effect on ovarian cancer, with the apoptotic death pathway. The activity of EA to target the apoptosis pathway was investigated by acridine-orange staining and DAPI staining experiments, gene expression profiles were examined by the qRT-PCR analyses, and changes in protein levels were determined by western blotting. The study showed that the number of apoptotic cells increased in EA-treated OVCAR-3 ovarian cancer cells compared to the control, according to the results of DAPI and acridine-orange staining methods. The qRT-PCR analyses revealed significant changes in the expression levels of 30 genes involved in the apoptosis pathway after treatment with EA. *CASP1, CASP3, CASP10,* and *TRADD* expression levels were significantly increased while *BIRC5* expression level was decreased, indicating that EA treatment induces apoptosis by increasing apoptotic signaling. Moreover, the expression levels of CASP3 and CASP9 proteins were also found to be increased. The findings indicate that EA induces apoptosis through both intrinsic (internal or mitochondrial) and extrinsic (extrinsic) pathways by regulating gene and protein expression levels. According to the study, EA has an anti-cancer activity on ovarian cancer by regulating the apoptosis pathway and EA can be novel drug candidate molecule for treatment of ovarian cancer.

Keywords: evernic acid, ovarian cancer, therapeutic activity, apoptosis

ORAL PRESENTATION

Determination of Chemical Compositions and Antioxidant Activity of *Prunus cerasifera*

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Abstract

Cherry plum, whose Latin name is *Prunus cerasifera* (*P. cerasifera*), belongs to the Rosaceae family. *P. cerasifera* fruits play an important role in human health and nutrition with their antioxidant content and other nutritional values [1-3]. *P. cerasifera* was extracted using methanol and acetone (40°C) for 45 minutes using ultrasounic bath (Heidolph, USA). For HPLC-DAD and antioxidant analysis, the filtered extracts were evaporated and dissolved in methanol. High performance liquid chromatography-diode array detection (HPLC-DAD) was used to analyse the phenolic composition of the extracts. Total phenolic content (TPC), total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) were used to characterise the antioxidant potential of the extracts. *P. cerasifera* methanol extract showed higher antioxidant activity than acetone extract. Different amounts of gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, ferulic acid and chlorogenic acid were detected in both extracts using HPLC-DAD.

Keywords: *Prunus cerasifera*, *Cherry plum*, antioxidant activity, HPLC-DAD

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ORAL PRESENTATION

The Effect of Extract Application Obtained from Catharanthus Roseus on POMP Gene Expression in AGS Cells

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Abstract

Gastric cancer ranks among the leading causes of cancer-related deaths worldwide and poses a significant threat to life today. One of the crucial factors involved in cancer development is proteins. The proteasome maturation protein (POMP) plays a role in the formation of the 26S proteasome, a component of the ubiquitin-proteasome pathway, and is a critical factor in the degradation of misfolded proteins. This project was conducted to investigate the effects of the extract of the plant *C. Roseus*, which contains over 200 alkaloids used in the treatment of various diseases, including cancer, on cell viability and POMP gene expression in AGS cells. AGS gastric cancer cells were cultured in a medium consisting of 89% Dulbecco's Modified Eagle Medium (DMEM), 10% Fetal Bovine Serum (FBS), and 1% Penicillin-Streptomycin at 37 ºC and %5 CO2. The AGS cells, seeded in 60 mm petri dishes at a density of 10^5, were treated with *C. roseus* extract at dilutions of 1:10, 1:100, and 1:1000 for 24 and 48 hours. Following the treatments, total RNA was isolated from AGS cells, and cDNA synthesis was performed. The expression of the POMP gene was analyzed using real-time polymerase chain reaction (RT-PCR). A significant decrease in POMP gene expression was observed at the end of 24 hours, while a significant increase in POMP gene expression was observed at the end of 48 hours.
It has been observed that, within 48 hours of application, the alkaloids present in *C. Roseus* induce degradation of tubulin proteins in AGS cells, resulting in decreased cell viability and increased POMP gene expression. The results obtained after 24 hours of application suggest that the inhibition of protein polymerization by the alkaloids present in the extract may lead to the accumulation of proteins within the cell, potentially inducing autophagy.

Keywords: C.Roseus, POMP, Gastric Adenocarcinoma

ORAL PRESENTATION

Is There a Role for the Endocannobinoid 2-Arachidonyl Glycerol in the Analgesic Effect of Meloxicam?

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Abstract

Monoacylglycerol lipase (MAGL) is an important enzyme that hydrolyzes the endocannabinoid 2-arachidonoylglycerol (2-AG), which plays a role in endogenous analgesia. Meloxicam is a non-steroidal anti-inflammatory drug used in all kinds of musculoskeletal pain and inflammatory conditions, especially rheumatoid arthritis, osteo arthritis and myalgia. However, the mechanisms of action of meloxicam in its pain-relieving effect have not been sufficiently elucidated. The aim of this study was to estimate the binding energy and binding sites of meloxicam with the MAGL enzyme. Molecular docking analyses were performed with AutoDock 4.0 to predict the possible binding sites of meloxicam on the crystal structure of MAGL enzyme (PDB ID: 6AX1). Before the docking analysis, the parameters of the enzyme and ligand (meloxicam) molecules were prepared with AutoDockTools (ADT). After 100 independent docking runs for the meloxicam molecule, all possible binding modes were clustered by the program and ranked for the selected agomelatine pose according to the binding free energy kcal/mol of the conformation with the best docking pose with the lowest binding free energy. The AutoDock 4.0-mediated best docking pose between ligand and enzyme was analyzed with BIOVIA Discovery Studio Visualizer 2016. The docking of meloxicam with human MAGL enzyme showed that meloxicam docked with a Gibss free binding energy of -8.17 kcal/mol, which is stronger than the threshold binding energy (-6.00 kcal/mol). The meloxicam molecule showed 7 different chemical bonds and 15 different amino acid interactions with the human MAGL enzyme. The results of the study indicate that meloxicam increases the endocannabinoid 2-AG by binding strongly to the MAGL enzyme. This study may offer a new perspective on 2-AG in the mechanism of analgesic action of meloxicam.

Keywords: . Meloxicam; MAGL; Molecular docking

ORAL PRESENTATION

Enhancing Anti-cancer Compounds in Madagascar Periwinkle through Magnetic Field

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Abstract

Madagascar Periwinkle (*Catharanthus roseus L.*) is an annual flowering ornamental plant contains more than 130 terpenoid indole alkaloids (TIA) used in the treatment of cancer, genitourinary system, kidney, liver, cardiovascular diseases. It is of great interest in the field of pharmacology, especially for its anticancer bisindole alkaloids (vinblastine, vincristine and vindesine) content. For this reason, many researchers have tried various plant tissue culture-based biotechnological interventions to change the alkaloid profile of *Catharanthus roseus* to date. Magnetic field (MF) treatment is one of the physical stimulants used to break seed dormancy, change germination percentage and speed, and affect plant metabolism, growth and development. The aim of this study was to induce the production of anticancer agents of vincristine and vinblastine in *Catharanthus rosesus* by using magnetic fields. Seeds exposed to 4.6 mT magnetic field for 2.44, 6.6, 19.8 and 33 seconds were harvested after 38 days of growth under in vitro conditions. Seed germination increased depending on the increasing magnetic field treatment. In the gene expression results, especially in samples exposed to magnetic field for 6.6 s, the expression level of ORCA3 gene along with D4H gene increased. While low level magnetic field treatment caused up-regulation of a few vinblastine and vincristine related genes such as ORCA3 and D4H, high level magnetic field treatment caused down-regulation of these genes. Different vincristine accumulation was detected between 2.44, 6.6, 19.8 and 33 seconds of exposure to 4.6 mT magnetic field. By FITR analysis, strong C=O bonds were determined in 2.44, 6.6- and 19.8-seconds magnetic field applications. It was determined that H bonds were broken in samples exposed to magnetic field for 33 seconds. In the continuation of our study, the transcriptional results will be compared after the product amount is determined by HPLC technique.

Keywords: Catharanthus roseus, Magnetic field, Vinblastine, Vincristine

ORAL PRESENTATION

Effects of Boron Dietary in Close-up Period on Calcium, Phosphorus and Magnesium Levels in Newborn Calf

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Abstract

In this study, we aimed to investigate the effects of different levels of boron supplementation to the diet during the close-up period and the first postpartum day on Calcium (Ca), Phosphorus (P) and Magnesium (Mg) levels in newborn calf. In our study, two experimental groups and one control group were formed. In the experimental group, 300 (T1) and 600 (T2) ppm boron was added to the close-up period ration (47.4% concentrate feed + 52.56% roughage) and the ration given on the first postpartum day (54.80% concentrate feed, 45.20% roughage). Ca, P and Mg values were determined by spectrophotometry using a biochemical autoanalyser (Biotecnica BT3000 plus, Italy) with commercial colorimetric test kits in calf blood samples taken at 24th and 48th hours following colostrum intake and boron analysis in TMR and calf blood samples were performed by ICP-MS. Boron level in the basal ration given in the close-up period was 378.13 ppb. Boron levels in the blood serum of calves born from the experimental groups were statistically significantly higher than the control group ($p<0.05$). 300 and 600 ppm boron supplementation to TMR in the close-up period significantly increased blood Ca values (12.09 ± 0.81; 12.31 ± 1.4; 11.31 mg/dL, T1, T2 and control, respectively) at 24 hours in newborn calves compared to the control group ($p<0,05$), Mg values (T1, T2 and control 2.74 ± 0.45; 3.05 ± 0.45; 3.24 ± 0.48 mg/dL, respectively) decreased significantly in the T1 group ($p<0.05$), but P levels (9.07 ± 0.77; 8.76 ± 0.95; 8.94 ± 0.98mg/dL in T1, T2 and control respectively) were not statistically significant. In addition, there was no statistically significant between the calf blood Ca, P and Mg values at 48th hour between the groups. As a conclusion, it was observed that the effect of boron supplement on Ca, P and Mg levels obtained through colostrum in the first milking was more effective than that obtained through colostrum in the second milking in postpartum.

Keywords: Boron, Calf, Ca, Mg, P

ORAL PRESENTATION

Lipid-based edible film synthesis using recovered lipid of coffee waste: A sustainable approach

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Abstract

Coffee is one of the most popular beverages in the world. Its production was reported as almost ten millions of tons annually. The abundant type of the waste is called Spent Coffee Ground (SCG) which is formed during the production of instant coffee and also as a result of brewing. The disposal of the waste is managed by incinerating or sending to landfills [1], however, these strategies result in serious problems related with the pollution of the environment. Regarding considerable amount of the waste, evaluation of SCG is important in terms of sustainability and circular bioeconomy. SCG contains cellulose, hemicellulose, proteins, lipid, lignin, polyphenols and caffeine [2]. These valuable components make SCG a potential zero-cost source for pharmaceutical, food, energy and agriculture areas. On the other hand, petroleum based packaging materials comprise another field that cause pollution to the environment. Recyclable and biodegradable materials are under research to replace plastic based ones. In this study, we aimed to recover lipid from SCG waste using ultrasound-assisted extraction with environmentally friendly green Deep Eutectic Solvents (DES) and to use recovered lipid in the synthesis of lipid based-edible films. Experimental design was also conducted by using Box-Behnken design using liquid-to-solid (LS), time and temperature as independent variables and lipid yield as the response. The lipid, recovered at the optimum conditions was used in the synthesis of the edible films. To our knowledge, it is the first time utilization of SCG lipid in the synthesis of lipid-based edible films, that were extracted with different types of DESs.

Keywords: Coffee waste, edible film, lipid recovery

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ORAL PRESENTATION

The effects of *Rubus discolor* root extract on lipid accumulation of in Hepg2 cells

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Abstract

The *Rubus* species exhibit potent antioxidant properties due to their high phenolic compound content. In our study was investigated in vitro antioxidant and anti-inflammatory effects of ethanol extract of *Rubus discolor* root (RDE). Subsequently, it was investigated that the effect of RDE on lipid accumulation in HepG2 liver cells. For this purpose, HepG2 cells were treated with oleic acid to induce lipid accumulation, and the effect of RDE on lipid accumulation was assessed with using Oil-Red staining and measuring intracellular triglyceride and cholesterol levels. The melatonin was used as a positive control. Enzymes involved in lipid synthesis, acetyl-CoA carboxylase (ACC1), fatty acid synthase (FAS), transcription factor sterol regulatory element-binding protein 1 (SREBP-1c) mRNA levels and antioxidant status were evaluated by RT-PCR. As dose-dependent, RDE significantly reduced lipid accumulation. It significantly suppressed ACC1, FAS, and SREBP-1c ($p<0.001$, $p<0.001$, $p<0.01$, respectively) at a dose of 50 µg/mL. The extract also exhibited in vitro antioxidant and potent anti-inflammatory properties. The results indicate that RDE has the potential to prevent lipid accumulation. These findings could provide a basis for further studies on the potential therapeutic effect of *Rubus discolor* in the treatment of NAFLD.

Key words:: *Rubus discolor*, HepG2, lipid metabolism, root extract, phytochemical contents

ORAL PRESENTATION

Dysbiosis and Hemorrhagic Shock: Respiratory Response

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Abstract

Dysbiosis refers to the imbalance or disruption in the normal composition of microbial communities, particularly in the gut microbiota. Hemorrhagic shock is a condition characterized by inadequate tissue oxygenation due to circulatory failure, triggering compensatory mechanisms, including respiratory adjustments. Recent studies highlight the gut-lung axis, emphasizing the bidirectional relationship between gut microbiota and respiratory health. While research indicates that gut dysbiosis can affect the respiratory system, its specific role in respiratory responses during the reversal of hypotension remains unclear. Six pregnant Sprague Dawley rats were divided into two groups: the dysbiosis group, treated with oral antibiotics (amoxicillin+vancomycin;n=3), and the control group, treated with oral saline (n=3) during the last week of pregnancy. Treatments continued until the offspring were weaned at 4 weeks of age. Dysbiosis was induced in the dysbiosis group offspring (n=7) by administering antibiotics for an additional 4 weeks, while control group offspring (n=7) received saline. At 12 weeks, respiratory parameters, including tidal volume (TV), respiratory rate (RR), and minute ventilation (RMV), were measured before and after hemorrhagic shock. Statistical analyses were performed using two-way RM-ANOVA followed by post hoc Bonferroni tests ($p<0.05$). According to the findings of the study, in the control group, TV decreased from 3 ml to 2.5 ml during hemorrhagic shock, while in the dysbiosis group, it dropped to 2.3 ml. In the control group, the RR increased from 70 breaths/min to 78 breaths/min following hemorrhagic shock, and was recorded at 75 breaths/min after one hour. In the dysbiosis group, the RR increased to a maximum of 74 breaths/min and was recorded at 73 breaths/min after one hour. As for the RMV values, in the control group, the baseline value of 210 ml/min decreased to 195 ml/min after hemorrhage and to 187.5 ml/min after one hour. In the dysbiosis group, these values were recorded as 170.2 ml/min and 167.9 ml/min, respectively.
These findings suggest that gut dysbiosis impairs the respiratory system's recovery following hemorrhagic shock, potentially limiting the compensatory mechanisms that restore blood pressure.
This research was funded by Bursa Uludag University Scientific Research Project Foundation, grant number TGA-2022-1216.

Keywords: Dysbiosis, Hemorrhagic shock, TV, RR, RMV.

ORAL PRESENTATION

Effect of cerium oxide nanoparticles alone and in combination with doxorubicin on the viability of ER-, PR- and HER2+ breast cancer cells

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Abstract

Previous studies have shown that cerium oxide (CeO) nanoparticles exhibit cytotoxic and anti-invasive properties for cancer cells while they may have a protective effect on normal cells. Protective effects have also been reported in breast cells exposed to irradiation. In this study, the effects of CeO nanoparticles, alone and in combination with doxorubicin (DOX), an effective chemotherapy agent widely used in the treatment of many types of cancer, including breast cancer, on the viability of ER-, PR-, and HER2+ breast cancer cell line MDA-MB-453 cells were investigated. Cells were treated with 0, 2, 5, 10, 25, 50, and 100 mM CeO nanoparticles and 0, 5, 25, 50, 100, and 250 µM DOX for 24, 48, and 72 hours. Cell viability was determined by MTT assay. Cell viability in cells treated with CeO nanoparticles decreased with increasing dose, and these decreases were statistically significant for all CeO nanoparticle concentrations at 72 hours ($p \leq 0.05$). Cell viability in cells treated with DOX was statistically significantly decreased ($p \leq 0.05$). To determine the possible synergetic effect of CeO nanoparticles on DOX, they were treated with 2 µM DOX and 1.5, 2, 3, 4, 6, and 8 mM CeO nanoparticles for 24 and 48 hours. At all treatment times, both DOX and DOX+CeO treatments decreased cell viability values statistically significantly compared to the control group ($p \leq 0.05$). Cell viability values were found to be lower than the viability values in cells treated with DOX alone in all treatments except 8 mM CeO+DOX after 48 hours of treatment and in all DOX+CeO treatments tested after 24 hours of treatment. These results show that CeO nanoparticles increase the effectiveness of DOX in breast cancer cells.

Keywords: Cerium oxide, doxorubicin, MDA-MB-453

ORAL PRESENTATION

The effect of the gut microbiota, a consortium of microorganisms, on brain health

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Abstract
The gut-brain axis acts as a two-way bridge between the gastrointestinal system and the nervous system. It has been explained that gut microbiota interacts with the brain through the gut-brain axis and has effects on human neurodevelopment. It is generally thought that gut microbiota regulates neurodevelopment in three ways: immune pathway, neuronal pathway and systemic pathway. Gut microbiota interacts with the brain by producing substances such as neurotransmitters and neuromodulators and microbial metabolites. In a state of dysbiosis, the balance of these substances and metabolites will change. Thus, it will contribute to neuroinflammation in central nervous system disorders by causing changes in intestinal barriers and immunological barriers. For example, microbiota-derived peptidoglycan fragments can cross the blood-brain barrier and affect responses such as social behavior, anxiety, and stress, which play a role in neurodevelopmental disorders such as autism spectrum. Fecal microbiota transplantation and antibiotic applications are used to explain brain-gut interactions. Many studies have shown that neurochemicals produced by intestinal bacteria affect behavior in humans and animals. According to a recent study, *Lactobacillus reuteri* supplementation improved intestinal microbiota and regulated metabolic functions in the brain. Another study reported that *Campylobacter jejuni*, one of the first causes of bacterial diarrhea, affected anxiety-related behaviors with the activation of sensory ganglia after intestinal application. With all this, it is thought that when we move to the other end of the bridge, the gut microbiota can also change. Intestinal permeability can change thanks to signaling molecules released from brain cells into the intestinal lumen. Therefore, this increase in intestinal permeability may affect the etiology of neurological diseases such as Alzheimer's and depression. As a result, understanding the brain-gut-microbiota interaction mechanism, which is quite cosmopolitan, will be enlightening in explaining the pathogenesis of many diseases, especially neurodevelopmental diseases. Thus, the intestinal microbiota, called the second brain, is very important for general body health. Therefore, elucidating all the mechanisms affecting the formation and change of microbiota is of great importance for a healthy microbiota and a healthy brain.
Keywords: Gut, Brain, Microbiota

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ORAL PRESENTATION

DNA Repair Mechanisms

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Abstract

DNA, like RNA or protein, is exposed to various chemicals. As the only permanent copy of the cell genome in living organisms, changes in the basic structure of DNA have far more important consequences than any other molecule in the cell. Mutations in the process of DNA replication can result from the insertion of the wrong bases. In addition, most chemical changes in DNA are caused both spontaneously and by prolonged exposure to radiation or chemicals. DNA is generally considered to be one of the intermediate sources of UV radiation DNA damage, and it is the form of DNA damage that has been most thoroughly investigated in the context of repair mechanisms. The fact that UV exposure in sunlight is the cause of almost all skin damage in humans is an important indicator of this. Such damage to DNA can stop transcription and replication and lead to an increased number of mutations. Cells have evolved mechanisms to repair damaged DNA to maintain genome integrity. This study was conducted to give detailed information about DNA repair mechanisms.

Keywords: DNA, RNA, Protein, Mutation, Radiation and Repair.

ORAL PRESENTATION

A benzothiazole derived chromenylium-cyanine fluorescent probe for the analysis of Hg^{2+}

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Abstract

Near-infrared (NIR) chemosensors occupy a prominent position in studies related to biological and environmental fields. Within this scope, chromenylium-cyanine compounds are particularly notable due to their exceptional optical properties, including high fluorescence quantum yields and absorption coefficients characteristics. These properties are similar to classical rhodamine-based sensors. Recent advancements have led to the development of chromenylium-cyanine-based chemosensors that not only exhibit spirolactam ring opening and closing properties similar to those of rhodamine but also possess extended absorption and emission wavelengths analogous to cyanine dyes. Mercury, despite its extensive historical usage, is recognized as a heavy metal with well-documented environmental and biological hazards. While mercury continues to find applications in various fields such as thermometers, manometers, and dentistry, it presents severe risks to living organisms. Thus, the detection of trace amounts of mercury is of considerable importance [1,2].
In this study, the **NIR20** compound was synthesized through the reaction of **CS2** and 2-aminobenzothiazole. The characterization of the **NIR20** compound was carried out using well-known analytical techniques, including ^{1}H NMR, ^{13}C NMR, HRMS, and FTIR. Trace mercury analysis was performed using Uv-Vis and fluorescence spectral methods with the **NIR20** compound.

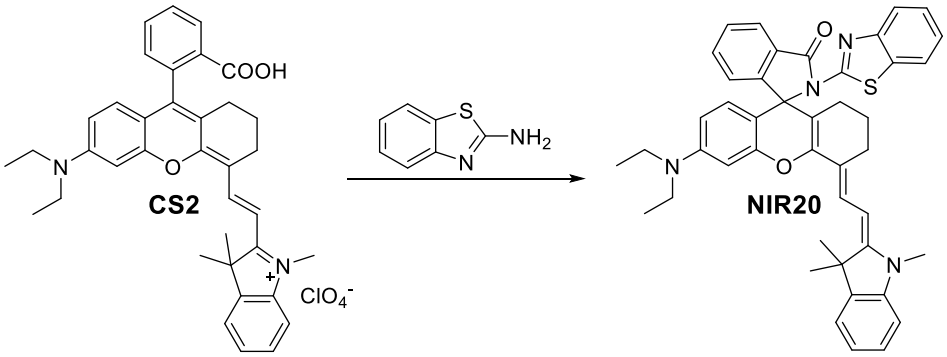

Figure 1. Synthesis of **NIR20** compound.

Keywords: Chromenylium-cyanine, mercury, probe

This work was supported by the Council of Higher Education (YÖK)-Istanbul Technical University (ITU) Research Fund YAP (Project No.: TGA-2023-43934).

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ORAL PRESENTATION

Construction of a new near-infrared and turn-on colorimetric probe based on chromenylium-cyanine platform with its crystal structure for determination of toxic Hg^{2+} ion

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Abstract

Heavy metal pollution is a major threat to human health and the ecosystem. In particular, the fact that mercury and its derivatives are difficult to degrade leads to the accumulation of this metal in organisms and its transfer to humans through the food chain. These metal ions can directly affect the human nervous system, liver, heart, brain and immune system even at ppm levels. Therefore, instantaneous and continuous monitoring of Hg^{2+} ions in environmental samples is essential.

Nowadays, near-infrared (NIR) and turn-on colorimetric probes attract great attention in the scientific area due to their superior properties such as low auto-fluorescence, high tissue penetration and visual detection possibility. Although there are several platforms such as BODIPY, pyrene, coumarin, quinone and squarine in the literature that can detect Hg^{2+} ions, most of them suffer from sensitivity, long detection time, absorption at low wavelength and are not suitable for visual analysis [1,2].

In this study, a NIR turn on and colorimetric chromenylium-cyanine-based chemical sensor decorated with bromophenyl-thiadiazole unit (CS-BTDA) that can selectively and sensitively detect toxic Hg^{2+} ions was synthesized (Figure 1) and its structure was elucidated by various spectroscopic methods including X-ray diffraction analysis method. This probe is expected to have superior properties that will solve all the analytical deficiencies in the literature.

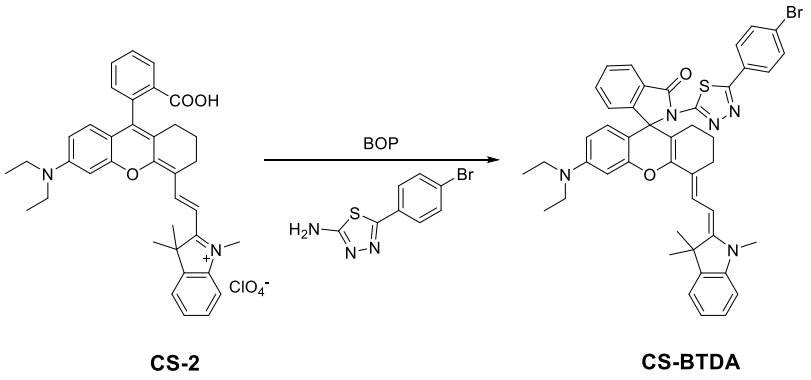

Figure 1. Synthesis of **CS-BTDA** compound

Keywords: Mercury, Chromenylium-Cyanine, Near-Infrared, Toxic, Colorimetric

This work was supported by the Council of Higher Education (YÖK)-Istanbul Technical University (ITU) Research Fund YAP (Project No.: TGA-2023-43934).

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ORAL PRESENTATION

Effects of *Laurus nobilis* fruit oil on germination of cress

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Abstract

Laurus nobilis L. (laurel) belongs to the Lauraceae family and has critical chemical components in different parts such as roots, stems, leaves and fruits. The most well-known and essential part of these components is the volatile oils found mainly in its leaves, while fixed oils are also found in its fruits. The effect of some chemical substances produced secondarily in plants on the development of another plant is defined as allelopathy. This study aimed to investigate the allelopathic effects of fixed oils of the laurel plant on cress (*Lepidium sativum* L.) a spice plant. For this purpose, different concentrations (0, 0.5, 1 and 5 mg ml^{-1}) of fixed oils obtained by soxhlet extraction from laurel fruits were prepared. Seeds of the Helen variety of cress were placed in petri dishes and watered with oil solutions, and their germination was ensured under controlled conditions. At the end of the experiment, germination percentage was calculated, and root and stem length and fresh and dry weight values were measured for developing plants. According to the statistical evaluation of the findings (ANOVA, $p<0.05$), it was observed that 1 and 5 mg ml^{-1} fixed oil concentrations had adverse effects on germination percentages, root and shoot lengths and fresh weight. In light of these, the allelopathic effect of laurel fixed oils on the germination stage of the cress plant was revealed.

Keywords: Allelopathy, laurel, cress, germination

ORAL PRESENTATION

Enhanced photocatalytic hydrogen evolution activity by in situ photodeposition of molybdenum sulfide on $NiTiO_3$

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Abstract

In the literature, numerous titanates, including $SrTiO_3$, $BaTiO_3$, $FeTiO_3$, and $NiTiO_3$, have been recognized for their photocatalytic properties. In contrast, $NiTiO_3$ possesses a significantly narrower band gap of approximately 2.18 eV compared $BaTiO_3$ and $SrTiO_3$[1]. Nickel titanate ($NiTiO_3$) is an emerging material in the field of photocatalysis. Its unique properties make it a promising candidate for photocatalytic applications, such as hydrogen production, environmental remediation, etc. In the process of photocatalytic hydrogen production, co-catalysts play a pivotal role in boosting the efficiency of the reaction. They are generally introduced to enhance specific steps, particularly by aiding the hydrogen evolution reaction (HER), improving charge separation, minimizing electron-hole recombination, and lowering the activation energy barrier. Noble metals such as platinum (Pt), palladium (Pd), and gold (Au) are frequently utilized for their superior capacity to accept photogenerated electrons and promote hydrogen production activity[2]. In this context, molybdenum disulfide (MoS_2) has become a promising co-catalyst due to its excellent catalytic activity, abundance, and lower cost relative to noble metals like Pt[2]. Upon light absorption by the main photocatalyst (e.g., TiO_2), electron-hole pairs are formed, and MoS_2, acting as a co-catalyst, captures the photogenerated electrons, facilitating proton reduction to produce hydrogen. In this study, an ilmenite type catalyst, $NiTiO_3$, was synthesized by co-precipitation method and characterized by X-ray photoelectron spectroscopy (XPS) and transmission electron microscope (TEM) for the structural and morphological properties, respectively. Then, the prepared $NiTiO_3$ was investigated for photocatalytic hydrogen production in the presence of eosin-Y photosensitizer dye. In order to increase the photocatalytic hydrogen production of $NiTiO_3$ photocatalyst, $(NH_4)2MoS_4$ was added to the reaction medium to obtain MoS_x structures by in situ photodeposition method on the catalyst surface.

Keywords: photocatalysis, hydrogen evolution, molybdenum sulfide

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ORAL PRESENTATION

Electrocatalytic hydrogen evolution by different size of tungsten sulfide

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Abstract

The use of fossil fuels today leads to the production of environmentally harmful carbon dioxide and other greenhouse gases, resulting in numerous adverse effects. It is evident that an urgent transition to a cost-effective and sustainable energy system is necessary. Hydrogen energy is widely considered one of the most promising candidates for clean and sustainable energy, with the potential to replace fossil fuels[1]. Additionally, excess energy generated from renewable sources can be used to facilitate the splitting of water. In this context, electrocatalytic hydrogen production stands out as a renewable method. Electrocatalysts are extensively used to lower electricity consumption and accelerate the reaction kinetics of the hydrogen evolution reaction (HER). To date, noble metal catalysts like platinum are known to exhibit the best HER activity[2]. However, their industrial applications are limited due to high costs and low reserves. Transition metal dichalcogenides (TMDs) have recently been extensively investigated as HER electrocatalysts due to their high activity and abundance in nature. However, the electrocatalytic properties of TMDs largely depend on the exposed edge sites and their conductivity. Therefore, the key factors in enhancing the HER performance of TMDs are increasing the density of exposed edge sites and improving their poor conductivity[3]. In this study, the electrocatalytic hydrogen production has been investigated by different sizes (micro and nano) of WS_2, a member of the TMD family. The overpotentials of the WS_2 electrocatalysts were determined using linear sweep voltammetry (LSV) by using potentiostat. Additionally, charge transfer resistances were examined using electrochemical impedance spectroscopy (EIS), and their capacitances were analyzed through cyclic voltammetry (CV) techniques. Herein, nano-sized WS2 was displayed improving electrocatalytic HER activity than that of micro-sized WS2 because of increasing active surface area.

Keywords: Electrocatalysts, Hydrogen Evolution, Tungsten Sulfide

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ORAL PRESENTATION

Hidrojellerin Bozunma Özelliklerini Belirlemek İçin Kombine QCM-AFM Sisteminin Geliştirilmesi

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Özet

QCM (Kuvars Kristal Mikro Terazisi), sensör yüzeyindeki kütle değişimlerini nano-gram hassasiyetinde ölçebilen kütleye duyarlı bir tekniktir. QCM sensörü doğal rezonansında titreşir ve titreşim sinyali kuvarsın piezoelektrik etkisi kullanılarak elektrik sinyaline dönüştürülür. Sensör yüzeylerine absorbe olan katmanlar rezonans frekansında kaymaya neden olur. QCM, hidrojel bozulmasını izlemek için kullanılabilir. QCM ayrıca yüzey elastikiyetiyle orantılı olan enerji harcanımı sinyalini de ölçebilir. Temel olarak, AFM bir numune yüzeyini taramak için çok keskin uçlu bir konsol kullanır. Uç-numune etkileşim kuvveti bir geri bildirim döngüsüyle sabit tutulur. Numune yüzeyi taranır ve her nokta için yükseklik verileri kaydedilir. Teknik olarak eş zamanlı QCM-AFM yaklaşımı uygulanabilirdir; QCM ve AFM aynı anda çalıştırılabilir. QCM sensörü, MHz aralığında sensör rezonans frekansında yanal olarak titreşir. AFM konsol, tamamen farklı frekans aralıklarında olan sıvıda yaklaşık 100 kHz'lik konsol rezonans frekansında dikey olarak titreşir. QCM sensörünün rezonans frekansını kilitlemek için PLL tabanlı QCM denetleyicisi kullandık. AFM ve QCM kilitlemeli amplifikatörler kendi temel frekanslarını kilitler, böylece teknikler karışmaz. Eşzamanlı QCM-AFM avantajları; AFM görüntüleri, yüzeyin yüksek çözünürlükle 3 boyutlu yapısını gösterir, QCM rezonans frekansı ve sönümlenme sinyali kayması, sensör yüzeyinde dağılan kütle ve elastikiyet tahminleri sağlar. AKM ve QCM teknikleri, malzemenin farklı özelliklerinin aynı anda araştırılmasına olanak sağlar. Bu çalışmada, yüksek frekans çözünürlüğüne sahip ve yumuşak malzeme araştırmaları için önemli olan enerji sönümlenme sinyalini ölçebilen PLL tabanlı bir QCM sistemi geliştirdik. QCM-AFM entegre edildi ve fizyolojik koşullarda deneyler yapmak için sıvı hücresinin sıcaklığının kontrol edilmesi sağlandı. Çalışmada, aljinat hidrojelinin bozunması eş zamanlı AFM/QCM sistemiyle başarılı bir şekilde gözlemlenebilmiştir.

Anahtar Kelimeler: AFM, QCM, hidrojel bozunumu, aljinat

ORAL PRESENTATION

Ethnobotanical characteristics of some villages of Araban district (Gaziantep, Türkiye)

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Abstract

This study was carried out to determine the ethnobotanical characteristics of plants found in some villages of Araban (Gaziantep) district in 2023. In this context, villages in the district were visited and interviews were held with local people. Ethical permission was received and a survey was conducted on the people living in villages. The research was conducted in 12 villages and with 50 participants. In addition, the local names of the plants, their usage patterns, purposes, parts used, usage patterns and collection times were determined within the scope of the study. As a result of the study, according to the purposes of usage of the taxa, 34 food-spice, 25 medicinal, 3 fodder, 2 fuel, 5 others (religion, trade, textiles, cosmetics). Among plant taxa are *Malva neglecta, Urtica dioica, Portulaca oleracea, Thymbra spicata, Sinapis arvensis, Papaver ,rhoeas, Teucrium polium, Capsella bursa-pastoris, Hordeum vulgare, Trifolium pauciflorum* plants are found out to be frequently used by the local people.

Keywords: Araban, Ethnobotany, folk remedies, traditional knowledge

ORAL PRESENTATION

Investigation of Bioinformatics Algorithms in Determination of Intron Retention

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Abstract

Intron retention (IR), one of the alternative types of splicing, occurs when an intron is inserted into the pre-mRNA and remains in the final mRNA. It has been reported that IR is much more frequent in mammals than previously thought and has been determined to affect the transcripts of most genes. Misregulation of alternative splicing is also associated with cancers and abnormal expression or mutations in splicing factors contribute to tumor formation. Because these events are difficult to measure reliably, they constitute a class of alternative splicing that is often neglected. This study aims to examine the IRFinder, IRFinder-S, iREAD, and Whippet algorithms, which are widely used in determining intron retention in RNA-seq data, and to determine the operational differences between the algorithms. As a result of the evaluation, it was observed that IRFinder and IRFinder-S algorithms use IRratio as the metric of intron retention, iREAD uses a measurement metric based on Entropy score, and the Whippet algorithm determines the retained introns according to Percent Splice In (PSI) values. In differential gene expression measurements, IRFinder, IRFinder-s algorithms used DEseq2, IREAD used edgeR and whippet used Delta PSI values. In addition, IRFinder-S was found to be effective in long read sequences.

Keywords: Intron Retention, IRFinder, IRFinder-S, IREAD, Whippet

ORAL PRESENTATION

Antifungal activities of AgNPs obtained from *Rheum ribes via g*reen synthesis

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Abstract

Nanoparticles (NPs) have unique physical and chemical properties due to their small size and high surface area-to-volume ratio. There are different methods to synthesize nanoparticles but the green synthesis method is a more economical and environmentally friendly method when compared to others. In the green synthesis method, plants have been frequently used due to their characteristics such as cheap, easily found and stabilizing effects. Many studies have shown that silver nanoparticles (AgNPs) from various plants have antimicrobial effects. In this study, we aimed to examine the antifungal effects of AgNPs obtained from *Rheum ribes* extract by green synthesis method. For this purpose, extract from peel of *R. ribes* was mixed with silver nitrate. Rr-AgNPs were characterized by zeta-sizer, UV-VIS (Ultraviolet and Visible) spectrophotometer and FE-SEM (Field Emission Scanning Electron Microscope). Then, antifungal effects of Rr-AgNPs with different concentrations (0.25, 0.5, 0.75, 1 mg/ml) on *Fusarium verticillioides*, *Ceratobasidium sp.*, *Fusarium oxysporum* were evaluated using the poisoned food method. It was observed that Rr-AgNPs had spherical shapes with maximum absorbance at 418 nm with a zeta potential of -17.5 mV. The maximum mycelial growth inhibition (67.67%) was observed in *Fusarium oxysporum* at 1 mg/ml, followed by *Ceratobasidium sp*. with 54.54% at 1 mg/ml and finally *Fusarium verticillioides* with 55.31% at 0.75 mg/ml. It has been determined that Rr-AgNPs have antifungal effects. Detail studies should be performed to analyze the antimicrobial properties of Rr-AgNPs and the potential usage should be evaluated in agriculture.

Keywords: Silver nanoparticle, green synthesis, *Rheum ribes*, antifungal activity

ORAL PRESENTATION

Impact of methoxyfenozide on oxidative stress markers in *Oncorhynchus mykiss*: a comparative study of gill, kidney, and liver tissues

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Abstract

Methoxyfenozide, a novel diacylhydrazine insecticide, functions as a potent agonist of the ecdysone receptor complex in lepidopteran insects, mimicking the molting hormone 20-hydroxyecdysone. This study aims to investigate the effects of methoxyfenozide on oxidative stress markers in the gill, kidney, and liver tissues of *O. mykiss* following 7, 14 and 21 days of exposure to sublethal concentrations. Oxidative stress was assessed by measuring thiobarbituric acid reactive substances (TBARS) and protein carbonyls as indicators of lipid and protein oxidation. In gill tissue, levels of TBARS and protein carbonyl showed ascending pattern reaching maximum of 229.88% and 739.13%, respectively. In kidney tissue, methoxyfenozide exposure caused significant rise in TBARS (347.85%) and protein carbonyl levels (727.39%) particularly at high insecticide concentration. Liver tissue also exhibited pronounced increase in TBARS and protein carbonyls with the percent change of 173.66% and 112.5% at 0.42 mg/L on 21 day. These findings highlight the substantial oxidative stress induced by methoxyfenozide in *O. mykiss* with the most severe effects evident at higher concentrations and prolonged exposure demonstrating its pro-oxidant potential.

Keywords: Methoxyfenozide, oxidative stress, *Oncorhynchus mykiss*, TBARS, protein carbonyl

Acknowledgment: The authors would like to thank Gaziantep University Research Fund for financial support (Project Number: FEF.DT.22.19).

ORAL PRESENTATION

Evaluation of neurotoxicity, oxidative stress, and apoptosis in aquatic toxicity studies

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Abstract

Biomarkers are defined as measurable cellular, biochemical, molecular and physiological changes and are widely used in toxicological studies as early warning signals indicating the sublethal toxicological effects of chemicals, target tissues and the general health status of the exposed organism. Neurotoxic potential of chemicals is primarily evaluated by the activity of acetylcholinesterase (AChE), a cholinergic enzyme functioning by hydrolyzing the neurotransmitter acetylcholine. Chemicals having anticholinesterase activity cause the inhibition of AChE leading accumulation of acetylcholine, resulting in persistent nerve signal transmission and a cascade of neurological disturbances. This situation negatively affects the organism's activities such as feeding, swimming, avoiding predators and spatial orientation, thus endangering its survival. Sodium potassium ATPase (Na^+,K^+-ATPase) is an enzyme that is localized in the cell membrane and plays an important role in osmoregulation by catalyzing the 2K/3Na antiport. Therefore, it is used as a biomarker in the evaluation of the negative effects of chemicals on osmoregulation. Oxidative stress is condition arises from an imbalance between reactive oxygen species. Enzymatic and non-enzymatic antioxidants function to eliminate the harmful effects of reactive species and prevent oxidative stress. When the activities of these enzymes are compromised, it reflects a reduced capacity to manage oxidative stress, leading to increased cellular damage. Lipid peroxidation is oxidative modification of membrane lipids and used as a key indicator of oxidative stress showing compromised cell integrity and altered membrane permeability. Oxidative modifications of protein side chains (lysine, arginine, proline and threonine) result in the formation of protein carbonyl derivatives, which cause changes in protein structure and function. The increase in protein carbonyl levels is also widely used as a valuable marker of reactive species induced stress. Apoptosis is programmed cell death that prevents inflammation and damage to surrounding cells by controlled breakdown of intracellular components. As an executioner caspase, caspase-3 orchestrates the final stages of apoptosis, leading to the dismantling and removal of damaged or unnecessary cells. Elevated caspase-3 activity indicates that cells are undergoing apoptosis, which can be triggered by severe oxidative damage or toxic exposure. Additionally, 8-hydroxy-2-deoxyguanosine serves as a marker for oxidative DNA damage signaling potential genomic instability and increased apoptosis. Overall, as an early warning system biomarkers are important in the monitoring of health and quality of aquatic environments together with valuable information they may provide regarding mechanisms of toxicity of different pollutants and stressors enabling timely intervention and mitigation measures.

Keywords: Biomarkers, neurotoxicity, oxidative stress, apoptosis

ORAL PRESENTATION

Potential Risks of Micropollutants in Vegetables Grown in Wastewater Irrigated Soils to Food Safety and Human Health

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Abstract

Micropollutants are inorganic and organic substances that can adversely affect the environment even in very small concentrations. They are produced in large quantities through human activities. The resulting group of micropollutants consists of substances that vary significantly in terms of toxicity, behaviour, treatment/purification techniques, etc. Factors such as the physicochemical properties of these compounds and the type of environment and location of the micropollutants (groundwater, surface water, sediment, wastewater treatment systems, drinking water facilities) determine the role of micropollutants in their fate. Wastewater contains many different micropollutants and when these waters are used for agricultural irrigation, they may contain pollutants that may pose a threat to human health. The greatest concern about the presence of these pollutants in soil is the evidence that they can be taken up by plants and incorporated into the plant body, and that they can accumulate not only in the roots but also in the edible parts of plants. Although the environmental effects of micropollutants are not yet well known, there are clear indications that they have long-term effects on the ecosystem. The reasons for this include their potential to accumulate in living organisms, their toxicity and their persistence in the environment. Therefore, these contaminants may enter food chains and constitute an exposure pathway for humans. The uptake of various pollutants by plants has become a global concern due to the direct and/or indirect involvement of the pollutants in plant metabolism and the changes in their nutritional values, which have harmful effects on the plants and the health of the people who feed on these plants. In this study, information was compiled on micropollution, especially in vegetables grown under the influence of wastewater, and the possible risks of these pollutants in terms of food safety and human health.

Keywords: Micropollutant, food safety, humman healthy, potential risks

ORAL PRESENTATION

Effects of pesticides on aquatic plants

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Abstract

Pesticides are chemical compounds used to protect crops and increase yields. However, these chemicals have the potential to harm both the environment and human health. The impact of pesticides on ecosystems is concerning, especially because they are transported through water bodies. Studies show that pesticides directly or indirectly cause significant adverse effects on aquatic organisms. Pesticides have serious adverse effects not only on target pests but also on non-target organisms living in aquatic ecosystems. Considering these effects of agricultural practices, more sustainable strategies should be developed for pesticide use. In this study, information is provided about the effects of pesticide pollution originating from agricultural activities on aquatic ecosystems, especially on aquatic plants. The effects of pesticides on aquatic organisms were examined through literature review, and toxicity levels on aquatic plants were evaluated.

Keywords: Pesticides, aquatic plants, off-target effects

ORAL PRESENTATION

In vitro evaluation of the effects of berberine on cell cycle and proliferation via the Cyclin D1/CDK4 /CDK6 axis in SH-SY5Y neuroblastoma cells

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Abstract

Berberine, a natural isoquinoline alkaloid, has shown significant antiproliferative effects in various cancer cell lines. This study investigates its effects on G1/S cell cycle regulation in SH-SY5Y neuroblastoma cells, focusing on the modulation of cyclin D1, CDK4 and CDK6 genes. These genes play a key role in promoting cell cycle progression from G1 to S phase by forming active complexes essential for the phosphorylation of the retinoblastoma protein (pRb), a key event for the initiation of DNA replication. The antiproliferative effects of berberine were determined by XTT assay and changes in mRNA expression were determined by real-time PCR. According to the XTT results, the IC50 value of berberine was determined to be 13 µM at 24h in SH-SY5Y neuroblastoma cells. These results indicate that berberine inhibits cell proliferation in a time- and dose-dependent manner in SH-SY5Y cells. Our results indicate that berberine treatment downregulates the expression of cyclin D1, CDK4 and CDK6 mRNA levels, resulting in cell cycle arrest at the G1 phase in SH-SY5Y cells. The suppression of these cell cycle regulators correlates with a significant decrease in SH-SY5Y cell proliferation, suggesting that berberine exerts its anti-proliferative effects by targeting the cyclin D1/CDK4/CDK6 axis. In conclusion, berberine may serve as a promising therapeutic agent in the treatment of neuroblastoma by specifically targeting critical regulators of the G1/S transition, leading to cell cycle arrest and reduced tumor cell proliferation. Further research is warranted to explore its potential in clinical applications.

Keywords: Berberine, neuroblastoma, SH-SY5Y cells, Cell Cycle

ORAL PRESENTATION

Makrohalkalı Fosfaza-Schiff Bazı Bileşiklerinin Sentezi Ve Yapısal Karakterizasyonu

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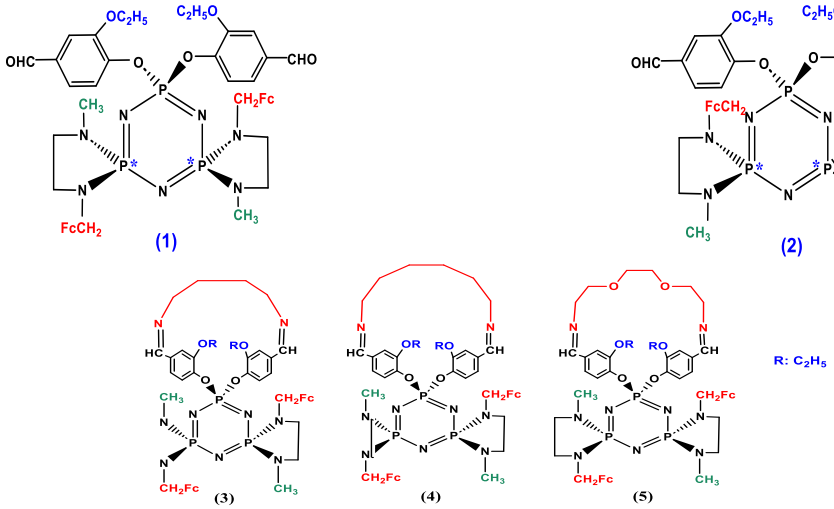
Özet

Hekzaklorosiklotrifosfazen (trimer, HCCP, $P_3N_3Cl_6$), fosfor ve azot atomlarının birbirine ardışık olarak bağlanması ile oluşan inorganik heterohalkalı bileşikler sınıfından olan halkalı fosfazenlerin bir üyesidir. Cl atomlarının bir veya birkaçının tek dişli ligandlarla yer değiştirmesiyle oluşan mono-, di-,tri-, tetra-, penta- ve hekza-sübstitüe bileşikler literatürde bilinmektedir [1-3]. Bu çalışmada, yeni etilvanillinato-sübstitüe trans/cis-dispiroferrosenilfosfazenler **(1 ve 2)** sentezlenmiştir. İlk aşamada, literatür yöntemine göre, ferrosenkarboksaldehit ile alifatik N-metiletilendiaminin etanol ortamındaki kondenzasyon reaksiyonları sonucunda Schiff bazı, bu Schiff bazının $NaBH_4$ ile indirgenmesinden ise monoferrosenildiamin bileşiği elde edilmiştir [4]. İkinci aşamada, sentezlenen ferrosenildiaminin HCCP ile THF ortamında 1:2 stokiyometrik oranda etkileştirilmesiyle trans- ve cis- dispiroferrosenilfosfazenler sentezlenmiştir. Sentezlenmiş olan trans/cis-dispiroferrosenilfosfazenin **(1 ve 2)** THF ortamında etilvanilinin potasyum tuzu ile etkileştirilmesiyle tamamen sübstitüe fosfazen bileşiği sentezlenmiştir. Elde edilen bis-etilvanillinato trans-ferrosenilfosfazen bileşiğinin **(1)** bütanol ortamında çeşitli diaminlerle [1,4-diaminobutan, 1,6-diaminohekzan ve 1,8-diamino-3,6-dioksaoktan] reaksiyonunda makrohalkalı fosfaza Schiff bazları **(3-5)** sentezlenmiştir. Ayrıca, yeni bileşiklerin yapısal karakterizasyon çalışmalarında tek boyutlu NMR, kütle ve IR spektroskopi teknikleri kullanılmıştır.



Anahtar kelimeler: Dispirofosfazenler, trimer, spektroskopi, etilvanillinatoferrosenilfosfazen

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ORAL PRESENTATION

Investigation of Electrocardiography Data in Dogs Found in Stray Animals Care and Rehabilitation Centre: Sivas Province Example

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Abstract

Dogs are social animals that spend their time searching for food in a wide range of environmental conditions. Taking the dogs from the environmental conditions and keeping them in restricted shelter areas leads to a decrease in their physical activity. The decrease in physical activity in dogs causes mental parameters to be negatively affected and even stress in dogs. Responses that occur in long-term stress situations affect physiological parameters. Decreased physical activity and stress are also effective on cardiac physiological parameters. The aim of the study was to investigate electrocardiography (ECG) parameters in dogs kept in shelter conditions. Within the scope of the study, ECG data were obtained from a total of 20 dogs, grouped by sex (male and female) and age (3 years old and under, and over 3 years old) and statistically compared by Mann-Whitney U test. No statistically significant difference was found between male and female groups in all electrocardiographic parameters ($P>0.05$). While there was a statistically significant difference between the age groups in terms of Q-T interval duration ($P<0.05$), there was no statistically significant difference in all other electrocardiographic parameters. In this study, ECG data of dogs in a stray animal care and rehabilitation centre were determined.

Keywords: Dog, Dog care and rehabilitation centre, ECG, Sivas

ORAL PRESENTATION

Investigation of Deafness in Dogs Found in Stray Animals Care and Rehabilitation Centre

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Abstract

Stray animals that have been domesticated by humans and found in the same environment and can establish social relationships with their own population and other living communities, especially humans, in nature and are ownerless. In this study, it was aimed to investigate the deafness of mongrel dogs in the Stray Animal Care and Rehabilitation Centre of Sivas Municipality. A total of 20 crossbred dogs in the Centre were examined for deafness with the Brainstem Auditory Evoked Response (BAER) test (20, 40, 60 and 80 decibels (dB)) and their hearing thresholds were obtained. Afterwards, the dogs were grouped as a single group without any discrimination according to general, and sex (male and female), whether the ear was cut or not, and age range (3 years old and under, and over 3 years old) and wave ranges of 20, 40, 60 and 80 dB of both ears in all groups, and the I-III-V. wave ranges of 80 dB in the general group were compared. In group comparisons, Mann Whitney U test was performed with SPSS v.25 package program since there were 2 groups in each group and the number was small. As a result of the study, 20 and 40 dB sound range could not be detected in 2 animals and 60 dB in 1 animal in the group over 3 years of age. As a result of the comparison of the groups, statistical significance was found in the 60 and 80 dB of left ear in the whether the ear was cut or not groups, and in all wavelengths of both ears in the age groups ($P<0.05$ and $P<0.01$). This study is the first study in which deafness screening was performed in different mongrel dogs in Sivas Municipality Stray Animal Care and Rehabilitation Centre. The study should be expanded both to increase the number of animals in the same Centre and to include stray dogs in different environments.

Keywords: BAER, Deafness, Stray Animals Care and Rehabilitation Centre, Sivas

ORAL PRESENTATION

Esans hammaddelerinin koltukaltı bakterilerine karşı etkilerinin incelenerek yeni esansların geliştirilmesi

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Özet

Koltuk altı kokusu insanların yaşamında büyük ölçüde rahatsızlık uyandıran ve sosyal yaşantısını önemli derecede etkileyen problemlerden biridir (James, 2020). Koku oluşumu, kokusuz doğal salgıların uçucu kokulu moleküllere mikrobiyal biyotransformasyonundan kaynaklanmaktadır. Bu mikrobiyotanın esas olarak *Staphylococcus, Corynebacterium, Micrococcus* ve *Propionibacterium* cinsi Gram-pozitif bakterilerden oluştuğu bilinmektedir (James vd., 2013). Kozmetik ürünlerinde kötü kokuyu engellemek amacı ile koltuk altı uygulamalarda kullanılan deodorantların içerisindeki antibakteriyel ajanlar, koltuk altı mikrobiyotasını bozmakta ve ciltte hassasiyet, tahriş ve birçok sorun ortaya çıkarmaktadır (Fredrich vd., 2013). Bu çalışma ise, antibakteriyel ajan kullanımını ortadan kaldırarak koltukaltı mikrobiyal florasını bozmayan esans tasarımlarının gerçekleştirilmesini sağlamıştır. Yapılan literatür araştırmaları sonucunda belirlenen 50 esans hammaddesinin *Staphylococcus* ve *Corynebacterium* cinsi bakterilere (*Staphylococcus epidermidis, Staphylococcus capitis Corynebacterium jeikeium Cornyebacterium striatum*) karşı antibakteriyel özellikleri disk difüzyon yöntemi ile belirlenmiş ve etki göstermeyen hammaddeler seçilmiştir. Seçilen bu hammaddeler kullanılarak esans tasarımları gerçekleştirilmiş ve esansların antibakteriyel özelliklerine bakılmıştır. Koltuk altı bakterileri tarafından oluşan kötü kokunun maskelendiği parfümörler tarafından olfaktif olarak onaylanmıştır.

Anahtar kelimeler: Koltuk altı bakterileri, disk difüzyon yöntemi, esans, olfaktif analiz

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ORAL PRESENTATION

Pesticides effects on fish antioxidant enzymes system

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Abstract

All aerobic organisms, including fish, require molecular oxygen (O_2) for energy synthesis and various metabolic processes. Nonetheless, the utilization of O_2 in physiological processes may result in the accumulation of oxidants and a reduction in antioxidants. This occurrence is referred to as oxidative stress. Oxidative stress is caused by a decrease in oxygen, resulting in the generation of reactive intermediates known as free radicals and non-radical reactive oxygen species (ROS). The disparity between ROS and the antioxidant defense system is termed oxidative stress. Elevated ROS can impair cellular defenses and influence many enzymes, proteins, lipids, and several biochemical processes, particularly the membrane ion transport system, potentially leading to DNA damage. All aerobic organisms, including fish, include an antioxidant defence mechanism to mitigate the detrimental effects of ROS, comprising: a) enzymatic antioxidant systems and b) non-enzymatic antioxidant systems. Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) constitute the enzymatic antioxidant system. Pesticides are substances that improve agricultural output by managing agricultural pests. Pesticides utilized in agricultural areas can infiltrate aquatic systems via multiple paths, exerting deleterious effects on non-target organisms, particularly fish. Pesticides can produce oxidative stress in fish. Research indicates that pesticide exposure elevates levels of CAT, SOD, GPx, and GR in fish tissues to mitigate oxidative stress and counteract free radicals.

Keywords: Fish, pesticides, antioxidant enzyme systems

ORAL PRESENTATION

Determination of UAE extraction yield of some bioactive phenolics in olive leaves with NADES liquids by HPLC-UV

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Abstract

Olive leaf is a rich source in terms of phenolic substance content and its use in traditional medicine. In this study, the yield of phenolic substances in extracts obtained from olive leaves using NADES (natural deep eutectic solvents) was compared with the data of traditional soxhlet method. In the research, UAE (ultrasonically assisted extraction) method was used in NADES mixture (choline chloride-lactic acid) and extracts were taken by Soxhlet method for method comparison. The extracts obtained were analysed with HPLC device and phenolic content was determined. Experimental design was performed using Design Expert 11 demo version and optimisation study was performed with 13 experiments by selecting NADES water ratio, temperature, olive leaf/NADES ratio as independent variables. The optimum extraction parameters were determined with the experimental data and the method was compared with the reference soxhlet method (70% ethanol 30% water) for 6 hours. While the total phenolic content was 2764.2 µg/g in the UAE method, this value was found to be 29949.4 µg/g in the Soxhlet method. The Soxhlet method is 10.8 times more effective than the UAE method in terms of extraction efficiency. However, considering the amount of solvent and time spent in the Soxhlet method, it was concluded that NADES extraction with UAE is an improvable method.

Keywords: Olive leaf, NADES, UAE, Soxhlet, HPLC analysis

Acknowledgment: This work was supported by the Scientific Research Project Department of Gazi University. Grant number: FGA-2023–8160

ORAL PRESENTATION

Precision Levodopa Sensing: An Electrochemical Sensor with Antibody-Modified Zinc Ferrite Nanoparticles and Acetylene Black for Enhanced Sensitivity

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Abstract

Levodopa is a key drug in the treatment of Parkinson's disease, as it serves as a precursor to dopamine, which is deficient in patients with this neurodegenerative disorder. The accurate detection of levodopa is critical for optimizing treatment regimens, personalizing medicine, and monitoring disease progression. In this study, an electrochemical sensor system was developed to detect levodopa using a screen-printed electrode (SPE) with a gold working electrode, a platinum counter electrode, and a silver reference electrode. The surface of the gold electrode was modified with acetylene black, providing a highly conductive and large surface area for enhanced electrochemical reactions. Zinc ferrite nanoparticles, further modified with amine groups, were immobilized onto the acetylene black to introduce magnetic and catalytic properties, further improving the sensitivity and selectivity of the sensor. Covalent immobilization of levodopa-specific antibodies on the modified surface enabled selective binding of the target molecule. Electrochemical Impedance Spectroscopy (EIS) was employed for surface characterization after each modification step, confirming successful immobilization and enhancement of the electrode's properties. Differential Pulse Voltammetry (DPV) was utilized for the quantitative analysis of levodopa across varying concentrations, demonstrating the sensor's capability to detect levodopa with high precision and sensitivity. This sensor platform offers a robust and efficient approach for levodopa detection, with potential applications in clinical monitoring and personalized Parkinson's disease treatment. The integration of acetylene black and zinc ferrite nanoparticles enhances the sensor's electrochemical performance, making it a promising tool for real-time monitoring of drug levels.

Keywords: Zinc ferrite nanoparticles, Electrochemical Sensor, Levodopa, Surface Functionalization

ORAL PRESENTATION

Modification of Disposable Pencil Graphite Electrodes and Their Use in Voltammetric Determination of Some Drugs and Pesticides

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Abstract

In this study, polyaniline-coated disposable pencil graphite electrodes (PGE) were prepared for the voltammetric analysis of azimsulfuron, a herbicide belonging to the pyrimidinylsulfonylurea. The electropolymerization of aniline on the surface of the PG electrodes was optimized to yield the most accurate results for the analysis of azimsulfuron. Under these conditions, the PG electrodes were subjected to an activation process consisting of five consecutive cycles using cyclic voltammetry in a 0.10 M H_2SO_4 supporting electrolyte within a potential range of -0.65 V to 1.10 V at a scan rate of 100 mV/s. After the activation process, pencil graphite electrodes were rinsed with distilled water and left to dry. For the electropolymerization of aniline on the PGE surface, a solution containing 0.2 mM aniline in 0.05 M H_2SO_4 was added to the electrochemical cell. The activated PG electrodes were connected to the BAS potentiostat to allow their surface to be coated with polyaniline using cyclic voltammetry. The electropolymerization was carried out at a scan rate of 100 mV/s with consecutive cycles within a potential range of -0.2 V to 1.2 V. The prepared electrodes were rinsed with distilled water and left to dry. The electrochemical characterization of polyaniline coated pencil graphite electrodes (PANI/PGE) was performed by cyclic voltammetry with scans between 0.6 V and -0.2 V (50 mV/s) by selecting the redox couple 0.1 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ in 0.10 M KCl for an ideal reversible reaction. Finally, validation parameters such as sensitivity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) for the detection of azimsulfuron was carried out on the constructed PANI/PGE electrodes.

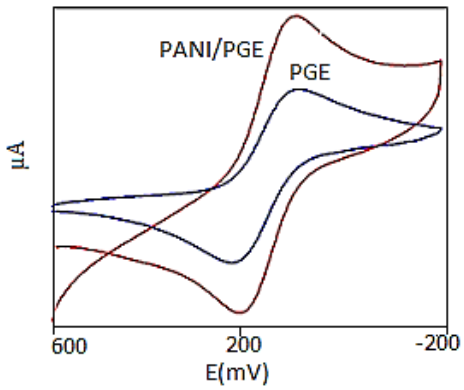

Figure 1. Cyclic voltammograms (CV) for $1.0x10^{-4}$ M $K_3[Fe(CN)_6]$ /$K_4[Fe(CN)_6]$ on PGE and PANI/PGE electrodes.

Keywords: Modified electrodes; Pesticides; Voltammetry

ORAL PRESENTATION

The effect of modern agricultural practices on natural flora elements

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Abstract

The existence of agricultural activities is almost as old as human history. People have primarily consumed what they have obtained from nature for many different reasons such as food, medicinal and aromatic, ornamental, enjoyable and as objects. However, the point that the world population and the needs of this population have reached today is too great to be solved by the method of collecting from nature. On the other hand, with the industrialization period, the soil and water quality of the areas where classical agricultural practices are carried out make it difficult to reach sufficient and healthy products. In addition, some synthetic products used in modern agricultural practices and some opportunities provided by agricultural mechanization (Deep plowing, Soil transportation, Artificial arrangement of soil structure, etc.) have increased the pressure on biodiversity as a result of the damage to natural habitats. Their fixed nature and lack of ability to move have made plants the first and most affected living beings by these harmful conditions. Plants react to these adverse conditions by adapting, moving away from the area and/or disappearing. Another harm of agricultural activities is that invasive plants, together with segetal flora, dominate agricultural areas and compete with natural flora elements. Since the impact of humankind on the natural environment due to agricultural areas is multidimensional, the direct and indirect effects of modern agricultural practices on natural habitats were investigated in the study. As a result of the compilation of literature studies, what these effects are, some suggestions were made on the precautions that can be taken.

Keywords: Modern agriculture, Natural, Flora, Environment, Impact

ORAL PRESENTATION

LC-HRMS ile *Teucrium melissoides* (Boiss.&Hausskn. Ex Boiss)'in Fenolik Profil Analizi ve Antioksidan Potansiyelinin Değerlendirilmesi

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Özet

Teucrium L. cinsi (Lamiaceae), Türkiye florasında 15'i endemik olmak üzere 47 takson ile temsil edilmektedir. Halk arasında farklı taksonların tonik, aromatik, diüretik, ekspektoran ve laksatif olarak, ayrıca gastrointestinal rahatsızlıklarda, sinir sistemi hastalıklarının tedavisinde, diyabet ve hemoroitte kullanımı kayıtlıdır. *Teucrium* taksonları üzerinde gerçekleştirilen fitokimyasal çalışmalarda neo-klerodan tip diterpenlerin yanısıra, flavonoitler, fenilpropanoitler, iridoitler, mono-, di-, tri- ve seskiterpenler ile bunların glikozitleri de izole edilmiştir (Topcu, 1997; Topcu, 1996). Yapılan biyoaktivite çalışmaları sonucunda ise bazı taksonların antioksidan, antiinflamatuvar, antiülseratif, hepatoprotektif, antidiyabetik, antimikrobiyal etkiler gösterdiği saptanmıştır (Jaradat, 2015; Ulubelen ve ark., 2000). Bu çalışmada daha önce üzerinde hiçbir fitokimyasal çalışma yapılmamış olan, Türkiye'de doğal yayılış gösteren *Teucrium melissoides* türünün gölgede kurutulan toprak üstü kısmı ile aseton ve metanol ekstreleri elde edildi. Elde edilen ekstrelerin antioksidan aktiviteleri metal bağlama ve CUPRAC yöntemleri, antiradikal aktiviteleri ise DPPH serbest radikal giderimi ve ABTS katyon radikali giderimi ile belirlendi. Ayrıca LC-HRMS ile metanol ekstresinin fenolik profili aydınlatıldı.

Anahtar kelimeler: *Teucrium melisoides*, LC-HRMS, Antioksidan.

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ORAL PRESENTATION

Glaziovine exhibits strong binding with target proteins in neurodegenerative diseases: drug repurposing approach

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Abstract

Glaziovine is a pro-aporphine alkaloid derived from the plant *Ocotea glaziovii* (Lauraceae) and has a wide range of pharmacological effects. Ethnobotanically, it has been used to treat digestive system disorders, fever, pain, and wounds. It has been registered under the trade name Suavedol® as a tranquilizer. Pharmacologically, glaziovine exhibits anxiolytic, analgesic, anti-ulcer, antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. However, studies on the drug repurposing potential of glaziovine for neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's) are limited. Molecular docking and pharmacokinetic studies play a crucial role in modern drug development processes, especially in optimizing existing drugs through drug repurposing approaches. This study aims to investigate the therapeutic potential of glaziovine for neurodegenerative diseases using molecular docking and pharmacokinetic analyses. Molecular docking analyses examined the interactions of glaziovine with proteins associated with Alzheimer's (APOE, PSEN2, APP, SorLA, CD33, and TrkB), Parkinson's (PARK2, PARK7 (DJ-1), UCH-L1, MAO-B, and LRRK2), and Huntington's (HTT and BDNF) diseases. The SWISS ADME tool was used to determine glaziovine's pharmacokinetic properties, including key parameters like absorption, distribution, metabolism, and excretion. Protein-protein interactions were analyzed using the STRING database. The study results revealed that glaziovine exhibited binding energies of -9.2 kcal/mol with HTT, -9.2 kcal/mol with MAO-B, -9.1 kcal/mol with PSEN2, and -9.0 kcal/mol with TrkB. This strong binding suggests that glaziovine may influence the pathophysiology of these diseases and could have therapeutic potential. Additionally, all proteins showed binding energies lower than -6.9 kcal/mol for the proteins studied. Glaziovine also exhibited drug-like properties according to Lipinski's Rule of Five, Egan, Ghose, Muegge, and Veber filters. According to the boiled-egg diagram, glaziovine cross the blood-brain barrier and well-absorbed by the intestines. These findings suggest that glaziovine could be a promising drug candidate for neurodegenerative diseases.

Keywords: Glaziovine, drug repurposing, molecular docking, neurodegeneration

ORAL PRESENTATION

Myrtle essential oil nanoemulgel formulations for the treatment of an inflammatory disease: recurrent aphthous stomatitis

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Abstract

Recurrent aphthous stomatitis (RAS) is an ulcerative disease of the soft tissue of the oral mucosa. The pathology is not exactly known, but, an increase in the factors such as TNF-α, IFN-γ and IL-6, IL-2 indicates that an inflammatory response occurs during the disease [1]. Nowadays, the use of phytoconstituents has grown in popularity. *Myrtus communis* L. (myrtle) is a source of essential oils that is characterized by a wide spectrum of biological activities [2]. However, several factors including low solubility, potential for allergic irritation, and low stability under external factors limit the use of essential oils as candidates for pharmacotherapeutic applications. Nanoemulsions are nano-drug carrier systems consisting of droplets of 10-200 nm in size. The drawbacks of essential oils could be eliminated by incorporating them into nanoemulsions [3]. Therefore, this study aimed to develop a nanoemulgel formulation containing myrtle essential oil for the treatment of RAS. Myrtle oil was selected due to its anti-inflammatory properties attributed to its 1-8 cineole compound and widespread availability of the plant in Türkiye. Nanoemulsions were prepared by the spontaneous emulsification method using myrtle essential oil, Capryol 90 as the oily phase, Tween 80 as a surfactant, and water. Minitab® Taguchi parameters were used to obtain the optimum nanoemulsion formulations, which were determined according to PDIs and droplet sizes. According to further characterization studies, the optimum formulation had a droplet size of 10.71±0.17 nm, PdI value of 0.25±0.02, zeta potential of -2.08±0.28, pH of 4.93±0.05. Also, it was stable after centrifuge testing and after storage 15 and 30 days at 4 °C and 25 °C. Then, nanoemulgel was prepared using Carbopol and its morphological and rheological properties were suitable for use in the oral mucosa. This study demonstrates that nanoemulgel containing myrtle oil presents a new approach for the treatment of inflammatory diseases such as RAS.

Keywords: *Myrtus communis* L., essential oil, nano drug carrier systems, nanoemulsion, nanoemulgel, recurrent aphthous stomatitis.

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ORAL PRESENTATION

Lithium Chloride Suppresses Colorectal Cancer Cell Survival and EMT via GSK3β Inhibition

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Abstract

Colorectal cancer (CRC) has the third-highest mortality rate globally, following lung and breast cancers. Despite advancements in treatments, CRC remains a leading cause of cancer-related deaths worldwide. Identifying novel therapeutic targets is crucial for improving outcomes in CRC. Glycogen synthase kinase-3β (GSK-3β), a serine/threonine kinase, has emerged as a potential target in various cancers. This study investigates the biological effects of lithium chloride (LiCl), a GSK-3β inhibitor, on colorectal cancer. We evaluated the effects of LiCl on CRC cells through qRT-PCR and cell viability assays. Publicly available datasets were also analyzed to assess GSK3β expression levels and their clinicopathological correlations in CRC patients. Our results indicate that LiCl at 20 nM reduces cell viability in CRC cells. Mechanistically, this effect may be attributed to the suppression of epithelial-mesenchymal transition (EMT), as evidenced by the decreased expression of mesenchymal markers TWIST and SLUG. Additionally, analysis of patient data from the Gene Expression Omnibus (GEO) revealed that GSK-3β is overexpressed in CRC compared to normal tissue and is associated with p53 mutation status. However, no significant association was found between GSK3β expression and poor clinical outcomes such as survival, advanced stage, or recurrence. In conclusion, our findings suggest that GSK3β is a promising therapeutic target in CRC, and LiCl may inhibit EMT in aggressive CRC profile. Further investigation is warranted to explore the potential of LiCl in combination with standard treatments in preclinical CRC models.

Keywords: Colorectal cancer, Glycogen synthase kinase-3β (GSK3β), lithium chloride, epithelial-mesenchymal transition (EMT), Wnt signaling.

ORAL PRESENTATION

Phenolic profiles of commercial propolis samples collected from different regions

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Abstract

Propolis, a naturally occurring resinous bee product, has many different biological and pharmacological properties thanks to its various bioactive compounds including phenolic acids and flavonoids. In this study, total phenolic content, total flavonoid content and antioxidant capacities of various commercial propolis extracts obtained from international markets were evaluated. For this purpose, 76 liquid propolis products (drop, spray, syrup and other liquids) sold in pharmacies, stores and online shopping websites from 18 different countries (Belgium, Brazil, Croatia, UEA, Estonia, France, Germany, Italy, Japan, Korea, Latvia, New Zealand, Romania, Russia, San Marino, Slovenia, Spain, USA) were collected between 2016 and 2019. According to the results, total phenolic content of the samples ranged between 0.1- 107.5 mg GAE/ml whereas total flavonoid contents were between 0.1-174.5 mg CE/ml. On the other hand, antioxidant capacity values, as measured by the CUPRAC method, ranged between 0.1 and 492.3 mg TE/ml. The results indicated that 18 propolis samples had an antioxidant capacity value higher than 50 mg TE/ml, and these samples were further examined for their phenolic profiles using HPLC-PDA system. These samples were observed to contain gallic acid, vanilic acid, caffeic acid, *p*-coumaric acid, trans-ferulic acid, protocatechuic acid, trans-cinnamic acid, naringenin, apigenin, pinocembrin and galangin. Among these phenolic compunds naringenin, apigenin and galangin were the ones detected in all samples. In summary, the results indicated a high variability in terms of phenolic content and antioxidant capacity of the collected propolis samples which is consumed by many people for their positive health effects. Establishing the necessary standards for the content and label of commercial propolis samples is essential from the consumer health and food safety aspects. In addition to the consumer concerns regarding the safety of the solvents used and the dose to be applied, preventing unfair competition is another critical point that needs to be considered.

Keywords: Propolis, antioxidant activity, phenolic compounds, HPLC-PDA

ORAL PRESENTATION

Investigation of *Borrelia burgdorferi* Seroprevalence in Tokat Province

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Abstract

Lyme disease or Lyme borreliosis is an infectious zoonosis caused by the spirochete *Borrelia burgdorferi*, which is more frequently seen in the Northern Hemisphere and spread by Ixodes type ticks. Lyme borreliosis is a multisystemic disease affecting the skin, musculoskeletal system, cardiovascular system and central nervous system. Although Turkey is similar to many European countries where Lyme borreliosis and tick-borne encephalitis have been reported in terms of geography and climate, the true incidence and prevalence of both diseases in our country are unknown. This study aimed to investigate the seroprevalence of tick-borne *B.burgdorferi* in Tokat province, which has a high tick population due to its geographical location and climatic conditions. In this retrospective cross-sectional study, *B.burgdorferi* IgG and IgM antibodies were screened using the ELISA method in serum and CSF samples taken from individuals with a history of tick contact or tick attachment between January and December 2022. A total of 78 individuals, 34 males and 44 females, were included in the study group. The ages of the cases were between 16-88 (mean age 42.9±15.2 years). 54 (69.3%) of the samples were sent from inpatient services. *B.burgdorferi* IgG antibodies were found to be positive in 5 (6.4%) of all patients, while they were found to be at borderline values in 1 case. *B.burgdorferi* IgM antibodies were detected positive in 22 (28.2%) of the cases. No seropositivity was detected in the CSF samples. As a result, in order to reduce tick exposure in our region for Lyme borreliosis, it is necessary to give importance to preventive health services for people in the risk group. Due to the detection of this pathogen in the region, albeit at a low frequency, it is recommended that cases that may be associated with Lyme disease be carefully evaluated in clinics.

Keywords: Lyme borreliosis; *Borrelia burgdorferi*; seroprevalence.

ORAL PRESENTATION

Investigation of the Therapeutic Effects of *Zingiber officinale* Roscoe (Ginger) on Vitiligo

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Abstract

Zingiber officinale Roscoe (ginger) is a plant belonging to the Zingiberaceae family. Ginger is one of the medicinal plants that grows naturally in various countries such as India, China, South East Asia, West Indies, Mexico and cultivated in other parts of the world. Ginger has been used by various civilizations such as Arab countries, China, European countries, Greece, India, Japan, Indonesia, Greece, India, Japan, Indonesia and America since ancient times for its antiemetic, antitussive, antiflatulent, cold, headache, antispasmodic, antihelmentic, antioxidant, analgesic, antimicrobial, antidiabetic, expectorant properties. Studies were carried out by gas chromatography, mass spectrometry and HPLC to find the active ingredients in Zingiber officinale Roscoe (ginger) and in this direction; active ingredients such as zingerol, gingerol, shogaol, paradol, zingiberene, alpha-farnesene, beta-bisabolene, luteolin, rutin were found. The aim of this study is to review the healing effect of *Zingiber officinale* Roscoe (ginger) on vitiligo by reviewing the studies conducted in the world and in our country.

Keywords: Zingiberaceae, Zingiber officinale, vitiligo, ethnobotany, treatment

ORAL PRESENTATION

Determination of dopamine on gold nanoparticle attached carbonaceous template by DPV

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Abstract

In this study, a method was developed for the determination of dopamine (DA), an important neurotransmitter involved in catecholamine signaling and neurotransmission (Erkal et al. 2016). A differential pulse voltammetry (DPV) method was established by preparing gold nanoparticle-supported carbonaceous electrodes (E.-Aytemur et al. 2019). The coal tar/typha tassel-mercaptophenyl-gold nanoparticle (CTP/TT-MP-Au) material was characterized using microscopy, spectroscopy, and electrochemical techniques.Using the DPV technique, a calibration study was conducted for the determination of dopamine in the concentration range of 5-100 µM. The equation of the developed method was Ip = 14.3576 + 0.2409[DA]. The limit of detection (S/N=3) was calculated as 1.02 µM, with an R^2 value of 0.9943, indicating high reliability. Additionally, the precision and accuracy of the method were confirmed through both intraday and interday measurements. An interference study was performed to assess various substances that could potentially affect DA measurement. The method was successfully applied to analyze commercial human serum and artificial urine samples, demonstrating its practical utility.

Keywords: dopamine, differential pulse voltammetry, coal tar, modified electrode

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ORAL PRESENTATION

Development of photo-crosslinkable pectin methacrylate (PecMA) hydrogels as pro-angiogenic scaffolds

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Abstract

Pectin is a polysaccharide found mostly in plant cell walls and has gained significant importance in tissue engineering applications because of its properties, such as being biocompatible, biodegradable, and having potential antimicrobial properties. Pectin is classified under two categories according to the degree of esterification: high methoxyl pectin (HMP) and low methoxyl pectin (LMP). LMP can conventionally be ionically crosslinked via the physical entrapment of divalent cations such as calcium. Recently, it has been reported that LMP can be methacrylated and photo-crosslinked in such a short time as an alternative crosslinking method. However, commercially available pectin is mostly sold as HMP. Therefore, a pre-process of de-esterification is necessary to obtain high methacrylation rates.

Accordingly, in this study, we synthesized LMP from commercially available HMP via alkali treatment. Then, LMP was methacrylated, and the de-esterification and methacrylation were characterized via proton nuclear magnetic resonance (^{1}H-NMR) and Fourier-transform infrared spectroscopy (FTIR). The effect of different photoinitiators (Irgacure 2959 and Lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP)) and their concentrations on the degree of gelation of PecMA via photo-crosslinking will be evaluated to create soft tissue engineering scaffolds before physical, chemical and mechanical characterizations. Finally, loading and release of 2-deoxy-D-ribose (2dDR), a simple sugar that possesses pro-angiogenic properties as a safe and cost-effective alternative to vascular endothelial growth factor (VEGF), will be assessed to create novel pro-angiogenic hydrogels, and its potential use in soft tissue engineering applications will be revealed via biological characterizations.

In conclusion, this study reveals that a novel pro-angiogenic photo-crosslinkable pectin-based hydrogel could be developed to be used in soft tissue engineering applications.

Keywords: pectin, pectin methacrylate, biomaterials, tissue engineering

Acknowledgements: We would like to thank Dr. Betül Aldemir Dikici for her guidance on the methacrylation protocol.

ORAL PRESENTATION

Hydroxyapatite-incorporated highly porous synthetic scaffolds for bone tissue engineering

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Abstract

Polycaprolactone (PCL) is a widely used biodegradable and biocompatible polymer. However, the hydrophobicity of this synthetic polymer limits cell-material interactions. Our research group has contributed to the literature by developing tetra-methacrylated polycaprolactone (4PCLMA) based emulsion-templated scaffolds and exploring their potential in bone tissue engineering (BTE). Despite these advancements, prior studies indicated a need for further enhancement of the material through surface functionalisation, such as plasma treatment or the coating with biological substances [1,2]. Alternatively, the bioactivity of biomaterials can be improved by creating composite materials that incorporate polymers/ceramics, such as hydroxyapatite (HA), which constitutes approximately 70% of natural bone. In this study, we aimed to develop nano-hydroxyapatite (nHA) containing polymerised high internal phase emulsions for BTE. 4PCLMA was synthesised and characterised using Nuclear Magnetic Resonance (NMR) spectroscopy. Scaffolds were fabricated via the emulsion templating method with nHA incorporated into either the internal or external phases of the emulsion. We investigated the effects of (i) surfactant concentration and (ii) nHA concentration using scanning electron microscopy (SEM) and contact angle measurements. Scaffolds exhibiting hydrophilic characteristics, large pore sizes, and open porosity were selected for further mechanical and biological characterisation. When nHA was added to the water phase of the emulsion, it was observed that the particles agglomerated within the scaffold pores. Conversely, when nHA was incorporated into the oil phase, some accumulation occurred on the polymer surface, though to a lesser extent. A 7.5% concentration of hydroxyapatite was successfully integrated into the water phase of the emulsion, while incorporation into the oil phase was limited to 2% due to emulsion stability constraints. Notably, nHA concentrations exceeding 5% significantly reduced the contact angle of the scaffolds. Following further characterisation, we anticipate that the design developed through this advanced characterisation will hold significant potential for treating bone tissue damage.

Keywords: emulsion templating, hydroxyapatite, polycaprolactone, biomaterials, tissue engineering

Acknowledgement: We would like to thank İzmir Institute of Technology Scientific Research Projects Coordination (Project code: 2022-İYTE-2-0025). IZTECH Integrated Research Center, The Materials Research Center.

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ORAL PRESENTATION

Methane Emissions from Ruminant Animals and their Effects on Greenhouse Gases

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Abstract

Methane, both enteric and manure-derived, is the most important greenhouse gas from ruminant animals, and its reduction can significantly reduce the carbon footprint of animal products and potentially contribute to climate change mitigation. Although options are limited, certain nutritional practices can significantly reduce enteric CH4 emissions in livestock. These practices can be broadly divided into two categories: dietary manipulation and feed additives. In the first category, feed selection and improved feed digestibility may be able to reduce enteric CH4 emissions. There is an opportunity to reduce enteric CH4 emissions by increasing the starch concentration in the diet. However, these practices may lead to reduced milk fat yield and farm profitability. Oil and oilseeds, which are effective in reducing CH4 emissions, have negative effects on rumen fermentation, feed intake, and milk production and composition. Scientific studies show that some feed additives, especially the CH4 inhibitor 3-nitrooxypropanol, can significantly reduce CH4 emissions from dairy and beef cattle. However, the long-term effects and external factors affecting the inhibitor's effectiveness should be further investigated and their use in the diet should be decided accordingly. At present, the feasibility of mass applications such as macroalgae is unknown. It is not fully known how both ration manipulation and feed additive addition interact with each other and whether there is synergy between feed additives with different effects. In addition, the effects of nutrition on greenhouse gas emissions from manure composition and storage have not been sufficiently studied. In general, methane reduction applications will provide consistent results and if new, effective and safe strategies are developed and implemented, up to 60% reduction in enteric CH4 emissions from ruminant animals can be achieved. In this review, some theoretical and practical information will be given about methane emission reduction and effects.

Keywords: enteric methane, greenhouse gas reduction, feed additive, ruminant animals

ORAL PRESENTATION

Usability of Animal Wastes that Cause Environmental Pollution as Feed Source

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Abstract

Processed animal protein sources, animal fats, milk and egg products and animal products such as expired food products constitute potential alternative feed sources. Every year in the world, Europe and Turkey, a large amount of animal fat and meat industry by-products are produced from slaughterhouses, dairies and facilities that produce food for human consumption. And these by-products and wastes are considered important elements that cause environmental pollution. In addition, tons of expired animal food products, if not utilized, pose both a significant economic loss and a significant environmental pollution threat. However, by-products and other animal products obtained from slaughterhouses and similar processing facilities can be converted into animal feed and added to the livestock production economy and their environmental damage can be prevented. As seen in a series of food crises in the recent past, inappropriate and irregular use of animal by-products and food waste can cause a major public health risk and a negative economy. For this reason, both in Europe and Turkey, there are serious restrictions on the use of animal by-products as feed for farm animals in order to obtain and use safe food. Especially in ruminant animal diets, the use of animal by-products has been banned in Europe and Turkey due to the increased risk of spongiform encephalopathies (mad cow disease). However, as a result of significant positive changes in the animal by-product processing industry and the development of valid diagnostic methods to test species-specific material in feeds, the European Commission has begun to reorganize strict rules regarding these animal by-products and has introduced new regulations regarding their use in poultry, taking into account feed hygiene. The purpose of this review is to define the situation regarding the obtainment and use of animal by-products in the feed industry, to identify new usage opportunities and to provide a new scientific approach to the use of these environmentally harmful residual by-products as a safe feed source, in accordance with the framework of EU feed and food legislation, and to ensure their use as alternative feed materials in animal feeding diets.

Keywords: Animal nutrition, animal by-products, alternative food sources, slaughterhouse residues

ORAL PRESENTATION

Physical, Nutritional and Sensory Properties of Gluten-Free Muffins Enriched with Melon Seed and Green Lentil Protein Isolate

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Abstract

With the growing prevalence of gluten sensitivities and celiac disease, there is an increasing demand for accessible, nutritious, and enjoyable gluten-free baked goods. Therefore, this study examines the nutritional, textural and sensory properties of gluten-free muffins enriched with melon seed and green lentil protein isolate. For this purpose, quinoa and rice flour control muffin (C) was compared with muffins containing quinoa substitutions with 10% green lentil protein isolate, referred to as protein control (PC). Furthermore, melon seed powder was added in varying quantities (5%, 10% and 15%) to increase the antioxidant activity and phenolic content (TPC) of the gluten-free muffins. The results demonstrated that the addition of green lentil protein isolate and 15% melon seed powder significantly increased the protein, ash and lipid contents of the muffins, while the carbohydrate content decreased. Volume and uniformity index of muffins increased significantly when green lentil protein isolate was added. An increase in hardness was observed with the addition of green lentil protein isolate, while the substitution of melon seed powder did not change the hardness of the gluten-free muffins. As the melon seed powder substitution ratio increased, the crumb and crust color changed significantly. Sample C (110.7±3.5 mg GAE/100 g; 15.1±1.8 mg CE/100 g, respectively) was found to contain lower amounts of TPC and flavonoid content compared to gluten-free muffins enriched with 15% melon seed powder (150.2±6.2 mg GAE/100 g; 19.4±0.5 mg CE/100 g, respectively). In terms of antioxidant activity, the gradual substitution of quinoa and rice flour with melon seed powder increased antioxidant activity by 1.8-fold. The taste and aroma of gluten-free muffins were not affected by the melon seed powder substitution and perceived as pleasant by the sensory panelists. These findings signify the potential of melon seed powder in enhancing the nutritional profiles and conferring health benefits to gluten-free muffins.

Keywords: Gluten-free muffins, melon seed, green lentil, functional food.

ORAL PRESENTATION

Effect of copper on antioxidant enzyme activities in tissues of *Oreochromis niloticus*

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Abstract

In this study, the effect of copper on antioxidant enzyme activities in the liver, kidney, muscle and gill tissues of Oreochromis niloticus was investigated. Fish were exposed to 0.6, 3.0 and 6.0 mg/L Cu concentrations for 30 days and catalase (CAT), superoxide dismutase (SOD) and glutamate pyruvate transaminase (GPT) activities were determined in their tissues. Significant changes were detected in antioxidant enzyme activities in the liver, kidney, gill and muscle tissues of O. niloticus when exposed to different concentrations of copper, compared to control values. Generally under the influence of copper, while a decrease was observed in CAT activity, an increase was observed in SOD and GPT activities.

Keywords: Copper, *Oreochromis niloticus*, tissue, antioxidant, toxicity.

ORAL PRESENTATION

The impact of nanoparticles produced by Green Synthesis on environmental health

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Abstract

In recent years, green synthesis has been increasingly preferred in the industry as an environmentally friendly and sustainable approach. One of the most important purposes of this synthesis method is to reduce the use of nanoparticle heavy metals and other harmful substances used in the production of chemical technologies. In addition, thanks to green synthesis, it is to minimize the formation of waste products and by-products and to eliminate their negative effects on the environment and living health. It is very important to reduce nanoparticle heavy metals that threaten the environment, nature and water with this method. In this study, the areas of use of nanoparticle heavy metals produced by green synthesis and their products and the studies conducted are compiled and presented.

Keywords: green synthesis, nanoparticles, health, environment

ORAL PRESENTATION

Çevre Dostu Bileşenlerin Kullanılmasıyla Kontrollü Salım Azot Gübrelerinin Hazırlanması

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Özet

Bitkiler için mutlak gerekli elementler bitki büyümesinde ve gelişmesinde önemli işlevlere sahiptirler. Bu bileşenlerin bitkilerce yeterli düzeyde alınamaması durumunda bitkilerin gelişimleri gerilemekte, buna bağlı olarak da alınan ürün miktarı ve kalitesi olumsuz etkilenmektedir. Kontrollü salımlı gübreler, bitkilere sürekli ve kontrollü olarak besin sağlayarak, besin kaybını azaltmakta, sera gazı emisyonu, toprak ve yer altı suyu kirliliği gibi olumsuz etkileri önlemektedirler. Kontrollü salım gerçekleştiren akıllı gübrelerin (CRF) geliştirilmesi, sürdürülebilir ve etkili gübrelerin üretilmesinde yeni bir potansiyel teknolojidir. Bu çalışmada kontrollü salımlı gübre hazırlamak için doğal bir polimer olan kitosan, azot kaynağı olarak üre ve farklı miktarlarda montmorillonit kullanılmıştır. Gübreler hazırlanırken; üre ve montmorillonit bir süre karıştırıldıktan sonra kitosan eklenmiş, homojen olana kadar karıştırılmış ve küre şeklini alması için sodyumtripolifosfat çözeltisine damlatılarak ortalama 2 mm çaplı küreler elde edilmiştir. Bu kürelerden azot salım deneyleri saf su ortamında ve 30oC sıcaklıkta incelenmiştir. Azot salım deneylerinde küreler saf su ortamına eklenerek belirli aralıklarla örnekler alınmış ve bu örneklerdeki azot miktarları UV spektrofotometrede 526 nm dalga boyunda absorbansları ölçülerek belirlenmiştir. Azot salım sonuçları montmorillonit oranı arttıkça azot salımının bir miktar azaldığını, kümülatif azot salım yüzdelerinin %20-25 arasında değiştiğini göstermiştir. Ayrıca montmorillonit içeren gübrelerden azot salımının montmorillonit içermeyen gübrelere göre azot salım yüzdesinin %70'lerden %20'lere azaldığını göstermiştir.

Anahtar Kelime: Kitosan, Montmorillonit, Gübre, Azot

Teşekkür: Yazarlar mali desteklerinden dolayı Gazi Üniversitesi Bilimsel Araştırma Projeleri Birimi'ne (FYL-2024-9458) teşekkür ederler.

1.2. FULL-TEXTS

ORAL PRESENTATION

Application of response surface methodology to optimize the process parameters for cotton dyeing with natural extract of saffron petals

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Abstract

The use of natural dyes for textile coloration has increased in the past few years due to their eco-friendly and non-toxic nature. The main aim of this research work is to determine the ideal conditions of dyeing the cotton yarn with natural dye extracted from saffron petals using the response surface methodology. Saffron petals have coloring compounds that can be extracted and used for dyeing of textiles. The saffron petals were taken from a local producer after harvesting stigmas for trade purposes. They remain as a waste and are deposited into environment. These petals were taken, dried and used in this experimental work. The natural dye extracted through traditional method was applied into the cotton fiber at different values of dyeing time (40-80 min), pH (5-10), temperature (90-110°C), dye concentration (0.2-7%) and amount of mordant (0-3.6g). The dye uptake of natural dye was evaluated by absorbance measurements, using UV-Vis spectrophotometer. The result of the dye uptake percentage was used in response surface methodology analysis. By RSM the dyeing conditions were determined as dyeing time of 61 minutes, pH of 9.6, temperature of dyeing 103°C, dye concentration of 4.87% and mordant amount 3.35g.

Keywords: natural dyeing, saffron petals, cotton fabrics, optimisation, RSM

INTRODUCTION

The textile industry is responsible for around 17-20% of industrial water pollution around the world. This pollution is mostly produced by dyeing processes that consume large amounts of synthetic dyes. A considerable amount of the consumed dyes remain in the wastewater, leading to several environmental and health problems (Vadood et. al., 2022; Rahman et. al., 2023; Werede et. al., 2021). Removal of these dyes using various technologies is the approach usually considered in the industry to reduce their effect on the environment. Another approach that has recently attracted a lot of attention is the revival of natural dyes in the textile industry (Vadood et. al., 2022). Natural dyes are renewable and eco-friendly products that render soft and soothing shades to textiles (Rahman et. al., 2023; Werede et. al., 2021; Benli et. al., 2022; Bhouri et. al., 2023). They are sourced from natural resources like insects, plants, minerals and microbes (Verma et. al., 2023, Rahman et. al., 2023; Baaka et. al., 2023; Bhouri et. al., 2023). Their use has numerous advantages including high bio-degradability and compatibility with the environment (Baaka et. al., 2023; Benli et. al., 2022). Moreover, recently the governments of many countries have started to encourage textile industries to use environmentally friendly substances with the vision to elevate the natural dyeing process from small scale, so called “handicraft” or homemade products to the industrial scale. This trend has intensified interest in textile dyeing with natural dyes, especially in Europe, over the past decade (Rahman et. al., 2023). Saffron (*Crocus sativus L.*) is one of the most valuable medicinal plants worldwide (Naim et. al.,2022; Kumar et. al., 2022; Kothari et.al., 2021). The plant belongs to *Crocus Sativus* genius. It is a plant that grows in warm countries like Italy, Iran, Afghanistan, India, Greece, France, Spain, Turkey, Morocco, etc. (Kumar et. al., 2022; Kothari et. al., 2021). It is cultivated also in Albania, in Elbasan region. In saffron production, great amounts of floral bio-residues are generated. After harvesting stigma the petals of the plant remain as a waste. So the saffron petal, as a by-product is available for free and in large amounts, compared to the saffron stigma (Naimet.al., 2022). The petals are taken and used as a source of natural colorant for dyeing of cellulosic yarn.

Cotton is the most widely used natural fiber in the textile and fashion industry owing to its unique properties, such as absorbency, comfort and soft handle (Vadood et. al., 2022; Guo et. al., 2020; Rahman et. al., 2022). Its structure consists of cellulose, a polymer constructed of monomeric glucose units (Pisitsak et. al., 2016). Natural dyeing of cotton is difficult due to the low affinity and weak fastness of the natural dyes in this fiber (Vadood et. al., 2022; Rahman et. al., 2023). The mordant dyeing method is often used to improve the substantivity and fastness properties of natural dyes (Rahman et. al., 2023).

Another problem in natural dyeing of textiles is the reproduction of the obtained hues, especially when a mixture of colorants or mordants is applied. To ensure the levelness of the hues and the reproducibility of the results, natural dyes require careful extraction and optimization of the dyeing procedure (Vadood et. al., 2022).

The usual approach to process optimization involves modifying one parameter at a time while keeping the others at their current levels. This allows the effect of a single parameter to be studied while keeping the overall optimization intact. On top of that, substantial amounts of time, effort, and experimentation are required. Furthermore, this method may provide inaccurate outcomes, such as the belief that interaction effects cannot be observed, making it difficult to ascertain process parameters performance (Pervez et. al., 2023). Optimization of dyeing is definitely the substantial method to determine the optimum condition in order to get highest dyeing uptake exhaustion. Response surface methodology is one of a perfect method to simulate the variables simultaneously. It has been widely used in process and product improvement because it can optimize the complex process and minimize the experimental number of trials (Hamid and Munaim, 2017). The central composite design is widely used experimental design method due to the selection of appropriate pivot points and the retention of the rotatability and sequentiality of the experimental data, which greatly improves its prediction accuracy (Guo et. al., 2020). The main aim of this study is to optimize the dyeing conditions of saffron dye extracted from saffron petals onto the cellulosic fiber. The significant factors such as dyeing time, dyeing bath concentration, dyeing temperature, mordant amount and pH of the solution have been identified and proceed further using central composite design (CCD) under RSM. The optimal conditions were determined to achieve the maximum dye uptake percentage during the dyeing process.

MATERIALS AND METHODS

Cotton preparation: The cellulosic fiber was sized in 4x10cm with 2g weight and prepared to be dyed with the best mordanting method (simultaneous-mordanting) for the cotton fibre. The method of mordanting and the alum mordant were determined by our previous experiments during the project.

Extraction of natural dye from saffron petals: The saffron petals were obtained from Victus Fed company. They were dried and prepared for extraction. The dye was extracted with traditional boiling method. After the extraction the mixture was allowed to cool down and was filtered to remove the petals. The extracted solution was kept in the dark box and used in the dyeing process.

Dyeing process: The dyeing experiments were performed according to the runs determined by RSM method. The cellulosic fiber was dyed with saffron extracts with simultaneous-mordanting method with liquor ratio of 1:75, while the other parameters were ranged as is showed in table 1. At the end, the absorbance of the extract remained in the dyeing bath was measured.

Absorbance measurements: The absorbance measurements were recorded by using UV-Vis spectrophotometer by using quartz cells with a path length of 1cm at a maximum wavelength of 265nm which was previously determined in our experiments. The dye uptake percentage was measured by using the absorbance of the dye solution before and after dyeing in three measurements. The percentage of the dye uptake (exhaustion) was calculated by using the equation given below:

$$E = \left(\frac{A_0 - A_f}{A_0}\right) x100 \qquad (1)$$

Where: A_0 is the absorbance of the dyeing solution before the dyeing process
A_f is the absorbance of the dyeing solution after the dyeing process

Experimental design and dyeing conditions optimization: The RSM method was used to determine the optimum conditions for a system with five variable. We have used five independent factors namely dyeing time (x1), dye concentration (x2), mordant amount (x3), dyeing temperature (x4) and pH (x5), while the dye uptake (y) was taken as a response. The number of experiments required for development of this design is N^k runs, where k indicates number of factors used in the study, N is the number of levels. A total full factorial design method includes 2^5=32 experiments for our study. The ranges of independent variables are summarized in table 1. The results of the experimental design were analyzed using Minitab18 statistical software to investigate the effects as well as the parameters and statistical plots. The interactions between independent variables were analyzed with analysis of variance (ANOVA) and the main effects of dye adsorptions were identified based on the P value with >95% of confidence level. The general form of the equation is as follows:

$$y=b_0 + \sum_{j=1}^{5} b_j x_j + \sum_{i<j} b_{ij} x_i x_j + \sum_{j=1}^{5} b_{jj} x_j^2 \qquad (2)$$

Where: y is the response factor, b_0 is the constant term, bj represents the linear term, bij is the coefficient of interaction, bjj is the quadratic coefficient.

Table 1. Independent variables and their levels used for response surface study

Factors	Unit	Levels				
		-2	-1	0	+1	+2
(x1) dyeing time	Minute	40	50	60	70	80
(x2) dye concentration	g/L	0.2	1.9	3.6	5.3	7
(x3) mordant amount	g	0	0.9	1.8	2.7	3.6
(x4) dyeing temperature	°C	90	95	100	105	110
(x5) pH	pH	5	6.25	7.5	8.75	10

RESULTS AND DISCUSSION

Model fitting and ANOVA results

In order to check the model adequacy, the experimental data were fitted to various models. According to the values of p and adjusted R-squared we can conclude that the dyeing process of cotton fibers with saffron petals extract was most suitably described by a quadratic model. The analysis of variance was used for measuring up the significance of the effect of the dyeing process variables and their interactions on the dye uptake as the response. The ANOVA results of applied quadratic model to the experimental design space of cotton fibers dyeing with extract of saffron petals are shown in table 2. According to the probability value of the independent variables, the significant or insignificant factors were determined. A probability value less than 0.05 indicates that the effect of this independent variable on the response (dye uptake) is significant. In this case x3, x1*x1, x2*x2 and x3*x3 are the significant independent factors on the dye uptake of cotton fibers, due to their low probability values and their high F-values. Represented model (eq.3) is the second-order polynomial equation obtained from regression analysis of the experimental data:

$$\begin{aligned} y = {} & -802 + 2.24\,x_1 - 7.05\,x_2 - 5.8\,x_3 + 15.32\,x_4 + 1.1\,x_5 - 0.02674\,x_1{*}x_1 \\ & - 1.069\,x_2{*}x_2 \\ & - 3.58\,x_3{*}x_3 - 0.0777\,x_4{*}x_4 - 0.920\,x_5{*}x_5 + 0.0930\,x_1{*}x_2 - 0.173\,x_1{*}x_3 \\ & + 0.116\,x_1{*}x_5 + 0.737\,x_2{*}x_3 + 0.957\,x_2{*}x_5 + 0.217\,x_3{*}x_4 + 1.47\,x_3{*}x_5 \qquad (3) \end{aligned}$$

This empirical model was used in the optimization and prediction of the dyeing uptake percentage within the range of variable factors in this experiment. The coefficient of determination was 0.945 and adjusted R^2 was 0.92. Since R^2 is near to unity, it means that the fitted empirical model is suitable for actual data and the model can explain the relation among variables.

Table 2. ANOVA results of fitted quadratic model to the experimental data of cotton dyeing process

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	17	1795.90	105.641	3.95	0.006
Linear	5	838.68	167.736	6.27	0.003
x1	1	13.78	13.783	0.52	0.485
x2	1	30.31	30.308	1.13	0.305
x3	1	769.78	769.783	28.77	0.000
x4	1	20.42	20.424	0.76	0.397
x5	1	4.38	4.381	0.16	0.692
Square	5	699.49	139.898	5.23	0.006
x1*x1	1	209.80	209.797	7.84	0.014
x2*x2	1	279.91	279.915	10.46	0.006
x3*x3	1	245.99	245.988	9.19	0.009
x4*x4	1	110.55	110.553	4.13	0.061
x5*x5	1	60.64	60.644	2.27	0.154
2-Way Interaction	7	257.74	36.819	1.38	0.289
x1*x2	1	40.00	39.999	1.50	0.242
x1*x3	1	38.91	39.906	1.45	0.248
x1*x5	1	33.47	33.472	1.25	0.282
x2*x3	1	20.33	20.331	0.76	0.398
x2*x5	1	66.23	66.227	2.48	0.138
x3*x4	1	15.29	15.292	0.57	0.462
x3*x5	1	43.51	43.507	1.63	0.223

The applied approximating model was examined by the residuals plot. Residuals are the difference between actual and predicted values for each point and show that how well the model satisfies the assumptions of the analysis of variance. The normal probability plot of the residuals for the data tests the hypothesis that the residuals have a normal distribution, where if the plot gives straight line, this means that the residuals have a normal distribution. From figure 1 it is clear that the points were almost distributed in a line, which indicated that the model was satisfactory and accurate.

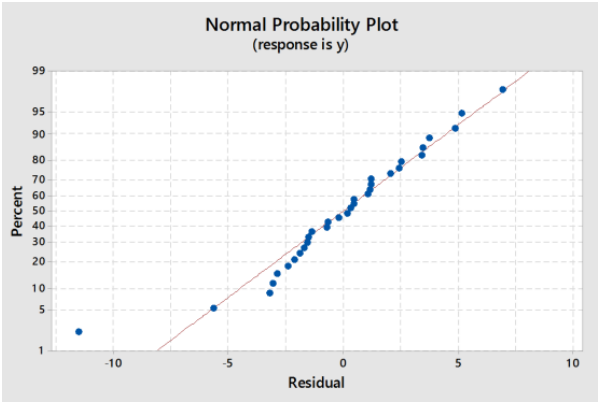


Figure 1. Normal plot of residuals

The normal probability plot of the standardized effects (figure 2) was used to verify the statistically main and interaction effects that were included in the model. According to the normal probability plot, the points located closer to the fitted line are not considered as significant, whereas points located far away from the line tend to be more significant. The main factors A and B on the left have a negative effect, while factors C and D lie on the right side and have a positive effect on the adsorption efficiency. The amount of mordant (point C) has the largest effect because its point lies farthest from the line. The second important factor is the dye concentration (point B), which is more significant than dyeing temperature (point D) and dyeing time (point A). Regarding the interaction terms, BE, CE, AB, AC, AE,BC, CD have significant effect on adsorption efficiency because they stay away from the line. The quadratic terms BB, CC, AA and DD have significant effects compared to two-way interactions.

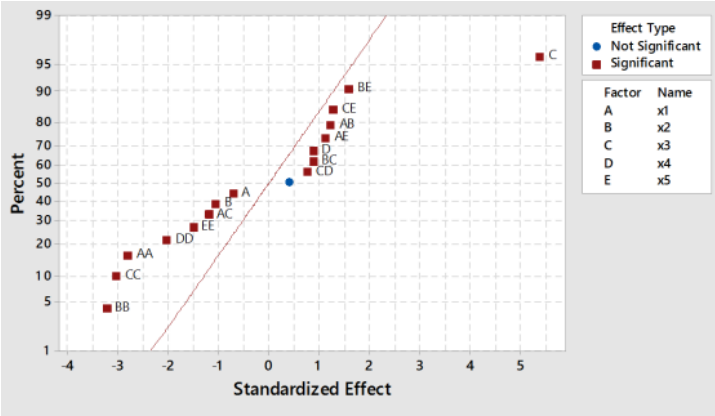


Figure 2. Normal probability plot of the standardized effects

The same indication is given clearer in the Pareto chart. The Pareto chart of this experimental design is presented in figure 3. In this figure it is shown the influence of the important factors on adsorption efficiency. The significance of the factors and their interactions was obtained by using Student t-test with 95% confidence interval. Amount of mordant represented the most significant effect on adsorption efficiency. The interaction effect between BB, CC, AA and DD have greater effects on adsorption efficiency. While other interactions between BE, EE, CE, AB, AC, AE, BC, CD and the main factors A, B and D have significant effect and are statistically significant at 95% confidence level. The experimental points are reasonably aligned, confirming normal distribution pattern.

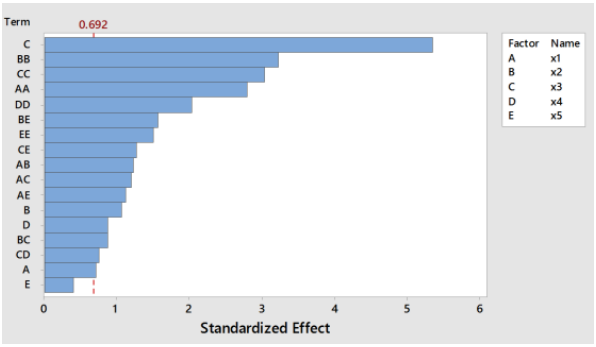
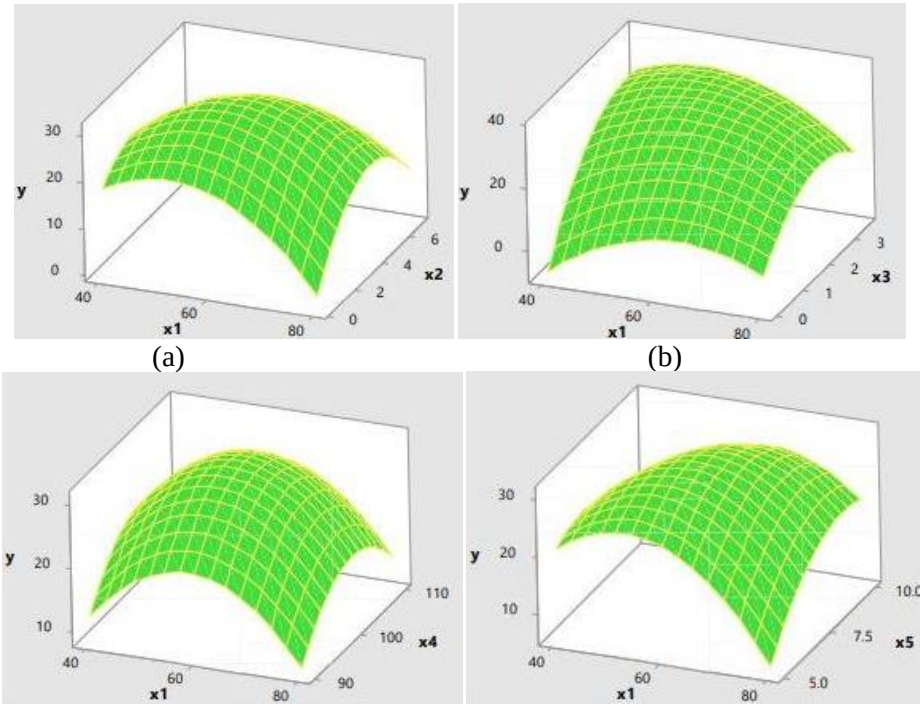


Figure 3. Pareto chart of the standardized effects of model variables

Three dimensional surface plots

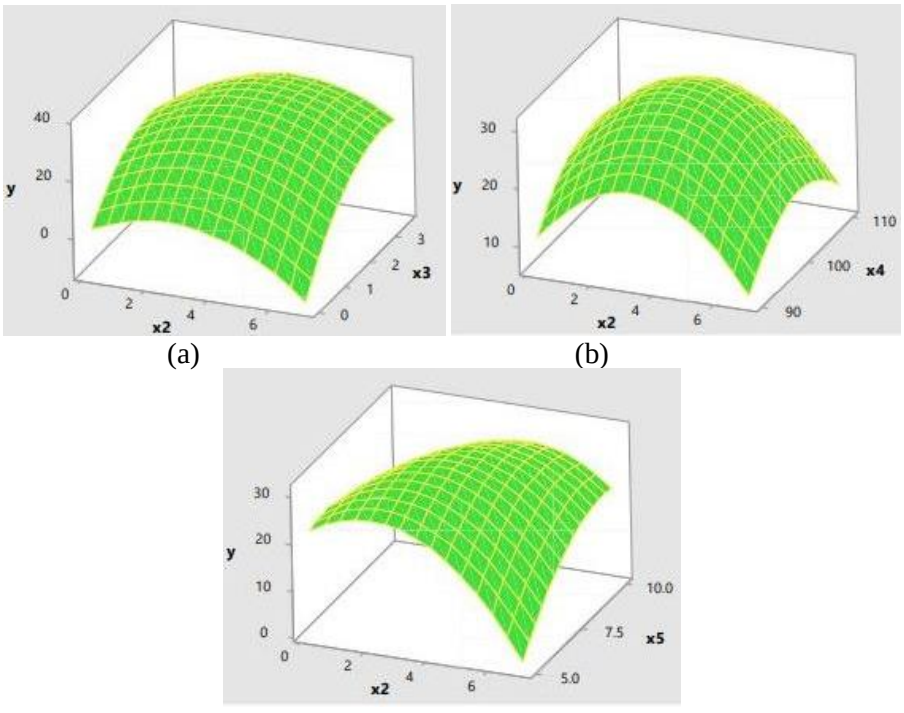
The effect of dyeing temperature, dyeing time, dye-bath concentration, amount of mordant and pH on dyeing uptake efficiency was further analyzed with the assistance of three-dimensional response surface plots in the views of quadratic model. Three dimensional surface plots obtained by using Minitab18 software are represented in figures 4, 5 and 6. In all response surface plots, three other variables which are not mentioned are in their midpoints.



(a) (b)

(c) (d)

Figure 4. The 3D surface plots of the dye uptake for (a) dyeing time and dye concentration, (b) dyeing time and mordant amount, (c) dyeing time and dyeing temperature, (d) dyeing time and pH



(a) (b)

(c)

Figure 5. The 3D surface plots of the dye uptake for (a) dye concentration and mordant amount, (b) dye concentration and dyeing temperature, (c) dye concentration and pH.

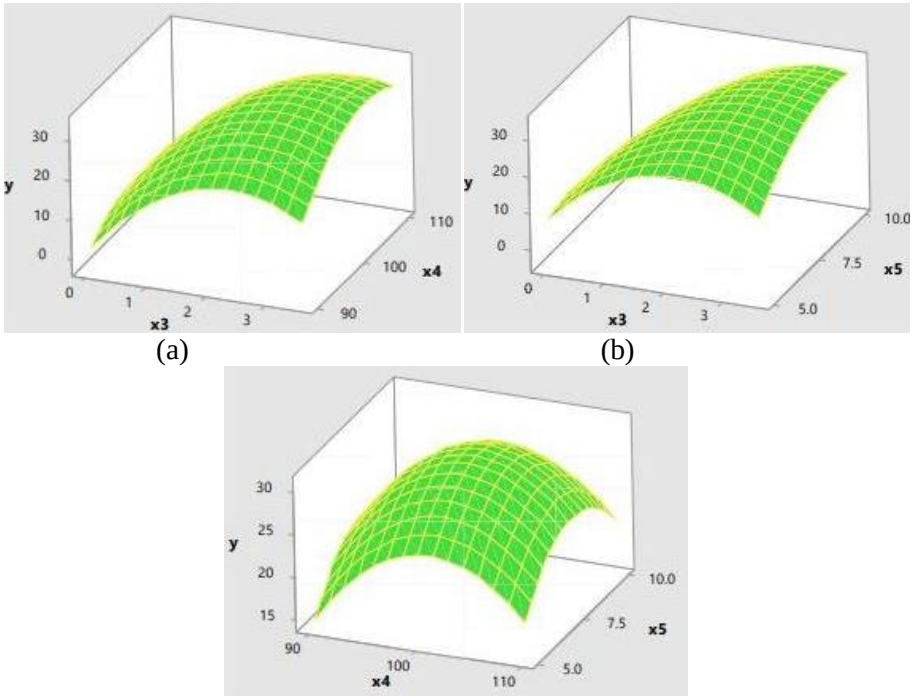


Figure 6. The 3D surface plots of the dye uptake for (a) mordant amount and dyeing temperature, (b) mordant amount and pH, (c) dyeing temperature and pH.

The effect of dyeing time and dye concentration, mordant amount, dyeing temperature and pH, illustrated in figures 4, 5 and 6, demonstrated that the dye uptake percentage decreased when dye-bath concentration, dyeing time and dyeing temperature values increased. The mordant amount has the highest influence in dye uptake, as it can be seen in figure 4 (b) and 5 (a). It can be observed also from table 2 that this factor is the most significant. Mordanting increases the interaction between the hydroxyl groups of cotton fibers and the flavonoind and phenolic groups present in the saffron petal extract used for dyeing. The increased amount of mordant leads to increased dye uptake. In all cases increasing the dyeing temperature and dyeing time reduced the dye uptake due to degradation of coloring compounds and due to desorption process that can occur. Increasing the initial dye concentration leads to an increase in mass gradient between the dye solution and the cotton fiber. This acts as a driving force leading to transfer of dye molecules from solution to fiber surface, but in high concentration values occurs the association of dye molecules so they can not penetrate into the fiber pores. High values of pH increase the dye uptake. This may be due to the protonisation of cotton fiber, which makes the dye fixation better. The 3D graphs show the simultaneous effects of factors on the response in which the highest points of the curved surface plots indicate the amounts of factors resulting in maximum dye uptake. These graphs are useful for establishing response values and operating conditions that are needed. From these graphs we can conclude that the dye uptake was higher near the optimum conditions of the dyeing process as it is mentioned in the optimization paragraph below.

Optimization of dyeing conditions

Optimization by using CCD method involved the selection of the target, and for our case was to maximize the response with desirability values of d=1.000 implying a response almost similar to the optimum. The optimum conditions for dyeing of cotton fiber were found to be dyeing time 61 minutes, dye concentration 4.87%, mordant amount 3.35g, dyeing temperature 103°C and pH 9.6 (figure 7). These values are close to the values obtained with traditional method of optimization, but in this case they are obtained with a reduced number of experiments and time. The good agreement between the predicted response and the experimental values means that the empirical model derived from RSM can be used to adequately describe the relationship between factors and response in this study (figure 8).

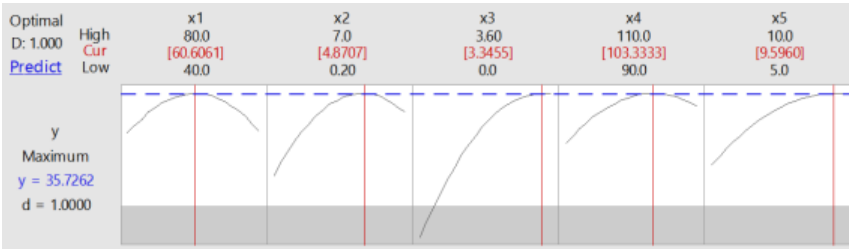


Figure 7. Optimum dyeing conditions for cotton fiber

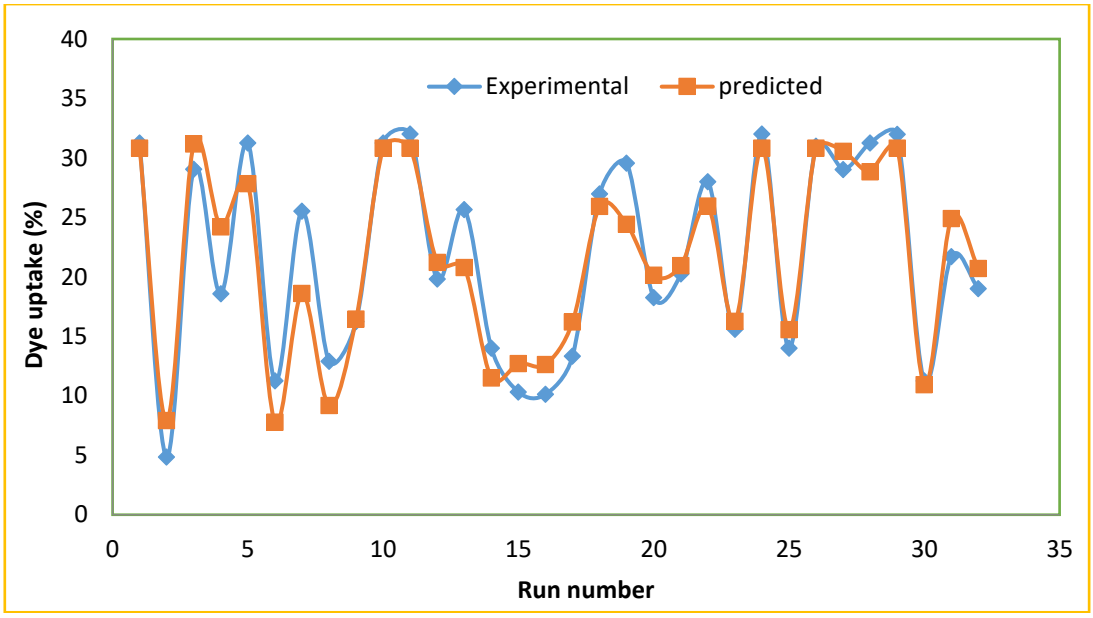


Figure 8. Relative deviation between experimental and predicted dye uptake for cotton fiber dyed with extract of saffron petals

CONCLUSIONS

Cotton fibers are successfully dyed with saffron petals extract as a natural dye. The dyeing conditions of cotton fibers were optimized by response surface methodology. The study identified the significant variables in affecting the percentage efficiency of saffron dye adsorption into cellulosic fibers. The ANOVA results of the quadratic model indicated that the mordant amount was the significant independent factor on the dye uptake as well as their interactions such as x1*x1, x2*x2, x3*x3 were significant model terms. The set of optimum conditions for the maximum uptake of saffron dye on cellulosic fiber were derived from statistical data. Their values were as follows: dyeing time 61 minutes, dye concentration 4.87%, amout of mordant 3.35g, dyeing temperature 103°C and pH 9.6. The R^2 value of 0.945 shows the satisfactory explanatory of the fitted model. The experimental values were in good agreement with the model predicted values. This study clearly showed that response surface methodology was one of the suitable methods to optimize the best operating conditions to maximize the dye uptake with reduced number of experiments and time.

ACKNOWLEDGEMENTS

The authors want to thank the VICTUS FED Company for providing the saffron petals.

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ORAL PRESENTATION

Comparative study on self-medication practice among Albanian undergraduate students

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Abstract

The practice of taking medication on one's own without a doctor's advice for diagnosis or treatment is known as self-medication. Because of their basic knowledge and easy access to medications as well as to social media undergraduate students are more likely to practice self-medication.
The current study aimed to determine and consequently compare the prevalence of self-medication among two different groups of Albanian undergraduate students, as well as what factors and conditions motivate this practice.
This cross-sectional study was conducted among undergraduate students of medical and non-medical programs from public and private universities in two different cities, in Albania. The study was carried out between October 2021 and March 2022 for the first group of the study population, and between November 2023 and April 2024 for the other one. A self-administered questionnaire with open-ended and closed-ended questions about demographics, years of education, and self-medication habits, as well as questions about attitudes and perceptions of the practice, was used to collect the data. Statistical Packages for Social Sciences (SPSS) version 21 was used to analyze the data.
The majority of the participants for both groups were female, respectively 66.5% and 78.8%. The mean age of the included participants for both groups was around 20.4 ± 1.9 years old. The majority of the participants were nonmedical students (64.6%) for the first group and medical students (79.7%) for the second group. 71% and 73.8% respectively for the first and second groups practiced self-medication due to the mild illness. Of all, 78.5% and 84.7% of students took analgesics/NSAIDs for self-medication, shifting to antibiotics, 48.8% and 27.1%. Headache (94.7% and 91.2%) followed by fever (38.3% and 45.9%) were stated by the students as the most common self-medication indications. It was discovered that the most popular sources of information were previous experiences and local pharmacist consultations.
The study findings showed that the most often indication for self-medication was headache whereas analgesics/NSAIDs were the most often medication used. Self-medication was mostly motivated by previous experiences, then by self-acquired knowledge, specifically by the second group where medical students dominated. It is recommended that students should be well-educated and aware of the negative effects of self-medication.

Keywords: self-medication practice, undergraduate students, medication, illness, attitude.

INTRODUCTION

Self-medication is the practice of giving oneself medication to prevent, treat, or cure an ailment whose identification and severity are usually unknown, either on one's initiative or on the advice of a third party (WHO, 2000). Using leftover medications from previously recommended treatment courses, as well as "over-the-counter" drugs or medications suggested by friends or family, can all be considered forms of self-medication (Tesfaye et al., 2020). Both developed and developing nations have high prevalence rates of self-medication (SM), which range from 25.6 to 73.6% (Loni et al., 2023). When properly applied, SM has the potential to save lives in emergencies, reduce long waiting times for appropriate medical attention, and save healthcare expenses for both the patient and the system. Conversely, SM may increase the risks of overusing medication, consuming it for a longer period, experiencing drug interactions, and polypharmacy (Araia et al., 2019).
Research indicates that undergraduate students are extremely unlikely to seek advice from medical professionals about their health, get treatment, or receive other healthcare services (Niwandinda et al., 2020). In recent years, with the increase of the influence of social media, students are turning more and more to the

internet for health-related information instead of consulting medical professionals. It makes undergraduate students more likely to use self-medication to manage ailments that they self-diagnose. Undergraduate students with and without medical backgrounds have different basic knowledge, particularly in the area of health (Shkreli and Tabaku, 2024).

Several research that looked into the practice of self-medication among various population groups of students were carried out in several countries. The findings of research carried out by Shah et al., 2021 and Malli et al., 2023 indicated that symptoms such as headaches, fevers, sore throats, cramps, and abdominal pain were common, as were illnesses like respiratory infections, pneumonia, eye infections, urinary tract infections, colds, and gastrointestinal disorders (Shah et al., 2021, Malli et al., 2023). Similarly, a variety of reasons for self-medication practices have been documented, including the mildness of the illness, pharmacological knowledge, saving time and avoiding long waiting times to visit a doctor, recommendations from friends, the practice's low cost, and previous experiences (Rahimisadegh et al., 2022). The most often utilized classes of pharmaceuticals for self-medication were analgesics, antipyretics, antibiotics, anti-acids, antimicrobials, antihistamines, common cold tablets and syrups, vitamins, and nutritional supplements (Banda et al., 2021).

The purpose of this study was to determine and consequently compare the prevalence of self-medication among two different groups of Albanian undergraduate students, as well as what factors and conditions motivate this practice.

MATERIALS AND METHODS

To determine how common is self-medication practice among Albanian undergraduate students a comparative cross-sectional study was conducted in two groups of population: group number one (G_1) composed of medical and nonmedical students from a public university in Korça and group number two (G_2) composed of medical and nonmedical students from public and private universities in Tirana. The study was carried out between October 2021 and March 2022 for the group of population G_1, and between November 2023 and April 2024 for the group of population G_2. Included students in the study were selected using a randomized selection approach, every student contacted indicated they would be willing to participate in the research. A self-administered questionnaire with open-ended and closed-ended questions about demographics, years of education, and self-medication habits, as well as questions about attitudes and perceptions of the practice, was used to collect the data. A pilot study with forty-three participants was conducted to assess and standardize the questionnaire. Statistical Packages for Social Sciences (SPSS), version 21 was used to analyze the collected data.

RESULTS

A total of 228 and 364 questionnaires were distributed to the undergraduate students of groups G_1 and G_2; and were completed and returned respectively 209 of group G_1 and 340 of group G_2, yielding a 91.7% response rate for group G_1 and 93.4% for group G_2. The mean ages of the students were 20.44 ± 2.09 years for group G_1 and 20.41 ± 1.81 years for group G_2. Most of the students enrolled in the study were female with the age group 18 to 22 years old. The majority of the students in group G_1 were nonmedical students (64.6%) whereas in group G_2 were medical ones (79.7%). The study`s findings demonstrated that 62.19% of participants from group G_1 and 75.58% of participants from group G_2 had a positive attitude towards self-medication practice. The detailed data on demographic characteristics and attitudes toward self-medication are presented in Table 1.

Table 1. Prevalence of attitude towards self-medication by demographic characteristics of participants

Variables			**Attitude towards self-medication**			
			N $_{G1}$ = 209		**N $_{G2}$ = 340**	
	N $_{G1}$= 209	**N $_{G2}$ = 340**	**Yes (n, %)**	**No (n, %)**	**Yes (n, %)**	**No (n, %)**
Female	139 (66.5%)	268 (78.8%)	85 (40.67%)	54 (25.83%)	200 (58.82%)	68 (20.0%)
Male	70 (33.5%)	72 (21.2%)	45 (21.53%)	25 (11.97%)	57 (16.76%)	15 (4.42%)
Age group						
18-22 years	179 (85.65%)	304 (89.42%)	111 (53.11%)	68 (32.53%)	234 (68.82%)	70 (20.59%)
> 22 years	30 (14.35%)	36 (10.58%)	19 (9.09%)	11 (5.27%)	23 (6.76%)	13 (3.83%)
Mean age ± SD	20.44 ± 2.09	20.41 ± 1.81				
Study program						
Medical students	74 (35.4%)	271 (79.7%)	54 (25.83%)	20 (9.56%)	205 (60.29%)	66 (19.41%)
Non-medical students	135 (64.6)	69 (20.3%)	76 (36.36%)	59 (28.25%)	52 (15.29%)	17 (5.01%)
Degree of the study program						
Master's degree	57 (27.2%)	68 (20.0%)	35 (16.75%)	22 (10.53%)	53 (15.59%)	15 (4.41%)
Bachelor degree	152 (72.8%)	272 (80.0%)	95 (45.45%)	57 (27.27%)	204 (60.0%)	68 (20.0%)

Note: G_1 group number 1, G_2 group number 2
N number of participants in the study

As shown in Figure 1, the majority of the participants from groups G_1 and G_2 practiced self-medication because their illness was mild (66% and 73.8%), emergency uses (33.5% and 35.6%%), and previous experiences (22.5% and 20.0%).

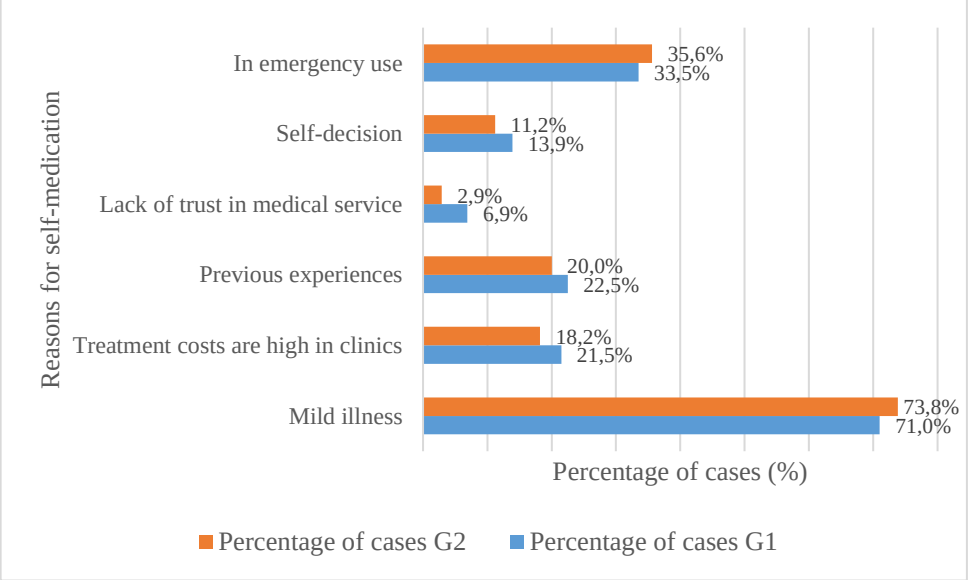

Figure 1. Reasons for self-medication practice

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The majority of the participants used non-steroidal anti-inflammatory drugs (NSAIDs) or analgesics 78.5% of students from group G_1 and 84.7% of them from group G_2; antibiotics 48.8% of students from group G_1 and 27.1% of them from group G_2; and herbal medication 54.1% of students from group G_1 and 25.3% of them from group G_2 (Table 2}

Table 2. Drugs commonly used for self-medication

	Classes of drugs used for self-medication			
Variables	**N G 1 = 209**		**N G 2 = 340**	
	Yes n (%)	**No (%)**	**Yes n (%)**	**No (%)**
Non-steroidal anti-inflammatory drugs NSAIDs	164 (78.5%)	45 (21.5%)	288 (84.7%)	52 (15.3%)
Antibiotics	102 (48.8%)	107 (51.2%)	92 (27.1%)	248 (72.9%)
Drugs used in gastrointestinal problems	22 (10.5%)	187 (89.5%)	48 (14.1%)	292 (85.9%)
Eye/ear drops	13 (6.2%)	196 (93.8%)	63 (18.5%)	277 (81.5%)
General tonics/syrups	51 (24.4%)	158 (75.6%)	60 (17.6%)	280 (82.4%)
Supplements/vitamins	67 (32.1%)	142 (67.9%)	83 (24.4%)	257 (75.6%)
Herbal drugs	113 (54.1%)	96 (45.9%)	86 (25.3%)	254 (74.7%)
Antihistamines	23 (11.0%)	186 (89.0%)	65 (19.1%)	275 (80.9%)

Headache was the most common self-medication indication among the students 94.7% of cases from group G_1 and 91.2% of cases from group G_2, fever 38.3% of cases from group G_1 and 45.8% of cases from group G_2, flu/cough/cold and general weakness following closely behind (Figure 2).

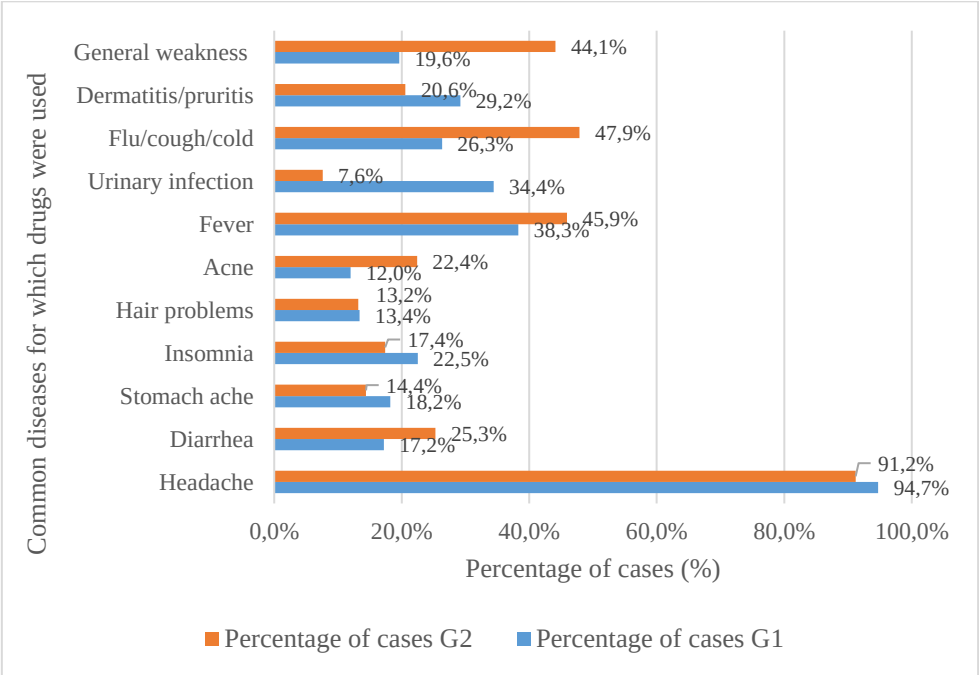

Figure 2. Common diseases for which medicines were used

Previous experiences (57.9% of cases from group G_1 and 52.9% of them from group G_2), consulting with a local pharmacist (47.4% of cases from group G_1 and 49.7% of cases from group G_2), knowledge from the study program (23.0% of cases from group G_1 and 48.8% of cases from group G_2), and other sources like friends, relatives, pamphlets, and the media were the most popular sources of information for self-medication (Figure 3).

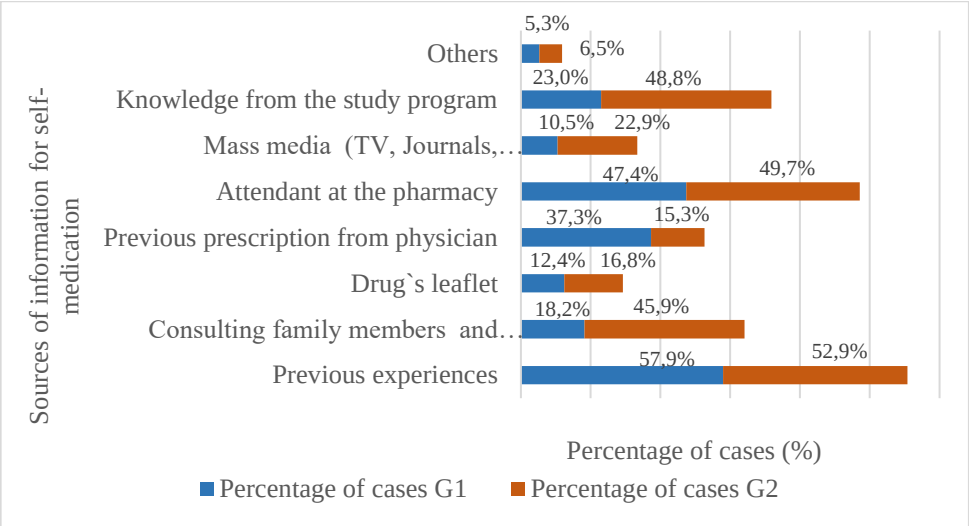

Figure 3. Sources of information for self-medication

DISCUSSION

The high rate of self-medication among undergraduate students may be caused by several factors, such as general knowledge of commonly used medicines and overconfidence coming from their advanced education. In addition to this, students are also encouraged to use medication without a doctor's prescription by reading news articles, medical books, brochures, or social media.

The findings of this research showed that the majority of enrolled students in both groups had a positive attitude toward the practice of self-medication (62.19% and 75.58%), which is higher compared to the results of the research (58.4%) conducted in Saudi Arabia in 2022 (Alduraibi and Altowayan, 2022). Compared to nonmedical students, the proportion of medical students who were well-versed in this subject was higher; as a result of variations in the curricula and educational courses offered in the field of healthcare.

The most common illnesses for which students practiced self-medication were headaches, flu/colds, fever, acne, and gastrointestinal problems. According to some published papers symptoms such as a common cold, headache, fever, vomiting, and hyperacidity can be treated without a doctor's advice (Loni et al., 2023, Castro-Catano et al., 2022).

Similar to earlier data, analgesics/NSAIDs (75.8% and 84.7%%) were the most often utilized class of medications (Zewdie et al., 2020, Siraj et al., 2022). This may be because the majority of responders thought analgesics were nontoxic medications that could be taken at any time and in any amount. On the other hand, analgesics are included in the list of over-the-counter medications, or those sold without a prescription.

The second-most-used medication for SM were antibiotics (48.8% and 27.1%), which were used is a higher degree by nonmedical students compared to medical ones. This could be from the fact that medical students are aware that antibiotics have side effects and should be prescript appropriately by physicians. Health science students in Northeast Ethiopia have reported a high rate of self-medication with antibiotics, 35.9% (Zewdie et al., 2020). Despite being prescription-only medications, antimicrobials are known to be taken inappropriately, especially in developing countries. Inappropriate use of antibiotics can result in treatment failure, severe medication responses, and the spread of antibiotic resistance. Discouragement of populations in the use of antibiotics during self-medication is very important, thus health education initiatives are essential in highlighting the dangers of improper antibiotics self-medication.

While convenience and cost-saving have been cited by some authors (Anwar et al., 2020, Daanish. et al., 2022), as the top reasons for self-medication, or the presence of long queues at health facilities and lack of time to visit the hospital (Banda et al., 2021), the current study indicated that mildness of illness and knowledge about the drug and disease were the key reasons for practicing self-medication (Anwar et al., 2020, Daanish. et al 2022, Banda et al., 2021).

The limitations of this study were the limited number of interviewed students; as well as the chance for responders to discuss among themselves while filling it the questionnaire.

CONCLUSION

In this comparative study, it was shown that a large percentage of Albanian undergraduate medical and nonmedical students had a positive attitude toward self-medication practice. For both groups, it was observed that the most common motivations for self-medication were mild illness and emergency use in which the students believed that seeing a doctor would be a waste of time. Analgesics (NSAIDs) were the most often utilized medications, followed by antibiotics and herbal medicine. The most often reported indications of self-medication included headache, fever, and flu/cough/cold. Self-medication was mostly motivated by previous experiences, then by self-acquired knowledge, specifically by the second group where medical students dominated. It is recommended that students should be well-educated and aware of the negative effects of self-medication, especially of the overuse of antibiotics; it is also suggested that legislative authorities should enhance restrictions regarding the dispensing of drugs.

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ORAL PRESENTATION

Green strategies boosting food sovereignty in Morocco

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Abstract
This study allows the strengthens capacities and skills of actors via Green strategies. This biologic strategy constitute the greatest pillar to accomplish the food sovereignty in Morocco. Indeed, green strategies can play in agriculture an important role in improving the standard of living of the rural population and it is central in the management of natural resources (water, land, climate and plant) in order to set up sustainable agriculture and development. In this context, it is necessary to prepare specialists who are capable of supporting the policies adopted by the Green Strategies; Green Morocco Plan (2008-2020) {15} and Green Generation (2020-2030) {16}, in terms of quality, food security, water saving and environmental protection. Indeed, agriculture provides 45% of national employment generates more than 65% of the income of rural households and enhances 8.7 million ha of the Useful Agricultural Area, of which 18% irrigated. By green strategy, apart from edible oil, good coverage ratio have been reached with sugar production 62%, milk/meat 70%, cereal products 70%, and vegetables/fruits around 100%.

Key words: Food sovereignty; Green Generation; Green Morocco Plan; Management of natural resources; Sustainable agriculture; Useful agricultural area.

INTRODUCTION

It is important to remember that Moroccan farmers tend to assimilate the principles of a sustainable agricultural system. Their approach goes beyond methodology to embrace a philosophy of coexistence with nature rather than its exploitation **{20}**. Traditional farmers would dispute the claim that resilient agriculture is the agriculture most closely associated with sustainable agriculture, as many of them also use methods they view as preserving or enhancing the natural resource base {1; 17}. Technology transfer is defined as the application of scientific knowledge (education and research) of agriculture to achieve desirable national goals, for example competitiveness, environmental sustainability, food security, and food sovereignty {12}. This interpretation is broader than the traditional interpretation, in which the term refers to the transfer of technology from one company or a country for commercial use elsewhere. This involves the development, communication and implementation of technological advances, primarily in the form of research, but also in demonstrations and other technological initiatives. {14; 21}.

The important question in this area should be: are **Green strategies** a farming system or a management strategy?. Both can be accepted, and each term adds a perspective that helps explain the complexity of this multidimensional concept. It is obvious that the term has different meanings depending on the context. Regardless, sustainability in agriculture means a system of policies, institutions, the private sector and communities providing goods and services while preserving natural resources and environment {8}. The objectives can be multipled and diverse depending on the specific time and place, including food security, the eradication of poverty, the development of local/national economies, the protection of cultural assets, etc. An essential condition for farmers to support sustainable agriculture is the use of conservative technologies and practices, minimizing dependence on external inputs and increasing local human and social capital {4; 10}.

The current situation is alarming, and we are currently experiencing five difficulties: a) Natural resource degradation, pollution and human health problems, b) Climate is changing c) Poverty and Food insecurity are still there, d) Economy is in trouble, and finally e) Population is increasing, {8}. To reach the main objectives of Sustainable agriculture, we have implemented these Green Strategies entitled: 1- Green Morocco Plan ‘GMP’ 2008-2020 {**15**}; and 2- Green Generation ‘GG’ 2020-2030 **{16}**. Otherwise, Sustainable Agriculture is a system, which involves farming system that are environmentally sound, profitable, productive and compatible with Socio-economic conditions (J. Pesek, 1994) {19}.

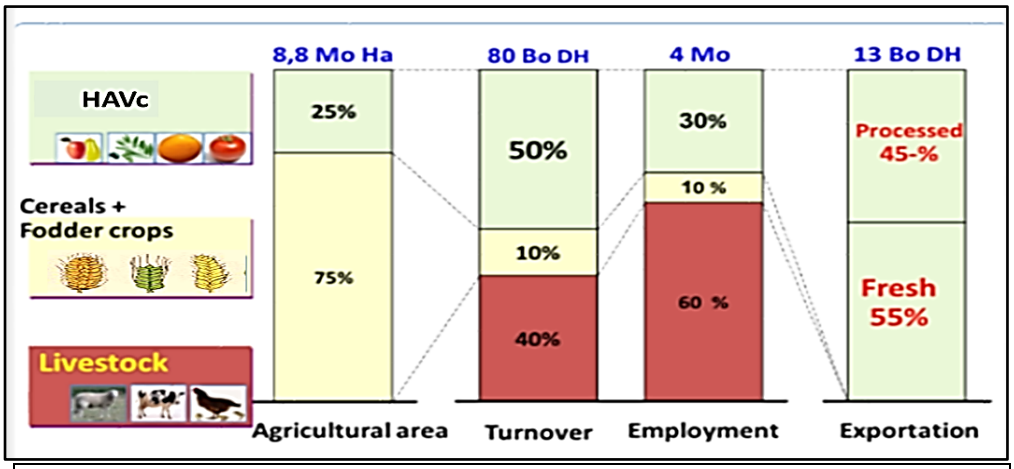
Sustainable production systems substantially reduce erosion, principally due to the use of sophisticated crop rotations and organic matter management techniques and groundwater contamination. The use of toxic materials in production is very low compared to conventional systems. So, the environmental and health problems associated with their use do not occur {11}. Energy use in sustainable system may be reduced by up to 60% depending on the region and production system. Many producers use older, sometimes rare, crop cultivars and animal breeds because they find them more appropriate in their production systems. Diversified crop production systems, windbreaks, and the more diversified landscape associated with sustainable agriculture system often contribute to improved and varied wildlife habitat{13; 2}.

MATERIALS AND METHODS

The Kingdom of Morocco is located in the north of Africa, 14 km far from Europe; its capital is Rabat, the population is approximately 37 million inhabitants, spread over a total area of 712,550 km² (71.26 million Ha), (BBC, 2023) {23}. In Morocco, the climate is typically Mediterranean on the coast with a mild winter (9 to 12°C) and moderately wet, coupled with a hot and dry summer (24 to 26°C). In the hinterland reliefs, precipitation can reach a maximum of more than 1500 mm per year with snow in winter. Precipitation and temperature are strongly influenced by the Atlantic Ocean to the west. The useful agricultural area is approximately 8.8 million Ha, the forest area is approximately 5.5 million Ha and pastures are approximately 21 million Ha, {16; 23}.

The gross domestic product GDP is increased to 122 billion euros. (Moroccan Dirham, MAD). In addition, GDP by sector of activity varies depending on agriculture: 14.8%, industry 29.1% and services 56.0%. The active population exceeds 10.399 million, of which 39.1% is in agriculture, 20.3% in industry and 40.5% in services. With all combined sectors , the unemployment rate is around 9.23%. Concerning nominal GDP, it should increase by 6% in 2024, bringing inflation measured by the implicit GDP index to 4.5% in 2023 after having recorded 3.1 % in 2022, (HCP, 2024) {5}.
Therefore, agricultural activities should record an increase of 2.5% in 2024, contributing to GDP growth of 0.3 points. Taking into account an improvement in maritime fishing activities, the primary sector should generate an added value improvement of 2.7% instead of an increase of 6.7% estimated for 2023. (HCP, 2023), {5}.

The low investment capacity was initially expressed by a very low participation of the banking system in finance, where just 18% of farmers benefit from granting loans. Then a weakness in the agro-industry fabric, which can barely reach 24% of industrial units and 30% of processing units, which strongly degrades the added value of the main agricultural sectors {24; 18}.
Concerning agricultural land, it shows excessive fragmentation (70% of agricultural holdings < 2 ha). However, it is with an apparent complexity of legal status, and a very low registration rate. Consequently, this alarming situation in 2008-2009 deprives farmers of all possibilities of investment and modernization. Thus, limiting their activity to food crops, in particular cereal farming, which occupies 75% of the usable agricultural area (UAA), and only represents 10 to 15% of total agricultural turnover (Figure 1). However, the high added value crops (HAVc) occupied only 25%.
Water resources influenced largely by climate change. Sometimes a prolonged water shortage with an abundance of structural drought continues to worsen until today, and is characterized by the weakness and irregularity of the rainfall {6; 3}.
This analytical observation has necessarily led to the undervaluation and overexploitation of surface and groundwater through an ineffective and inefficient irrigation system.



HAVc: high added value crops; **Mo Ha**: million hectare; **Bo DH**: billion Moroccan Dirham

Figure 1. Configuration of the Moroccan agricultural sector (MAPMDREF, 2008)

Despite the importance of agricultural areas (around 75% of the UAA) intended for cereal, fodder crops, this production has not benefited from the advantages of export to member countries. In addition, it benefited from barely 10% respectively in turnover and employment.
Thus, this technical-economic observation has led us to broadly and profoundly rethink Moroccan agricultural policy. Hence, a continuous green revolution with two phases, namely the importance of the launch of the Green Morocco Plan 2008-2020 strategy {15}, which is followed by the Green Generation strategy (2020-2030) {16}.

3.1. Green strategies for sustainable agriculture in morocco (2008- 2030)

To tackle the challenges of development, Morocco has established two Strategies as a green revolution in order to reach the main goal in the sustainable development. These green strategies are entitled: 1- Green Morocco Plan 'GMP' 2008-2020 {**15**}; 2- Green Generation 'GG' 2020-2030 **{16}**.
The design and implementation of these strategies are described and illustrated according to the following paragraphs.

3.2. Design of Green Morocco Plan GMP 2008-2020

Adopted by the Moroccan Government in April 2008, the Green Morocco Plan aims to make the agricultural sector as the main engine of the country's economic growth. To achieve its ambitious objectives, the Green Morocco Plan targets small and medium-sized family farms, entrepreneurial farms with large areas and access to capital. In few years, Morocco has considerably increased public investments allocated to the agricultural sector, which has notably resulted in an increase in cultivated areas.
The Green Morocco Plan GMP as a national strategy constitutes the best roadmap for a successful formula of sustainable agriculture. The different components of this project are shown in Table 1 below.

Table 1. Project of National agricultural program

Green Morocco Plan "GMP"	Pillar 1.	Pillar 2.	Cross-functional Project	Total
Nbr. of Projects	961	545		1506
Investment (Bo. MAD)	52	20	75	147
Nbr. Farmer	560.000	840.000		1.4 Mo

(MAPMDREF, 2008)

RESULTS & DISCUSSION

Analytical description of the Green strategies

Two pillars are based on two production models. The Green Morocco Plan "**GMP"** is divided into two pillars. The first one aims for the "aggressive development of high value-added agriculture" partly oriented towards export, while the second is intended for "solidarity support for small-scale agriculture". Large farms benefit from various supports: investment aid, rental of land with low prices over a long period in exchange for an investment commitment. Small farms benefit from subsidies to set up individual or collective projects (tree planting, olive crushing unit, etc.). Sometimes, these subsidies cover up to 100% of the cost of the investment. The first Pillar receives the majority of funding: when it was launched, the GMP planned to implement 1,506 projects (including 961 on Pillar I and 545 on Pillar II) for a total investment of MAD 147 billion; with 52 billion for Pillar I projects, 20 billion for Pillar II projects and 75 billion MAD for cross-functional actions **{16}.**

To achieve these objectives, the Green Morocco Plan 'GMP' is based on seven foundations, aiming to implement the principles of sustainable agriculture, according to three main components, namely:

a- Mode of intervention: As part of this approach, agriculture must become the main lever of growth in the next 10-15 years (foundation 1); then, adopting aggregation as a model for organizing agricultural sector, and the approval of aggregation as a regulatory mechanism (foundation 2); finally, ensuring the development of Moroccan agriculture as a whole without any exclusion, hence the interest of the two pillars P1 and P2 (Foundation 3) **{16}.**

b- Investment System: In this context, private investments must be encouraged with public assistance (foundation 4); then, adopting a contractual approach to realize the implementation of the Green Morocco Plan, the objective of which is the achievement of 1,500 projects (Foundation 5) **{16}.**

c- Sustainable Agriculture: Sustainability is a strategy aimed at supporting the development of Moroccan agriculture through the promotion and improvement of natural resources in accordance with the principles of sustainable agriculture (foundation 6). Finally, it would be beneficial to repair the overhaul of the sectoral framework (real estate, taxation, market, etc.) and to reform the infrastructure and logistics linked to good governance of sustainable development (foundation 7) **{16}.**

Specifically, this program plans to intensify agricultural production (P1); promote employment in rural areas (P2), create 1.5 million additional jobs; increase the value of agricultural exports (P1); and contribute to the fight against poverty in rural areas (Table 1).

Restrictions and obstacles

At the beginning of the launch of the Green Morocco Plan strategy, the growth rate of the Moroccan economy was 4.8% in 2009, an increase of 0.5 points compared to the estimate announced by the HCP, (2011). This development stems from the following actions; the 1.6% drop in volume in the added value of the agricultural sector (excluding fishing) compared to an increase of 30.4% in 2009; the 4.2% increase in the added value of other activity sectors (taken as a whole) compared to 0.8% a year earlier; and the 6.7% increase in volume of taxes net of subsidies affecting products instead of 4.6% of the previous year. This is how the growth rate of GDP excluding agriculture increased from 1.2% in 2009 to 4.5% in 2010 **{5}.**

Climate change is the main component of obstacles. After the significant drought in 2016, the Moroccan economy, which includes a favorable agricultural cycle, is expected to rebound in 2017. Driven by an above-average cereal harvest, economic growth increased by 4.3% in the first half of 2017 (compared to 1.6% during the same period in 2016). However, growth in non-agricultural activity remained less pronounced at 3.3% {5}.

Strengths and Features

The main retained factors are as follows: 1-The geographical location and proximity to the European market; 2- Dynamic national market; 3- Population growth; 4- Presence of qualified workers; 5- Proven benefits of numerous products (HAV); 6- Numerous efficient agricultural economic models (COPAG {6}, ANOC {3},…etc.).

Morocco's main exporting sectors are the automotive industry (28.4%), the agricultural and agri-food sector (21.8%) and phosphates, of which Morocco holds the largest reserves in the world. Tourism also plays an important role for the Moroccan economy representing 7% of GDP {5}. Indeed, Morocco attracts a large population of tourists with its natural wonders, its imperial cities, its climate, and the diversity of its multiple ecotourism composed of aromatic and medicinal plants, crafts and socio-cultural heritage (the case of the M' Goun Geopark) **{22}**. Population growth is often combined with the emergence of a highly skilled and ambitious workforce category for creativity and innovation at different scales; the know-how, interpersonal

skills and well-being. As an example, we cite successful transaction models, proven assets for many high value-added products (fruits and vegetables, etc.), and the success of numerous agricultural and food economic models such as COPAG, ANOC, COSUMAR **{7; 8; 10}**,…etc.

The actions of this program are distributed separately between pillar 1 (P1) and pillar 2 (P2), and concern approximately 1506 projects, with a budget of 147 million MAD aimed at approximately 1.4 million of farmers **(Table 1).**

The first pillar (P1) indicates modern agriculture with high added value favoring irrigated and fallow agriculture, which is preferred. The second pillar (P 2) indicates solidarity agriculture, which exists in mountain areas, oases and even rainfed agriculture.

Concretely, this plan shows an intensification of agricultural production; promotion of employment in rural areas; increasing the value of agricultural exports; contribution to the fight against poverty in rural areas; and improving the level of food security **(Table 2).**

Table 2. Action program of the main agricultural sectors

Agricultural sectors		**Area (1000) Ha**		**Production (T)**		**Budget (Bo. MAD).**	**Coverage ratio %**
Years		**2008**	**2020**	**2008**	**2020**	**2020**	
Sugar production		64	74	3.8 Mo-	4.2 Mo	4.85	62%
Oil product	**Sunf./ Rap.**	25	32	32	46 Thd	4	10%
	Pea. / Ses.	10	14	32	32	5	(950 CU), (75 PPU)
Cereal products		5400	4600	6.3 Mo	86 Mo	4,3 (p. subsidies)	70%
Production of	**Milk**	1.6 Mo Cows	1.8 Mo Cows	1.7	2.5 Bo.L	Tur: 6…/.. AV: 2,7	70%
	Meat	400.000 T	606.000 T	300	600 Thd	Tur : 9 …/ AV: 4,2	50 Mo WD

Sunf. : sunflower; **Rap**.: Rapeseed; **Ses.**=Sesame; Pea.=Peanut; **Mo**=Million; **Thd**=thousand; **Bo**= billion; **L**.=liter; **WD**= work days; **CU**= crashing unit; **PPU**= processing and packaging unit; **MAD**= Moroccan dirhams; **Tur**.: Turnover; **AV**.: Added value.

(MAPMDREF, 2020)

Economic impact

The GMP had a positive economic impact, as the gross domestic product (GDP) doubled from 65 billion MAD to more than 128 billion MAD. Concerning exportations, they have been multiplied by 2.4 to reach a satisfactory figure around 35 billion MAD, i.e. 80% of the programmed objective. Private investments experienced a significant increase, reaching a total amount of 63 billion MAD **{4; 16}.**

According to the new data, the coverage of basic agricultural products was able to reach, except for edible oil, a satisfactory level, ranging from 50 to 100%. This trend in the coverage rate particularly affecting the following sectors: sugar 62%; table oil 10%; meats and milk 70%, cereals 70%; fruits and vegetables around 100% (Table 2).

Social impact

On a social and household level, the GMP has enabled more additional working days up to +50 million days. In addition, this strategy was recorder an increase of 250 000 additional farmers, including woman, out of 1.5 million active farmers {16}. In other words, more than 30% of farmers were able to place themselves on the labor market, with a satisfactory level of exploration.

A dynamic of integration was well promoted by the GMP strategy, which was able to alleviate the problem of unemployment in rural areas by offering the opportunity to new beneficiaries; around 2.7 million small and medium-sized organizations to access the job market. Table 1, shows that farmers in P1 benefited four times (4 x) from the general budget compared to P2. However, farmers in P2 (60%) only benefit from 1/6 of the total budget. Others across-functional actions, i.e transversal projects; like infrastructure and logistic are largely intended for the two pillars together such as bridges, roads, commercial sites or markets, etc.

Environmental impact

The new technologies of the irrigation system and the drip system in particular, have made it possible to save a volume of great importance, which have been able to reach a volume of 2 billion m^3. This is a surplus to be exploited to fill the water deficit which has existed for several years and which continues to intensify in periods of drought. At the same time, this improvement was materialized with the expansion up to 3.7x of agricultural areas equipped with hydro-agricultural equipment to reach up to +547000 ha. In fact, the agricultural area irrigated by the drip system, increased from 145000 ha to 692000 ha, out of a global irrigated area 1.5 million ha. This situation results in an intensification of arboricultural areas with an increase of 450000 ha {10; 16}.

CONCLUSION

Indeed, this Green strategy model could be reproduced only in West Africa due to the decent agronomic, climatic, economic, social and political conditions. However, the Green Morocco Plan offers avenues for reflection or inspiration, such as the need for significant public investment for agriculture and food security (from State revenue and budgetary aid), the reaffirmed need to support family farming, the provision to farmers of financial resources and clear rules for accessing them. Indeed, the three components (Intervention, Investments and Sustainability) are retained in the foundations of the GMP that will be retained as an essential basis for defining a successful path to the establishment of sustainable agriculture, not only in Morocco, but also across Africa from West {**9; 16}**.

In fact, the GMP is a continuation of previous policies that protect the environment and strengthen natural resources (Chopin et al., 2021). It involves developing agriculture for promising sectors and according to a productivist approach (intensification of production through mechanization, irrigation, use inputs and genetic improvement) with a view to improving national food security and increasing agricultural exports. Apart from Oilseed products (10%), all results of GMP have shown a satisfactory coverage ratio with main agricultural sectors namely; sugar production 62%, meat/milk 70%, cereal products 70%, and around 100% of vegetables /fruits are reached.

To accomplish its mission in the development of sustainable agriculture, Morocco launched a second strategy called Green Generation spread over 10 years from 2020 to 2030, not yet accomplished, including here the main pillars and axes of implementation **{10; 16}**.

In the first pillar, it is important to continue the dynamism of agricultural development in order to achieve the consolidation of agricultural sectors (Resilient and eco-efficient agriculture), to make a modern and efficient distribution chain, and to establish a perfect quality, innovation and Green technology.

Concerning Pillar 2, the priority is giving to the human element, in order to set up a New generation of agricultural middle class; a New generation of young agricultural entrepreneurs, and finally reach a New generation of agricultural organization where the profession will be able to manage 30% of the public budget.

Through this second green generation strategy, which completes the first strategy of the Green Morocco Plan, it becomes necessary to arrive at the overall design of a sustainable agriculture capable of revitalizing the sustainable development system while based on the principles of the good governance.

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ORAL PRESENTATION

Deuterium and Oxygen-18 isotopic composition and Tritium content in thermal waters of Albania: Preliminary results

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Abstract

This research is focused on the O-18, D and T isotopic composition of the geothermal waters in Albania. For this purpose, geothermal waters were sampled from 13 sites (springs and wells) across Albania. The $\delta^{18}O$ and δD values indicate the presence of a complex hydrological system, where geothermal waters are primarily sourced from meteoric inputs and experience varying degrees of water-rock interaction at depth. The Peshkopia thermal spring is distinguished for the D and O-18 isotopic depletion showing that it is recharged by precipitation originating from higher elevations. On the contrary, the thermal waters from Ardenica zone show relative isotopic enrichment indicating that surface water suffered evaporation before entering the geothermal system. The deep and hot thermal waters from Kruja zone show a slight isotopic enrichment due to water – rock interaction. Tritium content in thermal waters allowed to distinguish deep circulating hot waters in Kruja (<0.6TU) from the springs of Peshkopia and Ardenica thermal zones (>0.6TU) which circulate at shallower depth and were mixed with meteoric waters during their ascent to the surface. These findings enhance the understanding of Albania's geothermal reservoirs and provide critical insights for the sustainable management and utilization of geothermal resources, particularly in the context of energy production and other aplications.

Keywords: Isotopic characterization, Oxygen-16, Deuterium, Tritium, Geothermal waters, Albania.

1. INTRODUCTION

Albania, located in a tectonically active region, contains several geothermal systems with considerable potential for renewable energy production and direct-use applications. Previous studies on Albania's geothermal resources have identified three distinct geothermal zones: i) Peshkopia, ii) Kruja, and iii) Ardenica geothermal zone (Eftimi and Frashëri, 2016). The majority of thermal springs (six) and deep hot water wells (two) are located within the Kruja geothermal zone, while the Peshkopia and Ardenica zones contain one and four thermal springs, respectively (Kodhelaj, 2023; Frashëri and Čermak, 2004). The water flow rate of the Peshkopia spring is approximately 14 liters per second, with a water temperature of around 43°C. In central Albania, several thermal springs emerge from the Kruja zone, with the most notable being the thermal springs in the "Llixhat e Elbasanit" area, known for their high water temperatures (> 66°C) and significant hydrogen sulfide content (>250 mg/l) (Xhomo et al., 2002; Dapi et al. 2023). Geothermal studies estimate that the water temperature at the "Llixhat e Elbasanit" reservoir formation exceeds 220°C (Dapi et al. 2023). Currently, Albania's thermal water resources are mainly used by a few health spas, where the most noted are at Peshkopi, in Elbasan area and Bilaj (Frashëri, 2010; Eftimi and Frashëri, 2018).

Geothermal fields in areas such as Peshkopi, Kruja, and Ardenica geothermal zone exhibit a wide range of geothermal water systems, characterized by different origins, temperatures, and depths (Jahja et al., 2024; Kodhelaj, 2023). To gain a comprehensive understanding of these systems and their long-term viability, isotopic characterization plays a vital role. The isotopes of oxygen, hydrogen and tritium offer critical insights into the dynamics of geothermal waters, providing essential information on their sources, interactions with surrounding geological formations, and thermal evolution (Sonney, 2010).

This article explores the application of Oxygen-16 (^{16}O), Deuterium (^{2}H) and Tritium (^{3}H) isotopes in the analysis of Albanian geothermal waters. These isotopic markers offer valuable insights into the sources of water, its age, interactions within geothermal reservoirs, all of which are crucial for the effective management and development of geothermal resources.

2. MATERIALS AND METHODS

2.1. Geological setting

The geological terrains of Albania, known as the Albanides, form a significant part of the Southern Alpine–Mediterranean mountain belt, situated between the Dinarides to the north and the Helenides to the southeast. These terrains consist of several geotectonic units (having their analogues in Dinarides and Helenides), predominantly extending from SSE to NNW direction (Fig.1), and are categorized as either Internal or External Albanides based on the presence or absence of Cretaceous transgressions and magmatic rocks (Xhomo et al., 2003; Eftimi and Frashëri, 2018).

The Internal Albanides comprise three main geological zones: a) *Korabi Zone*, which consists mainly of Paleozoic terrigenous metamorphic rocks; b) *Mirdita Zone*, which is mainly composed of Jurassic ophiolites; c) *Gashi Zone*, which includes Paleozoic metamorphic and volcanic rocks (Frashëri and Čermak, 2004).

The External Albanides, part of the South Adriatic sedimentary basin, are characterized by the lack of magmatic activity and exhibit continuous sedimentation from the Triassic to the Paleogene. These external zones, extending across western Albania and include the following geological zones (Fig.1) (Frashëri and Čermak, 2004): a) *Krasta-Cukali Zone* which consists of pelagic sediments and comprises two subzones: the Cukali subzone, featuring late Anisian rift volcanic rocks and Ladinian to late Cretaceous pelagic carbonates, and the Krasta subzone, composed of Albian-Cenomanian flysch, Upper Cretaceous pelagic limestone, and Upper Cretaceous–Eocene flysch; b) *Alps Zone*, characterized by upper Permian–lower Anisian terrigenous formations at its base, with Triassic platform limestones overlaying them. By the Jurassic, two subzones emerged: Malësia e Madhe, consisting of Jurassic–Upper Cretaceous platform limestone and Paleocene–Lower Eocene flysch, and Valbona, which contains Jurassic condensed carbonate sections and Maastrichtian flysch (Xhomo et al., 2002); c) *Kruja Zone*, composed of shallow-water platform-type limestones from the Upper Cretaceous to the Eocene, and Lower Oligocene flysch deposits; d) *Ionian Zone*, located in southwestern Albania, which represents a wide platform with shallow-water Triassic evaporites, Liassic carbonates, and pelagic Dogger–Late Eocene limestones, overlaid by Oligocene and early Miocene flysch formations (Kodhelaj, 2023); e) *Sazan Zone*, which consists of platform Triassic–Jurassic dolomites and shallow-water Cretaceous–Middle Oligocene limestones, covered by Miocene terrigenous sediments; f) *Adriatic and Albanian–Thessalonian depressions* that represent key geological units of the Albanides, filled with molasses deposits of the Serravallian–Pliocene and Middle Eocene–Pliocene, respectively (Eftimi and Frashëri, 2018).

2.2. Geothermal Waters in Albania

In Albania, that have a surface of 28,748 km², there are several hot and thermal waters, known since ancient times. Geological studies indicate that the presence of high mountain ranges and active fault systems facilitates the ascent of deep waters, which emerge at the surface as thermo-mineral springs (Eftimi and Frashëri, 2016). In Albania are distinguished three geothermal zones, characterized by low-enthalpy resources, which show different range of temperature, chemical composition, and depth of formation. Several significant thermal springs, rich in hydrogen sulfide (H_2S) and located in the Kruja zone, originate from deep karst aquifers (Kodhelaj, 2023; Eftimi and Frashëri, 2018).

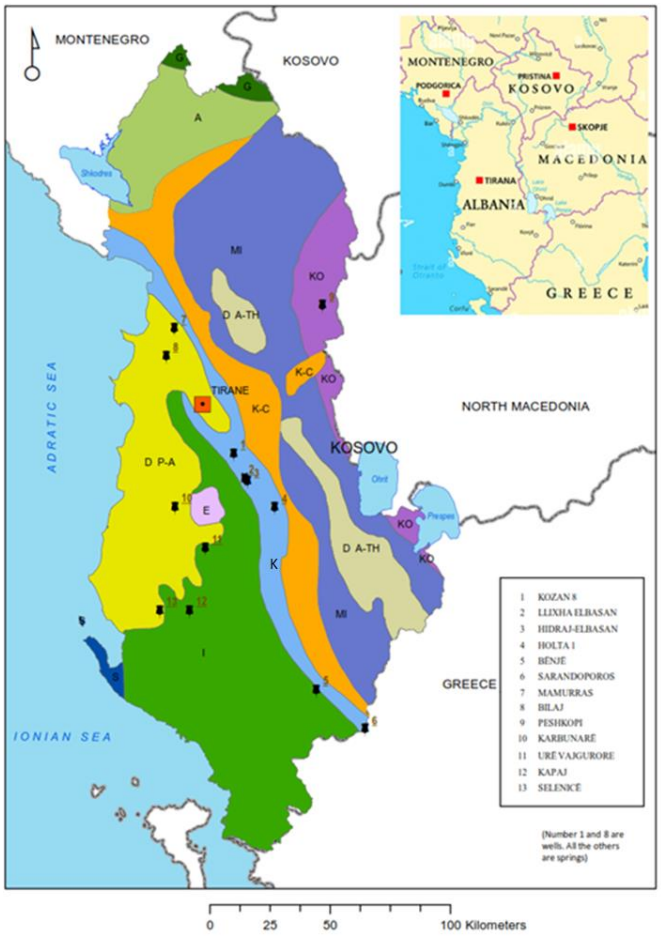

Figure 1. Tectonic zonation map of Albania where the sampled thermal water sites are shown (Jahja et al., 2024). Tectonic zones: K – Korabi; G – Gashi; A – Alps; MI – Mirdita; K-C – Krasta-Cukali; K – Kruja; I – Ionian; S – Sazani; D P-A – Peri-Adriatic Depression; D A-TH – Albanian – Thessalonian depression; E – Evaporites.

Thanks to their therapeutic properties, the thermal springs are mostly used for the treatment of various ailments at balneological centers of Peshkopi, Elbasan, Hidraj, Bilaj (Fig.2), but offer a promising energy source, as well (Kodhelaj, 2007; Kodhelaj and Bode, 2012). In particular, temperature and depth of Albania's geothermal waters vary significantly, influencing their potential uses (Jahja et al., 2024). The temperature of these waters spans from low-temperature systems (below 100°C) to higher-temperature systems (over 200ºC), depending on the geothermal gradient of each region (Kodhelaj, 2023; Dapi et al., 2023). Besides their traditional use for therapeutic purposes these waters maybe potentially used for heating buildings, greenhouses, and swimming pools and for electricity generation (Wator et al., 2020). Isotopic studies of geothermal waters, can provide deeper insights into the origin, recharge processes, and sustainability of Albania's geothermal systems, further supporting the country's potential for renewable energy development (Kodhelaj, 2007; Kodhelaj and Bode, 2012).

2.3. Sampling and isotopic analysis

In the frame of this study, 13 geothermal waters were sampled in August-October 2022 of which, 11 natural springs and 2 deep wells, located in Kruja, Peshkopia and Ardenica geothermal zones, as above mentioned (Fig.3).



Figure 2. Thermal spas a) Ure Vajgurore spring, b) Bilaj well, c) Sarandaporos spring

Temperature (T), pH, electrical conductivity (EC) and TDS, were measured in situ. The pH was measured with a pH meter type Testo 205 having an accuracy of ±0.02 pH, while its sensitivity is 0.01 pH. Electrical conductivity and TDS are measured with a Hanna type Conductivity Meter (HI 99300), having an accuracy of ±2% of the measured value, while its sensitivity is 1 µS/cm.



Figure 3. Geothermal waters: a) Sarandaporos spring; b) Hidraj-Elbasan well c) Holta spring

All water samples were filtered through 0.45 µm filter (cellulose acetate) on site. Samples were filled in polyethylene terephthalate (PET) bottles which had been rinsed with deionized water three times the day before sampling and twice more with the thermal water collected. At each water sample, concentrated HNO_3 was added to avoid precipitation. All the water samples were packed and sent to the Laboratory of Chemical and Isotopic Analysis at the Institute of Geosciences and Georesources, Pisa, Italy, where they were analyzed for the isotopic composition of O-18, D and Tritium.

The isotopic analyses of the geothermal samples were conducted at the Laboratory of Chemical and Isotopic Analysis at the Institute of Geosciences and Georesources, Pisa, Italy. O-18 analysis are conducted in a MAT 252 Mass Spectrometer manufactured by Finningan, using SMOW (Craig, 1961) as standard reference. Normalization rate is SMOW / SLAP, Average standard: GISP, while measurement error: 0.10. Analytical methodology was implemented referring to (Epstein and Mayeda, 1953; Craig, 1961). Deuterium (H-2) analysis are performed in a_Liquid Water Isotope Analyzer (LWIA) manufactured by Los Gatos Research (LGR), using SMOW (Craig, 1961) as standard reference. Normalization rate is SMOW / SLAP, average standard: GISP, while measurement error: 1.0. Analytical methodology was implemented referring to (Penna et al., 2010; Penna et al., 2012; Craig, 1961).

3. RESULTS and DISCUSSION

The results of the temperature, pH, electrical conductivity and TDS measured on site are summarized in Table 1. The temperature of the sampled waters ranges from 17.2°C to 63°C, with the hottest waters found in deep wells and some springs from the center of the Kruja zone. The pH values range from slightly acidic (6.26 – Llixha Elbasan) to slightly alkaline (7.02 - Sarandaporos spring). The total dissolved solids (TDS) content varies significantly, from 384.0 mg/l (Kapaj spring) to 14240 mg/l (Bilaj well).

Table 1. On-Site measurements and isotopic composition of O-18, Deuterium and Tritium

No.	Sampling points	Elevation asl[1] (m)	T (°C)	pH	ϰ (µS/cm)	TDS (ppm)	O-18	Deuterium V-SMOW	Tritium (TU)	Error[2] +/- TU
1	Kozan 8	174	63	6.47	7400	3928	-8.02	-54.3	0.0	0.3
2	Llixha Elbasan	142	54	6.26	11016	5848	-7.84	-53.5	0.1	0.3
3	Hidraj-Elbasan	252	55	6.29	10548	5564	-7.90	-53.2	0.3	0.4
4	Holta 1	248	24	7.24	3628	1916	-7.79	-52.7	0.4	0.3
5	Bënjë	319	30	7.21	2900	1526	-8.06	-55.1	1.4	0.4
6	Sarandoporos	389	29	7.92	1727	918	-8.11	-54.7	1.1	0.4
7	Mamurras	3	22	6.76	5324	2832	-6.76	-43.0	0.9	0.3
8	Bilaj	13	56	6.51	26856	14240	-7.06	-52.0	0.0	0.3
9	Peshkopi	647	19	7.82	5008	2656	-9.41	-64.9	0.8	0.4
10	Karbunarë	29	18.1	7.13	5100	2684	-5.39	-38.5	1.0	0.4
11	Urë vajgurore	33	17.4	7.56	1072	572	-6.86	-44.8	1.6	0.4
12	Kapaj	165	17.7	7.29	724	384	-6.66	-42.5	1.5	0.4
13	Selenicë	15	17.2	7.45	2548	1340	-6.18	-39.3	1.6	0.5

[2]Note: error 18O±0,10 / error D±1,0

The analytical results of O-18, D and T isotopic composition of the study geothermal waters are shown in Table 1, both O-18 and Deuterium show a wide isotopic variation (from -9.41 to -5.39 and from -64.9 to -38.5, respectively), while the content of Tritium ranges from 0 to 1.6 TU.
In the isotopic diagram of δD versus $\delta^{18}O$ (Fig.4) the isotopic composition of the sampled geothermal waters is confronted with the Global Meteoric Water Line (GMWL, Craig, 1961). Most of the sampled thermal waters fall above the GMWL indicating that the recharge source was meteoric water. The isotopic fractionations are calculated through the following formulas, based on the SMOW standard:

$$\delta^{18}O_{water} = \left(\frac{\left(\frac{^{18}O}{^{16}O}\right)_{water}}{\left(\frac{^{18}O}{^{16}O}\right)_{SMOW}} - 1\right) x\ 1000 \qquad \delta D_{water} = \left(\frac{\left(\frac{D}{H}\right)_{water}}{\left(\frac{D}{H}\right)_{SMOW}} - 1\right) x\ 1000$$

Variations of the deuterium and ^{18}O contents in thermal waters may be related to a) mixing in varying proportions of waters with different $^{18}O/^{16}O$ ratios, b) different degrees of isotopic exchange with rocks and c) steam losses during ascent of the fluid (Gat and Gonfiantini, 1981; Belhai et al., 2015; Dinh Chau et al., 2022). In the diagram of the D and O-18 isotopic composition (Fig. 4) the thermal waters from the three thermal zones are isotopically well distinguished. Thus, the thermal water of the Peshkopia spring is the most isotopically depleted, as shown by negative values of δD and $\delta^{18}O$. This isotopic depletion probably comes as a result of the high-altitude rainfall infiltration in the shallow underground depths. Slightly higher values of isotopic fractionation are observed in the springs of the Kruja Geothermal Zone, which may be dedicated to the cationic exchange of the geothermal waters with the minerals of the surrounding rocks. These waters have higher temperatures due to the deeper circulation and longer residence times, which appear to be responsible for their isotopic enrichment. Finally, the springs of the Ardenica Geothermal Zone are distinguished for the highest isotopic content of D and O-18 in all the sampled springs.

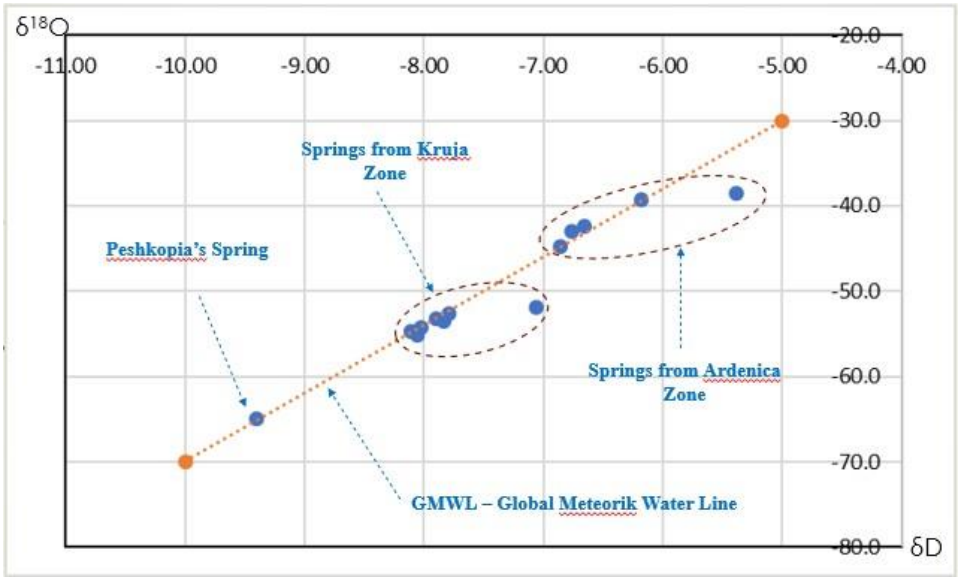

Figure 4. Isotopic composition of the geothermal waters in diagram of $\delta^{18}O$ and δD

This is a consequence of the ascending hot water mixing with fresh and colder waters that have undergone preliminary evaporation, as shown by the parallel variation of the δD and $\delta^{18}O$ values-according to a linear relationship.

In the Figure 5 is shown the variation of the Tritium content in all sites of the geothermal waters. Tritium (^3H) is the only radioactive isotope of hydrogen and is used to determine the age of geothermal waters and also to detect young - shallow water components in deep geothermal waters (Balderer et al., 2002). The global inventory of natural tritium produced by the interaction of cosmic radiation with nitrogen in the upper atmosphere (Craig and Lal, 1961) remains constant over time, as its production and decay are balanced. However, nuclear fusion bomb tests conducted until the early 1960s introduced high levels of tritium into the environment, with peak concentrations recorded in 1963 (GNIP station in Vienna; IAEA/WMO, 2019). During the bomb-test era, natural tritium variations were obscured by these elevated levels, but as the bomb-generated tritium decays, concentrations have nearly returned to natural levels (Palcsu et al., 2020; Yudong et al., 2021). This "tritium bomb peak" has been used in numerous studies to trace and date groundwater (Allison and Holmes, 1973), quantify recharge rates, and investigate ocean circulation patterns.

Taking a conventional value of 0.6 TU of Tritium content, it becomes possible to classify geothermal waters into two main groups:

1) Geothermal waters that circulate at shallow depths sampled in the following springs: Bënjë, Sarandoporos, Mamurras, Peshkopia, Karbunarë, Urë Vajgurore, Kapaj and Selenicë.

2) Geothermal waters that circulate at great depths: the Kozan and Bilaj wells, the springs of Llixhat Elbasan, Hidraj Elbasan and Holtë.

The water from the Kozan and Bilaj wells do not contain Tritium, because the geothermal waters circulate at greater depths, have a longer residence time and circulate upward to the surface through the column of the wells, thus not having contact with rich Tritium shallow waters. Since these two wells have zero content of Tritium, we may consider that the geothermal waters in these two wells are older than the time of the Tritium peak that was reached in the years 1960-1970. As a conclusion, low tritium concentrations suggest that many of Albania's geothermal systems are fed by older, deep-circulating waters that have not been recently recharged.

According to the variation shown in the Figure 6 there is an inverse relationship between Tritium content, Temperature and TDS. Lower Tritium concentrations correlate with higher Temperature and TDS values, and vice versa. At higher temperatures and at greater depths, the process of dissolution of the minerals of the surrounding rocks in the geothermal waters is encouraged, thus causing an increase in TDS. Waters that circulate in shallow depth, i.e. have a smaller residence time in the aquifer, result in a decrease in Temperature and TDS values, and an increase in Tritium concentration.

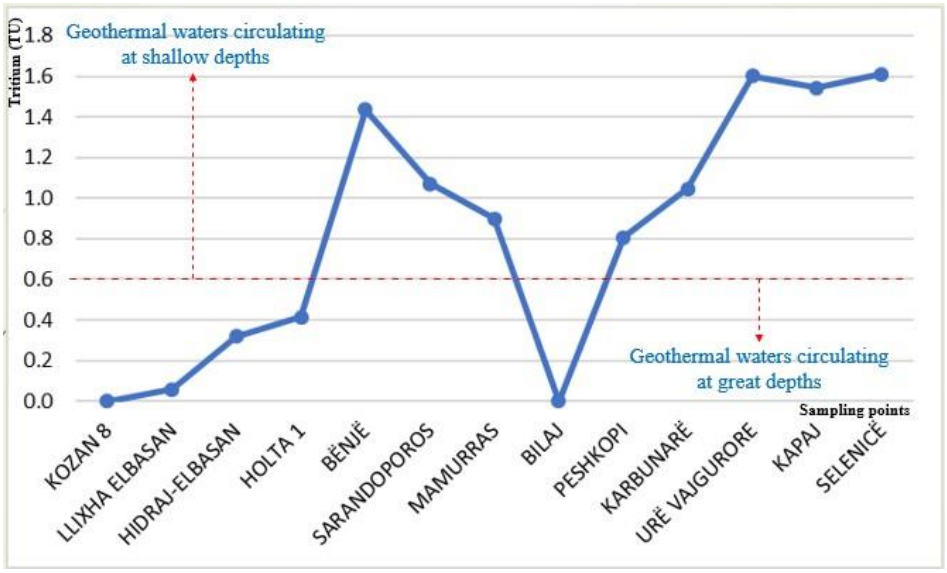

Figure 5. Variation of Tritium content in of the sampled geothermal waters

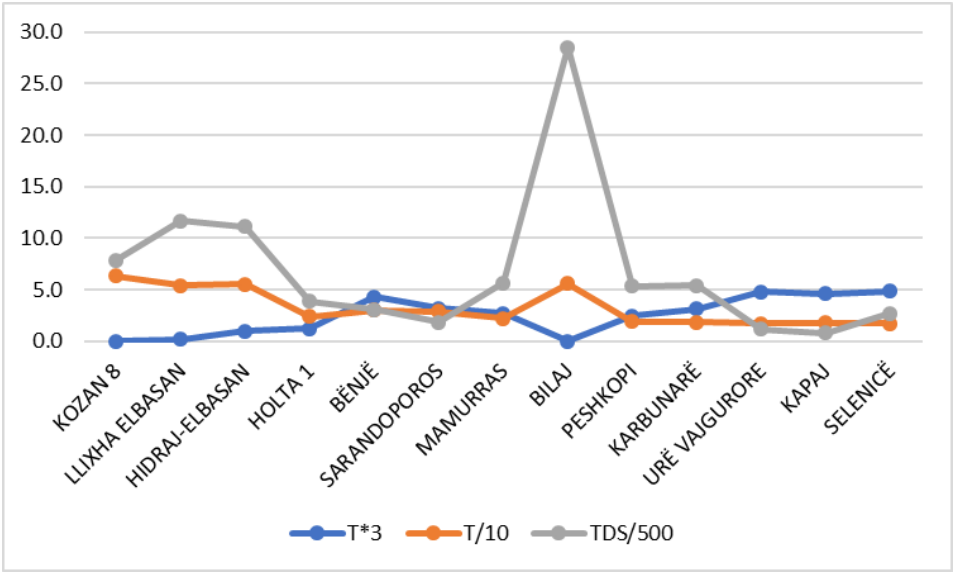

Figure 6. Variation of Tritium content, Temperature and TDS in all points of geothermal waters

CONCLUSIONS

The sampled thermal waters range in temperature from 17.2°C to 63°C, with the hottest water sites located in the central Kruja zone. pH levels range from 6.26 (Llixha Elbasan) to 7.92 (Sarandaporos spring), while TDS vary from 384.0 mg/l (Kapaj spring) to 14,240 mg/l (Bilaj well).

The isotopic composition of Oxygen-18, Deuterium, and Tritium shows significant variation, with $\delta^{18}O$ ranging from -9.41 to -5.39, δD from -64.9 to -38.5, and Tritium from 0 to 1.6 TU. Most sampled waters plot above the Global Meteoric Water Line (GMWL), indicating a primary meteoric origin. The Peshkopia spring is the most isotopically depleted, reflecting infiltration of high-altitude rainfall. In contrast, springs from the Kruja Geothermal Zone show slightly enriched isotopic values due to cationic exchange with surrounding rocks. These waters, which circulate at deeper geothermal systems and have longer residence times, exhibit higher temperatures and isotopic enrichment. The Ardenica Geothermal Zone springs have the highest $\delta^{18}O$ and $\delta^{2}H$ values due to the mixing of ascending hot water with cooler, fresher waters that have undergone preliminary evaporation.

By considering a conventional tritium content of 0.6 TU, two geothermal waters groups are distinguished: a) shallow-circulating thermal waters of the Bënjë, Sarandoporos, Mamurras, and Peshkopia springs; and b) deep-circulating waters, including Kozan and Bilaj wells, and springs from the Elbasan area. Low tritium

concentrations suggest that many of Albania's geothermal systems are fed by older, deep-circulating waters that have not been recently recharged.

Lower tritium concentrations correlate with higher temperatures and TDS values, reflecting increased dissolution of surrounding rock minerals at greater depths. Conversely, shallow waters with shorter residence times show lower temperatures, lower TDS, and higher tritium concentrations.

ACKNOWLEDGEMENTS

This study was carried out as part of a Ph.D. research project at the Polytechnic University of Tirana. The authors extend their gratitude to the research committee and the staff of the Laboratory of Chemical and Isotopic Analysis at the Institute of Geosciences and Georesources, Pisa, Italy, for their assistance in conducting the isotopic analysis of the geothermal water samples.

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ORAL PRESENTATION

Effect of *OPRM1* rs510769 single nucleotide polymorphism on impulsivity in opioid and methamphetamine co-users

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Abstract

In recent years, several studies have shown a significant increase in methamphetamine use among individuals with opioid use disorder (OUD). It is estimated that methamphetamine use may have become widespread to relieve the withdrawal symptoms seen in opioid users. Impulsivity is accepted as an important component of substance use disorders and can vary between addicted individuals. Thus, this study aimed to examine the effect of *OPRM1* rs510769 on impulsivity in a total of 105 opioid and methamphetamine co-users admitted to Ankara Training and Research Hospital AMATEM Clinic. *OPRM1* rs510769 was genotyped by PCR-RFLP. Impulsivity was measured by Barratt Impulsiveness Scale (BIS). The allele frequency of the *OPRM1* rs510769 polymorphism was determined in opioid and methamphetamine co-users as 74% for C allele and 26% for T allele, which is accordance with Hardy-Weinberg Equilibrium ($p>0.05$). The total score of BIS was 32.22±914. Although there was not a statistically significant difference between genotypes of the *OPRM1* rs510769 ($p=0.09$), opioid and methamphetamine co-users with TT genotype had lower BIS score (26±8.64) than those with CC (32.13±7.96) or CT (33.86±10.11) genotypes. This study revealed that *OPRM1* rs510769 seems to cause interindividual variability between opioid and methamphetamine co-users in view of impulsivity. However, this finding should be confirmed in future studies with larger and different populations.

Keywords: methamphetamine, opioid, *OPRM1*, PCR-RFLP

INTRODUCTION

Amphetamine is a psychoactive substance that produces a sense of euphoria, heightened energy and attention. Nevertheless, the use of this substance can also result in adverse effects, including anxiety and dysphoria, in some individuals, and it can lead to addiction (Brauer & de Wit, 1996; De Wit et al., 1986). Amphetamine exerts its effects by inhibiting the reuptake of dopamine, norepinephrine, and serotonin at the presynaptic level, while increasing synaptic dopamine levels (Schuldiner et al., 1993; Wallace & Connell, 2008). The reinforcing effect is regulated by opioid receptors (Mathon et al., 2006). The involvement of endogenous opioids in the amphetamine response has been demonstrated by the robust euphoric effect resulting from the combined administration of mu-opioid agonists and psychostimulants (Leri et al., 2003).

Heroin is a semi-synthetic substance that naturally contains opiates and is derived from morphine (Milella et al., 2023). A opioid use disorder is characterised by a persistent search for opioid substances, a persistent urge to use, a lack of control in limiting substance intake, and the development of negative emotional states when the substance cannot be accessed (Strang et al., 2020). Opioid use disorder is a recurrent and chronic disorder that occurs as a result of several factors, including genetic susceptibility, a history of heroin use, mental disorders, and environmental factors(Clarke et al., 2014; Strang et al., 2020).

In recent years, there has been increasing evidence of heroin and methamphetamine co-using in the USA and Mexico (Al-Tayyib et al., 2017; Glick et al., 2018; Meacham et al., 2016). The co-use of heroin and methamphetamine increased from 9% to 44% between 2015 and 2019 (Strickland et al., 2021). It is hypothesised that the observed increase may be attributed to a number of factors namely enhanced accessibility

to methamphetamine, a reduction in the cost of methamphetamine products, and the limitation of access to heroin and other opioids through the legal regulation of these substances. Another hypothesis is that individuals use heroin and methamphetamine together because the half-life of heroin is shorter than that of methamphetamine (Ciccarone, 2011). Other reasons for co-use include the desire to reduce opioid withdrawal, to replace heroin with a cheaper substance, to benefit from the synergistic effects of the two substances, and to avoid death due to overdose (Ellis et al., 2018; Lopez et al., 2021). However, studies have demonstrated an increased risk of overdose when methamphetamines are co-used with opioids (Korthuis et al., 2022).

Moreover, it is proposed that individuals who consume heroin and methamphetamine concurrently exhibit a distinctive profile. Glick et al. (2021) observed that 82% of those who used heroin and methamphetamine together were homeless or lacked consistent accommodation. Furthermore, these individuals were predominantly found to reside in rural areas, administer substances via injection, and present with significant mental health concerns (Jones, 2020; Strickland et al., 2019).

Impulsivity is defined by a tendency to act without sufficient consideration, a proclivity for seeking immediate thrills, a sense of haste, impatience, and a pursuit of immediate gratification (Whiteside & Lynam, 2003). In the literature, it is emphasised that impulsivity has numerous facets and that the methodologies employed for its assessment are of significant consequence (Perry & Carroll, 2008). However, in studies utilising disparate measurement techniques, it was observed that the impulsivity of individuals with substance use disorders was higher than that of healthy individuals (Sher et al., 2003).

Genes encoding opioid receptors are commonly studied in the area of substance use disorders. The mu-opioid receptor (MOR), whose ligand is beta-endorphin and which is encoded by the *OPRM1* gene, is the main target of heroin and other opioid analgesics, in addition to its endogenous ligand. Consequently, polymorphisms in the *OPRM1* gene are frequently subjected to investigation in studies of addiction (Levran et al., 2008). In a study of a Han Chinese population, a haplotype block including the *OPRM1* rs510769 intronic single nucleotide polymorphism (SNP) was found to be associated with response to heroin use (Zhang et al., 2007; Zhang et al., 2006). Similarly, a study of European-Americans showed that certain genotypes of the *OPRM1* rs510769 and *OPRD1* rs2236861 SNPs were associated with heroin addiction (Levran et al., 2008). The mu-opioid receptor is also involved in the rewarding effects of opioids and psychostimulants including methamphetamine. One study revealed that individuals carrying the G allele of the rs510769 polymorphism reported greater feelings of arousal following amphetamine use compared to individuals with the AA genotype. This study reported that genetic variations in the *OPRM1* gene were associated with the response to amphetamine. The results obtained elucidated the discrepancies in substance sensitivity and treatment success among individuals (Dlugos et al. (2011).

As heroin-methamphetamine co-use is becoming increasingly prevalent, and substance use disorders are linked to impulsivity, this study aimed to investigate the effect of the *OPRM1* rs510769 polymorphism on impulsivity in heroin-methamphetamine co-users.

MATERIAL AND METHODS

A total of 105 individuals who were concurrently using heroin and methamphetamine were enrolled in the current study. All individuals who were co-users of heroin and methamphetamine were admitted to the Ankara Training and Research Hospital (AMATEM Clinic) in Ankara, Turkey. The subjects were diagnosed with opioid use disorder and methamphetamine use disorder in accordance with the International Classification of Diseases-10 (ICD-10) diagnostic criteria and fulfilled the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria for heroin and methamphetamine use. The inclusion criteria for patients with opioid use disorder (OUD) and methamphetamine use disorder were as follows: (i) co-use of heroin and methamphetamine, (ii) absence of clinically significant comorbid psychiatric illness, (iii) absence of medication use for psychiatric illness.

Each subject who met the eligibility criteria for the study was provided with written informed consent. Sampling was performed in accordance with the principles set forth in the Declaration of Helsinki and with the approval of the institutional ethics committee (approval number and year: I3-220-21 in 2021) for the use of human subjects. Additionally, a brief questionnaire was utilized to gather socio-demographic data, including age, marital status, education, employment status, and the age of onset for heroin and meth use disorders. The Barratt Impulsivity Scale was employed to assess impulsivity.

Venous blood (2 mL) was collected from each subject and placed in tubes containing (EDTA) for DNA isolation. Genomic DNA was extracted from whole blood samples using the Thermo Scientific GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania) according to the manufacturer's instructions. The *OPRM1* rs510769 polymorphism genotyping was conducted using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP), as previously described by Özkan-Kotiloğlu (2023). Polymerase chain reaction (PCR) amplification was conducted on a Techne Tc 512 PCR System in a 25 µL reaction mixture, which consisted of 50 ng of genomic DNA, 4 mM of each dNTP, 10 pmol of each forward and reverse primer, 1.25 U of Taq DNA polymerase (Sugenomics, Turkiye), and 1X Standard Buffer. The size of the PCR products was 563 bp for rs510769, as confirmed by agarose gel electrophoresis. Subsequently, the 563 bp PCR products were subjected to digestion with *Ssp*I enzyme at 37°C for one hour. Undigested and digested PCR products were separated by gel electrophoresis on a 3% agarose gel, visualised by ethidium bromide staining under a UV illuminator, and then scanned and photographed using the Syngene Monitoring System (Figure 1).

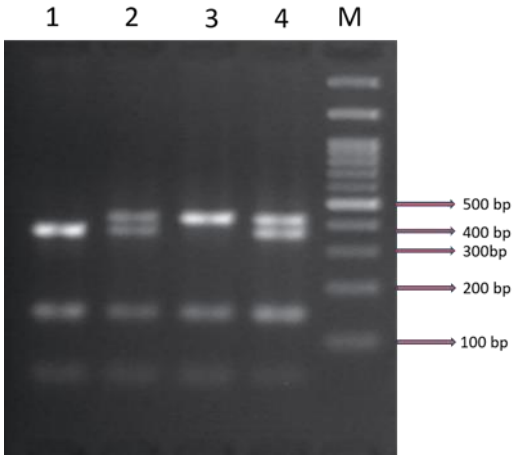

Figure 1. A representative agarose gel image of digested PCR products (563) bp) with *SspI* for the *OPRM1* rs510769 polymorphism. M: 100 bp ladder; Lane 1: homozygous polymorphic genotype (TT) (364 bp, 146 bp, and 53 bp); Lanes 2 and 4: heterozygous genotype (CT) (417 bp, 364 bp, 146 bp, and 53 bp); Lane 3: homozygous wild-type genotype (CC) (417 bp and 146 bp).

Statistical analyses were carried out using IBM SPSS Statistics for Windows, version 21.0. Categorical data were presented as numbers and percentages. In the case of numerical data, either the mean and standard deviation (SD) or the median and interquartile range (IQR) were provided, depending on whether the data were found to be normally distributed by the Kolmogorov-Smirnov test. Allele and genotype frequencies of *OPRM1* rs510769 were obtained through direct counting, and the Hardy-Weinberg equilibrium was assessed using the chi-square test. ANOVA analysis was used to evaluate The relationship between *OPRM1* rs510769 and impulsivity was evaluated by means of ANOVA analysis. A p-value of less than 0.05 was considered to indicate statistical significance ($p < 0.05$).

RESULTS

The socio-demographic characteristics of the subjects are presented in Table 1. Our population's mean age and weight were 30,28±5,03 (21-41) and 65,98±9,98 (45-90), respectively.

Table 1. Socio-demographic characteristics

Parameters	HM n	(%)	**Parameters**	HM n	(%)
Marital Status			**Occupation**		
Single	70	66,6	Working	50	47,6
Married	26	24,8	Not Working	55	52,4
Divorced	9	8,6	**Soldiering**		
Education			No	32	30,5
Primary	15	14,3	Yes	73	69,5
Secondary	48	45,7	**Soldiering Punishment**		
High School	37	35,3	No	87	82,8
University	5	4,8	Yes	18	17,1
Disciplinary Punishment			**Prison History**		
No	88	83,8	No	45	42,5
Yes	17	16,2	Yes	60	57,5
People who live together			**Probation**		
Alone	7	6,6	No	54	51,4
Partner	5	4,7	Yes	51	48,6
Wife/Husband	16	15,2			
Family	77	73,3			

Table 2. shows the genotype frequencies in heroin-meth users for the *OPRM1* rs510769 polymorphism. The frequencies of the CC, CT, and TT genotypes were 51.8%, 35.7%, and 6.3%, respectively. The genotype frequencies of the *OPRM1* rs510769 polymorphism were consistent with the Hardy-Weinberg equilibrium in patients ($\chi 2$=0.0009; p=0.98) (Table 2). The *OPRM1* rs510769 polymorphism was not found to be associated with heroin-meth users' impulsivity (p=0.095). Impulsivity levels according to genotypes are given in Table 3.

Table 2. The allele and genotype frequencies in heroin-meth users for the *OPRM1* rs510769 polymorphism

OPRM1 ***rs510769***	**HM (n=105)**		***p*-value**
	n	**%**	
CC	58	51,8	p>0.05
CT	40	35,7	
TT	7	6,3	
Variant allele freq.	26%		
HWE p-value	χ^2=0.0009; p=0.98		

Table 3. Impulsivity levels according to genotypes

Genotypes	Mean±SD (Min-Max)	ANOVA
CC	32,13±7,9 (15-47)	F=2,382 p=0,095
CT	33,87±10,11 (15-57)	
TT	26±8,64 (16-39)	

The association between *OPRM1* rs510769 genotypes and daily doses of heroin and methamphetamine and duration of use was also examined. There was no statistically significant association between *OPRM1* rs510769 genotypes and either the amount of heroin and methamphetamine used daily or the duration of use (Table 4.).

Table 4. Distribution of daily doses of heroin and methamphetamine and duration of use by genotype

Genotypes	Duration of Heroin Use Years (Min-Max)	Daily amount of heroin used mg (Min-Max)	Duration of Methamphetamine Use Years (Min-Max)	Daily amount of methamphetamine used mg (Min-Max)
CC	8,5 (5-12)	3 (1,37-5)	2 (1-7)	1 (0,5-1,12)
CT	10 (3-15)	3 (1,25-5)	1,5 (0,5-4,25)	1 (0,5-1,12)
TT	7 (7-13)	1,5 (1-5)	2 (2-3)	1,75 (0,5-3)
Kruskal-Wallis Test	x^2=0,624 p=0,732	x^2=0,418 p=0,811	x^2=1,465 p=0,481	x^2=2,206 p=0,332

DISCUSSION

The *OPRM1* gene is localised on chromosome 6 and is responsible for the coding of the mu-opioid receptor. The *OPRM1* rs510769 SNP is located in the first intron and involves a cytosine-to-thymine conversion (Al-Eitan et al., 2012). The evidence suggests that this SNP is associated with heroin and methamphetamine addiction (Dlugos et al., 2011; Levran et al., 2008). It is established that impulsivity is associated with substance use disorders and affects the treatment process (Kozak et al., 2019; Moeller & Dougherty, 2002). For this reason, this study examined, for the first time, the effect of the rs510769 SNP on impulsivity levels in Turkish heroin-methamphetamine users. No statistically significant relationship was observed between impulsivity and rs510769; however, individuals carrying the C allele showed higher levels of impulsivity. In order to establish a possible association, further studies with larger sample size are needed.

An analysis of the socio-demographic characteristics of individuals with substance use disorders indicated a tendency towards single status, unemployment, and low education levels (Al Abri et al., 2020; Erdoğan et al., 2022). In line with the literature, the majority of the participants in the current study were single, unemployed, and had a low level of education. The negative impact of substance use disorders on social functioning may explain this situation.

Semple et al. (2005) reported that individuals with high levels of impulsivity used higher doses of methamphetamine in the previous 30 days and posited a relationship between polysubstance use and impulsivity. The present study found no statistically significant difference in the daily amounts of heroin and methamphetamine used by individuals and the duration of use when these variables were compared according to genotype ($p > 0.05$). Dlugos et al. (2011) reported that individuals carrying the polymorphic genotype (AA) were less stimulated and felt euphoric by methamphetamine; therefore, these individuals may use a larger amount of amphetamine for stimulation than individuals carrying the G allele. In the present study, the per-day quantities of heroin and methamphetamine consumed by individuals with the CC genotype were 3 mg and 1 mg, respectively, while those with the TT genotype used 1.5 mg of heroin and 1.75 mg of methamphetamine. The findings of the current study indicated that individuals carrying the C allele exhibited higher impulsivity levels than those carrying the T allele. Although higher methamphetamine use among individuals with the TT genotype cannot be explained by impulsivity levels, it can be explained by the fact that individuals need to use more substances to feel more stimulated.

CONCLUSION

To our knowledge, this study is the first to examine the level of impulsivity at the *OPRM1* rs510769 polymorphism in heroin and methamphetamine users. The results showed no statistically significant association. However, the results need to be interpreted with caution given the limited sample size, and further larger studies are needed.

ACKNOWLEDGEMENTS

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) under the Grant Number 222S721. The authors thank to TUBITAK for their support.

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ORAL PRESENTATION

İmidacloprid Toksik Etkilerinin *Oreochromis niloticus* Beyin Dokusunda Antioksidant Enzimler Üzerinde Olan Etkileri

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Özet

Sucul ortam genellikle tarımsal kimyasalları, pestisitler ve kentsel faaliyetlerle sürekli olarak kirlenmektedir. Su kirliliği ise sucul canlılarda sağlığını da etkilemektedir. Tarımsal ve evsel ilaçlama programlarında çok kullanılan imidacloprid, neonikotinoid kimyasal sınıfında olan bir pestisid türüdür. Neonikotinoidler tarım alanlarında sulama yoluyla bitkiler üzerinden tarlalardan drenaj, akıntı yoluyla doğrudan salınmaları nedeniyle çevreyi yaygın olarak kirletmektedir. Çalışmada *Oreochromis niloticus*'un kontrol grubuyla birlikte imidacloprid maruziyet konsantrasyonları olan 1ppm, 2 ppm etkisinde 96 saat etki süresinde toksisite etkileri belirlenmiştir. Pestisid toksisite maruziyet süresi sonunda *Oreochromis niloticus*'un beyin dokusunda antioksidant sistem enzimatik koruma elemanları olan süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktivitelerinde olan değişimler araştırılmıştır. İmidacloprid gruplarının etkisinde *Oreochromis niloticus*'un beyin dokusunda 1ppm ve 2 ppm konsantrasyonlarda kontrol grubuna göre SOD enzim aktivitesinde sırasıyla %11, %24 artış, CAT enzim aktivitesinde kontrol grubuna göre sırasıyla %5, %8 artış olduğu belirlenmiştir. Araştırma sonuçları antioksidant enzimlerdeki değişimlerin aktivitesini artırarak pestisid toksisitesinin olumsuz etkilerine karşı koruyucu etkilerini arttırdığı düşünülmektedir.

Anahtar Kelimeler: İmidacloprid, Beyin, Süperoksit dismutaz, Katalaz

Effects of Imidacloprid Toxic Effects on Antioxidant Enzymes in *Oreochromis niloticus* Brain Tissue

Abstract

The aquatic environment is often constantly polluted by agricultural chemicals, pesticides and urban activities. Water pollution also affects the health of aquatic organisms. Imidacloprid, which is widely used in agricultural and domestic spraying programs, is a type of pesticide in the neonicotinoid chemical class. Neonicotinoids pollute the environment widely due to their direct release from plants in agricultural areas through drainage and runoff from fields. In the study, toxicity effects were determined for 96 hours of exposure time with the control group of *Oreochromis niloticus* under the effect of imidacloprid exposure concentrations of 1ppm and 2ppm. At the end of the pesticide toxicity exposure period, changes in the antioxidant system enzymatic protection elements superoxide dismutase (SOD) and catalase (CAT) enzyme activities in the brain tissue of *Oreochromis niloticus* were investigated. Under the effect of imidacloprid groups, it was determined that there was an increase of 11% and 24% in SOD enzyme activity and an increase of 5% and 8% in CAT enzyme activity in brain tissue of Oreochromis niloticus at 1ppm and 2ppm concentrations, respectively, compared to the control group. The research results are thought to increase the protective effects against the negative effects of pesticide toxicity by increasing the activity of changes in antioxidant enzymes.

Keywords: Imidacloprid, Brain, Superoxide dismutase, Catalase

GİRİŞ

İmidakloprid (IMI) bir neonikotinoid grubu pestisit türüdür ve özellikle mahsulleri etkileyen böcek ve zararlıların kontrolü için yaygın olarak kullanılmaktadır (Abd El-hameed ve ark., 2021). İmidakloprid yüzeylerde veya yeraltı sularında tarım sulamaları ve yağmurla birlikte karışmaktadır, uygulama veya püskürtme yöntemiyle tarım alanına dağılan etken madde su kütleleriyle birlikte akabilir; bu da su ortamında yüksek konsantrasyonlarda olmasına yol açabilir (Tišler ve ark., 2009).

Suda organizmaların organizmaların pestisit kirliliğine maruz kalması, hücrelerin ekzojen ve endojen kaynaklı reaktif oksijen türleri (ROS) üretimi arasında bir dengesizliğe yol açabilmektedir. Antioksidan savunma sistemleri ROS üretiminin denge de kalması ve canlıda hücre organizmalarında oksidatif tam hasarı önleyebilmektedir. Fakat sucul ortamın pestitler gibi toksik özellik gösteren kimyasallar ile kirlenmesi sonucunda antioksidant sistem yetersiz kalarak hücrelerin oksidatif hasara uğramasına neden olabilmektedir (Günal ve ark., 2020; Naiel ve ark., 2020).

SOD ve CAT gibi enzimler, ksenobiyotiklerin biyotransformasyonu sırasında üretilen ROS'un ortadan kaldırılmasında önemli bir rol oynar ve SOD/CAT sisteminin indüksiyonu, ROS'a karşı ilk savunma mekanizması olabilir (Lushchak, 2016). Çalışmada kontrol grubuyla birlikte imidacloprid maruziyet konsantrasyonları olan 1 ppm, 2 ppm etkisinde 96 saat etki süresinde toksisite etkileri *Oreochromis niloticus*'un beyin dokusunda antioksidant sistem enzimatik koruma elemanları olan süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktivitelerinde olan değişimler araştırılmıştır.

MATERYAL VE METOD

İmidaclporid'in ticari olarak Hekdivor 600 FS isimli ürünü alınmıştır. *Oreochromis niloticus* deney canlısında kontrol grubu ile beraber 1 ppm ve 2 ppm sublethal konsantrasyon dozlarını deney süreci 4 gün boyunca uygulanmıştır. Deneylerde kullanılan *O. niloticus* türünün ortalama boyu 11,2±0,68 cm, ağırlığı ise 21,34±0,65 gr olarak ölçülmüştür. Boyutları 40x40x40 cm olan cam akvaryumlarda dinlendirilmiş musluk suyu ile 40 L doldurulmuş ve akvaryumlar havalandırmaları merkezi sistem ile planlı bir şekilde havalandırılmaktadır.

Laboratuvar ortamında 12/12 saat fotoperiyodik period sistemi ile düzenli gece/gündüz uygulaması yapılmaktadır.

Deneyde kullanılan akvaryum su kalitesi parametreleri:
sıcaklık 21 ± 0.9 ºC,
pH 7,41 ± 0,56,
çözünmüş oksijen 6,48±0,79 mg/L,
alkalinite 234 mg/L $CaCO_3$,
sertlik 268 mg/L $CaCO_3$ olarak hesaplanmıştır.

Toksik deneylerde kimyasal miktarında azalmayı önlemek amacıyla 24 saat aralıklarla su değişimi ve toksik madde eklemesi yapılmıştır. 4 günün sonunda dekapitasyon yöntemiyle öldürülen balıklardan Beyin dokuları disekte edilerek çıkarılmıştır.Araştırma yapılmak üzere beyin dokusu fizyolojik tuz çözeltisi (%0.59 NaCl) ile yıkandıktan sonra biyokimyasal analizler için -20°C sıcaklıkta saklanmıştır. Beyin dokusu %1,17 KCl içeren pH 7.4, 0,1M sodyum-fosfat tamponunda, 1/20 oranında seyreltilerek 2 dk homojenize edilmiştir.

Homojenatların 16000 rpm'de +4 ºC'de 20 dakika sürede santrifüj edildikten sonra elde edilen süpernatantlar Sod enzim aktivitesi McCord & Fridovich (1969) yöntemi ve CAT enzim aktivitelerinde Beutler (1975) yöntemiyle olan aktivasyonda olan değişimler belirlenmiştir.

Ayrıca Bradford (1976) yöntemiyle protein miktarı belirlenerek istatiksel olarak hesaplamalar yapılarak deneysel süreçte olan enzimatik değişimler karşılaştırılmıştır. İstatiksel analizler SPSS 16 programında One-way Anova Duncan testi kullanılmıştır.

BULGULAR ve TARTIŞMA

Çalışmamızda, İmidacloprid gruplarının etkisinde *Oreochromis niloticus*'un beyin dokusunda 1ppm ve 2 ppm konsantrasyonlarda kontrol grubuna göre SOD enzim aktivitesinde sırasıyla %11, %24 artış, CAT enzim aktivitesinde kontrol grubuna göre sırasıyla %5, %8 artış olduğu belirlenmiştir. Yapılan çalışmalara göre SOD ve CAT enzim aktivitelerinde artış genel olarak hayatsal organların verdiği tepkiye göre değiştini göstermektedir. Etkileyen faktörlerden bir diğeri ise uygulama zamanının uzunluğu ve konsantrasyon oranı toksik etkiye cevabı değiştirmektedir.

Prochilodus lineatus'ta, imidacloprid'in 125 ve 1.250 ppm düzeyinde maruziyeti sonucunda SOD aktivitesinde artış ve CAT enzim aktivitesinde azalma belirlendi. Oluşan toksisite sonucunda süperoksit anyonlarının oluşumunun rolüyle enzim aktivitelerinde önemli artış gösterdiği raporlandı (Vieira ve ark., 2018). 7 ve 14 gün boyunca neonikotinoid tiametoksam etkisinde zebra (Danio rerio) balıklarında artan SOD enzim aktivitesi gözlemlendi (Yan ve ark., 2015).

Süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon-s-transferaz (GST) gibi antioksidan ve detoks edici enzimler, sinerjik bir şekilde oksidatif strese ve dokuya özgü hasara karşı koruma sağlamıştır. SOD, oksiradikalleri gidermek için önemli bir enzimdir ve süperoksit radikalini (O_2^-) hidrojen peroksit (H_2O_2) radikali ve oksijene dönüştürebilir Daha sonra, H_2O_2, Fe-protoporfirin içeren bir enzim türü olan CAT tarafından H_2O ve O_2'ye dönüştürülür (Su ve ark., 2014).

SOD ve CAT aktivitelerinin değişimleri oksidatif stresi tahmin etmek için önemli biyobelirteçlerdir. Bu iki enzim, aşırı ROS'u ortadan kaldıran ve organizmalar üzerindeki potansiyel toksik etkileri azaltabilen normal hücresel metabolizma olarak önemli bir rol oynar. Kim ve Hyun (2006) çalışmasına göre, antioksidan enzimler kirleticilerin artmasıyla birlikte aşırı ROS'u zamanında ortadan kaldıramadı, ROS'un biyolojik dengesi bozuldu ve antioksidan enzimlerin aktivitesini etkileyen ROS birikimine neden oldu. Bu arada antioksidan enzimler aşırı ROS'un sentezinden etkilendi ve hatta aktivitelerini engelleyerek oksidatif hasara neden oldu.

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ORAL PRESENTATION

Antibacterial Potential of *Stephanotis floribunda* Extract

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Abstract

Plants have been used for medicinal purposes from the past to the present. *S. floribunda* has many biological activities with the rich natural compounds. Nowadays, due to the negative side effects of synthetic preservatives, people have increased their tendency towards natural products and plants have begun to replace synthetic substances. In our study, the antibacterial activity of the water extract obtained from *S. floribunda* leaves on food-borne and clinical pathogenic bacteria was investigated to determine its potential use as a natural resource. The disc diffusion assay was first used to determine the antimicrobial activity of the extract. According to the results obtained from the activity of the extract at 0.5 mg/disc, 1 mg/disc and 2 mg/disc concentrations, the highest inhibition zone diameter was determined as 18.51 mm on *B. subtilis* RSKK 244 at 2 mg/disc. Then, the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentration values of the extract were determined using the micro-dilution method. *B. subtilis* RSKK 244 has the lowest MIC and MBC value of the extract with 6.25 mg/ml. Additionally, the extract has bactericidal activity on all tested bacteria. *S. floribunda* extract, which has high antimicrobial activity, may be used as a natural agent in the fight against pathogenic bacteria in the food, pharmaceutical and cosmetic industries.

Keywords: Antimicrobial activity, bactericidal activity, extract, natural agent, *Stephanotis floribunda*

INTRODUCTION

Since ancient times, humans have used plants to treat diseases caused by pathogenic microorganisms, and some of the medicinal drugs are still included as part of the treatment of various diseases (Barnes and Heinrich et al., 2004). Plants are the most abundant natural primary source of active drugs involved in the treatment process of preventing various diseases (Olasehinde et al., 2012). Additionally, most of the plants with wide application areas have promising activity against multiple disease causing pathogens (Ogbole et al., 2018). In recent years, natural compounds and plant extracts from plants have been investigated for their activity and potential treatment of diseases caused by pathogens (Kebede et al., 2021; Vaou et al., 2021). Although studies have been carried out on the antimicrobial activity of plants for years, research on the activities of different plants on various pathogens still continues.

S. floribunda (Madagascar Jasmine, Apocynaceae family), which is widely seen in tropical regions, is largely grown in greenhouses as cut flowers and ornamental plants (Dey et al., 2017; Van der Zee et al., 1999). Plants belonging to the Apocynaceae family are widely used for medicinal purposes (Islam and Lucky, 2019). *S. floribunda*, native to Madagascar, is now grown as a houseplant, although it was used for medicinal purposes in the past (Pott et al., 2002). The plant contains rich bioactive components such as flavonoids, alkaloids, terpenoids and steroids (Bhadane et al., 2018).

Many pathogenic microorganisms in the environment throughout human life reach the human gastrointestinal (GI) tract for various reasons. These pathogens colonize the GI tract, threatening health and increasing the risk of antibiotic resistance (Boyce et al., 2007). *E. coli* is among the most dangerous food-borne pathogens with its low infection dose. It is a dangerous pathogen that causes bloody diarrhea and hemolytic uremic syndrome (Bruyand et al., 2018). *Bacillus* species are difficult to remove from medical and pharmaceutical materials because they are resistant to heat, radiation and antibacterial agents. They are also known in food industries as spoilage organisms (Turnbull et al., 1996). *S. sonnei* is a dangerous intracellular pathogen that causes fever, bloody diarrhea, or acute infections. Their infections are especially fatal for children under 5 years of age

(Torraca et al., 2020). *Salmonella* is one of the major pathogens causing food-borne infections in humans (Herikstad et al., 2002). Salmonellosis causes significant economic losses in the food industry and threatens human health (Putturu et al., 2015). It is among the pathogens that can especially infect poultry and is important for poultry health and food safety (Carli et al., 2001).
In the study, the antimicrobial activity of the water extract obtained from *S. floribunda* leaves on some food and clinical pathogen test microorganisms was investigated to determine its potential for use in various industries.

MATERIALS AND METHODS

Preparation of Leaves Extract from *S. floribunda*

S. floribunda leaf samples were collected from the Alata Horticulture Research Institute (Mersin-Türkiye) (Figure 1). After the leaves were washed with distilled water, they were dried in an airy and sun-free environment. Then, the samples were ground using a waring blender. For extraction, 30 ml of water was added to 10 g of leaf sample and extracted using a water bath at 80°C for 12 hours (2 days). After extraction, the solvent was evaporated. Water extract from *S. floribunda* leaf was dissolved in dimethyl-sulfoxide (DMSO) and sterilized with a syringe filter (0.45 µm). The extract was stored in the refrigerator (+4˚C) until use.



Figure 1. *S. floribunda* leaf samples.

Test Microorganisms

The antibacterial activity of *S. floribunda* extract for disc diffusion assay and micro-dilution method was tested against four different food-borne and clinically derived pathogenic microorganisms. *Escherichia coli* ATCC 35218, *Bacillus subtilis* RSKK 244, *Salmonella enteritidis* RSKK 171 and *Shigella sonnei* Mu:57 were cultured in Nutrient-Broth (NB) medium at 37°C.

Disc Diffusion Assay

The antibacterial activity of *S. floribunda* extract on pathogens test microorganisms was determined using the disc diffusion test. The pathogens prepared at 0.5 McFarland concentration were spread by dropping 100 µl onto the agar medium. 5 µl (0.5 mg/disc), 10 µl (1 mg/disc) and 20 µl (2 mg/disc) samples taken from the extract were dropped onto sterile filter discs (diameter: 6 mm) placed in triplicate on the agar medium. Petri dishes were incubated at 37°C under appropriate conditions. At the end of the incubation, the inhibition zones formed around the discs were measured with a digital caliper.

2.4. Micro-dilution Method

The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentration values of *S. floribunda* extract against the test pathogens were determined using the micro-dilution method. The extract was diluted by half in tubes containing NB to obtain final concentrations (50–3.12 mg/ml). Then, the microbial suspension at a density of 0.5 McFarland was added to each tube separately. Tubes were incubated at 37°C under appropriate conditions. At the end of the incubation, the lowest concentration that prevented bacterial growth was determined as the MIC value of the extract. Then, 5 µl samples taken from the tubes were spot-dropped on the agar medium and left for incubation. The MBC value was determined as the lowest extract concentration at

which bacterial growth occurred in the solid medium at the end of incubation. If the MBC/MIC ratio was ≤4, it was determined as bactericidal, and if the MBC/MIC ratio was >4, it was determined as a bacteriostatic effect (Al-Shammari et al., 2022; Baj et al., 2020).

RESULTS AND DISCUSSION

In recent years, pathogenic microorganisms have been developing resistance to commercial antimicrobial agents and negatively affecting human health (Lucien et al., 2021). Plants have been used as therapeutic agents since ancient times and are the leading source for the production of contemporary medicines (Alexander et al., 2023).

In this study, the water extract was obtained from *S. floribunda* leaves to determine its biological activity. The results of disc diffusion test on pathogenic test bacteria at three different concentrations of the extract are presented in Table 1. The extract had an inhibition zone diameter of 9.77 mm, 12.59 mm and 18.51 mm on *B. subtilis* RSKK 244, which is the most sensitive strain at all concentrations (Figure 2). While the extract had no activity on *S. enteritidis* RSKK 171 at the concentration of 0.5 mg/ml, an inhibition zone diameter of 7.10 mm at 1 mg/ml and 10.07 mm at 2 mg/ml was obtained. It was determined that 2 mg/ml extract concentration had high antimicrobial activity on all tested pathogens.

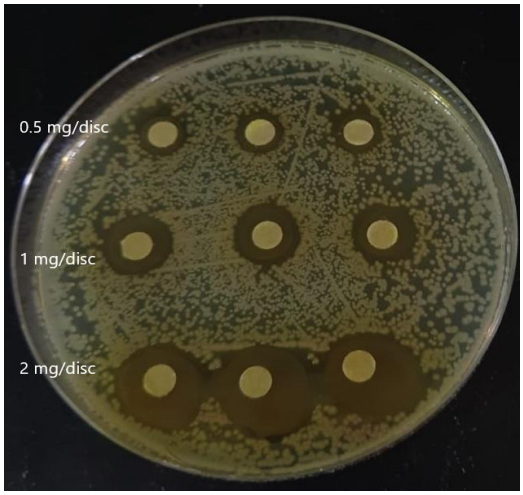

Figure 2. Disc Diffusion Assay Result of *S. floribunda* Water Extract on *B. subtilis* RSKK 244.

Table 1. Disc Diffusion Assay Results of *S. floribunda* Water Extract

Test Microorganisms	Inhibition Zone Diameter (mm±SD)		
	0.5 mg/disc	1 mg/disc	2 mg/disc
E. coli ATCC 35218	6.11±0.10	7.98±0.67	8.82±0.28
B. subtilis RSKK 244	9.77±0.78	12.59±0.19	18.51±0.61
S. enteritidis RSKK 171	NA	7.10±0.47	10.07±0.84
S. sonnei Mu:57	6.66±0.62	8.36±0.45	10.64±0.69

*NA: No Activity.

In a study, the antimicrobial activity of essential oil obtained using *Calycopteris floribunda* leaves was evaluated on *E. coli* and *B. subtilis* using the disc diffusion assay. The oil obtained from the leaf has a zone diameter of 8-10 mm on *E. coli* at a concentration of 1-2-4 µl and an inhibition zone diameter of 10-15 mm at a concentration of 6 µl. The oil has an inhibition zone diameter of 10-15 mm on *B. subtilis* at 4 µl and 6 µl concentrations (Liu et al., 2009).

MIC and MBC values of water extract from *S. floribunda* leaves were determined using the micro-dilution method and the results are presented in Table 2. The extract MIC values on pathogenic bacteria are between 6.25 mg/ml and 25 mg/ml, and its MBC values are between 6.25 mg/ml and 12 mg/ml. The most sensitive strain among pathogens to the extract was *B. subtilis* RSKK 244, with an MIC and MBC value of 6.25 mg/ml. Since the MBC/MIC ratio is ≤4, the extract has a bactericidal effect on the test microorganisms.

Table 2. MIC and MBC Values of *S. floribunda* Water Extract

Test Microorganisms	*S. floribunda* Extract		
	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
E. coli ATCC 35218	12.5	25	2
B. subtilis RSKK 244	6.25	6.25	1
S. enteritidis RSKK 171	25	25	1
S. sonnei Mu:57	12.5	12.5	1

*MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration.

CONCLUSION

Today, plants that have the potential to prevent the negative effects of synthetic additives can be important natural additives for various industries. In this study, the antibacterial activities of water extract from *S. floribunda* leaves on test microorganisms were determined and their potential for use in the food, pharmaceutical and cosmetic sectors was determined. With its high antibacterial and bactericidal activity, the extract has the potential to be a natural alternative to many currently used additives.

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ORAL PRESENTATION

Investigation of the Biological Activity of Fingered Citron Leaf Ethanol Extract on *Staphylococcus epidermidis* and *Listeria monocytogenes*

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Abstract

Recently, the infections have become an important problem that threatens public health. However, bacteria cause both diseases and product losses. The medicinal and therapeutic potential of plants constitutes an important resource in the development of new products. In this study, it was aimed to determine the antimicrobial activity of ethanol extract obtained from Fingered Citron leaf against *Staphylococcus epidermidis* ATCC 12228 and *Listeria monocytogenes* ATCC 7644 strains. The disc diffusion and micro-dilution methods were used to determine the antimicrobial activity of the extract on the strains. In the disc diffusion test results, the inhibition zone diameter of *C. medica* L. var. *sarcodactylis* leaf ethanol extract was 9.35 mm against *S. epidermidis* ATCC 12228 and 6.67 mm against *L. monocytogenes* ATCC 7644. The minimal inhibition concentration (MIC) of the leaf ethanol extract was determined as 5 µl/µg against *S. epidermidis* ATCC 12228 and 10 µl/µg against *L. monocytogenes* ATCC 7644. Minimal bactericidal concentration (MBC) values were determined as 20 µl/µg and 40 µl/µg. The results showed that *C. medica* L. var. *sarcodactylis* leaf ethanol extract has good antimicrobial activity and has the potential to be used as a natural alternative to synthetic additives in the pharmaceutical and food industries.

Keywords: Antibacterial, Extract, Fingered Citron, Natural additive

INTRODUCTION

Plants are valuable medicinally and have a great importance in the development of new products. Secondary metabolites found in plants provide unique resources for use in various fields. Additionally, different biological activities of various plant extracts such as antimicrobial, antidiabetic and antioxidant have been determined (Jain et al., 2019). In this context, Fingered Citron (*Citrus medica* L. var. *sarcodactylis)* is one of the oldest species of citrus fruits. Fingered Citron (FC), an important plant, belongs to the Rutaceae family (Wang et al., 2022; Huang et al., 2014). FC, which belongs to North-eastern India, began to establish its presence in China over time (Karp and Hu., 2018). This plant contains secondary methanolides such as coumarins, essential oils, flavonoids, terpenoids and polysaccharides (Xu et al., 2019; Xu et al., 2020). It is known for its high antioxidant activity (Li et al., 2019; Mahdi et al., 2019). It has been used for medical purposes, especially by combating lung problems, intestinal disorders and other ailments (Meena et al., 2011).

Recently, hospital-acquired infections pose a great danger to public health (Fürnkranz and Walochnik, 2021). The number of people affected by hospital-acquired infections worldwide is considered to be more than 1.4 million (Pittet and Donaldson, 2005; Tikhomirov, 1987). *Staphylococcus epidermidis* is a gram-positive bacterium found in the skin flora of humans and mucosal surfaces as well as in animals (Kleinschmidt et al., 2015; Namvar, 2014). *S. epidermidis* has a beneficial effect in helping people balance the microflora on the skin. However, it is an opportunistic pathogen and can cause nosocomial infections when it enters the human body through medical devices (Liu et al., 2020; Namvar, 2014; Otto, 2014).

Foods are products that can deteriorate quickly through contamination due to their unique characteristics and variable environmental conditions. This endangers the quality and safety of *food* (Ortega-Ramirez et al., 2014; Rawat, 2015). It shows that pathogens that cause product spoilage play a role in both the emergence of diseases and product losses (Bhuni, 2018; Tournas, 2005). One of the important pathogens that cause food contamination is *Listeria monocytogenes*. *L. monocytogenes* is the causative agent of a disease called Listeriosis. This type of pathogen can cause meningitis, sepsis and other clinical symptoms in newborns and people with weakened immune systems (Schlech III, 2019).

In our study, the antimicrobial activity of ethanol extract obtained from FC leaves on *S. epidermidis,* and *L. monocytogenes* strains was determined.

MATERIALS AND METHODS

Preparation of the Extract

FC leaf was obtained from Muğla (Türkiye) Köyceğiz Plant World in October 2022. The leaves were washed and then dried. Powdered leaf samples were ground with a Waring blender and then extracted with ethanol solvent using a hot water bath. The leaf samples were extracted with ethanol solvent at 70°C for 24 h. The solvents of the extracted samples were evaporated. The ethanol leaf extract was dissolved in dimethylsulfoxide (DMSO) and then sterilized with a 0.45 µm filter.

Preparation of Test Microorganisms

S. epidermidis ATCC 12228 was cultured in Nutrient Broth (NB) medium.and *L. monocytogenes* ATCC 7644 was cultured in Tryptic Soy Broth (TSB) medium for 24 hours at 37 ºC. Test microorganisms were washed twice with saline solution (SF). The bacterial suspensions were adjusted to 0.5 McFarland.

Determination of Biological Activity of FC Leaf Ethanol Extract

The biological activity of ethanol extract of FC was determined using the disc diffusion method. The prepared bacterial suspensions (0.5 McFarland) were spread on 100 µl agar medium. Then, sterilized discs with a diameter of 6 mm were placed on the agar medium in triplicate. 20 µl (2 mg/disc) of the extract was dropped onto the discs. The petri dishes were incubated at 37°C. After 24 hours of incubation, the inhibition zones were measured with a caliper.

Determination of Minimal Inhibition (MIC) and Bactericidal Concentrations (MBC) of FC Leaf Ethanol Extracts

The MIC and MBC values of the FC leaf ethanol extract were determined by micro-dilution method against test microorganisms. The prepared bacterial suspensions (0.5 McFarland) were added to the tubes containing extract and medium and vortexed. The tubes containing the mixture were incubated at 37°C for 24 hours. The concentration at which no growth occurred in the broth medium after incubation was determined as the MIC value. Then, the MBC value was determined by dropping the samples from tubes onto the appropriate solid medium. The petri dishes were incubated at 37°C (24 hours). At the end of the incubation period, the concentrations of the extracts that prevented bacterial growth on solid media were evaluated as MBC values of the extract.

RESULTS

The biological activity of FC leaf ethanol extract on *S. epidermidis* ATCC 12228 and *L. monocytogenes* ATCC 7644 was determined. The inhibition zones and MIC and MBC values of the *C. medica* L. var. *sarcodactylis* leaf ethanol extract on the test microorganisms are given in Table 1. It was determined that FC ethanol extract had an inhibition zone diameter of 9.35 mm on *S. epidermidis* ATCC 12228 and 6.67 mm on *L. monocytogenes* ATCC 7644. The MIC and MBC values of FC leaf ethanol extract on test microorganisms ranged from 5 µl/µg to 40 µl/µg. The MIC values of *S. epidermidis* ATCC 12228 and *L. monocytogenes* ATCC 7644 were determined as 5 µl/µg and 10 µl/µg. MBC values were recorded as 20 µl/µg and 40 µl/µg (Tablo 1). The results determined that FC ethanol extract provided a good preservative effect on the tested microorganisms.

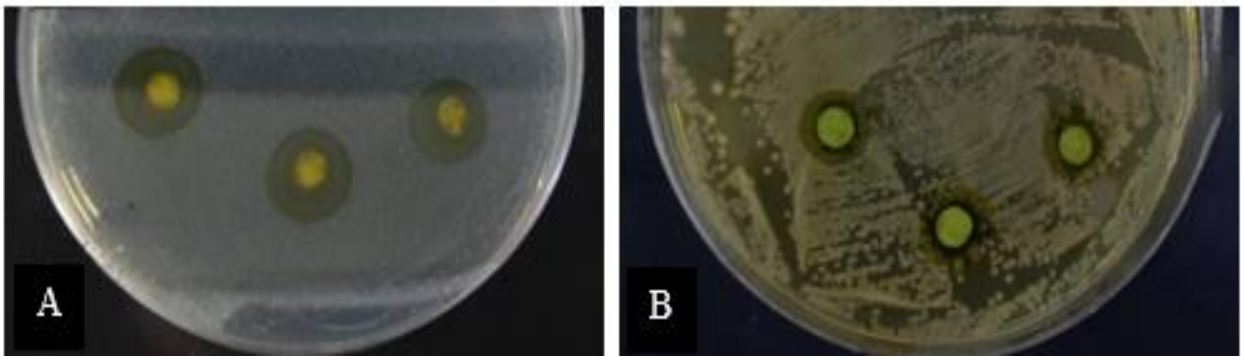

Figure 1. Petri Dish Image Obtained by Disk Diffusion Test of FC Leaf Ethanol Extract Against *S. epidermidis* ATCC 12228 (A) and *L. monocytogenes* ATCC 7644 (B).

Table 1. The Antimicrobial Activity of FC Leaf Ethanol Extract

Test microorganisms	Leaf Ethanol Extract		
	Inhibition Zone Diameter (SD±mm)	MIC (µl/µg)	MBC (µl/µg)
***S. epidermidis* ATCC 12228**	9.35±0.41	5	20
***L. monocytogenes* ATCC 7644**	6.67±0.29	10	40

*MIC: Minimal Inhibition Concentrations, MBC: Minimal Bactericidal Concentrations

In their study, Budiarti et al. (2021) determined the biological activity of ethanol extracts obtained from the *C. hystrix* using the disc diffusion method. The combination of *C. hystrix* leaf and bark extract tested was 12.5%, 25%, 50% and 75% (w/v). It was determined that the inhibition zone diameters of *C. hystrix* leaf and bark extract combinations on *S. epidermidis* ATCC 12228 varied between 14.6 mm and 28.3 mm.

In their study, Akarca and Baytal (2021) determined the biological activity of ethanol extracts obtained from four different citrus fresh leaves (*C. reticulata*, *C. lemon*, *C. paradisi* and *C. cinensis*). The inhibition zone diameters of *C. reticulata*, *C. lemon*, *C. paradisi* and *C. cinensis* leaf ethanol extract against *L. monocytogenes* ATCC 51774 were recorded as 15.10, 15.33, 16.65 and 11.70 mm, respectively. The MIC and MBC values of the extracts (*C. reticulata*, *C. lemon*, *C. paradisi* and *C. cinensis*) against *L. monocytogenes* ATCC 51774 were determined as 31.25, 117.19, 140.63, 35.16 and 117.19 µl/µg. MBC values were evaluated as 93.75, 93.72, 23.44 and 31.25 µl/µg. In our study, FC leaf ethanol extract generally had lower MIC and MBC values on *L. monocytogenes* than these tested citrus species.

CONCLUSION

In the study, the antimicrobial activity of *C. medica* L. var. *sarcodactylis* leaf ethanol extract against the test microorganisms *S. epidermidis* ATCC 12228 and *L. monocytogenes* ATCC 7644 was determined. The results showed that the leaf ethanol extract may have the potential to be a natural protective agent against infections caused by *S. epidermidis* ATCC 12228 and *L. monocytogenes* ATCC 7644. It has also been determined that it can lead to the development of new plant-based products by providing alternative options to synthetic agents in the pharmaceutical and food industries.

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ORAL PRESENTATION

Oreochromis niloticus Karaciğer Dokusunda Thiamethoxam'ın Maruziyetinin Akut Etkileri

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Özet

Neonikotinoidlerin hidrofilik olmaları sonucunda sucul sistemlerdeki kalıcılığını artırırken, hedef olmayan organizmalar için maruz kalma süresini de artırır. Neonikotinoid thiamethoxam'ın subletal toksisitesi *Oreochromis niloticus*'un kontrol grubuyla birlikte 1 ppm, 5 ppm etkisinde 48 saat boyunca toksisite etkileri belirlenmiştir. *Oreochromis niloticus*'un karaciğer dokusunda oksidatif hasara karşı koruyan antioksidant enzimleri süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktivitelerinde olan değişimler araştırılmıştır. 48 saat etkide *Oreochromis niloticus*'un karaciğer dokusunda 1 ppm ve 5 ppm thiamethoxam etkisinde kalan gruplarda kontrol grubuna göre SOD ve CAT enzim aktivitelerinde sırasıyla %27, %49 ve %13, %32 artış olduğu belirlenmiştir. Thiamethoxam toksisitesi sonucunda *Oreochromis niloticus*'un karaciğer dokusunda olan antioksidant enzimlerdeki değişimler toksik etkinin kısa sürede gösterebildiği ve önemli değişimlere neden olduğu belirlenmiştir.

Anahtar Kelimeler: Thiamethoxam, Karaciğer, *Oreochromis niloticus*

Acute Effects of Thiamethoxam Exposure on Oreochromis niloticus Liver Tissue

Abstract

Neonicotinoids are hydrophilic and therefore increase their persistence in aquatic systems, while also increasing the exposure time for non-target organisms. Sublethal toxicity of the neonicotinoid thiamethoxam Toxicity effects of 1 ppm and 5 ppm on *Oreochromis niloticus* with the control group for 48 hours were determined. Changes in antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) enzyme activities that protect against oxidative damage in the liver tissue of *Oreochromis niloticus* were investigated. It was determined that SOD and CAT enzyme activities increased by 27%, 49% and 13%, 32%, respectively, in the groups exposed to 1 ppm and 5 ppm thiamethoxam in the liver tissue of Oreochromis niloticus for 48 hours compared to the control group. As a result of thiamethoxam toxicity, changes in antioxidant enzymes in the liver tissue of Oreochromis niloticus were determined that the toxic effect could show itself in a short time and caused significant changes.
Key Words: Thiamethoxam, Liver, Oreochromis niloticus

GİRİŞ

Thiamethoxam (TMX) neonicotinoid grubuna dahil olan bir pestisid türüdür. Böcekleri kontrol altında tutmak amacıyla tarımsal ürün elde edilmesi aşamasında yaygın olarak kullanılan bir böcek ilacıdır (El Euony ve ark., 2020). TMX, çeşitli yağışlardan ve sulama kanallarından balıkların yaşadığı su kaynaklarına kolayca ulaşabilir ve olası balık toksisitesine neden olabilir. TMX'in maruz kalan balıklardaki toksikolojik etkileri üzerine birkaç çalışma bildirilmiştir (Baldisserav e ark., 2018).

Oksidatif hasarın belirlenmesi için kullanılan parametreler kirleticilerin eliminasyonu ve belirlenmesi önemli bir göstergedir. Oksidatif stres, reaktif oksijen türlerinin (ROS) aşırı oluşumunun hücrede detoksifiye etme sürecinin bozulduğu anlamına gelir (Li ve ark. 2010). ROS, lipitler ve proteinler gibi hücrede yapısal bileşenlerle reaksiyona girerek ve her ikisi yapıda da oksidatif hasar olarak ölçülen lipit peroksidasyonuna ve karbonil protein yapılarını son ürün olarak oluşumlarının ölücümü ile antioksidan sistem enzimleri olan süperoksit dismutaz (SOD) ve katalaz (CAT) gibi enzimler aracılığıyla hücrelerde aşırı ROS hasarına karşı korur. Antioksidan enzim aktivitelerindeki değişiklikler yüksek ROS seviyelerine işaret edebilmektedir (Min

ve ark., 2008). *Oreochromis niloticus*'un karaciğer dokusunda oksidatif hasara karşı koruyan antioksidant enzimleri süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktivitelerinde olan değişimler araştırılmıştır.

MATERYAL VE METOD

Thiamethoxam ticari olarak satılan sygenta marka Cruiser 350 FS ürünü alınmıştır. *Oreochromis niloticus* deney canlısında kontrol grubu ile beraber 1 ppm ve 5 ppm konsantrasyonlarında deney süreci 44 saat boyunca devam edilmiştir. Deneylerde kullanılan *O. niloticus* türünün ortalama boyu 10,87±0,69 cm, ağırlığı ise 19,56±1,16 gr olarak ölçülmüştür. 40x40x40 cm boyutta cam akvaryumlar dinlendirilmiş musluk suyu ile 40 L doldurulmuş ve akvaryumlarda merkezi sistem ile havalandırma yapılmıştır.

Laboratuvar ortamında 12/12 saat fotoperiyodik periyodu uygulanmıştır. Deneyde kullanılan akvaryum su kalitesi parametreleri ölçülerek belirlenmiştir.

Toksik deneylerde kimyasal miktarında azalmayı önlemek amacıyla 24 saat aralıklarla su değişimi ve toksik madde eklemesi yapılmıştır. 2 günün sonunda dekapitasyon yöntemiyle öldürülen balıklardan Karaciğer dokuları disekte edilerek çıkarılmıştır.

Karaciğer dokusu fizyolojik tuz çözeltisi (%0.59 NaCl) ile yıkandıktan sonra biyokimyasal analizler yapılana kadar -80 ºC'ye kaldırılmıştır. Karaciğer dokusu %1,17 KCl içeren pH 7.4, 0,1M sodyum-fosfat tamponunda, 1/10 oranında seyreltilerek 4 dk homojenize edilmiştir.

Homojenatların +4 ºC'de 16000 rpm'de 20 dakika santrifüjden sonra elde edilen süpernatantlar süperoksit dismutaz (McCord & Fridovich, 1969) ve katalaz Beutler (1975) enzim aktivitelerinde olan değişimler belirlenmiştir.

Ayrıca Bradford (1976) yöntemiyle protein miktarı belirlenerek istatiksel olarak hesaplamalar yapılarak deneysel süreçte olan enzimatik değişimler karşılaştırılmıştır. İstatiksel analizler SPSS 16 programında One-way Anova Duncan testi kullanılmıştır.

BULGULAR ve TARTIŞMA

Pestisitler, su kütlelerinde yaşayan balıklar da dahil olmak üzere ekonomik ve ekolojik açıdan önemli hedef dışı su türleri için başlıca sorun olarak bilinmektedir. Pestisitlerin ve çeşitli çevre kirleticilerinin toksik etkilerinin izlenmesi ve belirlenmesi hayati öneme sahiptir ve bu kimyasallar reaktif oksijen türlerinin (ROS) üretiminin artmasına neden olduğundan kritik öneme sahiptir (Naseer ve ark., 2020).

Çalışmamızda, 48 saat etkide Oreochromis niloticus'un karaciğer dokusunda 1 ppm ve 5 ppm thiamethoxam etkisinde kalan gruplarda kontrol grubuna göre SOD ve CAT enzim aktivitelerinde sırasıyla %27, %49 ve %13, %32 artış olduğu belirlenmiştir. Demirci ve ark. (2018) çalışmalarında, *Gammarus kischineffensis*'te TMX toksisitesi durumunda süperoksit dismutaz (SOD) ve katalaz (CAT) aktivitelerinde önemli bir artış olduğunu bildirdi. O. niloticus'un acetamiprid'e (Aceta) maruz kalması balıklarda oksidatif strese ve genetik hasara neden oldu. Bu çalışmada, Aceta'ya maruz kalma antioksidan enzimlerin, süperoksit dismutazın (SOD) seviyelerini önemli ölçüde azalttı (Hathout ve ark., 2021). Bu enzimlerin aktivasyonlarında olan artış, karaciğerin detoksifikasyon mekanizmaları sırasında serbest radikallerin üretiminin artmasıyla insektisitlerin neden olduğu oksidatif stresten kaynaklanıyor olabilir.

Karaciğer enzim takibi toksikolojik çalışmalarda önemli bir araçtır. Thiamethoxam toksisitesi sonucunda *Oreochromis niloticus*'un karaciğer dokusunda SOD ve CAT enzimlerdeki değişimler toksik etkinin önemli değişimlere neden olduğu belirlenmiştir.

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ORAL PRESENTATION

Exosomes: The Important Biomarkers in Breast Cancer

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Abstract

Exosomes are nano-dimensional, non-cellular cuts secreted by many cells and play important roles in intercellular communication. In breast cancer, through the horizontal transfer of various bioactive molecules, such as proteins and mRNAs, exosomes emerge as mediators of oncogenic information from cell to cell and play an important role in cancer progression. Exosomes are an important part of breast cancer liquid biopsy and play an important role in the development, progression, and formation of the tumor microenvironment. Exosomes released from breast cancer cells have been found to contribute to proliferation, metastasis, angiogenesis, and carcinogenesis. Various methods are being used to treat breast cancer, and new treatment options are being highlighted. Especially exosomes, as in multiple cancers, serve as an important biological factor in the early diagnosis and prognosis of breast cancer. Exosomes have strong potential as promising bio-reducers in new clinical applications for breast cancer and drug distribution systems.

Keywords: Biomarkers, Breast cancer, Exosomes.

INTRODUCTION

Breast cancer, caused by milk-producing cells, is one of the most common cancers among women worldwide. Although there are a variety of treatments for breast cancer, patients face many difficulties in this process (Alimirzaie et al., 2019). Traditional methods of cancer detection, such as liquid biopsy, are not comprehensive enough to reveal the entire genomic structure of breast tumors. However, in recent years, various biostimulants have played an important role in the diagnosis and treatment of breast cancer. Exosomes, one of these bio-enhancers, play an important role in liquid biopsy. With the introduction of new techniques, the application of liquid biopsy has improved, enabling the treatment of breast cancer, including early diagnosis, diagnostics, prognosis prediction, and early detection of eczema. Different components of tumor cells released into the bloodstream in breast cancer can be analyzed in liquid biopsy samples. Some of these include circulating tumor DNA (ctDNA), tumor cells (CTC), non-cellular RNA, tumor-trained platelets, and exosomes. These components are important to determine the stage and prognosis of breast cancer while facilitating early detection of the disease (Alimirzaie et al., 2019; Jabbari et al., 2020; Tang et al., 2022).

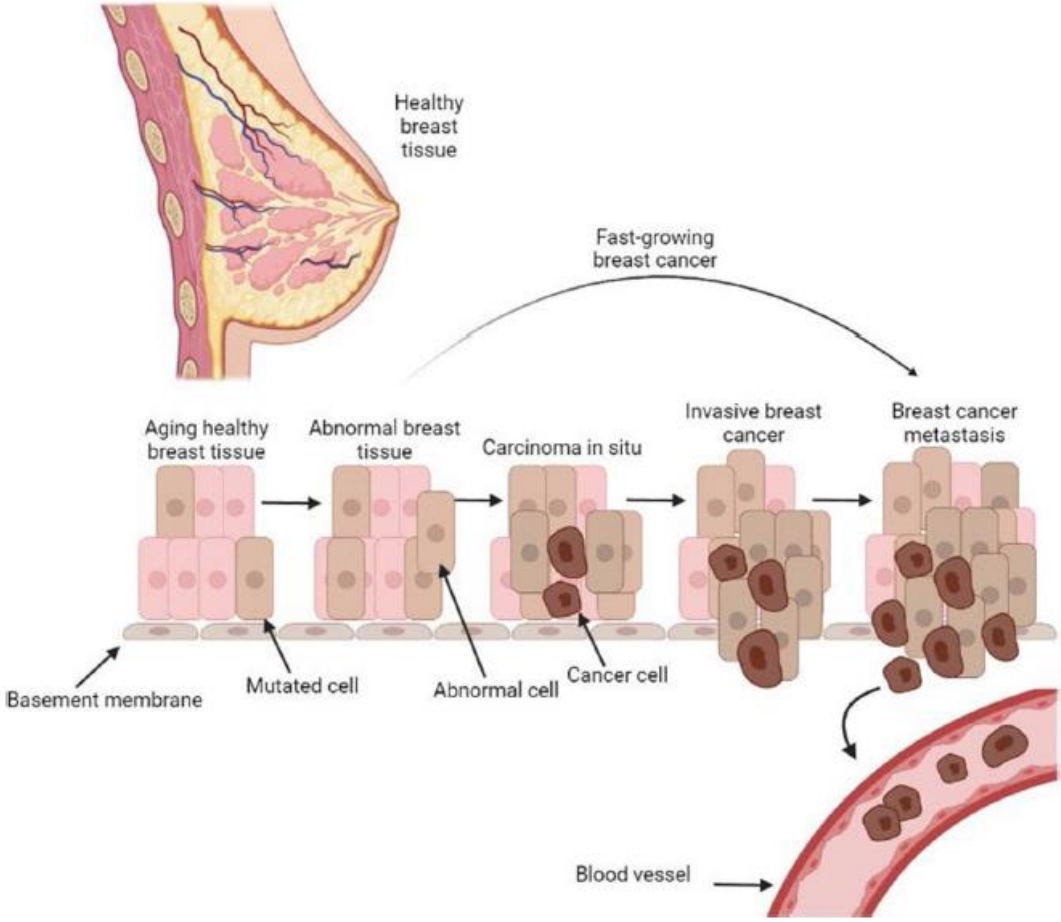

Figure 1. Breast cancer development and metastasis (Wekking et al., 2023)

All cells, including procaryotes and eucaryotes, release extra-cellular cells as part of their normal physiologies and during acquired abnormalities. These extracellular cells are divided into ectosomes and exosomes. Ectosomes are vesicles that compress the surface of the plasma membrane by outward splashing and contain microparticles and large cuts in the range of approximately 50 nm to 1 μm (Alimirzaie et al., 2019).

Exosomes are nano-dimensional membrane cuts consisting of lipid double layers released by various cell types and play an important role in intercellular communication (Jia et al., 2017). Exosomes are non-cellular cuts of endosomal origin with a diameter of approximately 40 to 160 nm (average ~100 nm). The invasion of the plasma membrane occurs through the formation of multicellular bodies that can contribute to the diversity of other intracellular cells and components of exosomes (Alimirzaie et al., 2019).

All extracellular cuts, including exosomes, contain many cell components such as RNA, DNA, lipids, metabolites, sitazolics, and cell surface proteins. Exosomes are responsible for removing more and more unnecessary components from cells to maintain cellular homeostasis from a physiological point of view. In exosomes, it shows a functional, targeted, mechanism-based accumulation of specific cellular components. Thus, exosomes are thought to have an important function in regulating intercellular communication (Kalluri ve LeBleu, 2020; Wang et al., 2020).

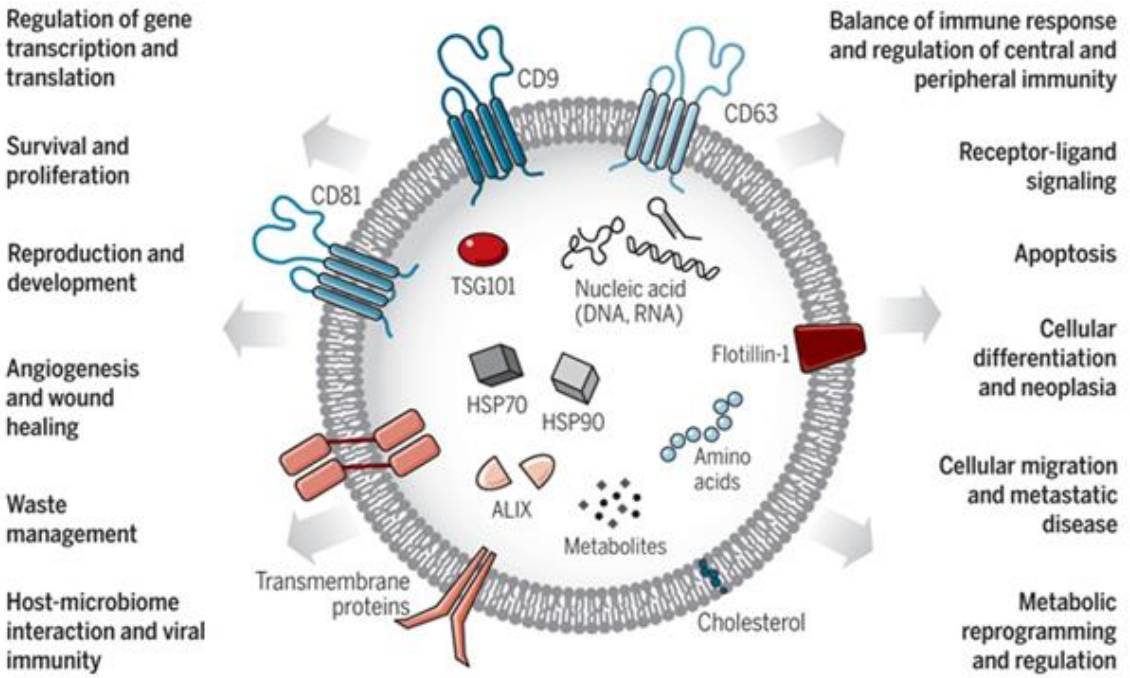

Figure 2. The distinctive features of exosomes (Kalluri ve LeBleu, 2020)

Exosomes play a crucial role in the advancement of breast cancer by acting as mediators of oncogenic information that travels from local and systemic cells to other cells through the horizontal transfer of numerous bioactive substances, including proteins and mRNAs (Jia et al., 2017). Exosomes, one of the extra-cellular cuts, carry biomolecules, such as lipids, proteins, and nucleic acids, and can generate signal transmission through the intercellular transmission of their contents. This mechanism promotes further invasion and proliferation of tumor cells, resulting in changes in the micro-environment. Stromal cells use exosomes to change the behavior of tumor cells (Lowry et al., 2015; Loric et al., 2023).

As a mechanism of short- and long-range intercellular communication in health and disease, they have an impact on several elements of cell biology (Kalluri ve LeBleu, 2020). Many studies in recent years have revealed that exosomes play an important role as biofertilizers. In various studies on breast cancer, the presence of exosomes is as important as a liquid biopsy (Alimirzaie et al., 2019).

EXOSOMES IN BREAST CANCER

Tumor cells secrete extra-cellular cells for intercellular communication. Non-cellular cuts and exosomes carry different proteins, lipids, and nucleic acids, affecting the function of target cells and acting as a biopreparator in tumor formation (Jabbari et al., 2020).

Yuan et al. (2021) stated that osteolytic bone metastasis develops over time in breast cancer patients, causing patients to be exposed to fractures, pain and spinal cord compression. They performed mouse xenograph models and intravenous injection of exosomes to analyze the *in vivo* role of exosomes released from breast cancer cells. The effects on osteoclast formation of exosomes secreted by mild metastatic MDA MB-231 and its high-metastatic subset SCP28 were confirmed by TRAP painting, ELISA, micro-computer tomography, histomorphometric analysis. To promote osteoclastogenesis, candidate exosomal miRNAs were globally scanned with RNA sequences, and qRT-PCR, western blot, confocal microscopy, and RNA sequence scans were performed to verify the function of the exosomatic myRNA. The study found that SCP28 tumor cells lead to increased osteoclast activity and decreased bone density. The exosomes released from the breast cancer cell played an important role in supporting breast cancer bone metastasis, which was associated with pre-metastatic niche formation through the transfer of miR-21 to the osteoclasts. They also noted that data from patient samples suggest that miR-21 has potential for clinical diagnosis and treatment of breast cancer bone metastasis.

Chen et al. (2021) studied the mechanisms underlying cancer-related fibroblasts (CAFs) tumor-propellant roles. CAFs derived from the patient and normal fibroblasts (NFs) are isolated from breast carcinomas and adjacent normal breast tissue. Exosomal microRNAs isolated by ultrasound and derived from CAF were scanned using new generation sorting technology. The MiR-500a-5p expression was evaluated by quantitative real-time polymerase chain reaction (qRT-PCR) and on-site hybridization. Tumor cell proliferation has been determined by MTT analyses, three-dimensional (3D) cultures, and tumor metastasis by *in vitro* Transwell analyses. The study revealed that CAFs significantly support the proliferation and metastasis of breast cancer cells through exosomal miR-500a-5p. The inhibition of miR-500a-5p, derived from CAF, is an alternative method for breast cancer treatment.

Angiogenesis covers a fundamental process of tumor formation, development, invasion, and metastatic proliferation. The extra-cellular vesicles released by tumors, especially exosomes, play an important role in angiogenesis and the progression of cancer (Han et al., 2022).

Han et al. (2022) isolated exosomes from breast cancer cells with high metastatic and low metastasic capacity. The pro-angiogenic effects of these exosomes have been evaluated *in vitro*, wound healing, rat arterial artery sphinctering, and *in vivo* Matrigel scab analysis. They used RNA sequencing, overexpression of various EPHA2 mutants, and small molecular inhibitors to analyze the angiogenesis-promoting effect of exosomal EPHA2, and its potential downstream mechanism. Finally, they used tumor cells that expressed EPHA2 at different levels to mimic exosomal secretion by tumour cells *in vivo*, creating xenographic tumor models, monitoring the metastasis of cancer cells, using the IVIS spectrum imaging system and computer tomography. As a result, they found that exosomes produced by breast cancer cells with high metastatic ability promote angiogenesis and metastasis.

Brain metastasis is known to be a major cause of death in breast cancer patients. The biggest event for brain metastasis is that cancer cells cross the blood-brain barrier. Lu et al. (2020), formed a series of metastatic breast cancer cells in the brain and evaluated the effect of exosomes on the blood brain barrier *in vitro* with transepitelyal/transendotelyal electrical resistance and permeability analyses. The study revealed that exosomes released from high levels of metastatic breast cancer cells in the brain have devastating effects on the blood-brain barrier, increasing the invasion of breast cancers.

Exosomes are produced by many cells just as they are released from cancer cells. Although exosomes are found in the serum of cancer patients, they are used for diagnosis and prognosis in cancer patients. Studies in recent years have revealed that the tears of non-cancer patients also contain exosomes. Inubushi et al. (2020), compared oncogenic miRNAs in exosomes isolated from tear fluids of five patients with metastatic breast cancer to tear exosoms of eight healthy volunteers. The findings revealed a higher rate of tear exosomes than serum exosomas (CD9, CD63). Tear exosomes performed quantitative reverse transcription polymerase reaction (qRT-PCR) and western blot analysis to clarify the state of miRNAs in the serum taken from patients with metastatic breast cancer. qRT-PCR and western-blot analysis revealed that miR-21 and miR-200c, specific to breast cancer, are expressed at higher levels in the tear exosomes of metastatic breast cancer patients, as opposed to those in healthy volunteers. These findings indicate that tear exosomes are a potential source of biopreparation for metastatic breast cancer and other cancers.

CONCLUSION

Exosomes, which play a crucial role in breast cancer, are emerging as vital biological factors in the early detection, diagnosis, prognosis, and treatment of various cancer types. These extracellular vesicles are involved in intercellular communication, transporting molecular information such as proteins, lipids, mRNA, and microRNAs, which makes them valuable biomarkers for detecting cancer at its earliest stages. The ability of exosomes to carry such molecular signatures means they can potentially be used to identify the presence of breast cancer before it is detectable through conventional imaging or biopsy techniques, allowing for earlier and more precise diagnosis. In addition to their diagnostic potential, exosomes have shown strong promise as bio-reductive agents, especially in the development of new clinical applications for breast cancer. Their small size and natural ability to penetrate tissues make them an ideal vehicle for drug delivery systems. Researchers are exploring ways to exploit this natural transport mechanism, allowing exosomes to deliver therapeutic agents directly to cancer cells with higher efficiency and specificity, potentially reducing side effects associated with traditional cancer treatments. This capability offers exciting possibilities for personalized medicine, where treatment can be tailored based on the molecular profile of an individual’s cancer.

As a result, exosomes represent a powerful tool for the future of healthcare. Their unique ability to transport molecular information across cell membranes, along with their potential as drug delivery systems and diagnostic biomarkers, makes them a transformative element in both cancer treatment and the management of other diseases. In the years ahead, exosomes are expected to play a crucial role in early disease detection, personalized therapies, and the development of innovative therapeutic strategies.

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ORAL PRESENTATION

Türkiye'den toplanan farklı propolis örneklerinin antibakteriyel aktivitesinin belirlenmesi

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Özet

Propolis, bal arıları tarafından bitkilerin yaprak, sap, tomurcuk gibi kısımları ile bitki nektarı ve poleninden toplanan reçineli maddelerin balmumu ve enzimlerle karıştırılmasıyla oluşan, 'arı tutkalı' olarak da adlandırılan doğal bir arı ürünüdür. Propolis %45-55 reçine, %25-35 bitkisel mumlar, %10 uçucu yağlar, %5 polen ve %5 organik bileşikler ve mineral maddelerden oluşur. Propolis içerdiği bileşenler ile antibakteriyel ve antifungal özellik gösterir. Propolisin içerdiği bileşenlerin miktarı toplandığı bölgeye ve hasat şekline göre değişiklik gösterir, bu nedenle farklı propolis örnekleri farklı şiddette antimikrobiyal aktivite gösterir. Bakteriler antibiyotiklere karşı dirençli olduğundan, doğal antibakteriyel etkiye sahip ürünlere ihtiyaç duyulmaktadır. Bu çalışmada, Bilecik, Ankara, Malatya, Düzce ve Balıkesir illerinden toplanan propolis örneklerinin antimikrobiyal aktivitesi disk difüzyon yöntemi kullanılarak belirlenmiştir. Bu amaçla, beş ilden beş farklı propolis örneği toplanmış ve %70'lik etil alkol çözeltisi ile ekstrakte edilerek antimikrobiyal aktiviteleri belirlenmiştir. Sonuçlar, farklı propolis örneklerinin farklı zonlarla farklı antimikrobiyal aktivite gösterdiğini ortaya koymuştur. Sonuçlardan, propolisin doğal bir antimikrobiyal ajan olduğu ve farklı uygulamalarda potansiyele sahip olduğu anlaşılmıştır.

Anahtar Kelimeler: Propolis, antimikrobiyal aktivite, bal arısı, disk difüzyon yöntemi

Antibacterial activity of different propolis samples collected from Türkiye

Abstract

Propolis is a natural bee product, also called 'bee glue', which is formed by honey bees by mixing resinous substances collected from parts of plants such as leaves, stems, buds, and plant nectar and pollen with beeswax and enzymes. Propolis consists of 45-55% resin, 25-35% vegetable waxes, 10% essential oils, 5% pollen, and 5% organic compounds and mineral substances. Propolis shows antibacterial and antifungal properties with the components it contains. The amount of components contained in propolis varies according to the region where it is collected and the way it is harvested, so different propolis samples show antimicrobial activity of different severity. Since bacteria are resistant to antibiotics, products with natural antibacterial effects are needed. In this study, the antimicrobial activity of propolis samples collected from Bilecik, Ankara, Malatya, Düzce and Balıkesir provinces was determined using the disk diffusion method. For this purpose, five different propolis samples were collected from five different city and extracted with a 70% ethyl alcohol solution and their antimicrobial activities were determined. The results showed that the different propolis samples showed different antimicrobial activity with different zones. It was clear from the results that propolis is a natural antimicrobial agent and had potential in different applications.

Keywords: Propolis, antimicrobial activity, honey bee, disc diffusion method

GİRİŞ

Propolis (arı tutkalı), bitkilerin farklı kısımlarından salınan salgılar ile arı salgıları ve balmumu ile karıştırılmış yapışkan reçinemsi doğal bir maddedir (Simone-Finstrom ve ark. 2017). Arılar bitki özütünü kendi tükürük salgıları ve balmumu ile karıştırdıklarında, propolis arıların kovanlarının çatlaklarını kapatmak için kullandıkları yapışkan, koyu renkli bir madde olarak oluşmaktadır (Martinotti ve Ranzato 2015). Propolis, M.Ö. 300 yılından bu yana besin maddesi ve genel sağlık için yararlı bir ürün olarak kullanılmaktadır (Wagh 2013). Propolis, çeşitli eski yazıtlarda tek başına veya diğer maddelerle karıştırılarak yaralanmaları iyileştirici bir ajan olarak tasvir edilmiştir (Martinotti ve Ranzato 2015). Propolisin serbest radikal temizleme, sitotoksisite ve antimikrobiyal aktivite gibi çeşitli özellikleri bulunmaktadır (Pasupuleti ve ark. 2017, Oryan ve ark. 2018, Elkhenany ve ark. 2019). Son yıllarda propolis, insan sağlığını iyileştirmek ve hastalıkları önlemek için içeceklerde bir takviye olarak geniş ölçüde kullanılmaktadır (Ramos ve Miranda, 2007). Propolis anti-viral, anti-enflamatuar, anti-bakteriyel (Sforcin vd. 2017), anestezik, anti-oksidan, anti-tümöral, anti-kanser, anti-fungal, anti-protozoal, anti-hepatotoksik, anti-mutajenik, anti-septik, sitotoksik aktivitesi vb. özellikleriyle bilinmektedir (Toreti vd. 2013, Sforcin 2016). Propolisin *in vitro* antibakteriyel aktivitesi, birçok Gram-pozitif ve Gram-negatif bakteri türüne karşı kanıtlanmıştır (Przybyłek ve Karpin 2019). Propolis antioksidan ve antimikrobiyal özellikleri, lipid oksidasyonunu geciktirme ve böylece gıda ürünlerinin raf ömrünü artırma potansiyeli nedeniyle gıda endüstrisi için de son derece önemlidir. Bu özellikler kafeik asit feniletil, flavanol, ester flavonoid, pinocembrin ve galangin gibi bileşenlerin varlığına atfedilir ve bu bileşenler bakteriyel RNA polimerazı inhibe ederek antimikrobiyal etki mekanizmasını göstermektedir (Funari ve Ferro 2006).

Propolisin farklı alanlarda kullanımı, botanik kökenleriyle ilişkili olarak kimyasal bileşiminin araştırılması son yıllarda popüler olmuştur. *Apis mellifera* tarafından üretilen propoliste fenolik bileşikler bulunmaktadır. Propolisteki temel fenolik bileşikler olan flavonoidler, arının yaşadığı ekolojik botanik ortamdan etkilenmektedir (Toreti ve ark. 2013, Becerra ve ark., 2019). Bal arılarının propolis yapmak için erişebileceği yapraklar ve yaprak tomurcukları üzerindeki lipofilik malzemeler, ziftler, yapıştırıcılar, zamklar yaralanma veya kesilme durumunda bitkiler tarafından üretilen maddelerdir (Ristivojevic´ ve ark. 2015). Propolisin bitki bazlı içeriği bileşimini belirlemektedir. Bu nedenle, propolisin özellikleri arıların yaşadığı yerdeki bitki örtüsü ile sıkı bir şekilde ilişkilidir (Bankova ve Marcucci, 2000). Propolis, oldukça karmaşık reçineli bir doğal üründür. Reçine (%50 ila %70), yağ ve balmumu (%30 ila %50), polen (%5 ila %10), B, C ve E vitaminleri, mineraller, şekerler, flavonoidler, fenol, amino asitler ve aromatik bileşikler gibi diğer maddeler propolisin bileşimini oluşturmaktadır (Ahangari ve ark. 2018). Çoğunlukla, kovan çevresinde gelişen ve bal arılarının gerekli salgıları topladığı bitki türlerinin çeşitliliği nedeniyle propolisin bileşimi son derece değişkendir (Aminimoghadamfarouj ve Nematollahi 2017, Drescher ve ark. 2019). Propolisin bileşimi rakım, aydınlatma, mevsimsel değişimler ve arıların beslenme alanlarından çok fazla etkilenmektedir (Silva-Carvalho ve ark. 2015). Propolisin kimyasal ve biyolojik bileşimi üzerine çok sayıda araştırma yapılmıştır. Bugüne kadar çeşitli coğrafi bölgelerden toplanan propolislerde 300'den fazla farklı bileşik tespit edilmiştir (Freires ve ark. 2016). Propoliste bulunan başlıca bileşenler flavonoidler, fenolikler ve aromatik maddelerin karışımlarıdır (Devequi-Nunes et al. 2018, Galeotti et al. 2018, Wozniak et al., 2019). Ayrıca propolis bazı yağlar, terpenler ve bal mumu da içermektedir. %30 esansiyel yağ, %10 polen, %5 diğer organik maddeler, bitki reçineleri, propolisin kalitesi, nektarın kalınlığı, viskozitesi ve kristalleşme yeteneği gibi fiziksel özelliklerini etkileyen ve aynı zamanda tadı, çözünebilirliği ve korunabilirliği gibi kullanımı gerekli özelliklerini etkileyen en önemli parametrelerindendir (SimoneFinstrom ve Spivak 2010).

İçermiş olduğu aktif bileşenler ile antimikrobiyal aktivite gösteren propolisin bileşimi toplandığı yıl, toplama şekli, flora vb. gibi nedenlerle değiştiğinden yapılan bu çalışmada Türkiye'nin farklı şehirlerinden aynı yıl toplanan ham propolis örneklerinin *E. coli* üzerine antibakteriyal aktivitesi tespit edilmiş ve kıyaslanmıştır.

MATERYAL VE METOT

Örneklerin toplanması ve propolis ekstraksyonu

Çalışmada kullanılan propolis örnekleri Ankara, Düzce, Malatya, Balıkesir ve Bilecik şehirlerinden 2022 yılında temin edilmiştir. Ham propolis örnekleri propolis tuzakları kullanılarak arıcılar tarafından toplanmıştır.

Propolis ekstraksiyonu

Çalışmada kullanılan ham propolis örnekleri %70'lik etil alkol çözeltisi ile ekstrakte edilmiştir. Bu amaçlar yaklaşık 1 g ham propolis örneği 10 mL %70'lik etil alkol çözeltisi (1:10 a/v) ile macerasyon tekniği kullanılarak ekstrakte edilmiştir. Maserasyon işleminin ardından oluşan karışım süzülmüş ve elde edilen propolis ekstraktı (süzüntü) kullanılana kadar +4 °'de saklanmıştır (Kolaylı ve ark., 2020).

Antibakteriyal aktivitenin tespit edilmesi

Çalışmada kullanılan gram negatif bir bakteri olan *Escherichia coli* (*E. coli*) Halk Sağlığı Genel Müdürlüğü Refik Saydam Ulusal Tip Kültür Koleksiyonu Laboratuvarından temin edilmiştir. Temin edilen yerli suş uygun koşullarda inkübe edilmiştir. Daha sonra hazırlanan ekstraktların *in vitro* antimikrobiyal aktiviteleri disk difüzyon metodu kullanılarak tespit edilmiştir. Ekstraktan bir miktar alınarak boş antimikrobiyal duyarlılık disklerine (OXOID) emdirilmiştir. Daha sonra diskler kurutulmaya bırakılmış ve kuruma işleminin ardından dikkatlice daha önce hazırlanmış olan petrilere yerleştirilmiştir. Hazırlanan petriler 37°C'de 24 saat boyunca inkübe edilmiş ve oluşan zon çapları ölçülmüştür (Kolaylı ve ark., 2020).

BULGULAR ve TARTIŞMA

Antibiyotikler geçmişten günümüze bakteriyel hastalıkların iyileştirilmesinde önemli bir yer tutmasına rağmen toplum tarafından bu ilaçlar önemsenmemiş ve bilinçsizce kullanılmıştır. Antibiyotiklerin fazla ve yanlış kullanımı, kalıntı sürelerinin takipsizliği ve çevresel bulaşma ciddi bir şekilde artan antimikrobiyal dirence ve buna bağlı olarak antibiyotiklere dirençli bakterilerin ortaya çıkmasına yol açmıştır. Bu durum her geçen gün etkili antibiyotik sayısının azalmasına ve dirençli patojen bakterilerin sayısında da artmaya sebep olmuştur. Dünya ülkelerinde olduğu gibi ülkemizde de deneme yanılma yöntemiyle bulunmuş doğal ürünler (şifalı bitkiler, hayvansal maddeler vb.) mikrobiyal hastalıkların tedavisinde kullanılmaktadır. Son yıllarda çoklu antibiyotik direncine sahip mikroorganizmaların artması yüzünden bu mikroorganizmaların neden olduğu enfeksiyonun tedavisi giderek içinden çıkılmaz hale gelmektedir. Bilinen tüm antibiyotiklere direnç geliştirmekte olan bakterilerde, ilaç dirençliliği artmakta ve yayılmaktadır. Bu nedenle ilaçlara alternatif olarak doğal antimikrobiyal maddelerin kullanılması önerilmektedir ve bu maddeler antimikrobiyal olarak kullanılmaktadır.

Mikroorganizmalar vücuda solunum, sindirim, deri kesikleri veya diğer insan ve hayvanlarla yakın temas yoluyla girmektedir. Bazı mikroorganizmalar vücut dokularını istila ederek (patojenler), diğerleri toksin denilen zehirli salgılarla hastalıklara neden olmaktadır. Bunlardan biride Enterobacteriaceae ailesinden *Escherichia coli* bakterisidir. *E.coli* gerek toplum kökenli gerekse hastane kaynaklı pek çok enfeksiyonun (idrar yolu enfeksiyonu, gastroenterit, sepsis ve menenjit gibi) sorumlu etkenidir. Özellikle toplum kökenli idrar yolu enfeksiyonlarının %80'den fazlasının ve çoğu hastanede kazanılmış idrar yolu enfeksiyonun etkenidir. Gelişmekte olan ülkelerde en önemli gastroenterit etkenlerden biridir. Genelde yeni doğanlarda menenjit ve gastroenterit dışındaki tüm enfeksiyonlar endojendir, kişinin normal biyotasındandaki *E. coli* suşları savunma mekanizmaları ortadan kalkarsa virülan hale gelebilir. Bu nedenle propolis gibi doğal ürünlerin gıda takviyesi olarak kullanılması önem arz etmektedir.

Yapılan bu çalışmada Türkiye'nin farklı illerinden temin edilen ham propolis örnekleri ekstrate edilmiş ve elde edilen ekstrartların disk difüzyon metoduna göre *E. coli* üzerine antibakteriyal aktivitesi tespit edilmiştir (Şekil 1).



Şekil 1. Propolis örneklerine ait antibakteriyal aktivite görselleri

Şekil 1 incelendiğinde, Bilecik yöresi propolisinin *E. coli* bakterisi üzerinde zon oluşumu gerçekleştirdiği ve bakterisid etki gösterirken zon çapından uzaklaştıkça az miktarda bakterinin çoğalmasını engellediği (bakteriyostatik) görülmüştür. Ankara yöresi propolisinin *E.coli* bakterisi üzerinde zon oluşumu gerçekleştirdiği ve bakterisid etki gösterirken zon çapından uzaklaştıkça az miktarda bakterinin çoğalmasını engellediği (bakteriyostatik) görülmüştür. Düzce yöresi propolisinin *E.coli* bakterisi üzerinde zon oluşumu gerçekleştirdiği ve bakterisid etki gösterirken zon çapından uzaklaştıkça az miktarda bakterinin çoğalmasını engellediği (bakteriyostatik) görülmüştür. Balıkesir yöresi propolisinin *E.coli* bakterisi üzerinde zon oluşumu gerçekleştirdiği ve bakterisid etki gösterirken zon çapından uzaklaştıkça az miktarda bakterinin çoğalmasını engellediği (bakteriyostatik) görülmüştür. Malatya yöresi propolisinin *E.coli* bakterisi üzerinde zon oluşumu gerçekleştirdiği ve bakterisid etki gösterirken zon çapından uzaklaştıkça az miktarda bakterinin çoğalmasını engellediği (bakteriyostatik) görülmüştür.

Literatür incelendiğinde propolisin farklı mikroorganizmalar üzerine antimikrobiyal aktivitesi olduğu oldukça açıktır (Majiene ve ark., 2007; Lu ve ark., 2005; Sforcin 2007; Kolaylı ve ark., 2020; Kolaylı ve ark., 2023). Kolaylı ve arkadaşları tarafından yapılan bir çalışmada propolis örneklerinin *Helicobacter pylorii* (*H. pylorii*) ve *Streptococcus agalactiae* mikroorganizmaları üzerine antimikrobiyal etki gösterdikleri ifade edilmiştir. Yapılan bu çalışmaya göre propolisin *Helicobacter pylorii* üzerine daha etkili olduğu vurgulanmıştır. Gonsales ve arkadaşları tarafından yapılan bir çalışmada ise propolisin etanolik ekstratlarının *Staphylococcus aureus* ve *Escherichia coli* üzerine antimikrobiyal aktivitesi belirlenmiştir. Bu çalışmaya göre propolis örnekleri *Staphylococcus aureus* büyümesini inhibe ederken *E.coli* büyümesini durdamamıştır. Yapılan bu çalışma ile Türkiye'nin farklı illerinden toplanan propolis örneklerinin de antimikrobiyal aktivitesi olduğu; ancak Şekli 1' de görüldüğü üzere farklı zon çaplarında bakterilerin üremesinin engellendiği açıktır. Bu yönüyle Türk propolisinin antimikrobiyal aktivitesinin oldukça iyi olduğu ifade edilebilir.

SONUÇ

Bal arıları tarafından üretilen doğal bir ürün olan propolis içermiş olduğu biyoaktif bileşenler ile oldukça iyi bir antimikrobiyal aktivite sergilemektedir. Bu özelliği ile propolis gıda takviyesi olarak veya gıda koruyucu olarak farklı alanlarda kullanım potansiyeline sahiptir. Yapılan bu çalışmada da Türkiye'nin beş farklı şehrinden toplanan propolis örneklerinin antimikrobiyal aktivite etkisi incelenmiş ve bu propolislerin *E.coli* üzerine farklı oranlarda antimikrobiyal etkisi olduğu tespit edilmiştir.

TEŞEKKÜR

Çalışmada kullanılan ham propolislerin temin edildiği arıcılara teşekkür ederiz.

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ORAL PRESENTATION

Beyaz yonca (*Trifolium repens* L.) yapraklarından hazırlanan ekstraktların insan eritrosit karbonik anhidraz izoenzimleri (hCA I ve hCA II) üzerine etkileri

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Özet

Karbonik anhidrazlar (CA, EC 4.2.1.1), günümüze kadar birçok canlıda varlığı tespit edilmiş olan ve karbon dioksitin (CO_2) bikarbonat (HCO_3^-) ve protona (H^+) dönüşümlü hidrasyon reaksiyonunu katalizleyen metaloenzimlerdir. İnsan vücudunda 15 farklı hCA izoenzimi mevcuttur. hCA I birçok dokuda yaygın olarak bulunur, retinal ve serebral ödem gibi bazı patolojik süreçlerde rol alır. hCA II de çeşitli dokularda bol miktarda bulunur ve glokom, epilepsi, ödem, yüksek irtifa hastalığı, böbrek bozuklukları ve vertigo gibi hastalıklarla ilişkilidir. Günümüze kadar hCA ilişkili hastalıkların tedavisi için sentetik ya da doğal kaynaklı birçok inhibitör rapor edilmiştir. Asetazolamid, metazolamid ve brinzolamid gibi sentetik inhibitörler klinikte de kullanılmaktadırlar. Ancak genel problem, inhibitörlerin tek bir hCA izoenzimine karşı seçici olmayıp diğerlerini de inhibe etmesidir. Sonuç olarak bu durum birtakım yan etkilerin ortaya çıkmasına neden olmaktadır. Beyaz yonca (*Trifolium repens* L.), baklagiller (Leguminosae) familyasından olan çok yıllık otsu bir bitkidir. Akdeniz bölgesi ve ılıman, subtropikal yüksek irtifa bölgelerinde yaygın olarak dağılış gösterir. Halk arasında değerli yem bitkileri ve aynı zamanda tıbben önemli otlar olarak bilinmektedir. Ülkemizde *Trifolium repens*, *Trifolium arvense*, *Trifolium pratense* gibi bazı Trifolium türleri, balgam söktürücü, antiseptik ve analjezik olarak ve romatizma ağrılarına karşı kullanılmaktadır. İlave olarak, beyaz yonca ekstresinin anti-inflamatuar, anti-kolinesteraz ve antioksidan aktivite gösterdiğini belirten çalışmalar da yayınlanmıştır. Bu çalışmada beyaz yonca yapraklarından hazırlanan ekstraktların hCA I ve hCA II üzerindeki inhibisyon etkilerinin *in vitro* olarak incelenmesi amaçlanmıştır. Bu kapsamda öncelikle beyaz yonca yaprakları güneş görmeyen bir ortamda kurutuldu ve su, metanol, etanol, etil asetat ve *n*-hekzan çözücüleri kullanılarak ekstraktları hazırlandı. Çözücüsü uzaklaştırılan ekstraktarın DMSO içerisinde 0,1 mg/mL lik stok çözeltileri hazırlandı ve hCA I ile hCA II nin esteraz aktivitesi üzerindeki inhibitör etkileri *in vitro* olarak incelendi. Esteraz aktivitesi, hCA enzimlerinin *p*-nitrofenil asetat substratını hidrolizlemesi ve açığa çıkan *p*-nitrofenol bileşiğinin miktarının 348 nm de ölçülen absorbansı ile belirlendi. İnhibitörsüz ortamdaki hCA esteraz aktivitesi %100 kabul edilerek inhibitörlü ortamdaki bağıl aktivite değerleri belirlendi ve %Bağıl Aktivite – [İnhibitör] eğrileri çizilerek IC_{50} değerleri hesaplandı. Sonuç olarak beyaz yonca yaprağı ekstraktlarının sadece hCA I izoenzimini inhibe ettiği, hCA II izoenzimine etki etmediği bulunmuştur. Ekstraktların hCA I inhibisyon potansiyeli *n*-hekzan > etil asetat > etanol > metanol > su şeklindedir. Azalan polarite ile inhibisyon etkisinin arttığı belirlenmiştir. Ekstraktların sadece hCA I izoenzimini inhibe etmesi izoenzim seçiciliği açısından kayda değer olup *in vivo* çalışmalar için potansiyel teşkil etmektedir.

Anahtar Kelimeler: Beyaz yonca, Ekstrakt, İnhibisyon, Karbonik anhidraz

Effects of extracts prepared from white clover (*Trifolium repens* L.) leaves on human erythrocyte carbonic anhydrase isoenzymes (hCA I and hCA II)

Abstract

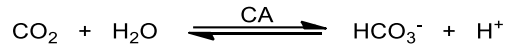
Carbonic anhydrases (CA, EC 4.2.1.1) are metalloenzymes that catalyze the hydration reaction of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and proton (H^+). They have been identified in many living organisms to date. There are 15 different hCA isoenzymes in humans. hCA I is widely distributed in many tissues and is involved in pathological processes such as retinal and cerebral edema. hCA II is also abundant in various tissues and is associated with diseases such as glaucoma, epilepsy, edema, altitude sickness, kidney disorders, and vertigo. To date, many synthetic or natural inhibitors have been reported for treating hCA-related diseases. Synthetic inhibitors such as acetazolamide, metazolamide, and brinzolamide are also used clinically. The

general problem, however, is that inhibitors are not selective against a single hCA isoenzyme but also inhibit others. As a result, this situation causes some side effects to occur. White clover (*Trifolium repens* L.) is a perennial herbaceous plant from the legume (Leguminosae) family. It is widely distributed in the Mediterranean and temperate, subtropical high-altitude regions. They are popularly known as valuable fodder plants and medicinally essential herbs. In our country, some Trifolium species, such as Trifolium repens, Trifolium arvense, and Trifolium pratense, are used as expectorants, antiseptics, analgesics, and against rheumatic pains. In addition, studies indicating that white clover extract shows anti-inflammatory, anti-cholinesterase, and antioxidant activity have also been published. The aim of this study was to investigate the *in vitro* inhibition effects of extracts prepared from white clover leaves on hCA I and hCA II. In this context, first, white clover leaves were dried in a sunless environment, and extracts were prepared using water, methanol, ethanol, ethyl acetate, and *n*-hexane solvents. After removing the solvent, stock solutions of the extract were prepared at 0.1 mg/mL in DMSO, and the inhibitory effects of hCA I and hCA II on the esterase activity were investigated *in vitro*. Esterase activity was determined by the hydrolysis of *p*-nitrophenyl acetate substrate by hCA enzymes, and the amount of released *p*-nitrophenol compound was measured by absorbance at 348 nm. The relative activity values in the medium with the inhibitor were determined by accepting the hCA esterase activity in the medium without the inhibitor as 100%, and IC_{50} values were calculated by plotting %Relative Activity—[Inhibitor] curves. Consequently, white clover leaf extracts inhibited only the hCA I isoenzyme and had no effect on the hCA II isoenzyme. The extracts' hCA I inhibition potential was as follows: *n*-hexane > ethyl acetate > ethanol > methanol > water. The fact that the extracts inhibit only the hCA I isoenzyme is noteworthy regarding isoenzyme selectivity and poses potential for *in vivo* studies.

Keywords: White clover, Extract, Inhibition, Carbonic anhydrase

GİRİŞ

Karbonik anhidrazlar (CA, EC 4.2.1.1), günümüze kadar birçok canlıda varlığı tespit edilmiş olan ve karbon dioksitin (CO_2) bikarbonat (HCO_3^-) ve protona (H^+) dönüşümlü hidrasyon reaksiyonunu katalizleyen metaloenzimlerdir (Şekil 1) (Alterio ve ark., 2012).



Şekil 1. Karbonik anhidraz enzimi tarafından katalizlenen reaksiyon.

CA enzimleri, canlı organizmalarda sekiz farklı gen ailesi (α-, β-, γ-, δ-, ζ-, η-, θ- ve ι- CA'lar) tarafından kodlanmaktadır. α gen ailesi tarafından kodlanan omurgalı CA enzimlerinin 16 farklı izoformu (I – XV) olup, sitozolik, mitokondriyal, salgı CA'ları ve membrana bağlı CA'lar olmak üzere dört ana sınıfa ayrılır. Bütün insan CA'ları (hCA'lar) α sınıfına ait olup, aktif bölgesinde, üç histidin kalıntısı ve bir su molekülü/hidroksit iyonu ile koordine Zn(II) iyonu bulundurur. Bu izoformların, moleküler özellikler, hücresel lokalizasyon, organ ve dokulardaki dağılım, ifadelenme seviyeleri ve farklı sınıf inhibitörlere karşı yanıt gibi özelliklerinin farklılıklar gösterdikleri bilinmektedir (Mishra ve ark., 2020).

CA katalizli CO_2 hidrasyon reaksiyonu, dokular ve akciğerler arasında CO_2 ve bikarbonat taşınımı, pH ve CO_2 homeostazı, çeşitli dokular ve organlarda elektrolit salınımı, glukoneogenez, lipogenez ve üregenez gibi biyosentetik reaksiyonlar, kemik resorpsiyonu, kalsifikasyon, tümör oluşumu ve çeşitli patojenlerin virülansı gibi pek çok fizyolojik ve patolojik süreçte rol alır (Supuran, 2017).

CA'lar üzerine yapılan araştırmalar, bu enzimlerin, glokom, obezite, osteoporoz, kanser, yüksek irtifa hastalığı, epilepsi, nöropatik ağrı ve uyku apnesi gibi hastalıklarla yakından ilişkili olduğunu ortaya koymuştur. Bu nedenle, hCA izoformlarının çoğu, inhibitörlerin tasarımı için önemli hedefler arasında yer almaktadır (Winum ve ark., 2006). CA I birçok dokuda yaygın olarak bulunur ve kesin fizyolojik işlevi gizemini korumasına rağmen, retinal ve serebral ödem gibi bazı patolojik süreçlerde rol alır (Gao ve ark., 2007). CA II de çeşitli dokularda bol miktarda bulunur ve glokom, epilepsi, ödem, yüksek irtifa hastalığı, böbrek bozuklukları ve vertigo gibi hastalıklarla ilişkilidir (Supuran, 2008). CA III oksidatif stres ile bağlantılı olup, romatoid artrit gibi çeşitli inflamatuar hastalıklarla ilişkilidir (Du ve ark., 2009). CA IV, glokom, felç ve retinitis pigmentosa için bir ilaç hedefi olarak, oldukça iyi bir şekilde çalışılmıştır (Datta ve ark., 2009). Mitokondriyal izoformlar olan CA VA ve CA VB' nin obezite ile ilişkili olduğu rapor edilmiştir (De Simone ve Supuran, 2007). CA VI nispeten daha az çalışılmış olmakla birlikte, diş çürüklerinin oluşumunda rol oynadığı bilinmektedir (Kivelä ve ark., 1999). CA VII, epilepsi için iyi çalışılmış bir hedeftir ve bu izoform, beyinde bilişle ilgili süreçlerde CA II ve XIV ile birlikte hareket edebilir (De Simone ve ark., 2009). CA VIII, nörodejeneratif bozukluklar ve zeka geriliği ile ilişkisi nedeniyle incelenmiş bir izoformdur. Ancak, katalitik

olmayan CA VIII, X ve XI izoformlarının, gerçek fizyolojik işlevlerine ilişkin çok az bilgi mevcuttur (Aspatwar ve ark., 2010). CA IX ve CA XII, hipoksik tümörler/metastazlar için iyi çalışılmış ilaç hedefleridir ve bunların inhibisyonu, tümör büyümesini, ilerlemesini ve metastazı aktif olarak kontrol eder. CA IX, çeşitli hipoksik tümör türlerinde hastalık gelişiminin değerli bir belirteci olarak kabul edilir. CA XII, CA IX ile kıyaslandığında, antikanser ilaç hedefi olarak daha az çalışılmış olmakla birlikte, bu izoformun kanser ilerlemesindeki rolünü ortaya koyan çalışmalar da rapor edilmiştir. Bunlara ilaveten, CA XII izoformunun epilepsi ve diğer merkezi sinir sistemi hastalıkları ile ilişkili olduğunu kanıtlayan bazı çalışmalar vardır (Pastorekova ve ark., 2006). CA XIII, sperm motilitesindeki rolü açısından incelenmiştir ve bu izoformun inhibisyonu, kontraseptif ajanlar geliştirmek için kullanılabilir (Lehtonen ve ark., 2004). Son olarak, CA XIV izoformunun, epilepsi ve bazı retinopati türleri için bir hedef olabileceği öngörülmüştür (Nagelhus ve ark., 2005). CA XV ise insanlarda ifadelenmemektedir.

Beyaz yonca (*Trifolium repens* L.), baklagiller (Leguminosae) familyasından olan çok yıllık ılıman otsu bir bitkidir. Akdeniz bölgesi ve ılıman, subtropikal yüksek irtifa bölgelerinde yaygın olarak dağılış gösterir (Chen ve ark., 2019). Halk arasında değerli yem bitkileri ve aynı zamanda tıbben önemli otlar olarak bilinmektedir. *Trifolium repens, Trifolium arvense*, *Trifolium pratense* gibi bazı *Trifolium* türleri, balgam söktürücü, antiseptik ve analjezik olarak ve romatizma ağrılarına karşı kullanılmaktadır. *T.repens* L.'nin kaynatılması ile elde edilen içecek ise ishal önleyici ilaç olarak kullanılmıştır (Mustafa ve ark., 2012). Beyaz yoncanın, bazı bölgelerde gıda olarak kullanımı da mevcuttur. Çin'deki Yili bölgesinin otlaklarında, beyaz yonca, arıcılık için önemli bal kaynaklarından biridir. Yine Çin'in Miao bölgesinde halk, pirinç şarabı eldesinde beyaz yonca kullanmaktadır. Şarap ile beraber ağız yoluyla alınan beyaz yoncanın, fazla ısıyı düşürmeye, idrarı temizlemeye ve ciltteki kaşıntıyı gidermeye yardımcı olduğu, haricen kullanımda ise nedeni bilinmeyen şişlikleri iyileştirmeye yardımcı olduğu bilinmektedir (Jing, 2014). Ayrıca beyaz yoncada bulunan flavonoidler, izoflavonoidler, biyokumarinler ve triterpenoid saponinlerin, östrojenik, antispazmodik ve balgam söktürücü özellikler gibi çeşitli biyoaktiviteler sergiledikleri bildirilmiştir (Leung ve Foster, 2003). İlave olarak, beyaz yonca ekstresinin anti-inflamatuar, anti-kolinesteraz ve antioksidan aktivite gösterdiğini belirten çalışmalar da yayınlanmıştır (Ahmad ve ark., 2020).

MATERYAL VE METOT

Çalışmada kullanılan beyaz yonca yaprakları Kütahya Dumlupınar Üniversitesi Evliya Çelebi Yerleşkesi'ndeki uıygun alanlardan toplandı. Ekstraksiyon çözücüleri (metanol, etanol, etil asetat ve *n*-hekzan) Fischer Scientific International Inc. (New Hampshire, USA) firmasından, kinetik ölçümlerde kullanılan *p*-nitrofenil asetat bileşiği Sigma-Aldrich (Missouri, USA) firmasından, stok inhibitör çözeltilerinin hazırlanması için kullanılan DMSO Carlo Erba Reagents GmbH (Emmendingen, Germany) firmasından, tampon çözelti hazırlamak için kullanılan TRIS ve H_2SO_4 ise Merck (Darmstadt, Germany) firmasından temin edildi. Enzim aktivite ölçümleri SHIMADZU UV-1700 PharmaSpec UV-Vis spektrofotometresi (Kyoto, Japan) kullanılarak gerçekleştirildi. Tampon çözeltilerin hazırlanması için Mettler Toledo SevenDirect SD-20 pH metre (Ohio, USA) kullanıldı. Öğütme işlemleri IKA A11 öğütücü (Staufen, Germany) kullanılarak yapıldı. Çalkalama işlemleri için DAIHAN ThermoStable IS-20R (Gangwon, South Korea) inkübatör kullanıldı.

Ekstraktların hazırlanışı

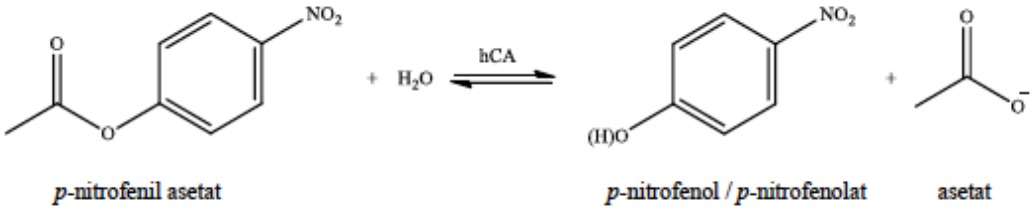
Yonca yaprakları güneş görmeyen bir ortamda oda sıcaklığında birkaç gün süreyle kurutuldu. Kurutulan yaprakların öğütülmesini takiben, öğütülmüş yaprak (g) : ekstraksiyon çözücüsü oranı (mL) 1:5 olacak şekilde 100 rpm de 25 °C sıcaklıkta 24 saat süreyle çalkalandı. Süspansiyon süzüldü ve berrak bir ekstrakt çözeltisi elde etmek amacıyla, süzüntü 5000 rpm de 20 dakika süreyle santrifüjlendi. Daha sonra süpernatant rotary evaporatörde düşük sıcaklık ve basınçta konsantre edildi. Elde edilen katı/kıvamlı kalıntı inhibisyon çalışmalarında kullanılıncaya kadar -20 °C de muhafaza edildi (Kaya ve ark., 2019).

Kinetik çalışmalar

İnhibisyon çalışmalarında kullanılan hCA I ve hCA II izoenzimleri insan eritrosit hücrelerinden Sepharose®4B-L-tirozin-*p*-aminobenzensülfonamit afinite kromaografisi tekniği kullanılarak, önceki çalışmalarımızda detaylı olarak açıklandığı şekilde, tek basamakta saflaştırıldı. Saflaştırılan enzimlerin saflık kontrolü SDS-PAGE ile yapıldı (Parmaksız ve Tunca, 2024; Özkul ve ark., 2024).

Saflaştırılan hCA I ve hCA II izoenzimlerinin esteraz aktiviteleri, *p*-nitrofenil asetat substratının hidrolizi sonucu açığa çıkan *p*-nitrofenol bileşiğinin absorbansının 25 °C de 348 nm dalga boyunda üç dakika süre ile ölçülmesi sonucu belirlendi (Şekil 2). Reaksiyon ortamı toplam hacim 3,0 mL olacak şekilde 50 mM pH 7,4 TRIS tamponu, 3 mM *p*-nitrofenil asetat substratı, hCA I / hCA II enzimi ve saf sudan oluşmaktadır. Aynı

ölçüm enzimsiz ortamda da tekrarlanarak *p*-nitrofenil asetat'ın otohidrolizi sonucu açığa çıkan *p*-nitrofenol miktarı belirlendi ve hCA aktivitesi için düzeltme yapıldı (Özkul ve ark., 2024).



Şekil 2. hCA enzimleri tarafından katalizlenen ester hidroliz reaksiyonu.

Yonca yaprağı ekstraktlarının karbonik anhidraz inhibitör özelliklerinin incelenmesi

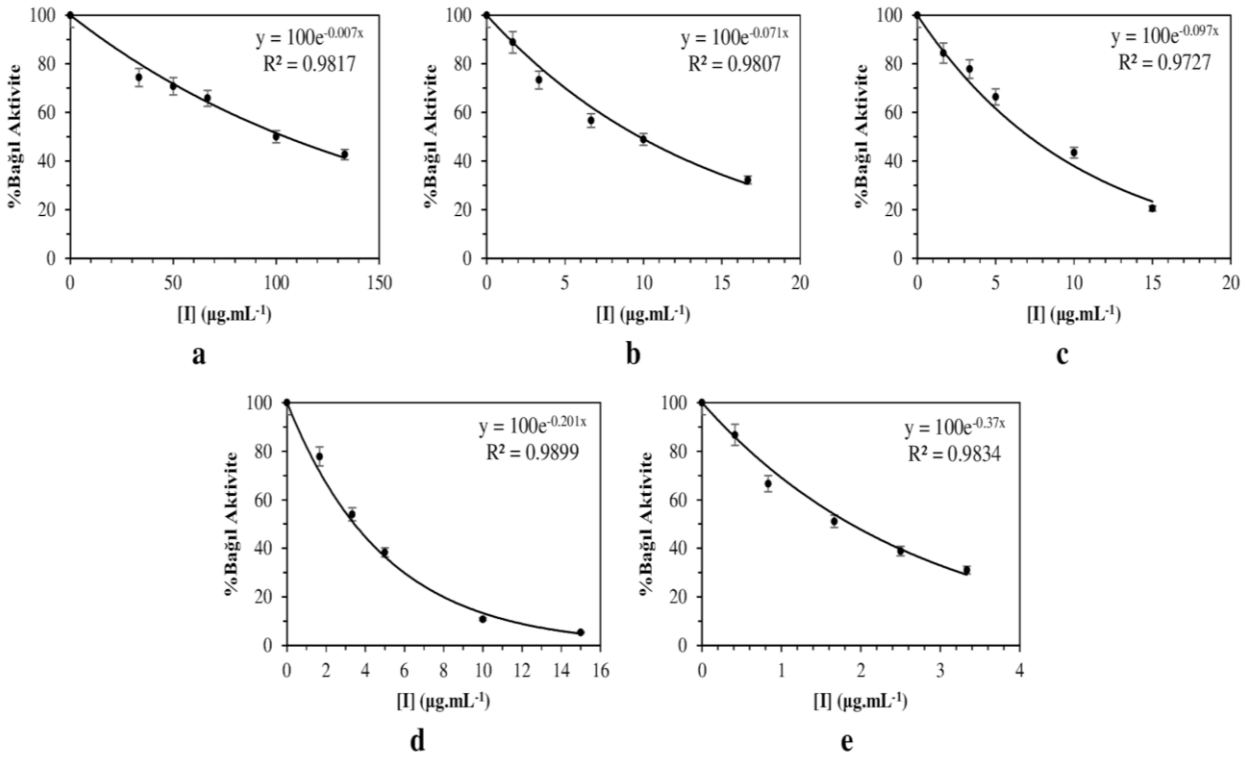
Beyaz yonca yaprağı kullanılarak hazırlanan ve çeşitli polaritelerde bileşenler içeren ekstraktların hCA I ve hCA II üzerindeki inhibisyon etkilerinin incelenmesi için ilk olarak DMSO içerisinde %0,1 (w/V) lik stok çözeltileri hazırlandı. Daha sonra farklı konsantrasyonlardaki inhibitör varlığında, hCA I ve hCA II nin esteraz aktiviteleri yukarıda açıklandığı şekilde ölçüldü. Ekstraksiyon çözücülerinin de DMSO içerisinde %0,1 (w/V) lik stok çözeltileri hazırlanarak aynı ölçümler tekrarlandı ve inhibisyon değerleri için düzeltme yapıldı. İnhibitörsüz ortamdaki enzim aktivitesi %100 kabul edilerek inhibitörlü ortamdaki bağıl enzim aktiviteleri hesaplandı. Microsoft Excel (Office 365) paket programı yardımıyla %Bağıl aktivite – [İnhibitör] eğrileri çizilerek, yeterli oranda inhibisyon etkisi gösteren bileşikler için IC_{50} değerleri hesaplandı (Okumuş ve ark; 2023).

İstatistik analiz

Tüm deneyler üç kez tekrarlandı ve ortalama ± standart sapma olarak ifade edildi. Veriler, SPSS paket programı yardımıyla (SPSS 13.0, SPSS Inc., Chicago, IL) çoklu karşılaştırmalar için tek yönlü varyans analizi kullanılarak analiz edildi. $p<0,0001$ istatistiksel olarak anlamlı kabul edildi.

BULGULAR VE TARTIŞMA

Çalışmamızda; balgam söktürücü, antiseptik, analjezik, antiromatizmal ve ishal önleyici olarak kullanılan beyaz yonca bitkisinin yaprağından hazırlanan ekstraktların, eritrositlerde yüksek oranda ifadelenen ve retinal/serebral ödem, glokom, yüksek irtifa hastalığı ve epilepsi gibi rahatsızlıklarla ilişkili olduğu bilinen hCA I ve hCA II izoenzimlerinin esteraz aktiviteleri üzerindeki etkileri *in vitro* olarak incelendi (Şekil 3). Bu kapsamda öncelikle hCA I ve hCA II izoenzimleri afinite kromatografisi ile yüksek verimde saflaştırıldı. hCA I; 1045 EU/mg spesifik aktivite ve %19,2 verimle 155,6 kat, hCA II ise 1916 EU/mg spesifik aktivite ve %26,1 verimle 301,1 kat saflaştırıldı.



Şekil 3. Beyaz yonca yaprağından hazırlanan ekstraktların hCA I esteraz aktivitesine etkileri (**a:** su, **b:** metanol, **c:** etanol, **d:** etil asetat, **e:** *n*-hekzan)

Çizilen eğrilerin denklemleri kullanılarak hesaplanan IC_{50} değerleri Tablo 1 de verilmiştir. Tablo 1 deki değerler incelendiğinde *Trifolium repens* L. yapraklarından hazırlanan ekstraktların hCA II izoenzimini inhibe etmedikleri, sadece hCA I izoenzimini inhibe ettikleri görülmektedir. Bu iki hCA izoformunun aktif bölgesinde bulunan amino asit kalıntıları birbirine çok benzemekle birlikte, farklılık gösteren kalıntılar da mevcuttur (Tablo 2). Dolayısıyla ekstraktarın izoenzim seçiciliklerini, farklılık gösteren bu amino asit kalıntılarının belirlemiş olması muhtemeldir.

Bir farmakolojik ajanın inhibisyon potansiyeline ilave olarak izoenzim seçiciliğinin olması, muhtemel yan etkilerinin en aza indirgenmesi açısından önem arz etmektedir. Çalışmamızda elde etmiş olduğumuz sonuçlar, yonca yaprağı kullanılarak doğal bir ilacın hazırlanması ve hCA I ile ilişkili hastalıklar olan retinal ve serebral ödem tedavisinde kullanılması durumunda bir diğer majör hCA izoenzimi olan hCA II izoformunun inhibe olmayacağını ve birtakım yan etkilerin görülme olasılığının daha az olacağını göstermektedir.

Tablo 1. Yonca yaprağı ekstraktlarının hCA I ve hCA II esteraz aktiviteleri üzerindeki inhibisyon etkileri.

Bileşik/Ekstrakt	**Esteraz IC_{50} (µg.mL^{-1})[a,b]**	
	hCA I	**hCA II**
Asetazolamit (AAZ)[c]	1,652±0,033	0,016±0,0001
Su	99,00±3,25	*İnhibe etmedi*
Metanol	9,76±1,01	
Etanol	7,14±0,74	
Etil asetat	3,44±0,89	
n-Hekzan	1,87±0,47	

[a]Ortalama±standart sapma değeri (n = 3). [b]p<0,0001 [c]AAZ kontrol bileşiği olup, IC_{50} değerleri Aydın ve ark. (2019)'dan alınmıştır.

Tablo 2. hCA I ve hCA II izoenzimlerinin aktif bölgelerindeki amino asit kalıntıları (Bhatt ve ark., 2017).

Kalıntı	hCA I	hCA II
7	Tyr	Tyr
62	Val	Asn
64	His	His
67	His	Asn
91	Phe	Ile
92	Gln	Gln
94	His	His
96	His	His
119	His	His
121	Ala	Val
131	Leu	Phe
135	Ala	Val
141	Leu	Leu
143	Val	Val
198	Leu	Leu
199	Thr	Thr
200	His	Thr
202	Pro	Thr

Tablo 1 de göze çarpan bir başka durum ise çözücü polaritesi azaldıkça artan inhibisyon potansiyelidir. Bu çalışma kapsamındaki en polar çözücü olan su kullanılarak hazırlanan ekstraktın IC_{50} değeri 99,00±3,25 $\mu g.mL^{-1}$ iken, en apolar çözücü olan *n*-hekzan kullanılarak hazırlanan ekstraktın inhibisyon potansiyeli yaklaşık 53 kat daha güçlü olup IC_{50} değeri 1,87±0,47 $\mu g.mL^{-1}$ dir. *n*-hekzan ekstraktının inhibisyon potansiyeli kontrol bileşiği olan ve klinikte de halen kullanılan AAZ bileşiği ile kıyaslanabilecek durumdadır. Azalan çözücü polaritesi ile artış gösteren inhibisyon potansiyeli, ortamda artış gösteren apolar bileşenlerin hCA I aktif bölgesinin hidrofobik amino asit kalıntıları ile daha sıkı etkileşime girdiklerini göstermektedir. Polar çözücülerin kullanıldığı ekstraktların inhibisyon potansiyellerinin nispeten zayıf olması, ortamdaki polar bileşenlerin aktif bölgedeki polar amino asit kalıntıları ile yeterince güçlü etkileşime giremediklerini göstermektedir. Özetle beyaz yonca yaprağı *n*-hekzan ekstraktının içerisindeki bileşenlerin kromatografik yöntemlerle ayrılıp tanımlanması ve güçlü inhibitör etkinin hangi bileşik veya bileşiklerden kaynaklandığının belirlenmesi ilaç geliştirme aşamalarında ileri basamaklar olan *in vivo* testler için kayda değer bir veri sağlayacaktır. Bu kısımda yapılan yapı-aktivite yorumları elde edilen inhibisyon sonuçlarına ve hCA I – hCA II izoenzimlerinin aktif bölge amino asit sekanslarına dayanarak yapılmıştır. Kesin bir yorum yapılabilmesi için ekstraklardaki bileşenlerin saflaştırılması, karakterize edilmesi, moleküler docking çalışmalarının gerçekleştirilmesi ve enzim-inhibitör komplekslerinin hazırlanarak X-ray tekniği ile yapılarının aydınlatılması gereklidir.

SONUÇ

Bu çalışmada beyaz yonca yaprağı ve farklı polaritelerde çözücüler (su, metanol, etanol, etil asetat ve *n*-hekzan) kullanılarak hazırlanan ekstraktların hCA I ve hCA II esteraz aktiviteleri üzerindeki etkileri *in vitro* olarak incelenmiş ve sadece hCA I izoenziminin inhibe edildiği bulunmuştur. hCA I için en güçlü inhibitörün ise *n*-hekzan kullanılarak hazırlanan ekstrakt olduğu belirlenmiştir. Doğada bol bulunan yonca bitkisinin izoenzim seçici ve güçlü inhibisyon potansiyeline sahip olması, yeni ve ekonomik ilaç etken maddesi olabilecek bileşiklerin ekonomiye kazandırılması açısından önem arz etmektedir.

TEŞEKKÜR

Çalışmamızı, 2021 yılı ikinci dönem 2209-A Üniversite Öğrencileri Araştırma Projeleri kapsamında destekleyen TÜBİTAK'a teşekkürlerimizi sunarız. (Proje No: 1919B012105233)

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ORAL PRESENTATION

Volatile aromatic compounds and consumer preferences of butter and honey-aromas added tiger nut oil oleogels prepared with beeswax oleogelator

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Abstract

This study involved in the production and characterization of oleogels from tiger nut oil flavoured with added butter and honey aromas with beeswax as organogelator. Instrumental color, free fatty acidity (FFA), and volatile aromatic compounds were measured and compared to the unflavoured control sample. In addition, one hundred voluntary consumers evaluated the oleogels for appearance/color, aroma, taste, spreadability and general acceptance. Colors of the oleogels were very similar (creamy-yellow tones) to each other, and this color is suitable for many prepared foods. The FFA of oleogels slightly exceeded the codex limit value. There was no statistical difference among the color and spreadability properties of the oleogel samples, but flavoured oleogels had higher scores than unflavoured oleogels in terms of consumer aroma and general acceptability perceptions. While the butter and honey aromas in flavoured oleogels was quantified with the volatile compounds analysis, all oleogels had common terpenic, pine and herbal defined aromatic compounds. Overall, it was suggested that tiger nut oil oleogels can be produced succesfully with different aromas. Further studies with food applications of the prepared oleogels are considered.

Keywords: tiger nut oil, beeswax, oleogel, aroma, volatile, consumer

INTRODUCTION

The isomerization of fatty acids from *cis* form to *trans* form, which occurs during the structuring of oils into the desired plasticity profile with traditional modification technique (hydrogenation) makes the increase of *trans* fatty acids in the oil inevitable. It has been proven that constant and excessive consumption of high amounts of saturated and *trans* fats increases the risk of obesity, diabetes and coronary heart diseases. Oleogels produced by the alternative oil structuring method 'oleogelation technique' have remarkabled at this matter. Lipid gels, in which the continuous liquid phase is oil, have been the subject of research in recent years as the most promising alternative to margarine, spreadable fats and shortenings (O'Brien, 2004; Naeli, 2022). Oleogels can be defined as three-dimensional oil networks created with food-grade organogelators as structured viscoelastic semi-solid systems. Many organogelators that can gel organic phases may not be suitable for use directly in food products, therefore the term "oleogelator" can be used instead of the term "organogelators" for gelling agents that provide structuring in edible vegetable oils. Oleogelators should be food-grade, GRAS (safe), natural, thermo-reversible, cheap, easily obtainable and economically applicable. Oleogels are a new alternative method for obtaining solid-fat-like vegetable oils that not contain *trans* and/or saturated fatty acids in food industry (Guo et al., 2023; Co and Marangoni, 2012). One of the most important factors affecting consumer acceptance of foods is flavour. Food researchers use the flavours and aromas during food processing to enhance their consumer acceptance and marketabilities. Even different physical delivery systems that control the flavour release rate have been developed (Huang et al., 2023). In this study, butter and honey-aromas, which are the most commonly prefered ones for spredable fats, were used for the production of tiger nut oil oleogel.
The study was aimed to prepare and characterize butter and honey-aromas enriched tiger nut oil oleogels. The purpose were to observe the effect of aroma addition on the consumer acceptance and volatile compound compositions. Further, the instrumental color and free fatty acidity of the samples were measured and all data were compared with unflavoured control sample.

MATERIALS AND METHODS

Materials

Tiger nut oil was collected by cold press technology in our laboratory (Keskin Uslu and Yılmaz, 2024). It was placed in an amber-colored bottle, flushed with nitrogen gas and stored in a cool ambient until oleogel production. Butter and honey aromas were bought from Aromsa Besin Aroma ve Katkı Maddeleri San. ve Tic. A.Ş. (Kocaeli, Türkiye). Beeswax was purchased from Kahlwax Co. (Kalh GmbH &Co., Trittau, Germany). All other chemicals and standards were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, USA) or Merck (Darmstadt, Germany).

Preperation of the oleogels

To prepare the 100 g of flavoured tiger nut oil oleogel, 89.5 g of tiger nut oil and 10 g of beeswax were weighed into a 100 mL sample container and completely melted by keeping them in a water bath at 80 °C for half an hour. Then, 0.5 g of butter and honey aromas were added and mixed for 20-25 seconds at 80 °C and 55 rpm in a shaking water bath (Termal Elektronik, 12420, Istanbul, Türkiye). After that, the mixture was cooled immediately to 20 ± 0.3 °C. Finally, the produced oleogels were kept in a refrigerator.

Color properties of the oleogels

The color properties of the oleogel samples were determined using a Minolta CR-400 (Konica Minolta Sensing, Osaka, Japan) colorimeter according to CIE standards. The L*, a*, b* values were recorded from several points of oleogel surface.

Free Fatty Acids of the oleogels

The free fatty acidity (FFA) values of the were measured with method Ca 5a-40 (AOCS, 1998).

Consumer tests of the oleogels

The oleogels were placed into transparent glass cups coded with numbers, and served to 100 volunteer consumers at room temperature, together with a slice of bread, a plastic knife, a piece of apple, water, and an expectoration cups. To score the consumer preferences, a 5-point hedonic scale (1＝dislike extremely to 5＝ like extremely) was applied. Each consumer tested the oleogel samples twice randomly on different days for appearance, smell, taste, spreadability, and general acceptance (Meilgaard et al. 1991).

Volatile aromatic compounds analysis of the oleogels

A Shimadzu GC-2010 Plus gas chromatograph equipped with an MS-QP2010 plus mass spectrometer (Shimadzu Corporation, Kyoto, Japan) was used to analyze volatile aromatic compounds of the oleogels. The method with minor modification of Krist et al. (2006) was followed. First, around 2 g of oleogel was weighted into a vial (15 mL, Clear PTFE/silicone septa cap, Supelco), and the vial placed into a water bath set at 60 °C. Then, it was kept for 15 min without fiber, and 30 min with fused silica SPME fiber CAR/PDMS（75 mm Fused Silica, Supelco Ltd., Bellefonte, PA, USA) inserted. The volatiles were picked up on a fused silica SPME CAR/PDMS (75 mm Fused Silica, Supelco Ltd., Bellefonte, PA, USA) fiber with the SPME technique. The volatiles adsorbed fiber was placed into the injection port of the GC, and then volatiles were desorbed at 250 °C for 5 min. The compound separation was achieved with an Rx-5Sil MS capillary column (30 m-0.25 mm × 0.25 mm; catalog no: Restek 13623, Restek, Bellefonte, PA, USA). Helium was the carrier gas at a flow rate of 1.61 mL/min. The volatiles adsorbed fiber was placed into the injection port of the GC, and then volatiles were desorbed at 250 °C for 5 min. The injector and detector were held at 250 °C, respectively. The ionization energy was 70 eV, and mass spectra analysis was performed in a scan mode between the 40 and 300 amu mass range. The analysis conditions were as follows: oven temperature, waiting at 40 °C for 2 min, heating to 250 °C (4 °C/min), and keeping at that temperature for 5 min. Volatile compounds were tentatively identified by Wiley and Nist mass spectra libraries. The identified and quantified volatiles presented with retention times and % area values.

Statistical analysis

The three oleogels were produced at two different times as two replicates, and all anaylsis for each replicate sample were accomplished in triplicate. The data presented were the means of six measurements with standard deviations. The Analysis of Variance (ANOVA), and Tukey's tests were completed for comparison. Statistical analysis were performed with Minitab v.16.1 software. The level of confidence was at least 95% in this study (Minitab, 2010).

RESULTS AND DISCUSSION

Color properties

As shown in Table 1, the CIE color values (L*, a*, b*) of the oleogels were very similar. The L* values (the luminosity) of the oleogels varied between 43.17 and 44.74. The a* values ranged from -4.20 to -3.92 indicating that all had some green color tones (negative a* values). Similarly, the b* value (level of yellowness) of the TBO-HO was the lowest (17.53), while TBO-BU had the highest value (18.64). It was stated that the L*, a* and b* color property of an oleogel was impacted by the liquid oil type, oleogelator type or concentration (Sharma et al., 2023). Since the oil type and oleogelator concentration were the same in all oleogels, slight difference in color values might be caused by the added aromas.

Free fatty acidity (FFA)

The measured FFA values of the oleogels were in the range of 2.93-3.82% linoleate (Table 1). According to edible vegetable oil codex, the consented upper limits of acid value for virgin oils must be 4.0 mg KOH/g oil (Codex, 2017). As the equivalence acid value of the measured FFA, it was observed that the FFA of oleogels slightly exceeded the codex limit value. It has been previously reported that there are some long-chain saturated FFA in the natural beeswax (around 13%). The enhanced FFA are thought to be originated from the beeswax oleogelator added (Aydeniz Güneşer et al., 2021).

Table 1. Color and free fatty acidity values of the oleogels prepared

	L*	a*	b*	Free fatty acid (linoleic %)
TBO	$44.16 \pm 0.56^{a\dagger}$	-3.92 ± 0.03^{a}	18.16 ± 0.57^{a}	$3.82^{a} \pm 0.31^{a}$
TBO-BU	44.70 ± 0.58^{a}	-4.20 ± 0.14^{b}	18.64 ± 0.58^{a}	$3.00^{b} \pm 0.17^{b}$
TBO-HO	43.17 ± 0.33^{b}	-4.12 ± 0.11^{b}	17.53 ± 0.42^{b}	$2.93^{b} \pm 0.11^{b}$

†Small letters within each column indicate significant differences among the samples for the mean ± SD values by one-way analysis of variance and Tukey's test ($p \leq 0.05$). TBO: tiger nut oil-beeswax oleogel, TBO-BU: tiger nut oil-beeswax-butter flavoured oleogel, TBO-HO: tiger nut oil-beeswax-honey flavoured oleogel

Consumer tests

Five sensory attributes evaluated by 100 volunteer consumers with the hedonic scale were shown in Table 2. The appearance of all oleogels were not different and above the neutrality (3.0) point. The aroma properties of TBO-BU and TBO-HO (4.56, 4.61) were higher than TBO (4.11). Among the oleogels, taste of TBO-HO was liked the most, while taste of the TBO was the least preferred. Still, the taste scores of the oleogels were above the neutrality point. The spreadability properties of the oleogels were determined to be 3.53, 3.45, 3.69 for TBO, TBO-BU and TBO-HO, respectively. Total acceptabilities of TBO-BU and TBO-HO samples (4.51 and 4.50) were higher than the TBO sample (4.05). Clearly, the TBO-HO was the most liked sample by the consumers (overall general acceptance score of 4.60). Overall, the flavoured oleogels were liked and accepted by the consumers. Yılmaz et al. (2015) proved that the flavours added to BW fish oil oleogels could improve the palatability of the products, and our study aggreed to that conclusion.

Table 2. The consumer test scores of the oleogels prepared

	TBO	TBO-BU	TBO-HO
Apperance/color	$3.84 \pm 0.19^{a\dagger}$	3.86 ± 0.17^{a}	3.98 ± 0.26^{a}
Aroma	4.11 ± 0.37^{b}	4.56 ± 0.39^{a}	4.61 ± 0.33^{a}
Taste	3.04 ± 0.39^{c}	3.86 ± 0.31^{b}	4.59 ± 0.44^{a}
Spreadability	3.53 ± 0.55^{a}	3.45 ± 0.28^{a}	3.69 ± 0.29^{a}
General acceptance	4.05 ± 0.33^{b}	4.51 ± 0.35^{a}	4.60 ± 0.29^{a}

†Small letters within each row indicate significant differences among the samples for the mean ± SD values by one-way analysis of variance and Tukey's test ($p \leq 0.05$). TBO: tiger nut oil-beeswax oleogel, TBO-BU: tiger nut oil-beeswax-butter flavoured oleogel, TBO-HO: tiger nut oil-beeswax-honey flavoured oleogel

Volatile Aromatics Compositions

The volatile aromatic compounds of TBO sample included 28, TBO-BU sample included 26 and TBO-HO sample included 32 identified molecules (Table 3). It was assumed that the differences in the number of volatile compounds could be due to the effects of added aromas. In TBO, limonene (85.11%), β-myrcene (3.07%), and nonanal (1.69%) were the most common volatiles. These compounds provide terpenic, pine, herbal, peppery, fresh, waxy, orange peel aroma attributes. The volatile compounds with higher amounts in TBO-BU sample were limonene (34.71%), diacetyl (30.67%), acetic acid (15.46%), n-butyric acid (10.63%), and 2-heptanone (2.25%), respectively. These compounds were usually described with terpenic, pine, herbal, butter, cheese, sharp, vinegar, rancid and sweet definitions. Finally, the most detected volatile compounds in the TBO-HO were limonene (63.25%), ethyl phenylacetate (24.03%), β-myrcene (2.01%), ethyl butanoate (2.05%) and acetic acid (1.11%). These compounds showed sweet, creamy, honey, terpenic, pine, herbal, peppery, sour and vinegar descriptors. In our previous study, the aromatic volatile composition of cold press TO was identified with 40 volatile compounds, and the most common of them were furfural (24.27%), 2,5-dimethyl pyrazine (15.86%), acetic acid (13.66%) described with almond, sweet, caramel, and roasted (Keskin Uslu and Yılmaz, 2024). Felicioli et al. (2019), reported that almost 82 volatile components of BW were identified, and the commonly detected compounds were ethanol, limonene and nonanal. These compounds were also observed in all TBO, TBO-BU and TBO-HO samples. The volatile composition data were in accordance with the consumer preference findings.

Table 3. The volatile aromatics compounds of the oleogels prepared

RT* (min)	Volatile compound	Aroma/Flavour Definition**	TBO		TBO-BU		TBO-HO	
			Mean Peak Area	Peak Value	Mean Peak Area	Peak Value	Mean Peak Area	Peak Value
1.174	β-Ionone epoxide	Fruity, sweet, berry, woody, violet	104307 ± 3128	0.18	92841 ± 1856	0.09	92914 ± 3718	0.13
1.633	Isobutyraldehyde	Fresh, aldehydic floral, green	34773 ± 8751	0.06	nd.	nd.	nd.	nd.
1.761	Diacetyl	Butter	nd.	nd.	31869313 ± 637186	30.67	nd.	nd.
1.813	Acetic acid	Sharp, sour, vinegar	763461 ± 53245	1.33	16056921 ± 963421	15.46	782987 ± 2349	1.11
1.896	Ethyl Acetate	Ethereal, Fruity, sweet, weedy, green	287557 ± 8626	0.50	nd.	nd.	206856 ± 82645	0.29
2.201	2-Butenal	Fresh, fruity	nd.	nd.	751489 ± 3765	0.72	nd.	nd.
2.250	3-Methylbutanal	Sour, fruity, chocolate	102659 ± 2053	0.18	nd.	nd.	72889 ± 2156	0.10
2.340	2-Methylbutanal	Musty, chocolate, nutty, furfural	88522 ± 17705	0.15	nd.	nd.	53411 ± 277	0.08
2.700	Pentanal	Fermented bready, fruity, nutty, berry	91008 ± 2275	0.16	nd.	nd.	nd.	nd.
3.062	Acetol	Butter, cream	nd.	nd.	136732 ± 34269	0.13	nd.	nd.
4.299	Hexane-2,3-dione	Creamy,buttery, fruity, toasted, brown caramellic	nd.	nd.	55858 ± 1787	0.05	nd.	nd.
4.611	Hexanal	Fresh,grassy, leafy, fruity, sweaty	637871 ± 9248	1.11	292427 ± 68751	0.28	nd.	nd.
4.660	Ethyl butanoate		nd.	nd.	nd.	nd.	1441980 ± 75845	2.05
5.141	2,4-Dimethylheptane	Chickpea seed	33239 ± 7682	0.06	nd.	nd.	28158 ± 6659	0.04
5.625	n-Butyric acid	Rancid, cheese, sweat, butter	nd.	nd.	11044823 ± 236991	10.63	nd.	nd.

6.896	3-Methylbutyl acetate	Sweet fruity, banana	nd.	nd.	nd.	nd.	308045 ± 72852	0.44
6.970	2-Methylbutyl acetate	Sweet banana, fruity ripe,tropical juicy, fruit	nd.	nd.	nd.	nd.	96649 ± 24771	0.14
7.277	2-Heptanone	Dairy, blue cheese	nd.	nd.	2339821 ± 55346	2.25	nd.	nd.
7.310	Styrene	Sweet, balsam, floral, plastic	171520 ± 4956	0.30	nd.	nd.	110712 ± 26183	0.16
7.692	Heptanal	Fresh, aldehydic fatty, green herbal, wine-lee, ozone	234352 ± 5424	0.41	nd.	nd.	nd.	nd.
7.928	2,4-Dimethylpentan-3-one		nd.	nd.	85666 ± 278	0.08	nd.	nd.
7.975	Butyrolactone	Creamy, oily, fatty caramellic	67740 ± 1561	0.12	nd.	nd.	nd.	nd.
8.104	2,6-Dimethylpyrazine	Cocoa nutty, brown coffee, butter milk	218512 ± 55982	0.38	161481 ± 41058	0.16	170091 ± 39556	0.24
8.751	alpha- Pinene	Woody-pyne, terpentine, spicy	145892 ± 35874	0.25	nd.	nd.	87790 ± 20683	0.12
10.234	Sabinene	Woody, terpene, citrus pine, spice	39071 ± 10012	0.07	nd.	nd.	52045 ± 1333	0.07
10.939	β- Myrcene	Peppery, terpene, spicy, balsam	1759011 ± 45658	3.07	1007602 ± 26071	0.97	1414580 ± 346855	2.01
11.255	2-Ethyl-6-methylpyrazine	Roasted potato	82608 ± 21643	0.14	nd.	nd.	71823 ± 1043	0.10
11.304	Hexanoic acid	Pungent, fruity	nd.	nd.	109928 ± 27989	0.11	nd.	nd.
11.370	Decane	Alcane	103655 ± 33758	0.18	56254 ± 14415	0.05	577119 ± 1468	0.82
11.484	Octanal	Aldehydic waxy, citrus, orange peel, green herbal, fresh, fatty	875044 ± 22486	1.53	151495 ± 38831	0.15	296667 ± 75605	0.42
11.626	Sabinene hydrate	Minty, eucalyptus, green terpene	118211 ± 298	0.21	62182 ± 1593	0.06	152166 ± 38994	0.22

11.798	4-Methyldecane	Sweet, balsamic	nd.	nd.	37403 ± 95856	0.04	54012 ± 1437	0.08
12.251	*p*-Cymene	Fresh, citrus, terpenic, wo odyspicy	140956 ± 35914	0.25	116212 ± 36323	0.11	104648 ± 26821	0.15
12.472	Limonene	Terpenic, pine, herbal, peppery	48786945 ± 12633	85.11	36064618 ± 91913	34.71	44508998 ± 110312	63.25
14.312	4,8-Dimethyl-1-nonanol	Strawberry-like	64028 ± 1631	0.11	66212 ± 1685	0.06	153700 ± 39159	0.22
14.482	1-Dodecene	Butter	71862 ± 1805	0.13	71679 ± 1846	0.07	96468 ± 24576	0.14
14.565	Allyl caproate		nd.	nd.	nd.	nd.	147906 ± 34986	0.21
14.892	2-Nonanone	Hot milk	nd.	nd.	1067569 ± 276233	1.03	nd.	nd.
15.431	Nonanal	Waxy, aldehydic, rose, fresh, orange peel	970960 ± 12075	1.69	265287 ± 68629	0.26	189785 ± 48936	0.27
15.521	3-Methylbutyl 3-methylbutyrate	Sweet fruity, green apple, estry	nd.	nd.	271763 ± 64051	0.39	nd.	nd.
18.981	Ethyl caprylate	Sweet	nd.	nd.	nd.	nd.	104253 ± 22368	0.15
19.099	Dodecane	Alkane	69775 ± 16501	0.12	51654 ± 16141	0.05	280626 ± 12256	0.40
19.297	Decanal	Soap, orange peel, butter	219906 ± 51677	0.38	nd.	nd.	47160 ± 11101	0.07
20.660	Ethyl phenylacetate	Sweet, honey, floral	nd.	nd.	nd.	nd.	16910686 ± 398483	24.03
21.115	Anisaldehyde	Sweet, mimosa, hawthorn	nd.	nd.	nd.	nd.	364149 ± 118566	0.52
23.514	5-Butylnonane	Sandalwood	nd.	nd.	nd.	nd.	82936 ± 19545	0.12
29.160	delta- Decalactone	Dairy, coconut	nd.	nd.	1232672 ± 290503	1.19	nd.	nd.

*RT: Retention time on HP 5MS column; Peak value unit: %, **Aroma definitions of the compounds were found on the web pages of https://www.flavournet.org/flavournet.html and www.thegoodscentscompany.com, nd: not detected

CONCLUSION

In this study, tiger nut oil oleogels were succesfully prepared with beeswax and flavoured with butter and honey aromas. The flavoured samples were compared with unflavoured control. The colors of the oleogels were quite similar. The FFA values of the oleogels were in the range of 2.93-3.82% linoleate. In general, consumers liked and accepted the flavoured oleogels more than the control sample. While terpenic, pine and herbal aromatics were felt as common aromatics in all samples, the butter and honey aromas were only detected in the flavoured samples. In conclusion, this study was proved that the unconventional tiger nut oil could be utilized in flavoured oleogel production to offer consumers new spreadable fat products. Further, these prepared oleogels can be used in different food formulations as solid fat sources.

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ORAL PRESENTATION

Morphologic differences of *in situ* generated molybdenum sulfide by changing substrates at liquid/liquid interfaces

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Abstract

Liquid/liquid interfaces is versatile electrochemical system formed two immiscible solutions, which provide successfully separation reactants and products as well as inhibited side-reactions. In this system, two phases consist of acidic aqueous solution and 1,2-dichloroethane (DCE) containing electron donor molecule, namely decamethylferrocene (DMFc). In this study, molybdenum sulfide occurred at the interface between two phases by reducing $(NH_4)_2MoS_4$ at aqueous phase using DMFc at organic phase. Herein, carbon-based substrate materials dispersed in aqueous phase and generated carbon-based nanocomposites at the interface between water and DCE. Morphological analyses of the generated composites have been performed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) techniques. This paper is focused the changing of size and shape of *in situ* generated molybdenum sulfide on different carbon-based substrates.

Keywords: liquid/liquid interfaces, electrodeposition, molybdenum sulfide, carbon-based composites

INTRODUCTION

The liquid/liquid interface strategy is a highly effective, simple, and direct method for producing nanocrystalline films of metals, metal chalcogenides, and metal oxides through self-assembly at the liquid-liquid interface. Self-assembly is a powerful approach for creating novel nanomaterial structures of significant academic interest. Nanomaterials in film form are particularly important due to their potential applications in nanodevices. This method typically involves a single step to form nanocrystalline films at the interface between two immiscible liquids, usually water and an organic solvent. The interface, which is nonhomogeneous and typically only a few nanometers thick, provides an ideal environment for the self-assembly and chemical manipulation of nanocrystals (Keti & Katharina, 2016; Rao & Kalyanikutty, 2008). The organic–aqueous (liquid–liquid) interface remains underutilized for materials synthesis. In this study, we illustrate the facile generation of ultrathin nanocrystalline films of metals, including gold and silver, as well as inorganic materials such as metals and metal chalcogenides, at the liquid–liquid interface. Notably, this method also yields single-crystalline films of specific metal chalcogenides. The gold films formed at the toluene–water interface consist of relatively monodisperse, closely packed nanocrystals. The characteristics and properties of these films are influenced by various reaction parameters, including reaction temperature, duration, reactant concentrations, mechanical vibrations, and the viscosity of the medium (Rao & Kalyanikutty, 2008). At the liquid–liquid interface, a diverse range of nanostructures is formed through the assembly and reaction of molecules and substances. This diversity is particularly pronounced in processes involving organic molecules, which exhibit flexible shapes and low structural symmetry. Among the various material processing techniques, thin-film deposition is one of the most effective and efficient methods. A thin film is an extremely thin, continuous layer of material—ranging from one atom to several microns in thickness—deposited on a substrate. Processing materials as thin films offers several advantages, including direct integration into multi-component or multi-layer devices, the ability to coat nearly any substrate, and significant material and cost savings. Thin-film technology is widely utilized in numerous everyday applications, such as batteries, transistors, solar cells, supercapacitors, fuel cells, optics, sensors, microelectronics, transparent electrodes, magnetic devices, coatings, corrosion protection, micro-mechanics, actuators, biological implants, magnetic shielding, catalysis, data storage media, flat panel displays, food packaging, electrochromism, and more. The key innovation of this technique lies in leveraging the liquid–liquid interface to synthesize materials

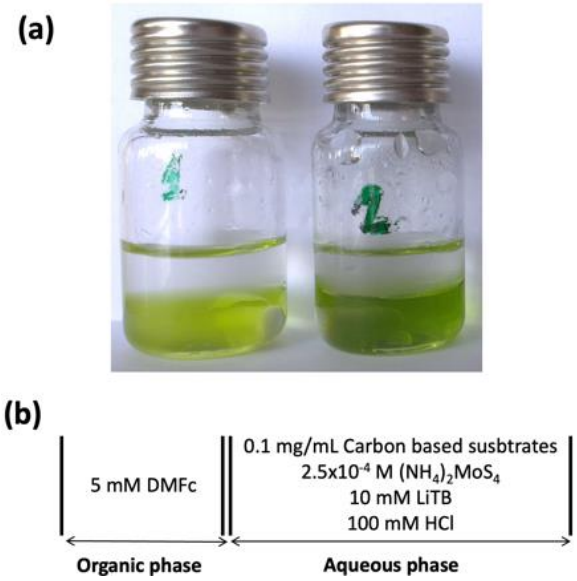
directly as thin films, combining synthesis and processing in a single-pot, single-step approach. This method holds immense potential for creating novel multi-component (composite) materials, enabling the production of thin films that are otherwise unattainable through other experimental approaches.

The liquid/liquid interface comprises two distinct phases: an aqueous phase and an organic phase. This interface functions as a working electrode, where catalysts are adsorbed to facilitate catalytic reactions. Compared to solid electrodes, the liquid/liquid interface offers significant advantages, including high reproducibility and repeatability in catalytic measurements. Additionally, this interface is considered defect-free relative to traditional solid/liquid interfaces because it allows for the separation of reactants, preventing unwanted direct interactions (Toth & Dryfe, 2015). Furthermore, the low ohmic drop observed at liquid/liquid interfaces results in clean voltammetric responses (Peljo & Girault). Electrochemical processes at these soft interfaces have proven beneficial in various applications, including chemical sensing, drug delivery, phase-transfer catalysis, and energy conversion systems, by emulating the properties of biological membranes (Reymond et al., 2000). Electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) offers a highly reproducible and defect-free surface, making it an ideal platform for measuring charge transfer reactions such as heterogeneous electron transfer, ion transfer, or proton-coupled electron transfer (PCET) (Mendez et al., 2010). This interface can be controlled by applying a Galvani potential difference to facilitate these charge transfer reactions (Méndez et al., 2010). The polarizable nature of ITIES enables a wide range of electrochemical applications, including sensors, macromolecule detectors, and energy-conversion systems (e.g., oxygen reduction, hydrogen evolution, and carbon dioxide utilization) (Hirunpinyopas et al., 2017; Lestari et al., 2014; Rastgar et al., 2014). Notably, ITIES is advantageous for the electrodeposition of nanoparticulate metals, such as for hydrogen evolution reactions (HER), due to its molecularly smooth surface (Aslan et al., 2015a; Nieminen et al., 2011; Platt & Dryfe, 2005). Nieminen *et al.* have explored the electrodeposition of platinum and palladium nanoparticles at this interface for proton reduction (Nieminen et al., 2011). Moreover, the soft interface is an effective platform for the electrodeposition of metallic nanoparticles, including platinum group metals, silver, and gold (Trojánek et al., 2007). Research conducted by Dryfe's group has successfully obtained platinum group noble metal nanoparticles on templates, with controlled structural and morphological characteristics (Ananthoju et al., 2019; Toth, Haigh, et al., 2015; Toth, Rodgers, et al., 2015). However, to date, our research group is the only one to report on the *in situ* electrodeposition of non-metallic molybdenum sulfide (MoS_x) at liquid/liquid interfaces (Aslan & Patir, 2021).

In this study, the experimental setup involved an acidic aqueous solution and 1,2-dichloroethane (DCE), which contained the electron donor molecule decamethylferrocene (DMFc). In this study, molybdenum sulfide was synthesized at the interface of these two phases by reducing $(NH_4)_2MoS_4$ in the aqueous phase using DMFc from the organic phase. Carbon-based substrates, including carbon nanotubes and graphene derivatives, were dispersed in the aqueous phase and formed carbon-based nanocomposites at the interface between the water and DCE. Morphological characterizations of the resulting composites were conducted using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). This study specifically examines the variations in size and shape of the *in situ* generated molybdenum sulfide when different carbon-based substrates are used.

MATERIALS AND METHODS

Two-phase reactions were performed in the 11 mL head-space glass flask. This reaction cell is firstly filled with 2 mL of organic phase including DMFc. Then, 2 mL of aqueous phased added onto the organic phase including HCl, lithium tetrakis(pentafluorophenyl)borate ethyl etherate (LiTB), $(NH_4)_2MoS_4$ and carbon-based substrates, which are played role as proton source, phase transfer catalyst, molybdenum sulfide precursor salt and substrate for composite materials, respectively. The concentration and combination of two-phase reaction are given in **Scheme 1a** as photographic and **Scheme 1b** as a schematic representation.



Scheme 1. (a) Photographic and **(b)** schematic representation of two-phase reactions (in the absence **(1)** and presence **(2)** of $(NH_4)_2MoS_4$ and carbon-based substrates).

RESULTS and DISCUSSION

This study is based on our previous studies about hydrogen evolution of this materials (Aslan & Patir, 2021; Emre Aslan et al., 2021; E. Aslan et al., 2021), however, the morphological comparison of these materials is reported for the first time. This study is based on *in situ* generated MoS_x particles at liquid/liquid interfaces in the absence and presence of carbon-based substrates, such as multi-walled carbon nanotubes (CNT) and reduced graphene oxide (rGO). Herein, MoS_x can be obtained by reduction of $(NH_4)_2MoS_4$. When both graphene oxide (GO) and $(NH_4)_2MoS_4$ added in the aqueous phase, they can be reduced by DMFc organic electron donor from organic phase to obtain rGO/MoS_x (**Equation 1 and 2**). In addition, CNT is the non-reactive material, when both CNT and $(NH_4)_2MoS_4$ added in the aqueous phase, they can be reduced by DMFc organic electron donor from organic phase to obtain CNT/MoS_x.

$$DMFc^{o} + (NH_4)_2MoS_4 \rightarrow DMFc^{+,o} + MoS_x \quad (1)$$

$$DMFc^{o} + GO \rightarrow DMFc^{+,o} + rGO \quad (2)$$

The morphological characteristics of the *in situ* electrodeposited MoS_x catalyst were analyzed using SEM and TEM. The SEM images (**Figure 1a**) of the *in situ* synthesized bare MoS_x, taken at 30,000x magnification, revealed a uniform spherical morphology. During the reaction, these spherical particles exhibited a tendency to agglomerate, with the agglomerated structures ranging in size from 50 to 80 nm. The TEM analysis (**Figure 1b**) further demonstrated a homogeneous distribution of the MoS_x particles, although they also displayed a tendency to aggregate. The particle size distribution observed through TEM ranged from 12 to 20 nm. The size discrepancies observed between the TEM and SEM images can be attributed to particle agglomeration, consistent with the amorphous MoS_x structures reported in the literature (Dinda et al., 2016).

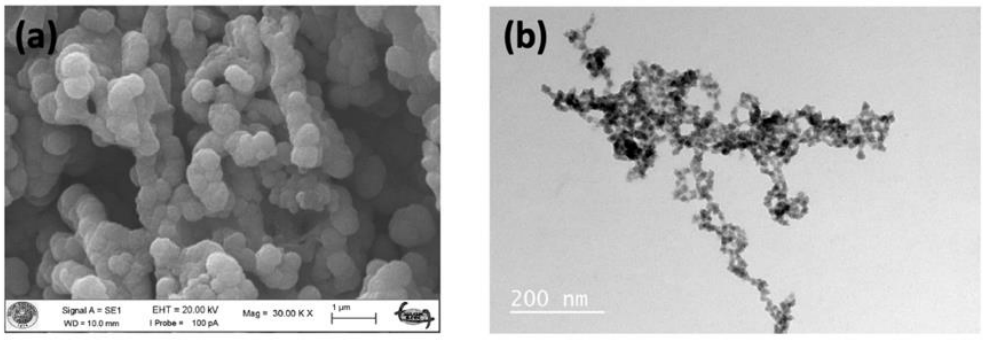


Figure 1. (a) SEM and **(b)** TEM images of *in situ* generated bare MoS_x at liquid/liquid interfaces.

The elemental distribution of the *in situ* electrodeposited MoS_x was analyzed using TEM, as depicted in **Figure 2**. The elemental maps in **Figures 2b**, **2c**, and **2d** reveal a uniform distribution of molybdenum (Mo) and sulfur (S) throughout the MoS_x structure. The inset table in **Figure 3** presents the elemental composition of MoS_x, derived from the energy-dispersive X-ray spectroscopy (EDS) spectrum shown in **Figure 3**. The sulfur-to-molybdenum (S:Mo) ratio in MoS_x, determined through three independent sampling experiments, was found to range between 2.6 and 2.9, which aligns with the expected ratio for amorphous MoS_x structures (Dinda et al., 2016). Additionally, the EDS spectrum confirmed the absence of impurities, indicating that the catalyst consists solely of Mo and S.

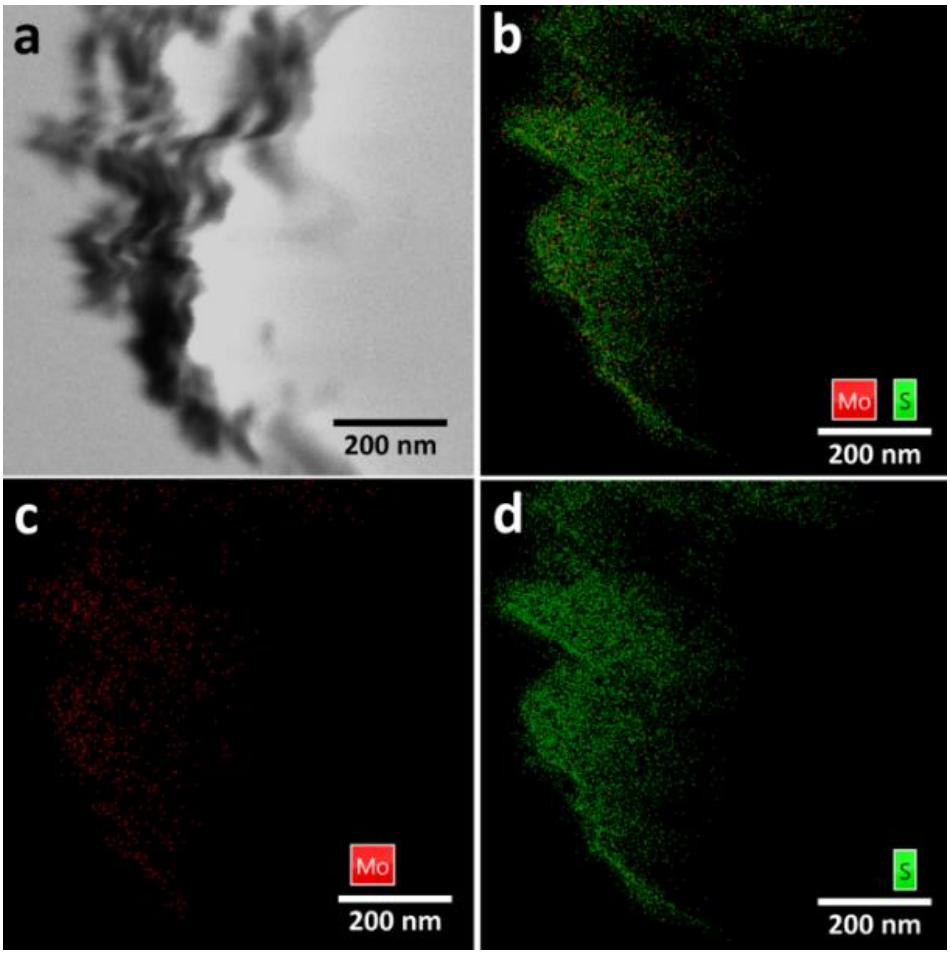
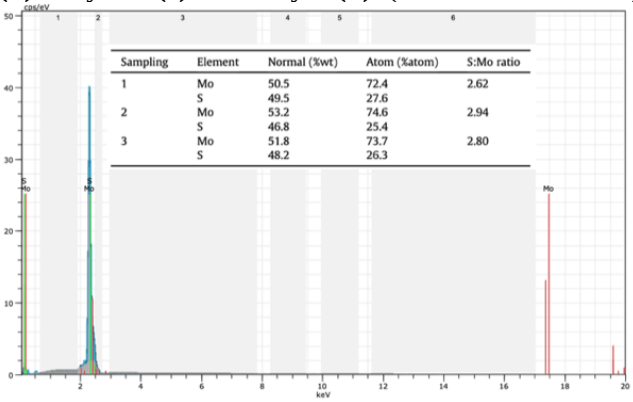


Figure 2. Elemental mapping of *in situ* generated MoS_x catalyst with original image **(a)**, both Mo and S **(b)**, only Mo **(c)**, and only S **(d)**. (Aslan & Patir, 2021)



Sampling	Element	Normal (%wt)	Atom (%atom)	S:Mo ratio
1	Mo	50.5	72.4	2.62
	S	49.5	27.6	
2	Mo	53.2	74.6	2.94
	S	46.8	25.4	
3	Mo	51.8	73.7	2.80
	S	48.2	26.3	

Figure 3. EDS spectra of bare MoS_x obtained from SEM-EDS. Inset: elemental analysis of bare MoS_x. (Aslan & Patir, 2021)

The morphological characteristics of the *in situ* synthesized rGO/MoS_x nanocomposite catalyst were examined using field emission scanning electron microscopy (FESEM) and TEM. The FESEM image of rGO/MoS_x, presented in **Figure 4a**, reveals that MoS_x particles are uniformly deposited with spherical shapes on the rGO nanosheets. Agglomeration of the MoS_x particles on the rGO surface is also evident. This agglomeration, along with the irregular shapes of MoS_x particles, is further illustrated in the TEM image (**Figure 4b**). The TEM analysis confirms that MoS_x particles on rGO predominantly exhibit spherical shapes with an approximate size of 10 nm, which is slightly reduced compared to the free-standing MoS_x particles (Aslan & Patir, 2021). This observation is consistent with the behavior of *in situ* generated catalysts on carbon-based substrates at liquid/liquid interfaces (Emre Aslan et al., 2016; Aslan & Patir, 2022; Emre Aslan et al., 2021). Additionally, some larger particles, around 30 nm in size, are observed, likely resulting from the aggregation of smaller particles due to reduced surface energy (Emre Aslan et al., 2016; Aslan et al., 2015a).

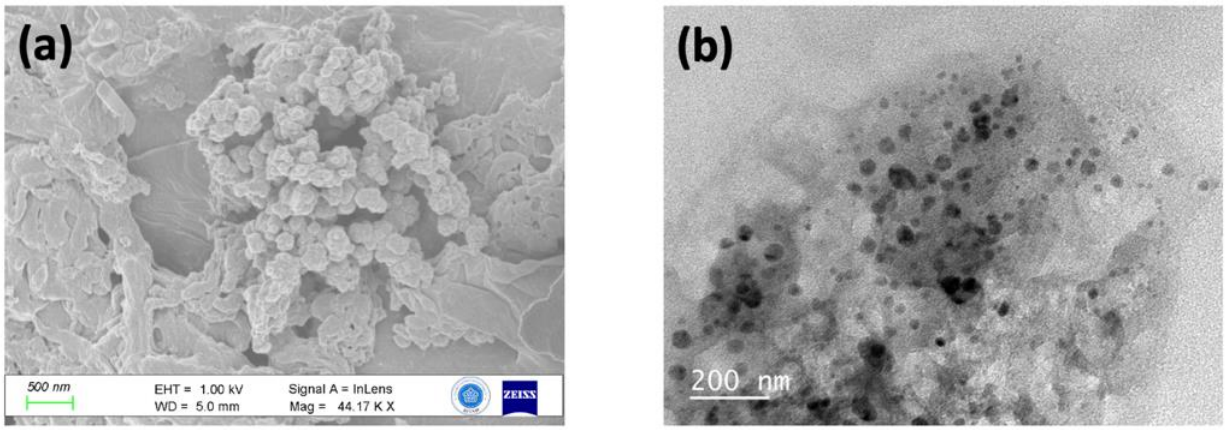


Figure 4. (a) SEM and **(b)** TEM images of *in situ* generated rGO/MoS_x at liquid/liquid interfaces.

Elemental mapping of the rGO/MoS_x nanocomposite catalyst was conducted using both FESEM and TEM techniques, as illustrated in **Figure 5**. The results clearly demonstrate that the MoS_x particles are uniformly distributed across the rGO nanosheets. The EDS spectrum obtained from these mapping studies, shown in **Figure 6**, indicates the absence of impurities in the rGO/MoS_x nanocomposite, with the exception of some gold peaks observed in the FESEM-EDS analysis, attributed to the gold coating applied prior to examination. The elemental ratio of sulfur to molybdenum (S:Mo) on the rGO/MoS_x nanocomposite was determined to be approximately 2.9, as indicated by the FESEM-based EDS analysis (see **Figure 6** inset). This ratio shows minimal variation compared to the elemental composition of the free-standing MoS_x particles (Aslan & Patir, 2021).

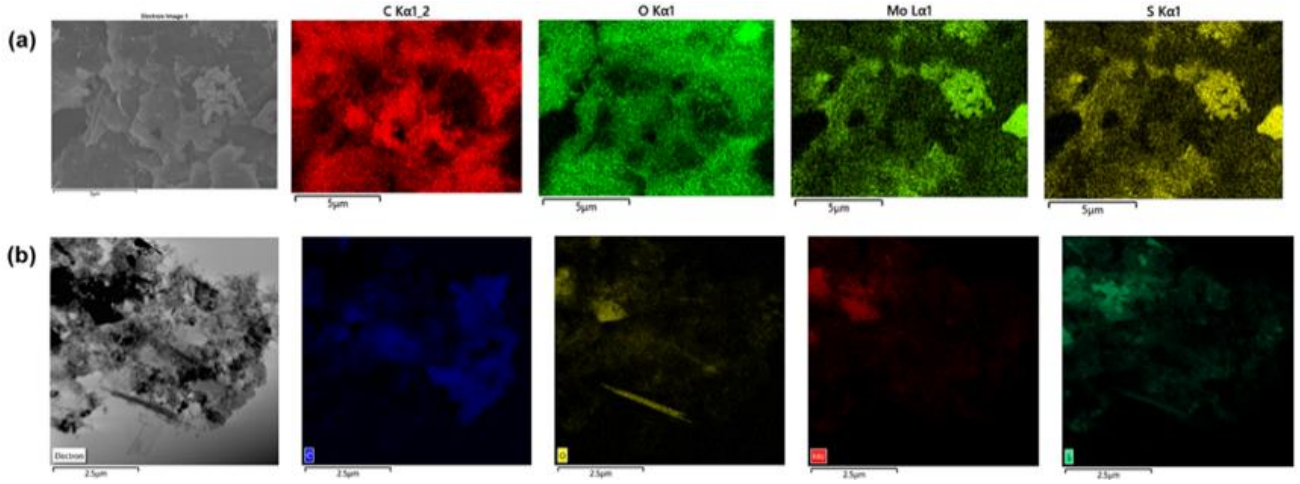
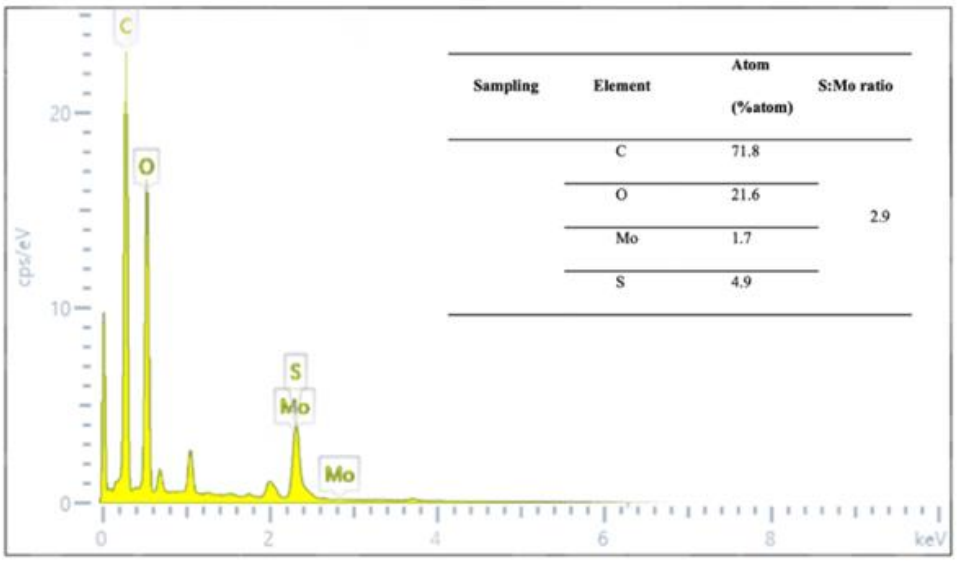


Figure 5. Elemental mapping results of rGO/MoS_x obtained from **(a)** SEM-EDS and **(b)** TEM-EDS. (E. Aslan et al., 2021)



Sampling	Element	Atom (%atom)	S:Mo ratio
	C	71.8	
	O	21.6	2.9
	Mo	1.7	
	S	4.9	

Figure 6. EDS spectra of rGO/MoS_x obtained from SEM-EDS. (E. Aslan et al., 2021)

The morphological characteristics of the CNT/MoS_x nanocomposite were analyzed using FESEM and TEM techniques. **Figure 7a** presents the FESEM images of the CNT/MoS_x nanocomposite at 30,000x magnification, revealing a uniform distribution of spherical MoS_x particles with some agglomeration on the CNT. However, compared to free-standing MoS_x (Aslan & Patir, 2021), the degree of agglomeration is reduced due to the stabilizing effect of CNT, which lowers the surface energy and stabilizes electrons (Emre Aslan et al., 2016; E. Aslan et al., 2016; Murthy et al., 2018). **Figure 7b** illustrates the TEM images, which show MoS_x particles on the CNT with ill-defined spherical and cubic-like shapes. The particle size of MoS_x on CNT is smaller compared to free MoS_x (Aslan & Patir, 2021), aligning with previous studies on *in situ* electrodeposited CNT composite catalysts (Emre Aslan et al., 2016; Aslan et al., 2015b). Additionally, zones of agglomeration were observed on the CNT, likely resulting from the amorphous nature of MoS_x (Dinda et al., 2016).

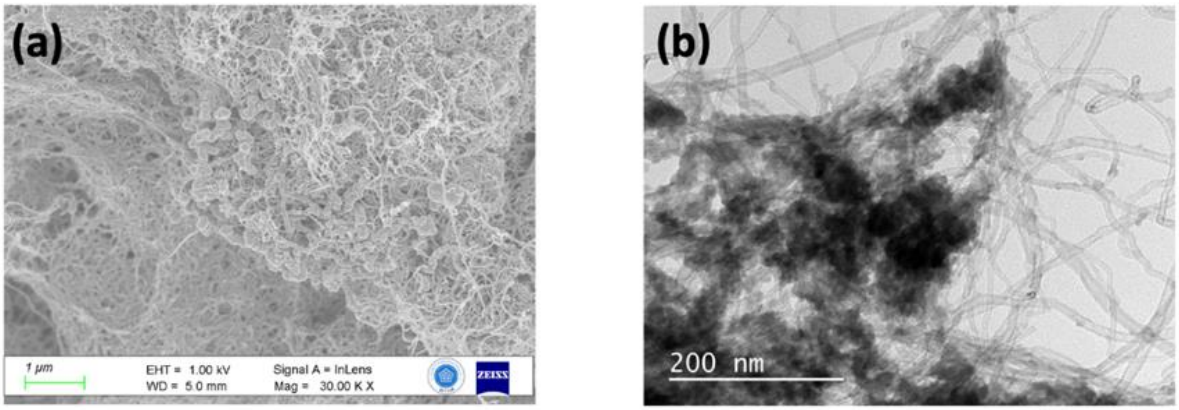


Figure 7. (a) SEM and **(b)** TEM images of *in situ* generated CNT/MoS_x at liquid/liquid interfaces.

Elemental mapping of the CNT/MoS_x nanocomposite was conducted using both FESEM and TEM techniques, as illustrated in **Figures 8a** and **8b**, respectively. These studies revealed a homogeneous distribution of MoS_x particles on the CNT, consistent with the EDS spectrum results obtained from these methods (**Figure 9**). The elemental ratio of S to Mo in the CNT/MoS_x nanocomposite was determined to be approximately 2.2 based on the FESEM-EDS analysis (**Figure 9**, inset table), indicating a decrease in the S/Mo ratio compared to free MoS_x (Aslan & Patir, 2021).

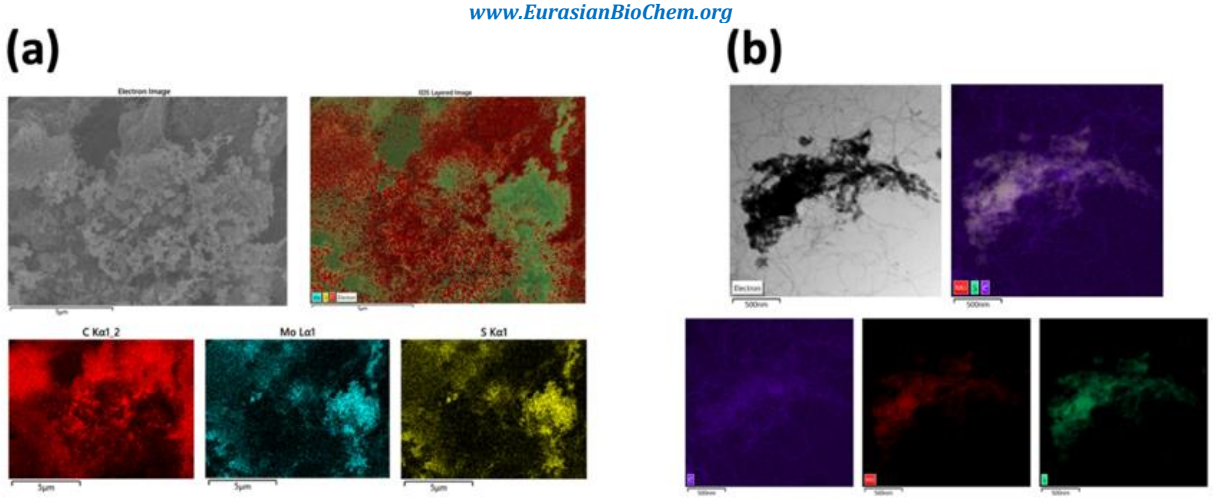


Figure 8. Elemental mapping results of CNT/MoS_x obtained from **(a)** SEM-EDS and **(b)** TEM-EDS. (Emre Aslan et al., 2021)

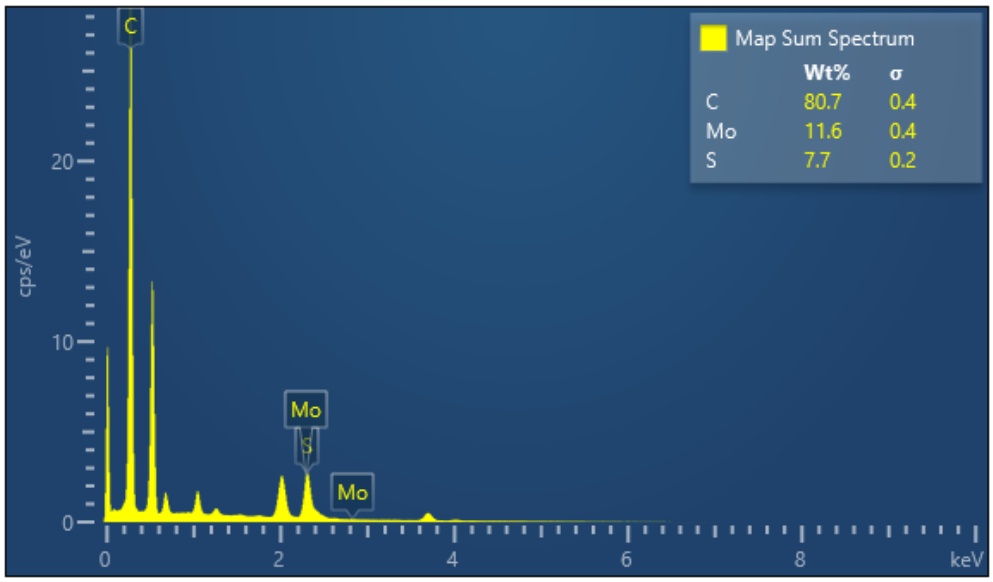


Figure 9. EDS spectra of CNT/MoS_x obtained from SEM-EDS. (Emre Aslan et al., 2021)

CONCLUSION

A spontaneous reduction process has been explored to prepare template-free *in situ* MoS_x grown on rGO and CNT during catalytic HER at liquid/liquid interfaces. The resulting free-MoSx, rGO/MoS_x, and CNT/MoS_x nanocomposites were characterized using SEM, TEM, and EDS analysis. The agglomerated free-MoS_x structures were approximately 20 nm in size, while the MoS_x particles formed uniformly on the rGO nanosheets and CNT with spherical shapes of around 10 nm. The presence of rGO and CNT inhibited aggregation and reduced the particle size of MoS_x. This study provides a new approach to developing molybdenum sulfide-based nanocomposites, which hold potential for various electrochemical applications. Furthermore, it suggests that molybdenum sulfide-based nanocomposite catalysts for HER at liquid/liquid interfaces could be prepared on different substrates in future research, paving the way for novel energy-conversion materials.

ACKNOWLEDGEMENTS

The author would like to thank you to Selçuk University for financial support. This study is part of the Selçuk University Scientific Research Projects (BAP) results (Grant number: 19401163)

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ORAL PRESENTATION

Kanserle mücadelede yeni hedef: Laktat dehidrogenaz A (5) izoformu

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Özet

Kanser, dünya genelinde en yaygın ölüm nedenlerinden biridir ve mevcut tedavi seçenekleri genellikle sınırlıdır. Bu nedenle, kanser tedavisinde yeni hedeflerin keşfi büyük önem taşımaktadır. Laktat dehidrojenaz A (LDHA) izoformu, son yıllarda kanser tedavisinde umut verici bir hedef olarak öne çıkmıştır. LDHA, glikoliz sırasında piruvatın laktata dönüşümünü katalize eder ve kanser hücrelerinde yüksek düzeyde eksprese edilir. Kanser hücreleri, hızla çoğalmak için yüksek miktarda enerjiye ihtiyaç duyar ve bu enerjiyi sağlamak için glikolize yönelirler. Warburg etkisi olarak bilinen bu olgu, kanser hücrelerinin oksijen varlığında bile anaerobik glikolizi tercih ettiğini gösterir. LDHA, bu süreçte kilit bir rol oynar ve tümör hücrelerinin enerji üretimini destekler. Çeşitli kanser türlerinde LDHA'nın aşırı ekspresyon gösterdiği ve bu ekspresyonun tümör hücrelerinin hayatta kalması ve metastazı için kritik olduğu bulunmuştur. LDHA inhibitörleri, kanser hücrelerinin enerji üretimini kesintiye uğratarak tümör büyümesini ve yayılmasını engelleyebilir. Bu inhibitörlerin klinik denemeleri, LDHA'nın kanser tedavisinde etkin ve güvenli bir hedef olabileceğini göstermektedir. Gelecekte, LDHA hedefli tedavilerin diğer kanser tedavileriyle kombinasyon halinde kullanımı ve bu kombinasyonların sinerjik etkileri araştırılmalıdır. Ayrıca, LDHA ekspresyonunun biyomarker olarak kullanımı ve hastaların tedaviye yanıtını öngörmedeki potansiyeli de incelenmelidir. LDHA, kanser tedavisinde yeni ve etkili bir hedef olarak dikkat çekmektedir ve bu enzime yönelik tedavilerin geliştirilmesi, kanserle mücadelede önemli bir adım olabilir.

Anahtar Kelimeler: Kanser, Laktat Dehidrogenez A, LDHA inhibitörleri

A new target in the fight against cancer: Lactate dehydrogenase A (5) isoform

Abstract

Cancer is one of the most common causes of death worldwide, and current treatment options are often limited. Therefore, the discovery of new targets in cancer treatment is of great importance. The lactate dehydrogenase A (LDHA) isoform has recently emerged as a promising target in cancer therapy. LDHA catalyzes the conversion of pyruvate to lactate during glycolysis and is highly expressed in cancer cells. Cancer cells require large amounts of energy to proliferate rapidly, and turn to glycolysis to provide this energy. This phenomenon, known as the Warburg effect, indicates that cancer cells prefer anaerobic glycolysis even in the presence of oxygen. LDHA plays a key role in this process and supports the energy production of tumor cells. It has been found that LDHA is overexpressed in various types of cancer and that this expression is critical for tumor cell survival and metastasis. By interrupting the energy production of cancer cells, LDHA inhibitors can inhibit tumor growth and spread. Clinical trials of these inhibitors show that LDHA can be an effective and safe target for cancer treatment. In the future, the use of LDHA-targeted therapies in combination with other cancer therapies and their synergistic effects should be investigated. Furthermore, the use of LDHA expression as a biomarker and its potential in predicting patients' response to treatment should also be examined. LDHA is attracting attention as a new and effective target in cancer treatment, and the development of therapies targeting this enzyme may be an important step in the fight against cancer.

Keywords: Cancer, Lactate Dehydrogenase A, LDHA inhibitors

GİRİŞ

Kanser, dünya genelinde en yaygın ölüm nedenlerinden biridir ve her yıl milyonlarca insanı etkilemektedir. Kanserin karmaşık doğası ve çeşitli türleri, tedavi sürecini zorlaştırmakta ve yeni tedavi yöntemlerinin geliştirilmesini zorunlu kılmaktadır. Mevcut tedavi yöntemleri genellikle cerrahi müdahale, radyoterapi, kemoterapi ve hedefe yönelik tedaviler gibi seçenekleri içerir. Ancak, bu tedavilerin etkinliği sınırlı olabilir ve çoğu zaman ciddi yan etkilere yol açabilir. Bu nedenle, kanser tedavisinde yeni hedeflerin ve tedavi stratejilerinin keşfi büyük önem taşımaktadır (Cairns ve Mak, 2016).

Son yıllarda, kanser hücrelerinin metabolik adaptasyonları ve bu adaptasyonların tümör gelişimindeki rolü üzerine yoğunlaşan araştırmalar artmıştır. Kanser hücreleri, hızla çoğalmak ve hayatta kalmak için normal hücrelerden farklı metabolik yolları kullanırlar. Bu hücreler, enerjiyi sağlamak için glikoliz yolunu tercih ederler ve bu süreçte laktat üretimi artar. Bu fenomen, Warburg etkisi olarak bilinir ve kanser hücrelerinin oksijen varlığında bile anaerobik glikolizi tercih ettiğini gösterir. Warburg etkisi, kanser hücrelerinin enerji ihtiyaçlarını karşılamak için glikoz tüketimini artırır ve laktat üretimini teşvik eder (Fadaka ve ark., 2017).

Laktat dehidrojenaz A (LDHA), glikoliz sırasında piruvatın laktata dönüşümünü katalize eden bir enzimdir. Bu enzim, kanser hücrelerinde yüksek düzeyde eksprese edilir ve hücrelerin enerji üretiminde kritik bir rol oynar. LDHA'nın aşırı ekspresyonu, kanser hücrelerinin hayatta kalmasını ve metastaz yapmasını kolaylaştırır. Bu nedenle, LDHA, kanser tedavisinde potansiyel bir hedef olarak büyük ilgi görmektedir (Zhang ve ark., 2022).

LDHA'nın inhibisyonu, kanser hücrelerinin enerji üretimini kesintiye uğratarak tümör büyümesini ve yayılmasını engelleyebilir. LDHA inhibitörleri üzerinde yapılan araştırmalar, bu enzim hedefli tedavilerin kanser tedavisinde etkili olabileceğini göstermektedir (Cairns ve Mak, 2016). Bu inceleme de, LDHA'nın kanser hücrelerindeki rolü, LDHA inhibitörlerinin geliştirilmesi ve klinik önemi üzerine mevcut literatürü gözden geçirildi. Ayrıca, LDHA hedefli tedavilerin gelecekteki araştırma alanları ve potansiyel uygulamaları hakkında bilgi verildi.

LDHA'nın Yapısal ve Fonksiyonel Özellikleri

LDH, dört alt birimden oluşan bir tetramerik enzimdir ve bu alt birimler A ve B olarak adlandırılır. LDHA, esas olarak kas dokusunda bulunur ve piruvatın laktata dönüştürülmesinde yüksek bir katalitik kapasiteye sahiptir. LDHB ise daha çok karaciğer ve böbrek gibi oksijen açısından zengin dokularda bulunur ve laktatı piruvata dönüştürme eğilimindedir. Kanser hücrelerinde, LDHA alt birimi genellikle baskındır ve bu da glikoliz yolunun devam etmesini sağlar (Fadaka ve ark., 2017).

LDHA'nın Kanser Hücrelerindeki Önemi

Kanser hücreleri, enerji üretimini sağlamak ve metabolik ihtiyaçlarını karşılamak için LDHA'ya büyük ölçüde bağımlıdır. LDHA'nın aşırı ekspresyonu, birçok kanser türünde gözlemlenmiştir ve bu durum, tümör hücrelerinin hayatta kalması ve yayılması için kritik bir rol oynar (Zheng, 2012). Örneğin, meme kanseri hücrelerinde LDHA ekspresyonunun arttığı ve bu artışın tümör agresifliği ile ilişkili olduğu bulunmuştur (Ni ve ark., 2024).

LDHA'nın aşırı ekspresyonu, laktat üretimini artırır. Laktat, tümör mikroçevresinde birikir ve bu da mikroçevrenin asidik hale gelmesine neden olur. Asidik mikroçevre, bağışıklık hücrelerinin işlevini bozar ve tümör hücrelerinin bağışıklık sisteminden kaçmasına yardımcı olur. Laktat ayrıca, hücre dışı matriksin (ECM) parçalanmasında rol oynayan metalloproteinazların (MMP'ler) aktivitesini artırır. Bu enzimler, tümör hücrelerinin çevre dokulara invazyonunu kolaylaştırır ve metastaz potansiyelini artırır (Li ve ark., 2023; Zhou ve ark., 2022).

LDHA ve Genetik Regülasyon

LDHA ekspresyonu, birçok kanser türünde çeşitli onkogenler ve transkripsiyon faktörleri tarafından düzenlenir. LDHA ekspresyonu, çeşitli onkogenler ve transkripsiyon faktörleri tarafından düzenlenir. MYC onkogeni, LDHA ekspresyonunu doğrudan artıran güçlü bir transkripsiyon faktörüdür. MYC, hücre proliferasyonunu ve metabolizmasını düzenler ve birçok kanser türünde aşırı eksprese edilir. MYC'nin LDHA üzerindeki etkisi, kanser hücrelerinin enerji ihtiyaçlarını karşılamada ve hızlı büyüme yeteneklerini sürdürmede kritik bir rol oynar (Zhao ve ark., 2022).

HIF-1 (Hipoksi İndüklenebilir Faktör-1), hipoksik koşullar altında LDHA ekspresyonunu artıran bir başka önemli düzenleyicidir. Tümör mikroçevresi genellikle düşük oksijen seviyelerine sahiptir ve bu da HIF-1'in aktif hale gelmesine neden olur. HIF-1, LDHA geninin transkripsiyonunu artırarak kanser hücrelerinin hipoksik koşullara adaptasyonunu destekler ve enerji üretimini sağlar (Zhao ve ark., 2022).

LDHA, aynı zamanda PI3K/AKT/mTOR yolakları gibi diğer onkogenik sinyal yolları tarafından da düzenlenir. Bu sinyal yolları, hücre büyümesi, proliferasyonu ve hayatta kalma süreçlerini kontrol eder.

PI3K/AKT/mTOR yolaklarının aktivasyonu, LDHA ekspresyonunu artırarak kanser hücrelerinin enerji üretimini ve metabolik esnekliğini destekler (Zhao ve ark., 2022).

LDHA İnhibisyonunun Terapötik Potansiyeli

LDHA inhibitörleri, kanser tedavisinde umut verici bir yaklaşım sunmaktadır. Bu inhibitörler, LDHA'nın katalitik aktivitesini bloke ederek laktat üretimini azaltır ve kanser hücrelerinin enerji metabolizmasını kesintiye uğratır.

Oksamat ve Diğer LDHA İnhibitörleri

Oksamat, LDHA'nın en bilinen inhibitörlerinden biridir. Oksamat, piruvatın bir analoğu olarak davranır ve LDHA'nın aktif bölgesine bağlanarak piruvatın laktata dönüşümünü engeller. Oksamatın, hem in vitro hem de in vivo modellerde kanser hücrelerinin enerji üretimini kesintiye uğratarak tümör büyümesini ve metastazı azalttığı gösterilmiştir (Chelakkot ve ark., 2023).

Piruvat analojileri, LDHA'nın aktif bölgesine bağlanarak enzimin normal substratları olan piruvat ve NADH ile yarışır. Bu inhibitörler, LDHA'nın katalitik döngüsünü bozarak laktat üretimini azaltır ve kanser hücrelerinin enerji metabolizmasını kesintiye uğratır. Oksamatın yanı sıra, piruvat analoğu olan FX11 de LDHA'nın NADH bağlanma bölgesine bağlanarak enzim aktivitesini inhibe eder ve kanser hücrelerinde metabolik stres oluşturur. Preklinik çalışmalarda, FX11'in çeşitli kanser türlerinde tümör büyümesini ve metastazı azalttığı gösterilmiştir (Ni ve ark., 2024).

NHI-1, başka bir NADH bağlanma bölgesi inhibitörüdür. NHI-1, LDHA'nın aktif bölgesine bağlanarak NADH ile yarışır ve enzim aktivitesini bloke eder. Bu inhibitör, kanser hücrelerinin enerji metabolizmasını kesintiye uğratarak hücresel stres ve apoptoza yol açar (Zhang ve ark., 2022). Galloflavin ise, LDHA'nın NADH ile etkileşimini engelleyerek enzim aktivitesini inhibe eder (Manerba ve ark., 2012).

LDHA İnhibitörlerinin Kombinasyon Tedavileri

LDHA inhibitörlerinin diğer kanser tedavileriyle kombinasyonu, tedavi etkinliğini artırmak için umut verici bir stratejidir. Klinik araştırmalar, LDHA inhibitörlerinin kemoterapi, radyoterapi ve immünoterapi gibi geleneksel kanser tedavileriyle kombinasyon halinde kullanılmasının tedavi etkinliğini artırabileceğini göstermektedir (Fadaka ve ark., 2017). Örneğin, oksamat ve doxorubicin kombinasyonu, kanser hücrelerinin hem enerji üretimini hem de DNA sentezini hedef alarak tümör büyümesini daha etkili bir şekilde inhibe edebilir (Zhang ve ark., 2022). Bu kombinasyon tedavileri, kanser hücrelerinin farklı metabolik yollarını hedef alarak, tedaviye direnç gelişimini önleyebilir ve tedavi sonuçlarını iyileştirebilir.

LDHA İnhibitörlerinin Geleceği

LDHA inhibitörlerinin geliştirilmesi ve klinik uygulamalardaki potansiyeli, kanser tedavisinde önemli bir adımı temsil etmektedir. Gelecekte, LDHA inhibitörlerinin daha geniş hasta popülasyonlarında test edilmesi ve bu inhibitörlerin diğer kanser tedavileriyle kombinasyonlarının araştırılması gerekmektedir. Ayrıca, LDHA inhibitörlerinin uzun vadeli etkinliği ve güvenliği konusunda daha fazla veri elde edilmelidir (Cairns ve Mak, 2016)

Yeni LDHA inhibitörlerinin geliştirilmesi, bu enzim hedefli tedavilerin klinik uygulamalarda daha yaygın kullanımını sağlayabilir. Moleküler dinamik simülasyonlar ve yüksek verimli tarama teknikleri gibi ileri teknolojiler, LDHA inhibitörlerinin tasarımını ve optimizasyonunu hızlandırabilir. Bu tür yaklaşımlar, daha etkili ve spesifik LDHA inhibitörlerinin geliştirilmesine katkıda bulunabilir (Mishra ve Banerjee, 2019).

Sonuç olarak, LDHA inhibitörleri, kanser tedavisinde umut verici bir yaklaşım sunmaktadır. Bu inhibitörler, kanser hücrelerinin enerji metabolizmasını kesintiye uğratarak tümör büyümesini ve metastazı engelleyebilir. Gelecekte, LDHA inhibitörlerinin klinik uygulamalarda daha yaygın kullanımı için daha fazla araştırma yapılması gerekmektedir.

Klinik Bulgular ve LDHA

LDHA'nın aşırı ekspresyonu, birçok kanser türünde kötü prognozla ilişkilendirilmiştir. Örneğin, meme kanseri, prostat kanseri, kolon kanseri ve akciğer kanseri gibi çeşitli kanser türlerinde yüksek LDHA seviyeleri, hastaların daha düşük sağkalım oranları ve daha agresif tümör davranışları ile ilişkilendirilmiştir (Zhang ve ark., 2022). Bu bulgular, LDHA'nın kanser tedavisinde hedeflenmesi gereken kritik bir molekül olduğunu göstermektedir.

Faz I Klinik Denemeler

Faz I klinik denemeler, yeni bir ilacın insanlardaki güvenliğini, tolere edilebilirliğini ve farmakokinetik profilini değerlendirmek için yapılan ilk çalışmalardır. Bu denemelerde, genellikle küçük bir hasta grubu kullanılır.

LDHA inhibitörleri için yapılan faz I klinik denemeler, bu inhibitörlerin kanser hastaları üzerindeki etkilerini değerlendirmiştir.

Örneğin, oksamatın faz I klinik denemesi, ileri evre pankreas kanseri hastalarında yapılmıştır. Bu denemede, oksamatın güvenliği ve tolere edilebilirliği değerlendirilmiş ve sonuçlar, oksamatın hastalar tarafından iyi tolere edildiğini ve minimal yan etkilere sahip olduğunu göstermiştir (Shiratori ve ark., 2019; Zhao ve ark., 2022). Ayrıca, oksamatın farmakokinetik profili de değerlendirilmiş ve ilacın vücutta nasıl dağıldığı ve metabolize edildiği incelenmiştir.

Faz II Klinik Denemeler

Faz II klinik denemeler, ilacın etkinliğini ve güvenliğini daha geniş bir hasta grubunda değerlendirmek için yapılır. Bu denemeler, ilacın belirli kanser türlerinde tümör büyümesini ve metastazı engelleme potansiyelini değerlendirir. Faz II denemelerde, ilacın etkinliği ve optimal dozajı belirlenir. LDHA inhibitörleri için yapılan faz II klinik denemeler, oksamatın ve diğer LDHA inhibitörlerinin çeşitli kanser türlerinde etkinliğini değerlendirmiştir. Örneğin, oksamatın meme kanseri ve kolon kanseri hastalarında yapılan faz II denemelerinde, oksamatın tümör boyutunu küçülttüğü ve hastaların yaşam kalitesini artırdığı gözlemlenmiştir (Shiratori ve ark., 2019; Zhao ve ark., 2022). Bu denemeler, oksamatın belirli kanser türlerinde terapötik potansiyelini ortaya koymuştur.

Faz III Klinik Denemeler

Faz III klinik denemeler, ilacın etkinliğini ve güvenliğini daha geniş hasta popülasyonlarında ve daha uzun sürelerde değerlendirmek için yapılır. Bu denemeler, ilacın diğer standart tedavilere karşı etkinliğini kıyaslar ve ilacın klinik uygulamalarda kullanılabilirliğini değerlendirir. LDHA inhibitörleri için yapılan faz III klinik denemeler, oksamat ve diğer LDHA inhibitörlerinin uzun vadeli etkinliğini ve güvenliğini değerlendirmiştir. Bu denemelerde, oksamatın kemoterapi ve radyoterapi gibi geleneksel tedavi yöntemleriyle kombinasyon halinde kullanımı incelenmiştir. Sonuçlar, oksamatın kombinasyon tedavileri ile kullanıldığında tedavi etkinliğini artırabileceğini ve yan etkileri azaltabileceğini göstermiştir (Shiratori ve ark., 2019; Zhao ve ark., 2022).

Biyomarker Geliştirilmesi ve Hastaların Seçilmesi

LDHA ekspresyon seviyeleri, hastaların tedaviye yanıtını öngörmede kullanılabilecek potansiyel biyomarkerlar olarak değerlendirilebilir. Gelecekteki araştırmalar, LDHA seviyelerinin biyomarker olarak kullanımını ve hastaların tedaviye yanıtını tahmin etmedeki rolünü incelemelidir. LDHA ekspresyonunun belirlenmesi, hastaların tedaviye yanıtını artırabilir ve tedavi sonuçlarını iyileştirebilir (Chelakkot ve ark., 2023; Zhou ve ark., 2022). Biyomarker gelişimi, LDHA inhibitörleri ile tedavi edilecek hastaların seçimini optimize edebilir. Bu, hastaların genetik ve moleküler profillerine dayalı olarak özelleştirilmiş tedavi stratejilerinin belirlenmesini sağlar. Bireyselleştirilmiş tıp yaklaşımları, hastaların tedaviye daha iyi yanıt vermesini ve tedaviye bağlı yan etkilerin azalmasını sağlayabilir (Chelakkot ve ark., 2023; Zhou ve ark., 2022).

Mekanistik Çalışmalar ve Moleküler Mekanizmaların Aydınlatılması

LDHA inhibitörlerinin kanser hücreleri üzerindeki etkilerini ve bu inhibitörlerin moleküler mekanizmalarını daha iyi anlamak için mekanistik çalışmalar yapılmalıdır. Bu çalışmalar, LDHA inhibitörlerinin hücresel enerji metabolizması, redoks dengesi, hücresel stres yanıtları ve hücresel ölüm yolları üzerindeki etkilerini incelemelidir. Mekanistik çalışmalar, LDHA inhibitörlerinin kanser hücrelerinde nasıl çalıştığını ve bu inhibitörlerin etkilerini artırmak için hangi yolların hedeflenebileceğini anlamamıza yardımcı olabilir (Cairns ve Mak, 2016).

Özellikle, LDHA inhibitörlerinin hücresel enerji üretimi ve metabolizması üzerindeki etkileri incelenmelidir. Bu inhibitörlerin hücresel NAD+ seviyeleri, ATP üretimi ve reaktif oksijen türleri (ROS) üzerindeki etkileri araştırılmalıdır. Ayrıca, LDHA inhibitörlerinin tümör mikroçevresi üzerindeki etkileri de incelenmeli ve bu inhibitörlerin tümör mikroçevresinin asidik doğasını nasıl değiştirdiği ve bağışıklık hücrelerinin aktivitesini nasıl artırdığı belirlenmelidir (Mishra ve Banerjee, 2019; Shiratori ve ark., 2019).

Bireyselleştirilmiş Tedavi Yaklaşımları

LDHA inhibitörlerinin bireyselleştirilmiş tıp yaklaşımlarıyla entegrasyonu, kanser tedavisinde önemli bir adımı temsil eder. Hastaların genetik ve moleküler profillerine dayalı olarak özelleştirilmiş tedavi stratejileri, tedaviye yanıtı artırabilir ve yan etkileri azaltabilir. Bireyselleştirilmiş tedavi yaklaşımları, hastaların tedaviye daha iyi yanıt vermesini ve tedavi sonuçlarının iyileşmesini sağlayabilir (Shiratori ve ark., 2019; Zhao ve ark., 2022).

Gelecekteki araştırmalar, LDHA inhibitörlerinin bireyselleştirilmiş tedavi yaklaşımlarında nasıl kullanılabileceğini ve bu inhibitörlerin hastaların genetik ve moleküler profillerine göre nasıl optimize edilebileceğini incelemelidir. Bu araştırmalar, LDHA inhibitörlerinin kanser tedavisinde daha geniş çapta ve daha etkili bir şekilde kullanılmasına katkıda bulunabilir (Shiratori ve ark., 2019; Zhao ve ark., 2022). Sonuç olarak,

LDHA inhibitörleri, kanser tedavisinde umut verici bir yaklaşım sunmaktadır. Gelecekteki araştırmalar, bu inhibitörlerin geliştirilmesi, kombinasyon tedavileri, biyomarker kullanımı, mekanistik çalışmalar ve klinik denemeler üzerinde odaklanmalıdır. Ayrıca, bireyselleştirilmiş tedavi yaklaşımları, LDHA inhibitörlerinin kanser tedavisinde daha etkili bir şekilde kullanılmasına katkıda bulunabilir.

Sonuç ve Öneriler

Laktat dehidrojenaz A (LDHA), kanser hücrelerinin enerji metabolizmasında merkezi bir rol oynar. Kanser hücreleri, glikozdan enerji üretmek için LDHA'ya güvenir ve bu süreçte LDHA, pirüvatı laktata dönüştürerek NAD+ üretimini sağlar. Warburg etkisi olarak bilinen bu durum, kanser hücrelerinin oksijen varlığında bile glikolizi tercih etmesine neden olur, bu da hücrelerin hızlı büyümesini ve çoğalmasını destekler. LDHA inhibitörleri, kanser tedavisinde umut verici bir yaklaşım sunar. Bu inhibitörler, LDHA'nın aktivitesini bloke ederek kanser hücrelerinin enerji üretimini durdurur ve tümör büyümesini engeller. Klinik denemeler, LDHA inhibitörlerinin hastalar tarafından iyi tolere edildiğini ve çeşitli kanser türlerinde tümör boyutunu küçülttüğünü göstermiştir. Gelecekte, LDHA inhibitörlerinin kemoterapi, radyoterapi ve immünoterapi gibi geleneksel tedavilerle kombinasyonu, tedavi etkinliğini artırabilir. Ayrıca, LDHA ekspresyon seviyeleri, hastaların tedaviye yanıtını öngörmede biyomarker olarak kullanılabilir. Bu, bireyselleştirilmiş tıp yaklaşımlarıyla entegre edilerek, hastaların tedaviye daha iyi yanıt vermesini sağlar. LDHA inhibitörlerinin klinik uygulamalardaki potansiyeli, daha fazla hasta popülasyonunda test edilerek ve etkinliği daha iyi anlaşarak artacaktır. Yeni inhibitörlerin geliştirilmesi, bu tedavilerin daha spesifik ve etkili olmasını sağlayacaktır. Sonuç olarak, LDHA inhibitörleri kanser tedavisinde umut verici bir yaklaşım sunmakta ve gelecekte daha yaygın kullanılmaları beklenmektedir.

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ORAL PRESENTATION

Türkiye'de yayılış gösteren *Stachys mardinensis* (Post) R. R. Mill (Lamiacea) türü üzerine yapılan çalışmalara bir bakış

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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. *Stachys* L. (Adaçayı), bu familyanın en büyük ve en önemli cinslerinden biridir. *Stachys* cinsi bu familyanın Lamioideae (Stachyoideae) alt familyasına ve *Stachydeae* Dumort. Tribusuna aittir. Bu cins ilk defa 1753 yılında Linnaeus tarafından "Species Plantarum" isimli eserde ele alınmıştır. Bu cins yarı-kozmopolit olup, Akdeniz ve Güneybatı Asya'nın ılıman bölgelerinde, Kuzey ve Güney Amerika ile Güney Afrika'da yayılış göstermektedir. Dünyada yaklaşık 370 tür ve 435 takson ile temsil edilmektedir. Türkiye' de ise 94 tür (121 takson) ile temsil edilen bu cinsin en çok yayılış gösterdiği ülke olması sebebiyle, cinsin gen merkezidir. Bu taksonlardan 52'si endemiktir. *Stachys* taksonları uçucu yağlar bakımından zengindir ve antimikrobiyal aktivite göstermektedir. *Stachys* türleri uçucu bileşenleri (Caryophyllene, germacrene D, α-pinene, linalool, β-pinene v.b) ve fenolik bileşenleri nedeniyle genellikle bitki çayı olarak tüketilmektedir. *Stachys mardinesis* ülkemizde tıbbı olarak kullanılmaktadır. Türkçe bilimsel adı "Kayapungu" olarak bilinmektedir. Bitki, diyabet, öksürük kesici, bronşit hastalığının tedavisinde kullanılmaktadır. Bu çalışmada, ülkemizde *Stachys mardinensis* türü üzerine yapılan taksonomik ve fitokimyasal çalışmalar derlenmiştir.

Anahtar kelimeler: Dağ çayı, Mardin, *Stachys*, taksonomi, tıbbi bitki

A review of the studies on *Stachys mardinensis* (Post) R. R. Mill (Lamiacea) species distributed in Turkey

Abstract

The Lamiaceae family has a cosmopolitan distribution and includes more than 7000 species belonging to 236 genera in the World. In Türkiye, the Lamiaceae family, called Ballıbabagiller, is represented by 586 taxa belonging to 46 genera. Taxa of this family are distributed mainly in the Mediterranean region of our country. *Stachys* L. (sage) is one of the largest and most important genera of this family. The genus *Stachys* belongs to the subfamily Lamioideae (Stachyoideae) of this family and Stachydeae Dumort, belongs to the Tribus. This genus was first discussed by Linnaeus in 1753 in her work titled 'Species Plantarum'. This genus is semi-cosmopolitan and is distributed in the temperate regions of the Mediterranean and Southwest Asia, North and South America and Southern Africa. It is represented by approximately 370 species and 435 taxa in the World. Turkey is the gene center of the genus, as it is the country where this genus, represented by 94 species (121 taxa), is most widespread. 52 of these taxa are endemic. *Stachys* taxa are rich in essential oils and demonstrate antimicrobial activity. *Stachys* species are generally consumed as herbal tea due to their volatile components (Caryophyllene, germacrene D, α-pinene, linalool, β-pinene, etc..) phenolic components.

Stachys mardinensis is used medicinally in our country. Its scientific name in Turkish is known as 'Kayapungu'. The plant is used in the treatment of diabetes, coungh suppressant and brochitis. In this study, taxonomic and phytochemical studies on the *Stachys mardinensis* species in our country were compiled.

Keywords : Mountain tea, Mardin, *Stachys*, taxonomy, medicinal plant

GİRİŞ

Türkiye, toprak ve iklim özellikleri bakımından farklılıklar gösteren coğrafi bölgelere sahip olması, Asya ve Avrupa kıtalarının kesişiminde bulunması, iki önemli Vavilov gen merkezinin (Yakın Doğu veAkdeniz) kesişiminde bulunması, üç tarafının denizlerle çevrili olması ve üç önemli fitocoğrafik bölgeyi (Akdeniz Avrupa-Sibirya ve İran-Turan) barındırması nedeniyle bitki çeşitliliği bakımından yeryüzünde önemli bir yere sahiptir. Son yapılan teşhislerle birlikte Türkiye'de, yaklaşık 10000 tür ve 12000'ine yakın bitki taksonu yetişmektedir (Avcı 2005). Türkiye Florası, Davis ve arkadaşlarının 1965-1988 yılları arasında yayınladıkları "Flora of Turkey and East Aegean Islands" adlı 10 ciltlik eserdir. ZamanlaTürkiye Florası'nda meydana gelen değişiklikler Türk botanikçileri tarafından 11. ciltte yayınlanmıştır (Güner ve ark. 2000). Türkiye'de 167 familya ve bu familyalara ait 1320 cins vardır. Ülkemizde sırasıyla Asteraceae, Fabaceae, Brassicaceae, Lamiaceae ve Caryophyllaceae familyaları içerdikleri takson sayısı bakımından en büyük familyalardır (Güner ve ark. 2012). Lamiaceae familyası için önemli bir biyolojik çeşitlilik merkezi olarak kabul edilen Türkiye'de, bu familyaya ait 603 tür ve toplam 782 takson yayılış göstermektedir. Bu taksonlardan 346'sı endemiktir (Celep, F., & Dirmenci, T. 2017).

Stachys L. Lamiaceae familyasının en önemli cinslerinden biridir. *Stachys* cinsi bu familyanın Lamioideae (Stachyoideae) alt familyasına ve Stachydeae Dumort. Tribusuna aittir. Bu cins ilk defa 1753 yılında Linnaeus tarafından "Species Plantarum" isimli eserde ele alınmıştır. Bu cins yarı-kozmopolit olup, Akdeniz ve Güneybatı Asya'nın ılıman bölgelerinde, Kuzey ve Güney Amerika ile Güney Afrika'da yayılış göstermektedir (Bhattacharjee, R., 1982). Türkiye' de 94 tür (121 takson) ile temsil edilen bu cinsin en çok yayılış gösterdiği ülke olması sebebiyle, cinsin gen merkezidir. Bu taksonlardan 52'si endemiktir. *Stachys* taksonları uçucu yağlar bakımından zengindir. Uçucu yağlarının antimikrobiyal aktivite gösterdiği tespit edilmiştir. Ayrıca uçucu yağlarının ana bileşiklerinin linoleik, oleik, palmitik ve stearik asit olduğu belirlenmiştir (Goren ve ark. 2011).

Cinsin etnobotanik (Halk tarafından) kullanımı Doğu'dan Batı Anadolu'ya kadar olan bölgede yaygın olarak görülmektedir. Ancak Türkiye'nin bazı bölgelerinde herhangi bir kullanıma rastlanmamıştır. Etnobotanik araştırmalara ve literatür taramasına göre *Stachys* L. türleri uçucu bileşenleri (Caryophyllene, germacrene D, α-pinene, linalool, β-pinene gibi) ve fenolik bileşenleri nedeniyle genellikle bitki çayı olarak tüketilmektedir (Satıl, F., & Açar, M. 2020). Bununla birlikte türün uçucu yağ bileşimi, cinsin Anadolu etnobotaniğinde çay olarak tüketilmelerinin başlıca nedenlerinden biridir (Altundag, E., & Ozturk, M. 2011). Bileşen kimyasalların sinerjik etkileri, çiçeklerinin ve toprak üstü kısımlarının Anadolu kültüründe tıbbi amaçlarla çay olarak kullanılmasının ana nedenlerindendir (Dönmez ve ark. 2011).

Türkçe bilimsel adı "Deliçay" olmasına rağmen, genellikle ülkemizde dağ çayı ve çay olarak adlandırılır. Bu bitkiler grip, soğuk algınlığı, bronşit, üst solunum yolları rahatsızlıkları, uykusuzluk, sakinleştirici ve kan basıncını düzenleme gibi tıbbi amaçlarla kullanıldığı bilinmektedir. Ayrıca mide ağrıları, diyabet ve alzheimer hastalığına karşı kullanımından başka ellerdeki yaraların iyileşmesi amacıyla, kök ve yaprakların bu yaraların üzerine sürülerek tedavi amaçla kullanıldığı tespit edilmiştir.

Özel bir kullanıma sahip olan cinsin; baş ağrısı için, bitki kaynatıldıktan sonra süzülür ve kınaya katılır. Daha sonra kına yakılır. Genellikle kadınlar tarafından kullanılır. Gıda olarak kullanımı ise tarhananın aromasının olması için, tarhana bu bitkinin üzerine serilir. Ayrıca çaya da aroma vermesi için, bitki yaprakları çayın içine bir miktar atılır. (Güner, Ö. & Akçiçek, E. 2015)

Bu derlemede *S. mardinensis*'in genel özellikleri derlenmiştir.

YÖNTEM

Mardin, Güneydoğu Anadolu Bölgesinde 39°56"-42°54" doğu boylamları ve 36°55"-38°51" arasında yer alır. Doğuda Şırnak ve Siirt, batıda Şanlıurfa, kuzeyde Diyarbakır ve Batman, Güneyde Suriye topraklarıyla çevrilidir (Şekil 1). Mardin 8.891 km^2 alana sahip ve denizden yüksekliği 1.082 metredir (Scheen ve ark. 2010).

Mardin'in güneydeki çöl ikliminin etkisi altında bulunması ve kuzeyde yüksek dağlarla çevrili olması serin hava kütlelerinin bölgeye girişini engellemesi nedeniyle, yazlar çok sıcak olup, bölgede tipi karasal iklim görülmektedir. Ancak; yörede mikro iklim özelliği de görülmektedir. Çünkü Derik, Nusaybin ve Savur ilçelerinde pamuk, fındık ve zeytin gibi ürünlerin yetişmektedir. Mardin ilinin meteorolojik verilerine göre; en fazla yağış Mart ayında 115.8 m, en yüksek sıcaklığın Temmuz ayında 42.5 °C ve en düşük sıcaklığın ise Şubat aynıda -2,6 °C olduğu belirlenmiştir. En yüksek nem oranı ise Ocak ayında % 76.1 olarak tespit edilmiştir.

Güneydoğu Anadolu Bölgesinde, Dicle ve Fırat havzaları arasında yer alan Mardin, çeşitli yeryüzü şekillerine sahiptir. Mardin, bütün jeolojik zamanları temsil eden kristal ve tortul kütle çeşitlerinden iç püskürük mahsullerine ve bunların değişmesinden meydana gelen sahalara varıncaya kadar çeşitli formasyonlara sahiptir. İlin yeryüzü şekilleri temelini I. zamanda kayalar teşkil eder. Jeolojinin II. zamanında kuzeydoğuda kalkerli yerler tebeşir devrinde oluşmuştur. III. zamanda Toros kıvrımları oluşurken, volkanlar oluşarak, faaliyete geçmiştir. Eski volkanların biriktirdiği lavlar Turabdin, Karacadağ gibi yapıları meydana getirmiştir. IV. zamanın alüvyon topraklarını ortadan bölmekte ve Diyarbakır havzasıyla Suriye çölü arasında bir eşik meydana getirmektedir.

Mezopotamya Ovası ile Suriye çölüne egemen bir konumda olan Mardin Eşiği, ova ile arasında yaklaşık 600-1000 m yükseklikte çok geniş bir alan oluşturmaktadır. Mardin Eşiğinin; Mazıdağı, Derik, Midyat, Savur ve Nusaybin yörelerinin iç kısımlarına doğru uzanan yüksek kesimlerinde meşe ağaçlarından oluşan topluluklar göze çarpar. Bu orman kısımlarında Sakız ağacı, Dişbudak, Söğüt ve Çınar ağaçlarına da rastlanılır. Ayrıca Nusaybin ve Savur ile Mardin dağlarının vadi boylarında yetiştirilen kavak, badem, bıttım, ceviz, sumak, kiraz ve mahleplerin dışında genelde yörede bozkırlar egemendir. Büyükdere Vadisi ile Gümüş Çayı Vadisinin birleşmesiyle genişleyen taban üzerinde yer alan Kızıltepe Ovası en geniş alana sahiptir. Akarsuların taşıdığı alüvyonlarla kaplı Derik, Kızıltepe, Mardin ve Nusaybin Ovaları kısa zaman içerisinde GAP'la birlikte bolluk ve berekete kavuşmayı beklemektedir. Suriye sınırı boyunca Mardin ve Nusaybin Ovaları (Mezopotamya) uzanır (Anonim 2022).



Şekil 1. Türkiye haritası üzerinde Mardin ilinin konumu

BULGULAR

Taksonomik ve Tıbbi Bulgular

S. mardinensis (Kayapungu) Ballıbabagiller (Lamiaceae) ailesinden bir türdür. Türkiye'de Yukarı Fırat, Dicle alt bölgelerinde doğal yayılış göstermektedir.

Bilimsel olarak *S. mardinensis* (Post) R.R.Mill şeklinde tanımlanır. *Stachys* cins adı Eski Yunancada *Stachys germanica*`yı tanımlayan *stákhus* kelimesinden türetilmiştir. Parantez içerisindeki kısaltmalar bu taksonu ilk yayınlayan kişiyi/kişileri, parantez dışındakiler ise ilk yayın ardından bu taksona revizyon yapan kişi/kişileri belirtir. Post kısaltması bilim insanı George Edward Post (1838-1909) için kullanılmıştır. R.R.Mill kısaltması bilim insanı Robert Reid Mill (1950-) için kullanılmıştır.

S. mardinensis Yarı odunsu çok yıllık bitki. Çiçek sapları 10-60 cm, genellikle dik, bazı zamanlar sarkık, tabanda kırılgan, basit veya dallı, yoğun yapraklı, genellikle yoğun ve belirgin şekilde tüylü uzunluğunda, uzun bezli tüyler ve kısa salgı tüyleri ile kaplı, bazen tabana doğru seyrek tüylü. Gövde yaprakları genellikle geniş oval, tabanda yuvarlak, kenarı kısmen dişli, nadiren dalgalı, ucu sivri, nadiren küt, tabanda kalp şeklinde, keçe gibi tüylü. Çiçek yaprakları sapsız, gövde yapraklarına benzer ama daha küçük, genellikle çiçek kümelerinden daha uzun. Çiçek kümeleri genellikle dağınık, nadiren en üst kısımda yoğun, (2-)4-8 çiçekli. *Çanak ± düzenli, huni şeklinde, tüylü; dişler neredeyse eşit, üçgen-mızrak şeklinde, ucu dikenli değil. Taç yapraklar sarımsı, tüp dışarıda, halka şeklinde. Nutletler yumurtamsı, üçgen, kenarlarında hafifçe kanatlı, genellikle tabana yakın, ucu hafifçe yuvarlak. Çiçeklenme mayıs ve temmuz ayları arasında, 450-1200 m yükseklikte, kalkerli kaya yamaçları, kaya ve uçurum yarıklarında yayılış göstermektedir.

S. mardinensis türünün etnobotanik kullanımına bakıldığında, bitkinin yapraklarından ve toprak üstü kısımlarından yapılan çay baş ağrısı, diyabet, öksürük ve bronşit hastalığı tedavisinde kullanılır. Ayrıca tarhana çorbası yapımında baharat olarak kullanılır (Güner, Ö. & Akçiçek, E. 2015)

***Stachys mardinensis* (Post) R. Mill** in Notes R.B.G. Edinb. 38:57 (1980). Syn:*Nepeta mardinensis* Post in Bull. Herb. Boiss. 7:159 (1899) (!); *Stachys glechomifolia* Nab. in Publ. Fac. Sci. Univ. Masaryk Brno 70:61 (1926)! Ic: Nab., op. cit. 63, t. 7(1926) (Şekil 1).



Şekil 2. *S. mardinensis* çiçek ve genel görünümü

Tür üzerine yapılan çalışmalar incelendiğinde, Kaya ve ark., (2017) yaptıkları fitokimyasal çalışmada *S. mardinensis* türünün içeriğinde menthyl asetat (15.3%), isomenthon (15.0%), pulegon (10%), spathulenol (7.0%), ve caryophyllene oxide (6.7%) for menthyl asetate (15.3%), isomenthon (15.0%), pulegon (10%), spathulenol (7.0%), and caryophyllen oxid (6.7%) tespit etmişlerdir. Güner ve Ferrer-Gallego *S. mardinensis* türünün de aralarında bulunduğu taksonomik çalışmada, türün İran örneklerinin, Türkiye'de yayılış gösteren örneklerden morfolojik olarak farklı olmadığını belirtmişlerdir. Karaboduk ve ark. (2014) üç *Stachys* taksonunun fitokimyasal içeriği ile ilgili yaptıkları çalışmada, *S. mardinensis* türünün en yüksek fenolik (225.10 mg GAE/g), ayrıca HPLC flavonoid analiz sonuçlarına göre de, *S. mardinensis*'in diğer *Stachys* türlerine göre daha yüksek kuersetin (26.5mg/g) ve kateşin (11.00 mg/g) içeriğine sahip olduğunu kaydetmişlerdir.

SONUÇ ve ÖNERİLER

Ekonomik değeri olan, endemik *S. mardinensis* türü ülkemize katkısının ortaya çıkarılması açısından bitkisel araştırmaların önemi çok fazladır ve bu çalışmaların artarak devam etmesi gerektiği de elzemdir.

Bu bitki zenginliklerinin tespit edilip korunması ve bunlardan daha iyi yararlanılabilmesi için yerel halkın bilimsel yöntemlerle eğitilmesi gerekmektedir. Bölgedeki popülasyonu düşük olan taksonlar da dahil olmak üzere tüm bitkilere yönelik potansiyel tehditler arasında ormanların tarıma dönüştürülmesi, otlatma, kentleşme, tarım kimyasalları ve orman yangınları yer alıyor.

Türkiye'nin biyolojik zenginliğinin tanınması ve korunması için, yapılan etnobotanik, taksonomik ve biyoaktivite çalışmaları büyük önem taşımaktadır ve teşvik edici rol oynamaktadır.

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ORAL PRESENTATION

Tüketicilerin Geleneksel Gıda Ürünlerini Satın Alma Tutum ve Davranışları: Çanakkale İli Örneği

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Özet

Geleneksel gıdalar, ülkelerin coğrafi koşulları, iklim, üretim imkanları ve geleneksel yaşam tarzının etkisi ile şekillenen ve uzun yıllardan beri üretilen gıda maddeleridir. Bu çalışmada, Çanakkale ili ve yöresindeki geleneksel gıda ürünleri hakkında tüketicilerin bilgi düzeyleri ile satın alma tutum ve davranışlarının incelenmesi amaçlanmıştır. Araştırma kapsamında, tüketicilerin sosyo-demografik yapılarını belirlemek ve geleneksel gıda ürünlerine yönelik tercihlerini ölçmek amacıyla yüz yüze anket çalışması gerçekleştirilmiştir. Ankete katılan 1014 kişiden %40,53'ü kadın, %59,47'si erkek olarak belirlenmiştir. Katılımcıların %50,39'unun evli olup, %32,25'inin 18-25 yaş aralığında, %31,85'inin ise 40-54 yaş aralığında olduğu tespit edilmiştir. Ayrıca, katılımcıların %68,24'ünün Çanakkale doğumlu olduğu, bu kişilerin yaklaşık %44,18'inin ise kökenlerinin kent merkezinin dışında olduğu gözlenmiştir. Katılımcıların %36,39'u yöreye ait bitkisel, hayvansal, süt ürünleri ve unlu mamullerin hepsini tükettiğini ifade etmiştir. Bununla birlikte, tüketicilerin %57,10 oranı ile süt ürünlerini her gün, diğer ürünleri ise sadece ayda birkaç kez tükettikleri belirlenmiştir. Katılımcıların %45,76'sı geleneksel gıdaları doğal olduğunu düşündüğü için, %17,65'i lezzetli olduğu ve %26,33'ü sağlıklı olduğunu düşündüğü için tercih ettiklerini ifade etmiştir. Katılımcıların büyük çoğunluğu (%63,41) geleneksel gıdaların teminini market/pazardan sağladığını bildirmiş, satın alma durumunda dikkat edilen ve tercih sebebi olan en önemli unsurun, fiyat olduğu belirlenmiştir. Ayrıca katılımcılar, geleneksel gıdalar hakkında çoğunlukla yakın çevrelerinden (%48,32) ve ürün etiket bilgilerinden (%49,81) bilgi sağladıklarını belirtmiştir. Sonuç olarak ülkemiz için çok yönlü avantajlara sahip olan geleneksel gıdaların, kültürel miras algısıyla yeni nesillere aktarılması ve ticari üretimlerinin önünün açılabilmesi için kapsamlı çalışmalar yapılması ve farkındalıkların artırılması gerekmektedir.

Anahtar Kelimeler: Geleneksel Gıdalar, Çanakkale, Tüketici Davranışları, Sosyo-demografik Özellikler

Consumer Purchase Behavior and Attitudes Towards Traditional Food Products: The Case of Çanakkale Province

Abstract

Traditional foods are food items shaped by a country's geographical conditions, climate, production capabilities, and traditional lifestyle, and they have been produced for many years. This study aims to examine consumers' knowledge levels, purchase attitudes, and behaviors regarding traditional food products from the Çanakkale province and its surroundings. To determine the socio-demographic structure of consumers and measure their preferences for traditional food products, a face-to-face survey was conducted. Of the 1014 participants, 40.53% were female and 59.47% were male. It was found that 50.39% of the participants were married, 32.25% were aged 18-25, and 31.85% were aged 40-54. Additionally, 68.24% of the participants were born in Çanakkale, and approximately 44.18% of these individuals had origins outside the city center. 36.39% of the participants stated that they consume all types of local plant-based, animal-based, dairy, and bakery products. However, it was

determined that 57.10% of consumers consume dairy products daily, while other products are consumed only a few times a month. 45.76% of the participants preferred traditional foods because they believe they are natural, 17.65% because they find them tasty, and 26.33% because they consider them healthy. The majority of participants (63.41%) reported obtaining traditional foods from markets/stores, and the most important factor considered in purchasing decisions was price. Additionally, participants indicated that they generally obtain information about traditional foods from their close circle (48.32%) and product label information (49.81%). In conclusion, it is necessary to undertake efforts to transfer traditional foods, which have multiple advantages for our country, to new generations as a cultural heritage and to facilitate their commercial production.

Keywords: Traditional Foods, Çanakkale, Consumer Behavior, Sociodemographic Characteristics

GİRİŞ

Geleneksel gıda ürünleri, tarih boyunca toplumların kültürel mirasını yansıtan ve nesilden nesile aktarılan değerli besin kaynakları olarak kabul edilmiştir. Bu ürünler, yerel tarımsal yöntemlerin, kültürel alışkanlıkların, coğrafi şartların ve kültürel birlikteliğin bir yansıması olarak hem beslenme hem de sosyal anlamda önemli bir rol oynamaktadır (Muhialdin ve ark., 2021; Schellhaas, 2016). Günümüzde ise küreselleşme ve modernleşmenin etkisi, tüketici davranış ve tutumlarıyla birlikte, geleneksel gıda ürünlerini satın alma dinamiklerini de etkilemektedir (Soleri ve ark., 2023).

Tüketici davranışları, bireylerin satın alma kararlarını şekillendiren psikolojik, sosyal ve ekonomik faktörlerin bir kombinasyonudur (Ramya ve Ali, 2016). Geleneksel gıda ürünleri söz konusu olduğunda, bu davranışlar kültürel bağlam, sağlık bilinci, çevresel endişeler ve ekonomik faktörler gibi birçok unsura bağlı olarak değişkenlik gösterebilir (Tyagi, 2018). Özellikle tüketicilerin, küresel iklim değişiminin negatif etkisi sonucu, organik ve yerel ürünlere artan ilgisi ve sağlıklı yaşam trendleri ile ilgili konularda artan farkındalıkları geleneksel gıdaların pazar payını da önemli derecede etkilemektedir (Dinç ve ark., 2022; Feldman ve Wunderlich, 2023; Künili ve ark., 2024).

Bu bağlamda tüketicinin geleneksel gıda ürünleri konusundaki davranışları ve bu ürünlere yönelik tutumlarını anlamak hem pazarlama stratejileri hem de gıda politikaları açısından büyük önem taşımaktadır (Feldman ve Wunderlich, 2023; Vlontzos ve ark., 2018). Tüketicilerin bu ürünlere olan ilgilerini, satın alma tercihlerini ve tercihlerini etkileyen faktörleri incelemek, gıda endüstrisinin geçmiş, günümüz ve gelecekteki yönelimlerini belirlemede ve sektör stratejilerinin planlanmasında önem arz etmektedir.

Bu çalışmada, Çanakkale ili ve yöresinde tüketicilerin geleneksel gıda ürünlerini satın alma davranışlarını ve bu ürünlere yönelik tutumlarının incelenmesi amaçlanmıştır. Bunun için, Çanakkale ilinde tüketicilerle yüz yüze anket çalışması gerçekleştirilmiştir. Anket kapsamında, katılımcıların sosyo-demografik özelliklerini belirlemeye yönelik sorular ve geleneksel gıda ürünlerini tercih ve tutumlarını belirlemeye yönelik sorular yer almıştır. Sonuç olarak, çalışmada geleneksel gıda ürünlerinin tüketim eğilimleri, bu eğilimleri etkileyen faktörler ve sektörel yansımalar ortaya çıkarılmaya çalışılmıştır.

MATERYAL VE METOD

Araştırmanın materyalini, anket verileri oluşturmaktadır. Araştırma 2022-2023 yılları arasında Çanakkale yöresi il merkezi ve ilçelerinde yapılmıştır. Toplam 20 sorudan oluşan anket çalışması yüz yüze olarak gerçekleştirilmiştir. Anket uygulamasına 18 yaş ve üzeri toplam 1014 kişi katılım göstermiştir. Elde edilen veriler ofis programları vasıtası ile düzenlenmiştir. Araştırmada elde edilen veriler aritmetik ortalama ve yüzde değerlerden yararlanılarak çözümlenmiştir. Sonuçlar grafikler halinde derlenmiş ve yorumlanmıştır.

BULGULAR VE TARTIŞMA

Araştırma Çanakkale ilini temsil edecek şekilde il merkezi ve ilçelerinde gerçekleştirilmiştir. Bu alanda katılımcılara, geleneksel gıda ürünlerini tercih ve tutumlarını belirlemeye yönelik sorular sorulmuş ve tüketici davranışları incelenmiştir. Bireylerin bilinç gelişiminde önemli rol oynayan sosyo-demografik özelliklerle ilgili elde edilen bilgiler Tablo 1'de özetlenmiştir. Elde edilen verilere göre, katılımcıların %40,53'ünün kadın %59,47'sinin erkek olduğu belirlenmiştir. Çalışmaya katılım gösterenlerin büyük çoğunluğu 18-25 (%32,25) ve 40-54 (%31,85) yaş arasında tespit edilmiştir. Katılımcıların %50,39 evli, %68,24 Çanakkale doğumlu ve % 55,82'si kent merkezi doğumlu olarak belirlenmiştir.

Tablo 1. Tüketicilerin sosyo-demografik yapılarının dağılımları (%)

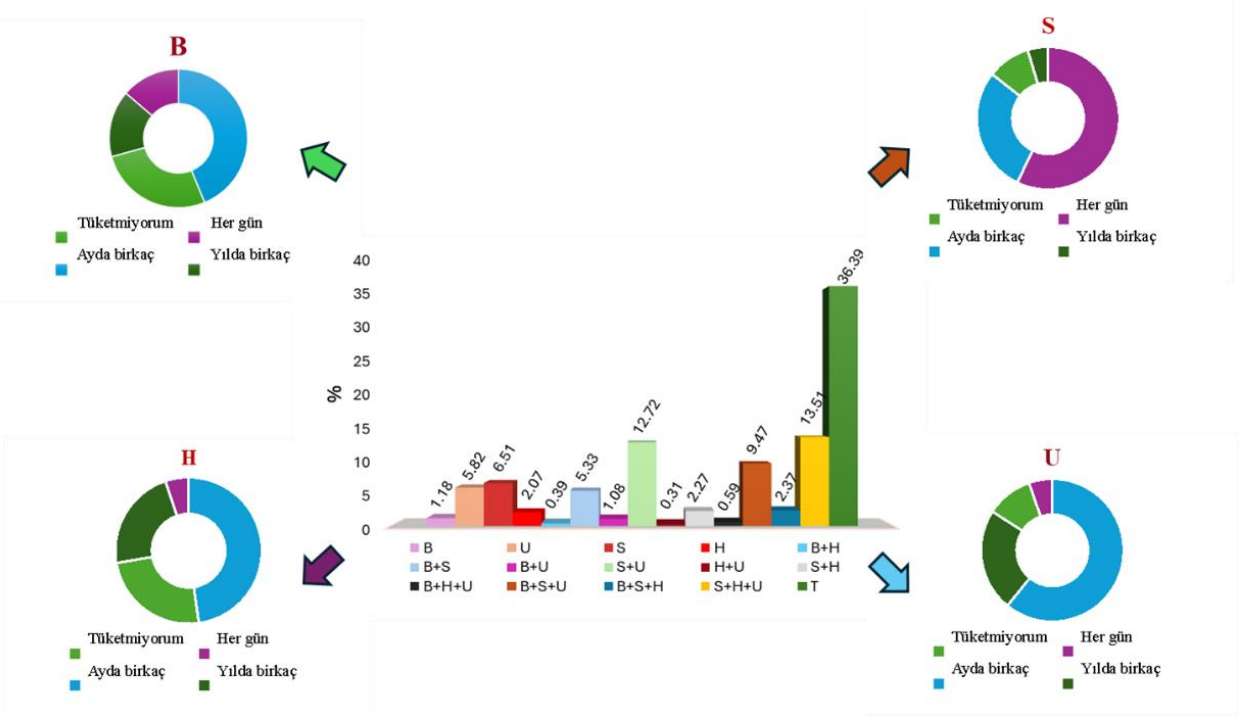
Gösterge	Oran	Gösterge	Oran
Cinsiyet		**Öğrenim durumunuz?**	
Kadın	40,53	Okur-yazar	3,35
Erkek	59,47	İlkokul mezunu	7,89
Yaş Grupları		Ortaokul	8,09
18-25	32,25	Lise	23,18
26-39	29,09	Ön lisans	11,74
40-54	31,85	Lisans	35,60
55+	6,80	Lisansüstü	10,16
Medeni Durum		**Ailenizin aylık ortalama hane geliri ne kadar?**	
Evli	50,39	<5.500* TL	10,36
Bekar	46,84	5.500-10.000TL	36,69
Diğer	2,76	10.000TL +	52,96
Çanakkale doğumlu musunuz?		**Mesleğiniz?**	
Evet	68,24	Çalışmıyor	26,53
Hayır	31,76	İşçi	26,33
Doğum yeriniz?		Memur	18,44
Kır kökenli	44,18	Özel sektör	24,75
Kent kökenli	55,82	Emekli	3,94

Tüketici davranışlarında bilinç gelişimi ve farkındalığın oluşumuna etki eden faktörlerin başında, eğitim durumu ve gelir dağılımı gelmektedir. Bu çalışmada da tüketicilerin öğrenim durumu ve gelir dağılım verileri incelendiğinde; lisans eğitimi alan tüketicilerin en fazla orana sahip olduğu (%35,60), ikinci sırada ise lise mezunlarının, %23,18 oranı ile, geldiği belirlenmiştir. Gelir seviyesi açısından ise, dönemin asgari ücretinin (5.500 ₺) 2 katı ve fazlası olan katılımcı sayısı %52,96 olarak belirlenmiş, asgari ücretli olanların ise %36, 69 oranında oldukları görülmüştür.

Genel tüketici profilinin belirlenmesinin ardından tüketicilere ilk olarak "Yöremize özgü geleneksel ürünlerden hangilerini tüketiyorsunuz?" sorusu ve ardından tüketim sıklığına dair "Yöremize özgü geleneksel ürünleri tüketim sıklığınız nedir?" sorusu yöneltilmiştir. Anket soruları kapsamında yöreye özgü geleneksel ürünler bitkisel (Biga biber turşusu, kuru bakla vb.), hayvansal (Gelibolu Sardalyası, konserve balık, vb.), süt ürünleri (Ezine peyniri, Biga yoğurdu, vb.) ve unlu mamuller (Çanakkale peynir helvası, Bayramiç tahin helvası, Biga cevizli lokumu, Biga peynir tatlısı vb.) olarak kategorize edilmiştir. Tüketim sıklığı ise "tüketmiyorum", "her gün tüketiyorum", "ayda birkaç kez tüketiyorum" ve "yılda birkaç kez tüketiyorum" şeklinde belirlenmiştir. Geleneksel ürün tüketim tercihi ve tüketim sıklığının belirlenmesine ait elde edilen sonuçlar Şekil 1'de verilmiştir.

Tüketicilerin %36,39'u yöreye özgü tüm ürün gruplarına ait gıdalardan tükettiğini belirtmiştir. Tüketimde geleneksel süt ürünleri, hayvansal ürünler ve unlu mamulleri tercih eden katılımcıların oranı %13,51, süt ürünleri ve unlu mamulleri tüketen katılımcıların oranı ise %12,72 olarak belirlenmiştir (Şekil 1). Katılımcıların tüketim sıklığına dair cevaplarında ise; ayda birkaç kez tüketilen ürünlerin bitkisel ürünler (%43,79), hayvansal ürünler (%47,73) ve unlu mamuller (%60,85) olduğu, geleneksel süt ürünlerinin ise, %57,10 oranında katılımcı tarafından her gün tüketildiği tespit edilmiştir (Şekil 1). Ayrıca, geleneksel bitkisel, hayvansal ürünler, süt ürünleri ve unlu mamulleri hiç tüketmediklerini belirten tüketicilerin oranları ise sırasıyla % 26,92, %24,56, %9,76 ve %10,55

olarak bulunmuştur. Elde edilen bu sonuçlar tüketim tercihi verileriyle doğru orantı göstererek anket verilerine verilen cevapların doğruluğuna işaret etmektedir.

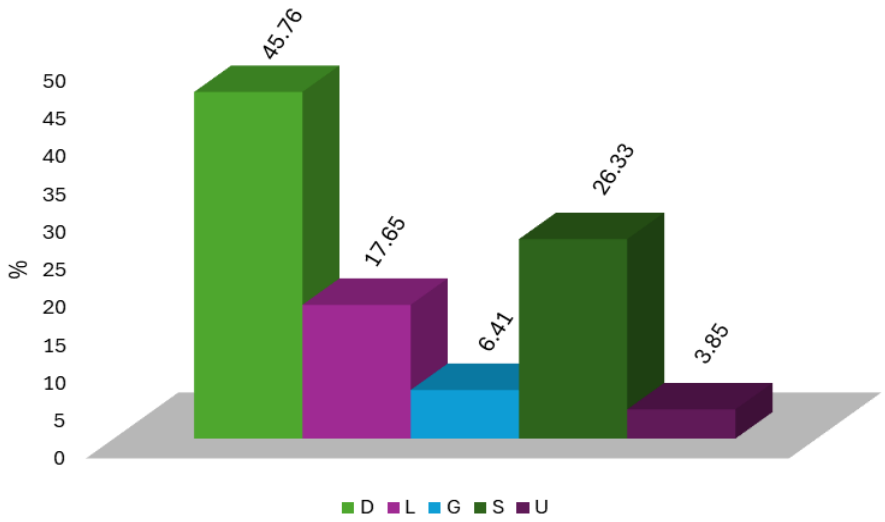


Not: B: Bitkisel ürünleri; U: Unlu mamulleri; S: Süt ürünlerini; H: Hayvansal ürünleri ve T: Tüm ürün gruplarını temsil etmektedir.

Şekil 1. Geleneksel ürünlerden hangilerinin tüketildiği ve tüketim sıklığı, verilerinin dağılımı.

Duru ve Seçer (2019) Mersin ilinde benzer bir çalışma yürütmüş ve tüketicilerin en çok satın aldıkları geleneksel gıdaların süt ürünleri (%65,00), hayvansal ürünler (%12,30), konserve ürünleri (%8,60), kuru gıdalar (%7,80) ve unlu mamuller (%6,00) olduğunu bildirmişlerdir. Ayrıca aynı çalışmada tüketim sıklığı ile ilgili olarak katılımcıların %46,10'unun bazen, %27,10'unun sık sık, %6,50'sinin her zaman ve %20,40'ının da nadiren geleneksel gıda satın almayı tercih ettiklerini tespit etmişlerdir. Sonuçlar bu çalışma kapsamında tüm grupları tüketen tüketicilerin ardından gelen yüksek oranda süt ürünleri tüketimi sonuçları ile uyuşmakta ve tüketim sıklığı ile ilgili sonuçlar ise benzerlik göstermektedir. Her iki çalışmanın sonuçları, toplumuza ait beslenme kültürünün temelinde göçebe yaşam kültürünün bulunması ve bu kültürde süt ve süt ürünlerinin tüketilen besin olarak birinci sırada yer alması ile örtüşmekte ve açıklanabilmektedir.

Geleneksel gıda kavramı ile ilgili olarak tüketicilerin aklına gelen ilk kavramların doğallık, köken ve yerellik, işleme ve özen ile duyusal özellikler olduğu ifade edilmektedir (Guerrero ve ark 2009). Çalışma kapsamında "Geleneksel gıdaları tüketme nedeniniz nedir?" sorusunda ise tüketicilerden "doğallık", "lezzet", "gelenek/alışkanlıklar", "sağlık", ve "ucuzluk" parametreleri üzerinden değerlendirme yapmaları istenmiştir. Tüketicilerin soruya verdikleri yanıtlara ait bulgular Şekil 2'de belirtilmiştir.

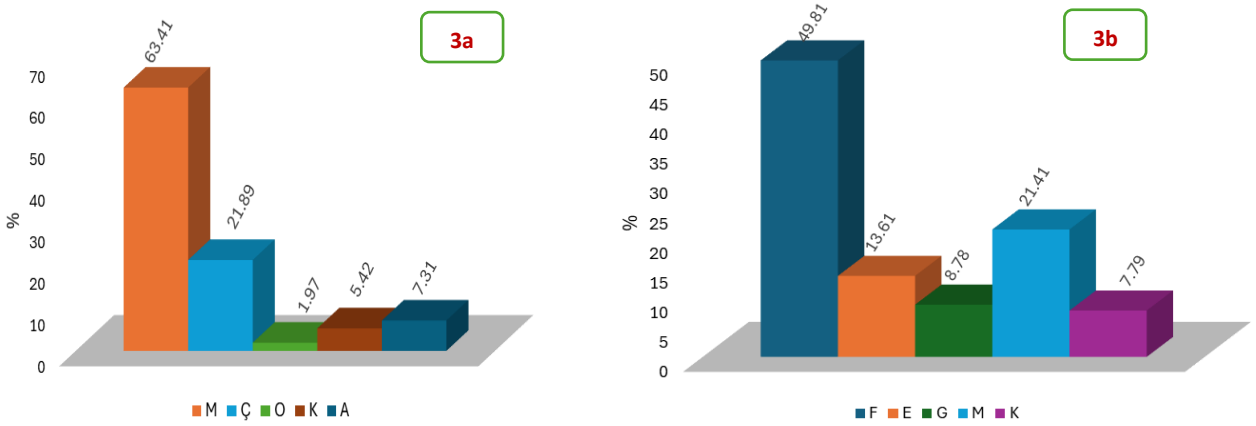


Not: D: Doğal; L: Lezzet; G: Geleneksel/alışkanlık; S: Sağlık; U: Ucuzluk yanıt gruplarını temsil etmektedir.

Şekil 2. Katılımcıların geleneksel gıdaları tüketme nedeni verilerinin dağılımı.

Tüketicilerin %45,76'sı geleneksel gıdaları doğal olduğunu düşündüğü için tükettiğini bildirmiştir. Tüketicilerin %26,33'ü geleneksel gıdaları sağlıklı olduğu, %17,65'i lezzetli olduğunu düşündüğü için tükettiklerini bildirirken, yaklaşık %10,26'sı ise gelenek/alışkanlıklar veya ucuzluk düşüncesinden dolayı tükettiklerini ifade etmişlerdir. (Şekil 2). Elde edilen sonuçlar tüketicinin genel olarak geleneksel gıdalar hakkında doğru bir bilgi birikimi ve analiz yeteneğine sahip olduklarını göstermektedir. Tüketicilerin geleneksel gıda ve tutumlarını ölçen bir başka çalışmada "Bir gıdaya geleneksel diyebilmemiz için kesinlikle doğal olması, üretimi esnasında herhangi bir katkı maddesinin kullanılmaması gerekir." ifadesine katılım durumları sorulmuştur. Çalışmaya katılan tüketicilerin bu ifadeye katılım oranı % 79,3 olarak tespit edilmiştir (Başaran, 2016). Başka bir çalışmada da doğallığa vurgu yapılarak tüketicilerin geleneksel gıdalarda inovasyonunda temel özelliklerde değişim yapılmadan fayda sağlayacak şekilde olması halinde, (örneğin paketleme, besleyicilik, kolaylık odaklı yenilikler) yüksek oranda "kabul edilebilir" olduğu belirtilmiştir (Guerrero ve ark., 2009). Yapılan başka bir çalışmada da tüketicilerin %87,9 oranında geleneksel gıdaları tükettikleri tespit edilmiş, tüketimi etkileyen değişkenlerin ise sırasıyla; aile büyüklerinin geleneksel gıda tüketimi, geleneksel gıdaların besleyici olması, sağlıklı olması ve merak faktörü, şeklinde olduğu belirlenmiştir (Onurlubaş ve Taşdan, 2017).

Tüketicilerin satın alma davranışları sosyo-demografik yapı ile yakından ilişkilidir. Bu davranışlar özellikle eğitim düzeyi ve gelir seviyesine göre şekillenebilmektedir. Bu çalışma kapsamında, tüketicilere yönetilen sorular arasında "Geleneksel ürünleri çoğunlukla nereden temin edersiniz?" ve "Geleneksel ürünleri satın alırken nelere dikkat edersiniz?" yer almış ve satın alma davranışları hakkında bilgi edinilmesi amaçlanmıştır (Şekil 3a, Şekil 3b).



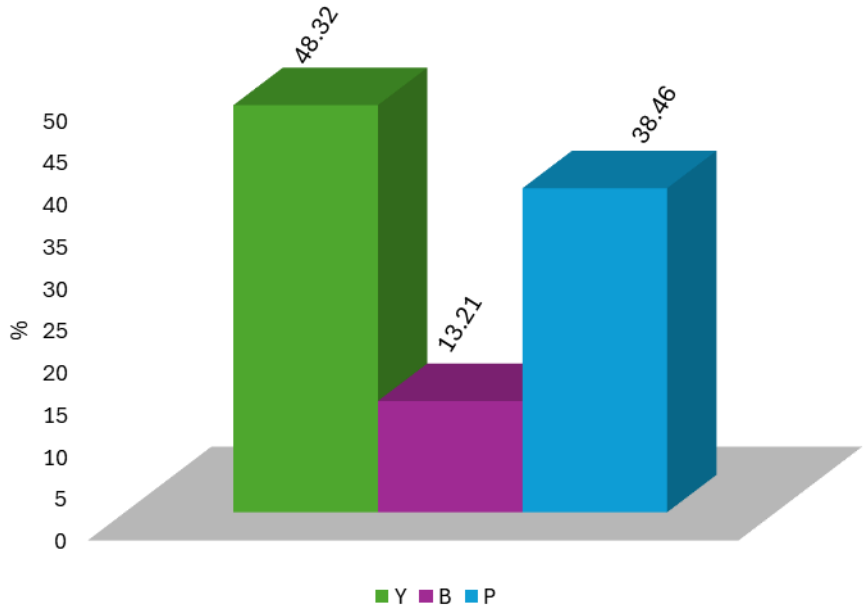
Not: M: Market/Pazar; Ç: Çiftçi/üretici; O: Online siteler; K: Kendi üretimi; A: Aile büyüklerinin üretimini ifade etmektedir.
Not: F: Fiyat; E: Etiket bilgisi; G: Güvenlik sertifikası; M: Marka; K: Kullanımının kolay olması değişkenini ifade etmektedir.

Şekil 3a. Geleneksel ürünlerin çoğunlukla nereden temin edildiği **3b.** Geleneksel ürünleri satın alırken katılımcıların hangi kriterlere dikkat ettiği konusundaki veri dağılımları

Katılımcıların geleneksel gıda temininde market/pazar (%63,41) ve çiftçi/üreticiyi (%21,89) daha çok tercih ettikleri, en az tercihi ise online sitelerden yana kullandıkları tespit edilmiştir. Geleneksel gıda temininde aile büyüklerinin etkisi %7,31 olarak, tüketicinin evde üretimi ise %5,42 olarak belirlenmiştir (Şekil 3a). Bu durum özellikle ankete katılım gösteren kadınların sayısının yarıya ulaşması ve çalışma durumlarıyla ilişkilendirilebilir. Yoğun iş temposunun, çekirdek aile düzeninde aile büyüklerini dışarıda tutması ve mutfakta daha çok kadının aktif olması beslenme konusunda alınan kararlarda kendini göstermektedir. Mersin ilinde yapılan bir çalışmada tüketicilerin %28,90'ı geleneksel gıda satın almada ilk tercihinin marketler olduğunu belirtmişlerdir (Duru ve Seçer, 2019). Ayrıca benzer bir çalışmada tüketicilerin en önemli gıda satın alma yerini market olarak belirlenmiş ve geleneksel ürünler için ayrı raf ve stant düzenlemeleri hakkında önerilerde bulunulmuştur (Taşdan ve ark. 2014). Başka bir çalışmada ise, tüketicilerin geleneksel gıdaları tipik lezzetinden uzaklaşmadan daha sağlıklı, hijyenik ve ambalajlı olarak, temin etmek istedikleri yönünde tespitler yapılmıştır (Ocak ve Önder, 2014). Bu durumda tüketicilerin market tercihi, yine eğitim düzeyi ve yaşam standartları paralelinde ortaya çıkan bir faktör olarak değerlendirilmiştir.

Gıdaların satın alınması sırasında tüketicinin hangi kriterlere dikkat ederek alım yaptığı bilgisi, piyasa iştirakçilerinin yönlendirilmesinde önemli rol oynamaktadır. Bu çalışmada tüketicilerin alım sırasında en çok "fiyata" (%49,81) dikkat ettikleri tespit edilmiştir. İçinde bulunduğumuz ekonomik zorluklar tüketiciyi buna mecbur bırakmaktadır. Fiyatı takip eden değişkenler arasında ise, bilinen marka (%21,41) ve ürünün etiket bilgileri (%13,61) ön plana çıkmakta, ürünün sahip olduğu güvenlik sertifikası (%8,78) ve kolay kullanımına (%7,79) ait kriter özellikleri en sonda yer almaktadır (Şekil 3b). Tekirdağ ili tüketicilerinin geleneksel ürünleri tercihleri üzerine yapılan bir çalışmada da tercihlerde lezzet, gıda güvenliği ve tazelik gibi faktörlerin yanı sıra fiyatın da önemli düzeyde etkili olduğu belirlenmiştir (Oraman ve ark., 2011). Öğrencilerle yapılan başka bir çalışmada ise, öğrencilerin geleneksel gıda satın alırken hijyen şartları, son kullanma tarihi, tat ve aroma, koruyucu madde içermemesi ifadelerini çok önemli buldukları tespit edilmiştir. Bununla beraber ürünün indirimli olması, promosyonlu olması, amblem ve logolu olması, üreten firma, ambalaj, fiyat, marka gibi kriterlerin de önemli olduğu, ifade edilmiştir (Keskin ve ark., 2021). Çalışmamızın ve diğer literatür verilerinin aksine Duru ve Seçer (2019), fiyatın geleneksel gıdaların satın alımında tüketiciler açısından önem arz etmediğini tespit etmiş ve %5'e kadar fiyat artışının tolere edilebileceğini bildirmişlerdir.

Ankete katılan bireylere "Geleneksel gıdalar ile ilgili bilgiyi nereden alırısınız?" sorusu da yöneltilmiştir. Sorunun, yakın çevre, basın ve yayın organları, paket bilgileri, satış temsilcileri ve sosyal medya üzerinden şıkları ile değerlendirilmesi beklenmiştir. Katılımcıların soru ile ilgili yanıtlarına ait bulguları Şekil 4'te şematize edilmiştir.



Not: Y: Yakın çevre; B: Basın ve yayın organları ile sosyal medya; P: Paket bilgileri ile satış temsilcilerini temsil etmektedir.

Şekil 4. Katılımcıların geleneksel gıdalar ile ilgili bilgiyi nereden aldıkları konusundaki verilerin dağılımı

Buna göre katılımcıların %48,32 ünün gıdaya ait bilgiyi, yakın çevresinden tavsiye ile aldığı tespit edilmiştir. Yakın çevrenin ardından tüketici paket bilgileri ile satış temsilcileri ile iletişim (%38,46) üzerinden bilgi alındığı ifade edilmiş, basın ve yayın organları ile sosyal medyanın geleneksel gıdalar konusunda yaklaşık %13,21 oranında kullanıldığı tespit edilmiştir. (Şekil 4). Bununla beraber Trabzon ili halkı üzerinde yapılan bir çalışmada, yazılı ve görsel medyanın tüketim davranışları üzerinde etkili olduğu bulunmuş, bu mecralarda çıkan olumsuz haberlerin tüketici üzerinde negatif etki yarattığı dolayısıyla basın ve yayın organlarının bilgi kaynağı olarak değerlendirildiği belirlenmiştir (Başaran, 2016).

SONUÇ

Dünya genelinde tek tipli beslenmeye doğru ilerleyen gıda sektörüne rağmen, tüketici nezdinde geleneksel gıdalar ve tüketimine doğru bir yöneliş görünmektedir. İnsanlar hazır gıdalardan ziyade yöreye özgü hammadde ve yöntemlerle üretilen gıdaları tercih etmeye başlamışlardır. Geleneksel gıdaların zengin olduğu ülkemizde de tüketicilerin tutum ve davranışlarında benzer değişiklikler gözlenmeye başlamıştır. Bu bağlamda planlanan bu çalışmada, tarihi ve kültürel birikimi çok eskilere dayanan Çanakkale yöresinde, tüketicilerin geleneksel gıda ürünleri konusundaki düşünceleri sorgulanmış, tutum ve satın alma davranışları incelenmiştir.

Çalışmanın sonucunda, yörede en fazla tüketilen geleneksel ürün grubunun süt ürünleri olduğu belirlenmiş, katılımcılar bu ürünleri daha çok doğal olduğuna, katkı maddesi içermediğine inandığı için tükettiğini ifade etmiştir. Katılımcıların geleneksel gıda ürünlerini tercih sebeplerinin, sadece sağlık ve lezzet kriterleri ile sınırlı kalmadığı, ekonomik durumla bağlantılı olarak da şekillendiği ortaya çıkmıştır. Günümüzde yaşanan ekonomik sorunlar halkın gıda alım kararında kendini göstermektedir. Dolayısıyla bu çalışmada da gıda satın alımında fiyat faktörünün tercih sebebi olarak ilk sırada yer aldığı belirlenmiştir.

Geleneksel gıda ürünleri, yalnızca beslenme ihtiyaçlarını karşılamakla kalmayıp, aynı zamanda kültürel kimliğin, toplumsal alışkanlıkların ve yerel ekonominin önemli bir parçası olarak değer taşımaktadır. Gelecekte geleneksel gıda üretimi, sürdürülebilir tarım uygulamaları ve yerel üretimin teşvik edilmesiyle desteklenmeli, bu konuda çalışmalar artırılmalıdır. Bu bağlamda, değişen tüketici eğilimlerini takip etmek ve bu eğilimlere uygun stratejiler geliştirmek de geleneksel gıda ürünlerinin gelecekteki başarısı için kritik öneme sahip olacaktır.

TEŞEKKÜR

Bu çalışmaya desteklerinden dolayı Çanakkale Onsekiz Mart Üniversitesi Bilimsel Araştırmalar Komisyonuna teşekkürlerimizi sunarız. Çalışma FLÖAP-2022-3993 no'lu proje tarafından desteklenmiştir. Bu çalışma için etik kurul izni Çanakkale Onsekiz Mart Üniversitesi, Lisansüstü Eğitim Enstitüsü Etik Kurulu, Bilimsel Araştırma Etik Kurulu'ndan alınmıştır (22.09.2022 Tarih 16/11sayılı Karar (E-84026528-050.01.04-2200218806)).

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ORAL PRESENTATION

Türkiye kıyılarında dağılım gösteren *Belone* spp. popülasyonlarının morfolojik özellikleri

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Özet

Belone cinsine ait güncel ve geçmiş çalışmalar incelendiğinde araştırmacıların cinsin Akdeniz ve Karadeniz havzasında dağılım gösteren taksonları hakkında ortak bir kanaate varamadıkları, dolayısıyla cinse ait alt türler tanımlandığı, tanımlanan bu taksonlardan bazılarının farklı araştırmalarda geçerli tür olarak kabul edilirken bazılarının ise sinonim olarak değerlendirildiği tespit edilmiştir. Gerçekleştirilen çalışma kapsamında Türkiye kıyısal sularında dağılım gösteren *Belone* türlerine ait popülasyonlar örneklenerek cins içindeki taksonların morfolojik özellikleri ortaya konmuş ve popülasyonlar arasındaki farklılıklar irdelenmiştirBu kapsamda, *B. belone* türüne ait Karadeniz popülasyonlarından (Doğu-Orta-Batı) 112, Marmara Denizi popülasyonuna ait 42 ve Ege Denizi popülasyonlarından (Kuzey-Orta-Güney) ise 85 birey olmak üzere toplam 239 örnek temin edilmiştir. *Belone svetovidovi* türüne ait Ege Denizi popülasyonlarından (Kuzey-Orta-Güney) 145 birey ve Marmara Denizi'nden ise 19 birey olmak üzere toplam 164 örnek temin edilmiştir. Morfolojik analizlerde 32 farklı karakter kullanılarak morfometrik analizler gerçekleştirilmiştir. Buna göre Karadeniz ve Ege Denizi popülasyonları arasında morfolojik farklılığa sebep olan karakterlerin sırasıyla "Maksil diş sayısı/göz çapı, Post orbital/HL ve Mandibul diş sayısı/göz çapı" olduğu tespit edilmiştir. Morfolojik analizlerde popülasyonlar arası benzerlik ilişkisi için Bray-Curtis benzerlik değerleri hesaplatılmış olup *B. belone* türü için en yüksek benzerlik orta Ege (OE) ve kuzey Ege (KE) (%99,4), en düşük benzerlik oranının ise doğu Karadeniz (DK) ve güney Ege (GE) popülasyonları (%98,6) arasında olduğu tespit edilmiştir. *Belone belone* popülasyonları arasındaki Bray-Curtis benzerlik değerlerine göre üretilen benzerlik kümelerinin MDS dağılımına bakıldığında Karadeniz, Marmara Denizi ve Ege Denizi'ne ait alt popülasyonlarında yaklaşık %95 oranda benzerlik olduğu görülmüştür. Elde edilen sonuçlar ülkemiz kıyılarında dağılım gösteren zargana popülasyonları arasındaki morfolojik farklılıkların türiçi varyasyon seviyesinde olduğunu göstermiştir.

Anahtar Kelimeler: Belone, zargana, Akdeniz, Karadeniz, morfoloji

Morphological characteristics of *Belone* spp. populations distributed along the Turkish coast

Abstract

The members of the family Belonidae, which usually live in the coastal areas of tropical and subtropical seas as epipelagic and neritic species, contain species of economic value that are in demand for both recreational and commercial fishing activities. A review of current and past studies on the genus Belone has shown that researchers have not been able to reach a common opinion on the taxa of the genus distributed in the Mediterranean and Black Sea basins, leading to the definition of many subspecies belonging to the genus, some of which have been accepted as valid species in different studies, while others have been considered as synonyms. A total of 239 specimens of *B. belone* were collected during the study, 112 from the Black Sea populations (East-Central-West), 42 from the Marmara Sea population and 85 from the Aegean Sea populations (North-Central-South). A total of 164 specimens of the species *Belone svetovidovi* were obtained, including 145 individuals from the Aegean Sea populations (North-Central-South) and 19 individuals from the Sea of Marmara.

In the morphological analyses, morphometric analyses were performed using 32 different characters. The characters causing morphological differences between the Black Sea and Aegean Sea populations were determined to be 'maxillary tooth number/eye diameter', 'post-orbital/HL' and 'mandibular tooth number/eye diameter'. In morphological analyses, Bray-Curtis similarity values were calculated for the similarity relationship between populations, and it was determined that the highest similarity for *B. belone* species was between the Central Aegean Sea (CA) and North Aegean Sea (NA) populations (99.4%), and the lowest similarity was between the Eastern Black Sea (EB) and South Aegean Sea (SA) populations (98.6%). The MDS distribution of the similarity clusters generated according to the Bray-Curtis similarity values between the *B. belone* populations showed that there was approximately 95% similarity in the Black Sea, Marmara Sea and Aegean Sea subpopulations.

Keywords: Belone, garfish, Mediterranean, Black Sea, morphology

GİRİŞ

Denizel ve tatlısu ekosistemlerinde toplamda 10 cins ve 44 türle temsil edilen Belonidae familyası (Fricke ve ark., 2023) Doğu Atlantik, Akdeniz ve Karadeniz ihtiyofaunasının önemli gruplarından birini oluşturmaktadır. Belonidae familyası üyeleri (Fricke ve ark., 2023) hem amatör hem de ticari balıkçılık faaliyetlerinde talep gören ekonomik değere sahip türler barındırır. Tropikal ve subtropikal denizlerin genellikle sahil bölgelerinde epipelajik ve neritik olarak yaşamlarına devam eden *Belone* cinsine ait türlerin hepsinde ovipar üreme görülür ve yumurtaları demersaldir (Akşıray, 1987; Collette, 2016).

Türkiye deniz balıkları listesinin verildiği son çalışmada; *Belone belone* (Linnaeus, 1761), *Belone svetovidovi* (Collette ve Parin, 1970) ve *Tylosurus acus* (Lacepède, 1803) olmak üzere 3 türün varlığından söz edilmektedir (Bilecenoğlu vd., 2014).

Belone cinsine ait güncel ve geçmiş çalışmalar incelendiğinde araştırmacıların cinsin Akdeniz ve Karadeniz havzasında dağılım gösteren taksonları hakkında ortak bir kanaate varamadıkları görülmektedir. Fricke ve ark. (2023)'e göre *Belone* cinsine ait *Belone acus, Belone belone ve Belone svetovidovi* olmak üzere 3 geçerli tür bulunmaktayken Froese ve Pauly (2023)' göre *Belone belone, Belone euxini* ve *Belone svetovidovi* olmak üzere 3 tür bulunmaktadır. Gerek uluslararası literatür, gerekse ulusal fauna çalışmaları incelendiğinde özellikle *Belone* cinsine ait çok sayıda alttür tanımlandığı, farklı araştırmalarda ise tanımlanan bu alttürlerden bazılarının geçerli tür olarak kabul edilirken bazılarının ise sinonim olarak değerlendirildiği tespit edilmiştir. Gerçekleştirilen çalışma kapsamında Türkiye kıyısal sularında dağılım gösteren *Belone* türlerine ait popülasyonlar örneklenerek cins içindeki taksonların morfolojik özellikleri ortaya konmuş ve popülasyonlar arasındaki farklılıklar irdelenmiştir.

MATERYAL VE METOT

Proje kapsamında elde edilen *Belone belone* ve *Belone svetovidovi* bireylerinin morfoloji analizlerinde Zorica ve Čikeš Keč (2011)' den yararlanılmış olup morfolojik incelemelerin detaylandırılması adına ilave karakter kullanılmıştır. Örnekleme bölgeleri ve istasyonlar Tablo 1 ve Tablo 2 de verilmiştir.

Morfometrik ölçümler ve meristik sayımlar için vücut bütünlüğünde herhangi bir deformasyon bulunmayan örnekler çalışılmıştır. Buna istinaden 143 adet *B. belone*, 114 adet ise *B. svetovidovi* bireyinin morfolojisi çalışılmıştır. Toplam boy, çatal boy, standart boy, maksil ve mandibul uzunlukları, baş boyu, pre-dorsal, pre-pelvik, pre-anal, pre-pektoral, pre-orbital post-orbital ve pelvik ile anal yüzgeç arası mesafeleri, yüzgeçlerin uzunlukları, göz çapı ve gözler arası mesafe, dorsal, pelvik ve anal yüzgeç kaidelerinden vücut yükseklikleri gibi toplam 32 karakter ölçülmüş, dorsal, anal, pektoral ve pekvik yüzgeç ışını, maksil ve mandibul diş, solungaç diken sayımları gerçekleştirilmiştir.

Tablo 1. *Belone svetovidovi* bireylerinin örneklendiği istasyonlar

Örnekleme Bölgesi	**İstasyonlar**	**Örnek sayısı**
Marmara Denizi	Boğaz/BGZ – Gemlik/GML	19
Kuzey Ege	Çanakkale/CNK – Altınoluk/ALT	35
Orta Ege	İzmir/IZM	73
Güney Ege	Bodrum/BDR – Didim/DM	37

Tablo 2. *Belone belone* bireylerinin örneklendiği istasyonlar

Örnekleme bölgesi	**İstasyonlar**	**Örnek Sayısı**
Karadeniz		**60**
Batı Karadeniz		33
	Kefken/KFK- Riva/RV	
Orta Karadeniz		19
	Samsun/SAM-Ordu/ORD	
Doğu Karadeniz		8
	Ardesen/ARD-Rize/RZ	
Marmara Denizi		**25**
	Marmara Ereglisi/MRE-Kemer/KMR	
Ege Denizi		**58**
Kuzey Ege		25
	Canakkale/CNK-Altinoluk/ALT	
Orta Ege		18
	İzmir/IZM	
Güney Ege		15
	Bodrum/BDR	

Elde edilen verilerin standardize edilmesi amacıyla standart boy ve baş boyuna oranlar kullanılmıştır. Ayrıca diş sıklığının tespit edilebilmesi amacıyla maksil ve mandibul toplam diş sayısı ile standart boy, maksil-mandibul uzunlukları ile diş sayıları ve göz çapı değerlerlerinin birbirlerine oranları belirlenmiştir.

Popülasyonlar arasında benzerlik ve farklılığa neden olan karakterler Primer7 ile belirlenmiştir (Clarke ve Gorley, 2015). Bray-Curtis Benzerlik matrisi uygulanan verilerde MDS (non-Metric Multidimensional Scaling – Metrik Olmayan Çok Yönlü Ölçümleme) ve SIMPER (Similarity Percentages –Benzerlik Yüzdesi) yöntemleri kullanılmıştır. Popülasyonları gruplandırmak içinse bir kümeleme yöntemi olan UPGMA (Aritmetik Ortalamalı Ağırlıksız Çift Grup Yöntemi) uygulanmıştır.

BULGULAR VE TARTIŞMA

Gerçekleştirilen morfolojik analizlerde *B. svetovidovi* bireylerinin 26,1-41,5 cm (ortalama 32,67 cm) standart boya sahip oldukları tespit edilmiştir. Kafa boyu 5,7-12,5 cm (ortalama 10,02 cm) arasında değişirken, göz çapının 0,7-1,1 cm (ortalama 0,9 cm) arasında değiştiği saptanmıştır. *B. svetovidovi* bireylerinin meristik karakterlerinde ise, dorsal yüzgeç ışın sayısının 14- 19 (ortalama 16,8) arasında değiştiği, anal yüzgeç ışın sayısının 18-25 (ortalama 21,1) arası değişkenlik gösterdiği, pektoral yüzgeç ışın sayısının 11-15 (ortalama 12,4) arasında değiştiği gözlemlenmiştir. Solungaç diken sayısı 31-50 (ortalama 41,4) arası değişirken, maksil diş sayısı 106- 312 (ortalama 189,2) ve mandibul diş sayısının ise 105- 440 (ortalama 286,6) arasında değişkenlik gösterdiği saptanmıştır.

B. belone bireylerinin ise 24,6-58,2 cm (ortalama 34,4 cm) standart boya sahip oldukları tespit edilmiştir. Kafa boyu 7,7-17,4 cm (ortalama 11,04 cm) arasında değişirken, göz çapının 0,7-1,5 cm (ortalama 0,97 cm) arasında değiştiği saptanmıştır. *B. belone* bireylerinin meristik karakterlerinde ise, dorsal yüzgeç ışın sayısının 15-20 (ortalama 17,1) arasında değiştiği, anal yüzgeç ışın sayısının 17-24 (ortalama 20,4) arası değişkenlik gösterdiği, pektoral yüzgeç ışın sayısının 10-14 (ortalama 12,5) arasında değiştiği gözlemlenmiştir. Solungaç diken sayısı 22-32 (ortalama 27,1) arası değişirken, maksil diş sayısı 64-178 (ortalama 122) ve mandibul diş sayısının ise 65-254 (ortalama 155,1) arasında değişkenlik gösterdiği saptanmıştır. Analiz edilen bireylere ait morfometrik ölçümler ve meristik özellikler Tablo 3, Tablo 4 ve Tablo 5' de verilmiştir.

Popülasyonlar arası benzerlik ilişkisi için Bray-Curtis benzerlik değerleri hesaplatılmış olup *B. belone* türü için en yüksek benzerlik orta Ege (OE) ve kuzey Ege (KE) (%99,4), en düşük benzerlik oranının ise doğu Karadeniz (DK) ve güney Ege (GE) popülasyonları (%98,6) arasında olduğu hesaplanmıştır (ŞEkil 1). *B. sevetovidovi* için hesaplanan Bray Curtis benzerlik değerlerine göre en yüksek benzerlik Orta Ege (OE) ve Güney Ege (GE) (%99,4), en düşük benzerlik oranının ise Kuzey Ege (KE) ve Marmara (M) popülasyonları (%98,6) arasında olduğu hesaplanmıştır (Şekil 2).

Çalışma kapsamında *Belone belone* ve *B. svetovidovi* türleri arasında morfolojik olarak benzerlik ve farklılıklar da incelenmiştir. Bu kapsamda türler arasındaki benzerlik ilişkisi için Bray-Curtis benzerlik değerleri hesaplatılmış olup en yüksek benzerlik orta Ege Denizi (OE) *B. svetovidovi* ile doğu Karadeniz (DK) *B. belone* arasında (%94,7), en düşük benzerlik ise *B. belone* ve *B. svetovidovi* Marmara Denizi popülasyonları arasında olduğu görülmüştür (%93,5) (Şekil 3). *Belone belone* ve *B. svetovidovi* popülasyonları arasındaki Bray-Curtis benzerlik değerlerine göre üretilen benzerlik kümelerinin MDS dağılımına bakıldığında tür içi popülasyonların yaklaşık %95 benzerlik göstererek kendi aralarında kümelendiği, iki türünde yaklaşık %90 oranda benzerlik gösterdiği görülmektedir (Şekil 4).

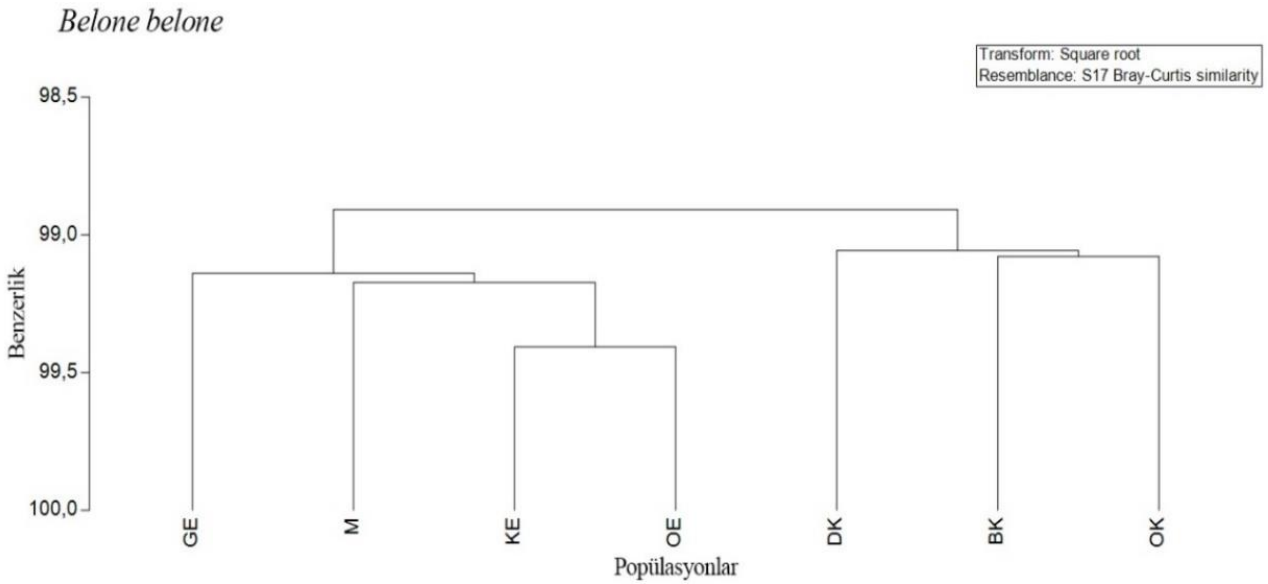
Yapılan morfometrik oranlara dayalı gerçekleştirilen SIMPER analizine göre *B. belone* ve *B. svetovidovi* türleri arasındaki arasında ortalama %5,78 fark hesaplanmıştır. Bu farklılığa neden olan karakterlerin ise türler arasındaki diş sayısındaki farklılıklara dayalı olduğu görülmüştür (Tablo 6).

Bu çalışmada *B. belone* türüne ait analiz edilen bireylerin dorsal yüzgeç ışın sayısının 15-20 (ort 17.1) arasında değiştiği tespit edilmiştir. Karadeniz ve Ege Denizi popülasyonlarında dorsal yüzgeç ışın sayısı ortalaması 17,2 tespit edilirken Marmara Denizi popülasyonunda 16,7 olarak belirlenmiştir. Farklı coğrafik bölgelerde *B. belone* ile yapılan çalışmalarda yukarda bahsi geçen meristik karakterlere ait değerlerin iç içe geçtiği gözlenmektedir**.** Bahsi geçen veriler ışığında hem *B. belone* türünün hem Akdeniz, hem de Karadeniz popülasyonları arasında tür seviyesinde morfolojik bir farklılık olmadığı, mevcut farklılığın ancak varyasyon sınırları dâhilinde kaldığı görülmektedir. *Belone belone* popülasyonları arasındaki Bray-Curtis benzerlik değerlerine göre üretilen benzerlik kümelerinin MDS dağılımına bakıldığında Karadeniz, Marmara Denizi ve Ege Denizi'ne ait alt popülasyonlarında yaklaşık %95 oranda benzer olduğu ve Karadeniz'de dağılım gösteren popülasyonun morfolojik olarak farklı bir popülasyon olmadığı kanaatine varılmıştır. Popülasyonlar arasında farklılığa sebep olan karakterlerin ise meristik karakterler yerine kafa ve diş ile ilgili olan metrik karakterler olduğu tespit edilmiştir.

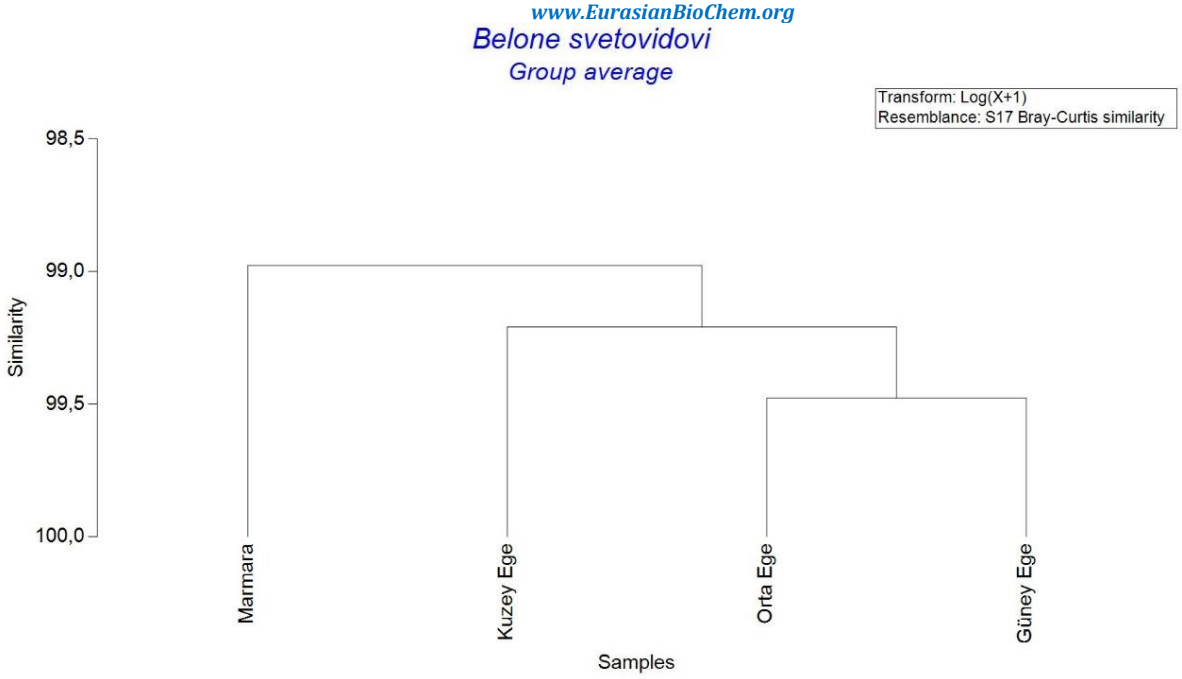
Çalışmada incelenen *B. svetovidovi* türüne ait popülasyonların Bray Curtis benzerlik değerleri Kuzey Ege – Marmara popülasyonu hariç popülasyonların %99 üzeri benzerlik gösterdiğini ortaya koymuştur. SIMPER analizine göre ise saptanan en yüksek farklılık ise Marmara Denizi ve Kuzey Ege Denizi popülasyonları arasında (%1,40) tespit edilmiştir. Buna ek olarak, popülasyonlar arasında farklılığa sebep olan karakterlerin başında

"İnterorbital/HL; Alt çene diş sayısı/mandibul uzunluğu; Head depth/HL" gibi genelde baş ile ilgili oranların yer aldığı tespit edilmiştir. Tekerrürlü gerçekleştirilen örnekleme çalışmalarında *B. svetovidovi* türünün popülasyon yoğunluğunun Orta Ege Denizi'nde diğer örnekleme bölgelerine göre daha yoğun olduğu, bu yoğunluğun ise güneye doğru azaldığı gözlenmiştir. Güney Ege ve Levantin kıyılarımızda yapılan tekrarlı örnekleme çalışmalarında *B. svetovidovi* türüne sadece Didim'de ulaşılabilmiştir. Gerçekleştirilen çalışmada *B. svetovidovi* türünün kıyılarımızdaki popülasyon yoğunluğunun Orta Ege Denizi'nde diğer örnekleme bölgelerine göre daha yoğun olduğu, bu yoğunluğun ise güneye doğru azaldığı gözlenmiştir. Levantin kıyılarımızdaki su sıcaklığının diğer denizlerimize nazaran daha fazla olmasının, bu türün güneydeki dağılımını etkileyen faktör olabileceği ve Levantin Denizi'ndeki sıcaklık değerlerinin demersal yumurtalayan bu türlerin yumurtlama substratları olabilen deniz çayırlarının da dağılımını da etkileyerek türün güneydeki dağılımını sınırladığı kantine varılmıştır.

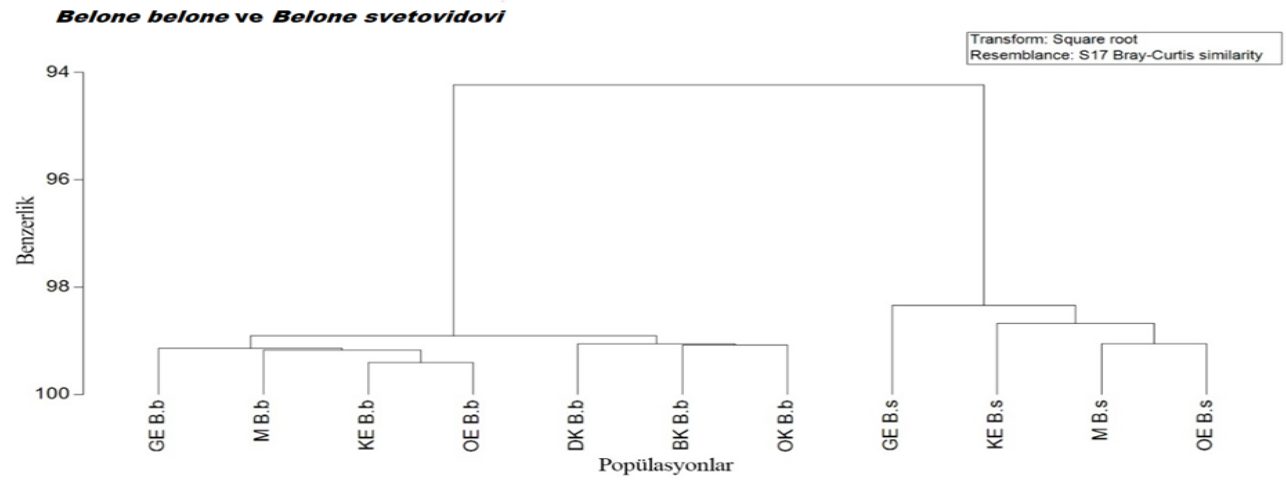
Çalışmadan elde edilen sonuçlar göstermiştir ki, ülkemiz kıyılarında dağılım gösteren Zargana popülasyonları arasında gözlemlenen morfolojik farklılıklar türiçi varyasyon seviyesinde olup, bu farklılıklar genelde kafa ile ilgili olan karakterlerden kaynaklanmaktadır. Bununla birlikte, morfolojik olarak çok benzeyen *B. svetovidovi* ve *B. belone* türleri solungaç diken sayısı, diş sayısı ve diş sayısının göz çapına oranı gibi karakterlerle kolaylıkla birbirinden ayırt edilebilmektedir.



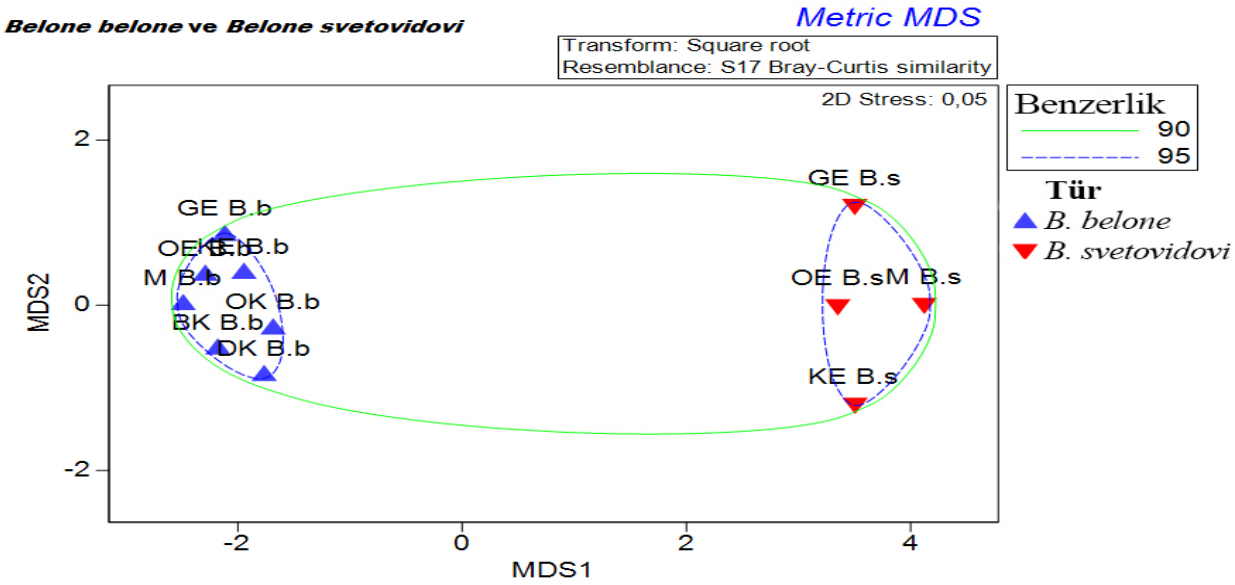
Şekil 1. *B. belone* popülasyonlarının morfolojik benzerliklerinin Bray-Curtis benzerlik dendogramı (DK: Doğu Karadeniz, BK: Batı Karadeniz, OK: Orta Karadeniz, M: Marmara Denizi, KE: Kuzey Ege Denizi, OE: Orta Ege Denizi, GE: Güney Ege Denizi popülasyonlarını ifade etmektedir).



Şekil 2. *B. svetovidovi* popülasyonlarının morfolojik benzerliklerinin Bray-Curtis benzerlik dendogramı



Şekil 3. *B. belone* ve *B. svetovidovi* popülasyonlarının morfolojik benzerliklerinin Bray-Curtis benzerlik dendogramı (B.b: *B. belone*, B.s: *B. svetovidovi*, DK: Doğu Karadeniz, BK: Batı Karadeniz, OK: Orta Karadeniz, M: Marmara Denizi, KE: Kuzey Ege Denizi, OE: Orta Ege Denizi, GE: Güney Ege Denizi popülasyonlarını ifade etmektedir).



Şekil 4. *B. belone* ve *B. svetovidovi* popülasyonları arasındaki morfolojik benzerlik kümelerinin mMDS dağılımı (B.b: *B. belone*, B.s: *B. svetovidovi*, DK: Doğu Karadeniz, BK: Batı Karadeniz, OK: Orta Karadeniz, M: Marmara Denizi, KE: Kuzey Ege Denizi, OE: Orta Ege Denizi, GE: Güney Ege Denizi popülasyonlarını ifade etmektedir).

Tablo 3. *B. belone* ve *B. svetovidovi* türlerine ait morfometrik oranlar

	Güney EGE		Kuzey Ege		Orta Ege		Marmara		Genel		
Belone svetovidovi	**Ortalama**	**std**	**Ortalama**	**std**	**Ortalama**	**std**	**Ortalama**	**std**	**Değişim aralığı (min-mak)**	**ort**	**std**
Dorsal ışın sayısı	17,43	0,87	16,68	1,17	16,70	1,29	16,64	1,21	14-19	16,8	0,4
Anal ışın sayısı	21,14	1,56	21,41	1,14	20,73	1,61	21,45	1,04	18-25	21,1	0,3
Pektoral yüz. ışın sayısı	13,05	0,86	12,32	0,89	12,28	1,67	12,27	0,79	11-15	12,4	0,4
Pelvik yüz. ışın sayısı	6,10	0,30	6,00	0,00	6,03	0,18	6,09	0,30	6-7	6,05	0,0
Solungaç dikeni sayısı	42,48	3,59	41,73	3,81	42,57	4,76	38,73	3,69	31-50	41,4	1,8
Üst çene diş sayısı	203,52	30,99	167,77	40,22	194,90	40,59	190,91	14,71	106-312	189,2	15,3
Alt çene diş sayısı	287,95	60,83	261,32	90,69	298,80	79,56	298,64	46,30	105-440	286,6	17,7

Tablo 4. *B. svetovidovi* bireylerine ait meristik özellikler

	Belone belone														***Belone svetovidovi***							
	KARADENİZ						**MARMARA**		**EGE DENİZİ**						**MARMARA**		**EGE DENİZİ**					
	Doğu		Orta		Batı				Kuzey		Orta		Güney				Güney		Orta		Kuzey	
Birey sayısı (n)	8		19		33		25		25		18		15		11		21		60		22	
	ort	std ±	ort	std ±	ort	std ±	ort	std ±	ort	std ±	ort	std ±	ort	std ±	ort	std ±	ort	std ±	ort	std ±	ort	std ±
HL/SL	28,2	1	29	1,8	29,8	4,4	30,6	1	30,6	3,7	30,4	1,6	28,1	2,4	29,5	2,1	29,3	1	28,6	1,8	27,6	3,6
Predorsal/SL	80,3	1	77,1	13,7	80,8	0,7	80,6	0,6	80,6	1	81,4	0,8	81,6	1,2	80,5	1,3	81,3	1,7	80,9	1,3	80,6	5,4
Prepelvik/SL	64,4	1,8	65,4	0,9	65,8	0,8	66,2	1,4	66,3	1,2	67,2	0,9	66,9	1,2	66,8	1,5	68,1	4	66,8	1,6	65,8	3,6
Preanal/SL	78	1,1	78,5	0,7	78,9	0,7	78,7	1	79,5	2,6	79,9	0,8	79,9	0,8	78,4	1	78,7	0,6	78,6	1,3	77,8	3,4
Prepektoral/SL	31,8	2,6	32,7	1,3	33	1,4	34,3	2,8	33,5	1,6	33,8	1,9	33,4	1,4	32,2	2,5	32,2	2,8	31,8	2,6	31	5
Pelvik-anal/SL	13,4	0,9	13	0,5	11,5	2,8	12,8	0,9	12,8	0,6	12	2	12,9	0,8	11,5	0,7	11,5	0,4	11,9	1	12	0,8
Pektoral Y. uzunluğu/SL	7	0,5	6,6	0,3	6,6	0,5	6,5	0,5	6,1	0,6	6,1	0,4	5,8	0,4	5,8	0,4	5,3	0,3	5,8	0,4	5,7	0,4
Pelvik Y. uzunluğu/SL	5,1	0,1	4,9	0,6	4,9	0,4	4,6	0,5	4,6	0,5	5	2,1	4,3	0,3	4,3	0,3	4	0,2	4,5	0,6	4,4	0,4
Kaudal Y. uzunluğu/SL	10	0,4	10	0,5	10,5	0,7	9,8	0,4	10	0,5	10,1	0,6	9,4	0,4	10	0,4	9,4	1	10,2	0,6	10,1	0,9
Kuyruk sapı yüksekliği/SL	1,7	0,2	1,8	0,2	1,9	0,3	1,9	0,3	2	0,3	1,9	0,2	2	0,1	2	0,1	1,7	0,1	1,8	0,2	2	0,4
Dorsal Y. kaidesinden vücut yüksekliği/SL	5	0,3	5,2	0,3	5,3	0,4	5	0,8	5,4	0,6	5,3	0,6	5,8	0,3	5,4	0,4	5,5	0,3	5,5	0,5	5,2	0,6
Pelvik yüzgeç kaidesinden vücut yüksekliği /SL	5	0,3	5,1	0,3	5,1	0,3	4,8	0,7	5,4	0,8	5,3	0,6	5,4	0,3	5,5	0,4	5,4	0,3	5,5	0,7	5,2	0,5
Anal Y. kaidesinden vücut yüksekliği /SL	5	0,3	5,1	0,3	5,1	0,3	4,9	0,7	5,3	0,5	5,1	0,5	5,6	0,2	5,4	0,4	5,3	0,3	5,4	0,5	5,1	0,5
Dorsal Y. taban uzunluğu/SL	12,3	0,4	12,6	0,6	12,5	0,8	12,1	1	12,3	0,6	11,8	0,8	12,2	0,6	12,8	0,8	12,1	0,5	12,1	0,7	12,3	1,1
Anal Y. taban uzunluğu/SL	14,6	0,6	14,8	0,7	14,5	1,2	13,8	0,8	14	0,7	13,5	0,7	14,1	0,6	14,9	0,6	14,6	0,7	14,8	0,9	15,3	1,5
Kafa yüksekliği/HL	14	1,3	13,6	0,8	13,9	0,7	12,7	1,4	14	1,1	14,5	1,2	14	0,8	14,9	1,6	13,6	0,8	13,7	2,2	12,6	2,9
Pre orbital/HL	68,5	3,2	69,9	1,5	70,1	2,4	70,9	2,4	70,2	2,8	70,2	1,6	72,2	2,5	66,7	15	71,1	0,9	69,8	5,8	65,9	7,7
Post orbital/HL	22,4	2,6	21,8	0,9	26,4	7,9	20,5	1,4	20,7	2,2	21,3	1,4	20,5	1,4	22,7	2,8	20,4	0,6	21,5	2,9	23,2	5,3
Göz çapı/HL	9,4	0,7	8,7	0,6	9	0,5	8,5	0,6	8,8	0,9	9,2	0,6	8,4	0,6	9,7	1,1	8,6	0,4	9,4	1	10,5	2,5
Gözler arası mesafe/HL	8	1	7,7	0,4	8,2	0,6	7,4	0,7	7,7	1,8	8,1	0,7	8,2	0,5	7,9	1,1	7,2	0,4	8,1	0,9	9,9	2,5
Maksil diş sayısı/maksil uzunluğu	18,9	3,5	21,3	3,6	18,7	2,8	19,6	6	18,4	3,6	19	2,2	19,4	2,6	35,4	2,7	31,2	4,9	34,5	6,8	37,5	5,3
Mandibul diş sayısı/mandibul uzunluğu	22,9	3,2	21,4	5,6	20,9	4,1	20,7	8,3	22,5	3,8	22,1	2,8	20,4	2,8	50,6	7,8	39,2	8,1	47	12,7	50,7	10,8
Maksil diş sayısı/göz çapı	129	23,1	135,3	22,2	128,6	17,6	127,1	31,1	121	20,8	120,6	18,2	124,7	17,6	212,3	24,1	225,9	38,3	202,1	41,7	188,8	45,8
Mandibul diş sayısı/göz çapı	168,4	19,2	158	39,9	161,9	29,4	155,4	59,6	164,9	26,4	156	23,8	159,8	16,4	333,5	63	320,1	71,2	309,5	82,2	295,7	107
Toplam diş sayısı/ SL	8,5	1,2	8	1,5	8,3	1,2	8,3	3,1	8	1,2	8,3	1,5	7,6	0,7	15,9	2,7	15	2,9	14,7	3,4	14,4	4

Tablo 5. *B. belone* bireylerine ait meristik özellikler

	Batı Karadeniz		**Doğu Karadeniz**		**Orta Karadeniz**		**Marmara**		**Kuzey Ege**		**Orta Ege**		**Güney Ege**		**Genel Ortalama**		
Belone belone	**Ort.**	**std**	**Ort.**	**std**	**Ort.**	**std**	**Ort.**	**std**	**Ort.**	**std**	**Ort.**	**std**	**Ort.**	**std**	**Değişim aralığı**	**Ort.**	**std**
Dorsal ışın sayısı	17,03	0,93	17,50	0,71	17,32	0,73	16,68	1,22	16,64	1,02	17,67	1,15	17,47	0,72	15-20	17,1	0,4
Anal ışın sayısı	20,85	1,37	20,50	1,32	20,21	1,20	20,20	1,10	20,20	1,30	20,28	0,87	20,33	0,87	17-24	20,4	0,2
Pektoral yüz. ışın sayısı	12,73	0,62	11,88	0,60	12,00	0,92	12,12	0,71	12,56	0,80	12,61	0,68	12,60	0,80	10-14	12,5	0,3
Pelvik yüz. ışın sayısı	6,09	0,29	6,00	0,00	6,17	0,37	6,12	0,32	6,04	0,20	6,00	0,00	7,00	0,00	6-7	6,2	0,3
Solungaç dikeni sayısı	26,85	2,19	27,25	1,98	27,53	2,06	25,56	2,40	27,44	1,77	27,22	2,10	27,80	1,51	22-32	27,1	0,7
Üst çene diş sayısı	122,42	15,98	115,13	26,09	118,79	21,20	108,72	22,12	118,08	17,36	142,67	17,99	128,47	16,21	64-178	122	10,9
Alt çene diş sayısı	154,64	29,71	149,25	21,35	139,05	36,74	132,36	45,40	161,00	21,41	184,44	25,04	164,87	15,96	65-254	155,1	17,3

Tablo 1. *B. belone* ve *B. svetovidovi* türleri arasında farklılığa morfolojik olan karakterler.

Belone belone* ve *Belone svetovidovi	
Ortalama Farklılık=5,78	
Karakter	**% Katkı**
Mandibul diş sayısı/göz çapı	33,61
Maksil diş sayısı/göz çapı	22,09
Mandibul diş sayısı/mandibul uzunluğu	14,40

TEŞEKKÜR

Bu çalışma TÜBİTAK 121Y553 numaralı proje tarafından desteklenmiştir.

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ORAL PRESENTATION

Investigation of the Inhibition Effects of Igepal CO-520 on AISI 405 Stainless Steel Corrosion in 1.0 HCl

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Abstract

Corrosion of metals and alloys is a very destructive process, which causes a grave economic loss to the whole world, and the elimination or at least inhibition is of utmost importance. One of the methods employed to inhibit the corrosion rate is the use of inhibitors. Herein, the corrosion inhibition ability of Igepal CO-520 on AISI 405 stainless steel in 1.0 M HCl was investigated by electrochemical impedance spectroscopy and linear polarization method. The surface morphology of AISI 405 stainless steel in 1.0 M HCl with the absence and presence (100 ppm) of Igepal CO-520 with scanning electron microscopy confirmed the fact that the SS-450 surface was covered with a defensive layer of absorbed Igepal CO-520 film. Chromium concentration is determined by atomic absorption spectroscopy. The results obtained from all the techniques are in good agreement.

Keywords: Igepal CO-520, Stainless steel, Corrosion inhibition, Surfactant.

INTRODUCTION

Corrosion is a universal and harmful phenomenon that almost all metallic materials are susceptible to [1]. Corrosion causes environmental problems and loss of human life in addition to the cost of structural damage and maintenance of pipelines, steel bridges etc., loss of working hours, decreased production and polluted products [2, 3]. Stainless steels due to, their excellent mechanical properties, and corrosion resistance are extensively used in diversified fields ranging from aviation to automobile, pharmaceutical, and construction to food industries [4, 5]. Type AISI 405 (UNS S 40500) is a ferritic stainless steel with low content of Ni, very low content of C, and 12-17% Cr content [6]. Mineral acids are much better cleaning agents than organic acids [7]. Acid solutions, especially hydrochloric acid, are very effective for pickling, cleaning, scale removal, acidification, and chemical and petrochemical procedures [8, 9]. The chloride anions have a vital role in the corrosion of steel [10]. Therefore, the preservation of metals and their alloys from this destructive effect is one of the issues with crucial importance in the world agenda awaiting immediate action for the industry [11].

Corrosion inhibitors are widely used in closed-loop systems due to their ease of use, low cost, and high efficiency [12]. Figure 1 indicates the structure of the Igepal surfactants, which are non-phenylethoxylates with ethoxy (EO) numbers of five for CO-520, 12 for CO-720, and 40 for CO-890 [13]. Igepal surfactants can be used in pharmaceutical, food, and cosmetic application due to their benign character against on humans [14]. Igepal CO-520 (pentaoxyethylene-glycol-nonyl-phenyl ether) is a non-ionic surfactant which contains 50% mol hydrophilic group [15-17].

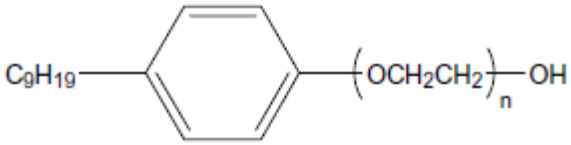

Figure 1. Non-ionic surfactants of the Igepal CO series

In this study, Igepal CO-520, a non-ionic surfactant, has been applied as a corrosion inhibitor. The inhibition efficiency of IgCO-520 for SS-405 in 1.0 M HCl was evaluated by electrochemical tests, such as EIS, and

linear polarization. The surface morphologies of SS-405 in the absence and presence of inhibitor were evaluated using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Chemicals

The non-ionic surfactant Igepal CO-520 ($(C_2H_4O)n \cdot C_{15}H_{24}O$, n~5) was a Sigma-Aldrich product (CAS Number: 68412-54-4, St. Louis, MO, USA) and used without purification. 1.0 M HCl which is the corrosive media was prepared by dilution of 37% HCl (analytical grade, CAS Number: 7647-01-0, St. Louis, MO, USA) with double distilled water (18 MΩ cm^{-1}). The experimental concentrations were within the range 5–100 ppm for Igepal CO-520. A 1000 ppm Cr (III) ($Cr(NO_3)_3$) stock solution in %2 HNO_3 was purchased from Merck (Darmstadt, Germany).

Stainless steel

This study is carried out to investigate the inhibition effect of Igepal CO-520 upon the corrosion of AISI 405 (UNS S 40500) stainless steel. The chemical composition of SS-405 was (wt%): C 0.08, Mn 1.00, Si 0.75, Cr 12.50, Ni 0.50, P 0.04, S 0.03, and Fe balance. The stainless steels were mechanically cut as eighteen 2.0 × 2.0 × 0.05 cm coupons. All the coupons were corroded using emery papers with different grades (400, 600, and 1200), polished with alumina (1 µm and then 0.5 µm), washed and degreased with double distilled water and acetone, respectively, and dried at ambient temperature. The coupons were weighted and kept in a desiccator before analysis.

Electrochemical measurements

All the electrochemical measurements were carried out by a PalmSens3 potentiostat. An Ag/AgCl electrode and a platinum wire were used as reference and auxiliary electrodes, respectively. The whole area of the stainless steel (working electrode) electrode was insulated with Teflon tape except for an area of 1 cm2 left open to act as an electrode surface exposed to the aggressive medium. The SS-405 electrode was kept immersed in the 1.0 M HCl solution until the open circuit potential was established before electrochemical experiments.

EIS measurements were performed in a frequency range of 100 kHz to 0.1 Hz with amplitude of 10 mV using AC signals at 25 °C.

Linear polarization curves were measured at 45 °C. Tafel polarization plots were obtained automatically in the potential range of about -300 to +300 mV at OCP with a scan rate of 0.5 mV/s. Tafel extrapolation method was used for the estimation of both the corrosion current (i_{corr}) and the corrosion potential (E_{corr}).

Scanning electron microscopy (SEM)

The surface morphology of specimens was examined after keeping in 1.0 M HCl solution for 120 h with and without 100 ppm of IgCo-520 by JEOL JSM–6060 LV.

Determination of Chromium

The absorbance of the colored solutions was measured by BioTek Epoch 2 (microplate reader) at the maximum absorption wavelength of 330 nm, and the concentration of chromium was determined from a calibration curve prepared by the use of standard solutions of concentrations of Cr (III) from 0.10 to 10.00 ppm by the use of AAS. An AAS (Perkin Elmer, AAnalyst 700), equipped with a chromium hollow cathode lamp and an air-acetylene flame atomizer was used. The wavelength, spectral bandwidth, and lamp current were 357.9 nm, 0.7 nm, and 25 mA, respectively [18].

RESULT AND DISCUSSION

Electrochemical measurements

EIS is a non-destructive surface method that gives us valuable information about the surface oxide layers and metal/solution interface, the protective layer on the metal surface significantly increases the impedance of the corrosion system together with charge transfer resistance (R_{ct}) [19].

The Nyquist plots for the corrosion of SS-405 in the absence and presence (5, 10, 25, 50 and 100 ppm) of IgCO-520 are shown in Figure 2.

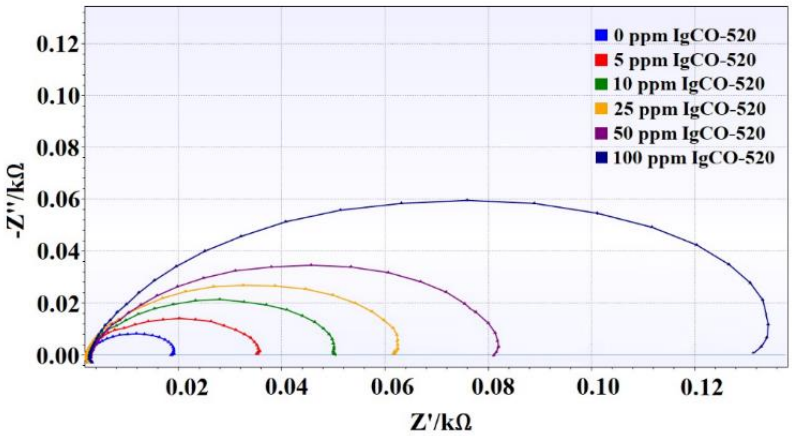

Figure 2. Nyquist plots for SS-405 in the absence and presence of different concentrations of IgCO-520 at 25 °C

The charge transfer resistance (R_{ct}), solution resistance (R_s), phase shift (n), and double-layer capacitance (C_{dl}) were obtained by fitting the Nyquist plots [20]. The *IE%* of the different concentrations of IgCO-520 for 12 h of immersion time at 25 °C was calculated by the following equation [21]:

$$IE\% = \left(\frac{R_{ct} - R'_{ct}}{R_{ct}}\right) \times 100 \quad (1)$$

where R'_{ct} and R_{ct} are charge transfer resistance of uninhibited and inhibited solutions, respectively. The calculated electrochemical parameters and *IE%* are tabulated in Table 1.

The appearance of a single semicircle loop in Figure 2 indicates that the corrosion of SS-405 and inhibition of IgCO-520 are mainly related to the charge transfer phenomenon [20]. The increase in the diameter of Nyquist semi-circle plots with the increased concentration of IgCO-520 is attributed to the formation of a defensive layer on the steel surface [22]. As seen in Table 1, the R_{ct} values increased with the increasing of concentration of IgCO-520. These increased R_{ct} values suggest that IgCO-520 adsorption upon the SS-405 surface decreases the number of active sites upon the surface thus obviate the corrosion process [23, 24]. The values of C_{dl} decreased with increasing IgCO-520 concentration. The decrease in local dielectric constant due to the adsorption of IgCO-520 at the metal/solution interface and/or the increase in the thickness of the electrical double layer can cause a decrease in C_{dl} [25, 26]. Larger R_{ct} and smaller C_{dl} values indicate the formation of an adsorbed film upon the steel surface in a corrosive medium [27]. The absorbed layer at the SS-405 surface increases the inhibition efficiency *IE%* by isolating it from the aggressive oxygen-containing medium (Table 1) [28].

Table 1. Electrochemical parameters and *IE%* obtained from EIS plots for SS-405 in 1.0 M HCl with and without IgCO-520

Temperature (°C)	Concentration (ppm)	R_s (Ω/cm²)	R_{ct} (Ω/cm²)	C_{dl} (μF/cm²)	n	*IE%*
25	0	2.47	17.05	100.05	1.00	0.00
	5	2.10	34.12	85.11	0.90	50.03
	10	2.50	48.80	63.23	0.92	65.06
	25	2.16	63.04	55.18	0.93	72.95
	50	3.03	80.20	40.26	0.89	78.74
	100	2.50	136.09	35.64	0.91	87.47

Figure 3 indicates the potentiodynamic polarization curves for SS-405 in 1.0 M HCl at different concentrations of IgCO-520 at 45 °C. The anodic and cathodic Tafel slopes (β_a and β_c, respectively), corrosion potential (E_{corr}), and corrosion current density (i_{corr}), are listed in Table 2.

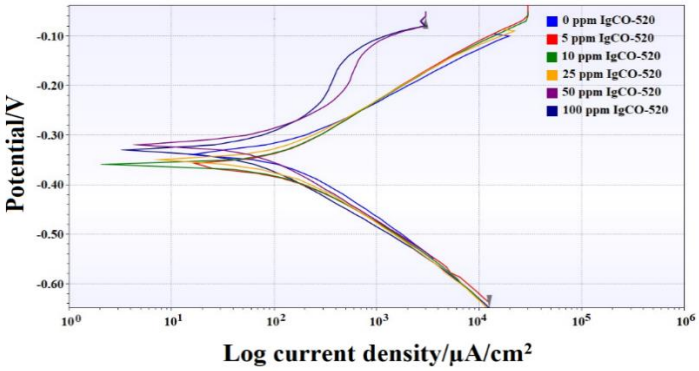

Figure 3. Potentiodynamic anodic and cathodic polarization curves recorded for SS-405 at a scan rate of 0.1mV/s at 45 °C in 1.0 M HCl without and with various concentrations of IgCO-520

The *IE%* was calculated by Equation 3 [29]:

$$IE\% = \left(\frac{i'_{corr} - i_{corr}}{i'_{corr}}\right) \times 100 \quad (2)$$

where i_{corr} and i'_{corr} indicate the corrosion current densities in the presence and absence of inhibitors, respectively.

As seen in Table 2, the addition of IgCO-520 to the 1.0 M HCl solution decreases i_{corr} and increases IE% values which clearly indicate the inhibition effect of the IgCO-520. The β_a and β_c values show slight changes in the presence and the absence of the inhibitor, which reveals the fact that the addition of an inhibitor does not cause any change in the mechanism of iron dissolution. The addition of the IgCO-520 does not significantly shift the corrosion potential, which demonstrates that the inhibitor behaves as a mixed inhibitors [30].

Table 2. Electrochemical parameters of SS-405 corrosion in 1.0 M HCl without and with the addition of different concentrations of IgCO-520 at 45 °C.

Temperature (°C)	Concentration (ppm)	E_{corr} (mV)	i_{corr} (μA/cm²)	R_p (Ω)	β_a (V/dec)	$-\beta_c$ (V/dec)	IE (%)
45	0	-0.334	145.23	230.60	0.107	0.138	0.00
	5	-0.356	76.02	275.60	0.122	0.115	47.66
	10	-0.360	62.32	277.90	0.122	0.105	57.09
	25	-0.342	50.08	264.70	0.111	0.136	65.52
	50	-0.310	35.65	418.40	0.077	0.123	75.45
	100	-0.324	27.63	548.00	0.084	0.110	80.98

Surface analysis by SEM

Surface morphological investigation of SS-405 in 1.0 M HCl was carried out by SEM. Figure 4a shows the polished surface of SS-450. Figure 4b and Figure 4c show the SEM images of the surface of SS-405 coupons after immersion in 1.0 M HCl for 120 h in the absence and presence of IgCO-520 (100 ppm), respectively.

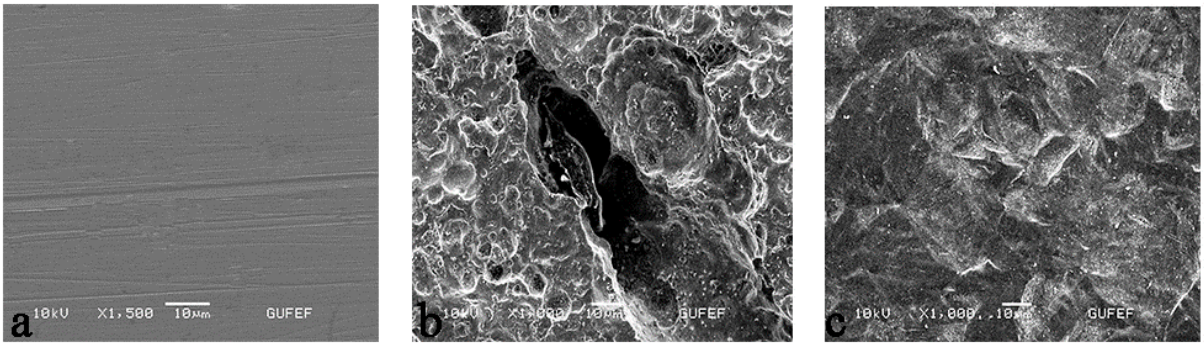

Figure 4. SEM images of SS-405: (a) polished coupon before immersion, (b) and (c) steel coupons in 1.0 M HCl at 65°C in the absence and presence of 100 ppm IgCO-520 for 120 h

The SEM images depicted in Figure 4b and Figure 4c indicate highly big and relatively low damage resulted on SS-405 surface in the absence and presence of IgCO-520, respectively. This verifies the fact that IgCO-520 has a good corrosion protection features due to its good coverage capacity [31]. The results of electrochemical measurements and SEM analysis are in good accordance.

UV-vis spectrophotometric and AAS analysis

During the immersion period the colors of test solutions were observed to change from transparent to green-blue. The UV spectra of the solution in the range of 300 to 700 nm revealed the fact the origin of this color change was most probably the Cr(III) dissolution from the steel coupons since SS-405 is mainly constituted with iron and chromium. Figure 5. shows the UV-Vis spectra of IgCO-520 (100 ppm of concentration in 1.0 M HCl), standard solution of Cr(III) (25 ppm of concentration in 1.0 M HCl), and test solutions (after 12 h immersion period).

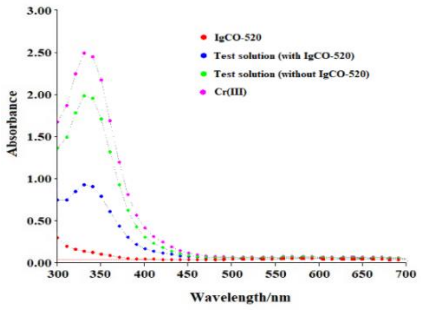

Figure 5. Adsorption spectra of IgCO-520, Cr(III) and test solutions

As seen in Figure 5, IgCO-520 (high inhibitor concentrations) gave no signal while the test solution and Cr(III) gave a peak at 330 nm. Dissolution of Cr(III) released into the test solutions may have caused this absorbance peak at the test solution.

For the last two decades, AAS has proven to be a very suitable tool for determining dissolved chromium in uninhibited and inhibited specimens in corrosive media. The results can then be employed to calculate *IE%* [32]. Fouda et al. used the following equation for *IE%*:

$$IE\% = \left(\frac{U-I}{U}\right) \times 100 \quad (3)$$

where *U* and *I* indicate the amount of dissolved metal determined in uninhibited and inhibited test solutions, respectively. Chromium was determined by AAS and Equation 3 was used for the calculation of *IE%*. Calibration curve for Cr was plotted by the use of a blank solution and nine standard solutions at concentrations of 0.1, 0,25, 0,50, 1.00, 2.00, 4.00, 6.00, 8.00 and 10 ppm. The regression equation was found to be A = 0.6044

(ppm) - 0.052 (R^2 = 0.991). The results are listed in Table 3. The analysis for Cr(III) has shown the presence of 0.28 – 6.98 ppm chromium.

Table 3. The content of chromium dissolved in 1.0 M HCl uninhibited and inhibited solutions containing (different concentrations of IgCO-520) of the investigated compounds.

Temperature/°C	Concentration of IgCO-520/ppm	Amount of chromium content	*IE%*
25	0	2.23	0
	5	1.11	50.22
	10	0.78	65.02
	25	0.51	77.13
	50	0.42	81.17
	100	0.28	87.44
45	0	5.48	0
	5	2.75	49.82
	10	2.21	59.67
	25	1.59	70.99
	50	1.41	74.27
	100	0.99	81.93
65	0	6.98	0
	5	3.44	0.72
	10	3.1	55.59
	25	2.57	63.18
	50	2.08	70.20
	100	1.92	72.49

CONCLUSIONS

IgCO-520 displays good inhibition characteristics for the corrosion of SS-405 in 1.0 M HCl medium. The corrosion inhibition efficiency is directly proportional to inhibitor concentration and reversely proportional to temperature. The values of R_{ct} increased while C_{dl} decreased with the increase in inhibitor concentration as obtained from Nyquist plot. The results of linear polarization measurements indicated that the surfactant investigated acts as mixed-type inhibitor. Results obtained from weight loss and determination of chromium are in reasonably good agreement. The theoretical calculations appear to be in good compliance with the experimental results.

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ORAL PRESENTATION

Voltammetric analysis of guaifenesin on glassy carbon electrode and its determination in pharmaceuticals

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Abstract

The electro-oxidation behaviour of the expectorant drug guaifenesin (GUF) was investigated using a glassy carbon electrode (GCE) through cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The oxidation of GUF was found to be irreversible and diffusion-controlled. A linear response was observed in the concentration range of 1×10^{-6} to 1×10^{-4} M, with a detection limit of 1.324×10^{-8} M for DPV in a 0.1 M phosphate buffer solution (PBS) at pH 2.0. The fully validated DPV method was successfully applied to determine GUF in pharmaceutical dosage forms, yielding satisfactory results.

Keywords: Guaifenesin, determination, differential pulse voltammetry, voltammetry.

INTRODUCTION

Guaifenesin (GUF) is a widely used expectorant for treating cough and congestion associated with bronchitis, the common cold, and other respiratory conditions (Albrecht et al., 2017). GUF works by thinning mucus, enhancing the lubrication of the respiratory tract (including the lungs, nose, and throat), and promoting mucus clearance (Balsamo et al., 2010). Additionally, it is used in surgery due to its additive effects with narcotics and its muscle relaxant properties (Sweetman, 2009).

Drug analysis techniques are crucial for drug formulation, pharmacodynamics, toxicology, stability, pharmacokinetics, and bioequivalency studies (Vais et al., 2015; Gupta et al., 2011). The redox properties and electron transfer mechanisms of drugs provide valuable insights into their pharmacological activity in vivo and their metabolic pathways (Xu et al., 2009; Özkan et al., 2003). While chromatographic (Vasudevan et al., 2000; Wilcox et al., 2000; Chen et al., 2005; Patil et al., 2014; Sharaf and Stiff, 2004; Singh et al., 2010) and spectrophotometric (Rele, 2016; Abdallah, 2010) methods have been used to monitor GUF levels, these techniques are often time-consuming, costly, and technically challenging, as they require preliminary sample preparation, like extraction. Voltammetric methods offer an efficient alternative for drug analysis, providing advantages such as high sensitivity, shorter analysis time, simplicity and lower costs. Previous studies on the voltammetric determination of GUF have utilized various electrodes, including the pyrolytic carbon film electrode (PCF) (Hadi, 2015), a multiwalled carbon nanotube/ionic liquid modified glassy carbon electrode (MWCNT/IL/GCE) (Gholivand and Khodadadian, 2014) a carbon nanotube bimetallic Au-Pt inorganic-organic nanofiber hybrid nanocomposite electrode (Au/Pt/NPs/NFs/CNT/GCE) (Gholivand et al., 2011), and a platinum electrode (Tapsoba et al., 2005).

In this study, we propose that the differential pulse voltammetric method offers a simple, fast, sensitive, and cost-effective approach for the voltammetric determination of GUF. The GCE was employed to achieve more sensitive and precise measurements of GUF. This practical electro-analytical method was successfully applied for the quantitative determination of GUF in pharmaceutical dosage forms using GCE.

MATERIALS AND METHODS

The pharmaceutical dosage form of GUF (Vicks Expectorant® syrup, 200 mg/15 mL) was obtained from a pharmacy and used as received, without further purification. GUF stock solutions (1.00×10^{-3} M) were prepared in nanopure water and stored at +4°C, shielded from light. For voltammetric analysis, working solutions were prepared by directly diluting the GUF stock solution with the chosen supporting electrolyte.

Britton-Robinson buffer (BRB) solutions with pH values ranging from 2.0 to 10.0 were prepared using 0.04 M CH_3COOH (Merck, Germany), 0.04 M H_3BO_3 (Aldrich, USA), and 0.04 M H_3PO_4 (Merck, Germany). PBS (0.1 M) were prepared using H_3PO_4 (Merck, Germany), Na_2HPO_4 (Aldrich, USA), and NaH_2PO_4 (Merck, Germany) at pH levels of 2-4 and 5.5-8.0, respectively. Acetate buffer solutions (AcB) (0.5 M) were prepared using CH_3COOH and $NaCH_3COO$ (Merck, Germany) at pH 3.5, 4.5, and 5.5. The final pH values were adjusted with 5 M NaOH (Aldrich, USA) solution.

Sartorius Arium proUV nanopure water (resistivity ≥18 MΩ·cm) and appropriate reagents were used to prepare the solutions.

A three-electrode electrochemical cell was used for all experiments, consisting of a GCE (Bioanalytical Systems, 3 mm diameter) as the working electrode, a platinum wire as the counter electrode (Bioanalytical Systems), and an Ag/AgCl electrode (Bioanalytical Systems, 3.0 M KCl) as the reference electrode. All electrochemical measurements were conducted using an Autolab PGSTAT128N potentiostat/galvanostat with Nova 11.0 software (Metrohm-Autolab, The Netherlands). The pH measurements were performed with a HANNA HI2211 pH meter (Romania), accurate to ±0.05 pH at room temperature. All experiments were conducted at room temperature (25 ± 1 °C).

A stock solution of GUF was prepared from Vicks Expectorant® syrup (200 mg/15 mL). The required volume of syrup was transferred to a 10 mL calibrated flask and diluted to the mark with bi-distilled water. This solution, equivalent to 1.00×10^{-3} M GUF, was then used for voltammetric analysis by directly diluting it in the selected supporting electrolyte.

RESULTS and DISCUSSION

To explore the electrochemical behaviour of GUF, CV, DPV, and square wave voltammetry (SWV) techniques were employed in AcB, BRB, and PBS across a pH range of 2.0 to 10.0. CV and DPV methods were utilized for quantitative analysis of GUF, while SWV was not feasible due to poor peak shapes in all buffer solutions. The electrochemical analysis of a 100 µM GUF solution on GCE was conducted using CV at a scan rate of 100 mV/s in 0.5 M AcB, 0.04 M BRB, and 0.1 M PBS over pH ranges from 2.0 to 10.0. The oxidation peak of GUF indicated an irreversible charge transfer process on the GCE, as evidenced by the absence of a cathodic peak in the reverse scan (Figure 1). Based on peak shape and peak current, PBS at pH 2.0 was selected as the optimal supporting electrolyte for subsequent experiments.

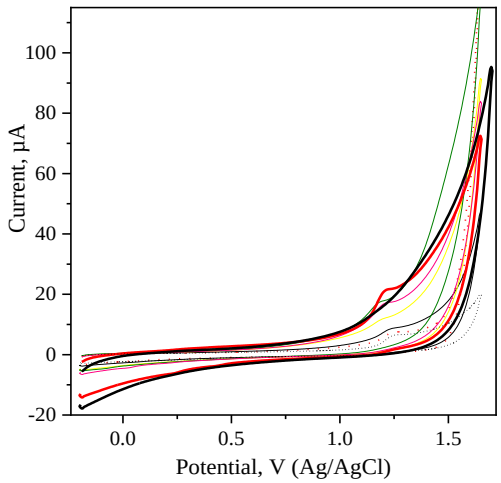

Figure 1. Cyclic voltammograms of GUF in (a) PBS at pH 7.0, (b) BRB at pH 8.0, (c) PBS at pH 5.5, (d) BRB at pH 7.0 and (e) pH 6.0. GUF concentration: 100 µM; scan rate 100 mV s^{-1}.

To determine whether the electro-oxidation process of GUF at the GCE was diffusion or adsorption-controlled, we examined the effect of scan rate on the oxidation peak current and peak potential. The scan rate study was conducted within the range of 5 to 100 mV s^{-1}. The peak potential of 100 µM GUF in 0.1 M PBS at pH 2.0 shifted to less positive values with increasing scan rate (Figure 2), which is characteristic of irreversible electrochemical reactions. A plot of the peak current (I_p) versus the square root of the scan rate ($\sqrt{v}$) produced a linear relationship. This demonstrated that the electro-oxidation process of GUF is diffusion controlled. The linear regression equation is provided below:

$$I_p = 0.216\nu^{1/2} + 0.1452 \quad r=0.998, n=6 \tag{1}$$

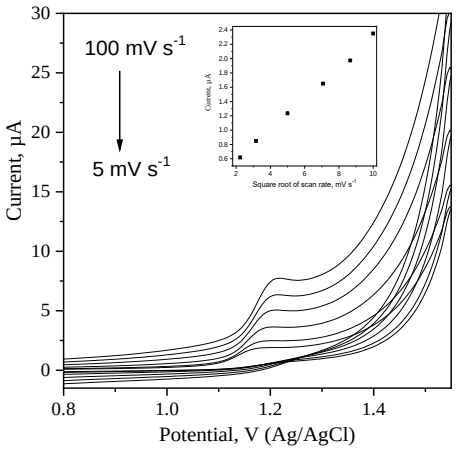

Figure 2. Cyclic voltammograms of 100 µM GUF in PBS at pH 2.0 at scan rates of 5, 10, 25, 50, 75 and 100 mV s^{-1} with GCE. Inset: The graph of the peak current of GUF *vs.* the square root of scan rate.

Quantitative analysis of GUF was conducted using the DPV method on the GCE. A calibration curve was created by plotting peak current against GUF concentration under optimal conditions. The peak current showed a strong linear correlation (r = 0.996) with GUF concentration across a range of 1.00 µM to 100 µM in the supporting electrolyte. The voltammograms of GUF are displayed in Figure 3. The linear regression equation derived from the calibration data is as follows:

$$I_p(\mu A) = 0.341\ \mu M - 0.199;\ r = 0.999\ (n=9)\ \text{for DPV} \tag{2}$$

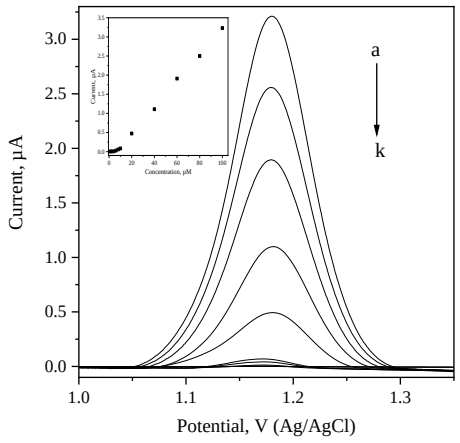

Figure 3. Differential pulse voltammograms of a) 100 µM, b) 80 µM, c) 60 µM, d) 40 µM, e) 20 µM, f) 10 µM, g) 8 µM, h) 6 µM, i) 4 µM, j) 2 µM and k) 1 µM in PBS at pH 2.0 for GCE. Inset: The graph of the peak current *vs.* GUF concentration.

The limit of detection (LOD) and limit of quantification (LOQ) were determined using the formulas 3s/m and 10s/m, respectively, where s represents the standard deviation of the peak currents from three measurements and m is the slope of the corresponding calibration curve (Riley and Rosanske, 1996; Ermer and Miller, 2005). The details of the calibration curve results obtained via DPV are presented in Table 1.

We evaluated the precision of the developed method through repeatability studies. A working solution of 4.00 × 10^{-5} M GUF in 0.1 M PBS at pH 2.0 was used for precision testing, with repeated measurements taken both on the same day and on different days, focusing on peak currents and peak potentials. Precision was quantified using five data points to calculate the % Relative Standard Deviation (RSD%) for the DPV method. The results,

presented in Table 1, indicate that the method exhibits excellent precision, repeatability, and reproducibility for both peak currents and peak potentials.

Table 1. Validation data of calibration lines for the quantitative determination of GUF by DPV on the GCE.

	Supporting electrolyte
	DPV
Linearity range (µM)	1.00-100
Slope (µA μM^{-1})	3.34×10^{-1}
Intercept (µA)	-0.150
Correlation coefficient	0.996
Limit of detection (µM)	1.324×10^{-2}
Limit of quantification (µM)	4.034×10^{-2}
Repeatability of peak current (RSD%)	0.122
Repeatability of peak potential (RSD%)	0.233
Reproducibility of peak current (RSD%)	0.994
Reproducibility of peak potential (RSD%)	0.267

The developed method was applied to determine GUF in Vicks Expectorant® syrup (200 mg/15 mL) by utilizing the relevant calibration curve. To evaluate potential interference from excipients, as well as to verify the selectivity, accuracy, and precision of the validated DPV method, recovery studies were conducted by spiking known amounts of GUF into the dosage form. The results, summarized in Table 2, confirmed that the proposed method is suitable for accurately and precisely determining GUF in pharmaceutical formulations without interference. The method is both simple and rapid, making the DPV approach highly recommended for analysing GUF in pharmaceutical products. Additionally, the results showed no significant differences between the values obtained with the DPV method and those reported by the manufacturer.

Table 2. The results of the determination of GUF from its pharmaceutical dosage form and recovery experiments in 0.1 M PBS solution at pH 2.0 by DPV on the GCE.

	Syrup (200 mg / 15 mL)
	DPV
Labelled claim (mg)	200.00
Amount found (mg)*	199.71
RSD%	0.191
Bias%	-0.145
Added (mg)	100
Found (mg)*	100.023
RSD% of recovery	0.806
Bias%	0.023

*Each value of the mean 5 experiments.

The analytical performance of the proposed method using GCE was compared with calibration data from voltammetric studies in the literature. For GUF, the LOD value obtained in this study was lower than those reported in other studies, as indicated in Table 3. The developed method provides a wide linear range, low detection limit, and cost-effectiveness, making it a highly effective approach for GUF analysis on GCE.

Table 3. Compared parameters obtained using different electrochemical sensors for the determination of GUF.

Electrode	Method	Linear range (M)	LOD (M)	Ref.
PCF	DPV	1.0×10^{-7} - 2.5×10^{-5}	2.3×10^{-8}	(Hadi, 2015)
MWCNT/IL/GCE	DPV	1.5×10^{-6} - 4.8×10^{-4}	8.5×10^{-5}	(Gholivand and Khodadadian, 2014)
Au-PtNPs/NFs/CNT/GCE	DPV	5.0×10^{-8} - 3.0×10^{-4}	1.75×10^{-8}	(Gholivand et al., 2011)
Platin electrode	DPV	1.0×10^{-4} - 3.03×10^{-4}	-	(Tapsoba et al., 2005)
Glassy carbon electrode	DPV	1.0×10^{-6} - 1.0×10^{-4}	1.324×10^{-8}	This work

PCF: Anodized nanocrystalline graphite-like pyrolytic carbon film electrode
MWCNT/IL/GCE: Multiwalled carbon nanotube-ionic liquid modified glassy carbon electrode
Au-PtNPs/NFs/CNT/GCE: Carbon nanotube bimetallic Au-Pt inorganic-organic nanofiber hybrid nanocomposite electrode

CONCLUSION

In this study, CV and DPV techniques were utilized to analyze GUF in pharmaceutical dosage forms. The DPV method exhibited a linear response within a concentration range of 1.00×10^{-6} to 1.00×10^{-4} M, with a detection limit of 1.324×10^{-8} M and a correlation coefficient of 0.996. This detection limit is lower than those reported for other voltammetric methods in the literature. Furthermore, the DPV method on GCE consistently produced reliable results for both peak current and peak potential of GUF. As results, this method demonstrated high sensitivity and selectivity for GUF analysis in pharmaceutical formulations, without requiring sample pre-treatment or lengthy extraction and evaporation procedures.

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ORAL PRESENTATION

Yeni bir peroksidaz kaynağı olarak çay tohumu: Kısmi saflaştırılması ve karakterizasyonu

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Özet

Peroksidazlar farklı amaçlarla çeşitli endüstriyel ve biyokimyasal uygulamalarda etkili bir şekilde kullanıldığı için, bu enzimin yeni kaynaklardan saflaştırılması ve karakterizasyonu ilgi görmektedir. Bu çalışmada, peroksidaz ilk kez çay tohumundan soğuk aseton çöktürmesi yöntemi uygulanarak %88,91 verimle 18,37 kat kısmi saflaştırılmıştır. Optimum pH ve sıcaklık değerleri sırasıyla 5,0 ve 40 ℃ olarak bulunmuştur. Çay tohumu POD'u için Km ve Vmaks değerleri guaiacol ve hidrojen peroksit için sırasıyla 1,75 mM ve 2,59 EU/mL.dk, 0,82 mM ve 3,79 EU/mL.dk olarak hesaplanmıştır. Enzim ısıl kararlılığı incelendiğinde 40 ℃ ve 50 ℃'de 60 dakikalık inkübasyon sonrasında aktivitesini %85 üzeri koruduğu tespit edilmiştir. Enzimin pH kararlılığı pH 4,0-7,0 aralığında 4 ℃ 24 saat sonunda aktivitelerinin %90 üzerinde koruduğu tespit edilmiştir. Çay tohumu POD'unun depolama kararlılığı 4 °C ve -20 °C bir ay boyunca incelenmiş ve her iki sıcaklıktada başlangıç aktivitesinin %96 üzerinde koruduğu tespit edilmiştir. Bu sonuçlar gösteriyorki çay tohumu peroksidazı endüstriyel ve biyoteknolojik uygulamalar için uygun maliyetli ve alternatif bir bitki peroksidaz kaynağı olarak hizmet edebilir.

Anahtar Kelimeler: Çay tohumu, Peroksidaz, Kısmi saflaştırma, Karakterizasyon.

Tea seed as a new source of peroxidase: Partial purification and characterization

Abstract

Since peroxidases are effectively used in various industrial and biochemical applications for different purposes, the purification and characterization of this enzyme from new sources is of interest. In this study, peroxidase was partially purified 18.37-fold with 88.91% yield by cold acetone precipitation method from tea seed for the first time. The optimum pH and temperature values were found to be 5.0 and 40℃, respectively. Km and Vmax values for tea seed POD were calculated as 1.75 mM and 2.59 EU/mL.min, 0.82 mM and 3.79 EU/mL.min for guaiacol and hydrogen peroxide, respectively. When the thermal stability of the enzyme was analysed, it was found that the enzyme retained more than 85% of its activity after 60 minutes of incubation at 40 ℃ and 50 ℃. The pH stability of the enzyme was found to maintain over 90% of its activity at the end of 24 hours at 4 ℃ in the range of pH 4.0-7.0. The storage stability of tea seed POD was investigated at 4 °C and -20 °C for one month and it was found that it retained 96% of its initial activity at both temperatures. These results indicate that tea seed peroxidase may serve as a cost-effective and alternative source of plant peroxidase for industrial and biotechnological applications.

Keywords: Tea seed, Peroxidase, Partial purification, Characterization.

GİRİŞ

Peroksidaz (POD; E.C. 1.11.1.7) prostetik grup olarak ferriprotoporfirin IX içeren oksidoredüktaz sınıfına ait enzimlerdir. Peroksidazlar bakteriler, mantarlar, algler, bitkiler, tohumlar ve hayvanlarda bulunurlar. POD, elektron alıcısı olarak H_2O_2 kullanarak çeşitli fenolik ve fenolik olmayan bileşiklerin (katekol, pirogallol, guaiakol, aromatik aminler, indoller ve sülfonatlar) oksidasyonunu katalize eder (Henriksen ve ark., 2001; Hamid ve Khalil-ur-Rehman, 2009; Pandey ve ark., 2017). Bitki peroksidazlarının geniş substrat spesifikliğine sahip olması nedeniyle birçok alanda yaygın olarak kullanılmaktadır. POD kullanıldığı bazı alanlar, biyoremidasyonunda (Basumatary ve ark., 2023), çeşitli klinik biyokimya uygulamaları (Fatima ve ark., 2007), biyosensor (Luo ve ark., 2011; Ghanavati vd. 2023), atık sularda boya uzaklaştırma (Karim ve ark., 2012;

Çelebi ve Altıkatoğlu, 2023) vb. sıralayabiliriz. Son yıllarda, peroksidaz enziminin, klinik, endüstriyel ve çevresel alanlardaki yaygın kullanımı nedeniyle yeni, ucuz biyouyumlu ve antioksidan olan kaynaklardan saflaştırılmasına ve karakterizasyonuna olan ilgi artmıştır (Altinkaynak ve ark., 2020).

Peroksidazlar bitkilerde çokça bulunmasına rağmen, ticari olarak temin edilip kullanılabilen peroksidaz temel kaynağı yaban turpu kökleridir (Yuzugullu Karakus ve ark., 2018). Bununla beraber farklı kaynaklardan farklı karakteristik özellikler sahip peroksidazların bulunması geniş potansiyel uygulamalarından dolayı yeni analitik yöntemlerin ve endüstriyel proseslerin geliştirilmesini katkı sunacaktır (Altın ve ark., 2017). Peroksidazlar, elma ve portakal tohumundan (Zia ve ark., 2011), Türk kara turpundan (*Raphanus sativus* L.) (Şişecioğlu ve ark., 2010), pirinç kepeğinden (Gautério ve ark., 2015), buğday (*Triticum aestivum* ssp. vulgare) (Altın ve ark., 2017), brokoli (*Brassica oleracea* var. *Italica*) (Thongsook ve Barrett, 2005), *Araujia sericifera* tohumu (Landi ve ark., 2019) gibi birçok kaynaktan saflaştırılıp karakterize edilmiştir.

Çay bitkisi (*Camellia sinensis* L.) dünyanın dört bir yanındaki bulunan, yaprağını dökmeyen bir çalı veya küçük ağaçtır. Çay Dünya'da en çok tüketilen içecekler arsında yer alır. Çayın popülerliğinin artması ile beraber çay tohumu üretimde de artış gerçekleşmiştir (Jo ve ark., 2012; Huang ve ark., 2019). Çay tohumunun içeriğinde saponinler (Chen ve ark., 2022), flavonoidler (Sekine ve ark., 1991), nişasta (Huang ve ark., 2019) ve yağ asitleri (Wang ve ark., 2011) gibi birçok biyolojik olarak aktif bileşiği içerdiği bilinmektedir. Çay tohumunun antimikrobiyal (Er ve Kayış, 2015), antioksidan, tirozinaz inhibitörü ve asetilkolinesteraz inhibitörü (Jo ve ark., 2012) olduğu literatürde rapor edilmiştir. Bununla beraber çay tohumunu literatürde gördüğümüz kadarıyla enzim kaynağı olarak kullanılmamıştır. Bu çalışmanın amacı endüstriyel bir enzim olan peroksidazın antioksidan, antimikrobiyal, çevredostu ve uygun maliyetli çay tohumundan kısmi saflaştırılması ve karakterizasyonudur.

MATERYAL VE METOT

Materyal

Çay (*Camellia sinensis* L.) tohumu Rize İli'ndeki yerel pazardan satın alınmıştır. Çalışmalar yapılıncaya kadar -20 °C saklanmıştır.

Ham enzim Ekstraktının Hazırlanması

Peroksidaz çay tohumundan Geng ve ark., (2001) önerdiği yöntemde küçük değişiklikler uygulanarak ekstrakte edilmiştir. 10 gr çay tohumuna 40 mL 50 mM pH 5,0 asetat tamponu eklenerek homojanitörde parçalanmıştır. Daha sonra dört katlı tülbentten süzülerek 10.000 rpm 4 °C de 1 saat santüfüj edilmiştir. Süpernatan enzim ekstraktı olarak kullanılmıştır (Geng ve ark., 2001).

Aseton Çöktürmesi ile Enzimin Kısmi saflaştırılması

Elde edilen ham enzim ekstraktına 20 mL'sine eşit hacimde soğuk aseton, manyetik karıştırıcıda yavaş yavaş karıştırılarak eklenmiştir. Proteinlerin çökelmesi için 4 °C'de 2 saat inkübe edilmiştir. 10.000 rpm'de 4 °C 30 dakika boyunca santifüj ediltikten sonra elde edilen çökelti, 10 mL 50 mM asetat tamponunda (pH 5,0) tekrardan çözülmüştür. 10.000 rpm'de 4 °C 10 dakika boyunca santifüj edildikten sonra süpernatan enzim çözeltisi olarak kullanılmıştır (Akatin, 2013).

Protein Tayini

Ham enzim ekstraktında ve kısmi saflaştırılan enzim çözeltisindeki protein miktarı Bradford yöntemi ile tespit edilmiştir (Bradford, 1976).

Peroksidaz Aktivite Tayini

Peroksidaz aktivitesi UV-VIS spektrofotometresi kullanılarak Şişecioğlu ve ark., (2010) yöntemine göre yapılmıştır. 3 mL kuvete 1 mL guaiakol (45 mM), 1 mL H_2O_2 (22,5 mM), 0,980 mL asetat tamponu (50 mM pH 5,0) ve 0,02 mL enzim ekstraktından eklenmiştir ve 470 nm'de 3 dakikadaki absorbans ölçülmüştür (Şişecioğlu ve ark., 2010).

Optimum pH

Çay tohumu POD'unun optimum pH değerini tespit etmek için 50 mM glisin-HCI tamponu pH (3,0), 50 mM asetat tamponu pH (4,0-5,0), 50 mM fosfat tamponu pH (6,0-7,0) ve 50 mM tris-HCI tamponu pH (8,0-9,0) gibi farklı pH değerlerinde ayrı ayrı aktivite çalışması gerçekleştirilmiştir. Elde edilen sonuçlarla pH- %bağıl aktivite grafiği çizilerek enzimin optimum pH belirlenmiştir (Geng ve ark., 2001).

Optimum Sıcaklık
Çay tohumu POD aktivitesi üzerine sıcaklığın etkisini belirlemek amacıyla 10-80 ℃ aralığında 10 ℃ sıcaklık artışıyla ayrı ayrı aktivite tayini yapılmıştır. Optimum pH da enzim aktivitesi çözeltileri 5 dakika su banyosunda farklı sıcaklık değerlerinde (10-70 °C) inkübe edilmiştir. Daha sonra reaksiyon ortamına enzim ilave edilerek aktivite denemeleri gerçekleştirilmiştir. Sonuçlar ile sıcaklık- %bağıl aktivite grafiği çizilerek optimum sıcaklık belirlenmiştir (Somtürk ve ark., 2014).

Km ve Vmaks Değerleri
Çay tohumu POD'ının substrat olarak guaiacol ve H_2O_2 için Lineweaver-Burk grafikleri kullanılarak Km ve Vmaks değerleri tespit edilmiştir (Lineweaver-Burk, 1934).

pH Kararlılığı
Çay tohumu POD'un pH kararlılığı incelemek amacıyla pH 4,0-5,0 asetat ve 50 mM pH 6,0-7,0 fosfat tamponu ile enzim çözeltisi birebir karıştırılarak optimum şartlarda enzim aktivitesi bakılmıştır. Daha sonra 4 ℃ inkübe inkübe edilmiş ve 24 saat sonunda aktivite ölçümleri yapılmıştır. Elde edilen sonuçlardan faydalanılarak zamana karşı % kalan aktivite grafiği çizilmiştir (Şişecioğlu ve ark., 2010).

Isıl kararlılık
Çay tohumu POD'unun ısıl kararlılığı belirlemek için enzim 40-70 °C de 10 sıcaklık artışlarla 30 ve 60 dakika boyunca inkübe edildikten sonra numuler oda sıcaklığına soğutulmuş ve optimum şartlarda enzim aktivitesi bakılmıştır. İnkübasyon uygulanmayan enzim aktivitesi %100 kabul edilerek zamana karşı % kalan aktivite grafiği çizilmiştir (Elsayed ve ark., 2018).

Depolama Kararlılığı
POD enzimi 50 mM asetat tamponu (pH 5,0) içinde ayrı ayrı 4 ℃ ve -20 ℃ muhafaza edilmiştir. POD'un depolama kararlılığını belirlemek amacıyla ilk günki enzim aktiviteleri %100 kabul edilmiş ve farklı periyodlarda enzim aktivitesi bakılarak yüzde kalan aktivite hesaplanmıştır (Yuzugullu Karakus ve ark., 2018).

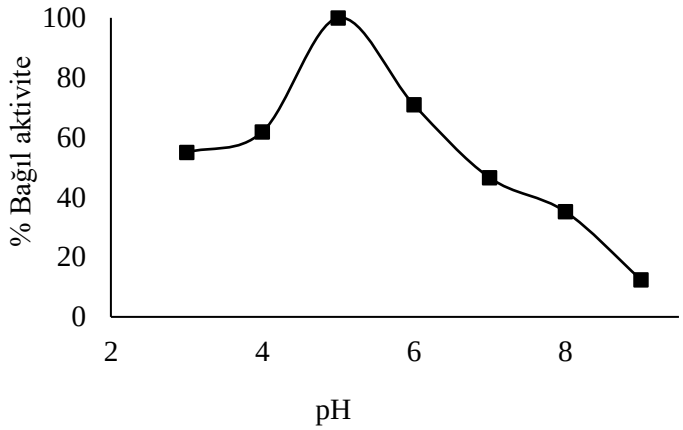
BULGULAR

Kısmi Saflaştırma
Çay tohumu POD'u soğuk aseton çöktürmesi yöntemiyle kısmi saf olarak elde edilmiştir. Peroksidazın verimi ve saflaştırma katsayısı sırasıyla % 88,91 ve 18,37 olarak hesaplanmıştır (Tablo 1).

Tablo 1. Çay tohumu POD'na ait kısmi saflaştırma tablosu

Saflaştırma Basamağı	Hacim (mL)	Toplam Aktivite (EU/mL.dk)	Toplam Protein (mg protein)	Spesifik Aktivite (U/mg protein)	Verim	Saflaştırma Katsayısı
Ham enzim ekstraktı	20	35,87	24,8	1,45	100	1
Aseton Çöktürmesi	10	31,90	1,2	26,58	88,91	18,37

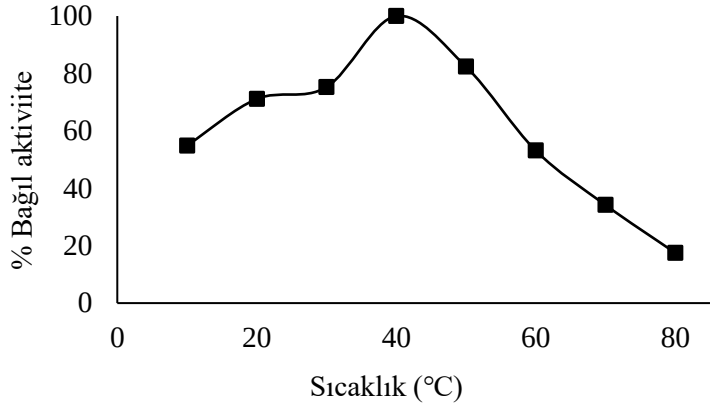
Optimum pH
POD'unun optimum pH değerini tespit etmek için pH (3,0-9,0) aralığında farklı pH değerlerinde ayrı ayrı enzim aktivitesi gerçekleştirilmiştir. Elde edilen değerlerden faydalanılarak optimum pH grafiği çizilmiştir. Çay tohumu POD'u için optimum pH'nın 5,0 olduğu tespit edilmiştir (Şekil 1).



Şekil 1. Çay Tohumu POD'nun optimum pH grafiği

Optimum Sıcaklık

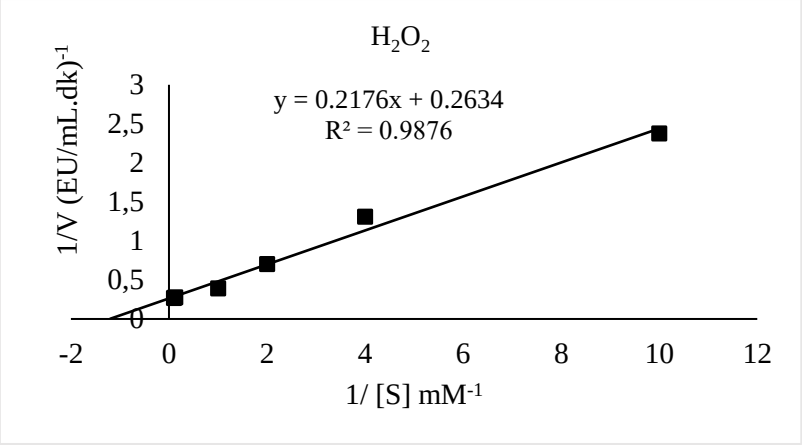
Çay tohumu POD'unun optimum sıcaklık değerini belirlemek amacıyla 10-80 ℃ sıcaklık aralığında 10 ℃ aralıklarla guaiacol substratı varlığında ayrı ayrı aktivite tayinleri gerçekleştirilmiştir. Elde edilen verilerden yararlanılarak optimum sıcaklık grafiği çizilmiştir. Çay tohumu POD'u için optimum sıcaklık değerinin 40 ℃ olduğu tespit edilmiştir (Şekil 2).



Şekil 2. Çay Tohumu POD'nun optimum sıcaklık grafiği

Km ve Vmaks Değerleri

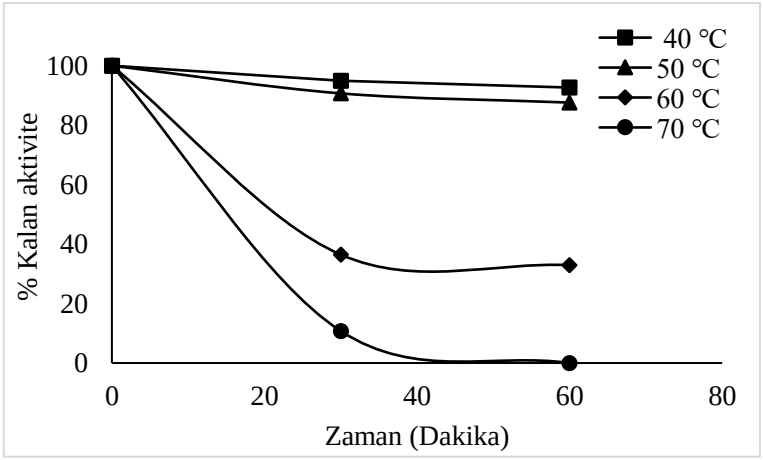
Çay tohumu POD'unun guaiacol ve H_2O_2 substratları için Km ve Vmaks değerleri Lineweaver-Burk grafiği çizilerek elde edilen denklemden yararlanılarak hesaplanmıştır. H_2O_2 için Km ve Vmaks değerleri sırasıyla 0,82 mM ve 3,79 EU/mL.dk olarak, guaiacol substratı için 1,75 mM ve 2,59 EU/mL.dk olarak hesaplanmıştır (Şekil 3).



Şekil 3. Çay Tohumu POD'unun H_2O_2 ve guaiacol substratları için Lineaweaver-Burk grafiği

Isıl kararlılık

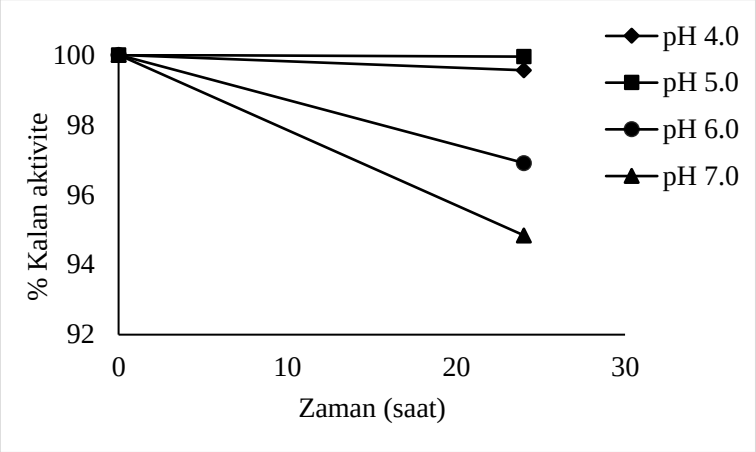
Çay tohumu POD'unun ısıl kararlılığı 40-70 ℃ sıcaklık değerlerinde 10 ℃ sıcaklık artışlarıyla 30 ve 60 dakikalık inkübasyonlardan sonra belirlenen optimum şartlarda ayrı ayrı enzim aktivitesi bakılarak test edilmiştir. Enzim 40 ℃ de 30 ve 60 dakikalık inkübasyon sonrasında aktivitesini %94,90 ve %92.64 korurken 50 ℃ de 30 ve 60 dakikalık inkübasyon sonrasında aktivitesini %90,68 ve %87,59 koruduğu tespit edilmiştir (Şekil 4). Enzim 60 ℃ de 30 ve 60 dakika inkübasyonlardan sonra aktivitesinin % 36,46 ve %32,93 koruyabilmiştir (Şekil 4). Enzim 70 ℃ de ise 30 dakika sonunda aktivitesinin neredeyse tamamını kaybetmiştir (Şekil 4).



Şekil 4. Çay tohumu POD'unun ısıl kararlılığı

pH Kararlılık

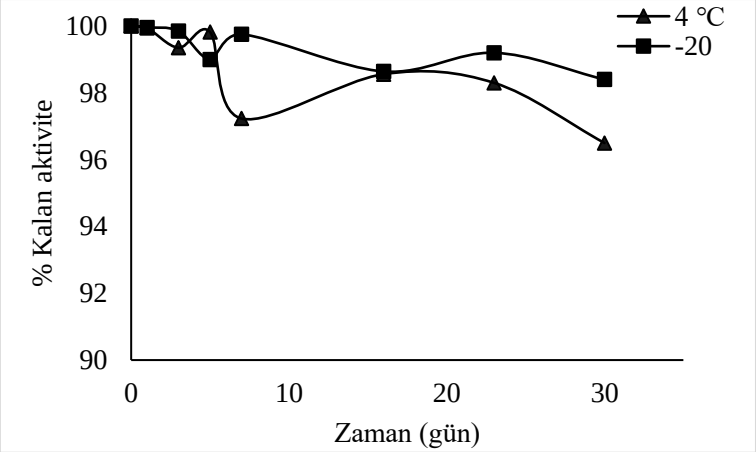
Çay tohumu POD'unun pH kararlılığı 4,0-7,0 aralığında 4 ℃ 24 saatinkübe edilerek incelenmiştir. Bu süre sonunda aktivite ölçümleri ayrı ayrı optimum şartlarda bakılarak test edilmiştir. pH 4,0-7,0 aralığında 4 ℃ 24 saat sonunda enzim başlangıç aktivitelerinin %90 üzerinde koruduğu tespit edilmiştir (Şekil 5).



Şekil 5. Çay tohumu POD'unun pH kararlılığı

Depolama Kararlılığı

Çay tohumu POD'unun depolama kararlılığı 4 °C ve -20 °C bir ay boyunca incelenmiştir. Enzimin 4 °C ve -20 °C bir ayın sonunda aktivitesini sırasıyla %96,5 ve %98,4 koruduğu tespit edilmiştir (Şekil 6).



Şekil 6. Çay tohumu POD'un depolama kararlılığı

TARTIŞMA

Çay tohumu POD'unun, ham enzim ekstraktı ve aseton çöktürmesi sonrası elde edilen enzim çözeltilerinde enzim aktivitesi ve protein tayini yapılarak hazırlanan saflaştırma tablosunda %88,91 verim ile 18,37 kat kısmi olarak saflaştırıldığı tespit edilmiştir. Daha önce peroksidaz enzimi farklı kaynaklardan faklı saflaştırma yöntemleri kullanılarak saflaştırılmıştır. POD, CM-sephadex katyon değişim kromatografisiyle pazı yapraklarından %14,4 verimle 35,5 kat (Bursal, 2013), *Sapindus mukorossi*'nin yapraklarından aseton çöktürmesi kullanılarak %97 verimle 4 kat ve ardında iyon değişimi kromatoğrafisi ile %22 verimle 14 kat (Singh ve ark., 2012), Sephadex-G-75 yöntemiyle elma tohumundan %39,99 verimle 8,34 kat ve portakal tohumundan %33,70 verimle 30,64 kat (Zia ve ark., 2011) saflaştırılmıştır.

Peroksidaz enzim aktivitesi, enzimin aktif bölgesinin konformasyonundan önemli derecede etkilenmektedir. Çünkü enzimler katalitik etkinliklerini, substratları aktif bölgelerine fiziksel ve kimyasal olarak bağlanarak başlattıkları için aktif kısmın şekli önemlidir. pH'daki değişimler, amino asitlerin polaritesinde değişikliklere yol açarak enzim aktif bölgesinin konformasyonunda yeniden düzenlemelere neden olurlar ve bu düzenlemeler sonucu enzim aktivitesi değişebilir (Ilesanmi ve Adedugbe, 2023). Çay tohumu POD'unun optimum pH'sı 5,0 olarak tespit edilmiştir (Şekil 1). Farklı kaynaklardan elde edilen peroksidazların farklı tampon sistemleri kullanılarak belirlenen optimum pH değerlerinde değişiklikler görülmüştür. Palmiye yağı (*Elaeis guineensis*

Jacq.) yaprağı POD'u için optimum pH 5,0 (Deepa ve Arumughan, 2002), karnabahar (*Brassica oleracea* L.) tomurcukları POD'u için optimum pH 5,0 (Köksal ve Gülçin, 2008), *Moringa oleifera* Lam yaprakları POD'u için optimum pH 5,0 (Agunbiade ve ark., 2021), Papaya (*Carica papaya*) Meyvesi POD'u için optimum pH 7,0 (Pandey ve ark., 2012) olarak tespit edilmiştir.

Enzim aktivitesi genel olarak sıcaklıktan etkilenmektedir. Enzim aktivitesi sıcaklık artışıyla belirli bir sıcaklık değerine ulaşıncaya kadar artmaktadır. Bu artışın nedeni, enzim aktif bölgesinin daha uygun konformasyonunu oluşturması, moleküllerin kinetik enerjisinin artması ve bu da enzimin aktif bölgesiyle substrat arasındaki etkileşimlerin artması olarak sıralayabiliriz. Bu artış enzimin maksimum aktiviteyi gösterdiği optimum sıcaklık değerine kadar devam eder. Sıcaklık daha fazla artırıldığında enzim denatürasyonundan dolayı enzim aktivitesi azalır (Kalsoom ve ark., 2015). Bu sebepten dolayı yeni kaynaklardan elde edilen enzimlerin optimum sıcaklık değerlerini belirlemek önemlidir. Çay tohumu POD'un optimum sıcaklığı 40 °C olarak tespit edilmiştir (Şekil 2). Literatürde daha önce yapılan çalışmalar değişik kaynaklardan elde edilen POD için optimum sıcaklığında değerlerinin değişkenlik gösterdiğini bildirmiştir. Farklı kaynaklardan elde edilen POD için optimum sıcaklık değerleri, *Zizania latifolia* için 40 °C (Luo ve ark., 2012), Türk kara turpu (*Raphanus sativus* L.) için 30 °C (Şişecioğlu ve ark., 2010), *Sapindus mukorossi*'nin yaprakları için 50 °C (Singh vd. 2012) olarak bulunmuştur.

Enzimler kendilerine özel kimyasal yapısından dolayı farklı substratlar üzerinde substratın yapısına ve kimyasal bileşimine göre değişik katalitik aktivite sergilerler. Michaelis-Menton sabiti Km (enzimin substrata ilgisi) ve Vmaks (enzimin maksimum aktivitesi) substrata göre değişkenlik gösterir. Bir enzimin belirlenen substratı için daha düşük Km ve daha yüksek Vmaks değerleri o enzimin substrata karşı ilgisini ve yüksek katalitik verime sahip olduğunu gösterir (Al-Senaidy ve Ismael, 2011). Yeni kaynaklardan elde edilen enzimlerin Km ve Vmaks değerlerinin belirlenmesi endüstriyel uygulamaları için yararlıdır. Çay tohumu POD'un Km ve Vmaks değerleri sırasıyla guaiacol için 1,75 mM ve 2,59 EU/ml.dk, H_2O_2 için 0,82 mM ve 3,79 EU/ml.dk olarak bulunmuştur (Şekil 3). Literatürde farklı bitki kaynaklarından elde edilen peroksidazlar için substrat olarak guaiacol ve H_2O_2 varlığında değişik Km ve Vmaks değerleri bildirilmiştir. Kırmızı lahana (*Brassica oleracea* var. *capitata* f. *rubra*) POD'un Km ve Vmaks değerleri sırasıyla 0.048 mM ve 1.46 $EU.mL^{-1}.dk^{-1}$ (guaiacol için) (Somtürk ve ark., 2014), Türk kara turpu (*Raphanus sativus* L.) POD'un Km ve Vmaks değerleri sırasıyla 0.036 ± 0.08 mM ve 38728.17 ± 1183.24 $EU.mL^{-1}.dk^{-1}$ (guaiacol için) (Şişecioğlu ve ark., 2010), Pazı (*Beta vulgaris* Subspecies *cicla*) yaprakları POD'un Km ve Vmaks değerleri sırasıyla 15.8 mM ve 3840 $EU.mL^{-1}.dk^{-1}$ (guaiacol için) (Bursal, 2013) olarak hesaplanmıştır.

Enzimim ısıl kararlılığı, enzimin elde edildiği kaynağa olduğu kadar deney koşullarına, ortamın pH'sına ve kullanılan substratın doğasına da bağlıdır (Elsayed ve ark., 2018). Çay tohumu POD'un yapılan ısıl kararlılık çalışmasına göre 40 °C ve 50 °C'de 30 ve 60 dakikalık inkübasyon sonra ısıl kararlılığını koruduğu görülmüştür (Şekil 4). Enzimin 60 °C'de 30 ve 60 dakikalık inkübasyon sonra ısıl kararlılığını koruyamadığı aktivitesini büyük ölçüde kaybettiği görülmüştür (Şekil 4). 65°C ve üzerindeki sıcaklığın etkisinin protein açılmasına neden olması ve bunun da peroksidatif aktivite kaybıyla sonuçlanması daha olasıdır. Literatürde 65 °C üzeri sıcaklıklarda enzimin üç boyutlu yapısında açılmaların muhtemel olduğu ve buda enzim aktivitesinde azalmaya neden olacağı bildirilmiştir (Rajan ve Murugan, 2010). Çay tohumu POD'unun 4 °C 24 saat inkübasyon sonrasın pH 4,0-7,0 aralığında aktivitesini %90 üzerinde koruduğu görülmüştür (Şekil 5). Bu değerler enzimin bu pH aralıklarında oldukça karalı olduğunu göstermiştir.

Depolama kararlılığı, enzimlerin ticari olarak çeşitli uygulamaları için önem arz etmektedir. Bu nedenle çay tohumu POD'unun depolama kararlılığını tespit etmek için 50 mM asetat tamponu (pH 5,0) içinde ayrı ayrı 4 °C ve -20 °C muhafaza edilmiştir. Enzim aktivitesi farklı zaman aralıklarında bir ay boyunca aktivite bakılmıştır. Çay tohumu POD'unun 4 °C ve -20 °C'de 30 gün sonunda sırasıyla %96,5 ve %98,4 koruduğu tespit edilmiştir (Şekil 6). *Amsonia kallus* peroksidazı -80 ve -20°C'demuhafaza edilmiş ve bir ay sonunda başlangıç aktivitesinin sadece %29-33 kaybetmiştir (Yuzugullu Karakus ve ark., 2018).

SONUÇ

Peroksidazlar klinik, endüstriyel ve çevresel alanlardaki yaygın kullanımı sebebiyle ticari olarak değerli bir enzimdir. Peroksidazların uygun maliyetli, çevre dostu çalışılmamış bitki kaynaklarındaki varlığının araştırılması yeni kaynak sağlaması bakımından önemlidir. Bu çalışmada çay tohumu bir peroksidaz kaynağı olarak ilk kez kısmi olarak saflaştırılmış ve karakterizasyonu yapılmıştır. Yapılan saflaştırma işlemiyle enzimin spesifik aktivitesi artmıştır. Yapılan depolama kararlılığı çalışması çay tohumu peroksidazının 4 °C ve -20 °C oldukça kararlı olduğu görülmüştür. Karakterizasyon basamağında çalışılan optimum pH, optimum

sıcaklık, Km ve Vmaks, pH kararlılığı, ısıl kararlılığı değerlerine göre çay tohumu peroksidazının endüstrinin farklı kollarında kullanılma potansiyeli olabileceğini göstermiştir.

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ORAL PRESENTATION

Beyond Earth: The Importance of Food and Nutrition in Space Exploration

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Abstract

The success of space exploration missions is increasingly linked to advancements in food and nutrition. Technological developments have shifted focus to preparing food for microgravity, emphasizing nutrient density, shelf life, optimized packaging, and preservation. Food products are now tailored to meet astronauts' balanced nutritional needs, minimized mission hazards, and efficiently use limited space. Ensuring a sufficient supply of fresh food is vital for long-duration missions and sustained human presence in space, addressing health risks from psychological stress, microgravity, and radiation. While food can be transported from Earth for short-term missions, long-duration missions to the Moon, Mars, and other distant destination-current research focuses-demand sustainable solutions like plant cultivation or alternative survival strategies. Scientists and researchers are working to create innovative food production systems that maximize nutritional quality, safety, and taste, while minimizing resource input, which could also offer benefits for Earth-based applications. Recently, the role of nutrition in maintaining astronauts' health and well-being during extended missions has become a critical area of research. Recently, the role of nutrition in maintaining astronauts' health and well-being during extended missions has become a critical area of research. Therefore, this review summarizes various aspects of space food and emphasizes important points of nutrition in space exploration.

Keywords: Nutrition, , health, food, space.

Introduction

Space food, specifically designed for astronaut consumption (Douglas et al., 2021), must be compact, easy to eat, and lightweight (Watkins et al., 2022). It also requires a long shelf life and the ability to remain stable in zero-gravity environments. Originally, space foods were created to meet the stringent demands of short missions and spacecraft constraints. In the 1960s, John Glenn, the first American astronaut to eat in space, consumed applesauce and xylose sugar tablets with water. However, as research advanced, extended missions highlighted the growing importance of food consumption (Liu et al., 2021). Consequently, it is vital to provide astronauts with nutritious, high-quality space food that meets their needs. The aim is to deliver foods that are both acceptable and palatable, closely resembling the taste of Earth-based foods (Pandith et al., 2023). During space missions, it is crucial for astronauts to consume sufficient food to meet their nutritional requirements and mitigate the negative impacts of space flight on the body (Douglas et al., 2021). For instance, microgravity leads to the loss of calcium, nitrogen, and phosphorus because it decreases bone density by disrupting the balance in bone remodeling due to alterations in bone cells (Genah et al., 2021). Additionally, space travel induces various physiological changes, including space motion sickness, fluid shifts in the body, congestion, and alterations in taste and smell. Space nutrition is essential for maintaining health and supporting growth during space missions (Oluwafemi et al., 2018). As distance from Earth increases, the regular resupply of food becomes impractical due to the constraints of planetary physics. Therefore, it is critical to ensure that space food is sufficiently nutritious to maintain the health of space explorers. Apart from providing nutritious food, the focus of missions has expanded over the years to include the development of a robust food system capable of sustaining long-duration spaceflight. Currently, food scientists are developing advanced technologies to enhance the culinary experience of astronauts in space. Additionally, a new generation of high-tech 3D food printers is being designed to satisfy both nutritional and taste requirements. These printers use slurries, additives, nutrients, and powders as ingredients, allowing the preparation of food with extended shelf life and optimal nutritional qualities through specialized software (Meral and Demirdoven, 2022). This innovation is expected to decrease the reliance on pre-packaged ready-to-eat meals in space shuttles (Park et al., 2012).

Research in space farming is advancing, with a focus on cultivating plants that maximize edible yield (Oluwafemi et al., 2018). NASA (National Aeronautics and Space Administration) has prioritized the ability to grow safe, fresh food in space to supplement astronauts' packaged meals. Space-grown crops, unlike those on Earth, encounter unique environmental conditions (Khodadad et al., 2020). While plants can survive in low gravity, a minimal amount is necessary for healthy growth, along with essential resources such as water, carbon dioxide, and nutrients (de Pascale et al., 2021). The first space-grown crop, Red Romaine lettuce, was successfully produced by astronauts (Jiang et al., 2020). Ongoing research includes cultivating plums, bell peppers, tomatoes, and other vitamin-rich staples. Additionally, there are improvements in space food products like dumplings, noodles, vacuum-sealed meals, dehydrated foods, brewed coffee, and tortillas, driven by growing demand (Song et al., 2012). Innovations in food technology are expected to accelerate space missions (Long et al., 2022).

Space research continues to influence food preservation, with new technologies and sustainable supply methods under development. Although astronauts now have access to a variety of foods, long-duration missions expose them to environmental changes that can lead to psychological health issues. Therefore, integrating food science with space psychology is crucial to creating foods that offer both psychosocial and physiological benefits (Douglas et al., 2021). Additionally, packaging plays a significant role, requiring designs that are easy to use, lightweight, and capable of extending the shelf life of food products (Long et al., 2022). This review provides a concise overview of the evolution of space food, its missions, nutritional aspects, and classification.

Evolution of Space Food

The shelf life of space food is crucial for long-duration missions, evolving from basic tubes and cubes to more sophisticated cuisine with technological advancements. Initial space food, tested during early missions like Mercury, was often unpalatable, consisting of pureed foods in aluminum tubes. The Gemini missions introduced solid foods, though challenges such as crumbs in spacecraft led to further innovations. Over time, packaging and preservation methods improved, especially during the Apollo and Skylab missions, where items like dehydrated foods and refrigeration were introduced. Research in space gardening has shown that some plants can grow in microgravity, leading to ongoing efforts in this area. The psychological and physiological well-being of astronauts, supported by nutritious and varied space food, has become a critical focus. Although fresh supplies are periodically delivered to space stations, ensuring a stable and nutritious food supply remains essential for long-term missions. Thus, space food must be carefully designed and packaged to maintain its nutrient content and freshness (Liu et al., 2020; Kordyum and Hasenstein, 2021).

The Characteristics of Space Food

Various foods are specifically created and processed for long-term space missions, addressing the unique needs of astronauts. Despite consuming 70% less food than on Earth, astronauts require meals that mimic Earth's cuisine while being free from bacterial contamination. Space food packaging and design differ significantly due to the microgravity environment, where floating crumbs or liquids pose serious risks. Beverages like coffee and juices are stored in powdered form and rehydrated with water through special tubes. Flexible packaging is favored for its efficiency in space, maximizing storage and ease of disposal (Oluwafemi et al., 2018). Key considerations in space food development include biological, operational, and engineering factors, such as preventing microbial growth, ensuring proper packaging, and maintaining the food's stability under extreme conditions. These factors are crucial in preserving the shelf life and safety of space food (Kim and Rhee, 2020).

Nutritional Strategies in Space Exploration Missions

The primary goal of space nutrition is to provide astronauts with meals suited for the space environment, aiming to minimize boredom and enhance food acceptance (Pometto and Bourland, 2003). However, the psychological and physiological changes in space, such as zero gravity and high-intensity radiation, can affect food intake and increase stress levels. These changes may lead to issues like poor digestion, motion sickness, and disruptions in the central nervous system, resulting in decreased food consumption and nutrient absorption (Douglas et al., 2020; Genah et al., 2021). Additionally, astronauts may experience nutrient deficiencies or excesses, such as the need for increased iron and calcium, while balancing the risks of renal stones and iron toxicity (Smith et al., 2012). Space food must address these challenges by adjusting nutrient content, such as lowering fiber and fat while increasing carbohydrates, and compensating for the lack of sunlight by providing Vitamin D through non-dairy alternatives (Bhatia et al., 2018). The psychological effects of space missions, including loneliness, anxiety, and irritability, also necessitate careful consideration in the development of space

food (Varese and Cane, 2017). Additionally, radiation exposure in space destroys B-vitamins and antioxidants like Vitamins A, C, and E, making it essential to develop protective and nutritious food options for astronauts (Bychkov et al., 2021).

Innovations in Space Food

Innovations in space food are critical for ensuring astronauts receive nutritious, safe, and palatable meals during long-term missions. The absence of proper refrigeration on space shuttles has driven efforts to grow fresh produce in space, particularly for missions to Mars expected in the mid-2030s (Tang et al., 2021). Space-grown crops like Red Romaine lettuce and radishes have already been successfully cultivated, demonstrating the potential to reduce reliance on pre-packaged foods and enhance the nutritional quality of diets during extended missions (Jiang et al., 2020; Bychkov et al., 2021). The development of space food systems faces significant challenges, including the need for extended shelf life to maintain food quality and support the crew's psychological well-being. For instance, the Vegetable Production System is a promising solution but comes with high costs and the risk of crop failure (Massa and Mitchell, 2012). Additionally, the limited space and weight constraints on spacecraft, such as the Orion capsule for the Mars mission, necessitate the use of compact, efficient food storage and preparation methods. To address these challenges, technologies like 3D food printing are being developed to create a variety of foods that meet both nutritional and taste requirements, though issues like speed, cost, and safety need further refinement (Park et al., 2012). Moreover, the physiological effects of space travel, including changes in taste perception and nutrient absorption, further complicate food preparation. For example, radiation exposure in space can degrade essential vitamins like A and C, necessitating careful nutritional planning to avoid deficiencies. Scientists are also exploring ways to maintain the psychological health of astronauts by ensuring that meals are not only nutritious but also enjoyable, which is crucial for long missions where monotony can lead to reduced food intake and increased stress (Pandith et al., 2023).

Overall, the advancement of space food systems is essential for the success of future missions, especially as we prepare for the challenges of long-term exploration of the Moon and Mars. These efforts are not only about survival but also about enhancing the overall well-being and performance of astronauts in the harsh conditions of space.

Conclusion

The development of space food systems is integral to the success and sustainability of long-term space missions. As exploration extends beyond Earth's orbit to destinations like Mars, the need for nutritious, safe, and psychologically supportive food becomes increasingly critical. Advances in food technology, including 3D printing and space farming, are paving the way for innovative solutions that address the unique challenges of space nutrition. These innovations not only ensure the physical health of astronauts but also support their psychological well-being, which is essential for maintaining morale and performance during extended missions.

Moreover, the ability to grow fresh produce in space and the development of efficient packaging and storage systems are crucial for reducing dependency on Earth-based supplies. However, significant challenges remain, including ensuring extended shelf life, preventing nutrient degradation due to radiation, and adapting food systems to the confined and weight-sensitive environment of spacecraft. Continued research and technological innovation are vital to overcoming these challenges, ultimately enabling successful human exploration of the Moon, Mars, and beyond.

As we consider the future of space exploration, we must not forget how important it is to provide astronauts with meals that are both nutritious and enjoyable. The evolution of space food is not just about meeting basic survival needs; it is about enhancing the overall quality of life in space, ensuring that astronauts remain healthy, motivated, and capable of performing at their best in the most challenging environments imaginable. The advancements in space food systems represent a crucial step forward in humanity's journey to the stars.

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ORAL PRESENTATION

Tüketime hazır sıvı soslarda mikroplastik varlığının araştırılması

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Özet

Mikroplastiklerin gıda ürünlerine geçişi, çevresel ve sağlık açısından önemli riskler taşımaktadır. Bu çalışma, soya sosu, balık sosu, istiridye sosu ve worcestershire sosu gibi yaygın kullanılan soslarda mikroplastik varlığını incelemeyi amaçlamıştır. Araştırma kapsamında, bu dört sos türünden piyasa üzerinde yaygın olarak bulunan ticari markalardan örnekler alınmış ve mikroplastik analizleri yapılmıştır. Sonuçlara göre, her 100 ml'lik sos örneğinde ortalama 1-10 mikroplastik parçacığı tespit edilmiş olup, parçacıkların boyutları 50 µm ile 300 µm arasında değişiklik göstermiştir. Parçacıklar genellikle fiber (lif), fragment (kırık), film (ince tabaka) ve granül (tanecik) şeklinde olup, renk olarak mavi, siyah, beyaz, şeffaf, kırmızı ve yeşil tonlarında görülmüştür. Mikroplastiklerin tür ve kaynakları üzerine yapılan analizlerde ise, en sık karşılaşılan polimer türleri polietilen (PE), polipropilen (PP) ve polistiren (PS) olarak belirlenmiştir. Bu bulgular, sos üretim süreçleri ve ambalaj malzemelerinin mikroplastik kirliliğine katkıda bulunabileceğini ortaya koymaktadır. Çalışma, mikroplastiklerin gıda güvenliği üzerindeki potansiyel etkilerini vurgulamakta ve gıda üreticileri ile tüketiciler için bu konuda dikkatli olmalarının önemini belirtmektedir. Ayrıca, mikroplastik kontaminasyonunun önlenmesi ve kontrol altına alınması için üretim süreçlerinin ve standartlarının gözden geçirilmesi önem arz etmektedir. Gelecek çalışmalarda da bu sorunun daha kapsamlı bir şekilde ele alınması için geniş ölçekli analizler yapılmasının ve daha etkin çözüm stratejilerinin geliştirilmesinin önemli olduğu düşünülmektedir.

Anahtar Kelimeler: İstridye sosu, Balık sosu, Worcestershire Sos, Soya Sosu, Mikroplastik

Investigation of microplastic presence in ready-to-use liquid sauces

Abstract

This study aimed to investigate the presence of microplastics in commonly used sauces such as soy sauce, fish sauce, oyster sauce, and worcestershire sauce. Samples from commercially available brands of these four types of sauces were analyzed for microplastics. The results indicated that an average of 5-10 microplastic particles were detected per 100 ml of sauce, with particle sizes ranging from 50 µm to 300 µm. The particles were generally found in the forms of fibers, fragments, films, and granules, and were observed in colors including blue, black, white, transparent, red, and green. Analysis of the types and sources of microplastics identified the most frequently encountered polymers as polyethylene (PE), polypropylene (PP), and polystyrene (PS). These findings suggest that the production processes and packaging materials of sauces could contribute to microplastic contamination. The study highlights the potential impacts of microplastics on food safety and emphasizes the importance of vigilance for both food producers and consumers. Additionally, it underscores the need for reviewing and improving production processes and standards to prevent and control microplastic contamination. Future research should involve more extensive analyses and the development of more effective strategies to comprehensively address this issue.

Keywords: Oyster Sauce, Fish Sauce, Worcestershire Sauce, Soy Sauce, Microplastics

GİRİŞ

Dünyanın karşılaştığı en önemli sorunların başında çevre kirliği ve buna bağlı küresel değişimler gelmektedir. Çevresel kirlilik nüfus artışı ile paralel artmakta, sanayi ve endüstriyel gelişimler bu süreci hızlandırmaktadır. Son yıllarda özellikle plastik kirliliği küresel bir çevre sorunu olarak büyük bir endişe kaynağı olarak öne çıkmaktadır. Farklı kaynaklardan meydana gelen plastikler, çevresel faktörlerin etkisi altında bozunmakta ve daha küçük parçalara ayrılarak doğada uzun süre bozulmadan kalabilen mikroplastikleri oluşturmaktadır. Bu küçük partiküller, özellikle deniz ve göl ekosistemlerinde birikim göstermekte ve su yoluyla gıda zincirine dahil olabilmektedir (Sun ve ark., 2018; Savoca ve ark., 2019). Dahası mikroplastikler, balıklar ve diğer deniz canlıları tarafından tüketildiklerinde, gıda ürünlerinde birikerek insanlara geçebilmektedir (Cho ve ark., 2019; Hermabessiere ve ark., 2019; Akhbarizadeh ve ark., 2020; Daniel ve ark., 2020; Sparks ve ark., 2021). Gıda ile insan vücuduna giren mikroplastiklerin sağlık üzerindeki etkileri henüz tam olarak anlaşılmamış olsa da bu durum potansiyel sağlık riskleri ve ekolojik dengede bozulmalar yaratma endişelerini beraberinde getirmektedir (Anderson ve ark., 2016; Smith ve ark., 2018; Sridhar ve ark., 2022). Bu sebeple, mikroplastik kirliliği ile mücadele etmek ve bu sorunun gıda güvenliği üzerindeki etkilerini araştırmak, çevre koruma ve halk sağlığı açısından kritik öneme sahiptir (Addo Ntim ve ark., 2018).

Soslar özellikle modern beslenme anlayışında yemeklerde tamamlayıcı ve lezzet artıcılar olarak kullanılan gıda bileşenleridir. Birçok kültüründe mutfağında önemli bir yer tutan soslar lezzet ve görünüm özellikleri ile geniş bir tüketici kitlesine hitap eden ürünlerdir (Lopetcharat ve ark., 2001; Sun ve ark., 2016; Nakano ve ark., 2018; Zang ve ark., 2020). Geçmişten günümüze gelen süreçte, balzamik sirke, mayonez-ketçap, nar ekşisi, soya sosu, domates sosu, çeşitli salça türleri gibi karasal kaynaklardan elde edilen soslar ve balık sosu, istiridye sosu ve worcestershire sosu gibi popüler denizel kaynaklardan elde edilen soslar sofralara zenginlik katmış ve hatta bazı kültürlerle özdeşleşmiştir (García-Casal ve ark., 2016). Ancak, bu sosların üretim süreçlerinde kullanılan hammadde ve paketleme malzemeleri mikroplastiklerin bu ürünlere bulaşmasına yol açabilir. Mikroplastiklerin gıda maddelerine geçişi, gıda güvenliği ve halk sağlığı açısından potansiyel riskler taşımaktadır.

Bu bağlamda, soslarda mikroplastik varlığının araştırılması hem tüketici sağlığını koruma hem de gıda üretim süreçlerinde gerekli iyileştirmeleri belirleme açısından büyük önem arz etmektedir. Bu çalışma, soya, balık, istiridye ve worcestershire sosları üzerinde yapılacak mikroplastik analizi ile, bu tür sosların potansiyel mikroplastik kirliliğini değerlendirmeyi amaçlamaktadır. Araştırmanın sonuçlarının, gıda endüstrisinde mikroplastik kirliliği ile mücadele stratejilerinin geliştirilmesine katkı sağlayacağı ve tüketicilere daha sağlıklı seçimler yapma imkânı sunacağı düşünülmektedir.

MATERYAL VE METOT

Materyal

Araştırmada materyal olarak, soya sosu, balık sosu, istiridye sosu ve worcestershire sosuna ait ticari ürünler piyasa üzerindeki yaygın markalardan temin edilmiştir. Numuneler analiz öncesinde karanlıkta ve oda sıcaklığında (25°C'de) muhafaza edilmiştir. Ayrıca hazırlık aşaması, analiz ortamı ve analiz prosedüründen kaynaklanabilecek kontaminasyonların önlenmesi adına ilgili mevzuatlar takip edilmiştir (Woodal ve ark., 2015; Masura ve ark., 2015).

Metot

Soslarda Mikroplastik Varlığının Analizi

Soslar GF/F (cam mikrofiber süzme kâğıdı, 0,7 µm göz açıklığı) filtre (Zhu ve ark., 2020) yardımı ile süzülmüştür. Sosların ambalajlarında olası parçacıkların kalmaması için ambalajlardan saf su geçirilmiş ve tekrar süzme yapılmıştır. Son olarak filtre kağıtları da saf su ile kalıntılardan ayrıştırılmıştır (Xu ve ark., 2022). Filtre kağıtları alüminyum folyo ile oda sıcaklığında ve direkt güneş ışığı almayacak şekilde kurutulmuştur (Masura ve ark., 2015). Kurutulmuş filtrelerdeki parçacıkların yoğunluklarına göre filtre üzerinde eşit aralıklara bölünerek alt örneklemesi yapılmış veya filtre üzerindeki tüm parçacıklar mikroskopta incelenmiştir. Plastik şüphesi olan tüm izole edilmiş parçacıklar FT-IR'ın okuma hücresine yerleştirilip 4000- 400 cm^{-1} dalga boyu aralığında okuma gerçekleştirilmiştir (Xu ve ark., 2022). Elde edilen dalga boyları kütüphane ile karşılaştırılıp herhangi bir plastik polimeri ile benzerlik yakalandığında plastik olarak tanımlanmıştır. Plastik olarak tanımlanan tüm parçacıkların ağırlıkları ve hacimleri belirlenerek, süzülen sos hacmi ile kıyaslanmıştır. Böylece her sosun birim hacimdeki plastik yükü bulunup bu durumda sosların kullanımına bağlı olarak ne kadar plastik tüketildiği ortaya konulmuştur.

TARTIŞMA VE SONUÇ

Genel Mikroplastik Bulguları

Mikroskop altında yapılan incelemelerde, her 100 ml'lik sos örneğinde ortalama 1-10 şüpheli mikroplastik parçacığı bulunmuştur. Parçacıkların boyutları 50 µm ile 300 µm arasında değişiklik göstermiştir. Parçacıklar genellikle fiber (lif), fragment (kırık), film (ince tabaka) ve granül (tanecik) şeklinde olup, renk olarak mavi, siyah, beyaz, şeffaf, kırmızı ve yeşil tonlarında görülmüştür.

Araştırmada kullanılan sosların hammaddeleri arasında yer alan balık, midye, istiridyenin yaşam ortamları ve bünyelerindeki mikroplastik yüküne dair bazı araştırmalar bulunmaktadır. İngiltere denizlerinde yapılan bir çalışmada, sekiz farklı istasyondan temin edilen deniz suyu ve midye (*Mytilus edulis*) örneklerinde, deniz suyunun her litresinde 1,5- 6,7 adet aralığında mikroplastik tespit edilirken, canlı midyelerde 0,9 adet/g, süpermarketlerde ise 1,4 adet/g mikroplastik tespit edildiği bildirilmiştir (Li ve ark., 2018). Midye (*Mytilus edulis*) ve istridye (*Crassostrea gigas*) örneklerinin kullanıldığı başka bir çalışmada ise her iki türde sırasıyla birey başına 0,61 ve 2,1 adet mikroplastik tespit edildiği belirtilmiştir (Phuong ve ark., 2018). Çalışmada ayrıca, mevsimlerin, örnekleme alanlarının ve kültür ya da doğal ürün olmalarının, birey başına tespit edilen mikroplastik sayısına etkisinin olmadığı rapor edilmiştir (Phuong ve ark., 2018). Basra Körfezi'nde yapılan bir çalışmada ise beş farklı istasyondan örneklenen ekonomik balık türleri ve ıstakoz türlerinde toplam 828 adet mikroplastik karakterize edilmiş, birey başına ortalama ise 7,8 ila 21,8 adet mikroplastik tespit edildiği rapor edilmiştir (Abbasi ve ark., 2018).

Kim ve ark. (2022), Kore'den temin ettikleri soya sosu örneklerinde, 12,3-78,7 parça/100 mL mikroplastik varlığı tespit etmişlerdir. Kore'de yapılan güncel bir çalışmada ise Kore geleneksel gıdaları ve sıklıkla tüketilen diğer gıda maddelerinde mikroplastik taraması yapılmış, aralarında soya sosu ve balık sosunun da bulunduğu birçok farklı gıda maddesinin tüketimi ile Kore halkının haftada ortalama 220-386 adet mikroplastiği bünyelerine aldığı tespit edilmiştir (Pham ve ark., 2023). Balık soslarında mikroplastik varlığı üzerine yapılan bir başka çalışmada ise, 1,3-4 parça L^{-1} olarak mikroplastik varlığına rastlanmıştır (Espiritu ve ark., 2024). Bu bulgular hammaddeden gelen mikroplastiklerin bazı sos gruplarında üretim metotları ile elemine durumunun göreceli olduğunu göstermektedir. Üretimde ek filtrasyon kullanılan sosların üretiminde mikroplastik varlığı azalırken, bazı sosların üretimlerinde mikroplastiklerin elemine edilmediğini, hatta üretimde ve ambalaj seçiminde yeterli özenin gösterilmemesi durumunda mikroplastik sayısının artabileceğini düşündürmektedir.

Mikroplastik Türleri ve Kaynakları

Mikroplastiklerin tür ve kaynakları üzerine yapılan analizlerde, en sık karşılaşılan polimer türleri polietilen (PE), polipropilen (PP) ve polistiren (PS) olarak belirlenmiştir. Bu polimer türleri genellikle gıda ambalajları, tek kullanımlık plastik ürünler ve sentetik tekstil ürünlerinden kaynaklanmaktadır. Pham ve ark. (2023) soya ve balık soslarının da dahil olduğu 90 numunede bulunan mikroplastiklerin PE, PP ve polietilen tereftalat (PET) olduğu tespit etmişlerdir. Espiritu ve ark. (2024) Filipinler'den temin edip analiz ettikleri balık soslarındaki mikroplastik tür ve kaynaklarının PP, PET ve Politetrafloroetilen (PTFE) olduğunu bildirmişlerdir. Sos içeren konserve deniz ürünleri üzerine yapılan bir çalışmada ise sekiz farklı renkte (mavi (%37,5), şeffaf (%30), siyah (%10), kırmızı (%7,5), sarı (%5), mor (%5), beyaz (%2,5) ve yeşil (%2,5)) mikroplastik gözlenmiş ve tür olarak plastik polimerler polyester (PES, %26), polipropilen (PP, %17), polietilen (PE, %17), suni ipek (%17), naylon (%13) ve polivinil (%9) olarak bildirilmiştir (Silva ve ark., 2024).

SONUÇ

Bu çalışmada, soya sosu, balık sosu, istiridye sosu ve worcestershire sosu gibi yaygın kullanılan dört farklı sos türünde mikroplastik varlığı araştırılmıştır. Yapılan analizler sonucunda, örneklenen soslardan bazılarında farklı tip ve miktarlarda mikroplastik parçacıkları tespit edilmiştir. Bu bulgular, sos üretim süreçlerinde kullanılan hammaddelerin ve ambalaj materyallerinin mikroplastik kirliliğine katkıda bulunabileceğini göstermektedir. Araştırmanın sonuçları, mikroplastiklerin gıda ürünlerine geçişinin önlenmesi için üretim süreçlerinde ve paketleme malzemelerinde dikkatli olunması gerektiğini ortaya koymaktadır. Mikroplastiklerin gıda güvenliği üzerindeki potansiyel riskleri göz önüne alındığında hem üreticilerin hem de tüketicilerin bu konuda bilinçli olması önem arz etmektedir. Gelecek çalışmalar, mikroplastiklerin gıda ürünlerinde daha kapsamlı bir şekilde incelenmesi ve mikroplastik kontaminasyonunun kaynağının belirlenmesi için daha geniş ölçekli araştırmaların yapılmasını gerektirmektedir. Ayrıca, gıda güvenliği standartlarının gözden geçirilmesi ve iyileştirilmesi, bu sorunun çözümüne katkıda bulunabilir. Toplum olarak bu konuda proaktif bir yaklaşım benimsemenin hem mevcut hem de gelecekteki sorunları önlemekte etkili olabileceği düşünülmektedir.

TEŞEKKÜR

Bu çalışmaya desteklerinden dolayı Çanakkale Onsekiz Mart Üniversitesi Bilimsel Araştırmalar Komisyonuna teşekkürlerimizi sunarız. Çalışma FLÖAP-2023-4504 no'lu proje tarafından desteklenmiştir.

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ORAL PRESENTATION

Immunobiotics: Exploring Antiviral Potential of Probiotics

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Abstract

Probiotics, a group of nonpathogenic microorganisms, have been historically incorporated into fermented foods to confer various health benefits. These beneficial bacteria, commonly belonging to the *Lactobacillus* and *Bifidobacterium* genera, are thought to improve nutritional outcomes and restore the microbial equilibrium of the human gut. The gut microbiota plays a pivotal role in bolstering the immune system through its diverse functions, including detoxification, antibody production, enhancement of natural killer cell cytotoxicity, reduction of inflammation, and colonization of enteropathogenic cells. Multiple studies have demonstrated that probiotics can enhance the immune cell function of the host. The molecular mechanisms underlying these effects involve interactions with epithelial cells, dendritic cells, and T-cells, leading to the induction of several cytokines, including TGF-β, IL-6, and IL-10. Additionally, bioactive compounds produced by probiotics, such as extracellular polysaccharides, bacteriocins, butyric acid, and dextran, have been shown to contribute to improved immune responses. For instance, dextran, an extracellular polysaccharide synthesized by certain probiotic strains, has been found to exhibit antiviral effects against infectious hematopoietic necrosis viruses. The antiviral activity of probiotics is attributed to their ability to stimulate the host immune response against viral infections. Due to their immunomodulatory properties, probiotics, particularly those belonging to the *Lactobacillus* genus, have been aptly termed 'immunobiotics.' Studies have demonstrated that *Lactobacillus* strains can enhance immune responses by increasing phagocytic activity, plasma lysozyme levels, complement activation, and the production of superoxide anions. The antiviral activity of various probiotics has been reported against both respiratory and alimentary tract viruses. On the other hand, the global health crisis posed by the COVID-19 pandemic has spurred an intensified search for innovative antiviral strategies to mitigate infection rates and accelerate recovery. Oral probiotics have been shown to exhibit antiviral effects and improve gut health to restore homeostasis, leading to their recommendation as adjunctive therapy for COVID-19.

Keywords: Probiotics, immune system, antiviral potential, immunobiotics

INTRODUCTION

While Louis Pasteur is credited with identifying the microorganisms responsible for fermentation processes in the early 20th century, a cornerstone of modern microbiology, Elie Metchnikoff was the first to explore the potential health benefits of these bacteria in humans. Inspired by observations of the long, healthy lives of Bulgarian peasants and their traditional dietary practices, Metchnikoff pioneered the concept of probiotics. While the term 'probiotic' has been defined in various ways by different researchers, one of the most widely accepted definitions was provided by the veterinarian Roy Fuller in 1989 as 'live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance' (Fuller, 1989). Today, the most accepted and widely used definition, established in 2001 by a joint FAO/WHO expert working group, defines probiotics as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'. This definition and the advancements in the field of probiotics were evaluated in 2013 by a panel of experts convened by the International Scientific Association for Probiotics and Prebiotics (ISAPP), which suggested a grammatical adjustment to the definition. The panel emphasized that the key concepts in the definition are 'live', 'microbial', and 'health benefits (Hill et al., 2014). These beneficial bacteria, commonly belonging to the Lactobacillus and Bifidobacterium genera, are thought to improve nutritional outcomes and restore the microbial equilibrium of the human gut (Table 1).

IMMUNOBIOTICS

The immune system serves as a critical defence mechanism against viral invasion, with innate and adaptive immune systems playing pivotal roles. The innate immune system, characterized by its rapid and non-specific response, acts as the first line of defence against invading pathogens. This system comprises macrophages, neutrophils, eosinophils, natural killer cells, epithelial cells, and M cells, which work together to eliminate pathogens through direct mechanisms (Muhialdin, 2021). Innate immune cells also secrete various cytokines, including type I interferon (IFN) and interleukins IL-1, IL-6, IL-12, and IL-16, which are instrumental in combating infections caused by pathogens and viruses (Akdis et al., 2016). On the other hand, the adaptive immune system is characterized by its slower but highly specific response. Memory B cells and memory T cells are critical players in this system, enabling the body to recognize and eliminate previously encountered pathogens (Muhialdin, 2021).

The gastrointestinal (GI) tract is the body's largest immune organ, housing a complex community of commensal microorganisms. The gut microbiome comprises approximately 160 bacterial species, although this diversity can vary based on environmental and genetic factors. While *Bacteroidetes* and *Firmicutes* predominate in the gut, *Proteobacteria* are also present in the lungs. A significant proportion of the body's immune cells, approximately 70-80%, are located within the GI tract, underscoring the profound connection between the immune system and the intestinal microbiome (Jabczyk et al., 2021).

Augmenting and stimulating the immune system can serve as a preventive measure against infections caused by diverse pathogens, including viral agents, due to their functions, including the detoxification of toxins, production of antibodies, enhance NKC cytotoxicity, reduce inflammations and prevent the colonization of enteropathogenic (Ashaolu et al., 2020). Probiotics have been shown to exert beneficial effects on the host immune system. Several studies have demonstrated that lactic acid bacteria (LAB) strains can enhance immune cell function, including splenic natural killer cell activity (Lee et al., 2017). The combination of LAB and yeast strains has been found to boost overall immune function (Zulkawi et al., 2017). Furthermore, *Lb. plantarum* LC01 has been shown to improve natural killer cell function (Kang et al., 2017). Moreover, *Lb. delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophiles*, and *Lb. casei* DN 114 001 have been shown to stimulate the production of proinflammatory cytokines (Meyer et al., 2007).

The molecular mechanisms underlying the immunomodulatory effects of probiotics involve interactions with epithelial cells, dendritic cells, and T-cells, leading to the induction of cytokines such as TGF-β, IL-6, and IL-10. Additionally, bioactive compounds produced by probiotics, including extracellular polysaccharides (dextran), surface exopolysaccharides (EPS) synthesized by *Bifidobacterium breve* UCC2003, bacteriocins, and butyric acid, have been shown to contribute to enhanced immune responses (Li & Gänzle, 2020).

The antiviral activity of probiotics is attributed to their ability to stimulate the host immune response against viral infections. Due to their immunomodulatory properties, probiotics, particularly those belonging to the *Lactobacillus* genus, have been aptly termed 'immunobiotics' (Villena et al., 2016). Studies have demonstrated that *Lactobacillus* strains can enhance immune responses by increasing phagocytic activity, plasma lysozyme levels, complement activation, and the production of superoxide anions. The antiviral activity of various probiotics has been reported against both respiratory and alimentary tract viruses.

According to results of an *in vitro* study (RAW264.7 cells) by Seo et al. (2020) it has been demonstrated that *Pediococcus pentosaceus* (CAU170229- 2 and CAU170230-3), *Weissella cibaria* (CAU170231-1 and CAU170231-3) isolates (from Kimchi) Stimulate NF-κB, IL-1β, TNF-α, and NO in macrophages against murine norovirus. Ermolenko and colleagues (2019) have found that Enterocin B from *Enterococcus faecium* L3 (isolated from dairy product) may stimulate interferon production and to enhance the humoral immune response against influenza virus (H3N2, H1N1) in both *in vitro* (MDCK cell line) and *in vivo* (female Balb/c mice) studies.

COVID-19 PANDEMIC and PROBIOTICS

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel RNA virus belonging to the *Coronaviridae* family, is responsible for coronavirus disease 2019 (COVID-19). Primarily affecting pulmonary tissues, COVID-19 is transmitted through respiratory droplets (Hung et al., 2021). The clinical manifestations of COVID-19 exhibit significant variability, ranging from asymptomatic or mild cases, often

observed in young individuals without comorbidities, to moderate cases characterized by pneumonia. Severe cases may be complicated by hypoxia, respiratory failure, multi-organ failure, and, in some instances, death.

SARS-CoV-2 is comprised of four structural proteins: spike glycoproteins (S), small envelope glycoproteins (E), membrane glycoproteins (M), and nucleocapsid (N), along with additional accessory proteins. The spike transmembrane protein utilizes angiotensin-converting enzyme 2 (ACE2) as its cell-entry receptor. Beyond its abundant presence in pulmonary tissue, ACE2 is also highly expressed in the gut. Consequently, enterocytes within human small intestinal organoids are readily infected by SARS-CoV-2. In the gut, ACE2 plays a pivotal role in regulating dietary amino acid homeostasis, innate immunity, gut microbial ecology, and susceptibility to colitis.

Moreover, ACE2 is linked to the activation of intestinal inflammation. Therefore, SARS-CoV-2 infections frequently manifest with gastrointestinal inflammation, presenting with a spectrum of clinical symptoms ranging from intestinal cramps and diarrhea to severe complications such as intestinal perforations (Hung et al., 2021). Disruptions in the gut microbiota can lead to elevated levels of ACE2 due to epithelial breakdown and inflammation.

The Chinese National Health Commission has advocated using probiotics to treat severe COVID-19 patients to maintain gut homeostasis and prevent secondary infections. Given that the standard treatment for COVID-19 often involves antibiotics and antiviral medications, which can disrupt the gut microbiome and contribute to diarrhea, probiotic therapy presents a dual role: not only as a potential therapeutic agent but also as a means of gut prophylaxis following antibiotic treatment (Jabczyk et al., 2021). Clinical studies investigating the administration of probiotics to COVID-19 patients have demonstrated reductions in disease duration and severity of symptoms, including fatigue, anosmia, dyspnea, nausea, vomiting, and other gastrointestinal complaints (Xavier-Santos et al., 2022). These findings suggest that probiotics may offer a promising adjunct therapy for COVID-19, helping to mitigate the severity of the disease and improve patient outcomes.

Putative mechanisms of probiotics to manage coronavirus infection: (Singh and Rao, 2021)

(A) Probiotic bacteria can hinder adsorption by directly binding to the virus and inhibiting entry into epithelial cells.

(B) Binding probiotic bacteria to the epithelial surface can cause steric hindrance and block the virus's attachment to the host cell receptor.

(C) Probiotic bacteria release antimicrobial substances (such as bacteriocins, biosurfactants, lactic acid, hydrogen peroxide, nitric oxide, organic acids) and intestinal mucins from mucosal cells, which can effectively inhibit virus proliferation.

(D) Virus neutralized by secretory antibodies like IgA.

(E) Upon virus attack in epithelial cells, probiotics mediate their antiviral effects by eliciting immune responses by activating macrophages and dendritic cells.

(F) The activation of the immune response leads to the differentiation of CD8+ T lymphocytes into CTLs, which are capable of destroying virus-infected cells.

(G) $CD8^+$ T lymphocyte cells differentiate into Th1, which activates phagocytosis through NK cells and macrophages, promoting pathogen killing.

(H) $CD4^+$ cells differentiate into Th2 cells, which induce B-cells' proliferation and produce antibodies like IgA, IgG, and IgM. CTLs, cytotoxic T-lymphocytes; Th1, T-helper cells type 1.

Table 1 Probiotic microorganisms (Salminen et al., 1998)

Lactobacillus spp.	*Lactobacillus cellobiosus*, *Lactobacillus delbrueckii*, *Lactobacillus brevis*
	Lactobacillus acidophilus, *Lactobacillus reuteri*, *Lactobacillus curvatus*
	Lactobacillus fermetum, *Lactobacillus plantarum*, *Lactobacillus jonhsonii*
	Lactobacillus rhamnosus, *Lactobacillus helveticus*
	Lactobacillus salivarius, *Lactobacillus gasseri*
Bifidobacterium spp.	*Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*
	Bifidobacterium thermophilum
Bacillus spp.	*Bacillus subtilis*, *Bacillus pumilus*, *Bacillus lentus*, *Bacillus licheniformis*
	Bacillus coagulans
Pediococcus spp.	*Pediococcus cerevisiae*, *Pediococcus acidilactici*
	Pediococcus pentosaceus
Streptococcus spp.	*Streptococcus salivarius* ssp. *thermophilus*, *Streptococcus intermedius*
Bacteriodes spp.	*Bacteriodes capillus*, *Bacteriodes suis*, *Bacteriodes ruminicola*, *Bacteriodes amylophilus*
Propionibacterium spp.	*Propionibacterium shermanii*, *Propionibacterium freudenreichii*
Leuconostoc spp.	*Leuconostoc mesenteroides* ssp. *mesenteroides*
Molds	*Aspergillus niger*, *Aspergillus oryzae*
Yeasts	*Saccharomyces cerevisiae*, *Candida torulopsis*

CONCLUSION

The dysbiosis of the gut microbiota has been implicated in the pathogenesis of numerous viral infections, particularly those affecting the gastrointestinal tract. Consequently, probiotic-based therapy is a promising strategy for managing these viral infections. Probiotics can modulate host immune responses and counteract the harmful effects of the cytokine storm associated with COVID-19. However, the optimal selection of probiotic species, whether administered alone or in combination, for combating viral infections remains an active area of research.

Understanding the host's gut microbiota composition, influenced by many factors, including age, lifestyle, dietary habits, and genetics, is crucial for selecting appropriate probiotic strains. Furthermore, investigating how the human gut microbiota responds to the introduction of new bacterial communities is essential for elucidating the mechanisms underlying the therapeutic effects of probiotics against viral infections.

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ORAL PRESENTATION

Lactobacillus plantarum'un *Vibrio harveyi* üzerindeki antagonistik etkisi: Midye ezmesi örneği

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Özet

Bu çalışmada, midye ezmesinde *Lactobacillus plantarum*'un starter kültür olarak kullanılmasının *Vibrio harveyi* üzerindeki antagonistik etkilerinin incelenmesi amaçlanmıştır. Fermente su ürünlerinin gıda güvenliğindeki rolünü vurgulamak amacıyla gerçekleştirilen bu araştırma, midye ezmesinde kullanılan *L. plantarum*'un, *V. harveyi*'nin üremesini etkili bir şekilde inhibe ettiğini göstermiştir. *L. plantarum* fermantasyon ortamında canlılığını 55 gün boyunca sürdürürken, *V. harveyi* fermantasyonun 2. Gününden sonra fermantasyon ortamındaki varlığını sürdürememiştir. Sonuç olarak, *L. plantarum* içeren fermente midye ezmesinin, patojenlerle mücadelede ve gıda güvenliğinin artırılmasında potansiyel bir araç olabileceği ortaya konmuştur. Bu bulgular, deniz ürünleri endüstrisinde fermente ürünlerin kullanımını teşvik edebilmekte ve probiyotik bakterilerin gıda güvenliği üzerindeki olumlu etkilerini desteklemektedir. Gelecek çalışmalarda ise bu probiyotiklerin diğer patojenler üzerindeki etkilerinin ve geniş ölçekli uygulama potansiyellerinin daha detaylı bir şekilde araştırması gerektiği düşünülmektedir.

Anahtar Kelimeler: Midye ezmesi, *Lactobacillus plantarum, Vibrio harveyi,* Fermantasyon, Gıda güvenliği, Antagonistik aktivite

Antagonistic effects of *Lactobacillus plantarum* on *Vibrio harveyi*: A mussel paste example

Abstract

This study aims to investigate the antagonistic effects of using *Lactobacillus plantarum* as a starter culture in mussel paste against *Vibrio harveyi*. Conducted to highlight the role of fermented aquatic products in food safety, this research demonstrates that L. plantarum used in mussel paste effectively inhibits the growth of *V. harveyi*. While *L. plantarum* maintains its viability for 55 days in the fermentation environment, *V. harveyi* cannot sustain its presence in the fermentation environment beyond the second day of fermentation. As a result, it has been shown that fermented mussel paste containing L. plantarum could be a potential tool in combating pathogens and enhancing food safety. These findings could encourage the use of fermented products in the seafood industry and support the positive effects of probiotic bacteria on food safety. Future studies should investigate the effects of these probiotics on other pathogens and their potential for large-scale application in more detail.

Keywords: Mussel paste, *Lactobacillus plantarum, Vibrio harveyi*, Fermentation, Food safety, Antagonistic activity

GİRİŞ

Günümüzde gıda güvenliği ve insan sağlığının korunmasında, gıda kaynaklı patojenlerle mücadele büyük önem taşımaktadır (Gallo ve ark., 2020; Bhattacharyya ve Das, 2022). Özellikle su ürünleri gibi yüksek besin değeri taşıyan ve dünya çapında yaygın olarak tüketilen gıdaların raf ömrü ve güvenlik konuları dünya çapında önemli bir yere sahiptir (Venugopal ve Gopakumar, 2017; Samanta ve Choudhary, 2019). Çünkü su ürünlerinin doğal yaşam ortamları ve bu ortamdan beslenme şekilleri su ürünlerinin mikrobiyal yükünü doğrudan etkilemektedir. Örneğin midyeler yaşadıkları su ortamını filtre ederek beslenen canlılar olarak, suda

bulunan mikrobiyal yükü bünyelerinde barındırabilmektedir (Çolakoğlu ve ark., 2020). Bu mikrobiyal yük içinde patojen mikroorganizmalar da bulunabilmekte ve insan sağlığını tehdit edebilmektedir. Bu mikroorganizmalar kontaminasyon kaynaklı enterik kökenli mikroorganizmalar olabileceği gibi küresel iklim değişimine bağlı olarak yaşam alanları genişleyen Vibrio spp. gibi bazı mikroorganizmalar da olabilmektedir (Künili ve ark., 2021; Künili ve Ateş, 2021). Bu nedenle, su ürünlerinin güvenli tüketimi her geçen gün daha da önemli bir sorun teşkil etmektedir (Samanta ve Choudhary, 2019; Mol ve Coşansu, 2022). Dolayısıyla bu tür patojenlerle mücadelede kullanılan yöntemler hem etkin hem de güvenli olmalıdır.

Fermentasyon, mikroorganizmaların kullanımıyla gıdaların kimyasal ve fiziksel özelliklerini değiştirebilen, besin kalitesini artırabilen ve raf ömrünü uzatan bir süreçtir (Ross ve ark., 2002; Hasan ve ark., 2014; Verni ve ark., 2022). Birçok gıda ürünü ile beraber su ürünlerinin de işleme ve korunmasında kullanılan popüler bir yöntemdir (Wang ve ark., 2023; Lee ve ark., 2024). Bu süreçte yer alan mikroorganizmalar, özellikle su ürünlerinin doğal yaşam ortamından edindikleri mikroorganizma yükünde bulunan patojenlerin kontrolü açısından büyük avantajlar sağlamakta, dolayısıyla patojenlerin neden olduğu sağlık risklerini azaltabilmektedir (Şanlier ve ark., 2019; Sharma ve ark., 2020). Su ürünlerinde kullanılan fermantasyon teknikleri, geleneksel yöntemlerin yanı sıra modern gıda işleme teknikleriyle de uyumlu hale getirilerek hem yerel hem de küresel gıda güvenliği ve sağlığına önemli katkılarda bulunmaktadır.

Son yıllarda geleneksel fermantasyon süreçleri probiyotikler ve starter kültürlerin ilavesi ile daha kontrollü ve etkin hale getirilmektedir (Kwofie ve ark., 2020; Patrignani ve ark., 2020; García-Díez ve Saraiva, 2021). Özellikle, laktik asit bakterileri gıda ürünlerinin fermantasyonunda starter kültür olarak kullanılarak hem besin kalitesini artırmakta hem de patojenik mikroorganizmaların kontrol altına alınmasına yardımcı olmaktadır (Behera ve ark., 2018; Zapaśnik ve ark., 2022; de Souza ve ark., 2023). Birçok çalışma laktik asit bakterilerinin ürettikleri metabolit ve parçalanma ürünleri ile gıda güvenliğini sağlayıcı özelliklere sahip olduğunu kanıtlamakta ve bu özelliğin, onların çeşitli gıda ürünlerinde kullanılmasını mümkün kıldığını vurgulamaktadır (Ayivi ve ark., 2020; Agriopoulou ve ark., 2020; Wang ve ark., 2021).

Bu çalışmada, ortamdan kolayca izole edilebilen *Lactobacillus (L.) plantarum* starter kültür olarak kullanılarak midye ezmesi üretilmiştir. Ülkemiz sularında artış gösteren Vibrio türlerinden, midyelerin doğal beslenme şekilleriyle bünyelerinde artış gösterebilecek *Vibrio (V.) harveyi* model patojen olarak seçilmiştir ve *L. plantarum*'un antagonistik aktivitesi incelenmiştir. Midye ezmesi, yüksek besin değeri ve geniş potansiyeli ile dikkat çekerken, starter kültürlerin bu ürün üzerindeki etkilerini incelemek, gıda güvenliğini artırmak ve alternatif koruma yöntemleri geliştirmek açısından önemlidir. Bu bağlamda, araştırmada midye ezmesindeki *L. plantarum*'un *V. harveyi* üzerindeki inhibe edici etkilerinin ortaya konulması hedeflenmektedir. Elde edilen bulguların hem su ürünleri endüstrisine hem de genel gıda güvenliği uygulamalarına önemli katkılar sağlayacağı düşünülmektedir.

MATERYAL VE METOT

Materyal

Araştırmada hammadde olarak kullanılan Akdeniz midyesi (*Mytilus galloprovincialis*) Çanakkale kıyılarından el ile toplanıp, buz aküleri ile soğutulmuş izolasyonlu örnek taşıma kutularında laboratuvara nakledilmiştir. Laboratuvara getirilen canlı midye örnekleri kum, çamur ve makroorganizmalardan arındırıldıktan sonra kabuk kısımları alkollü pamuk ile temizlenerek üretim için ön hazırlığı yapılmıştır. Ardından midye ezmesi üretimi için midyeler steril bıçak yardımı ile açılmış ve ayıklanmıştır. Üretimde kullanılacak baharatlar, tuz ve şeker yerel marketten temin edilmiştir.

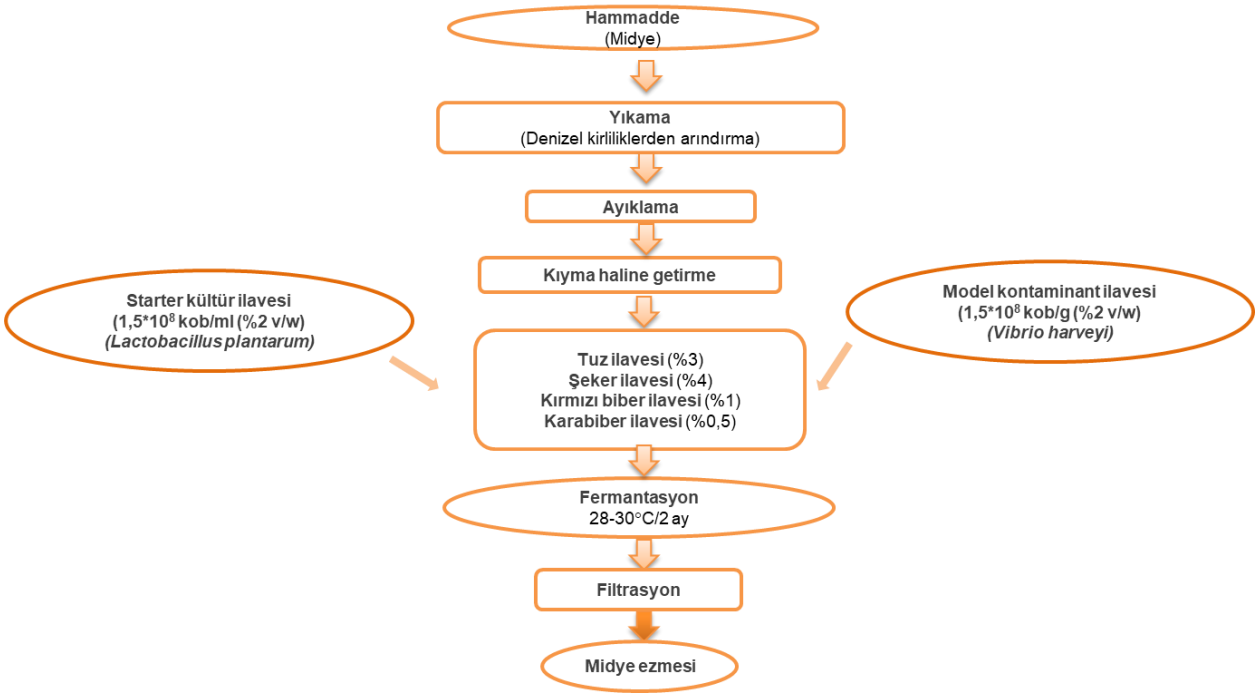
Metot

Mikroorganizmaların Midye Ezmesi Üretimine Hazırlanması

Suşların aktivasyonu için *L. plantarum*, 28-30°C'de 2 gün boyunca anaerobik koşullar altında De Man Rogosa ve Sharpe (MRS) besiyerinde iki kez kültüre edilmiştir. Bu arada, *V. harveyi*, 30°C'de 36 saat aerobik koşullar altında Thiosulfate Citrate Bile Sucrose (TCBS) Agar besiyerinde kültüre edilmiştir. Ardından, logaritmik büyüme fazındaki hücreler bir densitometre (BioSan, Türkiye) kullanılarak 0,5 McFarland birimi (yaklaşık $1,5\times10^8$ kob/ml) hücre yoğunluğuna ulaşmak için fizyolojik tuzlu suda süspanse edilmiştir.

Midye Ezmesi Üretimi

Midye ezmesi üretimi starter kültür ilavesi ile gerçekleştirilmiştir ve üretime ait şema Şekil 1'de verilmiştir. Kısaca, temizlenip ayıklanan midyeler homojen bir hamur elde etmek için parçalanmıştır. Ardından %3 tuz, %4 şeker, %1 kırmızı biber ve %0,5 karabiber ilavesi gerçekleştirilmiştir. Homojen karışım elde edilmesinin ardından süspanse edilen kültürler fermantasyon sürecini başlatmak ve model olarak kontaminasyon oluşturmak için (%2 v/w) için hazırlanmış ezme hamurlarına inoküle edilmiştir. Elde edilen karışım kavanozlara alınmış ve 28-30°C'de 2 ay süre ile fermantasyona bırakılmıştır. Fermantasyon süresince mikrobiyolojik analizler yapılarak mikroorganizmaların canlı kalma süreleri belirlenmiştir.



Şekil 1. Starter kültür (*L. plantarum*) ve model kontaminant ilavesi (*V. harveyi*) ile midye ezmesi üretim şeması

Mikrobiyolojik Analizler

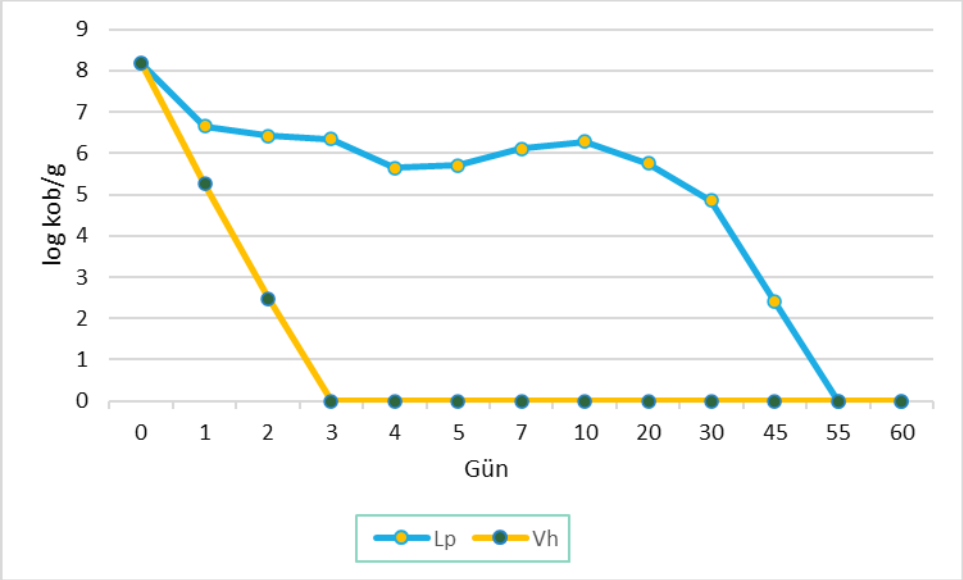
Midye ezmesi örneklerinden homojen olarak 10 g alınarak 90 ml peptonlu su (%2,5 NaCl ve %1 bakteriyel pepton) içerisine tartılmıştır. Tartım işlemi sonrasında örnekler Stomacher (Seward, 400)'de 4000 rpm'de 4 dakika homojenize edilmiştir. Homojen hale getirilen örneklerin yine %2,5 NaCl içeren peptonlu su içerisine 1ml'e 9 ml olacak şekilde, 10^{-6}'ya kadar desimal seyreltmeleri yapılarak, seyreltmelerden 1 ml alınmış aynı besiyeri bulunan 3 petriye dağıtılacak şekilde yayma plak yöntemine göre ekimler yapılmıştır (FDA-BAM, 1998). Ekimi yapılmış bakteri türleri ve inkübasyon koşulları ise aşağıdaki gibidir (Anonim, 2001).

Laktik Asit Bakteri Sayımı; MRS (de Man, Rogosa Sharpe) Agar besiyeri kullanılmıştır. Ekimi yapılan petri kapları 28°C'de 48 saat anaerobik (Aneorocult C) şartlarda inkübe edilmiştir. İnkübasyon sonunda oluşan bakteriler gözle sayılarak değerlendirilmiştir.

Vibrio spp Sayımı; Thiosulfate Citrate Bile Sucrose (TCBS) Agar besiyeri kullanılmıştır. Ekimi yapılan petriler 30°C'de 36 saat inkübasyona tabi tutulmuştur. İnkübasyon sonunda oluşan bakteriler gözle sayılarak değerlendirilmiştir. Tespit edilen sayılar seyreltme faktörü ile çarpılarak hesaplanmıştır (Harrigan ve McCance, 1976).

BULGULAR ve TARTIŞMA

Midye ezmesi üretimi hakkında yeterli literatür verisi bulunmamakla birlikte (Kilinc ve Sahin, 2015), ürün potansiyeli açısından dikkat çekmektedir. Bu çalışma kapsamında midye ezmesindeki *L. plantarum*'un *V. harveyi* üzerindeki antagonistik etkisinin incelenmesi, ürünün gıda güvenliğinin arttırılması ve alternatif koruma yöntemlerinin geliştirilmesi araştırılmıştır. Elde edilen sonuçlar Şekil 2'de verilmiştir.



Not: Lp, Starter kültür (*L. plantarum*) sayısını, Vh, model kontaminant (*V. harveyi*) sayısını ifade etmektedir.

Şekil 2. Midye ezmesindeki *L. plantarum*'un *V. harveyi* üzerindeki antagonistik etkisi

Elde edilen bulgulara göre, *L. plantarum* başlangıç konsantrasyonu 8,18 log kob/g olarak belirlenmiştir. Fermantasyon başlangıcından itibaren ilk 10 gün mikrobiyal yoğunluk korunmuş ve yaklaşık 6,65-6,27 log kob/g arasında değişim göstermiştir. 10. günden itibaren ise *L. plantarum*'un sayısındaki düşüş belirgin hale gelmiştir. Fermantasyon ortamında 55 gün canlılığını sürdüren *L. plantarum*, 30. günden sonra ise logaritmik olarak azalış göstermiştir. *L. plantarum*'un midye ezmesinde süren yaşam döngüsünün aksine *V. harveyi* sayısı fermantasyonun 1. gününde 5,26 log kob/g olarak belirlenirken, 2. gününde ani düşüş göstererek 2,48 log kob/g olarak tespit edilmiştir. Fermantasyonun 3. gününden itibaren ise canlılık gösterememiştir.

Tuzlu-fermente edilmiş karideslerden izole edilen yeni Lactobacillus (*L. plantarum* ve *Lactobacillus sakei*) suşlarının *Vibrio parahaemolyticus*'a karşı antagonist olarak probiyotik potansiyelinin belirlenmesi üzerine yapılan bir çalışmada iki türünde antagonistik aktivite gösterdiği bildirilmiştir (Le ve Yang, 2018). Fermente su ürünleri ve ilgili mikroorganizmalar ile başka araştırmaya rastlanmamış olmakla birlikte su ürünleri yetiştiriciliği ve hastalıklarının önlenmesi ile ilgili yapılan birçok çalışma mevcuttur. Çalışmalarda laktik asit bakterilerinin Vibrio spp. üzerinde antagonistik aktivite gösterdiği bildirilmiştir. Tank ve ark. (2018), tarafından yapılan bir çalışmada *L. plantarum* ve *Bacillus subtilis*'in *V. harveyi, V. parahaemolyticus* ve *Vibrio vunlificus* üzerinde antagonistik etki gösterdiği bildirilmiştir. Yapılan bir başka çalışmada, karideslerin sindirim sisteminden izole edilen *L. plantarum*'un *V. harveyi*'yi baskılayıcı etkisi in-vivo ve in-vitro olarak incelenmiş ve *L. plantarum*'un *V. harveyi*'yi yaklaşık 18-24 saatte baskıladığı ve canlının hayatta kalma süresini ve büyümesini olumlu yönde etkilediği belirtilmiştir (Kongnum ve Hongpattarakere, 2012).

SONUÇ

Bu çalışmada, *L. plantarum* içeren starter kültürle üretilmiş midye ezmesinin, *V. harveyi* üzerindeki antagonistik etkileri değerlendirilmiştir. Araştırma bulgularında, *L. plantarum*'un midye ezmesindeki etkili fermentasyon süreci ve yarattığı asidik ortamın *V. harveyi*'nin üremesini belirgin bir şekilde inhibe ettiğini düşünülmektedir. Bu sonuçlar, *L. plantarum*'un patojenlerle mücadelede potansiyel bir koruma aracı olarak kullanılabileceğini ve fermente deniz ürünlerinin gıda güvenliği açısından önemli bir uygulama olabileceğini ortaya koymaktadır. Bu bulgular, fermente su ürünlerinin, özellikle de probiyotik bakterilerle zenginleştirilmiş ürünlerin, gıda güvenliğini artırmak ve patojenleri kontrol altına almak için etkili bir yöntem olabileceğini desteklemektedir. Ayrıca, bu sonuçlar, deniz ürünleri endüstrisinde kullanılabilecek potansiyel yenilikçi yöntemlerin geliştirilmesine ve fermente ürünlerin daha geniş bir uygulama alanına sahip olmasına olanak tanımaktadır. Gelecek çalışmaların, bu tür probiyotiklerin diğer patojenler üzerindeki etkilerini ve genel gıda güvenliği üzerindeki potansiyel yararlarını daha kapsamlı bir şekilde araştırmasının faydalı olacağı düşünülmektedir.

TEŞEKKÜR

Bu çalışmaya desteklerinden dolayı TÜBİTAK'a teşekkürlerimizi sunarız. Bu çalışma 2209-A 2023 yılı 1. dönem çağrısı kapsamında 1919B012301475 no'lu proje ile desteklenmiştir.

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ORAL PRESENTATION

Coffee production utilizing the energy scenario application

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Abstract

This study delineates the product scenario of coffee (SCF), detailing the processing of coffee cherries and the energy requirements for drying and storage in the scenario coffee (ESCF). According to the scenario of coffee, there are four scenarios, two of which pertain to energy requirements. The application of SCFs indicates that the ESCF values were calculated to range from 3.37E+03 [kWh] to 8.41E+03 [kWh] for ESCF1, and from 4.64E+03 [kWh] to 1.16E+04 [kWh] for ESCF2. Additionally, for ESCF1, the values ranged from 1.21E+07 [kJ] to 3.03E+07 [kJ], and for ESCF2, from 1.67E+07 [kJ] to 4.17E+07 [kJ]. Manufacturers can reduce energy loss and decrease their carbon footprint by strategically positioning equipment. Efficient material handling can significantly reduce the labor needed to transport coffee beans and other elements during the manufacturing process. Coffee makers can enhance energy efficiency and decrease resource consumption by reducing waste and implementing recycling initiatives. Manufacturers can decrease the energy consumption in coffee production while upholding quality standards by optimizing the production process. Coffee makers can reduce expenses related to energy consumption and production costs through energy efficiency measures.

Keywords: coffee, energy, heat, process.

INTRODUCTION

Coffee production, a highly popular beverage in Turkey and globally, ranks as the second largest traded commodity worldwide, surpassed only by petroleum. The coffee industry generates significant quantities of toxic residues, resulting in serious environmental issues due to the high demand for this product (Batista et al., 2009; Esquivel & Jiménez, 2012; Gosalvitr et al., 2024; Mussatto et al., 2011). The process of transforming coffee beans from their raw state to their production in food factories involves several stages. Coffee can be produced in various forms, such as instant coffee, as documented by Gosalvitr et al. (2024) and Lopes et al. (2024).

Aside from coffee and coffee beans, there exist various forms and techniques of production, with numerous studies accessible in the literature. Lopes et al. (2024) evaluated instant coffee production using foam mat drying, focusing on optimal foaming conditions and analyzing kinetics and energy aspects. The production of conventional instant coffee necessitates the use of energy and water, resulting in the generation of coffee bagasse, which is a byproduct of the agricultural industry (Pazmiño et al., 2024). Gosalvitr et al. (2024) demonstrated that the sustainability ranking of various coffee beverages varies based on the specific functional unit being evaluated.

This study incorporates an energy analysis that assesses the relationship between the coffee production process and energy consumption. Numerous studies in the literature focus on the energy analysis of the coffee production process. Pazmiño et al. (2024) addressed the consequences of producing the necessary electricity employing an internal combustion engine fuelled by fossil fuels. According to Lee et al. (2024), blending spent coffee grounds and tea waste during torrefaction results in an increase in the higher heating value. Chavhan et al. (2024) analysed the kinetics and thermodynamics of coffee and tea waste pyrolysis to assess their potential as energy and carbon sources. Gosalvitr et al. (2023) conducted a comprehensive analysis assessing the environmental and economic sustainability of coffee production, identifying potential improvements in manufacturing and supply chain.

In addition, Zhang et al. (2024) established a highly efficient waste management strategy that effectively minimizes environmental contaminants while addressing pollution. Yeoh and NG (2022) evaluated the economic and environmental impact of biorefineries using spent coffee grounds, focusing on their size, location, and products. Sagita et al. (2022) analysed how the voltage gradient and process capacity impact the

heating pattern and energy consumption during temperature conditioning for coffee fermentation using ohmic heating.

Another concern pertains to the regulated management of water in the process of coffee cultivation. Water is a necessary component in the coffee production process, which is characterized by its high energy consumption. Gan et al. (2024) demonstrate that the utilization of coffee shell leads to a 3.2% enhancement in silicon production, a 6% reduction in carbon consumption, and a 2.5% decrease in energy consumption. Brommer et al. (2011) highlighted the ecological consequences of various approaches to coffee preparation.

Furthermore, the environment can be impacted by the carbon footprint resulting from coffee production. Hassard et al. (2014) analysed a study on the carbon footprint and energy consumption of six different coffee alternatives. Li et al. (2014) conducted a study on the analysis of the energy recovery potential from pyrolysis products of spent coffee grounds, which are a waste product of coffee. Pramulya et al. (2019) studied the life cycle of Gayo Arabica coffee, analysing material and energy flow, waste conversion, and biomass potential. Czekala et al. (2023) investigated the feasibility of converting waste from coffee production and consumption. Sisti et al. (2021) researched the factors that make coffee waste a valuable resource for recycling strategies aimed at achieving sustainable production of bio-based chemicals, materials, and fuels. Manrique et al. (2020) employed an analysis of the energy usage in a proposed coffee bean drying system that combines solar and biomass energy sources.

Upon a thorough study of the processes involved in coffee production, one can raise questions about the three stages of coffee production, energy consumption, and environmental factors. This study specifically examines the coffee production process and conducts an energy analysis. This study focused on the management and effectiveness of energy usage in coffee production with the aim of reducing negative impacts on the environment.

MATERIALS AND METHODS

This study conducted an energy analysis of the coffee production process by simulating a factory scenario. The quantities of products and energy consumed in standard coffee production are employed to evaluate the efficiencies of energy requirements. Prior to this, there was a detailed explanation of the coffee manufacturing process. The coffee production process encompasses several stages, including harvesting, separation, processing, and drying of the cherries, milling of the beans, cleaning, sorting, and grading, roasting, and the ultimate production of the coffee. Energy analysis can be defined as the examination of the energy usage involved in the coffee production process. The energy consumption for coffee production is influenced by factors such as the specific production processes employed, the type of coffee being produced, and the scale of production. These factors can impact the coffee production process due to variations in the structure of coffee beans.

Figure 1 depicts coffee cherries, which are harvested after five years, and their pulp and hull are extracted through wet or dry methods (Arya and Rao 2007; Mussatto et al. 2011). Robusta beans undergo dry processing, while Arabica beans undergo dry. Fermentation enhances coffee aroma (Batista et al., 2009; Esquivel & Jiménez, 2012; Mussatto, 2011).

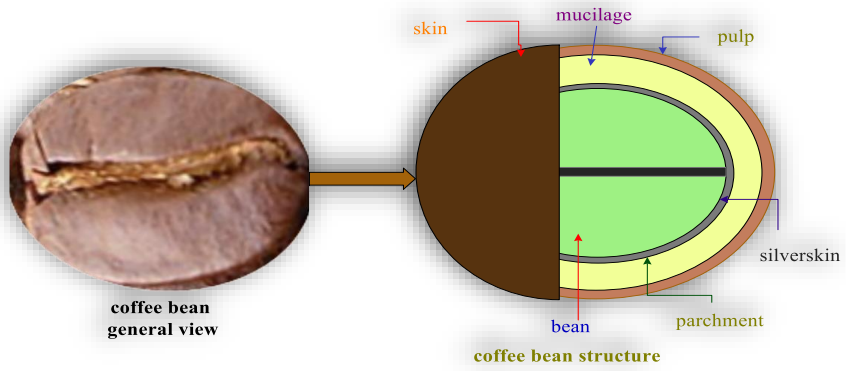

Figure 1. Coffee beans structure by drawing engineering concept draw program (Batista et al., 2009; Esquivel & Jiménez, 2012; Gosalvitr et al., 2023; Mussatto, 2011; Pandey et al., 2000; Zhang et al., 2024)

Process of the coffee production

Coffee production is very energy-intensive and involves many different stages. Before carrying out the energy analysis calculations, it is necessary to mention the processes involved in the production of coffee. The processes involved in the production of coffee are harvesting, sorting, drying of the cherries, grinding of the beans, cleaning, sorting and grading, and roasting (Figure 2).

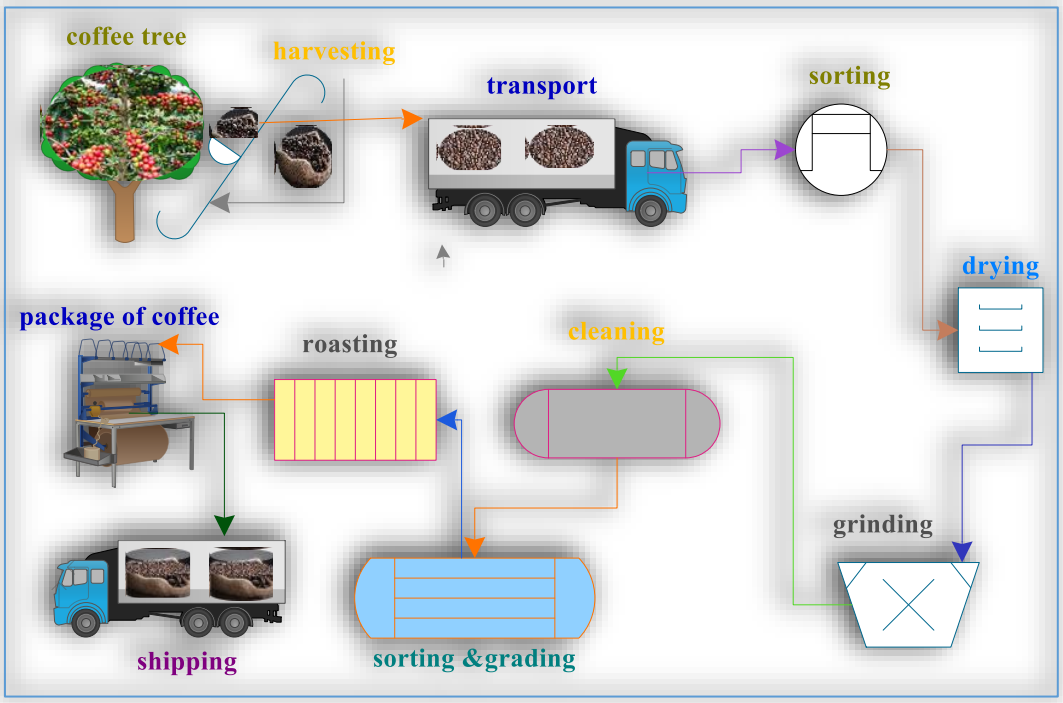

Figure 2. Process of the coffee production flow scheme by drawing engineering concept draw program

The coffee process can be delineated into six stages, as illustrated in Figure 2 as follows (Batista et al., 2009; Esquivel & Jiménez, 2012; Hassard et al., 2014; Gosalvitr et al., 2023; Mussatto, 2011; Pandey et al., 2000):

1. Harvesting process: The harvesting process in coffee production involves removing coffee fruit from trees using strip picking or selective picking methods, regardless of ripeness.
2. Sorting process: The second stage involves separating coffee cherries using wet and dry techniques, ensuring both underripe and perfectly ripe ones are separated from perfectly ripe ones.
3. Drying of the cherries process: Coffee cherries are processed and dried using three methods, which are dry, wet, and semidry. Dry-processed cherries are sun-dried, wet cherries are pulped, and semidry cherries are mechanically removed, resulting in a substantial body, earthy flavor, and moderate acidity.
4. Grinding of the beans process: Milling coffee beans involves hulling and polishing to remove fruit residue and enhance aesthetics, but temperature exposure can affect flavor.
5. Cleaning, sorting and grading process: The fifth step involves purification, categorization, and evaluation of materials, using air sorters, sieve shakers, gravity separators, color sorting, and final grading based on factors like size, altitude, and taste.
6. Roasting process: The sixth step in roasting involves using large commercial roasters to increase beans' temperature to 180°C to 230°C, causing expansion, color change, and roasted flavors.

The energy analysis of coffee production is derived from Figure 2. The coffee production process commences with the arrival of coffee beans and other necessary ingredients (raw materials) at the factory. Energy is utilized in the coffee production process. The factory consumes a substantial quantity of energy during this process.

Energy requirements of the coffee bean drying process for the production

The food industry's high water and energy consumption is a significant challenge. To conserve energy and water resources, effective control measures are needed. A hypothetical coffee factory relied heavily on food industry energy, and the heat of drying process can be evaluated using thermodynamic principles. This promotes sustainability and energy conservation. The total heat transfer of the processes ($\sum \dot{Q}$) was calculated based on the temperature changes in the process (thermal energy) from Eq. (1) to Eq. (3) using the 1st Law of Thermodynamics, as described as below (Çengel et al., 2019; Esen and Yuksel, 2013; Oztuna Taner, 2023; Oztuna Taner and Çolak, 2024):

$$\sum \dot{Q}\,[\mathrm{W}] = \sum \dot{P}\,[\mathrm{W}] + \Delta\dot{U}[\mathrm{W}] + \Delta\dot{K}E[\mathrm{W}] + \Delta\dot{P}E[\mathrm{W}] \quad (1)$$

$$\dot{Q}_{input}[\mathrm{W}] = \dot{m}_c \left[\frac{\mathrm{kg}}{\mathrm{s}}\right] \times C_p \left[\frac{\mathrm{J}}{\mathrm{kg}}\mathrm{K}\right] \times \Delta T[\mathrm{K}] \quad (2)$$

$$\sum \dot{Q}_{input}\,[\mathrm{W}] = \sum \dot{P}_{input}\,[\mathrm{W}] \quad (3)$$

Furthermore, the internal ($\Delta\dot{U}$), kinetic ($\Delta\dot{K}E$), and potential energies ($\Delta\dot{P}E$) were disregarded in the energy calculations due to their low magnitudes.

The specific energy consumption (En_{SEC}) can be calculated for the drying of the coffee from Eq. (4) as follows (Oztuna Taner, 2023; Oztuna Taner and Çolak, 2024; Lopez et al., 2024):

$$En_{SEC}\left[\frac{kJ}{kg}\right] = \frac{\dot{P}_{input}[W] \times t_{dry}[s]}{m_w[kg]} \quad (4)$$

This system exhibits a consistent and continuous output and is commonly regarded as an open system. The first law of thermodynamics is expressed by Eq. (5) as stated in the works of Çengel et al. (2019), Esen and Yuksel (2013) and Oztuna Taner (2023).

$$\sum \dot{Q} - \sum \dot{P} = \sum \dot{P}_{output} - \sum \dot{P}_{input} \quad (5)$$

The energy of the drying (E_{drying}) can be determined from water (m_w) and coffee (m_c) mass as well as latent (LH_w), water (C_w), and coffee (C_w) heat from the process. The latent heat can be assumed as 2260 [kJ/kg]. The energy of the drying can be evaluated from Eq. (6) as follows (Adams and Ghaly, 2007; Çengel et al., 2019):

$$E_{drying}[kJ] = \left\{LH_w\left[\frac{kJ}{kg}\right] \times m_w[kg]\right\} + \left\{m_c[kg] \times C_c\left[\frac{kJ}{kg}K\right] + m_w[kg] \times C_w\left[\frac{kJ}{kg}K\right]\right\} \times (T_{st}[K] - T_d[K]) \quad (6)$$

RESULTS and DISCUSSION

This study assesses the energy requirements of coffee production by assessing the energy utilized at each stage of the production process and dividing these values by the total output. To enhance the energy efficiency of coffee production, it is essential to minimize energy consumption at every stage of the production process. This can be accomplished by enhancing production processes, implementing new technologies, and utilizing energy requirements application. The coffee mass can be established for the energy analysis.

This case study can analyze four distinct instances of coffee production. The energy has been calculated based on certain assumptions for four distinct coffee production volumes. Based on the energy requirements identified in the literature, it was assumed that the energy consumption in coffee production, encompassing the final processes of drying, roasting, grinding, sorting, cleaning, and packaging, ranges from 6.73 to 9.27 [kWh/kg] for the coffee cherry process and drying with storage (Adams and Ghaly 2007; Hassard et al., 2014). The quantity of milk coffee ranges from 1000 kg to 2500 kg across the four scenarios. Green coffee beans intended for roasting may desiccate, yet moisture remains in the core. The initial moisture content of a harvested bean is approximately 50%, which diminishes to around 10% during processing and drying (Pramulya et al., 2019). The moisture content of the coffee bean is estimated to be approximately 80-85% (Mussatto et al., 2011). The subsequent assumptions are established to compute the masses of desiccated green coffee. The energy necessary to dehydrate 1 kilogram of coffee is 2700 kilojoules. The heat source (wood) necessary for drying the coffee is 1.0-1.5 kg of firewood with a density of 500 kg/m³, and the average heat

value is approximately 1.06 GJ. Consequently, 1 kg of coffee parchment combusts with a calorific value of 1450 [kJ/kg] (Adams and Ghaly 2007; Hassard et al., 2014).

Table 1 illustrates the thermal energy drying process for coffee across four scenarios. The heat energy required for the coffee drying process across four scenarios is as follows: SCF1 is 1.35E+06 [kJ], SCF2 is 2.03E+06 [kJ], SCF3 is 2.70E+06 [kJ], and SCF4 is 3.38E+06 [kJ].

Table 1. Drying of the heat energy for the coffee process for the 4 scenarios

Scenario of coffee	harvesting of the bean [kg]	input of the coffee bean (cherry raw) [kg]	output of the coffee bean (roasted) [kg]	drying of the heat energy [kJ]
SCF1	1000	500	450	1.35E+06
SCF2	1500	750	675	2.03E+06
SCF3	2000	1000	900	2.70E+06
SCF4	2500	1250	1125	3.38E+06

The energy requirement of the production process can be computed by the coffee production amount. Therefore, these processes, which are obtained for the energy analysis of the coffee production, are harvesting, sorting, and shelling of coffee beans; drying; cherry bean processing; roasting process. According to the scenario of coffee, there are 4 scenarios with 2 scenarios of the energy requirements.

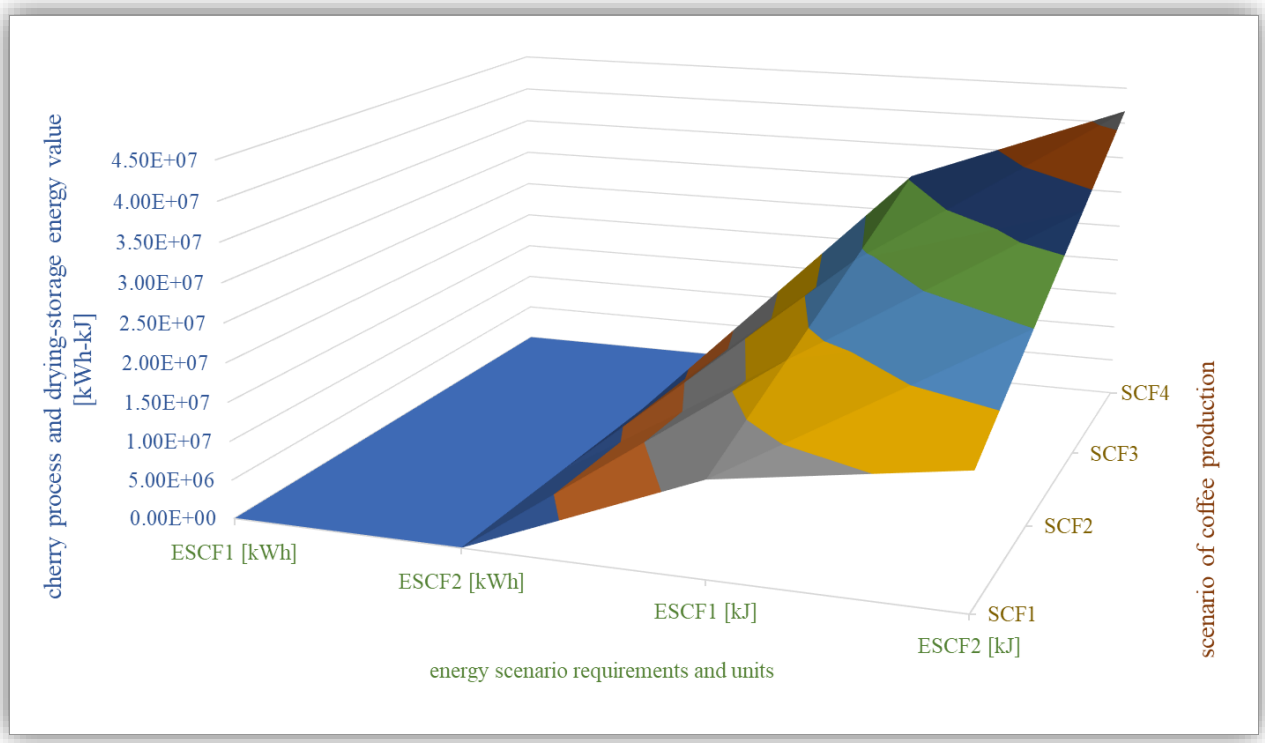

Figure 3. Scheme of the coffee cherry process and drying-storage energy requirement

Figure 3 indicates the coffee cherry process and drying-storage energy requirement of the scenario coffee (ESCF). According to the scenario of coffee, there are 4 scenarios with 2 scenarios of the energy requirements. The ESCFs were calculated from SCF1 to SCF4. According to SCFs application, these ESCF was computed from 3.37E+03 [kWh] to 8.41E+03 [kWh] for ESCF1 and from 4.64E+03 [kWh] to 1.16E+04 [kWh] for

ESCF2, as well as from 1.21E+07 [kJ] to 3.03E+07 [kJ] for ESCF1 and from 1.67E+07 [kJ] to 4.17E+07 [kJ] for ESCF2. The amount of heat that was transferred to dryers ranged from 1020 [MJ] to 1481 [MJ] for dry green coffee, according to Adams and Ghaly (2007). For the coffee cherry process and drying with storage, the energy requirements ranges from 6.73 to 9.27 (kWh/kg] (Adams and Ghaly 2007; Hassard et al., 2014).

Within the context of this scenario study, the SCF and ESCF of coffee production are utilized for the purpose of establishing the energy requirements that can be assumed to be available based on the literature.

CONCLUSION

By enhancing the processes that are employed, it is possible to reduce the amount of energy it takes to produce coffee. This can be accomplished through the implementation of various measures, such as enhancing the efficiency of the production process, minimizing waste, and refraining from production losses. Reducing the amount of energy that is used in the production of coffee can be accomplished with the help of new technologies. The implementation of automation systems, the utilization of intelligent production systems, and the utilization of energy-efficient machinery and equipment are all examples of measures that can be taken to accomplish this goal.

There is potential for a reduction in the amount of energy that is consumed during the production of coffee through the utilization of energy demand calculations. This is something that can be accomplished through the implementation of various measures, such as the utilization of heat recovery systems, the enhancement of lighting systems, and the setup of process control systems. The utilization of energy accounting in the production of coffee contributes to the reduction of production costs, the reduction of environmental impact, and the guarantee of production that is economically sustainable.

Utilizing energy in the production of coffee is a challenging endeavor; however, it has the potential to offer valuable insights into ways to enhance the industry's efficiency and commitment to sustainability. By implementing modifications to the manner in which coffee is transported, processed, and packaged, the industry has the potential to lessen its impact on the environment and increase the efficiency with which it produces coffee.

ACKNOWLEDGEMENTS

This study, which was presented at the 7th International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2024), is original research, constructed with the scenario study.

Conflict of Interest declaration: The author declares that she has no ties to or involvement with any organization or entity that has a financial enthusiasm for learning or materials discussed in this manuscript.

Author Contributions: OOT managed to design and carry out the research, as well as analyze the findings and write the subsequent manuscript. ***OOT*** created the original and oversaw the project.

NOMENCLATURE

Abbreviations

c	coffee
ESCF	energy requirement of the scenario coffee
SCF	scenario of coffee
w	water

Symbols and Units

$\dot{E}n_{input}$	energy input [W]
$\dot{E}n_{output}$	energy output [W]
$\dot{P}_{input}$	power input, [W]

$\sum \dot{Q}$	total heat transfer of the processes [W]
$\Delta \dot{K}E$	kinetic energy [W]
$\Delta \dot{P}E$	potential energy [W]
$\Delta \dot{U}$	internal energy [W]
C_c	coffee specific heat, $1600 \left[\frac{kJ}{kg}K\right]$
C_p	specific heat of the process $\left[\frac{\mathrm{J}}{\mathrm{kg}}\mathrm{K}\right]$
C_w	water specific heat, $4200 \left[\frac{kJ}{kg}K\right]$
E_{drying}	energy of the drying $[kJ]$
En_{SEC}	The specific energy consumption $\left[\frac{kJ}{kg}\right]$
LH_w	latent heat 2260 $\left[\frac{kJ}{kg}\right]$
$T_d[K]$	dead state of the temperature 293 [K]
$T_{st}[K]$	steam temperature, 373 [K]
m_c	coffee mass [kg]
m_w	water mass [kg]
t_{dry}	drying time, $[s]$
ΔT	difference of the temperature [K]

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ORAL PRESENTATION

SLC6A3 rs1809939 polimorfizmi ile eroin ve metamfetaminin birlikte kullanımı arasındaki ilişki

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Özet

Eroin ve metamfetamin birlikte kullanımı son yıllarda sürekli artmaktadır. Bu durumun temel motivasyonu, opioid yoksunluk semptomlarının metamfetamin ile azaltılmasıdır; çünkü metamfetamin opioidlere göre daha ucuzdur. Metamfetaminin opioid yoksunluğunu hafifletme etkisinin, beynin dopamin ödül sistemleri üzerindeki çift etkisiyle ilgili olduğu düşünülmektedir. Bu nedenle, bu çalışmada *SLC6A3* geninde bulunan rs1809939 polimorfizminin eroin ve metamfetamin birlikte kullanımındaki etkisinin belirlenmesi amaçlanmıştır. Bu çalışmaya toplam 99 eroin ve metamfetamin birlikte kullanan birey ile 94 sağlıklı birey dahil edilmiştir. *SLC6A3* rs1809939 genotipleme, polimeraz zincir reaksiyonu-restriksiyon parça uzunluğu polimorfizmi (PCR-RFLP) yöntemiyle gerçekleştirilmiştir. Kontrol grubunda AA, AG ve GG genotiplerinin frekansları sırasıyla %53,2, %43,6 ve %3,2 iken; eroin ve metamfetamin birlikte kullanan grupta bu frekanslar sırasıyla %48,5, %39,4 ve %12,1 olarak belirlenmiştir. Varyant alel (G) frekansı kontrollerde %25, opioid ve metamfetamin birlikte kullanıcılarında ise %32 olarak belirlenmiştir. Ancak, lojistik regresyon analizleri, *SLC6A3* rs1809939'un eroin ve metamfetamin birlikte kullanımı ile anlamlı bir ilişkiye sahip olmadığını ($p>0,05$) göstermiştir. Sonuç olarak, *SLC6A3* rs1809939'un Türk popülasyonunda eroin ve metamfetamin birlikte kullanımı ile ilişkili olmadığı gösterilmiştir.

Anahtar Kelimeler: Metamfetamin, eroin, *SLC6A3*, polimorfizm, PCR-RFLP, rs1809939

Association between *SLC6A3* rs1809939 polymorphism and the co-use of heroin and methamphetamine

Abstract

Heroin and methamphetamine co-use is constantly increasing in recent years. The primary motivation is that opioid withdrawal symptoms are reduced by methamphetamine, which is cheaper than opioids. Methamphetamine is thought to alleviate opioid withdrawal, possibly due to its dual effects on dopamine reward systems in the brain. Thus, this study aimed to determine the effect of rs1809939 polymorphism of *SLC6A3* gene on heroin and methamphetamine co-use. A total of 99 heroin and methamphetamine co-users and 94 healthy subjects were included in the study. *SLC6A3* rs1809939 were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The frequencies of AA, AG and GG genotypes were 53.2%, 43.6% and 3.2%, respectively, in controls, whereas they were 48,5%, 39.4% and 12.1%, respectively, in heroin and methamphetamine co-users. The frequency of variant allele (G) was lower in controls (25%) than in opioid and methamphetamine co-users (32%). However, logistic regression analyses showed that *SLC6A3* rs1809939 was not significantly associated with opioid and methamphetamine co-use ($p>0.05$). In conclusion, *SLC6A3* rs1809939 seemed not be associated with the co-use of heroin and methamphetamine in Turkish population.

Keywords: Methamphetamine, heroin, *SLC6A3*, polymorphism, PCR-RFLP, rs1809939

GİRİŞ

Madde kullanım bozukluğu, bireylerin yaşam kalitesini önemli ölçüde etkileyen genetik, çevresel ve psikolojik faktörlerin etkileşimiyle şekillenen bir halk sağlığı problemi olarak tanımlanmaktadır (Şahiner ve ark., 2023). Son yıllarda yapılan araştırmalarda, opioid kullanım bozukluğu olan bireylerde eroin ve metamfetamin birlikte kullanımı (EMBK) dikkat çekmektedir (Glick ve ark., 2021). Bu birlikte kullanımın ani artışının nedenleri henüz tam olarak bilinmese de olasılıklar arasında metamfetaminin erişilebilir olması ve bu maddelerin ortak kullanımında yarattığı etkiler örnek gösterilmektedir. Ellis ve ark. (2018) yaptığı çalışmaya göre, bir tedavi programına kayıtlı olan opioid kullanan bireylerde, metamfetamin ile birlikte kullanım oranlarınını 2011-2017 yılları arasında %18,8'den %34,2'ye yükseldiği bildirilmiştir. Bu programdaki katılımcıların %65'inde yaşam boyu metamfetamin kullanımı öyküsü var iken, %76,4'ü opioid kullandıkları sırada metamfetamin kullanımı olduğunu belirtmiştir. EMBK nedenleri incelendiğinde bazı bireyler bu kombinasyonun sinerjik yükseklikten dolayı olduğunu belirtmiş, diğerleri ise eroinin yarattığı depresif etkinin metamfetamin tarafından dengelendiğini ve metamfetaminin sağladığı uyarılmadan sonra ise eroin etkisiyle gevşemeye dönüştüğünü ifade etmiştir. Bunun yanı sıra, bazı bireyler, opioidlere erişim sağlayamadıklarında metamfetamini yoksunluk belirtilerini hafifletmek için kullandığını bildirmişlerdir (Ellis ve ark., 2018). Bu kullanım biçimi yoksunluk belirtilerinin yönetiminde metamfetaminin önemli bir rol oynadığını göstermektedir.

İnsan ve hayvanlar üzerinde yapılan bazı çalışmalara göre, opioidlerin aşırı tüketiminin dopamin salınımını azalttığı bulunmuştur. Birey dopamin salınımının azalmasıyla birlikte hem madde hem de madde dışı ödüllerle uyarılmaya çok daha az duyarlı hale gelmektedir. Başlangıçta kullandığı maddenin etkilerindeki öfori hissini artık yaşayamamakta ve yoksunluk belirtileri kendini göstermektedir (Volkow ve ark., 2016). Metamfetaminin kısa vadeli etkilerinden biri dopamin taşıyıcı proteinlerini (DAT) doğrudan etkileyerek, dopaminin sinaptik boşlukta birikmesine neden olmasıdır. Normal koşullarda, DAT dopaminin sinaptik boşluktan geri alımını sağlamaktadır. Ancak metamfetamin bu işlevi bloke ederek dopamin salınımı artmakta ve etkileri uzamaktadır (Greenwood ve ark., 2013; Riddle ve ark., 2006).

Opioid yoksunluğu sırasında, dopamin sistemi genellikle işlevini kaybetmekte veya bozulmaktadır. Metamfetaminin dopamin sistemini uyarması ile oluşan bu değişiklikler sonucu beyindeki ödül ve motivasyon sistemleri aktive edebilmektedir. Bu aktivasyon sonucunda opioid yoksunluk belirtilerini hafiflettiği düşünülmektedir. Bu nedenle dopamin taşıyıcı proteini kodlayan *SLC6A3* (*DAT 1*) geni dikkat çekmektedir (Li ve ark., 2006).

Bağımlılıkla ilgili genetik yatkınlığın doğasını anlamaya yönelik yapılan araştırmalarda, dopamin sistemindeki ilişkili genler önem arz etmektedir. *SLC6A3* genindeki varyasyonlar, dopamin düzeylerini ve dopamin sisteminin işleyişini etkileyebilmektedir (Van Der Zwaluw ve ark., 2009). Özellikle bu gendeki belirli polimorfizmler, alkol kullanım bozukluğu, bipolar bozukluk, majör depresif bozukluk, şizofreni, dikkat eksikliği ve hiperaktivite bozukluğu ve parkinson hastalığı gibi birçok durum ile ilişkilendirilmiştir (Reith ve ark., 2022). *SLC6A3* rs1809939 polimorfizminde bipolar bozukluk ile ilgili yapılan çalışma bulunmaktadır. Ancak bu polimorfizm ile EMBK arasındaki ilişkiyi inceleyen bir çalışmaya rastlanmamıştır. Dolayısıyla bu çalışmanın amacı *SLC6A3* rs1809939 polimorfizmin Türk popülasyonunda eroin ve metamfetamin kullanımı ile ilişkisini araştırmaktır.

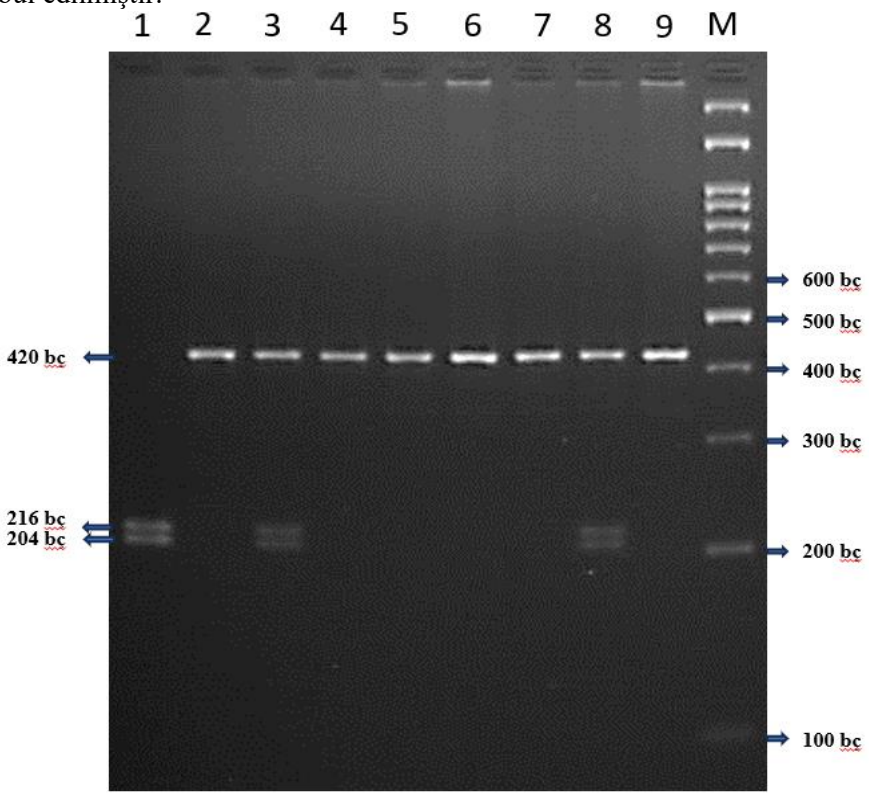
MATERYAL VE METOT

Bu çalışma Ankara Eğitim ve Araştırma Hastanesi, Alkol ve Madde Bağımlılığı Tedavi Merkezi'ne başvuru yapan DSM-V kriterlerine göre eroin kullanım bozukluğu tanısı ile birlikte metamfetamin kullanan 99 birey dahil edilmiştir. Eroin ve metamfetamin dışında kalan diğer yasa dışı bağımlılık yapan maddeleri kullanan kişiler çalışmaya dahil edilmemiştir. Kontrol grubu, daha önce veya günümüzde herhangi bağımlılık yapıcı madde kullanmamış 94 sağlıklı bireyden oluşmaktadır. Kontrol grubu ile, EMBK sahip hasta grubu cinsiyet ve sigara içme alışkanlığı açısından eşleştirilmiştir. Madde kullanım bozukluğu olan bireylerin dahil edilme kriterleri şu şekildedir: (i) 18-65 yaş aralığında olmak, (ii) sigara içiyor olmak, (iii) okur-yazar olmak. Dahil edilmeme kriterlerinde ise (i) ek bir psikiyatrik hastalık tanısı almış olmak, (ii) okuma yazma bilmemek bulunmaktadır.

Çalışmaya katılan her birey çalışma hakkında bilgilendirilerek onam formu alınmıştır. Örnekleme Helsinki Deklarasyonu ilkelerine uygun olarak yapılmıştır ve kurumsal etik komitesinden onay alınmıştır. Katılım sağlayan her bir bireye ait sosyo-demografik bilgiler (yaş, medeni durum, eğitim durumu, çalışma durumu, askerlik, cezaevi öyküsü, devam eden denetimli serbestlik dosyası), tıbbi öykü (kilo, boy, tıbbi hastalık öyküsü, ek psikiyatrik öyküsü, kullanmakta olduğu ilaç, sigara kullanımı) ve madde kullanımı ile ilgili (kullanım süresi, günlük/haftalık alınan miktar, kullanım şekli, ilk maddeyle tanışma yaşı, ailede madde kullanım öyküsü, maddesiz geçirebildiği en uzun süre) toplanmıştır.

EMBK bireylerden birer tüp (EDTA'lı) venöz kan örneği alınmıştır. Kan örnekleri, DNA izolasyonu aşamasından önce -20°C'de muhafaza edilmiştir. Genomik DNA, kan örneklerinden üretici firma tarafından önerilen yöntemle GeneJET Genomik DNA Saflaştırma Kiti (Thermo Fisher Scientific) kullanılarak izole edilmiştir. *SLC6A3* rs1809939 polimorfizminin genotiplemesi, Polimeraz Zincir Reaksiyonu (PCR) ve Restriksiyon Parça Uzunluk Polimorfizmi (RFLP) yöntemleriyle yapılmıştır. İleri (F) ve geri (R) primer dizileri sırasıyla 5'-AGTGTAATGCTCGGTGGGAG-3' ve 5'-TGAGCTGGGCCTGTGTAAAT-3'şeklindedir. PCR amplifikasyonu için karışım 25 µl olarak hazırlanmıştır: 10x PCR tamponu, 10 pmol/µl ileri ve 10 pmol/µl geri primerler, 1 U/µl Taq DNA polimeraz, 2 Mm DNTP ve 2 µl genomik DNA. PCR aşamaları ilk denatürasyonda 94°C'de 5 dakika, denatürasyonda 94°C'de 1 dakika, bağlanma 60°C'de 1 dakika, uzama 72°C'de 1 dakika ve son uzama 72°C'de 10 dakika şeklindedir. Çoğaltılmış 420 bç PCR ürünleri, 37°C'de 1 saat boyunca *MspI* enzimi ile kesilmiştir. Kesilen ve kesilmeyen PCR ürünleri, %3'lük agaroz jel elektroforezinde ayrılmış, UV ışık altında Etidyum Bromür (EtBr) ile görselleştirilmiş ve Syngene Jel Görüntüleme Sistemi aracılığıyla analiz edilip fotoğraflanmıştır (Şekil 1).

Çalışma sonuçlarının istatiksel analizleri SPSS programı kullanılarak gerçekleştirilmiştir. Öncelikle, verilerin normal dağılıp dağılmadığını belirlemek için Kolmogorov-Smirnov testi kullanılmıştır. Nicel verilerde, normal dağılım görülmediği için Ortanca (x̃) ve çeyrekler arası aralık (ÇAA, IQR: Interquartile Range) değerleri sunulmuştur. Normal dağılmayan örnekler için ikili grup karşılaştırılırken Mann-Whitney U testi (parametrik olmayan) uygulanmıştır. Nitel verilerde grup değişkenler için, ki kare testi (x^2) kullanılmıştır. Genotip ve alel frekanslarının Hardy-Weinberg dengesi hesaplanmıştır. *SLC6A3* rs1809939 ile eroin ve metamfetamin arasındaki ilişki ikili lojistik regresyon analizi ile modellenmiştir. $p<0.05$ istatistiksel olarak anlamlı olarak kabul edilmiştir.



Şekil 1. *SLC6A3* rs1809939 polimorfizmi için *MspI* enzimi ile kesilmiş agaroz jel görüntüsü. M: 100 bç ladder, 1.sütun (216 bç ve 204 bç) GG genotip, 2,4,5,6,7,9 sütunları (420 bç) AA genotip ve 3,8 sütunları (420 bç, 216 bç ve 204 bç) AG genotip.

BULGULAR

Çalışmada yer alan 99 EMBK'ına sahip ve 94 sağlıklı bireyin sosyo-demografik özellikler Tablo-1'de gösterilmiştir. Çalışmada yer alan EMBK bireyler ve kontrol grubunun ortanca yaşları sırasıyla 30.0 yıl (ÇAA: 26.0-34.00) ve 36.0 yıl (ÇAA: 27.00-42.00) olarak belirlenmiştir. Ortanca ağırlıkları EMBK bireylerde 65.0 kg (ÇAA: 60.0-72.0), sağlıklı bireylerde ise 82.0 kg (ÇAA: 75.0-92.0) iken, boy ortancaları sırasıyla 173.0 (ÇAA: 168.0-180.0) ve 178.0 (ÇAA: 173.0-180.0) olarak görülmüştür. Verilere göre, EMBK ve sağlıklı bireyler arasında yaş ve kilo açısından anlamlı bir fark var iken (p=0.001, p=0.001), boy açısından (p=0.007) anlamlı bir fark bulunmamıştır.

Tablo-1'de iki grup arasındaki ilişki değerlendirildiğinde, eğitim (p=0.001), çalışma durumu (p=0.001) ve medeni durum (p=0.001) arasında istatistiksel olarak anlamlı farklılıklar bulunmuştur. EMBK bireyler %14.1 oranında ilkokul, %44.4 oranın ortaokul, %36.4 oranında lise ve %5.1 oranında lisans mezunuyken, kontrol grubunda ilkokul mezunlarının oranı %9.6, ortaokul mezunlarının oranı %17.0, lise mezunlarının oranı %53.2, lisans mezunlarının oranı %16.0 ve yüksek lisans mezunlarının oranı ise %1.1 olarak belirlenmiştir. Çalışma durumu verilerinde, EMBK grubunun %48.5'i çalışıyor ve %51.5'i çalışmıyor iken, sağlıklı bireylerinde %87.2'si çalışıyor ve %9.6'sı çalışmıyor olarak bulunmuştur. Medeni durum açısından EMBK bireylerin %65.7'si bekar, %25.3'ü evli ve %9.1'i boşanmış/duldur. Kontrol grubunda ise %36.2'si bekar, %59.6'sı evli ve %1.1'i boşanmış/dul tespit edilmiştir.

Tablo-2'de *SLC6A3* rs1809939 polimorfizmi için EMBK ve sağlıklı bireylerdeki alel ve genotip frekansları verilmiştir. EMBK bireylerin A ve G allel frekansları sırasıyla %68 ve %32 iken, kontrol grubunda %75 ve %25 olarak bulunmuştur. Hem EMBK hem de kontrol grubunun genotip frekansları Hardy-Weinberg dengesinde olduğu görülmüştür (p>0.05).

TABLO-1 Eroin-metamfetamin kullanıcıları ve kontrol grubu sosyo-demografik özellikleri

Parametre		**EMBK (n=99)**		**Kontrol (n=94)**		**p-değeri**
Yaş (yıl)	x̃	30.0		36.0		0.001
	(ÇAA)	(26.00-34.00)		(27.00-42.00)		
Kilo (kg)	x̃	65.0		82.0		0.001
	(ÇAA)	(60.0-72.0)		(75.0-92.0)		
Boy (cm)	x̃	173.0		178.0		0.007
	(ÇAA)	(168.0-180.0)		(173.00-180.0)		
Eğitim		n	% frekans	n	%frekans	0.001
İlkokul		14	14.1	9	9.6	
Ortaokul		44	44.4	16	17.0	

Lise	36	36.4	50	53.2	
Lisans	5	5.1	15	16.0	
Lisans Üstü	-	-	1	1.1	
Çalışma Durumu	n	% frekans	n	% frekans	
Çalışıyor	48	48.5	82	87.2	0.001
Çalışmıyor	51	51.5	9	9.6	
Medeni Durum	n	% frekans	n	% frekans	
Bekar	65	65.7	34	36.2	
Evli	25	25.3	56	59.6	0.001
Boşanmış/Dul	9	9.1	1	1.1	

TABLO-2 Eroin-metamfetamin kullanıcıları ve kontrol grubunun genotipleri ve frekansları

***SLC6A3* rs1809939 Genotip**	**EMBK (n=99)**		**Kontrol (n=94)**		**p-değeri**
	n	**%**	**n**	**%**	
AA	48	48.5	50	53.2	p>0.05
AG	39	39.4	41	43.6	
GG	12	12.1	3	3.2	
Varyant alel frekansı	32%		25%		
HWE p-değer	$\chi2$=0.84; p=0.36		$\chi2$=2.5; p=0.11		

TARTIŞMA

Hem psikiyatrik hem de madde kullanım bozuklukları için genetik yatkınlığın belirlenmesi ile ilgili girişimlerde, dopaminerjik sistemde görev alan proteinleri kodalayan genler dikkat çekmektedir. Dolayısıyla, dopamin nörotransmisyonunda bulunan proteinleri kodlayan genlerin fonksiyonu veya düzenlenmesindeki genetik varyasyonlar ile, madde kullanım bozukluğuna verilen farklı yanıtların açıklanabileceği düşünülmektedir. Dopaminin düzenlenmesinde merkezi rol oynayan yapı dopamin taşıyıcılarıdır (DAT). DAT, Na^+/Cl^- bağımlı transmembran taşıma proteinleri ailesine ait bir proteindir ve dopaminin sinaptik boşluktan presinaptik nöronlara geri alınımını sağlayarak dopamin seviyelerini düzenlemektedir (Van Der Zwaluw ve ark., 2009). Bu nedenle, DAT seviyelerindeki farklılıklar, dopaminerjik ödül mekanizmasının da farklı çalışmasına sebep olabilmektedir (Giros ve ark., 1992). Bu da dopamin taşıyıcıyı kodlayan *SLC6A3* geninin madde kullanım bozukluğu üzerine etkilerini araştırmak için önemli bir aday haline getirmektedir (Van Der Zwaluw ve ark., 2009). Bildiğimiz kadarıyla bu çalışma, *SLC6A3* rs1809939 gen polimorfizminin EMBK ile ilişkisini Türk popülasyonunda inceleyen ilk araştırmadır.

SLC6A3 geni insan genomunun 5. kromozomunda bulunur ve 15 ekzondan oluşmaktadır (5p15.3), (Hamidovic ve ark., 2010). *SLC6A3* geninde birçok polimorfizm tanımlanmıştır ve rs1809939 bunlardan sadece biridir. Rs1809939 polimorfizmi, *SLC6A3* geninin 3'çevrilmemiş bölgesinde yer almaktadır (3'UTR) ve 3 'UTR'deki

yaygın olarak çalışılmış olan 40 bç'lik değişken sayıda tekrar eden (VNTR) 10 tekrarlı aleli ile güçlü bağlantı dengesizliği içinde olduğu bulunmuştur (Greenwood ve ark., 2013). *SLC6A3* gen varyantlarının çeşitli hastalıklarla ilişkili olup olmadığını gösteren çalışmalar bulunmaktadır. Greenwood ve ark. (2013) dopamin taşıyıcı gen varyantlarının bipolar bozuklukta, çocukluk çağı Dikkat eksikliği ve hiperaktivite bozukluğu (DEHB) özellikleri ile ilişkisini araştırdıklarında rs1809939 polimorfizminin aile örnekleminde ruh hali istikrarsızlığı için anlamlılık eğilimi göstermiştir (Greenwood ve ark., 2013). Hamidovic ve ark. (2010), *SLC6A3* gen varyantları üzerinde d-amfetaminin uyarıcı etkileri olup olmadığını araştırmışlardır. 152 hastanın kullanıldığı örneklemde, dört polimorfizm arasındaki ilişki değerlendirilmiştir (rs460000, rs3756450, rs37022 ve rs6869645). Rs460000 polimorfizminde C/C genotipine sahip bireyler A/A+A/C genotip grubuna göre ortalama iki kat daha yüksek uyarılma ve öfori hissi bildirmiştir (Hamidovic et al., 2010). Alkol kullanım bozukluğu üzerine yapılan birçok çalışmada dikkat çeken polimorfizm 40 bç'lik VNTR idi (Van Der Zwaluw ve ark., 2009). Le Strat ve ark. (2008) yaptığı çalışmada, *SLC6A3* VNTR 9 tekrarlı alelinin, alkol yoksunluk semptomları ile ilişki olduğunu bulmuştur. Stolf ve ark. (2014) tarafından yapılan araştırmada, kokain kullanan ve sağlıklı bireyler arasında, *SLC6A3* 3'UTR VNTR alel ve genotip frekansları karşılaştırılmış ve 3'UTR VNTR 10.10 genotipi iki grup arasındaki varyasyonda anlamlı bulunmuştur. Türk popülasyonunda *SLC6A3* rs1809939 polimorfizmi ile EMBK ilişkisini araştıran çalışmamızda, bu polimorfizm ile bağımlılık grubu arasında bir ilişki belirlenememiştir.

Yapılan çalışmalar, hem eroin hem de metamfetamin kullanan bireylerin tıbbi öykü ve sosyo-demografik özelliklerinde bazı risk faktörlerinden etkilendiğini göstermektedir. Madde kullanımının erken yaşlarda başlayıp devam ettiği ve kilo karşılaştırmasında kontrol grubuna kıyasla daha zayıf oldukları dikkat çekmektedir. Eğitim durumu, ortaokul ve lise düzeyinde daha yaygın olması madde bağımlılığı ile yakından ilişkisi olduğunu göstermektedir. Madde kullanımıyla ilgili medeni duruma bakılacak olursa, aile kurma zorluğu, bekar ya da aile üyeleriyle birlikte yaşama veya boşanmış olma durumu gözlemlenmiştir. Madde kullanım bozukluğunun iş yaşamı ile de ilişkili olduğu düşünülmektedir. Bağımlılığın getirdiği fiziksel ve zihinsel sağlık sorunları, sosyal ilişkilerin bozulması ve toplumdan izole olma, düşük iş motivasyonu ve iş performansından ötürü madde bağımlılığında işsizlik oranı daha yüksek olduğu söylenebilir. Dolayısıyla madde kullanım bozukluğu, bireylerin yaşam koşullarını olumsuz yönde etkileyebilecek bazı risk faktörleriyle ilişkilidir.

Ayrıca *SLC6A3* rs1809939 polimorfizmin genotip frekanslarının iki grup arasındaki analizlerinde, kontrol grubunun GG genotip frekansı %3.2 gözlemlenirken, EMBK bireylerde bu genotip %12.1 olarak analiz edilmiştir. Varyant alel G frekansı EMBK ve sağlıklı bireylerde sırasıyla %32 ve %25 olarak belirlenmiştir. Ancak lojistik regresyon analizleri, *SLC6A3* rs1809939'un EMBK ile anlamlı bir ilişkiye sahip olmadığını ($p>0,05$) göstermiştir.

EMBK ile *SLC6A3* rs1809939 polimorfizmi arasında anlamlı bir ilişki bulunamamış olması, bu madde kullanım türünün poligenik yapısına işaret etmekte ve bağımlılık davranışlarının birçok genetik faktörün ve çevresel etkileşimlerin bir sonucu olarak ortaya çıktığını düşündürmektedir.

SONUÇ

Sonuç olarak, bu çalışma, Türk popülasyonunda *SLC6A3* rs1809939 polimorfizminin EMBK ile ilişkisini araştıran ilk çalışmadır. *SLC6A3* rs1809939'un EMBK üzerinde anlamlı bir etkisi olmamıştır. Bu bulgular ışığında, gelecekteki araştırmaların daha geniş ve çeşitli örneklem grupları kullanılarak, farklı genetik varyantlar ve çeşitli risk faktörlerinin etkileşimlerini incelenmesi önerilmektedir.

TEŞEKKÜR

Bu çalışma, Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) tarafından 222S721 numaralı proje ile desteklenmiştir. Projeye verdiği destekten ötürü TÜBİTAK'a teşekkürlerimizi sunarız.

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ORAL PRESENTATION

Antioxidant activitiy of purple sweet potato anthocyanins and their anti-EMT, antimigratory effects on human bladder carcinoma 5637 cells

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Abstract

The epithelial-mesenchymal transition (EMT) is a potent process in the progression of cancer. Anthocyanins have prominent inhibitor activity on the EMT. Purple sweet potato anthocyanins (PSPAs) exhibit therapeutic activities in various cancers. However, their effects on the EMT of bladder cancer (BlCa) have not yet been completely revealed. In this work, we aimed to evaluate the antioxidant capacity of PSPAs and their effects on proliferation, migration and EMT of BlCa 5637 cells. Total anthocyanins of purple sweet potato grown in Hatay-Turkey were purified by chromatographic methods. Antioxidant features of PSPAs determined by DPPH and ABTS assays. The effects of PSPAs on cell proliferation were examined by WST-1 assay. Scratch-wound healing assay was implemented to investigate the efficacy of PSPAs on the migration. q-PCR was performed to evaluate the EMT inhibitory effects of PSPAs. For this purpose, the expressions of Snail, Vimentin and E-Cadherin were defined in 5637 cells. PSPAs exhibited strong DPPH (IC_{50}=5.06 µg/mL) and ABTS (IC_{50}=8.85±0.65 µg/mL) activities. Furthermore, the proliferation and migration of 5637 cells were remarkably inhibited by PSPAs. Additionally, PSPAs upregulated the E-Cadherin and downregulated Snail and Vimentin. In conclusion, our study revealed that PSPAs were effective on EMT, proliferation and migration inhibition of 5637 cells.

Keywords: Anthocyanin, Bladder Cancer, Epithelial-Mesenchymal Transition.

INTRODUCTION

Bladder cancer (BlCa) is a prevalent cancer in the urological system (Hwang et al., 2022; , Ma et al., 2021). The most common pathological subtype of BlCa is bladder urothelial carcinoma (BUC) (Moussa and Ali 2019; Yang et al. ,2021a). BUC, constituting nearly 90% of all BlCa cases, is the 10th most commonly diagnosed cancer type globally. In 2020, it was reported to cause 213,000 deaths and 573,000 new cases (Li et al., 2021; Ding et al., 2021). BUC is classified as non-muscle (NMIBC) and muscle-invasive bladder cancer (MIBC) (Yang et al., 2021a; Esmail et al., 2020). In MIBC, which is an advanced stage of BUC and accounts for 25% of all patients, the carcinoma invades the detrusor muscle in the bladder wall. MIBC tumors, which tend to grow and metastasize rapidly, are characterized by poor prognosis and low survival rates (Li et al., 2021, He et al., 2021). Consequently, there is a great need to develop new preventive, therapeutic strategies and more effective agents for BlCa (Luo et al, 2018; Zeng et al., 2020; Li et al., 2022; Zhang et al., 2021).

One of the molecular mechanisms leading to MIBC progression is epithelial to mesenchymal cell transition (EMT) (Li et al., 2021). Many studies have shown that EMT is potently related with aggressive BlCa behaviors such as recurrence, invasion, and metastasis (Yun and Kim, 2023; Cao et al., 2020). This situation raises the possibility of EMT as a rational therapeutic target in the therapy of BlCa (Kitagawa et el., 2021). The first and foremost regulator of EMT is E-Cadherin (Yun and Kim, 2023). E-Cadherin is a cell surface protein that is normally expressed in epithelial cells and mediates the cell-cell adhesion, thus protecting the textural and

physiological unity of epithelial tissues (Esmail et al., 2020). Various studies have proven upregulation of mesenchymal markers like P-Cadherin and N-Cadherin and downregulation of epithelial markers like E-Cadherin in MIBC tumors (Li et al., 2021). One of the mechanisms involved in the downregulation of E-Cadherin is the transcription factors that induce EMT, which consists of 3 families called Snail, Zinc Finger E-box Binding Homeobox (ZEB), Twist-Related Protein (TWIST). These transcription factors arrange E-Cadherin expression primarily by suppressing its promoter. However, it also positively regulates the expression of genes related with mesenchymal phenotypes such as Matrix Metalloproteinases (MMPs), α-Smooth Muscle Actin (α-SMA), N-Cadherin, Fibronectin, Vimentin. The transition to the mesenchymal phenotype confers migratory and invasive abilities on tumor cells, allowing them to spread to other regions (Vaquero et al., 2017). Therefore, the discovery of the molecular processes that induce EMT is crucial to prevent invasion and metastasis in cancer and to develop new treatment strategies (Yang et al, 2021b).

Many drugs and molecules with varying outcomes have been tried to control EMT. However, it remains a difficult therapeutic target for today's medicine (Avila-Carasco et al., 2019). It has been shown in many studies that natural plant compounds (NPCs) isolated from plants can arrange EMT via anti-fibrotic, anti-inflammatory or antioxidant mechanisms (Salehi et al., 2019; Liu et al., 2021). One group of NPCs reported to have inhibitory bioactivity on pathological EMT is anthocyanins, which are polyphenols commonly found in fruits and vegetables (de Arruda Nascimento et al., 2022). One of the vegetables with high anthocyanin content is purple sweet potato (PSP) (*Ipomoea batatas L.*). It has been determined that purple sweet potato anthocyanins (PSPAs) have antitumor effects in rectum (Hagiwara et al., 2002), colon (Lim et al., 2013), stomach (Hayashi et al., 2006) cancers. Few studies have also reported that PSPAs show chemopreventive and chemotherapeutic activity in BlCa (Li et al., 2018a; Li et al., 2028b). However, the effects and underlying mechanisms of PSPAs on the EMT and migration potential of BlCa have not yet been adequately elucidated.

In this study, we aimed to evaluate the efficacy of PSPAs on proliferation, migration and EMT of 5637 cells, and also to determine the antioxidant activities of total anthocyanins of PSP. In this context, total anthocyanins were extracted from PSP and purified by column chromatography. Their ABTS and DPPH activities were defined as antioxidant features. WST-1 and scratch-wound healing assays were used to investigate the effects of PSPAs on the proliferation and migration of 5637 cells, respectively. Moreover, qPCR was performed to evaluate the EMT inhibitory effects of PSPAs.

MATERIALS AND METHODS

Materials

The *Ipomoea batatas L*. (purple sweet potato) samples were obtained from Hatay province, Turkey, in 2022. These samples were frozen and stored at -18ºC before determining their anthocyanin content and antioxidant activity. Antioxidant assays were performed by a Shimadzu UV–Vis spectrophotometer. The used solvents and chemicals were purchased from Merck.

Extraction and purification of total anthocyanins

100 g of PSP sample was mixed with 2*250 mL of HCl (1%):methanol (15:85, v/v) solutions for 1 min by using a blender. The collected extracts were filtered on Whatman No. 1 paper under vacuum. The filtrate was condensed under vacuum at 35-40ºC by using rotary evaporator. The concentrated filtrate was loaded onto the column containing an Amberlite XAD-7 resin. For removing water-soluble components and polyphenols, it was washed with water (1000 mL) and ethyl acetate (500 mL), respectively. Total anthocyanins were eluted with methanol acidified with 0.1% trifluoroacetic acid (v/v) at last. This fragment was concentrated under vacuum.

Determination of total anthocyanin content (TAC)

The used method is based on the predominance of the colorless hemiketal forms of monomeric anthocyanins at pH 4.5 and the colored oxonium forms of them at pH 1.0. Accordingly, the difference in absorbance values measured at pH 4.5 and pH 1.0 is directly proportional to the anthocyanin concentration (Cemeroğlu and Yemenicioğlu, 2001). The pH of sample solutions were adjusted with potassium chloride buffer (0.025 M) and with sodium acetate buffer (0.4 M) for pH 1.0 and 4.5, respectively. These solutions were incubated for 15 min. and their absorbance were measured at 510 and 700 nm (Figure 1). The absorbance of the diluted sample (A) was calculated by using following equation 1;

$$A = [(A_{510} - A_{700})pH1.0 - (A_{510} - A_{700})pH4.5] \quad (1)$$

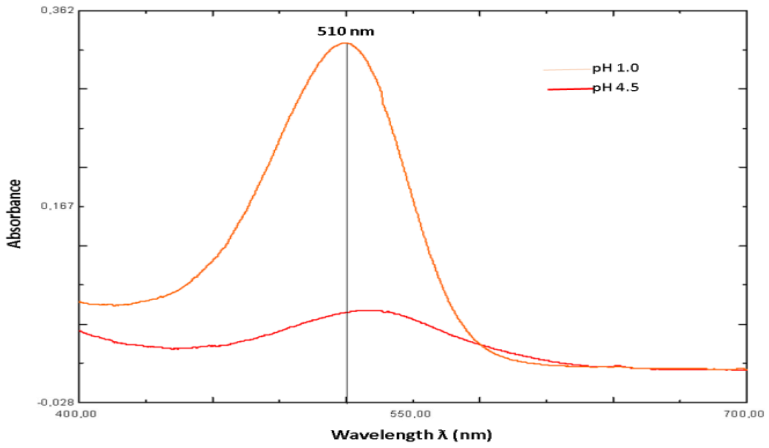

Figure 1. Maximum absorption wavelength in different pH environments.

The TAC of PSP was calculated as cyanidin-3-glucoside equivalents as by using following equation 2;

$$Anthocyanin\ pigment\ \left(\frac{mg}{L}\right) = \frac{A \times MW \times DF \times 1000}{a \times l} \quad (2)$$

where A is the absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, a is the molar absorptivity (26,900 L.mol^{-1} .cm^{-1}), and l is the cell path length (1 cm).

Antioxidant assays

DPPH assay

DPPH radical scavenging activities of total anthocyanins extracted from purple potato was measured according to the literature (El Cadi et al., 2020). The solutions of the sample were prepared in methanol at different concentration. 0.2 mL of these solutions was added to 3 mL of DPPH (0.05 mM) in 70% MeOH solution. After mixing and standing at room temperature for 30 min, the absorbance was determined at 517 nm. The DPPH activity is calculated according to following equation 3;

DPPH activity % = [(Abscontrol –Abssample) / Abscontrol] *100 (3)

The IC_{50} values of samples were calculated by using the graph of DPPH activity % against the extract concentration.

ABTS assay

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) scavenging activities of total anthocyanins were determined by using procedure reported in literature (Sonmez et al., 2019). 19.2 mg of ABTS (7 mM) and 3.3 mg K_2S2O_3 were dissolved in distilled water (5 mL). This ABTS solution was stored in dark for 18-24 h at rt. Its absorbance was immobilized to 0.70 ± 0.02 at 734 nm by dilution. After 6 min adding ABTS solution to sample solutions in MeOH at various concentration, their absorbances were measured at 734 nm. The absorbance decreases were used to determine their activities.

Cell culture

All cell lines used in the experiments were provided from American Type Culture Collection (ATCC). Human bladder cancer 5637 cells were maintained in Roswell Park Memorial Institute-1640 medium (RPMI, Capricorn Scientific, Germany). Human umbilical vein endothelial cells (HEK293T) used as normal epithelial cells were cultured in DMEM (Capricorn Scientific, Germany) medium. Both cell lines were supplemented with 1% Penicillin-Streptomycin (Sigma-Aldrich St USA) and 10% Fetal Bovine Serum (FBS, Gibco,

Invitrogen, USA). Cells were incubated at 37°C, in a humidified atmosphere including 95% humidity and 5% CO_2.

WST-1 assay

Cells were seeded in 96-well culture plates with $2x10^4$ cells in each well using 100 µL of medium and incubated for 24 h to ensure sufficient confluency. Cells were treated with different concentrations (0-1000 µg/mL) of PSPAs and incubated for 24 and 48 h. Then, the cell medium was removed and incubated by adding 10 µL of WST-1 Reagent (BioVision, CA) to the fresh medium with a final volume of 100 mL. After the incubation, cell viability analysis was performed by reading the absorbance values of the cells at 450 nm with a microplate reader (Agilent Technologies, USA). The viability of the control group that wasn't treated with PSPA was considered as 100% and the viability of the experimental cells was expressed as % compared to the control group.

***In vitro* scratch-wound healing assay**

For the wound healing assay, 5637 cells collected from the standard culture were seeded in a 6-well culture plate at a concentration of $1x10^6$ cells/well and then incubated for 24 h at 37°C and 5% CO_2. Each well was slid across the cell monolayer to create a linear wound with the p1000 pipette tip. Cells were treated with or without PSPA for 24 h. After the treatment, the culture medium was gently removed and 4% paraformaldehyde fixation was performed. The effect of PSPA concentrations on the cell migration process was observed with an Olympus inverted microscope (Olympus IX73, Japan).

Total RNA isolation and cDNA translation

For total RNA isolation, $1x10^6$ cells were seeded into 6-well culture plates and incubated for 24 h. RNA isolation was performed from cells which were removed by trypsinization according to the EXTRAzol Reagent (Blirt, Poland) kit protocol after the treatment of PSPAs at the most appropriate doses and hours determined by WST-1 analysis. Isolated RNA samples were quantitatively assayed with the Nanodrop spectrophotometer (Optizen, Korea) for purity and quantification. After the purity and quantity determination of the RNA samples, they were converted to cDNA in a T100 thermal cycler (Bio-Rad, Germany), in accordance with the OneScript® Plus cDNA Synthesis Kit (ABM, USA) protocol. cDNA samples were stored at -20°C for further analysis.

Quantitative Real-time PCR analysis

Snail, E-Cadherin and Vimentin mRNA levels were evaluated from cDNA samples specifically obtained using suitable commercially available primers. For each sample was used concentrations as following: 4 µL of cDNA, 0.8 µL of each forward/reverse primer, 10 µL of SensiFAST™ SYBR® No-ROX Kit (BioLine, USA) and 4.4 µL of DEPC water. qPCR was implemented by CFX Connect™ Real-Time system (Bio-Rad, Germany) and PCR profiles as follows: 1 cycle for 2 min at 95°C, 40 cycles for 5 sec at 95°C, 10 sec at 65°C, 2 min at 72°C. cDNA was amplified using primers for *Vimentin, Snail, E-Cadherin* and *ACTB* genes, which are: *Vimentin*, forward: 5′CTTCGCCAACTACATCGACA-3′, reverse: 5′GCTTCAACGGCAAAGTTCTC-3′; *Snail,* forward: 5′TCGTCCTTCTCCTCTACTTC-3′, reverse: 5′TTCCTTGTTGCAGTATTTGC-3′; *E-Cadherin,* forward: 5′ACGTCGTAATCACCACACTGA-3′, reverse: 5′TTCGTCACTGCTACGTGTAGAA-3′; *ACTB,* forward: 5'AGCGGGAATCGTGCGTG-3', reverse: 5'CAGGGTACATGGTGGTGGTGCC-3'. *β-Actin (ACTB)* was used as the reference gene and comparisons between the experimental groups were made compared to the control group. Relative expression was quantified using the $2^{-\Delta\Delta Ct}$ method. GraphPad Prism 9.0.0 was used to visualize the relative expression of related genes in experimental groups.

Statistical Analysis

All experiments were repeated 3 times and the results were evaluated with GraphPad Prism 8.0.0 (La Jolla, CA). Data are expressed as mean ± standard deviation (SD). Comparisons between treated and untreated samples were made with One Way analysis of variance (ANOVA) and post hoc Tukey Test. Gene expression results were analyzed with CFX Manager™ software and images were analyzed using ImageJ Version 1.53u program. Statistical significance was determined as $P<0.05$.Provide sufficient detail to allow the work to be reproduced. The uniform appearance will assist the reader to read paper of the proceedings book. It is therefore suggested to authors to use the example of this file to construct their papers. This particular example uses this paper format with 25 mm margins left, right, top and bottom.

RESULTS

PSPA exhibits a high DPPH and ABTS activity

In this study, the total anthocyanin content and antioxidant capacities of a PSP variety grown in Hatay-Turkey were investigated. Total anthocyanins content (TAC), DPPH and ABTS activity results of total anthocyanins extracted from PSP are given in Table 1. Our results showed that the extract from PSP contained 98.344 mg of cyn-3-glu/g extract of total anthocyanins. IC_{50} values are 5.06±1.57 µg/mL and 8.85±0.65 µg/mL, respectively, and have high DPPH and ABTS activities. DPPH activities are almost 2 times less than ascorbic acid (IC_{50} = 2.36±0.97 µg/mL), while ABTS activities are about 4 times stronger than the standard used ascorbic acid (29.23±0.77 µg/mL) (Table 1).

Table 1. Total anthocyanin content (TAC), IC_{50} and I % value of DPPH and ABTS activity of total anthocyanins extracted from purple sweet potato.

Sample	TAC (mg cyn-3-glu/g extract)	DPPH (I %)	DPPH assay (IC_{50}, µg/mL)	ABTS (I %)	ABTS assay (IC_{50}, µg/mL)
Purple Sweet Potato	98.344	79.17±0.35	5.06±1.57	84.52±5.93	8.85±0.65
Ascorbic acid	-	81.89±3.31	2.36±0.97	92.91±5.09	29.23±0.77

Shows selective cytotoxic activity in PSPA 5637 cells

PSPA-derived total anthocyanins significantly reduced the viability of human BlCa 5637 cells due to increasing dose. At the end of the treatment of 5637 cells at different PSPA concentrations (50, 100, 250, 500, 750 and 1000 µg/mL) for 48 hours; It showed that doses <250 µg/mL did not affect cell proliferation, while doses >500 µg/mL decreased cell viability <50% (Figure 2A). Therefore, a concentration of 250 µg/mL was chosen in other analyzes to investigate the anti-metastatic activity of PSPA in 5637.
Selected doses of PSPA showed cytotoxic activity in 5637, but cell viability did not fall below <50% even at the highest indicated concentration in non-cancerous HEK293T endothelial cells (Figure 2B). Our results highlighted that PSPA showed selective cytotoxicity in 5637 BlCa cells.

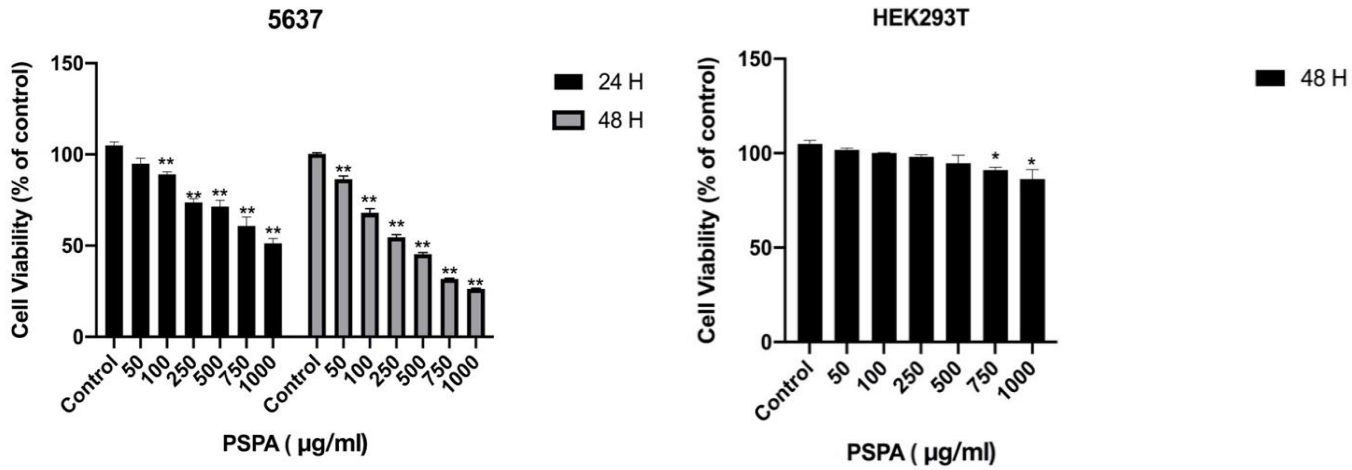

Figure 2. (A) Cell viability of 5637 cells treated with PSPA at different concentrations: 50, 100, 250, 500, 750 and 1000 µg/ml; **P<0.01 vs. control (BC cells). (B) Effect of PSPA on the viability of non-cancerous epithelial cells HEK293T. *P<0.05 vs. control.

The IC_{50} values of PSPAs were 976.8 µg/mL and 305.4 µg/mL at 24 and 48 h, respectively. IC_{50} values were significantly different at 24 and 48 h (Figure 3). Based on these results, proliferation was significantly decreased in 5637 cells in parallel with increasing PSPA treatment time ($P<0.05$) (Figure 3).

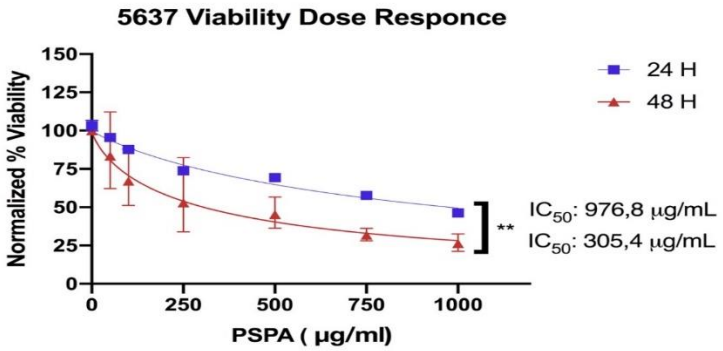

Figure 3. The half maximal inhibitory concentrations (IC50) for PSPA. All the values are expressed as mean ± standard deviation; **P< 0.01.

PSPA 5637 suppresses the migrating properties of BlCa cells

To evaluate the effect of PSPA on migration in 5637 BlCa cells, we performed a scratch-wound healing test. As shown in Figure 2A, PSPA showed weak cytotoxicity in 5637 cells even at the highest dose at 24 h of treatment. Therefore, incubation of the previously determined dose for 24 h was selected for cell migration exprement in order to see the wound closure. At the beginning of the test (0 h), approximately the same level of clearance zones were observed in the control and PSPA-treated (250 µg/ml) cells. After 24 h, control cells tended to migrate to the empty region, closing the gap region. However, PSPA treatment strongly inhibited cell migration at concentrations of 250 µg/ml. PSPAs significantly reduced the migration distance of 5637 cells by 68% compared to untreated cells after 24 h (Figure 4).

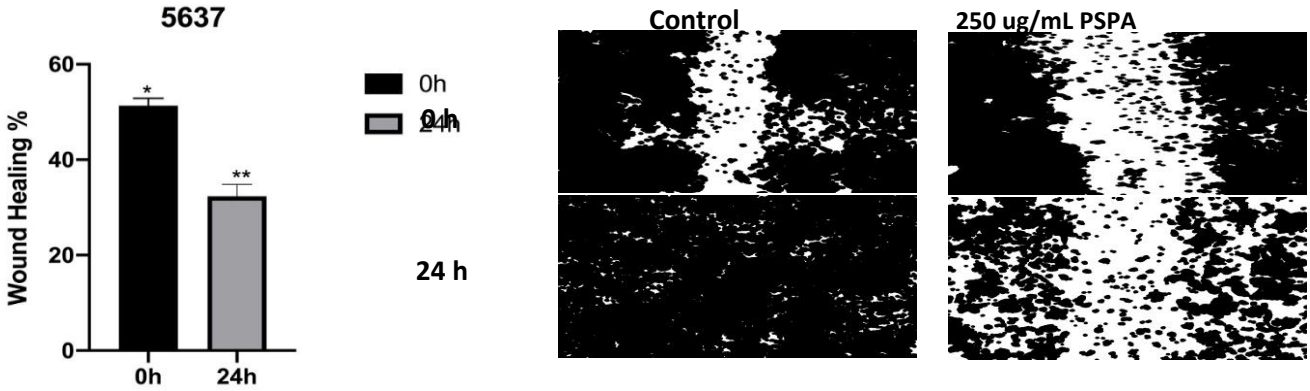

Figure 4. Effect of PSPA on cell proliferation and wound healing in 5637 cells. *P<0.05, **P<0.01 vs. control.

PSPA increases expression of epithelial biomarkers by reducing mesenchymal ability in 5637 cell. 5637 cells are characterized by a high N-Cadherin level and exhibit invasive activity (Adhim et al., 2011). In this study, we evaluated changes in the expression patterns of EMT-related biomarkers by RT-PCR to demonstrate that PSPA has inhibitory effects on EMT.

Our data showed a dose-dependent upregulation of the epithelial marker E-Cadherin expression in 5637 g7cells after PSPA treatment (Figure 5A). In addition, a statistically significant decrease in the expression of Snail, which is an important inducer of EMT and a potent suppressor of E-Cadherin, was also observed after 250 µg/mL PSPA treatment ($P<0.01$) (Figure 5B). revealed that it down-regulated the expression of the phenotype marker Vimentin (Fig. 5C).

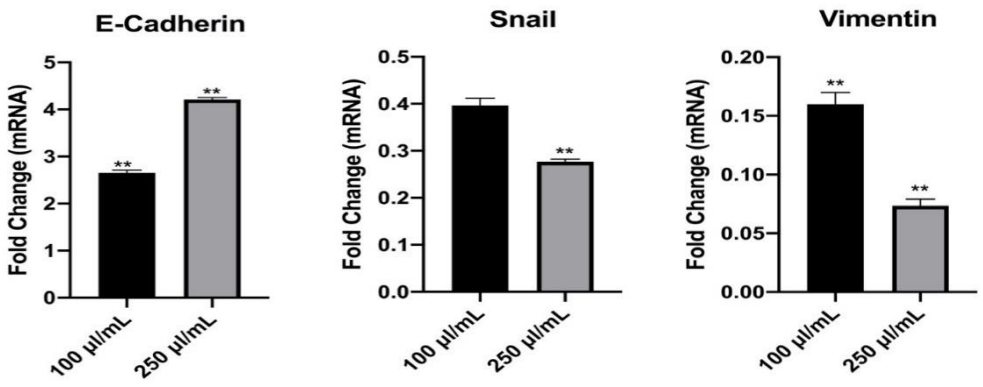

Figure 5. Expression levels of E-Cadherin (A), Snail (B) and Vimentin (C) determined by RT-PCR in 5637 cells treated with 100 and 250 µg/µL PSPA concentrations. **P<0.01 vs. control.

DISCUSSION

Today, obtaining effective chemo-preventive and chemotherapeutic agents from natural products for cancer treatment is of great interest worldwide (Miyake et al., 2012; Burton et al., 2015). Anthocyanins, a special group of flavonoids responsible for color in many fruits, flowers, are highly recommended for cancer prevention and management (Diaconeasa et al., 2020; Zhao et al., 2019). Their anti-tumor and anti-cancer activities emerging from in vitro studies are based on their anti-oxidant, phase II detoxifying enzyme stimulating, anti-proliferative, anti-inflammatory, anti-angiogenic, anti-invasive, anti-metastatic and apoptosis, autophagy, differentiation inducing activities (Wang and Stoner, 2009). Pigmented potato varieties such as red and purple potatoes are important sources of anthocyanins. In recent years, an increasing number of researches have focused on the characterization of the anthocyanin composition in purple potatoes. Their total anthocyanin composition (TAC) and individual anthocyanidin content (AC) vary greatly depending on the cultivar and the region or conditions in which they are grown. Besides, anti-oxidant activities determined with the ABTS, FRAP and DPPH assays, are highly corresponded with their TAC and AN content (Lachman et al., 2009). DPPH activity of methanol extracts of strawberry, raspberry, cherry and cranberry fruits was found to be 5.35 mg/mL, 8.43 mg/mL, 11.78 mg/mL and 12.21 mg/mL, respectively (Köroğlu et al., 2021). The total anthocyanins, extracted from purple sweet potato in this study, exhibited very stronger DPPH activity (IC_{50}= 5.06 µg/mL) than above reported fruits. It was reported that some extracts from various plants such as *Silene vulgaris*, *Rumex acetosella*, Achillea *millefolium* and *Hypericum orientale* exhibited ABTS activity with IC_{50} values ranging from 40.22 to 106.01 µg/mL (Kurt et l., 2018). The total anthocyanins extracted from PSP in this study, have higher ABTS activity (IC_{50} = 8.85±0.65 µg/mL) than above reported plants.

Studies aiming to evaluate the cytotoxic effects of anthocyanins are quite different, especially interms of the type of molecule, concentration and cell lines (de Arruda Nascimento et al., 2022). Various reports have shown that PSPAs can suppress the growth of cancer cells (Zhang et al., 2021; Guo et al., 2021; Wu et al., 2015). However, the extent to which plant extracts affect the growth of cell lines of different origins shows can be quite different (Garcia-Lazaro et al., 2020). Literature on the cytotoxic effects of PSPAs on BlCa cells are very limited. A study reported that the PSPAs reduced proliferation of BlCa BIU87 cell line and inhibited the growth of cells in a dose-dependent manner by inducing apoptosis (Li et al., 2018a). Another study researched the antitumor effect of PSPAs on BlCa 5637 and T24 cell lines and revealed that PSPAs reduced the viability of cells in a dose-dependent manner. They also suggested that the viability of non-cancerous bladder cells increased after 48 h incubation period with PSPAs and these cells exhibited a high Bcl-2/Bax ratio (Li et al., 2018b). With this study, we once again emphasized that PSPAs selectively inhibit the proliferation of BlCa 5637 cells and are not cytotoxic in non-cancerous HEK293T cells (Figure 2B). The reason for this apparently contradictory effect observed in cancerous and noncancerous cells may be due to the different intracellular microenvironments. The cytotoxic action mode of anthocyanins is not well-known. But, PSPAs have been reported to exert cytotoxic effects on BlCa cells by suppressing cell viability, exacerbating mitochondrial membrane potential collapse, promoting apoptosis and inducing cell cycle arrest (Lim et al., 2013; Li et al., 2018b).

Cell migration is a fundamental process for the invasion and metastasis of cancer cells (Tsakiroglou et al., 2019; Chen et al., 2006). 5637 BlCa cells exhibiting a trans-differential character from basal to lumen have a high migration ability. Migratory carcinomas show mesenchymal features with reduced apico-basal polarity and actin-rich cellular protrusions. EMT-type mechanisms are known to be involved in the migratory movement of these epithelia (Revenu and Gilmour, 2009). Our results suggested that the decreased migration of 5637 cells after PSPA treatment may be related to EMT inhibition.

EMT causes phenotypic changes that cause cells to lose their epithelial characteristics and acquire motile mesenchymal features (Mendez et al., 2010). Accordingly, EMT is strongly associated with aggressive BlCa behaviors such as recurrence, invasion and metastasis (Yun and Kim, 2013; Cao et al., 2020; Moussa et al., 2019). This raises the possibility of EMT being a rational therapeutic target in the treatment of invasive BlCa (Kitagawa et al., 2021). Anthocyanins have been described as a phytochemical that can modulate EMT-related signaling pathways (Salehi et al., 2019; Lu et al., 2013). Nevertheless, the EMT inhibitory activities of PSPAs have not yet been adequately elucidated in BlCa. Our study shows for the first time that PSPAs may have the potential to inhibit the EMT process in BlCa.

Downregulation of E-Cadherin, an epithelial marker and considered the keystone for initiating EMT, disrupts cell-cell contact and cells acquire the ability to migrate (Lo et al., 2017). Snail and Vimentin which are transcriptional factors that suppress E-Cadherin expression, are also important regulators of EMT and their high expression is associated with poor prognosis (Salehi et al., 2019). Vimentin is held responsible for the assembly of intermediate filaments to suppress epithelial markers and acquire a mesenchymal character in tumor cells (Liu et al., 2015). Vimentin is important mesenchymal marker and prognostic factor in BlCa (Zhao et al., 2014). It has been reported that highly metastatic BlCa cells isolated by in vivo selection show a high affinity to form invadopodia that help to perform mechanical and invasive action. Overexpression of Vimentin by cells in this subpopulation stabilizes invadopodia, an important molecular process for extravasation (Yonayema et al., 2014). In conclusion, we proved that PSPA reduced Vimentin expression in 5637 cells in correlation with its anti-migratative activity. In addition, we demonstrate that the possible potential of PSPA in the regulation of the Vimentin signaling cascade needs to be further clarified in terms of its pharmacological importance.

Members of the Snail family are critical inducers of EMT process progression and cell migration (Wu and Zhou, 2010). Accordingly, microRNAs with direct Snail target genes have been shown to reduce cell proliferation in BlCa, induce apoptosis and reduce the mesenchymal phenotype by promoting EMT repression (Xu et al., 2018). The development of drugs targeting Snail expression may be an effective strategy for inhibiting BlCa cell migration and invasion (Xu et al., 2016). It is known that many anthocyanins such as delphinidin-glucoside, cyanidin-glucoside and malvidin-glucoside found in PSPA modulate E-Cadherin expression by specifically targeting Snail (Lu et al., 2013). On this basis, our results support the hypothesis that Snail expression is targeted by PSPA to trigger mesenchymal-to-epithelial transition (MET) in 5637 cells. This section should explore the significance of the results of the work, not repeat them.

CONCLUSION

In conclusion, this present study elucidated for the first time the effects of PSPA on EMT and cell migration in 5637 cells. The ability of PSPA to suppress and modulate the EMT process and reduce the migratory potential of 5637 cells by supporting the epithelial phenotype should prevent metastatic spread of MIBCa. Compounds that induce MET may favor selection of metastatic tumor cells as a result of restoring epithelial properties. Therefore, it is extremely important that compounds that inhibit EMT are cytotoxic to tumors that already overexpress mesenchymal markers. In this respect, PSPA is a promising natural compound that inhibits EMT in 5637 cells while also selectively inhibiting their proliferation. It was determined that the methanolic extract obtained from PSP had high total anthocyanin content (98.344 mg cyn-3-glu/g extract) and they showed strong ABTS (IC_{50}= 8.85 µg/mL) and DPPH (IC_{50} = 5.06 µg/mL) activities as antioxidant properties. The obtained results show that PSPAs have also strong antioxidant activity, as well as significantly inhibitory activity against proliferation, migration and EMT of human BlCa 5637 cells. These results suggest that PSPAs may reduce the risk of BlCa and used to discover natural-based chemotherapeutic drug candidates for BlCa therapy.

ACKNOWLEDGEMENTS

In this manuscript, the relevant cell line in cell culture is Assoc. Prof. Serap Erkek's gift to our laboratory. We would like to express our gratitude to her for his support.

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ORAL PRESENTATION

Preparation of hydroxyapatite-silica composites for the efficient adsorption of quercetin

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Abstract

Quercetin is a flavonoid compound with strong antioxidant and anti-inflammatory properties. Research shows that quercetin could play a role as a protective agent against age-related diseases thanks to its antioxidant effects. However, studies on quercetin have shown that it is poorly absorbed and rapidly metabolized, which greatly reduces the bioavailability of quercetin. Because of that, the carrier material to which quercetin is applied plays an important role in the release of quercetin. For this reason, hydroxyapatite (HAP) was used in this study as it is inert, biocompatible, a biodegradable material and has unique bioactivity in its interaction with the body. However, the use of HAP alone is not efficient for drug delivery systems. Therefore, the combination of HAP with mesoporous SiO_2 will help to effectively dissolve hybrid nanoparticles. Our main objective in this study is to efficiently load the HAP/Si composite we synthesized with quercetin, investigate the effects of HAP and Si concentration on the efficiency of loading the drug, and subsequently achieve controlled release of quercetin. The prepared composites were analyzed using UV-VIS to calculate the drug loading and FTIR analysis was performed to investigate the chemical composition of the particles and the ratio of the HAP-Si quercetin complex to each other. At the end of the study, the highest encapsulation efficiency of 95.43% was obtained in the composite with a HAP:Si ratio of 3:2.

Keywords: Controlled drug release, quercetin, hydroxyapatite, silica

INTRODUCTION

Quercetin is a therapeutic agent for various diseases and has antidiabetic, anti-inflammatory and anticancer effects. However, pharmacokinetic limitations such as poor bioavailability, poor absorption, inadequate release and low bioavailability of quercetin have severely limited its potential. To overcome these limitations, various carrier systems have been developed and studies have been conducted to improve pharmacokinetic parameters, but insufficient data and progress have been made (Aghababaei et al. 2023).

Hydroxyapatites (HAP) are synthetic and biocompatible materials known for their biocompatibility, inertness and absence of organic matter (Zhang et al. 2012). However, the surfaces and bioactivity of HAPs are generally not at the desired level for drug loading and release studies. In addition, they dissolve rapidly in the acidic gastric environment. The active ingredient of the drug loaded in hydroxyapatite can dissolve rapidly in the gastric environment and release the drug suddenly into the environment, resulting in an burst release. Therefore, pure HAP is generally not used as the sole agent.

In recent years, HAP/silica (HAP/Si) composites have been found to have a larger surface area and higher bioactivity compared to pure HAP. Silicon dioxide is a bioglass-based material and can be used to increase the mechanical stability of hydroxyapatite frameworks and improve the long-term performance of the composite. The high chemical and thermal stability of silica, its large surface area and its compatibility with other materials allow its use in various applications (Vallet-Regí, et al. 2022). Therefore, HAP/Si composites are very suitable to increase the efficacy and bioavailability of quercetin (Budak et al. 2016).

In this study, HAP/silica composites were synthesized in different ratios and evaluated for quercetin loading properties for appropriate drug delivery.

MATERIALS AND METHODS

Materials

All chemicals used were analytical standard qualities. Quercetin (98%) and diammonium phosphate ($(NH_4)_2HPO_4$) are from Sigma Aldrich, USA; calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$ (98%) is from Carlo Erba, Italy and ammonia solution (NH_4OH) (20-25%) is from Tekk Kim. Türkiye.

Preparation of HAP samples

First, calcium nitrate ($Ca(NO_3)_24H_2O$) and diammonium phosphate ($(NH_4)2HPO_4$) were prepared in pure water with a Ca/P molar ratio of 1.67. The pH values of both solutions were adjusted to pH≥9 with ammonium hydroxide (NH_4OH) (0.1 M). Then the solutions were stirred for 15 minutes and then $(NH_4)_2HPO_4$ was slowly added to the prepared $Ca(NO_3)_24H_2O$ solution. The mixture was stirred for 4 hours under pH and temperature control and dried at 110° for 10 hours. The sample removed from the oven was then sintered at 600° for 4 hours.

Preparation of HAP/Si samples

Tetraethyl orthosilicate (TEOS) was chosen as the silica source. TEOS/ethanol solutions were prepared and added dropwise to the HAP suspension obtained in the previous step and stirred at 60°C for 12 hours and kept at room temperature. The ratio of HAP to Si was theoretically adjusted to 1:1, 2:1 and 3:2. The precipitate was then filtered and washed 3 times with distilled water. The resulting HAP/Si precipitate was dried at 110°C for 10 hours. Then the sample removed from the oven was sintered at 600°C for 4 hours.

Quercetin loading to HAP/Si composite

First, 100 ml quercetin solution (concentration 2 mg/ml) was prepared. Then 200 mg of the composite was added to the quercetin solution. The dispersion was stirred with a magnetic stirrer at 25°C for 48 hours and then centrifuged to obtain the quercetin-loaded composite. After centrifugation, the precipitate (quercetin-loaded composite) was separated from the solution, washed three times with pure water and dried. The separated solution was analysed by UV-VIS to calculate the efficiency of quercetin loading and calculated according to the following equations (1):

$$Drug\ loading\% = \frac{Actual\ amount\ of\ drug\ in\ composites(mg)}{Theoritical\ amount\ of\ drug\ in\ composites(mg)} \times 100 \quad (1)$$

Characterization

Fourier transform infrared spectroscopy (FTIR) was used to identify the bonding structures and chemical properties of the particles. The analysis was performed using a Thermo Scientific Nicolet IS10 FTIR spectrometer with an Attenuated Total Reflectance (ATR) accessory. The spectrometer scanned the wavenumber range from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} over 32 scans. The crystallinity of the samples was analyzed using Rigaku Miniflex X-ray diffraction (XRD) with CuKα radiation in the 0.02° (2θ) range of 5–80° at a scan rate of 2°/min.

RESULTS and DISCUSSION

Characterization

The spectral data examined in Figure 1 showed that the characteristic peaks of silicon dioxide (SiO_2) indicate Si–OH groups in the range of about 800 cm^{-1}. The OH peaks in the range of 3560 cm^{-1} indicate the presence of hydroxyapatite (HA) and SiO_2. In addition, the bands between 965-960 cm^{-1} reflect the n_1 and n_4 symmetric P-O stretching vibrations. The symmetric stretching vibrations of the Si-O-Si bonds were observed in the range of 810 cm^{-1}. The bands attributed to the stretching modes of the O–H bonds of the silanol (Si–OH) groups in the structure of the material were detected around 960 cm^{-1}. In addition, the characteristic bands of water molecules (water absorptions) were determined at a frequency of 873 cm^{-1}. Thus, the coexistence of HAP and silica components indicates that the material was successfully synthesized (dos Aptoslos et al. 2019; Abdian et al. 2023).

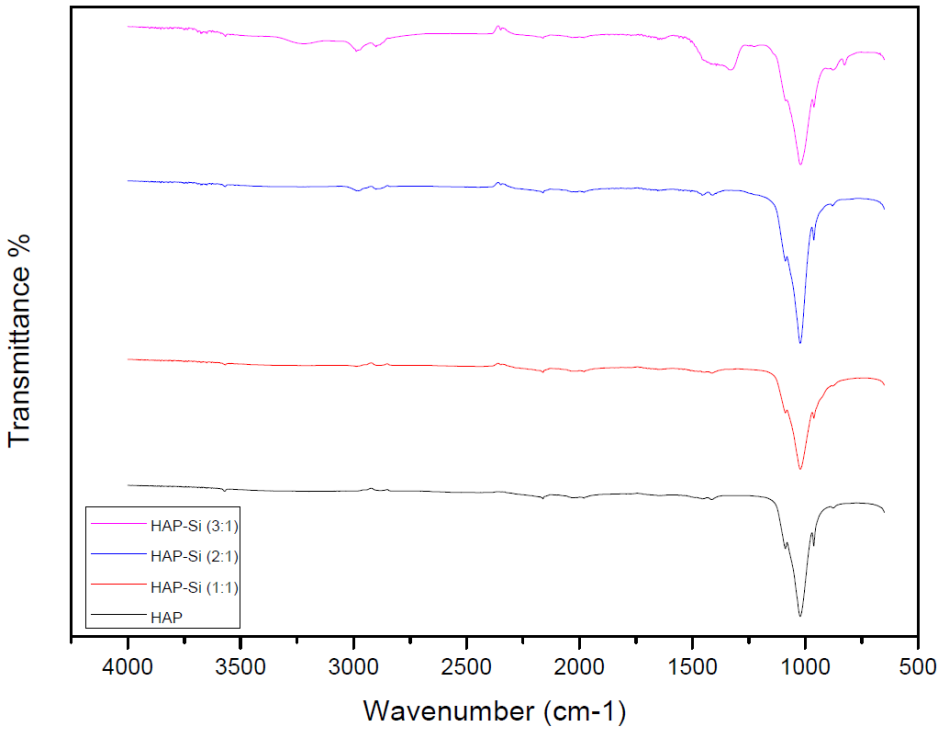

Figure 1. FTIR analysis of hydroxyapatite-silica composites

The XRD patterns of the composites showed that the materials retained their structure after modification with Si (Figure 2). All samples showed the same characteristic peaks for HAP at $2\theta^{o}$ = 32.30, 31.81 and 26.08 (Sezer and Hoşgün, 2023).

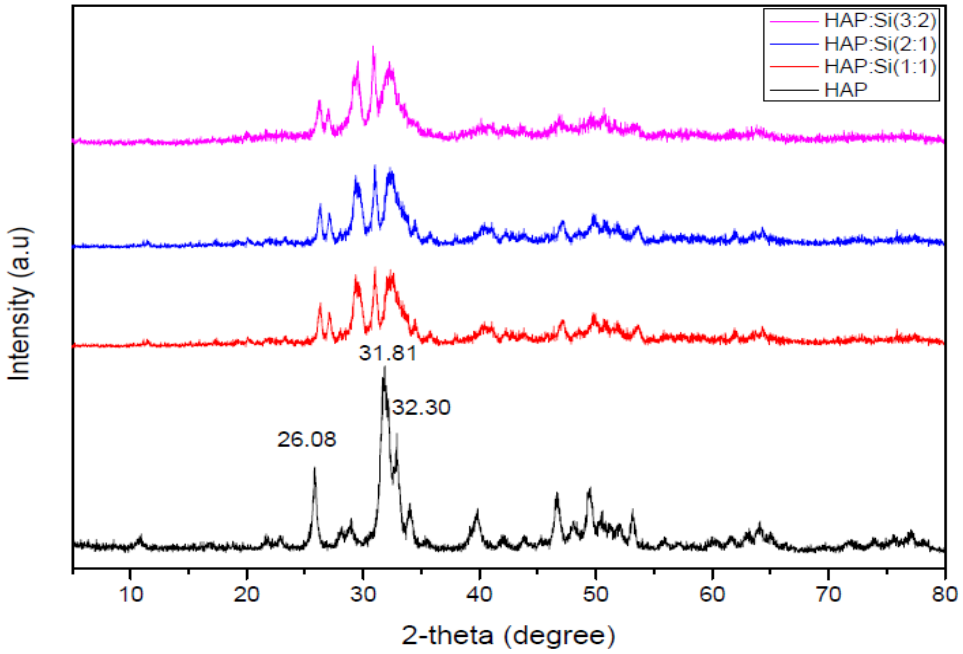

Figure 2. XRD patterns of hydroxyapatite-silica composites

3.2 Quercetin loading to HAP/Si composite

The changes in the percentage drug loading between the different composite types can be observed (Figure 3), which can be attributed to the influence of the hydroxyapatite and silica ratios in the composite components. The highest percentage of active substance loading among the composites was achieved with the HAP:Si (2:1) material at 95.4. While an encapsulation efficiency of 88.5% was achieved with the HAP-Si (1:1) composite, it was hypothesized that the balanced distribution of the hydroxyapatite and silica ratio in the HAP-Si (3:1)

composite shrunk the pores to prevent loading, thereby decreasing the drug loading efficiency to 80.3%. As expected, the HAP composite had the lowest loading efficiency of all composite types at 76.3%.

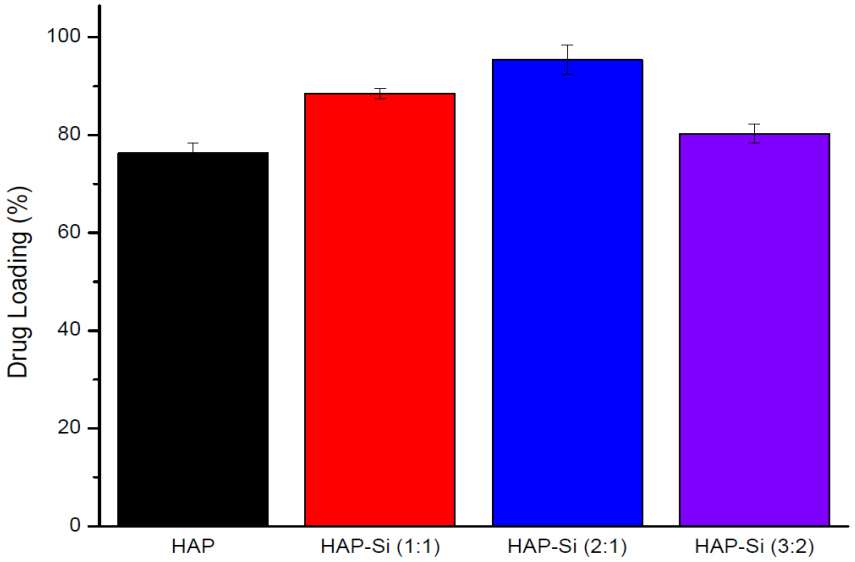

Figure 3. Drug loading (%) in the composites

CONCLUSION

Studies on quercetin have shown that it is poorly absorbed and rapidly metabolized, which greatly reduces the bioavailability of quercetin. For this reason, the carrier material on which quercetin is applied plays an important role in the release of quercetin. The use of HAP and silica composites as carriers for quercetin has significant potential to increase therapeutic efficacy and safety and provides a solid foundation for future research and applications. This study highlights that the combinations and characterization of HAP and silica composites in different ratios are crucial for optimizing the therapeutic effect and increasing bioavailability in biomedical delivery systems and shows that the HAP-Si composites are suitable and effective carriers for quercetin.

ACKNOWLEDGEMENTS

This study was supported by TÜBİTAK 2209-A Research Project Support Program for Undergraduate Students under grant number:1919B012319431 and Eskişehir Technical University Research Projects Commission under grant number:24LÖP153.

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ORAL PRESENTATION

Yapay Resif Batığının Hangi Biyolojik Özelliği Dalıcıyı Cezbeder?

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Özet

Yapay resifler koruma, beslenme üreme ve bir araya toplanma gibi doğal resiflerin bazı fonksiyonlarını taklit etmeleri için deniz tabanına veya yüzeyine kasıtlı olarak yerleştirilen yapılardır. Dünyada 50'den fazla ülkede farklı amaçlar için kullanılan yapay resifler, dalış ve olta balıkçılığı gibi rekreasyonel amaçlar için Amerika Birleşik Devletleri, Kanada, Avustralya, İsrail, Malta ve Türkiye gibi ülkelerde kullanılmaktadır.

Bu tez çalışmasında, Karaburun Yapay Batık Resiflerinden biri olan "9 Eylül" batığının hangi biyolojik özelliğinin dalıcılar tarafından tercih edildiği araştırılmıştır. Çalışmada çevrimiçi anket vasıtasıyla koşullu değer belirleme yöntemi kullanılmıştır. Çalışmada 9 Eylül batığının bir fotoğrafından Adobe Photoshop 2022 programı kullanılarak 8 kuramsal senaryo içeren fotoğraf oluşturulmuştur.

Ankete katılım sağlayan 156 kişinin %29'unu kadınlar, %71'ini erkekler oluşturmuştur. Katılımcıların %71'i iki yıldız ve üstü dalıcı sertifikasına sahipken, %8'i sadece tanıtım dalışı yapmış ve %6'sı hiç dalış yapmamış kişilerden oluşmuştur. Katılımcılar tarafından en çok tercih edilen biyolojik özellik batık etrafındaki biyoçeşitlilik (%48) ve balık bolluğu (%42) olarak belirlenmiştir. Katılımcıların demografik yapılarına ve dalış tecrübelerine göre biyolojik özellik seçiminde ve ödeme istekliliklerinde istatistiksel olarak anlamlı bir fark bulunmamıştır (P>0,05).

Anahtar Kelimeler: Yapay Resif Batığı, Rekreasyonel dalış, Koşullu değer belirleme yöntemi, 9 Eylül Batığı, Karaburun

Which Biological Attribute of Artificial Reef Wreck Attract Diver?

Abstract

Artificial reefs are structures that are deliberately placed on the seafloor or surface to mimic some of the functions of natural reefs, such as protection, feeding, reproduction, and concentrating populations of living marine resources. Artificial reefs, which are used for different purposes in more than 50 countries around the world, are used for recreational purposes (diving and angler fishing) in countries such as the United States of America, Canada, Australia, Israel, Malta and Turkey.
In this thesis, it was investigated which biological attribute of the 9 Eylül shipwreck, one of the Karaburun artificial wreck reefs, was preferred by the divers. In the study, contingent valuation method was used via an online questionnaire. Eight hypothetical scenarios were created using Adobe Photoshop 2022 programme from an original underwater photograph of the 9 Eylül wreck.
The results showed that, of the 156 people who participated in the survey, 29% were women and 71% were men. While 71% of the participants had two star or higher diver certification, 8% of total respondents were discovery diver and 6% of respondents were non-divers. The most preferred biological attributes of the wreck were determined as biodiversity (48%) and fish abundance (42%). There was no statistical difference on the preference of biological attributes and willingness to pay based on the demographic structure and diving experiences of the participants (P>0.05).

Keywords: Artificial wreck reef, recreational diving, contingent valuation method, 9 Eylül Batığı, Karaburun

GİRİŞ

Yapay resifler, koruma, beslenme, üreme ve bir araya toplanma gibi doğal resiflerin bazı fonksiyonlarını taklit etmeleri için deniz tabanına veya yüzeyine kasıtlı olarak yerleştirilen yapılardır (Fabi, 2015). Yapay resifler; önemli denizel habitatları korumak, yavru balıkların beslenmesi ve barınması için uygun alanlar yaratmak, küçük balıkçı takımlarının verimini arttırmak ve sportif balıkçılık ve dalış turizmini geliştirmek gibi amaçlara hizmet etmektedir (Fabi, 2015).

Yapay resifler günümüzde farklı amaçlar için 50'den fazla ülkede kullanılmaktadır. Ancak yapay batık resifleri, yani asli görevini tamamlamış uçak, gemi, tank gibi araçların kasıtlı olarak batırılmasıyla oluşan yapay resifler başta Amerika Birleşik Devletleri olmak üzere Avustralya, Kanada, Malta, Kıbrıs, İsrail ve Türkiye'de rekreasyonel dalışa hizmet etmek için yaygın olarak kullanılmaktadırlar. ABD'nde 1979'dan beri gaz platformları, gemiler ve diğer asli görevini tamamlamış araçlar yapay resif olarak kullanılmaktadır. Bu tip yapay resif uygulamaları ABD'de "gemiden resife" ve "platformdan resife" olarak adlandırılmıştır (Recfishwest, 2017). ABD yapay resif uygulamalarında öncü ülke olarak görülmektedir. ABD kıyılarında 2004 yılında 700'den fazla gemi rekreasyonel amaçlara hizmet etmek için yapay resif olarak kullanılmıştır (Pendleton, 2004). Günümüzde bu sayının çok daha fazla olduğu bilinmektedir.

Türkiye'de dalış turizmine katkı sağlamak ve canlılara yeni yuva oluşturmak amacıyla 2006 yılında Alanya'da batırılan gemi ile rekreasyonel amaçlara hizmet eden ilk yapay batık resifi oluşturulmuştur. Ardından 2007 yılında Bodrum Karaada'da iki gemi ve bir uçak batırılmıştır. Sonraki yıllarda ise çoğunluğu Akdeniz ve Ege Denizi'nde olmak üzere otuzun üzerinde araç (gemi, uçak, tank, vb.) deniz tabanına yerleştirilmiştir (Şensurat-Genç ve ark., 2017).

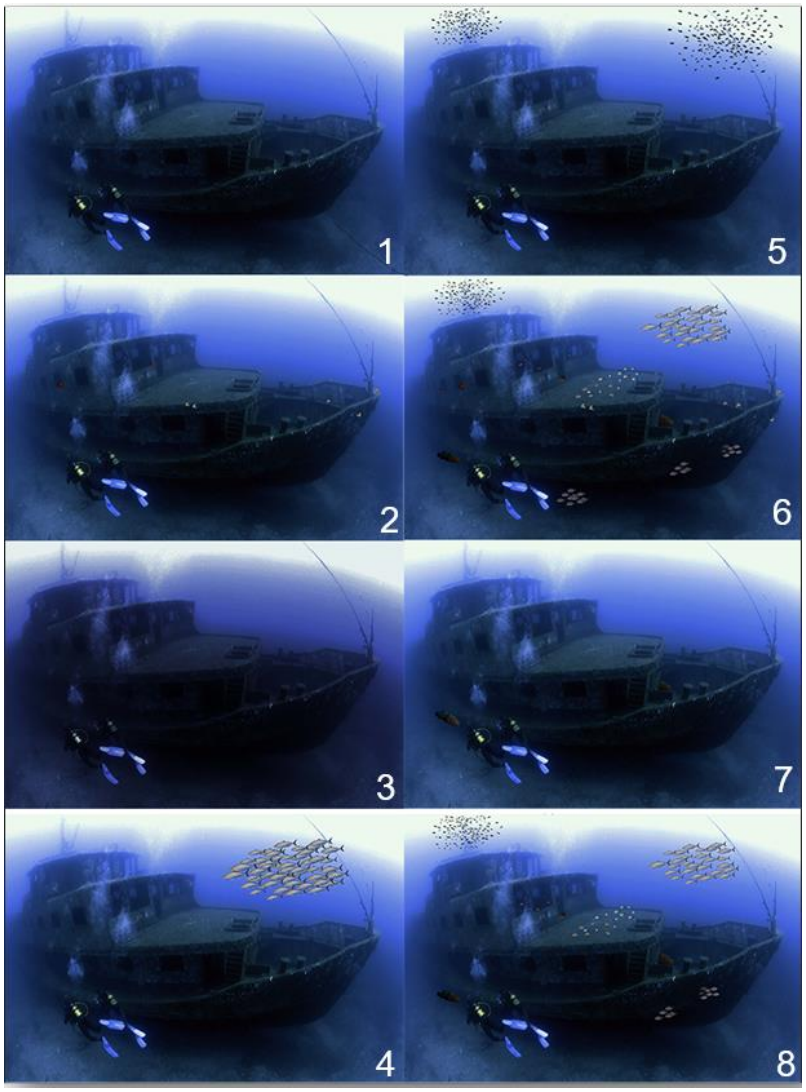
İnsanların, özellikle de turistlerin doğal manzaralara ve habitatlara değer verdiği bilinmektedir (Curtin, 2009). Günümüzde özel bir alan olarak görülen batıklar, dalıcıların en yeni motivasyon kaynaklarından birisidir (Edney, 2006). Mercan resifleri, batıklar, parklar gibi çevresel kamu mallarına kişilerin verdikleri değer kuramsal pazar ya da koşulların sunulduğu bir anket vasıtasıyla ortaya konulabilmektedir (Kirkbride-Smith, 2014). Bu tip değerlendirmelerin yapıldığı bazı çalışmalar denizel çevrenin biyolojik niteliklerinin (özellikle mercanlar ve balıklar) aletli ve aletsiz dalıcılar tarafından tercih edildiğini belirtirken (Shafer ve Inglis, 2000; Williams ve Poulinini 2000; Baht, 2013; Ramos ve ark., 2006; Uyarra ve ark., 2009; Leujak ve Ormond, 2007), bazıları da balık bolluğunun ve boyutunun mercanlara nazaran daha çok tercih edildiğini belirtmiştir (Uyarra ve ark., 2009; Leujak ve Ormond, 2007). Bu çalışmalarda dalıcıların biyolojik tercihlerinin dalış lokasyonuna, su berraklığı gibi çevresel koşullara (Leujak ve Ormond, 2007; Spurgeon, 1992) ya da sosyal durumlara (Shafer ve Inglis, 2000) göre farklılık gösterdiği ortaya konmuştur.

Koşullu değer belirleme yöntemi (CVM – Contingent Valuation Method), çevresel kaynaklarda meydana gelen iyileşme ve kötüleşmeler için bireylerin ödeme veya kabul eğilimlerini kuramsal senaryo eşliğinde doğrudan sorgulayarak öğrenmeye dayanan, bu şekilde çevresel kaynak kullanımları sonucu toplumsal refahta meydana gelen değişimleri, yani çevresel malların ekonomik değerini belirlemeye çalışan bir çevresel değer belirleme yöntemidir (Stolk ve Markwell, 2007). Bu analitik anket tekniği mal veya hizmetlerin pazarda mevcut olmayan parasal değerini kuramsal koşullarla ortaya koymaktadır (Brock, 1994).

Son yıllarda dalış turizminde popülaritesi giderek artan yapay batık resifleri ile ilgili sosyal ve ekonomik faydaların incelendiği çalışma sayısı oldukça azdır (Kirkbride-Smith, 2014). Bu tez çalışmasında Karaburun Yarımadası'nda 2016 yılında kasıtlı olarak batırılan "9 Eylül" batığının hangi biyolojik özelliklerinin dalıcılar tarafından tercih edildiği belirlenmeye çalışılmıştır. Ayrıca dalıcıların bu batığın ve bulunduğu denizel çevrenin korunması için ödeme istekliliğinde (WTP-Willingness to Pay) bulunup bulunmadıkları araştırılmıştır.

MATERYAL VE METOT

Tez çalışması, farklı seviyelerden dalıcıların ve daha önce hiç dalış yapmamış kişilerin katılımıyla Karaburun'da bulunan "9 Eylül" batığı üzerine gerçekleştirilmiştir. Mart-Haziran 2022 ayları arasında çevrimiçi anket vasıtasıyla yürütülen çalışmada, katılımcıların batıkların hangi biyolojik özelliğinden etkilendiğini belirlemek için "koşullu değer belirleme yöntemi" kullanılmıştır. Anket Google formlar üzerinde hazırlanmış ve uygulanmıştır. Ankette yer alan 8 farklı kuramsal biyolojik özelliğe sahip batık fotoğrafları "Adobe photoshop 2022" programı kullanılarak oluşturulmuştur. Fotoğraflar sırasıyla aşağıda belirtilen biyolojik ve çevresel özellikleri temsil etmektedir (Şekil 1.).



Şekil 1. Farklı biyolojik ve çevresel özellikleri temsil eden fotoğraflar; 1: Orijinal, 2: Mercan, 3: Derin, 4: Büyük Balık (Akya), 5: Küçük balık (Papaz), 6: Biyoçeşitlilik, 7: Tür (Orfoz), 8: Balık bolluğu

Çalışmada kullanılan anket 3 ana başlıktan oluşmaktadır: Demografik yapı (yaş, cinsiyet, eğitim), Dalış tecrübesi (dalış brövesi, yıl bazında dalış tecrübesi, yıllık ortalama dalış sayısı), Fotoğraf seçimidir. Ankette bulunan fotoğraflar batıklar etrafındaki farklı biyolojik özellikleri temsil etmektedir. Anketin son kısmında katılımcılara seçmiş oldukları fotoğraftaki gibi bir batığı korumak için kar amacı gütmeyen bir kuruma ödeme istekliliğinde (WTP) bulunup bulunmayacakları sorulmuştur. Katılımcıların demografik yapılarına ve dalış tecrübelerine göre yaptıkları fotoğraf seçimleri ve ödeme isteklilikleri arasındaki farklılıklar ki-kare testi ile analiz edilmiştir.

BULGULAR VE TARTIŞMA

Ankete toplamda 156 kişi katılmıştır. Katılımcıların büyük bir çoğunluğu erkeklerden oluşmaktadır (%70,5), bu oran geçmiş çalışmalarla (Musa ve ark., 2006; Uyarra ve ark., 2009; Sorice ve ark., 2007; Edney ve Spennemann, 2015) tutarlılık göstermektedir. Katılımcıların büyük bir çoğunluğu Kirkbride-Smith, (2014)'teki gibi 30'lu yaş grubunda (%40,4) yer almaktadır, Katılımcıların çoğunluğu (%82,7) yüksek eğitim seviyesine sahiptir ve bu durum yapay resifler üzerine yapılmış geçmiş çalışmaların sonuçlarına (Ditton et al., 2002; Stolk et al., 2005; Edney, 2012; Polak ve Shashar, 2013; Edney ve Spenneman, 2014) benzerlik göstermektedir. Katılımcıları büyük bir kısmı (%80,8) tam zamanlı çalışandır. Katılımcıları sahip oldukları dalış brövelerine göre incelediğimizde büyük bir çoğunluğunun 2* (%34,6) ve 3* (%24,4) dalıcılar oldukları görülmektedir (Tablo 1.).

Karaburun yapay batık resiflerinden biri olan *"9 Eylül"* bölgenin en çok tercih edilen dalış noktalarından biridir (Şensurat-Genç ve ark., 2022). Bu çalışma ile batığın en çok tercih edilen biyolojik özelliğinin biyoçeşitlilik (%48,1) ve bolluk (%41,7) olduğu belirlenmiştir. Katılımcıların büyük çoğunluğu balık ve omurgasız türlerinin bulunduğu biyoçeşitliliğin zengin olduğu ve balık tür zenginliği ile bolluğun fazla olduğu fotoğrafları tercih etmişlerdir. Ancak ankete katılan kişilerin demografik karakterlerinin ve dalış tecrübelerinin batığın cezbedici biyolojik özelliğinin seçiminde istatistiksel olarak anlamlı bir etkisinin olmadığı tespit edilmiştir ($p>0.05$). Polak ve Shashar (2013)'ın İsrail, Eilat'ta özel tasarımlı beton bir resif üzerinde gerçekleştirdiği benzer bir çalışmada biyoçeşitlilik en çok tercih edilen biyolojik nitelik olarak bulunmuştur. Ancak bizim sonuçlarımızın aksine balık bolluğu Polak ve Shashar (2013) tarafından en az tercih edilen nitelik olarak değerlendirilmiştir.

Tablo 1. Ankete katılanların demografik yapıları ve dalış tecrübeleri

Demografik yapı	Katılımcı sayısı	Dalış tecrübesi	Katılımcı sayısı
Cinsiyet		***Dalış Brövesi***	
Kadın	46	Tanıtım dalışı yapmış	12
Erkek	110	1* eğitim	1
Yaş		1* dalıcı	17
<20	0	2* eğitim	5
20-29	26	2* dalıcı	54
30-39	63	3* eğitim	6
40-49	52	3* dalıcı	38
50-59	12	1* eğitmen	9
>60	3	2* eğitmen	4
Eğitim düzeyi		3* eğitmen	0
İlkokul	0	Hiç dalış yapmamış	10
Ortaokul	1	***Dalış tecrübesi(yıl)***	
Lise	26	0	1
Lisans	80	1-5	53
Yüksek lisans	34	6-10	36
Doktora	15	11-15	20
Meslek		16-20	19
Öğrenci	17	>21	9
Yarı zamanlı	8	***Dalış sayısı(yıllık)***	
Tam zamanlı	126	0	19
İşsiz	5	1-10	48
Aylık gelir		11-20	26
<4250	13	21-30	20
4250	6	>31	43
4251-8500	22		
>8500	115		

Bu çalışmada ankete katılım sağlayan kişilerin %83'ü seçmiş oldukları fotoğraftaki batığın mevcut durumunun koruması için ödeme istekliliğinde olduklarında belirtmişlerdir. Katılımcıların demografik yapılarına (cinsiyet, yaş, eğitim, meslek, aylık gelir) ve dalış tecrübelerine (bröve, yıllık tecrübe, bir yılda gerçekleştirilen dalış sayısı) göre gösterdikleri ödeme istekliliği arasında istatiksel bir fark bulunamamıştır ($p>0.05$). Ancak kadınları %78'i 50 TL ve üzerinde ödeme istekliliğinde bulunurken, erkeklerde bu oran %67 olarak belirlenmiştir. Bunun yanı sıra acemi olarak tanımladığımız dalıcıların (1yıldız eğitim alan, 1 yıldız, 2 yıldız eğitimi alan) %65'i 50 TL ve üzeri ödeme istekliliğinde bulunurken, tecrübeli olarak tanımladığımız dalıcılarda (2 yıldız, 3 yıldız eğitimi alan, 3 yıldız, 1 yıldız eğitmen, 2 yıldız eğitmen) bu oran %74 olarak belirlenmiştir.

SONUÇ

Batıklar dalıcılar için hâlihazırda cezbedici yapılardır (Polak ve Shashar, 2012). Yapay resif batıkları, özellikle büyük donanma gemileri rekreasyonel dalıcılar tarafından en çok tercih edilen yapay resiflerdir (Polak ve Shashar, 2012; Ditton ve ark., 2002). Bu çalışma, insanların bir yapay batık resifinin biyolojik ve çevresel niteliklerini algılaması ve değerlendirmesi üzerinedir.

Yapay resiflerin cazibesi, doğal ve korunması gereken alanlar üzerindeki baskıyı azaltmada kritik bir öneme sahiptir (Polak ve Shashar, 2012). Halkın ilgisini çekmek ve denizler hakkındaki farkındalığı arttırmak için uygulanacak projeleri hazırlarken, potansiyel kullanıcılar tarafından en çok değer verilen biyolojik özelliğin bilinmesi önem taşıyacaktır. Orfoz ve akya gibi türe özgü koruma ve üretim çalışmalarından ziyade gelecekte gerçekleştirilecek sosyal farkındalık çalışmalarında ya da bilimsel araştırmalarda biyoçeşitliliğe odaklanmak hem denizel çevre için hem de biz nihai kullanıcılar için daha faydalı olacaktır.

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ORAL PRESENTATION

Alginate-carboxymethylcellulose particles prepared by ionotropic gelation for the efficient delivery of ciprofloxacin

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Abstract

Ciprofloxacin (CP) is an inexpensive broad-spectrum antibiotic that belongs to the fluoroquinolone category. Due to the low absorption rate of CP in the body, its oral bioavailability is a maximum of 70-80%. For this reason, it is essential to use high doses and frequently for treatment. However, CP has a dose-dependent toxicity that causes many undesirable side effects. The aim of this study was to perform encapsulation studies to increase the bioavailability and reduce the negative side effects of CP and to prepare a controlled oral release using the ionotropic gelation method. For this purpose, a biocompatible, non-toxic, highly biodegradable, environmentally friendly and cost-effective alginate (ALJ) and a carboxymethylcellulose (CMC) complex were selected as a drug carrier system and calcium chloride (CaCl2) was used as a gelling agent. In the study, ALJ-CMC concentrations were then evaluated for encapsulation efficiency and controlled drug release. The prepared macroparticles were analysed by UV-VIS to calculate the encapsulation. At the end of the study, the highest encapsulation efficiency was 91.5% and the highest cumulative release was 30%.

Keywords: Encapsulation, ciprofloxacin, alginate, carboxymethyl cellulose, ionotropic gelation

INTRODUCTION

Since its launch in 1987, ciprofloxacin (CP) has become one of the most widely used antibiotics in the world, especially in patients undergoing chemotherapy. Ciprofloxacin, a member of the fluoroquinolone category, is an effective broad-spectrum antibiotic that is active against Gramme-negative and Gramme-positive bacteria (Badawy et al., 2021; Igwegbe et al., 2021). The typical side effects of CP are severe headaches, insomnia, dizziness, nausea, tendonitis and tendon ruptures. Since its oral bioavailability is about 70%, it is administered in high and frequent doses, especially in cancer patients (Ball 1986; Drusano et al., 1986) and taking the drug in high doses and frequently leads to significant health problems for patients.

Over the past five years, researchers have conducted numerous studies on the transportation and delivery of ciprofloxacin to address these issues. In addition, various studies have shown that the specific carrier polymer and drug loading method play a critical role in determining the efficiency, release, stability, tolerability and targeted delivery of drugs in drug carriers. Ionotropic gelation is considered a cost-effective and simple method for drug loading among the various methods (Pedroso-Santana and Fleitas-Salazar, 2020). Alginate (ALJ) is a commonly used drug carrier polymer in this technique, known for its biocompatibility and sensitivity to pH. It readily forms gels with divalent cations, has a large pore size for high drug delivery capacity, and is cost-effective and user-friendly (Raus et al., 2021). Recently, ALJ has been used in drug delivery studies alongside carboxymethylcellulose (CMC), a biocompatible, viscosity-regulating, abundant and inexpensive polymer that remains stable over a wide pH range (Silva et al., 2019; Rahman et al., 2021; Morozkina et al., 2022; Ullah et al., 2024).

The aim of this study is to develop novel drug delivery systems that are biocompatible, affordable, easy to manufacture and suitable for large-scale production. This will be achieved by using the complex of alginate and carboxymethylcellulose, which has not been used for controlled release before, and the ionotropic gelation technique.

MATERIALS AND METHODS

Materials

All chemicals used were standard analytical grades. Carboxymethylcellulose sodium salt (CMC) (low viscosity) and sodium alginate (ALJ) (medium viscosity) are from Sigma Aldrich, USA; ciprofloxacin (98%) (CP) is from TCI, USA; calcium chloride ($CaCl_2$) is from Detsan Kim., Türkiye.

Preparation of CP-loaded ALJ-CMC Microspheres and Determine Encapsulation Efficiency

First, ALJ and CMC solutions prepared (Figure 1) at different concentrations (Table 1). These solutions, mixed in the ratio ALJ: CMC (w/w), are allowed to mix for 1 hour at room temperature using a magnetic stirrer to prepare the polymer solution. Then add 20 mg of CP to the solution and mix with a magnetic stirrer until it is completely dissolved. The prepared solution is then dropped into a 3% (w/v) calcium chloride ($CaCl_2$) gelation solution using an 18G syringe from a specific distance and at a specific rate. The solution is mixed for 30 minutes to allow the particles to harden. They are then filtered, and the particles are dried in an oven. Subsequently, 100 mg of the dry particles were weighed and placed in a PBS solution (pH=7.4) and shaken in a water bath at 37 °C for 24 hours to determine the encapsulation efficiency. After 24 hours, the solution was sonicated in an ultrasonic homogenizer at an amplitude of 20% (AM) for 5 minutes to ensure that all the active ingredient in the particles had been transferred to the solution. The solution was then centrifuged to separate the remaining solids from the solution and the supernatant was analysed by UV-VIS spectroscopy at 320 nm. The encapsulation efficiency values (%) were calculated using the following equation:

$$Encapsulation\% = \frac{Actual\ amount\ of\ drug\ in\ beads(mg)}{Theoritical\ amount\ of\ drug\ in\ beads(mg)} \times 100 \quad (1)$$

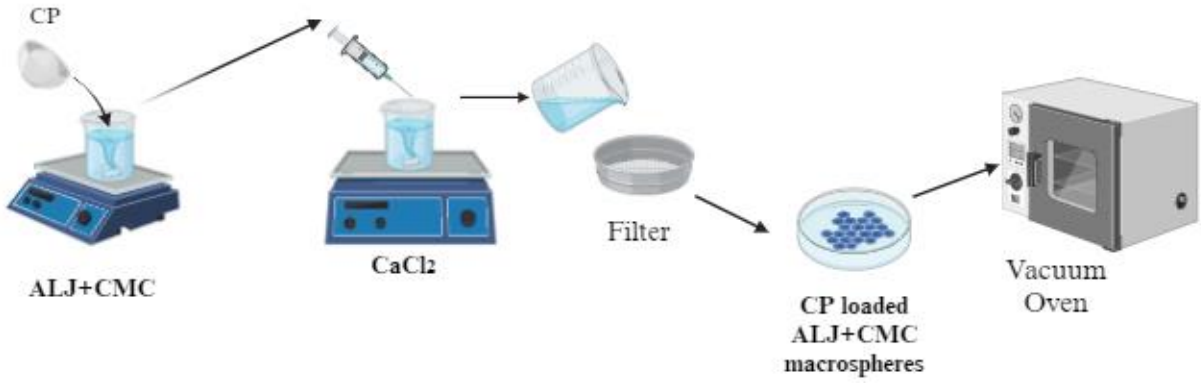

Figure 1. Graphical represent of preparing CP loaded ALJ/CMC macrospheres

Table 1. Experimental sets and their conditions

Formula Code	ALJ: CMC ratio	$CaCl_2$ (w/v)	CP (mg)
F1	1:1	3	20
F2	1:2	3	20
F3	2:1	3	20

Swelling studies

For the swelling studies, particles were randomly selected from each experimental setup and these particles were weighed. The selected particles were then immersed in a 10 ml solution of simulated gastric fluid (SGF) for 48 hours. At the end of the 48 hours, the particles were filtered and weighed. Calculations were then performed to determine the percentage of swelling according to equation (1).

$$Swelling\% = \frac{The\ mass\ at\ \ time\ t - Initial\ mass}{Initial\ mass} \times 100 \quad (2)$$

In vitro drug releases studies

For the controlled drug release studies, the initially dried and then weighed particles were selected and subjected to release in a SGF consisting of 0.1 N (pH=1.2) 100 ml HCl (containing 0.4 gr NaCl) at 37°C with stirring at 100 rpm using a shaking water bath for 4 hours. For the release calculations, samples of 4 ml were taken from the release medium at specific time intervals and an equal volume of fresh release solution was added to the medium. The samples were analysed at 354 nm by UV-VIS spectroscopy and the cumulative drug release was calculated.

Characterization

Fourier transform infrared spectroscopy (FTIR) was used to identify the bonding structures and chemical properties of the particles. The analysis was performed using a Thermo Scientific Nicolet IS10 FTIR spectrometer with an Attenuated Total Reflectance (ATR) accessory. The spectrometer scanned the wavenumber range from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} over 32 scans.

RESULT AND DISCUSSION

Characterization

The FT-IR spectrum (Figure 2) shows that a broad hydroxyl band around 3300 cm^{-1} was observed in all samples, originating from OH vibrations in water and polymer structures. The peak around 1600 cm^{-1} is related to symmetric and asymmetric vibrations of aromatic C=C or C=O ions in the structure of alginate and CMC. The peaks observed at 1020 cm^{-1} in alginate and CMC originate from C-O stretching in the structure of the two polymers. When examining the spectrum of CP, a band is observed at 3350 cm^{-1} which originates from H-N stretching in its structure. Bands were observed at 1200 cm-1 due to C-H stretching at 1478 cm^{-1} and the presence of C-F groups. When the spectra of drug-loaded and unloaded particles were examined, the CP-like bands observed especially between 1500 cm^{-1} and 600 cm^{-1} in drug-loaded particles indicate that the drug was successfully loaded into the particles. Moreover, the presence of OH, C=C, C=O and C-O groups in the drug-loaded particles proves that drug loading does not affect the bonding.

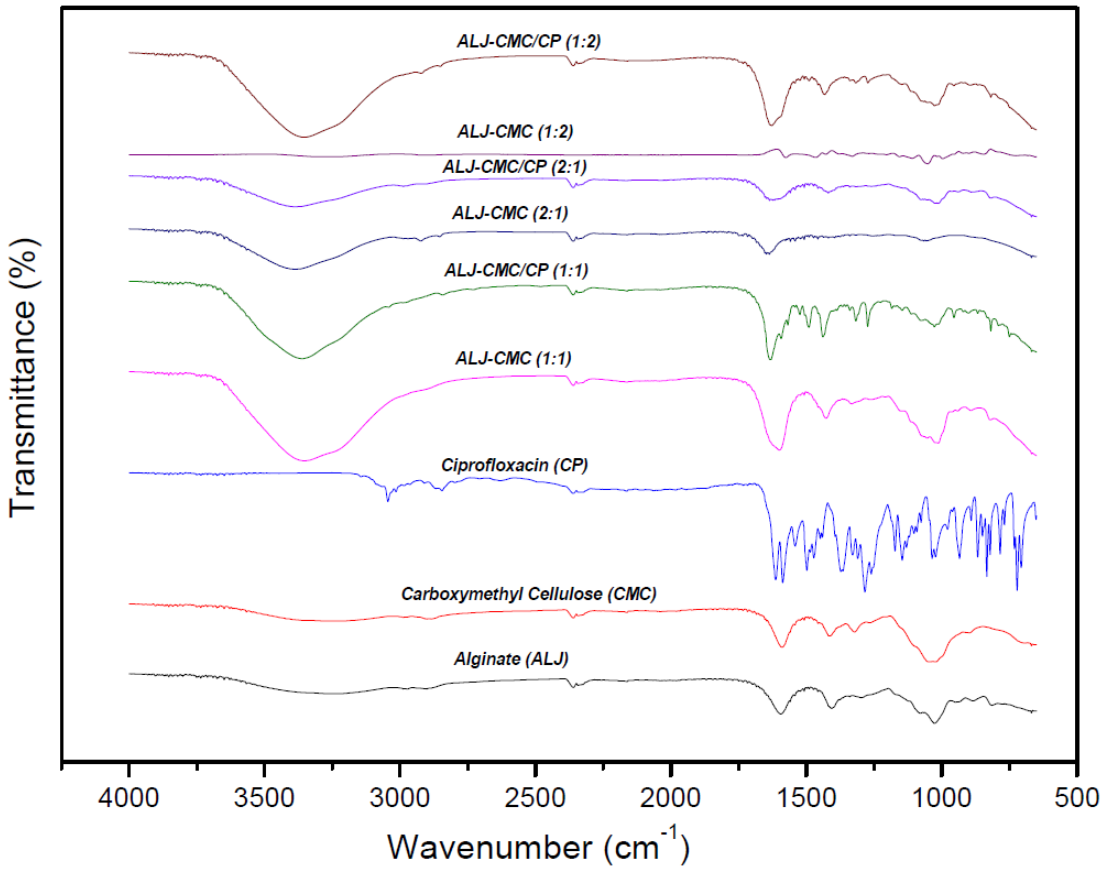

Figure 2. FT-IR spectra of hydroxyapatite-silica composites

Swelling studies

The swelling behaviour of the polymer matrix is directly related to the release of drugs from the matrix. The greater the swelling capacity of the polymer matrix, the more drug is theoretically released from the matrix (Hanna and Saad 2019). Examination of the results (Figure 3) shows that the highest swelling percentage is 120.5 achieved with F3 and the lowest is 113.1 with F2.

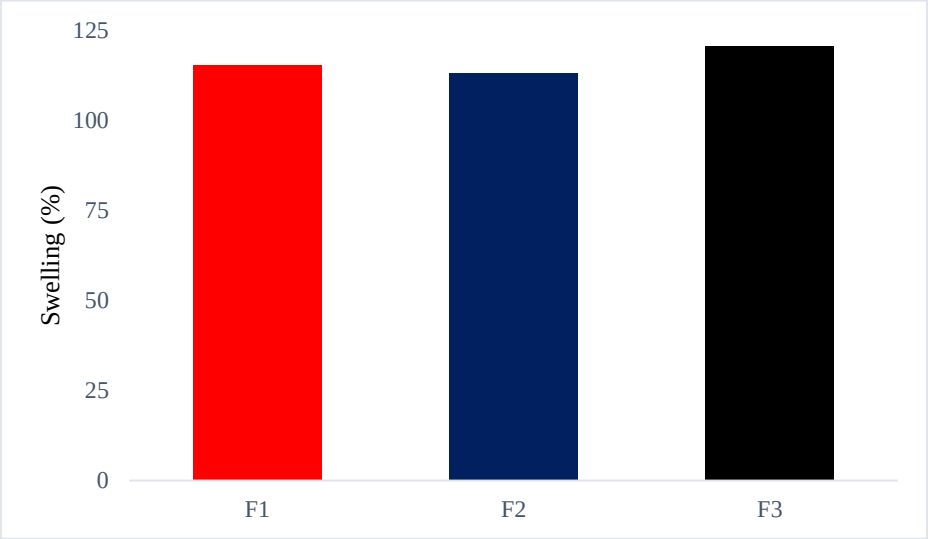

Figure 3. Swelling (%) behaviour of formulations

Drug loading and encapsulation efficiency of CP

The highest and lowest encapsulation efficiencies (Figure 4) were found to be 91.5% and 37.7%, respectively. The concentration at which the best loading occurred was observed at a ratio of 2:1, where the alginate concentration was high. In addition, the data obtained on the degree of swelling (Figure 2) support the results of encapsulation efficiency.

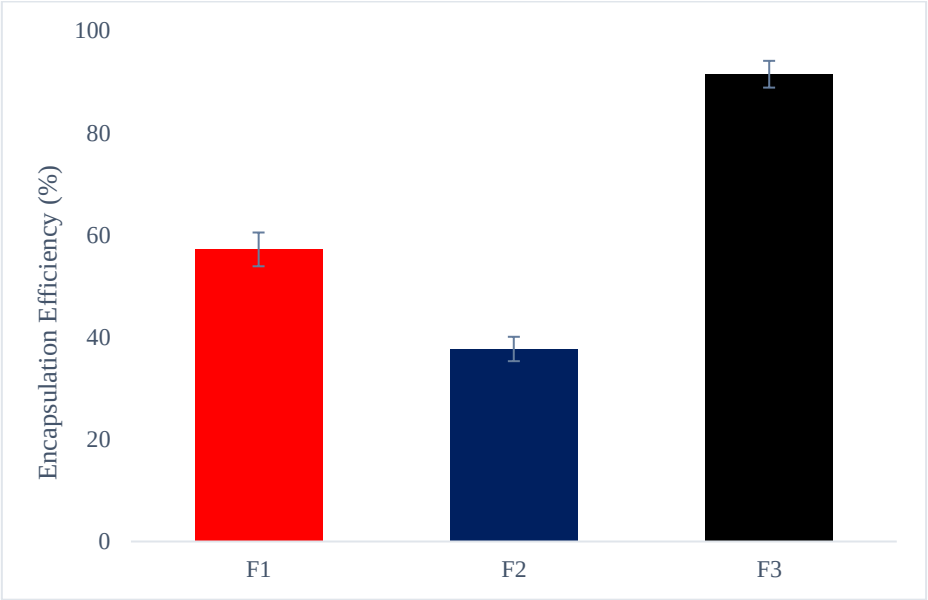

Figure 4. Encapsulation efficiency (%) of formulations

Controlled drug release studies

As can be seen in Figure 5, particles with a concentration of 1:1 (F1) released almost all of the drug in the first 15 minutes and reached a cumulative release of 30% at the end of the release. For particles with a concentration of 1:2 (F2), the burst release was reduced, and a more controlled release was achieved, reaching a cumulative release of 21% at the end of the release. At a concentration of 2:1 (F3), no burst release was observed, but a controlled release was achieved, although a cumulative release of 6% was achieved at the end of the release.

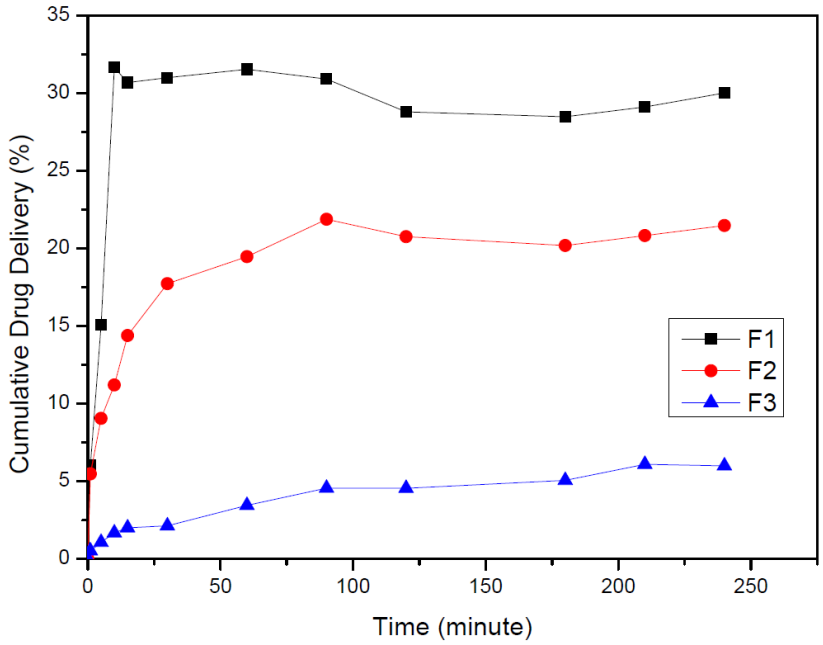

Figure 5. Controlled drug release

CONCLUSION

Due to the low absorption rate of CP in the body, its oral bioavailability is a maximum of 70-80%. For this reason, it is essential to use high doses and frequently for treatment. However, CP has a dose-dependent toxicity that causes many undesirable side effects. In this study, CP was loaded into alginate/carboxymethylcellulose macrospheres using the ionotropic gelation method. An attempt was made to find the best formulation by investigating the concentrations and ratios of ALJ and CMC polymers. The encapsulation efficiency increased with increasing alginate concentration. The highest encapsulation efficiency was achieved at 91.5%, while the highest cumulative drug release was 30%. This study shows that the undesirable side effects of CP can be avoided with this new drug release system.

ACKNOWLEDGEMENTS,

This study was supported by TÜBİTAK 2209-A Research Project Support Programme for Undergraduate Students under grant number:1919B01230456

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ORAL PRESENTATION

Pesticide Biodegradation: Comparing Theoretical Models with Experimental Data

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Abstract

This study investigates the biodegradation potential of five pesticides, namely carbofuran, chlorpyrifos, cyprodinil, malathion, and propanil under aerobic conditions. The research approach combines theoretical predictions using the BIOWIN models from the Estimation Programs Interface Suite with experimental data obtained from biodegradation experiments using biomass enriched from the water in a drinking water treatment plant. The study revealed varying degrees of agreement between theoretical estimations and experimental findings. Carbofuran and cyprodinil showed good correlation between predicted and observed biodegradation rates. Propanil demonstrated higher biodegradation potential in experiments than predicted by BIOWIN models. Malathion exhibited slightly slower biodegradation in experiments compared to model predictions. These findings highlight the importance of combining theoretical models with experimental validation in assessing pesticide biodegradation. The study underscores the complexity of predicting environmental fate for diverse chemical compounds and emphasizes the need for integrated approaches in environmental risk assessment and management of pesticides.

Keywords: Biodegradation, BIOWIN models, Drinking water, Pesticides

INTRODUCTION

Pesticides, often detected as micropollutants in drinking water treatment plant influents, pose a significant challenge to conventional water treatment processes (Jatoi et al., 2021; Sishu et al., 2022; Stehle & Schulz, 2015). Many of these facilities are not designed to effectively eliminate pesticides, potentially leading to human health risks. Biodegradation stands out as a crucial mechanism for removing micropollutants. This study aims to evaluate the biodegradation potential of five selected pesticides using BIOWIN models, a component of the EPI (Estimation Programs Interface) Suite program, and compare these theoretical predictions with experimental data. The selected pesticides are carbofuran (CBF), chlorpyrifos (CPF), cyprodinil (CYP), malathion (MLT), and propanil (PRP).

BIOWIN module is composed of six aerobic biodegradation prediction models, designed to estimate a substance's biodegradability under aerobic conditions in mixed microbial populations (US EPA,2012; Balakrishnan et al., 2020). Our research compares these model predictions with laboratory biodegradation experiments conducted using bacterial populations enriched from a drinking water treatment plant. This comparative analysis seeks to assess the accuracy and applicability of BIOWIN models in predicting real-world biodegradation processes for pesticides in water treatment contexts.

By bridging the gap between theoretical models and experimental data, this study aims to enhance our understanding of pesticide biodegradation in water treatment systems. The findings could contribute to improving risk assessment methodologies and inform the development of more effective strategies for pesticide removal in drinking water treatment processes.

MATERIALS AND METHODS

This study compares theoretical predictions of pesticide biodegradation with experimental data. The BIOWIN approach, which deconstructs molecules into fragments to assess biodegradability, was employed to generate numerical indicators of compound degradation likelihood (Pavan and Worth, 2008). These theoretical predictions were then compared with results from practical biodegradation experiments.

Experimental work utilized bacterial populations sourced from the Ömerli dam, Istanbul's primary drinking water reservoir. To enhance bacterial growth, water samples were enriched with a synthetic drinking water containing both organic and inorganic compounds. Biodegradation experiments were conducted in 200 mL volumes with a bacterial concentration of 500 mg/L volatile suspended solids (VSS) at 25°C.

Individual experiments for each pesticide were run over a 26-day period. Daily sampling and subsequent analysis using Liquid Chromatography-Mass Spectrometry (LC-MS/MS) allowed for precise monitoring of pesticide concentrations throughout the experimental duration. This comprehensive approach enabled a robust comparison between theoretical predictions and actual biodegradation rates observed under controlled laboratory conditions.

RESULTS AND DISCUSSION

The outputs of BIOWIN models are given in Table 1. According to the results, malathion is the only pesticide that has the capacity of rapid biodegradation. According to the primary biodegradation model (BIOWIN4) pesticides other than MLT can biodegrade in days to weeks, whereas biodegradation of MLT takes places in days. In addition, the BIOWIN 3 values, which indicates ultimate biodegradation, classifies CPF as refractory.

Table 1. BIOWIN outputs of studied pesticides.

	BIOWIN 1 (Linear Probability Model)	**BIOWIN 2** (Non-linear Probability Model)	**BIOWIN 3** (Ultimate Biodegradation Model)	**BIOWIN 4** (Primary biodegradation Model)	**BIOWIN 5** (MITI linear model prediction)	**BIOWIN 6** (MITI non-linear model prediction)	**Readily Biodegradability Prediction**
	> 0.5:fast biodegradation < 0.5: not to degrade fast		5-4.75: hours 4.75-4.25: hours to days 4.25-3.75: days 3.75-3.25: days to weeks 3.25-2.75: weeks 2.75-2.25: weeks to months 2.25-1.75: months < 1.75: refractory		> 0.5: rapidly biodegradable < 0.5: not to rapidly biodegradable		Yes or No
CBF	0.7244	0.8785	2.3184	3.5773	0.2435	0.1936	No
CPF	0.1928	1.0000	1.7442	3.2925	-0.0832	0.0014	No
CYP	0.6440	0.7018	2.4387	3.2821	0.0047	0.0310	No
MLT	1.2526	1.0000	2.9033	4.2944	0.6707	0.5691	Yes
PRP	0.4890	0.1934	2.2498	3.4078	0.2768	0.0749	No

The experimental results revealed varying biodegradation rates among the studied pesticides. In order of increasing removal percentage, the findings were as follows: CYP exceeded 51%, CBF reached 60%, MLT achieved 68%, and CPF attained 88% removal. Notably, PRP exhibited the highest biodegradation efficiency, with more than 99% removal.

Key findings reveal varying degrees of agreement between theoretical estimations and experimental results:

- CBF and CYP showed good correlation between predicted and observed biodegradation rates.
- PRP demonstrated higher biodegradation potential in experiments than predicted by BIOWIN models.
- MLT exhibited slightly slower biodegradation in experiments compared to model predictions.
- CPF showed significant biodegradation in experiments, contrary to its classification as refractory by BIOWIN models.

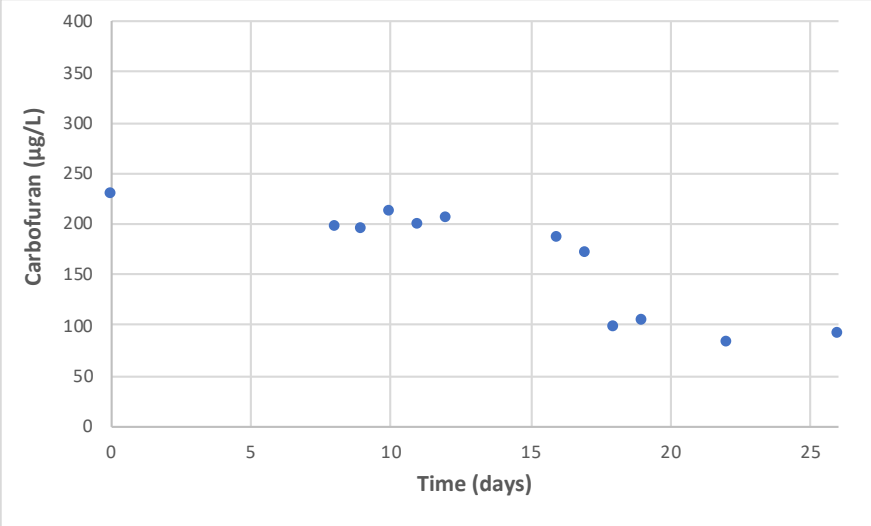

Figure 1. Biodegradation of carbofuran (CBF).

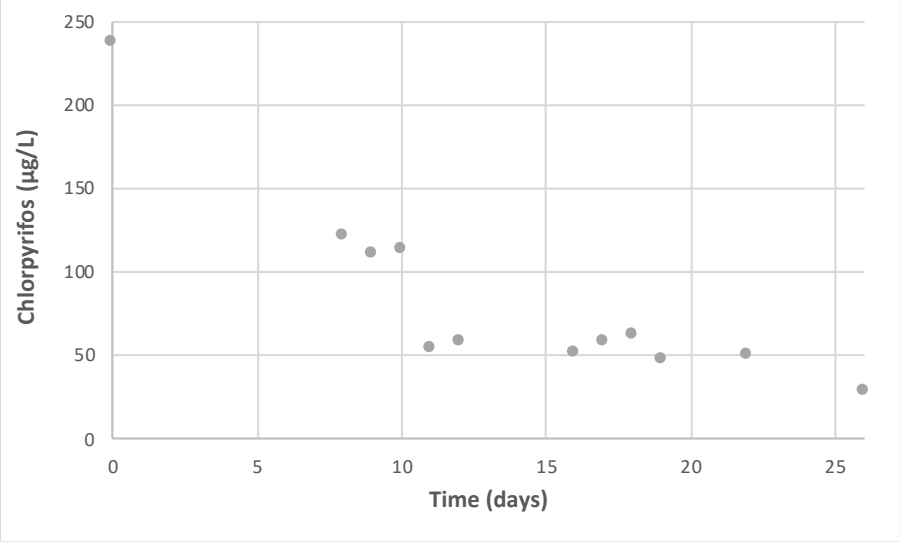

Figure 2. Biodegradation of chlorpyrifos (CPF).

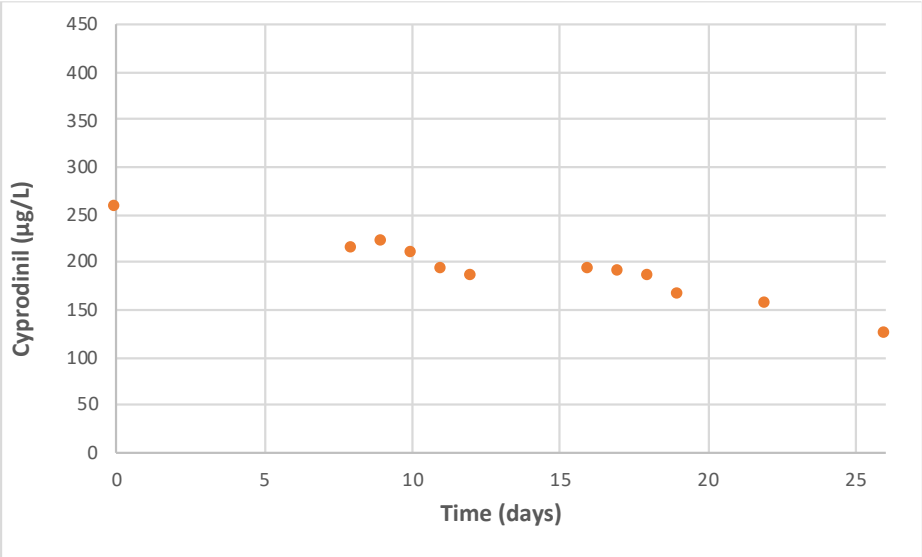

Figure 3. Biodegradation of cyprodinil (CYP).

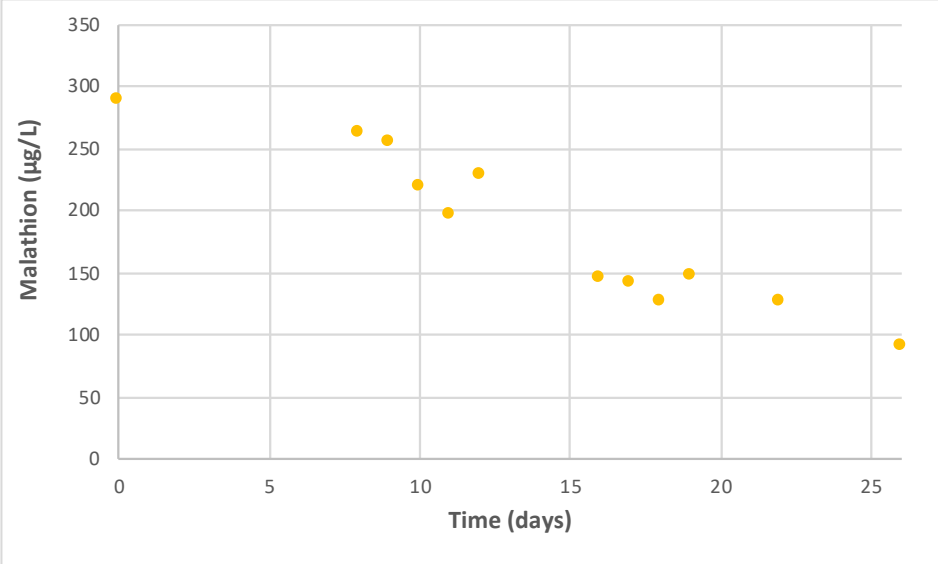

Figure 3. Biodegradation of malathion (MLT).

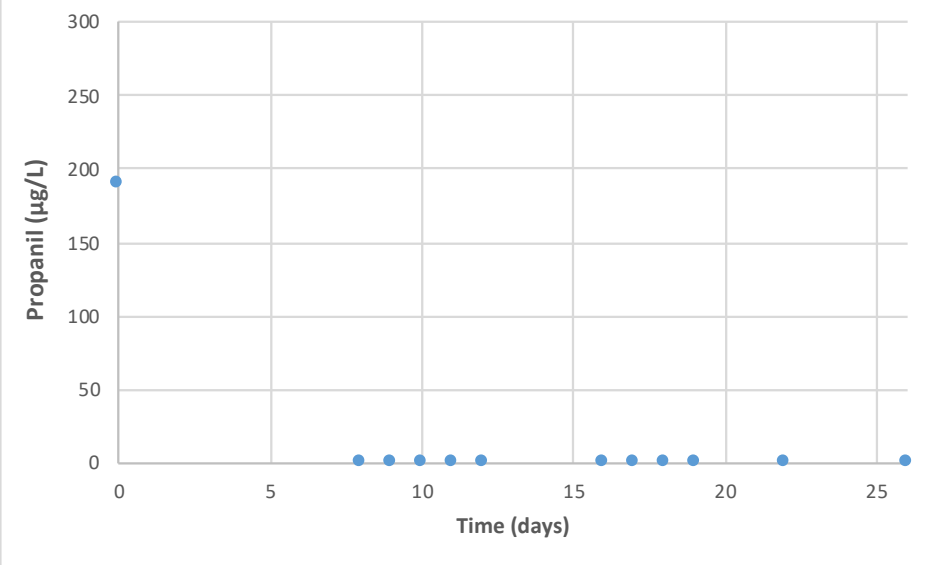

Figure 3. Biodegradation of propanil (PRP).

CONCLUSION

These experimental outcomes provide valuable insights into the actual biodegradation behavior of the selected pesticides under controlled laboratory conditions. The observed differences in degradation rates among the compounds highlight the complexity of pesticide biodegradation processes and underscore the importance of considering individual chemical properties when assessing environmental persistence and developing remediation strategies.

Comparing these experimental results with the BIOWIN model predictions reveals both consistencies and discrepancies, emphasizing the need for integrating theoretical models with empirical data to achieve a more comprehensive understanding of pesticide biodegradation dynamics in real-world scenarios.

ACKNOWLEDGEMENTS

This project is supported by Boğaziçi University Research Fund Grand Number: 19Y00D5.

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ORAL PRESENTATION

Farklı elma dokularından DNA izolasyonunun genomik DNA verimi ve kalitesi üzerine etkileri

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Özet

Organizmanın toplam genomunu temsil eden genomik DNA izolasyonunun etkinliği moleküler çalışmalar için oldukça önemlidir. Elma bitkisi (Malus domestica) DNA izolasyonu ve PCR amplifikasyonu gibi moleküler çalışmaları engelleyebilecek yüksek miktarda polisakkarit, polifenol ve diğer ikincil metabolitleri içerir. Bu çalışmada, her doku tipinden DNA elde etmek ve kalitesini belirlemek için tomurcuk, dal, meyve, meyve çekirdeği, meyve kabuğu ve yaprak olmak üzere altı farklı elma dokusundan DNA izolasyonu yapılmıştır. DNA ekstraksiyonu, bitki dokuları için optimize edilmiş standart bir CTAB DNA izolasyon protokolü kullanılarak gerçekleştirilmiştir. İzole edilen DNA, spektrofotometrik yöntemler ve agaroz jel elektroforezi ile DNA verimi, saflığı ve bütünlüğü açısından analiz edilmiştir. Elde edilen sonuçlar, dokulardan izole edilen DNA'nın hem miktar hem de kalitesinde farklılıklar olduğunu, genel olarak dal ve tomurcuk örneklerinden elde edilen DNA'ların hem konsantrasyon hem de saflık açısından en iyi sonuçları verdiğini ortaya koymuştur. Bu bulgular, ekstraksiyonda kullanılan doku tipinin elmalarda DNA izolasyonunun başarısını belirlemede önemli bir rol oynadığı ve bu çalışmanın sonuçlarının moleküler ıslah programları için yararlı bilgiler oluşturacağı öngörülmektedir.

Anahtar Kelimeler: Bitki dokusu, DNA izolasyonu, Elma, Malus domestica

Effects of DNA isolation from different apple tissues on genomic DNA yield and quality

Abstract

The efficiency of genomic DNA isolation, representing the organism’s total genome, is crucial for molecular studies. Apple (Malus domestica) contains high levels of polysaccharides, polyphenols, and other secondary metabolites that can hinder DNA isolation, PCR amplification, and subsequent molecular analyses. In this study, DNA was extracted from six different apple tissues—bud, branch, fruit, fruit seed, fruit skin, and leaf—to evaluate DNA yield and quality from each tissue type. DNA extraction was performed using a standardized CTAB DNA isolation protocol optimized for plant tissues. The isolated DNA was analyzed for yield, purity, and integrity using spectrophotometric methods and agarose gel electrophoresis. The results revealed that the quantity and quality of DNA isolated from different tissues varied, with DNA from branch and bud samples generally yielding the best results in terms of both concentration and purity. These findings suggest that tissue type plays a critical role in determining the success of DNA isolation in apples, which may provide valuable insights for molecular breeding programs.

Keywords: Plant tissue, DNA isolation, Apple, *Malus domestica*

GİRİŞ

Elma (Malus domestica) dünya çapında yaygın olarak yetiştirilen ve çok sayıda çeşide sahip olan meyve türlerinden biridir (Kikuchi ve ark., 2017). Var olan çok sayıda çeşidin karakterize edilmesi, sınıflandırılması ve moleküler markör teknolojisi kullanılarak yeni çeşitlerin kazanılması, tarımsal çalışmalar için oldukça önemlidir. Bu süreç genetik çeşitliliğin korunmasında etkilidir (Ateş Sönmezoğlu ve Kütük, 2014). Ayrıca, bitki çeşitliliğini ve adaptasyon yeteneğini artırarak, tarımsal verimliliği sürdürülebilir bir şekilde yükseltmeye

ve tarımsal üretkenliği artırmaya yönelik stratejiler geliştirilmesinde kullanılabilir (Dayteg ve ark., 2007; Abdel Latif ve Osman, 2017).

Moleküler ıslah çalışmalarının ilk adımı çeşitli bitki türlerinden genomik DNA izolasyonudur. Fakat, hücre lizisinden sonra DNA'ya bağlanabilen fenolik terpenoidler gibi ikincil bitki ürünleri sebebiyle üzüm (Vitis spp.), elma (Malus spp.), armut (Pyrus spp.) ve iğne yapraklılar benzeri yüksek polifenolik içeriğe sahip bitkilerden kaliteli DNA izole etmek zordur (Kim ve ark., 1997). Fenolik bileşikler, DNA ekstraksiyonu sırasında nükleik asitlerle etkileşime girerek DNA'nın bozulmasına yol açabilir. Bu nedenle, bu bitkilerden yüksek miktarda ve saf DNA elde edebilmek için klasik CTAB yönteminin (Doyle ve Doyle, 1990) bazı değişiklikler ile (Drábková, 2014) iyileştirilmesi gerektiği vurgulanmaktadır (Kiy ve Akbulut, 2024). Bununla birlikte, DNA ekstraksiyon yöntemlerinin farklı bitki türleri veya bitkinin farklı dokuları ile kullanıldığında daha ileri moleküler markör uygulamaları için farklı sonuçlar vereceği literatürde tartışılmıştır (Pipan ve ark., 2017).

Meyve ağacı türlerindeki yapraklardan elde edilen genomik DNA'lar için DNA profillemesi yapılmıştır. Örneğin, Cheng ve ark. (2003), Citrus türleri ve diğer tropikal/suptropikal meyveler için yüksek kaliteli DNA izolasyonunun zorluklarını ortaya koymuş ve bu zorlukların aşılması için suyla doymuş eter ve 1,25 M NaCl kullanılarak polisakkaritleri çıkarmak için bir saflaştırma adımı kullanan değiştirilmiş bir CTAB veya SDS prosedürü önermişlerdir. Bu yöntem kullanılarak, erik, nar, mango elma, hurma gibi meyve türlerinin genç yaprakları, yaşlı yaprakları, donmuş yaşlı yaprakları ve solmuş yaşlı yapraklar dahil farklı doku türlerinden DNA izolasyonu yapmışlardır. Ancak taze ve işlenmiş meyvelerden elde edilen DNA'lar için çok sayıda uygulama bulunmamaktadır (Yamamoto ve ark., 2006). Yamamoto ve ark.(2006), armutta taze ve işlenmiş meyveler için bir DNA profili yöntemi geliştirmişlerdir. Çalışmalarında genç yapraklardan izole edilen genomik DNA miktarı, taze meyve örneklerinden elde edilene göre çok daha fazla bulunmuştur. Genomik-tip yöntemi ile taze meyvelerden izole edilen DNA miktarı, yapraklardan izole edilenin sadece %4'ü kadardır. Bu da genç yaprakların, taze meyvelere kıyasla DNA izolasyonu açısından çok daha verimli bir doku olduğunu göstermiştir. Ancak araştırmadaki doku örneğine ve örnek alınması gereken zamana göre meyve ve yaprak dışındaki farklı bitki kısımlarından da DNA izolasyonu yapılması gerekmektedir.

Bu çalışmada elmanın altı farklı bitki dokusundan (tomurcuk, dal, meyve, meyve çekirdeği, kabuk ve yaprak) optimize edilmiş CTAB protokolü kullanılarak DNA izole edilmiştir. Spektrofotometrik yöntemler ve agaroz jel elektroforezi ile elde edilen sonuçlar incelenen tüm doku örnekleri için, izole edilen DNA'nın miktarı, saflığı ve bütünlüğü incelenmiştir.

MATERYAL VE METOT

Bitki Materyali

Bu çalışmada Karaman Tarım İl Müdürlüğü'nün elma koleksiyonundan temin edilen ve referans çeşitlerden biri olan Golden Delicious çeşidi kullanılmıştır. Genomik DNA izolasyonu için elma ağacının genç yaprakları, olgunlaşmış taze meyvesi, meyve kabuğu, meyve çekirdeği, yeşil tomurcukları ve ağaç dalı vasküler (kabuk) dokusu kullanılmıştır. Her örnek için kullanılan doku miktarı 100 mg olarak belirlenmiş, sıvı azotla öğütülmüş ve bekletilmeden kullanılmıştır.

DNA İzolasyon Metodu

DNA izolasyonu elma örneklerinin farklı dokularından Doyle ve Doyle (1990)'nın yöntemine göre bazı küçük değişikliklerle yapılmıştır. Likit nitrojenle öğütülmüş doku örnekleri üzerine 500 µl Liziz buffer (%2 (w/v) CTAB, 10 mM Tris-HCl (pH 8,0), 150 mM NaCl, 2 mM EDTA (etilendiamintetraasetik asit, pH 8,0), %1 β-merkaptoetanol) eklenmiştir. Ardından bir ünite Proteinaz K (Thermo Scientific) eklenmiş ve vortekslenmiştir. 80 µl % 10 SDS (Sigma-Aldrich) ardından 65 oC su banyosunda 1 saat tutulmuş ve ara sıra alt üst ederek karıştırılmıştır. Su banyosundan çıkarılan tüplere 2/3 hacim kloroform:isoamil alkol (24:1) (Merck) eklenerek, 5-10 dakika alt üst edilmiştir. 10.000 g'de 15 dakika santrifüj ardından süpernatant 2/3 hacim yani 2-propanol (Sigma-Aldrich) içeren yeni bir tüpe alınmıştır. Bu aşamada alt üst edilerek DNA gözle görülür hale getirilmiştir. 15 dakika 10.000 g'de santrifüj sonrası sıvı kısım döküldü. Pelet kuruduktan sonra 500 µl 1 x TE eklenmiş ve su banyosunda 65oC'de 2 saat tutulmuştur. Her bir tüpe 10 mg/ml RNase (Thermo Scientific) çözeltisinden 1 µl eklenmiş ve DNA 65oC'de su banyosunda 1 saat inkübe edilmiştir. Ardından kloroform: isoamil alkol (24:1) aşaması tekrar edilmiş ve örnekler 15 dakika 10.000 g'de santrifüj edilmiştir. Süpernatant 26 µl 5 M NaCl içeren yeni tüplere tüpe alınmış ve hafifçe karıştırılmıştır. Ardından örneklere % 96'lık soğuk etil alkol ilave edilmiş ve DNA çökeltilmiştir. 10.000 g'de 20 dakika santrifüj edilmiştir. Pelet % 70 soğuk etil

alkol ile dikkatlice yıkanmış ve kurutulmuş, son olarak kuruyan pelet 100 µl 1 x TE' içinde çözdürülmüş ve DNA'lar elde edilmiştir.

DNA Konsantrasyon ve Saflık Ölçümleri

İzole edilen DNA'nın konsantrasyonu ve kalitesinin belirlenmesi için jel elektroforez yöntemi ve spektrofotometrik tekniklerden faydalanılmıştır. Bu amaçla, örneklerin 230-260-280 nm dalga boylarında NanoDrop (Denovix, DS-11 Spektrofotometre) üç tekrarla kantitatif ölçümleri alınmıştır (Wilfinger ve ark., 2006). Genomik DNA'lar 1 x TBE tamponu içeren %1'lik agaros jellerde yürütülmüş ve jeller görselleştirme için etidyum bromür (20 mg/ml) ile boyanmıştır. Analiz sonrasında jeller UV transillüminatör (BioRad, ChemiDocTMMP Görüntüleme Sistemi) kullanılarak görüntülenmiştir.

BULGULAR ve TARTIŞMA

Agaroz jel elektroforezi, izole edilen DNA'nın miktar ve kalitesini değerlendirebilmek için moleküler biyoloji çalışmalarında en çok kullanılan rutin yöntemlerden biridir. Elde edilen bantlar, DNA miktarının ve bütünlüğünün değerlendirilmesine olanak tanımaktadır. Bu sebeple bu çalışmada örneklerin DNA'larının bant bütünlüğünün değerlendirilmesinde kullanmıştır.

Şekil 1'de farklı elma dokularına ait genomik DNA'ların %1'lik agaroz jel görüntüsü verilmiştir. Buna göre, ilk kuyucuk 1 kb DNA Ladder (Thermo Scientific), 1-12 arası kuyucuklar 2'şer tekrarlı olmak üzere sırasıyla tomurcuk, dal, elma çekirdeği, yaprak, taze meyve ve meyve kabuğu örneklerinden oluşmaktadır. Son kuyucukta ise negatif kontrol olarak dH2O bulunmaktadır. Agaroz jelde bant yoğunluğu bakımından en belirgin bant profilleri tomurcuk (kuyucuk 1-2), dal (kuyucuk 3-4) ve yaprak (kuyucuk 7-8) örneklerine aittir. Bununla birlikte 9. kuyucuktaki meyve dokusundan izole edilen DNA oldukça yoğun görülmektedir. Meyve kabuğundan izole edilen DNA'ya ait bantlar agaroz jelde daha düşük yoğunlukta görülmekle birlikte çekirdek DNA'ları için çok zayıf ve belirsiz bantlar elde edilmiştir. Genel olarak izole edilen tüm dokulardan agaroz jel görüntüsünde gözlemlenebilir bantlar elde edilmiş ve farklı dokulara ait bant profilleri bant yoğunluğu bakımından birbirlerinden farklılık göstermiştir.



Şekil 1. Farklı elma dokularından izole edilen DNA'lara ait agaroz jel (%1) görüntüsü, L: 1 kb Ladder, kuyucuklar sırasıyla; 1-2: tomurcuk, 3-4: dal, 5-6: meyve çekirdeği, 7-8: yaprak, 9-10 taze meyve, 11-12 meyve kabuğu, NK: negatif kontrol

A260/280 oranı DNA kalite ve saflığı hakkında bilgi vermektedir. 1,8 oranı daha yüksek saflıkta bir DNA varlığına işaret ederken bu oran 2'nin üzerine çıktığında RNA kontaminasyonundan bahsedilebilir (Ateş Sönmezoğlu ve ark., 2018; Aksoy ve Sönmezoğlu, 2022). Tablo 1 elmanın farklı dokularından izole edilen DNA'ların spektroskopik ölçümlerini göstermektedir. DNA saflığı ve kalitesini değerlendirmek amacıyla A260/280 ve A260/230 oranları her bir DNA örneği için en az üç tekrar ile ölçülmüş ve ortalama değerler hesaplanmıştır. DNA konsantrasyonlarına bakıldığında, en yüksek değerin dal (182.72 ng/µl) örneğinde, onu

takiben çekirdek (175.65 ng/µl) örneğinde elde edildiği görülmektedir. En düşük DNA konsantrasyonu ise meyve kabuğunda (12.23 ng/µl) tespit edilmiştir.

Agaroz jelde belirgin bir bant gösterse de meyve örneklerinin ortalama DNA konsantrasyonu (13.52 ng/µl) düşük bulunmuştur. Bununla birlikte, meyve örneklerinde (özellikle 9. kuyucukta) görülen parlak bant profili aynı zamanda yüksek oranda kontaminasyona işaret edebilir. Tablo 1'deki verilere göre, meyve örneklerinin A260/280 ve A260/230 oranları, protein ve fenolik bileşikler gibi diğer safsızlıklara ait kontaminasyonunun bir göstergesi olarak oldukça düşük bulunmuştur. Bu durum, agaroz jelde net bantlar görülse bile, sonraki PCR analizleri için kontaminasyon riski oluşturabileceği anlamına gelmektedir.

Çekirdek örneğinde ise yüksek bir DNA konsantrasyonu (175.65 ng/µl) elde edilmiş olmasına rağmen, agaroz jel görüntüsünde bantlar net ve belirgin değildir. A260/280 ve A260/230 oranları sırasıyla 1.19 ve 0.21 olarak oldukça düşük çıkmıştır. Bu sonuçlar, çekirdek örneklerinden izole edilen DNA'nın yüksek konsantrasyonlarına rağmen, kontaminasyon nedeniyle örneğin tüpte jelleşmesi ve pipetlemeden kaynaklanan sorunlar sebebiyle jel yükleme sırasında uygun miktarlarda alınamamasına bağlı olabilir. En iyi A260/280 oranları ise yaprak, tomurcuk ve dal örneklerinde elde edilmiştir. Benzer şekilde, A260/230 oranı da en yüksek değerleri dal ve tomurcuk örneklerinde göstermektedir.

Bu bulgular, özellikle doku türüne göre DNA izolasyon kalitesinin önemli ölçüde değişebileceğini göstermekte ve moleküler çalışmalarda uygun doku seçiminin kritik olduğunu ortaya koymaktadır.

Tablo 1. Elma dokularından izole edilen DNA'ların konsatrasyon ve saflık ölçümleri

Örnekler	Konsantrasyon (ng/ µl)	$A_{260/280}$	$A_{260/230}$
Tomurcuk	113.02	1.83	1.50
Dal	182.72	1.86	1.60
Çekirdek	175.65	1.19	0.21
Yaprak	35.48	1.81	1.08
Meyve	13.52	1.08	1.21
Meyve kabuğu	12.23	1.52	0.35

Cheng ve ark. (2003), çok yıllık odunsu bitkilerde, özellikle genç dokularda bile polisakkarit içeriğinin tarla bitkilerine kıyasla daha yüksek olduğunu ve bu polisakkaritlerin DNA izolasyonunu zorlaştırdığını, dolayısıyla klasik DNA ekstraksiyon yöntemleri olan CTAB ve SDS'nin bu bileşikleri etkili bir şekilde ortadan kaldıramadığını belirtmiştir. Bu çalışmada da, geleneksel CTAB yöntemi ile izole edilen DNA örneklerinde, özellikle taze meyve ve elma çekirdeği örneklerinin en yüksek protein kontaminasyonunu gösterdiği, bunu meyve kabuğunun izlediği tespit edilmiştir. Elma ve armut gibi odunsu türler, yüksek miktarda fenol ve polisakkarit içerir (Mitra ve Kootstra, 1993; Salzman ve ark., 1999). Bu durum, meyvenin olgunlaşma ve postklimakterik dönemlerinde biyosentezinin arttığı bilinen potansiyel müdahale edici maddelerin etkili olduğu olgun meyve dokuları için geçerlidir (Shellie ve ark., 1997; Gasic ve ark., 2004). Yamamoto ve ark. (2006), armut meyveleri için yaptıkları çalışmalarında, genç yapraklardan izole edilen DNA miktarının, taze meyve örneklerinden elde edilenden çok daha yüksek olduğunu bulmuşlardır. Bizim elde ettiğimiz sonuçlar da yine benzer şekilde taze meyve için daha yüksek protein ve diğer safsızlıkların varlığına işaret ederken genç yaprak, tomurcuk ve dallardan elde edilen DNA miktar ve kalitesi daha iyi bulunmuştur.

Bitki numuneleri genellikle türler arasında değişen yüksek miktarlarda ikincil metabolitler içerir (Pipan ve ark., 2018). Dolayısıyla farklı ticari kitler veya DNA ekstraksiyon yöntemleri farklı bitki türleri veya bitkinin farklı dokuları ile kullanıldığında daha ileri SSR uygulamaları için farklı sonuçlar verecektir (Derling ve ark., 2014; Pipan ve ark., 2017). Bu sebeple bu çalışmanın sonuçlarının elma bitkisi için farklı dokuların kullanımının CTAB izolasyon metodu ile DNA izolasyonuna dair çalışmalara katkı sağlaması beklenmektedir.

SONUÇ

Bu çalışmanın bulguları test edilen bitki dokuları arasında, dal ve tomurcuk örneklerinin en yüksek DNA konsantrasyon ve saflık seviyelerini sağladığını göstermiştir. Bu sonuçlar elma bitkisinden elde edilen bu dokuların moleküler analizler için uygun genomik DNA kaynakları olabileceğini göstermektedir. Bununla birlikte, DNA izolasyonunun hem nicelik hem de nitelik açısından verimliliğinin kullanılan doku türüne bağlı olarak önemli ölçüde değiştiği belirlenmiştir. Meyve, tohum ve meyve kabuğu örneklerinden izole edilen DNA, muhtemelen izolasyon işlemi boyunca tam olarak giderilemeyen polisakkaritler, polifenoller ve diğer ikincil metabolitlerin varlığından dolayı daha düşük konsantrasyonlar ve çok daha düşük saflık oranları sergilemiştir. Genel olarak izolasyon prosedürleri içerisinde en sık kullanılan bitki materyali olan yaprak dokusu orta düzeyde bir DNA konsantrasyonu verirken, kirleticilerin varlığını gösteren ve yüksek olmayan bir saflık göstermiştir.

Bu sonuçlar, özellikle belirli dokularda yüksek düzeyde fenol ve polisakkarit içeren elma (*Malus domestica*) gibi türler için, bitkilerde genomik çalışmalarda kullanıma uygun doku tiplerinin seçilmesinin önemini vurgulamaktadır. DNA izolasyon protokollerini doku tipine göre optimize etmek, PCR amplifikasyonu ve moleküler belirteç analizi gibi sonraki moleküler uygulamalarında yüksek kaliteli sonuçlar sağlamak açısından önemlidir. Gelecekteki çalışmalar daha geniş moleküler uygulamalar için DNA verimini ve saflığını artırmak amacıyla, özellikle meyve ve tohum gibi zorlu dokular için ekstraksiyon yöntemlerinin iyileştirilmesine odaklanabilir.

TEŞEKKÜR

Bu çalışma, Karamanoğlu Mehmetbey Üniversitesi Fen Bilimleri Enstitüsü'ndeki Fatma Özarslan'ın Yüksek Lisans Tezi'nden türetilmiştir. Bu araştırma, Karamanoğlu Mehmetbey Üniversitesi Hassas Tarım Uygulamaları ve Yenilikçi İşleme Teknolojileri İhtisaslaşma Koordinatörlüğü tarafından 01-HASTUYL-24 proje numarasıyla finanse edilmiştir.

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ORAL PRESENTATION

Investigation of the Cytotoxic Effect of *Pistacia terebinthus* Extract on Lung and Breast Cancer

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Abstract

One of the most common diseases today is cancer. One of the treatment methods used in the fight against cancer is phytotherapy. However, information pollution about plants is increasing day by day. For this reason, it is important to investigate the plants in nature through scientific studies and identify their active ingredients. In this study, it was determined in the literature studies that some subspecies of the terebinth plant, whose soap and coffee is used in our country, may have cytotoxic effects against cancer. In this regard, when the literature research was continued, it was determined that the fruit of the subspecies Pistacia terebinthus plant was not examined in terms of cytotoxicity in lung and breast cancer cells. It is believed that the study will contribute to the literature by obtaining data that will address this gap. Oily and non-oily extracts were obtained from the sample obtained by roasting and pressing Pistacia terebinthus fruits collected from Bodrum District of Muğla Province. The obtained extracts were examined in lung and breast cancer cells by evaluating substance exposures and cytotoxicity. Fibroblast cells were used as the control group. As a result, oily and non-fatty extracts were more effective on breast cancer cells compared to lung cancer cells.

Keywords: *Pistacia terebinthus*, phytotherapy, cancer treatment, cytotoxicity, plant extracts.

Introduction

There were 18.1 million cancer diagnoses and 9.6 million deaths due to cancer in 2018, and when the rates are compared with 2020, it is seen that the cancer burden on the world has increased, and if the necessary precautions against cancer are not taken, it is estimated that 22 million new cancer cases will occur annually in 2030 (Kabalak, 2023). Phytotherapy continues to be rapidly applied all over the world, despite its unpredictable side effects, in diseases such as cancer, which modern medicine cannot fully treat. As a result, the uncontrolled use of herbal medicine poses threats to traditional treatments, and this encourages countries to make legal regulations. What needs to be done is to determine the pharmacological and toxicological parameters of the plants used in phytotherapy applications, and to establish the standards of herbal medicines in terms of quality, safety and effectiveness with the determined parameters (Kurhan, Ekici, 2021).
It is considered an important advantage that herbal medicines have fewer side effects and show effectiveness through more than one pathway. Phytochemicals have been used for some time in cancer cases as monotherapy or combined therapy with other agents. It has an important effect in sensitizing cancer cells to other treatment methods and reducing the damage caused by these methods (Sever, Toy, Erdoğan, Gündoğdu, 2023).
Active herbal ingredients exert an anticancer effect as a chemotherapeutic agent by inducing apoptosis and cell cycle arrest in cancer cells. It also shows that herbal components can reduce side effects, strengthen anticancer responses, and sensitize cancer cells to chemotherapy drugs (Bushehri, et al., 2023).
Breast cancer is one of the most common types of cancer in women. In breast cancer treatment; One or more of the options of surgery, radiotherapy, chemotherapy and hormonal treatment are used. However, it is also known that some cancer patients use some Complementary/Alternative Therapies (CAM) in addition to conventional medical treatments. The study aimed to investigate the frequency of CAM use and types of CAM used in breast cancer patients and found that a significant portion of them used CAM methods, especially herbal treatment. Frequent use of CAMs during and after medical treatment may affect the results of medical treatment and interact with the chemotherapeutics used. For this reason, it is thought that the use of CAM should be examined in all breast cancer cases (Kurt, Keşkek, Çil, Canataroğlu, 2013).

In a retrospective and descriptive study conducted among older adults actively undergoing chemotherapy attending the Oncology DH Unit (COES) of the Molinette Hospital AOU Città della Salute e della Scienza in Turin (Italy), greater attention was paid to the use of phytotherapeutics to reduce the risks of toxicity and ineffective treatment. It was stated that the conscious use of these substances should be encouraged to ensure safe use and benefits (Allegra, et al., 2023).
Literature searches have shown that this species has been evaluated for a wide range of pharmacological properties, including antioxidant, antimutagenic, animogenic, antiviral, anti-inflammatory, gastrointestinal radiotherapy, accelerated and serum biochemical effects, antiatherosclerotic and antidiabetic, antiparasitic, antitumor, anticholinesterase, neuroprotective and wound healing properties. P. lentiscus contains various phytochemical components such as terpenoids, phenolics, fatty acids and sterols. As a result of the research, it was concluded that P. lentiscus is an important source for the natural expansion and commercialization of various pharmaceutical products, but the risk was taken into account and more preclinical and clinical studies were heard and run instead of better illumination (Pekacar, Orhan, 2022).
GC/MS analysis of essential oil from immature fruits of P. terebinthus led to the identification of seventy-two chemical components. The essential oil of P. terebinthhus produces time- and dose-dependent inhibition in human lung cancer (A549) cell lines (Najibullah, Ahamad, Sultana, Zafar, 2022).
In the study in which AgNPs were synthesized by the green method using the extract of P. terebinthus, ultraviolet-visible spectrophotometry, was applied to evaluate and verify the formation of antibacterial and antibacterial substances. The anticancer activity of the synthesized AgNPs was also examined. The results show that biogenic AgNPs can function as useful medicinal compounds (Nagmachi, et al., 2022). Pistacia terebinthus L. subsp on DMBA-induced breast cancer in 66 female Sprague-Dawley rats. The therapeutic effects of Palaestina (turpentine) and Rhus coriaria L (sumac) plants were investigated through biochemical analysis. As a result of the research, it has been shown that Pistacia terebinthus and Rhus coriaria plants can be used against breast cancer and reduce its negative consequences, and it has been stated that studies should continue for their safe medical applications (Erişir, Kireçci, Özşahin, Yılmaz, 2022).

This study measures the effects of Pistacia terebinthus flower extract on the amounts of lipid peroxidation (LPO), total protein, glutathione and enzyme activities in the blood. It is parallel to the effect of the extract used in the study on LPO levels in tissues, and shows that the plant species used in the study have positive effects on the activities of antioxidant molecules, as well as their protective effects against LPO (Türkoğlu, Çelik, Keser, Türkoğlu, Yılmaz, 2017).

Pistacia, a genus of flowering plants in the family Anacardiaceae, includes about twenty species; Among these, five are more popular, including P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus. Different parts of these species are used in traditional medicine for various purposes such as tonic, aphrodisiac, antiseptic, antihypertensive and treatment of dental, gastrointestinal, liver, urinary tract and respiratory disorders. Scientific findings have also revealed the broad pharmacological activities of various parts of these species, such as antioxidant, antimicrobial, antiviral, anticholinesterase, anti-inflammatory, antinociceptive, antidiabetic, antitumor, antihyperlipidemic, antiatherosclerotic and hepatoprotective activities, as well as their beneficial effects in gastrointestinal disorders. Various phytochemical components such as terpenoids, phenolic compounds, fatty acids and sterols have also been isolated and identified from different parts of Pistacia species (Bozorgi, et al., 2013).

Pistacia terebinthus soap has been shown to be used safely and effectively in the treatment of Erlotinib-induced skin toxicity in non-small cell lung cancer patients (Taştekin, 2017).

Considering the mechanism of action of the Pistacia lentiscus species of the terebinth plant, Pistacia lentiscus can be considered a promising source of natural antitumor agents and may have therapeutic potential in integrated oncology (Catalani, Palma, Battistelli, Benedetti, 2017). showed that treatment of immunocompetent mice with mastic oil (45 mg/kg body weight, intraperitoneally, 3 times per week for approximately 3 weeks) significantly inhibited tumor growth (Magkouta, et al. 2009).

METHOD

Collection and pressing of Pistacia terebinthus fruits
Menengiç fruits were obtained in the amount of 700 grams from the Menengiç (Pistacia terebinthus) Tree (Picture 1), which was diagnosed by the Bodrum District Directorate of Agriculture, from Yakaköy College Street, Bodrum District of Muğla Province, on 02.10.2023, and were roasted and pressed over medium heat for 30 minutes.



Picture 1. Photo examples of Pistacia terebinthus plant

The pressed residue was rested and the fixed oil was allowed to rise to the upper surface (Figure 2).



Picture 2. Pistacia terebinthus fruits and the pressed residue obtained from these fruits.

Extract preparation

Experiment materials:

- Glass balloon
- Beaker
- Precision scale
- 96% ethanol
- Heidolph rotavapor
- Pleated filter paper
- 123mg vials

Extract preparation was carried out by Dr. Karadeniz Technical University, Faculty of Pharmacy, Pharmacognosy Research Laboratory. Lecturer It was made by member Nurdan Yazıcı BEKTAŞ.
The oily part of the residue was decanted and taken into a separate beaker and 10 g was weighed and placed in the flask, called the "oily sample" (Picture 3).

The residue remaining at the bottom was shaken and weighed as 10 g, called “fat-free sample” and placed in the flask (Figure 3).



Picture 3. Oily and non-oiled sample

10 ml of 96% ethanol was added to two samples and extracted by rotating with the help of rotavapor under 350 mbar pressure at 40°C for 1 hour (Figure 4).



Picture 4. *Rotavapor (Heidolph)*

After 1 hour, the samples were filtered through pleated filter paper and the solvent was removed from the rotavapor device under low pressure at 40°C (Figure 5).



Picture 5. Filtering through filter paper

The dry residues (Figure 6) were weighed and the extract yield was determined as 10% for both samples.



Picture 6. Dry balances

123 mg of each of the dry residues was weighed and taken into vials (Figure 7) and stored at +4 °C until the experiments were carried out to be used in cytotoxic activity studies.



Picture 7. 123 mg vials

Experiments on examining the obtained extracts in lung and breast cancer cells were carried out by Lecturer Ebru KANIMDAN at Bezmialem Foundation University Faculty of Medicine Medical Biochemistry Laboratory.

Experiment materials:

• 25T flasks
• 175T flask
• DMEM, DMEM-F12, DMSO
• Oven
• Trypsin
• 96-well transparent plates
• Cancer (A549 and MCF-7) and normal (CCD-1072Sk) cell lines
• Esco CelCulture CO2 incubator
• Varioskan Flash
• ThermoFisher Varioskan Plate Reader to measure fluorescence intensity (Fl), time-resolved fluorescence (TRF), endpoint absorbance and luminescence, kinetic and spectral measurements in the UV/VIS/NIR range from suitable microplate formats.

Cell lines

Cancer (A549 and MCF-7) and normal (CCD-1072Sk) cell lines were commercially available in cell culture banks. Cells were first in 25T flasks, then in 175T flasks, by adding specific media for each cell (DMEM, DMEM-F12, respectively) and incubated in an oven at 37°C, 5% CO2 until they reached 80% density. Cells passaged with trypsin were seeded in 96-well transparent plates and the cells were incubated for 24 hours. After incubation, the cytotoxic dose for lung and breast cancer cells (A549 and MCF-7) was determined by treating the extracts at different concentrations prepared by diluting them with appropriate media containing DMSO <0.001 for 24 hours.

Evaluation of cancer activity with MTT test

MTT testing was performed to determine the potential antiproliferative and cytotoxic properties of Pistacia terebinthus oil against lung and breast (A549 and MCF-7) cancers. Testing was performed by the method described by Marquez et al. At the end of the incubation period, MTT (at 5 mg/mL) was added to each well and the cells were incubated for another 2–4 h until purple precipitates formed. Finally, MTT along with media were removed from the wells and washed with 1X PBS (200 µL). Additionally, DMSO (100 µL) was added and the plate was shaken for 5 min to dissolve formazan crystals. Each plate was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the IC50 value was calculated using GraphPad Prism 6.0 software (USA).

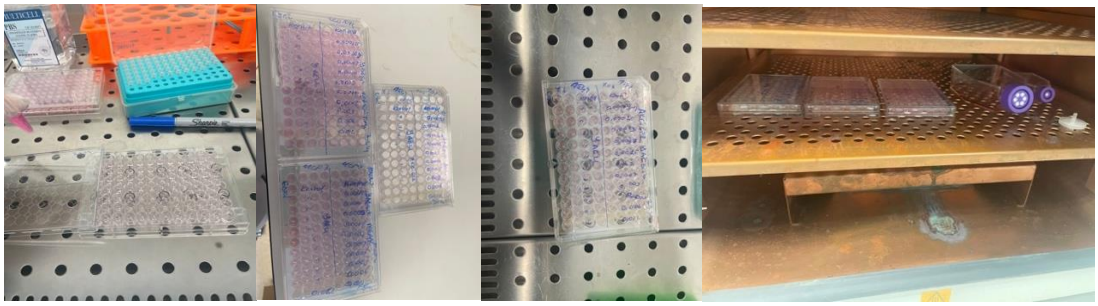

Figure 8. Photographs of cells before MTT test



Figure 9. Esco CelCulture CO_2 incubator for cell growth

Findings

According to the data obtained as a result of the experiments, the following findings emerged:

1. Extraction and Pressing Process:

• Terebinth (Pistacia terebinthus) fruits were collected.
• The collected fruits were roasted over medium heat for 30 minutes and pressed.
• The pressed residue is rested and the fixed oil is allowed to rise to the upper surface.

2. Extract Preparation:

• The lean and oily parts of the pressed oil were placed in separate beakers.
• 10 ml of 96% ethanol was added to both samples and extracted with the help of rotavapor at 40°C for 1 hour.
• Extract yield was determined as 10% for both samples.
• Dry residues were stored at +4 °C until the experiments were carried out.

3. Cell Lines and Culture:

• Cancer (A549 and MCF-7) and normal (CCD-1072Sk) cell lines were used.
• Cells were incubated in certain media under appropriate conditions and reached a certain density before use.

4. Cytotoxic Activity Studies:

• The cytotoxic dose was determined for cancer cells (A549 and MCF-7) by treating them with the extracts for 24 hours.
• Antiproliferative and cytotoxic properties of Pistacia terebinthus oil against lung and breast cancers were evaluated using the MTT test.

5. MTT Test Results:

• Figure 1 shows the dose-dependent variation in normal fibroblast (CCD-1072Sk) cell viability applied to different concentrations of both oily and aqueous extracts.

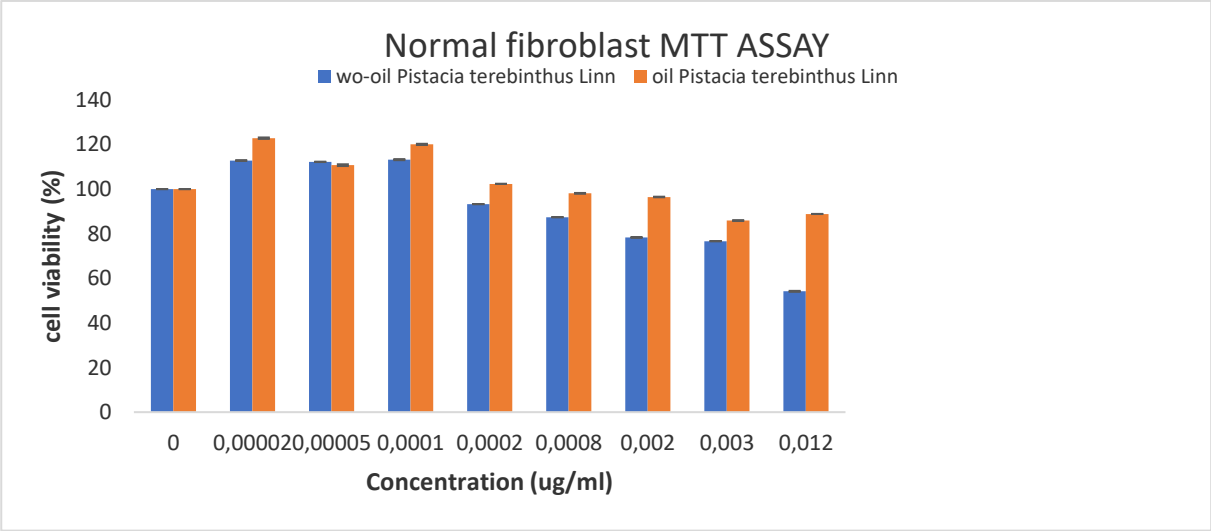

Figure 1. Changes in normal fibroblast (CCD-1072Sk) cell viability depending on the dose applied to both oily and aqueous extracts at different concentrations (0-0,0,12ug/ml).

Figure 2 shows the dose-dependent variation of lung and breast cancer (A549 and MCF-7) cell viability of oily extract at different concentrations.

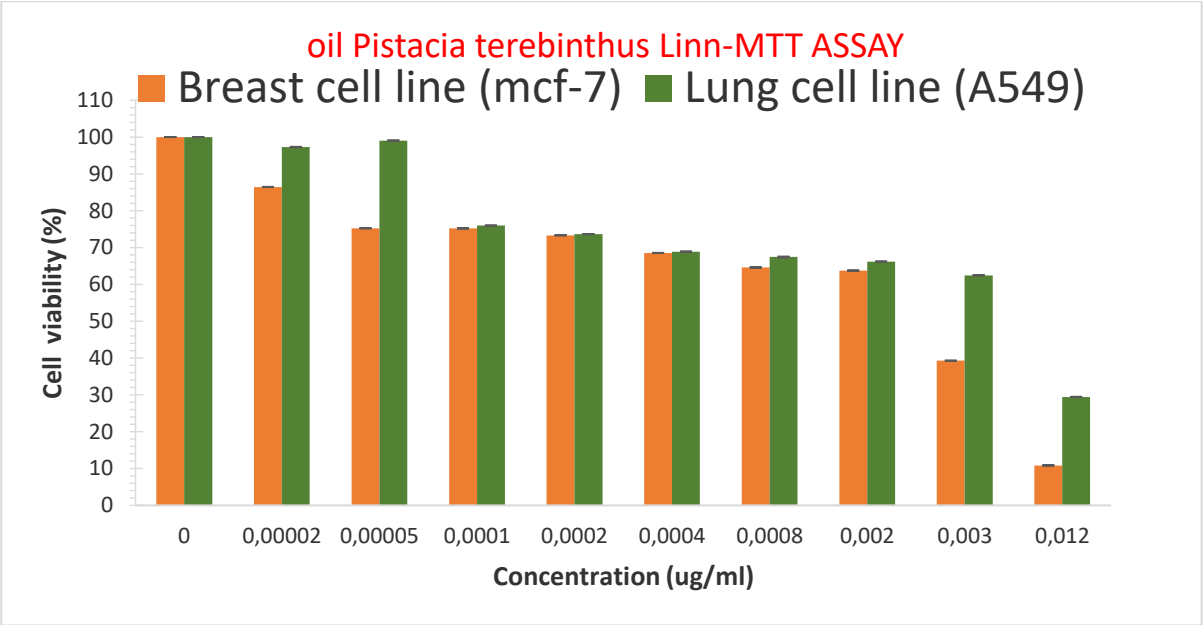

Figure 2. Changes in lung and breast cancer (A549 and MCF-7) cell viability to which oily extract was applied at different concentrations (0-0,0,12ug/ml) depending on the dose.

Figure 3 shows the dose-dependent variation of lung and breast cancer (A549 and MCF-7) cell viability of the aqueous extract at different concentrations.

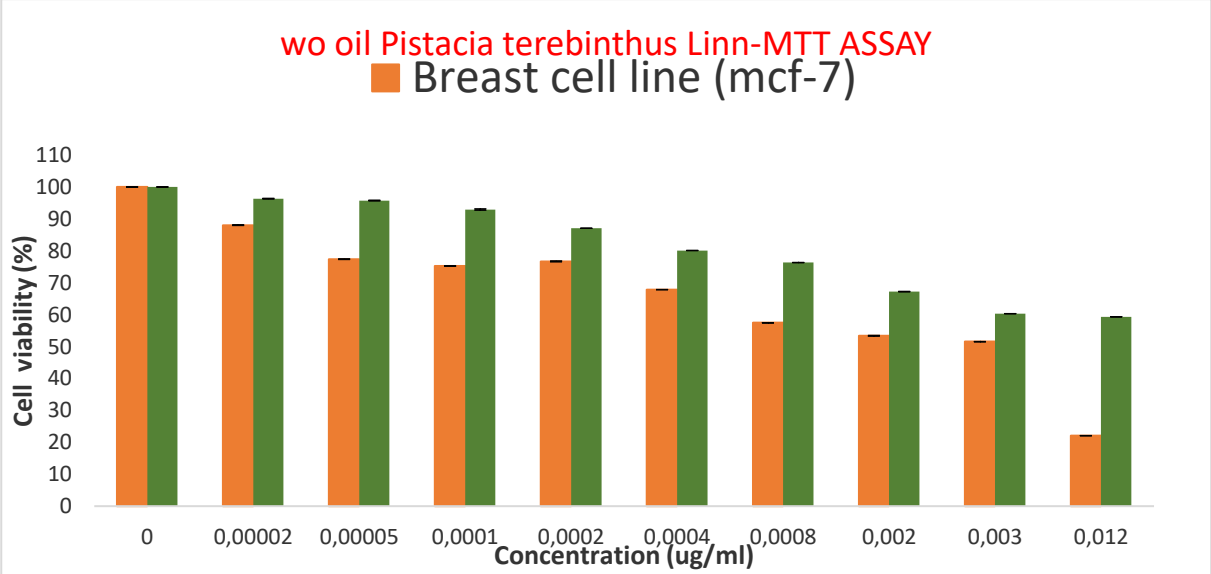

Figure 3. Changes in lung and breast cancer (A549 and MCF-7) cell viability depending on dose when aqueous extract was applied at different concentrations (0-0,0,12ug/ml).

n conclusion, the data obtained show that Pistacia terebinthus oil has potential antiproliferative and cytotoxic effects, especially against lung and breast cancer cells. These findings suggest that Pistacia terebinthus may have anti-cancer properties.

Result and Discussion

Pistacia terebinthus plant was extracted by two different methods: oily and oil-free. The effects of this extract on human breast cancer and lung cancer were analyzed by the MTT method. As a result of this analysis, the IC50 values of the oil-free Pistacia terebinthus extract on breast cancer and lung cancer cell lines were found to be 0.005247 ug/ml and 0.012856 ug/ml, respectively. The IC50 values of the oily Pistacia terebinthus extract on breast cancer and lung cancer cell lines were calculated as 0.004434 Ug/ml and 0.006264 Ug/ml, respectively. The Ic50 dose was not reached for both extracts in human normal fibroblast cells used as control cells. As a result of the study, it is predicted that both fatty and non-fatty extracts may be more effective on breast cancer compared to lung cancer, and this should be supported by new studies.

Suggestions

As a result of the study, it is thought that both fatty and non-fatty extracts may be more effective on breast cancer compared to lung cancer. This prediction, obtained by studying the effects of the extract on breast cancer over different periods of time, needs to be supported by new studies.

As a result, the recommendations of this study can be listed as follows:

1. Detailed Studies on Breast Cancer:

• The current study evaluated the effects of oily and non-oily Pistacia terebinthus extracts on lung and breast cancer cell lines. However, it may be recommended to examine the effects on breast cancer in more detail in future studies. This may provide an understanding of the effects and mechanisms of the extract on different subtypes of breast cancer cells.

2. Examination at Different Time Intervals:

• The recommendation of the study is to examine the effects of the extract on breast cancer cells at different time intervals. This may contribute to the understanding of time-varying effects by providing the possibility to evaluate the long-term effects of the extract as well as the determined IC50 values.

3. Investigation of Molecular Mechanisms:

• In the study, the effects of Pistacia terebinthus extracts on cancer cells were determined, but detailed information was not given about their molecular mechanisms. Therefore, further molecular studies may be recommended to understand the extract's intracellular interactions, gene expression changes and effects on signaling pathways.

4. Studies with Different Doses:

• IC50 values represent the dose at which half the effect on cell viability is observed. Determining the effects at different dose levels may help better understand the optimal therapeutic dose of the extract and possible side effects.

5. Transition to Clinical Studies:

• In order for the findings obtained to gain more reliability and be transferred to clinical application, transition to clinical studies may be considered after studies on animal models. This could evaluate whether Pistacia terebinthus extract plays a potential role in breast cancer treatment.

These recommendations may contribute to our better understanding of the effects of Pistacia terebinthus extract on breast cancer by ensuring that future studies are more comprehensive and informative.

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ORAL PRESENTATION

Parkinson hastalığı ile ilişkili bazı genlerdeki yüksek riskli zararlı SNP'lerin etkilerinin *in siliko* yöntemlerle belirlenmesi

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Özet

Nörodejeneratif bir hastalıkolan Parkinson hastalığı oldukça kompleks bir etiyolojiye sahiptir. Belli çevresel faktörlere karşı genetik yatkınlığı olan bireylerde, maruz kalınan çevresel faktörlerin kliniği ortaya çıkardığı düşünülebilir. Bununla birlikte genetik faktörlerin tek başına hastalığın etiyolojisinde çok önemli bir rol oynadığı bilinmektedir. Parkinson hastalığı için belirtilmiş genetik faktörler arasında mutasyonlar vep olimorfizmler yer almaktadır. Bu çalışmada, Parkinson hastalığı ile ilişkili olduğu düşünülen *SNCA, CD38, GBA, LRP10, EIF4G1, VPS35, PLA2G6, PINK1, LRRK2, UQCR1*ve*MMRN1* genlerinde bulunan tek nükleotid polimorfizmlerinin (SNP) çeşitli biyoinformatik araçlar kullanılarak proteinlerin fonksiyonu, yapısı, stabilizasyonu, evrimsel korunumu üzerine olan olası etkilerinin belirlenmesi amaçlanmıştır. Sonuç olarak *MMRN1* geni için rs1046993 (L982S), rs61734879 (N1075K), rs1432306000 (8G274), rs147438202 (R1057M), rs147438202 (G16R), rs147451161 (R1227L), rs148141919 (Q394R), rs149261966 (S1155R), rs189386556 (S414P), rs199716183 (Y1097H), rs200526047 (P651H), rs201006967 (P1140L), rs201761344 (N1084S), rs369001499 (M1100K), rs370732997 (D726Y), rs373754512 (D225G), rs374677318 (C592Y), rs376506594 (C211Y) polimorfizmlerinin zararlı etkil iolabileceği belirlendi. *UQCR1* geni için rs201911056 (R276C), rs145869559 (G312S), rs191821836 (R165S), rs200354059 (R378C) varyantlarının ve *LRRK2* geni için ise rs4640000 (P1262A), rs33995463 (L119P), rs150050676 (N246H) varyantlarının zararlı olabileceği belirlendi. Belirlenen zararlı etkili SNP'lerin çeşitli biyolojik süreçlerde rol alan proteinlerin yapısal ve fonksiyonel değişikliklerine neden olabileceği ve bu değişikliklerin sonucunda biyolojik süreçleri olumsuz olarak etkileyebilecekleri tahmin edildi. Bu çalışma da patojenik olarak tanımlanan missense SNP'ler Parkinson hastalığı için risk faktörü olarak değerlendirilmiştir. Bu patojenik missense SNP'lerin biyobelirteç olarak kullanılma potansiyelleri teşhis ve tedavi için bir temel oluşturabilir. Bu tez çalışmasından elde edilen sonuçların Parkinson hastalığı ile ilgili yapılacak *in vitro* ve *in vivo* ileri çalışmalar tarafından doğrulanması gerekmektedir.

AnahtarKelimeler: Parkinson hastalığı, SNP, *MMRN1, UQCR1, LRRK2*

Determination of the effects of high-risk deleterious SNPs in some genes associated with Parkinson's disease by *in silico* methods

Abstract

Parkinson's disease, which is a neurodegenerative disease, has a very complex etiology. In individuals with a genetic predisposition to certain environmental factors, it is conceivable that the environmental factors they are exposed to lead to the clinic. However, it is known that genetic factors alone play a significant role in the etiology of the disease. Mutations and polymorphisms are among the genetic factors specified for Parkinson's disease. It was aimed to determine the possible effects of single nucleotide polymorphisms (SNPs) in *SNCA, CD38, GBA, LRP10, EIF4G1, VPS35, PLA2G6, PINK1, LRRK2, UQCR1, MMRN1* genes on the function, structure, stabilization and evolutionary conservation of proteins using various bioinformatics tools. For the *MMRN1* gene, it was determined that rs1046993 (L982S), rs61734879 (N1075K), rs1432306000 (8G274), rs147438202 (R1057M) rs147438202 (G16R), rs147451161 (R1227L), rs148141919 (Q394R), rs149261966 (S1155R), rs189386556 (S414P), rs199716183 (Y1097H), rs200526047 (P651H), rs201006967 (P1140L), rs201761344 (N1084S), rs369001499 (M1100K), rs370732997 (D726Y), rs373754512 (D225G), rs374677318 (C592Y), rs376506594 (C211Y) polymorphisms may have damaging effects. It was determined that rs201911056 (R276C), rs145869559 (G312S), rs191821836 (R165S), rs200354059 (R378C) variants for

the *UQCR1* gene and rs4640000 (P1262A), rs33995463 (L119P), rs150050676 (N246H) variants for the *LRRK2* gene might be pathogenic. It was predicted that the identified missense SNPs may cause structural and functional changes in proteins involved in various biological processes and, as a result of these changes, adversely affect biological processes. In this study, the missense SNPs identified as pathogenic were evaluated as risk factors for Parkinson's disease. The potential use of these pathogenic missense SNPs as biomarkers may provide a basis for diagnosis and treatment. The results of this study need to be confirmed by further *in vitro* and *in vivo* studies in Parkinson's disease.

Keywords: Parkinson's disease, SNP, *MMRN1, UQCR1, LRRK2*

GİRİŞ

Parkinson hastalığı (PH), Alzheimer hastalığından (AH) sonra en yaygın görülen nörodejeneratif hastalıktır. Beyinde *substantia nigra'*da dopaminerjik nöronların kaybı ile meydana gelmektedir. Hastalığın klinik bulguları motor ve motor olmayan bulgulardan oluşmaktadır (Poewe ve ark., 2017). Motor bulgular; bradikinezi (hareketlerde yavaşlama), tremor (istirahat halinde titreme), rijidite (kaslarda sertlik), duruşu ve dengeyi koruyan reflekslerin bozulması şeklinde gözlenmektedir. Motor olmayan bulgular ise; ağrı, koku alma bozukluğu, uyku davranış bozukluğu ve mesane-bağırsak gibi sistemlerde görülebilen bozukluklardır (Aykaç, 2017).Parkinson hastalığında temel protein olan alfa-sinüklein (α–syn) birikmesi Lewy cisimciğinin oluşumuna neden olmaktadır. Parkinson hastalığına neden olan en sık görülen mutasyonların delesyon, duplikasyon olmasının yanı sıra tek nükleotid polimorfizmleri de oldukça önemlidir. Parkinson hastalığındaki genlerdeki yüksek risk taşıyan SNP'lerin etkilerinin *in siliko* olarak belirlenmesinin hastalığa yaklaşımda yeni bir yol olabileceği öngörülmektedir. Yapılan çalışmalarda SNP'lerin hastalıklar üzerine doğrudan etkilerinin olması, Parkinson hastalığında da yüksek risk taşıyan SNP'lerin hastalığın patogenezini ve tedavisini etkileyebileceğini göstermektedir. Böyle spesifik çalışmalar tedavisi zorlu olan Parkinson hastalığı üzerinde kolay çözümler sunabilir. Çalışmamızın amacı, Parkinson hastalığı ile ilişkili olduğu düşünülen bazı genlerdeki SNP'lerin etkilerini *in siliko* yöntemlerle belirlemektedir. Hastalığın tanı ve tedavisinde daha uygun ve daha hızlı olarak kullanılan *in siliko* yöntemler daha elverişli sonuçlar doğuracaktır.

MATERYAL VE METOT

Parkinson hastalığında, yüksek riskli missense SNP'lerin belirlenmesi için çok sayıda biyoinformatik veri aracı kullanıldı. *SNCA, CD38, GBA, LRP10, EIF4G1, VPS35, PLA2G6, PINK1, LRRK2, UQCR*1 ve *MMRN1* genlerindeki missense varyantların proteinler üzerindeki etkilerinin tahmin edilmesi için SIFT (Sortıng Intolerant From Tolerant- Toleransızı Toleraslıdan Ayırma) veri aracı kullanıldı. Genlerdeki Polimorfizm Fenotiplendirmesi verileri için PolyPhen-2, karşılaştırmalı ve yapısal evrimsel değerlendirmeye göre amino asit ikamelerinin insan proteinlerinin işlevi ve stabilitesi üzerindeki potansiyel etkilerini tahmin edilmesi için kullanıldı (Adzhubei ve ark., 2013).Potansiyel olarak patojenik veya zararlı SNP'leri tahmin etmek için Panther-Psep çevrimiçi aracı kullanıldı.İnsan genetiğindeki kodlayan ve kodlanmayan tek nükleotid varyantlarının hastalık üzerine etkilerinin tahmin edilmesi ve hastalıklarla ilişkili amino asit değişikliklerini tanımlamak için PhD-SNP çevrimiçi aracı kullanıldı (Capriotti ve diğerleri, 2017). Zararlı SNP'lerin tahmin edilmesi için SNPs&GO çevrimiçi aracı kullanıldı (Capriotti ve diğerleri, 2013). I-Mutant, genlerdeki mutasyonların protein stabilitesi üzerindeki etkilerinitahmin etmek amacıyla kullanılan veri aracıdır. I-Mutant2.0 biyoinformatik aracı ile missense SNP'lerin serbest enerji değişim değeri (Delta Delta G: DDG) üzerindeki etkileri tahmin edildi. Missense SNP'lerin filogenetik tahmini için Consurf çevrimiçi aracı kullanıldı. Protein ikincil yapı tahmini için SOPMA biyoinformatik çevrimiçi aracı kullanıldı. NetSurfP-3.0 çevrimiçi aracı protein yüzey erişilebilirliğinin tahmin edilmesinde kullanıldı. MutPred2 aracı amino asit değişimlerinin patojenik etkilerinin belirlenmesi amacıyla kullanılmıştır (Mottaz ve ark., 2010). Bu araç genel bir patojenite tahmini ve potansiyel olarak fenotipi etkileyen spesifik moleküler değişiklerin sıralı bir listesini sağlamaktadır.

BULGULAR

Zararlı Etkili Missense SNP'lerin Belirlenmesi

Parkinson hastalığı ile ilişkilendirilen *SNCA, CD38, GBA, LRP10, EIF4G1, VPS35, PLA2G6, PINK1, MMRN1, UQCR1* ve *LRRK2* genlerine ait SNP'lerin bilgileri NCBI veritabanından, missense SNP'ler ise dbSNP yazılım aracından alınmıştır. İlgili genlerin ürünleri olan protein sekansları FASTA formatında alınarak analizler yapılmıştır. Analizler sonucunda*CD38, GBA, LRP10, EIF4G1, VPS35, PLA2G6, PINK1* genleri, çalışmamızda kullanmış olduğumuz biyoinformatik programlarda el verişli sonuçlar elde edilemediği için

çalışmadan çıkarılmıştır. *MMRN1, UQCR1* ve *LRRK2* geni için tanımlanan zararlı missense SNP'ler sırasıyla tablo1, tablo2ve tablo3'te gösterilmiştir.

Zararlı Missense SNP'lerin Moleküler Etkilerinin Tahmini

SNCA, CD38, GBA, LRP10, EIF4G1, VPS35, PLA2G6, PINK1 genlerindeki sonuçlar çalışmamızdaki uygulama koşullarına elverişsiz olduğu için çalışmaya dahil edilmemiştir. *MMRN1, UQCR1* ve *LRRK2* genlerindeki missense SNP'lerin amino asit değişimlerinin patojenik moleküler etkilerinin belirlenmesi için MutPred2 veri aracı kullanıldı ve bu zararlı missense SNP'lerin değişmiş metal bağlama, disülfid bağ kaybı, heliks kaybı, glikolizasyon kaybı, metilasyon kaybı gibi değişiklikler ile ilişkili olduğu gösterilmiştir.

Zararlı Missense SNP'lerin Protein Stabilitesine Etkilerinin Tahmini

MMRN1, UQCR1 ve *LRRK2* genlerindeki zararlı missense SNP'lerin protein stabilitesine etkilerini tahmin etmek amacıyla I-Mutant 2.0 yazılım aracı kullanıldı.Serbest enerji değişim(ΔΔG)değerinin pozitif olduğu durumda mutasyonlu olan proteinin yüksek stabilitede bir etki gösterdiği sunulmaktadır. Serbest enerji değişim değerinin negatif olduğu durumda mutasyonlu olan proteinin düşük stabilitede bir etki gösterdiği sunulmaktadır. Bu durumlara bağlı olarak I-Mutant 2.0 sonuçları, protein stabilitesi üzerinde artma ya da azalma şeklinde belirlenmiştir.

Evrimsel Korunum Analizi

MMRN1, UQCR1 ve *LRRK2* genlerindeki missense SNP'lerin filogenetik korunum analizi için Consurf biyoinformatik aracı kullanıldı. ConSurfbiyoinformatik aracından elde edilen sonuçlara ve koruma puanlarına göre yüksek düzeyde, orta düzeyde ve değişken yani düşük düzeyde korunma şeklinde bir renk kodlama şeması bulunmaktadır. MMRN1 ve UQCR1proteinlerinin filogenetik korunum analizi sonuçları sırasıyla şekil1 ve şekil 2'de gösterilmiştir.

TARTIŞMA

Parkinson hastalığı, dünyaçapında 6 milyondan fazla insanı etkileyen ve 2040 yılına kadar yaygınlığının iki katına çıkması beklenen nörodejeneratif bir hastalıktır. Genetik varyasyonun, genel PH gelişme riskine yaklaşık %25 oranında katkıda bulunduğu tahmin edilmektedir (Day ve ark., 2021). Çok sayıda gen ve SNP'lerin Parkinson hastalığında önemli bir rol oynadığı bilinmektedir (Subramaniyanve ark., 2023). Bu bağlamda, bu çalışmamızda PH ile ilişkili olduğu düşünülen genlerdeki patojeniketkili missense SNP'ler belirlenmiştir. *MMRN1* geni için rs1046993 (L982S), rs61734879 (N1075K), rs1432306000 (8G274), rs147438202 (R1057M), rs147438202 (G16R), rs147451161 (R1227L), rs148141919 (Q394R), rs149261966 (S1155R), rs189386556 (S414P), rs199716183 (Y1097H), rs200526047 (P651H), rs201006967 (P1140L), rs201761344 (N1084S), rs369001499 (M1100K), rs370732997 (D726Y), rs373754512 (D225G), rs374677318 (C592Y), rs376506594 (C211Y) polimorfizmlerinin ppatojenik olduğu belirlendi. *UQCR1*geni için rs201911056 (R276C), rs145869559 (G312S), rs191821836 (R165S), rs200354059 (R378C) varyantlarının ve *LRRK2* geni için ise rs4640000 (P1262A), rs33995463 (L119P), rs150050676 (N246H) varyantlarının patojenik olduğu belirlendi. Bu patojenikvaryantların, protein stabilitesini, yüzey erişilebilirliğini, proteinin sekonder ve tersiyer yapısını değiştirerek ve allosterik bölge kaybı, heliks kaybı, ubikitilasyon kaybı gibi moleküler değişiklikler aracılığıyla PH patogenezini etkileyebileceği düşünülmektedir. Ancak literatürde bu varyantlarla ilgili herhangi bir çalışma mevcu tdeğildir. Elde etmiş olduğumuz sonuçların *in vitro* ve *in vivo* ileri çalışmalar tarafından doğrulanması gerekmektedir.

SONUÇ

Parkinson hastalığının değişken patolojik özellikleri ve çok faktörlü nedenlere sahip karmaşık bir nörolojik hastalık olduğu bilinmektedir. Bir hastalık sürecini etkileyen genetik faktörlerin anlaşılması, klinik bakım üzerinde önemli etkilere sahip olabilir. Bu genetik faktörlerin tanımlanması, nedensel genleri tanımlayabilir ve patogenez için önemli olan biyolojik yolları vurgulayabilir. Bu da söz konusu biyolojik yolları hedef alan terapötiklerin geliştirilmesine olanak sağlayabilir. Parkinson hastalığının mekanizmasının daha anlaşılır olmasında SNP'lerin rolünün büyük olduğu gösterilmiştir. Çalışmamızda, Parkinson hastalığı ile ilişkili olduğu düşünülen genlerdeki zararlı missense SNP'lerin protein yapısını ve fonksiyonunu değiştirerek hastalık patogenezinde etkili olabileceği gösterilmiştir. Çalışmamızın sonucunda da görülüyor ki Parkinson hastalığının tanısı ve tedavisi için patojenik missense SNP'ler çok önemli bir yere sahiptir. Parkinson hastalığı

ile ilgili yapılan çalışmamızda *MMRN1*, *UQCR1*, *LRRK2* genlerinde bulunan ve yüksek riskli olabileceği tahmin edilen SNP'lerin genotipleme araştırmaları için kaynak olabileceği düşülmektedir.

Tablo 1.*MMRN1* geni için belirlenen patojenik missense SNP'ler.

SNP ID	Nükleotid sübstitüsyonu	Amino asitdeğişimi	SIFT sonucu	SIFT skoru	PolyPhen-2 sonucu	PolyPhen-2 skoru	PANTHER-PSEP sonucu	PANTHER-PSEP skoru	PhD-SNP sonucu	PhD-SNP skoru	SNP&GO sonucu	SNP&GO skoru
rs1046993	T/C	L982S	Zararlı	0.005	Muhtemel zararlı	0.993	Muhtemel zararlı	0.5	Nötr	3	Nötr	3
rs61734879	C/A	N1075K	Zararlı	0.016	Muhtemel zararlı	0.993	Muhtemel zararlı	0.5	Hastalık yapıcı	2	Nötr	5
rs1432306000	A/G	G274R	Zararlı	0.005	Muhtemel zararlı	1.000	Muhtemel zararlı	0.57	Nötr	2	Nötr	7
rs147438202	G/A	R1057M	Zararlı	0	Muhtemel zararlı	1.000	Muhtemel zararlı	0.5	Hastalık yapıcı	7	Hastalık yapıcı	8
rs147438202	G/A	G16R	Zararlı	0	Muhtemel zararlı	0.054	Muhtemel zararlı	0.5	Hastalık yapıcı	3	Hastalık yapıcı	4
rs147451161	G/T	R1227L	Zararlı	0	Benign	1.000	Muhtemel zararlı	0.5	Hastalık yapıcı	6	Nötr	9
rs148141919	A/G	Q394R	Zararlı	0.028	Muhtemel zararlı	0.999	Muhtemel zararlı	0.5	Hastalık yapıcı	7	Hastalık yapıcı	8
rs149261966	T/G	S1155R	Zararlı	0	Muhtemel zararlı	1.000	Muhtemel zararlı	0.5	Hastalık yapıcı	5	Hastalık yapıcı	0
rs189386556	T/C	S414P	Zararlı	0.047	Muhtemel zararlı	1.000	Muhtemel zararlı	0.5	Nötr	4	Hastalık yapıcı	2
rs199716183	T/C	Y1097H	Zararlı	0	Muhtemel zararlı	1.000	Muhtemel zararlı	0.5	Nötr	3	Nötr	6
rs200526047	C/A	P651H	Zararlı	0.029	Muhtemel zararlı	1.000	Muhtemel zararlı	0.5	Hastalık yapıcı	0	Nötr	4
rs201006967	C/T	P1140L	Zararlı	0	Muhtemel zararlı	1.000	Muhtemel zararlı	0.5	Hastalık yapıcı	7	Nötr	5
rs201761344	A/G	N1084S	Zararlı	0.022	Muhtemel zararlı	0.780	Muhtemel zararlı	0.5	Nötr	4	Nötr	9
rs369001499	T/A	M1100K	Zararlı	0.014	Muhtemel zararlı	0.991	Muhtemel zararlı	0.5	Hastalık yapıcı	4	Nötr	3
rs370732997	G/T	D726Y	Zararlı	0.01	Muhtemel zararlı	1.000	Muhtemel zararlı	0.5	Hastalık yapıcı	4	Hastalık yapıcı	4
rs373754512	A/G	D522G	Zararlı	0.008	Muhtemel zararlı	1.000	Muhtemel zararlı	0.5	Hastalık yapıcı	5	Hastalık yapıcı	5
rs374677318	G/A	C592Y	Zararlı	0.027	Muhtemel zararlı	0.995	Muhtemel zararlı	0.5	Hastalık yapıcı	2	Nötr	3
rs376506594	G/A	C211Y	Zararlı	0.022	Muhtemel zararlı	1.000	Muhtemel zararlı	0.57	Hastalık yapıcı	9	Hastalık yapıcı	8

Tablo 2.*UQCR1* geni için belirlenen patojenik missense SNP'ler.

SNP ID	Nükleotid sübstitüsyonu	Amino asit değişimi	SIFT sonucu	SIFT skoru	PolyPhen-2 sonucu	PolyPhen-2 skoru	PANTHER-PSEP sonucu	PANTHER-PSEP skoru	PhD-SNP sonucu	PhD-SNP skoru	SNP&GO sonucu	SNP&GO skoru
rs201911056	G/A	R276C	Zararlı	0.014	Muhtemel zararlı	0.958	-	-	Hastalık yapıcı	5	Hastalık yapıcı	2
rs145869559	C/A	G312S	Zararlı	0	Muhtemel zararlı	1.000	-	-	Hastalık yapıcı	7	Hastalık yapıcı	6
rs191821836	G/T	R276C	Zararlı	0.001	Muhtemel zararlı	1.000	-	-	Hastalık yapıcı	6	Hastalık yapıcı	5
rs200354059	G/A	R378C	Zararlı	0.002	Muhtemel zararlı	1.000	-	-	Hastalık yapıcı	6	Hastalık yapıcı	2

Tablo 3. *LRRK2* geni için belirlenen patojenik missense SNP'ler.

SNP ID	Nükleotid sübstitüsyonu	Amino asit değişimi	SIFT sonucu	SIFT skoru	PolyPhen-2 sonucu	PolyPhen-2 skoru	PANTHER-PSEP sonucu	PANTHER-PSEP skoru	PhD-SNP sonucu	PhD-SNP skoru	SNP&GO sonucu	SNP&GO skoru
rs4640000	C/G	P1262A	Zararlı	0.007	Muhtemel zararlı	1	Muhtemel zararlı	0.13	Hastalık yapıcı	8	Nötr	7
rs33995463	T/C	L119P	Zararlı	0	Muhtemel zararlı	0.998	Muhtemel zararlı	0.13	Hastalık yapıcı	7	Hastalık yapıcı	8
rs150050676	A/C	N246H	Zararlı	0.036	Muhtemel zararlı	0.998	Muhtemel zararlı	0.13	Nötr	5	Hastalık yapıcı	6

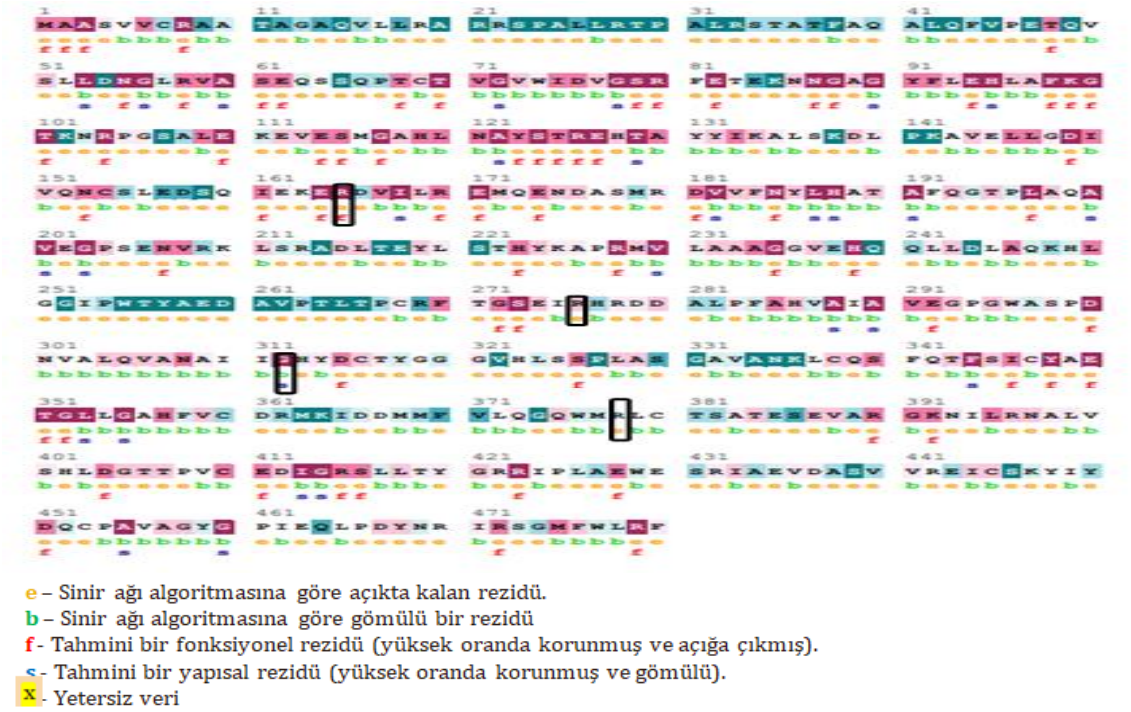


Koruma ölçeği:



e – Sinir ağı algoritmasına göre açıkta kalan rezidü.
b – Sinir ağı algoritmasına göre gömülü bir rezidü
f - Tahmini bir fonksiyonel rezidü (yüksek oranda korunmuş ve açığa çıkmış).
s - Tahmini bir yapısal rezidü (yüksek oranda korunmuş ve gömülü).
x - Yetersiz veri

Şekil 1.MMRN1 proteinine ait evrimsel korunum analizi. Koyu mavi kutular patojenik SNP'leri göstermektedir.



Şekil 2. UQCR1 proteinine ait evrimsel korunum analizi. Siyah kutucuklar patojenik SNP'leri göstermektedir.

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*Bu tam metin bildiri 1. Yazarın 2. Yazarın danışmanlığında gerçekleştirmiş olduğu yükseklisans tez çalışmasından üretilmiştir.

ORAL PRESENTATION

Malt Kalitesini Belirleyen Markörlerin Gradiyent PCR İle Optimizasyonu

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Özet

Arpa (*Hordeum vulgare* L.) dünyada ve ülkemizde temel olarak hayvan yemi ve malt sanayinde kullanılmak üzere yetiştirilmektedir. Arpa bünyesinde taşıdığı yüksek oranda nişasta ve malt enzimleri sayesinde malt üretiminde oldukça önemli bir yere sahiptir. Bu nedenle malt kalitesini iyileştirmek için ıslah çalışmaları gittikçe artmaktadır. Malt kalitesi tane protein içeriği, b-glucans kırılganlık ve diyastatik güç gibi birçok özelliğe bağlıdır. Bu bağlamda maltın kalitesini artırmak için bu özellikleri göz önünde bulundurularak ıslah çalışmaları yapılmaktadır. Moleküler markörler, karmaşık maltlık kalite özelliklerini incelemek için yararlı araçlardır ve ıslahçılara yeni çeşitlerin üretmelerini hızlandırmak için genetik bilgiyi sağlamaktadır. DNA markörleri ile moleküler tarama yapmanın ilk adımı PCR optimizasyonu için primerlerin bağlanma sıcaklığının belirlenmesidir. Bağlanma sıcaklığının belirlenmesinde yaygın olarak gradiyent PCR uygulaması yapılmaktadır. Bu çalışmada malt kalitesini belirleyen özelliklerle ilgili geliştirilen 14 primerin bağlanma sıcaklığı gradiyent PCR ile belirlenmiştir. Ayrıca sıcaklığın PCR optimizasyonu üzerindeki etkisini incelenmiştir. Elde edilen sonuçların markör destekli seleksiyon çalışmalarına ön veri sağlayarak ıslah çalışmalarının hızlandırılmasında katkı sağlayacağı sonucuna varılmıştır.

Anahtar Kelimeler: Arpa, malt, gradiyent PCR

Optimization Of Markers Determining Malt Quality With Gradient PCR

Abstract

Barley (*Hordeum vulgare* L.) is used both as animal feed and in the malt industry. Barley has a very important place in malt production due to its high starch and malt enzyme content. For this reason, breeding studies aimed at improving malt quality are increasing. The quality of malt depends on many properties such as grain protein content, b-glucans, Friability and diastatic power. In this context, breeding studies are carried out by taking these properties into consideration in order to increase the quality of malt. Molecular markers are useful tools for studying complex malting quality traits and provide breeders with genetic information to accelerate the production of new varieties. The first step in molecular screening with DNA markers is to determine the annealing temperature of the primers for PCR optimization. Gradient PCR is widely used to determine the annealing temperature. In this study the annealing temperatures of 14 primers developed for the characteristics determining malt quality was determined by gradient PCR. In addition, the effect of temperature on PCR optimization was investigated. It was concluded that the results obtained will contribute to the acceleration of breeding studies by providing preliminary data for marker-assisted selection studies.

Keywords: Barley, malt, gradient PCR

GİRİŞ

Arpa (*Hordeum vulgare* L.), gıda, yem ve biyoyakıt için yaygın olarak yetiştirilen bir serin iklim tahılıdır (Ghazy ve diğerleri, 2024). Arpa, mısır, pirinç ve buğdaydan sonra küresel üretim açısından dördüncü sırada yer almaktadır (Purugganan ve Fuller, 2009; Faostat, 2019). Anadolu'da yıllardır yetiştirilen arpa, ülkemiz tarımında buğdaydan sonra en çok ekimi yapılan tahıldır. Türkiye arpanın gen merkezlerinden biridir (Kün, 1996). Yetiştirme kolaylığı, zorlu ortamlara uyum sağlama yeteneği ve düşük gereksinimleri nedeniyle sınırlı kaynaklara sahip yetiştiriciler tarafından sıklıkla tercih edilen önemli bir kültür bitkisidir.

Türkiye'de yetiştirilen maltlık arpaların yemlik arplara göre hem ihraç edilme potansiyeli hem de fiyatları daha yüksektir. Bu nedenle ülkemizde yetiştirilen maltlık arpların üretim verimliliğini ve kalitesini artırmak önem arz etmektedir. Türkiye'de arpa ekimi, kuru tarım koşullarına sahip Doğu Anadolu ve Güneydoğu Anadolu Bölgesi yapılmaktadır. Bu da Türkiye'de arpaların birim alandan elde edilen tane verimin global ortalamadan daha düşük olmasına neden olmaktadır.

Arpa taneleri malt ve yem olarak kullanılan önemli bir tahıl bitkisidir. Arpa malt kalitesi, özellikle bira endüstrisi için çok önemlidir (Li ve diğerleri, 2023). Genotip-çevre etkileşimleri, çekirdek dolgunluğu, malt özütü ve diyastatik güç gibi malt kalite özelliklerini etkiler (Craine ve diğerleri, 2023). Maltta diyastatik gücü etkileyen en önemli faktörlerden biri protein oranıdır. Genel olarak yüksek protein oranına sahip arpa çeşitleri yüksek diyastatik güce sahip maltlar elde edilmesine olanak sağlamaktadır (Türker, 1977). Diyastatik güç ve protein oranı arasında doğru orantılı bir ilişki vardır, arpa tanesinde protein oranının artmasıyla beraber maltın diyastatik gücü aynı oranda artmaktadır. Bu ilişki Swanston (1979) elektroforez yöntemini kullanarak yaptığı analizde doğrulanmıştır. Aynı şekilde alfa amilaz aktivitesi ile arpa tanelerinin azot içeriği ve diyastatik güç arasında doğru orantılı bir ilişkinin olduğu başka çalışmalarda rapor edilmiştir (Hayter ve Riggs, 1973). Bunun yanı sıra değişen çevre koşullarının maltlık arpanın sahip olduğu fiziksel özellikleri üzerinde önemli değişikliklere neden olduğu bildirilmiştir (Ewertson, 1977; Atlı ve diğerleri, 1989). Hektolitre ağırlığı, tanelerin değerlendirilmesinde en güvenilir fiziksek kriterlerden biridir. Yüksek hektolitre ağırlığına sahip arpalar yüksek malt ekstreye sahip olduğu anlamına gelmektedir. Aynı zamanda, bin tane ağırlığının yüksek olması tane iriliğini, dolgunluğunu ve nişasta içeriğinin zenginliğine işaret etmektedir (Ewertson, 1977; Atlı ve diğerleri, 1989). Fox (2010)'un tarafından yapılan çalışmada tanenin fiziksel özellikleri ile malt kalitesi arasında bir ilişki olduğu bildirilmiştir. Ayrıca yapılan çalışmada tane büyüklüğünün 2.5-2.8 mm aralığında olması gerektiğini ifade edilmiştir. Başka bir çalışmada da bazı arpa çeşitlerinde bin tane ağırlığının 40.7-43.7 g arasında olduğu bildirilmiştir (Koçak ve diğerleri, 1992).

Moleküler markörler, karmaşık maltlık kalite özelliklerini incelemek için yararlı araçlardır ve ıslahçılara yeni çeşitleri geliştirmelerini hızlandıran genetik bilgiyi sağlar (Saygılı ve diğerleri, 2021). Moleküler markörler, türler ve popülasyon içindeki ve arasındaki genetik çeşitliliğin karakterizasyonu ve değerlendirilmesinde kullanılmıştır. Polimeraz zincir reaksiyonunun (PCR) keşfi basit dizi tekrarları (SSR), dizi etiketli siteler (STS) ve basit tekrarlı diziler arası polimorfizm (ISSR) gibi farklı moleküler markörlerin geliştirilmesini desteklemiştir. Bu moleküler markörler, genotip tanımlamasında, genetik karakterizasyonda, genetik haritalamada ve markör destekli seleksiyonda kullanılmaktadır (Kojima ve diğerleri, 2007; Pan ve diğerleri, 2008; Kandemir ve diğerleri, 2022).

Moleküler seleksiyon ve karakterizasyonda kullanılan PCR optimizasyonu için farklı primerlerin bağlanma sıcaklığının belirlenmesi önemlidir. Bağlanma sıcaklığının belirlenmesinde yaygın olarak gradiyent PCR uygulaması yapılmaktadır. Gradiyent PCR, spesifik moleküler markörleri çoğaltmak için PCR koşullarının optimizasyonunu sağlayarak özellikle markör destekli seleksiyonda (MAS) önemli bir rol oynamaktadır. Bu teknik, araştırmacıların kullandıkları primerlerin çalıştığı optimum sıcaklık koşullarının belirlemelerine olanak tanımaktadır. Böylece PCR için optimum koşulları belirleyerek amplifikasyon sürecinin verimliliğini artırabilir. Aynı zamanda gradiyent PCR ile tutarlı ve optimize edilmiş koşullar, farklı deneyler ve laboratuvarlarda tekrarlanabilir sonuçlar elde etmeye yardımcı olur. Gradiyent PCR, zamandan, reaktiflerden ve sarf malzemelerinden tasarruf sağlayarak optimizasyonu kolaylaştırır. Bu bağlamda yapılan bu çalışmada malt kalite özellikleri ile ilgili 14 adet markörün gradiyent PCR ile optimum çalışma sıcaklığı belirlenmiştir.

MATARYAL VE METOD

DNA İzolasyonu:

Genomik DNA izolasyonu için Yıldız çeşidine ait genç yapraklar kullanılarak aşağıda belirtilen Doyle ve Doyle (1987)'nin modifiye edilmiş CTAB protokolü kullanılmıştır.

- 100 mg arpa yaprağı sıvı azot içerisinde öğütülmüş, üzerine 500 µL tampon ve 20 µL proteinase K ilave edildikten sonra vortekslenmiştir.
- 40 µL %20 SDS eklenecek ve su banyosunda 65 °C 1 saat boyunca inkübe edilmiştir.
- İnkübasyon tamamlandıktan sonra her bir tüpe 2/3 hacim kloroform:isoaiml alkol (24:1) eklenmiş ve alt üst edilerek 10 dakika boyunca karıştırılmıştır.
- Sonrasında 10.000 rpm'de 15 dakika boyunca santrifüj edilmiştir.
- Elde edilen süpernatant 2/3 hacminde yeni bir tüpe aktarılıp ve 2-propanol eklenmiştir.
- Sonrasında 20 dakika boyunca 10.000 rpm'de santrifüj edilmiştir.

- Elde edilen süpernatant pelete zarar vermeden dökülmüştür.
- Elde edilen pelet kurutulduktan sonra 400 µL TE tamponu eklenip ve su banyosunda 65 °C'de 2 saat inkübe edildikten sonra tüpler +4 °C'de 1 gece tutulmuştur.
- Bir gece bekletilen tüpler su banyosunda 60 °C'de 3 saat inkübe edilmiştir. Sonrasında her bir tüpe 10 mg/µL RNase çözeltisinden 1 µL eklenmiş ve 65 °C'de su banyosunda bekletilmiştir.
- Sonrasında 400 µL kloroform:isoamil alkol eklenmiş ve 15 dakika boyunca alt üst edilerek karıştırılmıştır.
- Sonrasında 10.000 rpm'de 15 dakika boyunca santrifüj edilmiştir.
- Elde edilen süpernatantın üçte ikisi 100µL 1,2 M NaCl içeren yeni bir tüpe aktarılıp hafifçe karıştırılmıştır.
- Sonrasında % 96'lık 800 µL soğuk etanol eklenmiştir. Etanol eklendikten sonra tüpler hafifçe alt üst edilerek karıştırılmıştır.
- 10.000 rpm'de 20 dakika boyunca santrifüj edilmiştir. Elde edilen süpernatant dikkatlice dökülmüştür.
- Sonrasında elde edilen peletler soğuk % 70'lik alkol ile yıkanmış ve 2-3 saat boyunca kurutulduktan sonra 80 µL TE tamponu eklenmiştir
- Sonrasında tüpler +4 °C'de muhafaza edilmiştir.

DNA miktarı 260-280 nm dalga boylarında spektrofotometrik absorbans ile ölçülmüştür. İzole edilen DNA konsantrasyonu 50 ng/µL'ye ayarlanmıştır.

Gradiyent PCR:

Bu çalışmada kullanılan primerler Tablo 1'de verilmiştir. Her bir farklı primer dizisi için PCR işlemleri, Hokanson ve diğerleri (1998) ve Liebhard ve diğerleri (2002) 'nin çalışmalarında kullandıkları şartlara göre yapılmıştır. Gerçekleştirilen her reaksiyon için 250 nM primer, deoksinükleotidlerin her birinden 0.2 mM, 2.0 mM $MgCl_2$, bir ünite *Taq* Polimeraz enzimi ve 50-100 ng kalıp DNA kullanılmıştır. PCR işlemi; 94 °C'de bir dakika DNA'nın denatüre edilmesi işlemi, kullanılacak primere göre 50-65 °C arasında primerlerin bağlanması (annealing) işlemi, 72 °C' de bir dakika DNA zincirinin uzatılması işlemi ve 72 °C beş dakika son uzatma sirkülâsyonu şeklinde yapılmıştır. PCR işlemi sonucunda elde edilen ürünler % 1'lük agaroz jelde yürütülmüş ve UV altında görüntülenmiştir.

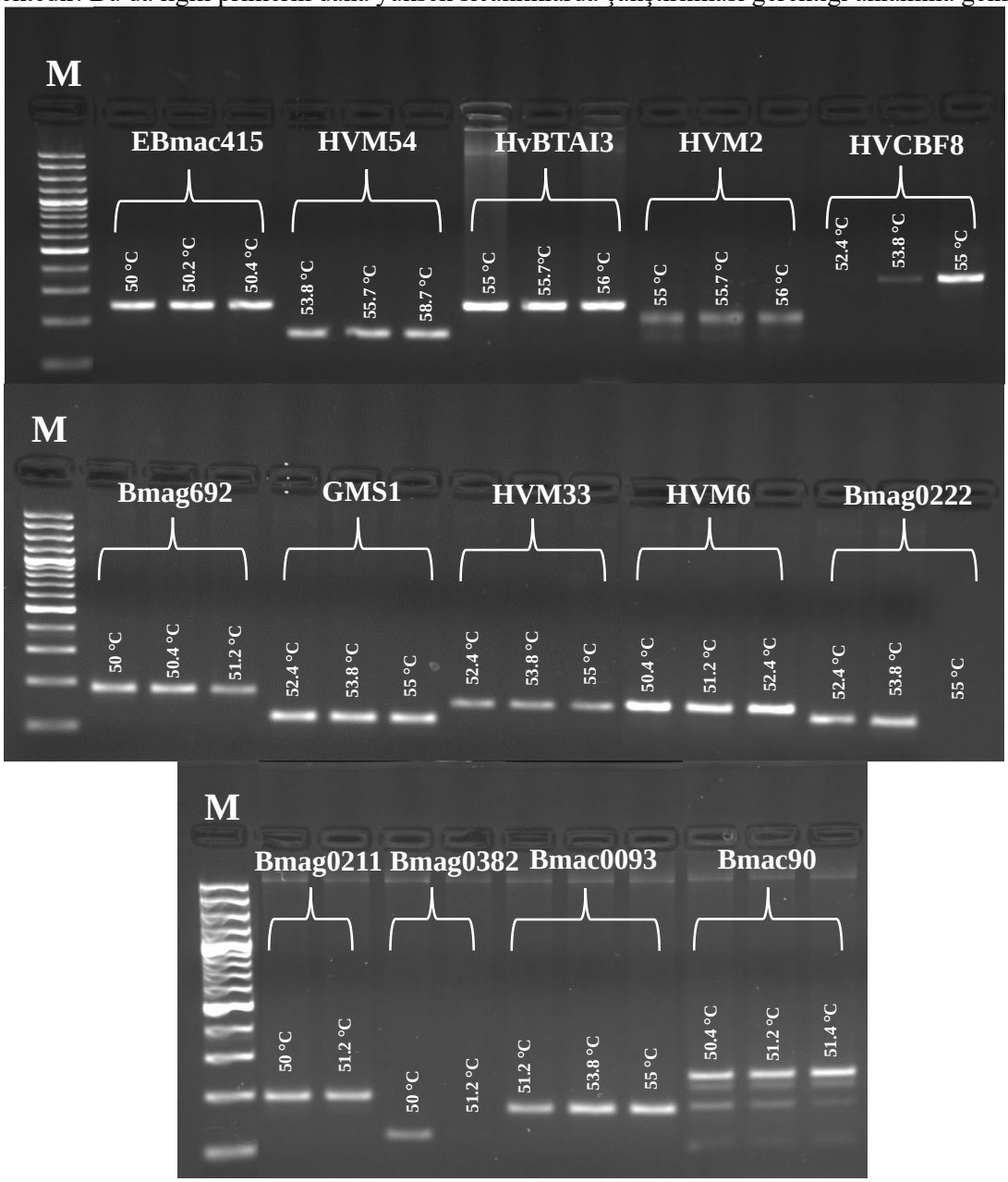
Tablo1. Çalışmada kullanılan primerlerin bilgileri

Primer Adı	Primer baz dizisi	Ürün bant uzunluğu (bç)	Primerin bağlı olduğu özellik	Referans
Bmag0211	5' ATTCATCGATCTTGTATTAGTCC 3' 5' ACATCATGTCGATCAAAGC 3'	174	Malt viskozitesi (Wort viscosity)	Laidò, 2008
Bmag0382	5' TGAAACCCATAGAGAGTGAGA 3' 5' TCAAAAGTTTCGTTCCAAATA 3'	109	Tane protein içeriği (grain protein content)	Laidò, 2008
Bmac0093	5' CGTTTGGGACGTATCAAT 3' 5' GGGAGTCTTGAGCCTACTG 3'	151	b-glucans Kırılganlık (Friability)	Laidò, 2008
HVM33	5' ATATTAAAAAAGGTGGAAAGCC3' 5' CACGCCCTCTCCCTAGAT 3'	157	Kırılganlık (Friability)	Laidò, 2008
HVCBF8	5' CAAGTTGCCGGTCGCGAC 3' 5'AACAGTTTCCTAATTGAGTGATGG 3'		Sıcak su ekstrakı (hot water extract)	Laidò, 2008
Bmag0222	5' ATGCTACTCTGGAGTGGAGTA 3' 5' GACCTTCAACTTTGCCTTATA 3'	179		Laidò, 2008
Bmac90	5' ACATCAACCCTCCTGCTC 3' 5' CCGCACATAGTGGTTACATC 3'	221	Malt extract, Diastatic power	Mei, 2012
HVM2	5' CAGGTGTCTAGTGGGTGCCT 3' 5' TTACATACCAAGGAGCAATCCC 3'	0,201	Malt extract, Diastatic power	Mei, 2012
Bmag692	5' GCAAGGTATCTCTTGTATTTTG 3' 5' TGGCATCTACAATCTAAAACA 3'	182	Malt extract	Mei, 2012
HVM54	5' AACCCAGTAACACCGTCCTG 3' 5' AGTTCCCTGACCCGATGTC 3'	159	Malt extract	Mei, 2012
EBmac415	5' GAAACCCATCATAGCAGC 3' 5' AAACAGCAGCAAGAGGAG 3'	274	Malt extract	Mei, 2012
HvBTAI3	5' GCCGCAGTCTCCTCCTCTTG 3' 5' ACTTGGCGAGGTATGGCTGC 3'	234	Alpha-amylase inhibitor	Mei, 2012
HVM6	5' CATGAATGAATGATTGGTTTTG 3' 5' CGCATCCGTATGTATGAGTAA 3'	175	Malt extract, Diastatic power	Mei, 2012

GMS1	5' CTGACCCTTTGCTTAACATGC 3' 5' TCAGCGTGACAAACAATAAAGG 3'	134	Malt extract, Diastatic power	Mei, 2012

BULGULAR VE TARTIŞMA

Bu çalışmada arpa yapraklarından genomik DNA izole edilmiş, gradiyent PCR ile amplifikasyon gerçekleştirilmiştir. Yapılan çalışma kapsamında maltın kalitesini etkileyen özellikler (maltın viskozitesi, kalitesi, tane protein içeriği, kırılganlığı, sıcak su ekstrakı, malt ekstrakı, diyastatik gücü ve alfa amlilaz inhibatörü) ile bağlantılı markörlerin optimum bağlanma sıcaklıkları belirlenmiştir. Şekil 1'de primerlere göre farklı saçaklıklarda yapılan gradiyent PCR ürünlerinin agaros jelde yürütülmesine dair bir kesit verilmiştir. EBmac415, HVM54, HvBTAI3, HVM2, GMS1, HVM33, HVM6, Bmag0211 primerler ile yapılan amplifikasyon sıcak değişiminden belirgin bir şekilde etkilenmemiştir. Ancak HVCBF8, Bmag692, Bmag0222, Bmag0382 ve Bmac0093 primerleri ile yapılan amplifikasyonlarda sıcaklığın etkisi belirgin bir şekilde görülmektedir. Elde edilen sonuçların çoğunda bağlanma sıcaklığının artırılması, beklenen büyüklükteki bantları daha belirgin hale getirmiştir. Ancak Bmag0382 primeri ile yapılan amplifikasyonda düşük sıcaklıkta (50 °C) ürün elde edilirken daha yüksek sıcakta (51.2 °C) ürün elde edilmemiştir. Bmac90 primeri ile yapılan amplifikasyon değerlendirildiğinde uygulanan 3 sıcaklıkta spesifik olmayan ürünler görünmektedir. Bu da ilgili primerin daha yüksek sıcaklıklarda çalıştırılması gerektiği anlamına gelmektedir.



Şekil 1. Gradiyent PCR ile denenen farklı bağlanma sıcaklıkları

Gradiyent PCR başta bağlanma sıcaklığı olmak üzere optimum $MgCl_2$, tampon ve primer konsantrasyonu gibi diğer modifikasyonların en uygun değerlerini optimize etmeyi sağlayan bir PCR çeşididir. Genel olarak düşük ısı spesifik olmayan ürünlerin meydana gelmesine neden olmaktadır. Yüksek ısı ise yanlış nükleotitlerin bağlanmasını önlemektedir. Bu nedenle primerlerin bağlanma ısısının optimizasyonu yapılması gereken optimizasyonların başında gelmektedir.

SONUÇ

Gradiyent PCR, primerlerin bağlanma sıcaklığını optimize etmeye yardımcı olan bir PCR çeşididir. Genel olarak markör destekli seleksiyon çalışmalarında ilk yapılan optimizasyonlardan biridir. Bu çalışmada malt kalitesini etkileyen primerler ile ilgili gradiyent PCR uygulaması yapılmış ve ilgili primerlerin en uygun bağlanma sıcaklıkları belirlenmiştir. Elde edilen sonuçlara bakıldığında bağlanma sıcaklığının artması ile amplifikasyon sonunda elde edilen bantların daha belirgin olduğu saptanmış, optimum çalışma sıcaklıkları belirlenmiştir. Ancak bazı durumlarda yüksek sıcaklık uygulandığında amplifikasyon olumsuz bir şekilde etkilenmiştir. Çalışmanın sonucunda elde edilen bulguların ileride yapılacak markör destekli ıslah çalışmaları için ön veri olarak kullanılabilirliği sonucuna varılmıştır. Bu da malt kalitesinin artırılmasına yönelik yapılacak ıslah çalışmalarının hızlanmasına yol açacaktır.

TEŞEKKÜR

Bu çalışma, Karamanoğlu Mehmetbey Üniversitesi Fen Bilimleri Enstitüsü'ndeki “Selami TUFAN “Yüksek Lisans Tez’inden türetilmiştir. Bu çalışma 16-YL-23 projesi kapsamında Karamanoğlu Mehmetbey Üniversitesi Bilimsel Araştırma Projeleri Koordinatörlüğü tarafından finanse edilmiştir.

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ORAL PRESENTATION

Determination of DPPH removal activity of *Amaranthus caudatus* L. grown with different fertilizer applications

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Abstract

In this study, it was aimed to examine the effects of applying organic and vermicompost fertilizers at different doses (half, recommended, double) to *Amaranthus caudatus* L. on the DPPH removal activity of the plant. This study was carried out in the research greenhouse of Karamanoğlu Mehmetbey University in 2022-2023. For the research, 7 different application groups were created along with the control group. Fertilizer application was made 3 times in total: before planting the seeds (6-hour treatment), when the plants were 15-20 cm tall, and during the flowering period. Plants that completed their vegetative period were harvested and stored under appropriate conditions.

Keywords: *Amaranthus caudatus*, Fertilizer, DPPH Activity

1. INTRODUCTION

Free radicals are chemical structures that contain one or more unpaired electrons in their terminal orbitals. Unpaired electrons in their outer orbitals greatly increase the reactivity of these structures with other structures. Free radicals can be positively charged, negatively charged or electrically neutral. These free radicals, which are harmful to living things, are eliminated by antioxidants. Antioxidant substances cope with these harmful compounds thanks to the phenolic compounds in their structure. Antioxidant substances neutralize free radicals by donating their electrons to them, and in the meantime, they do not turn into free radicals themselves. Antioxidants are divided into two classes: natural and artificial. Artificial antioxidants; butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and propylgallate (PG). Natural antioxidants are compounds found in plant and animal tissues that can be extracted. The most important natural antioxidants are tocopherols, flavonoids, phenolic acids, vitamin C, carotenoids, polyphenols and selenium. Interest in natural antioxidants is increasing due to the widespread belief that artificial antioxidants are carcinogenic (Doğmuş and Durucasu, 2013).

Amaranthus caudatus plant has become increasingly used both in research and as food in recent years due to the rich compounds it contains. When the studies are examined, it is seen that both the above-ground and underground parts of the *Amaranthus* plant contain significant levels of phenolic compounds. Thanks to the rich phenolic compounds it contains, it has a strong antioxidant property by fighting free radicals, which are extremely harmful to living things (Karamac et al., 2019).

2. MATERIAL AND METHODS

2.1. DPPH scavenging effect of *Amaranthus caudatus* L.

For the research, the tassel parts of the *Amaranthus caudatus* L. plant, grown by applying different fertilizer and dose adjustments, were cut and subjected to soxhlet extraction (10-12 hours). Methanol was chosen as the solvent component. Samples were prepared in different concentrations from the extracts obtained at the end of

the extraction, 0.5-1-2.5-5-10-25-50 mg/ml. 0.25 mL of 1 mM DPPH, 100 µL of sample, 2 mL of methanol were added and incubated for 20 minutes (Karamaç et al., 2019). Then, absorbance measurements of the samples were taken at 517 nm. DPPH removal activity was calculated as a percentage according to the formula below (Seyhan, 2019).

DPPH removal activity % = (Control Abs- Sample Abs)/Control Abs x 100

3. RESULTS AND DISCUSSION

Plants protect themselves against other pests thanks to the secondary metabolites they contain. In addition, these secondary metabolites obtained from plants provide many benefits for other living things due to the phytochemicals they contain. Free radicals formed in living things are very harmful components for living things. These phytochemicals, which play a role in clearing free radicals, are obtained and used from plants. *Amaranthus caudatus* L. has become a popular plant in recent years due to these valuable phytochemicals it contains (Jimoh et al., 2019). When the studies of Li, H. and others are examined, it is seen that the leaf, shoot, stem and flower extracts of the *Amaranthus caudatus* plant have a very valuable antioxidant capacity (Li et al., 2015). According to another study, the methanol extract of Amaranthus caudatus L. appears to have a strong DPPH scavenging effect with an IC50 of 77.5 ± 0.4 µg/ml (Kumar et al., 2011). These studies show that different fertilizers and doses applied to the plant affect the phytochemicals contained in the plant, and as a result, the antioxidant capacity of the plant changes. It was understood that among the applied fertilizers, the organic semi-group and vermicompost recommendation group, prepared to be 50 mg/ml, had the highest antioxidant capacity. Compared to the control group, the lowest antioxidant capacity was in the vermicompost recommendation and organic recommendation groups prepared at 0.5 mg/ml concentration. These studies show that the phytochemicals that plants contain are affected by the nutrients or other factors they are exposed to during their growth stages, and therefore their antioxidant capacities change positively or negatively.

Table 1. DPPH % inhibition data values of *Amaranthus caudatus* L.

Concentration (mg/ml)	Org/Half	Org/Recommended	Org/Double	Verm/Half	Verm/ Recommended	Verm/ Double	Control	Askorbic acid
0.5	9.08±0.50	2.87±0.10	9.46±0.11	8.15±0.19	2.61±0.04	5.24±0	4.3±0.12	58.4±0.77
1	15.2±1	15.67±0.07	19.34±0.03	16.39±0.14	17.85±0.16	15.96±0.03	15.93±0.07	87.2±1.8
2.5	58.13±0.07	46.67±0.04	56.95±0.03	47.42±0.07	34.84±0.09	39.22±0.05	33.33±0.07	
5	58.35±0.12	48.2±0.28	57.16±0.09	50.18±0.026	42.04±0.26	39.09±0.05	33.49±0.15	
10	59.22±0.07	58.44±0.04	74.07±0.09	59.98±0	77.75±0.08	65.82±0.04	61.52±0.04	
25	81.67±0.11	72.15±0.16	75.15±0.16	60.57±0.07	86.35±0.18	81.95±0.03	61.76±0.08	
50	89.95±0.02	80.11±0.04	77.16±0.07	63.11±0.02	88.22±0.12	85.47±0	69.88±0.4	

Each value was expressed as mean±Std. It was done in 3 repetitions. Data were tested with Duncan's Multiple Range (α=0.05).

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ORAL PRESENTATION

Investigation of the effects of different fertilizer treatments on the germination time of *Amaranthus caudatus* L.

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Abstract

In this study, it was aimed to investigate the effects of different doses (half, recommended, double) of organic, vermicompost and chemical fertilizers applied to *Amaranthus caudatus* L. on the emergence time (germination) and antioxidant capacity of the plant. This study was carried out in the Karamanoğlu Mehmetbey University research greenhouse in 2022-2023. 10 different treatment groups were formed for the research, including the control group. Fertilizer treatment was carried out 3 times in total, before planting the seeds (6-hour treatment), when the plants were 15-20 cm tall and during the flowering period. The plants that completed their vegetative period were harvested and stored under suitable conditions.

Keywords: *Amaranthus caudatus*, Fertilizer, Germination Time

1. INTRODUCTION

Many plant and living species have become extinct due to drought, which is one of the biggest problems of today. For these reasons, scientists have searched for species that are resistant to extreme conditions, can grow under difficult conditions, and produce abundant crops. Species within the Amaranthus genus are also quite resistant to infertile soils and arid climatic conditions and can withstand more drought than many cultivated plants (Temel et al., 2020).

Amaranthaceae Juss., also known as Amaranthaceae Juss., is a crowded family containing 180 genera and 2050-2500 species in the world. In Turkey, it is known with 33 genera. It is considered a difficult genus in terms of taxonomic studies due to its few and barely noticeable distinctive characters among its species. This plant can easily adapt to tropical and temperate environments. They are generally herbaceous, annual or perennial plants, short-lived, monoecious or dioecious, with spikes, compound panicles or cluster-shaped flowers at the top of the main axis (Uygur et al., 2021).

Grown for different uses, cockroaches are one of the oldest known edible grain sources and vegetables. It is also widely used as an ornamental plant. Its use as food in Mexico dates to 4000 BC. *A. caudatus* L., *A. cruentus* L. and *A. hypochondriacus* L. were domesticated as pseudocereals in South America and South Asia (Uygur et al., 2021).

Amaranth is also a good option for grains with its valuable nutritional components such as high levels of unsaturated fat, squalene and gluten-free proteins. Studies on Amaranth show that Amaranth seeds, oil and leaves have a lowering effect on blood pressure, cholesterol and blood sugar. In addition, antioxidant, anti-cancer properties and being a good food for celiac patients are among the benefits of Amaranth to human health (Boz, 2013).

2. MATERIAL AND METHODS

2.1. Surface sterilization of seeds

Amaranthus caudatus L. seeds to be used as research material were obtained from Tacir Depot. Before planting the seeds, surface sterilization was done by keeping them in 1% sodium hypochlorite (NaClO) solution for 1 minute and then washing them with sterile pure water (Ulusu and Şahin, 2020).

2.2. *Amaranthus caudatus* L. cultivation and fertilization

After surface sterilization of *Amaranthus caudatus* seeds, the seeds were kept in a mixture prepared at the rate of 100 mL of fertilizer per 1 liter of water for 6 hours before planting the seeds. Then, peat and garden soil were mixed in half, and the seeds were planted in the pots in April 2022, with 4 repetitions in each pot, every 3-4 days in the research greenhouse of Karamanoğlu Mehmetbey University (at 25-30 °C, in sufficient light and humidity conditions). grown under irrigation. In the study, 3 different fertilizers, including liquid synthetic, liquid organic and liquid vermicompost, were applied at 3 different rates (half dose, recommended dose and twice the dose), and a total of 10 treatment groups were created including the control group. After germination in the pots, the first fertilization was carried out when more than half of the plants reached 15-20 cm in height. The second fertilizer treatment was made when more than half of the plants started to bloom. The contents and amounts of fertilizers used in the study are given in Table 1.

Table 1. Fertilizer contents and amounts used

Treatment Number	Treatment Name	Chemical Composition	Half Dose	Recommended Dose	Double Dose
1	Control group	-	-	-	-
2	Liquid Synthetic (Chemical) fertilizer	N: P: K (%7: 7: 7)	1-1.5 L/ da	2-3 L/ da	4-6 L/ da
3	Liquid organic fertilizer (vegetable origin))	Total organic matter: 45% Organic C: 19.5% N: 3% K_2O:7	150-300 mL/da	300-600 mL/da	600-1200 mL/da
4	Liquid vermicompost manure (vegetable and animal origin)	Total organic matter: 5% N: 1% Humic+fulvicacid: 10%	0,75-1 L/da	1,5-2 L/da	3-4 L/da

2.3. Statistical analysis

Differences in control and treatment groups were analyzed by one-way ANOVA according to Duncan's multiple range test and were calculated at a significance value of $P<0.05$. In the study, 4 repetitions were made for each group (n = 4) (Duncan, 1955).

3. RESULTS AND DISCUSSION

3.1. Germination time (days)

Emergence time is defined as the time from planting the seeds until 70-80% emergence occurs in each pot. In the study, Amaranthus seeds were planted in each pot in 4 repetitions on 18.04.2022. In the study, it was observed that the first germination occurred on the 7th day. The latest germination time was on the 17th day. It was observed that the fastest germination among the treatment groups in terms of emergence time was in the chemical two-layer group with 7 days, followed by the vermicompost double layer and chemical recommendation group with 8 days. The longest germination time was 16 days with twice the organic dose and 14 days with the organic recommendation. Differences in the fertilizers, types and dosages applied in the study revealed statistical differences in terms of plant emergence time.

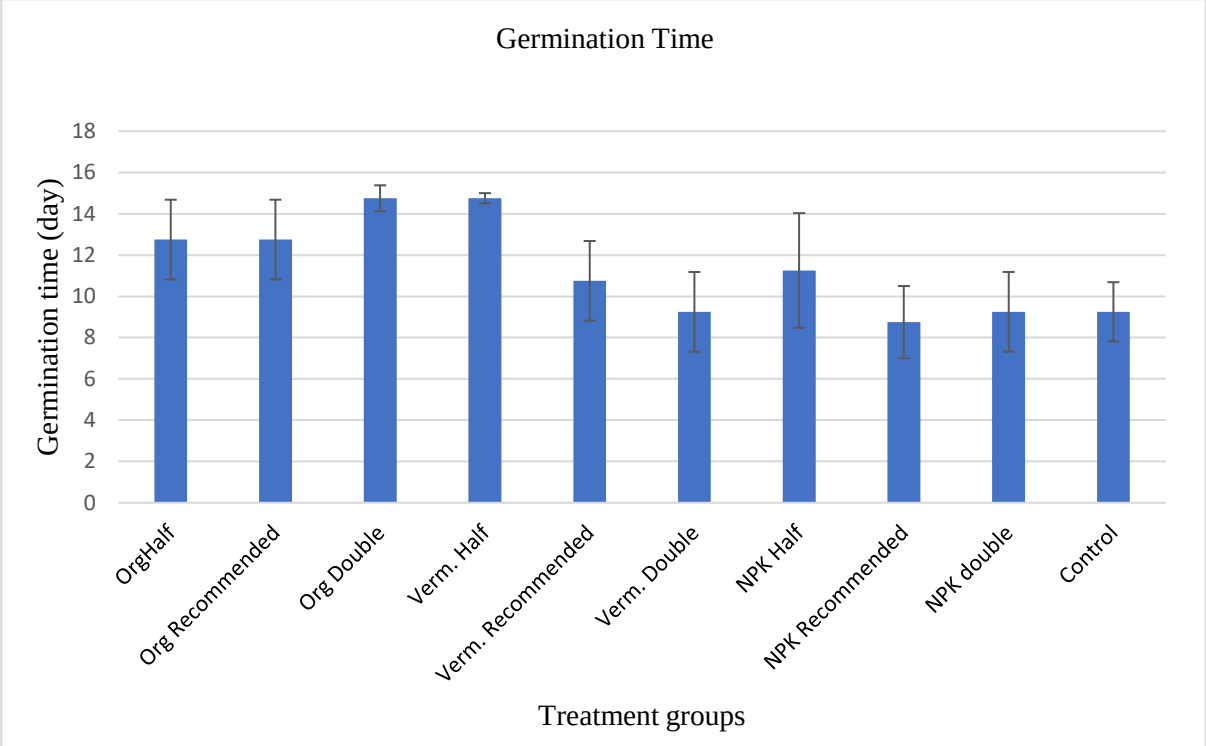

Figure 1. Average emergence (germination) times of treatment groups**.**

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ORAL PRESENTATION

Fabrication and thermoelectric properties of electro-blown poly(ε-caprolactone)/copper oxide fibers

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Abstract

Nanofibers have a wide range of applications due to their high surface area/volume ratio, high pore volume and diameters usually less than one micron. Therefore, it is important to develop new methods to produce nanofibers. One of these methods, electro-blowing, provides the opportunity to produce large amounts of nanofibers in a very short time and also saves energy by using air flow. In order to benefit from this advantage, in this study, poly(ε-caprolactone)/copper oxide fibers were produced by electro-blowing method using biocompatible and biodegradable poly(ε-caprolactone) and copper oxide nanoparticles synthesized at different ratios. The FTIR spectra of the synthesized copper oxide nanoparticles and fibers produced were taken. The surface morphologies of the fibers were examined with SEM and it was observed that the diameters of the fibers changed with the change in the amount of copper oxide. The electrical conductivity and Seebeck coefficient of the poly(ε-caprolactone)/copper oxide fibers were measured and their power factors were calculated with their results. The highest Seebeck coefficient of 52 μV/K was obtained from the fiber with the highest amount of copper oxide. Poly(ε-caprolactone)/copper oxide fibers were produced for the first time by the electro-blowing spinning method in this study and their thermoelectric properties were investigated for the first time in this study.

Keywords: Nanofiber, electro-blowing, poly(**ε**-caprolactone), copper oxide

INTRODUCTION

Poly(ε–caprolactone) (PCL) is a biodegradable and biocompatible polyester and has attracted considerable attention due to its favorable properties for producing nanofibers (Huner et al. 2023; Huner 2023). From past to present, PCL nanofibers have been produced using the electrospinning method and are still being produced. This method is a versatile technique used to create thin fibers from polymer solutions or melts under the influence of a high electric field. The diameters and morphological structure of electrospun nanofibers are affected by parameters such as applied voltage, distance between the nozzle and the collector, solvent, concentration and flow rate. The diameters of electrospun PCL fibers can vary from nanometers to micrometers depending on the process conditions. The formation of bead or smooth PCL nanofibers can also be affected by the process parameters. The advantages of PCL nanofibers make them attractive in many applications. For example, they are used as scales in tissues of this application, due to their mechanical strength, biocompatibility and controlled degradation rates. PCL nanofibers are suitable for drug delivery systems due to their high surface area (Huo et al. 2021), which allows for effective drug loading and controlled release.

Electroblowing is a technique frequently used in the field of material processing, especially in the production of nanofibers and nanostructured materials (Elnabawy et al. 2023; Omer et al. 2021). This technique can be adapted for both large-scale industrial production and small-scale laboratory experiments. It is scalable because it provides flexibility in producing nanofibers in various quantities. Compared to traditional fibers, nanofibers produced by electroblowing generally exhibit superior mechanical properties, increased strength and flexibility. Another advantage of electroblowing is that it can be more environmentally friendly and cost-effective compared to some other nanofiber production methods. There is less waste in the electroblowing method and less energy can be used. In addition, electroblowing is generally a safe and non-toxic process compared to some other production methods that may involve hazardous chemicals or high temperatures. These advantages make it a valuable technique in various fields, including biomedical engineering, environmental technology, and materials science.

Copper oxides are important materials with a wide range of applications, from catalysis (Jabłońska and Palkovits 2016) and semiconductors (El-Shafai et al. 2021) to pigments (Peng et al. 2022) and environmental remediation (Naz et al. 2020). Research into the synthesis of nanostructured copper oxides is also ongoing to exploit their unique properties for advanced technological applications. Copper oxide (CuO) nanofibers synthesized using electrospinning techniques (Dung et al. 2016) or other methods have attracted significant interest in scientific research due to their unique properties and diverse applications. They are generally promising in areas such as environmental remediation, energy storage, catalysis, and sensors.

Thermoelectric polymers are materials that can convert heat gradients directly into electrical energy or electrical energy into heat (Fan and Ouyang 2019). Therefore, they are valuable for energy harvesting, cooling applications, and flexible electronics. These polymers offer advantages such as flexibility, lightness, and ease of processing, but they often face challenges related to their thermoelectric performance due to their low electrical conductivity (Zhang et al. 2021). To overcome these, various synthesis methods or additives are incorporated into these polymers (Li et al. 2022).

One study addresses the challenges in device fabrication and performance for the thermoelectric properties of nanofibers while highlighting the potential of nanofibers in wearable and flexible applications (He and Wang 2024).

In this study, PCL/CuO nanofibers containing different amounts of CuO nanoparticles were produced using the electroblowing method, which has many advantages mentioned above. These nanofibers were characterized by FTIR spectroscopy and their surface morphologies were examined by SEM. The electrical conductivities and Seebeck coefficients of PCL/CuO nanofibers were measured and their power factors were calculated.

MATERIALS AND METHOD

Poly(ε-caprolactone) (PCL; Mw: ~80.000; Alfa Aeser), copper(II) nitrate hemi(pentahydrate) (Sigma Aldrich), ammonium hydroxide (NaOH; Sigma Aldrich), copper(II) chloride ($CuCI_2$; Sigma Aldrich), ethanol (Sigma Aldrich), and chloroform (Sigma Aldrich) were used without purification. All investigations used distilled water (DDI).

Synthesis of CuO Nanoparticles

CuO nanoparticles were synthesized by precipitation method as reported in the literature (Phiwdang et al. 2013). 0.1 M copper chloride ($CuCl_2$) and 0.1 M copper(II) nitrate hemi(pentahydrate) aqueous solutions were prepared. While this solution was stirred, 0.1 M NaOH solution was slowly added dropwise until the pH reached 7. Black precipitates were obtained and washed with deionized water and absolute ethanol several times. Then, the washed precipitates were dried at 80 °C and finally calcined at 500 °C for 2-3 h. The final product obtained was called CuO_x.

Fabrication of PCL/CuO Fibers

A 15% PCL solution was prepared in acetone-chloroform (1:9). The distance between the nozzle and the collector was set as 10 cm. The solution flow rate was used at 10 ml/h and 15 kV was applied. The air flow was 2 bar and the fiber was produced under air flow. This fiber was called PCL fiber. Fibers were produced under the same conditions from mixtures prepared by taking 5, 10 and 20% CuOx according to PCL and these were named as PCL/CuO_x-5, PCL/CuO_x-10 and PCL/CuO_x-20, respectively.

Characterization

A TENSOR27, Bruker Optik, GmbH Fourier transform infrared (FTIR) spectrophotometer was used to identify the bonds of molecules of all fibers obtained. ENTEK FPP 470 (measured using the four point probe technique) and ENTEK (SC 001) were used to determine the electrical conductivity and Seebeck coefficient at room temperature. The morphological studies of fibers using the Zeiss EVO® LS 10 Scanning Electron Microscope (SEM). For this, first, fibers were coated with gold 1.70 nm at 14 mA.

RESULTS and DISCUSSION

FTIR spectra of CuO_x, PCL and PCL/CuO_x fibers are given in Figure 1. The FTIR spectrum of CuO_x showed peaks around 843, 1363 and 1640 cm^{-1}. These are consistent with the literature (Akpomie and Conradie 2023) and especially the peak around 843 cm^{-1} is suggested to be due to Cu-O vibration. It was observed that PCL fiber has characteristic peaks of PCL. The peaks of PCL at ~ 2860 cm^{-1} and ~ 1720 cm^{-1} correspond to C-H

and C=O stretching, respectively (Janarthanan et al. 2019). The FTIR spectra of PCL/CuO_x fibers are similar to PCL due to the dominance of PCL peaks.

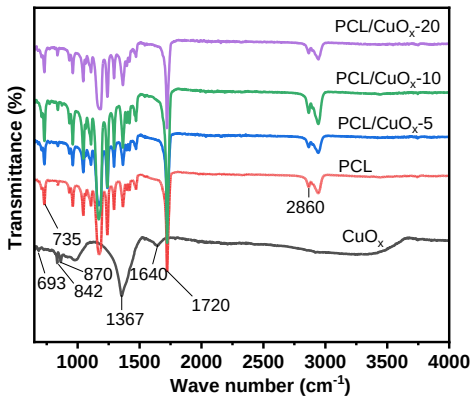

Figure 1. FTIR spectra of CuO_x, PCL, and PCL/CuO_x fibers

Electrical conductivity, Seebeck coefficient, and power factors of PCL/CuO_x fibers are given in Figure 2. The power factor (P) was calculated as the electrical conductivity (s) of the material multiplied by the square of the Seebeck coefficient (S) of the material ($P= s.S^2$) (Huner and Karaman 2022). Since PCL has no electrical conductivity (Chen et al. 2013), the thermoelectric properties of PCL fiber were not investigated and only the thermoelectric properties of PCL/CuO_x fibers were investigated. The electrical conductivities of the fibers with CuO_x as mass percentage of 5, 10 and 20% with respect to PCL are $8.5x10^{-7}$, $2.5x10^{-7}$ and $3.4x10^{-7}$ S/cm, respectively. Their Seebeck coefficients are 20, 25 and 52 µV/K, respectively. As the CuO_x ratio in PCL/CuO_x fibers increased, the Seebeck coefficient increased, although an irregular decrease in electrical conductivity was observed. In one study, the electrical conductivity for copper oxide (CuO) powder was reported as ~$1.5x10^{-4}$ S/cm (Mulla and Rabinal 2021) and in another study, it was reported as $5.3x10^{-2}$ S/cm (Sondors et al. 2024). In the same study, the Seebeck coefficient of copper oxide was reported as 632 µV/K. The Seebeck coefficient of copper oxide (Cu_2O) for powder and paste forms has been reported to be 656 and 539 µV/K, respectively (Andrei et al. 2014). In this study, the Seebeck coefficient of PCL/CuO_x fibers doped with PCL at different rates was measured and it was seen that the PCL/CuO_x-20 fiber with a CuO_x ratio of 20% by mass compared to PCL had the highest Seebeck coefficient. The power factor calculated with the results of electrical conductivity and Seebeck coefficient was also obtained as 2 $\mu W/mK^2$ from the highest PCL/CuO_x-20 fiber.

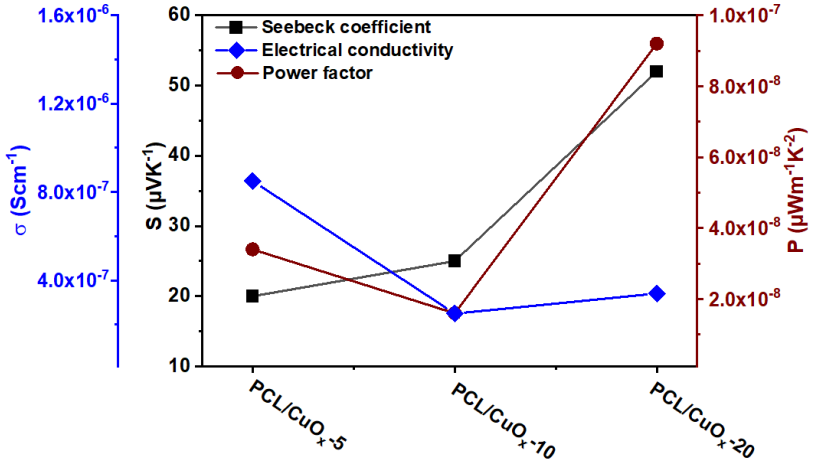

Figure 2. Electrical conductivity, Seebeck coefficient, and power factors of PCL/CuO_x fibers

SEM images of PCL and PCL/CuO_x fibers at 1.00 kX and 10.00 kX magnification are given in Figure 3. When the SEM images were examined, it was seen that all the materials were composed of straight and beadless fibers. Instead of regularly stacked fibers, they were composed of intertwined fibers. The number of nano-scale fibers in the electro-blown PCL fiber decreased with the addition of CuO_x, and the micro-scale fibers were more in the PCL/CuO_x-20 fiber containing the highest CuO_x content.

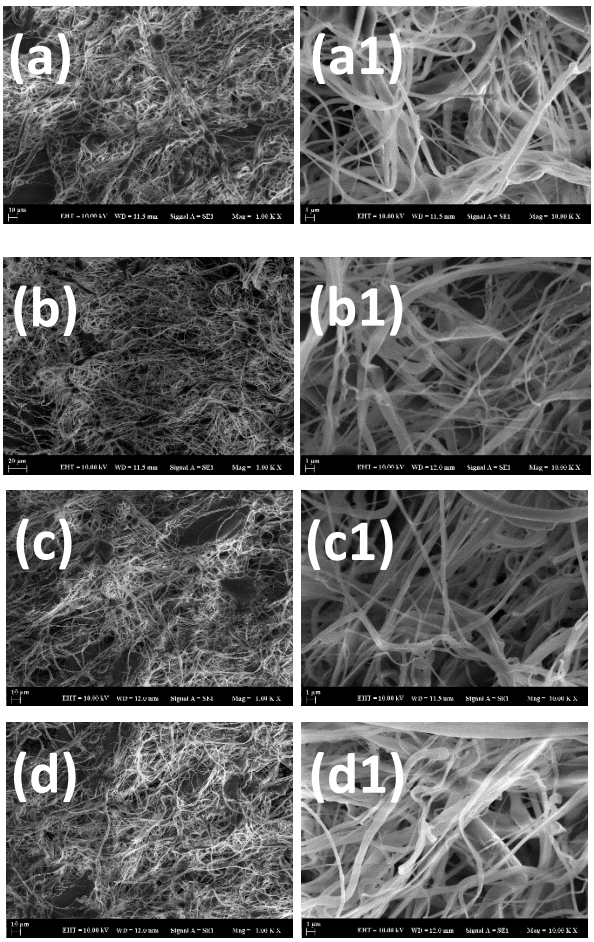

Figure 3. SEM images of a, a1) PCL, b, b1) PCL/CuO_x-5, c, c1) PCL/CuO_x-10, and d, d1) PCL/CuO_x-20

CONCLUSION

CuO_x nanoparticles were successfully synthesized. PCL/CuO_x fibers were produced by the electroblowing spinning method by mixing CuO_x nanoparticles with PCL in different ratios compared to PCL. These were characterized by FTIR spectroscopy. Electrical conductivities were measured, and the highest electrical conductivity value was obtained from the fiber with low CuO_x content. The highest Seebeck coefficient and power factor values were obtained from the fiber with the highest CuO_x content. According to SEM images of the fibers, it was seen that they were composed of beadless flat fibers, and CuO_x affected the fiber diameter. To improve the thermoelectric properties of these fibers produced by the electroblowing spinning method, which is a very fast method compared to the electrospinning method, it can be recommended to add a different additive with high electrical conductivity.

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ORAL PRESENTATION

Köpek oositlerinin *in vitro* maturasyonunda oviducttan toplanan hücrelerin etkisinin araştırılması

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Özet

Yardımcı üreme tekniği olan *in vitro* fertilizasyon (IVF), hastalıkların kontrol altına alınması, genetik materyalin korunması, nesli tükenme tehlikesi altındaki türlerin embriyo ve spermalarının saklanması, daha kaliteli oositlerin elde edilmesi gibi önemli avantajlar sunmaktadır. Dişi köpeklerde in vitro maturasyon (IVM) ve in vitro fertilizasyon gibi tekniklerin geliştirilmesi, nesli tükenmekte olan türlerin korunmasına ve gebelik oranlarının artırılmasına olanak sağlar. Bu tekniklerle, ölen veya kısırlaştırılmış köpeklerin ovaryumlarından oosit elde etmek ve *in vitro* koşullarda köpek embriyoları üretmek mümkündür. Ancak, köpeklerin üreme fizyolojisi diğer memelilere kıyasla farklıdır ve oositlerin in vitro olgunlaşma başarı oranı düşük kalmaktadır. Bu duruma özellikle köpek foliküler oositlerinin düşük mayotik kapasitesi sebep olmaktadır, bu da IVF çalışmalarını olumsuz etkilemektedir. Dişi köpeklerde oositler, germinal vezikül (GV) evresinde ovule edilir ve olgunlaşmaları için ovulasyondan sonra 2-3 gün süre gereklidir. Oositlerin tam olarak gelişebilmesi için çeşitli proteinler ve hormonlara ihtiyaç duyarlar. Bu nedenle, in vitro olgunlaşma sürecinde, memeli oositlerinin gereksinimlerini karşılamak için kültür medyumuna çeşitli katkı maddeleri eklenmektedir. Bu çalışmanın amacı, köpek oositlerinin in vitro maturasyon sürecinde medyuma oviducttan elde edilen hücrelerin eklenmesinin, kümülüs hücre genişlemesi üzerine olan etkisini incelemektir. Çalışma kapsamında, östrus aşaması bilinmeyen 1-3 yaşındaki melez dişi köpeklerin ovaryumları, rutin ovariohisterektomi operasyonu sonrası toplandı. Toplanan ovaryumlar, %0,9 serum fizyolojik solüsyonunda laboratuvara taşındı. Ovaryumlardan oositler, neşter yardımıyla Fosfat Tamponlu Tuz Çözeltisi (PBS) içerisine toplandı. Morfolojik olarak değerlendirilerek, A ve B sınıfına giren oositler maturasyon için seçildi. Oviduktlar, ovarian bursa'dan diseke edilerek medyuma alındı. Sonrasında ovidukt lümeni yıkanmasıyla elde edilen hücreler santrifüj işlemiyle toplandı. Bu hücreler, 100 µl'lik maturasyon medyumu içeren mikrodamlalarda, %5 CO2, %95'in üzerinde nem ve 38°C sıcaklıktaki CO2 inkübatörde 72 saat boyunca maturasyon sürecine tabi tutuldu. Süre sonunda, oositlerin çevresindeki kümülüs hücre genişlemeleri stereo mikroskop altında değerlendirildi. Bulgulara göre, in vitro maturasyon sürecinde ovidukt hücrelerinin kültüre eklenmesi, kontrol grubuna kıyasla kümülüs hücrelerinin gelişimini iyileştirmiştir.

Anahtar Kelimeler: İn vitro matürasyon (IVM), Köpek oositleri, Üreme fizyolojisi

The investigation of the effect of cells collected from the oviduct on *in vitro* maturation of dog oocytes

Abstract

In vitro fertilization (IVF), an assisted reproductive technique, offers significant advantages such as controlling diseases, preserving genetic material, storing embryos and sperm of species at risk of extinction, and obtaining higher quality oocytes. The development of techniques such as in vitro maturation (IVM) and in vitro fertilization in bitches allows the protection of endangered species and increasing pregnancy rates. With these techniques, it is possible to obtain oocytes from the ovaries of dead or neutered dogs and to produce dog embryos in vitro. However, the reproductive physiology of dogs is different compared to other mammals, and the success rate of in vitro maturation of oocytes remains low. This situation is caused especially by the low meiotic capacity of canine follicular oocytes, which negatively affects IVF studies. In bitches, oocytes are ovulated in the germinal vesicle (GV) stage and 2-3 days are required after ovulation for their maturation. Oocytes require various proteins and hormones to fully develop. Therefore, various additives are added to the culture medium to meet the requirements of mammalian oocytes during the in vitro maturation process. The

aim of this study was to investigate the effect of adding cells obtained from the oviduct to the medium during the in vitro maturation process of canine oocytes on cumulus cell expansion. Within the scope of the study, ovaries of 1-3 year old mixed breed bitches with unknown estrus stage were collected after routine ovariohysterectomy. The collected ovaries were transported to the laboratory in 0.9% physiological serum solution. Oocytes from the ovaries were collected into Phosphate Buffered Saline (PBS) with the help of a scalpel. Oocytes that were morphologically evaluated and classified as A and B were selected for maturation. Oviducts were dissected from the ovarian bursa and placed in the medium. Afterwards, the cells obtained by washing the oviduct lumen were collected by centrifugation. These cells were subjected to maturation process in microdrops containing 100 µl of maturation medium, 5% CO2, 95% humidity and 38°C CO2 incubator for 72 hours. At the end of the period, cumulus cell expansions around the oocytes were evaluated under stereo microscope. According to the findings, the addition of oviduct cells to the culture during the in vitro maturation process improved the development of cumulus cells compared to the control group.

Keywords: *In vitro* maturation (IVM), Canine oocytes, Reproductive physiology

GİRİŞ

Binlerce yıldır evcil köpekler insanlara birincil yoldaş hayvan olarak hizmet etmiştir (Vilà ve ark 1999, Leonard ve ark 2002, Savolainen ve ark 2002). Amerika Birleşik Devletleri'nde yapılan bir çalışmaya göre bir veya daha fazla evcil köpeğe sahip 30 milyondan fazla aile bulunmaktadır (Patronek ve Glickman 1994). Köpekler insanlarla yoldaş olmanın haricinde aynı zamanda insan hastalıklarını incelemek için ideal bir hayvan modelidir, çünkü köpeklerde genetik olarak 450'den fazla hastalık bildirilmiş ve bildirilen bu genetik hastalıklarının çoğu (yaklaşık 360) insanlarda klinik ve moleküler karşılıklara sahiptir; bu benzerlik diğer hayvanlar ile kıyaslandığında neredeyse iki kat daha fazladır (Patterson 2000, Sargan 2004, Shearin ve Ostrander 2010).

Köpekler monoöstrik hayvanlardır. Mevsimin östrusa gelmede etkisi yoktur. Seksüel siklus; proöstrus 3-17 gün, östrus 3-21 gün, metöstrus (diöstrus) 50-90 gün ve anöstrus 120-140 gün evrelerinden oluşur. Yılda iki defa ve 5-13 aylık aralarla (ortalama 7 ay) ovulatör folliküler dönem (proöstrus ve östrus) gösterirler. Her ovaryumda 4-5 adet follikül oluşur. Ovulasyon spontan olarak östrus başlamasından sonra 5 gün içinde, lüteinize edici hormon (LH) salınımından 36-50 saat sonrasına kadar gerçekleşir. Tüm foliküller ilk ovulasyonu takiben 24 saat içinde ovulasyonlar tamamlanır. Köpeklerde oositler primer oosit safhasında, mayoz tamamlanmadan önce, folikülden salınır. Birinci polar cismin atılması ve mayozun tamamlanması ovudukt içerisinde gerçekleşir. Köpeklerde oositler ovulasyon sonrası 2-3 güne kadar fertilize olamazlar. Spermatozoitler östrustaki köpeğin genital kanalı içinde uzun süre canlı kalabilirler. Altı veya 10 gün fertilize etme yeteneğini korurlar (Concannon 2011)

Köpekler için yardımcı üreme teknolojisi, kalıtsal hastalıkları ortadan kaldırmak ve nesli tükenmekte olan köpek türlerinin korunması için yeni ilaçların değerlendirilmesinde genomu düzenlenmiş köpek modelleri oluşturulmasında kritik bir rol oynayabilir. Ancak, köpekler için yardımcı üreme teknolojisi diğer türlere göre çok geride kalmıştır (Chastant-Maillard ve ark 2010). Köpek oositlerinin in vitro olgunlaşması (IVM) ve döllenmesi (IVF) üzerine ilk çalışma 30 yıl önce Mahi ve Yanagimachi (1976) tarafından bildirilmiştir. Bu çalışmada, üreme döngüsünün rastgele aşamalarında dişi köpeklerden elde edilen oositlerin yaklaşık %25'i mayoz bölünmeye devam edebilmiş ve 48-72 saat kültürlendikten sonra metafaz I (MI) veya MII aşamalarına geliştiği gözlenmiştir. Kültürlenmiş oositlerin in vitro tohumlanması, oositlerin %70'inin spermatozoa tarafından dezenfeksiyonu ve %20'sinin dekondense sperm başı(başları) içermesiyle sonuçlanmıştır. Bu rapordan bu yana çok az ilerleme kaydedilmiştir.

Bugüne kadar, in vitro fertilizasyondan elde edilen yavrular yalnızca in vivo olgunlaştırılmış (IVV) köpek oositleri ile elde edilmiştir (Nagashima ve ark 2015). Daha önceki çalışmalarda in vitro olgunlaştırılmış köpek oositleri kullanılarak IVF gerçekleştirilmiştir, ancak canlı doğum elde edilememiştir (Rodrigues ve ark 2004, Saikhun ve ark 2008). Bugüne kadar, IVF veya somatik hücre çekirdeği transferi ile in vitro olgunlaştırılmış köpek oositleri kullanılarak hiçbir yavru üretilmemiştir. Köpeklerin karakteristik üreme fizyolojisinde, köpek foliküllerinden gelen oositler hala germinal vezikül (GV) aşamasındadır ve metafaz II (MII) aşamasına ulaşmak ve dölleme yeteneğine sahip olmak için yumurta kanalında hala 54-72 saatlik olgunlaşmaya ihtiyaç duyarlar (Reynaud ve ark 2005). Bu özellikler köpek oositlerinin in vitro olgunlaşmasını daha zor hale getirir ve köpek yardımcı üreme teknolojisinin gelişimini büyük ölçüde sınırlamıştır. Bu nedenle, köpek türleri için yardımcı üreme teknolojisini ilerletmek için etkili bir in vitro olgunlaşma sistemi bulmak önemlidir.

Bu çalışmada, IVM aşamasında oviduktan toplanan hücreler IVM solüsyonuna eklenmiş ve köpek oositlerinin maturasyon sürecinde önemli bir faktör olan kümülüs hücre genişlemeleri incelenmiştir. Bu süreçte, maturasyonun iyileştirilmesi hedeflenmiştir.

MATERYAL VE METOT

Ovaryumların toplanması

Ovaryum bursasındaki ovaryumlar, barınakta yapılan ovaryohisterektomi sırasında östrus döngüsünün farklı aşamalarında dişi köpeklerden toplandı. Ovaryumlar, 37°C'de %1 penisilin streptomisin solüsyonu içeren %0,9 tuzlu suda tutuldu ve 4 saat içinde laboratuvara taşındı.

Oositlerin toplanması

Her ovaryum yağ ve kan damarlarından temizlendi ve daha fazla diseksiyon için Tyrode's lactate-Hepes ortamı (TL-HEPES) içeren bir petri kabına yerleştirildi. Yumurtalık dokusu, IVM için üçten fazla kümülüs hücresi katmanı ve koyu pigmentli oosit sitoplazması içeren kümülüs-oosit komplekslerini (COC) toplamak için tekrar tekrar dilimlendi. Aynı solüsyon içerisinde değerlendirilerek A ve B sınıfına giren oositler maturasyon için seçildi.

Ovidukt hücrelerinin toplanması

Ovaryohisterektomi sonrası ovaryumlar, 37°C'de %1 penisilin streptomisin solüsyonu içeren %0,9 tuzlu suda tutuldu ve 4 saat içinde laboratuvara taşındı. Oviduktlar hemen ovarian bursa'dan diseke edilerek, TCM-199 içeren başka bir petri kabına yerleştirildi. Ovidukt lümeni aynı medium ile yıkandı, hafifçe kazınarak ve sıkılarak Eppendorf tüpüne aktarıldı. Ovidukt hücreleri, Eppendorf tüpleri içerisinde 3500 rpm'de 1,5 dakika süreyle santrifüj (Nüve, NF200) edilerek yıkandı ve hücreler iki kez TCM-199 içinde süspansiyon haline getirildi. Üst faz (süpernatant) atıldı ve kalan pelletlere yaklaşık 100 µL maturasyon mediumu eklendi (Enginler ve ark 2014).

In vitro maturasyon

Mevcut çalışmada kullanılmak üzere temel IVM ortamı olarak %10 fetal sığır serumu (FBS), 0,2 mm pirüvat, 1 mg/ml sistein ve 50 µg/ml gentamisin ile desteklenmiş TCM-199 hazırlandı. 25-30 adet COC, 500 µl IVM ortamı ve 0,5 µg/ml FSH içeren dört kuyucuklu petrilerde (Nunclon, Nunc, Roskilde, Danimarka) 38°C'de, havada %5 CO_2 ile 72 saat boyunca maturasyona (Memmert, bırakıldı.

Kümülüs hücre gelişimin değerlendirilmesi

Kümülüs hücre genişlemesi, kalibre edilmiş Stereo mikroskop (ZEİSS, Stemi508) kullanılarak kümülüs komplekslerinin çapları yatay ve dikey olarak ölçülüp ortalaması alınarak değerlendirildi. Hücre çekirdeklerinde dejenerasyon tespit edilen oositler değerlendirme dışı bırakıldı.

İstatiksel değerlendirme

Kümülüs komplekslerinin in vitro gelişim düzeylerini karşılaştırmak için toplanılan veriler SPSS 26.0 programında independent samples T-testi uygulandı.

BULGULAR

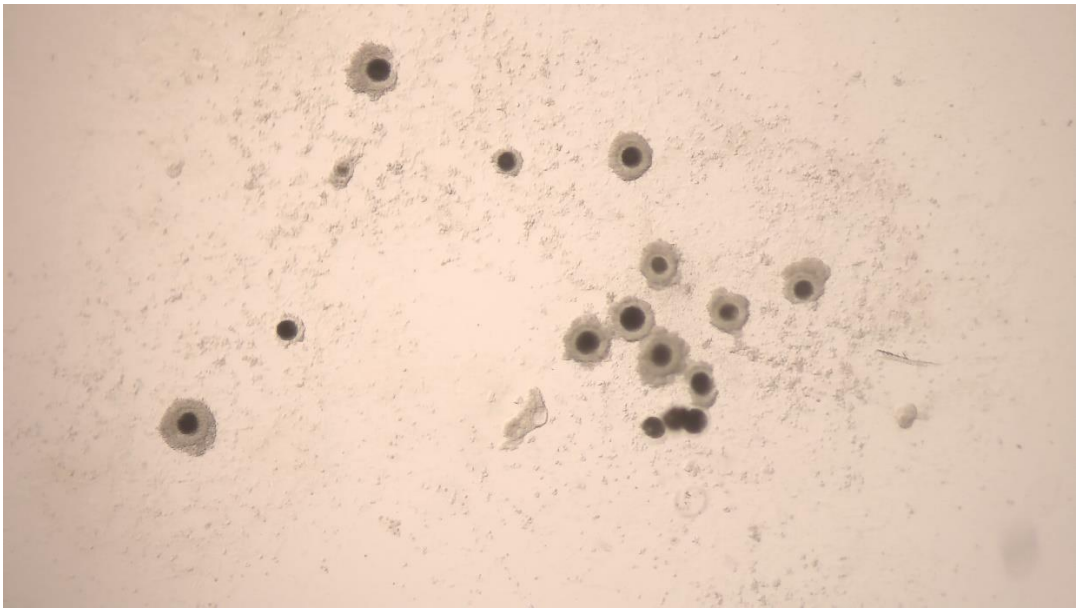
Kümülüs komplekslerinin çaplarının maturasyon süreci sonrasındaki karşılaştırılması sonucunda, Grup 1 (Kontrol) ve Grup 2 (Ovidukt Hücreleri Eklenmiş Maturasyon Solüsyonu Uygulanan Grup) arasında istatistiksel olarak anlamlı bir fark bulunmuştur ($P < 0,05$). Ovidukt hücreleri eklenen grubun kontrol grubuna göre kümülüs komplekslerinin çaplarının ortalama %13,18 daha geniş olduğu gözlemlenmiştir.

Tablo 1: Kümülüs komplekslerinin çaplarının maturasyon süreci sonrasındaki karşılaştırması yapılmıştır. Grup 1 (Kontrol), herhangi bir ovidukt hücresi eklenmeden yalnızca standart maturasyon solüsyonuyla incelenmişken, Grup 2'de maturasyon solüsyonuna ovidukt hücreleri eklenmiştir. (**P < 0,05**)

GRUP	Mean ± SEM diameter (µm) (range)
1	[a]188,1 ± 8,9 (35,7 - 49,9)
2	[b]212,9 ± 7,5 (36,5 - 50,8)



Figür 1: Grup 1 (Kontrol)



Figür 2: Grup 2 (Ovidukt Hücreleri Eklenmiş Maturasyon Solüsyonu Uygulanan Grup)

.TARTIŞMA

Birçok çalışmada, in vitro maturasyon ortamına belirli faktörler eklenerek köpek oositleri için potansiyel IVM teknikleri araştırılmıştır. Köpek oositleri üreme fizyolojisinde farklılıkları sebebiyle folikül içerisinde değil oviduktta olgunlaşmaktadır. Bu nedenle, yumurta kanalı epitel hücrelerinin in vitro köpek oosit olgunlaşmasında etkili olabileceği düşünülmektedir (No ve ark 2018). Bu çalışmada östrus evresi bilinmeyen köpeklerden toplanan ovaryumlardan elde edilen oositlerin gelişiminde oviduktan elde edilen hücrelerin katkısı değerlendirildi. Kümülüs komplekslerinin çaplarının maturasyon süreci sonrasındaki karşılaştırılması,

ovidukt hücrelerinin in vitro maturasyon üzerindeki etkisini ortaya koymaktadır. Grup 1 (Kontrol) ve Grup 2 (Ovidukt Hücreleri Eklenmiş Maturasyon Solüsyonu Uygulanan Grup) arasında istatistiksel olarak anlamlı bir fark bulunması ($P < 0,05$), ovidukt hücrelerinin kümülüs hücrelerinin gelişimine olumlu katkı sağladığını düşündürmektedir. Özellikle, ovidukt hücreleri eklenen grubun kontrol grubuna kıyasla kümülüs komplekslerinin çaplarının ortalama %13,18 daha geniş olması, bu hücrelerin maturasyon ortamında sağladığı potansiyel biyokimyasal veya yapısal destekle ilişkili olabilir. Bogliolo ve ark (2002) gerçekleştirdiği çalışma sonucunda, infundibulum ve ampulla bölgesinden toplanan oviduktal epitel hücrelerin kültür ortamına eklenmesinin, mayoz bölünme sürecine devam eden ve nükleer olgunlaşma sürecini tamamlayan (48.saat sonunda) in vitro köpek oositlerinin sayısını artırdığını göstermiştir.

Ovidukt hücrelerinin doğal ortamda oosit ve kümülüs hücreleriyle yakın temas içinde bulunması, onların gelişim süreçlerini destekleyici faktörler salgıladığını düşündürmektedir (Hunter 2012). Bu faktörlerin in vitro koşullarda da benzer bir etki göstermesi, özellikle köpek oositlerinin in vitro maturasyonu gibi zorlayıcı süreçlerde başarı oranlarını artırabilir. Elde edilen bu sonuçlar, ovidukt hücrelerinin eklenmesinin in vitro oosit maturasyonu çalışmalarında değerlendirilmesi gereken önemli bir faktör olduğunu göstermektedir (Lange-Consiglio ve ark 2017)(Luvoni ve ark 2005)

SONUÇ

Memelilerde oosit olgunlaşması, oositin içinde bulunduğu farklı anatomik ortamlar (folikül ve ovidukt) ile yakından ilişkilidir. Oositin somatik hücreler (granüloza, kümülüs ve ovidukt hücreleri) ile ya da germ hücreleri (spermatozoa) ile kurduğu biyolojik ve fiziksel etkileşimler, bu olgunlaşma sürecinde önemli rol oynar. Köpek oositlerinin in vitro ortamda başarılı bir şekilde geliştirilmesi için, düşük başarı oranlarını iyileştirebilecek etkili yöntemlerin geliştirilmesi gerekmektedir. Gerçekleştirilen bu çalışma sonucunda dişi köpeklerin oviduktlarından toplanan epitel hücrelerin in vitro maturasyon kültür ortamına eklenmesinin, köpek oositlerinin olgunlaşmasını ve kümülüs hücrelerinin gelişimini olumlu yönde etkilediği gözlenmiştir. Daha ileriki çalışmalarda oosit sayıları ve analiz yöntemleri arttırılmasının köpeklerdeki in vitro fertilizasyon probleminin çözümlerine katkı sağlayacağını düşünmekteyiz.

TEŞEKKÜR

Bu çalışma Aksaray Üniversitesi Embriyo Transferi Eğitim Uygulama ve Araştırma Merkezi tarafından finanse edilmiştir. Yazarlar, uygulamalara katkılarından dolayı kuruma ve yöneticilerine teşekkür etmektedir.

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ORAL PRESENTATION

Sıcak Havada Kızartma (Air Frying) Yöntemi ve Sağlık Üzerindeki Etkileri

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Özet

Sıcak hava ile kızartma (air frying), besin endüstrisinde düşük yağ içeriğine sahip yiyeceklerin üretimi için önemli bir çözüm olarak giderek daha fazla önem kazanmaktadır. Air fryer cihazları, içerisindeki fan ve sıcaklık sensörleri yardımıyla gıdaların sıcak hava sirkülasyonu ile hızlıca kızartılmasını sağlar. Bu süreçte yiyeceklerin dış yüzeyinde çıtır bir kızartma oluşturulurken, iç kısımları da eşit şekilde pişirilir. Air fryer ile yapılan pişirme işlemleri, azaltılmış yağ kullanımıyla daha sağlıklı yiyecekler sunarak; obezite, kardiyovasküler hastalıklar ve diyabet gibi kronik hastalıklarda azalma potansiyeli taşımaktadır.
Ayrıca, air fryer yöntemi, kanser gelişiminde rol oynayabileceği düşünülen akrilamid ve oksidasyon ürünlerinin oluşumunu azaltmaktadır. Çevresel açıdan da daha az yağ kullanılması ve kızartma sonrası atık yağ miktarının azalmasıyla air fryer, çevre dostu bir seçenek olarak değerlendirilmektedir.
Ancak, bu metotta bazı dezavantajlar da mevcuttur. Air fryer ile kızartmanın duyusal özellikler üzerindeki etkileri özellikle; kabuk oluşumu, lezzet, renk ve parlaklık gibi konularda geleneksel yöntemlerle çelişebilir. Ayrıca, pişirme süresinin uzun olması ve bazı çalışmalarda akrilamid düzeylerinde beklenen azalmaların görülmemesi de bu yöntemin sınırlamaları arasında sayılmaktadır.
Bu çalışma, air fryer yönteminin çalışma prensibini, avantaj ve dezavantajlarını, beslenme ve diyetetik alanındaki yerini ve bu konudaki güncel araştırmaları kapsamlı bir şekilde incelemektedir.

Anahtar Kelimeler: Air fryer, air frying, sıcak hava ile kızartma, pişirme yöntemleri, besin güvenliği

Air Frying Method and Its Effects on Health

Abstract

Hot air frying (air frying) has gained increasing importance in the food industry as a key solution for producing low-fat foods. Air fryer devices utilize fans and temperature sensors to rapidly fry food through hot air circulation. This process creates a crispy outer layer while evenly cooking the inside of the food. Air fryer cooking processes, by using reduced amounts of oil, can provide healthier meals, offering potential reductions in obesity, cardiovascular diseases, cholesterol, and diabetes-related chronic conditions.
Additionally, the air fryer method helps reduce the formation of acrylamide and oxidation products, which are thought to contribute to cancer development. From an environmental perspective, using less oil and reducing waste oil after frying makes air fryers an environmentally friendly option.
Nonetheless, several limitations are associated with this method. The sensory properties of air fryer-cooked foods, particularly crust formation, taste, color, and appearance, may differ from traditional methods. Furthermore, the longer cooking time and the inconsistent reduction in acrylamide levels reported in some studies are considered limitations of this method.
This study comprehensively examines the working principles of the air fryer method, its advantages and disadvantages, its role in the field of nutrition and dietetics, and the latest research on the subject.

Keywords: Air fryer, air frying, hot air frying, cooking methods, food safety

GİRİŞ

Air fryer, sıcak hava sirkülasyonu sayesinde yemeklerin pişmesini sağlayan, geleneksel kızartma yöntemlerine daha sağlıklı bir alternatif sunan bir cihaz olarak beslenme dünyasında dikkat çekmektedir. Bu teknoloji, yağ tüketimini azaltarak enerji alımını kontrol altında tutmayı hedefler ve bu nedenle obezite, kardiyovasküler hastalıklar ve diyabet gibi kronik rahatsızlıkların yönetiminde kullanılmaktadır. Air fryer yöntemi,

yiyeceklerde akrilamid ve oksidasyon ürünlerinin oluşumunu azaltma potansiyeline sahiptir (Zaghi ve ark., 2019). Bununla birlikte, air fryer kullanımının yiyeceklerin duyusal özellikleri ve besin değerleri üzerindeki etkileri, bu yöntemin yaygın kullanımı açısından dikkatle değerlendirilmelidir. Air fryer'ın çevresel etkileri de önemli bir avantaj olarak öne çıkmaktadır; daha az yağ kullanımı ve atık yağ miktarının azalması, bu cihazın sürdürülebilir bir seçenek olmasını sağlar (Andrés ve ark., 2013).

Sıcak Havada Kızartma (Air Frying)

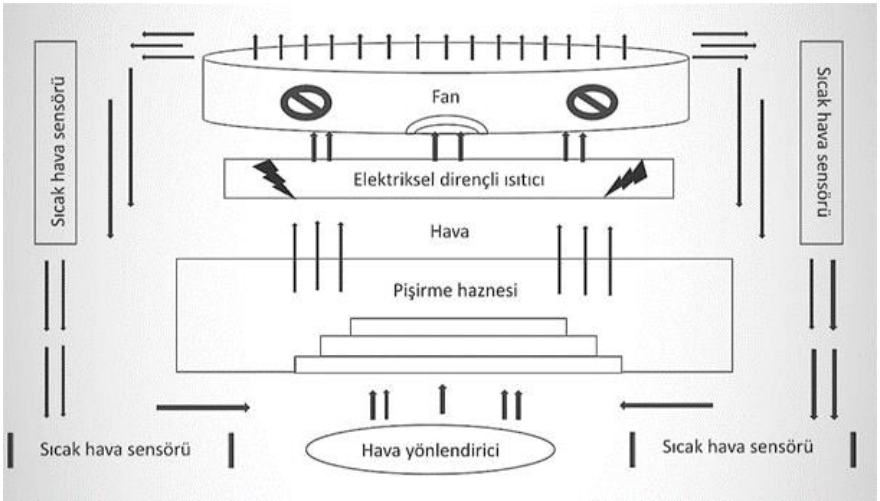
Sağlıklı ürünler hazırlamak için sıcak hava sirkülasyonunu kullanan yenilikçi bir yiyecek kızartma tekniği olan air frying teknolojisi, yiyeceklerin kızartılması veya pişirilmesi için sıcak havanın kullanıldığı bir yöntemdir. Bu sayede, klasik kızartma işlemine göre yaklaşık %80 daha az yağ kullanılarak yiyeceklerin hazırlanması mümkün olmaktadır. Bunun sonucunda, daha az kalori içeren ve bir kızartma veya pişirme yöntemi olarak bu teknoloji öne çıkmaktadır (Paul, 2023).

Air Fryer Tarihçesi

Sıcak havada kızartma prensibine dayanan air fryer, ilk kez 2010 yılında Hollandalı bir şirket tarafından piyasaya tanıtılmıştır. Tanıtımda cihazın ana amacı geleneksel kızartmaya kıyasla daha sağlıklı ürün elde etmek olarak açıklansa da yıllar içerisinde üretilen air fryerlar kızartmanın yanı sıra; közleme, ızgara, buharlı pişirme, hamur işleri pişirme gibi ek özelliklerle donatılmıştır. 2010'da air fryer cihazının piyasaya sürülmesi, ev tipi air fryer'ın popülerlik kazanmasına ve bu tarz pişirme cihazlarının daha geniş bir kitleye ulaşmasına yardımcı olmuştur (Zhao ve ark., 2021).

Air Fryer Çalışma Prensibi

Air fryer, yüksek hızlı sıcak hava dolaşımını kullanarak çalışan bir mutfak aletidir. Cihaz çalışmaya başladığında, sıcak hava üretir ve mekanik bir fan, yiyeceğin etrafında hızla dolaşarak yiyeceği kızartır ve üzerinde çıtır bir tabaka oluşturur (Tewari, Israni & Tolani, 2015). Air fryer cihazı genellikle beş ana parçadan oluşur: dayanıklı bir ısıtıcı, elektrikli bir fan, pişirme odası, hava akışı mekanizması ve sıcaklık sensörleri. Isıtıcının üstündeki büyük elektrikli fan, pişirme odasından havayı yukarı doğru çekerek güçlü bir dikey hava akışı oluşturur. Hava, elektrikli ısıtıcıdan geçerken ısı enerjisiyle yüklenir ve minimum hız ve enerji kaybıyla pişirme odasına taşınır. Hava akışı mekanizması, havayı dikey yönde yönlendiren ve ısıyı eşit şekilde dağıtan bir düzenek olarak altta yer alır. Sıcak hava, yiyeceklerin dış yüzeylerine etki ederken, onları kızartmak ve çıtır bir doku oluşturmak için gerekli olan yüksek ısıyı sağlar (Arslan ve ark., 2018). Bu yöntemde, yiyecekler genellikle ince bir yağ tabakasıyla kaplanır veya yağ püskürtülerek hazırlanır; bu da dış kabuk oluşumunu ve lezzeti artırır.



Şekil 1: Air fryer cihazının şematik diyagramı (Arslan ve ark., 2018).

4. Geleneksel Kızartma ile Sıcak Havada Kızartma (Air Frying) Yöntemlerinin Karşılaştırılması

Sıcak havada kızartma, geleneksel derin yağda kızartma yöntemine sağlıklı bir alternatif olarak öne çıkmaktadır. Bu teknik, yiyeceklerin sıcak hava ile temas ederek kızartılmasını sağlayarak yağ kullanımını minimumda tutar. Geleneksel kızartma yöntemleriyle karşılaştırıldığında, air fryer yöntemi genellikle daha az yağ emilimine neden olur ve bu da lipid bozulması ve oksidasyonun azaltılmasına katkı sağlar (Andrés ve ark.,

2013). Ayrıca, air fryer yöntemi, çevresel açıdan daha sürdürülebilir bir seçenek olarak görülmektedir; daha az yağ kullanımı ve atık yağ miktarının azalması, bu yöntemin çevreye olan olumsuz etkilerini sınırlamaktadır (Shaker, 2015).

Ancak bu yöntemin bazı dezavantajları da mevcuttur. Örneğin, air fryer ile kızartma işlemi genellikle daha uzun sürebilir ve bazı durumlarda geleneksel yöntemlere kıyasla daha fazla enerji tüketimi gerektirebilir (Teruel ve ark., 2015). Bazı çalışmalar air fryer yönteminde, yüksek sıcaklıklarda pişirilen gıdalarda oluşan ve potansiyel olarak kanserojen etkileri olabilen bir bileşik olan akrilamid oluşumunun tam olarak engellenmediğini ortaya koymuştur (Navruz- Varlı ve Mortaş, 2024).

Kimyasal özellikler açısından, air frying, derin yağda kızartmaya göre daha az yağ ve polar bileşik oluşumu sağlar. Bu da sağlık açısından daha az risk taşır (Zaghi ve ark., 2019). Ancak, bazı çalışmalarda sıcak havada kızartmanın geleneksel kızartmaya göre duyusal özellikler açısından bazı dezavantajları olduğu belirtilmiştir. Örneğin, lezzet ve doku farklılıkları, tüketicilerin air fryer ile kızartılan ürünler konusunda farklı beklentilere sahip olmasına yol açabilir (Wang ve ark., 2021; Dong ve ark., 2022).

Tablo 1. Geleneksel kızartma ile sıcak havada kızartmanın karşılaştırılması (Zaghi vd., 2019).

Analiz	Teknik	Sonuçlar					
Lipid bileşimi; bozulma göstergeleri; tokoferoller; toplam askorbik asit; βkaroten; antioksidan aktivitesi	Soya yağı ile geleneksel kızartma	Trans Yağ Asitleri	Tokoferoller	Toplam Askorbik Asit	β-karoten	Toplam Fenolikler	Toplam Antioksidan Aktivitesi
		0.026g/100g patates	4.60mg/100g	0.88mg/100g	167µm/100g	20.46mg gallik asit/100g	9.06mg gallik asit/100g
	Sıcak hava ile kızartma (Air frying)	0.004g/100g patates	1.13mg/100g	1.44mg/100g	167.5µm/100g	27.21mg gallik asit/100g	7.27mg gallik asit/100g

Ekipman performansı; örneklerin beslenme kalitesi; akrilamid içeriği	Soya yağlı geleneksel kızartma		Lipidler	Akrilamid içeriği	Enerji tüketimi	
					Pişirme süresi	Tüketim
			15.79g/100g	94.5 µg/kg	13 dakika	270.4 Wh
	Sıcak hava ile kızartma (Air frying)	Deney 1	9.25g/100g	209.5 µg/kg	20 dakika	233.0 Wh
		Deney 2	9.14g/100g	89 µg/kg	16 dakika	193.8 Wh

Patates kızartmasının yağının stabilitesi		Serbest yağ asitleri	Okside olmuş yağ asitleri	Kullanılan yağ miktarı	Absorbe edilen yağ miktarı
	Soya yağı ile geleneksel kızartma	Başlangıç değeri = %0,09	Başlangıç değeri = %0,002	Her 200 g patates için 2kg	%14,81
		Kızartma sonrası değer=%0,22	Kızartma sonrası değer=%0,11		
	Sıcak hava ile kızartma (Air frying)	Başlangıç değeri = %0,09	Başlangıç değeri = %0,002	30g/kg patates	%0,0025
		Kızartma sonrası değer=%0,12	Kızartma sonrası değer=%0,06		

Air Fryer Kullanımının Beslenme ve Diyetetikteki Yeri

Air fryer, beslenme ve diyetetik alanında sağlıklı pişirme yöntemleri arasında giderek daha fazla tercih edilmektedir. Geleneksel kızartma yöntemlerine kıyasla daha az yağ kullanarak yiyeceklerin pişirilmesini sağlamakta, bu da enerji kontrolüne ve yağ tüketiminin azaltılmasına önemli katkılar sunmaktadır. Air fryer kullanımı, özellikle vücut ağırlığı yönetimi, kardiyovasküler hastalıkların önlenmesi ve obezite tedavisinde etkili bir araç olarak değerlendirilmektedir (Wan ve ark., 2023). Diyetisyenler, düşük yağ içeriğine sahip yiyeceklerin hazırlanmasında air fryer'ı tercih etmektedir. Ve air fryer'ın sağladığı düşük yağ kullanımı sayesinde kalp sağlığı açısından olumlu etkiler elde edilmektedir. Geleneksel kızartma yöntemlerinde kullanılan yüksek miktarda yağ, obezite ve kardiyovasküler hastalık riskini artırırken (Hakami ve ark., 2014), air fryer bu riskleri azaltmakta ve sağlıklı bir alternatif sunmaktadır.

Air fryer, yağda eriyen vitaminlerin (A, D, E, K) daha iyi korunmasını sağlarken, suya duyarlı vitaminler (C vitamini gibi) üzerinde minimal kayıplara yol açmaktadır (Zaghi ve ark., 2019). Bununla birlikte, bu cihazla pişirilen sebzelerde daha az C vitamini kaybı gözlenmiştir, bu da vitamin korunumunda avantajlı bir yöntem olduğunu göstermektedir (Fontenot ve ark., 2019). Ayrıca, air fryer ile pişirilen yiyeceklerin protein yapısında minimal bozulmalar olduğu ve besleyici değerlerinin korunduğu tespit edilmiştir (Joshy ve ark., 2020). Trigliserid seviyelerinin düşmesi gibi metabolik sağlık üzerinde olumlu etkiler de gözlemlenmiştir (Feng ve ark., 2020). Ancak, yüksek sıcaklıkta pişirilen nişastalı gıdalarda akrilamid oluşumu gibi olumsuz etkiler gözlemlenebilir (Kayacier ve ark., 2014).

Air fryer'ın pratikliği ve hızlı pişirme kapasitesi, yoğun yaşam temposuna sahip bireylerin sağlıklı beslenme alışkanlıklarını sürdürmesine yardımcı olurken, düşük enerji yoğunluğu sayesinde kilo yönetimine de katkı sağlamaktadır (Teruel ve ark., 2015). Diyabet, kardiyovasküler hastalıklar ve obezite gibi sağlık sorunları olan bireylerde air fryer ile pişirilen düşük yağ ve enerji içeriğine sahip yiyecekler, bu hastalıkların yönetiminde destekleyici olabilir. Ayrıca, bu yöntem yüksek proteinli ve düşük karbonhidratlı diyetlerde besin değerlerini koruyarak kullanılabilir (Fikry ve ark., 2021).

Antioksidan ve Biyoaktif Bileşikler Üzerindeki Etkiler

Air fryer yöntemi, biyoaktif bileşiklerin ve antioksidanların korunmasında, geleneksel pişirme yöntemlerine kıyasla önemli avantajlar sunmaktadır. Özellikle fenolik bileşikler, flavonoidler, vitaminler (C, E, A) ve diğer antioksidan bileşenlerin korunması açısından bu yöntemin daha etkili olduğu görülmüştür. Bunun temel nedeni, air fryer cihazının daha düşük sıcaklıklarda ve daha kısa sürede pişirme imkanı sunmasıdır. Bu şekilde, ısıya duyarlı olan ve pişirme sırasında kolayca bozulan bileşiklerin korunması mümkün hale gelmektedir (Nandasiri ve ark., 2023; Salamattullah ve ark., 2021).

Özellikle polifenoller gibi biyoaktif bileşiklerin insan vücudunda oksidatif strese karşı güçlü bir savunma mekanizması oluşturdukları bilinmektedir. Bu bileşikler, antioksidan özellikleri sayesinde serbest radikalleri etkisiz hale getirir ve hücre hasarını önler. Air fryer yöntemi, bu bileşiklerin korunmasında ve biyoyararlanımının artırılmasında etkin bir yöntem olarak öne çıkmaktadır. Örneğin, sebzelerde yapılan çalışmalarda air fryer ile pişirmenin antioksidan kapasiteyi artırdığı ve fenolik bileşiklerin daha az bozulduğu gözlemlenmiştir Loganathan, ve Mohan, 2023; Šimurina ve ark., 2024)

Ancak, bu etkilerin yiyecek türüne ve pişirme koşullarına bağlı olarak değişebileceği unutulmamalıdır. Örneğin, nişastalı gıdaların yüksek sıcaklıkta pişirilmesi, bazı istenmeyen bileşiklerin (örneğin akrilamid) oluşumuna yol açabilir. Bu nedenle, air fryer kullanımı sırasında pişirme süreleri ve sıcaklıkların dikkatle ayarlanması önemlidir (Kayacier ve ark., 2014).

Bu bulgular, air fryer yönteminin sadece kilo yönetimi ve yağ tüketimini azaltma açısından değil, aynı zamanda biyoaktif bileşiklerin ve antioksidanların korunması açısından da önemli bir rol oynadığını göstermektedir.

Air Frying Yönteminde Besin Güvenliği ve Hijyen: Sağlıklı Pişirme Koşulları

Air fryer kullanımında besin güvenliğini sağlamak için, yiyeceklerin pişirme sırasında yeterince yüksek sıcaklıklara ulaşması gerekmektedir. Salmonella, Campylobacter ve Escherichia coli gibi patojen mikroorganizmalar, yiyeceklerde yaygın olarak bulunur ve yetersiz pişirme bu mikroorganizmaların gıdalarda bulunmasına neden olabilir (Doyle ve Erickson, 2012). Örneğin, tavuk gibi et ürünlerinde Salmonella ve Campylobacter'in tamamen yok edilmesi için iç sıcaklığın en az 75°C'ye ulaşması gerekmektedir (FDA, 2020).

Ayrıca, air fryerın düzenli temizliği ve doğru kullanımı da besin güvenliği için önemlidir. Yiyecek artıklarının air fryerda birikmesi, potansiyel çapraz bulaşma riskini artırabilir ve bu da gıda kaynaklı hastalıklara yol açabilir. Listeria monocytogenes gibi mikroorganizmalar, pişirme sonrası yiyeceklerle temas edebilir ve hastalık riski oluşturabilir (Salamattullah ve ark., 2021). Air fryer'ın her kullanım sonrasında uygun şekilde temizlenmesi ve hijyenik koşullarda saklanması, bu riskleri minimize etmek için gereklidir.

Air Fryerda Pişirme Süreleri ve Teknikleri

Air fryer'da doğru pişirme süreleri ve teknikleri, yiyeceklerin hem güvenli hem de lezzetli olmasını sağlamak açısından büyük önem taşır. Her yiyecek türü, belirli bir pişirme süresi ve sıcaklık ayarına ihtiyaç duyar. Örneğin, kırmızı et ürünlerinde Escherichia coli O157: H7 gibi patojenlerin tamamen yok edilmesi için iç sıcaklığın en az 71°C'ye ulaşması gerekmektedir (FDA, 2020). Yanlış pişirme teknikleri, yiyeceklerin dışının fazla pişmesine ve içinin çiğ kalmasına neden olabilir, bu da besin güvenliği açısından risk oluşturabilir.

Ayrıca, air fryer'da yiyeceklerin eşit şekilde pişirilmesi için doğru yerleştirme ve gerekirse pişirme sırasında karıştırma önemlidir. Yiyeceklerin homojen olarak pişmemesi, patojen riskini artırabilir ve gıda kaynaklı hastalıkların ortaya çıkmasına neden olabilir.

SONUÇ

Air fryer, sağlıklı pişirme yöntemleri arasında giderek daha fazla tercih edilen bir alternatif haline gelmiştir. Yağ tüketimini azaltması, ağırlık yönetimine katkıda bulunması ve pratik kullanım olanağı sunması, beslenme ve diyetetik alanında önemli bir yer edinmesini sağlamaktadır. Ancak, uzun vadeli sağlık etkileri ve çevresel sonuçları henüz tam olarak araştırılmamıştır. Bu nedenle, bilinçli kullanım ve potansiyel risklere karşı dikkatli olunması gerekmektedir.

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ORAL PRESENTATION

Sarı alıç (*Crataegus tanacetifolia*) sirkesinden izole edilen bakteriler tarafından bakteriyel selüloz üretimi ve karakterizasyonu

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Özet

Geleneksel sirkeler, alkol ve ardından asetik fermantasyonu yoluyla şeker veya nişasta içeren ham maddelerden doğal olarak üretilir. Bu çalışmada sarı alıç (*Crataegus tanacetifolia*) meyvelerinden geleneksel yöntemlerle üretilen sirkelerden bakteriyel selüloz (BC) üretebilme yeteneğine sahip bakteriler izole edilmiş ve bu bakterilerden Hestrin-Schramm besiyerinde üretilen BC'lerin fiziksel, kimyasal, yapısal ve termal özellikleri belirlemiştir. Çalışma sonucunda alıç sirkesinden izole edilen bakteriden yüksek oranda BC üretildiği (4,22 g/L), BC'nin yüksek termal stabiliteye, yüksek kristaliniteye (%67,95) ve ince lif çapına (53,48 nm) sahip olduğu belirlenmiştir. Bu çalışma, sarı alıç meyvelerinin doğal florasında BC üreten bakterilerin bulunduğunu ve bu meyvelerden elde edilen sirkelerin BC üretiminde kullanılabileceğini göstermiştir. İzolasyonu yapılan bakterilerin tanılaması, izole edilen bakterilerin BC verimini ve üretim kapasitesini artırmak için ortamı optimize edilmesi, en uygun kültür koşullarını belirlenmesi, bileşimin BC üretimi üzerindeki etkisini araştırılması ve üretim maliyetinin hesaplanması için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Sirke, Bakteriyel Selüloz, Karakterizasyon, Termal, SEM, XRD

Bacterial cellulose production and characterization by bacteria isolated from yellow hawthorn (*Crataegus tanacetifolia*) vinegar

Abstract

Traditional vinegars are naturally produced from raw materials containing sugar or starch by alcohol and then acetic fermentation. In this study, bacteria capable of producing bacterial cellulose (BC) were isolated from vinegars produced from yellow hawthorn (*Crataegus tanacetifolia*) fruits by traditional methods and the physical, chemical, structural and thermal properties of BCs produced by these bacteria in Hestrin-Schramm medium were determined. As a result of the study, it was determined that a high amount of BC was produced from the bacteria isolated from hawthorn vinegar (4.22 g/L), and BC had high thermal stability, high crystallinity (67.95%) and thin fiber diameter (53.48 nm). This study showed that BC-producing bacteria were supplied in the natural flora of yellow hawthorn fruits and that vinegars produced from these fruits could be used in BC production. Further studies are needed to identify the isolated bacteria, optimize the medium to increase the BC yield and production capacity of the isolated bacteria, determine the most appropriate culture conditions, investigate the effect of the composition on BC production and calculate the production cost.

Keywords: Vinegar, Bacterial Cellulose, Characterization, Thermal, SEM, XRD

GİRİŞ

Fransızca "vinaigre" (anlamı "ekşi şarap") kelimesinden türetilen sirke terimi, şarabın asidifikasyonu ile elde edilen sıvıyı ifade eder. Sirke, karbonhidratların alkol fermantasyonu ve etanolün asetik aside oksitlendiği asidifikasyon olmak üzere iki biyolojik işlemle üretilir (Silva ve ark., 2024). Sirke üretiminde, bir zamanlar "sirke anası" olarak adlandırılan bakteriyel selüloz (BC), hava-sirke ara yüzeyinde jelatinimsi bir zar olarak oluşur. Bu zar, UV radyasyonu ve susuz kalma gibi olumsuz çevre koşullarına karşı veya mikroorganizmalara karşı koruyucu bir bariyer görevi görerek veya oksijen bulunabilirliğini artırarak bakterilerin hayatta kalmasına yardımcı olur (Horue ve ark., 2023). BC, bitki selülozuna benzer glikoz ve hücre dışı bir polisakkarit birimlerinden oluşan bir biyopolimerdir. Ancak, bitki selülozundan farklı olarak, çeşitli mikroorganizmalar tarafından saf selüloz olarak üretilir (Saleh ve ark., 2024) ve lignin, hemiselüloz veya diğer ekstraktif malzemeler içermeyen selüloz nanofiber ağlarından oluşur (Leyun ve ark., 2024).

BC, bitki selülozundan daha yüksek kristalinite (> %70) (Horue ve ark., 2023), polimerizasyon derecesi (20.000 glikoz monomerine kadar) (Horue ve ark., 2023), su tutma kapasitesi (%99'a kadar) (Saibuatong, ve Phisalaphong, 2010) ve mekanik özelliklere (200 ila 2000 MPa çekme dayanımı ve 15-138 GPa'ya kadar Young modülü) (Horue ve ark., 2023) sahiptir. Bu özellikler BC'yi, değiştirilmiş özelliklere sahip yeni kompozitlerin üretimi için mükemmel bir hammadde haline getirir (Betlej ve ark., 2021). Benzersiz fizikokimyasal ve mekanik özellikleri nedeniyle BC, gıda, gelişmiş akustik diyaframlar, biyosensörler, doku iskelesi, paketleme, koruyucu kaplamalar, antimikrobiyal malzemeler ve esnek elektronikler (Zhou ve ark., 2023) gibi birçok alanda uygulanmıştır. Küresel BC üretim ve satış pazarı giderek artmakta olup 2016 yılında 207 milyona, 2019 yılında yaklaşık 250 milyona ve 2022 yılında yaklaşık 500 milyon ABD dolarına ulaşmış olup, pazarın 2024 yılında 570 milyon ve 2027 yılında 780 milyon ABD dolarını aşması öngörülmektedir (Rocha ve ark., 2023). Ancak yüksek maliyetler ve düşük verimler, BC'nin büyük ölçekli ticari üretiminin önünde engel teşkil etmektedir (Li ve ark., 2023) ve yüksek verimli BC üreten bakteriler sınırlıdır (Morgan ve ark., 2013). BC üreten izolatlar, meyveler, sebzeler, çiçekler, sirke ve fermente içecekler dahil olmak üzere birçok kaynaktan izole edilmiştir. *Pseudomonas, Acetobacter ve Gluconacetobacter* gibi başlıca BC üreticileri bildirilmiştir ve *Gluconacetobacter xylinus*, BC çalışmalarında yaygın olarak kullanılmaktadır (Urbina ve ark., 2021). BC'nin birçok uygulaması olmasına rağmen, düşük üretim verimliliği endüstriyel uygulamalar için bir sorundur. BC üreten suşların verimleri, endüstrinin talebini karşılamadığından, farklı kaynaklardan yüksek verimli BC üreten suşları araştırmak ve elde edilen BC' leri karakterize etmek gereklidir.

Bu çalışma, sarı alıç sirkelerinden BC üreten suşları izole etmeyi, bu suşların BC üretim kapasitelerini belirlemeyi ve üretilen BC'lerin özelliklerini karakterize etmeyi ve karşılaştırmayı amaçlamaktadır. Çalışmada, BC sentezleyebilen suşlar sarı alıç sirkesinden izole edildi. İzole edilen suşlar, statik koşullar altında Hestrin-Schramm (HS) ortamında BC üretmek için kullanıldı. Çalışmalar, farklı kaynaklardan ve farklı mikroorganizmalar tarafından üretilen BC'nin farklı özelliklere sahip olduğunu göstermiştir. Bu nedenle BC, Fourier dönüşümlü kızılötesi spektroskopisi (FTIR), taramalı elektron mikroskobu (SEM), X-ışını kırınımı (XRD), termogravimetrik analiz (TGA) ve farklı sıvılardaki çözünürlüğü ile karakterize edildi.

MATERYAL VE METOT

Materyal

Sarı alıç meyveleri yerel pazardan temin edildi. Tüm kimyasallar Sigma-Aldrich ve Merck KGaA tarafından sağlandı.

Sirke üretimi

Sirkeler geleneksel yöntemlerle üretildi. Sarı alıç meyveleri ayıklandı, temizlendi ve dilimlendi. Toplamda 1000×g meyve, 50×g toz şeker ve 2000 mL damıtılmış su ile 5 L'lik cam kavanozlarda karıştırıldı. Kavanozlar tülbent ile kapatılarak hava girmesi sağlandı. Karışım, yüzeyde toplanan meyve parçaları kavanozun dibine çökene kadar her 12 saatte bir karıştırıldı. Sirke üretimi için fermantasyon yaklaşık 8 hafta (yüzeyde oluşan sirke anası dibe çökene kadar) devam etti. BC üreten bakterileri izole etmek için sirke anası çıkarıldı. Sarı alıç sirkesi (HV) önce bir tülbentten ve ardından kaba filtre kağıdından süzüldü.

BC üreten bakterilerin izolasyonu

BC üreten bakteriler, geleneksel yöntemlerle üretilen sarı alıç sirkelerinden izole edildi. Sirke örnekleri (1 mL), %2 d-glikoz, %0,5 pepton, %0,5 maya özütü, %0,27 Na_2HPO_4 ve %0,11 sitrik asit içeren Hestrin-Schramm (HS) besiyerine aktarıldı. Daha sonra, selüloz üreten bakteriler 30 °C'de 18-24 saat inkübe edildi. Kültür besiyerinin seri seyreltileri HS agara plakalandı ve 30 °C'de 2 gün inkübe edildi. Daha fazla kültürleme ve analiz için birkaç koloni seçildi (Aydın ve Aksoy, 2014). Mikroorganizmaların morfolojileri ışık mikroskobu kullanılarak incelendi ve gram negatif suşlar Gram boyama ile seçildi. Beyaz-krem mukus yapısına sahip koloniler HS agarda birden fazla kültüre alınarak mikroorganizmalar saflaştırıldı. İzole edilen selüloz üreten bakteriler HS agar üzerinde 4°C'de saklandı.

BC üretimi

BC, sarı alıç sirkesinden izole edilen bakteriler kullanılarak üretildi. Bakteriler 10 mL HS besiyerine inoküle edildi ve statik koşullar altında 30 °C'de 48 saat kültürlendi. Bakteri kültürleri sterilize edilmiş (121 °C, 15 dakika) HS ortamına (200 mL) aktarıldı ve selüloz üretimi 28 °C–32 °C'de statik koşullar altında 14 gün boyunca sürdü. Besiyeri tabanına çöken BC ortamdan çıkarıldı, 10 dakika boyunca 4000×g'de santrifüj edildi ve kaynar suda 30 dakika boyunca %4 NaOH çözeltisinde tutuldu (Son ve ark., 2002). BC, NaOH'yi gidermek

için beş kez damıtılmış suyla durulandı, %2 asetik asit çözeltisinde tutuldu ve nötrleşene kadar damıtılmış suda tutuldu. Nötralize edilmiş BC etüvde kurutuldu (48 saat boyunca 50 °C) ve BC verimi g/L (kuru bazda) olarak belirlendi.

BC Analizleri

BC'nin çözünürlüğü farklı çözücülerde analiz edildi. Ayrıca, BC'nin FTIR, TGA, SEM ve XRD analizleri yapıldı. Çözünürlük için, 5 mL NaOH (30 °C ve 90 °C), metanol (30 °C ve 60 °C), aseton (30 °C ve 50 °C), dietil eter (30 °C), petroleum benzine (30 °C), hekzan (30 °C ve 60 °C), benzen (30 °C ve 80 °C) ve izopropil alkol (30 °C ve 80 °C), 250 mg BC ile 2 dakika inkübe edildi ve BC'nin çözünürlüğü değerlendirildi (Halib ve ark., 2012). FTIR analizi (4000–500 cm^{-1}), FT-IR Thermo Scientific Nicolet iS20 spektrofotometresi (ABD) kullanılarak gerçekleştirildi. SEM analizleri yüksek çözünürlüklü taramalı elektron mikroskobu (QUANTA 450 Field Emission Gun SEM) kullanılarak gerçekleştirildi. BC'nin lif çapları en az 10 BC lif çapının aritmetik ortalaması kullanılarak hesaplandı ve sonuçlar ortalama nm olarak ifade edildi. Numunelerin termal özellikleri SII Nanotechnology Exstar 7300 (ABD) ile 25 °C–650 °C arasındaki bir azot ortamında, 10 °C/dakika ısıtma hızında belirlendi. BC'nin kristal indeks değerlerinin belirlenmesi ve XRD analizleri Panalytical Empyrean Yüksek Performans Difraktometre Cihazı (Hollanda) kullanılarak gerçekleştirildi. XRD analizinde tarama 5°/dakika ve 2θ = 10°–50°'de Ni filtreli Cu X-ışını tüp cihazlarıyla gerçekleştirildi. BC'nin kristalinite derecesi (%) aşağıdaki curve-fitting yöntemi kullanılarak hesaplandı.

$$Crl\ (\%)\ (Curve\ Fitting\ Method)\ =\ Acryst/Atotal \times 100 \quad (1)$$

Acryst, kristalin bölgenin alanı ve Atotal, toplam alanı ifade etmektedir.

BULGULAR ve TARTIŞMA

Sarı alıç sirkesinden izole edilen bakteriler tarafından BC üretimi

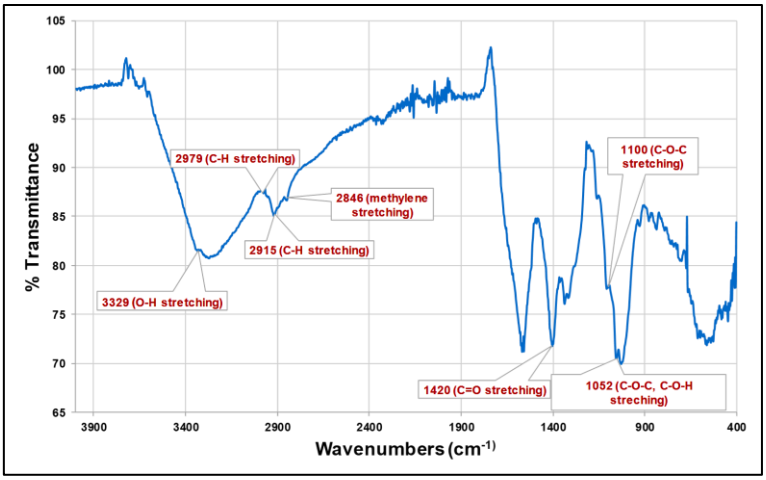
BC, sarı alıç sirkelerinden izole edilen bakteriler tarafından üretilmiştir. Bakteriler, statik koşullar altında (30 °C ve 48 saat) HS ortamında (10 mL) inkübe edildi. Toplamda, 1 mL bakteri kültürü 200 mL sterilize edilmiş (121 °C, 15 dakika) HS ortamına aktarıldı. Selüloz üretimi 28 °C–32 °C'de 14 gün boyunca devam etti. Sürenin sonunda, sarı alıç sirkesinden izole edilen bakteriler tarafından kültürlenen HS ortamında 4,22 ±0,20 g/L BC oluşumu gözlemlendi.

Castro ve ark. (2011) tarafından *Gluconacetobacter swingsii* ile HS broth ve ananas suyunda sırasıyla 2.1 g/L ve 2.8 g/L, Ramirez ve ark. (2018) tarafından *Komagataeibacter medellinensis* tarafından muz 4.81 g/L, mango 1.95 g/L ve peynir altı suyundan 2.37 g/L ve Güzel ve Akpınar (2019) tarafından ise *K. hansenii* GA2016 ile narenciye (limon, mandalina, portakal ve greyfurt) kabuklarından sırasıyla 2.06, 3.91, 2.33 ve 2.68 g/100 g kabuk oranında BC elde edildiği rapor edilmiştir. Çalışmamızda elde edilen veriler, diğer çalışmalarla karşılaştırıldığında, bu çalışmada incelenen bakteriden oldukça yüksek verimde BC elde edildiği belirlenmiştir. Verim ve üretim kapasitesini artırmak ve optimum kültür koşullarını belirlemek için daha ileri çalışmalara ihtiyaç vardır.

BC Analizleri

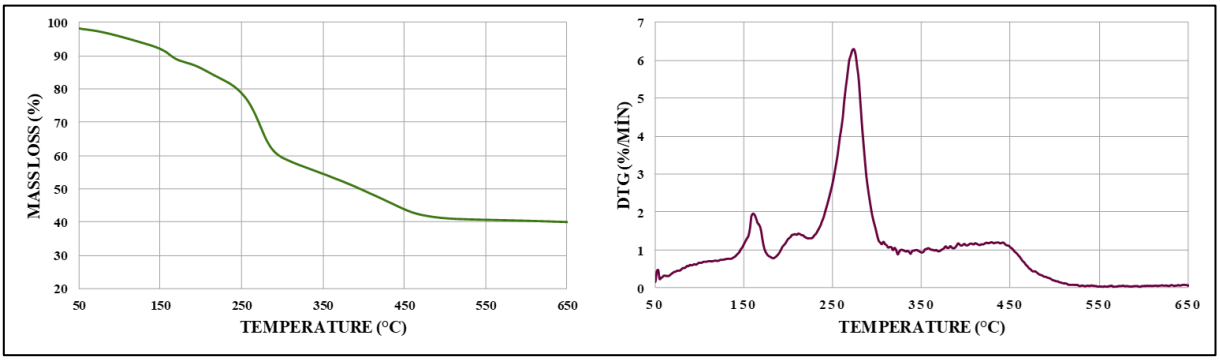
BC'nin çözünürlük analizi sonucunda, tüm BC örneklerinin aseton, metanol, dietil eter, petroleum benzene, heksan, benzen ve izopropil alkolde çözünmediği; 1 M NaOH'de (30 °C) kısmen çözündüğü ve 1 M NaOH'de (90 °C) çözündüğü gözlendi. Bitki selülozu ve BC, katı bir bazik yapıya sahip güçlü moleküller arası ve molekül içi hidrojen bağları nedeniyle yaygın çözücülerde zayıf çözünürlük gösterir (Utandi ve ark. 2019). Düşük çözünürlük, hava ve su filtrasyon sistemleri gibi çeşitli gazlara ve sıvılara karşı kararlılığın önemli olduğu uygulamalarda BC'ye avantaj sağlayabilir.

Selüloz karbon, hidrojen ve oksijen [$(C_6H_{10}O_5)_n$] içerir. Bu analizin amacı, BC örneklerindeki selülozun ana fonksiyonel gruplarını karşılaştırmaktı. BC örneklerinin FTIR spektrumları Şekil 1'de sunulmuştur. BC'nin karakteristik pikleri şu şekildedir. Hidroksil grupları: 3350 cm^{-1} ve 3400–3500 cm^{-1}, metilenin gerilme titreşimleri: 2800–2900 cm^{-1}, karbonil grupları: 1620–1640 cm^{-1} ve 1420–1440 cm^{-1}, C–O–C gerilmesi: 1160 cm^{-1} ve C–O gerilme titreşimleri: 1035–1060 cm^{-1}. C–H ve CH_2 bükülmesini temsil eden diğer selüloz pikleri yaklaşık ise 1300 cm^{-1} ve 1400 cm^{-1} (Halib ve ark., 2012). Çalışma sonucunda BC örneklerinin FTIR spektrumları, selüloz I'in spektrumlarıyla yakından eşleşmiş olup, ayrıca BC'nin FTIR spektrumları diğer çalışmalar ile tutarlı olduğu (Halib ve ark., 2012; Gayathry ve Gopalaswamy, 2014) belirlenmiştir.



Şekil 1. Sarı alıç sirkesinden izole edilen bakteriler ile üretilen BC'nin FTIR spektrumu

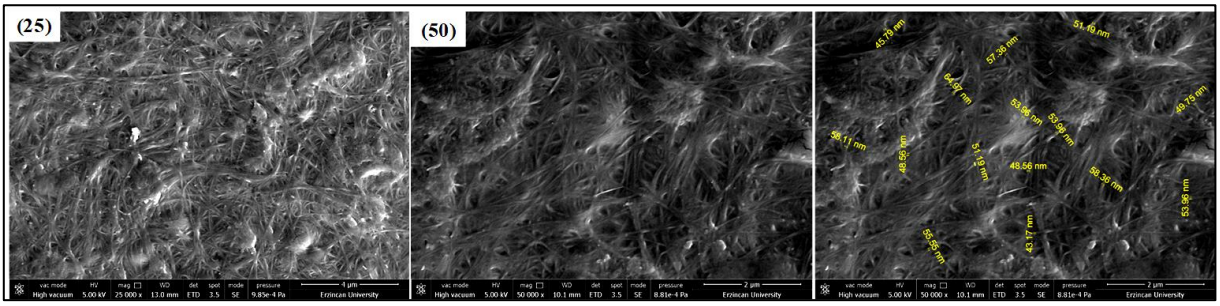
Termal analizler, biyomalzemelerin ön formülasyonunda, termal ayrışma reaksiyonlarını anlamak ve biyomalzemeler için depolama koşullarını belirlemeye yardımcı olmak için yaygın olarak kullanılır (Pasaribu ve ark., 2023). TGA, polimerik malzemelerin viskoelastik davranışını sıcaklık veya frekansa bağlı olarak inceler ve bir numunenin kontrollü bir sıcaklıkta ısıtıldığında veya soğutulduğunda ağırlık değişimini değerlendirir (Mamouri ve ark., 2023). BC'nin termogravimetrik (TG) ve türev termogravimetrik (DTG) eğrileri Şekil 2'de sunulmuştur. DTGmax, termal kararlılığın bir ölçütü olup maksimum kütle kaybı oranını temsil etmektedir. Bu değer BC için 274 °C olarak belirlenmiştir. BC'nin 650 °C'deki kütle kaybı ise %60 olarak hesaplanmıştır. Saf BC'nin termal bozunması, başlangıçta 117 °C–330 °C'de dehidratasyon ve ardından 350 °C–505 °C'de glikozil birimlerinin ayrışmasıyla oluşan iki ana ağırlık kaybı bölgesini içermektedir (Campano ve ark., 2016). *A. xylinum* tarafından üretilen BC için DTGmax değerleri 330 °C–370 °C (Halib ve ark., 2012), *K. medellinensis* tarafından üretilen BC için 355 °C, 327 °C ve 368 °C (Molina ve ark. 2018), *G. hansenii* tarafından üretilen BC için 290 °C (Costa ve ark., 2017) ve *K. hansenii* GA2016 tarafından üretilen BC için 228 °C–359 °C (Güzel ve Akpınar, 2019) olarak bildirilmiştir. 650 °C'de kütle kaybı, $T_{50\%}$ ve DTGmax değerleri karşılaştırıldığında, bu çalışmada üretilen BC'nin oldukça yüksek termal kararlılığa sahip olduğu sonucuna varılmıştır.



Şekil 2. Sarı alıç sirkesinden izole edilen bakteriler ile üretilen BC'nin TG ve DTG eğrileri

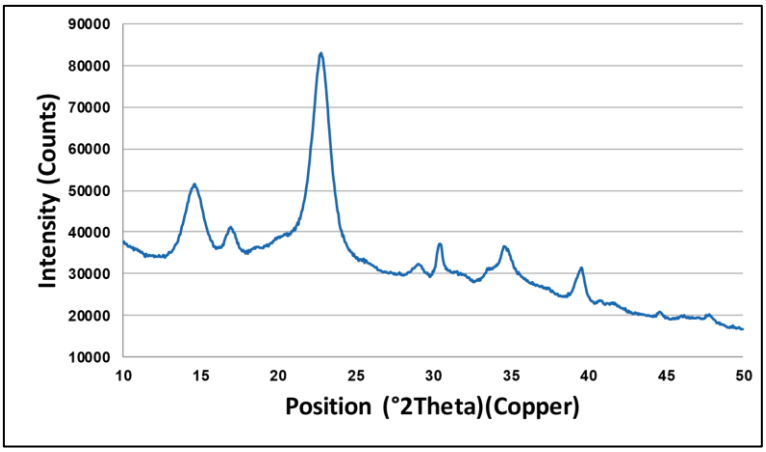
BC nanofibrilleri, yüksek su tutma kapasitesi, hidrofilisite, kristalinite ve kalıplanabilirlik gibi özellikler kazandıran <100 nm genişliğinde şerit şeklindeki fibrillerden oluşmaktadır (Gorgieva ve Trcek, 2019). BC'nin morfolojik özellikleri, SEM ile 25.000× ve 50.000× büyütmede incelenmiş olup (Şekil 3), BC'nin ortalama lif çapı 53,48 nm olarak hesaplanmıştır. SEM görüntüsü, selüloz fibrillerinin 3 boyutlu bir yapıya iç içe geçmesiyle oluştuğunu, BC membran pellikül yapılarının ince ve yoğun bir ağ oluşturan çubuk şeklindeki nanofiberler içerdiğini, BC katmanlarının dış ve iç yüzeylerinin görünüş olarak farklı olduğunu, BC'nin morfolojik yapılarının diğer çalışmalarda bildirilenlere benzer olduğunu göstermiştir (Güzel ve Akpınar, 2020;

Cheng ve ark., 2009; Martins ve ark., 2009). Daha ince lif çapı, BC'nin termal kararlılık, su tutma kapasitesi ve mekanik mukavemet gibi özelliklerini olumlu yönde etkileyen bazı avantajlar sağlamakta olup (Cheng ve ark., 2009) çalışma sonucunda BC'nin ince lif çapına sahip olduğu belirlenmiştir.



Şekil 3. Sarı alıç sirkesinden izole edilen bakteriler ile üretilen BC'nin taramalı elektron mikrografları (25) 25000x, (50) 50000x büyütme.

Selülozun kristalinitesi, selülozun fiziksel, mekanik, kimyasal ve morfolojik özelliklerini ve çevreyle etkileşimini ve dolayısıyla biyomedikal, tekstil ve elektronik gibi çeşitli uygulamalarda kullanımını etkilemektedir (Salem ve ark., 2023). XRD analizi, karmaşık ortam bileşenlerinin BC'nin yapısı ve kristalinitesi üzerindeki etkilerini araştırmak amacıyla yapılmakta olup, XRD, BC'nin mukavemet ve su tutma kapasitesi gibi makro özelliklerini ve performansını anlamak için faydalıdır (Digel ve ark., 2023). BC'nin XRD desenleri, yaklaşık 14,5°, 16,7° ve 22,7°'lik 2θ açılarında üç ana pik sergilemiş olup bu, BC'nin tipik bir selüloz I kristal formu olduğunu göstermektedir (Neelima ve ark., 2023) (Şekil 4). BC'nin kristallik derecesi (%) curve-fitting yöntemi kullanılarak hesaplanmış olup bu değer %67,95 olarak belirlenmiştir. Çalışmalarda, üretilen BC'lerin kristaliniteleri *A. tropicalis* tarafından %43 (Kaur ve Shah, 2022) ve *K. saccharivorans* tarafından %82,2 (Gopu ve Govindan, 2018) olarak bildirilmiştir. Sonuçlar BC'nin kristalinitesinin yüksek olduğunu ve literatürle uyumlu olduğunu göstermiştir.



Şekil 4. Sarı alıç sirkesinden izole edilen bakteriler ile üretilen BC'nin X-ışını difraktogramı

SONUÇ

Bu çalışmanın amacı, geleneksel yöntemlerle üretilen sarı alıç sirkesinden BC üreten bakterileri izole etmek, bu bakterilerin BC üretim kapasitelerini belirlemek ve üretilen BC'nin özelliklerini incelemekti. Çalışma sonucunda, sarı alıç sirkesinden izole edilen bakterilerin yüksek BC verimine (4,22 g/L) sahip olduğu, sarı alıç sirkesinden izole edilen bakteri tarafından üretilen BC'nin, yüksek termal stabiliteye ve kristaliniteye (%67,95) ve ince lif çapına (53,48 nm) sahip olduğu anlaşılmıştır. Çalışma, meyvelerden elde edilen sirkelerin BC üretmek için kullanılabileceğini ortaya koymakta olup, BC üreten bakterilerin farklı sirkelerden izole edilebilir olduğunu ve bu bakteriler tarafından üretilen BC'nin farklı özelliklere sahip olabileceğini göstermiştir. İzolasyonu yapılan bakterilerin tanılaması, izole edilen bakterilerin BC verimini ve üretim kapasitesini artırmak için ortamı optimize etmek, en uygun kültür koşullarını belirlemek, bileşimin BC üretimi üzerindeki etkisini araştırmak ve üretim maliyetini hesaplamak için daha fazla çalışmaya ihtiyaç vardır. Bu hususların ele

alınması, araştırma bulgularının önemini ve uygulanabilirliğini daha da artırabilir ve özellikle bu bakterilerden ticari BC üretiminin elde edilmesi açısından önemlidir.

TEŞEKKÜR

Bu çalışma Gümüşhane Üniversitesi Bilimsel Araştırma Projeleri Koordinatörlüğü tarafından desteklenmiştir (Proje Numarası: 20.B0433.02.01).

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ORAL PRESENTATION

Production and purification of protease enzyme from *Bacillus spizizenii*

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Abstract

In this study, protease enzyme production by *Bacillus spizizenii* and its partial purification by the method of ammonium sulfate precipitation was investigated. The best protease activity was obtained by growing the microorganism in a dextrose broth medium at 37 °C for 24 hours; the protein amount was 1.4 mg/mL, and the protease activity was 67±5 U/mL. In precipitation studies with the enzyme, a 1.54-fold purity and an activity yield of 85% were obtained at an ammonium sulfate concentration of 30% (w/w).

Keywords: *Bacillus spizizenii,* protease, partial purification

INTRODUCTION

Enzymes are biological macromolecules synthesized by living organisms. They are synthesized in protein structure specific to the gene sequence of each living system and play a role in the production and destruction of biochemical events. They accelerate the reactions they are involved in and since they are specific to the biomolecules called substrates in the reaction, they perform catalysis according to these structures and produce products. Enzymes and their functions have been used in many different sectors for centuries since they were defined. Examples of this are cheese, beer, wine, bread yeast, vinegar production. With technological developments, industrial production has increased over time and enzymes have taken part in biotechnological studies and developments as biochemical catalysts in these processes (GÜRZ UĞURLUER,2022).

The most important and largest class of commercial enzymes with industrial applications are the proteolytic enzymes. 50 percent of the global enzyme market are proteolytic enzymes that break down proteins. Enzymes in this class have long been used in the food, detergent and leather industries as well as in pharmaceuticals. They are also used as therapeutic agents in the field of medicine (. Peptidases, also known as proteases or proteinases, hydrolyze the amide bonds in the polypeptide structure of proteins. They play a crucial role in the breakdown of protein foods in the digestive system of all animals and are produced in large quantities by the stomach and pancreas. Other proteases originate from a variety of organisms, including bacteria, yeasts and plants. (GÜRZ UĞURLUER,2022).

Microbial proteases are one of the most important groups of enzymes of industrial importance. Proteolytic enzymes obtained from microorganisms of the genus *Bacillus* are the most important group among commercial enzymes. Among the microbial proteases, alkaline proteases, which are active and stable at alkaline pH (pH 8.0-12.0) and high temperatures (50-70 °C), account for the largest proportion. Alkaline proteases are used as a detergent additive in laundry detergents to remove proteinaceous stains such as blood, milk, sweat, grass, etc. (GÜRZ UĞURLUER,2022).

We present the synthesis and partial isolation of the extracellular protease from *Bacillus spizizenii* (ATCC6633), cultivated under finely tuned fermentation conditions to achieve a substantial enzyme yield.

MATERIALS AND METHODS

Determination of protease activity in agar media

Four different *Bacillus* strains (*B. subtilis*, *B. spizizenii* ATCC6633, *B. methylotrophicus* DSM28326, *B. pumilus* (Genebank No: KX592605)) were inoculated on skim milk agar medium with a pH of 5.0 using a line inoculation technique and incubated in an oven at 30ºC for 24 hours. At the end of the incubation, transparent

zones formed around the bacteria growing on the SMA medium due to hydrolysis of the skim milk. These transparent zones showed the presence of protease activity (Adinarayana et al., 2003).

Protease production

For the use of *B. spizizenii* (ATCC6633), Nutrient Agar was inoculated and incubated for 18 hours at 37 °C. and stored at +4 °C. For inoculation into the liquid medium to be used as enzyme production medium, the stock sample was transferred to nutrient broth and grown in liquid culture at 37 °C for 18 hours. Dextrose Broth and Nutrient Broth media, prepared at 2 liters each, were inoculated with 2 mL of the liquid medium and grown for 72 hours (GÜRZ UĞURLUER,2022)

Measurement of the protease enzyme activity

The enzyme activity of the crude enzyme extract of *B. spizizenii* was determined using a spectrophotometer (Cary 60, Agilent). To determine the protease activity, the reaction mixture was incubated with 1% (w/v) casein solution for 30 min at 60 °C with slow stirring. 0.8 mL of 1% casein was added to the reaction and blank liquid. 0.2 mL of enzyme was added to the reaction liquid (the blank liquid contained no enzyme). The samples were incubated in a water bath at 60°C for 30 min, then treated with 1 mL of trichloroacetic acid (TCA) and the reaction was stopped. Samples were placed on ice for 10-15 min. The samples were centrifuged at 9500 rpm for 20 min. After centrifugation, the supernatant was collected in the tubes and the enzyme activity was measured in a spectrophotometer at 280 nm. One unit of protease activity (1 U) is defined as the amount of enzyme required to produce 1 µg of tyrosine per minute at a pH of 10.5 (Öner, 2009). The enzyme activity was calculated according to the Eqs. (1) given below.

$$Enzyme\ Activity\ \left(\frac{U}{mL}\right) = \frac{\frac{OD660}{slope} \times reaction\ volume\ (mL) \times dilution\ factor}{Enzyme\ volume\ in\ cuvette\ (mL) x\ reaction\ time\ (min)} \quad (1)$$

To generate the tyrosine standard curve for the calculation of alkaline protease activity, solutions containing 0-50 µg/mL tyrosine in 50 mM NaOH-glycine-NaCl buffer at pH 10.5 were prepared. The reaction mixture to which a 50 mM NaOH-glycine buffer solution at pH 10.5 was added instead of tyrosine served as a blank. 0.5 mL from each solution with different tyrosine concentrations, was taken, 2.5 ml of 0.5 M $Na2CO_3$ and 0.5 ml of double-diluted Folin-Ciocaltcau reagent were added, mixed thoroughly and the reaction mixture was kept at room temperature for 30 min. The intensity of the blue color formed at the end of the time was determined by measuring the absorbance at 660 nm against the dye. The slope of the standard line (Figure 1) obtained by plotting the tyrosine concentration (mg/mL) against OD660 was determined and used to calculate the activity of the alkaline protease.

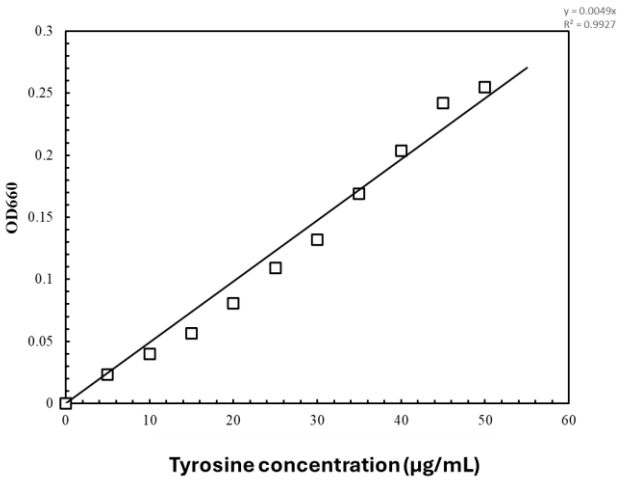

Figure 1. Tyrosine standard curve

Determination of protein amount

In this experiment, the Bradford method was used to determine the amount of protein. First, a standard curve was prepared with BSA (bovine albumin serum) to determine the protein of the enzyme produced by the microorganism. 20 µL of BSA solutions at different mg levels (2, 1.5, 1, 0.75, 0.5, 0.25, 0.125 mg) were taken and vortexed with 1 ml of Bradford solution. After 15 min of incubation, Simple Read was measured at 595 nm in a spectrophotometer and the slope was calculated from the standard curve (Figure 2).

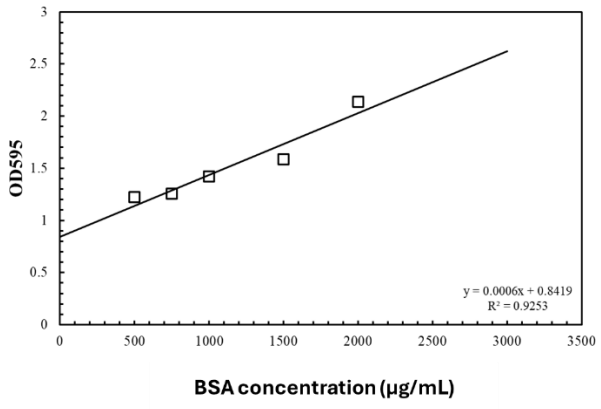

Figure 2. BSA standard curve

Partial purification of protease enzyme by ammonium sulfate precipitation

Since the protease enzyme produced by the strain *B. spizizenii* (ATCC6633) is an enzyme secreted outside the cell, the cells were removed from the medium at the end of fermentation by centrifugation, and the culture supernatant was stored at +4°C for enzyme purification.

In order to determine the most suitable ammonium sulphate saturation for the precipitation of the alkaline protease enzyme in the culture supernatant, precipitations at 20, 30, 40, 50, 60, 70 and 80 % ammonium sulfate saturation were tested. The suspension solution obtained at the end of the addition of ammonium sulfate was stirred for 3 hours. After this time, the protein aggregates contained in the suspension solution were precipitated by centrifugation at 12000 rpm for 55 min and the supernatant was separated from the precipitate. The precipitated protein (pellet) was dissolved in a minimum amount of 50 mM NaOH-glycine buffer solution with a pH of 10.5 and washed and dialyzed overnight with the same buffer solution. The ammonium sulfate precipitations and dialysis were all performed at +4°C. The protease enzyme activity and the protein content of the dialysate were determined as described above.

RESULTS AND DISCUSSION

The strains of *B. subtilis*, *B. spizizienii*, *B. methylotrophicus* and *B. pumilus* selected for the isolation of protease enzymes were tested for growth and enzyme synthesis on solid medium at 37 °C and pH 5.0(GÜRZ UĞURLUER,2022). The result of the study was that *B. subtilis* and *B. spizizienii* showed the broadest zone of activity (Figure 3). The strain *B. spizizienii* was selected for protease production because *B. subtilis* is widely used in protease production and there is no study on the protease of *B. spizizienii*.

At an incubation temperature of 37 °C, growth and enzyme production occurred at all pH values tested. At an incubation temperature of 37 °C, the tested *B. spizizienii* strain was found to be capable of growth and enzyme production at all pH values. In particular, the expansion of the activity zone at pH 5.0 is an indication that the strain synthesizes acidic enzymes.

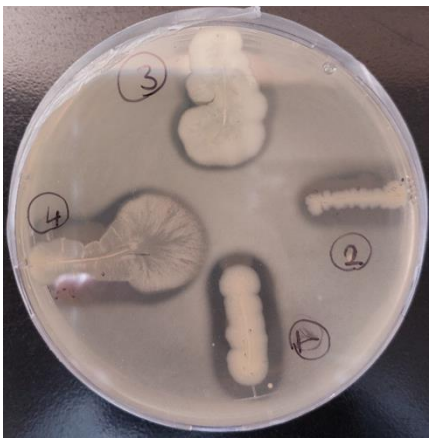

Figure 3. Colony and zone of *B. subtilis*, *B. spizizienii*, *B. methylotrophicus* and *B. pumilus* strains in the medium at pH 5.0 at 37 °C 24 hours

The narrowing of the zone diameters of *B. methylotrophicus* and *B. pumilus* indicates that the ability of the strain to synthesize the enzyme is weakened, even though it is growing.

After *B. spizizenii* was selected as the protease source, nutrient bread and grape sugar broth media were used to produce and quantitatively determine the activity of the protease enzyme to be obtained from this strain, and enzyme extract samples taken every 2 hours from both media were compared.

Protease activity was measured every 2 hours for 72 hours. In dextrose broth medium, the best values were observed after 24 hours (Figure 4). On the other hand, the best protease production was achieved after 32 hours with the strain grown in nutrient broth (Figure 5). When comparing the growth and activity results in different media, it was found that *B. spizizizenii* showed the best activity in Dextrose-Broth medium and 24 hours of growth. After establishing the conditions for enzyme production, the amount of strain enzyme protein produced was 1.4 mg/mL and the protease activity was 67±5 U/mL

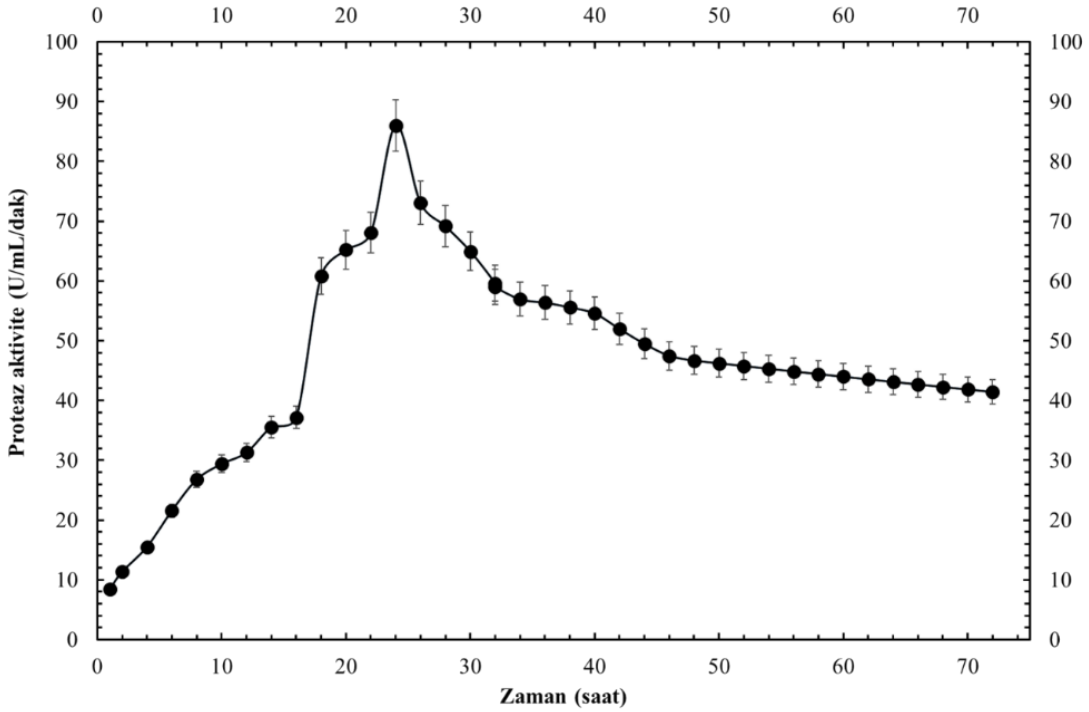

Figure 4. Protease activity graph of *B. spizizenii* grown in dextrose broth for 72 hours

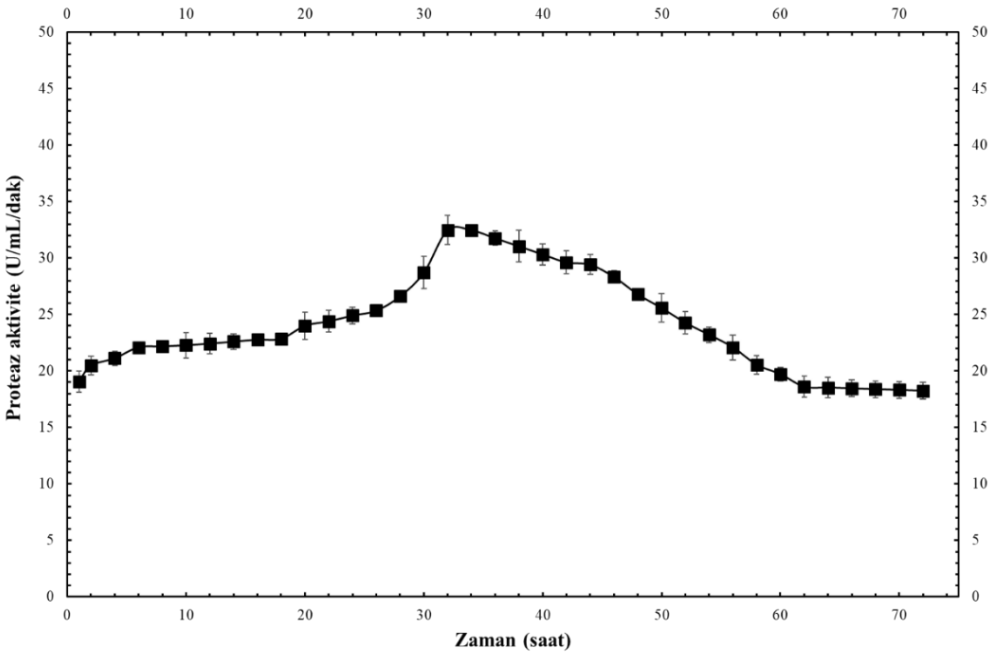

Figure 5. Protease activity graph of *B. spizizeni*i grown in nutrient broth for 72 hours

If we look at the studies on the production of protease enzymes in organisms of the genus *Bacillus*; Shafique et al. (2021) reported that the maximum protease production of strain *Bacillus subtilis* apr-IPL04 was reached after 72 hours (Ayvazoğlu, 2023). As a result of the optimization of the production conditions for protease production with the strain *Bacillus sp.* G17, the pH of the medium was set to 8.0, the incubation temperature to 40 ◦C and the incubation time to 72 hours. (Ayvazoğlu, 2023). The isolate of *Bacillus nakamurai* was incubated for 24 hours at 37 °C and 120 rpm on a shaker. (Iqubal et al. 2023) Enzyme production was performed by incubation at 37 °C and 120 rpm shaking speed for 54 hours. (KERİMAK ÖNER, 2009) A 1% (v/v) microbial liquid culture was inoculated into the medium and then incubated at 30 °C for 24 hours. (Yaman et al, 2020)

The ammonium sulfate precipitates were tested at 20, 30, 40, 50, 60, 70 and 80 % saturation in the culture supernatant. When comparing the precipitates, it was found that the 30% ammonium sulphate precipitates had higher purification coefficients at each percentage saturation value (Table 1). Accordingly, the partial purification step of the 30% ammonium sulphate precipitation of the protease enzyme from the supernatant at pH was completed with an 85% activity recovery and a purification fold of 1,5.

Table 1. Table for the partial purification of the protease enzyme isolated from the strain *Bacillus spizizenii*

Step	Purification fold	Activity recovery (%)
Crude extract	1.00	100
20% Ammonium sulfate precipitation	1.13	62
30% Ammonium sulfate precipitation	1.54	85
40% Ammonium sulfate precipitation	1.23	56
50% Ammonium sulfate precipitation	1.13	46
60% Ammonium sulfate precipitation	1.09	39
70% Ammonium sulfate precipitation	1.03	36
80% Ammonium sulfate precipitation	1.00	34

CONCLUSION

During the experiments, *B. spizizenii* was found to have protease activity that could be recovered by purification procedures. Characterization studies are ongoing, and it is expected that the role of *B. spizizenii* protease in industrial use will become more understandable.

ACKNOWLEDGEMENTS

This study has been funded by Kocaeli University (Project No: FYL-2023-3549).

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Ayvazoğlu Gizem, Department of Biotechnology August, 2023, Çukurova University Graduate School of Natural and Applied Sciences Master's Thesis Protease Production from Bacillus Sp. Protease Production from Bacillus Sp.

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Yiğit Şat[1] Esma, Nural Yaman[2] Belma, Gedikli[3] Serap, 2020 Heat and pH stable protease produced by a bacterium isolated from fields with high boron.

ORAL PRESENTATION

Elma (*Malus domestica*) meyve yan ürünlerinin değerlendirmesi

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Özet

Günümüzde elma, dünyada en çok yetiştirilen üçüncü meyvedir. Her yıl 50 milyon tondan fazla elma üretilmekte olup, toplam elma üretiminin yaklaşık %30'u meyve suyu ve elma şarabı gibi ana ürün olarak işlenmekte, bunun dışında büyük miktarda elma yan ürünleri oluşmaktadır. Elma yan ürünleri, diyet lifi, yağ asitleri, triterpenler veya polifenoller gibi biyoaktif bileşiklerin kanıtlanmış bir kaynağıdır. Elma suyu ve elma şarabı üretiminin bir yan ürünü olan elma posası, nutrasötikler ve pektin gibi değerli ürünlerin üretimi için sürdürülebilir bir hammadde olarak kullanılmaktadır. Elma posası, çeşitli sağlık avantajlarıyla ilişkilendirilen önemli miktarda antioksidan bileşik içermektedir. Bu nedenle, bu yan ürünlerden elde edilen değerli bileşenler gıda ve ilaç endüstrilerinde kullanılma potansiyeline sahiptir. Bu derlemede elma yan ürünlerinin değerlendirilme uygulamaları ve gıdalardaki potansiyel kullanımlarını içermektedir.

Anahtar Kelimeler: Atık, Değerlendirme, Elma

Evaluation of apple (*Malus domestica*) fruit by-products

Abstract

Today, apples are the third most cultivated fruit in the world. More than 50 million tons of apples are produced each year, and approximately 30% of the total apple production is processed as main products such as juice and cider, while a large amount of apple by-products are formed. Apple by-products are a source of bioactive compounds such as dietary fiber, fatty acids, triterpenes or polyphenols. Apple pomace, a by-product of apple juice and cider production, is used as a sustainable raw material for the production of valuable products such as nutraceuticals and pectin. Apple pomace contains significant amounts of antioxidant compounds that are associated with various health benefits. Therefore, valuable components obtained from these by-products have the potential to be used in the food and pharmaceutical industries. This review includes the utilization applications of apple by-products and their potential uses in foods.

Keywords: Waste, Evaluation, Apple

GİRİŞ

Gıda atıkları, hasat, dağıtım, işleme, perakende satış, restoranlar ve haneler dahil olmak üzere gıda tedarik zincirinin hemen hemen her adımında atılan organik atıklardır. Küresel olarak, insan tüketimi için üretilen yaklaşık 1,3 milyar ton her yıl boşa harcanmakta, bu da toplam gıda üretiminin üçte birini oluşturmaktadır (FAO, 2013). Her ne kadar küçük miktardaki gıda atıkları başka ürünlere çevrilse veya hayvan yemi olarak kullanılsa da, çoğu gıda atığı değerlendirilememektedir (Zhang ve ark., 2007). ABD'de, düzenli depolama alanlarına dökülen gıda atıkları, küresel ısınma açısından karbondioksitten 23 kat daha etkili bir sera gazı olan metan emisyonlarının büyük bir kısmından sorumludur. Hem ekonomi hem de nüfus büyümeye devam ettikçe, gıda üretimi ve tüketim miktarlarının da küresel olarak artması ve gıda atıklarının yönetiminin hem ekonomiye hem de çevreye sunduğu zorlukları artırması kaçınılmazdır (Cuéllar ve Webber, 2010).

Tarım-gıda sanayi yan ürünlerinin genel olarak bertaraf edilmesinin büyük maliyetlere ve çevresel sorunlara neden olması, bu ürünlerin değerlendirilmesine duyulan ihtiyacı artırmıştır (O'Shea ve ark., 2015). Kimyasal bileşimlerinin yanı sıra yüksek su içeriği ve büyük miktarlarda organik yük nedeniyle çabuk bozulan yapıları, verimli ve sürdürülebilir bir endüstriyel gelişmeyi destekleyebilecek maliyetsiz bir biyoaktif bileşik kaynağı sağlamaktadır (O'Shea ve ark., 2015). Nispeten yüksek karbonhidrat konsantrasyonları (şekerler ve nişasta) ve diğer besinler (azot ve mineraller) nedeniyle, gıda atıkları anaerobik sindirim veya fermantasyon yoluyla

bir dizi yenilenebilir kimyasal ve biyoyakıt üretiminde iyi bir substrat olarak tanımlanmıştır. Bunlara biyogaz (Antonopoulou ve ark., 2019), etanol (Matsakas ve ark., 2014), süksinik asit, laktik asit (Zhang ve ark., 2013) 2,3-bütandiol (Liakou ve ark., 2018) ve butanol (Huang ve ark., 2015) örnek olarak verilebilir.

Elma iyi bir biyoaktif polifenol rezervine sahip olup dünya çapında en çok tüketilen meyvelerden biridir ve çeşitli ürünlere işlenmektedir (Villamil-Galindo ve Piagentini, 2022; Ravindran ve ark., 2018). 2019-2020 verileri, kişi başına yıllık 21,9 kg elma tüketimini bildirmekte (He ve Liu, 2008; Kaeswurm ve ark., 2022) olup, epidemiyolojik kanıtlara göre, polifenoller gibi biyoaktif bileşiklerin varlığı nedeniyle elmanın düzenli tüketimi çeşitli sağlık yaraları sunmaktadır (Moni Bottu ve ark., 2022). Farklı elma çeşitleri genellikle elma suyu gibi işlenmiş ürünlerin üretiminde kullanılır. Ancak, kurutulmuş elma ürünlerinin üretiminde son yıllarda keskin bir artış meydana gelmiş olup, elmanın kurutulması işlemi, kabuğun çıkarılmasını gerektirmekte ve bu da tarımsal-endüstriyel atık olarak önemli miktarda elma kabuğu oluşumuna neden olmaktadır (Pastene ve ark., 2009). "Yemeye hazır" kültür nedeniyle, tarımsal-endüstriyel sektörün gıda işleme birimleri, çekirdek, kabuk ve çekirdek gibi %16-36 oranlarında kalıntıların önemli miktarda oluşmasına neden olmakta olup, bu durum katı atık yönetimi için büyük bir sorun oluşturmaktadır (McGhie ve ark., 2005).

ELMA YAN ÜRÜNLERİNİN DEĞERLENDİRİLMESİ

Elma dünya çapında en çok tüketilen meyvelerden biridir ve insan beslenmesinde fitokimyasalların ve antioksidanların başlıca kaynakları arasındadır (Massias ve ark., 2017). Dünya genelinde 7.500'den fazla elma çeşidi olup, bu çeşitlerin çoğu taze tüketim için yetiştirtilmekte, bazıları ise pişirilerek tüketme ya da şarap üretimi için yetiştirilmektedir. Şarap üretimi için yetiştirtilen elmalar genellikle taze olarak tüketilemeyecek kadar ekşi ve buruk özellikte olup, bu özellik tatlı elma çeşitlerinin veremeyeceği zengin bir lezzet vermektedir. Dünyada 2018 yılında 86 milyon ton elma üretimi gerçekleştirilmiş olup, Çin üretimde toplam üretimin %46'sını sağlamış, Türkiye ise 3.625.960 ton üretim ile dördüncü sırada yer almıştır (Tablo 1) (FAOSTAT, 2019).

Tablo 1. Elma Üretim Miktarları ve Oranları

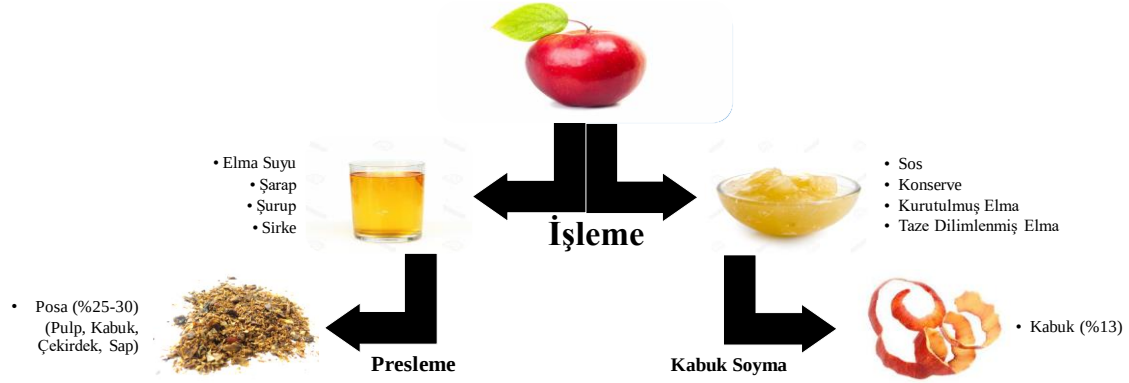
Ülke	Üretim miktarı (ton/yıl)	Oran (%)
Çin	39.233.400	%45.5
ABD	4.652.500	%5.4
Polonya	3.999.523	%4.6
Türkiye	3.625.960	%4.2
İran	2.519.249	%2.9
İtalya	2.414.921	%2.8
Hindistan	2.327.000	%2.7
Rusya	1.859.400	%2.2
Fransa	1.737.412	%2
Şili	1.727.277	%2
Diğer Ülkeler	22.045.555	%25.6
Toplam	**86.142.197**	**%100**

Bir elma yaklaşık olarak %86 su, %14 karbonhidrat, %0,3 protein, %0,2 yağ ve %0,2 kül içeriğine sahip olup, 100 g kabuklu bir elma yaklaşık 52 kalori ve orta düzeyde diyet lifi içeriğine sahiptir. Elmalar, fenolik bileşiklerin önemli beslenme kaynaklarıdır ve diğer meyvelerle karşılaştırıldığında güçlü antioksidan kapasiteye sahiptir (Sun ve ark., 2002). Elma polifenolleri çeşitli in vitro biyoaktivitelere sahiptir (Boyer ve Liu, 2004). Meyve pulpuna kıyasla genellikle meyvenin kabuğunda daha yüksek miktarlarda polifenoller, özellikle flavonol glikozitler bulunmaktadır (Khanizadeh ve ark., 2008). Endüstriyel faaliyetler sonucunda büyük miktarda elma yan ürünleri meydana gelmektedir. Bu yan ürünlerinin çoğu yaygın olarak hayvan yemi, toprak erozyonunu önlemek için organik malzeme ve günümüzde biyoyakıt öncüleri olarak kullanılmaktadır (Lucas-Torres ve ark., 2016). Elma meyvelerinin önemli kısımları Şekil 1'de sunulmuştur.



Şekil 1. Elma meyvelerinin önemli kısımları. A) olgun meyve, B) mezokarp, C) meyve kabuğu D) çekirdek

Elma suyu, şarap, reçel ve sirke üretiminde fazla miktarlarda elma kullanılmakta olup, üretim sonucunda yine oldukça yüksek miktarda elma posası olarak adlandırılan yan ürünler oluşmaktadır. Yılda oluşan elma posası miktarları, Almanya'da 200-250 bin ton, Japonya, İran ve Amerika, İspanya ve Yeni Zellanda'da 20 ila 160 bin ton arasında olduğu, dünya genelinde ise 3.6 milyon ton oluştuğu bildirilmiştir (Kammerer ve ark., 2014). Elma posası meyvenin çeşidi, yetiştiği yer ve üretim teknolojisine bağlı olarak değişmekle birlikte ortalama % 95 meyve kabuğu ve eti, % 2-4 çekirdek ve % 1 meyve saplarından oluşmaktadır. O'Shea ve ark. (2015) tarafından, elma posasının bileşimini % 9 nem, % 2,27 yağ, % 2,37 protein, % 1,6 kül, % 84,7 karbonhidrat olarak belirlemiş olup yine posanın % 5,6 nişasta, % 54,2 toplam şeker ve yüksek miktarda kalsiyum, potasyum ve magnezyum içerdiğini bildirilmiştir. Besin maddeleri açısından zengin olduğu için, günümüzün elma işleme endüstrisinde elma posasının atılması bir kaynak israfıdır. Elma endüstrisinin tipik yan ürünleri Şekil 2'de gösterilmektedir. Ayrıca, özellikle taze elma posası söz konusu olduğunda, çabuk bozulan ve biyolojik olarak parçalanabilir olması nedeniyle, büyük ölçekli modern elma işleme endüstrisinden gelen büyük miktardaki besleyici posa, ciddi bir çevre sorununa neden olabilir. Elma posasının başka ürünlere dönüştürülerek değerlendirilmesi çevresel etkiyi azaltabilir ve büyük ölçekli elma işleme endüstrisinin sürdürülebilir kalkınma gereksinimlerini karşılayabilir.



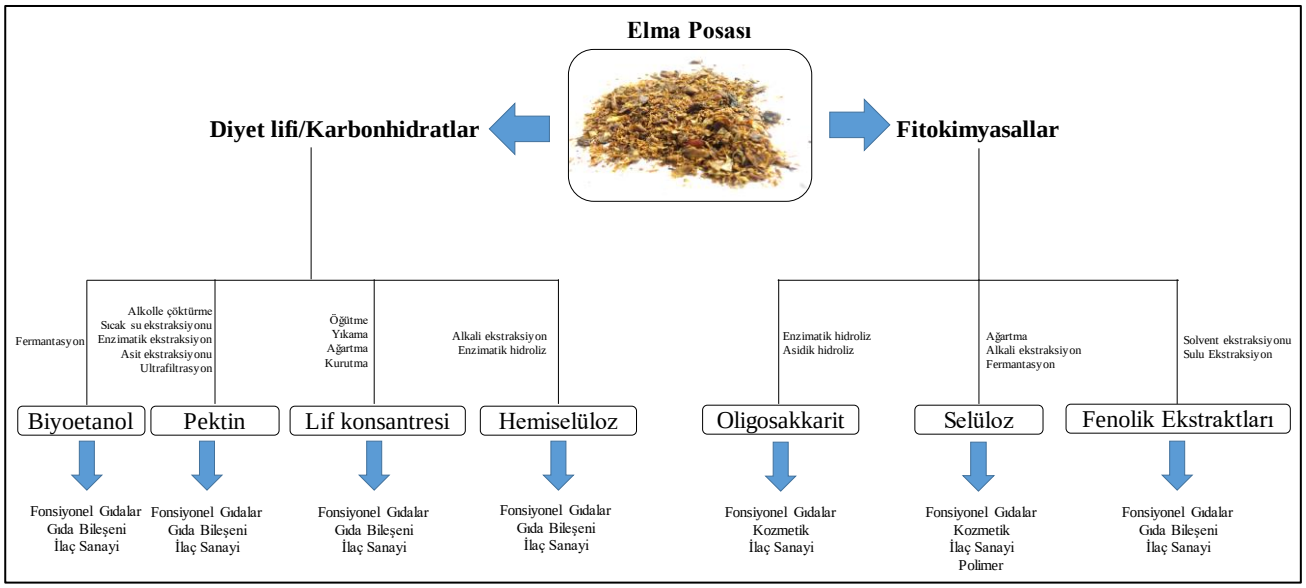
Şekil 2. Elma yan ürünleri

Elma posasının değerlendirilmesi için, hayvan yemi, organik asitler, enzimler, biyoetanol ve mikrobiyal fermantasyon yoluyla biyogaz üretimi için doğrudan kullanımı veya biyokompozitlerin bir parçası olarak yeni malzemelerin geliştirilmesi dahil olmak üzere çeşitli stratejiler önerilmiştir (Rust ve Buskirk, 2008). Bunun yanında, elma posası zengin bir sindirilebilir lif, pektin ve fenolik bileşik kaynağı (Walter ve ark., 1985) içermektedir. Yine de bu yan ürünler, değerli bileşenlerin kaynağı olarak kullanılabilen önemli bir yenilebilir fraksiyonlar sunmaktadır. Bunlara örnek olarak; hidroksisinamik asitler (klorojenik asit ve p-kumaroilkinik asit), flavan-3-oller (prosiyanidinler olarak bilinen büyük polimerlere epikateşin gibi monomerler), flavonoller (quercetin rutinosid, galaktosid, glukozit, ksilosid, arabinosid ve rhamnosid türevleri), dihidrokalkonlar (phloretin 2-O-glucoside ve phloretin 2-O-xyloglucoside) ve antosiyaninler (siyanidin 3-O-galaktosid) verilebilir (Çam ve Aaby, 2015).

Şekil 3, elma yan ürünlerini endüstriyel ürünlere dönüştürme olasılıklarını özetlemektedir. Elma posasının kullanımı ile ilgili birçok çalışma yapılmıştır. Çoğunlukla elma posası, mikroorganizmaların gelişimi için gerekli olan yüksek düzeyde karbonhidrat, vitamin, diyet lifi ve diğer birçok elzem besin maddesi içerdiğinden fermantasyon için hammadde olarak kullanılmıştır. Bu uygulamaların çoğunda, amaç hammaddelerde gelişen mikroorganizmaların sitrik asit, laktik asit, çeşitli enzimler (örneğin, selülazlar, hemiselülazlar, β-glukosidaz

ve pektinaz), biyopolimerler (örneğin kitosan, ksantan ve gum) ve hatta aroma bileşikleri gibi metabolik yan ürünleridir (Madrera ve ark., 2015).

Biyoyakıtlar, büyük popülasyonların artan enerji taleplerinin gereksinimleri için uluslararası bir eğilimdir. Bol, mevcut ve yenilenebilir bir doğal kaynak olarak, elma posası gibi tarımsal endüstriyel atıklar, biyoetanol üretimi için muazzam ticari potansiyele sahip gibi görünmektedir (Raganati ve ark., 2015). Ayrıca, bu tarımsal-endüstriyel atıkların biyoyakıt üretmek için kullanılması, yalnızca gıda ve gıda dışı uygulamalar için mahsul kullanımı arasındaki rekabeti değil, aynı zamanda atıkların bertaraf edilmesinin neden olduğu çevresel baskıyı da azaltabilir.



Şekil 3. Elma yan ürünleri değerlendirme yolları

Elmalar çeşit, olgunlaştırma, yetiştirme koşulları, depolama ve meyvenin bölümüne bağlı olarak farklı konsantrasyonlada ve profilde fenolik bileşiklere sahiptir. Araştırmalar organik elmaların geleneksel olarak yetiştirilen elmalara kıyasla daha yüksek oranda fenolik bileşiklere ve dolayısıyla daha yüksek antioksidan kapasitelere sahip olduğunu belirtilmiştir (Fratianni ve ark., 2007). Yapılan bir çalışmada meyvenin farklı bölümlerinin spesifik fenoliklere sahip olduğu ve genel olarak elma kabuklarının meyve etine kıyasla daha fazla fenolik bileşenlere sahip olduğu bildirilmiştir (Kalinowska ve ark., 2014). Bununla birlikte elma posası değerli bileşenlerinin ekstraksiyonu ile değerlendirilebilir. Çalışmalarda elma posasının polifenollerin, diyet liflerinin ve pektinin geri kazanımı için değerli bir kaynak olduğu bildirilmiştir. Sağlığı koruma kapasitesine sahip gıda bileşiklerine artan bir ilgi olmakta olup, bu bileşiklerin bitkilerden elde edilenlerine fitokimyasallar olarak ifade edilmektedir. Gıda ürünlerinde besin değeri bulunmayan bu bileşenler insan sağlığının korunmasında önemli bir rol oynamaktadır. Meyve ve sebzelerin fitokimyasalları güçlü antioksidan ve anti-proliferatif aktiviteye sahiptir. Bu fitokimyasalların ana gruplarından biri bitki fenolleridir ve birçok çalışmada elma posasının yüksek fenolik madde içerdiği bildirilmiştir. McCann ve ark. (2007) tarafından fenolikler açısından zengin elma atığı ekstraktını araştırıldığı çalışmada, elma fenoliklerinin ham ekstraktının DNA hasarına karşı koruduğu ve bariyer işlevini iyileştirdiği bildirilmiştir. Elma bileşiklerinin fenolik madde içeriklerinin farklı ekstraksiyon yöntemleri için değiştiği belirtilmektedir. Örneğin Reis ve ark. (2012) tarafından elma posasından fenolik madddeleri ekstrakte edilmesi için oda sıcaklığında su ekstaksiyonunun, %40 metanol ve %40 aseton akstraksiyonlarına kıyasla farklı fenolik, flavonoid ve antioksidan aktiviteye sahip olduğu bildirilmiştir. Solvent ekstraksiyonları fazla zaman almakta ve fazla miktarda solvent tüketimine neden olmaktadır. Ekstraksiyon aşamalarında metanol, etanol, aseton ve bunların su ile karışımları fenoliklerin geri kazanımı için kullanılmaktadır. Ekstraksiyon oranı ve verimini artırmak için ultrasonikasyon (Goula, 2013), süperkritik sıvı ekstraksiyonu (Massias ve ark., 2015), mikrodalga (Kala ve ark., 2016), hidrodinamik kavitasyon (Lohani ve ark., 2016), darbeli elektrik alanı (Lohani ve Muthukumarappan 2016) ve basınçlı sıvı-katı ekstraksiyonu (Franquin-Trinquier ve ark., 2014) gibi farklı yöntemler araştırmacılar tarafından bitki dokularında ya da elma atıklarında test edilmiştir.

Öte yandan, elma posası pektin bakımından zengindir, bu nedenle gıda, kozmetik ve ilaç endüstrisinde stabilizatör, jelleştirici ve koyulaştırıcı olarak yaygın olarak kullanılmaktadır. Son zamanlarda pektin, kardiyovasküler hastalık tedavisi, prostat kanseri hücrelerinin apoptozunun indüksiyonu, kolona özgü ilaç dağıtımı, anti-inflamasyon, probiyotik büyümeyi teşvik etmek ve hatta gözenekli malzemeler için yeni bir hammadde olarak kullanılmaktadır (Wang ve ark., 2014). Kumar ve Chauhan (2010), 2 farklı elma çeşidi kullanılarak elma posasından elde edilen pektinin lipaz inhibisyon etkisi gösterdiği bildirilmiştir. Schieber ve ark. (2003) tarafından, elma posasından elde edilen pektin ve polifenolleri ile ilgili çalışmada, posa içindeki pektinden polifenollerin uzaklaştırıldığı ve elde edilen baskın polifenol bileşiklerinin, floridzin, klorojenik asit ve kuersetin glikozitleri olduğu ve fenolik bileşiklerin uzaklaştırılmasının pektin renginin önemli ölçüde giderilmesini sağladığı bildirilmiştir. Ekstraksiyon sıcaklığı, basınç ve diğer teknoloji ile ilgili çalışma koşulları, çözücü (tip, polarite, moleküler ağırlık), katı/sıvı oranı, biyokütle partikül boyutu ve partikül boyutu dağılımı gibi faktörlerin ekstraksiyon kinetiğini etkilediği bilinmektedir. Çözünürlük özelliklerine bağlı olarak, pektin geleneksel olarak sulu çözücüler içinde elma posası gibi bitki dokularından ekstrakte edilmekte ve daha sonra organik çözücüler kullanılarak çökeltilerek geri kazanılmaktadır (Wikiera ve ark., 2015). Pektin ekstraksiyonu; yaygın olarak kullanılan geleneksel asit ekstraksiyonunun yanında, son yıllarda alternatif olarak; ultrasonik, mikrodalga, sub-kritik, yüksek basınç ve enzimatik ekstraksiyon gibi yöntemler ile gerçekleştirilebilmektedir. Geleneksel yöntem yerine bu yeni ekstraksiyon yöntemlerinin kullanılması, pektin verimini ve kalitesini artırmasının yanında, ekstraksiyon süresi, sıcaklığı ve toksik çözücülerin kullanımının azaltması gibi çeşitli avantajlar sunmaktadır (Zoghi ve ark., 2021).

SONUÇ

Atık yönetimi, emisyon azaltma veya hafifletmeden daha pratik bir stratejiye dönüşmüştür. Atık ürünler, uzun vadeli kalkınma için bir ihtiyaç olan ham kaynak olarak kullanılan uygulanabilir malzemeler olarak kabul edilmektedir. Elma bazlı içeceklerin modern endüstriyel üretimi, her yıl yüzlerce ton elma yan ürünü üretir ve bunlar genellikle atık olarak atılır. Elma yan ürünleri muazzam miktarlarda üretildiği ve yüksek oranda su içerdiği için, çürümeyi önlemek için derhal ele alınması gereken depolama sorunları vardır. Çöpleri katma değerli ürünlere dönüştürmek atıkları azaltabilir ve küresel toplum için uygulanabilir bir seçenek haline gelebilir. Modern elma işleme endüstrisi her yıl büyük miktarlarda elma yan ürünleri üretir. Bu nedenle çevresel etkileri azaltmak için bu yan ürünleri işlemek gerekir. Biyoaktif bileşiklerin ekstraksiyonu, iyi bir pektin ve polifenol kaynağı olduğu için elma yan atıklarının değerlendirilme yöntemlerine örnek olarak verilebilir. Bu yan ürünlerin güçlü antioksidan kapasitesi, antibakteriyel ve antiviral özellikleri, özellikle nutrasötik ve ilaç endüstrilerinde kullanılma potansiyeli sağlamaktadır. Böylece, insanlık tarihi boyunca önemli bir rol oynamış olan elma, tam olarak değerlendirilebilir ve bu da yeni katma değerli bir ürün haline gelebilir.

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ORAL PRESENTATION

Investigation of the Aqueous Extracts Inhibitory Activity of *Salvia viridis* on LPO Enzyme Activity

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Abstract

In recent years, the prominence of natural antimicrobial systems has increased in various areas including the food industry, cosmetics, and pharmaceuticals. In this regard, attention has been focused on the Lactoperoxidase (LPO) enzyme, which exhibits considerable potential as a natural antimicrobial system. On the other hand, natural plant extracts and components also provide essential sources of antioxidant, antimicrobial, and enzyme inhibition activities. *Salvia viridis* is a plant native to the Mediterranean region that has long been used to cure gum and throat infections. It has also been used to improve the quality of liquor in fermentation barrels. Despite its long-standing use for therapeutic purposes and as a food additive, there has been inadequate characterization of its biological activity. The effects of this plant, which also has antioxidant properties due to its high phenolic content, on the antibacterial LPO enzyme, which is important both in milk and human and animal metabolism, are unknown. Aqueous maceration, decoction, and infusion of the aerial portions of the *S. viridis* plant, were prepared to determine their effect on the LPO enzyme purified from buffalo bovine by affinity chromatografy. IC_{50} values were determined through the investigation of *S. viridis* extracts on the LPO enzyme activity. According to in vitro enzyme assays, IC_{50} values of *S. viridis* aqueous extract samples (maceration, decoction and infusion) were determined as 0.15, 0,74 and 1.30 mg/ml. Macerated samples demonstrated the strongest inhibitory activity with 0,15 mg/ml among these samples

Keywords: Lactoperoxidase, *Salvia viridis*, aqueous extract, maceration, decoction, infusion, enzyme inhibition

INTRODUCTION

Salvia species are a significant class of plants with medicinal properties that have been utilized across centuries and are still valued today. Ornate texts and paintings found in ancient tombs and monuments include the earliest documentation of its therapeutic qualities and its application in the treatment of various maladies (Nakipoglu and Oguz, 1990). The infusion of the aerial parts of the Salvia plant or its essential oil has been used for centuries to treat a variety of medical conditions, including colds, coughs, gingivitis, toothaches, sore throats, stomach and abdominal pains, diarrhea, diabetes, high blood pressure, rheumatism, and skin disorders. In addition, it has sedative, expectorant, and vascular constrictor properties (Skoula et al., 1999).

Salvia viridis L., also referred to as *Salvia horminum*, is the sole annual species of the Salvia genus found in Turkey. (Özdemir ve ark. 2009). *Salvia viridis L.*, naturally present in the Mediterranean region and also referred to 'red-topped sage', has been used in traditional medicine. Infusions of *S. viridis* shoots have been used in Turkey to treat some throat disorders, ulcers, intestinal spasms and gynaecological complications (Zengin et al., 2019) Besides being used in traditional medicine, its leaves, seeds, and essential oils are also used as a liqueur enhancer and for aromatizing some wines and beers. It is also recognized for its important honey production potential. (Coşge Şenkal, 2019). Its leaves, seeds and essential oils are used as a liqueur enhancer and for aromatizing some wines and beers. It is also recognised for its important honey production potential. throat disorders As an important source of nutrients produced by mammals, milk is an essential ingredient in the production of many dairy products. (Tanamati, F. et al, 202; Han et al, 2022). Milk contains bioactive substances and immune-boosting compounds important for neonatal and adulthood nutrition, among them bioactive proteins, bioactive lipids, lactoferrin (LF), lactoperoxidase, lysozyme, N-acetyl-ß-D-glucosaminidase (NAGase) and nisin. (Yılmaz, B. & Tosun H., 2013). Increasing identification of trace proteins in milk is revealing their involvement in many important biological functions. For instance, whey proteins, such as lactoperoxidase, play a role in immune defence, antimicrobial and antioxidation roles. (Li et al., 2018). Until recently, enzymatic activity in milk was regarded as undesirable, so more attention was paid

to inactivating enzymes rather than using them. However, studies in recent years have revealed that enzymatic modification of milk is possible. Enzymes, whether oxidative or hydrolytic, can be used to improve the flavour of milk by producing or enhancing the desired flavors by eliminating off-flavours or by preventing the development of off-flavors. LPO is mainly used for dairy applications to protect raw milk during storage and transport. The LPO system's antimicrobial agents found in milk inhibit many spoilage and pathogenic microorganisms, thereby improving milk microbiological quality.

With the growing awareness of the need to avoid the use of chemical preservatives in food preservation, natural anti-microbial systems are being explored, and one such system, the lactoperoxidase (LPO) system, is gaining importance. Lactoperoxidase acts as a catalyst for the oxidation of thiocyanate in the presence of H_2O_2 to produce intermediate products with antimicrobial effects. These products have broad-spectrum antimicrobial activity. They are effective against bacteria, mould and viruses.. (Yıldız, H. & Sert, S.,2008).
Preferring natural, plant-based antimicrobial preservatives is another alternative to reducing the use of synthetic preservatives in food applications. The antimicrobial and antioxidant properties of herbs, spices and their essential oils have been reported to have antimicrobial activity against food spoilage and foodborne pathogens. Salvia is one of many medicinal plants that have been used for many years for cooking, medicinal purposes and as a natural foodstuff preserving agent. (Bor et al., 2016; Gyawali, Hayek, and Ibrahim, 2015a; Gyawali and Ibrahim,2014; Raeisi et al., 2016; Raeisi, Ojagh, Sharifi-Rad et al., 2017). Due to their prevalence in the diet and their role in health and disease, there is increasing interest in the antioxidant effects of natural plant compounds.

S. Viridis, among the Salvia species with biological activity, is the least studied. The goal of this study was to determine the effect of extracts of this plant, which is used by the public for therapeutic purposes, on the activity of the LPO enzyme, which has an antibacterial effect in milk. For this purpose, maceration, infusion and decoction methods were used to extract the aerial parts of *S. viridis* because of their ease of preparation, widespread use and low cost (Grzegorczyk-Karolak & Kiss, 2018). The aim is to obtain high-yield, high-quality plant extracts; this is an important goal when considering medicinal plants. In the context of this information, our main question is how the extracts prepared from the plant in question affect the activity of the enzyme lactoperoxidase, which is important both in the food industry and in the field of pharmacy and medicine

MATERIALS AND METHODS

Materials

The chemicals used for this study were; 2,2'-azino-bis(3-ethylbenzthiazoline--6-sulfonic acid) (ABTS), Standard serum albumin, Ammonium sulfate, Hydrogen peroxide, Sodium chloride, dimethyl sulfoxide, Sodium hydroxide, Hydrochloric acid, Ethanol, Hexane and buffalo milk as enzyme source.

Preparation of plant material and extracts

Plants belonging to the species *Salvia viridis L.* were collected from rural areas of Adana province in April 2023 (Figure 1). After the aerial parts of the dried plant were ground to powder, they were macerated with distilled water at room temperature and both decoction and infusion were obtained at the same time. For this purpose, 1 gram of the drug was weighed separately in 10 mL of water and mixed occasionally and kept at room temperature for maceration and in a hot water bath for 2 hours for decoction and infusion. The water parts of the aqueous extracts were removed from the solvent by lyophilization and made ready for analysis and activity experiments.

Preparation and purification of lactoperoxidase enzyme

Buffalo milk was used as the source of LPO enzyme. The toluene-treated milk was then subjected to 60% ammonium sulphate precipitation and centrifuged at 15000 rpm for 45 minutes at +4 ºC. After centrifugation, the supernatant was discarded and the lower precipitate was collected. The precipitate obtained from the ammonium sulphate precipitation and dissolved in the equilibration buffer was slowly added to the Sepharose 4B-etilen diamin-4-tiyoureido-benzenesulfonamide affinity column equilibrated with 10 mM phosphate buffer (pH: 6.8). The column to which the sample was added was then washed with 400 mL of 25 mM phosphate buffer (pH: 6.8). This bound the lactoperoxidase enzyme to the column. Finally, 1 M NaCl / 25 mM Na_2HPO_4 (pH: 6.8) elution buffer was added to elute the lactoperoxidase enzyme (Bozdag et al., 2015).

Determination of lactoperoxidase enzyme activity

The activity of the lactoperoxidase enzyme was measured using the procedure established by some modifications of the Bardsley and Shindler method. This method is based on the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) chromogenic substrate with H_2O_2 and monitoring the increase in absorbance of the resulting coloured compound at 412 nm (Shindler & Bardsley, 1975).

In vitro inhibition studies

In order to determine the IC_{50} values of the plant extracts used in the study, the ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) substrate was studied for LPO under optimal conditions. First, the enzyme activity was determined in the medium without plant extract and obtained value was taken as 100% activity. The absorbances corresponding to the different concentrations of plant extracts were then read against the blank for LPO at 412 nm.

After plotting the % activity -- [I] graphs, IC_{50} values were calculated for each molecule using these graphs (Coddington A., 1970)

Tables and Figures



Figure 1. *Salvia viridis L.*

Table 1. Purification results of LPO from buffalo milk.

Purification steps	Total Volume (mL	Enzyme Activity (EU/mL)	Total Enzyme Activity (EU/mL·min)	Protein (mg/mL)	Total Protein (mg)	Specific Activity (EU/mg)	Yield (%)	Purification Fold
Homogenate	10	420.0	4199.9	489.35	4893.5	0.9	100	-
Affinity column	2	945.8	1891.5	0.346	0.692	2733.4	45.0	3184.9

Table 2. IC_{50} values for aerial part of *S. Viridis* aqueous extract used in this study.

Extraction Types	IC_{50} (mg/ml)	R^2
Maceration	0.15	0.9305
Decoction	0,74	0,9793
Infusion	1.30	0,9742

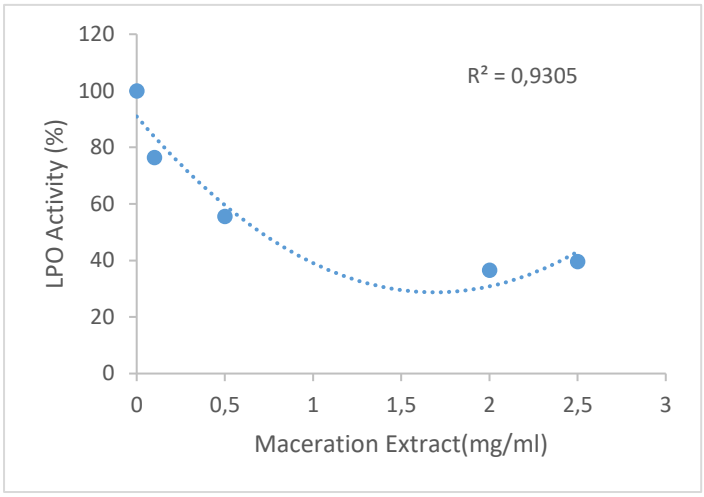

Figure 2. Effect of viridis extract at varying concentrations (0.5-2.15 mM) on buffalo milk lactoperoxidase.

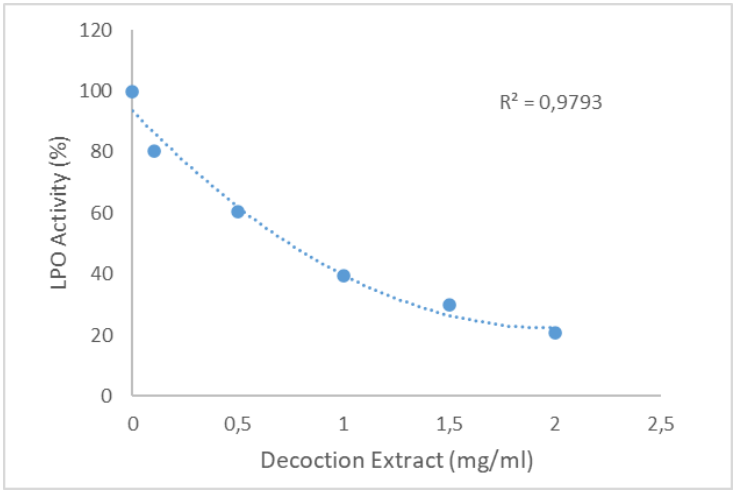

Figure 3. Effect of decocted S. viridis extract at varying concentrations (0.5-2.15 mM) on buffalo milk lactoperoxidase.

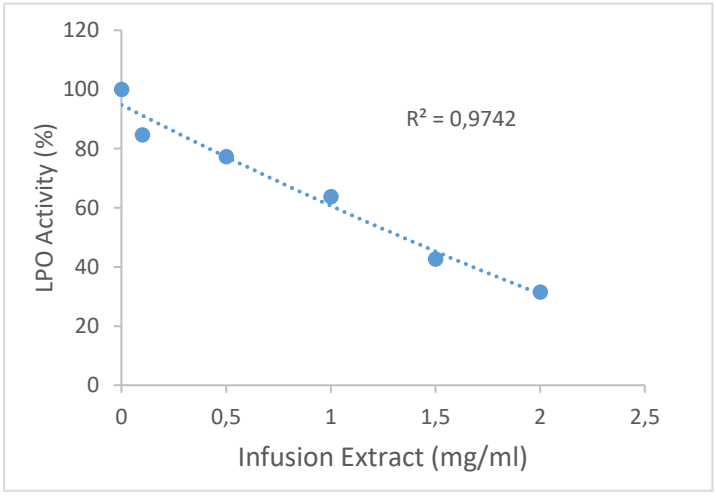

Figure 4. Effect of infused S. viridis extract at varying concentrations (0.5-2.15 mM) on buffalo milk lactoperoxidase.

RESULTS AND DISCUSSION

The biological value of the LPO enzyme is based on its role as a natural defence system against the invasion of micro-organisms. It has been reported that LPO inhibits biofilm formation in saliva, in addition to its widespread presence in milk (Uratasun et al., 2017). Besides its antimicrobial activity, LPO also plays a role in degrading various carcinogens and protecting animal cells from peroxidative effects (Magacz M., 2019; Nishioka, 1981LPO is also significant in the agricultural, veterinary, pharmaceutical and cosmetic industries, particularly in the dairy industry where it is used to protect milk during transport to milk processing plants. (Abul et al., 2023). In regions where mechanical cooling is not possible for technical and/or economic reasons, the LPO enzyme is used as an antimicrobial system naturally present in milk to prevent milk spoilage (Eyassu, et al., 2005). However, commercial dentifrices or oral care products that contain lactoperoxidase have been observed to be effective in inhibiting pathogen growth (MagaczM., 2019; Welk, et al., 2021). A patented study on powdery mildew, one of the world's most important plant diseases, showed that infection by the pathogen could be reduced by 90-95% as a result of using the LPO system with various modifications (Boots, et al., 2006). For the production of industrial products and for the health of living organisms, it is important to know under which conditions and with which molecules the LPO enzyme, which has such important functions, is inhibited or activated. For this reason, the focus of most research has been on low-budget purification assays of the LPO enzyme (Koksal et al., 2017; Otten, 1986).

To date, the purification of LPO enzyme from mammalian milk has involved a variety of chromatographic methods. Among these methods, affinity chromatography is an effective method for purifying a large number of proteins from very complex sources in a single step. In this study, to determine the effect of *S. Viridis* aquaeous extracts, LPO enzyme was purified from buffalo milk by affinity chromatography, yielding 45.0 % (Table 1LPO was purified 32.0-fold from human colostrum by chromatography, 1450.0-fold with a yield of 21.0% in a single step by immunoaffinity column chromatography. Furthermore, LPO was purified 53.0-fold with 49.50% yield from goat milk by using cation exchange chromatography and 13.90% yield from cow milk by CM-Sephadex C-50 and gel filtration chromatography (Köksal et al, 2017) LPO enzyme was purified from bovine milk with a yield of 34.67% using Amberlite CG-50H+ resin and Sepharose-4B-L-tyrosin-5-amino-2-methylbenzenesulfonamide affinity chromatography (Köksal & Alim, 2020).

In addition to sulfonamides, drugs and derivatives such as 3-amino-1,2,4-triazole (amitrole), mercaptomethylimidazole, salicylhydroxamic acid, drugs such as ketamine and bupivacaine, and some aromatic compounds such as L-ascorbic acid and folic acid have been reported to inhibit the LPO enzyme (Sharma et al., 2013). In a study investigating the effect of an ethanolic extract of *Leonurus cardiaca L.* on the LPO enzyme, certain constituents of *L. cardioca* were found capable of efficiently recovering the pseudohalogenating activity of lactoperoxidase (LPO).(Flemmig et al., 2015)

The antioxidative effects of natural phytonutrients are of increasing interest because of the widespread use of these compounds in the diet and the role they play in health and disease. *Salvia viridis* is the least studied Salvia species in terms of biological activity. Here, we present for the first time the effect of aqueous extracts of the *Salvia viridis* plant on LPO enzyme. The effects of aqueous extracts of *Salvia viridis* on LPO enzyme activity were investigated. In this study, the IC_{50} values for these aqueous extracts on buffalo LPO are determined and summarised in Table 2. IC_{50} plots of extracts are shown in Figure 2-4.

Following in vitro enzyme assay, IC_{50} values of *S. Viridis* aqueous extract samples (maceration, decoction and infusion) were determined to be 0.15, 0.74 and 1.30 mg/ml. The macerated samples showed the strongest inhibitory activity among these samples with an IC_{50} of 0.15 mg/ml. To investigate some biological activities of ethanolic root extracts of S. viridis obtained through various extraction methods—namely microwave-assisted extraction, maceration, supercritical fluid extraction, Soxhlet extraction, and ultrasonic-assisted extraction—the study examined the inhibitory effects on key enzymes associated with Alzheimer's disease (acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)), diabetes (α-amylase and α-glucosidase), and skin hyperpigmentation disorders (tyrosinase). It is reported that the root extracts of *S. viridis* demonstrated inhibitory activity against AChE, BChE, α-glucosidase, and tyrosinase, with a stronger effect on α-glucosidase than α-amylase, suggesting potential benefits over current hypoglycemic agents(Zengin et al., 2019b).

Previous studies have reported that *S. viridis*, like other sage species, contains abundant rosmarinic acid. Rosmarinic acid was reported to have several biological activities: anti-inflammatory, antioxidant, antimutagenic, antiviral, antibacterial, hepatoprotective, antitumour, and cardioprotective. On the other hand, the effect of rosmarinic acid against the LPO enzyme was determined as low micromolar(Gülçin et al., 2016).

CONCLUSION

This study has, for the first time, evaluated the LPO enzyme inhibitory properties of aqueous extracts of *S. viridis* aerial parts. The inhibitory properties of *S. viridis* aqueous aerial part extracts were found to be influenced by the choice of extraction technique. The present study will serve as a basis for future research on these species, as the Salvia species studied may be of interest to the pharmaceutical and food industry. To that end, additional experimental investigations are also required to understand the mechanisms of in vivo activity, bioavailability and the metabolic pathways implicated.

ACKNOWLEDGEMENTS

This study had the support of the Scientific Research Unit of Adana Alparslan Türkeş University of Science and Technology.

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ORAL PRESENTATION

Synthesis of Co/HTC Catalyst Derived from Hazelnut Shell for Efficient Hydrogen Production by Methanolysis

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Abstract

Biomass-based Co catalysts were prepared by an impregnation and chemical reduction method and used to catalyze the methanolysis of sodium borohydride ($NaBH_4$) for hydrogen production. At a solution temperature of 25 ºC, the methanolysis reaction can be effectively accelerated with a cobalt catalyst on a support of hydrothermally carbonized hazelnut shell (Co/HTC) and provides a desirable hydrogen generation rate, making it suitable for applications under the conditions of low environmental temperature (4.06 L min^{-1} g^{-1}). The catalytic activity of Co/HTC in $NaBH_4$ methanolysis can be further improved by suitable dissolution conditions of the methanol/water mixture. Increasing the methanol/water ratio increases the concentration of methanol in the mixture and thus the hydrogen generation rate (HGR). It was found that the reaction kinetics of methanolysis is faster than that of hydrolysis under the same experimental conditions. For the catalytic methanolysis reaction, the reusability of the catalyst over the Co/HTC catalyst was also investigated under optimized conditions.

Keywords: Hydrothermal carbonization, catalyst synthesis, methanolysis, hazelnut shell.

INTRODUCTION

Energy is mostly supplied by the combustion of fossil fuels which emits large amounts of greenhouse gases, thus causes to global warming. On the way towards a sustainable energy future, hydrogen appears to be the best energy carrier. However, the safe and efficient storage of hydrogen is still one of the most important and challenging problems in hydrogen economy (Çalışkan et al., 2010).

Nowadays, the major storage forms for hydrogen are high pressure gas and low-temperature liquid. These forms require high technology and equipment. In addition, hydrogen can be acquired only in hydrogen refueling stations. The limited storage forms increase the cost of hydrogen energy and seriously hinder the wide application of hydrogen energy. It is easy to on-site produce portable hydrogen through simple chemical reactions by using a chemical with high hydrogen storage density, for example, a metal hydride $NaBH_4$, KBH_4, LiH, or NaH or ammonia borane (Wang et al., 2018). Among these chemicals, sodium borohydride has been widely studied for its high gravimetric hydrogen density (10.8 wt%), high stability in alkaline solution, non-flammability, and low requirement for storage environment (Xu et al., 2012). Numerous metal catalysts with different formulations based on Ru, Pt, Co and Ni nanoparticles have been employed to extract hydrogen via hydrolysis of $NaBH_4$. Due to the slow reaction kinetics, low conversion at sub ambient temperatures, and limited solvation of components in the hydrolysis of $NaBH_4$, researchers continue to seek new and alternative reaction pathways for fast and controllable hydrogen production. For example, production of hydrogen from methanol and $NaBH_4$, shown in Eq. (1), provide a faster reaction rate than hydrolysis of $NaBH_4$ even at lower temperatures. Furthermore, $NaBH_4$ dissolution in methanol is 0.164g/mL and is thus higher than in water (Demirci et al., 2020).

$$NaBH_4 + 4CH_3OH \rightarrow 4H_2 + NaB(OCH_3)_4 \quad (1)$$

In order to control the methanolysis of $NaBH_4$ and achieve higher reaction rates, several different strategies have been undertaken such as use of catalysts, acid or elevated temperatures. It must be recognized that the use of acid and elevated temperatures is preferred due to environmental concerns and high operating cost, respectively. Consequently, use of catalyst for the methanolysis of $NaBH_4$ has gain more attention over the last decade (Bekirogluları 2024).

Hydrothermal carbonization (HTC) is a promising conversion method to fully valorize biomass constituents. HTC is also used for treating organic-rich wastewater such as olive-mill wastewater. With HTC, biomass is converted into gas, liquid and carbon-rich solid (hydrochar). Since the HTC reactions require water, wet biomass sources or moisture-rich biomass sources are more preferable feedstocks. The liquid fraction (hydrothermal carbonization liquid) mainly includes hydrolysis products of hemicelluloses and dissolved inorganic species. This liquid fraction can be reused or further processed to produce bio-based chemicals (Kabakcı and Baran 2019). HTC changes the original structure of the biomass by a series of chemical reactions (hydrolysis, dehydration, decarboxylation, aromatization and recondensation), which occur in the presence of water at low temperature (180–250 ºC) and under the pressure to keep water in liquid state (10–40 bar).

Hydrochar has several benefits over its original biomass, including higher carbon content, in recent years, bio-based hydrochars have been synthesized and further processed (mainly activated) in order to be used as support materials for heterogenous catalyst synthesis. In this study, the hydrochar obtained from hazelnut shell was used as a support material for synthesis effective catalyst for hydrogen generation via methanolysis of sodium borohydride.

MATERIALS AND METHODS

Materials

Potassium hydroxide pellets (>85%) were supplied by by Sigma-Aldrich (Taufkirchen, Germany. Cobalt(II) chloride hexahydrate (>97%) and sodium hydroxide pellets (>98%) were supplied by Sigma-Aldrich (Taufkirchen, Germany). All chemicals used were of analytical grade and distilled water was used for all syntheses.

Hydrothermal carbonization of hazelnut shells

6 g biomass and 24 mL deionized water were mixed in a stainless-steel autoclave. The autoclave was kept in an oven for 90 min at 220 ºC. By the end of the carbonization period, the autoclave was immediately cooled and the mixture was filtered. The hydrochar samples were dried in the oven at 105 ºC for 24 h. Dry hydrochar samples were mixed with KOH at a KOH/hydrochar ratio of 2. The hydrochar-KOH mixture was placed in a ceramic boat and then placed in a horizontal tubular furnace. The mixture was heated at a heating rate of 10 ºC/min up to 600 ºC, and kept at the same temperature for 1 h. After activation, samples were washed with 0.1 M HCl and deionized water, until the pH of the filtrate was 7. The samples were dried second time in the oven at 105 ºC for 24 h. Preparation method selected according to similar biomass hydrothermal carbonization studies (Kabakcı and Baran 2019).

Catalysts Synthesis

Carbon obtained by hydrothermal synthesis of hazelnut shells were used as support material for the synthesis Co catalysts using the impregnation and reduction method. The appropriate amount of $CoCl_2$ solution was prepared in stoichiometric ratio with distilled water. The cobalt content in the supported catalysts was adjusted to 20 % by weight. After impregnating the cobalt on the supports at 75 °C for one hour at 700 rpm, $NaBH_4$ solution was added dropwise for the chemical reduction process to reduce Co^{2+}. An excess amount of borohydride was used to achieve complete reduction with a molar ratio of $NaBH_4$:CoCl2 (5:1), and the reduced solution was stirred at 400 rpm for one hour at room temperature. Then the sample was filtered and washed with an ethanol/water mixture (volume ratio 1:1) to remove insoluble inorganics from the catalyst. The sample was placed in the oven at 105 °C for 24 hours. The resulting powder was black and was stored in a dark bottle to utilize the hydrolysis reaction.

Methanolysis Reaction

Methanol (Merck, 99.5%) was used as received in this work. The methanolysis reactions were carried out in a 50 mL jacketed reactor. In a typical H_2 generation experiment, 0.50 g $NaBH_4$ and 0.1 g catalyst were initially put into the reactor and the methanolysis reaction was initiated by injecting 10 mL methanol (or water, or the mixture of methanol and water) into the reactor. Because the evolved hydrogen could generate vigorous bubbles in the solution and enhance the contact between the reactant and the catalyst, no further stirring measure was taken during the reaction. A water displacement method was used to measure the cumulative volume of the generated hydrogen over time.

RESULTS AND DISCUSSION

Hydrolysis, methanolysis, and solvolysis (methanol-water mixture) of $NaBH_4$

Table 1. shows the reaction kinetics of hydrogen evolution by catalyzed hydrolysis, methanolysis and solvolysis (a methanol-water mixture with different volumetric ratios of methanol to water) of $NaBH_4$ at a solution temperature of 25 °C. As shown in Table 1, as the methanol/water ratio, i.e. the concentration of methanol in the mixture, is increased, the time of hydrogen generation increases. This indicates that the reaction kinetics of methanolysis is faster than that of hydrolysis under the same experimental conditions. The effect of the methanol/water molar ratio on the reaction kinetics could be related to the properties and structures of the water-methanol mixture, since the water-water interaction can play an important role in determining some properties of the water-methanol solution (Xu et al. 2012).

Table 1. Hydrogen generation rate of the reaction by hydrolysis, methanolysis, and solvolysis (methanol-water mixture with different volumetric ratios of methanol to water) at 25 °C.

Methanol:Water Ratio	Reaction Time (min)	Hydrogen Generation Rate (L min^{-1} g^{-1})
10:0	3.3	4.06
8:2	4.7	2.85
6:4	6.7	2.00
4:6	10.2	1.31
2:8	17.03	0.79
0:10	20.2	0.66

Although the amount of hydrogen produced is almost the same, the presence of water also has a negative influence on the kinetics of catalyzed methanolysis. However, this influence can be somewhat more complicated. In the case of a catalyzed reaction, methanol competes with water for adsorption on the catalyst surface. The O−H bond of water is more polarized than that of methanol, which leads to a stronger adsorption of water on the catalyst surface (Xu et al., 2010).

Reusability of the catalyst

The durability of catalysts is crucial for the application of $NaBH_4$-based hydrogen. The cyclic behavior of catalysts for the methanolysis of $NaBH_4$ over five cycles at a solution temperature of 25 ºC was performed under constant experimental conditions. After each experiment, the used catalyst was separated, rinsed with deionized water and dried again to obtain the same physical state as the fresh catalyst. In accordance with the reusability results, the hydrogen generation rate decreased during the five cycles. The corresponding hydrogen generation rates were again 4.06 L min^{-1} g^{-1}, 3.50 L min^{-1} g^{-1}, 3.17 L min^{-1} g^{-1}, 2.53 L min^{-1} g^{-1} and 1.80 L min^{-1} g^{-1}. At the end of the experiment, a significant slowdown in the reaction rate was observed, which could be attributed to partial blocking of the catalyst surface by sodium tetramethoxyborate and the relatively low concentration of $NaBH_4$ at the end of the reaction.

CONCLUSION

The methanolysis reaction can be effectively accelerated using Co/HTC, catalyst and provide a desirable hydrogen generation rate, which makes it suitable for applications under the circumstance of low environmental temperature, at solution temperature of 25 °C. With the increase of methanol/water ratio, i.e., the concentration of methanol in the mixture, the volume of generated hydrogen increases slowly, indicating that the reaction kinetics of the methanolysis is faster than that of the hydrolysis under the same experimental conditions. The highest hydrogen generation rate was obtained as 4.06 L min-1 g-1 with the methanol/water ratio of 10:0 at 25 ºC in the presence of 0.1 g Co/HTC and 0.5 g $NaBH_4$. The minimum hydrogen generation rate was obtained as 0.66 L min^{-1} g^{-1} with the methanol/water ratio of 0:10 at same experimental conditions. Reusability of the catalyst also investigated for the optimum reaction solution conditions. It was obvious that the hydrogen generation rate decreased during the five cycles. The corresponding hydrogen generation rates were decrease an obvious slow-down of the reaction rate at the end of experiment could be observed, which might be attributed to a partial blockage of the catalyst surface by sodium tetramethoxyborate and the rather low concentration of $NaBH_4$ at the end of reaction.

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ORAL PRESENTATION

New Applications of Hydrogen in the Food Industry

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Abstract

Consumers are aware of the health risks of synthetic additives and the effects of high-heat treatment on the nutritional and global quality of food products. Hydrogen (H2) was traditionally used in the manufacturing of margarine and shortening. Recently, many novel applications of H2 have been discovered in the food industry. In food drying, the inclusion of H2 in the drying atmosphere leads to the protection of the nutritional and sensory properties of the dried product (apricot and apple). In modified atmosphere packaging, the incorporation of H2 in the atmosphere of the food package leads to the protection of the quality and nutritional properties of food, such as strawberries, fish, and fresh cheese, with an extended shelf life. H2 showed other advantages for food products when it is used in the form of hydrogen-rich water (HRW) in food processes.

Keywords: Hydrogen, Food, hydrogen-rich water, Quality

INTRODUCTION

The deterioration of the quality of fresh food is a natural phenomenon that happens due to many chemical and biological factors, including microbiological and enzymatic activities. Oxidative reactions are one of the primary sources of food quality deterioration. The oxidative destruction of antioxidants, unsaturated fatty acids, vitamins, and pigments can shade the color and deteriorate the flavor and taste, which in turn shortens the shelf life of the food. Our team found that including hydrogen gas in some processes can help protect the nutritional and sensory properties of the food product and extend its shelf-life.

Food Drying

In the drying process, the inclusion of hydrogen gas in the atmosphere could preserve the color and antioxidants of fruits such as apricots (Alwazeer & Örs, 2019) and apples (Alwazeer, 2018) and herbs such as mint green tea leaves. This technique was called Reducing Atmosphere Drying (RAD). The color indices of the hydrogen-included atmosphere (RAD_{mix}) dried apricot slices were the closest to the fresh sample, while the freeze-dried slices were characterized by light and dully notes of color compared to a fresh sample. The antioxidant activity, total phenolic content, and total flavonoid content of RAD-dried apricot samples were the closest to fresh samples compared with hot air oven and vacuum-dried samples (Alwazeer & Örs, 2019). In another study, both apple and apricot slices were dried using RAD_{mix} (4% H_2, %5 CO_2, 91% N_2), which was compared with nitrogen (100%) and hot air drying. Results showed that the color parameters (L*, a*, b*) of RAD apple slices were the closest to the fresh ones besides the freeze-dried samples. However, the high increase in the L* value and the high decrease in the b* value of freeze-dried samples are reflected by the lightness of the yellowness of the sample color, which is considered undesirable for dried apples and apricots (Alwazeer, 2018).

Food Packaging

The packaging process aims to preserve the food product from external deteriorative conditions. The preservation of crops and foods under the atmosphere, different from air, is a technique that aims to prolong the shelf life of a product. This technique is called modified atmosphere packaging that generally uses a gaseous mixture of carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2) (Alwazeer, 2019). Our team revealed that the inclusion of hydrogen gas (H_2) with CO_2 and N_2 could preserve the nutritional and sensory qualities

of fruits, such as strawberries, and dairy products, such as fresh cheese and fish. This technique was called Reducing Atmosphere Packaging (RAP), which extended the shelf life of these food products. Different formulations of RAP were tested for preserving fresh cheese: [RAP 1 (90% CO_2/6% N_2/4% H_2), RAP 2 (50% CO_2/46% N_2/4% H_2)], modified atmosphere packaging (MAP) [MAP 1 (90% CO_2/10% N_2), MAP 2 (50% CO_2/50% N_2) during cold storage at + 4°C for 7 weeks. Results showed that the color and acidity of RAP1 cheese samples were the closest to the fresh sample. At the end of storage, the lowest mesophilic-aerobic bacteria counts were found for RAP1 samples, while the yeast-mold counts were the lowest for all RAP samples (Alwazeer et al., 2020).

In another product, rainbow trout fish was packaged under a hydrogen-included atmosphere (RAP) [RAP1 (50% CO_2 / 46% N_2 / 4% H2) and RAP2 (60% CO_2 / 36% N_2 / 4% H_2)] beside MAPs [MAP1 (50% CO2 / 50% N_2), MAP2 (60% CO_2 / 40% N_2)] and control (air). The fish samples were stored at 2°C for 15 days. RAP-packaged fish samples showed better results regarding TBARS and TVB-N (Bulut et al., 2023).

Rice milk

Our team revealed that when HRW was used for the preparation of rice milk from four varieties, the essential mineral content (Na, Mg, K, Ca, and Se), essential amino acids (Ile, Leu, and Met), desirable aroma, and sensory notes were improved (Zor et al., 2024).

Minced meat

Our team found that incorporating hydrogen-producing magnesium into minced beef meat allowed to control the microbial growth, restricted the formation of thiobarbituric acid reactive substances, restricted the formation of biogenic amines, limited the formation of the off-volatile compounds, and protected the whole quality of the product (Çelebi et al., 2024).

Butter

Recently, our team revealed that the incorporation of hydrogen gas into melted butter could improve microbial and chemical stability, enhance color (increasing yellowness), minimize the formation of biogenic amines (BAs), and produce no off-flavor or undesirable volatile compounds (Alwazeer et al., 2024). The results of the physicochemical parameters showed that the values of the butter samples incorporated with H_2 were similar to those of the control samples 30 days before. This technique was characterized by its application at normal conditions of temperature (40ºC) and pressure (1 atm) without adding any catalyst.

CONCLUSION

The integration of hydrogen in the food process can provide many advantages to the product. The preservation of the color, physicochemical properties, and microbial stability were reported in different hydrogen-processed food products. The use of hydrogen brings many advantages to both producers and consumers, such as its antioxidant properties, non-toxicity, natural notes, and low-cost equipment needs. The use of hydrogen needs, of course, precaution due to its explosion property with air.

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ORAL PRESENTATION

Bor Karbürün Yapısı, Özellikleri ve Biyomedikal Uygulamalarının Araştırılması

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Özet

Önemli yeraltı kaynaklarından olan bor madeni rezervinin yaklaşık %70'i ülkemizde bulunmaktadır. Doğal ve yarı işlem görmüş bor ürünlerinden 200 kadar bileşik üretilmektedir. Bu bileşiklerden amorf bor, boranlar, kristal bor, bor nitrür, bor hidrür, bor karbür en çok kullanılan ileri teknoloji bor bileşikleridir. Bor karbür (B_4C), bor ve karbon atomlarından oluşan, yüksek sertlik ve aşınma direncine sahip dünyadaki en kararlı bileşiklerden birisidir. Yüksek mukavemeti, yüksek erime ve kaynama noktası, düşük yoğunluğu vb. özellikleri sayesinde modern sanayi teknolojilerinde kritik bir malzeme olarak ön plana çıkmaktadır. B_4C düşük yoğunlukla uzay endüstrisi ve havacılık sektöründe, askeri alanlarda balistik zırhlarda, yüksek sertlik ve aşındırıcı özelliği ile metal işleme endüstrisinde zımpara ve taşlama makinelerinde kullanılmaktadır. B_4C yarı iletken özelliği sayesinde yüksek sıcaklık aralığında çalışan elektronik cihazlarda ve mükemmel nötron emebilme kapasitesi sebebiyle ise nükleer alanda kullanılır. B_4C antibakteriyel özellikleri ile endüstriyel boyaların bozulmasında fotokatalizör etkinliği sayesinde atık su arıtma sistemlerinde kullanılmaktadır. B_4C'ün biyomedikal alanlarda kullanımı sınırlı olsa da son yıllarda bu alandaki çalışmalar ivme kazanmıştır. B_4C'ün en heyecan verici uygulamalarından birisi kanser tedavisinde bor nötron yakalama terapisidir. Bor karbür nanopartikülleri biyosensörlerde biyolojik moleküllerin teşhisinde, ilaç salınımında ve hücrelerle etkileşim göstererek hedef hücre ve dokuların bölgesel tedavisinde kullanılmaktadır. Ayrıca, bor karbürün bildirilen biyouyumluluğu, hücre proliferasyonunu ve yara iyileşmesini teşvik etme kabiliyeti onun doku mühendisliğinde kullanım potansiyeline dikkat çekmektedir.

Anahtar kelime: Bor, Bor Karbür, Biyomedikal Uygulamalar

Investigation of Structure, Properties and Biomedical Applications of Boron Carbide

Abstract

Approximately 70% of boron reserves which is among the significant underground resources locates in our country. Nearly 200 compounds are produced from natural and semi-processed boron products. Of these compounds, amorphous boron, boranes, crystalline boron, boron nitride, boron hydride, boron carbide are the most widely used high-tech boron compounds. Boron carbide (B_4C) is a ceramic material consisting of boron and carbon atoms having high hardness and wear resistance is one of the most stable compounds in the World. It stands out as a crucial material in modern industrial technologies thanks to its unique properties, such as high strength, high melting and boiling points, low density. B_4C is used in the airspace industry and aviation sector with its low density, as ballistic armors in military fields, and in abrasive and polishing machines at the metal-processing industry due to its high hardness and abrasive features. B_4C is a ceramic material used in electronic devices operating in the high temperature range due to its semiconductor feature and in the nuclear field due to its neutron absorption capacity. Due to its efficiency as photocatalyst in the degradation of industrial dyes with antibacterial properties, B_4C is used in wastewater treatment systems. Although the use of B_4C in biomedical field is limited, the research studies in this area has gained a momentum recently. One of the most exciting applications of B_4C in the biomedical field is boron neutron capture therapy in cancer treatment. Boron carbide nanoparticles is utilized in biosensors for diagnosis of biological molecules, in drug delivery systems and in the treatment of targeted cells and tissues by interacting with cells. In addition, due to

its reported biocompatibility and ability to promote cell proliferation and wound healing, boron carbide attracts attention as a potential material to be used in tissue engineering.

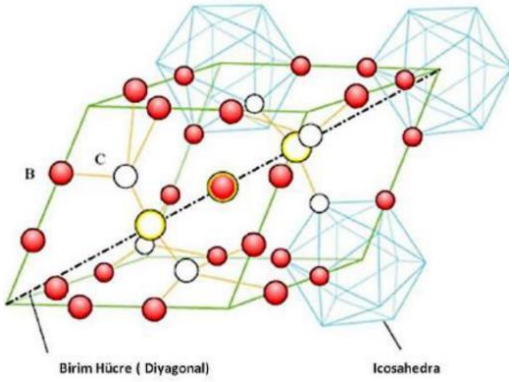
Keywords: Boron, Boron Carbide, Biomedical Applications.

1. GİRİŞ

Ülkemizin önemli yeraltı kaynaklarından bor mineralinin dünya rezervinden yaklaşık %72'si Türkiye'de bulunmaktadır. Borun tarihteki serüveni 1808 yılında H. Davy, J. L. Gay Lussac ve L. J. Thenard aracılığıyla merak edilip araştırmasıyla başlamıştır. Bu süreçte çeşitli bilim insanlarınca bor mineralini saflaştırma işlemine maruz bırakılarak %95-98 oranında saf bor elde edilebilmiştir. (Koç, 2024; Pekdemir, 2021). İnsanlar bor mineralinin çeşitli özelliklerini keşfetmeye başlamıştır. Birden çok üstün özelliğe sahip olan borun kullanım alanları genişleme sürecinde olup 400'den fazla modern sanayi ve endüstriyel uygulamalarda 200'den fazla türüyle varlığını devam ettirmektedir (Çarboğa, 2010). Bor ürünleri Türk Mühendis ve Mimar Odaları Birliği'nin yayınladığı rapora göre (2016), tabii ve rafine boratlar ve özel bor ürünleri olarak üç sınıfa ayrılmıştır. Tabii boratlar; tinkal kolemanit, üleksit gibi yalnızca saflaştırma ve sınıflandırmaya tabi tutulan bor cevherlerini içerirken rafine boratlar kimyasal işlemler sonucu elde edilen boraks, borik asit, sodyum perborat gibi bileşikleri içermektedir. Çoğunlukla borik asit veya bor oksitin temel öncü bileşenler olarak kullanıldığı, çeşitli kompleks prosesler ile üretilen ticari değeri yüksek özel bor bileşikleri bor karbür, bor nitrür, ferrobor, çinkoboratlar, fluoroboratlar, bor esterler, bor halojenürler vb. içermektedir (Türk Mühendis ve Mimar Odaları Birliği, 2016). Günümüzde ileri teknoloji uygulamalarında en yaygın kullanım alanına sahip olan bor bileşikleri ise amorf bor, kristal bor, bor oksit, bor karbür, hekzagonal bor nitrür ve kübik bor nitrürdür (Demirsar, 2007; Koçak, 2013).

2. Bor Karbürün Yapısı ve Özellikleri

Bor karbür parlak siyah renge ve elmas ile kübik-bor nitrürden sonraki en yüksek sertliğe sahip olan üçüncü malzeme olduğu için "kara elmas" şeklinde tanınmaktadır. Kimyasal formülü $B_{12}C_3$ olup sadeleştirilmesi ile B_4C halini almaktadır. Moleküler ağırlığı 55,26 g/mol'dür (Karabaş, 2006; Suri vd., 2010). Bor karbürün yapısı ve atomik düzenlemesi konusunda en kabul gören yapı rombohedral yapıdır ve köşelerinde ikosahedral olarak adlandırılan 12 atomlu (20 yüzlü) kristal yapılardan oluşur (Şekil 1). Bor karbür rombohedral kristal yapıya sahip olup bor-karbon bileşikleri arasında en kararlı olanıdır. Rombohedralin köşelerinde paylaşılan 3 atom ikosaheadral kümelere kovalent bağlar ile bağlanmıştır. Her bir karbon atomunun en az 4 bor atomuyla bağ yaptığı görülür (Khanra, 2007; Akarsu, 2009).



Şekil 1. Bor karbür rombohedral kristal yapısı (Cao, 2006).

Bor karbürün sertlik skalası 20-78 GPa arasında çeşitlilik göstermektir. Vickers sertliğinin genel kabul gören değeri 32 GPa bandındadır ve elastisite modülü 448 GPa olarak bildirilmiştir (Karabaş, 2006; Suri vd., 2010) . Yüksek sertliği sayesinde aşınma direnci de oldukça yüksek seviyededir. Malzemelerde sıcaklığın artması ile sertlikte düşüş gözlemlenir fakat bor karbürün sıcaklığı 300 K'den 1300 K'e çıktığında sertliğindeki düşüş oranı 1,4 katı bulurken aynı koşullarda bu oran bor nitrürlü seramikte 4'e ve tek kristal elmasta 2,5 kata kadar düşüş gösterir. Bu yönüyle ve elmas ve bor nitrüre göre daha ucuz olması tercih sebebi olmaktadır (Mohammad Sharifi vd., 2011). Bor karbürün stokiyometrik yoğunluğu 2,52 g/cm³ olarak hesaplanır. Mükemmel mukavemet değerlerine sahip olan bor karbürün gerilme mukavemeti 155 MPa, eğme mukavemeti 345 MPa ve basma mukavemeti 2,85 GPa değerindedir (Günay, 2017).

Bor karbür, yüksek termal genleşme, yüksek termodinamik stabilite, mükemmel termal iletkenlik ve nötron absorplama kapasitesi (600 barn) gibi seçkin özellikleri sayesinde modern sanayinin gözbebeği haline gelmiştir (Sokhansanj & Hadian, 2012; Suri vd., 2010). Bor karbür diğer karbür ve nitrürleri gibi erimeden direkt buharlaşma ile bozulma eğilimi göstermez. Bor karbür 2450 °C'de eriyerek sıvı faz oluşumu meydana getirmektedir (Karabaş, 2006). Kaynama noktası ise 3500 °C'den yüksektir. Kimyasal maddelere karşı üstün direnç gösteren bor karbür yalnızca HF, H_2SO_4, HNO_3 bileşikleri ile oluşturulan karışımlarda çok uzun sürelerde yavaş bir şekilde çözünebilmektedir. Bazı metal, metal hidrür ve oksitlerle borür meydana getirme olasılığı olup 3A ve 6A grubundan birkaç metalle, lantanitler ve aktinitler bor ve karbon elementi ile bor karbür fazlar oluşturur ama 4A ve 5A diborürleri bor karbürle tepkime göstermezler (Alp, 2015).

3. Bor Karbürün Üretim Yöntemleri

B_4C parçacıklarının sentezi için farklı yöntemler mevcuttur ve bunlar üretilen tozların karakteristik özellikleri ve maliyet açısından farklılıklara sebep olabilmektedir (Khanra, 2007). Araştırmacılar, bor karbür sentezinde bor karbürün yeterli kalitede olması, verimliliği ve çok yönlülüğünü göstermek için hala üretim yöntemleri incelemekte ve gelişimi için çaba gösterilmektedir (Suri et al., 2010).

3.1. Karbotermal İndirgeme Yöntemiyle Bor Karbür Üretimi

B_4C'ün keşfinden sonra saflaştırma işlemleri yapılmaya başlanmıştır ve ilk sentez çalışmasında saflık oranının %75'in altında kaldığı gözlemlenmiştir. 1933 yılında ise karbotermik indirgeme yöntemiyle %90 saflıkta kristal bor karbür üretilerek patenti alınmıştır (Haynes, 2016). B_4C parçacıkları sentez yöntemleri arasında araştırmalar kapsamında en yaygın olanı karbotermik indirgeme ve bor oksidin bir elektrik ark fırınında karbon ile reaksiyon vermesidir. Borik asidin karbotermik indirgeme işleminde borik asit ve bor trioksit bileşikleri karbon ile indirgenir. Belirli miktardaki borik asit ısılınca ortama su verip B_2O_3 bileşiği oluşmaktadır. B_2O_3 bileşiğinde karbon monoksit (CO) ile indirgenmesi 1400 °C'nin üzerindeki bir sıcaklıkta termodinamik bir tepkime ile gerçekleşir. Reaksiyonun hızını artırabilmek için fırının sıcaklığı 2000 °C civarına çıkarılabilmektedir. Bu sürecin gerçekleşmesi 16.800 kJ/mol enerji gerektiren endotermik bir tepkimedir (Suri vd., 2010). Tepkime sonunda elde edilen külçe haldeki bor karbür çok sert bir malzeme olduğundan dolayı öğütme işlemi sıcaklıkla gerçekleştirilmekte olup üretim maliyetini artırmaktadır. Endüstriyel ark ocaklarında malzemeye verilen sıcaklık 2200 °C civarındayken, sıcaklık eşit dağılmayıp malzemenin merkezinde 1200 °C'ye kadar düşebilmektedir. Bu sıcaklık farkı tepkimeye girmeyen reaktanları oluşturmakta ve malzemede safsızlıklar meydana gelebilmektedir. Külçe halindeki bor karbür öğütüldüğünde içerisinde hatırı sayılır safsızlıklar bulundurmaktadır. Ticari olarak üretilen bor karbür hem yeterince saf olmamakta hem de maliyeti sebebiyle alternatif üretim yöntemlerine başvurulmaktadır (Thévenot, 1990).

3.2. Kimyasal Buhar Biriktirme Yöntemi ile Bor Karbür Sentezi

Kimyasal buhar biriktirme yönteminde B_4C sentez işlemi; ince film kaplama gerektiren uygulamalar için gerçekleştirilir. Bu yöntemde kaplama oluşturacak başlangıç maddelerinin vakum ortamında termal, plazma ve ya lazer indükleme bibi yollarla gaz haline getirilmesi sonrası bir altlık malzemenin üzerine yönlendirilmesi ile kaplama gerçekleştirilmektedir (Karaman, 2007). Amorf-nanokristalin bor karbür yapılar, orto-karboran öncü bileşiği kullanılarak yüksek erime noktası ve nötron emilim kabiliyeti ile nükleer yakıt depolama uygulamaları için CVD tekniği ile ince film kaplamalar olarak üretilmektedir (Tu vd., 2020). Bu yöntemin dezavantajları korozif, toksik veya neme karşı hassas kimyasallar kullanıldığı için kapalı ortama ihtiyaç duyulur. Yüksek oranda madde sarfiyatı ve 300°C'nin altında reaksiyon sınırlı olmasıdır (Karaçay, 2008).

3.3. Sol-Jel Yöntemi ile Bor Karbür Sentezi

Sol-jel yöntemi ile bor karbür karbotermal indirme yöntemine göre daha düşük sıcaklıklarda sentezlenmektedir. Sol-jel yönteminin maliyeti nispeten daha düşüktür ve metal alkoksit veya inorganik öncüler kullanarak homojen saf ürün edilmesini sağlamaktadır (Najafi vd., 2018). Sol-jel yöntemiyle üretim yaparken bor elde etmek için borik asit, karbon elde etmek için ise PVA (Polivinil alkol) ve sitrik asit kullanılabilmektedir. Belirlenen oranlarda PVA ve borik asit çözdürülerek oluşturulan çözeltideki jel fazının kurutulması ile elde dilen tozun 750 °C'de piroliz işlemi sonucu bor karbür partiküllerinin sentezi bildirilmektedir (Karaçay, 2012). Tri-metil borat ve fenolik reçinenin bor kaynağı olarak kullanıldığı 60 °C'de gerçekleştirilen sol-jel reaksiyonu ile jel oluşumu, yaşlandırma ve kurutma sonrası 1270 °C'de sinterleme sonucu boyutu 30 nm'den düşük ve 154 m^2/g yüzey alnına sahip olan nano-boyutlu bor karbür üretimi bildirilmektedir (Najafi vd., 2018).

Diğer bor karbür sentez yöntemleri arasında; magnezotermik indirgeme, elementlerden sentez, buhar sentezi, polimerlerden sentez, sıvı faz reaksiyonu ve iyon ışın sentezi yöntemleri de literatürde mevcuttur (Suri vd., 2010).

4. Bor Karbürün Kullanım Alanları

B_4C yüksek stabiliteye sahip ve düşük maliyetli bir malzeme olduğu için pek çok uygulama için kullanım potansiyeli mevcuttur. Bor karbürün kullanım alanları içerisinde barındırdığı özelliklere göre şekillenmektedir. Yüksek sertlik, aşınma direnci ve düşük özgül ağırlığının bir araya gelmesiyle aşındırıcı malzeme olarak metal işleme teknolojilerinde, parlatma, taşlama ve kesme işlemlerinde, bilyalı değirmenlerde, tekerlek düzeltme aletleri, tel çekme kalıpları gibi pek çok alanda ön plana çıkmaktadır (Alizadeh vd., 2004; Tellioğlu, 2007). Sertliğinden faydalanarak kumlama ve su jeti başlıklarında sıklıkla tercih edilmektedir. Uzay ve havacılık sanayisinde (Bilgiç vd., 2024); uçak, helikopter, uzay mekiklerinde vb. savunma sanayisinde; tank, zırhlı araçlar için vazgeçilemez bir seramik malzemedir. Zırhlı araçlar ve helikopterlerde % 20 oranlarında hafiflik sağlaması daha kolay hareket ve fonksiyonel manevra yeteneği sağlamaktadır. Yüksek nötron yakalama kapasitesi sayesinde ise nükleer çalışmalarda koruyucu katman ve reaktör kontrol çubuğu olarak kullanılmaktadır (Skorokhod, 1998; Tu vd., 2020). Bor karbürün termoelektriksel özelliği 600-2200 °C aralığında ölçüm yapabilen yüksek sıcaklık termoçiftlerinde tercih edilmektedir (Skorokhod, 1998), ve yüksek sıcaklık yarı-iletken teknolojisinde kullanım potansiyeli yüksektir (Domnich vd., 2011). Sıcaklık değerlerin çok yüksek olduğu durumlarda kimyasal kararlığı sayesinde korozyon ve oksitlenme direnci gerektirecek çeşitli ekipmanlarda kullanılır. Bunlara ek olarak bor karbürün seramik matrisli kompozit malzemeler için takviye malzemesi olarak kullanımı söz konusudur (Sinha vd., 2002). Üstelik bor karbür toksit olmayışı ve biyouyumluluğuyla atık su arıtımında endüstriyel boya atıklarının bozunması için fotokatalizör olarak kullanılmaktadır (Singh vd., 2018).

5.1. Biyomedikal Uygulamalarda Bor Karbür

Bor karbürün biyomedikal alanlarda kullanımı sınırlı olmasına rağmen son yıllarda bu alandaki araştırmalar hız kazanmıştır. Bor karbürün biyouyumluluk çalışmaları incelendiğinde bor karbürlerin biyolojik özellikleri parçacıkların yüzey kimyasal bileşimine göre değişebildiği belirtilmiştir (Suri vd., 2010). Gerçekleştirilen *in-vitro* testlerde sentezlenen bor karbür tozunda, hücrelerde yüksek canlılık ve düşük sitotoksisite özellikleri tespit edilmiştir. Bunun yanı sıra, bor karbürün hidrofilik özelliği sayesinde proteinlerin, aktif kolajen peptitlerin, makrofaj ve fibroblast hücrelerinin bor karbür tozlarının yüzeyine kolayca tutunduğu görülmüştür (Werheit, 2016; Thevenot, 1990).

Bor karbür özellikle, yüksek nötron emilim kabiliyeti sayesinde tıbbi yönden kanser hastalığının tedavi yöntemlerinden birisi olan bor nötron yakalama terapisinde (BNCT) kullanılmaktadır. Boron nötron yakalama terapisi, kötü huylu ve hızlı yayılan tümörlerin tedavisinde yüksek bir etkiye sahiptir (Seoane & De Mattos-Arruda, 2014). Bu terapi yöntemi, ^{10}B izotipi ile etkileşimde bulunarak alfa parçacıkları ve geri yayılan Li-7 çekirdekleri üretilmesini gerçekleştirir (Barth, 2009). Bu parçacıkların hastalıksız hücrelere zarar vermeden kanserli hücrelerin nötronlarıyla etkileşime girerek yüksek enerjili parçacıklar oluşturması sonucu onların yok edilmesini sağlar. BNCT potansiyeli yüksek bir yöntem olmasına rağmen bor bazlı ajanların eksikliğinden doğan sıkıntılar mevcuttur. Bu sıkıntıların giderilmesi için nanoyapıdaki bor karbür BNCT için kullanım potansiyeli bulunan bir bileşen olarak araştırılmıştır (Hatanaka, 1994). BNCT teknolojisinde diğer bir çalışmada ise bor karbür nanopartiküller bağışıklık sistemi hücrelerinden olan makrofaj hücreleri içerisine eklenerek kanserli hücrelerin kontrollü olarak yok edilmesi sağlanmıştır (Wróblewska vd., 2024).

Bor karbürün diğer bir uygulama alanı ise ilaç salınım sisteminde karşımıza çıkmaktadır. Nanomalzemelerin, kanser hastalığında kullanılan ilaçların hastalıklı hücrelere gönderilmesinin olumsuz etkilerini azaltabileceği görülmüştür (Najafi & Zare, 2016). Yapılan bir çalışmada, saf NC_3 karbon benzeri bor karbür içerikli nanotüp (NC_3NT) nanomalzemesinin kanser tedavisinde kullanılan ilaç olan Florourasil (5FU) için ilaç taşıma kapasitesi araştırılmıştır (Kesharwani vd., 2012). Florourasil'in, NC_3NT yüzeyiyle etkileşimi hastalıklı hücrelerin asidik ortamında zayıfladığından, bu nano-taşıyıcı kullanıldığında 5FU'nun hedef kanser hücrelerinin etrafında salınımı amaçlanmıştır (Cao vd., 2022) ve elde edilen sonuçlar NC_3NT'nin 5FU taşıması için umut verici bir taşıyıcı potansiyelinin olduğunu göstermektedir. Diğer bir çalışmada, sol-jel yöntemi ile üretilen silika ile modifiye edilmiş bor karbür partiküllerinin farklı pH değerlerinde ilaç (denopezil) salınım kinetiği araştırılmıştır (Ozcan vd., 2023). Bir diğer çalışmada ise kanser tedavisi sürecinde kemoterapi evresinde kullanılan bir ilaç olan Melfalan (Mel) için hedefli ilaç salınımı bilgisayar destekli modelleme ile araştırılmıştır. Mel, DNA ve RNA üretimi inhibe edebilen ajanlardan biridir. Fakat kullanımı sonrası saç

dökülmeleri, mide bulantısı, enfeksiyon riskini artış ve yorgunluk gibi yan etkileri mevcuttur. Burada Mel ajanının yan etkilerini en aza indirip etkinliğini artırmak için nanomalzemeler ilaç taşıyıcı olarak kullanılmıştır. BC_3 nanoyapıları toksik olmaması, termal iletkenlik, yüksek oksidasyon direnci gibi üstün özelliklerinden dolayı tercih sebebi olmuştur. Çalışmalar sonucunda BC_3NT nanoparçacıkları Mel için ilaç taşıma aracı olarak başarılı sonuçlar sağlamıştır (Abolhasani Zadeh vd., 2022).

Bor karbür aynı zamanda hafifliği, yüksek sertliği, aşınma direnci, iyi bir kimyasal ve çevre kararlığı özellikleriyle biyosensör olarak Epinefrin (EP) tespitinde kitosan içine gömülü lakkaz enziminin tespiti için nanopartikül olarak kullanılmıştır. Grafitlenmiş karbon ile (GC) kaplanan B_4C (GC-B_4C) partiküller lakkazdan (Lac) nikel köpük (NF) elektroduna aktarılan elektronları aktarma aracı olarak kullanılmıştır. Üretilen Lac/GC-B_4C/NF elektrodu epinefrinin amperometrik tespitinde kullanılmıştır. Diğer nanomalzemelere göre kararlılığı ve iletkenliği ile Lac/GC-B_4C/NF elektrodu mükemmel performans göstererek sürecin ilerleyişini kolaylaştırdığı gözlemlenmiştir (Tang vd., 2024). Diğer bir biyosensör uygulamasında nefes alıp verme sırasında ortama yayılan uçucu organik moleküllerin tespiti aracılığıyla Coronavirus, diyabet, tüberküloz gibi hastalıkların teşhisinde kullanılabilecek biyosensörler araştırılmıştır. Bu kapsamda tek tabakalı grafit gibi yarı-iletken bor karbür yapılarının gaz sensörü olarak kullanımı araştırılmıştır ve soluk alıp-verme sırasında gaz adsorpsiyonunun öncesi ve sonrasındaki iletkenlikteki değişim ile biyoişaretçi olarak tanımlanan moleküllerin tespitinde önemli bir potansiyele sahip olduğu bildirilmiştir (Nosheen vd., 2022).

Bor karbürün doku mühendisliği alanında uygulamaları sınırlı olmasına rağmen güncel araştırma konuları arasındadır. Bu kapsamda, son zamanlarda gerçekleştirilen bir yara iyileşmesi araştırmasında lipoik asit ile konjuge edilen bor karbür ve bor nitrür nano-partiküllerin üstün antibakteriyel özellikler yanı sıra yara iyileşmesini tetikleyici performans sergilediği gözlenmiştir (Türkez vd., 2023).

6. Sonuçlar

İleri teknoloji bor bileşiği olan bor karbür yüksek sertlik ve kimyasal kararlılık, yüksek aşınma direnci, mükemmel mukavemet, nötron emilim kapasitesi, antimikrobiyal ve fotokatalizör etkinlik gibi üstün özellikleri sayesinde modern sanayi ve teknolojik ürünlerin araştırma ve geliştirme faaliyetlerinde göz bebeği olmuştur. Bor karbürün bu üstün karakteristik özellikleri onun son yıllarda henüz keşfedilmemiş yeni uygulama alanlarının ortaya çıkmasına yol açmaktadır. Bu kapsamda bor karbürün doku mühendisliği ve yenileyici tıp alanında tedavi ve teşhis yöntemlerini içeren çeşitli biyomedikal uygulamalarda yeni araştırmalara kapı aralayacağı düşünülmektedir. Bunlar arasında öncü uygulamalardan birisi bor karbür nanopartiküllerin yüksek nötron absorplama kapasitesiyle kanserli hücrelerin yok edilmesini hedefleyen bor nötron yakalama terapisi (BNCT) kapsamında kullanımıdır. Literatürde son yıllarda gerçekleştirilen araştırmalarda bor karbürün bildirilen biyouyumluluğu, hücre proliferasyonunu ve hücre yapışmasını teşvik etme kabiliyeti gibi özellikleri, yeni biyomalzeme araştırmaları için umut verici niteliktedir. Ayrıca, bor karbür nanopartiküllerin ilaç taşıcıyı olarak bölgesel, etkin tedavi için kullanımı ve bor karbür içeren biyosensörlerin diyabet ve nefes alıp verme kontrolünü sağlayıcı teşhis aracı olarak kullanımı bildirilmektedir. Sonuç olarak, bor karbürün nanopartikül halinde veya çeşitli kompozit formlarında biyomedikal uygulamalar için yenilikçi potansiyel bir malzeme olacağı öngörülmektedir.

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ORAL PRESENTATION

Acı badem (*Amygdalus communis* L.) çekirdeği etanolik liyofilize ekstresinin fitokimyasal içerik analizi ve biyolojik etkileri

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Özet

Bu çalışmada acı badem (*Amygdalus communis* L.) çekirdeği etanolik liyofilize ekstresinin fitokimyasal içeriğinin araştırılması amaçlandı. Toplanan kabuklu bademler kırılarak içleri çıkarıldı, kurutuldu ve blender ile toz haline getirildi. Ekstre elde etmek için 1:25 ağırlık/hacim (a/h) oranında %80 etil alkol kullanıldı. Watman kağıdı ile süzme işlemi tamamlandı, etanol evaporatör ile uzaklaştırıldıktan sonra örnekler -20°C dondurulduu. Dondurulan örneklerin suyu liyofilizatör cihazıyla uçurularak liyofilize ekstre elde edildi. Ekstrede toplam fenolik ve toplam flavonoit kapasiteleri ve LC-MS/MS ile kantitatif içerik analizi yapıldı. Elde edilen sonuçlara göre, ekstre verimi (% 5.28), toplam fenolik madde miktarı 19.77±0.0 mg/g gallik asit eş değeri ve toplam flavonoit madde miktarı 7.72±0.02 mg/g kertsin eş değeri olarak bulundu. LC-MS/MS ile analiz edilen etanolik ekstrede kinik asit, protokateşik asit ve protokateşik aldehit tespit edildi. *Amygdalus communis* etanol ekstraktında tespit edilen fenolik bileşiklerin çeşitli hastalıkların ilerlemesini yavaşlatma veya önlemede faydalı olabilecek alternatif antioksidanların kaynağı olabileceğini göstermektedir.

Anahtar Kelimeler: *Amygdalus communis*, ekstre, LC-MS/MS, toplam fenolik, toplam flavonoid

Phytochemical content analysis and biological effects of bitter almond (*Amygdalus communis* L.) seed ethanolic lyophilized extract

Abstract

This study was aimed to investigate the phytochemical composition of the bitter almond (*Amygdalus communis* L.) seed ethanolic lyophilized extract. The collected almonds with shell were broken, their insides were gained, dried, and then blended to get a powder. The extract was prepared using 80% ethanol at a weight/volume (w/v) ratio of 1:25. The filtration process was completed with a whatman paper, the ethanol was removed by using an evaporator and the samples were frozen at -20°C. The water of the frozen samples was evaporated with a lyophilizer device to obtain lyophilized extract. Total phenolic and total flavonoid capaticities were determined and quantitative content analysis of the extract was performed using LC-MS/MS in the extract. According to the obtained results, the extract yield was 5.28%, total phenolic content was determined by mg/g gallic acid equivalent (GAE) as 19.77±0.01 and total flavonoid content was determined by mg/g quercetin equivalent (QE) as 7.72±0.02. Quinic acid, protocatechuic acid and protocatechuic aldehyde were detected in the ethanolic extract analyzed by LC-MS/MS. The phenolic chemicals found in the ethanol extract of *Amygdalus communis* may provide an alternate supply of antioxidants that can be helpful in delaying or preventing the progression of several diseases.

Keywords: *Amygdalus communis*, extract, LC-MS/MS, total phenolic, total flavonoid content

GİRİŞ

Anavatanı Orta ve Batı Asya olan bademin (*Amygdalus communis* L.) bugün dünyanın dört bir yanında kültür ve yabani formları bulunmaktadır. Türkiye bademin gen merkezlerinden biri olup, kültürü yaklaşık 4000 yıl öncesine dayanmaktadır. Bademler makro/mikro besin ve fitokimyasallar açısından zengin kaynaklardır. Bademin tatlı ve acı olmak üzere iki formu bulunmaktadır. Badem, lipid, protein, karbonhidrat ve vitamin açısından zengin bir besindir. Ayrıca, badem E vitamini, bakır, magnezyum, yüksek kaliteli protein ve polifenoller açısında da zengindir (Javaid ve ark., 2019). Badem içeriğinde bulunan bileşenler antioksidan,

stres önleyici, hepatoprotektif, antideprasan, immün sistemi uyarıcı, lipid peroksidasyonunu düşürücü, kalp koruyucu, hafızayı güçlendirici ve yaşlanma karşıtı gibi çeşitli farmakolojik özelliklere sahip olduğu belirlenmiştir (Mushtaq ve ark., 2015; Özcan, 2023). Acı badem (*Amygdalus communis* L.) esansiyel yağları yaraları, hemoroidleri ve saç dökülmesini iyileştirmek, eklem ağrılarını hafifletmek, doğumu kolaylaştırmak ve saçları güçlendirmek için yüzyıllardır kullanılmaktadır (Moradi ve ark., 2017). Bu çalışmada, Acı badem (*Amygdalus communis* L.) çekirdeğinin etanolik liyofilize ekstresinin LC-MS/MS ile fitokimyasal içerik analizi, total fenolik ve flavonoid ve biyolojik etkileri araştırıldı.

MATERYAL VE METOT

Çalışmada kullanılan acı bademler (*Amygdalus communis* L.) Ağustos ayı 2022 yılında Mardin ilinin Dargeçit ilçesi yöresinden Doç. Dr. Abdulahad DOĞAN tarafından toplandı. Ekstre hazırlanması Dogan ve ark. (2018) göre yapıldı. Özetle, toplanan bademler gölgelik ortamda kurutulmaları sağlandıktan sonra bademler kırılarak içleri alındı, blender yardımı ile toz haline getirilen badem içleri 40 g örneği 1 L % 80'lik etanol içinde 2 saat süreyle çalkalayıcıda karıştırıldı. Kaba kurutma kağıdından süzüldü. Süzülen örnekler enjektör yardımıyla 0.45 µm'lik hidrofilik filtreden geçirildi. +37 °C'de evaporatör yardımıyla çözücüden arındırılan yoğunlaştırılmış fraksiyonlar -85 °C sıcaklık ve 50 millitor basınç altında liyofilizatör cihazında kuruyana kadar bekletildi. Elde edile liyofilize ekstrelerin verim%'si hesaplandı ve ilgili deneylerde kullanıldı.
Toplam fenolik miktar tayini Singlet ve Rossi (1965) metoduna göre yapıldı. Toplam flavonoid miktar tayini Woisky ve Salatino (1998) metoduna göre yapıldı. Acı badem çekirdeğinden hazırlanan % 80'lik etanol ekstresinin fitokimyasal içerik tanımlanması ultra yüksek performanslı sıvı kromatografi (UHPLC) cihazı ile daha önce validasyonu yapılan 53 fitokimyasalın taranması esasına göre yapıldı (Yılmaz, 2020). Bu 53 bileşiğin 3 tanesi bitki bileşiminde bulunmayan ticari olarak satın alınan saf madde iç standartlardır (IS). Bunlar sırasıyla, ferulik asit D1 (20 mg/L), rutin D2 (1 mg/L) ve kersetin D3 (5 mg/L) dir.

BULGULAR ve TARTIŞMA

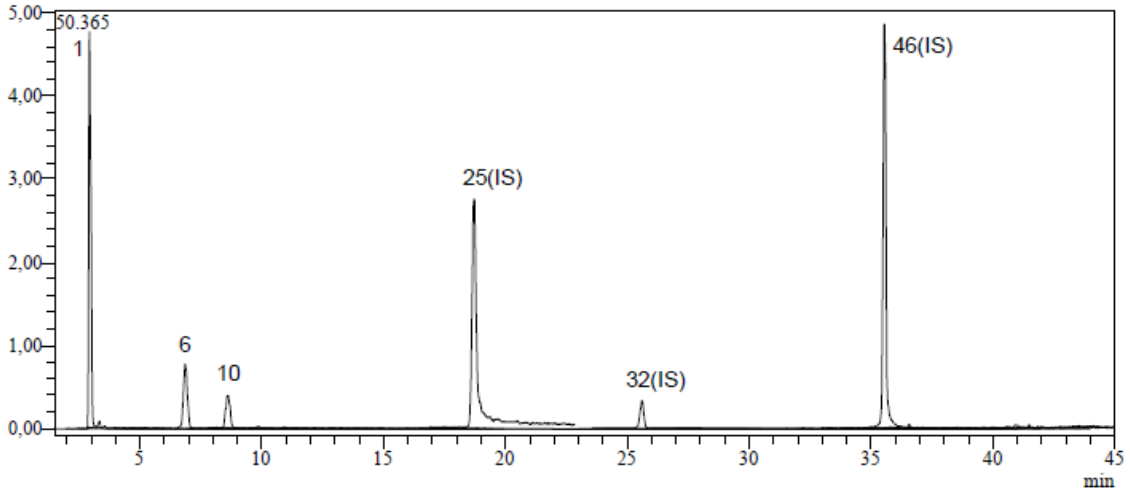
Tablo 1'de acı badem (*Amygdalus communis* L.) içi etanolik liyofilize ekstresinin verim yüzdesi, total fenolik ve flavonid miktarları verilmiştir. Solvent olarak kullanılan % 80 etanol ekstraksiyon işleminde ekstre verimi % 5.28 olarak belirlendi. Bitki ekstresindeki total fenolik madde tayini gallik asite eş değer (GAE) olarak mg fenolik madde/g ekstre 19.77 ± 0.01 olarak hesaplandı. Benzer şekilde total flavonoid madde tayini için kersetin eş değer olarak mg flavonoit madde/g ekstre 7.72 ± 0.02 olarak hesaplandı.

Tablo 1. Acı badem (*Amygdalus communis* L.) içi etanolik ekstresinin verim yüzdesi, total fenolik ve flavonoid içerikleri

Bitki	Çözücü	Ekstre verimi (%)	Toplam Fenolik (mg Gallik asit /g ekstre)	Toplam Flavonoid (mg Kersitin/g ekstre)
Amygdalus communis L.	% 80 etanol	% 5.28	19.77 ± 0.01	7.72 ± 0.02

Tablodaki her değer ortalama ± standart hata (n=3) olarak sunulmuştur.

Yapılan bir çalışmaya göre, acı badem (*Prunus amygdalus* var. amara) içi toplam fenolik ve flavonoid içerikleri sırasıyla 21.94 ± 0.29 ve 21.52 ± 0.14 µg/mL olarak bulunmuştur (Guici El Kouacheur ve ark., 2023). Başka bir çalışmada, badem çekirdeği ekstresinde toplam fenolik ve flavonoid içeriklerini sırasıyla 674.40±1.20 ve 22.98±0.95 mg /g olarak belirlenmiştir (Kernel, 2014). Yapılan çalışmalarda tespit edilen total fenolik içeriğinin bizim bulgularımıza yakın oldukları ancak total flavonoid içerik miktarının ise biraz faklılık gösterdiği belirlendi. Bu farklılık muhtemelen bitki türünün farklı oluşu, toplanma yeri/zamanı ve ekstraksiyon yöntemlerinin farklılığı etkili olmuş olabilir.



Şekil 1. Acı badem etanolik ekstresinin LC–MS/MS kromatogramı

LC-MS/MS analizleri yöntem validasyonu yapılmış ve çoğunluğunu fenolik ve falavonoidlerin oluşturduğu 53 fitokimyasal bileşik ve 3 tane iç standarda (IS) göre yapıldı. **1: Kinik asit**. 2: Fumarik asit. 3: Akonitik asit.4: Gallik asit. 5: Epigallokateşin. **6: Protokateşik asit**. 7: Kateşin. 8: Gentisik asit. 9: Klorojenik asit. **10: Protokateşik aldehit.** 11: Tanik asit. 12: Epigallokateşin galat. 13: 1,5-dikaffeoilkinik asit. 14: 4-OH Benzoik asit. 15: Epikateşin. 16: Vanilik asit. 17: Kafeik asit. 18: Siringik asit. 19: Vanilin. 20: Siringik aldehit. 21: Daidzin. 22: Epikateşin galat. 23: Piceid. 24: p-Kumarik asit. 25: Ferulik asit-D3-IS. 26: Ferulik asit. 27: Sinapik asit. 28: Kumarin. 29: Salisilik asit. 30: Sinarosid. 31: Miquelianin. 32: Rutin-D3-IS. 33: Rutin. 34: İzokersitrin. 35: Hesperidin. 36: o-Kumarik asit. 37: Genistin. 38: Rosmarinik asit. 39: Ellagik asit. 40: Kozmosiin. 41: Kuersitrin. 42: Astragalin. 43: Nikotiflorin. 44: Fisetin. 45: Daidzein. 46: Quercetin-D3-IS. 47: Quercetin. 48: Naringenin. 49: Hesperetin. 50: Luteolin. 51: Genistein. 52: Kaempferol. 53: Apigenin. 54: Amentoflavon. 55: Chrysin. 56: Acacetin. IS: İç standart.

Elde ettiğimiz LC-MS/MS sonuçlarına göre; Amygdalus communis etanolik liyofilize ekstresinde kolonda geliş zamanına (RT) göre kinik asit (5.391 µg/g), protokateşik asit (0.264 µg/g) ve protokateşik aldehit (0.082 µg/g) kayda değer miktarda bulundu (Şekil 1).

Daha önce badem ekstresinde yapılmış LC-MS/MS içerik analizinde protokateşik asit miktarı 0.32–1.20 mg/g aralığında olduğu rapor edilmiştir (Özcan, 2023). Benzer bir bir tür olan şeftali çekirdeğinde de kinik asit, protokateşik asit ve protokateşik aldehit varlığı belirlenmiştir (Koprivica ve ark., 2018). Kinik asit, protokateşik asit ve protokateşik aldehit katekol türevleridir ve antiradikal aktiviteleri ile ilişkili geniş terapötik etkilere sahip olduğu rapor edilmiştir (Aree, 2022; Benali ve ark., 2022; Shahidi ve ark., 2019).

SONUÇ

Bu çalışmada acı badem (*Amygdalus communis* L.) etanolik liyofilize esktresinin total fenolik içeriği bakımından zengin olduğu ve badem yapısında bulunan kinik asidin, protokateşik asit ve protokateşik aldehit içerikleri nedeniyle doğal bir antioksidan kaynağı olabileceğini gösterdi. İleriki çalışmalarda bu bitkinin farmasötik uygulamaları için güvenliğini doğrulamak amacıyla preklinik, toksikolojik ve ileri moleküler çalışmalar yapılmasında fayda vardır.

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ORAL PRESENTATION

Comparison of the Cytotoxicities of Some Mono-Functional Half-Sandwich-Ru(II)-Para-Cymene Complexes Against YKG-1 and HUVEC Cells

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Abstract

Glioblastoma, which accounts for approximately 47% of central nervous system cancers, is a highly lethal brain tumor with limited chemotherapy options. The clinical use of platinum-based drugs (such as cisplatin, oxaliplatin, and carboplatin) in cancer treatment is limited due to the fact that many tumor types develop resistance to these drugs and the agents cause severe side effects. Ruthenium [Ru (II)] complexes, on the other hand, present a strong alternative to platinum-based chemotherapy agents thanks to their low side effects and versatile biological activity. In our study, we synthesized 12 different half-sandwich (HS) mono-functional Ru(II)-para-cymene complexes containing biguanide/biguanide-sulfonamide/guanidyl urea as secondary ligands. These synthesized compounds were tested in vitro on a glioblastoma cancer cell line (YKG-1) and a healthy human cell population (HUVEC), and the IC_{50} values of the substances were calculated to range between 139 and 777 µg/mL. In addition, when the cytotoxicity values obtained for each complex in two cell lines were compared with the Two-Way ANOVA test, it was concluded that 11 out of 12 different complexes exhibited higher cytotoxicity on YKG-1 than they did on HUVEC ($p<0.05$). In the light of these findings, we concluded that of the 12 complexes studied, 11 might have the potential kill tumor cells without harming the healthy cell population when used in appropriate doses, suggesting that they could be potentially useful as agents in cancer treatment.

Keywords: Glioblastoma, biguanide, biguanide-sulfonamide, guanyl, MTS

Acknowledgement: This work was supported by TUBITAK with the Project Number of 122 Z 455.

Introduction

Cancer is defined as the uncontrolled, continuous proliferation of healthy body cells and their spread to other parts of the body (Brown et al., 2023). According to a study published in 2024, it was reported that there were 9.7 million cancer-related deaths and an estimated 20 million new cancer patients in 185 different countries (Bray et al., 2024).

Glioblastoma (GBM) is a type of cancer that originates from glial cells in the brain and is the most common and deadly brain cancer among adults. Despite radiotherapy, surgical intervention and chemotherapy treatments, the survival rate in glioblastoma is quite low (Omuro et al., 2013). In addition, almost all tumor types recur, and the majority of recurrent tumors develop resistance to chemotherapy (Stupp et al., 2005). The number of chemotherapy drugs developed for the treatment of brain tumors has been very limited (Liu et al., 2023). The most important reason for this is the difficulty of the evaluated molecules in passing the blood-brain barrier (Wang and Yakisich, 2014). For this reason, the need for new treatment methods and effective new drugs in the treatment of brain tumors continue (Alkis et al., 2023).

Nowadays, the use of metal complexes in treatments has become promising in cancer treatment. In particular, the success of platinum-linked drugs used in various types of cancer has increased the interest in metal complexes (Ndagi et al., 2017). The potential success of these complexes largely depend on the the fact that metal ions may have a wide range of oxidation states and large coordination numbers. This feature provides an almost unlimited structural and chemical space for chemical arrangements. In addition, the ligand structures of the metal ions can also vary. This makes it possible to direct the physicochemical properties of the complexes and contributes to the pharmacological activity of the metal complexes (Wang et al., 2018).

Although platinum-linked cancer drugs (e.g. cisplatin, carboplatin, and oxaliplatin) are widely used in cancer treatment, these drugs cause resistance in many tumor types and their clinical use is limited due to various side effects (such as nephrotoxicity and neurotoxicity) (Giorgi et al., 2022). However, ruthenium complexes have the potential to be an alternative to platinum-linked chemotherapy agents thanks to their wide range of biological activities with lower side effects. In addition, ruthenium complexes can effectively bind to the serum transferrin receptor, thus provide a higher rate of transport to the tumor site through some receptor-mediated mechanisms (Liu et al., 2006). Besides, ruthenium complexes have been shown to penetrate the blood-brain barrier (Lu et al., 2022)

Many studies have shown that ruthenium-containing compounds exhibit inhibitory effects such as reducing proliferation, cell migration and invasion, as well as inducing apoptosis in various cancer types (Popolin et al., 2017; Bugarcic et al., 2008; Kalaivani et al., 2013). In one of the recent studies, for instance, Kumar and colleagues demonstrated that ruthenium complexes reduced the colony formation ability of brain tumor cells (LN-229) by 75% (Kumar et al., 2020).

In the present study, we aimed to reveal the cytotoxic effects of 12 different metal complexes synthesized in the Inorganic Chemistry laboratory of Kahramanmaraş Sutcu Imam University, , on both healthy (HUVEC) and cancerous (YKG-1) cells and thus investigate their anti-cancer potentials.

MATERIALS and METHODS

Cell Culture

Glioblastoma (YKG-1) cells and healthy human umbilical cord endothelial cells (HUVEC) purchased from American Type Cell Culture (ATCC) were cultured in DMEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin-streptomycin in an incubator at 37°C and 5% CO_2 pressure.

Preparation of Synthesized Compounds

The compounds synthesized in solid form were dissolved in suitable solvents (Table 1.1). Stock solutions were prepared, and working concentrations were prepared by diluting them to the specified concentrations before application to the cells.

Table 1: Concentrations (µg/mL) and Solvents of Compounds Applied to Cells

Synthesized Compounds	Concentration (µg/mL)	Solvent
Complex 1	0,200,400,600,800,1000	DMSO
Complex 2	0,200,400,600,800,1000	DMSO
Complex 3	0,100,200,300,400,500	DMSO
Complex 4	0,200,400,600,800,1000	DMSO
Complex 5	0,50,100,150,200,250	DMSO
Complex 6	0,200,400,600,800,1000	DMSO
Complex 7	0,200,400,600,800,1000	DMSO
Complex 8	0,200,400,600,800,1000	DMSO
Complex 9	0,200,400,600,800,1000	DMSO
Complex 10	0,100,200,300,400,500	DMSO
Complex 11	0,200,400,600,800,1000	DMSO
Complex 12	0,200,400,600,800,1000	DMSO

Determination of cytotoxicity and IC_{50} value

The IC_{50} values of the synthesized metal complexes (Table 1) on HUVEC and YKG-1 cells were determined using the MTS (Methylthiazol Tetrazolium Assay) kit (Promega, USA). IC_{50} values were calculated by making some modifications to the protocol described by Cömertpay et al. (2022). Briefly, HUVEC and YKG-1 cells were seeded separately in 96-well plates with 5000 cells per well. The plates were incubated overnight to allow the cells to adhere to the surface, and the next day, both cell lines were treated with medium containing different concentrations of complexes (Table 1). After 24 hours of treatment, 10 µL of MTS was applied to each well and the plates were incubated again for 4 hours. At the end of 4 hours, absorbances were measured at 490 nm via a spectrophotometer (Spectramax; BMG Labtech., Offenburg, Germany). The viability percentages of the cells were determined, these values were visualized as an XY graph with GraphPad Prism software, and IC_{50} values were calculated.

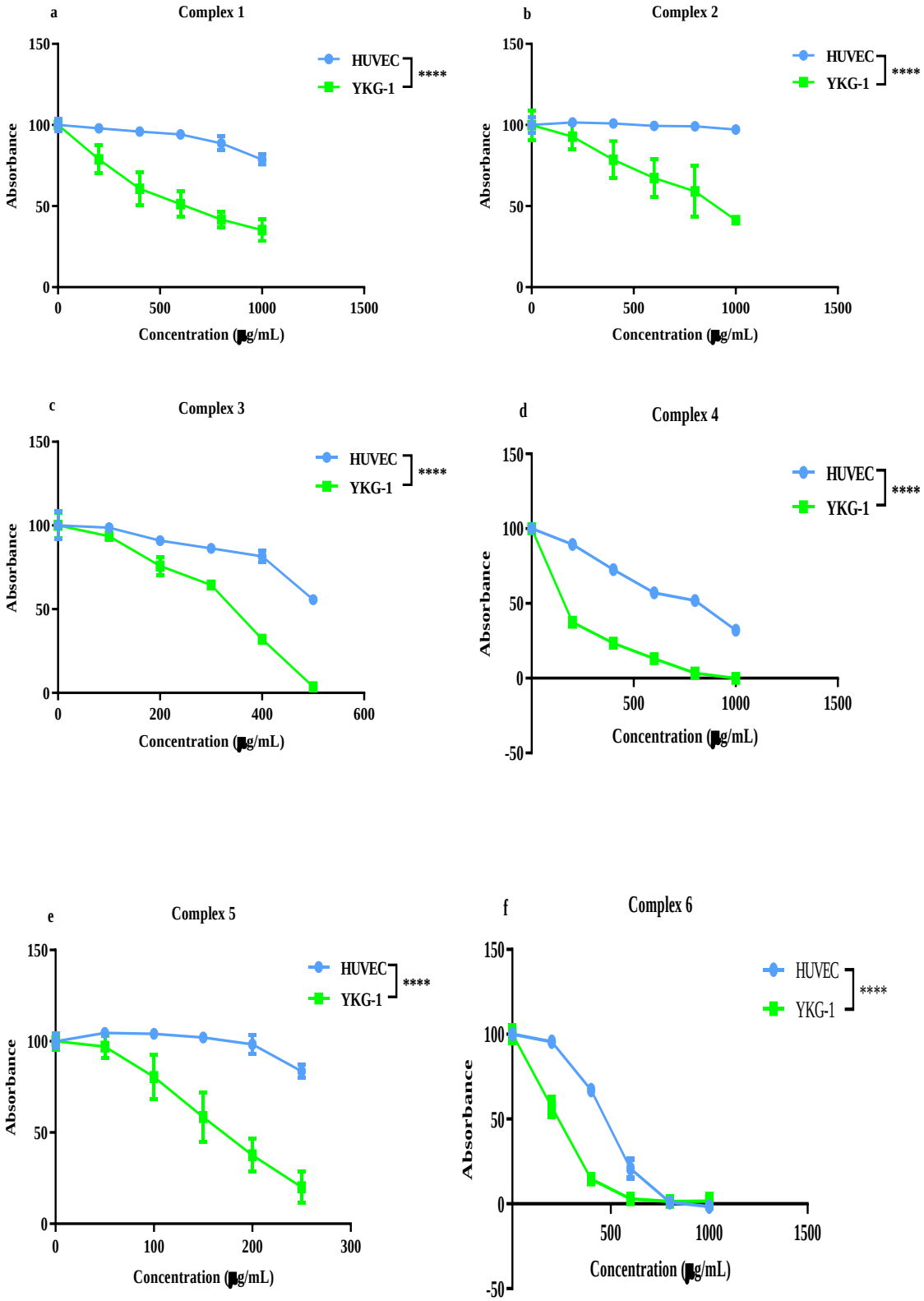
Statistical analysis

Statistical analyses were performed with GraphPad Prism (CA, USA) software. IC_{50} values were determined using the 'EC50 Shift' function in 'Nonlinear Regression' under the 'Analysis' tab. Comparisons of the effects of the compounds on HUVEC and YKG-1 cells were made by Two-Way ANOVA. Only p-values of 0.05 or less were considered significant. The symbols *, **, *** and **** were used to indicate p-values lower than (or equal to) 0.05, 0.01, 0.001 and 0.0001, respectively.

Results

Cytotoxicity and IC_{50} values of compounds

At the end of the experiments, the cytotoxic effects of each synthesized complex (Table 1) on both HUVEC and YKG-1 cells were determined. Cytotoxicity graphs were drawn for both cell populations and IC_{50} values were calculated. When the obtained comparative graphs were analyzed statistically, it was revealed that 11 of the 12 different compounds (complex 1,2,3,4,5,6,7,9,10,11,12) exhibited statistically higher lethal effects on YKG-1 cells than on HUVEC cells (Figure 1.a,b,c,d,e,f, g, i, j, k, l.). However, complex 8 showed a similar effect on both cell groups despite a calculated statistical significance (Figure 1h.).


a
Complex 1
HUVEC
YKG-1
****
Absorbance
Concentration (µg/mL)
b
Complex 2
HUVEC
YKG-1
****
Absorbance
Concentration (µg/mL)
c
Complex 3
HUVEC
YKG-1
****
Absorbance
Concentration (µg/mL)
d
Complex 4
HUVEC
YKG-1
****
Absorbance
Concentration (µg/mL)
e
Complex 5
HUVEC
YKG-1
****
Absorbance
Concentration (µg/mL)
f
Complex 6
HUVEC
YKG-1
****
Absorbance
Concentration (µg/mL)

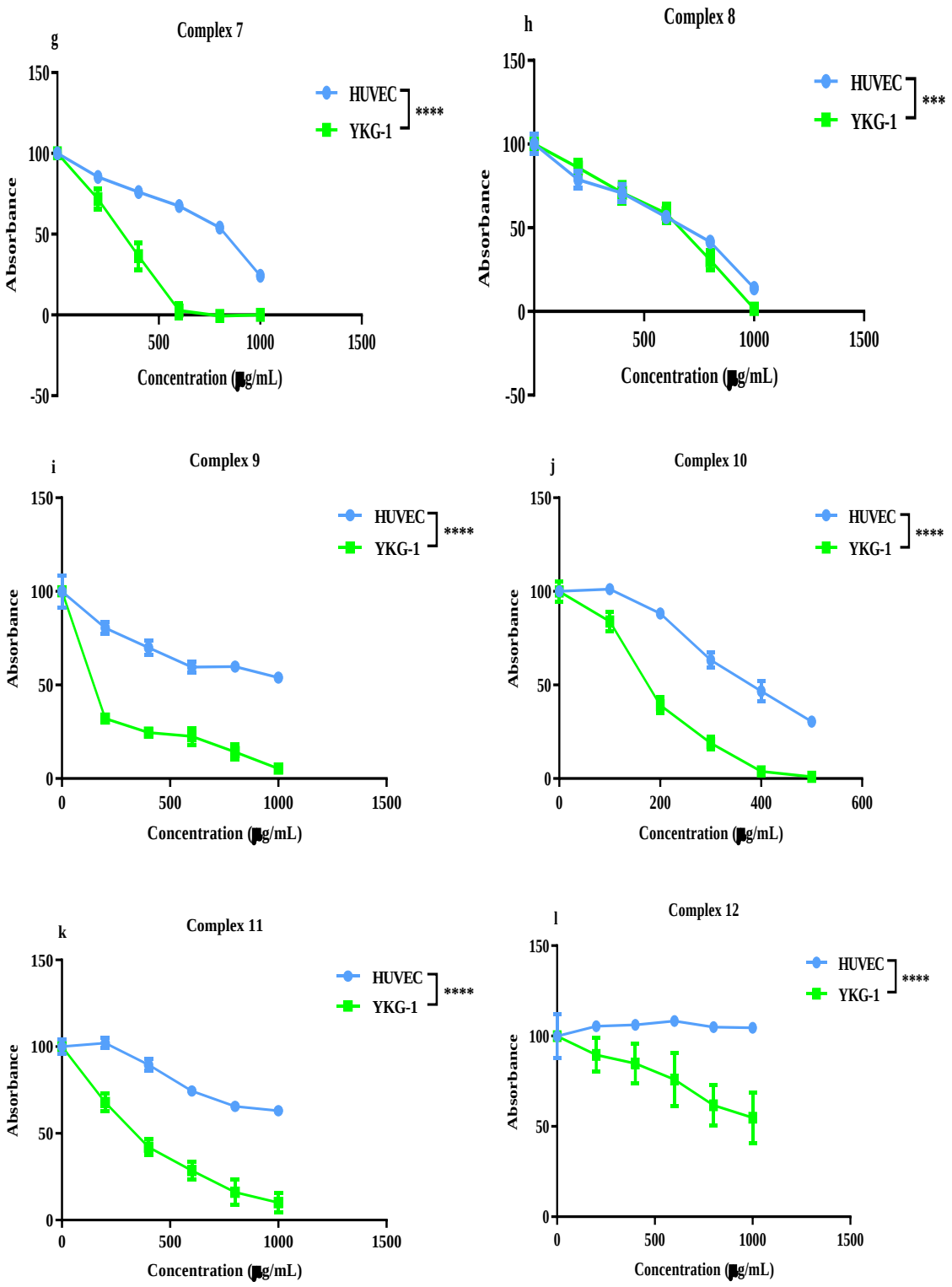


Figure 1: Comparative cytotoxicity graphs obtained from HUVEC and YKG-1 cells for the following compounds: **a.** Complex 1, **b.** Complex 2, **c.** Complex 3, **d.** Complex 4, **e.** Complex 5, **f.** Complex 6, **g.** Copmlex 7, **h.** Complex 8, **i.** Complex 9, **j.** Complex 10, **k.** Complex 11, and **l.** Complex 12. Green Line: YKG-1; Light Blue Line: HUVEC.

IC_{50} values for each compound were determined separately for both HUVEC and YKG-1 cells using cytotoxicity graphs (Table 2.). Upon examination of the obtained IC_{50} values, the highest value of 777.3 µg/mL was obtained from the complex 1 on HUVEC cells, while the lowest value of 139.4 µg/mL was obtained from the complex 9 on YKG-1 cells. When all values were carefully reviewed, a result consistent with the comparative graphs was observed: 11 out of 12 compounds (complex 1,2,3,4,5,6,7,9,10,11,12) had lower IC_{50} values on YKG-1 cells compared to HUVEC cells, whereas the complex 8 showed a higher IC_{50} value.

Table 2.: IC_{50} Values (µg/mL) of 12 Different Compounds Obtained from HUVEC and YKG-1 Cells Using Cytotoxicity Graphs.

Compounds	HUVEC	YKG-1
Complex 1	777,3	323,7
Complex 2	NA	541,3
Complex 3	414,4	338,9
Complex 4	484,8	159,8
Complex 5	214,4	155,9
Complex 6	477,1	229,8
Complex 7	679,9	324,7
Complex 8	599,2	655,9
Complex 9	265,8	139,4
Complex 10	298,9	174,8
Complex 11	492,7	299,4
Complex 12	NA	567,3

N/A: Not applicable because the compound did not show a lethal effect on the cells, even at the highest concentrations.

DISCUSSION

In this study, the lethal effects of 12 different metal complexes on healthy (HUVEC) and cancerous (YKG-1) cells were examined. In general, this examination revealed that YKG-1 cells were more cytotoxically affected by metal complexes than HUVEC cells. The observation was in agreement with the report authored by Hartinger and others where it is mentioned that guanidine and Ru(II) complexes may have strong anticancer potentials (Hartinger and Dyson, 2009). Besides, it has been shown in previous studies that Ru(II) complexes exhibit cytotoxic effects on various cancer cells (Süss-Fink, 2010).

As a reult of our experiments, it is seen that complex 2 and complex 12 have almost no cytotoxic effect on HUVEC cells, while presenting a significant lethal effect on YKG-1 cells. Complex 1, complex 4 and complex 7 exhibited higher lethal effects on YKG-1 cells than on HUVEC cells, the IC_{50} values obtained from Complex 3, complex 5, complex 6, complex 9, complex 10 and complex 11 were closer to each other. Therefore, it may easily be suggested that of all 12 complexes studied, complex 2 and complex 12 are the most effective compounds while complex 8 is the least effective.

When we examined the IC_{50} values we obtained, we realized that they varied in a wide range between 139.4 µg/mL and 777.3 µg/mL. The fact that the values are in such a wide range indicates that they may show different cytotoxic effects depending on the structural differences of the compounds. However, this observation was not unexpected, as one study in the literature reported similar IC_{50} values in human cells consistent with our findings (Vock et al., 2006).

Conclusion

As a result of all the studies, it may be concluded that 11 of the 12 different metal complexes investigated might have the potential to be used against glioblastoma. Nevertheless, as this study is based solely on in vitro evaluations, further research is required before these complexes can be considered for advanced clinical applications.

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ORAL PRESENTATION

Tübülin İnhibisyonu için Potansiyel Yeni 3-(4-(4-Florofenil(Karbonil/Sülfonil)Piperazin)-1-il)-1-(3,4,5-Trimetoksifenil)Propan-1-on Yapısı: Moleküler Yerleştirme ile *in silico* Yaklaşım ve Sentez Çalışmaları

Hilal ZIVALI[1*] (*Orcid: https://orcid.org/0000-0002-3240-9383*), Ahmet Buğra AKSEL[1] (*Orcid: https://orcid.org/0000-0001-5090-6474*), Zechra IMPRAM[2], Ahsen POLAT[2], İnci Selin DOĞAN[1] (*Orcid: https://orcid.org/0000-0003-4949-1747*)

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Özet

Hücre büyümesindeki kontrol mekanizmalarının, düzgün çalışmaması sonucu ortaya çıkan kanser, yılda yaklaşık 20 milyon ölümle dünya çapında önemli bir hastalık iken, gelişmiş ülkelerde ikinci önde gelen ölüm nedeni haline gelmiştir. Bu nedenle yeni, etkinliği yüksek, yan etkileri az, daha basit molekül yapısına ve sentez prosedürüne sahip bileşiklere ihtiyaç duyulmaktadır. Antikanser bileşiklerin temel hedefi, duyarlılaşmış kanser hücrelerinin programlanmış ölüm oranını artırmak olup, bu amaçla topoizomeraz, tübülin, aromataz, Poli (ADP-riboz) polimeraz (PARP) vb. çeşitli enzimlerin inhibisyonu hedeflenebilmektedir. Mikrotübüller, hücre şeklinin korunması, hareketliliğin düzenlenmesi, veziküllerin ve mitokondrinin taşınması ve hücre bölünmesi gibi sayısız hücresel süreçteki önemli rolleri nedeniyle antikanser ajanların gelişimi için oldukça çekici bir terapötik hedef olarak ön plana çıkmaktadır. Mikrotübül inhibitörleri arasında, kolşisin bağlanma bölgesiyle etkileşen bileşiklerin çoğunun 3,4,5-trimetoksifenil (TMP) halkasına sahip olduğu görülmektedir. Bu halkanın, aktif bölge ile hidrojen bağları ve π-π etkileşimlerinin kurulması için önemli olduğu birçok çalışmada bildirilmiştir. Piperazin halkası, doymuş siklik yapısının sunduğu iyileştirilmiş çözünürlüğü ve bulundurduğu heteroatomların bağlanma bölgesinde yaptığı iyonik bağlar ve hidrojen bağı etkileşimleri nedeniyle, birçok antikanser ajanın yapısında bulunmaktadır. Flor atomu da, ilaç adayı bileşiklerinin etkinliklerine, membran geçirgenliğine, metabolizmaya katkısı olduğundan kullanımı oldukça yaygındır. Bunun yanında hacimsel ve elektronik özellikleri sebebiyle medisinal kimya için çok önemli bir biyoizoster olarak ön plana çıkmaktadır. Bu proje çalışmasında 4-florofenil yapısına sahip 3,4,5-TMP-piperazin halkası taşıyan 2 adet yeni bileşik sentezlenmiştir. Sentezlenen bileşiklerin yapıları ^{1}H-NMR, ^{13}C-NMR ve HRMS spektral yöntemleri kullanılarak karakterize edilmiştir. Yapıları doğrulanan ilaç molekülü adayı bileşiklerin hedef enzim/reseptör bölgesinde yaptığı etkileşimler moleküler modelleme yöntemleri ile belirlenmiştir.

Anahtar Kelimeler: Tübülin, 3,4,5-Trimetoksifenil, Moleküler yerleştirme

The potential of 3-(4-((4-fluorophenyl(carbonyl/sulfonyl)piperazin-1-yl)-1-(3,4,5-trimethoxyphenyl)propan-1-one as a new scaffold for tubulin inhibition: in silico approach guided by molecular docking and synthesis studies

Abstract

Cancer, which occurs as a result of improper functioning of the control mechanisms in cell growth, has become the second leading cause of death in developed countries. At the same time, it is an important disease worldwide with approximately 20 million deaths per year. For this reason, there is a need to obtain new compounds with high efficacy, low side effects, and simpler molecular structure and synthesis procedures under the conditions of our country. The main target of anticancer compounds is to increase the programmed death rate of sensitized cancer cells. For this purpose, inhibition of various enzymes such as topoisomerase, tubulin, aromatase, Poly (ADP-ribose) polymerase (PARP), etc. can be targeted. Microtubules stand out as a desirable therapeutic target for the development of anticancer agents due to their important roles in numerous cellular processes such as maintenance of cell shape, regulation of motility, transport of vesicles and mitochondria, and cell division. Among microtubule inhibitors, most of the compounds that interact with the colchicine binding site have a 3,4,5-trimethoxyphenyl (TMP) ring. It has been reported in many studies that this ring is important for the establishment of hydrogen bonds and pi-pi interactions with the active site. The use of fluorine atoms for compounds that may be drug candidates is quite common due to its contribution to

potency, metabolism, membrane permeability, and metabolism. In addition, it stands out as a very important bioisoster for medicinal chemistry due to its volumetric and electronic properties. In this project study, 2 new compounds bearing a 3,4,5-TMP-piperazine ring with the 4-fluorophenyl structure were synthesized. The structures of the synthesized compounds were characterized using ^{1}H-NMR, ^{13}C-NMR and HRMS spectral methods. The interactions of the drug molecule candidate compounds at the target enzyme/receptor site were determined by molecular modeling methods.

Keywords: Tubulin, 3,4,5-Trimethoxyphenyl, Molecular docking

GİRİŞ

Hücre büyümesi kontrol mekanizmalarının düzgün çalışmaması sonucu ortaya çıkan kanser, yılda yaklaşık 20 milyon ölümle dünya çapında önemli bir hastalık iken, gelişmiş ülkelerde ikinci önde gelen ölüm nedeni haline gelmiştir. Bunun yanında, kanserin ülkemizde ve dünyada hem mortalite oranı, hem de ekonomik açıdan tedavi maliyeti gün geçtikçe artmaktadır. Bu nedenle, etkinliği yüksek, yan etkileri az, daha basit molekül yapısına ve sentez prosedürüne sahip yeni bileşiklerin ülkemiz şartlarında elde edilmesine ihtiyaç duyulmaktadır (Romagnoli ve ark., 2020). Mikrotübüller, mitoz sırasında kromozomların ayrılmasını sağlayan dinamik yapılar olup, bu süreç mikrotübül hedefleyici ajanlar tarafından bozulabilir. Bu ajanlar, tübülin polimerizasyon inhibitörleri ve mikrotübül ayrışma blokerleri olarak iki gruba ayrılır.

Son yıllarda hem doğal hem de sentetik moleküllerden oluşan çeşitli tübülin inhibitörleri tanımlanmış, klinik kullanım için güçlü ve geliştirilmiş özelliklere sahip küçük moleküllerin gerekliliği vurgulanmıştır (Yang ve ark., 2019).

Epotilon, paklitaksel ve vindesin uzun süredir piyasada olsa da, ilaç direnci ve üretim zorlukları nedeniyle klinik kullanımları sınırlıdır. Paklitaksel, vinka alkaloitleri ve kolşisin gibi doğal bileşikler, mikrotübüllere spesifik bölgelerden bağlanarak etki eder. Özellikle kolşisin bağlanma bölgesi, yeni bileşiklerin geliştirilmesi için önemli bir hedeftir. Ayrıca, bazı tübülin inhibitörlerinin tümör hücrelerinin damarlanmasını bozarak etki gösterdiği bildirilmiştir (Wang ve ark., 2019; Romagnoli ve ark., 2019).

Tübülin, kolşisin bağlayıcı proteinlerden oluşur ve kolşisin bağlanma bölgesi α1 ve β1 alt birimleri arasında yer alır. Bu bölge, ligand etkileşimleri için ana bölgedir ve üç hidrofobik cep ile iki hidrofilik bölge içerir. Hidrofobik cep I derin bağlanma modu bileşikleri için hedefken, hidrofobik cep II ana kolşisin bağlama cebi olarak bilinir. Kombretastatin A ve benzeri inhibitörler, bu bölgeyle etkileşim kurarak mikrotübül dinamiklerini bozabilir (Wang ve ark., 2022). Bu bilgiler, tübülinin yapısında bulunan kolşisin bağlanma bölgesi hakkında önemli bilgiler sunmaktadır. Bu bölge, tübülin inhibitörlerinin etki mekanizmaları üzerinde önemli bir rol oynar ve bu bağlanma bölgesine özgü etkileşimleri hedeflemek, mikrotübül dinamiklerini etkileyerek kanser tedavisinde potansiyel bir strateji olarak ön plana çıkmaktadır.

Mikrotübül inhibitörlerinde, metoksi ve hidroksi gruplarının aktivite için kritik olduğu (Ebenezer ve ark., 2022), özellikle trimetoksifenil grubunun değişmesi durumunda hücresel aktivitenin kaybolduğu bilinmektedir (Lee ve ark., 2017). Bu farmakofor grup, kolşisin bağlanma bölgesiyle etkileşim kurar. Stilbenoid türevi olan kombretastatin A-4 ve podofilotoksin, trimetoksi sübstitüenti içerir ve bu grup, kolşisin bağlanma bölgesinde hidrojen bağları aracılığıyla etki gösterir. Kombretastatin molekülündeki cis çifte bağın piperazin halkasına dönüştürüldüğü çalışmalar, bu halkaya sahip türevlerin antikanser aktivite gösterdiğini ortaya koymuştur (Yang ve ark., 2021).

Piperazin halkası, kolşisin bağlanma bölgesinde yeni etkileşimler sağlayarak bileşiklerin çözünürlüğünü artırmış ve metabolizmaya karşı daha güçlü bir profil sergilemiştir (McLoughlin ve ark., 2020).

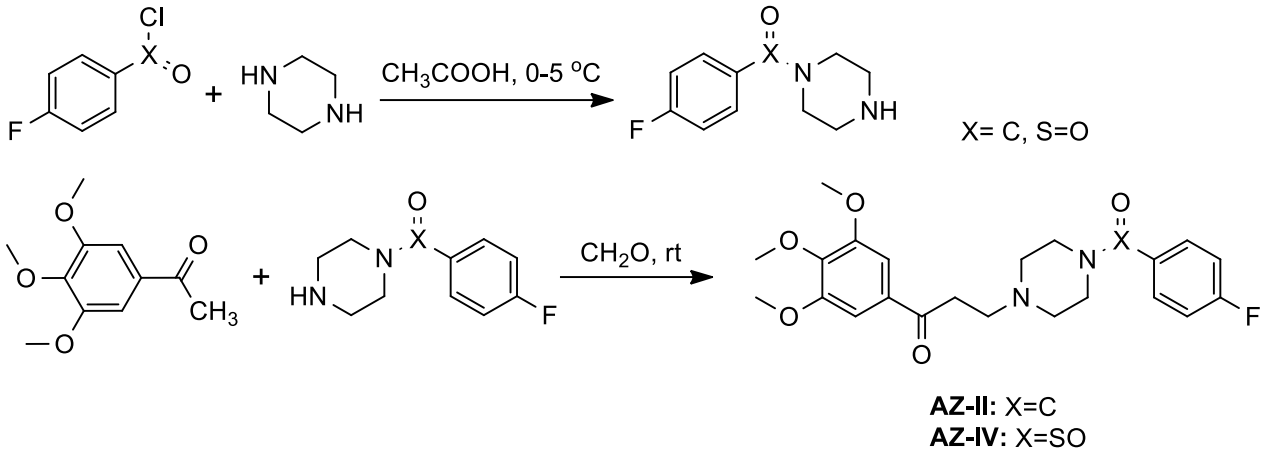
Antikanser aktivite kapsamında 3-(4-((4-florobenzoil)piperazin-1-il))-1-(3,4,5-trimetoksifenil)propan-1-on ve 3-(4-((4-florobenzensülfonil)piperazin-1-il))-1-(3,4,5-trimetoksifenil)propan-1-on bileşikleri sentezlenmiştir. Sentezlenen bileşiklerin HRMS, ^{1}H-NMR, ^{13}C-NMR spektrumları ile yapı aydınlatması gerçekleştirilmiştir.

MATERYAL VE METOT

Materyal

Aseton, etanol, formaldehit, kloroform, 3,4,5-trimetoksiasetofenon, piperazin, 4-florobenzoil klorür, 4-florosülfonil klorür, potasyum hidroksit, sodyum bikarbonat, tiyonil klorür, paraformaldehit, etilasetat, dioksan/THF, HCl, DCM, TEA Sigma-Aldrich ve Merck firmasından temin edilmiştir.

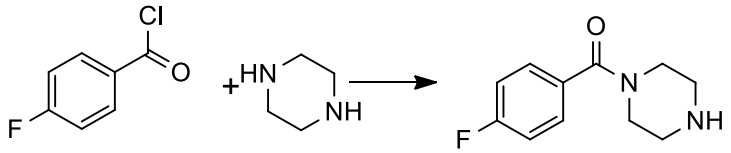
Sentez Yöntemleri



Şekil 1. Bileşiklerin genel sentez yöntemi

(4-Florofenil)(piperazin-1-il)metanon (AZ-I) Bileşiğinin Sentezi

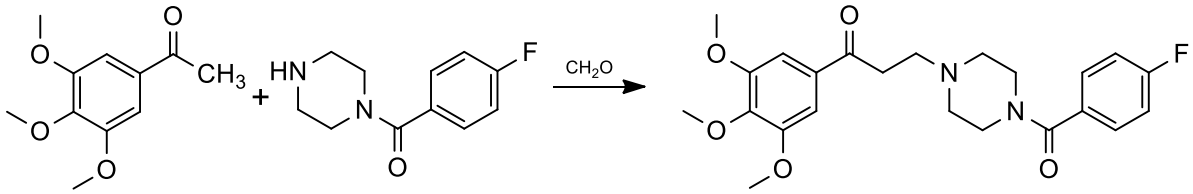
Reaksiyon balonunda, piperazin (100 mmol) üzerine, asetik asit (4 ml) eklenir. Damlatma hunisinde, 4-Florobenzoil klorür (5 mmol) ve asetik asit (1 ml) karışımı hazırlanır ve reaksiyon ortamına damla damla eklenir. Reaksiyon 0-5 °C'de 30 dk boyunca karıştırılır ve İTK kontrolü sonlandırılır. Reaksiyon ortamı % 40'lık NaOH çözeltisi ile nötralize edilir, vakum altında su içeriği uçurulur. DCM ve etil asetat ile yıkama yapılıp süzülür. Eter ile katılaştırılır ve ürün kolon kromatografisi yöntemi ile saflaştırılır (Li ve ark., 2007) (Şekil 2).



Şekil 2. Piperazin ve 4-florobenzoil klorür **AZ-I** bileşiğinin sentezi

3-(4-((4-Florobenzoil)piperazin-1-il))-1-(3,4,5-trimetoksifenil)propan-1-on (AZ-II) bileşiğinin sentezi

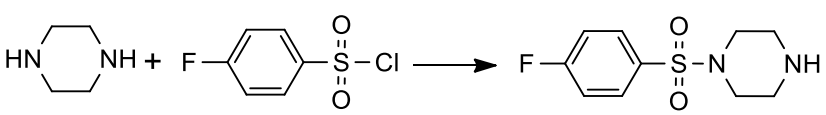
3,4,5-Trimetoksiasetofenon (0,48 mmol) ve **AZ-I** (0,48 mmol) reaksiyon balonuna alınarak etanol içerisinde karıştırılır. Üzerine formaldehit (1,44 mmol) eklenir. Reaksiyon takibi İTK ile yapılır. Etanol uçurulur. Elde edilen ham ürün kolon yöntemi ile saflaştırılır (Martínez-Esparza ve ark., 2001) (Şekil 3).



Şekil 3. 3,4,5-Trimetoksiasetofenon, formaldehit ve **AZ-I**'den **AZ-II** bileşiğinin sentezi

(4-Florobenzensülfonil)(piperazin-1-il)metanon (AZ-III) bileşiğinin sentezi

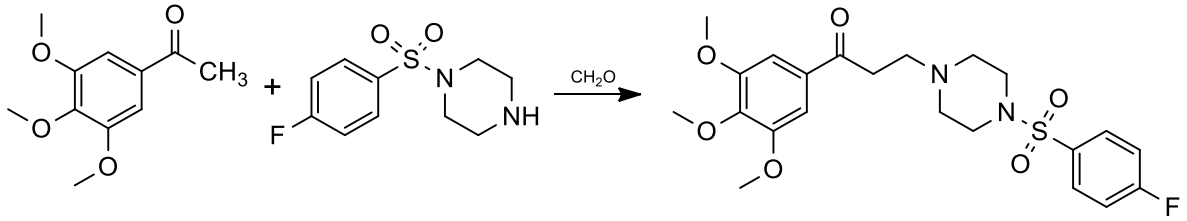
AZ-I bileşiği sentez yönteminde 4-Florobenzensülfonil klorür (5 mmol) kullanılarak reaksiyon tamamlanır (Handerson ve ark., 2011) (Şekil 4).



Şekil 4. Piperazin ve 4-florobenzensülfonil klorürden **AZ-III** bileşiğinin sentezi

3-(4-(4-florofenilsülfonil)piperazin-1-il)-1-(3,4,5-trimetoksifenil)propan-1-on (AZ-IV) bileşiğinin sentezi

3,4,5-Trimetoksiasetofenon (0,48 mmol) ve **AZ-III** (0,48 mmol) reaksiyon balonuna alınır. Üzerine formaldehit (1,44 mmol) eklenir. Reaksiyon ortamına çözücü olarak bir miktar etanol eklenir. Reaksiyon takibi İTK yöntemi ile yapılır. Reaksiyon ortamındaki etanol uçurulur. Elde edilen ham ürün kolon kromatografisi yöntemi ile saflaştırılır (Osorio-Nieto ve ark., 2023) (Şekil 5).



Şekil 5. 3,4,5-Trimetoksiasetofenon, formaldehit ve **AZ-III**'den **AZ-IV** bileşiğinin sentezi

Moleküler Modelleme Çalışmaları

Moleküler modelleme çalışmaları, bu bileşiklerin, tübülin enzim aktif bölge amino asitleriyle etkileşimlerini moleküler düzeyde tespit etmek amacıyla Autodock Vina programı kullanılarak (Trott ve Olson, 2010) gerçekleştirilmiştir. Tübülin enzimine ait x-ray yapısı (PDB: 5YL2), RCSB Protein Data Bank veri tabanından indirilerek Discovery Studio 2021 programında kristal yapısındaki su molekülleri silinip; Autodock Tools 1.5.7 programında yanlış ve eksik atomların düzeltilmesi, polar hidrojen atomlarının eklenmesi ve Kollman enerji minimizasyonu işlemleri yapılarak hazırlanmıştır. Enzim x-ray krisal yapısına bağlı inhibitör bileşiğin koordinatları Discovery Studio 2021 programı kullanılarak belirlenip, Autodock Tools 1.5.7 programında aktif bağlanma bölgesi için 20x20x20 $Å^3$'lük bir kutu (grid) oluşturulmuştur. Elde edilen grid dosyası moleküler docking çalışmalarında kullanılmak üzere kaydedilmiştir. Schrödinger programının Maestro arayüzünde çizilen bileşikler, LigPrep (Schrödinger Release 2023-2: LigPrep, Schrödinger, LLC, New York, NY, 2023) modülü kullanılarak varsayılan değerlerle üç boyutlu yapılarına geçirilmiştir. Uygulanacak moleküler docking protokolünü doğrulamak amacıyla tübülin enziminin x-ray kristal yapısında yer alan koligantlar ilgili enzim aktif bölgelerine yeniden yerleştirilmiş ve bu işlem sonucu elde edilen konformasyonlar ilgili koliganta ait biyoaktif konformasyonlar ile çakıştırılarak protokolün doğruluğunu gösterir düzeyde RMSD değerine (0,230 Å (PDB: 5YL2)) ulaşılmıştır. Bileşiklerin tübülin aktif bölgesine yerleştirilmesi, önceden kaydedilen grid dosyası üzerinden Autodock Vina programının Vina modülü kullanılarak gerçekleştirilmiştir. Elde edilen sonuçlar, XP GScore değerleri ile ifade edilen bağlantı enerjileri ve etkileşim türleri temel alınarak değerlendirilip, uygun bağlantı modları belirlenmiştir.

Analitik Yöntemler

Erime Derecesi Tayinleri: Bileşiklerin erime dereceleri "Thermo Scientific Capillary Melting Point Apparatus'' erime derecesi tayin cihazı ile saptanmıştır.

İnce Tabaka Kromatografisi: TLC alüminyum 20x20 cm Kieselgel 60 F254 (Merck) hazır plaklar kullanılmıştır.

Saflaştırma Yöntemi: Kolon Kromatografisi

Kloroform: Metanol [98: 2]

Kloroform: Metanol [99: 1]

Spektrofotometrik Analizler

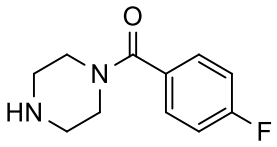
^{1}H-NMR Spektrumları: Bileşiklerin ^{1}H-NMR spektrumları, Gazi Üniversitesi Merkez Laboratuvarı'nda, dötero dimetilsülfoksit (DMSO-d_6, Merck) içerisindeki yaklaşık %10'luk çözeltileriyle Bruker Avance neo 500 MHz FT spektrometre cihazı ile alınmış, δ (ppm) skalasında değerlendirilmiştir.

^{13}C-NMR Spektrumları: Bileşiklerin ^{13}C-NMR spektrumları, Gazi Üniversitesi Merkez Laboratuvarı'nda Bruker Avance neo 125 MHz FT spektrometre cihazı ile alınmış, δ (ppm) skalasında değerlendirilmiştir.

BULGULAR

Kimyasal Çalışmalar

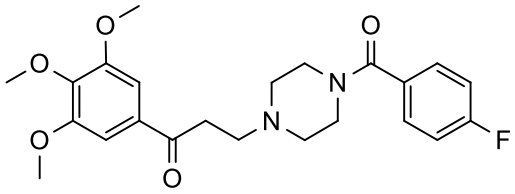
(4-Florofenil)(piperazin-1-il)metanon (AZ-I)



AZ-I bileşiğinin saflaştırılması için kolon kromatografisi $CHCl_3$:MeOH (98:2) olarak kullanılmıştır. Sarımsı renkte kristal toz görünümlü bir maddedir. Verim: %80 EN: 68-68.8 ^oC

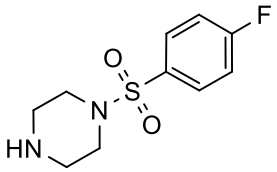
$C_{11}H_{13}FN_2O$ için HRMS (m/z) $[M+H]^+$ Hesaplanan: 209.1090; Bulunan: 209.1513

3-(4-((4-Florobenzoil)piperazin-1-il))-1-(3,4,5-trimetoksifenil)propan-1-on (AZ-II)



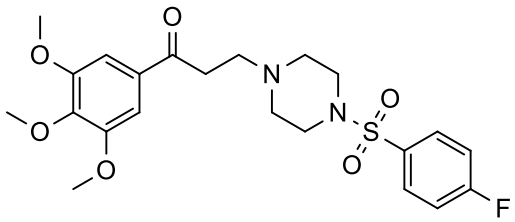
3AZ-II bileşiğinin saflaştırılması için kolon kromatografisi $CHCl_3$:MeOH (99:1) olarak kullanılmıştır. Bu bileşiğin saflaştırma işlemleri devam etmektedir. Bu bileşiğe ait tahmini spektrum verileri Şekil 6'da gösterilmiştir. Verim: %50 EN: 92-92.8 ^oC $C_{23}H_{27}FN_2O_5$ için HRMS (m/z) $[M+H]^+$ Hesaplanan: 431.1982; Bulunan: 431.2645

(4-Florobenzensülfonil)(piperazin-1-il)metanon (AZ-III)



AZ-III bileşiğinin saflaştırılması için kolon kromatografisi $CHCl_3$:MeOH (98:2) olarak kullanılmıştır. Sarımsı renkte kristal toz görünümlü bir maddedir. Verim: %24. EN: 108-108.6 ^oC. $C_{10}H_{13}FN_2O_2S$ için HRMS (m/z) $[M+H]^+$ Hesaplanan: 245.0760; Bulunan: 245.1246

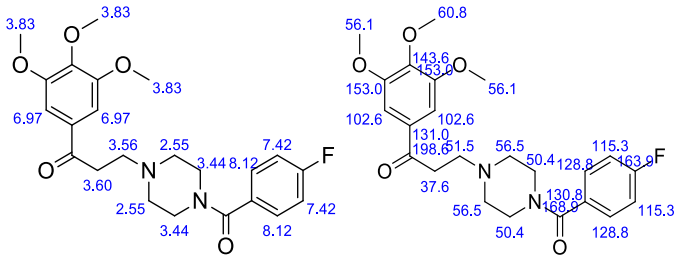
3-(4-(4-Florofenilsülfonil)piperazin-1-il)-1-(3,4,5-trimetoksifenil)propan-1-on (AZ-IV)



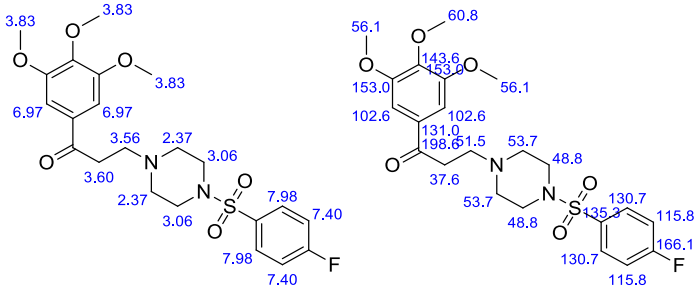
AZ-IV bileşiğinin saflaştırılması için kolon kromatografisi $CHCl_3$:MeOH (98:2) olarak kullanılmıştır. Bu bileşiğin saflaştırma işlemleri devam etmektedir. Bu bileşiğe ait tahmini spektrum verileri Şekil 7'de gösterilmiştir. Verim: %24. EN: 167.2-167.6 ^oC. $C_{22}H_{27}FN_2O_6S$ için HRMS (m/z) $[M+H]^+$ Hesaplanan: 467.1653; Bulunan: 467.1705.

^{1}H NMR (DMSO-*d*6, 500 MHz) δ ppm 2.81 (2H, t, *J*= 11.3 Hz, CH_2-piperazin), 3.25 (2H, br s, CH_2-piperazin), 3.67 (4H, br s, CH_2), 3.75 (3H, s, OCH_3), 3.80 (4H, t, *J*= 12.4 Hz, CH_2-piperazin), 3.90 (6H, s, OCH_3), 7.28 (2H, s, Ph-CH), 7.56 (2H, t, *J*= 8.7 Hz, Ph-CH), 7.88-7.90 (2H, m, Ph-CH).

*__AZ-II__ ve __AZ-IV__ bileşiklerinin spektral analizleri devam etmektedir. Tamamlanınca bildirilecektir.



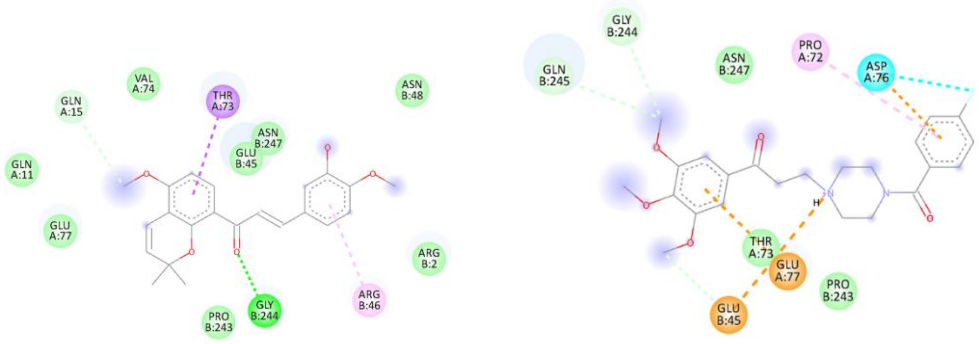
Şekil 6. AZ-II Bileşiğinin tahmini ^{1}H-NMR ve ^{13}C-NMR spektrumları



Şekil 7. AZ-IV Bileşiğinin tahmini ^{1}H-NMR ve ^{13}C-NMR spektrumları

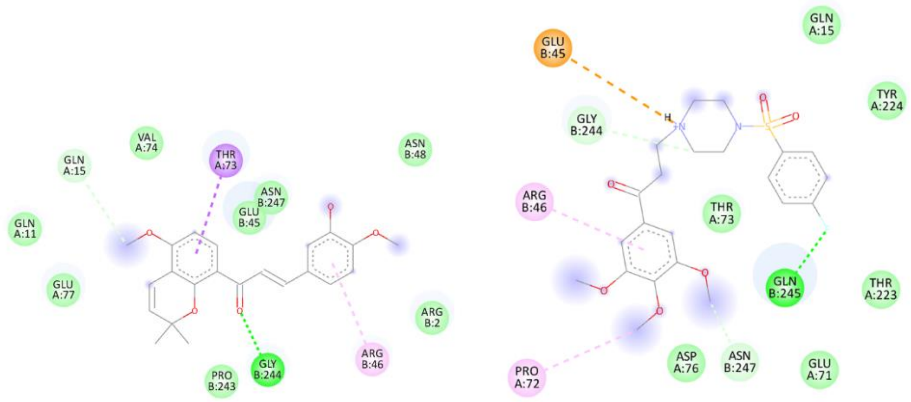
Moleküler Modelleme Çalışmaları

Proje kapsamında sentezlenen ilaç olabilecek moleküllerin SwissADME hedef tahmini modülü kullanılarak potansiyel olarak hangi hedef bölge için uygun olabileceği araştırılmıştır. Veri tabanından elde edilen bilgiler, bileşiklerimizin yapısal olarak tübülin inhibitörü bileşikleri %80-90 oranında benzerliğe sahip olduklarını ve tübülin inhibitörü ajanlar olabileceğini göstermiştir. Moleküler modelleme çalışmalarında tübülin x-ray kristal yapısı (PDB: 5YL2) (Yang ve ark., 2018) kullanılmıştır. Kristal yapısındaki koligantın (8WU) bağlanma skoru -6.8 kkal/mol olarak hesaplanmıştır. Yine tübülin inhibitörü olarak bildirilmiş olan Kombrastatin A4 molekülünün (IC_{50}=3 µM) bağlanma skoru -5.7 kkal/mol olarak hesaplanmıştır. Proje kapsamında bildirilen **AZ-II** ve **AZ-IV** kodlu bileşiklerin sırasıyla -7.5 kkal/mol ve -7.0 kkal/mol bağlanma skorlarına sahip oldukları hesaplanmıştır. Moleküler kenetleme pozları incelendiğinde **AZ- II** kodlu bileşiğin 3,4,5-TMP halkası üzerinden Gln245, Gly244 ve Glu45 amino asitleriyle hidrofobik etkileşimler kurduğu, Glu45 ile piperazin azotu üzerinden yük transfer etkileşimi; 4-florofenil halkası üzerinden Pro72 ile pi-alkil; Asp76 ile yük transfer etkileşimi ve molekülün yapısındaki flor atomu ile halojen bağı etkileşimi kurduğu görülmüştür (Şekil 8).



Şekil 8. Tübülin enzimi aktif bölgesinde koligant ve AZ-2 kodlu bileşiğin (PDB: 5YL2) meydana getirdiği etkileşimler. Hidrojen bağları, hidrofobik etkileşimler, pi-sigma bağları, yük transfer etkileşimleri ve halojen bağları sırasıyla yeşil, pembe, mor, turuncu ve turkuaz kesikli çizgiler ile gösterilmiştir.

AZ-IV kodlu bileşiğin 3,4,5-TMP halkası üzerinden Arg46, Pro72 ile pi-alkil; Asn247 ile hidrofobik etkileşimler kurduğu, Glu45 ile piperazin azotu üzerinden yük transfer ve Gly244 ile hidrofobik etkileşimleri; 4-florofenil halkası üzerinden Gln245 ile van der Waals etkileşimi kurduğu görülmüştür (Şekil 9).



Şekil 9. Tübülin enzimi aktif bölgesinde koligant ve AZ-4 kodlu bileşiğin (PDB: 5YL2) meydana getirdiği etkileşimler. Hidrojen bağları, hidrofobik etkileşimler, pi-sigma bağları ve yük transfer etkileşimleri sırasıyla yeşil, pembe, mor ve turuncu kesikli çizgiler ile gösterilmiştir.

TARTIŞMA

Bu çalışmada, 3-(4-((4-florobenzoil)piperazin-1-il))-1-(3,4,5-trimetoksifenil)propan-1-on ve 3-(4-((4-florobenzensülfonil)piperazin-1-il))-1-(3,4,5-trimetoksifenil)propan-1-on bileşikleri sentezlenmiş ve potansiyel aktiviteleri moleküler modelleme yöntemleri ile araştırılmıştır. Moleküler modelleme çalışmalarında tübülin x-ray kristal yapısı (PDB: 5YL2) kullanılmıştır. Kristal yapısındaki koligantın (8WU) bağlanma skoru -6.8 kkal/mol olarak hesaplanmıştır. Tübülin inhibitörü olarak bildirilmiş olan Kombrastatin A4 molekülünün (IC_{50} = 3 µM) bağlanma skoru -5.7 kkal/mol olarak hesaplanmıştır. **AZ-II** ve **AZ-IV** kodlu bileşiklerin sırasıyla -7.5 kkal/mol ve -7.0 kkal/mol bağlanma skorlarına sahip oldukları görülmüştür. Bu yeni bileşiklerin tübülin enzimine referans bileşiklere göre daha iyi bağlandıkları ispatlanmıştır (Tablo 1).

Tablo 1. Bileşiklerin serbest bağlanma enerjileri

Bileşik Adı	**Tübülin enzimi (PDB: 5YL2)**
AZ-II	-7.5 kkal/moll
AZ-IV	-7.0 kkal/mol
Koligant (8WU)	-6.8 kkal/mol
Kombrastatin A4	-5.7 kkal/mol

SONUÇ

Bu çalışmada, 2 yeni 3-(4-((4-florofenil(karbonil/sülfonil)piperazin-1-il)-1-(3,4,5-trimetoksifenil)propan-1-on yapısına sahip bileşik elde edilmiştir. Bileşiklerin yapıları ^{1}H-, ^{13}C-NMR ve IR ile aydınlatılmıştır. Sentezlenen bu yeni bileşikler antibakteriyel, antineoplastik, antiasetilkolinesteraz, antiviral, antiprotozoal ve antiinflamatuar olmak üzere birçok potansiyel biyolojik aktiviteye sahip olmaları bakımından önem taşımaktadırlar. Elde edilen moleküler modelleme sonuçları bileşiklerin tübülin inhibitörü olabileceğini göstermiştir. **AZ-2 ve AZ-4** kodlu bileşikler, literatürde bildirilen Kombrastatin A4 molekülüne göre ve tübülin X-ray kristal yapısındaki (PDB: 5YL2) koliganta (8WU) göre çok daha üstün bir profil sergilemiştir. Çalışmada elde edilen bilgiler bu seriye ait bileşiklerin sayısının artırılıp biyolojik aktivite bulgularıyla desteklendiğinde yeni çalışmalar için ilham verici olabileceğini göstermiştir.

TEŞEKKÜR

Bu çalışma, 2209-A Üniversite Öğrencileri Araştırma Projeleri Destekleme Programıyla TÜBİTAK tarafından (Proje kodu: 1919B012305574) desteklenmiştir.

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ORAL PRESENTATION

Synthesis of Nanocrystaline Cellulose From Watermelon Peel Albedos and Analysis of Its Surface Properties by Inverse Gas Chromatography

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Abstract

Recently, interest in the development of more environmentally friendly and biodegradable materials has increased significantly due to growing environmental concerns. Due to their low cost, biodegradability and good mechanical properties, plant fibres have replaced synthetic fibres in the production of composite materials. Watermelon peel is one of the most underutilised waste materials that has potential for the production of functional ingredients due to its high fibre content. This study was conducted to synthesise and characterise cellulose and nanocrystalline cellulose from watermelon albedo. The physicochemical and surface properties of the produced materials were characterised using the inverse gas chromatography (IGC) method. For the IGC studies, the retention times of n-hexane, n-heptane, n-octane, and n-nonane were measured under infinite dilution conditions between 40 and 70°C. The dispersive component of the surface energy (γ_{sd}) of the watermelon peel albedo was calculated. The dispersive surface energy analysis showed that the dispersive surface energy component decreases with increasing temperature. We conclude that the albedo of watermelon rind could be the main source of natural cellulose and nanocrystalline cellulose materials for food ingredient applications.

Keywords: Nanocrystaline Cellulose, Watermelon Peel, Inverse Gas Chromatography, Characterization

INTRODUCTION

Increasing environmental concerns and the depletion of fossil fuels have led to an ever-growing demand for the development of sustainable and environmentally friendly biocomposites. Cellulose, the most abundant natural polymer derived from sources such as plants, wood and bacteria, has recently gained greater attention. Researchers have developed various methods for extracting cellulose from plants, bacteria, algae, etc., such as mechanical, chemical and chemical treatments combined with mechanical treatments. Cellulose nanocrystals (CNC), cellulose nanofibrils (CNF) and microcrystalline cellulose (MCC) have been extracted and used for various applications such as food packaging, water purification, drug delivery and in composite materials (Poulose et al., 2022). Various methods (usually in combination) such as milling, high pressure homogenization, cryocrushing, steam explosion, ultrasonication, enzymatic pretreatments and chemical treatments are used for the isolation of nanocellulose from plant fibers (Pandi et al., 2021).

Nanocellulose is a 1D nanomaterial with a diameter that is usually in the nanoscale and a length of several hundred nanometers to a few micrometers, which is composed of cellulose chains. It has three hydroxyl groups in each anhydroglucose unit, which gives the nanocellulose a hygroscopic character and accordingly leads to a relatively easy dispersion of the nanocellulose in water. The numerous hydroxyl groups in the cellulose chain promote the formation of intermolecular hydrogen bonding between the polysaccharides, which facilitates the formation of the crystalline domain in the nanocellulose. All these properties endow the nanocellulose with renewability, biocompatibility, biodegradability, high aspect ratio, excellent mechanical properties, superior thermal stability and light transparency, making the nanocellulose potentially applicable as a reinforced nanomaterial for composites and coatings, as well as a building block for packaging, release films, biomedicine and transparent nanopaper. As a result, the interest of academia and industry in nanocellulose has greatly increased and is becoming a hot research topic in the field of materials science (Zhuo et al., 2017).

The analysis of the physicochemical surface properties of cellulose-based fillers is crucial for maximizing fibre-matrix interfacial adhesion to achieve excellent mechanical performance. Among the various techniques

that can be used to study the surface properties of cellulose-based materials, inverse gas chromatography (IGC) has proven to be a successful technique for characterizing surface activity, evaluating the efficiency of surface modification methods and predicting the behavior of the final products. IGC is a versatile approach to evaluate surface energy, acid-based properties and surface heterogeneity. These key parameters are used to (i) evaluate the efficiency of different surface treatments to add new functional groups, (ii) determine the compatibility of cellulosic materials with various polymer matrices to ensure optimal fibre-matrix interaction, and (iii) predict the performance of cellulose-reinforced composites in actual applications.

In this study, the IGC method was used to surface analyze different forms of cellulose composites from watermelon peel albedos on understanding the surface properties of cellulose-based composites and pave the way for new applications.

MATERIALS AND METHODS

Composite Synthesis

To obtain NCC, the biomass was first dried at 90 ºC for 6 hours, ground and sieved. The 90 nm sieved particles were dried a second time at 90 ºC for 6 hours and mixed with a toluene-ethanol mixture for 24 hours at room temperature. After the mixture was filtered, the solid phase was washed with distilled water. In this way, wax and oils were removed. Repeated bleaching with acetic acid, sodium chlorite and water was then carried out. After bleaching, the lignin was removed with acetic acid and decolorization was carried out with sodium chlorite. The residue was mixed with a potassium hydroxide mixture. The resulting cellulose was separated by centrifugation and washed with distilled water. This removed hemicellulose and silica. The cellulose obtained was placed in a sulphuric acid solution (%60) and shaken in a water bath. The hydrolysis was stopped by adding cold water, the volume of which was ten times the mixture. The resulting suspension was centrifuged. NCC was dialyzed against water to remove residues. NCC was prepared by sonication of the suspension in an ice bath and dried in a refrigerator. Biomasses and the NCC obtained from these biomasses are analyzed in the study. The produced NCC will be used for polymer composites.

Characterization

Characterization of Biomass

To determine the moisture content, the samples were stored in an oven at 103±2 °C and weighed for 2 hours to allow the moisture to be evenly distributed in the sample until a constant value was obtained. Before starting a test, the required amounts of seed were warmed up to room temperature and the moisture content was calculated using the following equation;

$$M\ (\%) = \frac{W1-W2}{W1} * 100 \quad (1)$$

In the equation, M is the moisture content of the melon, and W1 corresponds to the initial weight, W2 to the final weight of the biomass.
To determine the ash content in the biomass, the sample is heated to a very high temperature of about 600 °C to remove all moisture and volatile matter (as vapour) as well as organic matter (as carbon dioxide and nitrogen oxides). The ash content (dry matter) is calculated using the following equation;

$$A\ (\%) = \frac{A1}{S} * 100 \quad (2)$$

In the equation, A is the ash content of the sample, and A1 corresponds to the ash content, S corresponds to the dry quantity of the sample in the oven.

Surface characterization

Inverse gas chromatography (IGC) was used to measure the surface adsorption of some probes (n-alkanes on a series (n-C5 to n-C8) cyclohexane and benzene) on synthesized zeolites. The chromatographic peak was measured with an Agilent 7890A gas chromatograph. High purity nitrogen with a flow rate of 40 mL/min was used as carrier gas. The analyzes were performed with a 2 m long stainless steel column with an inner diameter of 5.35 mm. The dispersive component of the surface free energies (γ_{Sd}), was also calculated.

The net retention volume VN is calculated using the following equation;

$$V_N = F_0 . \frac{T}{T_0} . \frac{3}{2} \left[\frac{\left(\frac{P_g}{P_ç}\right)^2 - 1}{\left(\frac{P_g}{P_ç}\right)^3 - 1} \right] . (t_A - t_0) \quad (3)$$

In the equation, To is the retention time of the substance that does not interact with the solid in the column, and tA is the retention time of the solvent in the column. Pg denotes the inlet pressure, Pç the outlet pressure of the column. Fo is defined as the flow rate of the carrier gas at the column outlet, measured at room temperature. T_0 is the temperature read from the thermometer, T is the temperature of the flow rate of the purge gas (Aşkın and Bilgic, 2005). Dispersive component of surface energy γ_{Sd} calculated at different temperatures using IGC for all samples.

RESULTS AND DISCUSSION

The results of watermelon peel albedo, moisture, ash, volatile matter and fixed carbon are given in Table 1.

Table 1. Content analysis of watermelon albedos

Moisture (%)	0.17
Volatile content (%)	69.19
Carbon (%)	12.61
Ash (%)	18.03

The analysis of the basic content revealed that the moisture content, volatile matter content, fixed carbon content and ash content of the watermelon rinds were 0.17; 69.17; 12.61 and 18.03 percent, respectively.

The total surface energy of the solid (γ^{St}) is made up of two components: the dispersive surface energy (γ_{sd}) and the specific surface energy or acid/base component (γs^{AB}). The dispersive component represents the surface energy due to non-polar van der Waals interactions at the surface, while the specific surface energy represents the surface energy due to polar/acid–base interactions (hydrogen bonds, ionic bonds, metallic interactions). The total surface energy is simply the sum of the dispersive and specific components (Ali et al., 2013). To calculate the total surface energy $RTlnV_N$ - $a(\gamma_L^d)^{0.5}(Å^2 mJ^{0.5} m^{-1})$, the graph for the albedo of the watermelon rind in the temperature range of 40-70 °C was constructed according to IGC calculations (Figure 1).

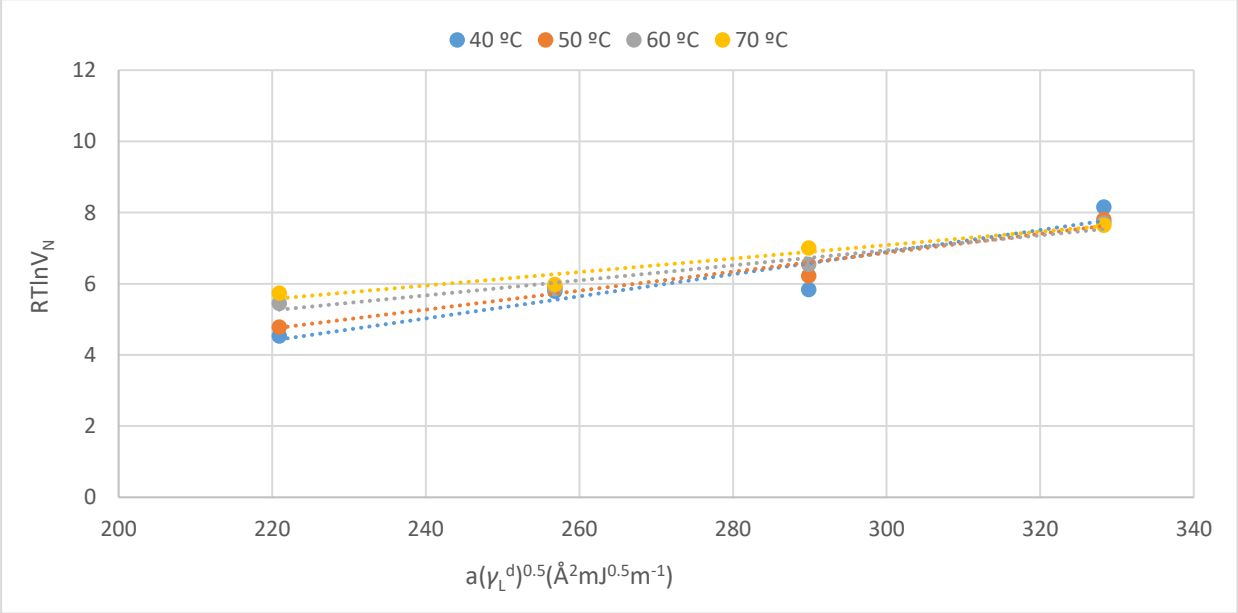

Figure 1. $RTlnV_N$ - $a(\gamma_L^d)^{0.5}(Å^2 mJ^{0.5} m^{-1})$graph for watermelon rind albedo in the temperature range of 40-70 °C.

The γ_{sd} values at different temperatures for the albedo of watermelon rinds analysed by inverse gas chromatography are listed in Table 2.

Table 2. Dispersive component of surface energy (γ_{Sd}) (mJ/m^2)

40ºC	**50ºC**	**60ºC**	**70ºC**
6.649	4.888	3.070	2.477

The results show that the surface free energy of the tested samples increases with increasing column temperature. It can be seen that the dispersive component gradually decreases. The values of the dispersive component of the surface energy, γ_{sd}, were calculated using the Schultz method. Examined by inverse gas chromatography, the γ_{sd} values decrease with increasing temperature for all samples (Fig. 1). Also RTlnVN–a.$(\gamma L\ d\ )^{1/2}$ The graphs showed good linearity for all articles.

CONCLUSION

In this study, the distribution component of the surface energy, the γ_{sd} values for the albedo of the watermelon rinds, was calculated using the Schultz method. For the sample analysed by reverse gas chromatography, the γ_{sd} values decrease with increasing temperature. In addition, the RTlnVN – a.$(\gamma_{sd})^{1/2}$ plots showed good linearity for the albedo of the watermelon peel.

The use of biomass as a source of renewable energy, bio-based materials or green chemistry reagents has increased in recent years. The green synthesis approach appears to be a cost-effective, non-toxic and environmentally friendly alternative suitable for large-scale production. In particular, interest in lignocellulosic biomass is increasing as it offers the advantage of a short production cycle while sequestering carbon.

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ORAL PRESENTATION

Diagnosis of Colorectal Cancer Using Newly Developed Cross-Over Local Binary Patterns and Transfer Learning Methods

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Abstract

Detecting the tumoral region is critical in the cancer diagnostic process. A recent study found that 2 million people died from lung cancer and 1 million from colon cancer. One of the most accurate ways for cancer diagnosis is a core detection and classification study based on pathology images. The primary purpose of such a study is to detect the tumor and learn more about its activity. There are certain complications while applying the pathology method to the patient. During the pathological procedure application phase, the patient may experience side effects and a variety of difficulties. However, it is incredibly difficult for pathology experts to properly assess the acquired samples. This study compared the results of different strong image processing technologies utilized in the cancer diagnostic procedure. The study compares transfer learning methods and the created local binary pattern method. The study focused on spectral histopathology pictures of the tumoral region. The analysis was carried out using the extremely effective LC25000 dataset, which comprises lung and colon cancer pathology images. The study's outcomes were as follows: 94.57% accuracy, 90.91% kappa, 94.6% precision, 94.6% recall/sensitivity, 94.6% F1 score, and 98.8% ROC area.

Keywords: Colorectal cancer, Cross-Over Local Binary Patterns, Deep Learning, Image Processing

INTRODUCTION

Cancer, one of today's most lethal diseases, is described as the fast multiplication of aberrant cells without visible symptoms. The World Health Organization (WHO) reported a detailed survey in 2020 that included nearly 150.000 persons from worldwide. According to this survey, the colon cancer global occurrence was nearly 20%. Colon cancer has the fourth highest global incidence. When colon cancer is analyzed in terms of cancer-related death rates worldwide, it is found to be 9% (https://gco.iarc.fr/, 2024). The main causes of colon cancer are poor nutrition, a lack of fiber, smoking, and alcohol consumption. According to the World Health Organization's official website, the global mortality toll from colon cancer was 600,000 in 2020, with this figure expected to approach 1 million by 2040.

The assessment of pathology reports is the most accurate way for cancer diagnosis. The study included an examination of pathology pictures for lung and colon cancers. Expert physicians must examine pathology photos, which is time-consuming and difficult. A physician with insufficient experience in the field is more likely to make poor decisions, which can lead to the patient's death. From this standpoint, the proper interpretation of pathology images is critical. Today, picture enhancement studies, particularly when deep learning (DL) algorithms are utilized, yield excellent results. This provides for more successful findings as compared to manual analyses performed by medical specialists. Classical computer imaging systems can struggle to deal with complex scenarios like disease identification, organ recognition, and bacterial colony categorization (Abiyev et al., 2018). However, one of the most common challenges in deep learning research is the necessity for huge labeled training data sets. For this goal, public web pages containing medical picture data sets have been made available, specifically for academic research. Classical computer imaging systems can struggle to deal with complex scenarios like disease identification, organ recognition, and bacterial colony categorization (Abiyev et al., 2018). However, one of the most common challenges in deep learning research is the necessity for huge labeled training data sets. For this goal, public web pages containing medical picture data sets have been made available, specifically for academic research.

In computer-aided image enhancement studies, in addition to achieving high accuracy rates, time savings are unavoidable. CNN-based deep learning approaches are at the cutting edge of image-enhancing techniques. The LC25000 data set contains data on colon cancer and lung cancer disorders organized into five different classifications. The data collection contains various histopathological photos for each class. The carcinoma type has a significant mortality rate among the primary causes of invasive cancer (Zullig et al., 2017). The study continues as follows.

RELATED WORKS

The study, conducted by González-Castro et al., applied more than one image-enhancing approach to pathology images. The study's results from various approaches were later re-evaluated using machine learning technologies. Kappa values were investigated as a result of re-evaluating the image enhancement approaches presented in the paper using machine learning methodologies. The study's LBP technique results were re-evaluated using the random forest machine learning method, yielding a Kappa value of 34.3 (González-Castro et al., 2020). In another investigation, the researchers employed four different effective image enhancement techniques. Nishio and Nagashima conducted their research using three different LBP-derived methodologies, one of which they created. The researchers questioned the area under the curve (AUC) value in each of the four approaches they tested. The random local binary pattern (RLBP) method had the greatest AUC value of 0.725 among the three distinct LBP-derived methods utilized for comparison (Singh et al., 2024).

In another significant study, the LBP method was applied on histological tissues obtained from tumoral regions. As a result of the application, area under the curve (AUC) = 0.995 and kappa = 0.90 were obtained. Turkki R. et al., who conducted the study, reported that extremely remarkable results were obtained in this study (Hage Chehade et al., 2022). In a hybrid study inspired by the LBP method, researchers examined two separate pathology data sets containing colorectal tissue and prostate tissue samples. With the MSBP-Net method developed by the researchers, the colorectal cancer classification F1-score value was 0.7225 and the prostate cancer classification F1-score value was 0.8553 (Hage Chehade et al., 2022). In 2019, researchers worked on medical images with a method derived from the LBP method. In this study, Murugappan and Sabeenian developed a hybrid method using the LBP method. In the study they conducted on colon cancer images, the researchers reported that they achieved 93% accuracy in the entire study (Murugappan et al., 2019).

USED ALGORITHMS

ResNet101

The ResNet101 method is developed from the ResNet method and consists of 101 levels. It outperforms several other approaches of transfer learning. Reducing the parameters is another essential factor that improves the efficiency of this strategy (Hu et al., 2021; Nawaz et al., 2023).

EfficientNetV2

It is a method derived from the EfficientNet method by Tan et. al. It was developed in 2019. The EfficientNetV2 method uses the maximum number of parameters. In this way, the effectiveness of the method also increases (Tan et al., 2021).

DenseNet201

DenseNet201 method is a method derived from DenseNet method. Especially in ResNet method, gradient flow in ResNet architecture was improved. Also DenseNet201 method compresses network topology (Mohandass et al., 2024).

Developed Cross-Over Local Binary Patterns

The developed LBP method is based on the classical LBP method, and the computation is done by comparing each pixel surrounding the core pixel to the pixel opposite it (180 degrees). The value acquired by comparing each pixel to the pixel in its symmetry is allocated as the new value for the central pixel. The novel LBP approach we proposed was called the Cross-Over LBP (CO-LBP) method. Figure 1 shows the architectural structure of the CO-LBP method.

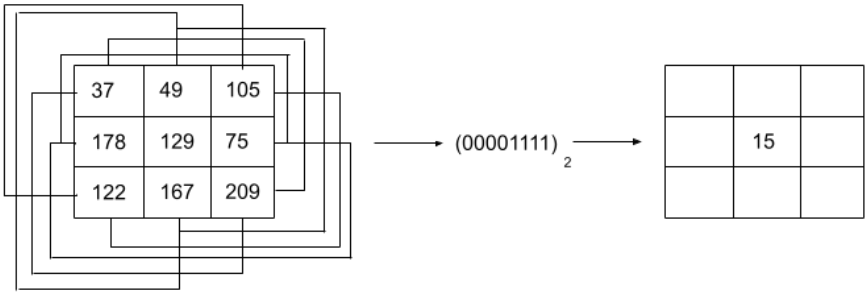

Figure 1: Architecture of CO-LBP method

The comparison method produces an 8-digit binary number. The center pixel value is set to the base-10 equivalent of the acquired integer. Figure 1 depicts the sub-image considered and the comparative results obtained. The following formula 1 represents the CO-LBP method.

$$CO - LBP = \sum_{i=0}^{P} c(P_i - P_c)2^t \quad (1)$$

According to the above formula, the comparison process proceeds as follows.

Pc = S(37>209), S(49>167), S(105>122), S(75>178), S(209>37), S(167>49), S(122>105), S(178>75) = 15

In the CO-LBP method, when the radius is accepted as 1, the obtained value is 15. In Figure 2 below, if the centre pixel (value 129) is considered and the radius is determined as 2,

Pc = S(28>84), S(174>195), S(207>64), S(147>67), S(84>28), S(195>174), S(64>207), S(67>147) = 60

With the CO-LBP method we developed, the center pixel value obtained by taking the radius as 2 is 60. If the default radius is set as 3;

Pc = S(39>148), S(38>218), S(193>122), S(157>139), S(148>39), S(218>38), S(122>193), S(139>157) = 60

It is obtained as. If the default radius is set to 4;

Pc = S(34>83), S(239>64), S(190>64), S(29>28), S(83>34), S(64>239), S(64>190), S(28>29) = 120

34	169	136	207	239	201	203	183	190
126	39	57	145	38	49	152	193	32
58	39	28	139	174	29	207	204	185
125	38	48	37	49	105	67	174	156
28	139	67	178	129	75	147	157	29
48	37	218	122	167	209	216	38	41
67	178	64	174	195	26	84	204	129
218	122	167	209	218	204	169	148	139
64	174	195	26	64	20	226	59	83

Figure 2: Pixel values of a sample image

As can be seen in the newly developed CO-LBP, a new pattern was obtained with each change of the radius. In the new method we proposed, a maximum of 4 radius values were considered around the central pixel, and a new pattern was obtained each time the Radius value was changed.

DATA AUGMENTATION AND PREPROCESSING

A large number of samples in the data set is critical for the successful completion of image processing analysis studies. Large data sets are required to accomplish this task. However, massive datasets are not always available. Many technical data augmentation approaches exist to increase the number of training datasets. These methods also help to create some new classes in the dataset. Increasing the amount of data is particularly critical in avoiding overfitting. The data augmentation software creates numerous variations by rotating the original images, randomly rotating them horizontally, or zooming them to a specific level, resulting in significant changes to the images' properties.

RESULTS AND DISCUSSIONS

The dataset considered for the study includes 25000 images of colon and lung cancer patients. The dataset evaluated in this work was analyzed using three different CNN-based algorithms. The trials were conducted on a PC equipped with an Intel i9 CPU 2.90GHz processor, 32.0 GB RAM, and an NVIDIA Quadro P2000 GDDR5 4 GB graphics card. The data was randomly divided by 30-70%, and the splitting process was carried out with various seeds.

	1						2						3						4					
	Test Accuracy	F1 Score	Kappa	ROC	Recall	Precision	Test Accuracy	F1 Score	Kappa	ROC	Recall	Precision	Test Accuracy	F1 Score	Kappa	ROC	Recall	Precision	Test Accuracy	F1 Score	Kappa	ROC	Recall	Precision
ResNet50	**67.53**	**67.54**	**35.08**	73.09	**67.53**	67.59	55.73	52.73	11.35	63.82	55.73	57.59	51.53	37.97	2.82	62.58	51.53	61.12	54.6	43.0	9.42	59.15	54.6	**75.55**
ResNet101	62.53	59.65	25.01	72.73	62.53	67.49	58.27	50.14	14.98	64.26	58.27	**70.45**	**59.0**	**53.73**	**17.11**	62.38	**59.0**	65.33	**58.27**	**50.0**	**14.97**	**61.07**	**58.27**	70.94
EfficientNet-V2	63.8	60.6	27.38	74.0	63.8	**70.13**	**60.07**	**59.68**	**20.68**	65.27	**60.07**	60.97	50.4	39.69	1.91	58.71	50.4	53.42	52.4	41.39	5.35	60.03	52.4	60.97
DenseNet201	56.67	49.92	13.59	70.83	56.67	64.85	50.53	33.93	0	50.0	50.53	25.54	54.6	43.8	6.37	62.93	54.6	62.45	50.8	34.23	0	50.0	50.08	25.8
MobileNet	67.2	66.65	34.42	**75.84**	67.2	68.45	59.07	52.72	18.29	**67.63**	59.07	59.87	58.0	49.68	14.9	**63.4**	58.0	**71.18**	56.6	46.14	10.91	59.78	56.6	72.3

Figure 3: the results of TL methods to analyze the CO-LBP method pre-processed colorectal cancer histopathological images

The highest test accuracy result was 67.53%, the highest F1 score result was 67.54%, and the highest kappa value was 35.08% obtained with the ResNet50 method with radius = 4 (n = 4). The MobileNet technique produced the highest ROC value of 75.84% when n = 4. The highest recall result was 67.53%, and the highest precision result was 75.55% obtained with the ResNet50 method when n = 4 (Figure 3). The summary of all results is displayed in Table 1.

The features were examined using some of the most well-known TL methods. In this study, the results obtained from the CO-LBP method are very successful.

Table 1: Summary of analysis studies performed with TL methods after the preprocessing stage of colorectal cancer images with the CO-LBP method.

	Test Accuracy	F1 Score	Kappa	ROC	Recall	Precision
ResNet50	Step 1 (67.53)	Step 1 (67.54)	Step 1 (35.08)		Step 1 (67.53)	Step 4 (75.55)
MobileNet				Step 1 (75.84)		

Table 2 shows a comparative table of the outcomes of this investigation and other recent studies. The results produced with the developed CO-LBP approach were quite successful.

Table 2: comparative results of recently conducted studies and this study

Studies	Used methods	Accuracy (%)	Kappa (%)	Precision (%)	Recall / Sensitivity (%)	F1 Score (%)	ROC Area (%)
(Tahir et al., 2023)	LBP+VGG16	99		99	98.8	98.8	
(Vuong et al., 2022)	MSBP	87.32			84.5	84.34	91.90
(Murugappan et al., 2019)	MGRLBP	93.02			92.27		
(Eresen et al., 2020)	LBP	65.38			84.6%		
(Kumar et al., 2022)	LBP	98.6		98.63		98.5	
(Sasmal et al., 2021)	LBP	90.12					91
(Yazdi et al., 2020)	LBP	90.8					
(González-Castro et al., 2020)	LBP	88	64.7		88.9		
Proposed study	CO-LBP	94.57	90.91	94.6	94.6	94.6	98.8

(HOG* = Histogram oriented gradient)

CONCLUSION

The study analyzed photos from the entire LC25000 dataset to diagnose colorectal cancer. The study's primary goal is to evaluate photos of colorectal cancer patients using binary-based, extremely effective image-enhancing technology. The study involves the recently developed CO-LBP technique. Since its conception, the local binary pattern technique has resulted in numerous versions that have gained interest in image improvement research. Although the local binary pattern algorithm serves as the foundation for many different algorithms, several of these methods have yielded excellent results. The characteristics generated by the CO-LBP approach were re-evaluated using the majority of TL methods. The reclassification process had an extraordinarily high success rate. The most successful outcome was obtained by identifying the ResNet50 approach. One of the study's most remarkable findings is the expansion of beneficial outcomes achieved within its scope. Future study will include model training with multiple datasets of histological images of colorectal cancer, as well as efforts to further refine it.

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ORAL PRESENTATION

Omega 6 / omega 3 oranın insan ve hayvan sağlığı üzerine etkileri

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Özet

Günümüz dünyasında kötü beslenme veya beslenme yetersizliği sıkça karşılaşılan önemli bir sorundur. Kötü beslenme veya yetersiz beslenme sonucu gelişim bozuklukları, vücut fonksiyonlarının düzenli çalışamaması ve bireyin hastalıklara açık hale gelmesi kaçınılmazdır. Sağlıklı yaşam, büyüme, gelişme, zihinsel ve bedensel fonksiyonlarının sürekliliği yeterli ve dengeli beslenme ile sağlanabilir. Özellikle esansiyel yağlar, bu fonksiyonların devamı için vazgeçilmezdir. Üzerinde oldukça fazla çalışma yapılan omega 3(ω-3) ve omega 6(ω-6) yağ asitlerinin fonksiyonlarının yanı sıra ω-6/ ω-3 oranın belli bir dengede olması ile metabolizmanın daha düzenli çalıştığı birçok çalışma ile kanıtlanmıştır. Geçtiğimiz yüzyılın ortalarında Avrupa'da bu yağ asitlerinin oranı yaklaşık 1/1 iken günümüzde 30/1 seviyelerine ulaşmıştır. Benzer durum dünyanın diğer bölgelerinde de yaklaşık olarak aynıdır. İdeal oran konusunda belirli bir ortak görüş olmasa da 1/1 ve 4/1 arasında vücut fonksiyonlarına bağlı olarak ideal oranlar değişmektedir. ω-6'nın çok fazla alınmasıyla birlikte inflamatuar etkilerde artış ve bu artışa bağlı hücre yapısında bozulma, ağrı, depresyon, kalp damar hastalıkları gibi kronik hastalıklar daha sık görülür. ω-3 ise ω-6'nın bu olumsuz etkilerini engelleyerek antienflamatuvar, antiperoksidatif, antioksidan vb. etkileriyle dikkatleri üstüne çekmektedir. Hem insanlarda hem de hayvanlarda ω-6/ ω-3 oranın yükselmesiyle oluşan olumsuz etkilerin, oranın düşmesiyle tekrar normale döndüğünü gösteren oldukça fazla çalışma vardır. Ayrıca bu oranın takviyeler ve beslenme ile düşürülmesi ile kronik hastalıklarda kullanılan ilaçların kullanımının azaldığını belirten çalışmalar da bulunmaktadır.

Anahtar Kelimeler: Omega 6, omega 3, antienflamatuvar, EPA, DHA

Giriş

İlk insandan günümüze kadar geçen süreçte yaşamın devam edebilmesi için vazgeçilmezimiz olan beslenme çok büyük değişikliklere uğradı. Zamana ve çevreye göre değişen beslenme alışkanlıklarımız teknoloji ve endüstrinin gelişmesiyle daha hızlı değişti. Bu süreç beraberinde birçok olumsuzluğu getirdi. Gıdaların yapılarının bozulması, daha fazla ve daha hızlı üretim ve kazanç için doğal ürünler yerine kimyasalların kullanılması bu olumsuzlukların belirgin olmasına ve doğrudan genel sağlığımızın bozulmasına yol açtı. Bu değişimden hemen tüm organik maddeler etkilendi.

Beslenmedeki en önemli organik maddelerden biri olan yağlar, sadece yüksek enerji kaynağı olmakla kalmayıp; yağda çözünen vitaminleri bulundurmaları, taşıma, koruma, dengeleme gibi birçok metabolik olayda görev alır. Yağların temel yapı taşları olan yağ asitlerinin birçoğu metabolik faaliyetlerle oluşabildiği gibi esansiyel olarak almamız gereken ve oldukça önemli yaşamsal fonksiyonları olan yağ asitleri de mevcuttur. Esansiyel yağ asitlerinden en önemlileri doymamış yağ asitleri olan Omega 3(ω-3) ve Omega 6(ω-6)'dır. Doymamış yağ asitlerini içeren özel bir bileşik olan ω-3 özellikle hücre gelişimini ve kas aktivitesini düzenler. Esansiyel olarak alınan ω-3 aynı zamanda vücuttaki yağların enerjiye dönüştürülmesine de yardımcı olur. Omega 6(ω-6) esansiyel olarak alınan ve hücre yapısının oluşmasına katkıda bulunan önemli bir doymamış yağ asididir. Hemen hemen tüm hücrelerin yapımına katıldığı gibi ω-6 yağ asitlerinin miktarının arttığı durumlarda fizyolojik olarak yapılan incelemelerde protrombik, kan viskozitesinde artış, vazospazm, vazokonstriksiyonda azalma gözlenmiştir. Ayrıca ω-6 yağ asitleri oranındaki artış trombüs, aterom, alerjik ve iltihaplı bozuklukların yanında duyarlı kişilerde hücre çoğalmasına yol açar. Her ikisi de esansiyel ve çoklu doymamış yağ asitleri (PUFA) olan ω-3 α-linolenik asit (ALA, 18:3ω-3), ω-6 çoğu diyette en yüksek miktarlarda linoleik asit (LA, 18:2ω-6) ve araşidonik asit (AA, 20:4ω-6) şeklinde bulunur. Bitkilerde sentezlendikleri için LA ve ALA büyük miktarlarda, öncelikle bitkisel gıdalarda bulunur. Örneğin, birçok tohum, kuruyemiş ve bitkisel yağ LA ve AA açısından zengindir; bunlar arasında aspir, ayçiçeği ve kabak çekirdeği ve soya fasulyesi yağları bulunur. Keten tohumları ve keten tohumu yağı gibi iyi ALA kaynaklarıdır (Djuricic ve ark., 2021). Yağlı balıkların önemli ölçüde ω-3 kaynağı olduğu da göz ardı edilememelidir.

Araşidonik asit eikonozidlerin biyosentezi için yaygın endojen öncülüdür ve fosfolipaz A2'nin etkisiyle hücre membran fosfolipdlerinden salınır. Araşidonik asit siklooksijenaz, lipoksioksioksijenaz veya epoksijenazlar yardımıyla sırasıyla prostaglandinler, lökotrienler ve endoperoksidazlar gibi hızla çeşitli lipid mediyatörlerine dönüşür. Bu nedenle diyetle alınan ω- 6 miktarındaki artış başta inflamatuar etkiyi tetikler. Memeli hücreleri, omega 3 desaturaz enzimleri bulunmadığı için ω-6'yı ω-3'e dönüştüremez. AA ve LA birikimi sonucu oluşan olumsuz etkiler ALA ile tersine döndürülmektedir. ω-6 ile ω-3'ün fizyolojik etkileri hemen hemen zıt yönlüdür. Bu nedenle omega-6/omega-3 esansiyel yağ asidi (EFA) oranının 1/1 olması daha sağlıklıdır. Birçok bilgi kaynağı, 150 yıl öncesinde insanların omega-6/omega-3 esansiyel yağ asidi (EFA) oranı 3-1/1 olan bir diyete sahip olduğunu bildirir. 20. Yüzyılın ortalarında endüstrinin gelişmesiyle birlikte insan ve hayvanların diyetlerindeki bu oranın 15/1–16.7/1 olduğunu ileri sürmektedir. Günümüzde bu oranın Avrupa ve Amerika'da 30/1 hatta gelişmemiş ülkelerde çok daha fazla olduğu düşünülmektedir. Güncel diyetler omega-3 yağ asitleri bakımından yetersizdir ve insanların evrimleştiği genetik olarak belirlenmiş diyete kıyasla aşırı miktarda omega-6 yağ asidi içerir. Aşırı miktarda omega-6 çoklu doymamış yağ asitleri (PUFA) ve çok yüksek omega-6/omega-3 oranı, kardiyovasküler hastalıklar, kanser ve inflamatuar ve otoimmün hastalıklar dahil olmak üzere birçok hastalığın patogenezini desteklerken, aynı zamanda omega-3 PUFA içeriğini artırmanın (düşük omega-6/omega-3 oranı) baskılayıcı bir etkisi vardır (Kirshenbauer, 1960; Simopoulos, 2002). Araştırmacıların bir kısmı omega-6/omega-3 oranın azaltılması ile hastalıkların sağaltımının doğrudan bağlantılı olduğunu bildirmişlerdir.

Farelerde beslenmenin ani kardiyak ölümler üzerine etkilerinin araştırıldığı bir çalışmada omega 3'ten zengin takviye edilen grupta ani kardiyak ölümde %45 oranında bir azalma görülmüştür. Omega-6 ve omega-3 yağ asitleri için toplam alımla ilgili diyet verileri olmamasına rağmen, ani ölümdeki fark büyük olasılıkla eikosapentaenoik asit (EPA)(ω-3) ve dokosaheksaenoik asit (DHA)(ω-3) artışından ve hücre zarı fosfolipitlerindeki AA'nın azalmasından kaynaklanmaktadır. AA'dan türetilen prostaglandinler proaritmiktir, oysa EPA'dan türetilen karşılık gelen prostaglandinler değildir (Li ve ark., 1997). Singh ve arkadaşları (2002) yaptıkları benzer çalışmada ani kardiyak ölümlerin ve ölümcül olmayan miyokard enfarktüslerinin azaldığını bildirmişlerdir. Hem normal sıçan kardiyomiyositlerinin hem de insan meme kanseri hücrelerinin, yuvarlak solucan *Caenorhabditis elegans*'tan elde edilen omega-3 yağ asidi desatürazını kodlayan cDNA ile beslendiğinde, Omega-6 yağ asitlerinin Omega-3 yağ asitlerini oluşturduğu açıkça göstermiştir. Böylece, omega-6 LA, omega-3 ALA'ya dönüşürken; AA, EPA'ya dönüştürülmüştür. Bu nedenle omega-6'nın omega-3 PUFA'ya oranı 1/1'e yakın olduğu görülmüştür (Kang ve ark., 2001). Daha ileri çalışmalar, omega-3 desaturazın varlığında kanser hücrelerinin apoptotik ölüme uğradığını, buna karşın daha yüksek omega-6/omega-3 oranına sahip kontrol kanser hücrelerinin çoğalmaya devam ettiğini göstermiştir (Ge ve ark., 2002).

Bartram ve arkadaşları (1993;1995) rektal epitel hücre çoğalmasını ve PGE 2 biyosentezini bastırmak için balık yağı takviyesi verilen çalışmalarında omega-6/omega-3 oranı 2,5/1 olduğunda olumlu sonuç elde edildi ancak aynı mutlak balık yağı alımı seviyesi ve 4/1'lik bir omega-6/omega-3 oranı ile elde edilmedi. Daha yakın zamanda, Maillard ve arkdaşları (2002) bir vaka kontrol çalışmalarında meme yağ dokusundaki omega-3 asitlerinin meme kanseri riski üzerinde koruyucu bir etkiye sahip olduğunu belirtmişlerdir.

Omega 3 ve omega 6 yağ asitlerinin solunum üzerine etkileri konusunda yapılan çalışmalarda Omega6/ omega 3 oranının 1/1'e yaklaştıkça olumlu etkilerin de arttığı bildirilmiştir. Laitinen ve ark., 1993; Broughton ve ark., 1997)

Omega-3 indeksi, stabil koroner arter hastalığı olan hastalarda inflamasyonla (CRP ve IL-6) ters orantılı olduğu bulunmuştur (Farzaneh-Far ve ark., 2009) Başka bir çalışmada, 60 yaşın üzerindeki kişilerde omega-3'lerin inflamasyonu (CRP ve IL-6) azalttığını ve insülin duyarlılığını iyileştirdiğini bulunmuştur (Tsitouras ve ark., 2008).

LA açısından zengin yağ asitleri ile karşılaştırıldığında, 3 ay boyunca ALA'dan zengin yağ saitleri içeren gıdaları tüketmenin CRP'yi (%38), serum amiloid A'yı (%23,1) ve IL-6'yı (%10,5) azalttığı tespit edilmiştir (Rallidis ve ark., 2003). Dahası, LA açısından zengin margarinle karşılaştırıldığında, ALA açısından zengin margarin (toplam ALA alımı yaklaşık 6-8 g/gün) 1 ve 2 yıl sonra CRP'yi önemli ölçüde düşürdüğü bir başka çalışmada bildirilmiştir (Bemelmans ve ark., 2004). Faintuch ve arkadaşları (2007) 30 gram keten tohumu ununun (5 g ALA/gün) CRP'yi, serum amiloid A'yı, beyaz kan hücresi sayısını ve fibronektini önemli ölçüde azalttığı bulmuştur; bu da keten tohumunun obezitede kronik düşük dereceli inflamasyonu baskılamada faydalı olabileceğini düşündürmektedir.

Omega 6 yağ asitlerinin proinflamatuar etkilerinin ve omega 3 yağ asitlerinin anti inflamatuar etkilerinin araştırıldığı 2 ayrı çalışmada omega-6'nın omega-3 ile değiştirilmesinin veya omega6/omega 3 oranının azalmasıyla inflamasyonu azalttığını gösterilmiştir (Tulk ve Robinson, 2009; Nelson ve Hickey, 2004)

Benzer şekilde fareler üzerinde yapılan farklı çalışmalarda (Yamashita ve ark., 2005; Wang ve ark., 2009) omega6/ omega 3 oranının azalmasıyla farelerde aterosklerozu ve trombogenezin azaldığı bildirilmiştir.

Stoll ve arkadaşları (1999) EPA ve DHA'nın remisyonu uzattığını, yani bipolar bozukluğu olan hastalarda nüks riskini azalttığını gösteren çalışmaların yanı sıra Tiemeier ve arkadaşları (2003) yaptıkları çalışmada Depresif bozuklukları olan deneklerin ruh hali üzerinde omega 3 yağ asitlerinin doğrudan bir etkisi olduğunu ileri sürmüşlerdir.

Özetle bahsi geçen oran farklı hastalıklarda farklı oranlarda daha etkilidir. Kardiyovasküler hastalıkların ikincil önlenmesinde, 4/1 oranı her nedene bağlı ölüm oranında %70'lik bir azalma ile ilişkilendirilmiştir. Kolorektal kanserli hastalarda 2,5/1 oranı rektal hücre proliferasyonunu azaltırken, aynı miktarda omega-3 PUFA ile 4/1 oranının hiçbir etkisi olmamıştır. Meme kanseri olan kadınlarda daha düşük omega-6/omega-3 oranı daha düşük risk ile ilişkilendirilmiştir. 2-3/1 oranı romatoid artritli hastalarda inflamasyonu baskılamış ve 5/1 oranı astımlı hastalarda faydalı etki göstermişken, 10/1 oranı olumsuz sonuçlar doğurmuştur. Bu çalışmalar, optimum oranın spesifik hastalığa bağlı olarak değişebileceğini düşündürmektedir. Bu, kronik hastalıkların multigenik ve multifaktöriyel olması gerçeğiyle tutarlıdır. Bu nedenle, omega-3 yağ asitlerinin terapötik dozunun, genetik yatkınlıktan kaynaklanan hastalığın şiddetine bağlı olması oldukça olasıdır. Batı toplumlarında ve gelişmekte olan ülkelerde yaygın olan ve dünyanın geri kalanına yayılan birçok kronik hastalığın riskini azaltmak için omega-6/omega-3 yağ asitleri oranının daha düşük olması tercih edilir. (Simopoulos, 2002).

Sonuç

Kronik rahatsızlıkların çoğu -kardiyovasküler hastalık, diyabet, kanser, obezite, otoimmün hastalıklar, romatoid artrit, astım ve depresyon- tromboksan A 2 , lökotrien B 4 , IL-1β, IL-6, TNF ve C-reaktif protein üretiminin artmasıyla ilişkilidir. Bu faktörler omega-6 yağ asidi alımındaki artışlarla artar ve omega-3 yağ asidi alımındaki artışlarla azalır. İnsanlık tarihinin çok büyük kısmında omega-6/omega-3 EFA oranının yaklaşık 1 olduğu dönem son 100-150 yılda hızla değişerek 30/1 oranına kadar yükselmiştir.

Çalışmalar, omega-6/omega-3 açısından dengelendiğinde arka plan diyetinin ilaç dozunu azalttığını göstermektedir ilaç tedavisi ile diyet seçimleri arasında tamamlayıcılık potansiyeli olduğunu, omega-3 yağ asitleri alımının artırılmasının ve omega-6 yağ asitleri alımının azaltılmasının ilaç tasarrufu sağlayan etkilere yol açabileceğini öne sürmektedir

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ORAL PRESENTATION

Preparation and *in-vitro* characterization of Eudragit® based vaginal nanofiber formulations

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Abstract

Nanofibers can be an excellent alternative to classical dosage forms in vaginal applications due to flexibility, high loading capacity, high mucoadhesive strength, high surface area/volume ratio, and high encapsulation efficiency. Eudragit®s, which are different synthetic polymers composed of methacrylate monomers, are preferred for vaginal drug delivery systems for controlled drug release. The aim of this study was to develop and in vitro evaluation of Eudragit® based nanofibers for vaginal drug delivery. Nanofiber formulations were prepared at different concentrations in ethanol:dimethylformamide (DMF) (7:3). Eudragit E100 (EE100) was used as polymers to prepare electrospinning solutions. Polymer solutions were characterized by surface tension, viscosity and conductivity. Electrospinning method was used for producing nanofiber. The contact angle measurements of formulations were measured by optical tensiometer. Mechanical and mucoadhesive properties of nanofiber formulations were evaluated using Texture Analyzer. The viscosity, surface tension and conductivity values of the polymer solutions were found to be in a suitable for electrospinning. Viscosity values increased with increasing polymer content. All formulations had hydrophobic properties with contact angles values above 90°. Mechanical properties of formulations were increased with increasing polymer concentration. The highest mucoadhesion value was obtained for formulation E4 (40% Eudragit®). It was seen that Eudragit-based formulations were suitable for vaginal applications.

Keywords: electrospinning, nanofiber, vaginal drug delivery

INTRODUCTION

Vaginal drug delivery systems have a promising and potentially important role in terms of their ability to exert local or systemic effects (Subi et al., 2024). Nanofiber is a very useful dosage form for vaginal drug delivery and provides several advantages such as prolonged release, reduced side effects, bioadhesive properties and localized drug delivery for vaginal drug administration (Tuğcu Demiröz et al., 2021).

Drug delivery systems such as nanoparticles, liposomes and dendrimers are used in vaginal drug delivery (Ensign et al., 2014). Among different drug delivery systems, nanofibers are also useful in enhancing the release, biodistribution and retention of drug substances in the vagina (Cazorla-Luna et al., 2021).

Nanofibers can be produced using various techniques such as solution blowing, centrifugal spinning, pressurized gyration, phase separation and electrospinning (Tuğcu Demiröz et al., 2020). Electrospinning method is an easy, versatile, economical, productive, and applicable in different areas (Saar and Tuğcu, 2023). Nanofibers can be easily produced by electrospinning with synthetic or natural polymers (Turanlı et al., 2019).

Cationic methacrylate polymer Eudragit E100 (EE100) was selected as the polymer carrier for the preparation of electrospun nanofiber (Alasino et al., 2012). EE100 is a pH-dependent polymer and acts by swelling when the pH is lower than 5 (Baranauskaite et al., 2019).

Aim of this study was to produce different concentrations of EE100 fibers and to determine the appropriate nanofiber formulation for vaginal application. Contact angles, mucoadhesive and mechanical properties of nanofibers produced with electrospinning were evaluated for vaginal use.

MATERIALS AND METHODS

Materials

Eudragit® E100 was a generous gift from Evonik (Germany). N, N-Dimethylformamide (DMF) and ethanol were purchased from Sigma Aldrich (St. Louis, MO). All chemicals were of analytical grade.

Preparation of nanofiber formulations

Nanofibers were prepared by electrospinning method. Ethanol:DMF (7:3) solvent system was chosen to dissolve the polymers. Electrospinning solutions were prepared by dissolving polymers with different concentrations at room temperature. Codes and content of the nanofiber formulations were shown in Table 1.

Table 1. Codes and content of the nanofiber formulations

Codes	Polymer Concentration (%)	Voltage (kV)	Feed rate (mL/sa)	Distance (mm)	Rotating Speed (rpm)
E3	30	19	1	125	100
E4	40	21.5	1.7	130	100
E5	50	20	1.7	135	100

Characterization studies of polymer solutions

Viscosity, conductivity and surface tension of the polymer solutions were characterized before electrospinning. The viscosity of polymer mixtures was measured using the Brookfield viscometer. Conductivity measurements of the polymer mixtures were carried out using a conductometer. The pendant observation drop method was used to measure the surface tension (Attension, Theta Lite, Finland).

Electrospinning process

Nanofiber formulations were prepared using a single nozzle equipment electrospinning (Inovenso Ltd, NE300, Turkey). Polymer mixtures were loaded into the pump using 10 ml syringes. Different voltage values were tested to produce each formulation and production was carried out at voltages where continuous jet formation occurred. Nanofibers were collected in the collector at a rotation speed of 100 rpm. Production parameters for each formulation are given in Table 1.

Mechanical characterization

Mechanical properties of nanofiber formulations carried out using Texture Analyzer (TA.XT. PlusTexture Analyzer, Stable Micro Systems, UK) equipped with mini tensile grip. Nanofibers were cut into 1 cm × 3 cm samples and attached to the apparatus. Tensile strength (MPa) and elongation at break (%) were calculated from the strain-stress graphs. At least three samples were tested.

Contact angle measurements

The contact angle measurements of formulations were carried out by Attension Theta Lite optical tensiometer. The nanofiber formulations were placed on a convex sample holder. Distilled water was used to form the drops and their contact angle values were measured using device software.

Ex-Vivo Mucoadhesion Studies

The work of mucoadhesion of formulations was measured using the TA.XT. Plus Texture Analyzer with cow vaginal tissue. Nanofibers were attached to the upper probe of the device and vaginal tissue was placed on the bottom. The work of mucoadhesion was calculated from the area under the force-distance curve (Saar and Tuğcu-Demiröz, 2023).

RESULTS AND DISCUSSION

Characterization of polymer solutions

Characterization results of polymer solutions were given in Table 2. It was observed that the conductivity, viscosity, and surface tension values differed due to the polymer concentration. The highest viscosity was observed in the E5 formulation. The polymer solutions were suitable for the electrospinning method.

Table 2. Characterization of polymer solutions

Codes	Conductivity (µS/cm)	Surface Tension (µN.m^{-1})	Viscosity (cP.s)
E3	12,65±0,29	24,30±0,09	272,67±12,05
E4	11,26±0,24	24,29±0,65	527±0
E5	10,24±0,11	24,69±0,39	2004±0

Mechanical characterization of nanofibers

Characterization result of formulations were shown in Table 3. The tensile strength and elongation at break values of nanofiber formulations were increased depending on the EE100 concentration. The mechanical properties of nanofibers can depend on numerous factors such as the polymer molecular weight, concentration, processing parameters, and environmental conditions (Alharbi et al., 2023). The increase in the polymer concentration caused a change in the mechanical properties of the nanofibers.

Table 3. Characterization results of nanofiber formulations

Codes	Tensile strength (MPa)	Elongation at break (%)	Contact Angle (°)	Work of Mucoadhesion (mJ/cm^2)
E3	0,954±0,048	1,990±0,827	118,58±0,86	0,038±0,005
E4	0,933±0,106	4,247±0,582	95,21±3,84	0,047±0,016
E5	2,257±0,107	9,727±1,087	111,15±3,45	0,043±0,006

Contact angle measurements of nanofibers

The contact angle value indicates the wettability of the nanofiber. The contact angle value higher than 90° is indicative of the hydrophobicity of the polymer (Table 3). In their study, Abdel-Rahman et al. determined the contact angle of the nanofiber formulation produced with EE100 as 136° (Abdel-Rahman et al., 2020).

Ex-vivo mucoadhesion studies of nanofibers

The work of mucoadhesion values of the nanofiber formulations are shown in Table 3. The highest mucoadhesion value was observed in the E4 formulation. The positive charge present in EE100 nanofibers may explain the stronger contact with the mucosa and provide the necessary residence time for the drug in the vagina (Turanlı and Acartürk, 2021).

CONCLUSION

We evaluated EE100 fibers with different concentrations as a vaginal delivery system for vaginal use. Contact angles, mechanical and mucoadhesive properties of nanofibers for vaginal application were investigated. We showed that the mucoadhesive and mechanical properties of the fibers changed depending on the EE100 concentration. It was found that the E5 formulation has superior properties compared to the other formulations. It was concluded that the E5 nanofiber formulation could be a promising alternative for vaginal delivery of different drugs.

ACKNOWLEDGEMENTS

This study was supported by Gazi University Scientific Research Projects Coordination Unit under grant number 02/2020-17.

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ORAL PRESENTATION

Investigation of the Potential of Furniture Industry Waste as Biomass Source

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Abstract

Excessive consumption and use of fossil fuels (coal, oil and natural gas) causes air pollution and greenhouse gas emissions. Unlike fossil fuels, biomass energy, one of the renewable energy sources, has attracted increasing attention in recent years. Biomass can be used as a renewable energy source because it is easily available and has environmentally friendly properties. Main biomass sources can be classified as agricultural products, animal waste, forest waste, food waste, and industrial waste. In our country, wastes such as hazelnuts, walnuts, rice husks, sawdust and corn cobs can be evaluated as biomass sources. It is of great importance to evaluate furniture industry waste, which is especially produced as a large amount of waste, as a biomass source. In this study, the potential of using furniture industry wastes (fir tree sawdust and medium density fiberboard) as biomass sources was investigated. In order to evaluate furniture industry wastes as a biomass source, both energy and physicochemical characterization of the wastes are very important. For this reason, firstly proximate (%ash, volatile matter and fixed carbon) and ultimate (%C, H, N, S and O) analyses of the wastes were carried out. Secondly, the energy potential (High heating value) of the wastes was examined and evaluated. The carbon content of fir tree sawdust (FS) and medium density fiberboard (MDF) products were measured as 46.29% and 44.79% respectively. One of the most important reasons for the high heating value (HHV) of the products is that they have high carbon content. The high heating values of FS and MDF products were determined as 17.87 and 18.97 MJ/kg.
According to the results obtained in the study; furniture industry wastes can be used as biomass fuel material because they have high heating value and low ash content.

Keywords: Biomass, Biofuel, Furniture Industry Waste

INTRODUCTION

Global energy demands are increasing due to increasing population and industrialization. Oil, coal and natural gas represent fossil fuel resources. The decreasing use of fossil resources causes an increase in renewable energy resources. Due to these developments, studies on the development of alternative energy sources have accelerated in recent years. It is important that alternative energy sources are renewable for a clean environment. In recent years, studies have been carried out on the evaluation of waste in energy production. For this reason, the use of agricultural, forest and industrial wastes as biomass sources is increasing (Al-Hamamre, 2017; Demir 2022).

Compared to fossil resources such as coal, oil and natural gas, which contain hydrocarbons, biomass is a clean, abundant, cost-effective, CO_2-free, low-sulfur, easily stored and non-polluting renewable material that can be used for heat and fuel production. Biomass has a faster life cycle than fossil fuels and is a sustainable energy source. Biomass energy is one of the fourth largest potential energy sources in the world and is considered as the energy carrier of the future. At the same time, biomass energy meets more than 10% of the world's global energy demand (Masnadi vd., 2014; Vu Ly vd., 2015). Biofuels can be obtained using different biomass materials. The main biomass sources can be classified as wood waste, agricultural waste, food waste and industrial waste (Al-Hamamre, 2017). In recent years, forest biomass has been used for energy production.

Forest biomass is usually produced by wood product industries (paper mills, furniture industry and sawmills (Martinez., 2021). Furniture manufacturing is one of the oldest industrial enterprises in the world. With the increase in technological developments, furniture production has also increased. In the furniture production industry, a large amount of wood waste is generated after the cutting and sanding processes of wood panels, the raw materials of which are wood panels. These wood wastes should be used in energy production.

Reconstituted panels and sawdust, especially those produced as waste, such as MDF (medium density fibreboard), have also been investigated in recent years (Özçifçi ve özbay, 2012; Pinho, 2023).

In this study, the potential of usability of furniture industry waste as a biomass source was investigated. In order to evaluate furniture industry wastes as a biomass source, both energy and physicochemical characterization of the wastes were carried out. For this reason, firstly proximate (%ash, volatile matter and fixed carbon) and ultimate (% C, H, N, S and O) analyses of the wastes were carried out. Secondly, the energy potential of the wastes (High heating value) was examined and evaluated. In addition, after evaluating the energy potential of furniture production waste, the results were compared with similar studies in the literature.

MATERIALS AND METHODS

Preparation of raw Furniture Industry Waste

In this study, two different furniture industry wastes were used as raw materials. One of them is fir tree sawdust (FS). The other is medium density fiberboard (MDF) powder. These wastes were first dried in an oven at 100°C for 10 hours. The dried biomass waste was reduced to small pieces by passing through a steel blade grinder. Those that reached a certain size (below 250 μm) were used for measurement. The image of the furniture industry waste used in the experiment is shown in Figure 1.

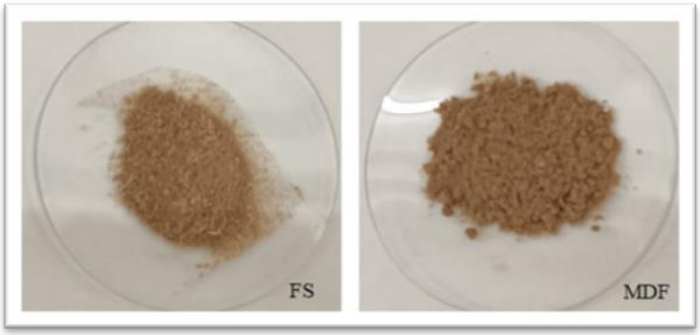

Figure 1. Waste generated in furniture manufacturing

Proximate and Ultimate analysis of Furniture Industry Waste

Proximate analysis (%ash, volatile matter and fixed carbon) and ultimate analysis (%C, H, N and S) were performed in the characterization of FS and MDF wastes. In addition, the equation given below was used to determine the HHV of FS and MDF waste (Sheng and Azevedo, 2005).

$$HHV = -1.3675 + 0.3137C + 0.7009H + 0.0318O \quad (1)$$

RESULTS AND DISCUSSION

Biomass wastes that can be used in energy production can be classified as forest wastes, agricultural wastes, municipal wastes and industrial wastes (Bardhan vd., 2021). In this study, the potential of using different furniture industry wastes (FS and MDF) as biomass materials was investigated. Firstly, the properties of the wastes in terms of ash, volatile matter and high heating value were examined and evaluated.

Because it is very important to investigate these parameters that determine the quality of biomass. Proximate and Ultimate analysis results of FS and MDF products are presented in Table 1. Proximate analysis of biomass sources are important parameters that directly affect the combustion process. Biomass with high ash content is not preferred as fuel. Biomass with high carbon content is mostly preferred as a biomass source (Taşar, 2021). The volatile matter content of FS and MDF products was calculated as 79.26% and 78.13%, respectively. The volatile matter content of biomasses reported in the literature varies between 50-94%. Ash content in the literature is between 0.1-25% (Wang vd., 2021). The ash values of FS and MDF biomasses are given in Table 1. As can be seen from the results, it can be said that the ash values of both FS and MDF products are lower than the results reported in the literature.

Table 1. Proximate and ultimate analysis of products

Parameter	FS	MDF
Proximate analysis (wt.%)		
Volatile Matter	79.26	78.13
Ash	0.3	1.34
Fixed Carbon[a]	20.44	20.53
Ultimate analysis (wt.%)		
C	46.29	44.79
H	4.49	6.93
N	-	3.26
S	-	-
O[a]	49.22	45.02

[a]fark

Ultimate analysis results of some biomass wastes have been reported in the literature. According to the ultimate analysis results, Nitrogen (N) and Sulfur (S) values of biomass vary between 0-8.65% and 0-2.01%, respectively (Wang vd., 2021). S and N values in biomass are expected to be low in order not to create greenhouse gases. As can be seen from the results, there is no %S in the content of the products (FS and MDF). In addition, the FS product has no %N content. The %N content of the MDF product is consistent with the literature. In this study, the fuel properties of furniture industry wastes (FS and MDF) were compared with different biomass types. For this purpose, high heating value comparison (HHV) was made between FS and MDF products and different biomass types. Considering the analysis results, the potential of waste products to be used as biofuels compared to different types of biomass was evaluated. The high heating value (HHV) results calculated using the elemental analysis results of FS and MDF products are presented in Table 2.

Table 2. Comparison of HHV values of biomasses

Biomass Types	**HHV (MJ/kg)**	**Referance**
FS	17.87	This research
MDF	18.97	This research
Forest waste	18.94	Zengin et al., 2020
Wood Dust	18.90	Zengin et al., 2020
Eucalyptus	20.00	Martinez et al., 2021
Oak wood waste pellets	19.681	Yıldız and Topkoç, 2023
Waste Furniture	19.99	Dang et al., 2023

The HHV values of the furniture industry wastes used in this study are presented in Table 2. Fiberboards are generally used in the furniture industry. Approximately 90% of these consist of pure biomass. In addition, they have a high heating value (at least 17 MJ kg^{-1}) due to their low moisture content (Dąbrowska-Salwin, 2017). The high heating values of FS and MDF products were calculated as 17.87 and 18.97 MJ/kg, respectively. The reason why the high heating value of the MDF product is higher than that of FS is due to the low amount of oxygen. The HHV value of raw FS and MDF wastes is comparable to the HHV values of other woody biomasses reported in the literature (Table 2). In addition, HHV values of different furniture biomass wastes have been reported in the literature. For example, in a study reported in the literature, HHV values of furniture factory waste dust and poplar wood sawdust were reported as 18.23 and 17.23 MJ/kg, respectively (Taşar, 2021). It can be said that the results obtained in this study are compatible with the literature. As a result, the high HHV values of FS and MDF products show their suitability as biomass material in the energy sector.

CONCLUSION

The aim of this study is to investigate the energy usage potential of abundant furniture industry waste. The evaluation of biomass resources for energy production is of great importance. For this purpose, furniture industry wastes were used. Firstly, ash and volatile matter analyses of these biomasses were performed. Proximate analysis results showed that FS and MDF products had low ash content. In addition, the HHV values of FS and MDF wastes were calculated as 17.87 and 18.97 MJ/kg. These results show that furniture industry waste has the potential to be used in energy production as biomass.

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ORAL PRESENTATION

A different perspective on COVID-19 disease diagnosis with the developed quad star local binary pattern method

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Abstract

In recent years, disasters have occurred in practically every part of the world, causing significant hardships. COVID-19 began in China and has since gone worldwide. The Reverse Transcription Polymerase Cain Reaction (RT-PCR) approach is the most definitive way to diagnose this extremely dangerous disease. Although the outcome of the treatment is certain, it is an exceedingly expensive method in terms of both cost and processing time. This condition compels the quest for other procedures that can produce faster outcomes. An image enhancement study is being conducted on lung pictures taken from COVID-19 patients. High accuracy rates have been achieved, particularly in experiments conducted using deep learning and machine learning approaches. The study looked at texture analysis methods, which are part of the image enhancement process. Among these methods, the local binary pattern (LBP) method stands out. Because of the LBP method's tremendous success, numerous effective derivative approaches arose from it. The study introduced the quad star local binary pattern (QS-LBP) method developed from the standard LBP method. The features extracted from the images enhanced with the QS-LBP method were examined using some well-known machine-learning techniques. The investigation obtained performance values of 99.21% accuracy, 96.2% kappa, 99.8% ROC area, 99.6% F-Measure, 99.5% precision, and 99.8% recall.

Keywords: COVID-19, Quad Star Local Binary Patterns, Deep Learning, Image Processing

INTRODUCTION

The COVID-19 disease, which spread as a pandemic all over the world and has severely harmed the healthcare system in particular, also effected a significant negative influence on the economies of many countries. Detecting the COVID-19 virus and diagnosing the condition early on is particularly useful in controlling it. This illness has spread quickly because it is transmitted via the respiratory tract. COVID-19, which mostly affects the respiratory system, spreads via coughing, sneezing droplets, and other environmental encounters. The virus's most common symptoms include fatigue, fever, cough, lack of appetite, lethargy, discomfort, coughing, and diarrhea. Since 2020, the COVID-19 viral virus has claimed the lives of about 7 million individuals worldwide (https://data.who.int/dashboards/covid19/deaths, 2024). In addition, approximately 800 million people worldwide have been diagnosed with this diagnosis (World Health Organization, 2024).

The most frequent approach for diagnosing COVID-19 disease is the Reverse Transcription Polymerase Chain Reaction (RT-PCR). Although the RT-PCR procedure is effective, the findings take approximately 6-9 hours. However, one of the most significant disadvantages of this procedure is the high cost of the kits used. The limited sensitivity of the RT-PCR technology causes inaccurate results in some circumstances. Image enhancement technologies are another method used to diagnose COVID-19. Recent successful investigations, particularly using deep learning (DL) and machine learning (ML) methods, seem promising.

The main motivation source of this study is listed below:

- In the proposed method, the feature extraction and generalization ability can be improved and the comparison can be made using more global datasets;
- It has the ability to distinguish not only Covid-19 normal cases but also different conventional pneumonia types;
- It can be quickly generalized on more comprehensive datasets in terms of data number;

The structure of this article is Related Works, Used Methods, Performance Evaluation, Data Acquisition, Results and Discussions, and Conclusion, respectively.

RELATED WORKS

Researchers recently used two separate datasets to identify early identification of COVID-19 disease. The second dataset had images of 476 patients, whereas the first dataset contained images of 230. The researchers offered two distinct CNN models: 23 layers and 54 layers. The researchers also used the local binary patterns (LBP) approach with both datasets. The greatest F-1 value obtained in their investigation was 0.98, with the highest AUC value being 0.99 (Yasar & Ceylan, 2021). Another recent study, published in 2024, evaluated CT and X-ray pictures of COVID-19 patients. The researchers devised a hybrid approach that incorporated a variety of image enhancement technologies. The LBP method is also among the methods used. In the valuable study reported, the success rate was determined as 95.47%, the precision value as 94.71%, and the recall value as 96.28% (Medeiros et al., 2024).

Another study on the preliminary diagnosis of COVID-19 disease presented a hybrid technique. In the study, researchers ran some analyses on images of COVID-19 patients. The researchers' proposed hybrid technique yielded high performance. The high-performance value achieved has an exceptionally high accuracy rate of 94.2% (Ju et al., 2024). In a useful study conducted in 2020, researchers investigated the preliminary diagnosis of COVID-19 disease. In the study, the researchers developed a hybrid technique. The proposed hybrid method included LBP, GLCM, and HOG algorithms. Their study had a very high success rate. The hybrid study indicated an accuracy rate of 99% (Rohmah & Bustamam, 2020).

Another study published in 2021 used machine learning to assess the retrieved attributes. The LBP approach is used to improve the dataset, and the features obtained are analyzed using machine learning algorithms. The researchers employ many methods throughout the analysis phase, including bag of trees, kernel extreme learning machine, KNN, and SVM (Saygılı, 2021). In (Liu et al., 2023), researchers created a deep CNN-supported model for Covid-19 that obtained 93.8% accuracy. In the same study, another model constructed by the researchers offered a hybrid strategy comprising of a pre-trained CNN model and the MLP method, which achieved 98.3% accuracy.

DEVELOPED QUAD-STAR LOCAL BINARY PATTERN

Local binary patterns (LBP), presented in the literature by Ojala et al. in 2001, are particularly important among image-enhancing methods (Ojala et al., 2001). This method, which has been used in statistical approaches to texture analysis for a quarter of a century, is exceptionally quick. In addition to being fast, the LBP method stands out from many other picture improvement methods by requiring fewer resources. This incredibly effective method has inspired the development of other derivative methods, each with greater strength than the previous one.

The Quad-Star LBP (QS-LBP) method is proposed as part of the study. The architecture of the developed derivative approach is depicted in Figure 1. The QS-LBP method proposed in the study comprises two distinct models. The characteristics obtained in both models were re-evaluated using machine learning methods, and the one with the best performance result was chosen as a result within the scope of the study.

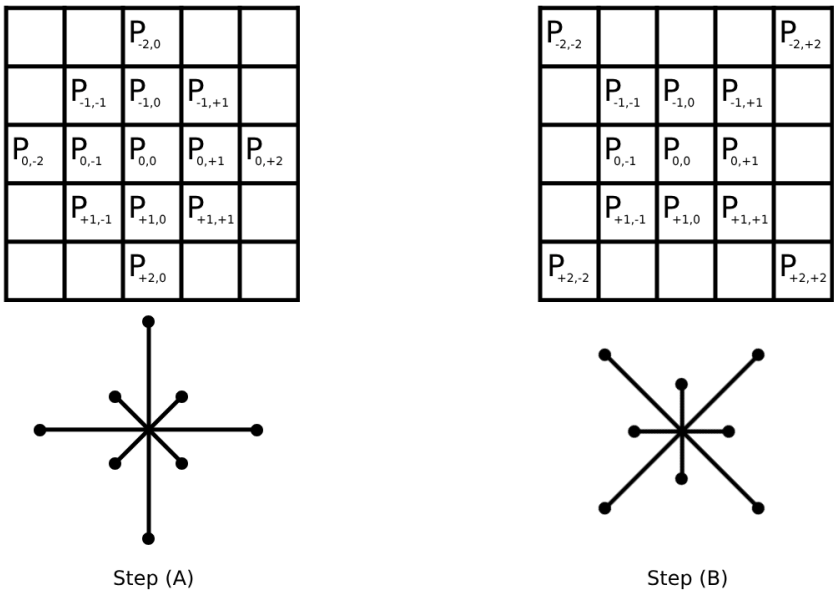

Figure 1: The architecture of QS-LBP

If the step is one, then $P_c = S(P_{(-1,-1)} > P_{(-2,0)})$, $S(P_{(-2,0)} > P_{(-1,+1)})$, $S(P_{(-1,+1)} > P_{(0,+2)})$, $S(P_{(0,+2)} > P_{(+1,-1)})$, $S(P_{(+1,-1)} > P_{(+2,0)})$, $S(P_{(+2,0)} > P_{(+1,-1)})$, $S(P_{(+1,-1)} > P_{(0,-2)})$, $S(P_{(0,-2)} > P_{(-1,+1)})$ relation is performed

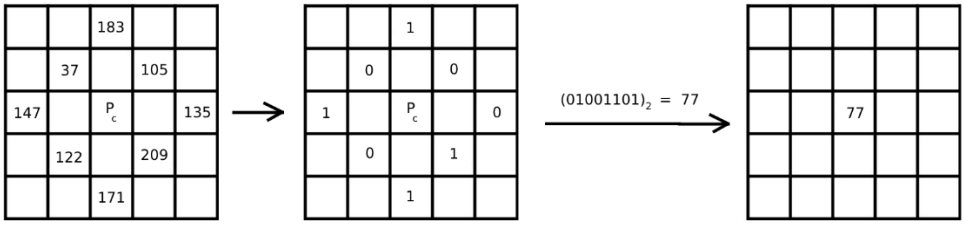

Figure 2: Demonstration of Model 1

Considering the architecture of the QS-LBP method, its application to a sample 5x5 pixel image piece to be taken on the image is shown in Figure 2. The new pattern to be obtained as a result of comparing the relevant pixel values in the 5x5 image considered in the figure is shown.

MACHINE LEARNING METHODS

Random forest algorithms

Breiman et al. proposed the random forest algorithm, which is a very successful tree-based technique. Each tree classifier in the algorithm votes for the most popular class. Each classifier is built with a random vector that is sampled independently of the input vector. The random forest classifier builds a tree at each node by combining or randomly selecting features. All instances are classified according to the class that received the most votes among all tree predictors in the forest (Breiman, 2001).

IBk

It is the equivalent of the KNN algorithm in the Weka software. The KNN algorithm specifies the dataset's existing instances using n-dimensional numerical characteristics. When the KNN algorithm evaluates an example from the dataset, it searches the pattern space for the "k" training examples that are the closest to the example. Predictions from many neighbors can be weighted based on their distance from the test example. In this situation, two separate formulas are used to translate the distance into weight. The amount of training instances stored on the classifier side can be changed and controlled (S. Vijayarani & M. Muthulakshmi, 2013).

Optimized Forest

The optimal forest algorithm belongs to the genetic algorithm family. It is based on the decision forest algorithm. The genetic algorithm is used to encode the population structure of 20 chromosomes in the structure. The generated population undergoes crossover and mutation processes using the roulette wheel technique. The chromosomes with the highest utilization are then picked using the elitist strategy. To prevent chromosome degeneration, the algorithm selects the first 20 chromosomes from 40 individuals. In this way, a sequential search strategy is developed to determine the optimal community accuracy for each individual (Ampomah et al., 2020).

PERFORMANCE EVALUATION

A set of performance values is used to analyze the output of machine learning algorithms. The following performance parameters will be employed in the study: confusion matrix, accuracy, precision, sensitivity, recall, specificity, and F1 score. Each analysis is evaluated based on true positive (TP), true negative (TN), false positive (FP), and false negative (FN) values. In the dataset, TP refers to the value that is acknowledged as a true positive and is associated with a positive result following analysis. The TN number relates to a real negative value in the analytical phase. FP is a value defined as negative in the dataset but considered as a false positive during the analysis process.

$$Accuracy = \frac{TP+TN}{TP+FP+TN+FN} \quad (1)$$

Precision: it corresponds to how many of the positively predicted values are positive. Precision value is considered a very important value, especially in cases where the cost of false positive estimated values is high.

$$Precision = \frac{TP}{TP+FP} \quad (2)$$

Recall: this measurement is very close to the sensitivity value, in other words, it is the ratio of those correctly classified among positive values. It corresponds to how successful a prediction is made between the situations defined as positive in the dataset.

$$Recall = \frac{TP}{TP+FN} \quad (3)$$

F-1 Score: Corresponds to the harmonic mean of Precision and Recall values.

$$F1\ Score = 2 * \frac{Precision*Recall}{Precision+Recall} \quad (4)$$

Receiver Operating Characteristic (ROC) curve and the Area Under the ROC Curve: The ROC curve and the area under the ROC curve (AUC) are the graphical representation of the true positive rate (TPR – recall) versus the false positive rate (FPR). Each point on the curve corresponds to a different threshold value. It is an extremely important and powerful visualization tool for interpreting the results obtained in the study (Everton Gomede, 2023).

$$TPR = \frac{TP}{TP+FN} \quad (5)$$

$$FPR = \frac{FP}{FP+TN} \quad (6)$$

Kappa: Cohen's kappa corresponds to the scalar measure of the accuracy value proposed by Cohen in 1960. It is the unit of measurement used for the accuracy of classifiers. Assuming that A corresponds to reality and B corresponds to the classifier, kappa evaluates the degree of accuracy between the classifier and reality (Ben-David, 2008).

DATASET

The images studied in the study were collected from a publicly available website. The images in the dataset were developed by two study teams from Qatar University and Dhaka University. The dataset was developed in partnership with two separate centers in Pakistan and Malaysia. The collection is divided into files labeled

normal, viral pneumonia, COVID-19, and lung opacity images. The dataset includes 10,192 normal, 6012 lung opacity, and 1,345 viral pneumonia images, as well as the associated lung masks, which were combined with 3,616 COVID-19-positive patients.

RESULTS AND DISCUSSIONS

Tissue feature extraction of chest images was performed as part of a study to aid in the early detection of COVID-19. The dataset under consideration contains a substantial number of images. The amount of data contained in the dataset is critical for a model to be trained. The images included in the study were first enhanced using the proposed QS-LBP method and then evaluated using machine learning techniques. The QS-LBP method has two models. Table 1 shows the examination of the model 1 outcomes using machine learning methods.

Table 1: Results obtained when the developed QS-LBP method is model 1

	Precision (%)	Recall (%)	F-Measure (%)	ROC Area (%)	Kappa (%)	Accuracy (%)
IBk	99.2	99.1	99.2	96.5	92.9	98.53
OP	98.8	99.7	99.3	99.7	93.52	98.7
RF	98.8	99.8	99.3	99.7	93.7	98.74

The outcomes of the images enhanced with the created QS-LBP method were analyzed using some machine learning algorithms. The top performance values obtained as a consequence of the analysis performed with the three most prominent machine learning algorithms are as follows: precision 99.2%, recall 99.8%, F-measure 99.3%, ROC 99.7%, kappa 93.7%, and 98.74%. The QS-LBP method yielded the highest precision value for model 1 with optimized forest and random forest, as well as the highest recall value, F-measure value, ROC curve, kappa value, and accuracy value with random forest.

Table 2: Results obtained when the developed QS-LBP method is model 2

	Precision (%)	Recall (%)	F-Measure (%)	ROC Area (%)	Kappa (%)	Accuracy (%)
IBk	99.2	99.2	99.2	96.5	93.29	98.62
OP	98.8	99.8	99.3	99.8	93.8	98.77
RF	98.8	99.8	99.3	99.8	93.75	98.76

The features extracted from images enhanced with model 2 of the QS-LBP method were examined using the three most prevalent ML methods. The top performance values obtained as a consequence of the analysis are as follows: 99.2% precision, 99.8% recall, 99.3% F-measure, 99.8% ROC, 93.8% kappa, and 98.77%. The QS-LBP method yielded the highest precision value for model 2 with optimized forest and random forest, as well as the highest recall value, F-measure value, ROC curve, kappa value, and accuracy value (Table 2). Table 3 shows the highest values obtained from both models of the QS-LBP method.

Table 3: The highest performance values obtained as a result of two models

	Precision (%)	Recall (%)	F-Measure (%)	ROC Area (%)	Kappa (%)	Accuracy (%)
Model 1	98.8	99.8	93.3	99.7	93.7	98.74
Model 2	98.8	99.8	99.3	99.8	93.75	98.77

The developed QS-LBP method was compared with other studies in the literature for the diagnosis of Covid-19 disease. The comparative results are shown in Table 4 below.

Table 4: Comparison of Literature

Studies	Precision (%)	Recall (%)	F-Measure (%)	ROC Area (%)	Kappa (%)	Accuracy (%)
(Medeiros et al., 2024)	94.71	96.28	95.44			95.47
(Mubarak et al., 2022)	96.4		97.5	97.4		97.5
(Shankar & Perumal, 2021)	94.85		93.2	93.5		94.08
(Shankar et al., 2023)			97.93			98.06
(Senan et al., 2021)				97.51		99
(Öztürk et al., 2021)	96.73		93.99			94.23
Proposed study	**98.8**	**99.8**	**99.3**	**99.8**	**93.75**	**98.77**

The designed QS-LBP method and the hybrid method made using ML algorithms produced extraordinarily high results, as shown in the comparative table. When compared to other recent research in terms of accuracy, it is clear that the study undertaken has a very high value. When assessed using criteria such as ROC area and Kappa, which are critical in the analysis of a study, the acquired ROC area and Kappa values likewise performed admirably. These results demonstrate the effectiveness of the developed QS-LBP method.

CONCLUSION

Texture analysis-based approaches have demonstrated great success in image enhancement research. LBP holds a significant role in texture analysis methods. Current investigations into the diagnosis of Covid-19 disease, the effects of which can still be felt now, are ongoing. The study included the development of a new method based on the standard LBP method for the early detection of COVID-19 illness. The features obtained using the developed QS-LBP method were re-analyzed using ML algorithms. The created hybrid method used quite substantial outcomes. In the next phase of the project, the created QS-LBP method will be applied to datasets with larger data volumes, and the findings will be improved.

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ORAL PRESENTATION

Isolation of Isoquinoline Alkaloid (6-acetonyl-5,6-dihydrosanguinarine) from the *Chelidonium majus* L.

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Abstract

Chelidonium majus L. (Papaveraceae) is a medicinal herb used in various traditional systems of medicine to treat ulcers, cancer, oral infections, liver disorders, chronic bronchitis, and asthma. The primary objective of this study was to isolate bioactive alkaloids from *Chelidonium majus*, a plant known for its rich alkaloid content and medicinal properties. For this aim, the methanol extract (5 g) was suspended in acidified water with acetic acid (5%) and then partitioned with ethyl acetate. The organic layer was discarded. The remaining water layer was partitioned three times with dichloromethane after neutralizing with ammonia. The organic layer was combined and evaporated. The resulting amorphous solid (1 g) was separated over Sephadex LH-20 using methanol as the mobile phase. 38 fractions were collected in 10 mL volumes. According to TLC analysis, 31-34 fractions with similar profiles and containing single spots were combined and recrystallized in acetone. The chromatographic separation process resulted in the isolation of 6-acetonyl-5,6-dihydrosanguinarine with high purity. The structural elucidation of the isolated compound was achieved using spectral analysis such as NMR, LC-MS/MS, and UV. The separation process applied in this study can be recommended as a rapid isolation technique for bulk production of 6-acetonyl-5,6-dihydrosanguinarine.

Keywords: *Chelidonium majus* L., Alkaloid, 6-acetonyl-5,6-dihydrosanguinarine, Isolation, Column Chromatography, LC-MS/MS

INTRODUCTION

Natural products have long been a valuable source for the discovery of novel bioactive compounds, particularly alkaloids, which are secondary metabolites known for their wide range of pharmacological properties. Among the plants that have garnered interest in this domain, *Chelidonium majus* L. (Celandine) holds a prominent position due to its diverse alkaloid content This perennial herb, belonging to the family Papaveraceae. Its stems can reach up to 90 cm in height, are branched, and sparsely covered with fine hairs. The leaves consist of 5 to 7 broad leaflets, with the terminal leaflet often divided into three parts and all leaflets having crenate lobes. The flowers measure 2 to 2,5 cm in diameter, while the capsule ranges from 3 to 5 cm in length. The seeds are black and have a white appendage.

C. majus is a medicinal herb used in various traditional systems of medicine to treat ulcers, cancer, oral infections, liver disorders, chronic bronchitis, and asthma (Móricz et al., 2015). Extensive phytochemical investigations of *C. majus* have revealed the presence of a wide variety of alkaloids, including benzophenanthridines, isoquinolines, protopines, protoberberines, aporphines, and quinolizidine. In addition to alkaloids, the plant contains various aromatic and aliphatic acids. Recent studies have also identified several caffeic acid esters. Furthermore, *C. majus* contains saponins, flavonoids, and other bioactive compounds such as choline, tyramine, and histamine (Cho et al., 2006; Hădărugă & Hădărugă, 2009; Kopyt'ko et al., 2005; Song et al., 2002). In addition to traditional uses of *C. majus*, the presence of antibacterial (Miao et al., 2011), antifungal (Matos et al., 1999), antiviral (Tan et al., 1991), anthelmintic (Bodalski et al., 1958), antiulcer (Kim et al., 2012), hepatoprotective (Mitra et al., 1996), hypolipidemic (Zarei et al., 2014), radioprotective (Song et al., 2003), antioxidant (Heo et al., 2013), anti-inflammatory and analgesic (Çitoğlu et al., 2009; Lenfeld et al., 1981), anti-arthritic(Lee et al., 2007), anti-Alzheimer (Kuznetsova et al., 2001), immunomodulatory

(Khmel'Nitskaia et al., 1998), natriuretic and antidiuretic (Koriem et al., 2013), anticancer (Karamova et al., 2011), and antispasmodic (Boegge et al., 1996) pharmacological activities has also been reported.

In this study, 6-acetonil-5,6-dihydrosanguinarine was successfully isolated from *C. majus* through a series of chromatographic techniques. The structural elucidation of the isolated compound was achieved using spectral analysis such as NMR, LC-MS/MS, and UV. Given the potential therapeutic applications of sanguinarine derivatives, the isolation of this acetonyled form offers new opportunities for bioactivity studies and drug development. This research aims to explore the biological significance of 6-acetonyl-5,6-dihydrosanguinarine further, evaluating its pharmacological properties and potential as a lead compound for future drug discovery efforts. Additionally, understanding its biosynthetic pathway in *C. majus* could offer insights into the production of related alkaloids with enhanced biological activity.

MATERIALS AND METHODS

Plant Material and Extraction

Chelidonium majus L. (Papaveraceae) aerial parts were obtained from Cumalıkızık village, Bursa, Turkey (July-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). *C. majus* aerial parts (500 g) were macerated in triplicate with 70% methanol. The methanol extract (5 g) was suspended in acidified water with acetic acid (5%) and then partitioned with ethyl acetate. The organic layer was discarded. The remaining water layer was partitioned three times with dichloromethane after neutralizing with ammonia. After basification of the extract, the formation of a precipitate was observed. The precipitate was filtered using filter paper, and the solution was subjected to liquid-liquid extraction with dichloromethane. The organic layer was combined and evaporated.

Isolation of 6-acetonyl-5,6-dihydrosanguinarine

The resulting amorphous solid (1 g) was separated over Sephadex LH-20 using methanol as the mobile phase. 38 fractions were collected in 10 mL volumes. According to TLC analysis in Methanol: $CHCl_3$ (4:6) solvent system, 31-34 fractions (Figure 1) with similar profiles and containing single spots were combined and recrystallized in acetone (Figure 2). The chromatographic separation process resulted in the isolation of 6-acetonyl-5,6-dihydrosanguinarine with high purity. The structural elucidation of the isolated compound was achieved using spectral analysis such as NMR, LC-MS/MS, and UV.

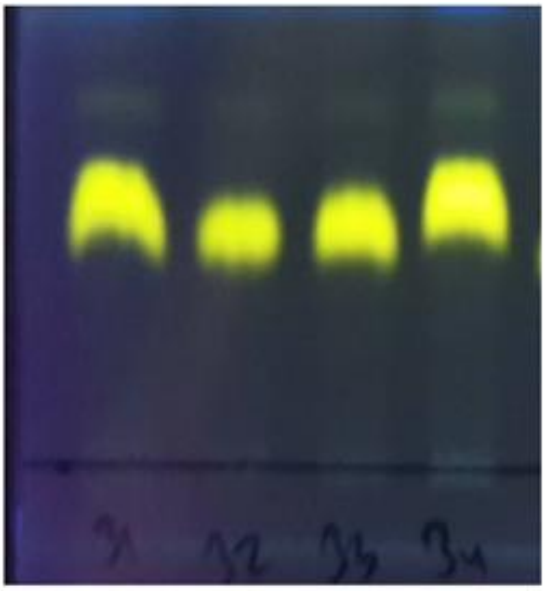

Figure 1. TLC view of Sephadex LH-20 column 31-34 (6-acetonyl-5,6-dihydrosangunerin) fractions (under 366 nm UV light)



Figure 2. Crystal structure of 6-acetonyl-5,6-dihydrosangunerine

RESULTS and DISCUSSION

6-acetonyl-5,6-dihydrosangunerine (Figure 3); obtained as a colorless crystal. (27 mg). The molecular formula was determined as $C_{23}H_{19}NO_5$ based on the negative LC-MS/MS ion (Figure 4) observed at m/z 388.1 $[M-H]^-$ (calculated. for $C_{23}H_{18}NO_5$ 388.1).

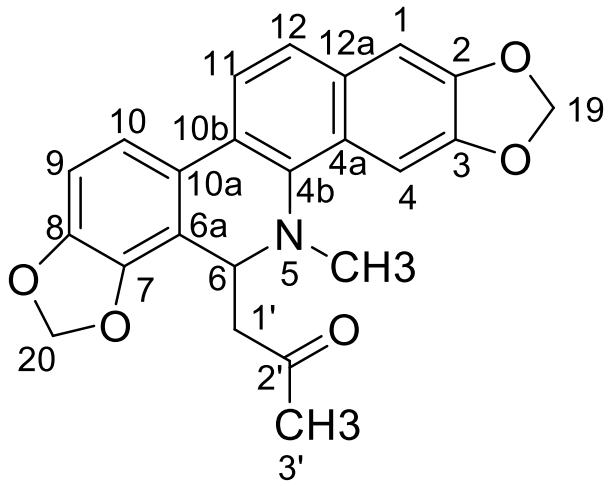

Figure 3. Chemical structure of 6-acetonyl-5,6-dihydrosangunerine

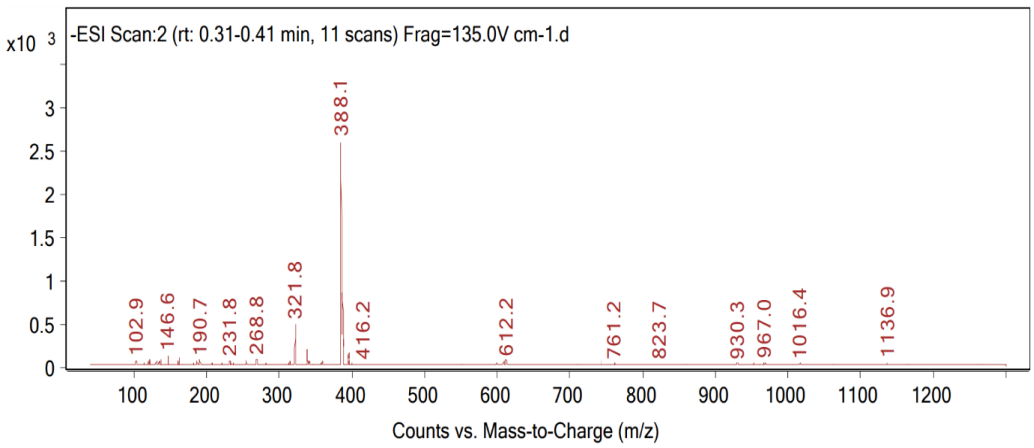

Figure 4. LC-MS/MS spectrum of 6-acetonyl-5,6-dihydrosangunerin

When the ^{1}H-NMR spectrum (Figure 5) of the compound was examined [^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.09 (^{3}H, s, H-3'), 2.34 (^{1}H, dd, *J* = 12.6, 8.1 Hz, H-1'b), 2.67 (^{3}H, s, N-CH_3-5), 2.87-2.83 (^{1}H, m, H-1'a), 4.97-4.79 (^{1}H, m, H-6), 6.08 (^{4}H, s, H-19 ve H-20), 6.88 (^{1}H, d, *J* = 8.2 Hz, H-9), 7.13 (^{1}H, s, H-1), 7.36 (^{1}H, d, *J* = 8.2 Hz, H-10), 7.51 (^{1}H, d, *J* = 8.6 Hz, H-12), 7.55 (^{1}H, s, H-4), 7.72 (^{1}H, d, *J* = 8.6 Hz, H-11)], two different AB systems were observed as doublets in the aromatic region at 6.88 (H9) ppm/7.36 (H10) ppm and 7.51 ppm (H11)/7.72 (H12) ppm with interaction constants of *J* = 8.2 Hz and *J* = 8.6 Hz, respectively. At 7.13 (H1) and 7.55 (H4) ppm, the AX system was observed as a singlet. At 6.08 ppm, two different oxymethylene signals connected to the aromatic rings in the molecule showed overlapping signals. The two methyl signals observed in the spectrum resonated as singlets at 2.09 (H-3') and 2.67 (N-CH_3) ppm. The H1'b proton was observed as a doublet of a doublet at 2.34 ppm with interaction constants of *J*=12.6, 8.1 Hz, while the H1'a and H6 protons were observed as multiplets at 2.87-2.83 and 4.97-4.79 ppm, respectively.

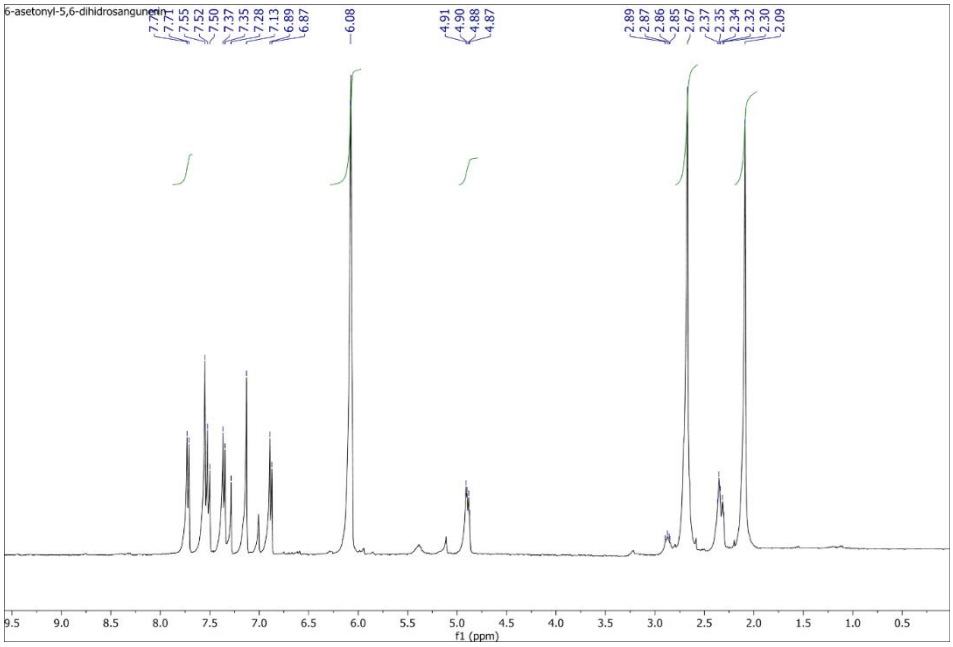

Figure 5. ^{1}H-NMR spectrum of 6-acetonyl-5,6-dihydrosangunerin

Upon examination of the ^{13}C-NMR spectrum (Figure 6) of the compound, 23 carbon signals were observed [^{13}C-NMR (100 MHz, $CDCl_3$) δ 30.3 (C-3′), 43.1 (N-CH_3), 46.6 (C-1′), 54.5 (C-6), 100.6 (C-19), 101.1 (C-20), 101.6 (C-4), 104.4 (C-1), 107.6 (C-9), 116.5 (C-10), 120.0 (C-11), 123.5 (C-10b), 124.0 (C-12), 124.7 (C-4a), 125.7 (C-10a), 127.5 (C-6a), 131.0 (C-12a), 139.2 (C-4b), 144.3 (C-7), 147.2 (C-8), 147.6 (C-2), 148.3 (C-3), 207.3 (C-2′)], corresponding to the expected number of carbons in the molecule. Among these, one signal was attributed to a carbonyl carbon at 207.3 ppm (C-2′). Seven aliphatic carbons were identified, including four non-oxygenated aliphatic carbons at 30.3 ppm (C-3′), 43.1 ppm (N-CH_3), 46.6 ppm (C-1′), and

54.5 ppm (C-6), along with two oxymethylene carbons at 100.6 ppm (C-20) and 101.1 ppm (C-19). In the aromatic region, six protonated carbon signals were observed at 101.1 ppm (C-4), 104.4 ppm (C-1), 107.6 ppm (C-9), 116.5 ppm (C-10), 120.0 ppm (C-11), and 124.0 ppm (C-12). Additionally, ten quaternary carbon signals were detected at 123.5 ppm (C-10b), 124.7 ppm (C-4a), 125.7 ppm (C-10a), 127.5 ppm (C-6a), 131.0 ppm (C-12a), 139.2 ppm (C-4b), 144.3 ppm (C-7), 147.2 ppm (C-8), 147.6 ppm (C-2), and 148.3 ppm (C-3). The downfield shift of the methyl signal at 43.1 ppm further supports the assignment of this signal to an N-CH_3 group. The results found are consistent with the literature results (Kim et al., 2013)

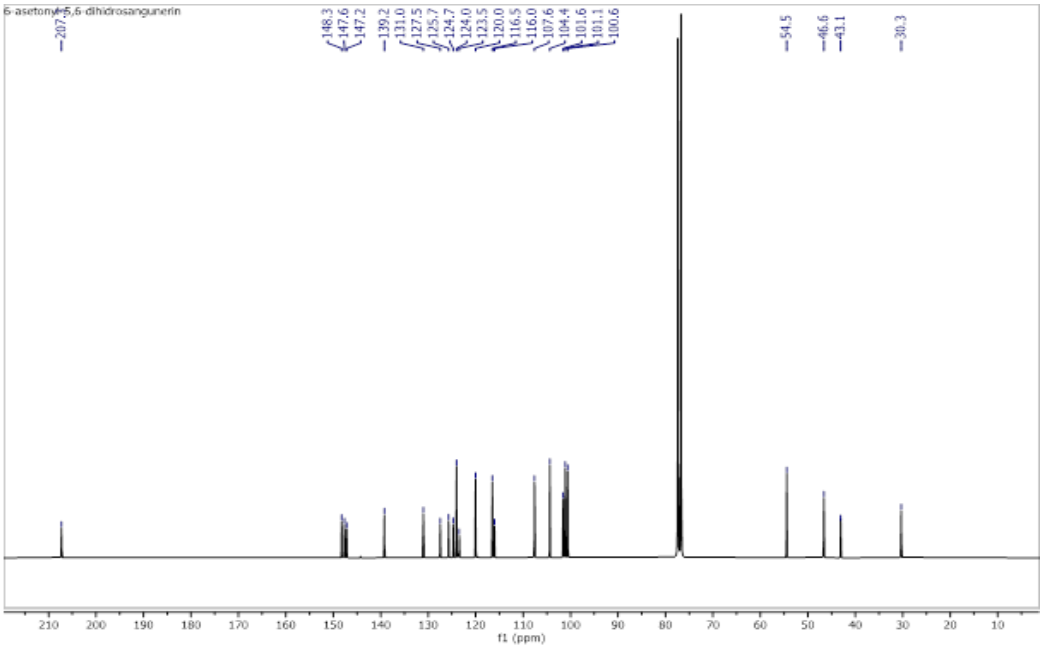

Figure 6. ^{13}C-NMR spectrum of 6-acetonyl-5,6-dihydrosangunerin

The UV spectrum of the compound (Figure 7), recorded in chloroform, shows absorption bands at λ_{max} 254, 280, and 336 nm, indicating the presence of aromatic rings in the structure.

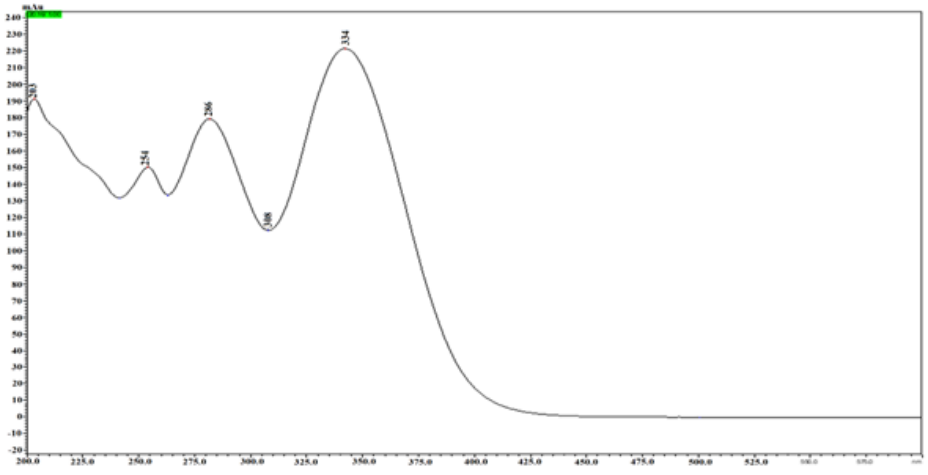

Figure 7. UV spectrum of 6-acetonyl-5,6-dihydrosangunerin

CONCLUSION

The alkaloid 6-acetonyl-5,6-dihydrosanguinarine was successfully isolated from *Chelidonium majus* using an one-step fractionation over Sephadex LH-20 column chromatography and recrystallization. The structure of the isolated compound was elucidated through comprehensive spectroscopic analyses, including NMR, LC-MS/MS, and UV spectroscopy, confirming the presence of aromatic rings and key functional groups. This work highlights an efficient and reproducible method for isolating 6-acetonyl-5,6-dihydrosanguinarine for large-scale purpose, a compound with significant potential for further pharmacological evaluation. The findings contribute to the growing knowledge of isoquinoline alkaloids, emphasizing their importance in drug discovery and therapeutic applications. Future studies should focus on exploring the biological activities of this compound and investigating its potential as a lead molecule for drug development.

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ORAL PRESENTATION

Investigation of cytotoxic effects of fruit and leaf extracts of *Mespilus germanica* (medlar) plant growing in Zonguldak province on MCF-7 cells.

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Abstract

Cancer is one of the most important health problems of our time and is the second leading cause of death after cardiovascular diseases. Due to the high mortality rates due to cancer and the side effects of chemotherapy used in treatment, the development of alternative treatment methods is a reality and, in this context, the discovery of new drugs/therapeutics for cancer is important. For this purpose, a great deal of research has recently been conducted on the use of plant extracts, which are of great medical importance, in cancer treatment. These traditional treatment methods, which are generally applied in addition to drug treatment, aim to contribute to the treatment process and restore the person to health. In this study, it was aimed to determine the cytotoxic effect of the fruit and leaf extracts of *Mespilus germanica* (medlar) plant collected from Zonguldak region of Turkey on human breast cancer cell line (MCF-7). In the study, both fruit and leaf aqueous extracts of medlar plant were applied to MCF-7 human breast cancer cell line at concentrations of 0.1, 1, 10, 100 µg/mL separately. After 24 hours of incubation, cytotoxic effects of both extracts on human breast cancer cell line were determined by MTT (3-(4,5-dimethylthiazol-2-yl)-diphenyl tetrazolium bromide). Results are shown as % viability. It was determined that medlar fruit and leaf extracts significantly reduced MCF-7 cell viability ($p < 0.05$) and also IC_{50} values of the extracts in MCF-7 cells were calculated. In conclusion, medlar fruit and leaf extracts can be considered as a therapeutic agent due to their *in vitro* cytotoxic activity on MCF-7 human breast cancer cells.

Keywords: *Mespilus germanica*, Breast cancer, Cytotoxicity, Cell culture

INTRODUCTION

Cancer represents a heterogeneous group of diseases characterized by a series of genomic and epigenomic changes (Taylor et al., 2017) and expressed by the disruption of highly complex molecular pathways (Sivanandam et al., 2010; Varkaris et al., 2014). This heterogeneity is one of the most important factors that complicate the treatment of cancer cases and slows down the development of molecular targeted therapy as a form of precision medicine. In this context, it is very important to analyze the developmental stages of the cancer process well and to apply the right treatment for the person to regain their health. Unfortunately, there is no definitive treatment method for this disease, which involves a highly complex process. Among cancer treatments, anti-cancer drugs such as chemotherapy, radiotherapy, surgery and targeted therapies are used (Huang et al., 2010). Considering the complex process of cancer and the different physiological characteristics of individuals, the discovery of more effective drugs or the production of effective drugs in the treatment of these diseases is a very important area of research. Natural compounds such as flavonoids and phenolic acids that can be found in plant species play an important role in the discovery and development of new drugs for the treatment of various diseases (Safari and Ahmady-Asbchin, 2019).

Mespilus germanica (Medlar) is an often overlooked fruit from the family *Rosaceae*, which consists of over 3000 species and is found mainly in the non-tropical region of the northern hemisphere of the earth (Popović-Djordjević et al., 2023). The medlar fruit is brown and reddish in color. Their diameter varies from 1.5 to 3 cm and their weight varies from 10-80 g (Browicz and Mespilus, 1972; Aygün and Tasci, 2013). It grows poorly in frost-free areas, on rocks and in poor soils. The medlar has long leaves, its leaves and flowers are similar to those of apples. The medlar has gained value in human consumption and is of commercial importance in recent years (Baird and Thieret, 1989). Medlar is a late ripening fruit and is consumed for nutritional purposes. It is also used to treat constipation, as a painkiller, diuretic, and to get rid of kidney and bladder stones (Baytop

1999; Glew et al., 2003). Organic acids such as linoleic acid, palmitic acid, malic acid, citric acid (Glew et al., 2003; Ayaz et al., 2008), sugars such as fructose and glucose, amino acids, aspartate and glutamate (Glew et al., 2003), potassium and volatile compounds (Pourmortazavia and Ghadirib, 2005) were detected at high levels in medlar fruits.

In this study, we aimed to determine the anticancer effects of aqueous extracts of medlar fruits and leaves collected from Karapınar town of Zonguldak in Turkey, on human breast cancer MCF-7 cells.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

Human breast cancer cells (MCF-7) were used as cell type in the study. Cells were fed using DMEM medium (Sigma-Aldrich, USA; prepared by adding 10% FBS, 100U/mL penicillin and 0.1 mg/mL streptomycin). Cells were maintained in 75 cm^2 culture flasks and placed in a humidified incubator (5% CO_2, 37°C). The medium was changed every 3-4 days and cells were passaged when they reached 80-90% density.

Test materials and MTT Assay

The medlar leaves collected from the Karapınar town of Çaycuma district of Zonguldak province were dried and ground, and the fruits were freeze-dried and extracted separately with water. Medlar fruit and leaf aqueous extracts were prepared in DMEM medium at concentrations of 10, 20, 40 and 80 µM. The cytotoxic effect of fruit and leaf extracts on human breast cancer cell line was tested by MTT (3-(4,5- dimethylthiazol-2-yl)-diphenyl tetrazolium bromide) method. Confulent cells were removed from the bottom of the flasks using Trypsin-EDTA solution and counted under a microscope. Cells were seeded in 96-well plates with $15x10^3$ cells per well. The seeded cells were incubated in a CO_2 incubator at 37°C for 24 hours. Following incubation, the mediums were changed and different concentrations of the prepared fruit and leaf extracts were added to the wells containing the cells and left to incubate for 24 hours (Koran et al., 2017). Then, the medium in the wells was removed and MTT solution (5 mg/mL) prepared in sterile PBS was added to each well and the plates were incubated for 3 hours. Afterwards, the solution in the wells was removed and incubation was completed by adding 100 µL DMSO to each well. The optical density of the cells in the plates was read with a spectrophotometer at a wavelength of 570 nm (Mosmann 1983). The absorbance values obtained from the control wells were averaged and this value was considered as 100% viable cells. Percent (%) viability values were determined by proportioning the absorbance values obtained from the wells treated with medlar fruit and leaf aqueous extracts to the control absorbance value.

Statistical Analysis

IBM SPSS Statistics 22.0 for Windows package program was used in the analysis. Normal conformity to the distribution was examined by Shapiro Wilk test. Quantitative variables between groups One-way analysis of variance was used for comparisons. When significant differences were determined, multiple comparisons were performed with Tukey's test. Data are expressed as mean ± standard error (SH) A value of $p<0.05$ was considered statistically significant.

RESULTS

The cytotoxic effects of medlar plant and leaf aqueous extracts on human breast cancer cell lines are shown in Figure 1 and Figure 2, respectively. Accordingly, it was determined that all applied concentrations of both extracts significantly ($p<0.05$) decreased cell viability depending on the dose compared to the control. In addition, the IC_{50} value of *M. germanica* leaf extract was calculated as 32.08 µg/mL and the IC_{50} value of the fruit extract was calculated as 48.73 µg/mL.

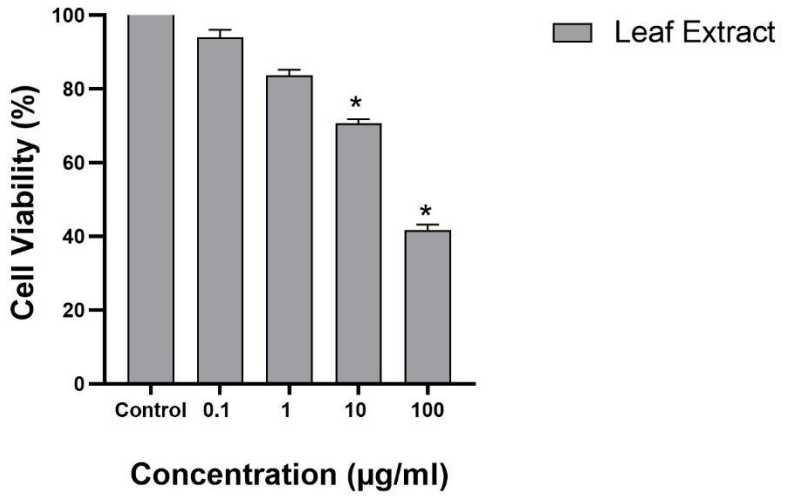

Figure 1. The % change in viability of human breast cancer cells (MCF-7) treated with medlar leaf extract concentrations for 24 hours (*p<0.05)

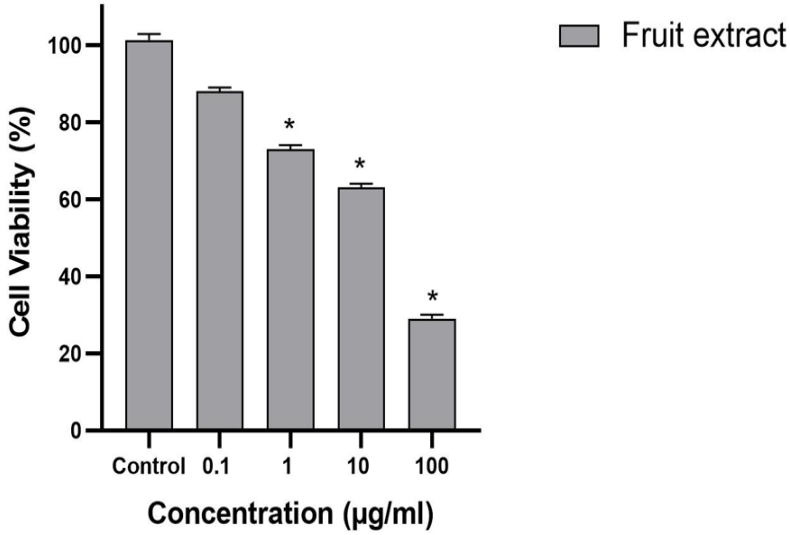

Figure 2. The % change in viability of human breast cancer cells (MCF-7) treated with medlar fruit extract concentrations for 24 hours (*p<0.05)

CONCLUSION

In conclusion, the fruit and leaf extracts of the medlar plant collected from the Zonguldak region have the potential to be a therapeutic agent candidate due to their in vitro cytotoxic activities in human breast cancer MCF-7 cells. However, more comprehensive studies are needed to clarify in detail the molecular mechanisms by which medlar fruit and leaf extracts cause cell death and to determine the level of biocompatibility.

ACKNOWLEDGEMENTS

This study was supported by TUBITAK 2209-A University Students Research Projects Support Program.

Project No: 1919B012107250.

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ORAL PRESENTATION

Impact of Physical and Psychsocial Environment on Students' Achievement

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Abstract

The learning environment plays a significant role in students' academic performance and overall well-being. This study explores the impact of both physical and psychosocial elements within educational settings, highlighting factors such as classroom design, resource availability, social interactions, and emotional support. Drawing on academic theories and field research, the study emphasizes how these environments contribute to student success. The physical environment, including infrastructure and spatial arrangements, is examined alongside the psychosocial environment, which encompasses social and emotional dynamics in schools. The study also addresses problems arising from these environments and offers practical solutions to improve learning spaces, with the aim of enhancing educational outcomes for all. Insights from teachers in Tirana and Maminas illustrate the practical implications of these factors and underscore the need for targeted improvements in both environments.

Keywords: learning environment, physical environment, psychosocial environment, student achievement, school violence, social and emotional learning (SEL).

INTRODUCTION

The concept of "learning environment" has been defined in various ways, reflecting different interpretations of its scope and significance. For Joyce & Weil (1996), the learning environment encompasses all elements that have the potential to foster learning, whether they are social, psychological, or pedagogical. However, it is also defined as the setting or atmosphere in which learning occurs, directly influencing the effectiveness of the learning process (Tessmer & Harris, 1992). According to Che Ahmad et al. (2013), the learning environment refers to the physical spaces where learning happens, including classrooms, science labs, workshops, fields, or even offices. Ramsden (1991) highlighted that the learning environment is closely tied to students' experiences within their educational context. This is because it is the space where students and educators come together for a set period to engage in learning activities (Biggs, 1999). Biggs introduces the concept of "constructive alignment," which is a teaching approach that ensures what is taught, how it is taught, and how it is assessed are all aligned with the intended learning outcomes.Therefore, the environments created during these activities are considered a crucial aspect of the teaching and learning process (Puteh et al., 2014).The learning environment, recognized as a key element in the teaching and learning process (Puteh et al., 2014), represents a series of influences from the environment that shape students' learning outcomes, whether in terms of skills, cognitive development, or behavior. The physical aspects of a classroom, such as lighting, seating arrangement, and noise levels, significantly affect students' ability to concentrate and perform well academically. Psychosocial factors like the teacher-student relationship, peer interactions, and the overall emotional climate in the classroom are crucial for fostering a conducive learning environment. Both physical and psychosocial environments are optimized to enhance students' learning outcomes. Fraser argues that students' perceptions of their classroom environment can influence their motivation, engagement, and academic achievement. He promotes the idea that educators should regularly assess and modify the classroom environment to ensure it meets the needs of all students, thus supporting inclusive and effective learning.(Che Ahmad et al., 2013; Fraser, 1998). This aligns with Saad's (1997) perspective, which suggests that the learning environment encompasses aligns with the idea that creating a well-rounded and supportive learning environment is key to enhancing student achievement. A recent study by Vujic, Vukovic, and Nikolic

(2021) examines the effects of classroom environments on student achievement specifically within Balkan schools. The study found that both physical elements, such as classroom design and lighting, and psychosocial factors, like teacher-student relationships, significantly impact student performance.
Interestingly, the research also noted regional differences, underscoring how cultural and socioeconomic contexts influence the effectiveness of learning environments. This aligns with our understanding that optimizing both physical and psychosocial aspects of the learning environment can enhance student outcomes. The regional context of this study highlights the importance of considering local factors when designing effective learning environments. Based on this study, educators and policymakers in similar regions should focus on both infrastructural improvements and fostering positive classroom climates to support student success.

Physical Environment plays a critical role in student achievement, health, and well-being.
Extensive research suggests that the built environment of classrooms profoundly impacts academic performance, behavior, and emotional well-being. A well-designed classroom fosters positive behaviors, emotions, and cognitive processes essential for effective learning.

- Color: Color significantly affects students' perception and learning outcomes. Cold hues, like blue, can enhance concentration and calmness, while warmer colors, like red, may stimulate excitement and improve memory retention (Salim, Husein, & Baper, 2021). A balanced approach, such as using neutral walls with vibrant accents, creates a stimulating yet focused learning environment (Oruikor, 2023).
- Lighting: Natural light contributes to physical and mental comfort, enhancing mood, energy levels, and focus (Oruikor, 2023). Adequate lighting, including LED lights, supports cognitive processes and academic performance (Palau & Recalde, 2020). In specialized classrooms, like biology labs, sufficient lighting is critical for detailed work, such as microscope observations.
- Air Quality and Ventilation: Proper ventilation is essential for a healthy learning environment, especially in biology classrooms where chemicals may be present (Oruikor, 2023). Good air quality prevents health issues that can impede concentration and learning. Adequate ventilation and air quality directly influence students' health and academic performance (Ordway, 2023).
- Noise Control: Noise can hinder learning by affecting concentration and cognitive processes. Excessive noise increases stress and reduces academic performance. Effective noise control, including sound-absorbing materials and strategic classroom placement, supports better focus and cognitive functioning (Astolfi et al., 2019). In specialized settings like biology labs, maintaining a quiet environment is crucial for effective work and discussion (Oruikor, 2023).
- Furniture and Layout: Classroom furniture and layout are key to an effective learning environment. Flexible arrangements that accommodate various teaching methods and group activities enhance interaction and engagement. Ergonomic furniture reduces discomfort and distractions, while effective space utilization improves focus. In biology labs, furniture must support practical activities and be adaptable to different learning styles (Tobia, Sacchi, Cerina, Manca, & Fornara, 2020).
- Personalization: Personalized learning environments address diverse learning styles and foster a supportive educational experience. Incorporating elements that reflect students' identities and interests, such as displays of student work, enhances engagement and self-worth (Mötteli, Grob, Pauli, Reusser, & Stebler, 2023). In biology classrooms, this might include student projects or models relevant to the curriculum.
- Number of students: Furthermore, global evidence strongly supports the advantages of smaller class sizes, including improved academic outcomes. Research such as the Tennessee STAR (Student Teacher Achievement Research) study, conducted between 1985 and 1989, randomly assigned students from kindergarten to third grade into either small or large classes. The results showed that students in smaller classes (13-17 students) scored about 5 percent, higher on standardized math and

reading tests compared to those in larger classes. This effect was particularly pronounced in the early grades and had lasting benefits as students progressed through school. Similarly, research from the Los Angeles Unified School District found that students who spent more time in smaller classes generally achieved higher levels in reading and language, with larger improvements also noted in mathematics, although students with limited English proficiency showed less pronounced gains (Barrett, Treves, Shmis, Ambasz, & Ustinova, 2019).

- By considering these factors, educators can create classrooms that support learning and promote student well-being, especially in specialized environments like biology labs.
- Budget constraints are a significant barrier for many schools. High-quality ergonomic furniture, advanced lighting systems, and state-of-the-art ventilation can be prohibitively expensive, especially for underfunded institutions. Another challenge lies in space limitations. Older school buildings may not provide the physical space needed to implement ideal layouts. These spatial constraints can limit the ability to arrange furniture effectively or integrate specialized areas like lab stations in science classrooms. As a result, educators may be forced to prioritize certain aspects of classroom design over others, potentially sacrificing elements that could enhance student engagement and collaboration.

Furthermore, resistance to change among educators and administrators can hinder the adoption of new classroom designs. Educators who are accustomed to traditional, teacher-centered environments may be reluctant to shift towards more flexible,
student-centered layouts. This resistance can stem from a lack of familiarity with new teaching methods or concerns about the effectiveness of non-traditional arrangements (Lomba Portela, Dominguez-Lloria, & Pino-Juste, 2022).
In summary, the physical environment of a classroom significantly impacts student achievement, health, and well-being. Key elements such as color, lighting, air quality, noise control, furniture, and personalization each play a crucial role in creating an effective learning environment. Research consistently shows that thoughtful design in these areas can enhance student focus, comfort, and engagement, ultimately leading to improved academic outcomes.
However, implementing these design elements is not without its challenges. Budget constraints, space limitations, and resistance to change can all impede the adoption of ideal classroom designs. To address these challenges, educators and administrators should consider several strategies like:

- Exploring funding opportunities
- Invest in training programs for educators to familiarize them with new classroom layouts and teaching methods.

By addressing these challenges and proactively implementing effective design strategies, schools can create environments that support and enhance student learning, health, and overall success.

Psychosocial Environment: In addition to the tangible aspects of classroom design, such as color, lighting, and layout, the psychological impact of the physical environment on students is equally significant. The classroom environment influences not only students' physical comfort but also their emotional and cognitive states, which in turn affect their academic performance and overall well-being. Understanding these psychological effects is crucial for creating a supportive and productive learning environment.
The psychosocial learning environment is crucial to students' educational experiences, extending beyond just the physical aspects of the classroom. This environment encompasses the social and emotional dimensions, such as teacher-student interactions, peer relationships, and the overall classroom climate. These elements are fundamental to effective learning and student motivation. A positive psychosocial environment can enhance student engagement, interest in subjects like science, and overall academic performance.
Key factors include student involvement, the quality of teacher feedback, and the classroom atmosphere, all of which contribute to feelings of safety, support, and encouragement essential for successful learning (Fraser & Walberg, 1981). Such an environment fosters effective teaching and learning, impacting both the

psychological and social aspects of students' lives (UNESCO, n.d.).

Key elements influencing the psychosocial school climate include the quality of personal relationships, instructional methods, classroom management, and the prevalence of school-based violence. Positive relationships between students and teachers, supported by effective parental involvement and collaborative practices, are particularly significant (UNESCO, n.d.). Research shows that the quality of the teacher-student relationship is a stronger predictor of student success than teaching methods alone, highlighting the teacher's role as a motivator and communicator (Ballantine, 1997; Jamaluddin et al., 2021).

However, maintaining a positive psychosocial environment can be challenging, especially in the face of school violence. Whether physical, psychological, or sexual, and whether occurring in person or online, violence significantly undermines the creation and preservation of a supportive and healthy school environment. Such violence severely affects students' physical and mental well-being, impairs their learning abilities, and negatively impacts educational outcomes. Victims and witnesses of school violence are more likely to skip school, achieve lower grades, or even drop out. They are also at a higher risk for low self-esteem, depression, anxiety, and other mental health issues. Data from PISA 2018 and TIMSS 2019 reveal that higher exposure to bullying correlates with poorer performance in reading, mathematics, and science. Certain student characteristics, including physical appearance, nationality, race, socioeconomic status, gender, indigenous status, sexual orientation, disability, migrant status, HIV status, and orphanhood, increase the risk of experiencing school-based violence. Globally, physical appearance is the most common reason for bullying, particularly among female students, followed by race, nationality, or color. Bullying related to sexual orientation and gender identity is also widespread, with many LGBT students facing homophobic or transphobic violence in schools. Indigenous children are particularly vulnerable due to social exclusion.

To address these challenges, evidence-based Social and Emotional Learning (SEL) programs have proven effective in supporting students' academic success and reducing bullying and violence. By fostering skills such as resilience, self-awareness, collaboration, empathy, and respect, SEL programs not only enhance students' school experiences but also prepare them for life beyond the classroom. SEL improves school attendance, engagement, and motivation, reduces negative behaviors, and enhances both student and staff mental health by lowering stress, anxiety, and depression (UNESCO, n.d.).

To enhance the psychosocial environment and address school-based violence effectively, several strategies and policies are crucial:

- Comprehensive Policies and Laws:

Developing national and school policies or laws that address school-based violence, including codes of conduct, digital safety guidance, and frameworks for staff and students, is a priority.

- Improving Data Collection:

Systematic monitoring of school-based violence, including violence based on sexual orientation and gender identity, is essential. This can be achieved by incorporating relevant questions into school censuses or using large-scale assessments and specific surveys. Data collection methods should be child-sensitive and confidential, and rigorous evaluation of existing programs is necessary to adjust activities effectively.

- Ensuring Adequate Teacher Training:

Teachers should be equipped to teach social-emotional learning (SEL), manage classrooms, and address school-based violence. Successful strategies include positive classroom management and providing various discipline approaches. Allocating female teachers has proven effective in reducing violence in some regions.

- Developing Appropriate Curricula:

Curricula should address school-based violence through civics, life skills, and comprehensive sexuality education. Textbooks and learning materials should promote healthy, safe, and non-violent relationships. Education outcomes should include SEL and student well-being, with successful programs being sequenced, focused, and actively engaging students.

- Reporting Mechanisms:

Clear, safe, and accessible reporting channels are crucial for addressing incidents of violence, assisting victims, and measuring incident prevalence. Examples include helplines, online reporting, "happiness and sadness" boxes, and using girls' clubs as safe spaces for reporting sexual violence (UNESCO, n.d.).

MATERIALS AND METHODS

To gain practical insights into the impact of physical and psychosocial aspects of the learning environment on students and teachers, interviews were conducted with three high school biology teachers from two different locations: Tirana and Maminas. Specifically, two teachers from "Eqerem Çabej" High School in Tirana and one teacher from "Kajo Karafili" High School in Maminas, Durrës, were interviewed. The objective was to understand how these factors shape academic outcomes and emotional well-being in both urban and rural settings.

RESULTS

Impact of physical resources on learning outcomes and emotional well-being:

- Teachers in Tirana reported that access to modern resources like smart boards and digital tools enhances student engagement and confidence. However, urban challenges such as resource inequality and competitive pressures sometimes create stress among students.
- In Maminas, the lack of up-to-date textbooks and digital tools limits learning experiences and affects student motivation, leading to frustration and negative emotional impacts.

Role of school design in students' focus and concentration:

- In Tirana, well-designed classrooms with natural light and ventilation support better focus and concentration. However, overcrowded classrooms pose a distraction.
- Maminas teachers noted that traditional classroom designs with less focus on lighting and ventilation lead to concentration difficulties, especially during warmer months.

Influence of relationships with colleagues and leadership on emotional well-being:

- Tirana teachers experienced mixed support from school leadership and felt overwhelmed by high expectations and competitive pressures. Collaboration with colleagues provided some emotional support, but there was a need for improved communication with administration.
- The Maminas teacher described a supportive and collaborative environment with colleagues and leadership, which fostered a strong sense of belonging and emotional support.

Impact of Noise Levels on the Learning Environment and Concentration:

- In Tirana, noise from traffic and construction often disrupts lessons and affects concentration.
- In Maminas, while the rural setting is quieter, occasional noise from agricultural activities or student play can be distracting. Overall, the lower noise levels are beneficial for maintaining classroom focus.

Role of outdoor spaces in maintaining students' mental health and emotional balance:

- Tirana teachers noted limited access to outdoor spaces, contributing to student stress due to insufficient opportunities for physical activity and breaks.
- In Maminas, available outdoor spaces such as playgrounds and gardens support physical activity, helping students manage stress and return to class more focused and emotionally balanced.

Students' sense of safety and its effect on well-being and motivation

- Teachers in Tirana observed that while physical safety is adequate, psychological safety can be compromised by peer pressure and competitive stress. Enhanced emotional support services were recommended to improve student security and motivation.
- The Maminas teacher reported that students feel safe within the school grounds, though the lack of proper facilities can be concerning. Strong community ties foster a sense of belonging and emotional safety, supporting student motivation.

DISCUSSION and CONCLUSION

The concept of the "learning environment" plays a pivotal role in shaping students' academic achievement and emotional well-being. The physical environment—including factors such as color, lighting, air quality, ventilation, noise control, furniture, layout, personalization, and student numbers—affects students' learning experiences and outcomes. Urban schools like those in Tirana benefit from advanced resources and infrastructure, yet face challenges such as overcrowding and high noise levels. Conversely, rural schools in Maminas experience fewer distractions and stronger community support but contend with limited resources and outdated classroom designs. Addressing these barriers through improved infrastructure, better resource distribution, and thoughtful classroom design is essential for optimizing learning environments in both contexts. The psychosocial environment, encompassing teacher-student interactions, peer relationships, and the overall classroom climate, is equally crucial. Maintaining a positive psychosocial environment is challenging, especially in the face of school violence. Evidence-based Social and Emotional Learning (SEL) programs have proven effective in supporting students' academic success and reducing bullying and violence. To enhance the psychosocial environment and effectively address school-based violence, comprehensive policies, improved data collection, adequate teacher training, appropriate curricula, and robust reporting mechanisms are necessary.

Insights from interviews with teachers in Tirana and Maminas reveal a clear divide between urban and rural learning environments. In conclusion, creating an optimal learning environment requires a holistic approach that addresses both physical and psychosocial factors. Tailoring strategies to the specific needs of urban and rural settings and implementing effective SEL programs and policies can significantly enhance students' educational experiences and overall well-being.

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ORAL PRESENTATION

TiO_2 ile Modifiye Edilmiş *Pistacia* Polenlerinin Kullanımı ile Süperkapasitör Elektrot Yapımı

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Özet

Son zamanlarda, birçok ülkenin öncelikli araştırma konuları arasında, alternatif enerji kaynakları ve sistemleri üzerine araştırma ve geliştirme faaliyetleri yer almaktadır. Süperkapasitörler bu enerji ihtiyacını karşılayacak umut vaat eden yeni alanlardan biridir. Özellikle metal TiO_2 toksik olmayan ve birçok uygulaması bulunan eşsiz bir yarı iletkendir.

Bu çalışma kapsamında, *Pistacia* polen mikrokapsülleri üzerinde 3 boyutlu TiO_2 nanoyapılar hidrotermal yöntem ile üretilmiştir. Bu malzemelerin morfolojisi taramalı elektron mikroskobu (SEM), gözeneklilikleri Brunauer-Emmett-Teller analizi (BET) ve kristal yapıları X-Işını Difraksiyon spektroskopisi (XRD) ile karakterize edilmiştir. Toz formdaki malzemeler, 300 ^oC, 360 ^oC, 430 ^oC ve 500 ^oC' de ısıl işlem yapılarak, süperkapasitör elektrot aktif malzemesi olarak kullanılmıştır. Elde edilen elektrotların elektrokimyasal özellikleri çevrimli voltametri (CV) ve uzun süreli dolma-boşalma testleri (GCD) ile ayrıntılı bir şekilde incelenmiştir.

Anahtar Kelimeler: metal oksit; süperkapasitör; sporopolen; nanoyapı

Fabrication of supercapacitor electrodes using TiO2 modified Pistacia pollens

Abstract

Recently, research and development activities on alternative energy sources and systems have been among the priority research topics of many countries. Supercapacitors are one of the promising new areas to meet this energy demand. Specifically, the metal TiO2 is a unique semiconductor that is non-toxic and has many applications.

Within the scope of this study, three-dimensional TiO2 nanostructures were produced on Pistacia pollen microcapsules using the hydrothermal method. The morphology of these materials was characterized by scanning electron microscopy (SEM), their porosity by Brunauer-Emmett-Teller (BET) analysis, and their crystal structures by X-ray diffraction (XRD) spectroscopy. The materials in powder form were subjected to thermal treatment at 300°C, 360°C, 430°C, and 500°C and used as supercapacitor electrode active materials. The electrochemical properties of the obtained electrodes were examined in detail using cyclic voltammetry (CV) and long-term charge-discharge tests (GCD).

Keywords: metal oxide; supercapacitor; sporopollen; nanostructure

GİRİŞ

Günümüzde artan enerji talepleri nedeniyle enerji üretimi ve depolama teknolojilerine ihtiyaç artmaktadır. Nüfus artışı ve teknolojinin gelişmesi kaynak arayışına neden olmuştur. Bu doğrultuda enerjinin bilinçsizce kullanımı küresel ısınma ve ekolojik sorunları doğurmuştur. Bu sorunların azaltılması amacıyla fosil yakıtları yerine yenilenebilir enerji kaynakları ve üretilen enerjinin en verimli şekilde depolanması önem kazanmıştır. Enerjinin elektrokimyasal olarak depolanmasında süperkapasitörler hızlı şarj-deşarj olma, güç kapasiteleri, uzun ömürlü ve çevre dostu özelliklere sahip olma nedeniyle son yılların gözdesi olmuştur.

Süperkapasitörlerin, biyo-teknoloji ile birleşimi, süperkapasitörlerin elektrokimyasal performansını geliştirmek için nanoyapılı malzemelerin üretimine yönelik çalışmalar büyük önem kazanmıştır. Biyo-nanoteknoloji kullanılarak farklı nanoyapılı morfolojilere sahip yüksek performanslı elektrot malzemelerin sentezine odaklanılmıştır. Doğada farklı boyut ve şekillerde nano yapılı malzemeler bulunmaktadır. Nano

yapılar üretmek için polen (Bu vd., 2013), böcek, kelebek kanatları, bakteri, virüs, alg ve mantar (Zhang vd., 2017) gibi yapıların iyi biyo-şablonlar olabileceği düşünülmektedir. Süperkapasitör elektrot malzeme üretiminde, biyolojik nanoyapılar yüksek yüzey alanı ve gözeneklilik özelliklerinden dolayı tercih edilmişlerdir. Daha büyük yüzey alanı sağlamak için küçük boyutlu nanomalzemeler tercih edilmektedir. Nanoyapılı malzemelerin üretimi sırasında karşılaşılan sorunlardan biri malzemeler yüzey enerjilerini azaltmak için kümeleşme eğiliminde olmak istemeleridir. Bu problemin önüne geçebilmek için genellikle bir şablon üzerinde malzemelerin büyütülmesi istenir (Atalay vd., 2020).

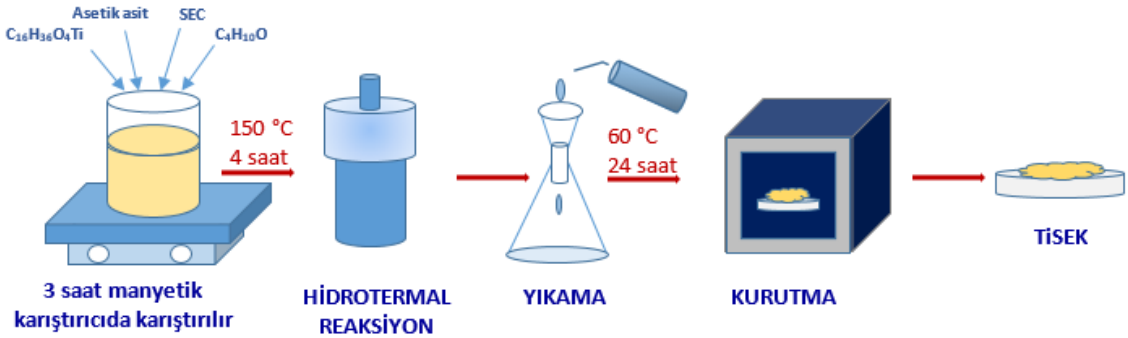
Bu çalışmada doğada bol miktarda bulunan, yenilenebilir, çevre dostu ve kolay elde edile bilen polenler biyoşablon olarak kullanılmıştır. Çalışmalarımızda, *Pistacia* polenleri kullanıldı ve Gaziantep Fıstık Araştırma Enstitüsü'nden temin edilmiştir Polen yapısında bulunan yağ, protein, selüloz gibi organik maddeleri arındırmak için, uygun aseton ve fosforik asit ekstraksiyon işlemleri ile içi boş, üç boyutlu sporopolen ekzin kapsülleri (SEK'ler) elde edilmiştir. Ekstraksiyon işleminden sonra girintili çıkıntılı yapıda olduğu, yüzey alanı ve gözenek hacminde önemli ölçüde artış olduğu gözlenmiştir. Böylece biyoşablon olarak SEK kullanımı ile daha az etkin malzeme kullanılarak süperkapasitör elektrodu geliştirilebilir.

Litaratür taramalarında nanotüp (Nguyen vd., 2017), nanoçubuk, nanoparçacık, nanotel (Guo vd., 2017) ve nanoflaks (Anandhi vd., 2017), yapılı TiO_2 malzemeler farklı yöntemler kullanılarak üretilmiştir ve çevre, elektronik, adsorbsion, kataliz ve süperkapasitör elektrodu gibi çok farklı uygulamalarda kullanılmışlardır. Çalışmalarımızda hidrotermal yöntem kullanılarak, *Pistacia* SEK' leri üzerine büyütülen metal oksit TiO_2 malzemelerin üretimi ve enerji depolama için süperkapasitör elektrotların tasarımında aktif malzeme olarak üretilmiştir. Literatür taramalarında sınırlı sayıda bitki poleninin kullanıldığı çalışmalar bulunmaktadır. Ayrıca *Pistacia* poleni kullanılarak nanoyapılı metal oksit kaplamaları ile ilgili çalışmalara rastlanılmamıştır.

DENEYSEL YÖNTEMLER

iki aşamalı asetoliz metodu ile ekzin mikrokapsüllerin polenlerden izolasyonu yapılmıştır. İlk aşamada amaç polenleri yağlardan arındırılmış, ikinci aşamada ise polenlerin intinleri ve protoplazmaları yok edilmiştir. SEK ektraksiyon işlemlerinin detayları daha önceki çalışmalarımızda verilmiştir (Atalay vd., 2020; 2022).

Pistacia SEK' leriden hidrotermal yöntem ile TiO_2 içerikli mikro küreler üretmek için susuz 0,5 M titanyum (IV) butoksit, 4M n-butanol alkol ve 4M asetik asit çözeltileri hazırlanarak, bu çözeltiye SEK' ler eknerek ardından 20 dk ultrasonik işleme tabi tutularak Titanyum kaplı SEK' ler (TiSEK) elde edilmiştir. Kaplamada izlenen aşamaların şematik gösterimi Şekil 1' de verilmiştir. 100 ml hidrotermal reaktörde 4 saat boyunca 150 °C 'de hidrotemal reaksiyon gerçekleştirilmiştir. Hidrotermal işlem sonrasında elde edilen toz yapı saf su ve etanol ile yıkanıp, 9000 rpm' de 30 dk santrifüjlendikten sonra, dipte kalan çökelek alınarak 60 °C' de 24 saat kurutuldu elde edilen malzemeler, tüp fırında 1 °C/dk ısıtma hızı ile 300 °C, 360 °C, 430 °C ve 500 °C sıcaklıklarda 3 saat ısıl işleme tabi tutulmuştur.



Şekil 1. Hidrotermal yöntem ile TiSEK' lerin elde edilme aşamalarının şematik gösterimi.

Toz formda, elde edilen TiSEK, elektrot aktif madde olarak kullanılmış ve üretilen elektronlar kütlece %75 aktif madde, %15 asetilen black ve %10 PVDF' den oluşmaktadır. Glovebox içerisinde, Zr_2O_3 havanda TiSEK ve asetilen black, 45 dk öğütüldükten sonra, üzerine PVDF eklenerek 30 dk daha öğütülmüştür. Elde edilen karışımdan 5 mg alınarak üzerine ~0,3 ml NMP eklenip bulamaç yapıldıktan sonra Ni foam üzerine mikropipet ile damlatılmıştır. Ardından 90 °C' de 20 mb vakumda 24 saat boyunca kurumaya bırakıldı. Elde edilen TiSEK elektrotlar, çalışma elektrodu olarak kullanılmıştır. Üç elektrotlu elektrokimyasal hücrede karşı elektrot olarak platin, referans elektrot olarak ise düşük sızıntılı seramik uçlu Harvard Apparatus marka Ag/AgCl elektrodu kullanılmıştır. Elektrolit olarak 6 M KOH kullanılarak, çalışma elektrodunun elektrokimyasal kapasitif özellikleri araştırılmıştır.

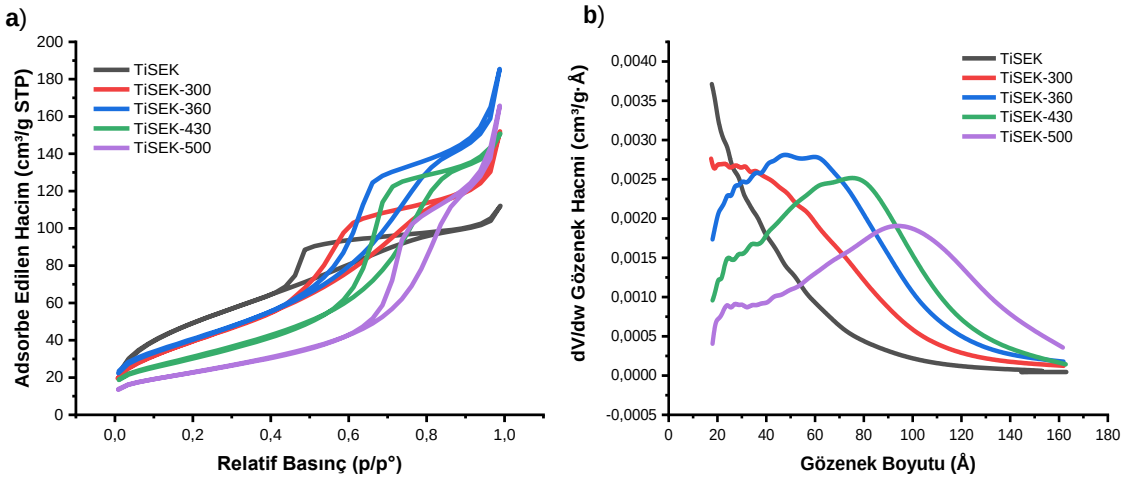
SONUÇLAR VE TARTIŞMA

TiSEK toz numunelerin farklı ısıl işlemler sonrasında elde edilen yüzey alanı, gözenek hacmi ve gözenek boyutu Tablo 1' de verilmiştir.

SEK' in yüzey alanının ve gözenek yoğunluğundaki artış Tablo 1' de açıkça görülmektedir. TiSEK için yüzey alan değeri 183.5 m^2/g ve gözenek hacim yoğunluğu 1126.7x10^{-4} cm^3/g olarak belirlenmiştir TiSEK' lerin ısıl işlem sıcaklığı arttıkça yüzey alanının azaldığı, buna karşın gözenek boyutunun arttığı gözlenmektedir. *Pistacia* polen yüzey alanı hidrotermal işlemle 12.1 m^2/g dan 183.5 m^2/g ve gözenek hacim yoğunluğu 142.3x10^{-4} cm^3/g' dan 1127x10^{-4} cm^3/g'a artmıştır. Aynı zamanda, ısıl işlem sıcaklığının arttırılması ile yüzey alanının dereceli olarak azaldığı, fakat en yüksek gözenek hacim yoğunluğunun ise 360 °C de 2185.9 x10^{-4} cm^3/g olduğu gözlenmiştir. Yüzey alandaki azalmaya rağmen gözenek hacmi önemli ölçüde artmıştır. Gözeneklilikteki artışın elektrokimyasal performasta etkisinin büyük olacağı düşünülmektedir. TiSEK' lerin adsorbsiyon/desorbsiyon grafikleri değerlendirildiğinde Şekil 2a' da TiSEK, TiSEK-300, TiSEK-360 numunelerin H3 histerezisine sahip Tip IV izoterm eğrisine, TiSEK-430 ve TiSEK-500 numunelerin adsorblama gücünün düşük olduğu Tip V izoterm eğrisine benzediği gözlenmektedir. 360 °C' de yüzey alan 149.2 m^2/g gözlenirken, 430 °C' de 114.6 m^2/g olmuştur. Buna karşın gözenek yoğunluğu 430 °C de 2057.5x10^{-4} cm^3/g olarak elde edilmiştir. Numunelerde gözenek dağılımının 2 ile 16 nm aralığında olduğu gözlenmiştir (Şekil 2b).

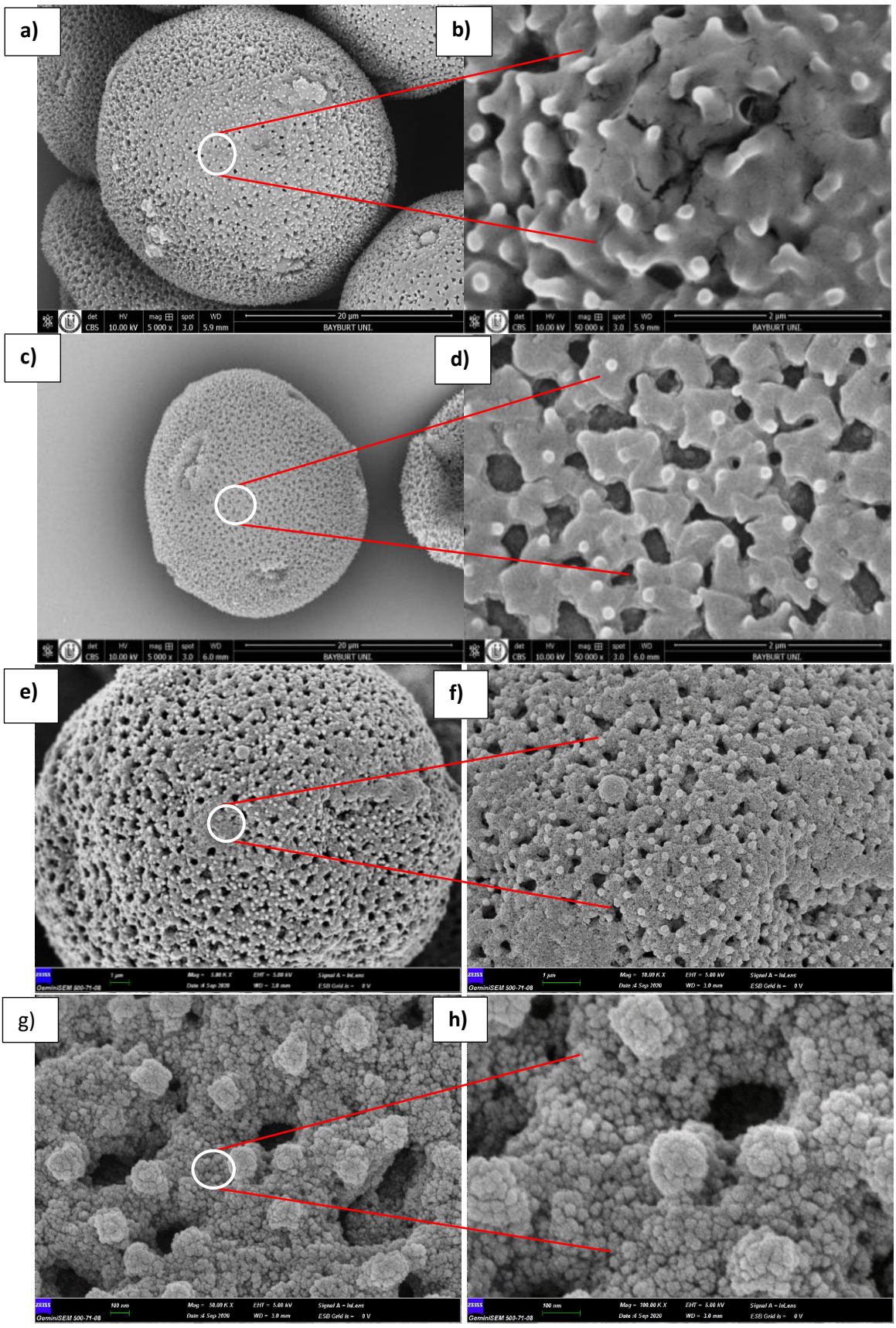
Tablo 2 . Farklı ısıl işlem uygulanmış TiSEK' ler için BET analiz sonuçları.

Numune İsmi	Isıl İşlem Sıcaklığı (°C)	Yüzey Alanı (m^2/g)	Gözenek Hacmi (x$10^{-4}$$cm^3/g$)	Gözenek Boyutu (Å)
SEK	-	12,1	142,3	50,5
TiSEK	-	183,5	1126,7	35,3
TiSEK-300	300	150,4	1735,1	43,1
TiSEK-360	360	149,2	2185,9	50,3
TiSEK-430	430	114,6	2057,5	57,9
TiSEK-500	500	83,3	1823,8	70,8

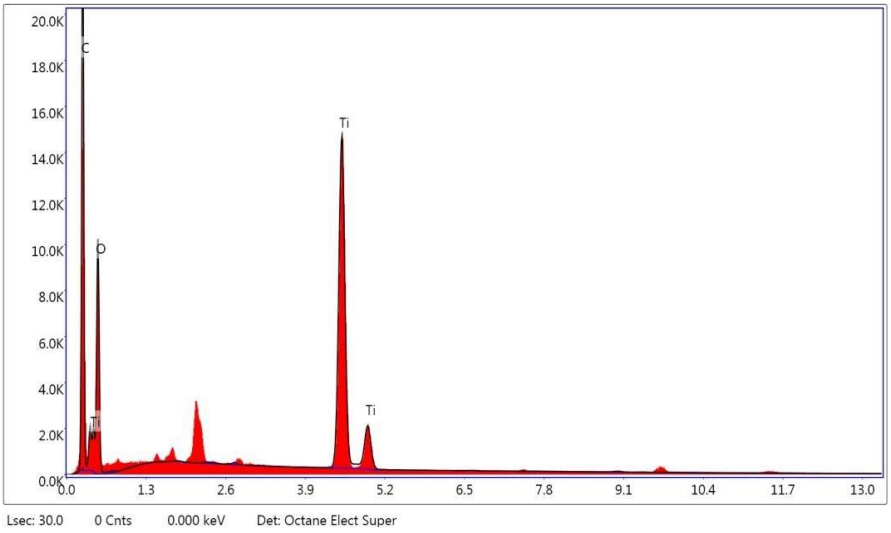


Şekil 2. Farklı ısıl işlem sıcaklıkları için TiSEK numunelerin: a) Adsorbsiyon/desorpsiyon izotermi, b) Gözenek dağılım grafikleri.

Şekil 3 a-b' de işlem görmemiş *Pistacia* polen SEM görüntüleri görülmektedir. Görüntülerde polenlerin gözenekli küresel bir yapı sergilediği ve yüksek büyütmelerde yüzeyin girintili çıkıntılı oluşumu gözlenmektedir. SEK' lerin 5000 kat ve 50000 kat büyütmeli SEM görüntüleri Şekil 3c-d' de görülmektedir. SEK yüzeyinde 2 ile 10 nm boyutlu tanecikli yapıların yer aldığı görülmektedir. Hidrotermal reaksiyon sonrası elde edilen TiSEK' lerin farklı büyütmelerdeki SEM görüntüleri Şekil 3e-h' de verilmiştir. Hidrotermal işlem sonrası SEK' lerin yüzeylerinin TiO_2 ile kaplandığı ve nanogözenekli yapıya sahip olduğu SEM görüntülerinden açık bir şekilde görülmektedir (Şekil 3e-h).

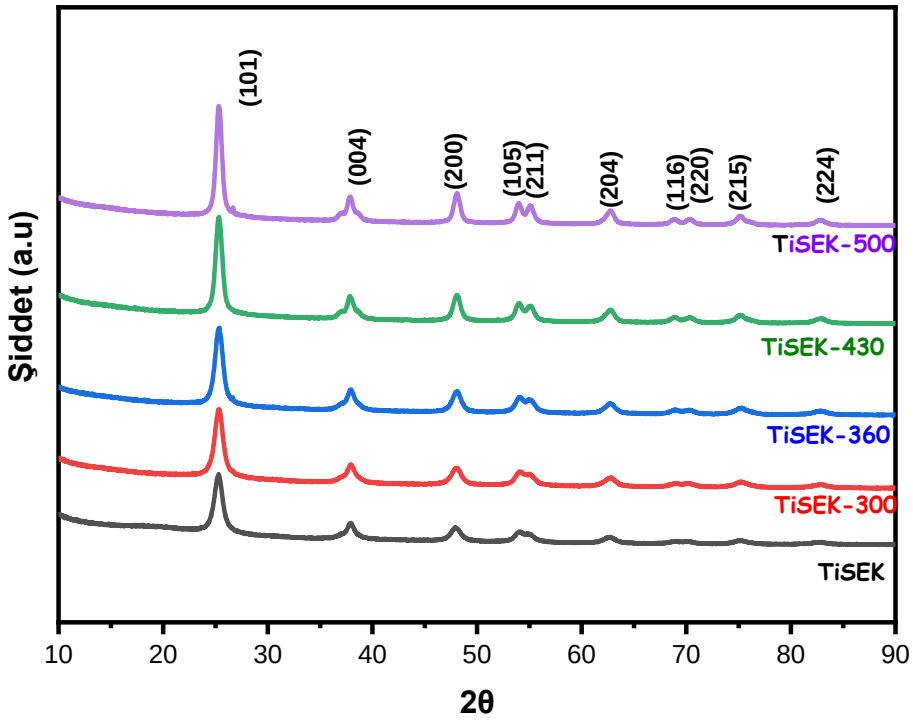


Şekil 3. a-b) İşlem görmemiş *Pistacia* polenlerin, c-d) SEK' lerin, e-h) Hidrotermal reaksiyon sonrası elde edilen TiSEK malzemenin, farklı büyütmelerdeki SEM görüntüleri.



Şekil 4. Hidrotermal reaksiyon sonrası elde edilen TiSEK malzemenin EDX spektrumu

Şekil 4' te hidrotermal reaksiyon sonrası elde edilen TiSEK malzemenin EDX analiz spektrumu verilmiştir. Analiz sonucunda SEK' lerin yapılarında Ti ve O varlığı belirlenmiştir.

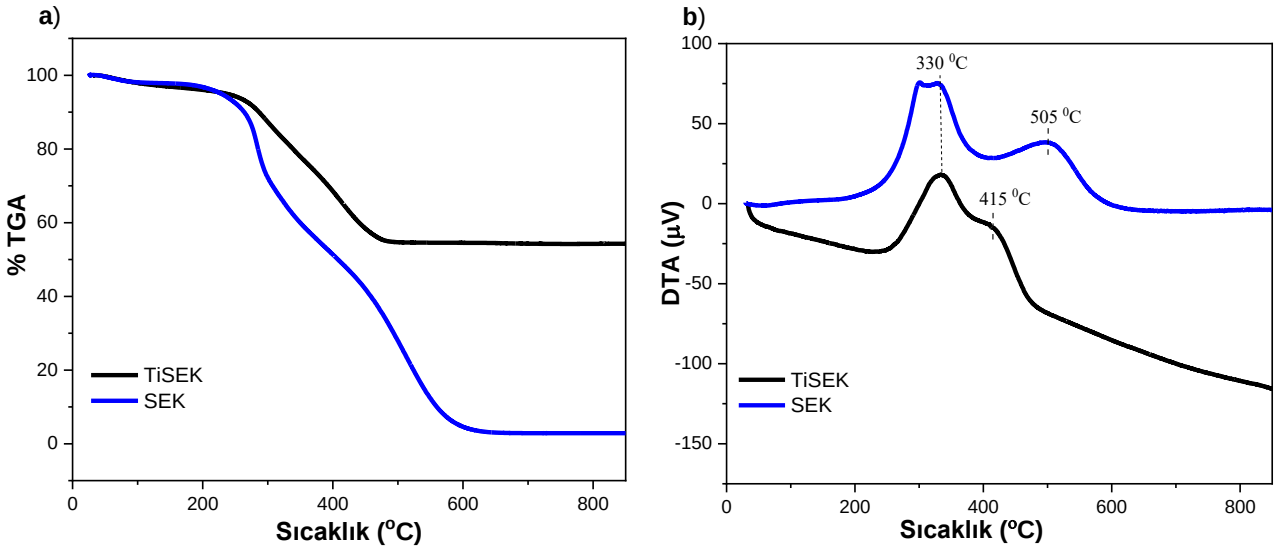


Şekil 5. Hidrotermal yöntem ile SEK üzerine büyütülmüş Ti içerikli TiSEK numunelerin XRD spektrumları

Farklı ısıl işlemlere tabi tutulan TiSEK malzemelerin XRD spektrumları Şekil 5' de verilmiştir. TiSEK malzemelerin 25.28°, 37.80°, 48.04°, 53.89°, 55.06°, 62.68°, 68.76°, 70.30°, 75.02° ve 82.65°' lerde gözlenen pikleri TiO_2' nin anataz yapısına ait (101), (004), (200), (105), (211), (204), (116), (220), (215), (224) düzlemlerine karşılık gelmektedir. [(PDF#21-1272) kristografi standardın]. XRD grafiğinden nanoparçacık boyutu Debye Scherrer denkleminden hesaplandı (Eş.1). TiSEK, TiSEK-300, TiSEK-360, TiSEK-430, TiSEK-500 malzemeler için kristal boyutları sırasıyla 3.06 nm, 3.04 nm, 4.86 nm, 5.73 nm, 5.23 nm bulunmuştur. Sıcaklığın artmasıyla parçacık boyutu artmıştır. Denklemde *D*; nanometre cinsinden ortalama kristal boyutunu, K; kristal form faktörünü (0.9), λ; X-Ray ışınının dalga boyunu (λ = 0.15406 nm' dir), β; maksimum saçılma pikinin yarı yüksekliğinin sahip olduğu genişliği (FWHM), θ; pik noktasını (Bragg açısı) ifade etmektedir.

$$D = \frac{K \times \lambda}{\beta \times \cos\theta} \qquad (1)$$

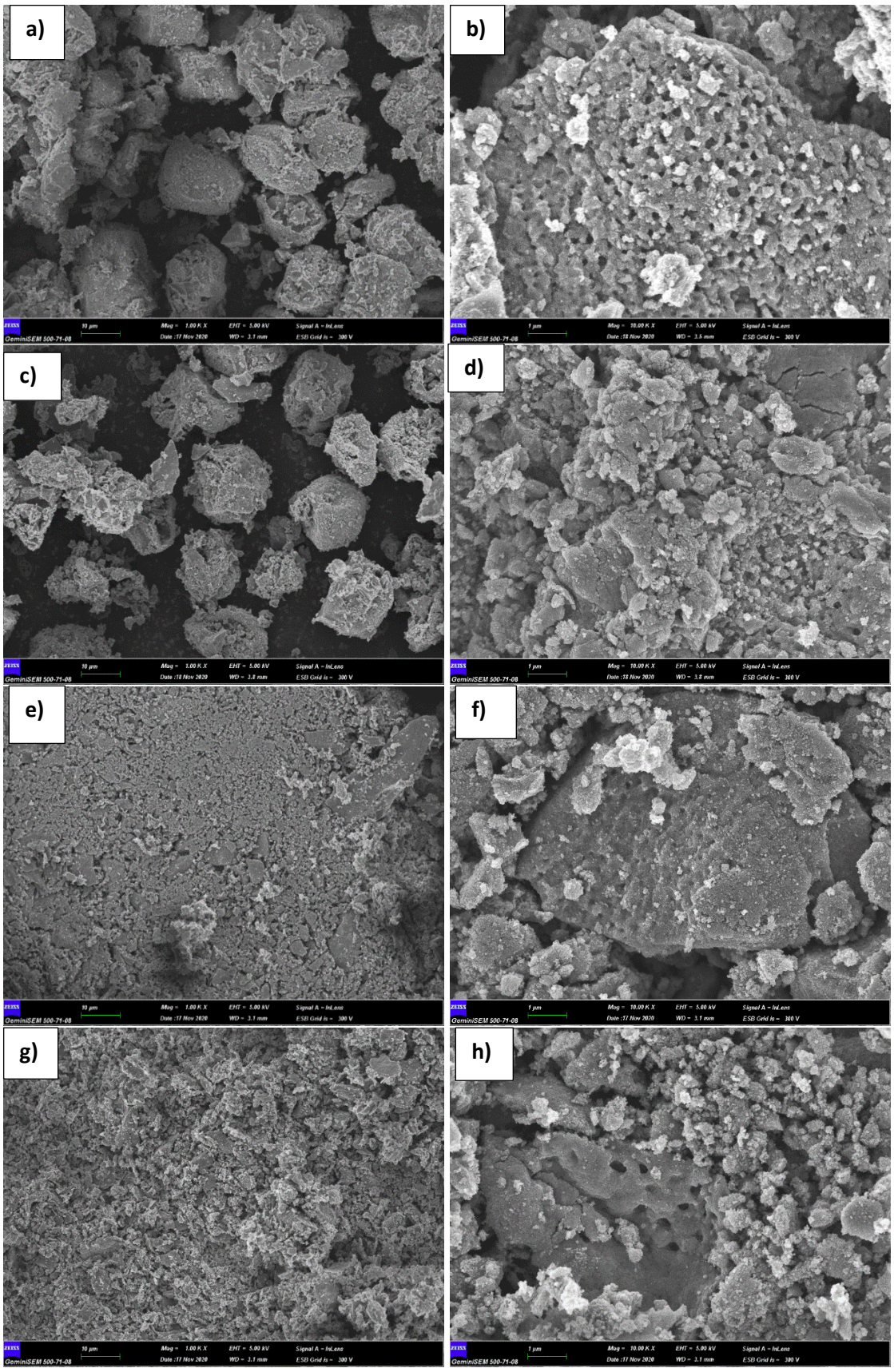
TiO_2 fazının oluşum sıcaklığını belirlemek için hava ortamında termogravimetrik analiz ve diferansiyel termal analiz SEK ve TiSEK numunelerinde gerçekleştirilmiştir (Şekil 6). SEK' in TGA eğrisinde 4 farklı bölgede kütle kaybı görülmektedir. İlk olarak 30-200 °C aralığında malzemede bulunan su ve etanolün uzaklaştığını yaklaşık %3 lük kütle kaybını göstermektedir. Daha sonra 3 farklı bölgeye tekabül eden 200-630 °C aralığında kütle kaybı malzemede bulunan yağ, protein, karbonhidratların ve ekzin tabakanın bozulmasıyla yaklaşık %93 olarak belirlenmiştir. TGA eğrisinde görülen kütle kayıpları DTA eğrisinde ekzotermik pikler olarak karşımıza çıkmaktadır (Şekil 6b).



Şekil 6. SEK ve hidrotermal reaksiyon sonrası elde edilen TiSEK a) TGA, b) DTA grafikleri.

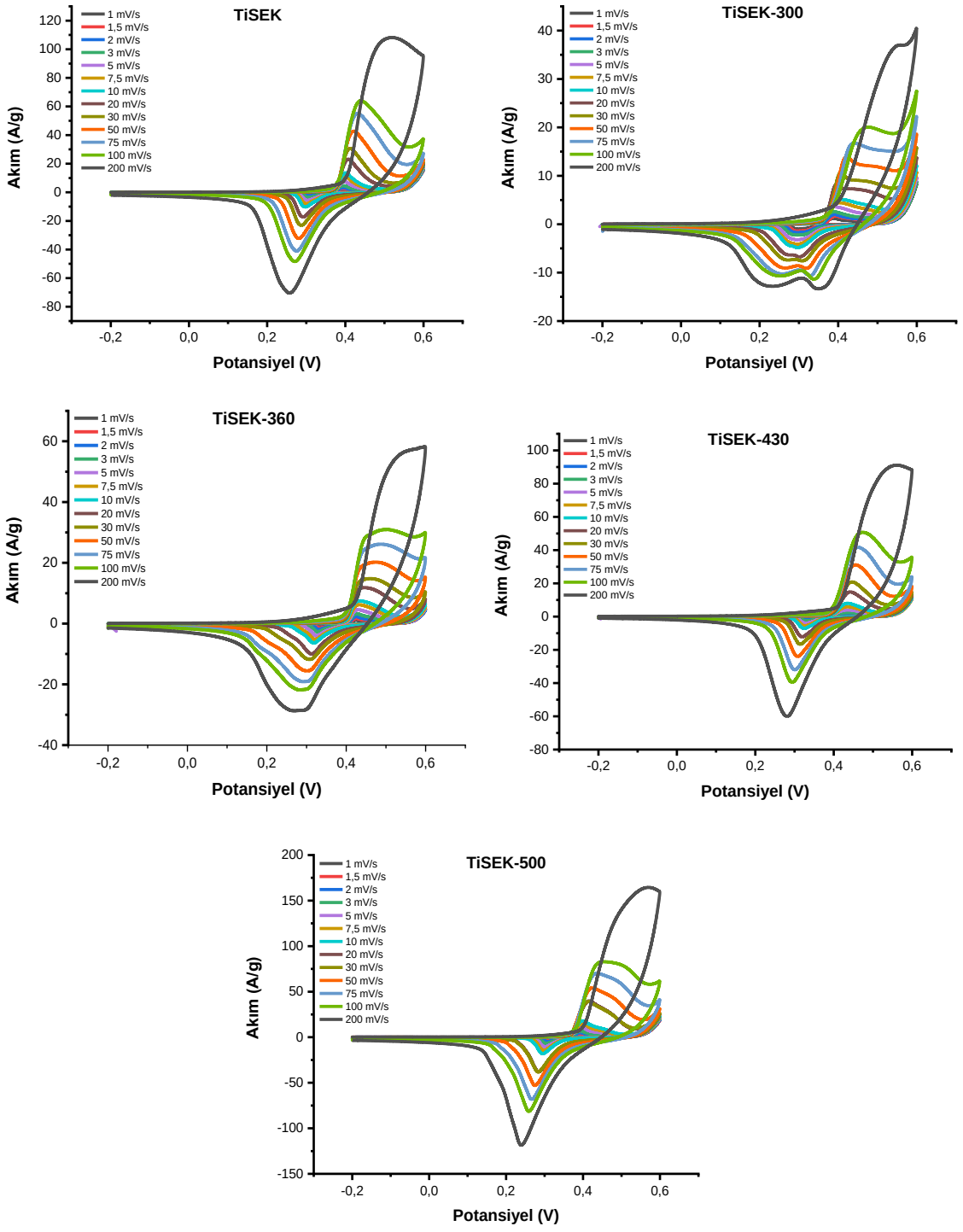
TiSEK numunenin TGA eğrisinde 3 bölgeden birincisi olan 30-200 °C aralığında kütle kaybı yaklaşık %3,95' dir. 200-490 °C arasında gerçekleşen ardışık kütle kayıpları SEK' in ve titanyumun dekompozesinden kaynaklanmaktadır ve %40 civarındadır. 30-1000 °C aralığında gerçekleştirilen ölçüm sonucunda numunenin yaklaşık %55' lik kütlesi korunmuştur. SEK için bu değer yaklaşık %3' tür. Şekil 6b' de verilen DTA eğrisinde 330 °C ve 415 °C görülen ekzotermik pikler SEK' te ve titanyumda gerçekleşen dekompozisyonu göstermektedir.

300 °C, 360 °C, 430 °C ve 500 °C ısıl işleme tabi tutulan numunelerin morfolojisindeki değişimler Şekil 7' de farklı büyütmelerdeki SEM görüntüleri verilmiştir. Hidrotermal işlem sonrası polen morfolojisi kısmen korunmakta ve polen yüzeyinde homojen bir kaplama görülmektedir. Fakat ısıl işlem sıcaklığının artması ile birlikte polen yüzeyindeki nanoboyutlu küresel taneciklerin, küreselliklerini yitirdiği ve tanecik boyutlarının küçüldüğü görülmektedir. Ayrıca polen morfolojisi 430 °C ve 500 °C' lik ısıl işlem sonrası yok olmuştur (Şekil 7e-f ve 7g-h). Fakat polen parçalarının izleri ve üzerinde büyüyen TiO_2 nanoyapıları içeren mikro yapılar halen görülmektedir.



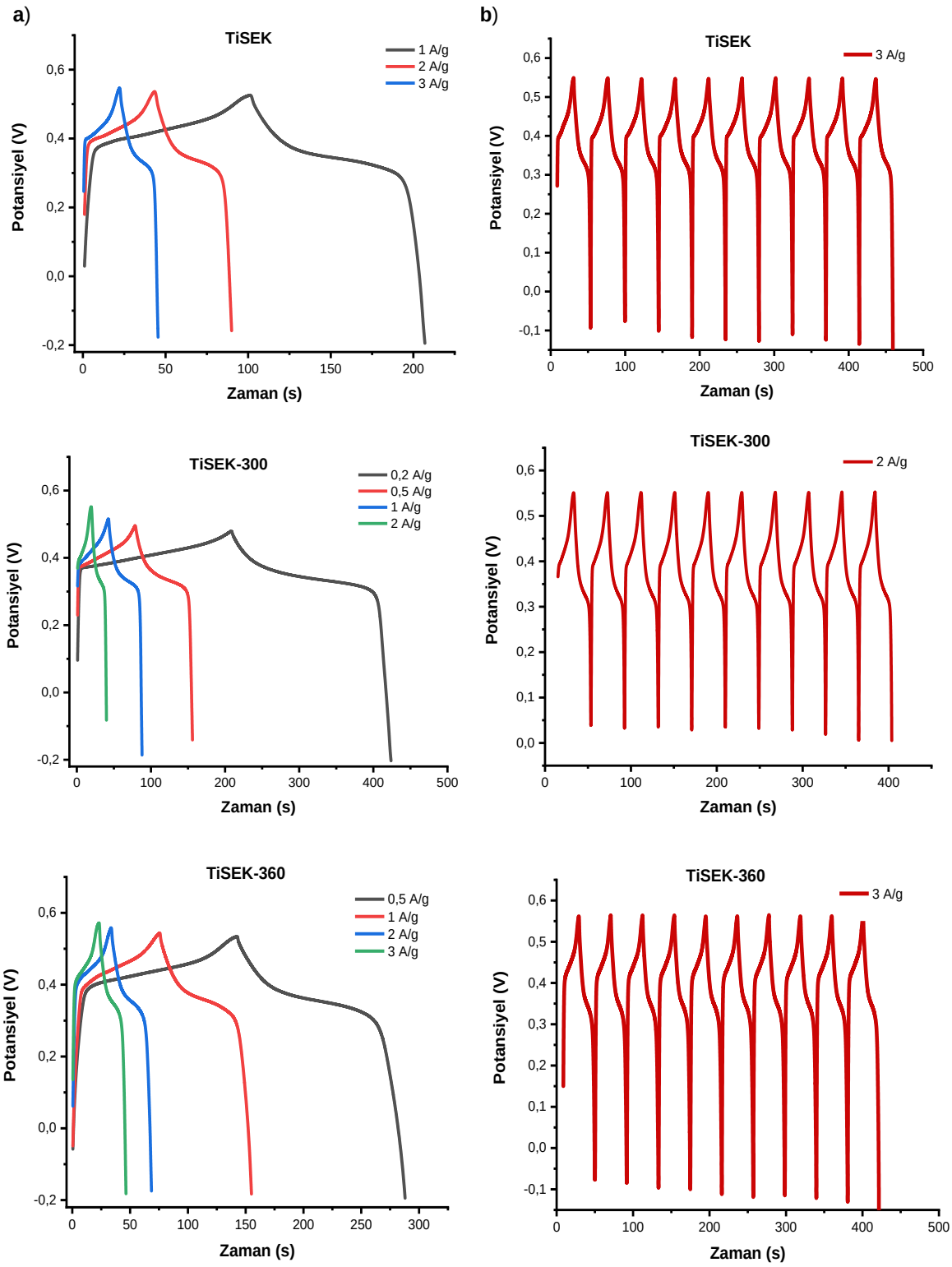
Şekil 7. TiSEK numunenin: a-b) 300 °C, c-d) 360 °C, e-f) 430 °C, g-h)500 °C' de ısıl işlem sonrası sırası ile 1000, 10000 kat büyütmeli SEM görüntüleri.

TiSEK ve bunların farklı ısıl işlem uygulanmış olanlarına 6M KOH elektrolitte -0.2V ile 0.6V potansiyel aralığında, 1-200 mV/s tarama hızlarında CV' leri alındıktan sonra elektrokimyasal empedans ölçümleri, daha sonra farklı akım yoğunluklarında dolma-boşalma testleri yapılmıştır. Yapılan testlerin grafikleri aşağıda detaylı olarak verilmiştir.

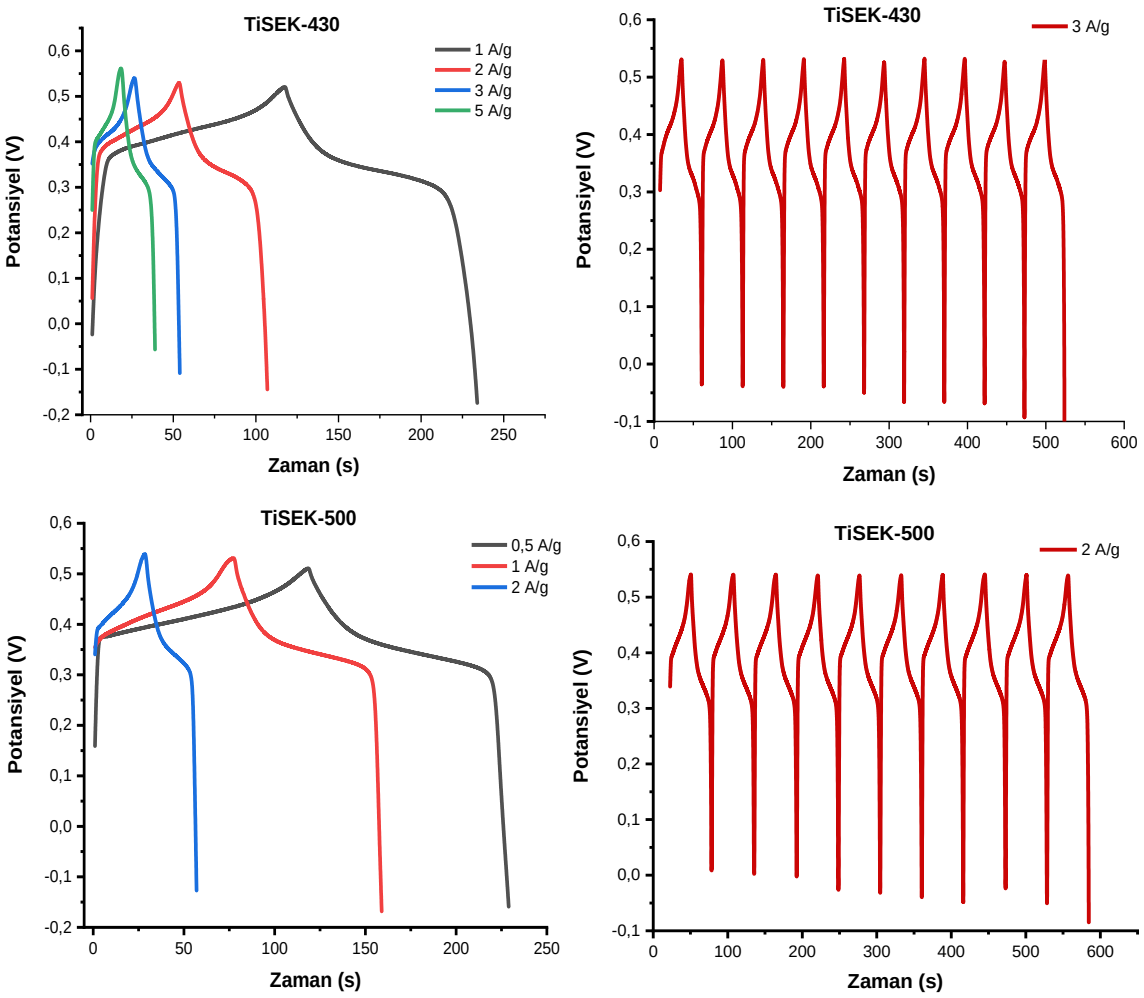


Şekil 8. Farklı tarama hızlarında farklı ısıl işlem sıcaklıklarındaki toz TiSEK elektrotlarının CV eğrileri.

Şekil 8' de farklı ısıl işlem sıcaklıkları için TiSEK elektrotlarının CV eğrileri görülmektedir. Toz malzemeye uygulanan sıcaklığın, oluşturulan elektrotların çalışma potansiyel aralığını önemli ölçüde etkilediği CV ölçümlerinden görülmektedir. CV eğrilerinin benzer şekillere sahip olduğu ve artan tarama hızı ile anodik ve katodik pik akım değerlerinin arttığı görülmektedir. Buda redoks reaksiyonlarının gerçekleştiğini ve elektrotların beşinin de pseudokapasitif davranış sergilediğini göstermektedir.



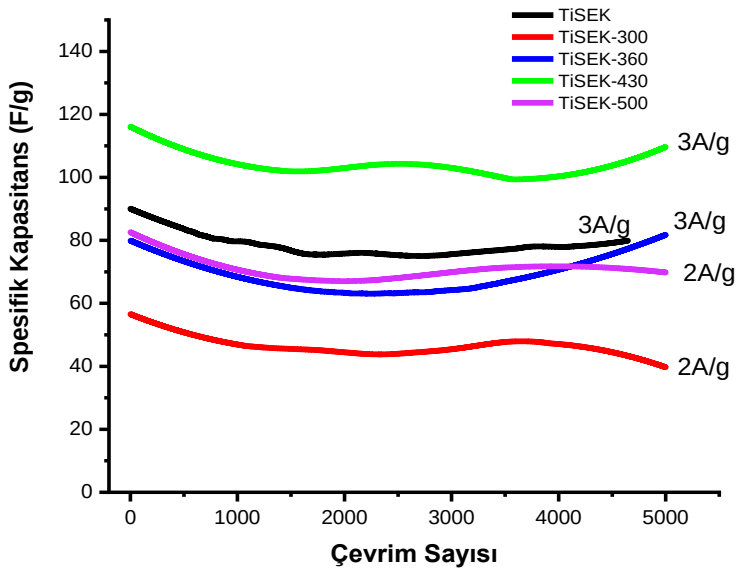
Şekil 9. Farklı ısıl işlem sıcaklıklarındaki TiSEK elektrotların: a) Farklı akım değerleri için CD, b) İlk on CD eğrileri.



Şekil 9. (Devam ediyor)

Her bir elektrotun farklı akım yoğunlukları için davranışları Şekil 9a' da ve ilk on dolma-boşalma eğrisi Şekil 9b' de verilmiştir. Her beş elektrot için akım değerinin artmasına karşın dolma-boşalma süresinde azalma görülmektedir (Şekil 9a). TiSEK-300 kodlu elektrot 0-0.55 V aralığında, TiSEK-430 kodlu elektrot ise -0.1-0.53 V potansiyel aralığında çalışmaktadır. Şekil 9b' de ilk on dolma-boşalma eğrileri testere diş görünümü süperkapasitör özelliğine sahip olduğunu göstermektedir.

Farklı ısıl işlem sıcaklıklarındaki toz TiSEK elektrotların dayanabildikleri en yüksek akım değerlerinde uzun süreli dolma boşalma testleri en az 5000 çevrim için yapılmıştır. Elektrotların spesifik kapasitan eğrileri Şekil 10' da verilmiştir. Yapılan uzun süreli ölçümlerde TiSEK-430 kodlu numune için maksimum spesifik kapasitansı 138.49 F/g olduğu, 5000 çevrim sonrasında kapasitansın %81' ini koruyarak 112,9 F/g' a düştüğü gözlenmiştir. TiSEK, TiSEK-300, TiSEK-360 ve TiSEK-500 kodlu dört elektron için maksimum spesifik kapasitans değerleri sırası ile 95,8 F/g, 69,3 F/g, 85,9 F/g ve 89,8 F/g olarak hesaplanmıştır. TiSEK-430 kodlu elektrotun maksimum enerji yoğunluğu 6 Wh/kg ve güç yoğunluğu 21.6 kW/kg olarak hesaplanmıştır.



Şekil 10. Farklı ısıl işlem sıcaklıkları için toz TiSEK elektrotların spesifik kapasitans eğrileri.

SONUÇ

Bu çalışmada, hidrotermal yöntemle biyo bazlı nanoyapılı içi boş TiO_2 mikroküreler üretilmesi ve bunların enerji alanında kullanımına yönelik araştırmalar için yüzey alan parametreleri iyi olan *Piscatia* poleni kullanılmıştır. Antep fıstığı araştırma enstitüsünden elde edilen *Pistacia* polenlerinden, SEK' ler iki aşamalı asidoliziz metoduyla yeşil mutakabata uygun olarak elde edilmiştir.

Toz formdaki materyalden hazırlanmış beş farklı numune arasında, 430 ^oC de ısıl işlem görmüş numuneyi içeren TiSEK-430 elektrotun, 3 A/g' da spesifik kapasitansının yapılan uzun sureli ölçümlerde, maksimum 138.49 F/g değerine sahip olduğu görülmüştür.

TEŞEKKÜR

Bu çalışma TUBITAK tarafından 218M267 ve İnönü Üniversitesi BAP FYL-2020-2196 proje numarası ile desteklenerek gerçekleştirilmiştir.

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ORAL PRESENTATION

The Growing Major Concern: Shigellosis

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Abstract

Foodborne disease is a significant public health issue in both developed and developing countries. An outbreak is defined as an incident where two or more individuals experience similar illness after consuming a common food source. Bacteria account for 60% of foodborne illness cases that require hospitalization. Among these, infection with *Shigella* species is a major cause, largely due to the bacteria's low infectious dose and its ability to spread through person-to-person transmission via the fecal-oral route. Shigellosis is a gastrointestinal infection caused by one of four species of *Shigella* bacteria: *Shigella sonnei*, *S. flexneri*, *S. boydii*, and *S. dysenteriae*. Humans serve as the primary reservoir for these bacteria, and infection occurs when someone ingests food, water, or materials contaminated by human feces, often through direct contact with contaminated hands or objects. Secondary infections are common due to the bacteria's low infectious dose. The incubation period typically lasts 1-2 days, and symptoms can range from mild to severe. Common signs of shigellosis include diarrhea (which may be bloody or prolonged for more than three days), fever, abdominal pain, and discomfort, along with tenesmus—a frequent urge to pass stool without being able to do so. In low-income regions, shigellosis primarily affects children, whereas in high-income areas, international travelers. In recent years, the growing resistance of Shigella to first- and second-line antibiotics has become a significant concern.

Keywords: *Shigella* spp., Foodborne pathogen, Shigellosis

INTRODUCTION

Shigella species are Gram-negative, non-spore-forming, rod-shaped bacteria belonging to the family *Enterobacteriaceae* (Lampel and Maurelli, 2003, Levine et al., 2007). These bacteria are facultative intracellular pathogens that primarily infect humans and other primates. The first discovery and identification of the bacteria responsible for bacillary dysentery, later named *Shigella*, was made by Kiyoshi Shiga in the late 19th century (Ud-Din and Wahid, 2014). In subsequent decades, various strains were identified, all closely related to the nonpathogenic bacterium *Escherichia coli*. The genus *Shigella* was classified to differentiate pathogenic strains of high clinical importance from less-pathogenic or nonpathogenic strains, based on biochemical, serological, and clinical characteristics (Schroeder et al., 2008).

The *Shigella* genus consists of four species: *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D). These species are further divided into several serotypes based on variations in their O antigens. *S. flexneri* is the most prevalent cause of infections, followed by *S. sonnei*. In contrast, *S. dysenteriae* is responsible for epidemic outbreaks and the most severe form of dysentery, accounting for most fatal cases of shigellosis. However, recent comparative genomic studies challenge this traditional classification, showing that *Shigella* species are closely related to *Escherichia coli*, rather than being a separate genus (Johnson, 2000). For example, the genetic difference between *S. flexneri* and *E. coli* K-12 is approximately 1.5%, a small contrast compared to the 15% difference seen in *Salmonella enterica*, another close relative of *E. coli* (Løbersli et al., 2016). Additionally, diarrheagenic enteroinvasive *E. coli* (EIEC) shares many biochemical traits, key virulence factors, and clinical symptoms with *Shigella* spp. Although EIEC does not entirely meet the definition of the *Shigella* genus, genomic analyses reveal it is more closely related to *Shigella* spp. than to commensal *E. coli* strains (Schroeder et al., 2008).

Comparative genomics demonstrates that *Shigella* species and enteroinvasive *E. coli* (EIEC) evolved from multiple *E. coli* strains through convergent evolution. Within this framework, three main *Shigella* clusters have been identified, each containing strains from the traditionally defined species. Although *S. sonnei* and certain *S. dysenteriae* strains are more distantly related to these main clusters, they still align with *E. coli*. The divergence of these three *Shigella* clusters from *E. coli* occurred between 35,000 and 270,000 years ago, while *S. sonnei* emerged more recently, separating from the others about 10,000 years ago. Since EIEC retains more traits of commensal *E. coli* than *Shigella* spp., it likely acquired its virulence mechanisms more recently, reflecting an earlier stage in the evolutionary path of *Shigella* spp. Despite these evolutionary insights, no new nomenclature has yet been established to encompass both evolutionary and phenotypic classifications for *Shigella* (Schroeder et al., 2008, Løbersli et al., 2016).

Shigellosis, caused by *Shigella* species, is a widespread infectious disease, particularly prevalent in many developing nations. It leads to approximately 80 million cases of bloody diarrhea and results in around 700,000 deaths each year. The infection disproportionately affects vulnerable groups such as children under five, the elderly, and those with weakened immune systems, who are more likely to experience severe symptoms. Though individuals of any age can contract *Shigella* infections, these groups face the highest risk (Ud-Din and Wahid, 2014, Wang et al., 2019). Protective immunity against *Shigella* can develop after repeated exposures to the bacteria (Barnoy et al., 2010). However, prior infections do not confer immunity to other *Shigella* serotypes. This immunity is primarily serotype-specific, driven by antibodies targeting the O antigen of the lipopolysaccharide, a key component of the bacterial cell wall. As the O antigen differs across serotypes, immunity remains restricted to the specific serotype involved in the initial infection (Altuntaş et al., 2018). The majority of cases occur in developing countries, where poor hygiene and water quality contribute to the spread of the disease. *S. flexneri* is the dominant serogroup in these regions, while *S. sonnei* is more common in developed countries. The infection spreads primarily via the fecal-oral route, often requiring as few as 10 to 100 bacteria to cause illness, making it highly contagious. This low infectious dose is partially due to *S. flexneri*'s acid resistance mechanisms, which help it survive stomach acid. Additionally, *Shigella* species can suppress the production of antimicrobial peptides, which are critical for defense against bacterial pathogens in the intestinal mucosa. After surviving the stomach and small intestine, the bacteria colonize the large intestine, where they cause infection (Bardhan et al., 2010).

Shigellosis is a gastrointestinal infection marked by diarrhea, and its most severe form is caused by *Shigella dysenteriae* serotype 1. *Shigella sonnei* typically leads to milder illness, while *S. flexneri* and *S. boydii* may cause either mild or more severe symptoms (Ud-Din and Wahid, 2014). Additionally, entero-invasive *E. coli* (EIEC) can induce dysentery, and due to similar pathogenic characteristics between EIEC and *Shigella* species, it can be challenging to distinguish them through routine diagnostic tests (Hendricks et al., 2020). Outbreaks of *S. dysenteriae* are often associated with epidemics and can result in fatal outcomes. Factors such as inadequate water supply, poor sanitation, and household environments prone to fly infestations are significant environmental contributors to shigellosis transmission. The disease can also lead to severe complications, including lethal toxic encephalopathy (Ekiri syndrome) and hemolytic uremic syndrome (HUS). The severity of clinical symptoms depends on factors like the *Shigella* serotype, the infectious dose, and the host's immunity and age. After an incubation period of 1 to 7 days (typically around 3 days), symptoms may last for 1 to 2 weeks (Lampel and Maurelli, 2007, Bardhan et al., 2010). Early signs include watery diarrhea, fever, and fatigue, but in more severe infections, particularly with *S. dysenteriae* serotype 1, patients may experience dysentery, characterized by painful, frequent stools with blood and mucus, along with abdominal cramping, nausea, and vomiting (Niyogi, 2005, Nygren et al., 2012). All *Shigella* species can cause acute bloody diarrhea (Ud-Din and Wahid, 2014). In most cases, the disease resolves on its own, and fatal outcomes are uncommon, except for *S. dysenteriae* serotype 1, which has a mortality rate of up to 20% (Lampel and Maurelli, 2003). Long-term complications may include Reiter's syndrome (reactive arthritis), especially following *S. flexneri* infection, and hemolytic uremic syndrome following *S. dysenteriae* serotype 1 infection (Warren et al., 2006). Without treatment, children can experience prolonged illness, which increases the risk of chronic diarrhea, malnutrition, and spreading the infection to family members. The increasing resistance of *Shigella* to commonly used antibiotics has made treatment more challenging. Although ceftriaxone, azithromycin, and quinolones were once effective, reports of multidrug-resistant *Shigella* strains are on the rise worldwide (Kara et al., 2015, Shoja et al., 2023).

Shigella has emerged as a significant public health issue due to the increasing prevalence of strains resistant to multiple antibiotics, highlighting the need for ongoing surveillance of this pathogen. Vaccination is considered the most practical preventive strategy for controlling shigellosis; however, no safe and effective vaccine has yet been developed. One of the promising candidates for future vaccine development involves outer membrane vesicles. Shigella species cause bloody diarrhea by invading and damaging the epithelial lining of the colon. This results in the formation of small ulcers, inflammatory exudates, and the infiltration of inflammatory cells (polymorphonuclear leukocytes, PMNs), leading to blood and mucus in the stool. The bacteria are highly sensitive to environmental changes and die quickly after being excreted, especially when exposed to drying or direct sunlight (Gaurav et al., 2013).

Shigella spp. growth and persistence in food are affected by various factors, including temperature, pH, salt concentration, and the use of preservatives. Research indicates that S. flexneri can thrive better at lower temperatures, higher pH levels, and lower sodium chloride concentrations. The optimal temperature range for the growth of these bacteria is between 6–8°C and 45–47°C. They become inactive quickly at approximately 65°C. However, Shigella spp. can endure for long durations under freezing conditions (-20°C) or when refrigerated (4°C) (Warren et al., 2006).

After contaminated food is consumed, Shigella spp. need to withstand the stomach's acidic environment and penetrate the epithelial cells of the colon to establish an infection. Once inside the colonic epithelial cells, these bacteria replicate and move to neighboring cells, resulting in cell death. This process causes inflammation and ulceration of the colon, leading to the characteristic bloody mucoid diarrhea associated with Shigella infections (Lightfoot 2003, Montville and Matthews 2005, Warren et al., 2006). Limited published data exists regarding Shigella presence in food. However, some international studies have detected Shigella spp. in various food items (Castillo et al., 2006).

Much of the understanding regarding the mechanisms of Shigella pathogenesis comes from research on S. flexneri. The infection process initiated by this invasive bacterium involves several stages. To penetrate the intestinal mucosa, S. flexneri must traverse the intestinal epithelium, which serves as a barrier against both harmless and harmful bacteria. During the early stage of infection, S. flexneri does not breach the epithelial barrier from the top; instead, it prompts uptake by microfold cells (M cells) and is subsequently transported across the epithelial layer. M cells are specialized cells that constantly sample the gut contents and transfer them to the nearby mucosal lymphoid tissue, where immune responses can begin. This mechanism of utilizing M cells aligns with observations that S. flexneri has minimal interaction with the apical surface of polarized epithelial cells, preferentially entering through the basolateral side (Avakh et al., 2018, Wang et al., 2019).

DIAGNOSIS

Diagnosis of shigellosis is based on stool culture, where the presence of *Shigella* species can be confirmed through microbiological techniques. Molecular methods such as PCR are also employed for faster and more specific identification. However, differentiating *Shigella* from Enteroinvasive *Escherichia coli* (EIEC) can be challenging due to the similarity in their clinical presentation and pathogenic mechanisms (Bardhan et al., 2010, Lee and Kong 2016).

PREVENTION AND CONTROL

Preventing the spread of *Shigella* is particularly challenging due to the bacteria’s low infectious dose and the fecal-oral transmission route. Improved sanitation, access to clean water, and proper hand hygiene are critical in reducing the risk of shigellosis outbreaks. Health education on food safety, especially in areas prone to outbreaks, is also essential (Lee and Kang, 2016).

One of the most promising strategies for long-term control of shigellosis is vaccination. While several vaccine candidates, including conjugate vaccines and live attenuated strains, are under development, there is currently no widely available vaccine for *Shigella*. Given the rise in antibiotic-resistant strains, vaccination remains a key focus in the global effort to control shigellosis (Ud-Din and Wahid, 2014).

CONCLUSION

Shigellosis continues to be a major public health issue, particularly in regions with inadequate sanitation and hygiene. The disease can range from mild to severe, with potentially fatal complications in vulnerable populations. With the increasing prevalence of antibiotic resistance, the development of effective preventive measures, particularly vaccines, is urgently needed. Until then, public health interventions focusing on improved hygiene, sanitation, and access to clean water remain the most effective means of controlling the spread of shigellosis.

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ORAL PRESENTATION

Assessment of antioxidant activity and phytochemical composition of Turkish sumac (*Rhus coriaria* l.) leaves

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Abstract

This manuscript presents a comprehensive study on the antioxidant potential and phytochemical composition of Turkish Sumac (*Rhus coriaria* L.) leaves. Sumac is a Mediterranean plant widely distributed in different parts of the world, known for its traditional medicinal uses and culinary applications. In this research, we aimed to evaluate the antioxidant activity, total anthocyanin, phenol, and flavonoid content of Sumac leaves to shed light on its potential health benefits. Sumac leaves were dried in the shade and then extracted with methanol. The total anthocyanin content was determined using a spectrophotometric method based on the pH differential technique. The determination of total phenol content involved the use of the Folin-Ciocalteu reagent, while the estimation of total flavonoid content was conducted through the aluminum chloride colorimetric assay. The antioxidant activity of Sumac leaf extract was assessed using various methods, such as DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay, and ferric reducing antioxidant power (FRAP) assay. When the results were evaluated, Sumac leaves were found to have high antioxidant activity (DPPH IC_{50}=0.019 µg/ml; ABTS IC_{50}= 0.0052 µg/ml; FRAP 0.015 trolox equivalents). While its total phenol content is found to be very high, it is predicted that its antioxidant activity is due to its high phenolic content (TPC of sumac: 251.29 mg gallic acid/g extract). In light of all this, it is thought that sumac leaves as well as fruits have high antioxidant effects and have a good therapeutic potential for various diseases.

Keywords: Sumac, total phenol, anthocyanin, flavonoid, antioxidan

INTRODUCTION

Sumac, *Rhus* sp. (Anacardiaceae) belonging to the genus has more than 150 species worldwide. At 1-3 meters in height, *R. coriaria* is a shrub or small tree. 9-15 leaflets on imparipinnate leaves. The blooms are small and greenish, and the inflorescence is an erect, compact panicle. Sumac fruits is a drupe that has a sour taste, is hairy, and turns dark red when mature (Perrone et al., 2022).

Traditionally, sumac has been used in different cultures for its health benefits. It is used in folk medicine to treat indigestion, anorexia, bleeding and hyperglycemia. Sumac fruits are also believed to be useful as a tonic and diuretic, in pharyngeal cold infections, dysentery and diarrhea (Asgarpanah and Saati, 2014). *R. coriaria* also has various biological activities, including anticancer, antioxidant, antidiabetic, anti-inflammatory, anti-microbial and cardiovascular disorders (Elagbar et al., 2020). Many phytochemical compounds are found in sumac. One of the first studies, conducted in 1896, discovered that the leaf extract contained myricetin and gallic acid. In a recent comprehensive study into the phytochemical content of fruit extract, 211 phytoconstituents including tannins, terpenoids, anthocyanins, and (iso)flavonoids were found (Alsamri et al., 2021). These compounds are recognized for their antioxidant, anti-inflammatory, and anti-cancer properties, making them of considerable interest in the field of natural medicine (Rahman et al., 2021).

Antioxidants, as pivotal contributors to health, play a crucial role in neutralizing free radicals within the human body. Free radicals, generated through metabolic processes or environmental factors, can lead to oxidative stress, contributing to the development of chronic diseases (Yadav et al., 2016). Sumac's strong phytochemical profile provides an effective choice for reducing oxidative stress and the health effects that go associated with it (Alsamri et al., 2021).

With a specific focus on measuring the total anthocyanin, phenol, and flavonoid content, our study aims to investigate the antioxidant activity of Turkish Sumac leaves in this context. Our aim is to add to the increasing amount of information on the possible health benefits of this plant resource by explaining the complex biochemical makeup of sumac leaves.

MATERIAL AND METHODS

Plant material

R. coriaria leaves were collected from Güzelsu village, located in Antalya province of Turkey in July 2022. Sumac leaves was determined by a botanist specialist (Osman Tugay, Professor at Department of Biology), works in Selcuk University. Voucher specimen was kept in Selçuk University, KNYA (Herbarium of Selçuk University, herbarium code: 26563).

Preparation of the extracts

Sumac leaves ground into a fine powder using a laboratory style miller after plant material had dried in the shade. Ten grams of powder were soaked in 100 mL methanol for 30 min in ultrasonic bath and filtered with Whatman filter paper No. 1. The filtrates were concentrated under vacuo using a rotary evaporator (Buchi, Switzirland) at 40°C. Three times around, the process was carried out. The extract was refrigerated until it was needed for in vitro tests. Extract yield is %3.45.

Total phenol (TPC), flavonoid (TFC), and total anthocyanin (TAC) contents

Spectrophotometric techniques, such as Folin-Ciocalteu (Clarke et al., 2013) and aluminum chloride (Yang et al., 2011) were used to measure the total phenol and flavonoid content of the sumac leaf methanol extract. In milligrams of gallic acid equivalents per gram of dry extract weight (mg GAE g^{-1} DW), the total phenol content was reported. The amounts of total flavonoids were determined using the mg equivalency of quercetin over the weight of the dry extract (mg QE g^{-1} DW). For total anthocyanin, the pH method used by Fredes et al was used and the outcomes are presented in terms of mg of cyanidin-3-glucoside equivalent g^{-1} extract (Fredes González et al., 2014).

Antioxidant activity

Plant-derived materials and extracts must have their antioxidant capacities assessed using techniques that take the antioxidant activity mechanism into account. Thus, the antioxidant ability of the extracts was evaluated in this study using the ferrous reducing power (FRAP) and free radical scavenging assays (DPPH and ABTS). The extracts' capacity to scavenge free radicals was assessed using the DPPH and ABTS reduction spectrophotometric assays (Re et al., 1999; Clarke et al., 2013). The reducing power of the extracts was controlled the FRAP assay (Yang et al., 2011).

Statistical data

All of the bioactivity tests were carried out in triplicate throughout the experimental protocols. The means of the three parallel assessments were used to compile the findings. To display the correlation, one-way ANOVA and the Tukey's test were employed. In statistical terms, a significance threshold was established, and a difference was deemed noteworthy if the $p<0.05$.

RESULTS AND DISCUSSION

Scientific researchers have reported that both fruits and leaves of sumac have antioxidant activities. Antioxidant activity studies have been carried out more widely, especially on *R. coriaria* fruits than its leaves (Elagbar et al., 2020; Öz et al., 2023). It was determined that the tannin fractions of these samples had a strong antioxidant capacity (Öz et al., 2023). Similarly, our findings revealed significant antioxidant activity in sumac leaves extract, as evidenced by the DPPH, ABTS, and FRAP assays (Figure 2). TAC was measured at 1.27 mg cyanidin 3-glycoside g^{-1} dried, TPC at 251.29 GAEs mg g^{-1}, and TFC at 7.48 QEs mg g^{-1} (Table 1, Figure 1).

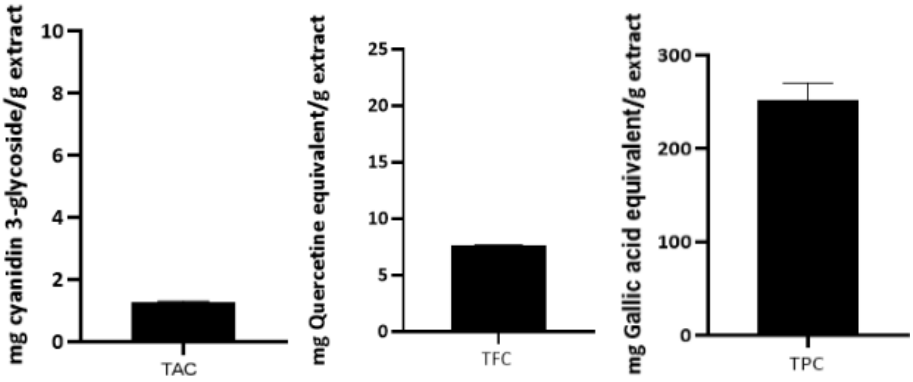

Figure 1. TAC, TFC, and TPC of Sumac Leaves Extract

Table 1. Sumac Leaf Yield, TPC, TFC, and TAC Amounts, And Antioxidant Activities of Sumac Leaves Methanol Extract[a]

Extract/ Reference	Extract yield (g g^{-1}%)	TPC (*m*g GAEs g^{-1})[b]	TFC (*m*g QEs g^{-1})[c]	TAC (*m*g CEs g^{-1} [d])	FRAP (mg TE g^{-1}[e])	Antioxidant activity (IC_{50} µg/ml)	
						DPPH	ABTS
Sumac leaves extract	%3.45	251.29 ± 18.8	7.48 ± 0.16	1.27 ± 0.03	0,501 ± 0,109	0.019 ± 0.005	0.0052 ± 0.003
BHT	-	-	-	-	-	0.036 ± 0.006	-
Trolox	-	-	-	-	-	-	0.0028 ± 0.002

a: The information was presented as the means ± standard deviations of three parallel calculations.
b: GAEs. Gallic acid equivalents (y = 1,3044x + 0,1364 gallic acid (mg) (r^2 = 0.990)
c: QEs. quercetin equivalents (y = 0,0382x - 0,2224 quercetin (mg) (r^2 = 0.990).
d: Cyanidin-3-glucoside equivalents
e:TE: Trolox equivalents

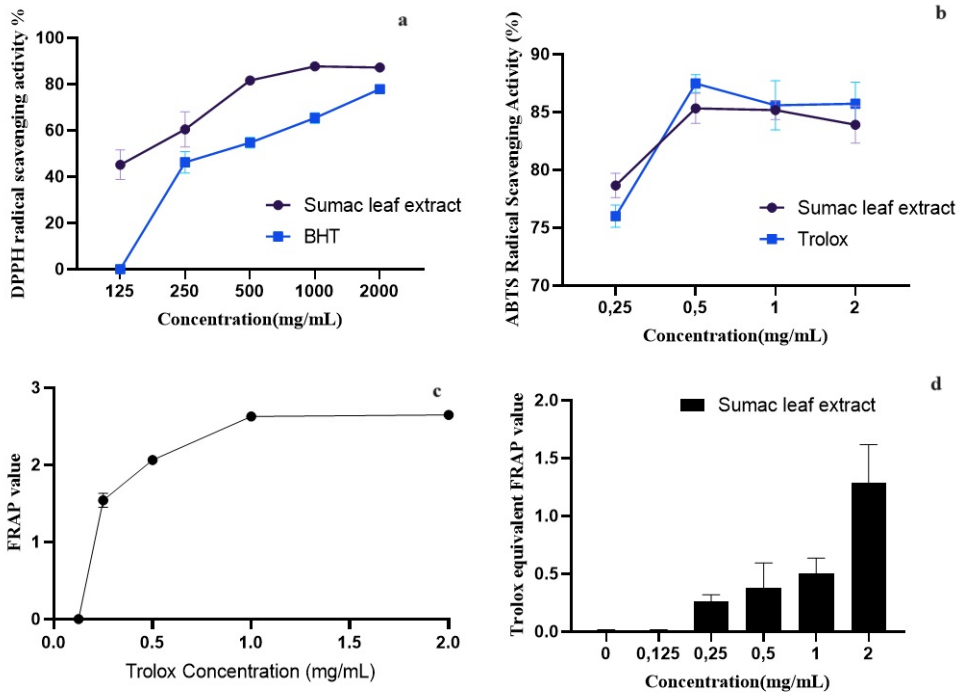

Figure 2. Antioxidant Activity of Sumac Leaves Extract. A: DPPH Radical Scavenging Activity; b: ABTS Radical Scavenging Activity; c: FRAP Value of Trolox; d: Trolox Equivalent FRAP Value of Sumac Extract

Concentration-dependent (0.25 mg mL^{-1}-2 mg mL^{-1} range) inhibition percentages of sumac leaves are given in Figure 3. Lower concentrations were used to calculate IC_{50} values. In both ABTS and DPPH methods, sumac leaves showed high antioxidant activity even at low concentration.

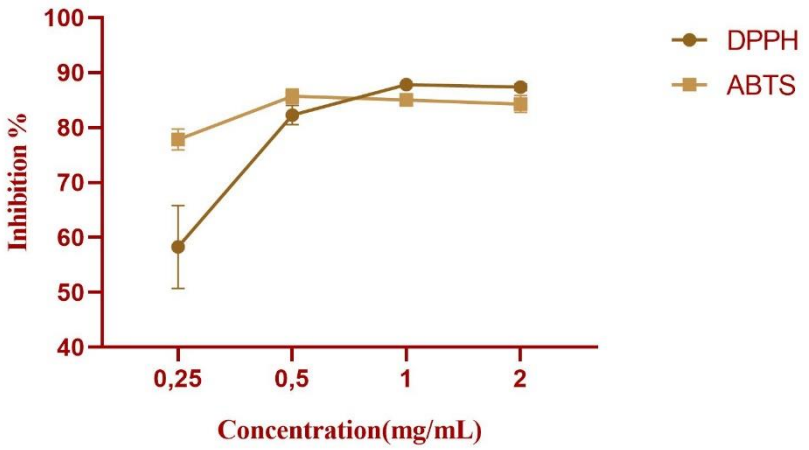

Figure 3. ABTS and DPPH Radical Scavenging Activity of Sumac Leaves Extracts

The correlation study aimed to establish a quantitative relationship between the antioxidant activity and the content of polyphenolics in the extracts. Table 2 and Figure 4 presents the findings of this study. A positive correlation was observed between the DPPH radical scavenging capacity and the anthocyanin and flavonoid content, with correlation coefficients of r=0.87 and r=0.47. This suggests that the ability of the extracts to scavenge DPPH radicals is, to some extent, more attributable to their anthocyanin content. Moreover, in terms of ABTS radical scavenging potential, the study found a strong positive correlation with total phenolic content (TPC) (r=0.97). This indicates that the ability of the extracts to scavenge ABTS radicals is more closely associated with their phenolic content rather than their flavonoid content. Additionally, FRAP value exhibited positive correlations with both TAC (r=0.98) and TFC (r=0.93). This suggests that the anthocyanin and flavonoid compounds present in the extracts play a significant role in the iron reducing activity of the extracts, as indicated by their FRAP values. In summary, the results of the correlation study highlight the importance of polyphenolic compounds, particularly flavonoids, in contributing to the antioxidant activity of the extracts, as demonstrated by their scavenging abilities against DPPH and ABTS radicals, as well as their iron reducing potential as measured by FRAP values.

Table 2. Pearson's Correlation Coefficients Between Phenolic Compound Contents (TPC, TFC and TAC) and Antioxidant Activity in DPPH, ABTS, FRAP Assays (at 1 mg mL^{-1} concentration)

	DPPH[a]	ABTS[b]	FRAP[c]	TAC[d]	TFC[e]	TPC[f]
DPPH	1	-	-	-	-	-
ABTS	-0.999	1	-	-	-	-
FRAP	0.767	-0.745	1	-	-	-
TAC	0.875	-0.858	0.982	1	-	-
TFC	0.472	-0.441	0.927	0.840	1	-
TPC	-0.974	0.966	-0.892	-0.962	-0.659	1

[a] % Scavenging activity in DPPH assay, [b]% Scavenging activity in ABTS assay, [c] Trolox equivalent FRAP value, [d]Total anthocyanin content, [e]Total flavonoid content, [f]Total phenolic content.

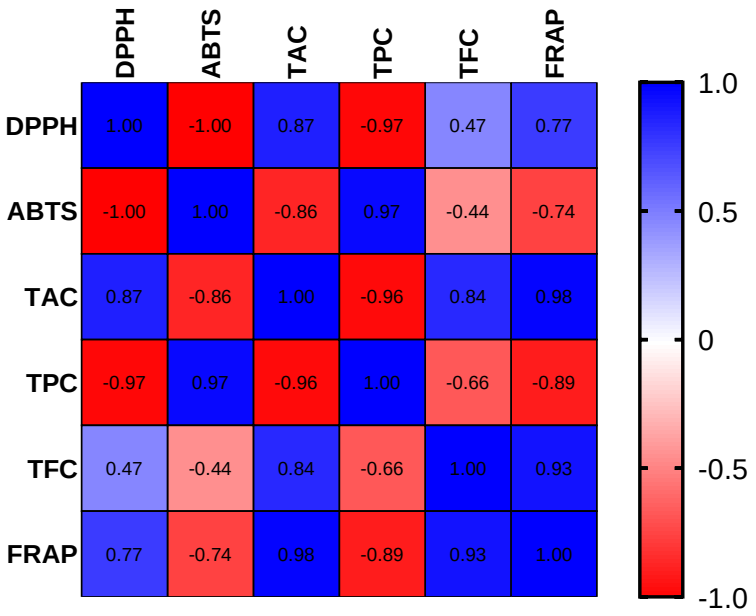

Figure 4. A Heatmap of Correlation Matrix About Phenolic Compound Contents (TPC, TFC, and TAC) and Antioxidant Activity in DPPH, ABTS, FRAP Assays

In a study conducted by Al-Boushi et al., (2014) Syrian sumac leaves were collected in August and September (two different months). TPC was found to range from 171.18 to 193.15 mg GAE g^{-1} extract. TAC content is given as 3.92 (September) and 4.21 (August) mg cyanidin 3-glycoside g^{-1} dried extract (Al-Boushi et al., 2014). Compared to our study, Turkish Sumac leaves were found to have higher TPC content and lower TAC content. In the previous study, the aqueous-acetone extract of Sumac leaves was found to have TPC of 48.7 mg of GAE 100 mg^{-1} fractions and TFC of 13.5 mg of QE 100 mg^{-1} fractions (Gabr and Alghadir, 2015).
In another study, it was observed that *R. coriaria* leaf ethanol extract showed high antioxidant activity in the DPPH (IC_{50} = 0.02 mg mL^{-1}) method (Jamous et al., 2018). BHA (IC_{50}= 0.006 mg mL^{-1}) and ascorbic acid (IC_{50}= 0.004 mg mL^{-1}) were shown to have weaker DPPH radical scavenging activity than *R. coriaria* leaf methanol extract (IC_{50}= 0.002 mg mL^{-1}) in a prior study. *R. coriaria* had higher FRAP (0.155 mM FeSO4 mg^{-1} extract) values in the same investigation, however these values are lower than those of the substances ascorbic acid (5.92 mM Trolox mg^{-1}) and BHT (1.1 mM $FeSO_4$ mg^{-1}) (Taskin et al., 2020). Another study found that Sumac extracts had higher antioxidant activity than BHT (Langroodi et al., 2018). Similarly, in our study, the DPPH radical scavenging activity of the sumac extract (IC_{50}= 0.019 µg mL^{-1}) was higher than the positive control (BHT) (IC_{50}= 0.036 µg mL^{-1}), while the ABTS radical scavenging activity (IC_{50}= 0.0052 µg mL^{-1}), although notable, was lower than the positive control (Trolox) (IC_{50}= 0.0028 µg mL^{-1}). Our results, consistent with previous studies, showed that sumac leaves have high antioxidant activity.
Previous phytochemical investigations have reported the existence of pentagalloyl glucose and gallotannins derived from gallic acid in sumac leaves. Additionally, the presence of flavones, tannins, anthocyanins, and organic acids has been identified in these studies (Shabestarian et al., 1998; Zannou et al., 2022). In this study, the phenolic components of sumac were found to be very high in terms of gallic acid equivalent. This explains the high free radical scavenging activity of DPPH (IC_{50}=0.019 µg mL^{-1}) and ABTS (IC_{50}= 0.0052 µg mL^{-1}).

Conclusion

This study provides valuable insights into the antioxidant potential and phytochemical composition of Sumac leaves. The findings underscore the significance of Sumac as a natural reservoir of bioactive compounds that may offer potential health advantages. Further research is warranted to explore the specific mechanisms and therapeutic applications of Sumac in promoting human health.

Acknowledgments

We would like to thank Professor Doctor Osman Tugay in identifying plant material and preparing the herbarium sample.

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ORAL PRESENTATION

Effect of Exogenous Strigolactones Application on Antioxidant Enzyme Activity of Pepper

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Abstract

Pepper is one of the vegetable species used in human nutrition and produced widely both in our country and around the world. Pepper is sensitive to environmental stress factors such as salinity, drought, heavy metals etc. during growth. Strigolactones (SLs) are a type of terpene lactone derived from carotenoids and recognized as a new type of plant hormone. SLs have the ability to tolerate various abiotic stresses in plants. Plants have an effective antioxidant system that both limits the formation of ROS and detoxifies the formed ROS. Enzymes such as superoxide catalase (CAT), peroxidase (POD), super oxide dismutase (SOD), glutathione reductase (GR), glutathione S-transferases (GST), glucose-6-phosphate dehydrogenase (G6PD), ascorbate peroxidase (APX) constitute the enzymatic components of this system. In this study, it was determined how SL influences the antioxidant enzyme activity (CAT, POD, SOD, GR, GST, G6PD, APX) of pepper. For this purpose, we used GR24, the synthetic analogue of SL. Exogenous SL applications with concentrations of 0 uM, 10 uM and 20 uM GR24 were made during the seedling period. The results of the study pointed out that SL applications elevated the enzyme activity investigated. The highest activities were obtained with the 20 uM GR24. In conclude GR24 can be used to elevate activity of the antioxidant enzymes in pepper during seedling growth in especially stress condition.

Keywords: Strigolactones, pepper, antioxidant enzyme activity.

INTRODUCTION

Pepper (*Capsicum annuum* L.) is a significant vegetable species extensively cultivated in Turkey and World. Besides serving as a fundamental component of human diet, it possesses substantial economic importance. The pepper plant is highly susceptible to environmental stressors during its growth and development phases. Abiotic stressors, including drought, salinity, and heavy metals, can harm plants significantly, resulting in growth inhibition and decreased yield (Aktas et al., 2015). Therefore, improving plant tolerance to stress conditions is essential for agricultural sustainability.

Strigolactones (SLs) are a class of carotenoid-derived phytohormones produced in the plastids and cytoplasm. SLs function as molecular signals, facilitating plant adaptation to fluctuating environmental conditions (Kapoor et al., 2024). SLs are active phytohormones synthesized by enzymes that bind to their receptors, characterized by their reversibility, specificity, and high affinity (Yao et al., 2016). SLs are predominantly synthesized in roots and exuded into the rhizosphere, although minor amounts have also been detected in other plant tissues. Strigolactone production in roots exceeds that in the aerial portions of the plant (Visentin et al., 2016; Xie 2016). Presently, over a thousand kinds of SLs have been documented in plants, however more than 30 natural SLs have been recognized, including strigol, strigyl acetate, sorgolactone, alectrol, orobanchyl acetate, and orobanchol (Xie 2016; Yoneyama et al., 2018).

Multiple abiotic challenges negatively impact plant growth and development, prompting diverse hormonal responses in plants to mitigate these impacts. SLs modulate plant growth and development in response to various abiotic challenges (Lv et al., 2018; Ling et al., 2020; Sedaghat et al., 2020; Bhoi et al., 2021). SLs play roles in various plant growth processes, including root development, interactions with root-associated microorganisms, seedling development, shoot gravitropism, stem morphology, flower development, leaf shape, and senescence (Saeed et al., 2017; Zhang et al., 2018). Oxidative stress generates reactive oxygen species that impede essential metabolic activities in plants, including photosynthesis, water and nutrient transport, signaling, and reproduction, hence posing a significant threat to their existence. Strigolactone buildup has been observed in stressed plant cells, prompting the production of osmolytes and antioxidants to sustain homeostasis (Banerjee and Roychoudhury 2018). Under stress situations, SLs collaborate with phytohormones to facilitate regulatory networks for plant adaptation (Mostofa et al., 2018). Strigolactones perform dual roles both as an endogenous and exogenous signaling molecule.

Plants generate reactive oxygen species (ROS) by initiating defense mechanisms in response to environmental stresses. While this may result in cellular damage, a robust antioxidant defense mechanism in plants mitigates the generation of reactive oxygen species (ROS) and facilitates their detoxification. Numerous enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), glutathione S-transferases (GST), glucose-6-phosphate dehydrogenase (G6PD), and ascorbate peroxidase (APX), are integral to the antioxidant defense system (Mittler, 2002). The capacity of SLs to modulate the activity of these enzymes is crucial for enhancing plant performance, particularly under abiotic conditions such as drought, salt, and heavy metal toxicity (Marzec and Muszynska 2016).

This study aimed to examine the effects of varying concentrations of GR24, a synthetic strigolactone analogue, on the activities of antioxidant enzymes (SOD, CAT, POD, GR, GST, G6PD, APX) in pepper and to ascertain its role in enhancing potential stress tolerance against abiotic stress conditions.

MATERIALS AND METHODS

Plant material and experimental design

In this study, pepper (*Capsicum annum* L. cv. Yalova Carliston) seeds were used as plant material and the experiment was carried out in a controlled greenhouse using pots. The temperature and humidity of the greenhouse were controlled by a sensor and kept at an average temperature of 24 (±2) °C during the day and 18 (±2) °C at night. Humidity was kept around 50 (±5) per cent. Pepper seeds were first sown in peat and when three true leaves appeared, the seedlings were transplanted into 1.5 litre pots, one seedling per pot. A medium consisting of garden soil, peat and sand in a ratio of (3: 1: 1, v: v: v), was placed in the pots. SL applications were made at 0 uM, 10 uM and 20 uM GR24 concentrations during the seedling period. GR24 was prepared by dissolving in acetone and Tween-20 (0.1%) was added to the GR24 solution to ensure better penetration into the leaves of the plants. SL was applied to the seedlings as foliar spray every 5 days for a total of 3 times. In this study, which was planned according to the random plots experimental design, each treatment was replicated 3 times and 8 plants were used in each replication. Fresh leaves required for antioxidant enzyme activity were frozen in liquid nitrogen and stored at -80 °C.

Antioxidant enzyme activities

The antioxidant enzyme activities (catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and Ascorbate Peroxidase (APX)) were measured on fresh leaf samples using the techniques of Shams et al., (2020) and Dadasoglu et al., (2021). GR enzyme and Glutathione-S-transferase (GST) enzyme was determined according to Chikezie et al., (2009) and the results are presented as enzyme units per g fresh leaves (Eu/g leaf). Glucose-6-phosphate dehydrogenase (G6PDH) enzyme was determined according to Landi et al., (2016). The absorbance values of the samples were measured at 340 nm by monitoring $NADP^+$ and the results were presented as enzyme units per g fresh leaves (Eu/g leaf).

Statistical analysis

The statistical program SPSS was used to analyze the data, One-way ANOVA were made and Duncan multiple range test (DMRT) tests were used to evaluate the differences among the means.

RESULTS

The effect of SL treatment on SOD enzyme activity in pepper seedlings is shown in Figure 1.A. In the control group, SOD activity was measured at the lowest level (~50 Eu/g leaf). 10 µM SL treatment showed a significant increase compared to the control group (~100 Eu/g leaf). This increase was statistically significant (** $p < 0.01$). 20 µM SL treatment showed the highest level of SOD activity (~200 Eu/g leaf). 20 µM SL provided a statistically very significant increase compared to both the control group and 10 µM SL treatment (**** $p < 0.0001$).

The effect of different SL concentrations on CAT enzyme activity in pepper seedlings is shown in Figure 1.B. In the graph, it is observed that 10 µM and 20 µM SL applications increased CAT enzyme activity compared to the control group. CAT activity was measured at the lowest level (~50 Eu/g leaf) in the control group. 10 µM SL application showed a significant increase compared to the control group (~100 Eu/g leaf). This increase was statistically significant (*** $p < 0.001$). 20 µM SL application: A further increase in CAT activity was observed (~125 Eu/g leaf), but the difference between 10 µM SL and 20 µM SL was not statistically significant.

Figure 1.C. shows the effect of different SL concentrations on POD enzyme activity. In the control group, POD activity was measured at the lowest level (~50 Eu/g leaf). In 10 µM SL treatment, it showed a significant increase compared to the control group (~ 200 Eu/g leaf). This increase was statistically very significant (**** $p < 0.0001$). In 20 µM SL treatment, a much higher increase in POD activity was observed (~400 Eu/g leaf). The 20 µM SL treatment exhibited statistically significantly higher POD activity compared to both the control group and the 10 µM SL treatment (**** $p < 0.0001$).

Figure 1.D. shows the effect of SL treatments on GR enzyme activity. GR activity was measured for the control group, 10 µM SL and 20 µM SL treatments. In the control group, GR activity was measured at a low level (~50 Eu/g leaf). 10 µM SL treatment showed a significant increase in GR activity compared to the control group (~600 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). In 20 µM SL treatment, GR activity increased further and reached the highest level (~1200 Eu/g leaf). 20 µM SL treatment was statistically significant (**** $p < 0.0001$) compared to both the control group and 10 µM SL treatment.

The effect of SL treatments on GST enzyme activity is shown in Figure 1.E. In the control group, GST activity was measured at a low level (~50 Eu/g leaf). In 10 µM SL treatment, a significant increase in GST activity was observed compared to the control group (~200 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). In 20 µM SL treatment, a higher increase in GST activity was observed (~250 Eu/g leaf). 20 µM SL treatment provided a statistically significant increase compared to both the control group and 10 µM SL treatment (**** $p < 0.0001$ and *** $p < 0.001$).

Figure 1.F. shows the effect of SL treatments on G6PD enzyme activity. In the control group, G6PD activity was measured at the lowest level (~100 Eu/g leaf). In 10 µM SL application, it showed a significant increase compared to the control group (~1500 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). In 20 µM SL treatment, G6PD activity increased further and reached the highest level (~2000 Eu/g leaf). The 20 µM SL treatment produced a statistically significant difference compared to both the control group and the 10 µM SL treatment (**** $p < 0.0001$).

The effect of SL treatment on APX enzyme activity is shown in Figure 1.G. APX activity was measured at the lowest level (~100 Eu/g leaf) in the control group. 10 µM SL application showed a significant increase compared to the control group (~1500 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). In 20 µM SL treatment, APX activity increased further and reached the highest level (~2000 Eu/g leaf). 20 µM SL treatment produced a statistically significant difference compared to both the control group and 10 µM SL treatment (**** $p < 0.0001$).

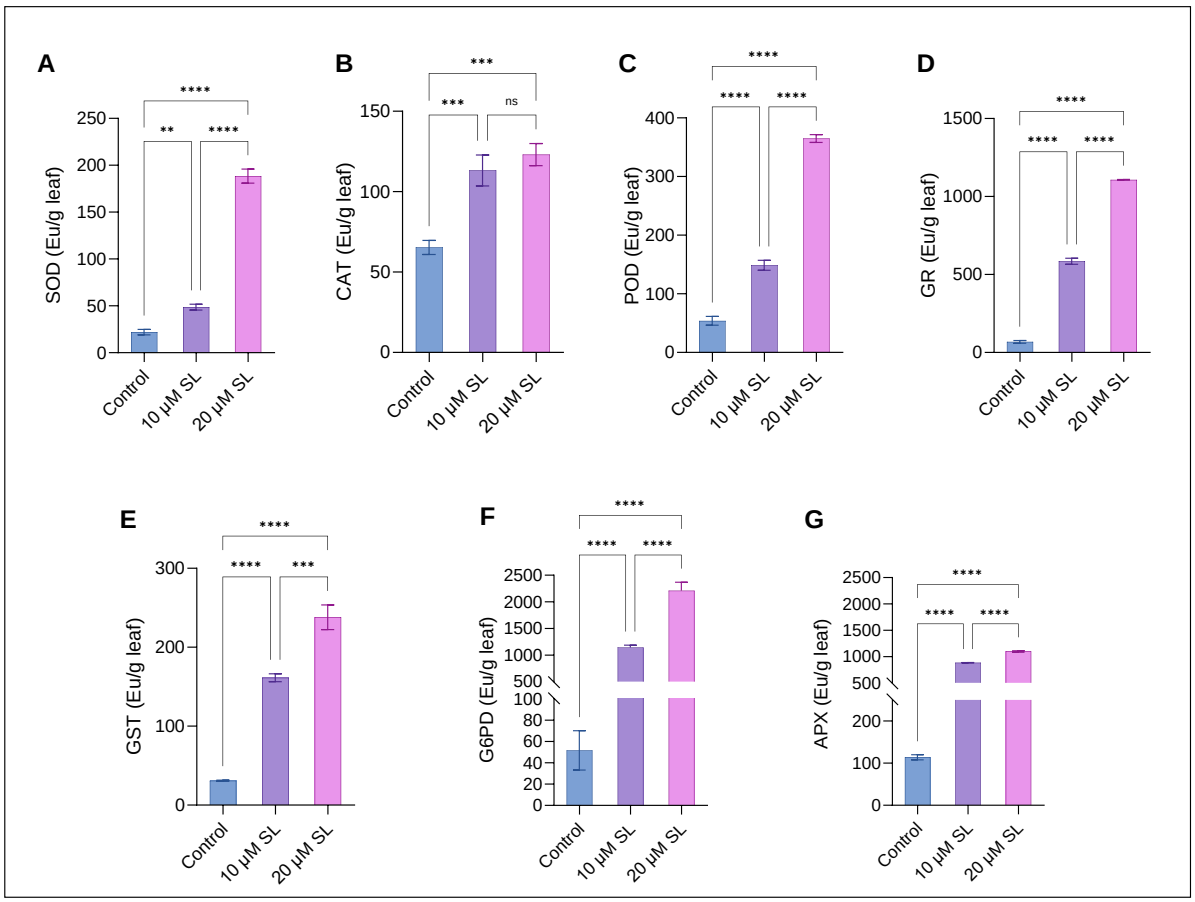

Figure 1. The antioxidant activity in leaves of pepper according to treatments. (A) SOD (Eu /g leaf), (B) CAT (Eu /g leaf), (C) POD (Eu /g leaf), (D) GR (Eu /g leaf), (E) GST (Eu /g leaf), (F) G6PD (Eu /g leaf), (G) APX (Eu /g leaf). ns: nonsignificant **: $p \leq 0.01$, ***: $p \leq 0.001$ and ****: $p \leq 0.0001$.

DISCUSSION

Oxidative stress in plants causes oxidative damage to cellular membranes, lipids, proteins and DNA, resulting in the formation of reactive oxygen species (ROS) that adversely affect plant growth and development. Abiotic stress factors cause oxidative stress by increasing ROS production in plants and plants have various defense mechanisms to cope with this stress (Mittler, 2002). In this study, the effects of SL applications on antioxidant enzyme activity in pepper seedlings were investigated. The results obtained revealed that SLs can increase the resistance against environmental stress conditions by strengthening the oxidative stress defence mechanism in plants. In a study conducted by Zulfiqar et al. (2021) in *Helianthus annuus* L. plant, it was reported that SL application increased SOD, CAT, POD enzyme activities under salt stress. In another study, SL application was performed under drought stress and it was reported that POD, CAT, SOD and APX antioxidant enzyme activity increased (Sedaghat et al., 2017). In a study in cucumber under salt stress, exogenous SL application significantly reduced O2-, H_2O_2 and -OH content in leaves (Zhang et al., 2022). In a different study, exogenous SL treatment was reported to eliminate ROS production by increasing SOD, POD and CAT enzyme activities in rice (Ling et al., 2020). It was reported that SL application effectively increased glutathione peroxidase (GPX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), APX and GR enzyme activities in barley under cadmium stress (Qiu et al., 2021).

The activities of GR, GST, and G6PD were dramatically increased by SL treatments. GR facilitates the preservation of intracellular redox equilibrium by converting oxidized glutathione (GSSG) back to reduced glutathione (GSH) (Noctor and Foyer, 1998). GST participates in the detoxification mechanisms of plants and is crucial in the detoxification of reactive oxygen species (Edwards et al., 2000). G6PD preserves redox equilibrium within the cell by facilitating NADPH synthesis in the pentose phosphate pathway and bolsters antioxidant defense throughout this process (Cramer et al., 2011). The observed elevations in these enzymes with SL applications signify the beneficial impacts of strigolactones on the redox equilibrium of plants.

In a study in *Arabidopsis*, exogenous SL application was proven to be a positive regulator of stress response by increasing tolerance to drought and salt stress (Ha et al., 2014). This study shows that strigolactones can increase antioxidant enzyme activities in pepper seedlings and improve plant resistance against environmental stress conditions. The potential of SLs to be used as biostimulants in agricultural production may play an important role in strategies to grow plants resistant to abiotic stress factors.

CONCLUSION

This study examined the impact of SLs on the activity of antioxidant enzymes in pepper seedlings. The findings indicated that SL administered at both 10 µM and 20 µM concentrations markedly enhanced the activity of CAT, POD, SOD, GR, GST, and G6PD enzymes. The application of 20 µM SL exhibited the maximum enzymatic activity and demonstrated that SL enhanced the plants' defense mechanisms against oxidative stress. The findings indicate that strigolactones may provide a significant protective function against abiotic stress in plants. The capacity of SLs to enhance plant growth by elevating antioxidant enzyme activity, particularly under environmental stressors like drought, salinity, and heavy metals, where oxidative stress prevails, is crucial for agricultural productivity. Strigolactones possess the potential to serve as biostimulants in vegetable cultivation, including peppers, and may aid in the formulation of plant growth methods that exhibit enhanced resilience to environmental stressors. Future research on the practical implementation of SL technologies in agriculture, together with the identification of optimal dosages and administration timings, will enhance our comprehension of the potential advantages of these hormones in agricultural productivity.

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ORAL PRESENTATION

N-acetyl-L-cysteine (NAC) Influences Antioxidant Enzyme Activity of Tomato

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Abstract

Tomato, one of the most produced vegetables in the world, has significant potential for food and commercial use. Tomato plants are sensitive to changing environmental factors such as drought and temperature during growth. Recent studies have shown that exogenous NAC is effective in reducing adverse effects from biotic and abiotic environmental stresses. N-acetyl-cysteine (NAC), a derivative of the amino acid cysteine, is considered an antioxidant due to its scavenging function of reactive oxygen species (ROS). Plants have an effective antioxidant system that both limits the formation of ROS and detoxifies the formed ROS. Enzymes such as superoxide catalase (CAT), peroxidase (POD), super oxide dismutase (SOD), glutathione reductase (GR), Glutathione S-Transferases (GST), Glucose-6-phosphate dehydrogenase (G6PD), Ascorbate Peroxidase (APX) constitute the enzymatic components of this system. In this study, it was determined how N-acetyl-L-cysteine (NAC) influences antioxidant enzyme activity (CAT, POD, SOD, GR, GST, G6PD, APX) of tomato. With this aim, 0 uM, 125 uM, 250 uM and 500 uM N-acetyl-L-cysteine solutions were foliar applied to tomato seedlings. The findings of the study showed that foliar NAC applications increased the enzyme activity investigated with increasing concentrations of NAC. The highest activities were obtained with the 500 uM NAC. In conclude NAC can be used to elevate activity of the antioxidant enzymes in especially stress condition.

Keywords: N-acetyl-cysteine (NAC), tomato, Antioxidant Enzyme Activity

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), one of the most commonly grown vegetable species worldwide, is a vegetable species whose production and consumption is still increasing due to its antioxidant and anticancer properties (Raiola et al., 2014). However, its productivity is hindered by a wide range of abiotic stresses and therefore the creation of stress-resistant crops is very important for agricultural sustainability. Adverse environmental factors such as extreme temperatures, salinity or drought cause particularly biochemical and physiological consequences (Gerszberg and Hnatuszko-Konka 2017). Adverse environmental conditions trigger the production of reactive oxygen species (ROS), which can be extremely harmful to plant cells and cause oxidative damage and inactivation of enzymes. To minimize the damage, cells produce antioxidants that scavenge ROS (Atkinson et al., 2011).

N-acetylcysteine (NAC), an organosulfur compound obtained from *Allium* plants, is known as a source of sulfhydryl groups (Nissar et al., 2013; Colak et al., 2015). NAC is the precursor of glutathione (GSH), which has a direct effect on the cell membrane by reducing cysteine or other disulfides to regulate cell membrane functions (Yuce et al., 2024). NAC is known as a powerful antioxidant and free radical scavenger, as well as being used as a pharmaceutical drug and dietary supplement for humans (Sun et al., 2014).

Determining the capacity of NAC to regulate antioxidant enzyme activities is very important for agricultural sustainability and production of crops resistant to adverse environmental conditions. Therefore, the aim of this study was to determine the effects of different NAC treatments on superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), glutathione S-transferases (GST), glucose-6-phosphate dehydrogenase (G6PD), and ascorbate peroxidase (APX) antioxidant enzymes and to reveal the potential stress tolerance of NAC against adverse environmental conditions.

MATERIALS AND METHODS

In the study, tomato (*Solanum lycopersicum* L. cv. H2274) seeds were used as experimental material. Seedling experiments were carried out in pots under semi-controlled greenhouse conditions (25±2/18±2 ºC day/night temperature and 65±5 relative humidity). Tomato seeds were first sown in a peat: perlite mixture in 216-well viols. A mixture of garden soil: peat: sand (2:1:1, v:v:v) was prepared for 2.5 liter pots and 100 kg per hectare fertilizer (15:15:15; N:P:K) was mixed into this mixture. Later, when they reached a size of 2-3 true leaves, homogeneous seedlings were transferred to the prepared pots. The pots were placed randomly in the greenhouse according to the randomized plots trial design. Different doses of NAC (0, 125, 250, 500 µM) were applied 2 days after the tomato seedlings were transferred to the pots and a total of 3 applications were made as foliar sprays every 5 days. Fresh leaves required for antioxidant enzyme activity were frozen in liquid nitrogen and stored at -80 °C.

Antioxidant enzyme activities

The antioxidant enzyme activities (catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and Ascorbate Peroxidase (APX)) were measured on fresh leaf samples using the techniques of Shams et al., (2020) and Dadasoglu et al., (2021). GR enzyme and Glutathione-S-transferase (GST) enzyme was determined according to Chikezie et al., (2009) and the results are presented as enzyme units per g fresh leaves (Eu/g leaf). Glucose-6-phosphate dehydrogenase (G6PDH) enzyme was determined according to Landi et al., (2016). The absorbance values of the samples were measured at 340 nm by monitoring $NADP^+$ and the results were presented as enzyme units per g fresh leaves (Eu/g leaf).

Statistical analysis

The statistical program SPSS was used to analyze the data, one-way ANOVA were made and Duncan multiple range test (DMRT) tests were used to evaluate the differences among the means.

RESULTS and DISCUSSION

The effect of NAC treatment on SOD enzyme activity in tomato seedlings is shown in Figure 1.A. In the control group, SOD activity was measured at the lowest level. 500 µM NAC treatment showed a significant increase compared to the control group (~5000 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). The SOD enzyme, which belongs to the group of metalloenzymes, converts $O^{2.-}$ into O_2 and H_2O_2, leading to the regulation of the redox state of the plant cell (Sharma et al., 2012; Lightfoot et al., 2017).
The effect of different NAC concentrations on CAT enzyme activity in tomato seedlings is shown in Figure 1.B. In the graph, it is observed that 250 µM and 500 µM NAC applications increased CAT enzyme activity compared to the control group. CAT activity was measured at the lowest level in the control group. 500 µM NAC application showed a significant increase compared to the control group (~15000 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). CAT enzyme occurs in the peroxisome where they accelerate the detoxification of ROS and help split H_2O_2 into a useful form of H_2O and O_2 without requiring high amounts of energy (Sedaghat et al., 2017).
Figure 1.C. shows the effect of different NAC concentrations on POD enzyme activity. In the control group, POD activity was measured at the lowest level. In 500 µM NAC treatment, it showed a significant increase compared to the control group (~ 9000 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). Particularly, the increase in POD enzyme in 500 µM NAC application may indicate its role in ROS homeostasis and also in strengthening the cell wall.
Figure 1.D. shows the effect of NAC treatments on APX enzyme activity. APX activity was measured for the control group, 125 µM NAC, 250 µM NAC and 500 µM NAC treatments. In the control group, APX activity was measured at a low level (~100 Eu/g leaf). 500 µM NAC treatment showed a significant increase in NAC activity compared to the control group (~800 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). The H_2O_2 scavenger APX enzyme reduces H_2O_2 to water using ascorbate as a specific electron donor (Radwan et al., 2010).
Figure 1.E. shows the effect of NAC treatments on GR enzyme activity. In the control group, GR activity was measured at a low level (~100 Eu/g leaf). 500 µM NAC treatment showed a significant increase in GR activity compared to the control group (~4000 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$).

The GR enzyme is another enzyme involved in the ascorbate-glutathione cycle and its activity is necessary to maintain glutathione levels, which is involved in the reduction of dehydroascorbate (Noctor and Foyer 2016). The effect of NAC treatments on GST enzyme activity is shown in Figure 1.E. In the control group, GST activity was measured at a low level. In 500 µM NAC treatment, a significant increase in GST activity was observed compared to the control group (~20000 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). GST enzymes are centrally positioned in the glutathione network of redox control and cellular detoxification mechanisms. Known roles of GST enzymes include detoxification of toxic substances through conjugation with glutathione, alleviation of oxidative stress, and participation in hormone transport (Labrou et al., 2015).

Figure 1.F. shows the effect of NAC treatments on G6PD enzyme activity. In the control group, G6PD activity was measured at the lowest level (~100 Eu/g leaf). In 500 µM NAC application, it showed a significant increase compared to the control group (~8000 Eu/g leaf). This increase was statistically significant (**** $p < 0.0001$). G6PD is the key enzyme that catalyzes the first reaction in the pentose phosphate pathway, which is the oxidation of glucose-6-phosphate to 6-phosphogluconolactone, thus providing energy to all cells by maintaining glucose levels (Hsieh et al., 2013).

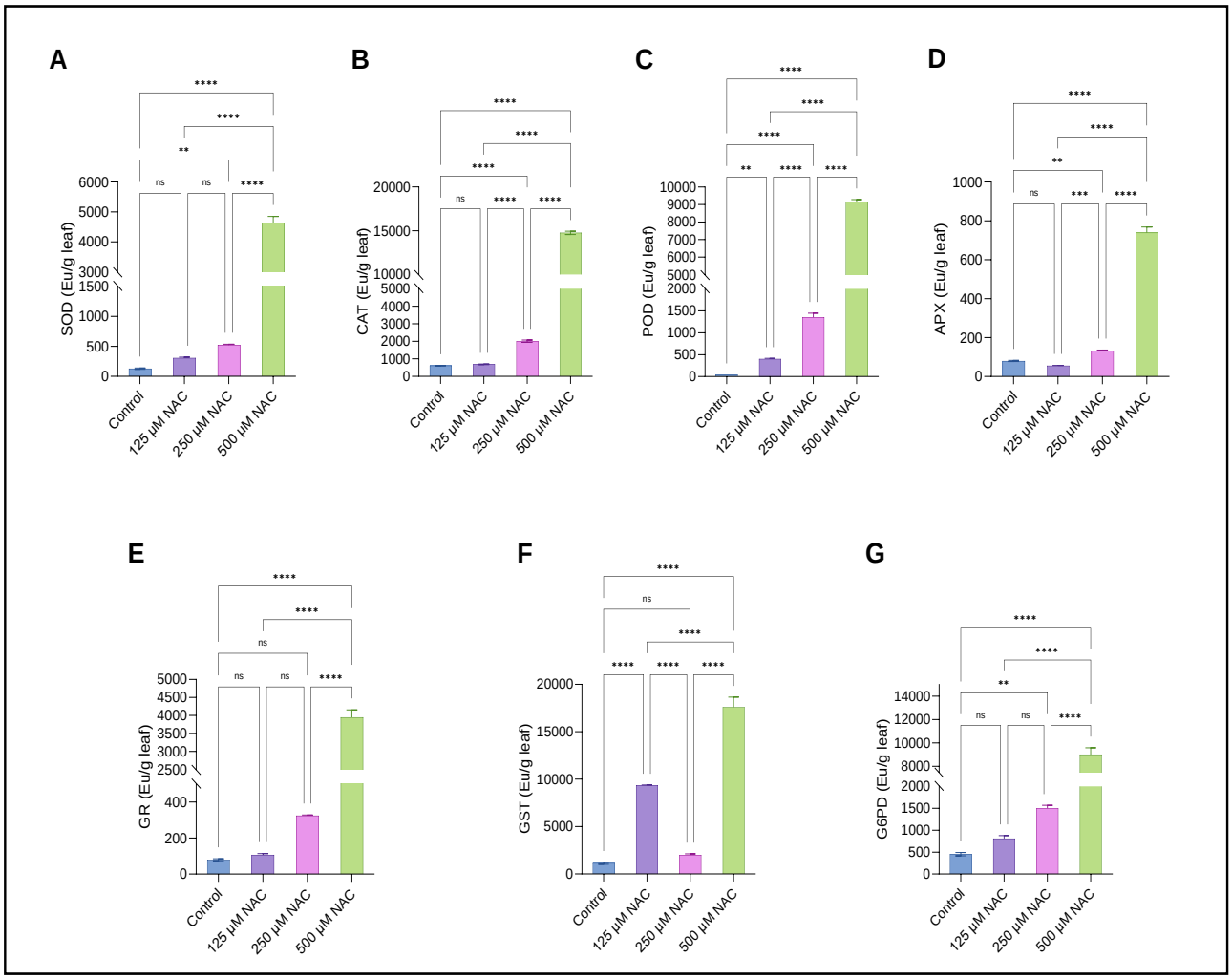

Figure 1. The antioxidant activity in leaves of tomato according to treatments. (A) SOD (Eu /g leaf), (B) CAT (Eu /g leaf), (C) POD (Eu /g leaf), (D) APX (Eu /g leaf). (E) GR (Eu /g leaf), (F) GST (Eu /g leaf), (G) G6PD (Eu /g leaf), ns: nonsignificant **: $p \leq 0.01$, ***: $p \leq 0.001$ and ****: $p \leq 0.0001$.

Adverse environmental conditions create oxidative stress in plants, which causes oxidative damage to cell membranes, lipids, proteins and DNA, leading to the formation of ROS, which negatively affects plant growth and development. Abiotic stress factors cause oxidative stress by increasing ROS production in plants, and plants have various defense mechanisms to cope with this stress (Parveen et al., 2019). In this study, the effects of NAC applications on antioxidant enzyme activity in tomato seedlings were investigated. The results obtained revealed that NACs can increase resistance to environmental stress conditions by strengthening the oxidative stress defense mechanism in plants.

Sun et al., (2014) suggests that under Cd stress, NAC may protect barley seedlings against Cd-induced damage by directly and indirectly scavenging reactive oxygen species and maintaining the stability and integrity of cell structure. In particular, NAC addition significantly reduced $O^{\cdot -2}$ accumulation and lowered Cd-induced $^{\cdot}OH$ content, especially in the roots of barley (Sun et al., 2014). Glutathione (GSH) is considered an important endogenous antioxidant defense against oxidative stress. NAC, considered as a source of sulfhydryl groups, can stimulate GSH synthesis, increase GSH-S-transferase activity, promote detoxification and act as a potent free radical scavenger when interacting with ROS (Nissar et al., 2014).

CONCLUSION

This study examined the impact of NACs on the activity of antioxidant enzymes in tomato seedlings. The findings indicated that NAC administered at especially 500 µM concentrations markedly enhanced the activity of CAT, POD, SOD, GR, GST, and G6PD enzymes and demonstrated that NAC enhanced the plants' defense mechanisms against oxidative stress. The findings indicate that NAC may provide a significant protective function against abiotic stress in plants.

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ORAL PRESENTATION

Purinerjik P2X3 Reseptörünün Meloksikam ile Etkileşimi: *İn Silico* Çalışma

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Özet

Meloksikam, primer dismenore, postoperatif ağrı, romatoid artrit ve osteoartrit semptomlarını azaltmak için kullanılan steroid olmayan bir anti-inflamatuar ilaçtır. Ağrı semptomları içeren birçok durumda, iyonotropik purinerjik (P2X3) reseptör nosiseptör aktivasyonuna neden olur. Ancak, Meloksikam ve P2X3 reseptörü arasındaki etkileşim bilinmemektedir. Bu çalışmada, Meloksikamın P2X3 reseptörü üzerindeki inhibisyon profili belirlendi ve Gibbs serbest bağlanma enerjisinin -6,19 kcal/mol olduğu bulundu. Bu sonuçlar, Meloksikamın P2X3 reseptörü için yüksek bir afiniteye sahip olduğunu göstermektedir. Meloksikamın P2X3 reseptörü ile etkileşimi, Meloksikamın ağrı semptomlarını azaltmadaki etki mekanizmasına ışık tutabilir.

Anahtar kelimeler: Meloksikam, P2X3, Moleküler Docking, *in Silico*

Purinergic P2X3 Receptor Interaction with Meloxicam: An *In Silico* Study

Abstract

Meloxicam is a nonsteroidal anti-inflammatory drug used to reduce symptoms of primary dysmenorrhea, postoperative pain, rheumatoid arthritis, and osteoarthritis. In many conditions involving pain symptoms, the ionotropic purinergic (P2X3) receptor causes nociceptor activation. However, the interaction between meloxicam and the P2X3 receptor is unknown. In this study, the inhibition profile of meloxicam on the P2X3 receptor was determined and the Gibbs free binding energy was found to be -6.19 kcal/mol. These results suggest that meloxicam has a high affinity for the P2X3 receptor. The interaction of meloxicam with the P2X3 receptor may shed light on the mechanism of action of meloxicam in reducing pain symptoms.

Key words: Meloxicam, P2X3, Molecular docking, *in Silico*

GİRİŞ

Meloksikam, romatoid artrit ve osteoartrit tedavisi için geliştirilmiş yeni bir nonsteroid anti-enflamatuar ilaçtır (NSAID). İnflamatuar yanıtta rol oynayan siklo-oksijenazın (COX-2) indüklenebilir izoformuna karşı, inhibisyonu gastrik, renal ve diğer yan etkilerle ilişkili olan bu enzimin yapısal formuna (COX-1) göre daha fazla *in vitro* ve *in vivo* inhibitör etkiye sahiptir. Hayvan modellerinde diğer NSAİİ'lere benzer veya onlardan daha iyi anti-enflamatuar etkilere ve daha büyük bir terapötik orana (ülserojenik potansiyel: adjuvan artritte etkinlik) sahiptir (Nobel ve Balfour, 1996). Meloksikamın, özellikle en düşük terapötik dozunda COX-2 tercihli olduğu ve enflamatuar hücrelerde prostanoid sentezini inhibe ederek anti-enflamatuar olduğu gösterilmiştir. COX-2 tercihli olduğu için, seçici olmayan NSAİİ'lere göre daha az gastrointestinal toksisiteye sahip olması beklenir. Meloksikamın osteoartritteki klinik çalışmalarında, daha az klinik gastrointestinal semptom ve daha az perforasyon, obstrüksiyon ve kanama ile piroksikam, diklofenak ve naproksen kadar etkili olduğu meta-analiz ile bulunmuştur. Periferik ödem ve hipertansiyon gibi advers olaylar geleneksel NSAİİ'lerle benzer oranda meydana gelmiştir (Fleischmann ve ark. 2002). Tercihli bir siklooksijenaz-2 (COX-2) inhibitörü olan Meloksikamın oral formülasyonları, osteoartrit, romatoid artrit, ankilozan spondilit ve iskelet-kas kaynaklı çeşitli ağrı sendromlarının (örn. bel ağrısı) tedavisinde uzun süredir kullanılmaktadır. Bununla birlikte, bu preparatlar zayıf çözünme oranı ve sonuç olarak yavaş etki başlangıcı nedeniyle akut ağrı tedavisi için nadiren endikedir. Yakın zamanda intravenöz (IV) NanoKristal Kolloidal Dispersiyon formülasyonunun piyasaya sürülmesi, bu ilacın perioperatif dönemde kullanılma olasılığını ortaya çıkarmıştır (Bekker ve ark. 2018).

Nöropatik ağrı, çeşitli hastalıkların yaygın bir klinik semptomudur ve hastaların fiziksel ve ruhsal sağlığını ciddi şekilde etkiler. Nöropatik ağrının karmaşık patolojik mekanizması nedeniyle, ağrının klinik tedavisi zordur. Bu nedenle, araştırmacılar arasında nöropatik ağrı için potansiyel terapötik stratejileri keşfetmeye

yönelik ilgi giderek artmaktadır. Çok sayıda çalışma nöropatik ağrı gelişiminin sinir iletimi ve ilgili sinyal molekülleri ile ilişkili olduğunu göstermiştir. P2X3 reseptörleri (P2X3R), sinirsel bilgi iletimine ve ilgili sinyal yollarına katılan, merkezi sinir sistemini hassaslaştıran ve nöropatik ağrının gelişiminde kilit rol oynayan ATP'ye bağlı iyon kanallarıdır (Dong ve ark. 2022). Hasarlı veya iltihaplı dokulardan salınan ATP, primer afferent nöronlar üzerinde ifade edilen P2X reseptörlerine etki edebilmektedir. Ortaya çıkan depolarizasyon, merkezi olarak ağrı olarak yorumlanan aksiyon potansiyellerini başlatabilmektedir. P2X3 alt birimleri, bazıları kapsaisine de duyarlı olan küçük çaplı, primer afferent nöronların bir alt kümesinde bulunur. Homo-oligomerik kanallar oluşturabilir veya P2X2 alt birimleriyle hetero-oligomerler halinde bir araya gelebilirler. P2X3 içeren reseptörler için seçici antagonistlerle yapılan çalışmalar, P2X3 alt birim seviyelerini azaltmak için antisens oligonükleotidlerle yapılan deneyler ve P2X3 nakavt farelerin davranışsal testleri, P2X2/3 reseptörünün kronik inflamatuar ağrı ve nöropatik ağrının bazı özelliklerinin sinyalizasyonunda bir rolü olduğunu göstermektedir (Hsieh ve ark. 2012).

Moleküler modelleme, moleküllerin reaksiyonlarını tanımlamak ve olası makroskopik özelliklerini tahmin etmek için kullanılır. Deneylerden elde edilen veriler bilgi sağlar. Ancak biyolojik sistemdeki etki mekanizmasını ortaya koymazlar. Bu mekanizmalar, biyolojik sistemlerin yapıları kullanılarak bilgisayar modellemesinde incelenebilir (Menchaca ve ark. 2020).

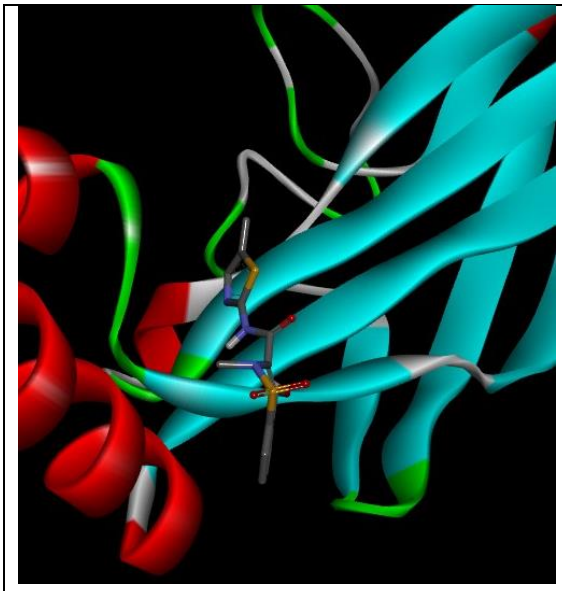
Bu çalışmamızın amacı, Meloksikamın nöropatik ağrı üzerindeki analjezik etki mekanizmasına ışık tutmak için P2X3 reseptör ile olan bağlanma afinitesi ve bağlanma modlarını bilgisayar destekli moleküler docking modellemesi ile ortaya çıkarmaktır.

MATERYAL VE METOT

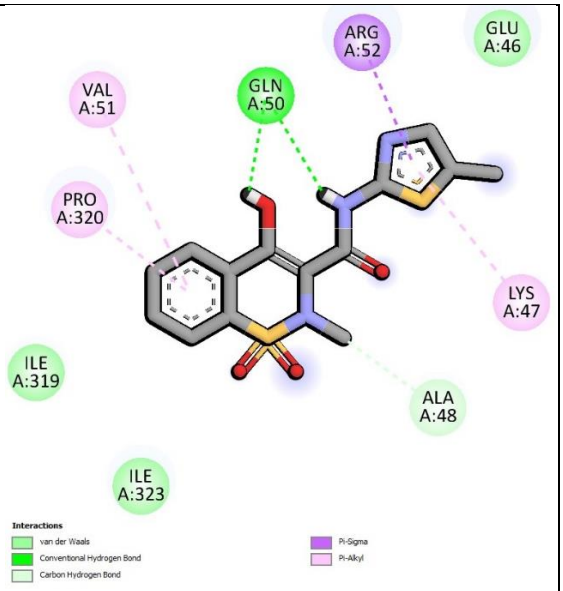
Moleküler docking analizleri AutoDock 4.0 (Sanner1999) kullanılarak Meloksikam'ın P2X3 reseptörü kristal yapısı üzerinde olası bağlanma bölgelerini tahmin etmek için gerçekleştirildi (PDB kodu: 5SVJ). Hedef (reseptör) molekülü olarak 2.98 Å çözünürlüklü P2X3 kristal yapısı seçildi. AutoDockTools (ADT), reseptör ve ligand molekülleri, docking analizini başlatmadan önce parametreleri hazırlamak için kullanıldı. Polar olmayan hidrojenler birleştirilirken P2X3 ve Meloksikam moleküllerindeki polar hidrojen atomları tutuldu. Gasteiger yükleri daha önce Ricci ve Netz (2009) ve Nasab ve ark. (2017) tarafından açıklandığı gibi ADT ile hesaplandı. Moleküler docking deneyi sırasında, ligandın tüm dönebilir bağlarının dönmesine izin verildi ve daha sonra hazırlanan reseptör ve ligand yapıları, PDBQT formatında kaydedildi. 0.375 Å ızgara aralığı ile 60 x 60 x 60 Å noktalı bir ızgara kutusu (grid box) boyutu ayarlandı. Dokingler en fazla 150 bireyden oluşan bir başlangıç popülasyonu kullanılarak 25 GA (Genetic Alghorithm) yürütme, $5x10^5$ enerji değerlendirme sayısı ve maksimum 27.000 nesil çalışmasından oluşturuldu. Popülasyona sırasıyla mutasyon ve geçiş oranları olarak seçilen 0,02 ve 0,8 değerleri uygulandı. Meloksikam molekülü için 100 bağımsız docking çalışmasından sonra, tüm olası bağlanma modları program tarafından kümelenmiş ve en düşük bağlanma serbest enerjisine sahip en iyi kenetlenme pozuna sahip konformasyonun bağlanma serbest enerjisi kcal/mol temelinde Meloksikam'ın seçilen pozu için sıralandı. AutoDock 4.0 kullanılarak ligand ve reseptör arasında elde edilen en iyi kenetlenme pozu BIOVIA Discovery Studio Visualizer 2016 kullanılarak analiz edildi (Husunet ve ark. 2021).

BULGULAR

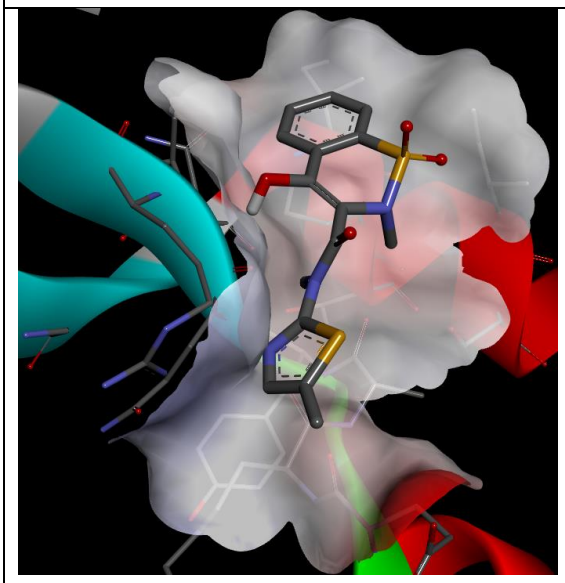
Kcal/mol olarak gibbs serbest bağlanma enerjilerine dayanan hedef molekül (P2X3) ile Meloksikam'ın aminoasit (aa) etkileşimi ve kimyasal bağ çeşitleri (Şekil 1.) gösterildi. En düşük negatif serbest bağlanma enerjisi (ΔG binding) -6.19 kcal/mol olduğundan, Meloksikam'ın P2X3 ile eşik bağlanma enerjisinden daha kuvvetli bir etkileşiminin olduğu görüldü. Meloksikam ve P2X3'ün en iyi kenetlenme pozları şekil 1'da gösterildi. Meloksikam moekülü, P2X3 reseptörü ile 9 farklı aa ve 5 farklı kimyasal bağ etkileşimi göstermiştir.



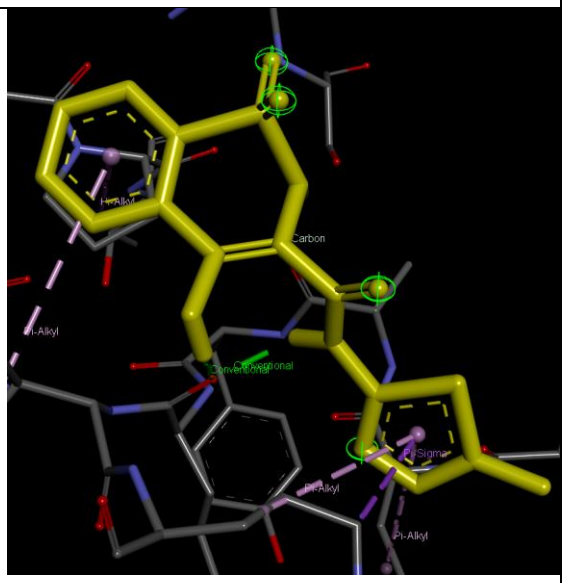
1-a



1-b



1-c



1-d

Şekil 1. Meloksikam ve P2X3 arasındaki Docking etkileşiminin gösterimi. 1a: En iyi 3D Docking pozu, 1b: 2D aa etkileşimi ve kimyasal bağ çeşitleri, 1c: 3D elektiriksel alan etkileşimi, 1d: Ligand etkileşimi pozu

TARTIŞMA

Yaptığımız moleküler docking sonuçları incelediğinde Meloksikam molekülü ile P2X3 reseptör bağlanma motifindeki (RBM) amino asit rezidüleri GLN50 ile güçlü hidrojen bağı etkileşimleri gözlenirken ILE 319, ILE323 ve GLU46 rezidü ile van der Waals etkileşimi, ALA48 ile karbon-hidrojen bağı gözlenmiştir. Ayrıca, Meloksikamın LYS47, VAL51 ve PRO320 aa ile Pi-Alkil bağı yaparken ARG52 aa ile Pi-Sigma yaptığı belirlenmiştir. Bağlanma sınır eşik enerji -6.0 kcal/mol olarak kabul edilmektedir. Meloksikam-P2X3 Gibss serbest bağlanma enersi -6.19 kcal/mol olarak tespit edilmiştir. Bu enerji eşik bağlanma enerjisinden daha kuvvetli olduğu için bulduğumuz sonuçlar anlamlı kabul edilmiştir. Bu sonuçlar yapılacak *in vitro* çalışmalar için ön veri sunması yönüyle önemli kabul edilebilir. Daha önce böyle bir çalışma olmaması çalışmamızın özgünlüğünü göstermekte literatüre yeni veriler sağlamaktadır.

SONUÇ

Bu sonuçlar Meloksikamın P2X3 reseptörüne yüksek afinitesi olduğunu göstermektedir. Meloksikamın P2X3 reseptörüyle etkileşimi, Meloksikamın ağrı semptomlarını azaltmadaki etki mekanizmasına ışık tutabileceği düşünülmektedir.

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ORAL PRESENTATION

İnterferon gamayı kodlayan *IFNG* genindeki yanlış anlamlı tek nükleotid polimorfizmlerinin olası etkilerinin *in silico* olarak değerlendirilmesi

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Özet

Bu çalışmada viral enfeksiyonlara karşı bağışıklık yanıtının önemli bir bileşeni olan interferon gamayı kodlayan IFNG genindeki tek nükleotid polimorfizmlerinin (SNP) olası zararlı etkileri hesaplamalı yöntemler ile araştırılmıştır. Öncelikle, IFNG genindeki SNP'ler arasından amino asit değişimine sebep olanlar belirlenmiştir. Bu amino asit değişimlerinin interferon gamanın yapısı, fonksiyonu ve stabilizasyonu üzerindeki etkileri çeşitli yazılım araçları kullanılarak tahmin edilmiştir. Sonuç olarak, R152Q ve T4I amino asit değişimlerinin kullanılan tüm yazılım araçları tarafından zararlı olabileceği belirlenmiştir. Evrimsel korunum analizi sonuçlarına göre, R152Q ve T4I varyantlarındaki yabanıl rezidünün korunmuş olabileceği tahmin edilmiştir. Ayrıca, gen-gen analizi sonuçlarına göre; IFNG geninin en çok etkileşimde olduğu 5 genin IFNGR1, IFNGR2, PSMB10, PTPN6 ve PRKCD olduğu belirlenmiştir.

Anahtar Kelimeler: Biyoinformatik analiz, IFNG, İnterferon gama, Tek nükleotid polimorfizmi

In silico evaluation of the possible effects of missense single nucleotide polymorphisms in the *IFNG* gene encoding interferon gamma

Abstract

In this study, the possible deleterious effects of single nucleotide polymorphisms (SNPs) in the IFNG gene encoding interferon gamma which is an important component of the immune response against viral infections were investigated using computational methods. First of all, among the SNPs in the IFNG gene, those causing amino acid changes were determined. The effects of these amino acid changes on the structure, function, and stabilization of interferon gamma were estimated using various software tools. As a result, it has been determined that R152Q and T4I amino acid changes may be harmful by all software tools used. According to the results of the evolutionary conservation analysis, it was predicted that the wild residue in the R152Q and T4I variants might be conserved. In addition, it was determined that the 5 genes in which the IFNG gene interacts most are IFNGR1, IFNGR2, PSMB10, PTPN6, and PRKCD, according to the results of the gene-gene analysis

Keywords: Bioinformatics analysis, IFNG, Interferon gamma, Single nucleotide polymorphism

GİRİŞ

İnterferonlar (IFN'ler), bağışıklık yanıtının koordinasyonunda merkezi role sahip olmakla beraber, İnterferonlar (IFN'ler), bağışıklık yanıtının koordinasyonunda merkezi role sahip olmakla beraber, antiviral, antitümör ve immünomodülatör nitelikleri olan pleiotropik sitokinlerdir (Castro vd., 2018; Gresser, 1990). IFN protein ailesi, virüslere ve diğer patojenlere karşı bağışıklık tepkisinde önemli roller oynar (Galbraith vd., 2022). Tip I interferon (IFN-α ve IFN-β), virüsle enfekte olmuş hücreler tarafından salgılanır. Tip II interferon veya interferon gama (IFN-γ), belirli aktivasyon koşulları altında timüs kaynaklı (T) hücreler tarafından ve doğal öldürücü (natural killer) hücreler tarafından salgılanır (Boehm vd., 1997). IFN-γ, hem virüs replikasyonunu kontrol etmede hem de enflamatuar hasarı indüklemede rol oynayabilen viral enfeksiyonlara karşı bağışıklık yanıtının önemli bir bileşenidir (Lee vd., 2013). IFN-γ antiviral savunma için gereklidir. IFN-γ viral replikasyonunu inhibe edebilir ve T hücreleri tarafından sitokin üretimini aktive ederek sitotoksik T lenfosit öldürme aktivitesini arttırır (Basheer vd., 2022; Levy & García-Sastre, 2001). Çalışmalar, IFN-γ'nın COVID-19'un sitokin fırtınasında kritik bir rol oynayabileceğini göstermektedir (Choi vd., 2022). IFN-γ 12. kromozomda (12q15) yer alan *IFNG* geni tarafından kodlanmaktadır. Bu gendeki mutasyonlar, viral,

bakteriyel ve parazitik enfeksiyonlara ve çeşitli otoimmün hastalıklara karşı artan bir duyarlılık ile ilişkilidir (*NCBI Database*, t.y.).

Genomdaki en yaygın varyasyon biçimi, tek nükleotid polimorfizmidir (SNP). SNP'ler, genomda sabit bir konumdaki tek bir nükleotidin bir başkasıyla ikame edildiği DNA varyantlarıdır (Orr & Chanock, 2008). Bir polimorfizm, proteinin katalitik fonksiyonunda, stabilitesinde ve/veya ifade seviyesinde değişikliklerle sonuçlanan fonksiyonel etkilere sahip olabilir (Dos Santos vd., 2021; Kelada vd., 2003). Hesaplamalı yöntemler kullanarak genetik varyasyonun etkilerini tahmin etmek ve anlamak, genetik çalışmalarda SNP seçimi ve hastalığın moleküler temelini anlamak için giderek daha önemli hale gelmektedir (Mooney, 2005). Bu çalışma, *IFNG* geninde yer alan ve amino asit değişimine neden olan SNP'lerin interferon gamanın yapısı, fonksiyonu ve stabilizasyonu üzerindeki olası etkilerini hesaplama yöntemleri ile araştırmak amacıyla yapılmıştır.

MATERYAL VE METOT

Bu çalışmada, *IFNG* geninde yer alan SNP'lere NCBI dbSNP (*NCBI DbSNP*, t.y.) veri tabanından erişilmiştir ve SNP'lerin sebep olduğu amino asit değişimleri ve pozisyon bilgileri kaydedilmiştir. *İn silico* analizlerde kullanılmak üzere insan interferon gama proteininin UniProtKB kodu ve FASTA formatlı dizisi UniProt (*UniProt Database*, t.y.) veri tabanından temin edilmiştir.

SNP'lerin olası etkilerinin tahmin edilmesi için kamuya açık ve çevrimiçi erişim sağlanabilen çeşitli yazılım araçları kullanılmıştır. SNP'lerin fonksiyonel etkilerinin tahmininde SIFT, PolyPhen-2 (HumVar ve HumDiv), SNAP2, PANTHER; protein stabilizasyonu üzerindeki azaltıcı/artırıcı etkilerinin tahmininde I-Mutant ve MUpro; yabanıl ve varyant tip amino asit rezidülerinin özelliklerinin karşılaştırılmasında ve proteinin üç boyutlu yapısının analizinde Project HOPE yazılım aracı kullanılmıştır. SNP'lerin sebep olduğu amino asit değişimlerinin evrimsel korunum analizleri ConSurf Server yazılım aracı kullanılarak yapılmıştır. Ayrıca GeneMANIA yazılım aracı kullanılarak gen-gen etkileşimleri incelenmiştir. Tablo 1'de bu araçların detayları verilmiştir.

Tablo 1. *İn silico* analiz araçları

Yazılım aracı / sunucu	Tahmin	URL	Referans(lar)
SIFT (Sorting Intolerant From Tolerant)	dizi homolojisi ve amino asitlerin fiziksel özellikleri	(*SIFT Sorting Intolerant From Tolerant*, t.y.)	(Ng & Henikoff, 2001)
PolyPhen-2 (Polymorphism Phenotyping v2)	basit fiziksel ve evrimsel karşılaştırmalı değerlendirmeler	(*PolyPhen-2 (Polymorphism Phenotyping V2)*, t.y.)	(Adzhubei vd., 2010)
SNAP2	"sinir ağı" adı verilen bir makine öğrenme cihazına dayalı	(*SNAP2*, t.y.)	(Hecht vd., 2015) (Bromberg & Rost, 2007)(Hecht vd., 2013)
PANTHER (Protein Analysis Through Evolutionary Relationships)	model organizmaların filogenetik ağaçlarını kullanarak gen ve protein fonksiyonunun çıkarımı	(*PANTHER*, t.y.)	(Thomas vd., 2022)
I-Mutant Suite (Predictor of effects of single point protein mutation)	destek vektör makinesi tabanlı	(*I-Mutant Suite*, t.y.)	(Emidio Capriotti, Fariselli, & Casadio, 2005) (Emidio Capriotti, Fariselli,

			Calabrese, vd., 2005) (E. Capriotti vd., 2006)
MUpro	destek vektör makinesi ve sinir ağları tabanlı	(*MUpro*, t.y.)	(Cheng vd., 2006)
Project HOPE	bir dizi web hizmetinden ve veri tabanından bilgileri toplayarak proteindeki nokta mutasyonunun yapısal etkilerini tahmin eder	(*Project HOPE*, t.y.)	(Venselaar vd., 2010)
ConSurf Web Server	amino asitlerin evrimsel korunumunu hesaplamak için Bayes yöntemi kullanılarak, korunum puanları tahminleri için güven aralıkları elde edilir	(*ConSurf Web Server*, t.y.)	(Ashkenazy vd., 2016) (Celniker vd., 2013) (Ashkenazy vd., 2010)
GeneMANIA	otomatik olarak seçilen ağırlıklandırma yöntemi (varsayılan): ağ ağırlıkları giriş gen listesinin (gen sayısının 5'ten az ya da 5 veya daha fazla olmasına göre) boyutuna göre seçilir	(*GeneMANIA*, t.y.)	(Warde-Farley vd., 2010)

BULGULAR VE TARTIŞMA

İnsan interferon gama proteinini (UniProtKB-P01579) kodlayan *IFNG* geninde (Gene ID: 3458) bulunan toplam 2730 varyanttan 90 tanesinin amino asit değişimine sebep olan yanlış anlamlı (missense) SNP olduğu belirlenmiştir. Bu SNP'lerin olası zararlı etkileri SIFT, PolyPhen-2 (HumVar ve HumDiv), SNAP2 ve PANTHER yazılım araçları ile analiz edilmiş ve tüm yazılım araçları tarafından zararlı olan 2 tane SNP olduğu tahmin edilmiştir. Bunlar rs371849964 (T4I) ve rs377736305 (R152Q) polimorfizmleridir (Tablo 2). 2014 yılında yayınlanan ve interferon gamanın genetik varyantları üzerine yapılan bir çalışmada, *IFNG* genindeki sinonim olmayan SNP'lerin protein işlevi üzerindeki tahmini etkileri rapor edilmiştir (veriler Ensembl web sitesinden temin edilmiştir). Buna göre *IFNG* geninde, protein işlevi üzerine zararlı olduğu tahmin edilen varyasyonlar arasında rs371849964 (T4I) ve rs377736305 (R152Q) polimorfizmleri de yer almaktadır. Bu polimorfizmlerin benzer şekilde SIFT ve Polyphen yazılım araçlarına göre zararlı olarak tahmin edildiği bildirilmiştir (Smith & Denning, 2014).

Amino asit dizisinin değişimine neden olan yanlış anlamlı genetik varyasyonlar, proteinlerin aktivitesi, stabilitesi, hidrojen bağı ağı, konformasyonel dinamikleri ve diğer birçok fizyolojik açıdan önemli özellikleri üzerinde bir etkiye sahip olabilir. Bir protein dizisindeki yalnızca bir rezidünün değişimi, birçok patolojik durumla bağlantılı olabileceği gibi hastalıklara yatkınlığı ve ilaç tedavisini etkileyebilir (Stefl vd., 2013). Ayrıca, amino asit dizisini değiştiren DNA varyasyonları protein stabilitesini artırarak veya azaltarak protein fonksiyonunu değiştirebilir, bu da proteinin çalışması için gereken konformasyonel değişiklikleri önleyebilir (Sanavia vd., 2020; Stefl vd., 2013). Hesaplamalı stabilizasyon tahmin edicileri yanlış anlamlı varyantların etkilerini anlayabilmek için alternatif bir yaklaşım sunmaktadır. Bu tahmin ediciler, mutasyon üzerine katlanma veya protein etkileşimi enerji değişikliklerini (Gibbs serbest enerjisindeki değişiklik – kısaca $\Delta\Delta G$) değerlendirmek için geliştirilmiş programlardır (Gerasimavicius vd., 2020). Bu çalışmada tüm yazılım araçları tarafından zararlı olarak tahmin edilen rs371849964 (T4I) ve rs377736305 (R152Q) polimorfizmlerinin protein stabilizasyonu üzerine etkileri I-Mutant ve MUpro yazılım araçları ile analiz edilmiştir. Sonuç olarak, R152Q amino asit değişiminin her iki yazılım aracında da protein stabilitesinde azalışa neden olabileceği belirlenmiştir. T4I amino asit değişiminin ise I-Mutant yazılım aracına göre protein stabilitesinde azalışa, MUpro programına göre ise artışa sebep olabileceği tahmin edilmiştir (Tablo 2).

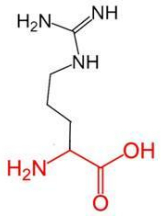
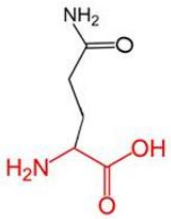
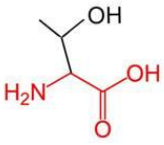
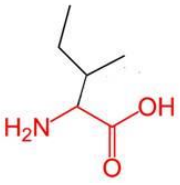
Tablo 2. SNP'lerin olası zararlı etkilerinin sonuçları

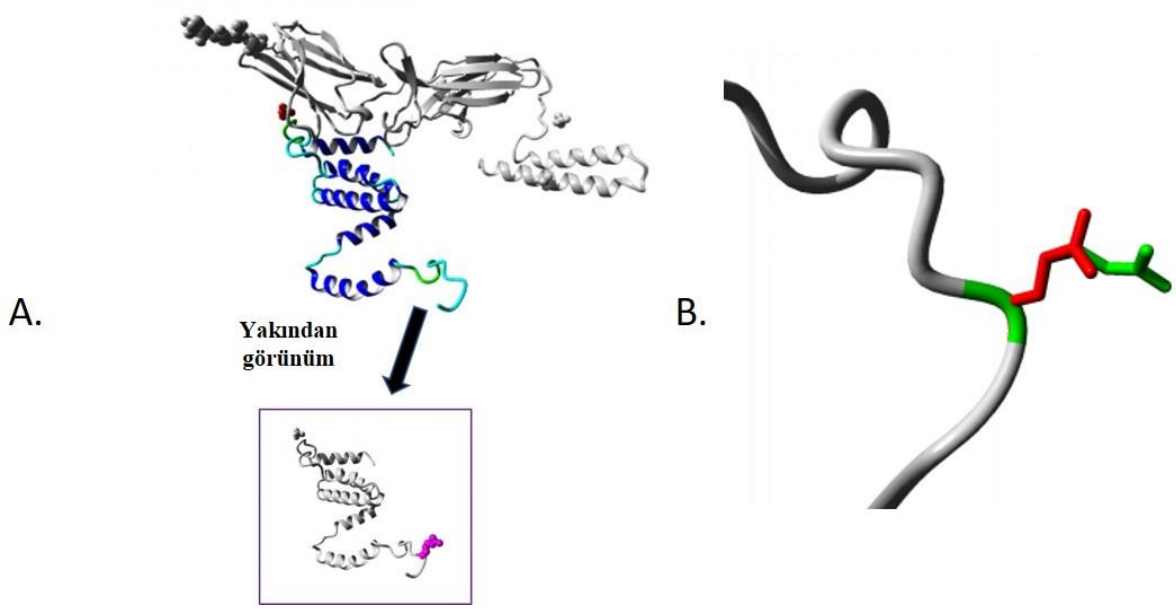
SNP ID	rs371849964	rs377736305
Nükleotit Değişimi	G>A	C>T
Amino Asit Değişimi	T4I	R152Q
SIFT	Zararlı	Zararlı
SIFT Skoru	0.008	0.008
PolyPhen-2 HumDiv	Muhtemel Zararlı	Muhtemel Zararlı
PolyPhen-2 HumDiv Skoru	0.994	1.000
PolyPhen-2 HumVar	Olası Zararlı	Muhtemel Zararlı
PolyPhen-2 HumVar Skoru	0.865	0.986
SNAP2	Etki	Etki
SNAP2 Skoru	45	77
SNAP2 Beklenen Doğruluk	%71	%85
PANTHER	Olası Zararlı	Olası Zararlı
Pdel (zararlı etki olasılığı)	0.5	0.5
I-Mutant	Azalış	Azalış
I-Mutant Güvenilirlik İndeksi (G.İ.) değeri	1	7
I-Mutant ΔΔG değeri	-0,11	-0,77
MUpro	Artış	Azalış
MUpro ΔΔG değeri	0.16063935	-0.60997373

ΔΔG değeri: serbest enerji değişimi

Project HOPE yazılım aracından temin edilen yabanıl ve varyant rezidülerin özellikleri (boyut, yük, hidrofobiklik) Tablo 3'te verilmiştir. Ayrıca R152Q polimorfizminin Project HOPE tarafından oluşturulmuş modelleri Şekil 1'de verilmiştir. T4I polimorfizmi için ise yapısal bilgi eksikliği nedeniyle, HOPE resim oluşturamamıştır.

Tablo 3. Project HOPE ile amino asit değişimlerinin karşılaştırılması (Venselaar vd., 2010)

Amino Asit Değişimi	**Açıklama**	**Yabanıl Rezidü (YR)**	**Varyant Rezidü (VR)**	**Karşılaştırma**
R152Q	152. pozisyondaki arjinin amino asidinin glutamine dönüşümü			Boyut: VR<YR Yük: YR pozitif, VR nötral
T4I	4. pozisyondaki treonin amino asidinin izolösine dönüşümü			Boyut: VR>YR Hidrofobiklik: VR>YR



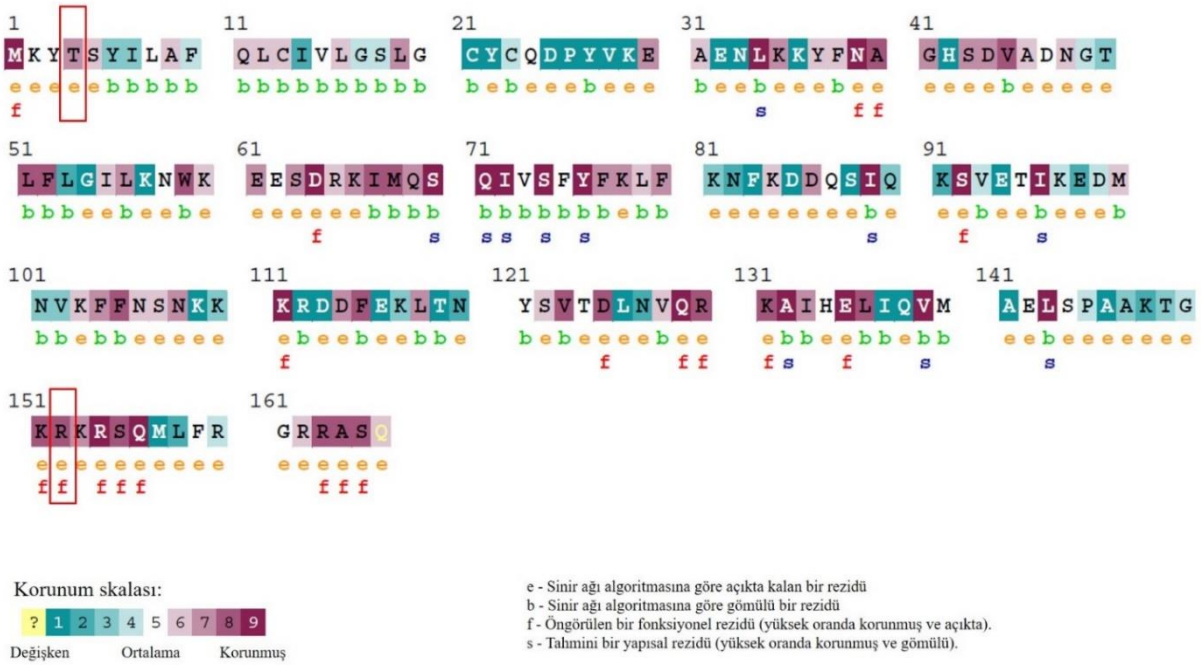
Şekil 1. R152Q polimorfizmi için A) Proteinin şerit sunumundaki modeli B) Polimorfizm bölgesinin yakından görünümü (Yan zincirler yabanıl rezidü için yeşil varyant rezidü için kırmızı renk ile gösterilmiştir) (Venselaar vd., 2010)

Aşağıda T4I ve R152Q pozisyonları için yabanıl ve varyant tip amino asitlerin özellikleri değerlendirilmiştir:

T4I amino asit değişimi 4. pozisyondaki treonin amino asidinin izölösin amino asidine değişimini ifade etmektedir. Treonin bir proteinin hem içinde hem de yüzeyinde bulunabilen bir amino asittir. Treoninler, protein fonksiyonel merkezlerinde oldukça yaygındır. Sahip olduğu hidroksil grubu (Bkz. Tablo 3), oldukça reaktiftir ve çeşitli polar substratlarla hidrojen bağları oluşturabilir. Hidrofobik bir amino asit olan izolösin ise proteinin hidrofobik çekirdeklerine gömülmeyi tercih eder. İzolösinin yan zinciri çok reaktif değildir. Bu sebeple, substrat tanımada bir rol oynayabilmesine karşın, kataliz gibi protein fonksiyonlarında nadiren doğrudan yer alır (Betts & Russell, 2003). Sonuç olarak, bu pozisyonda treonin amino asidinin izolösin amino asidine ikamesi ile daha hidrofobik bir rezidü ortaya çıkar. Bu durum, hidrojen bağlarının kaybına ve/veya doğru katlanmanın bozulmasına neden olabilir. Varyant rezidünün daha büyük olması ise çıkıntılara sebep olabilir. Ayrıca, mutasyon sinyal peptidi içinde bulunur. Bu peptidin bu dizisi önemlidir, çünkü diğer proteinler tarafından tanınır ve genellikle olgun proteini oluşturmak için bölünür. Sinyal peptidine dahil edilen yeni rezidü, özellikleri bakımından orijinal olandan farklıdır. Bu durumun sinyal peptidinin tanınmasını bozması mümkündür (Venselaar vd., 2010).

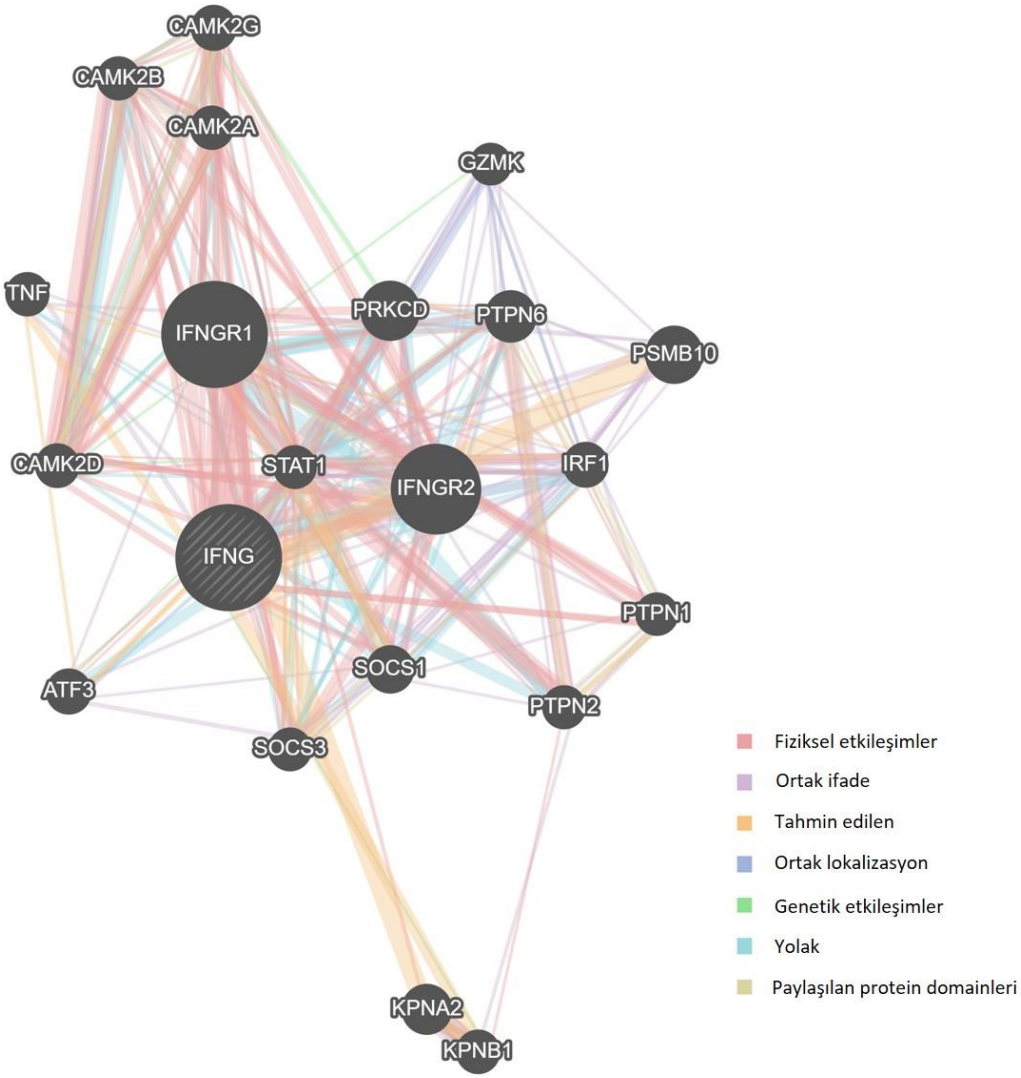
R152Q amino asit değişimi 152. pozisyondaki arjinin amino asidinin glutamin amino asidine değişimini ifade etmektedir. Arjinin, pozitif yüklü, polar bir amino asittir. Pozitif yük, negatif yüklü protein olmayan atomlarla etkileşime girebileceği anlamına gelir. Arjinin genellikle proteinin yüzeyinde olmayı tercih eder. Ancak amfipatik doğasından dolayı yan zincirin bir kısmının gömülü olduğu anlaşılmaktadır. Arjinin, yan zincirinde karmaşık bir guanidinyum grubu içerir (Tablo 3). Bu grup fosfatlar üzerindeki negatif yüklü grupları bağlamak için ideal olan bir geometriye ve yük dağılımına sahiptir (çoklu hidrojen bağları oluşturabilir). Arjininler, proteinin aktif veya bağlanma bölgelerinde oldukça sık görülür. Glutaminler de sıklıkla proteinin aktif veya bağlanma bölgelerinde yer alır. Polar yan zinciri, diğer polar veya yüklü atomlarla etkileşimlerde rol oynar (Betts & Russell, 2003). Sonuç olarak, bu pozisyonda arjinin amino asidinin glutamin amino asidine ikamesi ile pozitif yüklü olan rezidü nötral olan bir rezidüye yerini bırakmıştır. Yabanıl rezidünün yükü kaybolmuştur. Bu durum, diğer moleküller ile etkileşimlerin kaybına neden olabilir. Ayrıca varyant rezidünün boyutunun daha küçük olması olası bir dış etkileşim kaybına neden olabilecektir (Venselaar vd., 2010).

Bir proteindeki amino asit rezidülerinin korunum analizi, bunların önemini ve nesiller boyunca sınırlandırılmış evrimini anlamaya yardımcı olur (Shafie et al., 2021). Bu çalışmada, ConSurf sunucusu evrimsel korunum analizinde kullanılmıştır. T4I ve R152Q varyantlarındaki yabanıl rezidünün ConSurf sunucusu tarafından elde edilen evrimsel korunum analizleri Şekil 2'de verilmiştir. Sonuçlar incelendiğinde, 4. pozisyondaki treoninin korunum skalasının 7 olduğu ve sinir ağı algoritmasına göre açıkta kalan bir rezidü olduğu belirlenmiştir. 152. pozisyondaki arjininin korunum skalasının ise 8 olduğu belirlenmiştir. Ayrıca bu rezidünün açıkta kalan bir rezidü olduğu ve öngörülen bir fonksiyonel rezidü olduğu (yüksek oranda korunmuş ve açıkta) tespit edilmiştir. Sonuç olarak her iki rezidünün ConSurf sunucusu ile elde edilen korunum skalasına göre "korunmuş" rezidüler olabileceği belirlenmiştir (Şekil 2) (*ConSurf Web Server*, t.y.).



Şekil 2. T4I ve R152Q için evrimsel korunum sonuçları (*ConSurf Web Server*, t.y.) (Yabanıl amino asitlerin bulunduğu pozisyonlar kırmızı çerçeve içine alınmıştır)

Şekil 3'te *IFNG* geninin GeneMANIA yazılım aracı tarafından elde edilen gen-gen etkileşim ağı verilmiştir. *IFNG* geninin 20 gen ile etkileşimine bakılmıştır. Dolayısıyla Şekil 3'te toplam 21 ilişkili gen ve aralarında toplam 393 bağlantı olduğu görülmektedir. *IFNG* geninin en çok etkileşimde olduğu 5 genin ise *IFNGR1, IFNGR2, PSMB10, PTPN6* ve *PRKCD* olduğu belirlenmiştir (*GeneMANIA*, t.y.).



Şekil 3. *IFNG* için elde edilen gen-gen etkileşim ağı (*GeneMANIA*, t.y.)

SONUÇ

Bu çalışmada, interferon gamayı kodlayan *IFNG* genindeki toplam 90 adet amino asit değişimine sebep olan varyantın olası etkileri hesaplamalı yöntemler ile incelenmiştir. Biyoinformatik analiz sonuçlarına göre, T4I (rs371849964) ve R152Q (rs377736305) amino asit değişimlerinin tüm yazılım araçları tarafından zararlı olabileceği tahmin edilmiştir. Bu varyantların *IFNG* genindeki SNP'ler üzerine yapılacak olan çalışmalarda öncelikli olarak ele alınması önerilmektedir.

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ORAL PRESENTATION

Effects of thymoquinone on cell proliferation and oxidant-antioxidant status in uterine leiomyosarcoma cell line

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Abstract

Thymoquinone (TQ) the main active ingredient of Nigella sativa, is one of the promising phytochemicals that has attracted attention due to its pharmacological and therapeutic properties. It has been extensively studied for its anti-cancer properties, demonstrating growth inhibition in various cancer cell types, including prostate, breast, gastric, bladder, colon, and lung cancer. Additionally, TQ has shown potential in reducing the toxic side effects associated with conventional chemotherapy. This study investigated whether TQ could exhibit antiproliferative and antioxidant activity in *in vitro* cell models of uLMS. Cell viability was evaluated by CCK-8 assay on SK-UT-1 cell line. Rel Assay kits were used to calculate TOS (total oxidant status), TAS (total antioxidant status), and OSI (oxidative stress index) values. CCK-8 assay results demonstrated that TQ significantly decreased the proliferation of SK-UT-1 cells in a dose-dependent manner with IC50 value of 15.44 µM at 24 h. In addition, 100 µM of TQ reduced cell proliferation by over 90% in SK-UT-1 cells. Treatment with TQ increased level of TAS in SK-UT-1 cells compared with untreated control samples, although not quite significantly. Compared with untreated control samples, there were decrease in TOS and OSI in SK-UT-1 cells, although not quite significantly. This study report that TQ elicits potent *in vitro* anticancer activity against uLMS by reducing cell proliferation. Overall, these results suggest that TQ may be a promising therapeutic agent for the treatment of uLMS.

Keywords: Oxidative Stress, Phytochemical, Thymoquinone, Uterine leiomyosarcoma

1. INTRODUCTION

Uterine leiomyosarcoma (uLMS) is a rare and highly aggressive cancer that originates from the smooth muscle cells of the uterus (Denu et al., 2024). 25-36% of uterine sarcomas and 2% to 5% of all uterine-related malignancies are uLMS (Brahambe et al., 2014; Byar & Fredericks, 2022). The age of onset is usually 40-50, and the incidence increases rapidly after menopause. (Brahambe et al., 2014; Nagao et al., 2023). Regardless of the stage of the disease, its prognosis is poor due to its high rates of spread and recurrence and its resistance to treatment (Roberts et al., 2018; Byar & Fredericks, 2022). The risk of recurrence is 50% to 70% due to early lymphovascular invasion and spread to many extra pelvic sites (Roberts et al., 2018; Nagao et al., 2023). While the basis of treatment for early-stage patients is hysterectomy and bilateral salpingo-oophorectomy, chemotherapy and radiotherapy are recommended as a treatment option for metastatic and advanced-stage patients (Thanopoulou et al., 2014; Bose et al., 2022; Giannini et al., 2023). However, the treatment efficacy of FDA-approved agents (such as doxorubicin, eribulin, trabectedin, and pazopanib) is still limited (Nagao et al., 2023). In addition, due to the lack of sufficient information about the etiopathogenesis of uLMS, the very high recurrence rate after resection, resistance to radiotherapy and/or chemotherapy treatments, and the lack of effective results, a specific, effective strategy for treatment is still unknown (Greco et al., 2023). Therefore, research is needed to develop new targeted treatments, especially in advanced-stage and relapsed patients.

Natural compounds, particularly those derived from plants, are increasingly recognized for their essential role in the discovery of innovative therapeutics targeting a wide array of disease pathologies. Their relevance in modern pharmacology is underscored by their ability to match the efficacy of synthetic pharmaceuticals while exhibiting a more favourable safety profile with fewer adverse effects. This dual advantage positions natural compounds as promising candidates in the quest for safer and more effective treatment options (Okesola et al., 2022; Greco et al., 2023).

Thymoquinone (TQ), the main active ingredient of Nigella sativa, is one of the promising phytochemicals that has attracted attention due to its pharmacological and therapeutic properties (Ballout et al., 2018). In recent years, interest in TQ has increased due to its pharmacological properties such as antioxidant, anti-diabetic, anti-inflammatory, anti-fibrotic, anti-hypertensive, antihistamine, anti-microbial, anti-convulsant, analgesic, renal protective, anti-neoplastic, hepatoprotective, immunomodulatory, and spasmolytic (Khan et al., 2019; Zhao et al., 2023). TQ has been extensively studied for its anti-cancer properties, demonstrating growth inhibition in various cancer cell types, including prostate (Alshyarba et al., 2021), breast (Alobaedi et al., 2017), gastric (Feng et al., 2017), bladder (Zhang et al., 2018), colon (Zhang et al., 2016), and lung cancer (Yang et al., 2015). Additionally, TQ has shown potential in reducing the toxic side effects associated with conventional chemotherapy (Farooq et al., 2021). TQ exhibits anti-cancer properties by playing a role in various cellular processes and many mechanisms have been proposed (Alandağ et al., 2022). The mechanisms include arrest of the cell cycle at G1/S and G2/M, antiproliferation, prevention of cell migration and metastasis, prevention of oxidative stress by regulating reactive oxygen species, prevention of inflammation, increase in the expression of tumor suppressor genes, suppression of the expression of proto-oncogenes, induction of cell death mechanisms, and many other processes (El-Far et al., 2022; Zhou et al., 2022; Abutayeh et al., 2024). Research particularly focuses on oxidative stress and the induction of apoptosis. TQ reduces oxidative stress by affecting free radicals. It increases the activity of antioxidant enzymes that protect cells against oxidative damage (Deng et al., 2024). Consequently, it can mitigate oxidative DNA damage induced by free radicals, thereby lowering the risk of cancer development (Alandağ et al., 2022). TQ also triggers apoptosis by activating intrinsic-extrinsic apoptotic pathways, inducing caspases, decreasing NF-κB and IKKα/β phosphorylation, decreasing the levels of anti-apoptotic proteins and increasing the levels of pro-apoptotic proteins (Alandağ et al., 2022; Abutayeh et al., 2024).

TQ demonstrates significant anti-cancer properties across various cancer types, including its ability to inhibit cell growth and reduce the toxicity of conventional therapies. These findings highlight its potential as a promising therapeutic agent in cancer treatment, particularly for challenging malignancies like leiomyosarcoma. Thus, this study, for the first time, investigated the effects of thymoquinone on cell proliferation and oxidant-antioxidant status in uterine leiomyosarcoma cell line.

2. MATERIALS AND METHODS

2.1. Cell Culture

The SK-UT-1 cell line was cultured in Dulbecco's Modified Eagle Medium enriched with 10% fetal bovine serum and 1% penicillin (100 U/mL)/streptomycin (0.1 mg/mL) under standard conditions of 37°C in a humidified incubator with 5% CO2. Subculturing was performed in T-flasks two to three times weekly, with cells being harvested for assays once they reached approximately 90% confluency. The SK-UT-1 cells were incubated with TQ (Sigma Aldrich, USA) at the indicated concentrations for 24h.

2.2. Cell Viability

The antiproliferative effect of TQ was analysed by Cell Counting Kit 8 (CCK8, Elabscience, USA) assay according to the manufacturer's protocol. The SK-UT-1 cells were cultured in 96-well plates in media for 24 hours, and on the following day, cells were treated with media only (control) or increasing concentrations of TQ (12.5-200 µM) for 24 h. CCK-8 solution (10 µL/well) was added to each well, and the plates were incubated at 37°C for 1 h. Then, the absorbance was read at 450 nm with a microplate reader The half maximum inhibitory concentration (IC50) of TQ was determined via non-linear regression by using GraphPad Prism (version 9.4.1). The percentage of SK-UT-1 cell viability was calculated using the following formula:

Cell Viability (%) = [(Treatment group OD450 - Blank well OD450) / (Untreated group OD450 - Blank well OD450)] ×100%.

All experiments were performed using IC50 dose of thymoquinone obtained from cell viability assays for SK-UT-1 cells.

2.3. Total Oxidant Status Assay

TOS levels in cell supernatants were determined using method developed by Erel (Erel, 2005) with the Total Oxidant Status Assay Kit (Rel Assay Diagnostics, Türkiye) according to the manufacturer's instructions. TOS

value was calculated according to the following formula and the results were expressed in µmol hydrogen peroxide equivalents/L.

TOS= [(ΔAbsSample)/(ΔAbsStandard) X Concantration of standard]

2.4. Total Antioxidant Status Assay

The level of TAS was investigated with the Total Antioxidant Status Assay Kit (Rel Assay Diagnostics, Türkiye) according to the manufacturer's instructions. TAS value was calculated according to the following formula and the results were expressed in mmol Trolox equivalents/L.

TAS= [(ΔAbs H2O) - (ΔAbs Sample) / (ΔAbs H2O) - (ΔAbs Standart)]

2.5. Calculation of the Oxidative Stress Index (OSI)

The Oxidative Stress Index (OSI), which is considered an indicator of oxidative stress levels, is a unitless parameter obtained by expressing the ratio of TOS values to TAS values. In the calculation process, TAS was first converted from its unit of mmol Trolox equivalents/L to µmol Trolox equivalents/L.

The OSI was then calculated using the formula OSI = [(TOS, µmol H2O2 equivalents/L) / (TAS, µmol Trolox equivalents/L) × 100] (Acar et al., 2012).

2.6. Data Analysis

Prism software (v9.4.1; GraphPad) was used for statistical analysis. Differences among the experimental groups were assessed by using Mann–Whitney U test for comparing two groups. One-way ANOVA with Dunnett's post hoc test was used for comparison of more than two groups (ns $P > .05$, * $P \leq .05$, ** $P \leq .01$, *** $P \leq .001$, and **** $P \leq .0001$).

3. RESULTS

3.1. Antiproliferative Effect of Thymoquinone Treatment on SK-UT-1 Cells

The effects of TQ first were determined on cell proliferation in *in vitro* cell models of uLMS. SK-UT-1 cells were treated with various concentrations of TQ (12.5-200 µM) for 24h. Antiproliferative effect of TQ was assessed by using the CCK-8 assay. As shown in (Fig. 1A), TQ significantly decreased the proliferation of SK-UT-1 cells in a dose-dependent manner. More importantly, the effects were observed at doses above 12.5 µM (Figure 1A) and the IC50 value was 15.44 µM (95% CI: 7.987-24.44 µM) (Figure 1B). Thymoquinone was highly active in inducing cytotoxicity, with an efficacy of over 90% at concentrations of 100 µM.

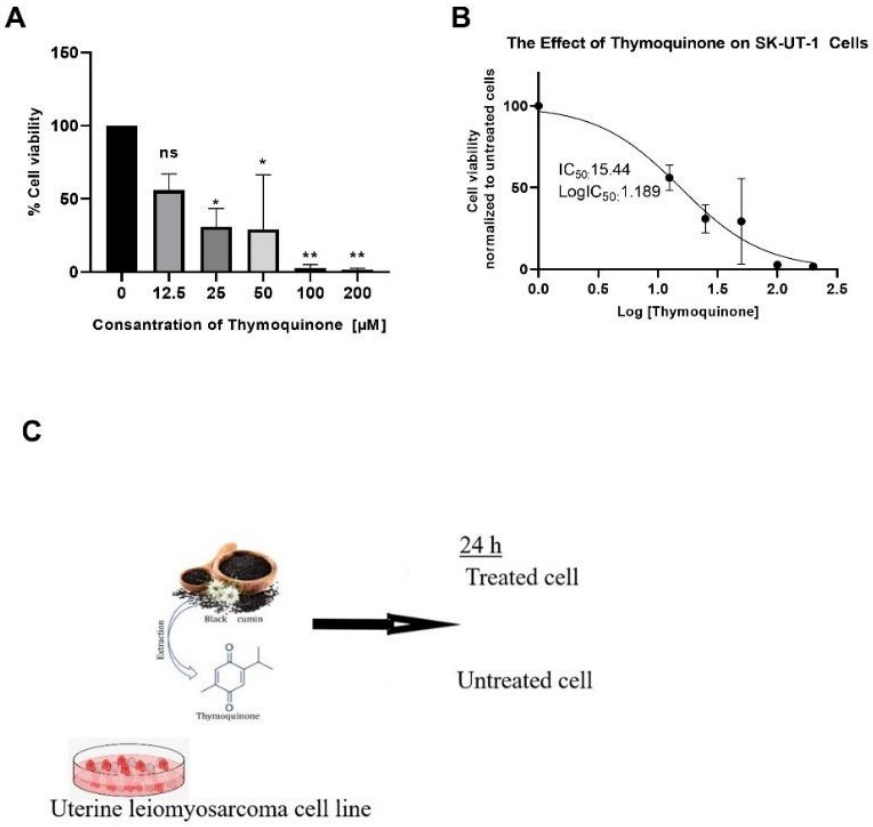

Figure 1. *In vitro* antiproliferative effect of thymoquinone on SK-UT-1 cells at 24 h post-stimulation. (A) The effect of thymoquinone on cell viability. (B) Dose-response curve of cell viability with respect to treatment with different concentrations of thymoquinone and (C) Experimental design for thymoquinone treatment. CCK-8 results are expressed as cell viability percentage (%) normalized to untreated SK-UT-1 cells. The data represent the mean values ± SD. Statistical comparisons were performed using the One-way ANOVA followed by Dunnett's multiple comparisons post-test (ns $P > .05$, * $P \leq .05$, ** $P \leq .01$, *** $P \leq .001$, and **** $P \leq .0001$).

3.2. Effects of Thymoquinone Treatment on Oxidant-antioxidant Status in SK-UT-1 Cells

Treatment with TQ increased level of TAS in SK-UT-1 cells from 1.183± 0.199 to 1.447± 0.044 mmol Trolox equivalents/L compared with untreated control samples, although not quite significantly (Fig. 2A; P=.2). Treatment with TQ reduced levels of TOS in SK-UT-1 cells (from 52.29 ± 4.103 to 41.69 ± 7.597 µmol hydrogen peroxide equivalents/L) compared with untreated control samples, although not quite significantly (Fig. 2B; P=.4). Compared with untreated control samples, there was a decrease in OSI in SK-UT-1 cells (from 4.845± 1.296 to 2.958 ± 0.487), although not quite significantly (Fig. 2C; P=.4).

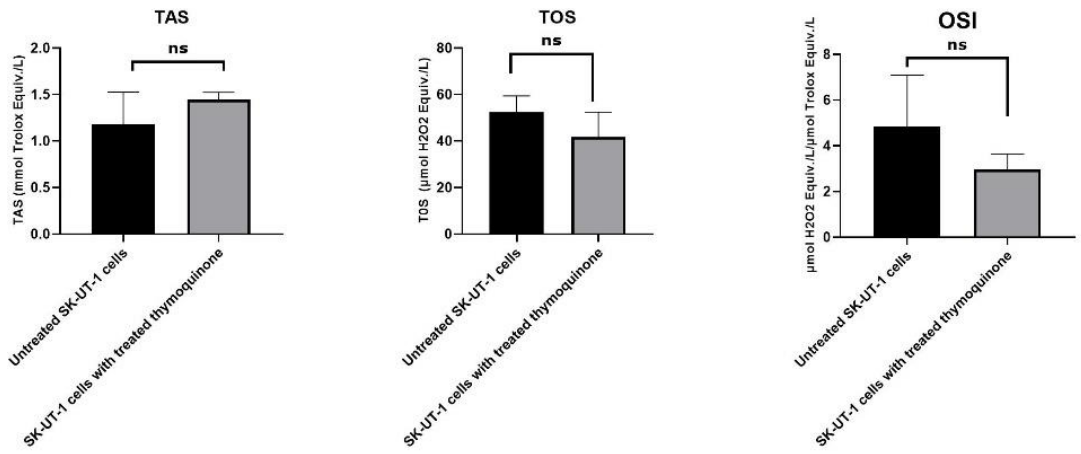

Figure 2. Effects of thymoquinone treatment on oxidant-antioxidant status in SK-UT-1 cells. The data represent the mean values ± SD. Statistical comparisons were performed using the Mann–Whitney U test (two tailed) (ns $P > .05$, * $P \leq .05$, ** $P \leq .01$, *** $P \leq .001$, and **** $P \leq .0001$).

4. DISCUSSION

A key component of black cumin is TQ, a small molecule known for its low toxicity and potential therapeutic effects against various diseases, such as diabetes, cancer, and neurodegenerative disorders (Mahmoud & Abdelrazek, 2019). It demonstrated significant anti-cancer activity against a range of human cancers while exhibiting minimal toxicity to normal cells (Rajput et al., 2013). TQ exhibits *in vitro* antitumor effects by inducing apoptosis, ROS generation, and arrest of cell cycle. TQ contains a lipophilic quinine compound in its structure, which facilitates the entry of the molecule into the cell structure and increases its effectiveness (Mahmoud & Abdelrazek, 2019).

In the present study, the *in vitro* anti-cancer effect of TQ, for the first time, was investigated in uterine leiomyosarcoma. CCK-8 assay results demonstrated that TQ significantly decreased the proliferation of SK-UT-1 cells in a dose-dependent manner with IC50 value of 15.44 µM at 24 h. This result was comparable to those reported for different cancer types for example, breast cancer (T-47D and MDA-MB-468 cell lines) with IC50 values were18.06 µM and 12.30 µM for 48 h, respectively (Rajput et al., 2013), gastric cancer (SGC-7901 cells) and pancreatic cancer (FG/COLO357) with IC50 values were 25 µM (Feng et al., 2017) and 73 µmol/L for 24 h (Torres et al., 2010), respectively.

Recent reports have demonstrated that the levels of key oxidative stress biomarkers, such as protein carbonyls, thiols, nitrate/nitrites, and 8-hydroxy-2′-deoxyguanosine are elevated in preoperative serum samples from patients with uterine leiomyomas compared with those without leiomyomas. These findings suggest oxidative stress is an important factor in the pathogenesis of leiomyomas (Santulli et al., 2013). In addition, previous studies have reported the leiomyoma cells have an impaired antioxidant system (Fletcher et al., 2013). Fletcher et al. demonstrated that leiomyoma cells exhibited an increased oxidative stress characterized by elevated expression levels of important oxidative stress regulators, inducible nitric oxide synthase and myeloperoxidase (Fletcher et al., 2017). Numerous studies have demonstrated that TQ enhance antioxidant defense by inducing cytoprotective enzymes, including superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR). These enzymes play crucial roles in scavenging hydrogen peroxide and superoxide radicals, as well as in preventing lipid peroxidation thereby protecting cells from oxidative stress-induced damage (Sayed-Ahmed et al., 2010; Kassab & El-Hennamy, 2017). These results demonstrate that treatment with TQ increased level of TAS in SK-UT-1 cells compared with untreated control samples, although not quite significantly. Compared with untreated control samples, there were decrease in TOS and OSI in SK-UT-1 cells, although not quite significantly. Additional studies are needed to comprehensively determine whether TQ exhibits antioxidant activity in SK-UT-1 cells. These comprehensive approaches will help to fully elucidate the mechanisms underlying the antiproliferative effect of TQ in SK-UT-1 cells and may provide new insights into its potential therapeutic applications.

5. CONCLUSIONS

This study report that TQ elicits potent *in vitro* anticancer activity against uterine leiomyosarcoma by significantly reducing cell proliferation. Future research should prioritize elucidating the underlying action mechanisms of TQ and exploring its efficacy in clinical settings. Further investigation of thymoquinone may pave the way for innovative treatment strategies that improve patient outcomes and minimize side effects.

Ethical Approval: Since it is a cell culture study, it does not require ethics committee approval.

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ORAL PRESENTATION

SII, Trombosit ve MPV obezitede inflamasyonu belirleyebilir

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Özet

Obezite vücutta yağ dokusunun artması olup, düşük dereceli inflamasyonla karakterize kronik, multisistemik, multifaktöriyel, epigenetik bir hastalıktır. Yaygınlığı dünya çapında artmaktadır. Bu nedenle önemli bir halk sağlığı sorunudur. Obezitedeki en önemli sorun enerji alımı ve harcaması arasındaki dengesizliktir. Bu çalışmada amacımız obezitede artan inflamasyonu, yeni tanımlanan inflamasyon markerleri ile incelemek ve yeni markerlerin obezite takibinde kullanılabilirliğinin tartışıldığı literatür bilgisine katkı sağlamaktır .Obezite nedeniyle polikliniğimize başvuran 362 hastanın verileri kullanıldı. Başvuran hastaların öncelikle çıplak ayakla boy uzunlukları ölçüldükten sonra antropometrik ölçüm için bioimpedans cihazı (BIA) kullanıldı. Bu cihazla kilo, beden bitle indeksi (BMI) (kg/m 2) yağ yüzdesi,(%F) yağ ağırlığı (FM), yağsız ağırlık (FFM) tespit edildi, Elde edilen yağ ağırlığı ve yağsız ağırlık boy uzunluğunun metrekaresine bölünerek yağ ağırlığı indeksi (FMI) ve yağsız ağırlık indeksi(FFMI) hesaplandı. Başvuran hastaların kan parametreleri incelenerek nötrofil, lenfosit, trombosit, MPV (mean platelet volume) verilerine ulaşıldı. Bu verilerden SII (SII=platelet x neutrophil/ lymphocyte) hesaplandı.

Antropometrik ölçümlerden elde edilen verilerle kan parametrelerinden elde edilen verilerin istatistiksel analizi yapıldı .

SII özellikle %F, FM ve FMI ile güçlü istatistiksel olarak pozitif sonuç gösterdi ve vücut yağ kompozisyonu ile ilişkili olarak sistemik inflamasyon için bir belirteç olma potansiyelini göstermektedir. Trombosit %F, FM, FMI ile güçlü istatistiksel olarak pozitif sonuç gösterdi. Vücut yağ kompozisyonu ile ilişkili olarak artması sistemik inflamasyon için bir belirteç olma potansiyelini göstermekle birlikte, yağsız ağırlıkla (FFM) da anlamlı sonuç elde edildi.

MPV ile antropometrik ölçümler arasında anlamlı bir korelasyon tespit edilemedi. Bu bulgu MPV' nin bu bağlamda sınırlı bir rolü olduğunu düşündürmektedir.

Obezite sınıflaması yaparak incelediğimizde yüksek riskli (sınıf III) obezite grubunda SII ve trombosit sayısının istatistiksel olarak anlamlı arttığı, MPV 'nin azaldığı tespit edildi.

Bu bulgular, sistemik enflamasyon ve hematolojik sağlığın kapsamlı bir değerlendirmesi için antropometrik ve hematolojik ölçümlerin entegre edilmesinin faydasının altını çizmektedir.

Anahtar kelimeler: Obezite, SII (systemic immunoinflammatory index), trombosit, MPV .

SII (systemic immunoinflammatory index), Platelet and MPV can determine inflammation in obesity

Abstract

Obesity is a chronic disease characterized by increased fat tissue and low-grade inflammation. The prevalence of obesity is increasing worldwide. Therefore, it is an important public health problem. The most important problem in obesity is the imbalance between energy intake and expenditure.
Our aim in this study is to examine the increased inflammation in obesity with newly defined inflammation markers and to contribute to the literature knowledge where the usability of new markers in obesity follow-up is discussed. Data of 362 patients who applied to our clinic due to obesity were used. After the barefoot heights of the patients were measured, a bioimpedance device (BIA) was used for anthropometric measurement. With

this device, weight, body mass index (BMI) (kg/m2), fat percentage (%F), fat mass(FM), and fat-free mass (FFM) were determined. The obtained fat mass and fat-free mass were divided by the square meter of the height to calculate the fat mass index (FMI) and fat-free mass index (FFMI).
Blood parameters of the patients were examined and neutrophil, lymphocyte, thrombocyte, MPV (mean platelet volume) data were obtained. SII (SII=platelet x neutrophil/ lymphocyte) was calculated from these data. Statistical analysis of the data obtained from anthropometric measurements and blood parameters was performed.
SII increased statistically significantly with specially with %F, FM and FMI, indicating its potential as a marker for systemic inflammation in relation to body fat composition.
Platelet increased statistically significantly with % F, FM, FMI. Its increase in relation to body fat composition indicating its potential as a marker for systemic inflammation.
No significant correlation was found between MPV and anthropometric measurements, suggesting a limited role of MPV in this context.
When we examined the obesity classification determined that SII and platelet count increased statistically significantly and MPV decreased in the high-risk (class III) obesity group.
These results considered that the utility of integrating anthropometric and hematological measurements for a comprehensive assessment of systemic inflammation and hematological health.

Keywords: obesity, SII (systemic immunoinflammatory index), platelet, MP

GİRİŞ

Latince kelime kökü "ob" (durmadan, bütünüyle) "esus" (yeme) "obesus (şişman olana kadar yemek) /obesitas (çok şişman veya fazla kilolu olma durumu)" kelimeleri; günümüzde İngilizce'de "obese/obesity" şeklinde, dilimizde ise obez (çok şişman) obezite (çok şişmanlık)" olarak kullanılmaktadır (Muratdağı ve Karadeniz, 2022). DSÖ (Dünya Sağlık Örgütü) tarafından BMI (body mass index) e göre obezite sınıflandırılmış olup BMI= 30 ve üzeri olanlar obezite sınıfına dahil edlmiştir (Obez Sınıf 1 30.00 – 34.99, Obez Sınıf 2 35.00 – 39.99 , Obez Sınıf 3 ≥ 40.00) (Dönder ve Ünalan 2018). Günümüz tanımı ile obezite vücutta yağ dokusunun artmasıyla gelişen low grade inflamasyonla karakterize, multifaktöriyel, multisistemik, epigenetik, kronik bir hastalıktir (Mayoral ve ark. 2020). Dünyada ve ülkemizde sıklığı giderek artmaktadır (Ural ve ark, 2018). Obezitede en önemli problem enerji alımı ve harcanması arasındaki dengesizliktir (Pi-Sunyer FX.,2002).

Bu çalışmada amacımız, obezitede artan inflamasyonu, yeni tanımlanan inflamasyon markerları ile incelemek ve yeni markerların obezite takibinde kullanılabilirliğinin tartışıldığı literatür bilgisine katkı sağlamaktır.

MATERYAL VE METOT

Çalışmamızda obezite nedeniyle başvuran 362 kadın hastanın verileri kullanıldı. Hastaların öncelikle çıplak ayakla düz bir zeminde boy uzunlukları ölçüldü. Daha sonra bioimpedans cihazında (Tanita-BC418) tüm vücut analizi yapılarak kilo, beden kitle indeksi (BMI), yağ yüzdesi, (%yağ) yağ ağırlığı (FM9 , yağsız ağırlık (FFM) tespit edildi.
Bu analizden elde edilen verilerden FMI (fat mass index= fat mass/ height m^2), FFMI (fat free mass index= fat free mass index/ height m^2) hesaplandı. Başvuran hastaların kan parametreleri incelenerek nötrofil, lenfosit, trombosit ve MPV verilerine ulaşıldı. SII (sistemik immuninflamatuar index = platelet x neutrophil/lymphocyte) hesaplandı. Antropometrik ölçümlerden elde edilen verilerle kan parametrelerinden elde edilen verilerin istatistiksel analizi yapıldı.

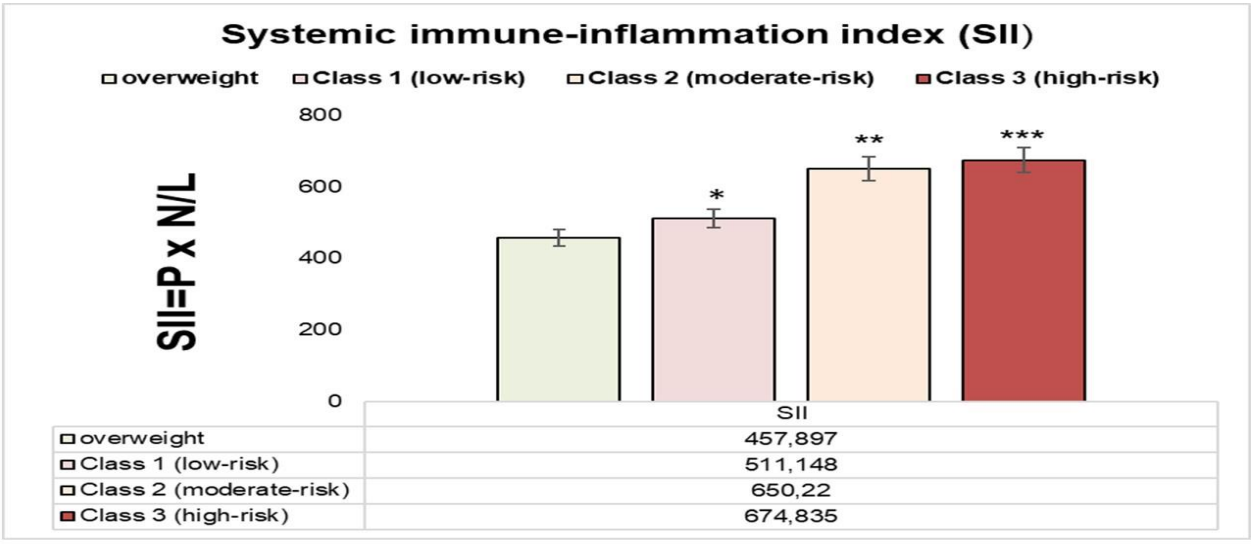
Tablo 1. Antropometrik ölçümlere ait veriler.

	Age (Year)	Height (cm)	Weight (kg)	BMI (kg/m²)	%F	FM	FFM	FMI	FFMI
Mean	41.6	160	112	43.9	46.1	52.3	58.6	20,5	22,9
Standard deviation	11,5	6.00	25.0	9,75	6,39	16,2	9,33	6,38	3,59
Minimum	15	144	60.9	24,3	4,8	17,8	41.2	6,76	15,2
Maximum	72	186	200	73.2	58.3	96.6	98.4	38.7	36.9
Shapiro-Wilk p	0.015	0.032	0.019	0.016	< .001	0.021	< .001	0.016	< .001

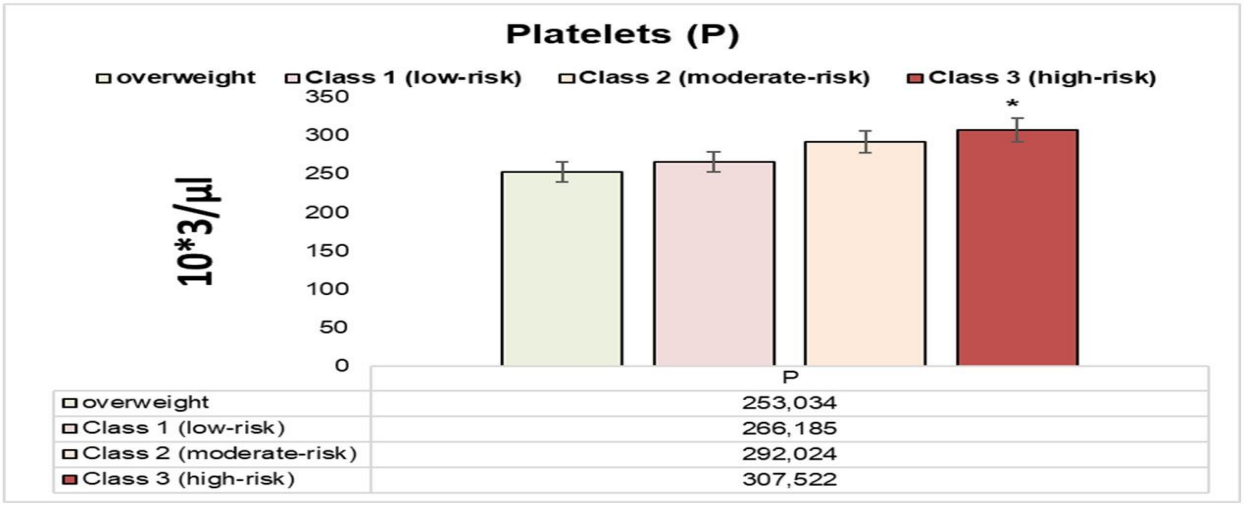
Tablo 2. . Antropometrik ölçümle ve Hematolojik Parametreler arasındaki korelasyonu gösteren tablo.

		P	%F	FM	FFM	FMI	FFMI	SII	MPV
P	Pearson's r	—							
	p-value	—							
%F	Pearson's r	0.241***	—						
	p-value	< .001	—						
FM	Pearson's r	0.266***	0.858***	—					
	p-value	< .001	< .001	—					
FFM	Pearson's r	0.162**	0.328***	0.727***	—				
	p-value	0.004	< .001	< .001	—				
FMI	Pearson's r	0.261***	0.864***	0.968***	0.661***	—			
	p-value	< .001	< .001	< .001	< .001	—			
FFMI	Pearson's r	0.170**	0.346***	0.684***	0.881***	0.732***	—		
	p-value	0.003	< .001	< .001	< .001	< .001	—		
SII (P × N/L)	Pearson's r	0.599***	0.240***	0.246***	0.126*	0.234***	0.114*	—	
	p-value	< .001	< .001	< .001	0.027	< .001	0.046	—	
MPV	Pearson's r	-0.047	-0.090	-0.091	-0.071	-0.095	-0.088	-0.053	—
	p-value	0.408	0.118	0.113	0.215	0.096	0.124	0.346	—
bmı	Pearson's r	0.216***	0.687***	0.912***	0.805***	0.951***	0.896***	0.168**	—
	df	312	349	349	348	345	345	313	—
	p-value	< .001	< .001	< .001	< .001	< .001	< .001	0.003	—

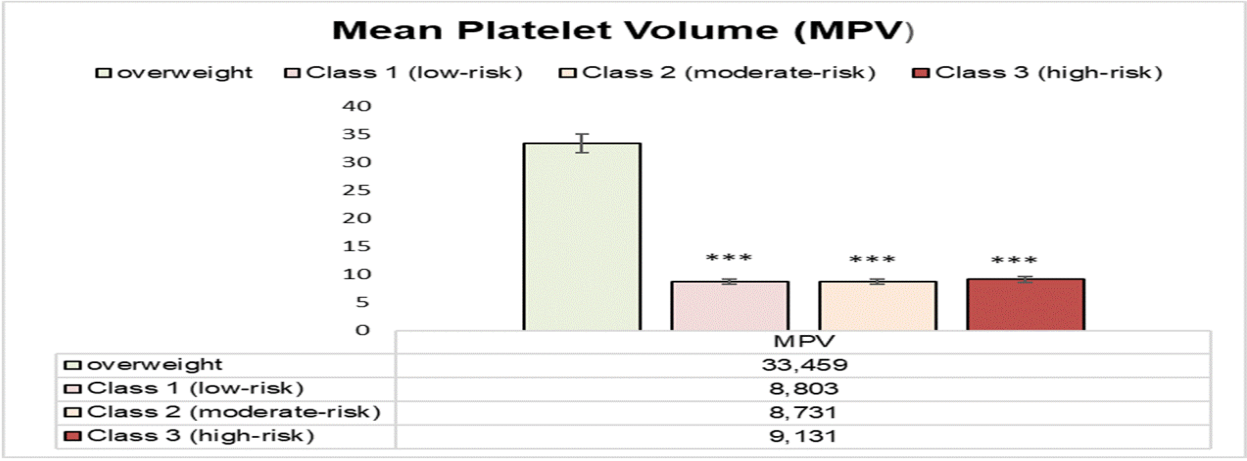
“Pearson's r”: Pearson korelasyon katsayısı (r), iki değişken arasındaki doğrusal ilişkinin gücünün ve yönünün bir ölçüsüdür. * p < .05, ** p < .01, *** p < .001”: Sistemik-immün-inflamasyon indeksi (SII), Ortalama trombosit hacmi (MPV), P (trombosit), BMI (Vücut kitle indeksi).



Şekil 1. Obezite sınıflamasına göre SII nin değişimini gösteren şekil. *p < .05 ve **p < .01 ve p<0.001 istatistiksel olarak anlamlılık seviyesidir



Şekil 2. Obezite sınıflamasına göre trombosit değerlerinde değişimi gösteren şekil.



Şekil 3. Obezite sınıflamasına göre MPV değerlerinde değişimi gösteren şekil.

BULGULAR V TARTIŞMA

Antropometrik ölçümlere ait elde edilen veriler tablo 1 de görülmektedir. Vücut kitle indeksi (BMI), yağ yüzdesi (%F), yağ ağırlığı(FM), yağsız ağırlık(FFM), yağ ağırlığı indeksi (FMI), yağsız ağırlık indeksi (FFMI) 0.001'den küçük p-değerleri "$p<0.001$" istatistiksel olarak anlamlıdır(Tablo 1) .

Trombosit sayısı ve SII, BMI (vücut kitle indeksi), yağ yüzdesi (%F), yağ ağırlığı (FM), yağ ağırlığı indeksi (FMI) güçlü pozitif korelasyon göstermektedir. Trombosit ve SII, obezitede yağ dokusunun artmasına bağlı inflamasyonu gösteren güçlü belirteçler olabilir. MPV ile antropometrik ölçümler arasında anlamlı bir ilişki elde edilemedi. Bu bulgu MPV'nin bu bağlamda sınırlı bir rolü olduğunu düşündürmektedir (Tablo 2).

SII,obezite derecesi ile ilşikili olarak anlamlı artış gösterdi ve böylece güçlü bir belirteç olma düşüncesini destekledi (Şekil 1). Trombosit ,sınıf 3 obezite grubunda anlamlı olarak arttı, ileri derecede obezitede trombosit artışı inflamasyon için bir belirteç olabilir (Şekil 2). MPV obezite derecesi ile anlamlı olarak azaldı (Şekil 3). MPV obezite derecesi ile ilişkili olarak bir belirteç olabilir.

SONUÇ

Çalışmamız, antropometrik ölçümler ve hematolojik parametreler arasında anlamlı korelasyonlar olduğunu ortaya koymuştur. Gruplar arasında SII, trombosit, MPV değerleri önemli farklılıklar göstermiş olup, bu parametrelerin çeşitli BMI kategorileri arasında enflamatuar ve hematolojik durumları değerlendirmek için önemli olabileceğini göstermektedir.

Bulgularımız obezitede sistemik enflamasyon ve hematolojik sağlığın kapsamlı bir değerlendirmesi için antropometrik ve hematolojik ölçümlerin entegre edilmesinin faydasının altını çizmektedir.

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ORAL PRESENTATION

Neurosteroids

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Abstract

Previously, it was known that steroids were synthesized by the gonads, adrenal glands, and placenta. It is known that steroids bind to intracellular receptors and affect genes. In addition to these steroids, it has been reported that some steroids can also be produced in the brain, and that they can interact with receptors on the cell surface, such as ligand-gated ion channels, and thus change neuronal excitability and affect brain functions. These steroids are called neurosteroids. This review focuses on the definition, synthesis, and effects of neurosteroids on certain behaviors and diseases.

Keywords: Brain, Hormones, Neurosteroids.

INTRODUCTION

It was previously known that steroids were synthesized by the gonads, adrenal glands, and placenta. It is known that steroids bind to intracellular receptors and affect genes. In addition to these steroids, it has been reported that some steroids can also be produced in the brain, and that they can interact with receptors on the cell surface such as ligand-gated ion channels, changing neuronal excitability and affecting brain functions. These steroids are called neurosteroids (Kartalcı and Eşel, 2004). In fact, steroids synthesized in the brain were first revealed by Baulieu and his colleagues in 1980 (Corpechot et al., 1981). Steroid hormones, which are produced in organs such as the adrenal glands, placenta, and gonads and can easily cross the blood-brain barrier, play an important role in the growth, development, and maturation of the brain (Stoffel-Wagner, 2001). It is generally known that steroid hormones act as transcription factors in the regulation of gene expression by binding to intracellular receptors (Rupprecht and Holsboer, 1999). However, some steroids have been found to interact with certain neurotransmitter receptors and their subunits [such as ligand-gated ion channels] on the surface of the cell and alter neuronal excitability. Steroids with this property have been called 'neuroactive steroids' (Paul and Purdy, 1992). While the time required for the genomic effects of steroids requires a period of minutes to hours due to the protein biosynthesis required, the regulatory effects of neuroactive steroids occur in much faster periods such as milliseconds or seconds (McEwen, 1991). There are some studies showing that neurosteroids are secreted in the brain as well as in the placenta, adrenal glands and gonads. One of these was demonstrated by Baulieu and colleagues in rats where the peripheral tissues that synthesize steroids were removed. Baulieu and colleagues observed that even after removing the gonads and adrenal glands of these animals, newly synthesized steroids were still present in the central and peripheral nervous systems (Corpechot et al., 1981; Corpechot et al., 1983). In addition, the concentrations of some steroids, such as dihydroepiandrosterone (DHEA) and pregnenolone sulfate (PREGS), were found to be higher in the brain than in plasma (Corpechot et al., 1981). Another example is that enzymes necessary for steroid production, such as P450 side chain cleavage (P450ssc) and aromatase, have also been demonstrated in the brain (Stoffel-Wagner, 2001). Neurosteroids are thought to control many behaviors such as depression, sleep, stress, and eating disorders (Biggio et al., 2001), as well as their effects on embryonic axonal and dendritic growth, neuroprotection, and myelination (Baulieu, 1998; Tsutsiu, 2006). In addition to these, neurosteroids have been reported to play a role in many diseases and mental disorders such as Alzheimer's disease (Bernardi et al., 2000), mood disorders, anxiety disorders (Bicikova et al., 2000), post-traumatic stress disorder, memory modulation, the effects of alcohol (Khisti et al., 2002), and attention deficit (Spivak et al., 2000).

Synthesis of Neurosteroids

The basis of neurosteroid production is the conversion of cholesterol into mineralocorticoids, glucocorticoids and sex hormones by the catalysis of certain enzymes in the mitochondria of neurons and glial cells. The mechanism of this conversion is the same in peripheral steroidogenic organs and the brain (Mellon et al., 2001). Tsutsui and Yamazaki found that the concentration of pregnenolone in the brains of adult birds was higher

than in peripheral tissues using specific radioimmunoassay. Since the pituitary glands of the subjects they used in their experiment were removed, they think that this result is independent of the peripheral organs. They also demonstrated the existence of cytochrome P450 scc, which catalyzes the formation of pregnenolone from cholesterol, with the western immunoblot technique they performed (Tsutsui et al., 2006). Similar results were obtained in ringed pigeons (Clark et al., 1999). The same results were supported by immunohistochemical staining in birds (Usui et al., 1995). It is understood from these studies that P450scc catalyzes the synthesis of pregnenolone from cholesterol in the brain and the presence of this enzyme shows that pregnenolone synthesis is also carried out in the brain. The synthesis of progesterone in the brain is catalyzed by the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) (Mason, 1993). The presence of 3β-HSD in the mammalian brain has been demonstrated biochemically (Weidenfeld et al., 1980; Akwa et al., 1993; Kabbadj et al., 1993). The same results were also obtained by biochemical studies in birds. The presence of 3β-HSD in the brain shows that pregnenolone is converted to progesterone here (Mellon et al., 2001). Studies in birds have shown that not only pregnenolone but also progesterone is synthesized in the brains of these animals (Tsutsui et al., 2006). Biochemical analyses in all parts of the brains of adult birds have shown that the enzymatic effect of 3β-HSD is at higher levels in the telencephalon and diencephalon than in the mesencephalon (Ukena et al., 1999a). Analyses in the brains of rats have also shown that this enzymatic activity is at a very high level in the olfactory bulb, striatum, cortex, thalamus, hippocampus, hypothalamus, septum and cerebellum (Guennoun et al., 1995). The conversion of progesterone to androgen is achieved by the enzyme P45017α lyase. The presence of this enzyme has been determined in the brains of mammals and birds by biochemical studies (Compagnone et al., 1995; Kohchi et al., 1998). Conversion of androgen to testosterone occurs by the activity of the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) (Matsunaga et al., 2002). Conversion of testosterone to estrogen occurs by the activity of the enzyme P450 aromatase (Schlinger and Callard, 1987, 1989a, c). In biochemical tests, P450 aromatase was determined more in the diencephalon than in other parts of the brain (Matsunaga et al., 2002).

Myelination

Progesterone, a neurosteroid, has been shown to provide myelination in damaged sciatic nerves in rodents, and it has also been reported that its rate of myelin formation increases in in vitro Schwann cell culture and that it provides myelination in damaged nerves in vivo (Koenig et al., 1995). Yarım et al. investigated the effect of progesterone on demyelination caused by Canine Distemper Virus (CDV). In this study, cerebellar lesions were found in five dogs with acute and chronic disease. Moth-bite-like demyelination areas were detected in the white matter and immunoreactivity was observed in the substantia abla. The severity of demyelination was found to be mild in five dogs, moderate in two dogs and severe in three dogs. When progesterone levels in the cortex, corpus callosum and medulla oblongata were compared with the control groups, no significant difference was observed. In contrast, progesterone levels were found to be significantly lower in the cerebellum of dogs infected with CDV compared to controls (Yarim et al., 2007).

In another study conducted by Yarim and Kabakci, 3B-HSD distribution was examined in the cerebellum of dogs infected with Canine Distemper Virus and it was reported that 3B-HSD immunopositivity increased in astrocytes in demyelinated areas of the cerebellum in CDV infection (Yarim and Kabakci, 2002). As a result, the increase in the amount of 3B-Hydroxysteroids in astrocytes in demyelinated areas in this study suggested to the researchers that progesterone production could be increased in order to ensure remyelination in this region (Yarim and Kabakci, 2002).

3B-HSD was detected at high levels in normal steroidogenic glands (testis, ovary and adrenal glands). Progesterone is secreted by the ovary and placenta in addition to peripheral tissues such as skin and liver (Mason, 1993) and is one of the most important reproductive hormones at birth. Progesterone and pregnenolone are called neurosteroids in the central and peripheral nervous system. It has been reported that progesterone is synthesized by Schwann cells to form new myelin after damage to the peripheral nervous system (Koenig et al., 1995). Thus, progesterone plays an important role in the regeneration of the nervous system. Progesterone and pregnenolone can be synthesized by astrocytes, oligodendrocytes and neurons in the central nervous system. It is known that both neurosteroids have neuroprotective effects by demonstrating the presence of 3β-HSD in the spinal cords of rats (Jung-Testas et al., 1989; Dupont et al., 1994; Guennoun et al., 1995; Kohchi et al., 1998; Zwain and Yen, 1999). The presence of 3β-HSD in astrocytes indicates that pregnenolone is converted to progesterone here (Zwain and Yen, 1999). Oligodendrocytes are more active than astrocytes in the production of pregnenolone. However, oligodendrocytes are less active in the formation of progesterone, which is necessary for the formation of myelin protein (Jung-Testas et al., 1994; Zwain and Yen, 1999). Purkinje cells have steroidogenic enzymes such as 3β-HSD and P450 and can produce neurosteroids (Tsutsui, 2006). Karayigit et al. reported in their study that after experimentally induced

demyelination in the corpus callosum, cornu ammonis and cerebellar superior peduncle regions of the brain, there was a significant increase in the 3B-HSD level during demyelination in these regions. They showed that spontaneous myelin loss was repaired in the same regions 6 weeks after the damage. The researchers suggested that progesterone levels may have increased to provide myelination after demyelination in the brain (Karayigit and Yarım, 2018).

It has been observed that after a lesion caused by cold, rapid degeneration occurs in the frozen regions and distal parts of the axon and its associated myelin sheath, however, the intact basal lamina provides a suitable environment for regeneration, and it has been reported that proliferation and myelination begin in Schwann cells one week after surgery. Two weeks later, it reaches one-third of its normal width. Pregnenolone and progesterone levels remain high in the damaged parts of the nerves and it has been stated that the level remains high even 15 days after the lesion (Koenig et al., 1995).
The role of progesterone in myelin repair was observed after two weeks when trilostone, which blocks 3β-HSD that converts pregnenolone to progesterone, or alternatively RU486, which acts as an antagonist at progesterone receptors, was applied to the lesioned area, and there was a decrease in the thickness of the myelin sheaths. The inhibitory effect of trilostone was reversed by the simultaneous use of progesterone, and therefore did not cause toxicity. Baulieu was able to achieve remyelination with high doses of pregnenolone or progesterone (Baulieu 1998).

Organogenesis
DHEA and DHEAS are thought to act as very powerful regulators in the regulation of neurite growth and, in addition, the functions of various neurotransmitter receptors. Neurite growth is more advanced in regions where P450c17 is synthesized, and while DHEA causes dendritic growth and branching, it has been reported that it causes axonal growth in certain regions, and in addition, allopregnenolone has been reported to cause axonal regression in the developing hippocampus. It is thought that neurosteroids synthesized in the nervous system develop and direct synaptic connections and axonal growth in the developing brain tissue. (Compagnone and Mellon 1998).

Neuroprotective Effects
In humans, the ratio of DHEA and DHEAS is very high during development. It has been reported that their levels decrease with age, stress or some diseases (Orentreich et al., 1992). Because it has been stated that old age and stress cause a degeneration in neuronal utilization. It has been reported that these and many other neurosteroids have a neuroprotective effect in events resulting in neurodegeneration or ischemia (Mellon and Griffin, 2002). In many recent studies, it has been determined that neurosteroids have effective neuroprotective functions. In both cerebral and spinal cord ischemia, DHEA reduces neuronal and glial damage. It has also been stated that DHEA has a protective effect on hippocampal neurons in glutamate-related neurotoxicity (Kimonides et al., 1998).

It has also been reported that pregnenolone and progesterone reduce primary and secondary spinal cord damage and cause an improvement in neuronal recovery. The improvement in locomotor activity in animals with spinal cord contusion with progesterone treatment is paralleled by the continued effect of progesterone on myelination and less loss of white matter on the side of the injury. Similarly, the improvement in locomotor activity with pregnenolone treatment also reduces histopathological changes (Mellon and Griffin, 2002). Allopregnenolone also reduces the irreversible changes in intracellular Ca^{2} concentration in glutamate-containing hippocampal neurotoxicity. (Frank and Sagratella; 2000). In addition, it has been reported that it reduces the infracture area in ischemia in both females and males of rats given progesterone both chronically and acutely, as well as brain edema after trauma. Based on these results, it is thought that neurosteroids have a protective effect against acute and chronic neurotoxicity and delay or prevent cell death in neurons through many mechanisms. (Mellon and Griffin, 2002). It is thought that the level of aromatase, which catalyzes the biosynthesis of estrogen from procursor androgens, increases after damage to the brain and that aromatase has a neuroprotective effect. The effect of aromatase on neurodegeneration caused by the effects of neurotoxins has been evaluated. In mice, non-neurotoxic domoic acid caused significant neuronal loss in the hippocampal hilus of mice whose gonads were removed. In addition, the aromatase product and its own metabolite, estradiol, as well as testosterone, an aromatase substrate, protected hilar neurons from domoic acid. These studies may show that aromatase conversion of testosterone to estradiol may produce a neuroprotective effect of testosterone. In addition, while low doses of domoic acid in aromatase knock-out mice (a type of mouse with genetically reduced levels of aromatase in the brain) can cause significant neuronal loss, it does not have the

same effect in control mice. This indicates that aromatase deficiency increases the susceptibility of hilar neurons to neurotoxic degeneration. The neuroprotective effect of aromatase was tested in male rats with the aromatase inhibitor fadrozole and kainic acid injection. Kainic acid is a good neurotoxin for hilar neurons in rats. Fadrozole increases the neurodegenerative effect of kainic acid in male rats and this effect is reduced by the activity of estradiol. In addition, the neuroprotective effect of testosterone against kainic acid in castrated male rats was blocked by fadrozole. These findings indicate that the neuroprotective effect of aromatase is due to the effect of testosterone, which is the precursor of estradiol. In conclusion, the role of local cerebral aromatase in neuroprotection was indicated by the intracerebral effect of fadrozole, which increases the effect of kainic acid, which causes neurodegeneration in the hippocampus of male rats. These findings indicate that aromatase deficiency reduces the neurodegeneration threshold and local cerebral aromatase is neuroprotective. Brain aromatase may therefore be a new target in the therapeutic approach to neurodegenerative diseases (Azcoitia et al., 2001).

Alzheimer's Disease

To demonstrate the pathophysiological importance of neurosteroids in Alzheimer's disease, the concentrations of pregnenolone, pregnenolone sulfate (PREGS), dihydroepiandosterone, dihydroepiandosterone sulfate (DHEAS), progesterone and allopregnenolone in the nervous system tissues such as hippocampus, frontal cortex, striatum, hypothalamus and cerebellum of elderly patients with and without Alzheimer's disease were compared. When the brain tissues of patients with Alzheimer's disease were compared with the control groups, a decrease in the concentration of all neurosteroids was observed. PREGS and DHEAS were found to be low in the cerebellum and striatum, and at the same time, a significant decrease in the level of DHEAS was observed in the hypothalamus. PREGS and cortical β-amyloid peptides in the striatum and cerebellum.

CONCLUSION

In addition to steroid hormones released from the adrenal glands, gonads and placenta, steroid hormones are also released in the brain. These hormones play a major role in the development and normal functioning of the brain, and many diseases related to the nervous system, such as Parkinson's and Alzheimer's, occur when these hormones are deficient. Further studies on neurosteroid hormones will shed light on the pathogenesis and treatment methods of central nervous system diseases that occur when these hormones are deficient.

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ORAL PRESENTATION

Nitric Oxide Functions and Relationship Disease

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Abstract

Nitric oxide (NO) is a gaseous messenger molecule synthesized from L-arginine and molecular oxygen by three different NO synthases, namely neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) forms. NO has been widely recognized as an important signaling molecule in many physiological processes. The initial identification of NO as an endothelium-derived relaxing factor generated great interestin its function in vascular biology. Over the following years, however, the focus on NO research rapidly expanded from the vascular system to its role in immunity and inflammation, the nervous system, pregnancy, aging, and cell death. Many studies suggest that excessive or abnormal production of NO plays a crucial role in neuronal cell death assocciated with neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, as well as various conditions of vascular dysfunction. This review focuses on NO functions and increases or decreases in nitric oxide in diseases.

Keywords: Nitric Oxide, eNOS,iNOS, nNOS.

INTRODUCTION

Since its characterisation in the early 1980s by Furchgott, Ignarro and others (Martin et al., 1985; Ignarro et al., 1987; Ignarro et al., 1987;) nitric oxide (NO) has been widely recognised as an important signalling molecule in many physiological processes. The initial identification of NO asendothelium-derived relaxing factor (Palmer et al., 1987) generateda great interest in its function in vascular biology. Oversubsequent years, the focus on NO research rapidly expanded from the vascular system to its role in immunity and inflammation, cell death, cell survival, and aging, to name but a few. Of particular interest is its role within the nervous system and its function in neuronal signalling. NO was first identified to be present in the central nervous system by the discovery of one of its synthesising enzymes, neuronal nitric oxide synthase (nNOS), within the mammalian brain. Aside from its production through nNOS, NO can also be synthesised through activation of either one of the two other nitric oxide synthases termed endothelial nitric oxidesynthase (eNOS) and inducible nitric oxide synthase (iNOS) (Alderton et al., 2001). NO is responsible to regulate a diverse range of physiological and cellular processes including endothelial cell migration, proliferation, extracellular matrix degradation, and angiogenesis (Cooke et al.,2002). NO is a key component in endothelium-dependent regulation of vascular tone, platelet function, angiogenesis and mitogenesis that are crucial in cardiovascular physiology (Naseem et al., 2005).

nNOS

In the past years, an increasing number of reports have confirmed the significance of nNOS in a variety of synaptic signalling events. Neuronal NOS has been implicated in modulating physiological functions such as learning, memory, and neurogenesis (Zhou et al.,2009). In the central nervous system (CNS), Retrograde communication across synaptic junctions is presumed to be involved in memory formation, and there is evidence that inhibitors of NOS impair learning and produce amnesia in animal models (Holscher et al.,1992;). There is also evidence that NO formed in the CNS by nNOS is involved in the central regulation of blood pressure (Togashi ey al.,1992). Blockade of nNOS activity in the medulla and hypothalamus causes systemic hypertension (Toda et al.,2009). In the periphery, many smooth muscle tissues are innervated by nitrergic nerves, i.e. nerves that contain nNOS and generate and release NO (Förstermann and William, 2012).

Abnormal NO signalling is likely to contribute to a variety of neurodegenerative pathologies such as excitotoxicity following stroke, multiple sclerosis, Alzheimer's, and Parkinson's diseases (Steinert., 2010) Hyperactive nNOS, stimulated by massive Ca2+ influx into neuronal cells, has been implicated in N-methyl-D-aspartate receptormediated neuronal death in cerebrovascular stroke.46 Under those conditions, NO can

contribute to excitotoxicity, probably via peroxynitrite activation of mitochondrial permeability transition. High levels of NO can also produce energy depletion, due to inhibition of mitochondrial respiration and inhibition of glycolysis (Lipton et al.,1993) Some disturbances of smooth muscle tone within the gastrointestinal tract (e.g. gastro-oesophageal reflux disease) may also be related to an overproduction of NO by nNOS in peripheral nitrergic nerves (Tøttrup et al. 1991).

eNOS

NO produced in the endothelium via the enzyme eNOS is an important vasoactive compound (Fleming et al 2003., Panza et al.,1995). NO is responsible to regulate a diverse range of physiological and cellular processes including endothelial cell migration, proliferation, extracellular matrix degradation, and angiogenesis (Panza et al.,1995). NO is a key component in endothelium-dependent regulation of vascular tone, platelet function, angiogenesis and mitogenesis that are crucial in cardiovascular physiology (Naseemet al., 2005). A potent antiinflammatory agent, NO inhibits leukocyte interactions with the vessel wall, thereby reducing pathological inflammation and thrombosis (Kubes et al.,1991). eNOS knockout mice studies showed that leukocyte adherence to the vessel wall is elevated 10-fold. Also NO inhibits inflammation in various vascular disease models like myocardial infarction, glomerulonephritis, lung injury, and stroke (Jones et al.,1999). Thereby NO controls inflammation and thrombosis in part by regulating vesicle trafficking. NO decreases granule trafficking from the Golgi to the plasma membrane by targeting a key component of exocytic machinery. Loss of endothelial function due to phosphorylation and sub-cellular localization of eNOS (Fulton et al.,2002; Michel et al 1997), has been implicated in a number of cardiovascular diseases, making regulation of eNOS through posttranslational modifications, a promising therapeutic target (Tai e al.,2004).

iNOS

Inducible NOS is not usually expressed in cells, but its expression can be induced by bacterial lipopolysaccharide, cytokines, and other agents. Although primarily identified in macrophages, expression of the enzyme can be stimulated in virtually any cell or tissue, provided that the appropriate inducing agents have been identified28. Once expressed, iNOS is constantly active and not regulated by intracellular Ca2+ concentrations (Förstermann et al., 1994; Holscher etall., 1992).

Inducible NOS, when induced in macrophages, produces large amounts of NO, which represent a major cytotoxic principle of those cells (Nathan et al., 1991). Higher concentrations of NO, as produced by induced macrophages, can directly interfere with the DNA of target cells and cause strand breaks and fragmentation (Wink et al.,1991). A combination of these effects is likely to form the basis of the cytostatic and cytotoxic effects of NO on parasitic microorganisms and certain tumour cells . Interestingly, non-immune cells can also be induced with cytokines to release amounts of NO large enough to affect the neighbouring cells. Cytokine-activated endothelial cells, for example, have been shown to lyse tumour cells and induced hepatocytes can use NO to kill malaria sporozoites. Inducible NOS activity is likely to be responsible for all of these effects (Li et al., 1991)

The high levels of NO produced by activated macrophages (and probably neutrophils and other cells) may not only be toxic to undesired microbes, parasites, or tumour cells, but—when released at the wrong site—may also harm healthy cells. In vivo, cell and tissue damage can be related to the NO radical itself or an interaction of NO with O2 2† leading to the formation of peroxynitrite (ONOO2). The large majority of inflammatory and autoimmune lesions are characterized by an abundance of activated macrophages and neutrophils. Significant amounts of NO can be secreted by those cells, leading to damage of the surrounding tissue (Kröncke et al., 1991). Inducible NOS-derived NO is also likely to be involved in non-specific allograft rejection (Langrehr et al., 1991)

Inflammatory neurodegeneration contributes to a number of brain pathologies. Mechanisms by which activated microglia and astrocytes kill neurons have been identified in cell culture. These mechanisms include the activation of the phagocyte NADPH oxidase in microglia and expression of iNOS in glia. This combination produces apoptosis via $ONOO^-$ production. Inducible NOS-derived NO also synergizes with hypoxia to induce neuronal death because NO inhibits cytochrome oxidase. This can result in glutamate release and excitotoxicity (Kanwar., 2009).

Lastly, excessive NO production by iNOS plays a crucial role in septic shock. This condition is characterized by massive arteriolar vasodilatation, hypotension, and microvascular damage. Bacterial endotoxins usually initiate the symptoms. A number of mediators such as platelet-activating factor, thromboxane A2, prostanoids, and cytokines such as interleukin-1, tumour necrosis factor-a, and interferon-g are elevated in septic shock and have been implicated in its pathophysiology. However, the fall in blood pressure is predominantly due to excess NO production by iNOS induced in the vascular Wall (Lange et all.2009).

Expression in Central Nervous System Diseases in Animals

In a 2020 study, it was reported that nNOS expression in the brain was higher than in control tissues in listeriosis disease observed in ruminants (Karayigit and Dincel 2020). Another study showed that eNOS and iNOS expression in the central nervous system increased in listeriosis disease. In addition, it was stated that iNOS and eNOS expressions may be responsible for the apoptosis that occurs in the medulla oblongata region of the brain in these findings (Karayigit 2018). In another study, it was explained that immunohistochemical eNOS and iNOS expressions were higher in the brains of experimental group animals in encephalitis induced experimentally in mice with toxoplasmosis (Dincel and Atmaca 2015).

The increase in NO levels observed naturally and experimentally induced encephalitis in animals has been directly associated with the diseases. As a result, it is thought that they play an absolute role in the pathogenesis stages such as the initiation and progression of encephalitis formed in diseases. On the other hand, it is stated that the high NO expression levels obtained from these studies trigger oxidative stress in cells and as a result may be closely related to cell death that occurs in encephalitis.

CONCLUSION

In conclusion, detailed information was given about the functions of nitric oxide in this review. In addition, it was emphasized from which cells it can or cannot be released under physiological and pathological conditions. On the other hand, its roles in very current diseases that affect the central nervous system of animals were mentioned. Further research of nitric oxide in similar diseases is of great importance for understanding the pathogenesis of the diseases and creating appropriate treatment protocols.

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ORAL PRESENTATION

Cucurbita pepo L. yetiştiriciliği yapılan tarımsal alandan mikroorganizma izolasyonu tanımlanması ve tarımsal ürünün atıklarının mikrobiyal yolla değerlendirilmesi

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Özet

Çerezlik kabak *Cucurbita pepo* L. 'nin ülkemizde oldukça geniş bir tarım alanı bulunmakta ve hasat sonrasında yüksek oranda atık bırakılmaktadır. Bu sebeple *Cucurbita pepo* L. atıklarının mikrobiyal olarak değerlendirme potansiyeli araştırılmıştır. *Cucurbita pepo* L. yetiştiriciliği yapılan ve yine bu alanlara hasat sonrası kabak posasının bırakıldığı tarlalardan alınan toprak örneklerinden mikrobiyal izolasyon yapılmış ve mikroorganizmaların kabak posasında gelişim ve atığı kullanım potansiyelleri incelenmiştir. Ayrıca karbon kaynağı olarak posaları kullanan mikroorganizmaların biyoplastik üretim yeteneğine sahip olup olmadıkları belirlenmiş ve biyoplastik üretim yeteneği bulunan mikroorganizmalar moleküler olarak tanımlanmıştır.
Çalışma kapsamında Eskişehir Çifteler ilçesinden Nisan 2019 tarihinde alınan iki farklı toprak örneğinden seri dilüsyon ve yayma plak metodu kullanılarak izolasyon işlemi gerçekleştirilmiştir. İzolasyon sonrası gelişen mikroorganizmaların biyoplastik üretim yetenekleri, karakterizasyon ve tanımlanması mikroskobik ve moleküler yöntemler kullanılarak gerçekleştirilmiştir. Ayrıca tanımlanan mikroorganizmaların tek karbon kaynağı olarak seçilen çerezlik kabak posasını kullanım ve bu tarımsal atıktan biyoplastik üretim yetenekleri belirlenmiştir.
Çalışma sonucunda iki farklı örnekten toplam 127 izolat saflaştırılmış ve biyoplastik üretim yeteneklerinin belirlenmesi amacıyla yapılan tarama sonucunda 12 izolatın biyoplastik üretim yeteneğine sahip olduğu belirlenmiştir. Tek karbon kaynağı ile biyoplastik üretim denemelerinde 5 izolatın kabak posasını kullanarak biyoplastik üretebildiği belirlenmiştir.
Bu sonuçlar neticesinde tarımsal atıkların mikroorganizmalar tarafından biyoplastik üretiminde değerlendirilerek hem ekonomik açıdan kazanç sağlanması hem de tarımsal atıkların çevre üzerindeki olumsuz etkilerinin azaltılarak çevre kirliliğinin önlenmesi amaçlanmaktadır.

Anahtar Kelimeler: Tarımsal atık, çerezlik kabak posası, biyoplastik

Isolation and identification of microorganisms from *Cucurbita pepo* L. farming area and evaluation of agricultural product wastes through microobiological processes

Abstract

Cucurbita pepo L. has a very large agricultural area in our country and a high amount of waste is left after harvest. For this reason, the potential of microbial utilization of *Cucurbita pepo* L. wastes was investigated. Microbial isolation was performed from soil samples taken from fields where *Cucurbita pepo* L. was grown and pumpkin pulp was left after harvest, and the growth of the isolated microorganisms in pumpkin pulp and their waste utilization potential were investigated. In addition, it was determined whether the microorganisms using the pulp as a carbon source were capable of bioplastic production, and bioplastic producing microorganisms were molecularly identified.
Within the scope of the study, isolation was carried out from two different soil samples taken from Eskişehir Çifteler district in April 2019 using serial dilution and spreading plate method. The bioplastic production capabilities, characterization, and identification of microorganisms developed after isolation were carried out using microscopic and molecular methods. In addition, the ability of the identified microorganisms to use

pumpkin pulp as the single carbon source and to produce bioplastic from this agricultural waste was determined.
As a result of the study, a total of 127 isolates were purified from two different samples and 12 isolates were found to be capable of bioplastic production as a result of screening to determine their bioplastic production ability. In the bioplastic production experiments with a single carbon source, it was determined that 5 isolates were able to produce bioplastic using pumpkin pulp.
As a result of these findings, it is aimed to evaluate agricultural wastes in bioplastic production by microorganisms and to transform them into products with biotechnological potential both in terms of economy and to reduce the negative effects of agricultural wastes on the environment and to prevent environmental pollution.

Keywords: Agricultural waste, pumpkin pulp, bioplastic

GİRİŞ

Her türlü tarımsal üretim sonrası oluşan istenmeyen atıklar tarımsal atık olarak tanımlanmaktadır. Bu atıklar tarımsal faaliyet sonucu üretilen veya hasat edilen bitkilerin tarladan kaldırma sonrası arta kalan sap, kök, gövde, etli meyve gibi kullanılmayan kısımlarını oluşturmaktadır (García vd., 2019). Ülkemiz de bir tarım ülkesi olduğundan ve oldukça fazla tarım arazisi bulunduğundan, tarımsal ürün açısında oldukça zengin bir çeşitliliğe sahiptir ve bu ürünlerin işlenmesi sonucu yüksek miktarlarda çeşitli tarımsal atıklar açığa çıkmaktadır (Baran ve ark., 1995)
Bu atıkların başında da çerezlik kabak atıklarının oranı oldukça önemli bir paya sahiptir. Çünkü *Cucurbita pepo* L. ve benzer türlerin tarımının yapılması ülkemizde önemli bir sektör haline gelmiştir. Özellikle İç Anadolu Bölgesi'nde oldukça yoğundur (Yanmaz, 2014). Ancak hasat sırasında kabak çekirdekleri alınmakta ve diğer geriye kalan kabuk, meyve gibi tüm kısımlar artık olarak bırakılmaktadır. Bırakılan, çürüyen veya küflenen bu tarımsal atıklar kirliliğe sebep olmaktadır. (Hashemi ve Razzaghzadeh, 2007, Konca, 2014). Ayrıca bu yöntem toprağın, yer altı sularının ve çevrenin kirlenmesine neden olmaktadır (Sümer ve ark., 2016; Dai ve ark., 2018) Bu sebeple bu atıkların çevreye zarar vermeden yok edilmesi kritik bir konu haline gelmiştir. Atıkların fayda sağlayacak şekilde kullanılmasında mikroorganizmalar en önemli rollerden birine sahiptir. Yoğun tarımsal üretim sonucu oluşan atıklar, güvenli bir şekilde mikroorganizmalar tarafından kompostlama, enzimatik süreçler, anaerobik parçalanma ve biyoteknolojik değerli ürün üretimi gibi süreçlerle değerlendirilebilir.
Cucurbita pepo L. atıklarınını mikrobiyal süreçlerle değerlendirilmesi ile ilgili çok çalışma bulunmamakta ve yapılan çalışmalarda da daha çok yem ve hayvan besin maddesi eldesi üzerinedir (Yılmaz ve ark.,2016).
Bu sebeple çalışma *Cucurbita pepo* L atıklarını kullanan mikroorganizmaları belirlemeyi ve belirlenen mikroorganizmaların hem kabak posalarını kullanarak atık yok etme hem de biyoteknolojik olarak oldukça değerli ürünler olan biyoplastiklerin düşük maliyetli üretimini gerçekleştirebilme potansiyellerini belirlemeye yönelik bir öncü olmayı amaçlamaktadır.

MATERYAL VE METOT

Örnekleme yapılan alan

Mikrobiyal çalışmaların gerçekleştirilmesi için Eskişehir Çifteler ilçesinden Nisan 2019 ayında *Cucurbita pepo* L. tarımı yapılan 2 farklı tarladan yaklaşık 15-20 cm derinlikten steril torbalara toprak örnekleri alınmıştır.

Mikroorganizmaların İzolasyonu

Toprak örneklerinden mikroorganizma izolasyonu için Gliserol-yeast extract agar (GYEA) ve Nutrient agar (NA) besiyerleri kullanılmıştır. Toprak örnekleri seri dilüsyon yöntemi kullanılarak yayma plak yöntemi ile NA ve GYEA besiyerine inoküle edilmiştir. NA besiyerine inoküle edilen örnekler 37 °C'de, GYEA besiyerine inoküle edilen örnekler 30 °C'de 24-48 saat inkübasyona bırakılmıştır. Inkübasyon sonrası gelişen koloniler saflaştırılmış ve %20'lik gliserol stokları içerisinde -85 °C'de muhafaza edilmiştir.

İzolatların Biyoplastik Üretim Yeteneklerinin Belirlenmesi

NA ve GYEA besiyerinden saf olarak izole edilen izolatların biyoplastik üretme yeteneklerinin belirlenmesi amacıyla izolatlardan preparatlar hazırlanmıştır. Hazırlanan preparatlara sudan black boya (0,3 g Sudan Black 100 ml %70'lik etanol ile çözündürülür) ile muamele edilerek 20 dk bekletilmiştir. 20 dakika sürenin sonunda su ile yıkanan preparatlar 2 dk boyunca Safranin boya ile boyanmış ve yıkama sonrası 100x objektifte incelenmiştir (Burdon ve ark., 1942; Phanse ve ark., 2010).

Mikroorganizmaların Tanımlanması

Bakterilerin moleküler tanımlanması amacıyla DNA ekstraksiyonu yapılmıştır. Bu amaçla kalıp DNA olarak kullanılmak üzere izole edilen mikroorganizmalardan tek koloni alınmış 200 µl steril distile su içerisinde karıştırılarak resüspanse edilmiş ve 96 °C'de 10 dakika Appiled Biosystems® Thermal Cycler da hücrelerin lize olması sağlanmıştır. Sonrasında 12.000 rpm'de 10 dakika santrifüjün ardından süpernatant yeni steril ependorfa aktarılarak, kalıp olarak kullanılmak üzere -20 °C'de muhafaza edilmiştir. (Anton vd., 1999; Dyall-Smith, 2004).

Elde edilen kalıp DNA'dan tanımlama amacıyla 16S rRNA spesifik primerler (27F ve 1492R) ile PZR (Polimeraz Zincir Reaksiyonu) kurulmuştur. PZR reaksiyonu kurmak için ileri primer olarak 27F, geri primer olarak ise 1492R, kalıp DNA ve Biolabs OneTaq® Quick-Load®2X Master Mix (M0486S) kullanılmıştır. 16S r RNA amplifikasyonu için Bacter 50 programı kullanılmıştır.

Biyoplastik Sentezinden Sorumlu Sentaz Genlerinin Amplifikasyonu

Biyoplastik ürettiği mikroskobik olarak belirlenen izolatlarda sentaz genlerinin saptanması amacıyla PCR reaksiyonları hazırlanmıştır. PHA üreten bakterilerde sentaz genlerinin amplifikasyonu için Sınıf 1-F, sınıf 1-R ve sınıf 2-F sınıf 2-R primerler (Zaki,2018); PHA sentaz geni varyasyonlarını belirlenmesi amacıyla, B1R, B1F ve B2R primerleri (Shamala ve ark., 2003); PHB biyosentezinde görev alan PHB sentaz geninin amplifikasyonu için PDTL-F, PDTL-R ve RCT-F, RCT-R primer setleri kullanılmıştır (Nehra ve ark., 2017).Amplifikasyonlar için Tablo 1 belirtilen programlar kullanılmıştır. PCR sonucu elde edilen ürünler %1 lik agaroz jele 1X'lik TAE (Tris-Asetik asit-EDTA) tamponu içinde 5V/cm akım uygulanarak gözlenmiştir.

Tablo 1: Biyoplastik Sentezinden Sorumlu Sentaz Genlerinin Amplifikasyon Koşulları

PHA sentaz geni (Sınıf 1F-R, Sınıf 2 F-R primerleri) spesifik PZR reaksiyon koşulları	
95 °C…….5 dakika (Denatürasyon)	
95 °C…….2 dakika 60 °C…….30 saniye 72 °C……..…2 dakika	35 döngü
72 °C……..10 dakika 4 °C……. Süresiz	
PHA sentaz geni varyasyonları (B1-F B1-R ve B1-F,B2-R primerleri) PZR reaksiyon koşulları	
94 °C…….4 dakika (Denatürasyon)	
94 °C…….30 saniye 60 °C…….45 saniye 72 °C……..…1 dakika 15 saniye	30 döngü
72 °C……..10 dakika 4 °C……. Süresiz	
PHB sentaz geni (PDTL ve RCT primer setleri) spesifik PZR reaksiyon koşulları	
94 °C…….3 dakika (Denatürasyon)	
94 °C…….30 saniye 54 °C…….45 1 dakika 72 °C……..…1 dakika	36 döngü
72 °C……..10 dakika 4 °C……. Süresiz	

Tarımsal atık olarak belirlenen Kabak posası içerik analizi

Çerezlik kabak atıkları steril koşullar altında numune şartlarında istenen özellikte ve miktarda hazırlanmıştır. Tarımsal Atığın içerdiği ham protein, karbonhidrat, toplam şeker, şeker çeşitleri, nitrit ve nitrat miktarlarının belirlenmesi için Tübitak Bursa Test ve Analiz Laboratuvarına gönderilmiştir.

Tanımlanan izolatların kabak agarlı ortamda gelişimi ve biyoplastik üretim yetenekleri
Tanımlanan ve standart besiyerinde biyoplastik üretme yeteneğine sahip izolatların kabak atığını kullanıp kullanmadıklarını ve biyoplastik üretimi potansiyelini test etmek için besiyeri içeriklerine kabak posası ilave edilerek kabak agar besiyeri hazırlanmış ve izolatlar inoküle edilerek 30 °C ve 37 °C'de 24-48 saat inkübasyona bırakılmıştır. İnkübasyon sonucunda gelişim gösteren izolatlardan sudan black boyama için preperatlar hazırlanmış ve boyama sonucunda tarımsal atıkları karbon kaynağı olarak kullanabilen izolatların biyoplastik üretim yetenekleri tespit edilmiştir.

BULGULAR

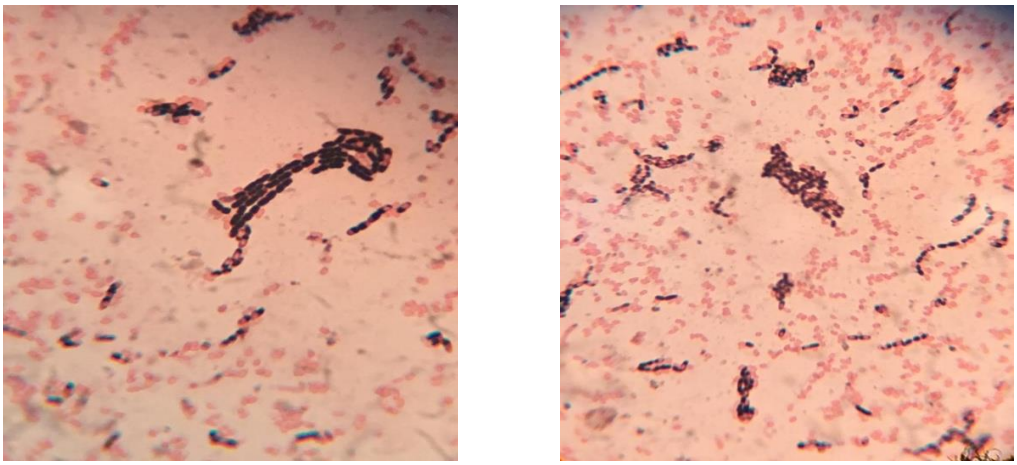
İzolasyon

Eskişehir Çifteler ilçesinden alınan toprak örneklerinden GYEA'dan 64 izolat saflaştırılmıştır. Aşağıdaki Şekil 1'de seçilerek saflaştırılan izolatların besiyerlerindeki görüntüsü verilmiştir.



Şekil 1: Saflaştırılan izolatların petri görüntüsü

Biyoplastik Üreten İzolatların Taranması
Biyoplastik taraması için Sudan Black boyama yöntemi kullanılmış ve başarılı sonuç elde edilmiştir. 127 izolat saflaştırılmış ve biyoplastik üretim yeteneklerinin belirlenmesi amacıyla yapılan tarama sonucunda 12 izolatın biyoplastik üretim yeteneğine sahip olduğu belirlenmiştir. Biyoplastik taramasında granül tespit edilen bazı izolatların görüntüleri şekil 2'de verilmiştir.



Şekil 2: Sudan Black boyama mikroskop görüntüsü

Biyoplastik üretim yeteneğine sahip izolatların tanımlanması
İzole edilen mikroorganizmalardan biyoplastik üretimi taraması gerçekleştirildikten sonra yoğun granül tespit edilen 12 izolatın tanımlanması amacıyla izolatların 16S rRNA genlerinin polimeraz zincir reaksiyonu ile amplifikasyonu izole edilen kolonilerin Bacteria spesifik primerler ile gerçekleştirilen 16S PCR sonucunda yaklaşık 1500 bazlık ürün elde edilmiştir. DNA ekstraksiyonları yapılan ve PZR ile 16S rRNA geni amplifiye edilen izolatların direkt olarak dizi analizleri hizmet alımı olarak yaptırılmıştır. Dizi analizinde 27F ve1492R

primerleri kullanılmıştır. Dizi analizinden sonra dizilenen örnekler NCBI veri tabanında bulunan referans örneklerle karşılaştırılarak Tablo 2'de belirlenmiştir.

Biyoplastik Sentaz Genlerinin Belirlenmesi

PHA ve PHB sentaz genleri hedefli primerler ile yapılan PZR'lar sonrası elde edilen ürünler %1'lik agoroz jelde yürütülmüştür. PZR sonrası Sınıf-1ve Sınıf 2 primer setlerinde 505-550 bp'lik ürün varlığı, B1 primer setinde 600 bp'li ürün varlığı, B2 primer setinde 380 bp'lik ürünün varlığı, PDTL ve RCT primer setlerinde 1089 bp'lik ürün varlığı ile izolatların sentaz genlerine sahip olup olmadığı tespit edilmiştir. Sınıf-1 primer setinde VA18, VA22 numaralı izolatlarda pozitif sonuç görülmüştür. B1-B2 primer setinde pozitif sonuç gözlenmezken; PDTL ve RCT primer setleri ile kurulan PZR sonucu da KA24 izolatında PHB sentaz genine sahip olduğu belirlenmiştir (Tablo 2)

Tablo 2: Tanımlanan izolatlar, kabak atıklarında gelişim ve biyoplastik üretim yetenekleri değerlendirmesi

Tanımlanan İzolatlar			**Kabak Atığında Gelişim ve Biyoplastik Üretimi**		**Sentaz Genleri**		
İzolat Kodu	**Benzerlik Gösteren Tür**	**Eşleşen Baz Sayısı ve Referans Numarası**	**Kabak Agar Gelişim**	**Biyoplastik Üretim Yeteneği**	**Sınıf 1 Sınıf 2**	**PDTL RCT**	**B1 B2**
VB15	*Paenibacillus tundrae* strain GMXG-8 16S ribosomal RNA gene, partial sequence	1221/1259(97%) MF101179.1	NEGATİF	NEGATİF	-	-	-
VB21	*Bacillus mojavensis* strain 261AG9 16S ribosomal RNA gene, partial sequence	1427/1429(99%) KF811046.1	POZİTİF	NEGATİF	-	-	-
VA23	*Bacillus simplex* strain AsT25 16S ribosomal RNA gene, partial sequence	1429/1429(100%) KX866680.1	POZİTİF	NEGATİF	-	-	-
VA24	*Pseudomonas* sp. Brn9 gene for 16S ribosomal RNA, partial sequence	1337/1345(99 LC420197.1	NEGATİF	NEGATİF	-	-	-
KA21	*Bacillus simplex* strain X7 16S ribosomal RNA gene, partial sequence	1426/1427(99%) KJ586280.1	POZİTİF	NEGATİF	-	-	-
VA7	*Pseudomonas frederiksbergensis* strain BZ27 16S ribosomal RNA gene, partial sequence	1388/1399(99%) HQ588846.1	POZİTİF	POZİTİF	-	-	-
VA18	*Pseudomonas frederiksbergensis* strain BZ27 16S ribosomal RNA gene, partial sequence	1371/1378(99%) HQ588846.1	POZİTİF	POZİTİF	PHA sentaz geni pozitif	-	-
VA22	*Pseudomonas* sp. strain F-14 16S ribosomal RNA gene, partial sequence	1375/1390(99%) MG266302.1	POZİTİF	POZİTİF	PHA sentaz geni pozitif	-	-
VA25	*Paenarthrobacter nitroguajacolicus* strain Q5 16S ribosomal RNA gene, partial sequence	1394/1396(99%) MK719879.1	POZİTİF	POZİTİF	-	-	-
KA18	*Bacillus simplex* strain WBY-7 16S ribosomal RNA gene, partial sequence	1423/1424(99%) MH012051.1	POZİTİF	NEGATİF	-	-	-
KA24	*Arthrobacter* sp. B86 16S ribosomal RNA gene, partial sequence	1194/1382(86%) HQ877777.1	POZİTİF	POZİTİF	-	PHB sentaz geni pozitif	-
KA34	*Pseudarthrobacter oxydans* strain D161 16S ribosomal RNA gene, partial sequence	938/950(99%) MF681932.1	POZİTİF	NEGATİF	-	-	-

Tarımsal atık olarak belirlenen Kabak posası içerik analizi

Tübitak Bursa Test ve Analiz Laboratuvarı'ndan elde edilen sonuçlara göre kabak posasının ham protein, karbonhidrat, toplam şeker, şeker çeşitleri, nitrit ve nitrat tayin sonuçları tabloda verilmiştir.

Tablo 3: Kabak posası içerik analizi

Parametre	Birim	Deney Sonucu Ortalama± s
Ham Protein	g/100g	0.95 ± 0,03
Karbonhidrat	g/100g	3,89 ± 0,26
Toplam Şeker	g/100g	1,90 ± 0,15
Glukoz	g/100g	0,85 ± 0,01
Fruktoz	g/100g	0,82 ± 0,01
sakkaroz	g/100g	0,08 ± 0,01
Maltoz	g/100g	0,17 ± 0,02
Nitrat	mg/kg	<50
Nitrit	mg/kg	18 ± 2

Tanımlanan izolatların kabak agarlı ortamda gelişimi ve biyoplastik üretim yetenekleri

Hazırlanan kabak agar besiyerine tanımlanan izolatların inkübasyonu sonucu; VB21, VA23, KA21, VA7, VA18, VA22, VA25, KA18, KA24, KA34 kodlu izolatları gelişim göstermiş ve VA7, VA18, VA22, VA25, KA24 kodlu izolatların biyoplastik üretimi pozitif sonuç vermiştir.

TARTIŞMA ve SONUÇ

Çalışma kapsamında, ülkemizde uzun yıllardan beri yapılmakla birlikte 2004 yılından sonra üretim alanı ve miktarında daha hızlı bir artış olan çekirdek kabakçılığı atıkları kullanılmıştır. Çekirdek kabakçılığı yapılırken çekirdekler dışında diğer tüm kabağa ait materyaller atık olarak kalmaktadır. Kabak atıkları ile oluşturulan minimal besiyerleri ile yapılan denemelerinden pozitif sonuçlar alınmıştır. İzole edilen mikroorganizmalardan 10 izolatın kabak posasını kullanabildiği belirlenmiştir.

Yüksek oranda açığa çıkan *Cucurbita pepo* L. tarımsal atıkları, ekonomik anlamda üretimde büyük sorun teşkil eden karbon kaynağı hammadde ihtiyacına da alternatif çözüm olabileceği sonucuna varılmıştır. Kabak posalı ortamda gelişim gösteren 10 izolattan 5 tanesinin kabak posalı ortamda biyoplastik üretimi yapabildiği belirlenmiştir. Ayrıca ileriye yönelik çalışmalar için biyoplastik sentaz genlerinin varlığı araştırılmış ve 3 izolatta pozitif sonuç elde edilmiştir. Elde edilen bulgular tarımsal kabak atıklarının giderilmesinde mikroorganizmaların kullanılabileceğini ve biyoteknolojik ürünlerin düşük maliyetli olarak atıklar kullanılarak elde edilebileceğini göstermiştir.

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ORAL PRESENTATION

Ardışık kesikli reaktörlerde azot ve fosfor giderimi: Gümüşhacıköy Atıksu Arıtma Tesisinin incelenmesi

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Özet

Atıksuların alıcı ortamlara deşarjı sonrasında en çok karşılaşılan problemlerden biri ötrofikasyondur. Bu problemin önlenmesi için ötrofikasyonun asıl sorumlusu olan azot ve fosforun atıksulardan giderilmesi gerekmektedir. Dünya genelinde, bu konudaki çalışmalar Sürdürülebilir Kalkınma Amaçları'ndan "Temiz Su ve Sanitasyon" kapsamı altında doğrudan ele alınmakta ve desteklenmektedir. Azot ve fosfor gibi besi maddelerinin giderilmesinde en efektif ve ekonomik çözüm ise biyolojik arıtımdır. Yalnızca karbonlu organik madde giderimi yapan konvansiyonel biyolojik arıtma tesisleri ile kıyas yapıldığında organik maddenin yanı sıra nütrient giderimi de yapan sistemlerin projelendirilmesi ve işletilmesinin daha zor olduğu kabul edilen bir gerçektir. Bu sebeple tasarım aşamasında güvenilir ve yeterli veri toplanması, uygun olabilecek proseslerin belirlenerek alternatifler arasından en uygun yönteme kantitatif bir değerlendirme yaparak karar verilmesi gereklidir. Ardışık kesikli reaktörler, değişen işletme koşullarına kolayca uyum sağlayabilmesi ve nütrient giderimi için ek ünitelere ihtiyaç duymaması sayesinde, düşük yatırım maliyeti ve esnek işletme imkanı sunarak öne çıkmaktadır.
Bu çalışmada, Gümüşhacıköy (Amasya) Atıksu Arıtma Tesisi örneği üzerinden ardışık kesikli reaktörlerin atıksu arıtımındaki üstünlük ve kısıtları ortaya konulmuştur. Ayrıca ardışık kesikli reaktörlerle azot ve fosfor giderim mekanizması açıklanmıştır.

Anahtar Kelimeler: Ardışık Kesikli Rekatör, İleri Biyolojik Atıksu Arıtımı, Nütrient Giderimi

Nitrogen and phosphorus removal in sequencing batch reactors: Investigation of Gümüşhacıköy Wastewater Treatment Plant

Abstract

One of the most common problems encountered after the discharge of wastewater into receiving (water) body is eutrophication. To prevent this problem, it is necessary to remove nitrogen and phosphorus, which are the main causes of eutrophication, from wastewater. Globally, studies on this issue are directly addressed and supported under the framework of the Sustainable Development Goal "Clean Water and Sanitation". The most effective and economical solution for the removal of nutrients such as nitrogen and phosphorus is advanced biological treatment. Compared to conventional biological treatment plants that only remove carbon-based organic matter, it is a well-known fact that the design and operation of systems that also remove nutrients are more complex. Therefore, it is essential to collect reliable and sufficient data during the design phase, identify potentially suitable processes, and determine the most appropriate method through a quantitative evaluation of the alternatives. Sequencing batch reactors are distinguished by their flexibility, which results from their ability to adapt to changing operating conditions, and their cost-effectiveness, attributed to the absence of a need for additional units for nutrient removal.
In this study, the advantages and limitations of sequencing batch reactors in wastewater treatment are demonstrated through the example of the Gümüşhacıköy (Amasya) Wastewater Treatment Plant. Additionally, the nitrogen and phosphorus removal mechanisms of sequencing batch reactors are explained.

Keywords: Sequencing Batch Reactor, Advanced Biological Wastewater Treatment, Nutrient Removal

GİRİŞ

Su, canlıların varlığı devam ettiği sürece ihtiyaç duyulan en önemli doğal kaynaklardan biridir. Son yüzyılda üstel artış gösteren dünya nüfusu ve buna paralel gelişen sanayileşmeye bağlı olarak içme ve kullanma suyuna olan talebin artması, sınırlı su kaynaklarının hızla tükenmesine ve kullanılmış su miktarının da artmasına sebep olmaktadır (Ayaz, 2022). Yeterli düzeyde arıtılmadan deşarj edilen atıksular günümüz dünyasında önemli çevre sorunları arasında yer almaktadır. Bu sebeple su ve atıksuyun yönetimi açısından, çeşitli arıtma teknolojileri uygulanıp geliştirilerek, çevresel sürdürülebilirlik artırılmalıdır (Yaşar, 2023). Aynı zamanda atıksuların içerisinde bulunan azot ve fosfor gibi besin maddeleri alıcı ortamlarda ötrofikasyona sebep olmakta, çözünmüş oksijen seviyesinin düşmesine, balık ölümlerine, biyolojik çeşitliliğin azalmasına yol açmakta ve canlı sağlığı için bir tehdit oluşturmaktadır (Coşkun ve ark., 2018). Gelişmiş ülkelerde nütrientler için deşarj standartları son yıllarda daha sıkılaşmıştır. Deşarj standartlarını sağlayacak arıtma proseslerinin, güvenilir, düşük maliyetli ve kolay uygulanabilir alternatiflerinin geliştirilmesine yönelik çalışmalar artmaktadır (Taşlı, 1996). Biyolojik nütrient giderim prosesleri, atıksuyun içeriğinde olan azot ve fosforun arıtımı için geliştirilen, anaerobik, anoksik ve aerobik evrelerin kullanıldığı aktif çamur proseslerinin farklı modifikasyonlarıdır. Evsel atıksuların biyolojik arıtımında karbon ve nütrient giderimini yapabilmek adına çeşitli konfigürasyonlar üzerinde çalışma ve araştırmalar yapılmıştır. Öne çıkan prosesler, anaerobik/anoksik/aerobik (A2O), Cape Town Üniversitesi (UCT), Bardenpho, Virginia tipi (VIP), Johannesburg ve ardışık kesikli reaktörlerdir (AKR). (Uysal ve Üstünyıldız, 2016). Aktif çamur sistemlerinde nütrient giderim verimlerinin karşılaştırılması üzerine yapılan bir çalışmada azot gideriminde ilk sırada Bardenpho (5 kademeli) prosesi yer alırken bu prosesin akabinde AKR ve UCT proseslerinin olduğunu belirtmiştir. Aynı şekilde fosfor gideriminde ilk sırada UCT prosesinin olduğu, ardından Bardenpho (5 kademeli) ve AKR prosesleri geldiği belirtmiştir (Coşkun ve ark., 2018). Aktif çamur prosesi, dünya çapında kentsel atıksu arıtma tesislerinin çoğunluğu tarafından atıksu arıtımı için benimsenen geleneksel bir tekniktir. Son yıllarda, aktif çamur prosesinin geliştirilmiş bir biçimi olan ardışık kesikli reaktör, sağladığı avantajlardan ötürü yaygın olarak kullanılan bir sistem haline gelmiştir. AKR'ler, atıksuyun doldurma ve boşaltma prensibiyle arıtıldığı anaerobik-aerobik biyoreaktörler için entegre sistemlerden biridir. Klasik bir AKR prosesi temel olarak sırasıyla beş adımdan oluşur: doldurma, reaksiyona sokma, çökeltme, boşaltma ve bekleme evreleridir (Alagha ve ark., 2020). AKR sisteminde dengeleme, biyolojik arıtma ve ileri arıtım gibi işlemler zaman kontrollü bir şekilde tek bir tank içerisinde meydana gelmektedir. Fakat geleneksel aktif çamur sisteminde bu prosesler birden fazla üniteye ihtiyaç duyularak gerçekleşmektedir (Vijayan ve ark., 2017).

Ardışık kesikli reaktör sistemleri nitrifikasyona ek olarak denitrifikasyon ve fosfor giderimini de iyi bir performansla gerçekleştirebilir. İşletme ve bakım sorunları çok az olmakla birlikte sistem operasyonel esnekliğe sahiptir ve bütün işlemlerin tek bir tankta gerçekleştirilmesinden ötürü daha az yer kaplamaktadır (EPA, 2000). Tüm bu avantajlarına rağmen bu sistemlerin bazı zayıf yönleri de bulunmaktadır. Aynı tank içerisinde çamur çöktürme işlemi de gerçekleştiğinden, kullanılan havalandırma sistemlerinde tıkanma veya arızalanma gibi sorunlar meydana gelebilir. Ayrıca sistemin çalışma prensibi gereği dengeleme tankına ihtiyaç duyulmaktadır, bu da AKR'leri ancak düşük debili atıksuların arıtımı için uygun bir seçenek yapmaktadır (Manav, 2006).

Bu çalışmada, Gümüşhacıköy (Amasya) Atıksu Arıtma Tesisi örneği üzerinden ardışık kesikli reaktörlerin atıksu arıtımındaki üstünlük ve kısıtları ortaya konulmuştur. Ayrıca ardışık kesikli reaktörlerle azot ve fosfor giderim mekanizması açıklanmıştır.

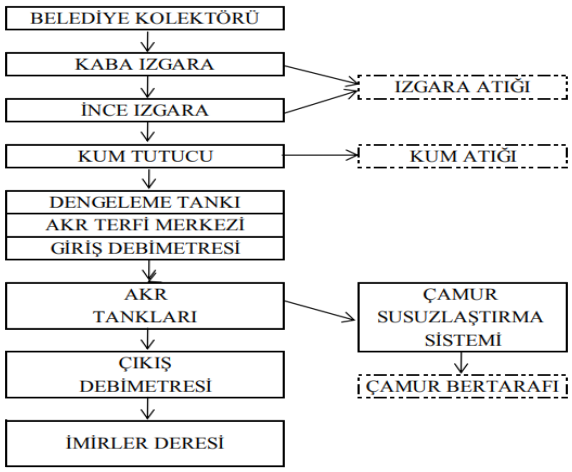
MATERYAL VE METOT

Bu çalışmada incelenen Gümüşhacıköy Atıksu Arıtma Tesisi, Amasya ili'ne 70 Km uzaklıkta bulunan Gümüşhacıköy İlçesi'nde bulunmaktadır. Atıksu arıtma tesisi 2037 yılı için 5692 m^3/gün, 2052 yılı için 6785 m^3/gün kapasiteli olarak inşaatı tamamlanmış ve 2023 yılında işletmeye alınmıştır. Çalışma alanı Şekil1.'de gösterilmektedir.



Şekil 1. Çalışma Alanı

Tesis kaba ızgara, ince ızgara, kum tutucu, dengeleme havuzu ve iki adet ardışık kesikli reaktörden oluşmaktadır. Tesis akış şeması Şekil 2.'de gösterilmektedir.



Şekil 2. Tesis Akış Şeması

Gümüşhacıköy Atıksu Arıtma Tesisi'ne kolektör hattından gelen atıksu ilk olarak kaba ızgara ve ince ızgara ünitelerinden geçerek kum ve yağ tutucu ünitesine geçmektedir. Sonrasında atıksu fiziksel arıtımını tamamlayarak pik debileri dengeleme amaçlı yapılan dengeleme havuzuna geçer. Buradan da son olarak atıksu biyolojik arıtımını tamamlamak üzere paralel işletilen iki adet AKR tankına terfilendirilir. Arıtılan suyun deşarjı Gümüşhacıköy İlçesi'nde bulunan İmirler Deresi'ne yapılmaktadır.

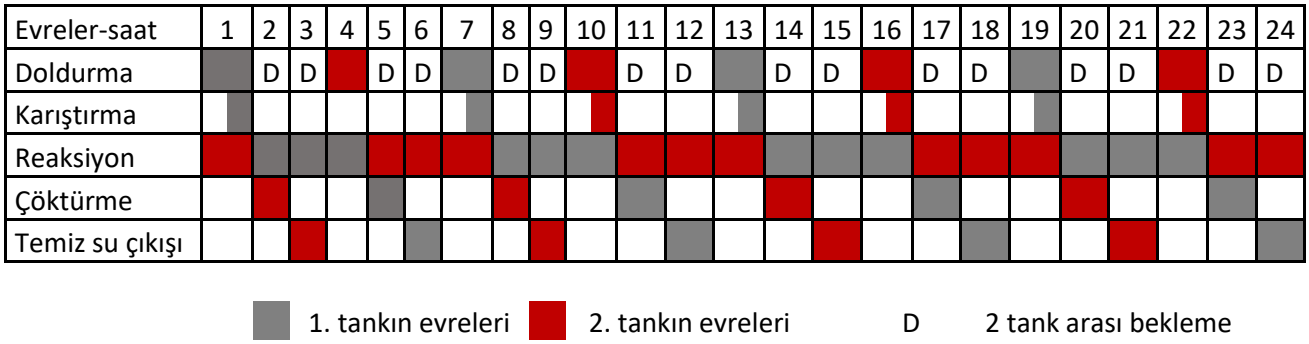
BULGULAR ve TARTIŞMA

Son yıllarda AKR'ler hem endüstriyel hem de evsel atıksu arıtımında yaygın bir şekilde kullanılmaktadır. AKR'lere ait uygulamalar her geçen gün artmaktadır. Örnek olarak Almanya'daki atıksu arıtma teknolojileri arasında AKR prosesinin kullanım oranı %1,3'ü bulmaktadır (Topaloğlu ve ark., 2009).

Gümüşhacıköy Belediyesi tarafından arıtma tesisi olarak belirlenen bölgede tarım arazilerinin yoğunluğundan ötürü kamulaştırma problemi yaşanmış ve kısıtlı arazide daha küçük ölçekli bir arıtma tesisi yapabilmek adına AKR prosesi tercih edilmiştir.

Gümüşhacıköy Atıksu Arıtma Tesisi'nde karbonlu organik maddenin yanısıra nütrient giderimi ve katı sıvı faz ayrımı tek bir tank içerisinde gerçekleştirilmektedir.AKR tankının bir döngüsü altı saatten oluşmaktadır. İlk bir saat tankın dolum evresidir. Bir saatin son yarım saatinde havasız karıştırma yapılır. Ve bu aşamada anaerobik ortam oluşturulur. Fosfor gideriminde temelde anoksik ve/veya anaerobik ortamda sıvı fazda organizmaların hücre içinde depoladıkları fosfatı ortama bırakmaları suretiyle fosfor konsantrasyonunun artması ve aerobik ortamda sıvı fazdaki fosfatın organizmalar aracılığıyla tutularak fosfat konsantrasyonunun azaltılması prensibiyle çalışır (Akın, 2002). Döngünün ikinci ve üçüncü saatinde blowerlar devreye girer. Bu aşamada aerobik ortam oluşmaktadır. Bu şekilde fosfor giderimi sağlanmış olur. Ardından bu evredeki başka

bir amaç nitrifikasyonu gerçekleştirmektir. Döngünün dördüncü saatinde ise blowerlar kapatılır ve anoksik koşullar oluşturulur. Burada denitrifikasyon gerçekleşir ve azot giderimi tamamlanmış olur. Beşinci saatte ise su dinlenmeye bırakılır ve çamur çöktürülür. Altıncı saatte ise arıtılan atıksu İmirler Deresi'ne deşarj edilir. Şekil 3.'te AKR tanklarının evreleri gösterilmektedir.



Şekil 3. AKR Tankının evreleri

Gümüşhacıköy Atıksu Arıtma Tesisi'nde en çok karşılaşılan sorunlardan biri de sık sık difüzörlerin bozulması veya tıkanmasıdır. Bunun yanısıra klasik aktif çamur sistemlerine oranla daha geç çamur oluşumu gözlenmiştir. Herhangi bir kimyasal ilavesi gerekmediğinden ekonomik açıdan da avantaj sağlamaktadır.

SONUÇ

Atıksu arıtma tesislerinin deşarj kriterlerini sağlaması ve atıksuyun yeniden kullanılabilir hale getirilmesi için proses seçimi, tasarım ve projelendirilme aşamalarına önem verilmeli ve gelişen teknolojiler takip edilmeli ve uygulanmalıdır. İşletme döneminde karşılaşılan problemleri minimum düzeye indirgeyebilmek için düzenli bir şekilde tesisin bakım ve onarımı yapılmalı, tesislerde çalışan personelin yetkinliği artırılmalıdır.

AKR'ler hem laboratuvar ölçeğinde kolay kurulumu sayesinde herhangi bir araştırmada araç olarak kullanılabilirliği yönünden, hem de atıksu arıtımında sağladığı kolaylıklar ele alındığında güvenilir, ekonomik ve pratik bir sistem olmasından dolayı sadece endüstriyel atıksular için değil evsel atıksuların arıtımında da azot ve fosfor gideriminde yaygınlıkla kullanılabilir.

Günümüz dünyasında azalan su kaynakları; suyun daha verimli kullanılmasını ve kullanılan suyun geri dönüşümünü gerekli kılmaktadır. Bu amaçla yenilikçi teknolojiler geliştirildiği kadar mevcut sistemlerin veriminin artırılmasına yönelik çalışmalar da yapılmaktadır. Bu alanda, mevcut arıtma teknolojilerinin AKR'ler ile entegrasyonu sayesinde sürdürülebilir çözümler ortaya çıkmaktadır. AKR'lere doğal adsorbanların ilavesiyle veya sabit biyofilm sitemlerin entegre edilmesiyle karbon ve azot giderim verimlerinin iyileştiği görülmüştür (Gürtekin, 2008; Mohamad ve Işık, 2024). Benzer bir yaklaşımla, granüler çamur kullanılan AKR'lerde çökelme veriminde artış sağlanmıştır (Mağden, 2010).

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ORAL PRESENTATION

Influences from Solvents on Li-Ion Insertion in Molten Salt MXenes

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Abstract

In this study, we investigated the effect of surface termination groups of MXenes, a member of two-dimensional nanomaterials, on Li-ion intercalation and the Li ion storage mechanism in organic electrolytes. Tailoring of the MXene material surface was successfully achieved using a new method, molten salt etching technique. In our comprehensive analysis, we explore how organic electrolytes affect the capacitive behaviour and charge storage dynamics of the stratified MS-MXene, characterized by its distinct layered structure. Our results demonstrate that solvents with lower polarity enhance capacitive performance during both positive and negative charge cycles, whereas solvents with higher polarity exhibit pronounced asymmetric charging. Electrochemical assessments indicate that the charging in these non-protonic electrolytes is predominantly driven by cations, with solvents playing a role in higher apolar electrolytes. In summary, our research suggests that solvents that are both larger and less polar contribute to saturation between the layers of MS-MXene electrodes, leading to more balanced charging but reduced energy storage capacity.

Keywords: MXene, 2D material, Li-Ion batteries, Organic Electrolytes, Molten Salt Etching.

INTRODUCTION

The development of a secure and scalable methodology for the fabrication of MXenes represents a critical yet challenging objective that has persisted since the initial successful synthesis of these materials in 2011(Naguib et al. 2011, Arole et al. 2022). Discovered initially in 2011 by Barsoum and Gogotsi and others, MXenes have emerged as a prominent group of 2D materials (Matthews et al., 2022), spurring extensive research. These 2D transitional metal carbides, nitrides, and carbonitrides, collectively referred to as 'MXenes,' exhibit significant potential in various applications (Maleski et. al., 2022, Anasori et. al., 2017), including energy storage, electromagnetic interference shielding, sensing (Shahzad et. al. 2016), electronics (Iqbal et. Al., 2020), and biomedicine. MXenes stand out in the 2D material family due to their versatility, offering a broad selection of transition metals, a range of thicknesses for individual flakes, tunable surface chemistry (Lee et. Al., 2017, Melianas et. Al. 2021), and the capacity to accommodate different species within their layers. MXenes are typically produced from a layered ternary carbide/nitride structure, referred to as MAX phases (Meng et. Al., 2018, Mojtabavi et. Al., 2019). These compounds feature a two-dimensional nanostructure with a conductive core of transition metal carbides or nitrides (Wei et. Al., 2021, Wang et. Al., 2023). This core structure allows for the natural attachment of various functional groups like –OH, –O, –F, –Cl, among others. (Alhabeb et. Al., 2017). For this process, selectively removing group A elements (like Al, Ga, Sn) from the MAX phase's carbide/nitride structure with HF acid is necessary, resulting in a layered, accordion-like formation (Ghidiu et. Al., 2014). This structure can then be exfoliated using ultrasonic waves or manual shaking to create extremely thin MXene sheets. Agents such as LiCl, TBAOH, and DMSO are often employed to aid in exfoliation by reducing the van der Waals forces between sheets. Due to the dangers of HF, alternative methods like combining fluorides (for instance, LiF, NaF, NH4, HF2) with HCl are used to produce MXenes (Feng et. Al., 2017, Liu et. Al., 2017). However, the use of F-based aqueous etchants in processing presents considerable toxicity and corrosivity risks, which can impede mass production. (Lakhe et. Al., 2019) Numerous methods for synthesizing MXenes have been explored, yet only wet chemical etching with fluorine-rich acids and Lewis acid molten salt (MS) etching have proven to be scalable. These techniques have garnered significant interest for the MXenes they produce (Zhang et. Al., 2024). However, efforts to separate MS-MXenes into single layers often result in thicker, multilayered lamellas instead of the desired single-layer flakes (Liu et. Al., 2022,

Arole et. Al., 2022 and Arole et. Al., 2021) While these techniques struggle with scaling up, a more rapid synthesis approach for a wide variety of MXenes has been developed. This method involves etching MAX phases of different compositions in molten salts that exhibit Lewis acid properties, marking a significant advancement in the field (Li et. Al., 2020). This method can be employed at high temperatures in the air, thanks to the shielding effect of the molten salt, yielding MXenes with tuneable inter-layer spacing and surface groups. This research introduces a secure and uncomplicated approach to separate MXene layers produced through the MS technique, employing LiCl—a harmless, readily available, and cost-effective salt—as the separating agent. The use of LiCl successfully separates the MXene layers while preserving their original surface chemistry, overcoming a previously difficult hurdle.

Conversely, the advancement of supercapacitors hinges on a solid grasp of charging processes. This knowledge unveils the reasons behind the superior performance of certain electrode and electrolyte pairings and may pave the way for enhanced efficiency. Building upon this, Simon and colleagues delved into the electrochemical boundary of the stratified MS-$Ti_3C_2Cl_2$ interfaced with Lithium bis(trifluoromethanesulfonyl)imid (LiTFSI) within an acetonitrile electrolyte, employing an organic electrolyte methodology. In our comprehensive analysis, we explore how organic electrolytes affect the capacitive behavior and charge storage dynamics of the stratified MS-MXene, characterized by its distinct layered structure. Our results demonstrate that solvents with lower polarity enhance capacitive performance during both positive and negative charge cycles, whereas solvents with higher polarity exhibit pronounced asymmetric charging. Electrochemical assessments indicate that the charging in these non-protonic electrolytes is predominantly driven by cations, with solvents playing a role in higher apolar electrolytes. In summary, our research suggests that solvents that are both larger and less polar contribute to saturation between the layers of MS-MXene electrodes, leading to more balanced charging but reduced energy storage capacity.

MATERIALS AND METHODS

Synthesis of Multilayer MS-MXene

MS-MXene electrode films obtained with the molten salt approach were produced as stated in the article titled Delamination of Chlorine-Terminated MXene Produced Using Molten Salt Etching by Teng Zhang et al (Zhang et. Al., 2024). In summary:

$Ti_3C_2Cl_2$ MXene was synthesized by selective etching of the Al layers from the $Ti_3C_2Cl_2$ MAX phase precursor using a molten salt etchant with the following reactions:

$$2Ti_3AlC_2(s)+3ZnCl_2(l)\rightarrow 2\ Ti_3C_2\ (s)+2AlCl_3(g)+3Zn(s) \quad (1)$$

$$2\ Ti_3C_2\ (s)+2ZnCl_2(l)\rightarrow 2\ Ti_3C_2Cl_2(s)+2Zn(s) \quad (2)$$

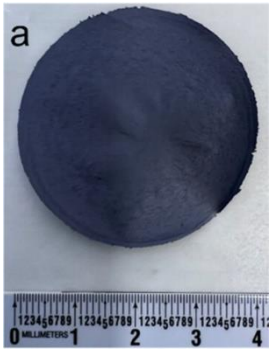
During the reaction, Zn2+ ions are transformed into solid Zn through a reduction process, creating a molten Zn layer around the MXene layers. Concurrently, Al is converted into AlCl3, which then vaporizes at approximately 660 °C. The Zn, now in a liquid state, predominantly vaporizes and condenses on the cooler end of the tube. The absence of oxygen or water during this process results in a consistent –Cl termination on the MXene produced. Upon returning to ambient temperature, the liquid Zn crystallizes into nanoparticles and flakes adjacent to the MXene layers. Any remaining Zn metal within the newly formed $Ti_3C_2Cl_2$ MXene is effectively removed using 12 M HCl solution.

Intercalation of MS-MXene

Zhang and colleagues found that MXene with chlorine termination, synthesized via the MS technique, is capable of delamination when treated with lithium salts in anhydrous solvents like dimethyl sulfoxide. The hydrophobic nature of MS-MXene means that it resists the intercalation of hydrated cations within its layers. Therefore, it's essential to carry out the delamination in an environment devoid of moisture to avert the formation of a hydration shell around the lithium ions. The procedure was conducted inside a glovebox filled with argon gas. Initially, 1.2 grams of LiTFSI were mixed into 10 milliliters of DMSO within a 20-milliliter glass container, ensuring complete dissolution by stirring with a PTFE-coated magnetic stirrer for ten minutes in a sealed vial. Subsequently, 3 grams of MS-$Ti_3C_2Cl_2$ in multilayer form were introduced to this mixture and agitated at a speed of 1000 revolutions per minute over a period of 24 hours to facilitate intercalation.

Solvent Exchange of Delaminated MXene Solution and Freestanding Film Preparation

MS-MXene layers, once separated with LiTFSI, can be evenly distributed in various polar solvents including DMF, NMP, acetonitrile, DMSO, propylene carbonate, and 2-propanol through a process of solvent exchange in a normal atmospheric setting. Taking IPA as a case in point, the solution of MS-$Ti_3C_2Cl_2$, which had been delaminated using NMF, was densified using a centrifuge operating at 11,200 RCF for a duration of 10 minutes. Additionally, MXene films that were not attached to any substrate were created employing diverse delamination techniques, utilizing vacuum-assisted filtration across a Celgard 3501 membrane. (Picture1).



Picture 1. (a) Digital photograph of MS-$Ti_3C_2Cl_2$ MXene freestanding film.

RESULTS and DISCUSSION

Electrochemical Characterizations

To analyze the inconsistency and difference between chlorine termination and MXenes with conventional termination groups, we performed electrochemical testing of a 7-micron thick MXene film coating to minimize ion transport limitations due to electrode architecture. In all electrochemical tests performed with the Swagelok cell design, 3-electrode system was used, with Ag wire as the reference electrode and carbon film as the counter electrode. In the current work, stainless steels were used as current collectors, instead of glassy carbon electrode. In the conducted experiments, MXene electrodes were fabricated consistently, maintaining uniformity in electrode surface area, thickness, mass ratio, and contact quality with the current collector. Consequently, variations in the outcomes of electrochemical characterization can be attributed solely to the disparities in the electrolytes used. Organic electrolytes prepared with 1 M LiTFSI in dimethyl sulfoxide, acetonitrile and propylene carbonate were used as electrolyte solution to facilitate the intercalation of Li ions into hydrophobic layered MS- $Ti_3C_2Cl_2$ and $Ti_3C_2T_x$ layers respectively. A series of electrochemical characterizations were performed to evaluate the electrochemical performance of vacuum-dried MS- $Ti_3C_2Cl_2$. Figure 1. a to 1. c show shows cyclic voltammetry profiles of the MS- $Ti_3C_2Cl_2$ and $Ti_3C_2T_x$ MXenes recorded at 1 mV s^{-1} with different negative cut-off potentials in three different organic electrolytes. (1 M LiTFSI in DMSO, PC and ACN).

To enhance the energy density of supercapacitors (SCs), nonaqueous electrolytes are frequently employed to broaden the potential window. Since energy density is directly proportional to the square of the cell voltage, considerable research efforts are directed towards developing electrolytes that are stable, highly conductive, and capable of operating over a wide potential range. Transitioning from aqueous to organic electrolytes can elevate the voltage from 1 V to between 2.5 and 2.7 V for electric double-layer capacitors (EDLCs). Organic electrolytes frequently utilized are conductive salt solutions, for instance, tetraethylammonium tetrafluoroborate ($TEABF_4$) and related compounds, along with lithium hexafluorophosphate ($LiPF_6$) dissolved in either acetonitrile (ACN) or propylene carbonate (PC). The preference often leans towards PC because of its superior flash point and reduced toxicity relative to ACN. (Nasrin et. Al., 2021).

When the $Ti_3C_2T_x$ MXene electrodes were tested in this configuration, the operation potential window was extended to -2.5 V (Open Circuit Voltage (OCV) to −2,5 V versus Ag) (Figure 1. a-f).

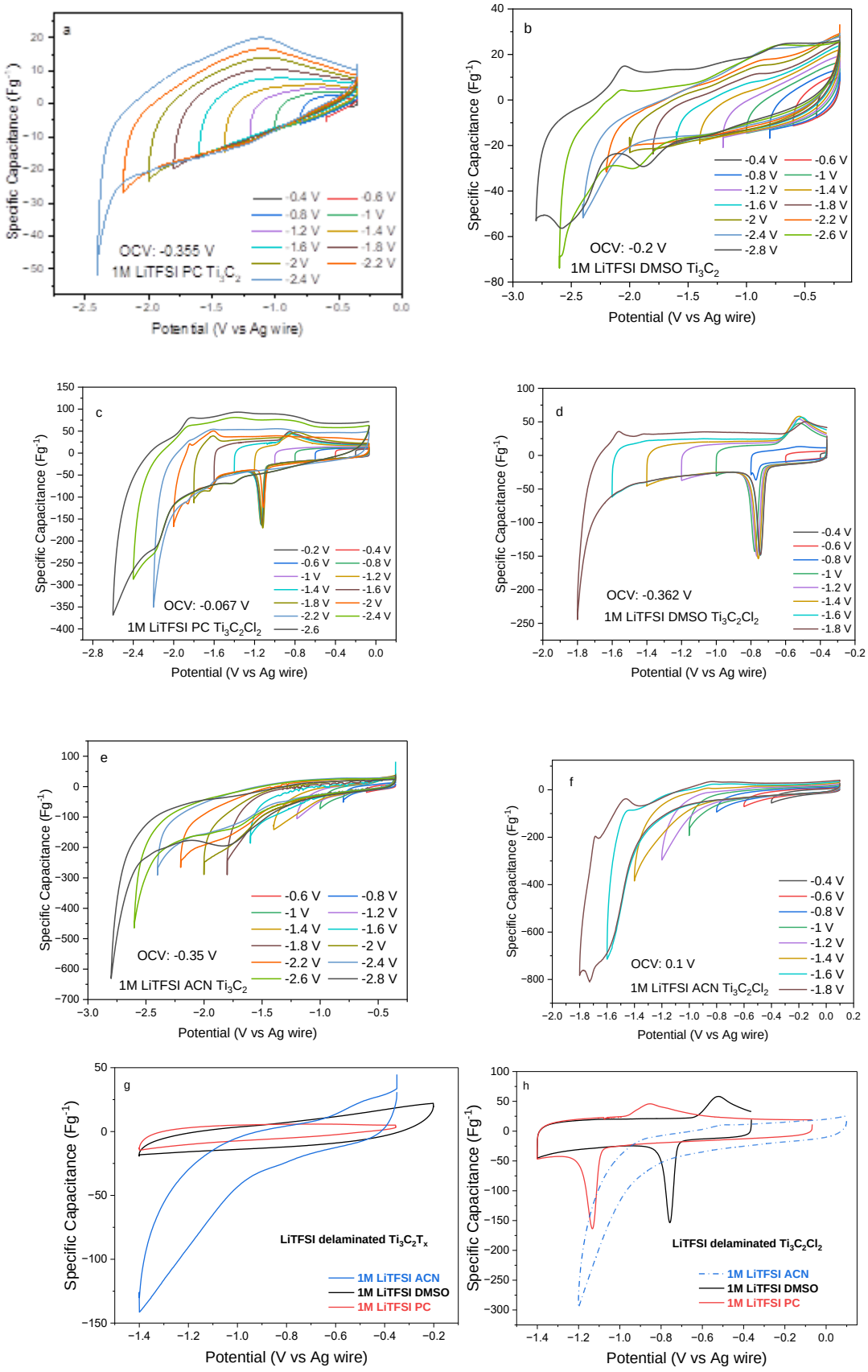


Figure 1. a) Cyclic voltammetry voltage window profiles for $Ti_3C_2T_x$ of 1M LiTFSI PC electrolyte, b) $Ti_3C_2T_x$ for 1M LiTFSI DMSO electrolyte, c) LiTFSI delaminated MS- $Ti_3C_2Cl_2$ for 1M LiTFSI PC electrolyte, d) LiTFSI delaminated MS- $Ti_3C_2Cl_2$ for 1M LiTFSI DMSO electrolyte, e) $Ti_3C_2T_x$ for 1M LiTFSI AC, f) LiTFSI delaminated MS- $Ti_3C_2Cl_2$ for 1M LiTFSI ACN, g) comparision of $Ti_3C_2T_x$ in three different electrolytes and h) comparision of MS-$Ti_3C_2C_2$l in three different electrolytes

In this study, we explore the boundaries of pseudocapacitive charge storage by examining the rate, capacitance, and voltage window with the use of MS- $Ti_3C_2Cl_2$ and $Ti_3C_2T_x$ materials. Additionally, we illustrate the efficiency of electrode design strategies in facilitating energy storage and rapid delivery at extremely high rates.

The investigated electrodes in EDLCs exhibited a maximum operating voltage of -1.4 V, a figure that stands on the lower side when juxtaposed with the voltages of other EDLCs using ACN-based and carbonate-based electrolytes as documented in existing studies (Liu et. Al., 203, Wang et.al., 2020 and Wang et. Al., 2019). However, this voltage surpasses the 1.2 V mark typical of EDLCs employing aqueous electrolytes. The operational voltage threshold for ACN-based electrolytes is primarily hindered by the breakdown of acetonitrile, which begins at potentials exceeding -0.8 V versus Ag, despite acetonitrile being recognized for its superior electronic conductivity as seen in Figure f. Interestingly, such degradation has not been observed in carbonate-based and DMSO electrolytes, making it an unexpected finding. With these considerations, EDLCs were carefully constructed and subjected to initial tests, incorporating a safety cut-off for both the electrodes and the entire device to ensure safe operation parameters. Figure 1 g and h show a comparison of cyclic voltammograms (CVs) at 1 mV s^{-1} for MS- $Ti_3C_2Cl_2$ and $Ti_3C_2T_x$ electrodes tested in 1 M LiTFSI organic electrolytes with different solvents, DMSO, ACN and PC. Surface terminations on MXene surfaces are known to significantly affect their electrochemical performance, yet the underlying mechanisms remain unclear. Liu et al. reported that introducing the O surface group activates the pseudocapacitive redox reaction of Cl-terminated Ti_3C_2 MXene in non-aqueous electrolytes. (Li et. Al. 2021). The electrochemical profile of this MXene is notably distinct from the established characteristics of traditional MXenes synthesized through etching in HF- or fluoride-based electrolytes, commonly referred to as HF-MXenes, when utilized in non-aqueous electrolytic solutions. The OCVs are −0.2 V, −0.355 V and −0.35 V for MS- $Ti_3C_2Cl_2$ and −0.362 V, −0.067 V and −0.1 V for $Ti_3C_2T_x$ versus Ag for DMSO, PC and ACN -based electrolytes for, respectively.

The voltage window ranges of MS- $Ti_3C_2Cl_2$ and $Ti_3C_2T_x$, respectively, in all non-aqueous electrolytes except ACN, are -1.4 V. Although acetonitrile is among the organic solvents with the highest dipole moment and conductivity, it was observed that its electrochemical stability was low under negative potential. ACN's easy decomposition makes it unsuitable for use in future tests.

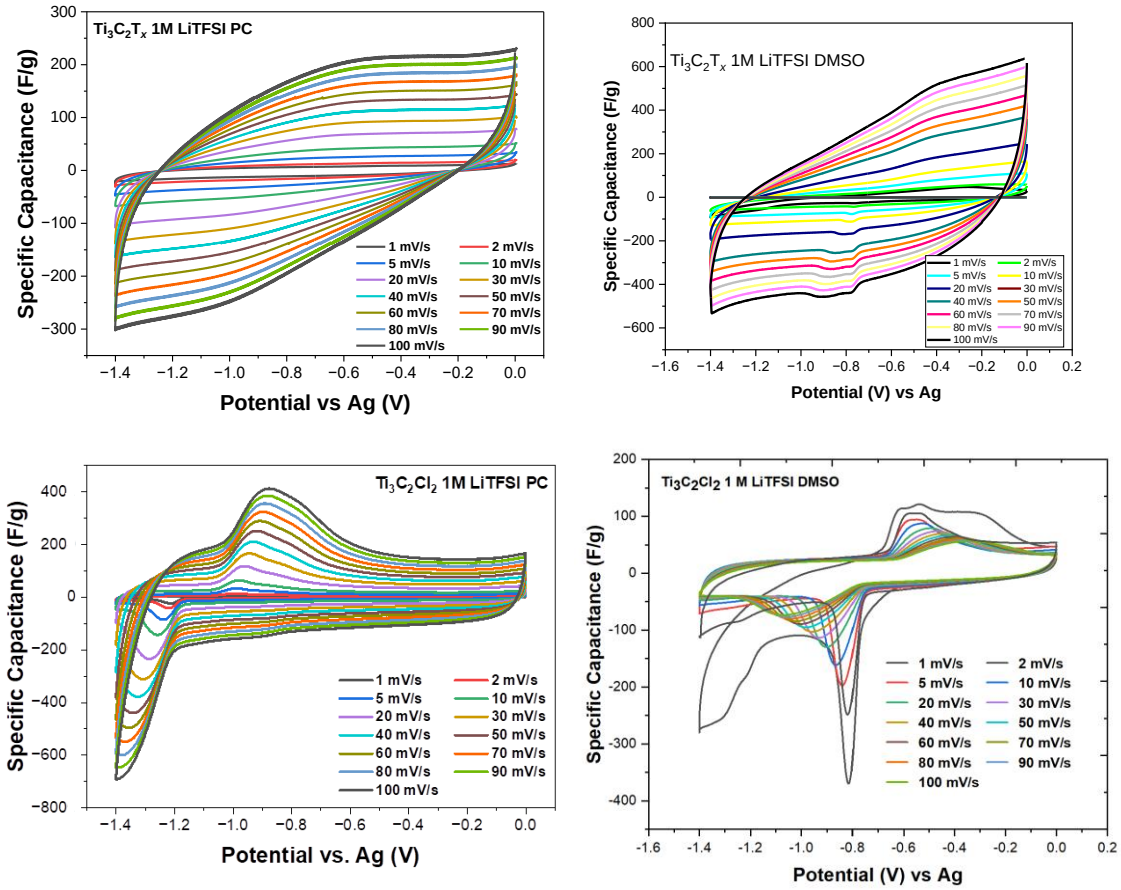


Figure 2. a) Cyclic voltammetry profiles for $Ti_3C_2T_x$ of 1M LiTFSI PC electrolyte, b) $Ti_3C_2T_x$ for 1M LiTFSI DMSO electrolyte, c) LiTFSI delaminated MS- $Ti_3C_2Cl_2$ for 1M LiTFSI PC electrolyte, d) LiTFSI delaminated MS- $Ti_3C_2Cl_2$ for 1M LiTFSI DMSO electrolyte

MXenes possess a layered structure that is partially delaminated, which allows for both ion intercalation and electric double-layer (EDL) formation to contribute to charge storage. The delamination of nanosheets through sonication, a common method for creating free-standing MXene films, modifies the balance between these two storage contributions. Voltammograms of MS-$Ti_3C_2Cl_2$ and $Ti_3C_2T_x$ in LiTFSI/organic solvent electrolytes exhibit distinct features, with two pairs of symmetric peaks during cycling, suggesting that the charging process is primarily governed by rapid redox reactions linked to intercalation in Figure 2c-d. $Ti_3C_2T_x$ electrodes show more like rectangular cyclic voltammetry (CV) curves, characteristic of EDL capacitor electrodes in Figure 2 a-b. It can be concluded that the interlayer distance does not change during Li intercalation and desertification, preventing the interlayering of large organic molecules and dissolved ions due to the small ionic radius of Li^+ (0.9 Å) and the limitation of nonpolar organic solvent interaction of the hydrophilic $Ti_3C_2T_x$ electrode. The specific capacitance values indicate that the interactions of organic solvents with hydrophilic HF-MXene electrodes are relatively low. In contrast, when MS-MXene electrodes are used in nonaqueous electrolytes, the resulting capacitive cyclic voltammetry (CV) curves are not the typical shape. Taking the MS-$Ti_3C_2Cl_2$ electrode in a nonaqueous DMSO Li^+ electrolyte as an example, there is a noticeable cathodic peak at -0.8 V and an anodic peak at -0.5 V (Figure 2d). This is in addition to a consistent capacitive current, roughly 100 F/g, spanning from the open circuit voltage (OCV) to -1.4 V against Li/Li^+. When considering the electronic structure of MXene during the charge/discharge process with aqueous electrolytes, it's observed that electrons occupy what were once vacant states within MXene, which results in its reduction. However, in nonaqueous electrolytes, the solvation energy is considerably less compared to hydration, which promotes partial desolvation at the interface between MXene and the electrolyte. This leads to a hybridization of the atomic orbitals of the intercalated cations with those of the MXene.

Additionally, the Li^+ intercalation/deintercalation in MS-$Ti_3C_2Cl_2$ MXene occurs sequentially, leading to multiple sets of redox peaks that correspond to the Li^+ intercalation within the interlayer slits as shown in Figure 2d different peaks are clearly visible. In contrast, the charge storage in $Ti_3C_2T_x$ MXene follows a

constant current relative to the applied potential, akin to the behavior seen in pseudocapacitive materials, with nearly uniform current throughout the reduction and oxidation stages within a potential window of OCV to -1,4 V versus Ag.

Our research reveals the pivotal role of electrolyte solvent selection in influencing the lithium-ion intercalation dynamics within $Ti_3C_2T_x$ and MS-$Ti_3C_2Cl_2$ MXene electrodes when immersed in organic electrolytes. When cycling with a lithium bis(trifluoromethylsulfonyl)amine (LiTFSI) in propylene carbonate (PC) electrolyte, lithium ions within the $Ti_3C_2T_x$ interlayers are completely desolvated, which fosters a charge storage process primarily through pseudocapacitive intercalation. However, substituting PC with acetonitrile (ACN) or dimethyl sulfoxide (DMSO) markedly reduces capacitance, attributed to lithium ions drawing solvent molecules into $Ti_3C_2T_x$'s interlayers. This observation defies the anticipated outcomes derived from carbon supercapacitor studies, wherein ACN is presumed superior due to its high ionic conductivity. Our findings underscore the necessity of tailoring electrolyte composition to electrode material to steer the charge storage mechanism and secure enhanced electrochemical performance.

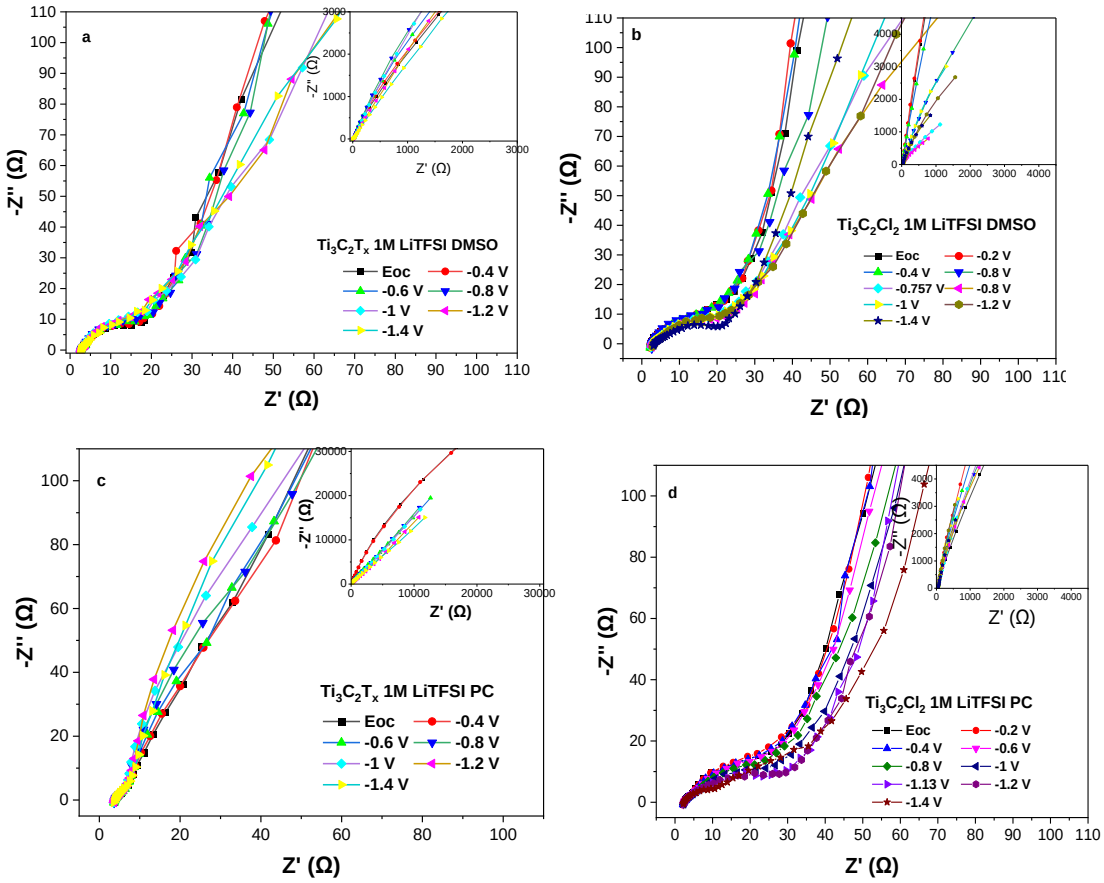


Figure 3. b) The electrochemical impedance spectroscopy plots recorded at various bias potentials. The Nyquist plots for a) $Ti_3C_2T_x$ of 1M LiTFSI PC electrolyte, b) $Ti_3C_2T_x$ for 1M LiTFSI DMSO electrolyte, c) LiTFSI delaminated MS-$Ti_3C_2Cl_2$ for 1M LiTFSI PC electrolyte, d) LiTFSI delaminated MS- $Ti_3C_2Cl_2$ for 1M LiTFSI DMSO electrolytes.

Electrochemical impedance spectroscopy (EIS) measurements were conducted for each of the three electrolytes at open circuit voltage (OCV) and at various bias potentials relative to Ag to evaluate the electrochemical performance of the MS-Ti3C2Tx MXene material (Figure 3. a-d). The Nyquist plots consistently exhibited a high-frequency semicircle followed by a rapid increase in the imaginary component of the impedance at low frequencies. The high- frequency semicircle corresponds to a charge-transfer resistance of approximately 20 Ω cm², which is nearly three times greater than that observed in a porous MXene electrode within a propylene carbonate-based electrolyte (Liu et. Al., 2017). The equivalent series resistance, determined from the real-axis intercept of the Nyquist plots in Figure 3. a-d, follows an increasing order for the solvents studied: DMSO and then PC, consistent with the trend of decreasing electrolyte conductivity (Wang et. Al., 2015). At OCV, the $Ti_3C_2T_x$ electrode exhibits typical capacitive behavior in both DMSO- and PC-based electrolytes, with a small high-frequency loop indicative of interfacial impedance at the electrode/current-collector interface, and a pronounced low-frequency rise. The inset of Figure 1. c reveals a mid-frequency region with a 90° slope, suggesting similar ion diffusion constraints for both PC and DMSO

systems at their resting potentials. Conversely, the EIS plots at anodic and cathodic peak potentials show a departure from ideal capacitive behavior in the low-frequency range, with an almost uniform rise in both the imaginary and real components of the impedance (Come et. Al., 2012). This indicates that the solvent in the DMSO system facilitates redox-like reactions and lithium-ion transport within the MS-$Ti_3C_2Cl_2$ electrode, which is unexpected given the high ionic conductivity of water-based electrolytes. Furthermore, when EIS spectra were obtained at the maximum negative potential for each system (Figure 3. d), all Nyquist plots exhibited deviations from capacitive behavior in the low-frequency region and a marginally increased ion diffusion resistance (45° linear segment) compared to the OCV spectra. These findings align with the notion that the $Ti_3C_2T_x$ electrode's charge-storage mechanism is not solely capacitive.

The steep slope of the imaginary components at lower frequencies indicates that the kinetics of capacitive charge storage are more likely than those of a diffusion-controlled process. This inference is supported by the lack of a Warburg region, typically observed as a 45-degree line, in the mid-frequency range. Upon closer examination of the low-frequency domain, which is indicative of the charge storage mechanism, it is observed that the MS-MXene electrodes exhibit the most significant deviation from the ideal 90-degree phase angle. This deviation corroborates the CV signatures previously noted for these electrodes. Notably, a pronounced divergence is detected at the potentials corresponding to the onset of lithium intercalation within DMSO and PC electrolytic mediums (Figure 3. c-d), which is attributable to CV voltammograms that are reminiscent of redox activity.

CONCLUSION

The initial phase of the research initiative, encompassing the novel synthesis of MXene and the fabrication of electrodes, has been executed with success, culminating in the conduction of electrochemical assays via a battery half-cell configuration. These assays have elucidated that MXenes endowed with a novel hydrophobic termination moiety exhibit enhanced interaction with aprotic organic solvents as opposed to polar organic solvents. Electrochemical analyses have elucidated the significant influence of termination groups on MXenes on the mechanisms underlying charge storage. Additionally, it has been observed that non-aqueous electrolytes exhibit interactions with MS-MXenes that parallel the interactions noted with traditional MXenes in aqueous electrolyte solutions. Our study highlights the critical importance of choosing the right electrolyte solvent to affect the dynamics of lithium-ion intercalation in $Ti_3C_2T_x$ and MS-$Ti_3C_2Cl_2$ MXene electrodes in organic electrolytes. Specifically, using a lithium bis(trifluoromethylsulfonyl)amine (LiTFSI) in propylene carbonate (PC) electrolyte leads to complete desolvation of lithium ions within the $Ti_3C_2T_x$ layers, promoting charge storage mainly via pseudocapacitive intercalation. In contrast, replacing PC with acetonitrile (ACN) or dimethyl sulfoxide (DMSO) significantly decreases capacitance due to the entrapment of solvent molecules by lithium ions within the $Ti_3C_2T_x$ layers. This contradicts expectations based on carbon supercapacitor research, which suggests ACN should be superior because of its high ionic conductivity. These results emphasize the need to customize electrolyte composition according to the electrode material to direct the charge storage process and improve electrochemical performance. According to the Electrochemical Impedance Spectroscopy measurements, a detailed analysis of the low-frequency region, which reflects the charge storage process, reveals that the MS-MXene electrodes demonstrate a notable departure from the perfect 90-degree phase angle. This discrepancy supports the cyclic voltammetry findings previously associated with these electrodes. Importantly, a distinct deviation is observed at the potentials where lithium begins to intercalate in DMSO and PC electrolytes. This is consistent with the CV voltammograms, suggesting redox reactions are occurring. This study also presents a systematic approach to designing and pairing electrode materials with optimized electrolytes.

ACKNOWLEDGEMENTS

The research was sponsored by The Scientific and Technological Research Council of Türkiye (TÜBİTAK). J. Wang and Y. Zhang from Drexel University are acknowledged for helping with material characterization.

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2. POSTER PRESENTATIONS
2.1. ABSTRACTS

POSTER PRESENTATION

Preparation of mesoporous TiO_2 with enhanced crystallinity and evaluation of photocatalytic performance under UV irradiation

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Abstract

Thermally stable mesoporous TiO_2 nanoparticles can be synthesized by different routes. In this work, mesoporous titania was synthesized by an eco-friendly, simple, and fast-effective method [1], using titanium butoxide as a precursor and eucalyptus leaf extract solution as a reducing and capping agent to avoid the use of hazardous chemicals.For phase conversion from amorphous TiO_2 into crystalline anatase titania, the obtained material was soaked in water at elevated temperature for a prolonged time. This is an alternative process to the conventional calcination methods, which cause irreversible collapse of the porous structure during thermal treatment and drastically decrease the surface area [2, 3]. This is confirmed by BET analysis (the surface area of TiO_2 nanoparticles calcined at 500°C is 5.0646 m^2/g). The aim of our work is the preparation of porous TiO_2 with both a large surface area and enhanced crystallinity, and the evaluation of photocatalytic activity for the degradation of methylene blue as a model organic dye under UV irradiation (365 nm). The crystalline nature and structural formation of titanium dioxide NPs biosynthesized were confirmed by the X-ray diffraction technique, and functional groups of TiO_2 materials were confirmed by FT-IR spectroscopy [4, 5]. The results revealed that the biosynthesized material, treated in water at 90°C for 24 hours, exhibited an enhanced rate of photocatalytic degradation of the toxic dye (MB). A comparative study with biosynthesized TiO_2 calcined at 500°C was carried out for the oxidation of MB under UV (365 nm). Heat treatment in water could be used as a simple, mild, yet effective method to transform sol-gel derived amorphous TiO_2 nanostructures into a porous anatase phase with high surface area and controllable crystallinity.

Keywords: soaked in water, eco-friendly, enhanced crystallinity, *Eucalyptus* leaf, photocatalytic activity.

POSTER PRESENTATION

Synthesis, Characterization, Biological, and Molecular Docking Studies of (*Z*)-*N*-Substituted-4-(Pyridin-2-yl)-6-(1*H*-Pyrrolo[2,3-*b*]Pyridin-4-yl)Pyrimidin-2-Amine

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Abstract

A series of fifteen (*Z*)-*N*-substituted-4-(pyridin-2-yl)-6-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)pyrimidin-2-amine, was designed, screened computationally and synthesized. Structures of the compounds, were elucidated using spectroscopic methods like FT-IR, NMR (1H and 13C), and mass spectroscopy. The compounds were tested for antibacterial and antifungal potential and represented significant antibacterial and antifungal potential. The compounds were also studied for cytotoxicity in terms of per cent viability of cells against HepG2 cells and found to be in the range of 70–80% up to 100 µM. The molecular docking was also carried out to observe the formation of H-bond and binding affinities against the receptor GlcN-6P and observed that only the amino acid residues like GLU315, SER316, ASP474, ALA520, and ASN522 were able to form the H-bond with compounds with binding affinity (–8.5 to –6.7 kcal/mol).

Keywords: Pyrimidine, indole, antimicrobial, MTT assay, molecular docking

Acknowledgment: The author, Dr. Mohammad Arshad, is highly thankful to Dr. Hamza M. Maghraby (The Dean) and Dr. Talal Maghrabi (The Vice Dean), Al-Fayha College, Al-Jubail, Saudi Arabia for providing the necessary support to accomplish this study.

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POSTER PRESENTATION

Acetominophen retention by an industrial by-product as a biosorbant

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Abstract

Organic pollution from the pharmaceutical industry represents a major challenge for the environment and public health due to the accumulation of non-biodegradable products. To solve this problem, adsorption is a key technology that uses absorbent materials. This study used deffated date stones, a common natural residue in Algeria, to evaluate their adsorption capacity for paracetamol present in pharmaceutical industry wastewater. Physico-chemical parameters were used to characterize the functional groups on the surface of date stones, including Fourier transform infrared spectroscopy, the point of zero charge. Different operating parameters were studied in this work, such as contact time, initial concentration, temperature, pH and ionic strength. The adsorption kinetics perfectly follows the pseudo-second order model and Langmuir isotherm because of the high correlation coefficient R^2=0.999 and 0.937, respectively. The maximum adsorption capacity was 6.09 mg/g at the best yield of 63% in an equilibrium time of 10 minutes. The data showed that maximum adsorption occurs at pH = 7, and room temperature, allowing us to suggest that the process is very beneficial cost-effective. After the thermodynamic study, it was found that the process was favorable and spontaneous ($\Delta G < 0$), exothermic ($\Delta H < 0$) and physical in nature.
All the results showed that defatted date stones used as a biosorbent can be a very promising alternative for the elimination of PARA, thus contributing to the valorization of this agricultural by-product.

Keywords: Deffated date stones, valorization, paracetamol, biosorption, wastewater.

POSTER PRESENTATION

GC-MS-based molecular networking for ranking phytoconstituents as antifungals against phytopathogen

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Abstract

The search for effective antifungal agents from natural sources is critical in managing phytopathogenic fungi. This study investigates the antifungal potential of essential oils from twelve Asteraceae species collected in the Bogotá Plateau. Phytochemical distribution was analyzed using GC-MS-based molecular networking to classify compound types. Antifungal selection was performed through covariate statistics using single-Y orthogonal partial least squares (OPLS) analysis. The antifungal activity was assessed via a fumigant assay against *Fusarium oxysporum* to evaluate the mycelial growth inhibition on PDA solid cultures. The findings revealed that essential oils from four species exhibited significant antifungal activity against *F. oxysporum*, with IC_{50} above than 5 µL/L air. Seven compounds were identified as potential contributors to this antifungal effect. Among these, a terpinenol-like compound stood out as a primary candidate. This compound was individually tested against *F. oxysporum*, demonstrating strong antifungal activity and validating the covariate-based relationship suggested by the single-Y OPLS analysis. These results underscore the potential of essential oils from Asteraceae species as sources of novel antifungal agents. The identification of specific compounds responsible for antifungal activity provides a promising direction for future research and development in plant-based fungicides. The findings highlight the importance of integrating advanced analytical techniques and statistical methods in natural product research to discover and validate bioactive compounds. *The present study is a product derived from the project EXT-CIAS-3854 financed by Vicerrectoria de Investigaciones at UMNG, validity 2023.*

Keywords: GC-MS, Molecular Networkig, Covariate statistics, Asteraceae, Essential oils.

POSTER PRESENTATION

Structure-based virtual screening for selecting chitin synthase-targeting agents

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Abstract

A relevant pathogenic fungus responsible for significant agricultural losses worldwide is *Fusarium oxysporum*. Its control remains a major challenge due to the limited efficacy of existing antifungal agents and the development of resistant strains. In this study, we explored the antifungal bioactivity of a custom-made chemical library consisting of over 300 phenolic compounds, with a focus on identifying novel agents with fungistatic/fungicide effects against *F. oxysporum*. Using a combination of in vitro bioassays and structure-based virtual screening (SBVS), we aimed to uncover potential molecular targets and lead compounds for further development. The initial bioactivity screening revealed that ten compounds exhibited potent antifungal activity, with IC_{50} values below 30 µM. These active compounds demonstrated a significant capacity to inhibit the growth of *F. oxysporum*, positioning them as promising candidates for antifungal development. To elucidate potential mechanisms of action, SBVS was performed against chitin synthase, a key enzyme involved in the biosynthesis of chitin, an essential component of the fungal cell wall. The SBVS results identified three prenylphenolic compounds from the active subset that showed strong binding affinity to the chitin synthase enzyme, suggesting a possible mode of action. These compounds were further evaluated for their binding interactions, revealing critical residues involved in the stabilization of the enzyme- inhibitor complex. The identified interactions provide valuable insights into the molecular basis of inhibition and offer a foundation for rational design and optimization of chitin synthase inhibitors. *The present study is a product derived from the project EXT-CIAS-3854 financed by Vicerrectoria de Investigaciones at UMNG, validity 2023.*

Keywords: Molecular Docking, Chitin synthase, Virtual Screening.

POSTER PRESENTATION

Effect of essential oil of *Artemisia absinthium* plant against some bacterial strains

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Abstract

Aromatic plants represent a great source of molecules with therapeutic properties. In addition to primary metabolites, they produce other metabolites called secondary metabolites including essential oils. The therapeutic properties of essential oils have been known since ancient civilizations.*Artemisia absinthium* (Asteraseae) is a perennial, herbaceous and aromatic plant . It is native to Eurasia and North Africa. In Algeria it is called « Chehaîba » . *A.absinthium* essential oil was extracted by hydrodistillation using Clevenger-type apparatus for 3 hours.The yield of extraction was 0.54% (w/w). The antimicrobial activity of the isolated oil was evaluated by paper disc diffusion method, revealing that *Pseudomonas aeruginosa (*ATCC 27853) and isolated *Pseudomonas aeruginosa* were resistant to the essential oil . *A. absinthium* EO also showed moderate and moderate-to-weak antimicrobial activities on strains of *Escherichia coli* (ATCC 25922), Staphylococcus aureus (ATCC 25923), *Candida albicans* (ATCC 10231) and isolates of *Klebsiella pneumoniae* and *Serratia marcescens.*

Keywords: Essential oil, *Artemisia absinthium*, antibacterial activity

POSTER PRESENTATION

Characterization of green inhibitors extracted from some indigenous plants as a good solution for the environment, human health, as well as the protection of metals from corrosion.

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Abstract

For centuries, many plants used in alternative medicine have shown anti-inflammatory, antibacterial and anti-cancer properties, as well as antioxidant activity. These important properties are due to their chemical structure. The compounds in these structures contain heteroatoms such as nitrogen, phosphorus, sulphur, oxygen, or cyclic rings with delocalized electrons. So, the extracts of plant materials such as: roots, leaves, or fruits contain polyphenols, glycosidic compounds, triterpenes, tannins, flavonoids etc. These chemical structures have shown to be successful in the protection of metals or metal alloys from corrosion. This is why extraction from plant materials of a group of corrosion inhibitors, called green inhibitors is performed in this work. These types of inhibitors are easily extracted and at low costs. At the same time, they demonstrate high biodegradability and are environmentally friendly. The method of extraction and the solvent use can be selected in accordance with the chemical composition of the extracts. The green inhibitors presented in this research are the alcoholic extracts of walnut (Juglans regia L.) fruit septum, Ulmus L. leaves and Arundo Donax leaves. All these plants are native Albanian plants, and the last one is quite widespread. The dried plant materials were extracted with ethanol 70% for 24 hours, under reflux at 25°C. The prepared extracts have been analyzed with spectral methods such as: ATR-FTIR, UV-VIS and HPLC, that identified the presence of gallic acid, quercitrin, isoquercitrin and catechins in them. The material which was used for the testing of green inhibitors was iron Fe B500, often used in construction. The performed tests indicate a good protective efficiency for these extracts, as green inhibitors. The purpose of this research is to prepare these green inhibitors and to characterize their chemical composition.

Keywords: green corrosion inhibitors, environmental protection, spectral methods.

POSTER PRESENTATION

Application of *Ulmus* L. and *Arundo donax* leaf extracts as corrosion inhibitors for steel (HRS) Fe B500B)

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Abstract

The destruction of metal structures from corrosion brings catastrophic economic and environmental consequences. Penetration of acidic media into the concrete reinforcement (caused by condensation in coastal urban areas) through chemical or mechanical cracks in the metal surface, is the cause of corrosion of the reinforcement and further pollution of the environment by corrosion products. Mitigation of corrosion impact upon the environment is very important, especially using corrosion inhibitors. The synthetic compounds which are reported to have good anticorrosive activity are highly toxic to the environment. The challenge of the worldwide corrosion science groups is to find corrosion inhibitors that protect the environment from corrosive products pollution, being friendly, non-toxic, and acceptable to it. This challenge is the motivation of the scientific work in the working group of the corrosion laboratory, concretely the investigate of alcoholic extract of Ulmus L. leaves and Arundo Donax leaves as green inhibitors for concrete armor in acidic solution in presence of Cl- ions. The material under investigation is (HRS) Fe B500B, commonly used in Albanian construction as concrete reinforcement. Acidic medium is 1M HCl solution. The protection efficiency of the extracts has been studied in a competitive way: with the method of weight loss, electrochemical (potentiodynamic polarization) and thermodynamic, in the temperature's range 298-318K. The electrochemical methods at 25 ºC, represent inhibition efficiency respectively 90.53 % in the presence of 100 mg/L of extract in synergy with KI for Ulmus leaves extract and 87.17 % in the presence of 50 mg/L of extract for Arundo Donax leaves. Refereeing the results of weight loss method at 25 ºC, the inhibition efficiency was respectively 81.82 % in the presence of 100 mg/L of extract in synergy with KI for Ulmus leaves extract and 76.92 % in the presence of 50 mg/L of extract for Arundo Donax leaves. The inhibition efficiency of the extracts decreased with increasing of temperature in the range of 298–318 K.

Keywords: Green inhibitors, Ulmus L. and Arundo Donax leaf extracts, corrosion protection.

POSTER PRESENTATION

Investigation of the relation between surfactant head group size and formation of different types of lyotropic structures in binary surfactant/dye systems: A small-angle X-ray scattering study

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Abstract

Lyotropic phase properties of novel mixtures of some dodecylalkylammonium bromide surfactants (DAABr) in the mixtures of 1-dodecanol (DDeOH)/Sunset Yellow dye (SSY)/water were investigated in the frame of this study. DAABr surfactants were chosen as dodecyldimethylammonium bromide (DDMABr), dodecyldimethylethylamonium bromide (DDMEABr) and dodecyltriethylammonium bromide (DTEABr). In lyotropic mixtures, those surfactants were used in the binary form, i.e., DDMABr/DDMEABr and DDMEABr/DTEABr to control the average number of the methylene ($-CH_2$) groups in their head groups (n_{avg}) at the micelle surfaces. By changing the relative concentrations of each surfactant in the mixtures at constant total surfactant and mixture compositions, lyotropic mixtures presenting lamellar, nematic, and hexagonal phases were obtained. Small-angle X-ray scattering (SAXS) was mainly performed to evaluate micellar structure parameters by using a model proposed in the literature [1,2]. The results indicate that there is a close relation between the stabilization of different lyotropic structures and the n_{avg} values.

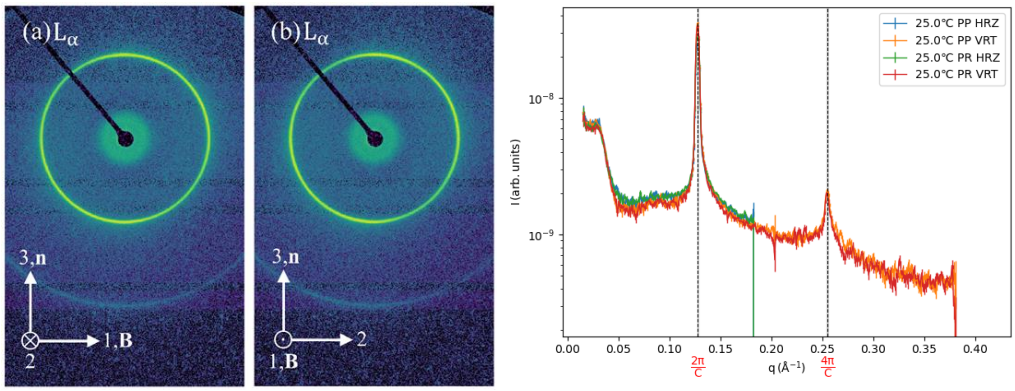

Figure. (*Left*) The 2D small-angle X-ray scattering (SAXS) patterns of the lamellar (L_α) phase (n_{avg}: 2.00). The pattern (a) was measured at 25.0 °C at perpendicular, PP, position. The pattern (b) was obtained at the same temperature at parallel, PR, position. For other samples, similar diffraction patterns for nematic and hexagonal phases were observed and will be presented in the conference. (*Right*) Frame integration of the patterns of the lamellar phase at 25°C for the PP and PR positions.

Keywords: Lyotropic liquid crystals, nematic, lamellar, hexagonal, small-angle X-ray scettering.

Acknowledgement: The study subjected to this presentation was financially supported by TUBITAK (grant no: 121Z084) and under preparation for publishing in a SCI/SCI-Exp journal (in 2024).

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POSTER PRESENTATION

Development of immunoassay for pesticides butachlor and acetochlor on the base of polyclonal antibodies

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Abstract

Chloroacetanilide pesticides (butachlor (BUT), acetochlor (AC)) were widely used in agriculture. The advent of organic farming and new studies on the toxicity of pesticides entering the soil led to a ban on the use of these preparations in the European Union. However, there is a need to determine their residual amounts in soil and water in rice-growing regions. Immunochemical methods based on specific antigen-antibody interaction are proposed for routine screening. Complementarity of analytes to recognition centres of antibodies makes these interactions unique and specific. As well as butachlor and acetochlor are low-molecular compounds, their conjugates with carrier proteins were obtained for immunization. In this work, the synthesizes derivatives of the BUT and AC with mercaptosuccinic (MSA) and mercaptopropionic (MPA) acids were conjugated by the methods of activated esters with bovine serum albumin (BSA) and soybean trypsin inhibitor (STI). Chinchilla rabbits were immunized with the obtained preparations and four blood serum collections were carried out. A total of 6 conjugates and 24 antiserum preparations were obtained. All 144 preparations were tested in a competitive enzyme-linked immunosorbent assay (ELISA) with two analytes – butachlor and acetochlor. The analytical characteristics were obtained for all pairs of reagents and optimal combinations were selected based on the calibration curves. The lowest detection limit for butalor was observed in the system with an immobilized conjugate with BSA-BUT-MPA and antiserum to STI-BUT-MPA, and was 0.8 ng/ml. In the case of acetochlor, the combination of BSA-AC-MSA on a solid phase and antiserum to STI-AC-MPA was optimal. In this case, the detection limit was 2.9 ng/ml. Based on the results obtained, modifications of hapten molecules were identified that make it possible to obtain the best preparations of specific antibodies for immunoanalytical purposes.

Keywords: immunoassay, butachlor, acetochlor, ELISA, conjugate.

This work was financially supported by the Russian Science Foundation (grant 23-46-00018).

POSTER PRESENTATION

Rhenium(V) complexes with picolinic acid derivatives – synthesis and chemical characterization

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Abstract

Rhenium has a broad spectrum of oxidation states in coordination compounds. However the most important state for application in medicinal chemistry is Re(I) with $[Re(CO)_3]^+$ core, while rhenium(V) and rhenium(VII) complexes are mostly used as catalysts[1]. Rhenium(V)-oxo complexes with chloride, triphenylphosphine and N,O ligands (picolinate or 8-hydroxy quinoline derivatives) have been described in literature as good catalysts for the catalytic epoxidation of cyclooctene and perchlorate reduction. Literature crystallographic data showed that the most stable isomer for those compounds was with the two chlorido ligands *cis* to each other, which was also confirmed by DFT calculations[2].
In this study we synthesized five new Re(V)-oxo complexes (C1 – C5) with general formula $[ReOCl_2PPh_3$(L1-5)], where L1-5 are 5-bromo, 6-bromo, 6-chloro, 6-fluoro and 5-hydroxo picolinic acid. All complexes were synthesized starting with $[ReOCl_3(PPh_3)_2]$ and corresponding ligand in acetonitrile with reflux for 24h and all of them precipitated as green powders. All complexes were characterized with ^1H, ^{13}C NMR and IR spectroscopy and structures of two crystalized complexes were additionally confirmed by X-ray analysis. Green crystals of those two complexes were obtained from acetonitrile solutions. Like most of the dichlorido-oxido-(triphenylphosphine)-rhenium(V) complexes, in those two complexes (C4 and C5) the two chlorido ligands are *cis* to each other, with the phosphine *trans* to one of them. The rhenium complex exhibits a distorted octahedral geometry with the picolinate ligand chelating the rhenium center through the nitrogen atom and one oxygen atom of the carboxylic acid group in an almost flat chelate. The Re-oxido ligand bond length is similar to the Re-oxido complexes previously reported, with the oxido ligand *trans* to the oxygen donor of the ligand[3]. Complex C5 co-crystalized with a phosphine oxide molecule and crystal structure showed an intermolecular hydrogen bonding between the hydroxy group and a phosphine oxide molecule.

Keywords: rhenium complex, picolinic acid, X-ray analysis

Acknowledgments: This work was supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia, contract numbers 451-03-66/2024-03/200168 and 451-03-66/2024-03/200288. We gratefully acknowledge the support from the European Union's Horizon Europe program under the Marie Skłodowska-Curie Actions grant agreement No. 101086373.

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POSTER PRESENTATION

Could cocoa (*Theobroma cacao* L.) extracts have applications in the treatment of cancer and inflammation?

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Abstract

Theobroma cacao L., also known as cocoa, has been used by human especially in diet for millennia. Nonetheless, current research has shown its therapeutic properties, notably during the treatment of inflammatory diseases and cancer. The aim of this review is to show a comprehensive review analyses the effect of *Theobroma cacao* extracts combined with gold and silver nanoparticles against cancer and inflammation. The diverse activity can be associated with its chemical profile, including alkaloids, flavonoids and polyphenols [1]. *In vitro* studies have shown that cocoa extract, has multiple biological effect like apoptosis induction, inhibition of cancer cells proliferation and signalling pathway modulation in different cancer cell lines [2,3]. Extracts also reduce side effects of known chemotherapeutic, enhance their efficacy and lower tumour growth *in vivo* [4]. The anti-inflammatory treatment are based of the abilities held by *Theobroma cacao* extract including inflammatory mediators modulation, NF-*k*B signalling inhibition and macrophage polarisation regulation [5, 6]. Mentioned activities have been observed in both animal and cellular models of inflammation [7]. This review suggests that cocoa extracts combined with conventional chemotherapy can lower organ toxicity induced by treatment.

Keywords: *Theobroma cacao*, anticancer, anti-inflammatory, polyphenols, *in vitro* studies, *in vivo* studies.

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POSTER PRESENTATION

Photocatalysis by advanced TiN/Cu-films inactivate *E*-Coli bacteria under low-intensity: A comparative study: DCMS, DCMSP and HIPIMS

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Abstract

Antimicrobial surfaces are of significant interest due to their potential to reduce or prevent hospital-acquired infections (HAIs) caused by antibiotic-resistant bacteria that can survive on hospital surfaces for prolonged durations. The objective of this study is to prepare, evaluate and optimize the high power impulse magnetron sputtering (HIPIMS) deposition of thin TiN/Cu-films on Cotton-textile and the comparison with films deposited by DC-magnetron sputtering (DCMS) and DC-magnetron pulsed sputtering (DCMSP) leading to *E. coli* inactivation. At the same time, the HIPIMS sputtered textile needed a) a shorter sputtering time and b) a lower amount of TiN /Cu on the textile for fast bacterial inactivation compared to DCMS and DCMSP. The *E. coli* complete inactivation occurred within 3 hours on DCMS sputtered samples and within 2 hours with DCMSP. HIPIMS-sputtering at 5 Amp for 80s led to complete *E. coli* bacterial inactivation within 1 hours loaded with Ti 0.03% Ti an amount much lower than the other sputtering modes. HIPIMS induced a larger Ti/Cu-coverage of the Cotton allowing for a more extended migration of electrons/charges between the Ti-nanoparticles and the bacteria in the order HIPIMS > DCMSP > DCMS. The evidence is presented by redox reactions Ti^{4+}/Ti^{3+} occurring in the photocatalyst during bacterial inactivation. X-ray photoelectron spectroscopy (XPS) was used to determine the surface atomic concentration of N, Ti, and Cu on the textile as a function of the *E. coli* inactivation time providing evidence for the bacterial oxidation on the TiN/Cu-textile.

Keywords: photocatalysis, sputtering, TiN /Cu-textile, *E.* coli.

POSTER PRESENTATION

The changes in root vegetables microbiota after ozonation process

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Abstract

Introduction: Root vegetables, such as carrots, parsley, and celeriac, are an important part of the diet. However, their close contact with soil exposes them to contamination by various microorganisms, including human pathogens and food spoilage microorganisms. The ozonation process, as a method of disinfection, is gaining popularity in the food industry.
Aim of the study: The objective of the study was to determine the impact of the ozonation process on the microbiota of selected root vegetables
Methods: Solid microbiological media on plates were used in the study. Samples for analysis were collected using sterile swabs moistened in sterile saline solution. Surface inoculation was performed using the streak method for each of the analyzed root vegetables, both before and after ozonation. The plates were incubated at 37°C for bacteria for 24–48 hours, and at 28°C for fungi for 24–48 hours. The ozonation process was carried out using an ozone generator with an ionizer [OZONE INSTRUMENT ZY-H103 by Eltom].
Results: The ozonation process was more effective against Gram-negative bacteria isolated on MacConkey Agar, especially bacteria from the *Enterobacteriaceae* family and the *Pseudomonas* genus. A significant reduction in colonies on the Rodac ConTact Test medium, which measures the total microbial count, was also observed after the ozonation process. Fungi, including both yeasts and molds, proved to be more resistant to the effects of ozone compared to bacteria.
Conclusions: Bacteria are more sensitive to ozone than yeasts or fungi, with Gram-positive bacteria being more susceptible than Gram-negative bacteria.

Keywords: root vegetables, ozonation, bacteria, fungi

POSTER PRESENTATION

Could flavonoids be effective topoisomerase inhibitors for future cancer treatment?

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Abstract

Plant-derived secondary metabolites known as flavonoids are of great interest due to their potential anticancer properties [1]. This review examines the ability of flavonoids to inhibit DNA topoisomerases, essential enzymes in DNA replication, transcription and cell division [2]. The review presents the classification of flavonoids, their biosynthesis and natural sources of occurrence. It then delves into the molecular mechanisms underlying the anticancer effects of flavonoids, highlighting their interactions with topoisomerases. The review offers a detailed analysis of both laboratory and animal studies demonstrating topoisomerase inhibitory effects of various flavonoids and their derivatives. Key findings highlight the ability of flavonoids to act as catalytic inhibitors, poisons or DNA intercalators, affecting both type I and type II topoisomerases [3][4]. The structure-activity relationships of flavonoids were investigated in relation to their topoisomerase inhibition efficacy. This review highlights the potential of flavonoids as promising candidates for the development of novel topoisomerase inhibitors that may have a profound impact on cancer treatment [5]. However, it also identifies the need for additional studies to fully understand the complex interactions between flavonoids and topoisomerases in cellular environments.

Keywords: flavonoids, anticancer agents, DNA topoisomerase inhibition, in vitro studies, in vivo studies

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POSTER PRESENTATION

Box-Behnken Optimization of Beta Glucan Nanoparticles Synthesis Conditions

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Abstract

Cancer is a fatal disease resulting from abnormal cell proliferation and spread. Genetic, environmental and lifestyle factors play a role in the development of cancer. Conventional treatments often cause side effects. In recent years, smart drug delivery systems such as immunotherapy, hormone therapy, angiogenesis inhibitors have made significant progress in cancer treatment. These systems minimise side effects by enabling direct delivery of drugs to tumour cells.The evaluation of the usability of nanoparticles obtained from polysaccharides such as beta glucans as drug delivery systems in cancer treatment may allow the development of a new and effective method. Beta glucans are known to enhance natural immune responses against cancer cells by increasing the activation of immune system cells, induction of apoptosis (programmed cell death) in cancer cells and antiinflammatory effects, emphasising their antitumoural effects. These effects may help fight cancer by controlling the growth and spread of cancer cells. The aim of this study is to synthesise beta glucan nanoparticles to be used as a smart drug carrier in cancer treatment. For this purpose, optimization of three important nanoparticle synthesis parameters (temperature, time, NaOH concentration) using Box-Behnken experimental design was performed. The nanoparticles obtained as a result of the optimization study will be characterised by TEM, DLS and BET analyses and the suitable nanoparticle will be selected as the drug carrier for different cancer treatment drugs.

Keywords: Yeast betaglucan, optimization, nanoparticles,cancer

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POSTER PRESENTATION

Investigation of GDF9 Gene Exon II Polymorphism in Central Anatolian Merino Sheep

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Abstract

In sheep breeding, the number of lambs to be born at a birth varies depending on many factors. In addition to genetic structure, environmental factors, feed amount, feed quality, and health services are important parameters affecting the number of lambs. It would be better to develop and work on breeding programs by considering the genotypes of the sheep to improve the ovulation rate in terms of the number of lambs to be obtained. It has been observed that mutations determined in some major genes (BMP1B, BMP15 and GDF9), which are members of the TGFβ superfamily that affect the ovulation rate, cause multiple lambing or infertility according to the inheritance patterns. In this study, mutations in GDF9 gene exon II were investigated by DNA sequence analysis method in Central Anatolian Merino sheep bred in various enterprises in Eskisehir Province Sivrihisar District of the Central Anatolian Merino Sheep Breed Sub-Project carried out with the support of the Ministry of Agriculture and Forestry.After DNA isolation from blood, PCR assay was performed with the relevant primers and the PCR products were visualized on agarose gel electrophoresis. PCR products were sequenced using the automated DNA Sequencer and genotypes were determined. By comparing and aligning the obtained sequences with the gene bank data, two synonymous (A>G; G>A) mutations and four missense (A>G; G>C; C>A; G>A) mutations for exon II were detected in the studied population. This study determined mutations that will affect the fertility and the number of offspring per birth of the animals examined. It aims to contribute to increasing the number of lambs at birth by obtaining data that will guide breeding studies.

Keywords: Central Anatolian Merino, DNA sequences, fecundity genes, GDF9.

This study was supported by a grant from Scientific Research Projects of Eskişehir Osmangazi University (Project No: FLO-2024-3026; Project coordinator: Dr M. Kaya).

POSTER PRESENTATION

Investigation of GDF9 Gene Exon I Polymorphism in Central Anatolian Merino Sheep

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Abstract

In sheep breeding, the number of lambs to be born at a birth varies depending on many factors. In addition to genetic structure, environmental factors, feed amount, feed quality, and health services are important parameters affecting the number of lambs. It would be better to develop and work on breeding programs by considering the genotypes of the sheep to improve the ovulation rate in terms of the number of lambs to be obtained. It has been observed that mutations determined in some major genes (BMP1B, BMP15 and GDF9), which are members of the TGFβ superfamily that affect the ovulation rate, cause multiple lambing or infertility according to the inheritance patterns. In this study, mutations in GDF9 gene exon I were investigated by DNA sequence analysis method in Central Anatolian Merino sheep bred in various enterprises in Eskisehir Province Sivrihisar District of the Central Anatolian Merino Sheep Breed Sub-Project carried out with the support of the Ministry of Agriculture and Forestry. After DNA isolation from blood, PCR assay was performed with the relevant primers and the PCR products were visualized on agarose gel electrophoresis. PCR products were sequenced using the automated DNA Sequencer and genotypes were determined. By comparing and aligning the obtained sequences with the gene bank data, G>A missense mutation for exon I were detected in the studied population. This study determined mutations that will affect the fertility and the number of offspring per birth of the animals examined. It aims to contribute to increasing the number of lambs at birth by obtaining data that will guide breeding studies.

Keywords: Central Anatolian Merino, DNA sequences, fecundity genes, GDF9.

This study was supported by a grant from Scientific Research Projects of Eskişehir Osmangazi University (Project No: FLO-2024-3010; Project coordinator: Dr M. Kaya).

POSTER PRESENTATION

A preliminary study of the effect of *Chlorella sp.* and *Porphyridium cruentum* on hepatic CYP1A1 activity

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Abstract

7-ethoxyresorufin O-deethylase (EROD, CYP1A1) is a critical enzyme in the metabolism of a wide range of substances such as polycyclic aromatic hydrocarbons, aromatic amines, steroids, and fatty acids. Understanding and modulating EROD activity might have profound effects on health outcomes and therapeutic strategies. *Chlorella sp*. and *Porphyridium cruentum* are both valuable microalgae with distinct bioactive compounds that offer various health benefits. *Chlorella sp.* is known for its high antioxidant content, including vitamins C and E, beta-carotene, and chlorophyll. On the other hand, *Porphyridium cruentum* is a potent source of antioxidants, with its sulfated polysaccharides, pigments, and polyunsaturated fatty acids contributing to its ability to combat oxidative stress. These bioactive compounds with antioxidant properties can potentially influence the overall antioxidant defense system of organisms, which might indirectly affect EROD activity. Since CYP1A1-mediated metabolism can generate reactive oxygen species, antioxidants from both of the microalgae could mitigate oxidative damage. When a literature review is done, it seems that there are almost no direct studies about the relationship between EROD activity with both *Chlorella sp.* and *Porphyridium cruentum*. For all of these reasons, we aimed to examine the effect of methanol and acetone extracts of Chlorella sp. and Porphyridium cruentum on the activity of EROD in the liver of rats in the present study. As a result of our experiments, we found that methanol extracts of *Chlorella* sp. inhibited EROD activity at the highest level. Specific studies would be needed to determine whether *Chlorella sp.* and *Porphyridium cruentum* compounds have such effects on EROD activity.

Keywords: EROD activity, CYP1A1, hepatic, *Chlorella sp.*, *Porphyridium cruentum*

POSTER PRESENTATION

Determination of Nitrosamine Impurities in Medicines

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Abstract

Nitrosamines are extremely harmful carcinogenic, mutagenic and teratogenic impurities that have been detected in drugs recently. These impurities can be included in the drug substances by being formed by the reagents, catalysts, solvents or raw materials used in the production process, or they can also occur after the drugs are produced, depending on the storage conditions, packaging materials and the decomposition processes of the active substances. In this study, it was aimed to determine the amounts of N-nitrosodimethyl amine (NDMA) and N-nitrosodiethyl amine (NDEA) in drugs containing sartan and its derivatives. Medicines containing sartan and its derivatives as active ingredients such as valsartan, losartan, telmisartan, olmesartan, candesartan, irbesartan were purchased from pharmacies. NDMA and NDEA amounts of drugs were determined by headspace GC-MS. The highest NDMA concentrations were found in drugs with losartan active ingredient (67.05 ± 0.15mg kg^{-1}), and the highest NDEA concentrations were found in drugs with candesartan (22.48 ± 0.06 mg kg^{-1}) and olmesartan (20.13 ± 0.06 mg kg^{-1}) active ingredient. The NDMA contents in each tablet were between $3{,}6.10^{-4}$ - $1{,}7.10^{-2}$ mg and the NDEA contents were between $3{,}3.10^{-4}$ - $8{,}8.10^{-3}$ mg. The acceptable daily intake limits specified by the European Medicines Agency and the Food and Drug Administration are 96 ng/day for NDMA and 26.5 ng/day for NDEA. It was observed that the values obtained were above the acceptable intake limits even if one tablet was taken per day.

Keywords: Drug, headspace GC-MS, N-nitrosodiethyl amine (NDEA), N-nitrosodimethyl amine (NDMA), sartan

POSTER PRESENTATION

Determination of Phthalates in Pharmaceuticals

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Abstract

Phthalates are used as functional excipients in many oral pharmaceutical formulations. They are most commonly used as a plasticizer in enteric film coating materials or as a matrix binder in tablets, capsules, dragees and granules. Due to their plasticizing properties, phthalates are also included in soft gelatin capsule formulations. They are also used to control the viscosity of certain liquid formulations. Today, it is known that phthalates are among endocrine disrupting chemicals. Studies conducted on animals have revealed that phthalates have many negative effects on health. It has been determined that phthalates cause many diseases, especially heart, urological, endocrine and genital diseases, and play a role in the development of testicular and prostate cancer in men and breast cancer in women. It is extremely important to know which drugs contain endocrine disrupting chemicals such as phthalates, which threaten human health, and in what quantities. Within the scope of this study, the phthalates and their amounts contained in drugs used in anti-inflammatory, painkillers, anti-acids, cholesterol and blood pressure were determined. Then, the phthalate amounts in each tablet of the examined drugs were calculated. Maximum exposure rates and hazard indexes were also calculated to determine the potential risks arising from the phthalates exposure due to the drugs used. In the drug samples examined, it was determined that total phthalate concentrations varied between 0.209 and 4.800 mg kg^{-1}, and the amounts in a single tablet varied between $2.7.10^{-5}$ and $1.5.10^{-3}$ mg/tablet. The highest concentrations of total phthalate were found in cholesterol (4.800 mg kg^{-1}) and blood pressure lowering drugs (4.442 mg kg^{-1}). The calculated hazard index values were found to be less than one. This showed that the phthalates detected in the drugs examined did not pose a risk to human health.

Keywords: Phthalate, medicine, pharmaceutical, endocrine disruptor, GC-MS

POSTER PRESENTATION

In vitro effects of dapagliflozin on liver: A preliminary study

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Abstract

It is important that antidiabetics present a good hepatic safety profile due to nonalcoholic fatty liver disease (NAFLD), one of the leading causes of chronic liver disease worldwide is mostly associated with type 2 diabetes (T2DM). Dapagliflozin, the first globally approved sodium-glucose cotransporter 2 (SGLT2) inhibitor in the treatment of T2DM, reduces blood glucose levels by an independent mechanism of insulin. Several studies reported inconsistent results on the hepatic effects of dapagliflozin. This preliminary study aimed to determine the appropriate drug concentrations needed to treat HepG2 cells to assess the biochemical and cell death-related effects of dapagliflozin. Firstly, concentrations of dapagliflozin to be applied were selected according to the Cmax (0.5µM) value, and the literature information was considered. Cells were treated with a wide range of final concentrations (0.5-100µM) for 24 hours (at least n=3) followed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) cell viability test. Subsequently, 0.5, 10, and 50µM final concentrations were examined for 48 and 72 hours separately. Any final concentration of dapagliflozin did not inhibit the 50% of cell viability for any time, therefore IC50 value could not be established in the HepG2 cell line. This study was supported by the Health Institutes of Türkiye (TUSEB) (grant no: 2023-A4-04-38071) and the Research Foundation of Marmara University (grant no: TYL-2024-11306).

Keywords: Dapagliflozin, SGLT2 inhibitor, HepG2, MTT, Cell viability

POSTER PRESENTATION

Development and Validation of RP-HPLC Impurity Method for Safinamide Mesylate Impurities and Isomeric Impurities in its Tablet Dosage Form

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Abstract

Parkinson's disease is a neurological disorder characterized by involuntary and uncontrolled movements. Patients experience tremors and difficulty with coordination while performing daily activities. Proper medication, such as Safinamide, can help control tremors and other motor issues. It operates as a selective MAO-B inhibitor, enabling an increment in dopamine levels within the brain (Sivanandy et al., 2022). Managing impurities in medications is vital for ensuring patient safety. Analytical techniques such as High-Performance Liquid Chromatography (HPLC) are commonly employed for detecting, identifying, and quantifying impurities in medical products. Isomeric impurities are challenging to identify using reversed-phase chromatography; therefore, isomer separation and quantification are typically conducted with normal-phase chromatography (Chhabra et al., 2013). However, this study uses a straightforward method for determining para and ortho isomers of safinamide mesylate using RP chromatography. A reverse-phase liquid chromatographic impurity method has been developed and validated for safinamide mesylate and its isomers in tablet formulation. Separation was carried out using an RP-18 column with a mobile phase composed of methanol and phosphate buffer (pH 6.5) at a ratio of 47.5:52.5, pumped at a flow rate of 0.8 ml/min. Detection occurred at a wavelength of 224 nm, with the column maintained at 25°C. The validated analytical method investigates the degradation behaviour of safinamide mesylate tablets under stress conditions such as acidic, basic, oxidative, and thermal. The validated HPLC method identified related impurities and degradation impurities without any interference. The developed method is well-suited for use in the pharmaceutical industry due to its simplicity in analysing impurities in tablet formulations containing the active ingredient Safinamide Mesylate.

Keywords: Parkinson’s Disease, Safinamide Mesylate, Isomeric impurities, RP-HPLC, Degradation

Acknowledgement: This study was supported by the Research Fund of Istanbul Technical University (Project number: 45901) and Ali Raif İlaç San. Tic. A.Ş.

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POSTER PRESENTATION

Characterization of 7-Ethoxyresorufin *O*-deethylase activity (EROD) in *Asparagus officinalis* L. extracts

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Abstract

There is evidence in the literature that the simultaneous use of medicinal drugs and several foods can cause food-drug interactions (1). The genus *Asparagus* L. includes approximately 300 species, which are mainly distributed in the Mediterranean region, Southern Europe, Anatolia, Asia, Africa, and Europe (2). It is noted that several *Asparagus* species are used to treat rheumatism, arthralgia, epilepsy, urinary system diseases, stomach ailments, and respiratory system diseases such as bronchitis, pharyngitis, laryngitis, colds, and flu. The edible young shoots of *Asparagus officinalis* L., known as "kuşkonmaz", are widely consumed as vegetables in salads, meals, and soups (3, 4). Cytochrome P450 (CYP450) is a group of enzymes expressed in different organs, mainly in the liver, and involved in xenobiotic and drug metabolism. Among CYP enzymes, CYP1A1 is responsible for the metabolism of many drugs such as caffeine, theophylline, paracetamol, riociguat, granisetron, axitinib, erlotinib, and conivaptan (3). Upon review of the literature, there are no studies assessing the efficacy of *A. officinalis* on CYP1A1. The objective of this study was to evaluate the activity of methanol and ethyl acetate extracts obtained from the young shoots of *A. officinalis* on CYP1A1. The effects of 5 mg/mL doses of methanol and ethyl acetate extracts prepared from young shoots of *A. officinalis* on CYP1A1 enzyme activities in rat brain microsomes were investigated using the 7-Ethoxyresorufin *O*-deethylase (EROD) assay. EROD activity is measured by observing the CYP1A1-mediated deethylation of the substrate 7-ethoxyresorufin to generate the product resorufin, which can be fluorometrically monitored. Caffeine was used as a standard, whereas dimethyl sulfoxide, in which the extracts were dissolved, was chosen as a control. Methanol and ethyl acetate extracts from young shoots of *A. officinalis* inhibited CYP1A1 enzyme activity by 81% and 73%, respectively (caffeine: 63%). The study predicted that *A. officinalis*, which is widely consumed as food, affects CYP1A1 activity. *A. officinalis* should be carefully consumed with drugs metabolized by the CYP 1A1 isozyme.

Keywords: food-drug interaction, *Asparagus officinalis* L., EROD assay, CYP 1A1.

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POSTER PRESENTATION

Investigation of the effect of the presence of Hofmeister ions on the properties of ionic surfactants in micellar solutions

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Abstract

Electrolytes affect the micellization properties of ionic surfactants [1]. When they ionize in water, they give ions to the bulk solution. Depending on kosmotropic and chaotropic properties of these ions, which determine their sequence in Hofmeister series of ions, surfactant head groups interact them differently at micelle surfaces. Those kinds of interactions have been widely investigating in the literature [2]. In the present study, we aim to investigate the effect of existence of Hofmeister ions in the intermicellar region on the micellization of an ionic surfactant. For this purpose, the effect of LiBr and KBr, where Li^+ and K^+ is classified as kosmotrope and chaotrope, respectively, on the micellization properties of a chaotropic ionic surfactant dodecyltrimethylammonium bromide (DTMABr) by determining critical micelle concentration (cmc) of DTMABr in the absence and presence of those electrolytes from electrical conductivity measurements. Furthermore, the effect of the concentration of both electrolytes on cmc of DTMABr was also investigated.

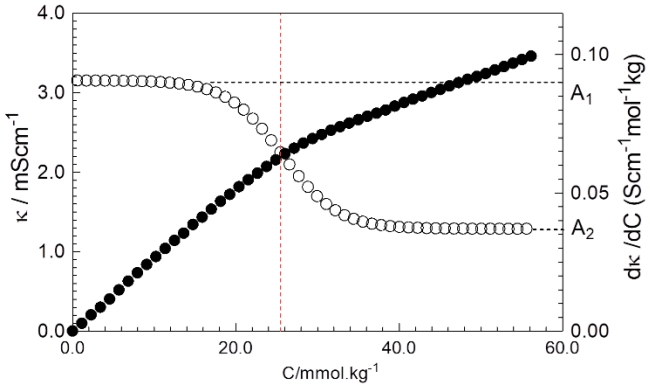

Figure. A representative graph of specific conductivity (κ) – total surfactant concentration (C). The data shown by (●) and (○) correspond to the change of κ and first derivative of κ - C (dκ/dC) with C, respectively. The dashed red line shows the cmc of the surfactant. The degree of counterion ionization can be calculated from A_2/A_1.

Keywords: Micellization, surfactant, Hofmeister ions, conductivity.

Acknowledgement: The study subjected to this presentation was financially supported by TUBITAK-1001 program (grant no: 122Z515) and under preparation for publishing in a SCI/SCI-Exp journal (in 2024).

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POSTER PRESENTATION

Effect of the dielectric constant of the medium on the micellization properties of an ionic surfactant in the presence of an electrolyte

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Abstract

It is well-known that being an important physical and chemical property of liquid, dielectric constant may control interactions between solute and solvent, and solvation microstructure [1]. Surfactants have amphiphilic character (hydrophilic and hydrophobic) and form various aggregate structures, so-called 'micelles', in bulk solutions due to their self-assembly behavior. They are widely used in some industrial and biotechnological applications [2,3]. In this study, we have studied the dielectric properties of potassium dodecanoate (KDD) micelle aggregates at constant KDD and electrolyte concentrations. Two organic solvents were chosen: ethylene glycol (EG) and formamide (FA). The micellization properties of KDD were studied at different polar solvent/water (in mol%) mixtures. The results indicated that the dielectric constant of the medium affects the micellization properties of KDD surfactant, Figure.

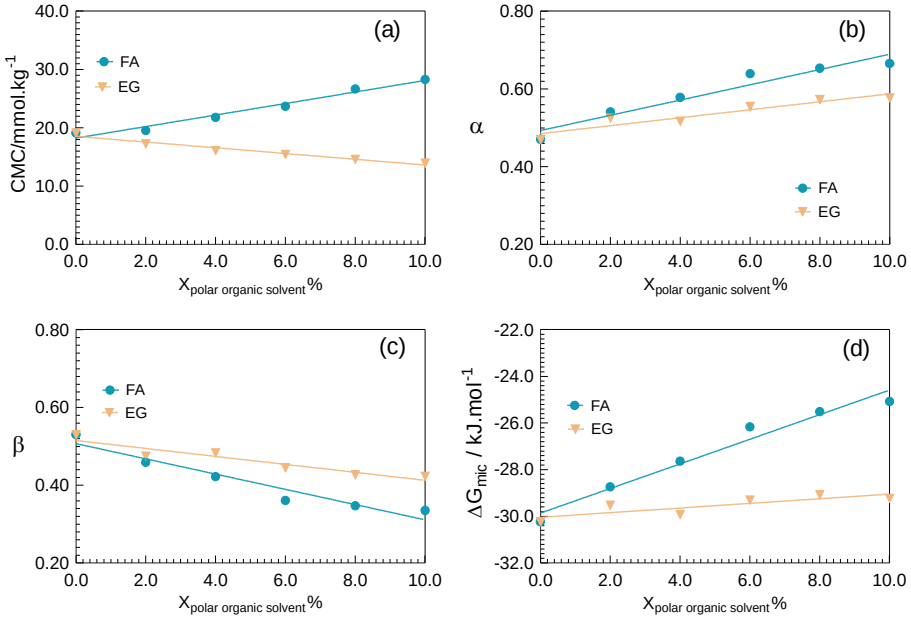

Figure. Change of some micellization properties of KDD in different polar organic solvent/water solutions: (a) critical micelle concentration, cmc, (b) degree of counterion ionization, α, (c) degree of counterion binding, β, and (d) micellization Gibbs free energy, ΔG_{mic}.

Keywords: Micellization, surfactant, dielectric constant, conductivity.
Acknowledgement: *The study subjected to this presentation was financially supported by TUBITAK-1001 program (grant no: 122Z515) and under preparation for publishing in a SCI/SCI-Exp journal (in 2024).*

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POSTER PRESENTATION

Dependence of the formation of lyotropic uniaxial and biaxial nematic phases on the existence of Hofmeister ions in the intermicellar region

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Abstract

Some experimental studies in the literature showed that Hofmeister ions present at the micelle surfaces play a crucial role on obtaining different nematic phases [1]. In this study, lyotropic nematic phase properties of some potassium dodecanoate (KDD)/electrolyte/decanol (DeOH)/water mixtures were studied to investigate the effect of the existence of Hofmeister ions in the intermicellar region by using KF, KCl, KBr, KI and KSCN as electrolytes. All these 1:1 type electrolytes include Hofmeister anions. The nematic-nematic phase transitions were determined by laser conoscopy technique, which is a very useful to evaluate the phase transitions from the temperature dependence of the birefringences of the nematic phases [2], Figure 1. The textural analysis was performed by a polarizing optical microscope, Figure 2. The results indicated that the existence of Hofmiester ions in the intermicellar region affect on the formation of different nematic phases and nematic-nematic phase transitions.

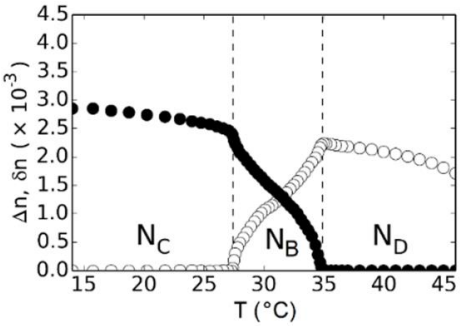

Figure 1. Temperature dependence of the nematic phases for KDD/KCl/DeOH/water mixture. The second-order nematic-nematic phase transitions were observed as predicted by Landau theory [3].

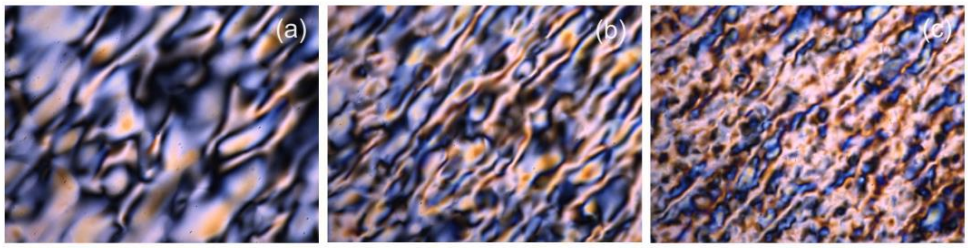

Figure 2. Texture of the nematic phases of KDD/KCl/DeOH/water mixture: (a) 40°C, discotic nematic, N_D, (b) 30°C, biaxial nematic, N_B, (c) 25°C, calamitic nematic, N_C. Sample thickness: 0.3 mm. Objektive: 10x.

Keywords: Lyotropic nematic phases, birefringes, phase transitions, laser conoscopy.

Acknowledgement: The study subjected to this presentation was financially supported by TUBITAK-1001 program (grant no: 122Z515) and under preparation for publishing in a SCI/SCI-Exp journal (in 2024).

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POSTER PRESENTATION

Recycling Management Practices In Local Governments

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Abstract

Packaging waste refers to the material that, after being used to prevent damage during the transfer of goods, becomes waste once the process is completed. This type of waste is predominantly recyclable and reusable. Recycling, as opposed to discarding materials post-use, plays a crucial role in environmental protection and economic development. The rapidly growing population leads to increased demand for raw materials, which in turn exacerbates ecological imbalances. Effective recycling practices contribute to maintaining the natural balance by reducing the need for raw materials. Additionally, recycling and reusing waste offer substantial energy savings. Despite the significant emphasis placed by countries and local governments on recycling and zero-waste initiatives, research indicates that achieving the desired efficiency remains challenging due to practical implementation issues. Şişli District in Istanbul, recognized for its high levels of education and environmental awareness, has undertaken various initiatives for the separate collection of packaging waste at the source for over two decades. Nonetheless, despite extensive educational efforts, the district has not achieved the desired level of success. The Şişli Neighbor Card Project, which has been implemented in the district for an extended period, has notably improved the separate collection of packaging waste at the source, particularly in households. However, the overall efficiency of this initiative has not reached the anticipated levels.

Keywords: zero waste, rcycle, reuse, energy saving, sustaniable waste management

Acknowledgement: This study was supported by İstanbul, Şişli Municipality, Antalya Muratpaşa Municipality

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POSTER PRESENTATION

Fruit anatomy of four subspecies of *Heracleum sphondylium* L.

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Abstract

Heracleum sphondylium L. (Apiaceae) is native to Türkiye with four subspecies. These subspecies are *H. sphondylium* subsp. *cyclocarpum* (K.Koch) P.H.Davis, subsp. *montanum* (Schleich. ex Gaudin) Briq., subsp. *ternatum* (Velen.) Brummitt, and an endemic subsp. *artvinense* (Manden.) P.H.Davis. The aim of this study is to determine the anatomical features of four subspecies of *H. sphondylium* and to reveal their similarities and differences. The Apiaceae family's anatomical fruit characteristics are important for taxonomy and plant identification. Therefore, mature fruit specimens were collected from different areas in Türkiye. Fruits were kept in glycerin for at least one week, and then at least three fruits were transversely sectioned by hand with razor blades. Alcian blue and basic fuchsin were used to stain the sections. Sections were mounted in glycerin and observed under light and stereo microscopes. The lengths and widths of mericarps, endosperms, vascular bundles, vallecular, and commissural vittae were measured by ImageJ software. Commissural vittae and mericarp shapes in the transverse sections, hypendocarp, and rib oil ducts showed similarities between subspecies. Meanwhile, fruit indumentum, dorsal ribs, vallecular vittae shape, and existence of druse crystals in the endosperm found to be the distinguishing characteristics.

Keywords: *Heracleum sphondylium*, Apiaceae, Fruit anatomy.

POSTER PRESENTATION

Antipsikotik ilaç etken maddelerinden brekspiprazolün ilgili bileşikler analizinin RP-HPLC ile belirlenmesi

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Özet

Brekspiprazol psikiyatrik hastalıkların tedavisinde kullanılan antipsikotik yatıştırıcı ilaç gruplarından biridir. Dopamin D2 ve serotonin 1A reseptörlerine karşı kısmi agonist etki gösterir. Esas olarak şizofreninin tedavisinde kullanılmakla birlikte majör depresif bozukluk için ek bir tedavi seçeneği olarak da kullanılabilmektedir. Diğer tipik ve ikinci nesil antipsikotik ilaçlarda sıkça görülen yan etkiler ile karşılaştırıldığında daha düşük risk taşıdığı görülmüştür (Das vd., 2016). Bir ilaç maddesinin çeşitli çevresel koşullar altındaki saflık profilini ve davranışını bilmek çok önemlidir (Bhaskar vd., 2020). Bunlar stres testleri ile görülebilir. Stres testleri; ilaçların aşırı koşullara maruz bırakılarak bozunma ürünlerinin oluşumunu hızlandıran süreçlerdir. Bu çalışmalardan elde edilen bilgiler genellikle kimyasal stabiliteyi belirlemek, bozunma yollarını tespit etmek, bozunma ürünlerini tanımlamak, saklama koşullarını ve raf ömrünü değerlendirmek, yardımcı maddelerin uyumluluğunu belirlemek ve seçici analitik yöntemlerin geliştirilmesine olanak sağlamak için kullanılır (Roberto de Alvarenga Junior ve Lajarim Carneiro, 2019). Geliştirilen metotda bozundurma çalışmalarıyla ilgili spesifik koşullar ve yöntemler ICH Q1A(R2) kılavuzu esas alınarak; asidik, bazik, oksidatif ve termal ortam şartları altında çalışıldı. Ayrım C18 kolon ile sağlanarak mobil fazın %0.1 fosforik asit tamponu ile asetonitril ve metanolün hacimce 80:20 oranında kullanıldığı, değişen gradient program ile gerçekleştirildi. Metodun akış hızı 1 ml/dk'ya ayarlanarak 225 nm dalga boyunda çalışmalar yapıldı. Bu çalışmada brekspiprazol etken maddesine zorlaştırılmış bozundurma çalışmaları(stres testleri) uygulanarak, bir ters fazlı yüksek performanslı sıvı kromatografisi ile ilgili bileşiklerinin ayrımı gerçekleştirildi. Böylece brekspiprazolün bozunma ürünleri ve diğer bağlı madde safsızlıkları ile girişim yapmadığı özgül bir analitik metodun geliştirilmesine katkı sağlandı.

Anahtar kelimeler: Brekspiprazol, Bozundurma, HPLC

Teşekkür: Bu çalışma Ali Raif İlaç San. Tic. A.Ş. tarafından desteklenmektedir.

Kaynaklar:

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POSTER PRESENTATION

Determination of antimicrobial activity of *Digitalis ferruginea* subsp. *ferruginea*

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Abstract

Antimicrobial activity studies have increased with the understanding of how important microbial resistance is for public health. The discovery that natural agents efficient against increasing resistance are effective at this point is one of the reasons for the increase in these studies. Especially the fact that the most effective natural antimicrobial compounds are found in plants is the reason why plants are preferred in these studies. Their ability to act on various microorganisms is another important reason for the use of plants. *Digitalis ferruginea* subsp. *ferruginea*, also found in Turkiye and popularly known as beehive, is also used in bioactivity studies. *D. ferruginea* subsp. *ferruginea* is a robust plant belonging to the *Plantaginaceae* family. It is a perennial species distributed in the Black Sea region and western parts of Turkiye. Studies have shown that it is rich in phenolic component content. It is known that compounds established to be effective such as rutin and ferulic acid are predominant. In this study, the antimicrobial activity of *D. ferruginea* subsp. *ferruginea* was evaluated using the disk diffusion test. A total of 40 strains were used for the test. In the study carried out with ethanol extract, the disks were impregnated with 40 µl and 160 µl amounts. The highest antimicrobial effects were observed in standard isolates of *Staphylococcus epidermidis* DSMZ 20044 (18-24 mm), *Staphylococcus aureus* ATCC 25923 (12-19 mm) and multidrug-resistant *Staphylococcus aureus* (15-20 mm). Scientific studies of *D. ferruginea* subsp. *ferruginea* can be improved by using the ability of *Pseudomonas* species to metabolize the rutin component. The bacterium *Pseudomonas fluorescens* P1 (0-11 mm) used in this study and the result obtained can be a start to guide future studies.

Keywords: *Digitalis ferruginea* subsp. *ferruginea*, antimicrobial activity, disk diffusion test

POSTER PRESENTATION

Study of salivary lithiasis

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Abstract

Introduction: This study reports the chemical composition and morphological aspects of salivary stones analyzed by infrared spectroscopy, and their correlation with some characteristics of the patients studied, like age, symptoms and radiographic aspects. Materials and Methods: Our study focused on salivary stones collected after surgical excision performed in the dental pathology department of the dental clinic of Monastir and the ORL department of Fattouma Bourguiba university hospital. Five patients were the study population, consisting of (4 women and one man), the patients were all adults. The Analysis by infrared spectroscopy Fourier transform has identified the different types of salivary calculi, their forms and their biological components. Results: The clinical signs reported by 4/5 of the patients were pain during meals and swelling of the gland. The diagnosis of this pathology in the population studied was mainly clinical and radiological. The key examination was the bi-digital palpation of the stone and the management consisted of the surgical removal of the sialolith. Chemical analysis of the collected stones revealed two ovoid stones, two irregular stones and only one compact stone. The consistency of all the stones was hard, the aspect of the section was mainly concentric, and the nucleus was not detectable. The composition of the surface consisted mainly of proteins. The section was made up of protein and hydroxyapatite. Conclusion: Our results showed the main clinical signs and radiographic aspects of salivary stones as well as the biological aspects of the collected salivary stones; their shape, their density and their chemical composition.

Keywords : Lithiasis, saliva, salivary gland, salivary stone

POSTER PRESENTATION

Preparation of novel cardanol based polyurethane dispersions as fluorine free water repellents for textile Applications

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Abstract

Due to fluoro carbon based water repellents´ toxic effects on human health and environment, eco-friendly water repellents based on non-fluorine chemistries are extremely attracted by researchers. Cardanol, a byproduct of cashew nut industry, is a renewable resource containing mixture of monohydroxyl phenol with long saturated hydrocarbon chain in metaposition, which leads this material having good flexibility and hydrophobic nature. In this study, different types of waterborne polyurethane (PU) dispersions were synthesized by using HDI, IPDI and different types of cardanol based polyols via prepolymer mixing method. The chemical structure of obtained dispersions were determined by FTIR, thermal properties were investigated by DSC. Thereafter, dispersions were characterized in terms of solid content, viscosity, pH, mean particle size and particle size distribution. Moreover, film forming behaviour of the resulting dispersions was examined. Finally, the obtained dispersions were coated on textile surfaces and their water repellency properties were determined with the AATCC 22 Water Repellent:Spray test method.

Keywords: fluorine free water repellents, water repellency, textile coating applications

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POSTER PRESENTATION

Salinomycin'in yumurtalık kanser hücreleri üzerindeki anti-kanser etkilerinin *in vitro* incelenmesi

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Özet

Kontrolsüz hücre çoğalması ile karakterize edilen kanser, dünya genelinde önde gelen ölüm nedenlerinden biridir. Genellikle ileri evrelerde teşhis edilen yumurtalık kanseri, beş yıllık sağkalım oranı %50'nin altında olan kötü bir prognoza sahiptir. Benzer şekilde, agresif bir beyin tümörü olan glioblastoma multiforme (GBM) de sadece %5'lik bir sağ kalım oranına sahip olup, hastaların çoğu beş yıldan uzun süre hayatta kalamamaktadır. Kemoterapi ve cerrahi gibi geleneksel tedaviler yaygın olarak kullanılsa da, ilaç direnci ve tümörün tekrarlaması tedavi başarısızlığına yol açmakta ve bu durum yeni tedavi yaklaşımlarına olan ihtiyacı ortaya koymaktadır. Salinomisin, doğal bir antibiyotik olarak kalsiyum ve demir dengesini bozarak apoptoz ve ferroptozu indükleyebilen umut verici bir ajan olarak tanımlanmıştır. Bu çalışmanın amacı, Salinomisin'in in vitro ortamda yumurtalık kanseri (MDAH-2774) ve glioblastoma (U-87) hücre hatları üzerindeki anti-kanser etkilerini araştırmaktır. Çalışmada, ilacın sitotoksiteyi, kalsiyum salınımını ve apoptoz/ferroptoz belirteçlerinin ifadesini değerlendirerek hücre ölümünü indükleme potansiyeli incelenmiştir. Hücre canlılığı MTT testi ile değerlendirilmiş, hücre içi kalsiyum seviyeleri ise Fura-2 AM floresan boyası kullanılarak ölçülmüştür. Apoptoz ve ferroptoz yollarında rol alan anahtar proteinlerin ifadesi, Western blot analizi ile analiz edilmiştir. Sonuçlar, Salinomisin'in hem MDAH-2774 hem de U-87 hücrelerinde doz bağımlı sitotoksisite gösterdiğini ortaya koymuştur. Salinomisin ve Thapsigargin ile yapılan tedavi sonrası artan hücre içi kalsiyum seviyeleri, kalsiyum homeostazının bozulduğunu göstermektedir. Ayrıca, Western blot analizleri, apoptoz (p53 ve bcl-2) ve ferroptoz (GPX4 ve FTH1) yollarının aktive olduğunu doğrulamıştır. Sonuç olarak, Salinomisin, yumurtalık ve glioblastoma kanser hücrelerinde hem apoptoz hem de ferroptozu indükleyerek önemli bir terapötik ajan potansiyeli göstermektedir. Bu bulgular, ilaç direncini aşmada ve kanser tedavi sonuçlarını iyileştirmede Salinomisin'in önemli bir rol oynayabileceğini vurgulamaktadır.

Anahtar Kelimeler: Yumurtalık kanseri, Glioblastoma, Salinomisin, Apoptoz, Ferroptoz, Kalsiyum homeostazı

POSTER PRESENTATION

The antimicrobial activity of *Amanita muscaria*

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Abstract

Throughout the history of humanity, mushrooms have been utilized not only as a source of food but also for therapeutic purposes due to their medicinal properties. Many mushrooms found worldwide have served as the source of the earliest drugs used in the treatment of diseases, laying the foundations of modern medicine. Today, numerous medications are derived from compounds of fungal and plant origins, emphasizing the importance of investigating the potential medicinal benefits of plants and fungi. In this study, the antimicrobial effects of a mushroom species known as *Amanita muscaria*, commonly distinguished by its red cap with white spots, were examined. The disk diffusion test, a widely used technique to measure the inhibitory effect of a substance on different microorganism types, was employed to evaluate the antimicrobial potential of the mushroom. The ethanol extract of *Amanita muscaria* was tested in amounts of 30 µl, 60 µl, and 150 µl in the disk diffusion test conducted on 18 strains. The results demonstrated that the mushroom exhibited significant antimicrobial activity against certain Gram-positive bacteria, such as *Bacillus subtilis* and *Enterococcus faecium*. Notably, an inhibition zone of 8 mm was observed at all concentrations against *Bacillus subtilis*. A 10 mm inhibition zone was recorded against *Listeria monocytogenes* at the highest concentration. However, no inhibitory effect was observed against Gram-negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella*. These results indicate that *Amanita muscaria* shows promise as a potential antimicrobial agent against specific Gram-positive bacteria, meriting further in-depth research in the future.

Keywords: *Amanita muscaria*, Mushroom, Disk diffusion test, Antimicrobial activity, Microorganism

POSTER PRESENTATION

Development of graphene based electrode materials for redox flow desalination batteries

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Abstract

With increasing population and industrialization, the demand for energy and clean water resources continues to increase day by day. One fourth of the water on earth is fresh water resources; when the portion of these resources that can be used by humans is evaluated, only 0.3% of the total water on earth is usable. Therefore, desalination of water from salty water comes to the forefront. Among the existing desalination systems, reverse osmosis method is mostly used, but this method is costly. In recent years, the potential of electrochemical systems such as capacitive deionization as an alternative to this system has been examined [1]. Redox flow desalination battery systems (RFDB) are emerging to solve energy storage and desalination systems in a single system [2]. These systems are composed of electrodes with high surface area and conductivity and solvents containing redox components. Graphene oxide is one of the most frequently encountered chemicals as an electrode component with these two desired properties [3]. Methyl viologen (MV) and 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (4-OH TEMPO) redox active components in NaCl solution were used as electrolyte components in redox flow batteries and showed high capacitive value and high retention [4]. Deep eutectic solvents are solvent systems that can act as both solvent and electrolyte components, are easy to prepare and environmentally friendly [5]. In this study, for the first time, MV and 4-OH TEMPO redox active components dissolved in DES and graphene oxide were used together in a RFDB. Raman spectroscopy, X-ray photoelectron spectroscopy and scanning electron microscopy were used for characterization. The electrochemical performance of the electrodes was optimized by Cyclic Voltammetry and Electrochemical Impedance Spectroscopy. The redox flow battery performance of the prepared system was analyzed by charge-discharge method. The salt concentration during charge-discharge of the RFDB system was monitored using the total dissolved solids value.

Keywords: Desalination, redox flow battery, graphene based electrode, deep eutectic solvent.

Acknowledgements: This study was supported by the Scientific and Technological Research Council of Türkiye (TUBITAK) (Project Number: 121Y181).

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POSTER PRESENTATION

Evaluation of substrate and product toxicity on *Rhodopseudomonas palustris* TIE-1

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Abstract

As climate change necessitates sustainable alternatives, microbial electrosynthesis is gaining attention for its possible sustainable approach to the production of value added products. *R. palustris* is a versatile purple bacterium capable of microbial photoelectrosynthesis which is a process that uses light and a poised electrode reduce CO_2 into complex organic molecules via the Calvin-Benson-Bassham cycle [1]. Additionally, the bacterium can grow in various metabolic modes: photoautotrophic, photoheterotrophic, chemoheterotrophic, and chemoautotrophic. This study aims to evaluate the potential of using *R. palustris* TIE-1 as a microbial cell factory for synthesizing *N*-heterocycles, which are crucial building blocks used for pharmaceuticals and agrochemicals [2]. An imine reductase catalyzes the *N*-heterocycle synthesis from diamines and dicarbonyls. However, these compounds are known to be possibly toxic to microorganisms, requiring toxicity threshold investigations [3, 4]. This study evaluates the toxic effects of various diamines, dicarbonyls, and *N*-heterocycles on *R. palustris* TIE-1 by cultivating it heterotrophically under different concentrations while monitoring cell growth via optical density. Pyrrolidine concentrations up to 3 mM were non-lethal, while 10 mM was toxic to sustain growth. 20 mM piperazine resulted only in a significant decrease in growth, but 50 mM was to toxic to sustain growth of *R. Palustris* TIE-1. Ethylenediamine was toxic above 10 mM. *R. palustris* TIE-1 was able to grow at 0-1 mM methylglyoxal but no growth was observed at 2 mM methylglyoxal. Experiments with oxygen levels indicated that slightly opened caps improved growth rates. The study highlights the need to understand substrate and product concentrations for optimizing *N*-heterocycle synthesis and suggests exploring fed-batch operations and possibly product removal strategies to mitigate toxicity.

Keywords: *R. palustris*, *N*- heterocycles, Whole-cell biocatalysis, Substrate/Product Toxicity

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POSTER PRESENTATION

Metal Nanotaşıyıcı Sistemler ve Fenoksazin Türevlerinin Etkileşiminin Moleküler Modelleme Yoluyla İncelenmesi

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Özet

Sığır ve/veya insan hemoglobininin o-aminofenol ve türevleri ile reaksiyonu sonucu üretilen fenoksazin türevleri Phx-1 (2-amino-4,4α- dihydro-4α,7-dimethyl-3H-phenoxazine-3-one), Phx-2 (3-amino-1,4α-dihydro-4α,8-dimethyl-2H-phenoxazine- 2-one), ve Phx-3 (2-aminophenoxazine-3-one)' ün antikanser etkisi, yapılan bilimsel çalışmalarla kanıtlanmıştır. Bu nedenle potansiyel antikanser ilacı olarak kullanılabilirliği araştırılmaktadır. Fenoksazin türevlerinin kanser hücreleri üzerinde hücre büyümesini inhibe ettiği ve apoptozu indüklediği gözlemlenmiştir. Nanotaşıyıcılar, ilaçların hedeflenen bölgelere iletilmesi amacıyla kullanılan bir yöntemdir. Nanoteknoloji sayesinde kanser tedavisinde kemoterapinin yan etkileri azaltılabilir ve ilaçlar doğrudan hedef bölgelere yönlendirilebilir. Nanopartiküller, moleküler ve hücresel süreçlerle etkileşime girmelerine olanak tanıyan yüksek bir yüzey-hacim oranına sahiptir ve bu da onları ilaç dağıtım sistemleri için uygun kılar. Metal nanopartiküller, ilaçların hedeflenen etki bölgelerine yönlendirilmesi amacıyla önemli bir araştırma alanı olarak dikkat çekmektedir. Gümüş, altın, platin gibi metallerden oluşan nanopartiküllerin ilaç dağıtım sistemlerinde kullanımı yaygın bir araştırma konusudur. Gümüş nanotaşıyıcıların, antikanser ajanlarıyla kombinasyonu, tedavinin etkinliğini artırırken yan etkileri minimize etme potansiyeli sunmaktadır. Bu nanotaşıyıcılar, ilaçların daha düşük dozlarda kullanılmasını sağlayarak sağlıklı hücreler üzerindeki toksik etkilerini de azaltabilir. Özellikle yüksek yüzey alanı ve ayarlanabilir yüzey kimyası, onları ilaç taşıyıcıları olarak ideal hale getirmiştir. Yapılan çalışmalar, AgNP'lerin ROS üretimi ve hücresel stres yoluyla kanser hücrelerinde apoptozu tetiklediğini, böylece tümör hücrelerinin çoğalmasını engellediğini göstermiştir. Bu çalışmada, başlangıç yapılarını elde etmek için Fenoksazin türevlerinin ve gümüş nanotaşıyıcıların üç boyutlu modellerinin oluşturulması ve konformasyonel analizlerinin yapılması için Spartan20 yazılımı kullanılmıştır. Analiz sonucunda Phx-1 için 30 konformer, Phx-2 için 27 konformer, Phx-3 için 2 konformer ve AgNP için 13 konformer elde edilmiştir. Bu moleküller Yoğunluk Fonksiyoneli Teorisi (DFT) yaklaşımı ile Gaussian16 yazılımı yardımıyla optimize edilmiştir. Fenoksazin türevlerinin hesaplanması için zayıf etkileşimleri dikkate alan ωB97XD fonksiyoneli 6-311++G(d,p) baz seti ile birlikte kullanılmıştır. Gümüş nanotaşıyıcılar için ise LANL2DZ baz seti tercih edilmiştir. Hesaplamaların kolaylığı açısından hesaplamalar önce gaz fazında (vakumda) gerçekleştirilmiştir; daha sonra ise belirlenen en kararlı yapılar için hesaplamalar Polazirable Continuum Model (PCM) yaklaşımı kullanılarak sulu çözeltiyi temsil eden ortam içinde tekrarlanmıştır.

Anahtar Kelimeler: Fenoksazin, AgNP, Gümüş Nanotaşıyıcı; DFT, Moleküler Modelleme

Teşekkür: Bu çalışma TÜBİTAK 2210/A programı kapsamında desteklenmiştir. Bu araştırmada yer alan hesaplamaların bir kısmı TÜBİTAK ULAKBİM, Yüksek Başarım ve Grid Hesaplama Merkezi'nde (TRUBA kaynaklarında) gerçekleştirilmiştir.

POSTER PRESENTATION

Development of a high accuracy and high sensitivity analytical method for the determination of sulfonamide derivative antibiotic residues in food matrixes using green extraction strategies

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Abstract

This study aims to determine sulfonamide derivative antibiotic residues in honey samples using deep eutectic solvents (DES) and to develop an analytical method for their determination in the HPLC system by validating key performance parameters. In beekeeping, drugs are used to strengthen bees against external factors and ensure production at maximum efficiency. However, the use of antibiotics in treatment may lead to residues in the produced honey. These residues are a significant concern as they can cause various side effects, including the transmission of antibiotic-resistant bacteria to sensitive individuals, potential allergic reactions, immunopathological effects, reproductive disorders, and even carcinogenicity (e.g., sulfamethazine, oxytetracycline, furazolidone). Accurately and reliably detecting residues of sulfonamide drugs used to protect bees and preventing their harmful effects on consumer health are key objectives of this study. The development of a new analytical method is also important for contributing to quality control processes in the food industry. The European Union Commission has established a "Maximum Residue Level" (MRL) based on research, which has been declared in the Turkish Food Codex regulation as the maximum residue limits for pharmacologically active substances that may be found in animal products. Due to honey's structure, which contains proteins, carbohydrates, acids, and minerals, detecting and isolating antibiotic residues poses challenges. Therefore, prior to analysis, eco-friendly, green solvents will be used for preliminary purification, and the residues will be determined using High-Performance Liquid Chromatography (HPLC). In this research, deep eutectic solvents were preferred for the purification stage as they are non-toxic, recyclable, non-volatile, and cost-effective, contributing to a sustainable cycle in the literature. This study investigates the extraction of sulfonamides, which are difficult to extract with hydrophobic solvents, using DES to leverage their high solubility. These solvents are favored due to their stability, ease of use, biodegradability, low toxicity, and low cost. Trace amounts of sulfonamides extracted from the matrix will be concentrated and prepared for measurement without losing their natural structure.

Keywords: Sulfonamide, Honey, Residue, HPLC, Antibiotic,green solvents

POSTER PRESENTATION

Gelatin nanofibers for cephalexin delivery

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Abstract

In recent years, a series of global problems, such as insufficiency of natural resources and environmental pollution, have emerged with the increasing world population. In order to overcome these problems, it is very important to use the existing natural resources at the optimum level and to evaluate the waste in the best way. Obtaining natural polymers from animal wastes is also an important step to prevent environmental pollution and evaluate the existing resources. In this study, electrospun nanofibers were produced using bovine gelatin obtained from animal wastes . Gelatin/cephalexin-loaded nanofibers were produced with cephalexin used as a model drug. The surface morphologies of these nanofibers were examined with SEM, and the cumulative release (%) profile of cephalexin-loaded nanofibers was examined. The effect of gelatin used at different concentrations on the surface morphology and cephalexin release was evaluated.

Keywords: Bovine gelatin, cephalexin, nanofiber

POSTER PRESENTATION

Rat Spermlerinin Kriyoprezervasyonunda Sulandırıcıya İlave Edilen Melatonin ve Koenzim Q10'un Spermatalojik Özellikler Üzerine Etkisi

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Özet

Sunulan çalışmada, Rat spermlerinin dondurulabilirliği üzerine %23 yumurta sarısı, %8 laktoz monohidrat ve %10 TRİS aminometan içeren spermatozoon dondurma medyumuna ilave edilen farklı dozlardaki Melatonin (0,5 mM, 1 mM, 2 mM) ve Koenzim Q10 (25µM, 50µM, 75 µM)' un etkileri araştırıldı. Araştırmada 10-16 haftalık yaşta, 56 ergin Wistar Albino ırkı erkek rat kullanıldı (n:8). Ratlar servikal dislokasyonla sakrifiye edilerek spermatozoonlar elde edildi. Spermatozoonlar çalışma medyumlarına ilavedilerek 40 dakika ekilibrasyona bırakıldı. Ekilibrasyona sonrası Sıvı azot seviyesinden 4 cm yukarıda sıvı azot buharında dondurulduu ve sıvı azota daldırıldı. Dondurulmuş spermatozoonlar 37℃ su banyosunda 30 saniyede çözdürüldü. Çözünme sonrası motilite, ölü spermatozoon oranı, plazma membran bütünlüğü, anormal akrozom oranı, HTF (Human Tubal Fluid) içerisinde 90 dakikaya kadar en iyi motilite dayanıklılık (longevity) süresi ve apoptotik hücre oranı bakımından değerlendirildi. Dondurma çözdürme sonrası %12.90±1.34'lık kontrol grubu ile karşılaştırıldığında en yüksek motilite %24.58±1.68 ile 1 mM Melatonin ve %24.23±1.95 ile 25 µM Koenzim Q10 gruplarında tespit edildi *($p<0.01$)*. Ölü spermatozoon oranı %75.47±2.27'lik kontrol grubu ile kıyaslandığında en düşük oran %63.08±4.89 ile 1 mM Melatonin grubunda tespit edildi *($P<0.01$*). Anormal akrozom oranları bakımından kontrol grubu ile araştırma grupları arasında önemli bir fark bulunmadı *($p>0.01$*). Sağlam plazma membran bütünlüğü oranları %35.50±1.82'lik 1 mM Melatonin ve %31.31±1.93'lik 25 µM Koenzim Q10 gruplarında, %18.21±1.36'lik kontrol grubundan daha yüksek bulundu *($p<0.01$)*. Çözünme sonrası, 90 dakikanın sonunda en iyi longevity süresi, kontrol grubu ile karşılaştırıldığında 1 mM Melatonin ve 25 µM Koenzim Q10 grubunda belirlendi (*$p<0.01$)*. Apoptoz oranları değerlendirildiğinde 1 mM Melatonin ve 25 µM Koenzim Q10 grupları, kontrol grubuna göre anlamlı derecede daha düşük olduğu tespit edildi (*$p<0.01$)*. Sonuç olarak Rat sperm sulandırıcısına 1 mM Melatonin veya 25 µM Koenzim Q10 ilavesinin dondurup çözdürme sonrası motilite, canlı spermatozoon, sağlam plazma membran bütünlük oranlarını ve longevityi artırdığı apoptotik hücre oranını azalttığı ve böylece rat spermasının kriyoprezervasyon medyumunda başarılı bir şekilde kullanılabileceği belirlendi.

Anahtar Kelimeler: Rat Sperm analizi, Apoptozis, Kriyoprezervasyon, Antioksidan, Melatonin, Koenzim Q10

POSTER PRESENTATION

The anti-proliferation effect of epigenetic agent SGI-1027 against a triple-negative breast cancer cell line

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Abstract

Triple-negative breast cancer (TNBC) is the most resistant to therapy among breast cancer types; hence, research into new treatment options for TNBC is critical. DNA methylation, an epigenetic modification that occurs in mammals via DNA methyltransferase (DNMT) enzymes, can lead to diseases including cancer. Therefore, the reversibility of DNA methylation mechanisms enhances the therapeutic value of DNMT inhibitors. SGI-1027, a recently developed DNMT inhibitor, has been shown in various studies to be effective in treating of various human malignancies. In this investigation, we compared the therapeutic effect of the DNMT inhibitor SGI-1027 on TNBC cells to that of luminal-type breast cancer cells. Estrogen-positive breast cancer cell line MCF7 and TNBC cell line MDA-MB 231 were used to investigate the effect of SGI-1027 on cell proliferation. The cell proliferation was determined using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. SGI-1027 was used to treat MCF7 cells for 24 and 48 hours at concentrations of 20, 40, 80, 100, and 200 µM, whereas MDA-MB 231 cells were treated at 10, 20, 40, and 80 µM. MCF-7 cells exhibited the IC50 value of 88.6 mM for 24 h and 30 mM for 48 h. On the other hand, MDA-MB 231 cells showed an IC50 value of 270.8 mM for 24 h and 20.9 mM for 48 h. Our preliminary data showed that SGI-1027 had an anti-proliferation effect on MDA-MB-231 after 48 hours. The effects of SGI-1027 on the DNA methylation profile and gene expression will be investigated further. Thus, it aimed to develop a novel drug strategy by assessing the efficacy of a new agent that might be useful in the treatment of TNBC. This study was supported Research Foundation of Marmara University (grant no: TYL-2024-11188).

Keywords: SGI-1027, DNA methylation, MDA-MB 231, MTT, cell proliferation

POSTER PRESENTATION

Determination of Clopidogrel Levels by Liquid Chromatography Mass Spectrometry Method

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Abstract

Clopidogrel, a thienopyridine, exerts its effects by selectively binding to adenylate cyclase-coupled ADP receptors on platelet surfaces. This selective action, a significant feature of its pharmacological profile, provides confidence in its targeted effect. It is used to reduce atherosclerotic events, including myocardial infarction, ischemic stroke, and vascular death, in patients with atherosclerosis. Additionally, it is widely used in acute coronary syndromes and following the placement of intravascular stents. Acute or chronic use of clopidogrel can lead to bleeding, a significant side effect that may result in drug discontinuation or non-compliance with therapy. Our objective is to develop a rapid, specific, and reproducible method for the quantitative determination of clopidogrel in serum. 300 µL of the sample were transferred to Eppendorf tubes, with the addition of 100 µL of internal standard and 600 µL of methanol. The mixture was vortexed for 30 seconds and centrifuged at 14.000 rpm for 10 minutes. The supernatants were collected into clean glass tubes and dried under nitrogen at 45°C. The residues were reconstituted in 200 µL of a methanol:water (20:80%, v/v) mixture. 40 µL was injected to the LC-MS/MS system. The proposed method has been validated for a linear range of 5-5000 ng/mL with a correlation coefficient (r^2) better than 0.995. For clopidogrel the limits of detection and quantification were 5 and 20 ng/mL, respectively. The retention time was 2.57 minutes with a total run time of 5 minutes. The intra- and inter-assay imprecision ranged under 11%. The mean recovery was 95.9% and matrix effect values were less than 11.7%. A rapid, economical, simple, accurate, and sensitive method for clopidogrel has been developed. This method is suitable for routine analysis of clopidogrel.

Keywords: Therapeutic drug monitoring, tandem mass, clopidogrel, adverse effect, LC-MS/MS.

POSTER PRESENTATION

A Rapid, Robust and Reliable Measurement Method For The Quantitation of Lansoprazole

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Abstract

Lansoprazole, a benzimidazole derivative with antisecretory and antiulcer activities, inhibits acid pump activity in the final stage of the enzymatic process. This inhibition occurs by suppressing H+ and K+-adenosine triphosphatase, thereby reducing acid secretion from parietal cells. This agent is used to treat short-term erosive reflux esophagitis, gastric and duodenal ulcers, and NSAID-induced ulcers. Additionally, it is effective in treating healed reflux esophagitis and duodenal ulcers, Zollinger-Ellison syndrome, and eradicating Helicobacter pylori in combination therapies. Common side effects of lansoprazole include nausea, vomiting, itchy skin, constipation, and dryness in the mouth and throat. More serious and rare side effects reported include joint pain, skin rashes, and severe diarrhea. Our objective is to develop a rapid, specific, and reproducible method for the quantitative determination of Lansoprazole in serum. After transferring 250 µL of the sample into Eppendorf tubes, 100 µL of internal standard and 600 µL of acetonitrile were added. The mixture was vortexed for 30 seconds and then centrifuged at 12.000 rpm for 10 minutes. The supernatants were transferred to clean glass tubes and evaporated under nitrogen gas at 40°C. The residues were reconstituted in 200 µL of acetonitrile:water (50:50%,h/v) mixture. Subsequently, 20 µL of this solution was injected into the LC-MS/MS system. The method was linear for lansoprazol in the 5-10000 ng/mL range with an r2 value greater than 0.996. Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined to be 5 and 15 ng/mL, respectively. %CV values for intra-day and inter-day precision studies were 4.8% and 8.7%, respectively. The total run time was 5 minutes. A practical, economical and reliable measurement method has been developed to measure lansoprazole levels. Based on the obtained data, the completed method validation study indicates that lansoprazole is now ready for clinical use.

Keywords: Therapeutic drug monitoring, tandem mass, Lansoprazole, adverse effect, LC-MS/MS.

POSTER PRESENTATION

Effect of coenzyme Q10 on the viability of human urothelial cells

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Abstract

Coenzyme Q10 is an antioxidant that plays a role in protecting cell membrane structure, providing energy conversion in the cell and reduces the effect of free radicals. In this study, the effect of coenzyme Q10 on the viability of human urothelial cells was investigated.
In our study, hTU1 cells, an hTERT-immortalized human urothelial cell line, were used. The cells were cultured in a keratinocyte-SFM medium. Coenzyme Q10 was dissolved in sterile PBS and filtered. Cells cultured in 96 wells were treated with Coenzyme Q10 at concentrations of 0, 5, 10, 25, 50, 100, 250, 500 and 1000 µM for 24, 48 and 72 hours. Cell viability was determined by MTT test.
Coenzyme Q10 decreased urothelial cell viability at all treatment times. Cell viability values at concentrations of 50 µM and above were found to be statistically significant in the 72-hour treatment compared to the control group, while cell viability values at concentrations of 100 µM and above were found to be statistically significant in the 24 and 48-hour treatments ($p \leq 0.05$). These results show that coenzyme Q10, which has antioxidant properties and is responsible for energy production, can be toxic to urothelial cells due to increased concentration.

Keywords: Coenzyme Q10, hTERT-hTU1, cell viability

POSTER PRESENTATION

Osteopontin: A therapeutic candidate for neurodegenerative diseases

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Abstract

Osteopontin is a multifunctional protein that is expressed and secreted in many cell types and plays a role in cell adhesion, invasion, migration, chemotaxis, prevention of apoptosis, and anchorage-independent growth of tumor cells. Osteopontin has received increasing attention due to its role in the pathophysiology of several degenerative, autoimmune, inflammatory diseases and cancer. Modular structural motifs in osteopontin that provide interaction surfaces for integrins and CD44 variant receptors elicit signal transduction, cytokine, and chemokine effects.In addition to its proinflammatory and proatherogenic functions, osteopontin is a potent physiological inhibitor of mineralization. Osteopontin is accepted as a potential biomarker in cardiovascular and central nervous system diseases and cancer. Osteopontin is a powerful sensor that responds to acute or cumulative damage that disrupts homeostasis in inflammatory neurodegenerative disorders. Recent studies have demonstrated that osteopontin promotes tissue damage and the repair mechanisms triggered by the inflammatory response. The disparate effects observed attributed, at least in part, to the presence of distinct functional domains within the osteopontin molecule that become accessible following cleavage by thrombin or metalloproteases. Elevated plasma and cerebrospinal fluid osteopontin levels have been observed in individuals with multiple sclerosis and Alzheimer's disease, as well as in their respective experimental models. Under normal conditions, osteopontin expression levels are low in the brain. However, osteopontin expression is significantly increased in macrophages/microglia and astrocytes in Parkinson's disease, hypoxia-ischemia brain injury, Alzheimer's disease, and multiple sclerosis demonstrating a neuroprotective effect. Osteopontin plays a role in maintaining homeostasis in the central nervous system by facilitating the recruitment of immune cells via tissue-level signals and by regulating proinflammatory signaling via glial-neuronal interactions, thereby providing neuroprotection. A deeper understanding of the molecular signaling pathways involved in osteopontin exerting its neuroprotective effects will contribute to developing osteopontin as a potential therapeutic target in treating neurodegenerative diseases.

Keywords: Osteopontin, neuroprotective, neurodegenerative.

POSTER PRESENTATION

Yüksek pH Ortamında Kararlı Esans Hammaddelerin Sentezlenmesi ve Endüstriyel Uygulamaları

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Özet

Esanslar, parfüm, kozmetik ve temizlik ürünleri gibi pek çok endüstriyel alanda temel bileşenler olarak önemli rol oynamaktadır. Özellikle kozmetik ürünlerinde kullanılan esansların ürün içindeki stabiliteleri, tüketici memnuniyeti ve ürün performansı açısından büyük önem arz etmektedir. Farklı pH aralıklarında esansların kararlılığı kritik bir konu olup, bu zorlu koşullarda esansların stabilitesinin sağlanması önemli bir araştırma alanı olarak karşımıza çıkmaktadır. Örneğin, yüksek pH'a sahip ürünler, özellikle konsantre çamaşır suyu gibi bazik ortamlar, esans stabilitesini olumsuz etkileyen zorlayıcı koşullar sunmaktadır. Bu sorunu çözmek adına esans formülasyonlarında kullanılacak hammadde seçimi önem arz etmektedir. Buna göre, bu çalışma kapsamında bazik koşullarda kararlı olabileceklerini düşündüğümüz Asetal bileşikleri hedef esans hammaddeleri olarak seçilmiştir. Böylelikle, farklı koku notalarına sahip 10 asetal bileşiği sentezlenmiş ve konsantre çamaşır suyu bazında koku ve stabilite açısından değerlendirmeye alınmıştır. Sonuç olarak, 6 asetal bileşiğinin test edilen şartlarda etkinlik gösterdiği belirlenmiştir. Elde edilen bulgular, bazik ortamlarda stabil kalabilen esans formülasyonlarının geliştirilmesine yönelik önemli veriler sunmakta olup, bu alanda gelecekte yapılacak araştırmalara katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Asetaller, Bazik Ortam, Kozmetik Ürünler, pH

POSTER PRESENTATION

Detection of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected from Passerine birds of Middle Black Sea Region of Turkey

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Abstract

Various species of ticks that may be infected by zoonotic pathogens are known to parasitize passerine birds. These migratory and domestic birds may be reservoirs of tick-borne pathogens such as *Borrelia burgdorferi* s.l. that cause Lyme disease that is important for animal health and Public Health. Lyme disease is a multisystemic zoonotic disease with worldwide distribution. The aim of this study was to determine the potential role of passerine birds in maintenance of *B. burgdorferi* s.l. by detection of the spirochete in *Ixodes (I.) ricinus* ticks collected from resident and migratory birds in Samsun and Sinop regions during the period of 2023-2024 based on *flaB* gene targeted-PCR. Various species of passerine birds were included in our study. The ticks collected from the birds were determined to be belonged to the genera *Rhipicephalus*, *Haemaphysalis*, *Hyalomma* and *Ixodes*, *I. ricinus* being the most common species among the collected ticks. A total of 489 *I. ricinus* (blood-sucking ticks not included) species ticks were studied and 23 DNA extracts were obtained. According to *flaB* gene-PCR results, the rate of *B. burgdorferi* was determined as 16.6% on tick basis and 17.4% on DNA extract basis. The most common tick hosts that were infected with *Borrelia burgdorferi* s.l. were *Turdus merula* and *Cettia cetti*. The results show that passerine bird species are likely to carry *I. ricinus* species ticks infected with *B. burgdorferi* s.l. Therefore, birds that move short or long distances may have an important role in transmission of *B. burgdorferi*-infected ticks to animals and humans.

Keywords: Passerine birds, *Ixodes ricinus*, *Borrelia burgdorferi* sensu lato, Lyme disease.

This study was supported by Ondokuz Mayis University Scientific Research Grant no PYO.VET.1908.23.008

POSTER PRESENTATION

Medical Challenges and Emergencies in Space Missions: A Review of Historical Incidents and Health Risks

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Abstract

Space missions present unique medical challenges due to the microgravity environment, radiation exposure, and isolation, leading to various physical and psychological conditions. This review summarizes the major medical events encountered on space missions that have raised awareness and led to prevention strategies and possible solutions. During the Apollo 7 mission (1968), astronauts experienced severe cold symptoms, exacerbated by the microgravity environment, highlighting the risks of even minor infections in space. Similarly, during Skylab 3 (1973), astronauts experienced muscle and bone loss, emphasizing the need for in-flight exercise protocols. Apollo 15 (1971) astronaut James Irwin encountered irregular heart rhythms during his moonwalk, illustrating potential cardiovascular risks in space. The STS-1 mission (1981) saw the onset of space motion sickness, causing nausea and vomiting among astronauts, leading to the development of countermeasures. One of the most tragic incidents occurred on the Soyuz 11 mission (1971), where a sudden cabin depressurization resulted in the death of the entire crew. This event underscored the critical importance of pressure control in spacecraft. Long-duration missions aboard the Mir Space Station (1990s) further confirmed significant muscle and bone degradation, driving the development of advanced exercise equipment on space stations. On the International Space Station (ISS), several astronauts developed vision impairment due to fluid shifts in microgravity, a condition now known as vision impairment intracranial pressure syndrome. ISS missions have widely reported psychological issues such as stress, depression, and anxiety, underscoring the importance of mental health support. Finally, a case of deep vein thrombosis (2016) aboard the ISS highlighted the need for preparedness for medical emergencies, even in space. These medical events demonstrate the diverse challenges faced in space missions, as well as the need for ongoing development of medical care strategies to safeguard astronaut health during long-duration spaceflight.

Keywords: Space medicine, Medical emergencies in space, Microgravity

POSTER PRESENTATION

Voltammetric determination of ascorbic acid using palladium nanoparticle doped carbonaceous electrode

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Abstract

In this study, a mixture of equal amounts of raw coal tar pitch (r-CTP) and glass fiber (GF) was pyrolyzed in a high-temperature furnace at 1000°C for 1 hour, with a heating rate of 10°C/min under nitrogen atmosphere (Ç.-Yeter et al. 2021). The resulting carbonized material was ground using a Spex-type grinder (Retsch, PM100), then placed in a beaker where palladium nanoparticles (PdNPs) were synthesized on it (Zheng et al. 2016). The obtained material was denoted as Pd/CTP-GF. Pd/CTP-GF was characterized using SEM and XRD. The Pd/CTP-GF modified glassy carbon (GC) electrode was characterized by electrochemical cyclic voltammetry (CV). A method was developed using differential pulse voltammetry (DPV) on the Pd/CTP-GF modified GC electrode for ascorbic acid in the concentration range of 0.01-1 µM. The detection limit (S/N=3) of the developed method was determined to be 5 nM. The intraday and interday precision and accuracy of the method were evaluated. The interference effects of dopamine, uric acid, and some cations on ascorbic acid were also investigated. Ascorbic acid was successfully analyzed in orange juice samples with a recovery value of over 95%.

Keywords: Ascorbic acid, differential pulse voltammetry, palladium nanoparticle, carbonaceous electrode

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POSTER PRESENTATION

Bölgemizde yetişen Endemik *Stachys rupestris* türünün kimyasal içeriği ve biyolojik aktivitelerinin belirlenmesi

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Özet

Lamiaceae familyası, özellikle Akdeniz ülkelerinde ve ılıman iklim kuşağında yer alan birçok ülkede yetişen 200 kadar cins ve 3000'in üzerinde türü içeren zengin bir familyadır. Bu familyaya ait bitkilerin çoğu antik çağlardan bu yana halk ilacı olarak çeşitli hastalıkların tedavisinde kullanılmalarının yanı sıra, tıpta, gıda endüstrisinde, parfümeri ve kozmetikte yer alan bitkilerdir. Dünya üzerinde 224 cins ve yaklaşık 5600 tür ile temsil edilen kozmopolit bir familyadır. Türkiye Florası'nda ise Lamiaceae familyası 45 cins, 565 tür ve toplam 735 takson ile temsil edilmektedir (Duman ve ark., 2005). Dünyada ve ülkemizde geniş yayılış gösteren Labiatae familyasının önemli bir cinsi *Stachys* L. cinsidir. Bu cinsin orjini Akdeniz fitocoğrafik bölgesidir. Stachys cinsi içerdiği 300 civarındaki tür ile Lamiaceae familyasının en büyük cinslerinden biridir. Bu cins, kozmopolit olup Akdeniz ve Güneybatı Asya'nın ılıman bölgelerinde merkezlenmiş, ikincil yayılış merkezleri olarak da Kuzey ve Güney Amerika ile Güney Afrika göze çarpmaktadır. Tür sayısı bakımından cinsin iki merkezi bulunmaktadır. Birisi Güney ve Doğu Anadolu, Kafkasya, Kuzeybatı İran ve Güney Irak'ı içine alan bölge, diğeri ise Balkan Yarımadası'dır. Cinsin türlerinin büyük çoğunluğu kayalık alanlarda, özellikle de kalker kayalıklarda yayılış göstermektedir (Bhattacharjee, 1980). Bu çalışmada Andırın - Kahramanmaraş'tan bitki örnekleri toplanmış ve bitkinin yer üstü kısımları kullanılarak etanol ekstresi hazırlanmıştır. Ekstraktın sekonder metabolitleri bir LC-HRMS analizi ile belirlendi. Ayrıca toplam fenolik ve flavonoid içerikleri hesaplanmış ve in vitro antioksidan aktivite çalışmaları yapılmıştır. Buna göre 36 farklı bileşik tanımlanmış ve bunların arasında ana bileşen olarak verbascoside (640.528 µg analit / g özü) bulunmuştur. Yüksek toplam fenolik içeriği (66.80 ± 2.60 µg PES / mg özü) ve toplam flavonoid içeriği (32.85 ± 1.66 µg qes / mg özü) ile karakterize edilen ekstrakt, üç farklı deneyle gösterildiği gibi önemli antioksidan aktivite sergilemiştir: DPPH serbest radikal temizleme (IC50: 26.92 ± 0.89Katyon radikal temizleme (IC50: 18.53 ± 0.10 g / ml ve CUPRAC aktivite yöntemleri (A0.5: 27.12 ± 0.92g / ml). Bu çalışmanın Türkiye'deki Stachys türleri üzerine yapılan araştırmaların ilerlemesine katkıda bulunması ve tıbbi uygulamalara yönelik gelişimlerini desteklemesi amaçlanmıştır.

Anahtar Kelimeler: *Stachys rupestris,* LC-MS/MS, antioksidan,fenolik bileşik.

POSTER PRESENTATION

Coumarin-Embedded DMPC and DMPG Lipid Membranes: A Raman Spectroscopy Approach

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Abstract

Phospholipids are the primary components of most cell membranes. When immersed in water, they form a bilayer due to their amphiphilic nature, which includes a hydrophilic head group connected by a glycerol backbone to hydrophobic long acyl chains. Molecular interactions with phospholipid membranes are crucial for several biological processes, including cell signaling, enzyme control, and drug and toxin absorption. Considering the significance of these interactions, creating a model system that permits exploration of molecular interactions at lipid bilayer interfaces might reveal new insights into these mechanisms. Zwitterionic dimyristoylphosphatidylcholine (DMPC) liposomes are widely used as a model system of biomembranes because lecithins are the major components of most mammalian membranes. Anionic dimyristoylphosphatidylglycerol (DMPG) is not a main phospholipid in mammalian cell membranes, but it plays a significant role in bacterial membranes and altering surface charge density. It may also be found in mitochondrial and chloroplast inner membranes. Raman spectroscopy has been regarded as an effective method for investigating the molecular organization of phospholipids in relation to biomembranes. One of the key advantages of this method is the ability to quantitatively examine some of the observed spectrum patterns in terms of conformational and structural organization. In this study, the interactions between coumarin and liposomes of DMPC and DMPG were studied using Raman spectroscopy. Coumarin was selected as a representative phenolic compound. Raman spectra of pure DMPC and DMPG bilayers with 5 and 10 mol% coumarin were obtained at 400-3200 cm^{-1}. When 5 and 10 mol% coumarin was added to pure DMPC and DMPG liposomes, conformational changes in the hydrophobic chain structure, the depth of penetration in the bilayer, and the type of interaction were all found. As a result, coumarin's physicochemical characteristics are mostly determined by the charge of the lipid's head group. Thus, coumarin's effects on model cell membranes could be applied to biological membranes, supporting the compound's potential utility in the prevention and treatment of a variety of diseases.

Keywords: Coumarin, DMPC, DMPG, Raman spectroscopy.

2.2. FULL-TEXTS

POSTER PRESENTATION

Study of drug-drug interactions in pediatric hospital medical records

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Abstract

Drug-drug interactions (DDI) are a contributing factor to drug-use-related issues. Drug-drug interactions may have an impact on the anticipated therapeutic result. Keeping up with the numerous reports of novel drug interactions has proven to be challenging for medical practitioners. Verifying the extent and nature of potential drug interactions in pediatric medical records of patients admitted to the pediatric ward of a regional hospital in Albania is the goal of this study. The study includes a retrospective and descriptive analysis for children diagnosed with respiratory disorders between January and December of 2023, a period of one year.

The study included 100 medical records of pediatric patients, ranging in age from 1 month to 14 years. Pathologies of the digestive and respiratory systems were the most common diagnoses mentioned. The Medscape database was used to find medication interactions. One hundred medical records were examined. According to the medical record, a total of three to eight prescription medications were prescribed. Medication interactions were detected in just 18 out of 100 records. Of all contacts, 28% of cases had low interactions, 62% had moderate interactions, and 10% had major interactions. There were twenty diagnoses made, eight of which related to problems with the respiratory system. A regular evaluation of the clinical protocol is necessary to evaluate the risk factors for adverse reactions and toxicity, which include the number of prescription drugs administered and their dosage. Drug interactions are more likely to occur and may result in responses if more medicines are given. negative consequences, toxicity, and extended hospital stays.

Keywords: *Medical records, drug-drug interactions, drugs, diagnosis*

INTRODUCTION

A reasonable prescription for medication should consider the dose that will provide the desired therapeutic effect with the least amount of side effects. Combining two or more medications can enhance the overall therapeutic outcome. On the other hand, as more medications are given, there is a greater chance of drug-drug interactions, which can result in toxicity, negative side effects, and extended hospital stays. The World Health Organization defines an adverse drug reaction as a detrimental, inadvertent, or undesirable drug reaction that happens at dosages used in humans for treatment, diagnosis, or prevention. Adverse drug reactions can be caused by a variety of circumstances, such as age, disease severity, and multiple drug therapy.

Specifically, during childhood development, a drug's pharmacokinetic and pharmacodynamics properties can alter, making youngsters more susceptible to negative effects than adults.

Furthermore, renal function reaches full maturity by one year of age, however it is unknown if transport proteins have a role in active renal excretion and the absorption of several medications during childhood. Newborns and small neonates have lower levels of total plasma proteins, including albumin, which raises the percentage of some medicines and affects tissue distribution. Furthermore, in late childhood, gastrointestinal motility reaches adult values after decreasing in neonates. The quantity and dosage of medications supplied are risk factors for toxicity and adverse responses; keeping an eye on these variables necessitates ongoing clinical protocol reviews.

MATERIALS AND METHODS

The study includes a retrospective and descriptive analysis for children diagnosed with respiratory disorders between January and December of 2023, a period of one year. The study included 100 pediatric medical records, ranging in age from 1 month to 14 years. The gastrointestinal tract and respiratory systems were the primary focus of the diagnoses mentioned. Data from medical materials was compiled using a two-part structure.

Part 1: contains information on diagnosis, polymedication and drug-drug interactions.

Part 2: contains information on drugs used, doses and dosing regimens.

The Medscape database was used to find medication interactions. The degree of action in these interactions was then categorized. Drug interactions were categorized as significant when there was an immediate medical risk assessment and necessary action to mitigate the interaction. When an interaction led to a worsening of the patient's condition or side effects and necessitated changing the medication, it was deemed moderate. A small interaction resulted in an unpleasant response and clinical symptoms, but no medication change was necessary. The medications were deemed contraindicated when it was not advised to use them all at once.

RESULTS

Table 1. Prevalence of diagnoses in the studied cards

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Diagnostic	Percentage %
Acute bronchiolitis	12
Bronchial asthma	6
Bronkopneumoni dexter	5
Unspecified bronchopneumonia	1
Pneumoni e paspecifikuar	5
Tracheobronchitis	4
Purulent tonsillitis	3
Catarrhal angina	15
Adenoidit	1
Urinary infection	2
Febrile convulsion	1
Varicella	1
Pielonefritis	1
Diarrhea	14
Abdominal colics	4
Enterovirus	3
Cystitis	1
Gastroenteritis	9
Vomiting	4
State of lipothymia	1
Unspecified tonsillitis	7
Total	**100**

The highest prevalence represents catarrhal angina with 15% and the lowest prevalence represents unspecified bronchopneumonia, adenoid, lipothymia, cystitis, pyelonephritis, varicella and febrile convulsion with 1%

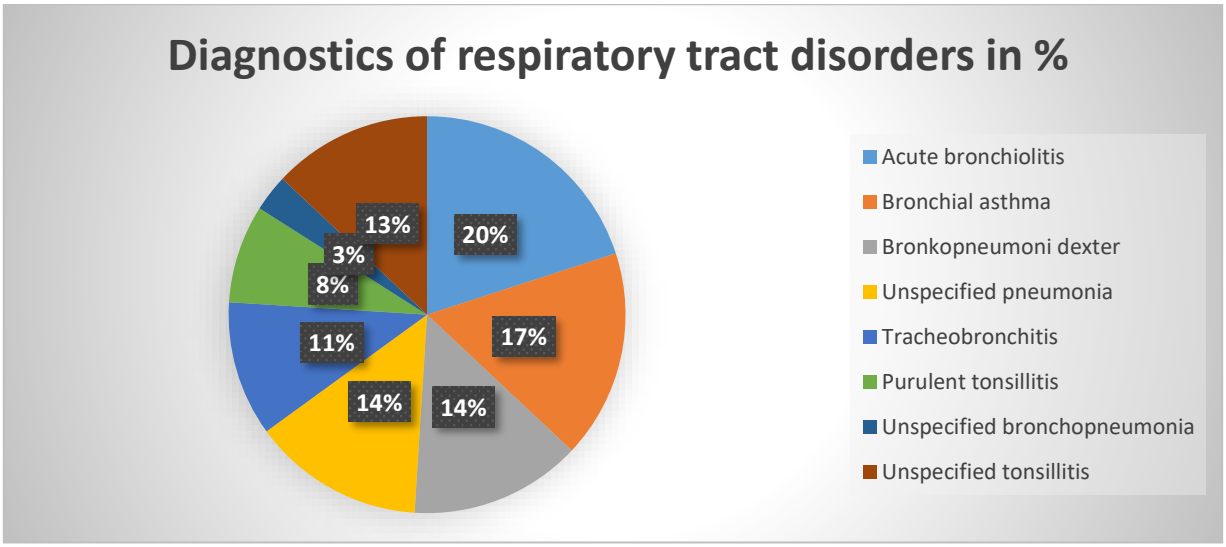


Figure 1: Prevalence of respiratory system diagnoses

In 100 medical records in total, a total of 20 diagnoses were recorded, among which 8 diagnoses belonged to the respiratory system diorders.

Table 2. Frequency of prescribed drugs

Pharmatherapeutic Class	Prescribed drugs	%
Nonsteroidal anti-inflammatory drugs (NSAID)	Paracetamol, Ibuprofen	6
Cephalosporins	Ceftazidime, Cefuroxime, Ceftriaxone	19
Other antibiotics	Amikacin, Ampicillin	3
Antihistamines	Desloratadine, Loratadine	1
Benzodiazepines	Diazepam	1
Corticosteroids	Prednisolone, Budesonide	18
Inhibitors of HCl secretion	Ranitidina	1
Adrenergic agonists	Adrenalina	1
Others	Acetylcysteine, Vitamins, Magnesium sulfate, Solutions (Ringer's, saline, glucose)	50

The drugs that are prescribed the least in the pediatric charts that we studied are adrenergic agonists, antihistamines, benzodiazepines, and HCl secretion inhibitors with 1% and the drugs that are prescribed more are the other drugs with 50%

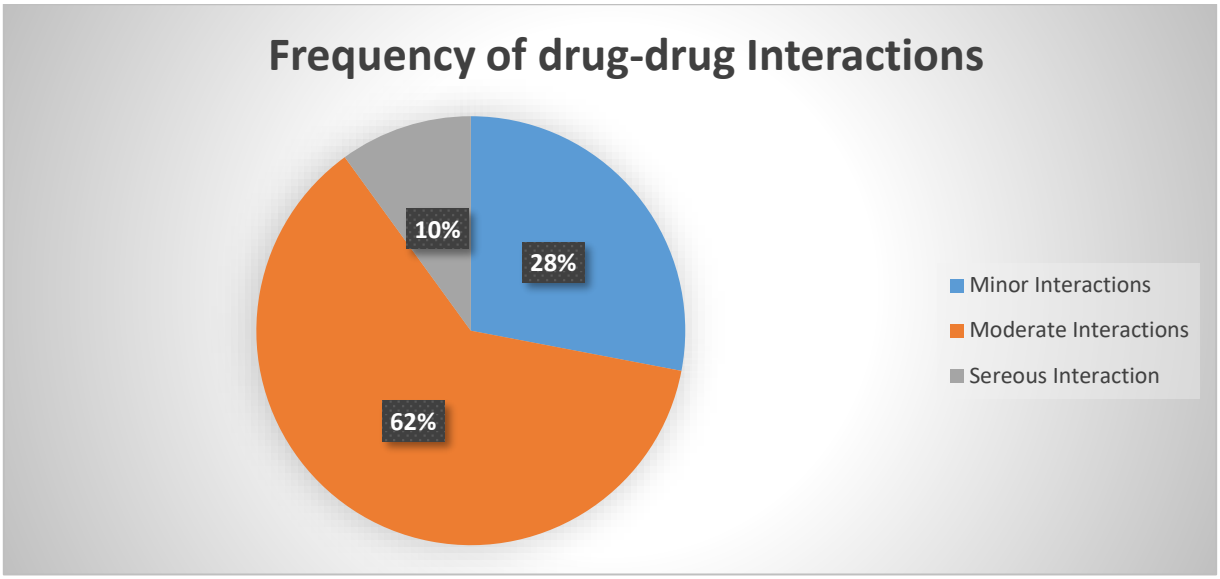
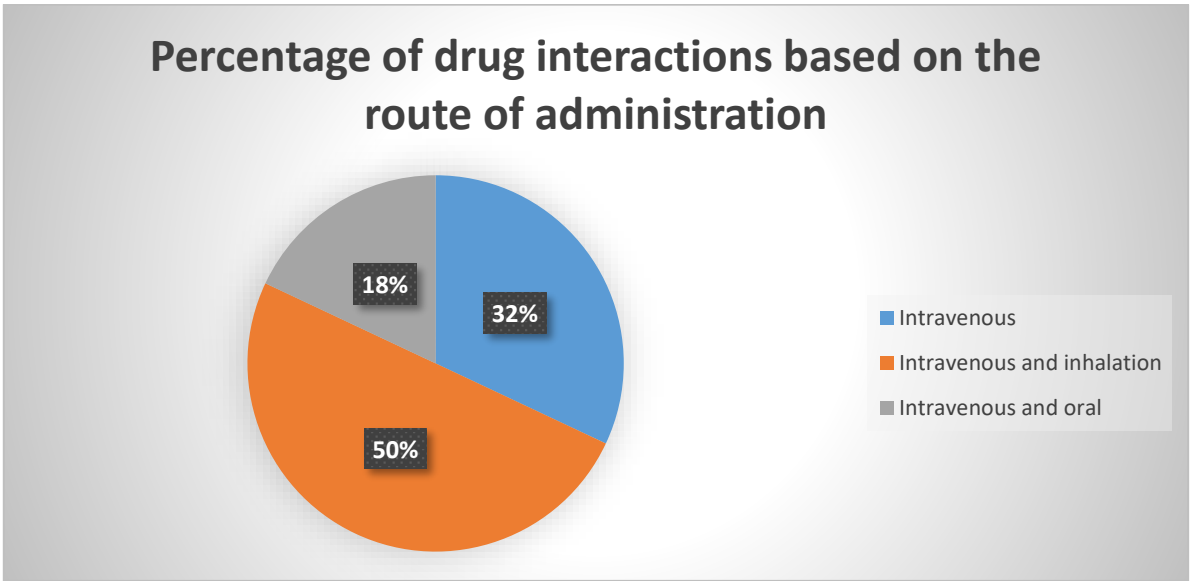


Figure 2: Frequency of drug interactions

According to our study, in 100 medical records, drug interactions were found in only 18 of them. Among these 18 cases, only 12 cases of drug interactions related to the respiratory apparatus were found (1 to 3 interactions for each medical record). Among these 12 cases, 26 medium interactions and 13 low interactions were found. It turns out that there are 10% of serious interactions, 62% of medium interactions and 28% of minor interactions, where the highest percentages represent medium interactions.

Figure 3; Percentage of drug interactions based on the route of administration



This study showed that there is a possibility of possible interactions between the drugs administered in the following ways**:**
1) Intravenous (32%) where we mention Prednisolone + Aminophylline, Sol. Glucose + MgSO4
2) Intravenous and Inhalation (50%) overthinking Prednisolone + Budesonide, Adrenaline + Ventolin, Aminophylline + Budesonide
3) Intravenous and oral (18%) where we mention Sol.Ringer + Ibuprofen

CONCLUSION

One hundred medical records were examined. There were twenty diagnoses in all on these cards, eight of which related to the respiratory system. Ten medical records had bronchiolitis, seven had unidentified tonsillitis, four had bronchial asthma, three had dexter bronchopneumonia, three had purulent tonsillitis and tracheobronchitis, two had unidentified pneumonia, and one had bronchopneumonia. There were three to eight prescriptions for each medical document, for a total of twenty prescriptions.

The most popular methods of delivery were inhalation, oral, and intravenous. Cephalosporins and corticosteroids were the most often recommended medications.

Only eighteen out of the hundred cards had medication interactions, according to studies. Merely 12 instances of medication interactions associated with the respiratory system were identified from these 18 cases (one to three interactions per medical record). There were 13 low interactions and 26 medium interactions among these 12 cases. Low interactions were thus detected in 28% of cases, moderate interactions in 62% of cases, and major interactions in 10% of cases out of all interactions.

Prednisolone, aminophylline, budesonide, adrenaline, and ventolin are the medications that have shown the greatest number of drug interactions, according to this investigation.

To avoid drug interactions, the prescription should contain the fewest number of medications possible.

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POSTER PRESENTATION

Investigation and Quantification of Methylome Products containing in Human dentine by ^{1}H-NMR

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Abstract

Methylome products occur by adding methyl groups into DNA or amino acids. The metabolites are potential biomarkers for age estimation in forensic science. Teeth is an endurable tissue and has remained in the explosion and decomposition process. This study aimed to use nuclear magnetic resonance spectroscopy (^{1}H-NMR) techniques to quantify methylome products in the dentin layer. The second molar powder (n=70) was prepared and hydrolyzed. Then, methylome products were investigated and quantified by ^{1}H-NMR. 3-Methylguanine, creatine, and N-methyl-L-glutamic acid were found to have the highest level presenting 15.74±14.58, 5.82±6.08, and 1.9±2.47 µM, respectively. These methylome products might be utilized for estimating sex and chronological age.

Keywords: Methylome Products, Dentine, Quantitative Nuclear Magnetic Resonance Spectroscopy, Forensic metabolome

INTRODUCTION

Methylome products are metabolites that produced from the methylation of DNA or amino acids. These processes are depended on age and chemical exposure and are involve biochemical changes in the body (Bekaert, Kamalandua, Zapico, Van de Voorde, & Decorte, 2015).

Age determination is a crucial process for establishing their identity, specifically in civil and criminal litigation. Various methods and techniques from different fields are used under available remains condition. For deceased, age estimation can be done through X-rays of bone joints 4th rib, pubic symphysis, cranial sutures, and limb bones (Krishan, Kanchan, & Garg, 2015). However, post-mortem changes, fire damage and explosions can destroy the sample leading to limitations for these methods. then, alternative methods those be essential for age determination in forensic identification.

Dentine, a layer underneath the enamel of the tooth, consists of approximately 72% inorganic matter, 20% organic matter, and 8% water by weight (Diez et al., 2021). Its exceptional hardness makes dentine an invaluable asset in age estimation studies, facilitating comprehensive chemical analysis. One of the organic compounds found in dentine, dl-aspartic acid, has been recognized as a biomarker (Ogata et al., 2022). Nonetheless, there is a dearth of knowledge regarding other potential biomarkers, emphasizing the necessity for additional research to unearth supplementary compounds that are advantageous in the field of forensic science.

Proton-Nuclear Magnetic Resonance (^{1}H-NMR) technique is a widely used technique for analyzing the quality and quantity of chemicals. It is particularly valuable for studying the chemical compounds present in tooth tissue and distinguishing between proteins and DNA that impact tooth tissue repair (Ni & Nicolella, 2005). Furthermore, it offers valuable insights into the molecular context and interactions involving DNA sequences in human genetics (Márquez-Ruiz, González-Herrera, Luna, & Valenzuela, 2020). In criminal cases, this technique is especially advantageous for conducting comprehensive forensic analyses.

The objective of this study is to quantify methylome products in the dentin layer using ^{1}H-NMR spectroscopy at a frequency of 500 MHz the quantification of these chemicals, combined with the ^{1}H-NMR

technique, can be used to estimate sex and chronological age, thereby enhancing the capabilities of forensic research.

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 teeth sample collection

Seventy non-carious permanent second molars (35 males and 35 females) aged 20 years and older were collected as samples. The teeth samples were obtained from the Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, Thailand. This research was approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University (FOR-2565-09049). The teeth samples were cleaned using a solution of 0.9% normal saline and distilled water to remove any remaining blood (Maulani & Auerkari, 2020). The dried samples were then stored in plastic bags at -20 °C until further use.

2.3 Dentine powder preparation

The dentin samples were washed with saline and distilled water following the protocol established by Pellegrini et al. The tooth samples were horizontally cut into 1 mm thick slices using a precision saw model Isomet 1000 with a diamond blade. Subsequently, the finished samples were treated to remove the tooth enamel, using a high-speed diamond drill bit. Both steps of sample preparation were carried out under coolant conditions to prevent heat generation.

2.4 ^{1}H-NMR spectroscopy analysis

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 1 mL 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

Thirty nine methylome products are available, including 3-methylthymine, N-methyl-DL-alanine, 1-methylcytosine, 6-methyluracil, N(4)-methylcytosine, 2-amino-6-methoxypurine, 2-aminoisobutyric acid, N-methyl-L-glutamic acid, methylamine, N-methylalanine, N-Methyl-D-aspartic acid, L-arginine, N2-methylguanine, 6-amino-5[N-methylformylamino]-1-methyluracil, N-methylvaline, sarcosine, S-methylmethionine, creatine, 3-methylguanine, 7-methyladenine, N-methyl-L-proline, gamma-glutamyl-S-methylcysteine, anserine, methyrosine, alpha-Methylphenylalanine, N-methlyphenylalanine, methylhistidine, 5-methylcytosine, 1-methylhistidine, 9-methyladenine, 3-methyladenine, 7-methylguanine, 6-methyladenine, 1-methyladenine, N-acetyl-1-methylhistidine, 5-Acetylamino-6-formylamino-3-methyluracil, 1-methylguanine, 3-methylcytosine, and 2-O-methylcytosine. The identification of each biomolecule was determined using The Human Metabolome Database (HMDB) (Wishart et al., 2013) and the analysis involves utilizing the Cluster Midpoint, peak sequence (Coupling type), number of hydrogen atoms (H's), atomic number, and Coupling constant (*J*). The Coupling constant refers to the distance between peaks resulting from spin-spin coupling of neighboring protons. It is crucial to ensure chemical compatibility with the database. Specifically, the chemical shift values of each Cluster midpoint must not deviate by more than 0.02, and the same number of occurrences should be observed. Subsequently, the concentration of each chemical can be calculated, following the method outlined (Choi, Myoung, Seo, & Ahn, 2021)

$$C_A = \frac{I_A}{I_{TSP}} \times \frac{H_{TSP}}{H_A} \times C_{TSP}$$

C_A represents the concentration of the substance.
I_A denotes the intensity value of the substance.
I_{TSP} signifies the intensity value of TSP.
H_{TSP} corresponds to the hydrogen atom of TSP.
H_A refers to the hydrogen atom of the substance.
C_{TSP} represents the concentration of TSP.

2.6 Statistics analysis

The concentration of each chemical is reported as the mean ± standard deviation. The data distribution is assessed using the Kolmogorov-Smirnov test. When comparing the means of age groups, Pearson's correlation coefficient is employed if the data is normally distributed. However, if the data is not normally distributed, Spearman's correlation coefficient is used. A statistical significance level of 95% confidence ($p < 0.05$) is considered significant.

RESULTS and DISCUSSION

In this study, out of a total of 70 dental tissue samples, dentin was extracted, pulverized, and digested with hydrochloric acid. The resulting compounds were analysed using the ^{1}H-NMR technique to determine their types and quantities. The study successfully identified a total of 39 products associated with the DNA and amino acid methylome, and the results are shown in Table 1.

Table1: DNA and amino acid methylome products in human dentine samples.

Methylome Products	Chemical shift	HMDB ID
1. 3-methylthymine	0.02	HMDB 0245938
2. N-methyl-DL-alanine	0.03	HMDB 0255173
3. 1-methylcytosine	0.04	HMDB 0243939
4. 6-methyluracil	0.06	HMDB 0247093
5. N(4)-methylcytosine	0.07	HMDB 0247144
6. 2-amino-6-methoxypurine	0.08	HMDB 0245002
7. 2-aminoisobutyric acid	1.48	HMDB 0001906
8. N-methyl-L-glutamic acid	2.58	HMDB 0062660
9. Methylamine	2.59	HMDB 0000164
10. N-methylalanine	2.74	HMDB 0094692
11. N-Methyl-D-aspartic acid	2.82	HMDB 0002393
12. L-targinine	3.16	HMDB 0029416
13. N2-methylguanine	3.26	HMDB 0006040
14. 6-amino-5[N-methylformylamino-1-methyluracil	3.29	HMDB 0059771
15. N-methylvaline	3.74	HMDB 0061716
16. Sarcosine	3.78	HMDB 0000271
17. S-methylmethionine	3.85	HMDB 0038670
18. Creatine	3.92	HMDB 0000064
19. 3-methylguanine	3.97	HMDB 0001566
20. 7-methyladenine	4.04	HMDB 0011614
21. N-methyl-L-proline	4.34	HMDB 0094696
22. gamma-glutamyl-S-methylcysteine	4.54	HMDB 0031863
23. Anserine	7.14	HMBD 0000194
24. Methyrosine	7.19	HMDB 0014903
25. alpha-Methylphenylalanine	7.38	HMDB 0029223
26. N-methlyphenylalanine	7.40	HMDB 0029224
27. Methylhistidine	7.69	HMDB 0000479
28. 5-methylcytosine	7.80	HMDB 0002894
29. 1-methylhistidine	7.89	HMDB 0000001
30. 9-methyladenine	7.92	HMDB 0240713
31. 3-methyladenine	8.01	HMDB 0011600
32. 7-methylguanine	8.10	HMDB 0000897

33. 6-methyladenine	8.18	HMDB 0002099
34. 1-methyladenine	8.28	HMDB 0011599
35. N-acetyl-1-methylhistidine	8.29	HMDB 0240340
36. 5-Acetylamino-6-formylamino-3-methyluracil	8.34	HMDB 0011105
37. 1-methylguanine	8.49	HMDB 0003282
38. 3-methylcytosine	8.55	HMDB 0011601
39. 2-O-methylcytosine	8.81	HMDB 0004339

The NMR chromatograms are depicted in Figures 1 and 2. They have been categorized into 25 types of DNA methylome and 14 types of amino acid methylome.

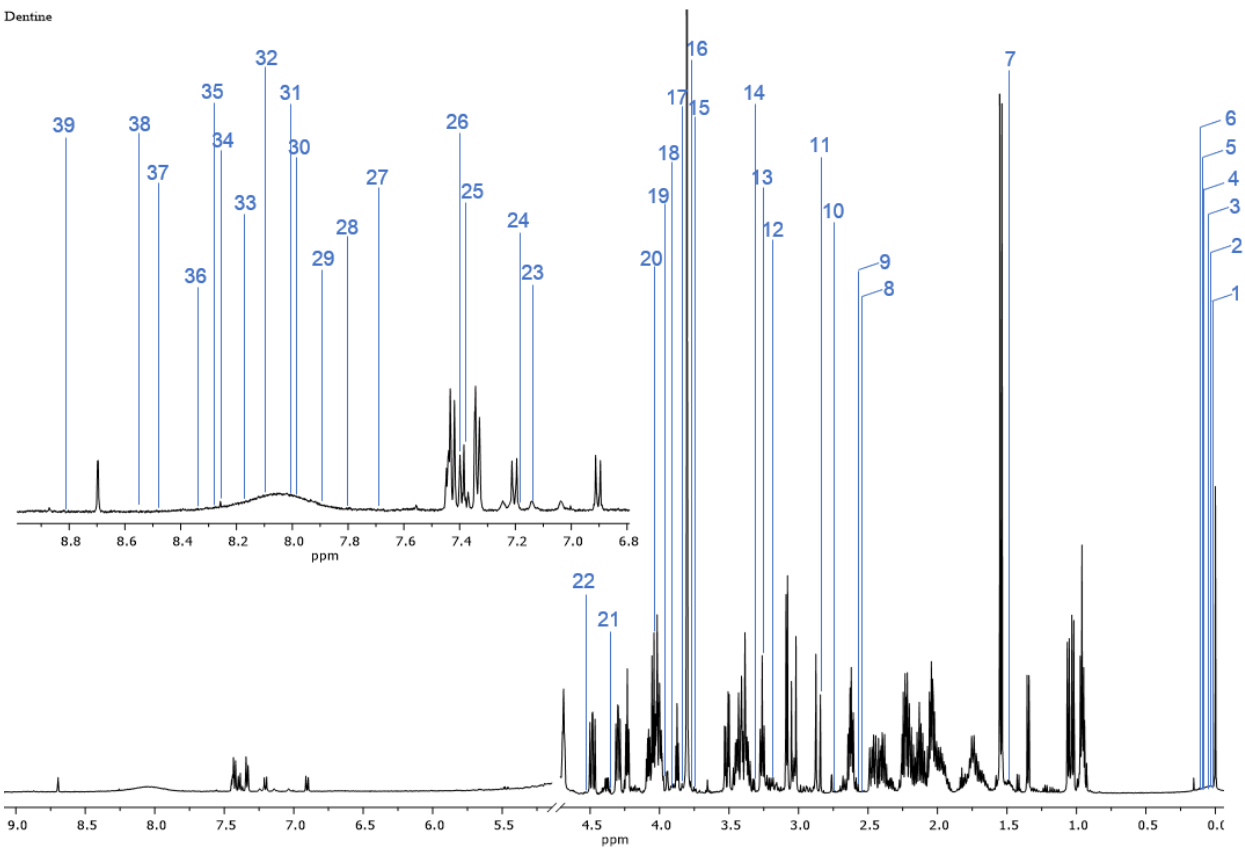

Fig.1 Chromatograms of typical 500 MHz 1H-NMR spectra of hydrolyzed dentine are presented in Figure A and B, displaying the 0.00-5.00 and 5.00-8.50 ppm regions, respectively. The main signals in the spectra have been assigned to the following compounds: 3-methylthymine (1), N-methyl-DL-alanine (2), 1-methylcytosine (3), 6-methyluracil (4), N(4)-methylcytosine (5), 2-amino-6-methoxypurine (6), 2-aminoisobutyric acid (7), N-methyl-L-glutamic acid (8), methylamine (9), N-methylalanine (10), N-methyl-D-aspartic acid (11), L-arginine (12), N2-methylguanine (13), 6-amino-5[N-methylformylamino]-1-methyluracil (14), N-methylvaline (15), sarcosine (16), S-methylmethionine (17), creatine (18), 3-methylguanine (19), 7-methyladenine (20), N-methyl-L-proline (21), gamma-glutamyl-S-methylcysteine (22), anserine (23), methyrosine (24), alpha-Methylphenylalanine (25), N-methlyphenylalanine (26), methylhistidine (27), 5-methylcytosine (28), 1-methylhistidine (29), 9-methyladenine (30), 3-methyladenine (31), 7-methylguanine (32), 6-methyladenine (33), 1-methyladenine (34), N-acetyl-1-methylhistidine (35), 5-Acetylamino-6-formylamino-3-methyluracil (36), 1-methylguanine (37), 3-methylcytosine (38), and 2-O-methylcytosine (39).

Thirty-nine methylome products especially 3-Methylguanine, creatine, and N-methyl-L-glutamic acid were found to be at the highest-level presenting in dentine layer. Thirty-nine methylome products especially 3-Methylguanine, creatine, and N-methyl-L-glutamic acid were found to be the highest level presenting 15.74±14.58, 5.82±6.08, and 1.9±2.47 mM, respectively (Figure 2).

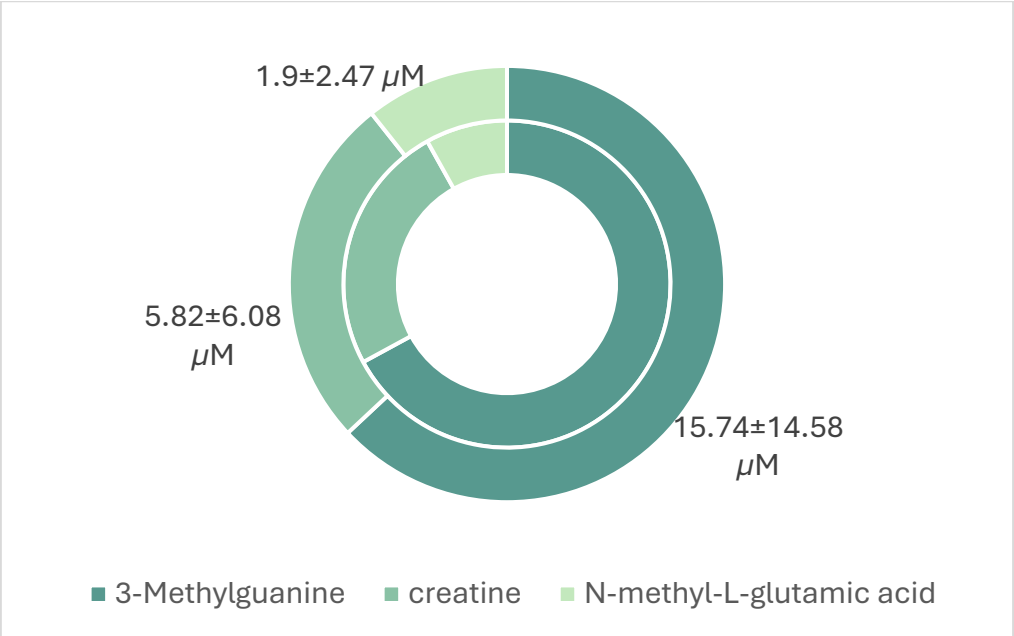

Fig. 2 Top 3 most common substances and amounts

NMR spectroscopy was selected as an alternative technique because it requires only milligram-level samples and can identify or specify the details of various chemical compounds. However, there is still a lack of correlation between the obtained data and various variables to enable analysis and prediction. Therefore, this research sought to find the relationship between the amount of substances and age, as age is an important variable in identifying individuals. This research analyzed chemicals associated with the methylation process, which increases with age. This finding is consistent with the research of Maulani and Auerkari (2020), who studied the importance of DNA methylation in age estimation using different sample types with STR profiling techniques. Their analysis indicated that DNA methylation is a good option for estimating the age at the time of death for forensic profiling. Furthermore, the amount of DNA methylation demonstrated specificity in the quantity of DNA methylation at different age ranges. This is the first research in Thailand that applied ^{1}H-NMR combined with metabolomics techniques in forensic science research. However, the information and conditions of bone and teeth are limited especially, underlying diseases, postmortem intervals leading to change of DNA methylome levels (Paczkowski & Schutz, 2011).

CONCLUSION

^{1}H-NMR spectroscopy technique can identify a total of 39 methylomes products. 3-Methylguanine, creatine and N-methyl-L-glutamic acid were the highest-level chemicals contained in dentine samples. These chemical compounds and their metabolites can be applied for biological age estimation. The acquisition of information from bone and teeth samples from individuals deceased, but also presents research opportunities and valuable insights for case investigations.

ACKNOWLEDGEMENTS

We are grateful to Dr. Rattakorn Pernsuwan, Wirintra Watcharanone, Kanjana Inthong, Atchara Pothikunapatg, Preeyajak Chinwaraphat for collecting the teeth samples. and the Fundamental Fund (FRB646006) for supporting the NMR analysis.

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POSTER PRESENTATION

Synthesis of a new cholesteryl consisting liquid crystalline compound

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Abstract

Cholesteryl chloroformate is one of the most important liquid crystal materials. It was modified with aromatic functions to give new molecules a long-lasting LC. In this study, a new Cho-LC8 compound was synthesized starting from Cholesteryl chloroformate and 8-(4-cyanobiphenyl-4'-oxy) octan-1-ol (LC8) in the presence of triethylamine (TEA) as an acid scavenger at room temperature. The mesomorphic properties of the LC was studied by polarized optical microscope (POM).

Keywords: Cholesteryl chloroformate Liquid Crystal, Mesogen

INTRODUCTION

Liquid crystals are quite interesting compounds and they are used in many areas from visual technologies to sensor applications (Dierking, 2003; Andrienko, 2018).
Cholesterol-based mesogens are the first known liquid crystalline materials. They are an important source of focus with their innovations and potential contributions to this field. Even though their derivatives are perhaps the most studied mesogens, naturally since they are the first species found in more than a century ago, still with their chirality and uniqueness, they continue to be promising for new studies in many areas, from visual technologies to electronic device development (Dunmur and Sluckin, 2014; Yelamaggad et al., 2008).
Because of their biological structures, cholesterol-derived materials are also used in many different fields. Their chiral backbones and lipid characters, which are a result of their biological structure, also help them find many biocompatible applications such as gene transfer.

In this study, cholesterol-based liquid crystalline compound was synthesized and liquid crystal properties of the compound was determined using physical and chemical methods.

MATERIALS AND METHODS

4-hydroxy-4’-biphenylcarbonitrile (Sigma Aldrich), 8-chloro-1-octanol (Sigma Aldrich), Ethyl alcohol (Merck), Cholesteryl chloroformate (Sigma Aldrich), N,N-dimethylformamide (Sigma Aldrich), dimethyl sulfoxide (DMSO) (Sigma Aldrich). All materials are commercially available and were used as received.

Synthesis of 8-(4-cyanobiphenyl-4′-oxy) octan-1-ol (LC8)

4′-((8-hydroxyoctyl)oxy)-[1,1′-biphenyl]-4 carbonitrile (LC8) synthesized according to the literature (Korkmaz et al., 2017).

Synthesis and characterization of Cho-LC8

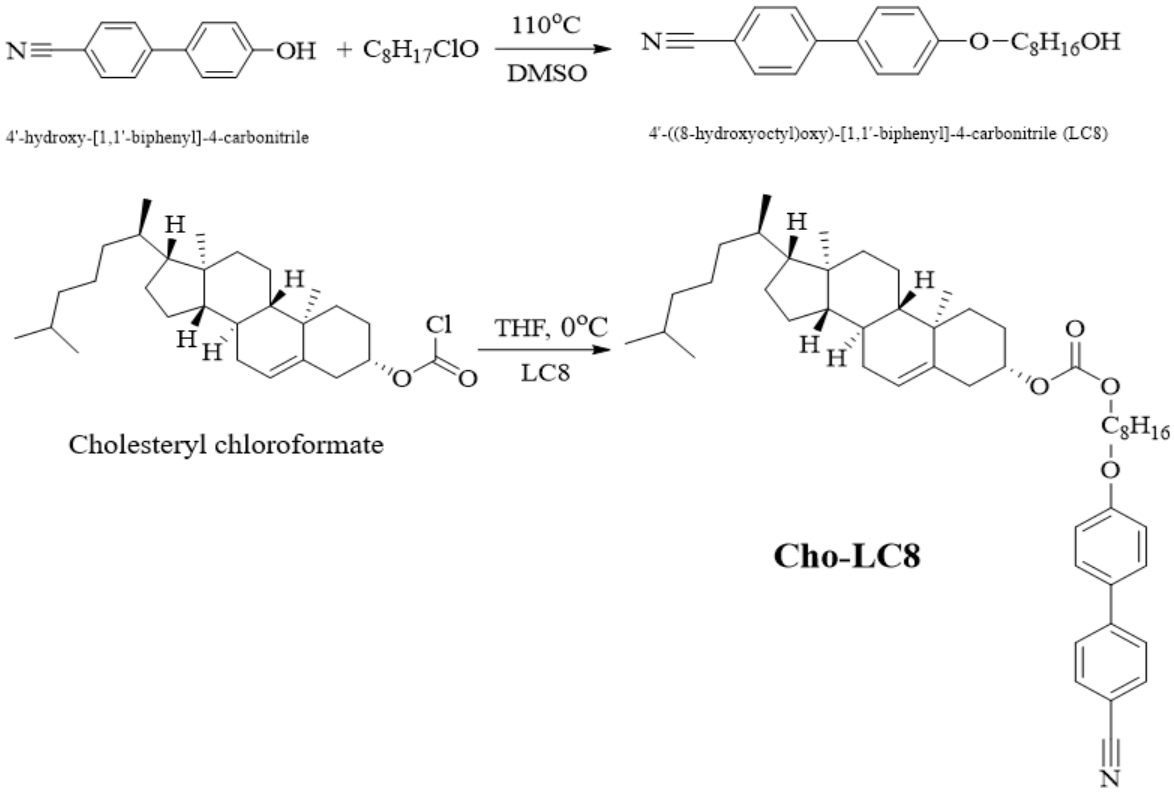
0.403 g (1.25 mmol) of LC8 was dissolved in 15 mL of THF and 0.20 mL triethylamine was added into this solution. After that, 0.56 g (1.25 mmol) of Cholesteryl chloroformate in 15 mL of THF solution was added slowly with a dropping funnel at 0 °C. And then, reaction was proceeded at room temperature for 24h. At the end of the reaction, the reaction mixture was poured into iced water and precipitated mass was filtered. The

obtained product recrystallized with ether, dried under vacuum and white crystals were obtained. Yield: 63 %.

RESULTS and DISCUSSION

Preparation of Cho-LC8

Cho-LC8 was synthesized starting from cholesteryl chloroformate and LC8 in the presence of triethylamine (TEA) as an acid scavenger at room temperature (Scheme 1).



Scheme 1. Synthesis of Cho-LC8

Spectrophotometric characterization of the compound was investigated by FTIR spectrophotometer. According to the FTIR spectrum of Cho-LC8 (Figure 1), characteristic ester carbonyl peak was observed at 1759 cm^{-1}. Aromatic peaks of Cho-LC8 at between 3322 cm^{-1} and aliphatic C-H stretching peaks of Cholesterol at between 2855 cm^{-1} - 2933 cm^{-1} can also be observed. Also, the peak at 2235 cm^{-1} belongs to characteristic CN bond.

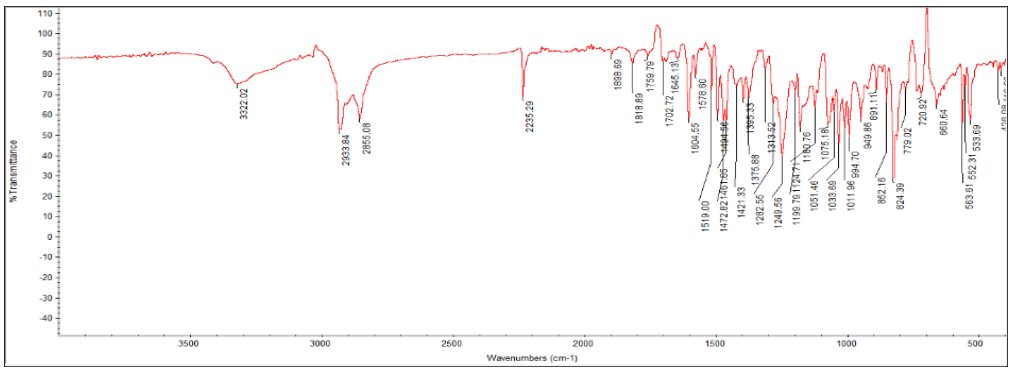
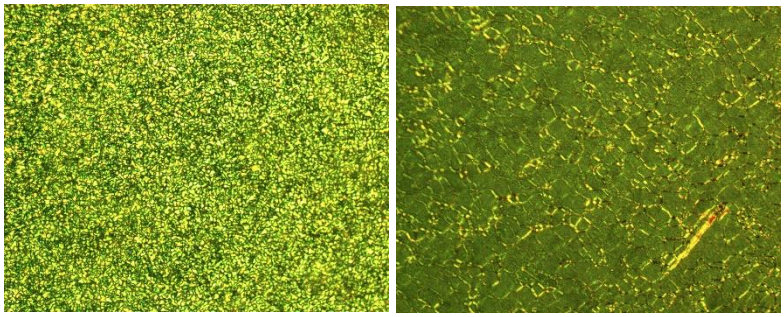


Figure 1. FTIR spectrum of the Cho-LC8

Liquid crystal properties

The mesomorphic properties of the Cho-LC8 was examined with polarized optical microscope by heating and cooling at 10 degrees per minute. Cho-LC8 mesogen has enantiotropic polymorphism those shows nematic texture. During heating process (Figure 2(a)), Cho-LC8 gives nematic liquid crystalline phase at 71 °C. Also, a new nematic cholesteric texture was observed at 38 °C during cooling (Figure 2(b)). At 153 °C, isotropic phase is observed.



(a) (b)

Figure 2. POM pictures of Cho-LC8 (400x)

Conclusion

In this study, a new cholesteryl derivative liquid crystalline compound Cho-LC8 was prepared. Liquid crystalline properties were investigated by POM. Phase transition temperatures were observed with a polarized optical microscope (POM) both heating and cooling process. Cho-LC8 shows enantiotropic polymorphism.

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POSTER PRESENTATION

Nutrition and obesity in Aldent university students

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Abstract

The condition of extra fat accumulation is called overweight. Measuring a person's weight and height and determining their body mass index (BMI)—weight (kg)/height2 (m2)—allows for the diagnosis of overweight and obesity. The waist circumference and other measurements can aid in the diagnosis of obesity. The body mass index is a proxy measure of fatness. For newborns, children, and adolescents, there are several BMI categories according to age and gender that define obesity. The study included 334 students, or 13.03% of the total, and was cross-sectional and of simple size. That is, 173 students in the Master of Science (Msc) and Integrated Master Study programs (PI) and 161 students in the Bachelor of Studies (Ba) program. The sampling was stratified and done at random. On February 20, 2023, the students who were chosen as a sample showed up at their school. Anthropometric measurements were taken and the pre-prepared questionnaires were completed by them. Variants were aggregated using SPSS version 21. According to the information gathered, 27.3% of respondents in the FSHMT are overweight or obese, whereas 72.7% of respondents are in good health. Obesity is heavily influenced by genetics and diet.

Keywords: *Obesity, Overweight, BMI, Malnutrition, Metabolic Disease*

INTRODUCTION

The World Health Organization defines overweight and obesity in adults as follows: overweight is defined as having a BMI of 25 or higher, while obesity is defined as having a BMI of 30 or higher. Age must be taken into account when defining overweight and obesity in children.
In 2022, 16% of adults globally who were 18 years of age or older were obese. Between 1990 and 2022, the prevalence of obesity more than doubled globally.
In 2022, more than 390 million kids and teenagers between the ages of 5 and 19 were overweight. From only 8% in 1990 to 20% in 2022, the prevalence of overweight (including obesity) among children and adolescents aged 5-19 has increased significantly. Both boys and girls have seen an increase in overweight people: in 2022, 21% of males and 19% of girls were overweight.
In 1990, just 2% of children and adolescents aged 5 to 19 (or 31 million young people) were obese; by 2022, however, 8% of children and adolescents (or 160 million young people) were obese.
The dangers obesity and overweight pose to one's health are becoming more widely known and understood. An estimated 5 million fatalities from noncommunicable diseases (NCDs) such as cancer, diabetes, cardiovascular disease, neurological disorders, chronic respiratory illnesses, and digestive disorders occurred in 2019 as a result of having a higher-than-optimal BMI (1).
Childhood and teenage obesity impacts the health of these individuals immediately and is linked to an increased risk of developing multiple non-communicable diseases (NCDs), including type 2 diabetes and cardiovascular disease, as well as an earlier onset of these conditions. Obesity in children and adolescents has detrimental psychosocial effects; it impairs quality of life and academic performance and is exacerbated by stigma, discrimination, and bullying. Obese children have a higher chance of becoming obese adults and are more likely to have NCDs as adults. he obesity epidemic's financial effects are equally significant. The global expenses of obesity and overweight are expected to exceed US$ 18 trillion by 2060 and US$ 3 trillion annually by 2030, respectively, if nothing is done (2). Lastly, a problem that was formerly exclusive to high-income countries is rapidly becoming worldwide due to the rise in obesity rates in low- and middle-income countries, notably among lower socioeconomic categories. The WHO reports that since 1975, the incidence of obesity has tripled. 13% of adults in 2016 were obese, while 39% of adults were overweight. Overweight or obese, 41 million children under the age of five. The abnormal or excessive buildup of adipose tissue that might impair one's health and quality of life is referred to as obesity and overweight. Diet is one of the main factors contributing to obesity. Along with a sedentary lifestyle, excessive consumption of saturated fats and carbohydrates (sugars) is one of the main causes of overweight and obesity.

MATERIALS AND METHODS:

This research was cross-sectional in design. A survey was employed, incorporating closed-ended inquiries about diet and heredity factors. After that, the anthropometric measurements were completed, and SPSS 2 was used to analyze the data. The overall number of students in 2023–24 was 2505, of which 1199 (48%) were enrolled in the Ba study programs and 1306 (52%) in the Msc and Msc integrated study programs. These percentages were determined using calculator.net, and the sample size is 334 students, or 13.03% of the total, with a 95% confidence level and a 5% margin of error. Therefore, 173 students in the Master of Science and MSc (PI) programs and 161 students in the Bachelor study program A stratified random sampling procedure was used. Since February 20, 2023, is the first day of the second semester and, based on statistics from the secretary, the day with the highest attendance in an auditorium, we used those pupils as a sample. Anthropometric measurements for the collected survey data and also the data were assembled using SPSS 21.

RESULTS

Table 1: Gender data of sample

	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
MALE	202	59.8	59.8	59.8
FEMALE	136	40.2	40.2	100.0
Total	338	100.0	100.0	

338 students were asked about their nutritional status. The variables used are gender, BMI, the quality of their food intake, whether they take alcohol or not, and whether they have obesity running in their family. All the variables are compiled and the results are gotten through SPSS.
Based on the results that were gotten through SPSS 21, 202 students are male, resulting in 59.8%, and then 136 are female, resulting in 40.2%. When it comes to their BMI, 18.6% resulted to be underweight, 39.9% resulted to be of normal weight, 41.4% resulted to be overweight. Obese were very little, resulting in 2%.

Table 2: BM of sample

	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
UNDERWEIGHT	63	18.6	18.6	18.6
NORMAL WEIGHR	135	39.9	39.9	58.6
OVERWEIGHT	140	41.4	41.4	100.0
Total	338	100.0	100.0	

When asked about their food intake and the quality of the food they consume, 81.4% of the students answered that they eat very healthy food, and then 18.6% answered that they do not eat healthy food.

Table 3: Food intake

	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
GOOD	275	81.4	81.4	81.4
BAD	63	18.6	18.6	100.0
Total	338	100.0	100.0	

When asked about alcohol intake, 18.6% of the students answered that they consume alcohol socially, and then 81.4% of the students answered that they do not consume alcohol at all.

Table 4: Alcohol intake

	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
YES	63	18.6	18.6	18.6
NO	275	81.4	81.4	100.0
Total	338	100.0	100.0	

When asked about whether obesity runs in their family, 20% of the students answered that obesity does run in their family, and 80% of the students answered that obesity does not run in their family.

Table 5: Obesity in family

	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
YES	67	19.8	19.8	19.8
NO	271	80.2	80.2	100.0
Total	338	100.0	100.0	

We correlated two variables, BMI and alcohol intake, using the Pearson coefficient, which resulted to be positive at 0.01 level, which means that the students who consume alcohol socially and who drink more than others are to weight more and to have, consequently, a higher BMI.

Table 6: Correlations

		BMI	ALCOHOL INTAKE
BMI	Pearson Correlation	1	.793**
	Sig. (2-tailed)		.000
	N	338	338
ALCOHOL INTAKE	Pearson Correlation	.793**	1
	Sig. (2-tailed)	.000	
	N	338	338

**. Correlation is significant at the 0.01 level (2-tailed).

Two other variables were correlated BMI and hereditary obesity, which resulted to be significant at 0.01 level using the Pearson coefficient. So, it means that the students who have obesity running in their family are to have a higher BMI and to be more overweight compared to others who do not have obesity running in their families.

Table 7: Correlations of obesity in family

		BMI	OBESITY IN FAMILY
BMI	Pearson Correlation	1	-.518**
	Sig. (2-tailed)		.000
	N	338	338
OBESITY IN FAMILY	Pearson Correlation	-.518**	1
	Sig. (2-tailed)	.000	
	N	338	338

**. Correlation is significant at the 0.01 level (2-tailed).

Two other variables were correlated BMI and hereditary obesity, which resulted to be significant at 0.01 level using the Pearson coefficient. So, it means that the students who have obesity running in their family are to have a higher BMI and to be more overweight compared to others who do not have obesity running in their families.

Table 8: Correlations of food intake

		BMI	FOOD INTAKE
BMI	Pearson Correlation	1	-.793**
	Sig. (2-tailed)		.000
	N	338	338
FOOD INTAKE	Pearson Correlation	-.793**	1
	Sig. (2-tailed)	.000	
	N	338	338

**. Correlation is significant at the 0.01 level (2-tailed).

CONCLUSION

According to the statistics collected, 27.3% of respondents in the FSHMT are overweight or obese, whereas 72.7% of respondents are in good health. The level of obesity is influenced by genetics and diet

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Journal: British Journal of Nutrition / Volume 131 / Issue 1 / January 2024Published online by Cambridge University Press: , pp. 134-142

POSTER PRESENTATION

The impact of stress on students' academic performance, age 18-25 years in the Aldent University, Medical University

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Abstract

This study assessed the impact of stress on the academic performance of students in the Aldent University, Tirana , Albania. Stress is a major source of problems that students face during their university education. Academic pressure is one of the factors that causes students to fail. Sharma, Parasar and Mahto (2017) define stress as the mental response and action by hormonal signaling, the perception of danger triggers and automatic response system.
The aim of this study is to examine the effect of stress on the academic performance of Aldent University students and the associated risk when stress is not managed.
The study was carried out in the city of Tirana (for a period of two months). For this study a sample of 73 students in the age group of 18 to 25 years old was used out of six hundred and sixty-nine (669) students in the bachelor and master program in nursing. The selection of students was done randomly.
In the realization of this study are used several methods, among which the main was the exploration of theoretical findings and various materials from the internet, such studies carried out the correlation between stress and it"s impact on the academic perfomance. The instrument of data collection was a questionnaire which consisted of 15 questions that focus on collecting data on students experience regarding stress and academic performance. The data were processed statistically, through SPSS.
From examining the rapport between stress and academic performance, it turned out that there was a statistically significant negative connection between stress and academic performance. The higher the stress levels, the lower the academic results of the students. It was concluded that stress has a negative impact on the academic performance of students.

Key words: Academic performance, stress, managing stress, students.

INTRODUCTION

Academic achievement represents performance outcomes that indicate the extent to which a person has accomplished specific goals that were the focus of activities in instructional environments, specifically in school, college, and university. School systems mostly define cognitive goals that either apply across multiple subject areas (e.g., critical thinking) or include the acquisition of knowledge and understanding in a specific intellectual domain (e.g., numeracy, literacy, science, history). Among the many criteria that indicate academic achievement, there are very general indicators such as procedural and declarative knowledge acquired in an educational system, more curricularbased criteria such as grades or performance on an educational achievement test, and cumulative indicators of academic achievement such as educational degrees and certificates.

MATERIALS AND METHODS

In the realization of this study are used several methods, among which the main was the exploration of theoretical findings and various materials from the internet, such studies carried out the correlation between stress and it"s influence on the academic perfomance of students. The data was gathered from a sample of 73 students in the age group of 18 to 25 years old was used out of six hundred and sixty-nine (669) students in the bachelor's and master program in nursing. The selection of students was done randomly.
The study reveals this context through the literature used to complete this paperwork and by means of a questionnaire from 1 - 20 February 2022. These interviews, questionnaire and observations were carried out at the "Aldent University" a private university in the city of Tirana, during the students' free hours.
Sampling: : To realize this study i managed to secure a sample of 73 students, 87,7% of respondents are girls and 12.3% are boys. 92% of the respondents turn out to be aged 20-25 years and 8% 18 years old.

This division was made with the idea of seeing if does exist a correlation between stress and it"s impact on the academic performance of students. In the realization of this study are used several instruments, such as: closed questionnaire with students, monitoring, among which the main was the exploration of theoretical findings and various materials from the internet, such studies conducted regarding the importance of stress and its impact on the academic performance of students. The interview consisted of 15 questions. The interviews were analyzed according to the answers received.

It"s purpose was to provide more specific information about the raised hypothesis, for its validation or not. At the beginning, on the distribution day after the pilot phase, was made clear to the students that they would be anonymous, as well as the student was free whether they wanted to complete it or not.

The most important ethical issues: All respondents who were interviewed aware of the purpose of the interview. The names of the interviews were not used in the study. To any person was clarified the nature of the study, it's aim and responsibility of each party. To all those who cooperated were asked sincerity in answering and that the study would preserve anonymity. All collaborators were explained to the confidentiality of the study.

RESULTS

Table 1. Distribution of students by age group in number and percentage.

Age	**No. of students**	**Age in %**
≥ 18	15	8 %
20-25	67	92 %

Table 2. Distribution of students by gender in number and percentage.

Gender	**No. of students**	**%**
Female	63	87.7 %
Male	10	12.3 %

Table 3. Distribution of students according to the type of program, bachelor or master.

Program	No. of students	%
Bachelor in Nursing	50	67.7 %
Master in Clinic Nursing	23	32.4 %

Table 4. Distribution of students according to residence.

City	**No. of students**
Tirana	39
Fier	2
Lushnje	1
Berat	2
Other	29

53.4% of respondents live in Tirana, 2.7% in Fier, 1.4% in Lushnje, 2.7% in Berat, and 39.7% in another city in Albania.

Table 5. Distribution of students according to stress sympotms.

Diagnostic	**Yes**	**No**
Fatigue	71.9 %	9.6%
Insmonia	83.6%	16.4%
Changes in appetite (loss of appetite or constant eating)	89 %	11%
Inability to concentrate in class	82.2 %	17.8 %

Table 6. Distribution according to stress symptoms, such as low mood and sadness.

Have you ever had low mood, sadness and anxiety ?	**%**
Yes	42.5%
No	4.1%
Sometimes	53.4%

42.5% of respondents report that they had low mood, sadness and anxiety continuously, 4.1% refer to having had mood changes, sadness and 53.4% report that they have sometimes had a low mood, sadness or worry.

Table 7. Distribution according to stress effect on the academic performace of students.

Do your stress level effect your academic performance?	No. of students	%
Yes	64	86.5%
No	9	13.5 %

CONCLUSION

The first section dealt with the analysis and presentation of demographic data collected from the respondents. This is the preliminary analysis which focuses mainly on the profile of respondents. By this preliminary analysis, inference is made on the respondents' age, gender, program of study, residential status This analysis is very important as it enables the researchers have background information about the participants.

The study revealed that majority of students in the Aldent University,Ba and Master in Nursing studies are within the age group of 20-25 (92%). The demographic data also revealed that the female population dominated the sample 82.7 %. The results obtained from the analyses of the data on the symptoms of stress revealed that the following signs; loss of appetite, sleeplessness, irritability, inability to concentrate in class, fatigue, nervousness were all experienced by the respondents. This confirms the assertion by Agolla and Ongori [14] that due to pressure many university students suffer from fatigue, irritability, loss of appetite, insomnia and sometimes depression. The study further revealed that academic stress affects students' performance. Some identified effects of stress by the respondents during the survey were poor performance in exams, weak memory, lack of appetite for learning, low attendance in class, sleepless nights, and lack of interest in extracurricular activities. The result show that 86.5% of respondents answered that stress affect heir academic perfomance and 13.5 % does not affect their academis performance.

The researchers observed that higher levels of stress could result in poor academic performance. Students who suffer from acute stress may experience negative physical and mental health outcomes which could affect their academic performance. Even though stress can sometimes have a positive impact on students' academic performance, the findings show that to a large extent, it affects students negatively. The inability of students to cope with stress often leads to poor academic performance.

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POSTER PRESENTATION

Determination of total fat and total polyphenols in four types of nuts

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Abstract

Nuts are a good source of amino acids, proteins, fat, polyphenols, minerals, fibres, etc., and their content varies according to species, geographic position, and harvest year as well as on storage conditions after harvest. Nuts are well known for their nutritional values and also for their positive health effects against different serious diseases like diabetes, cardiovascular problems, cancer, etc. Four types of shelled nuts were used to perform this study, walnuts, hazelnuts, almonds and peanuts, provided directly on free market and randomly selected. Unshelled and grounded samples were analyzed for moisture, total oil and total polyphenol content. Moisture content was determined by oven drying, total fats were determined using Soxhlet extraction method and total polyphenols were determined according to the spectrophotometric method described by Ivanova et.al (2010). Peanuts resulted with the highest moisture content (2.95 %) and hazelnuts resulted with the lower moisture content (1.14%). Walnuts and hazelnuts had the highest total fat content, respectively of 66.16 and 66.62 %, and almonds and peanuts had lower total fat content, respectively of 48.60 and 40.74%. Total polyphenols content resulted higher on walnuts (3667.92 mg/100 g dry matter), followed by peanuts with 371.47 mg/100 g dry matter, almonds with 234.16 mg/100 g dry matter and hazelnuts with the lower total polyphenols content 171.14 of mg/100 g dry matter. Soxhlet extraction method used in our study gave considering high yield of total fat, and referred on literature almost the same yields can result by using other extraction methods including green methods.

Keywords: nuts, total fat, total polyphenols, Soxhlet extraction

INTRODUCTION

Nuts have a wide consumption rate, while almonds and walnuts have the highest consumption rate accounting respectively of 31% and 19% in world range, and Asia has been the top consumption region (32%) followed by Europe (31%) and North America (20%) (INC Nuts & Dried Fruits Statistical Yearbook 2022/23). This high trend of nuts consumption is related to their nutritional values because they are rich in amino acids, fatty acids, proteins, vitamins, polyphenols, minerals, etc., (S.K. Chang et al.2016). Nuts contain considering amounts of unsaturated fatty acids thus they are rich in antioxidants compounds (Jelena Rajić et.al 2022). Several studies have reported health benefits because of nuts consumption, like reducing chances of cardiovascular diseases, reduction of cholesterol levels, blood pressure, oxidative stress and protection from some types of cancer (Nogales-Bueno et al. 2021).

However, other factors like cultivar type, environmental conditions, agronomic practice, harvest conditions and storage conditions, can significantly affect levels of the main constituents of nuts: proteins, vitamins, fat, bioactive compounds, minerals, etc., (A. Wojdyło et.al 2022).

The aim of this study was determination of total fat and polyphenols content in four types of nuts with high consumption rate, in order to evaluate their chemical composition for these two main components and their nutritional values.

MATERIALS AND METHODS

To perform this study four type of shelled nuts were used, walnuts, hazelnuts, almonds and peanuts. They were provided on the market and randomly selected. The four types of shelled nuts were codified, grounded and then analyzed for moisture, total fat and total polyphenol content.

For moisture content assay, 5 grams of each type of nuts were ground and oven dried on 50 to 70^{0}C for about 6 hours and moisture content was calculated based on weight loss before and after heat treatment (AOAC 1995).

Soxhlet extraction method was used for total fat content assay. In this method, 5 grams pasta from each type of nuts, was dehydrated with potassium sulphate anhydrous, dried up to a constant weight on thermostat on 103^{0}C, and then fat extraction was performed by using petrolium ether on Soxhlet extraction system (Soxhlet Behr Labor) for 4 hours.

To prepare extracts for total polyphenols assay, 5 grams of ground sample (from each type of nuts) were twice extracted for 15 minutes with 20 mL ethanol/water (80/20 v/v) containing HCl (0.1/10 v/v) to prevent polyphenols oxidation and then put on an ultrasound bath in room temperature and mixed for 30 minutes on magnetic stirrer. After centrifugation (3000 rpm for 10 minutes), supernatants from 2 extractions were combined to a final volume of 25 mL with distilled water. Provided extracts were filtered on filter membrane 0.45 µm and left in dark till spectrophotometric determination of polyphenols.

Total polyphenols assay was performed by using the method described by Ivanova et.al.2010. 25 mL flask was filled with 2.5 mL extract, 12.5 mL of distilled water, 2.5 mL Folin–Ciocalteu reagent, after 3 minutes 2.5 mL sodium carbonate 7.5%, that is then filled up to the mark sign with distilled water. İt is then left in dark for 40 minutes, in room temperature, to determine optical density on 750 nm against a water blank, by using a SPECORD 40 spectrophotometer. The result were exspressed as gallic acid equivalent (GAE).

Calibration curve was created by using water solution of gallic acid of 50 – 500 mg/L concentrations.

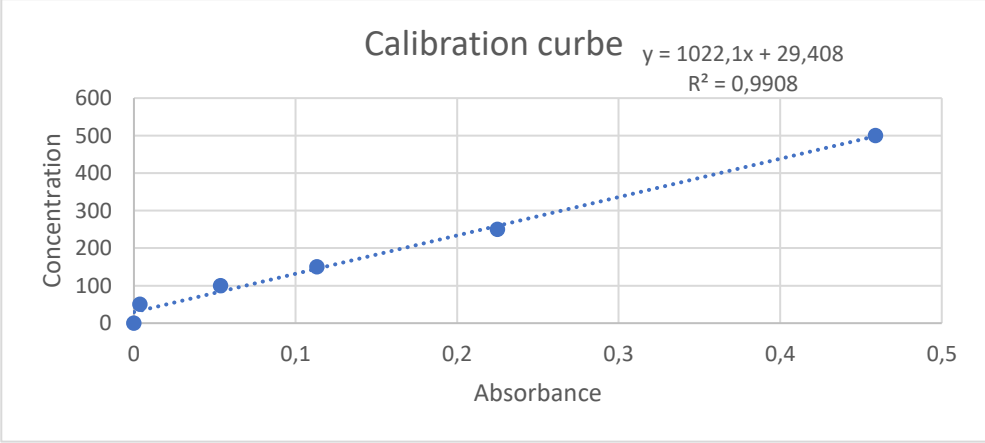

Figure 1. Calibration curve for total polyphenols determination

RESULTS AND DISCUSSION

Data presented on Table 1, represent the results obtained from Moisture and Total Fat (lipid) assay of the four types of nuts (walnuts, hazelnuts, almonds and peanuts). Peanuts resulted with the highest value of moisture content (2.95 %) followed by almonds (2.44%), walnuts (2.30 %) and hazelnuts resulted with the lowest moisture content. All these low values of moisture content are typical for the analyzed types of dry fruits (nuts). Sahri Yanti et. al 2021 have reported values of 2.42% moisture content on peanuts.

Among the four types of analyzed nuts hazelnuts and walnuts resulted with te highest values of total fat (%) respectively with 66.62 and 66.16 %. While analyzing five varieties of walnuts (Chandler, Franquette, Howard, Lara and Tulare) by using soxhlet extraction method with petrolium ether as extraction solvent, Nogales-Bueno et al. 2021, obtained similar values varying from 65.58% to maximum values of 68.86 %.
Sahri Yanti et.al 2021 have reported 40.08% total fat content on peanuts when extracted with Soxtec 8000^{TM} at 135^0 C, n-hexane 50 mL, boiling time 40 min and sample weight of 3 grams.

When discussing fatty acid content in differnet type of nuts, Emilio Ros and Jose´ Mataix (2006) have reported that among nuts walnuts and hazelnuts had the highest value of fatty acids (respectively of 65.20 and 60.80 g/100 gram) and almonds and peanuts had lower values of fatty acids (respectively 50.6 and 49.2 g/100 gram).

Table 1. Moisture (%) and Total Fat (%) content of analyzed samples

Sample	Moisture content (%)			
	Measure 1	**Measure 2**	**Measure 3**	**Mean**
Walnuts	2.30	2.40	2.18	2.30
Hazelnuts	1.15	1.14	1.13	1.14
Almonds	2.44	2.46	2.42	2.44
Peanuts	2.94	3.01	2.90	2.95
Sample	**Total Fat content (%)**			
	Measure 1	**Measure 2**	**Measure 3**	**Mean**
Walnuts	66.14	66.23	66.12	66.16
Hazelnuts	66.61	66.64	66.63	66.62
Almonds	48.58	48.62	48.6	48.60
Peanuts	40.75	40.73	40.73	40.74

Traditional extraction methods, including cold press and solvent extraction, have been widely used to extract oil and fat of nuts. Cold press extraction method does not use any solvent so it is more safety for environment and the most commonly extraction solvent used are hexane or petroleum ether (Masoodi, L, et.al 2022) that can extract high amounts of oils and fats but poses environmental concern and thermal breakdown of selected compounds (Castro, M.D. Let.al 2010).

Total polyphenols content results obtained are presented in Table 2. The data show that walnuts had the highest polyphenols content of 3667.92 mg/100 g dry matter, followed by peanuts containing 371.47 mg/100 dry matter, almonds with 234.16 mg/100 g dry matter and hazelnuts resulted with the lowest polyphenols content of 171.14 mg/100 g dry matter.

Table 2. Total polyphenols (mg/100g dry matter) content of analyzed samples

Sample	**Absorbance 1**	**Absorbance 2**	**Absorbance 3**	**Absorbance (mean)**	**Total Polyphenols (mg/100 g dry matter)**
Walnuts	0.038	0.045	0.046	0.04	3667.92
Hazelnuts	0.14	0.129	0.147	0.14	171.14
Almonds	0.211	0.195	0.195	0.20	234.16
Peanuts	0.334	0.337	0.333	0.33	371.47

High values of polyphenols content in walnuts of 3733 ± 1190 mg/100 g dry matter, have also been reported by Zujko, Małgorzata & Witkowska, Anna. (2011), when using Singleton-Rossi method, resulting in high values of FRAP antioxidant values as well (59.17 ± 17.6 mmol/100g dry mass). The same authors have reported that hazelnuts contained 550 ± 130 mg/100 g dry matter that was higher that polyphenols content in peanuts (460 ± 90 mg/100 g dry matter).

When using Folin-Ciocalteu reaction, Bolling BW et al. 2010 reported polyphenols content (mg/100 g) in descending order from walnuts to hazelnuts, almonds and peanuts with the lowest content.

CONCLUSION

All the four types of analyzed nuts resulted in considering levels of total fat content, similar to other studies. The Soxhlet extraction method used, even not included on green extraction methods because of petroleum ether usage, can still be used as a good extraction method to obtain high amount of fat that can be further used for qualitative and quantitative analyses.
Because of relatively high total fat and polyphenols content, the four types of nuts can serve as a a good source of energy and antioxidants.

ACKNOWLEDGEMENTS
We would like to thank the staff of Food Research Center, near our Faculty for their help and support in reaching the aims of this study.

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POSTER PRESENTATION

Preparation of poly (2-methacryloxy ethyl trimethyl ammonium chloride) hydrogel for removal of Remazol Black B from aqueous solutions

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Abstract

The interaction between polymer and dye leading to polymer-dye complex formation exhibits many interesting and important practical features. In this study, a hydrogel was prepared by crosslinking copolymerization of 2-methacryloxy ethyl trimethyl ammonium chloride with methylene bis acrylamide (MBA) in aqueous solution, using potassium persulfate as a radical initiator at 80 ^{0}C. Dye extraction experiments were carried out by interacting wetted gel samples with aqueous dye solutions at room temperature. Capacities were determined by colorimetric analysis of the residual dye contents. The hydrogel swelled in water showing that reasonable high dye sorption capacity 600 mg per gram of gel was achieved.

Keywords: Hydrogels, dye removal, poly (2-methacryloxy ethyl trimethyl ammonium chloride)

INTRODUCTION

Polymeric gels have been used in chemical separation (Bicak N et.al, 2000; Matsuyama H et.al, 1996) biomedical engineering, etc. (Friends CD et.al,1992; Bruinsma GM et.al, 2001). Hydrogels are prepared mostly by crosslinking copolymerization with a water-soluble divinyl monomer. Methylene bis-acrylamide (MBA) is the most common used and commercially available co-monomer for crosslinking of water-soluble monomers. Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade (Karadag E,1998). Though many dyes are inert and non-toxic at the waste concentrations discharged into surface waters, some are harmful to aquatic life in rivers where they have been discharged. Moreover, the color they impart is very undesirable to the water user. Therefore, colour removal from waste-water is a major environmental problem.

Many sorbents were used for dye removal. For example, chitosan, monocarboxymethylated chitosan (MCM-chitosan) (Vandevivere, P.C,1998) and rice husk (Sun, Q., Yang, L, 2003) were used as a sorbent for removal of dyes from aqueous solutions. However, most of them are not regenerable, such as bagasse pith (Ravi Kumar, M.N.V, et all, 1998) or eucalyptus bark (Ghoreishi, S.M., Haghighi, R., 2003). Good sorption capacities for reactive dyes (60-420 mg.g^{-1}) are found for quaternized organic materials such as (Jain, A.K ,2003) rice husks and coconut husk Ho, Y.S., McKay, G ,2003). The interaction between polymer and dye leading to polymer–dye complex formation exhibits many interesting and important practical features.

Coulombic, hydrophobic, and steric interactions are major factors governing the thermochemical and dynamic aspects of complex formation. The interactions of the hydrogels with dyes are of principal interest. Considering the environmental requirements, the development of water-based colored polymer materials is one of the modern targets for the chemical industry.

MATERIALS AND METHODS

Methlene bisacrylamide (MBA), 99% (Merck), 2-methacryloxy ethyl trimethyl ammonium chloride (Merck), potassium persulfate, 99% (Fluka). All chemicals used were analytical grade.

Preparation of the hydrogel

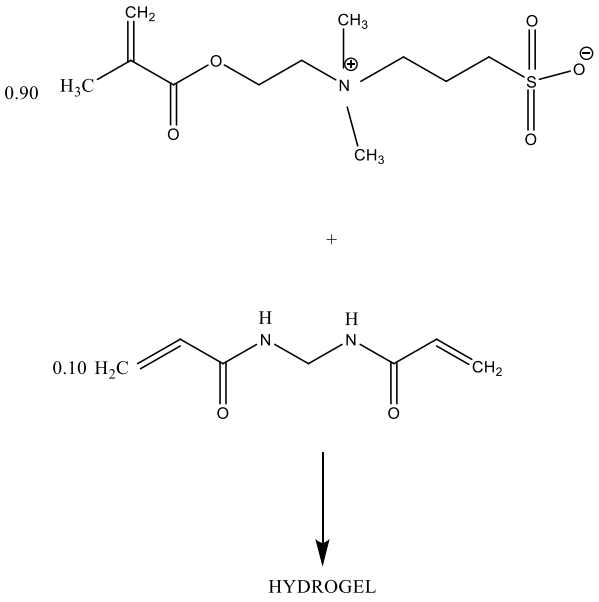
2 g of 2-methacryloxy ethyl trimethyl ammonium chloride and 0.13 g of MBA were placed in a flask attached to a reflux condenser and a nitrogen inlet. An appropriate amount of water was added to the flask, to obtain a total monomer concentration of 40%. Then, 0.42 g $K_2S_2O_8$ was added to the solution and nitrogen was flushed through the solution. The mixture was stirred until gelation proceeded at 80^0C. The formed gel was washed with excess water and acetone respectively. The product was dried under vacuum at room temperature for 24 hr. The yield was 1.8 g.

Dye uptake measurements of the hydrogels

Dye capacity of the hydrogels were determined after determining optimum sorbent quantity and mixed 15 mg of polymer sample with 10 ml aqueous dye solution (20-1500 ppm). In these experiments Remazol black B Everzol was used. The mixture was stirred for 24 h and then filtered. The dye concentrations were determined colorimetrically at 597 nm. The dye loading capacities were calculated from the initial and final dye contents of the solution. Also, dye sorption kinetic experiments were performed using 15 mg hydrogel and 10 mL (250 ppm dye). The mixtures were stirred with a magnetic stirring bar and aliquots of the solution (5 ml) were taken at appropriate time intervals for the analysis of the residual dye contents by the method described. Dye sorption kinetic experiments were also studied and

RESULTS AND DISCUSSION

In this study, hydrogel was prepared starting from gelation with 2-methacryloxy ethyl trimethyl ammonium chloride monomer (90 % mol) , MBA (10 % mol) as crosslinking agent and potassium persulfate as initiator at 80 ^{0}C (Scheme 1). Hydrogel swelling ratio was found as 677.5%.



Scheme 1. Preparation of Hydrogel

Extraction of dyes

Batch experiments of dye sorption were performed at room temperature by depending on different initial dye concentrations. Residual dye contents were determined by colorimetrical analysis and dye sorption capacities were calculated difference from non-adsorbed and adsorbed dye concentrations. Dye sorption capacity of the hydrogel was found about 578 mg / g hydrogel (Fig.1).

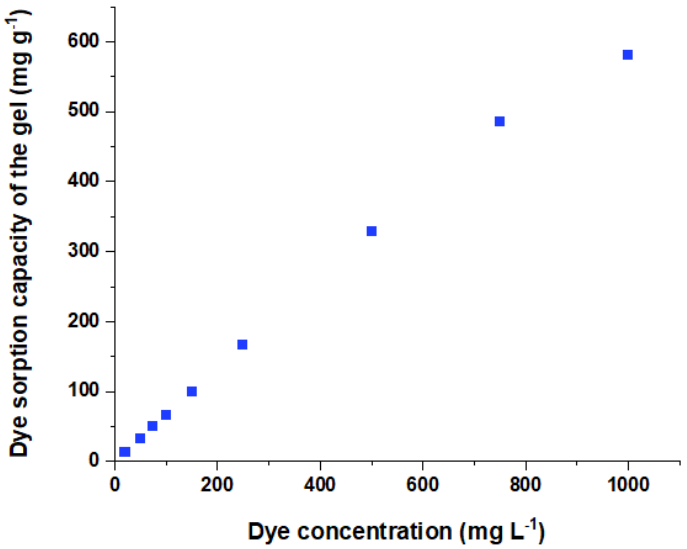


Figure 1. Dye sorption capacity of the hydrogel

In the adsorption isotherm experiments, Freundlich and Langmuir isotherm models were used for dye sorption of the hydrogel. Langmuir model is suitable with the highest correlation factor value according to Freundlich isotherm model (Figure 2).

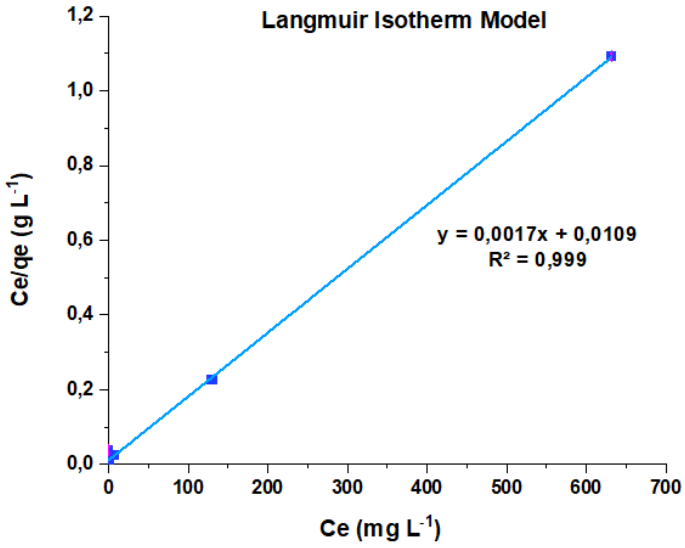


Figure 2. Langmuir isotherm model of the hydrogel

In aqueous solutions, the synthetic reactive dye was dissolved and the sulfonate group of the reactive dye was dissociated and converted to anionic dye ions:

$DyeSO_3Na + H_2O \rightarrow DyeSO_3^- + Na^+$ (1)

The adsorption process then proceeded due to the electrostatic interaction between these two counterions:

$-NH^{+3} + DyeSO_3^- = -NH^{+3-}\ O_3SDye$ (2)

İsoelectric point of the hydrogel was determined and found as 7. pH depending dye sorption experiments were performed and maximum dye sorption capacityof the hydrogel was found at pH=7 (Table 1).

Table 1. Maximum dye sorption capacities of the sorbent depending on pH

pH	Capacity (mg dye / g gel)
2.0	177.95
3.0	259.28
4.0	265.30
5.0	287.32
6.0	373.61
7.0	578.00
8.0	500.52
9.0	460.42

Dye sorption kinetics of the hydrogel

This material is able to remove Remazol Black B from diluted aqueous dye solutions which are highly important. Batch kinetic sorption experiment was performed with diluted dye solutions (250 ppm) to investigate the efficiency of the hydrogel. Dye loading capacity–time plot in Figure 3 shows that within about 60 min of contact time, the dye concentration falls to zero.

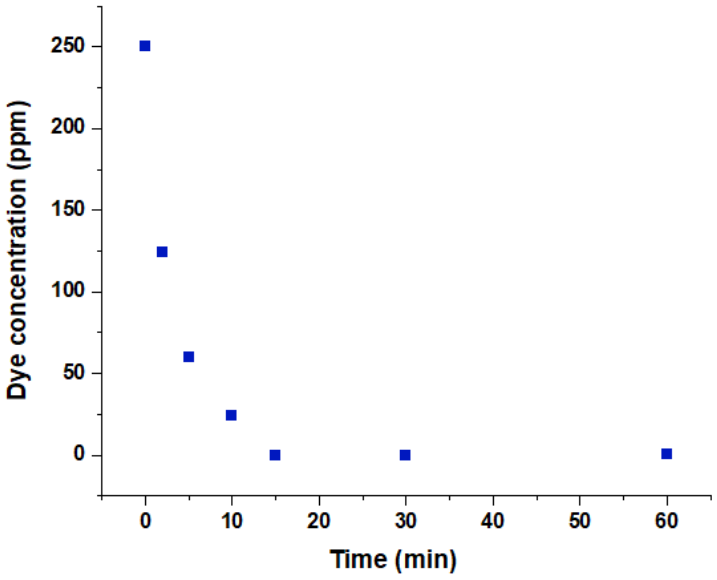


Figure. 3. Dye sorption kinetics of the hydrogel

CONCLUSION

The hydrogel is highly effective in removal of Remazol Black B from water and the hydrogel format makes the material of interest as a column packing material for technological use. Under non-buffered conditions, the dye uptake capacity is around 578 mg dye / g polymer.

ACKNOWLEDGEMENT: This research has been supported by the Istanbul Technical University (Project number: TYL-2024-45979)

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POSTER PRESENTATION

Vairimorpha ceranae as the main cause of bee colony losses in the northern part of Albania

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Abstract

During the year 2021-2023, the losses of bee colonies in Albania were analyzed. Colony losses resulted in 23% (Korro, et al 2023). During these years, in cooperation with students of the Faculty of Veterinary Medicine, 123 samples were collected from 37 bee parks in the districts of Tropoja, Kukesi, Dibra and Shkodra. These samples were analyzed for the presence of Vairimorpha ceranae and Vairimorpha apis (previously called Nosema). Our study shows that 95% of the samples had the presence of Vairimorpha ceranae. The presence of Vairimorpha ceranae was identified in all the bee parks, where we collected the samples. The study found the presence of Vairimorpha ceranae in all the districts included in the study. The study did not find any cases with Vairimorpha apis, different from the results of previous years that had resulted in the presence of Vairimorpha apis. The study was focused on the northern part of Albania, being initiated by the information of the beekeepers about the losses in the bee colonies that they had found during the winter season. The study was the first to be carried out in these districts and confirms the concerns of beekeepers about the losses in bee colonies in recent years.

Keywords: Vairimorpha ceranae, Vairimorpha apis, bee, Albania, losses of bee

Introduction

During the last 10 years, large losses of bee colonies (Apis mellifera) have been recorded in Europe and throughout the globe. Colony losses have occurred for various reasons, but researchers have listed among the most important causes *Varroatosis, Nosematosis* or *Vairimorpha* as it is now called and it can be caused by *Vairimorpha ceranae* and *Vairimorpha apis*, viral diseases in bees, poisoning by neonicotinoid pesticides that are used today in developed agriculture, as well as infections caused by bacteria. (Willmer et al., 2017). Beyond doubt, Varroa mites has been evaluated as a destructive machine of bees, but Nosematosis caused by Vairimorpha ceranae and Vairimorpha remains another important factor in the damage of bee families, not only in Europe but also in other continents (Martín-Hernández et al., 2018) For years, infections by Vairimorpha ceranae and Vairimorpha apis have been found mixed, and often in the monitoring of bee colonies, not only one species is found, but both types of Vairimorpha (Topolska, G et al., 2018).

In view of the difficulties in diagnosing Nozematosis caused by Vairimorpha ceranae, the disease is often neglected by beekeepers but sometimes also by veterinarians, and this leads to the great spread of the infection, destroying bee colonies (Ewa Danuta Mazur and Ana Maria Gajda 2022). The life cycle of Vairimorpha apis is about 5 days, while the life cycle of Vairimorpha ceranae is only 3 days, making the latter more widespread and harmful to bee colonies (Urbieta-Magro et al 2019). Due to the high pathogenicity of Vairimorpha ceranae that focuses on the degeneration of the bee intestines, as well as the high rate of spread, attention to the fight against this infection remains an emergency in Apis mellifera bee populations. (Galajda, R et al., 2021).

2. Materials and Methods

During the years 2021-2023, based on the information of beekeepers about the death of bees in their parks, 123 samples were collected in 37 bee parks. For each sample, 30 worker bees were taken from the moment of entering the hive or inside the hive. The samples were taken based on beekeepers' concerns about the unexplained death of bees. The samples were stored in the laboratory of Bee and Wildlife Diseases at the Faculty of Veterinary Medicine, Agricultural University of Tirana.

The abdomens of 30 honey bees were separated and macerated in about 2.5 mL of distilled water using a mortar and pestle and successively 27.5 mL of distilled water was added to a final volume of 1 mL per bee. A drop of this suspension was examined under a light microscope at 400× magnification to evaluate the presence of Vairimorpha spp.spores. Vairimorpha spp.-positive samples were analysed for V. apis and Vairimorpha

ceranae species identification after DNA extraction from 1 mL homogenate using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with a lysozyme pre-incubation step. The yield and purity (260/280 and 260/230 nm absorbance ratios) of DNA were determined using the Nanodrop™ OneC (Thermo Fisher Scientific, Waltham, MA, USA) spectrophotometer. DNA was stored at −20 ◦C until use. Negative controls (negative process control—NPC: water for molecular biology applications instead of the sample) were included in each extraction session.

3. Results

Our study resulted that from 123 samples collected in the bee parks with cases of bee deaths, 117 samples resulted positive with Vairimorpha ceranae.

Table1 present the positive cases according to the samples collected

No	Places of bee parks where the samples were collected	No. of samples collected	Results with the presence of V. ceranae	Results without the presence of V.or V. apis
1	Tropoja	30	28	2
2	Kukesi	30	29	1
3	Dibra	33	31	2
4	Shkodra	30	29	1
	Total	123	117	6

The samples were taken in two years and were divided into two seasons: In the spring and in the summer

The results came out almost the same in the two years, with a slightly higher level in the spring than in the autumn..

Discussions:

The study is initial in these districts and it identified V. ceranae., which was not identified in previous years. In the past years, the cause has been identified as V. apis. This leaves room to discuss whether V. ceranae existed before, or was confused with V. apis, since the diagnostic methods applied before were microscopic methods, making it impossible to identify V. apis (Martín-Hernández et al., 2011). The application of the molecular method enables the differentiation of Vairimorpha ceranae from Vairimorpha apis. The research results enable the identification of the main cause of the loss of bee colonies in this area and enable research in other areas of Albania to identify the cause of bee losses. The study results also solve the doubts of beekeepers, why there is a loss of bee colonies in their parks, at a time when the treatments for varroa mites have been carried out. (Higges, M et al., 2008)

Conclusion

The study is the first of its kind in the northern areas of the country to identify Vairimorpha ceranae in bee farms, using coherent diagnostic methods that allow differentiation from Vairimorpha apis. The study identified Vairimorpha ceranae as the main cause of bee colony losses in this area, orienting beekeepers to the correct treatment of the disease and eliminating suspicions of other possible causes. The study can serve as a model to conduct similar research in other areas of Albania to identify if Vairimorpha ceranae is widespread in other areas of the country.

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POSTER PRESENTATION

Effects of the different sulfur doses on yield and yield components of soybean

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Abstract

Sulfur fertilizer has been identified as a key factor in optimizing plant growth, and enhancing the seed yield. It was aimed to determine the effects of different S rates on yield and yield components of soybean in this study. The study was conducted in Hatay, Eastern Mediterranean, Türkiye, in 2022 according to a randomized complete block design with three replications. Agronomic traits were found to be significant for sulfur doses except for the number of branches per plant. The highest number of pods per plant, plant height, 100-seed weight, and also seed yield were observed in S30 treatment while S45 treatment had the maximum value for the first pod length. Finally, the present study indicated that sulfur positively affected the yield components of soybean, especially with S30 treatment which increased seed yield to 487.19 kg da^{-1}.

Keywords: Seed yield, soybean, sulfur.

INTRODUCTION

Soybean (*Glycine max* L.), belonging to *Fabaceae*, is one of the most important legume crops with significant economic and nutritional importance worldwide. It also has roles in both human and animal nutrition. Soybean seeds contain approximately 20% oil and 40% protein (Dawood et al., 2013; Rady et al., 2015). Soybean provides a key source of protein and income for smallholder farmers (Pagano and Miransari, 2016).

In 2022, soybean covered approximately 133.8 million ha area in 115 countries. The world's peanut production was about 348.9 million t, averaging about 2.6 t per hectare. Brazil came to the forefront in production, with about 35% of overall world production, followed by the USA having 33% share and Argentina having 13% (FAO, 2024). In that year, Türkiye produced about 155 thousand t of soybean, averaging about 4.1 t per ha on 38 thousand ha area. Provinces Adana, Kahramanmaras, and Mersin, located in the Mediterranean region, led the first three places with 84% of total production (TUIK, 2024).

Fertilization is accepted as the major factor in plant nutrition. Besides, the availability of plant nutrients is affected by the type, amount, and treatment method of fertilizer, which also influences biochemical and physiological mechanisms in plants (Darakeh et al., 2021; Yan et al., 2021, Zhou et al., 2021). Sulfur (S) nutrition has been identified as a key factor in optimizing plant growth, particularly in chlorophyll production, protein synthesis, and oil quality. Efficient S utilization is critical for achieving high yield and maintaining crop health. This makes S an indispensable nutrient in the cultivation of crop, directly influencing both the quality and quantity of the harvest (Ariraman and Kalaichelvi, 2020; Vinothini et al., 2022).

Yigit et al. (2021) conducted a study to determine the effects of sulfur fertilization on soybean growth and found that agronomic traits were not affected by sulfur. However, it was reported that sulfur treatments increased the yield and improved the yield components (Zhao et al., 2008). Similarly, Mahal et al. (2022) observed that the grain yield of corn and soybean showed a significant positive response to S fertilizer.

The objective of this study was to evaluate soybean yield and yield components' response to different S fertilizer doses.

MATERIALS AND METHODS

The field experiment was conducted of Hatay Mustafa Kemal University Agricultural Research Field (36°18'19.9"N 36°13'29.4"E; 83 m) in the Eastern Mediterranean of Türkiye during the growing season of 2022. Soybean cultivar Nazlican was used as plant material. The pH of the soil used in the study was slightly alkaline (pH ~8.3). Lime content (~4%) and organic matter (~1%) of the soil was poor. There were no differences between the studied year and long term about total precipitation and average temperature.

Experiments were conducted in Randomized Complete Block Design (RCBD) with three replications. Each plot was 5 m long and had four rows with 70 × 5 cm spacing. Di-ammonium phosphate (18% N, 46% P_2O_5) fertilizer was used at a rate of 25 kg da^{-1} before sowing, and 20 kg urea (46% N) per da was used in R1 stage for the top fertilizer. The treatments of S (0, 15, 30, and 45 kg S per hectare) were also applied with DAP. Soybean seeds were inoculated with *Brady rhizobium* spp. just before sowing. Sowing was performed on June 20, 2022. Hand weeding was performed with the emergence of the plants. Besides, the springer irrigation system was used for the irrigation (Fig. 1). Manuel harvests were performed on October 3, 2022. Harvests were performed from two inner rows by taking into consideration side effects.



Figure 1. General view of the experimental area

Plant height, the first pod length, number of branches per plant, number of pods per plant, 100-seed weight, and seed yield were measured over 10 plants randomly selected from each plot following the harvest. For 100-seed weight, 4 × 100 seed groups were taken from each plot and they were weighed, and averaged. Seed yield was determined through weighing the seeds of all plants of a plot except side effect rows (Sahin and Isler, 2021).
Experimental data were subjected to analysis of variance (ANOVA) in accordance with "RCBD" with the aid of SPSS 22 software. Means were compared with the aid of Duncan's multiple range test.

RESULTS and DISCUSSION

All investigated parameters were found to be significant ($p < 0.01$) for S doses except the number of branches per plant according to ANOVA (Table 1). Plant height varied between 86.25-90.24 cm while the first pod length ranged from 8.50 cm to 12.84 cm. The highest value for plant height was observed in S30 treatment with the value of 90.24 cm. Unlike plant height, the maximum value for the first pod length was obtained on S45. Burkitbayev et al. (2021) reported that S-containing treatments increased plant height compared to non-treated groups in the studied years. Similarly, Yigit et al. (2021) observed that agronomic traits were not affected by S fertilizer. However, S enhanced the plant height and the first pod length and reported that the maximum and minimum plant height were 92.10 cm and 43.25 cm, respectively.
The number of branches and pods per plant varied between 2.35-3.27 and 55.90-70.23, respectively. Even if the branch number was found not to be significant, the highest value was observed in S15 treatment. Similar to plant height, S30 came to the forefront with the maximum pod number with the value of 70.23. All S treatments got higher values than the non-treated group. Yigit et al. (2021) reported that values of pod number ranged from 23.11 to 43.55. Boydak et al. (2018) concluded that the pod number of soybean varied between 19.46 and 72.10. The present study had a similar result with their study.

Table 1. Mean values of yield and yield components of soybean according to sulfur doses

	Plant Height (cm)	The First Pod Length (cm)	Number of Branches per Plant	Number of Pods per Plant	100-Seed Weight (g)	Seed Yield (kg da^{-1})
Control	86.60±1.32 c	11.51±1.78 b	2.35±0.18	55.90±3.34 d	14.34±0.76 b	381.59±25.59 d
S15	88.96±4.97 b	11.73±0.62 b	3.27±0.47	63.89±3.03 c	16.36±0.67 a	449.58±30.41 b
S30	90.24±2.12 a	08.50±0.12 c	2.79±0.37	70.23±4.93 a	16.87±0.37 a	487.19±63.91 a
S45	86.25±1.31 c	12.84±1.29 a	2.60±0.35	67.68±1.94 b	14.84±0.42 b	401.54±33.60 c
Mean	88.01±1.32	11.14±0.69	2.75±0.18	64.43±2.21	15.60±0.40	429.98±21.53
Block	ns	ns	ns	ns	ns	ns
S	**	**	ns	**	**	**

ns: non-significant; **: $p < 0.01$

The maximum and minimum 100-seed weights were found to be 16.87 g and 14.34 g, respectively. S15 and S30 treatments came to the forefront with the highest values. Seed yield ranged from 381.59 kg da^{-1} to 487.19 kg da^{-1}. As expected, S30 treatment also had the highest value like 100-seed weight. Yigit et al. (2021) observed that seed yield was not significantly affected by S fertilizer. However, it was reported that 100-seed weight and seed yield were significantly affected by S fertilizer (Mahal et al., 2022).

CONCLUSION

The S treatments at different rates showed positive influences on plant characteristics except the number of branches per plant. Increasing S doses enhanced the plant growth and improved the seed yield to 487.19 kg da^{-1}. Therefore, the results suggested that 30 kg S per ha (S30 treatment) was suitable for agricultural productivity purposes because it presented a higher seed yield in soybean.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Cem Tufan AKCALI and Mr. Mehmet Cihan CAYMAZER for their assistance during the study. Also, Mr. Ahmet Emrullah CIKAR who passed away because of the earthquake that occurred on February 6, 2023, will always be remembered.

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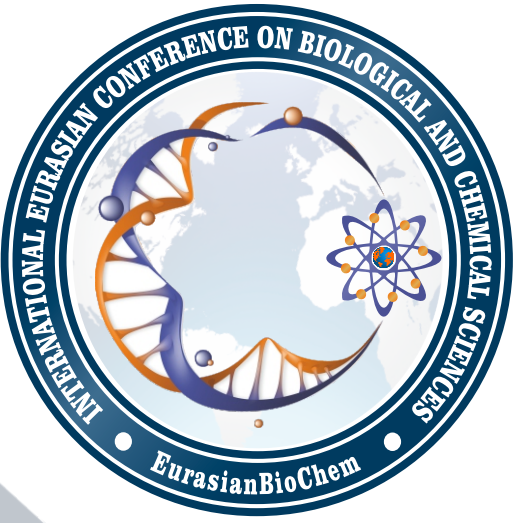

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