International Journal of Secondary Metabolite
2026, Vol. 13, No. 3, 666–682
https://doi.org/10.21448/ijsm.1764418
Journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

Evaluation of prebiotic activities of *Salvia* species as potential functional components for intestinal health

Mevlüde Nur Dilgen [1,2*], **Betül Aydin** [1], **Murat Koç** [2]

[1]Gazi University, Faculty of Science, Department of Biology, 06500 Ankara, Türkiye
[2]Ankara Yıldırım Beyazıt University, Department of Biotherapeutic Products Research and Development, Public Health Institute, 06010, Ankara, Türkiye

ARTICLE HISTORY

Received: Aug. 14, 2025
Accepted: Apr. 28, 2026

KEYWORDS

Salvia,
Prebiotic,
Probiotic,
Antioxidant,
Hydrophobicity.

Abstract: Some *Salvia* species have long been used in traditional medicine for digestive disorders. This study investigated the prebiotic potential of water and ethanol extracts of *Salvia fruticosa*, *S. huberi*, *S. sclarea*, and *S. aethiopis* on probiotic bacteria *Lactobacillus fermentum* ATCC 14931 and *L. plantarum* ATCC 8014. Among the tested species, the extracts of *S. huberi* and *S. sclarea* exhibited the highest prebiotic activity. The ethanol extract of *S. huberi* showed prebiotic activity scores comparable to commercial prebiotics. Under simulated gastrointestinal conditions, the *S. huberi* water extract significantly increased the survival of *L. fermentum*, while the *S. sclarea* water and ethanol extracts provided the highest viability rate for *L. plantarum*. Both species provided the highest cell surface hydrophobicity values and strong post-fermentation antioxidant activity*; S. huberi* water extract reached the radical scavenging rate. The present *in vitro* findings suggest that *S. huberi* and *S. sclarea* are promising candidates with potential prebiotic properties that may contribute to probiotic viability and gut health.

INTRODUCTION

The genus *Salvia* L., a member of the Lamiaceae family, represents one of the most important and comprehensive groups of medicinal and aromatic plants (Russo *et al*., 2013). Members of the genus *Salvia* have been shown to possess several beneficial properties, including antimicrobial, antioxidant, antitumor, antidiabetic, anxiolytic, and anti-inflammatory activities (Cui *et al*., 2015). Evidence presented indicates that the rich pharmacological potential of *Salvia* species can be exploited, particularly in research on their prebiotic properties. Prebiotics are a category of nutrients that nourish probiotics within the gut microbiota. It is well-documented that prebiotics have health benefits, and as such, they are considered an attractive therapeutic agent with the potential to improve human well-being (Davani-Davari *et al*., 2019). This is achieved by increasing the abundance of certain bacterial strains and regulating host lipid metabolism, thereby contributing to improved host health (Gibson & Roberfroid, 1995). Moreover, there is evidence to suggest that they promote intestinal health, thereby reducing the incidence of diarrhea, stimulating the immune response, and increasing mineral absorption

*Corresponding Author: Mevlüde Nur DİLGEN nurtopal92@gmail.com Gazi University, Faculty of Science, Department of Biology, 06500 Ankara, Türkiye

The copyright of the published article belongs to its author under CC BY 4.0 license. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/

e-ISSN: 2148-6905

(Rastall & Gibson, 2015). On the other hand, probiotics are defined as live microorganisms that confer health benefits to the host when administered in adequate amounts (Maftei *et al*., 2024). Probiotics have been shown to contribute to the maintenance of a healthy balance of gut microflora, which is essential for the proper functioning of the digestive system and the absorption of nutrients.

Probiotics have been shown to play a pivotal role in the prevention and treatment of various digestive disorders, including infectious diarrhea, antibiotic-associated diarrhea, and irritable bowel syndrome (IBS) (Al-Habsi *et al*., 2024). The immune system is modulated by probiotics, which enhance the production of antibodies and stimulate immune cells, thereby improving resistance to infection (Gul & Durante-Mangoni, 2024). There is a growing body of evidence to suggest that they may offer benefits in the management of cardiovascular diseases, obesity, and type 2 diabetes (Lau & Quek, 2024). Probiotics have been investigated for their possible anticancer properties, particularly in regard to the prevention of colon cancer, through the modulation of gut microbiota and the production of bioactive compounds. Recent studies suggest a potential relationship between probiotics and the gut-brain axis, with implications for mental health conditions such as depression and anxiety (Latif *et al*., 2023). The synergistic relationship between prebiotics and probiotics is pivotal in promoting the diversification and enhancement of gut microbiota. This in turn, has been associated with improved immune responses, optimal digestive function, and even positive effects on mental health.

The utilization of plant extracts as prebiotics represents a novel and rapidly expanding research domain, owing to their demonstrated capacity to modulate gut microbiota and impart health benefits. Plant extracts, which are abundant in bioactive compounds such as polyphenols, phenolic compounds, and dietary fibers, have been shown to stimulate the growth of beneficial gut bacteria while concomitantly inhibiting the growth of potentially harmful microorganisms (Rodriguez-Sojo *et al.*, 2023). The utilization of plant extracts has been demonstrated to facilitate the proliferation of probiotics by establishing an optimal environment and supplying essential nutrients. For instance, it has been demonstrated that *Zizyphus lotus* extracts can enhance the aggregation and adhesion of probiotics. This is a critical process for their successful colonization and activity within the gastrointestinal tract (Quldchikh *et al*., 2020). Another study showed that *Curcuma xanthorrhiza* extract significantly stimulated the growth of probiotic *Lacticaseibacillus paracasei*. The combination of pineapple juice and *Curcuma xanthorrhiza* extract resulted in the highest lactic acid production and antioxidant capacity, indicating a synergistic effect in promoting probiotic growth (Mashudin *et al*., 2024).

Milutinović *et al*., (2021) determined that extracts derived from the herbal medicines *Hypericum perforatum* and *Achillea millefolium* exhibit a stimulatory effect on the growth of the probiotic microorganisms *Saccharomyces boulardii* and *Lactiplantibacillus plantarum*. Notably, the findings indicate that *A. millefolium* extract was particularly effective, as evidenced by a 130-fold increase in the growth of *S. boulardii*. These results suggest that the polyphenolic compounds present in *A. millefolium* could contribute to a substantial prebiotic effect, potentially enhancing the proliferation of beneficial probiotic species.

Microbial transformation of phenolic compounds can be achieved through fermentation by probiotic bacteria. The process varies considerably among the specific species and strains of lactic acid bacteria involved (Morais *et al.*, 2019). It has been shown that probiotics can use enzymatic activity pathways for the release of phenolic compounds, which are then released in a more biologically active form than glycosides (Delgado *et al.*, 2019). It has been demonstrated that the utilisation of plant extracts can promote the proliferation of probiotics by attenuating oxidative stress, thereby enhancing their survival rates. Phenolic compounds derived from plant sources have been observed to contribute to gut health and facilitate the growth of probiotics (Holkem *et al.*, 2023). Additionally, the incorporation of these bioactive compounds into delivery systems has been shown to enhance the stability and efficacy of probiotics when

embedded within food matrices. This synergistic relationship between plant phenolic compounds and probiotics suggests a promising approach for achieving health benefits.

This study aimed to investigate the prebiotic potential of four *Salvia* species (*S. sclarea*, *S. huberi*, *S. fruticosa*, and *S. aethiopis*) grown in Türkiye, which are known for their rich bioactive profiles and pharmacological relevance. Water and ethanol extracts of these species were evaluated using multiple *in vitro* approaches to assess their ability to promote probiotic growth and enhance bacterial survival under gastrointestinal conditions. To our knowledge, this is the first comprehensive study comparing the prebiotic activities of these *Salvia* species.

MATERIAL and METHODS

Collection and Storage of Plant Samples

In June and July of the year 2023, all plants were collected and subsequently identified at the species level. The location information of the plants are respectively: *S. sclarea* L. localities and herbarium record information: Eskisehir: Tepebasi, above Kizilinler village, Ca. 890 m, UTM, 36 S 0278850 – 4394361, Koç 3820. *S. fruticosa* Miller locality and herbarium record information: Antalya-Alanya, going to Akseki Sadiklar Village, roadside, 800 m, UTM, 36 S 392751 – 408876, Koc 3589. Localities and herbarium record information of *S. huberi* Hedge: Erzincan: Cayirli, near the village of Bolukova, Ca. 1620 m, UTM, 37 S 0591355 – 4418895, 10.07.2020, Koç 3875, and finally, *S. aethiopis* L. locality and herbarium record information: Sivas-Zara, between Tepekoy and Begendik Villages, 1500 m, UTM, 37 S 0416949 – 4414771, Koç 3588. All samples were dried and stored.

Preparation of Plant Extracts

The plant extracts were obtained using the maceration extraction method. The powdered plant samples were water-extracted with distilled water at a ratio of 1:10 in a shaking water bath at 60 °C for 1 hour. To prepare ethanol extracts, the powdered plant samples were stored in the dark for seven days at a ratio of 1:10 ethanol and plant material. The samples were filtered through filter paper. Excess water was removed from the extracts in an evaporator.

Preparation of Bacterial Strains

Lactobacillus fermentum ATCC 14931 (recently reclassified as *Limosilactobacillus fermentum*) and *Lactobacillus plantarum* ATCC 8014 (recently reclassified as *Lactiplantibacillus plantarum*) were used as probiotic bacterial strains. *Escherichia coli* ATCC 25922 strain was used in the study. *L. fermentum* and *L. plantarum* probiotic bacteria strains were grown in DE MAN, ROGOSA, and SHARPE (MRS) medium at 37 °C for 24 hours. *Escherichia coli* (*E. coli*) strain was grown in Tryptic Soy Broth at 37 °C for 24 hours. Bacterial cultures were stored at -20 °C in 40% a 40% glycerol-medium mixture.

Determination of the Prebiotic Potential of Plant Extracts

Probiotic bacterial cultures were inoculated into MRS broth (without glucose) with 2% glucose, inulin, fructooligosaccharide (FOS), or plant extract, and incubated at 37 °C. *E. coli* ATCC 25922 (the initial bacterial concentration 10^8 CFU/mL) was grown in M9 medium under the same carbohydrate and extract conditions. Viable cell counts were determined by spread plating on MRS agar for lactic acid bacteria and tryptic soy agar for *E. coli*, followed by incubation at 37 °C for 24 h. *E. coli* is a commonly used reference organism in prebiotic studies and is essential for calculating the prebiotic activity score (PAS), which compares probiotic growth with that of an enteric strain. The total number of viable bacteria in 1 mL was calculated as colony-forming units (CFU/mL).

Salvia extracts are expected to increase the viability of the probiotic bacteria *L. plantarum* and *L. fermentum* in the intestinal flora, but not the enteric strain *E. coli*. Therefore, the prebiotic activity score indicating the prebiotic effect was determined. To calculate the PAS, glucose (negative control), inulin, and fructooligosaccharide (FOS) (positive control), and plant extracts

(experimental group), were used as carbon sources (Thuaytong & Anprung, 2011). This score is calculated according to (Eqs. (1)).

Prebiotic activity score (PAS):

$$\mathrm{PAS} = \left[\frac{(\text{probiotic log CFU ml}^{-1}\text{on the prebiotic at 24 h} - \text{probiotic log CFU ml}^{-1}\text{on the prebiotic at 0 h})}{(\text{probiotic log CFU ml}^{-1}\text{on glucose at 24 h} - \text{probiotic log CFU ml}^{-1}\text{on glucose at 0 h})}\right] - \left[\frac{(\text{enteric log CFU ml}^{-1}\text{on the prebiotic at 24 h} - \text{enteric log CFU ml}^{-1}\text{on the prebiotic at 0 h})}{(\text{enteric log CFU ml}^{-1}\text{on glucose at 24 h} - \text{enteric log CFU ml}^{-1}\text{on glucose at 0 h})}\right] \quad (1)$$

If specific carbon sources are well metabolized by lactic acid bacteria, and are not metabolized by other gut bacteria, the prebiotic activity score will be positive. Also, the higher the score, the higher the prebiotic activity.

Effect of Extracts on Probiotic Bacteria in A Simulated Gastrointestinal Environment

Simulated gastric juice (SGJ) and intestinal juice (SIJ) were prepared according to the methods described by Skenderidis *et al*. (2019). To prepare SGJ, the final concentration of the pepsin solution in 0.5% sterile saline was adjusted to 0.22% (pH 2). To prepare simulated intestinal juice (SIJ), bile extract was used at a final concentration of 0.33%. A total of 10 mL of the 1% test group was added to 90 mL of sterile SGJ and SIJ, resulting in a final concentration of 0.1%. This concentration was selected based on previous studies demonstrating that low extract levels are effective for prebiotic screening while avoiding inhibitory or toxic effects on probiotic bacteria (Huebner *et al*., 2007). The control group was treated with 10 mL of sterile distilled water. To this mixture, 1 mL (v/v 1%) of each probiotic bacterial culture (at a concentration of 1×10^8 CFU/mL) was added and incubated at 37°C. Bacterial viability was assessed at specific time intervals (SGJ: 0, 120, 240 minutes; SIJ: 0, 30, 90, 180 minutes) as CFU/mL.

The Effect of Extracts on Antioxidant Activities of Probiotic Bacteria

Active probiotic bacterial cultures were separately inoculated in MRS liquid medium with or without 2 % plant extract or standard prebiotics (inulin and FOS) and incubated at 37 °C. After incubation, bacterial cultures were centrifuged at 4000 rpm, and cell-free supernatants were obtained. The antioxidant activity of the cell-free filtrates was determined by DPPH free radical scavenging activity assay (Braca *et al*., 2001). For this purpose, a freshly prepared DPPH solution was added to 500 µL of filtrate and incubated in the dark for 30 minutes. The absorbance of the samples was determined using a spectrophotometer with a wavelength of 517 nm. Percent inhibition values for DPPH free radical scavenging activity of the samples were calculated using the following equation (Eqs. (2)).

$$\%inhibition = \left\{\frac{(Absorbance\ control - Absorbance\ sample)}{Absorbance\ control}\right\} \times 100 \quad (2)$$

Effect of Plant Extracts on Cell-Surface Hydrophobicity of Probiotic Bacteria

Active probiotic bacterial cultures were inoculated into MRS broth with 2% plant extract or standard prebiotics (Inulin and FOS) and incubated at 37 °C. After 24 h of incubation, the culture was centrifuged, and the cell pellet was washed and resuspended in sterile saline. The optical density of the cell suspension was adjusted to 0.3 at a wavelength of 600 nm using a spectrophotometer. 0.25 mL of n-hexadecane was added to the cell suspension, vortexed, and allowed to stand at room temperature for 10 min. The optical density (OD) of the aqueous phase was determined on a spectrophotometer at a wavelength of 600 nm. Bacterial culture grown in broth without extract was used as a control group (Jankute *et al*., 2017) (Eqs. (3)).

The hydrophobicity index (HI) was calculated using the following equation:

$$HI = \frac{OD(initial) - OD(final)}{OD(initial)} \times 100 \quad (3)$$

Phenolic Compounds Utilised by Probiotic Bacteria During Incubation

The total phenolic compounds in the culture medium before and after incubation were determined by the Folin-Ciocalteu method using gallic acid standard (Singleton & Rossi, 1965). 0.1 mL culture supernatant was mixed with 0.2 mL Folin-Ciocalteu reagent (1:1, folin reagent: water) and kept at room temperature for 3 minutes. Subsequently, 2 % sodium carbonate was added to the reaction mixture and allowed to incubate for one hour. Following the incubation period, the absorbance of the samples was determined using a spectrophotometer with a wavelength of 760 nm. The same procedure was applied to the gallic acid solution prepared at different concentrations, and a standard curve was constructed. According to this curve, the total phenolic compounds of the cultures were calculated as mg gallic acid equivalent in one mL of culture (mg GAE/mL culture).

Statistical Analyses

All experiments were conducted using three independent experimental replicates. Each sample was measured twice as technical replicates, and the average value of the technical replicates was used as a single statistical unit. Data are presented as mean ± standard deviation (SD) with n = 3. Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for multiple comparisons. For the simulated gastric juice and simulated intestinal juice tests, analyses were performed separately for each bacterial strain and each time point. Statistical significance was set at the $p < 0.01$ level. Exact p-values were reported where available, and comparisons with $p \geq 0.01$ were not considered statistically significant.

RESULTS and DISCUSSION

Prebiotics are selectively metabolized by some microorganisms in the gastrointestinal tract. Accordingly, prebiotics modify the composition of microorganisms in the gastrointestinal system. Furthermore, the presence of prebiotics facilitates the proliferation of certain microorganisms, enabling growth and fermentation processes. The objective of this study was to quantify the extent to which selected *Salvia* species express prebiotic activity in conjunction with specified Lactobacillus strains and to ascertain the extent to which a medicinal and aromatic plant can be utilized as a prebiotic.

Evaluation of the Prebiotic Potential of Plant Species

The prebiotic activity score (PAS) is a widely accepted *in vitro* parameter that quantifies the ability of a substrate to selectively stimulate probiotic growth relative to an enteric bacterium (Huebner *et al*., 2007). This score is established through *in vitro* fermentation studies in which the growth of specific bacterial strains is observed in the presence of different prebiotic substrates (Figueroa-Gonzalez *et al.*, 2019). In the present study, PAS values of *Salvia* extracts were compared with those of conventional prebiotics, namely inulin (-0.18 ± 0.03) and fructooligosaccharides (FOS) (-0.20 ± 0.01). The results (Figures 1 and 2) demonstrated statistically significant differences between the plant extracts and standard prebiotics at the predefined significance level of $p < 0.01$.

The most important finding of this study was the high prebiotic activity observed in the ethanol extracts of *S. huberi* (0.72 ± 0.03) and *S. Sclarea* (0.71 ± 0.01). These extracts showed the highest PAS values against *L. fermentum* (Figure 1), while the ethanol extract of *S. huberi* also exhibited significant activity against *L. plantarum* (PAS = 0.71 ± 0.01) (Figure 2). These results suggest that ethanol-based extraction may be more effective in recovering bioactive components that support probiotic growth (Dilgen *et al*., 2025). In contrast, the ethanol extract of *S. fruticosa* yielded a negative PAS value (−0.23 ± 0.01) against *L. plantarum* (Figure 2). This situation is due the inability of bacteria to metabolize the compounds contained in the plant extract, resulting in a negative PAS value Sutherland *et al*. (2009).

Variations in PAS values reported in the literature further support the influence of plant matrix, extraction method, and bacterial strain specificity. For instance, Ab Azid *et al*. (2024) reported markedly higher PAS values (1.44–1.90) for freeze-dried fruit powders using *Bifidobacterium* strains, highlighting the impact of both substrate composition and microbial model selection. (Aydın, 2024) examined the prebiotic activity scores of *Tripolium pannonicum* extract and commercial prebiotics. Accordingly, *L. plantarum* and *L. fermentum* reported PAS values of 0.90 and 0.40, respectively. The difference in the results may be due to the use of different plant species, bacterial strains, and extraction methods. Similarly, enhancement of probiotic viability has been reported for herbal-enriched yogurt formulations containing thyme, rosemary, basil, and mint, where bioactive polyphenols were suggested to contribute to probiotic stimulation (Bayram *et al.*, 2024). Other studies have also demonstrated strain-dependent responses, including increased PAS values for *Lactobacillus bulgaricus* and *L. acidophilus* in the presence of specific plant extracts (Chae *et al*., 2024; Shargh *et al*., 2012).

Taken together, these findings indicate that the prebiotic potential of plant extracts is strongly influenced by phytochemical composition, solvent polarity, and probiotic strain selection. Within this context, *in vitro* evidence from the present study suggests that ethanol extracts of *S. huberi* and *S. sclarea* possess notable prebiotic potential, exceeding that of conventional prebiotics such as inulin and FOS under the tested conditions. Based on these promising results, further *in vitro* and *in vivo* investigations are warranted to better elucidate the prebiotic potential of *Salvia huberi* and *S. sclarea* extracts and their possible applicability as functional food ingredients.

Effect of Plant Extracts on Probiotic Viability in Simulated Gastric and Intestinal Juice

The effect of plant extracts on the viability of probiotic bacteria in simulated gastric juice (SGJ) and simulated intestinal juice (SIJ) has become a topic of significant interest due to their potential to enhance probiotic survival under gastrointestinal conditions. Numerous studies have investigated the inclusion of plant extracts in probiotic formulations to improve the stability and viability of probiotics. These extracts have shown protective properties contributing to increased probiotic survival rates when exposed to simulated gastric environments.

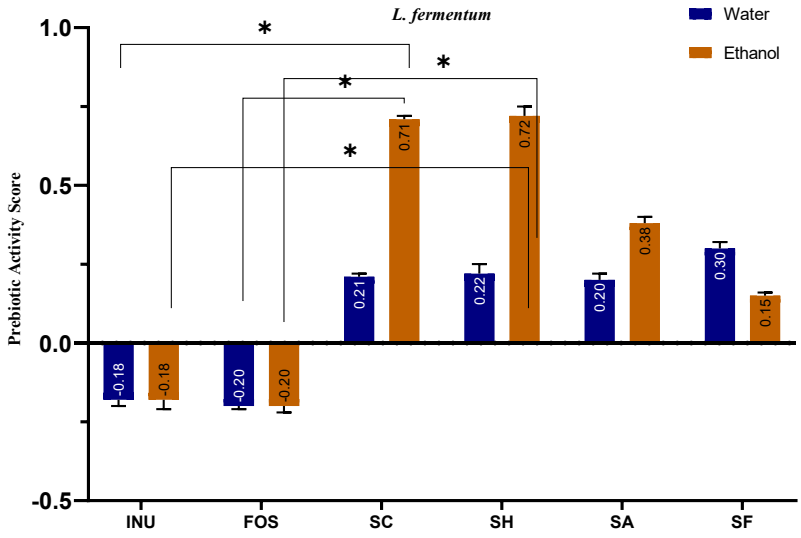


Figure 1. *Prebiotic activity scores of plant extracts and commercial prebiotics (L. fermentum). S. sclarea (SC), S. huberi (SH), S aethiopis (SA), S. fruticosa (SF), Inulin (INU), Fructooligosaccharide (FOS). Values are presented as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Duncan's multiple range test. Brackets indicate statistically significant pairwise comparisons at p < 0.01.*

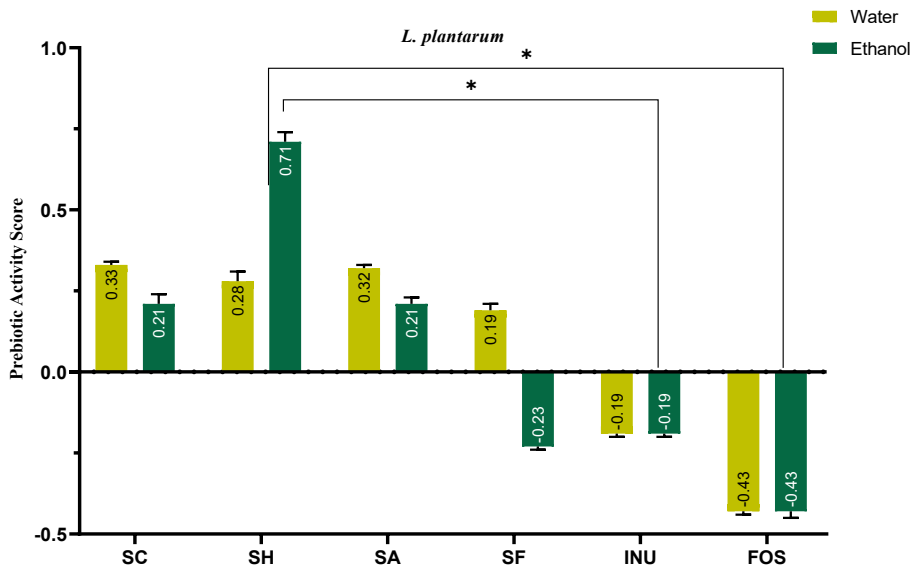


Figure 2. *Prebiotic activity scores of plant extracts and commercial prebiotics (L. plantarum). S. sclarea (SC), S. huberi (SH), S. aethiopis (SA), S. fruticosa (SF), Inulin (INU), Fructooligosaccharide (FOS). Values are presented as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Duncan's multiple range test. Brackets indicate statistically significant pairwise comparisons at p < 0.01.*

Simulated Gastric Juice

In this experimental protocol, conducted using *L. fermentum* and *L. plantarum*, reductions in bacterial viability were evaluated in simulated gastric juice after 120 and 240 min. Among the tested samples, the *S. fruticosa* water extract (SFW) demonstrated a notable protective effect on *L. plantarum*, resulting in higher survival rates at both time points compared to commercial prebiotics (inulin and FOS) (see Table 1). When water and ethanol extracts were compared, water extracts consistently showed a stronger protective effect on bacterial viability than ethanol extracts. Similar protective effects of plant-derived substrates have been reported in the literature. Shehata *et al*. (2019) demonstrated that the addition of moringa, fennel, and green tea extracts significantly improved the survival of *L. plantarum* DSM 20205 under simulated gastric conditions, with moringa extract showing the highest efficacy. Likewise, Dimitrellou *et al*. (2025) reported that the incorporation of apple fiber into yogurt increased the viability of *Lactobacillus casei* ATCC 393 during simulated gastrointestinal digestion, enhancing survival in both gastric and intestinal environments. Differences between these studies and the present findings may be attributed to variations in plant matrix composition, polyphenolic profiles, and the physicochemical properties of the tested substrates.

For L. *fermentum*, sage extracts have demonstrated even stronger prebiotic effects. Specifically, *Salvia huberi* water extract (SHW) significantly increased the survival rate of *L. fermentum* at both 120 and 240 minutes compared to commercial prebiotics and other plant extracts (Table 1). These results are consistent with previous findings, which showed that *L. fermentum* is relatively acid-tolerant and can maintain its viability under simulated gastric conditions (Castorena-Alba *et al*., 2018). In one study, the polyphenolic content of *S. huberi* water extract was reported, and higher phenolics were found compared to *S. fruticosa*, *S. sclarea*, and *S. aethiopis*. The presence of polyphenols may have contributed to the stronger prebiotic effect of this plant species (Dilgen *et al*., 2025). Furthermore, protective strategies containing natural bioactive compounds have been reported to improve the survival of this strain (Van Engeland *et al*., 2025).

Overall, *in vitro* evidence from the present study suggests that selected *Salvia* water extracts have the potential to support probiotic survival under simulated gastrointestinal conditions.

However, further *in vitro* mechanistic and in vivo studies are required to confirm their applicability in food systems and probiotic formulations.

Simulated Intestinal Juice

When the viability of *L. plantarum* was evaluated in simulated intestinal juice (SIJ) at 30, 90, and 180 min, the highest survival rates were observed in the presence of *S. sclarea* water extract (SCW) and *S. sclarea* ethanol extract (SCE), compared with commercial prebiotics (FOS and inulin) (Table 2). These findings indicate that certain *Salvia* extracts may enhance the survival of *L. plantarum* under simulated intestinal conditions.

For *L. fermentum*, the highest survival was recorded in the presence of the *S. fruticosa* ethanol extract (SFE) (Table 2). Overall, survival values observed for *L. plantarum* were higher than those observed for *L. fermentum* under SIJ conditions, suggesting strain-dependent differences in intestinal stress tolerance.

These differences could arise from inherent biological factors, including variations in cell wall structure and thickness, membrane composition, expression of stress-response proteins, and specific interactions between bacterial surface components and bioactive compounds present in the *Salvia* extracts. Previous studies have reported that *L. plantarum* exhibits higher resistance to gastrointestinal stress compared with several other probiotic strains, likely due to its robust stress-adaptation mechanisms (Stasiak-Różańska *et al*., 2021). Similarly, enhanced probiotic survival under simulated intestinal conditions has been demonstrated following the use of plant-based protective substrates, such as red onion peel extract incorporated into alginate microparticles (Raddatz *et al*., 2022).

In summary, the present in vitro findings suggest that selected *Salvia* extracts possess potential prebiotic-like activity by improving the survival of *L. plantarum* and *L. fermentum* under simulated intestinal conditions, in some cases surpassing the performance of established prebiotics such as FOS and inulin. Nevertheless, these results are limited to in vitro models; therefore, further studies, particularly in vivo trials and human intervention studies, are required to confirm prebiotic efficacy and to elucidate the precise mechanisms responsible for the observed protective effects.

Table 1. *% decrease values of 120th and 240th minute viability in L. plantarum and L. fermentum in SGJ.*

Plant Species	*L.plantarum*		*L.fermentum*	
	120	240	120	240
SCW	86.90±4.04^{b}	99.28±0.32^{b}	50.33±8.96^{b}	93.19±0.84^{abc}
SHW	93.33±3.31^{bc}	99.81±0.05^{c}	31.54±8.64^{a}	90.06±9.00^{ab}
SAW	90.15±2.50^{b}	99.33±0.36^{b}	78.15±2.10^{e}	92.82±3.04^{abc}
SFW	58.76±10.63^{a}	97.51±0.34^{a}	86.67±1.80^{f}	92.81±3.26 ^{abc}
SCE	100.00±0.00^{c}	100.00±0.00^{c}	95.51±3.08^{f}	99.56±0.30^{c}
SHE	100.00±0.00^{c}	100.00±0.00^{c}	89.84±1.06^{f}	98.86±0.47^{c}
SAE	100.00±0.00^{c}	100.00±0.00^{c}	90.08±1.06^{f}	97.85±1.45^{bc}
SFE	100.00±0.00^{c}	100.00±0.00^{c}	92.05±3.91^{f}	99.39±0.38^{c}
GLU	100.00±0.00^{c}	100.00±0.00^{c}	68.23±3.39^{cd}	93.38±2.62^{abc}
INU	100.00±0.00^{c}	100.00±0.00^{c}	60.32±4,69^{bc}	89.67±2.36^{a}
FOS	100.00±0.00^{c}	100.00±0.00^{c}	57.00±4.24^{b}	92.90±0.47^{abc}
CNTL	100.00±0.00^{c}	100.00±0.00^{c}	87.00±4.24^{e}	92.42±2.51 ^{abc}

Given as mean ± standard deviation (S.D.). Different lowercase letters (a-f) in the same column indicate significant differences ($p < 0.01$) (n=3). *S. sclarea* water (SCW), *S. huberi* water (SHW), *S. aethiopis* water (SAW), *S. fruticosa* water (SFW), *S sclarea* ethanol (SCE), *S. huberi* ethanol (SHE), *S. aethiopis* ethanol (SAE), *S. fruticosa* ethanol (SFE), Glucose (GLU), Inulin (INU), Fructooligosaccharide (FOS), Control (CNTL, without extract or standard prebiotics).

Table 2. % decrease values of 30th, 90th and 180th minute viability in *L. plantarum* and *L. fermentum* in SIJ.

Plant Species	*L.plantarum*			*L.fermentum*		
	30	90	180	30	90	180
SCW	24.00±5.66^{b}	61.50±4.95^{b}	79.25±8.84^{a}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{b}
SHW	63.33±4.71^{e}	85.83±1.18^{d}	93.67±1.41^{bc}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{b}
SAW	43.50±6.36^{c}	82.50±4.95^{d}	94.63±0.05^{bc}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{b}
SFW	21.00±5.66^{b}	81.25±1.77^{c}	92.50±2.83^{b}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{b}
SCE	4.00±5.66^{a}	44.50±4.95^{a}	82.25±1.77^{a}	94.84±2.58^{c}	98.98±0.45^{c}	100.00±0.00^{b}
SHE	29.67±5.18^{b}	89.00±1,41^{d}	94.92±0.59^{bc}	94.91±0.25^{c}	98.00±0.90^{c}	100.00±0.00^{b}
SAE	53.50±4.95^{d}	83.50±3.53^{d}	95.38±0.31^{bc}	60.23±6.62^{b}	93.89±0.78^{b}	99.92±0.03^{b}
SFE	100.00±0.00^{f}	100.00±0.00^{e}	100.00±0.00^{c}	44.44±7.85^{a}	46.04±3.24^{a}	99.28±0.31^{a}
INU	100.00±0.00^{f}	100.00±0.00^{e}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{b}
FOS	100.00±0.00^{f}	100.00±0.00^{e}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{b}
GLU	52.50±3.53^{f}	100.00±0.00^{e}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{b}
CNTL	100.00±0.00^{f}	100.00±0.00^{e}	100.00±0,00^{c}	100.00±0.00^{c}	100.00±0.00^{c}	100.00±0.00^{b}

Given as mean ± standard deviation (S.D.). Different lowercase letters (a-f) in the same column indicate significant differences ($p < 0.01$) (n=3). *S. sclarea* water (SCW), *S. huberi* water (SHW), *S. aethiopis* water (SAW), *S. fruticosa* water (SFW), *S sclarea* ethanol (SCE), *S. huberi* ethanol (SHE), *S. aethiopis* ethanol (SAE), *S. fruticosa* ethanol (SFE), Glucose (GLU), Inulin (INU), Fructooligosaccharide (FOS), Control (CNTL, without extract or standard prebiotics).

pre-print

Cell-Surface Hydrophobicity Results of Probiotic Bacteria After Fermentation

Cell-surface hydrophobicity is an important indicator of probiotic adhesion capacity and plays a key role in gastrointestinal colonization and competitive exclusion of pathogens through interactions with host epithelial cells (Farid *et al.*, 2021). In this context, the present study evaluated the effect of *Salvia* extracts on the cell-surface hydrophobicity of *L. fermentum* and *L. plantarum* during fermentation. The results showed that the highest hydrophobicity index in *L. fermentum* was observed in the presence of *S. sclarea* ethanol extract (SCE) and *S. huberi* water extract (SHW), while in *L. plantarum*, the highest values were recorded for *S. aethiopis* ethanol extract (SAE) and *S. huberi* (SHW) (Figure 3). These findings indicate that the impact of plant extracts on bacterial surface properties is strain and extract-dependent.

Variability in cell-surface hydrophobicity among *Lactobacillus* strains has been widely reported in the literature. Haritash & Kaushik (2009) demonstrated differing adhesion capacities among *L. johnsonni* LA1 (47%), *L. acidophilus* LA7 (57-58%) and *L. plantarum* (37.1-37.7%), highlighting intrinsic strain-specific differences in surface characteristics. Similarly, Lau *et al.* (2025), showed that the addition of *Acca sellowiana* bark extract significantly increased hydrophobicity in *Limosylactobacillus reuteri* compared with *Lactobacillus paracasei*, suggesting that plant polyphenolic composition and bacterial surface chemistry play crucial roles in extract–bacteria interactions.

Further evidence supports the strong hydrophobic response of *L. plantarum* to plant-derived bioactive compounds. Sadeghi *et al.* (2022) reported that *L. plantarum* and *L. casei* exhibited the highest hydrophobicity among tested strains, while Murugesan *et al.* (2025) demonstrated a dose-dependent increase in *L. plantarum* hydrophobicity following treatment with *Plectranthus amboinicus* extract. These observations suggest that differences in cell wall structure, stress-response mechanisms, and affinity for phenolic compounds may account for the enhanced hydrophobic response of *L. plantarum* observed in the present study.

Overall, these findings suggest that specific *Salvia* extracts, particularly those obtained using ethanol or water as solvents, may enhance the cell-surface hydrophobicity of probiotic strains *in vitro*, potentially contributing to improved adhesion properties and probiotic functionality.

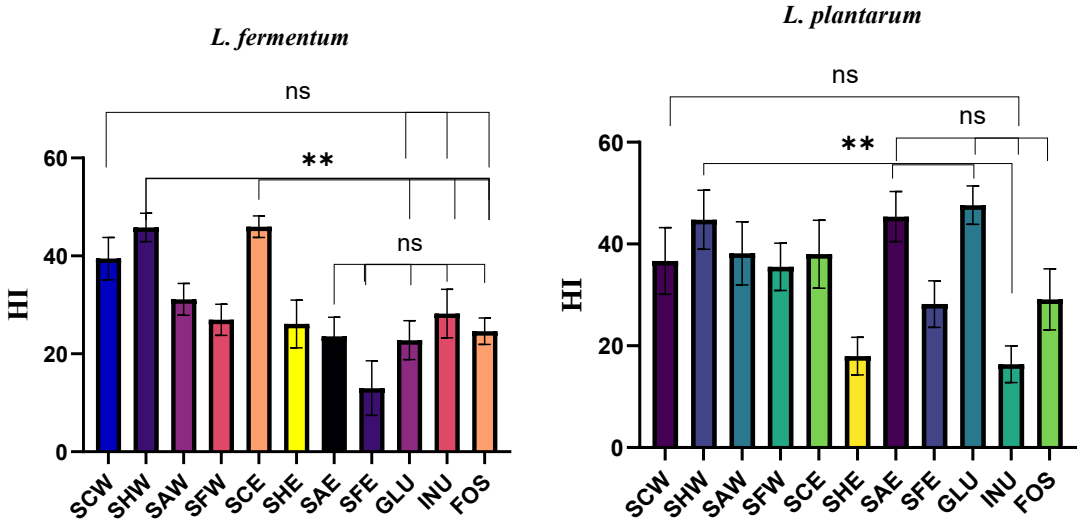


Figure 3. *Hydrophobic index values of L. plantarum and L. fermentum bacterial cultures incubated in the presence of extracts and standard prebiotics. Values are presented as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Duncan's multiple range test.* $^{**}p < 0.01$*; ns: not significant. S. sclarea water (SCW), S. huberi water (SHW), S. aethiopis water (SAW), S. fruticosa water (SFW), S sclarea ethanol (SCE), S. huberi ethanol (SHE), S. aethiopis ethanol (SAE), S. fruticosa ethanol (SFE), Glucose (GLU), Inulin (INU), Fructooligosaccharide (FOS).*

Evaluation of Antioxidant Activities of Probiotic Bacteria After Fermentation

Antioxidants reduce and counteract oxidative stress by neutralizing free radicals, which are unstable molecules that can damage cells and prevent many diseases (Feng & Wang, 2020). Probiotics have been reported to show antioxidant properties to protect against various diseases by reducing oxidative stress (Kim *et al.*, 2022). In addition, the evaluation of antioxidant activity in probiotic bacteria post-fermentation provides significant insight into the potential health benefits and functional properties of these microorganisms (Aziz *et al.*, 2023). In this study, the inhibition of DPPH radical and the extent to which plant extracts were affected after incubation were determined (see Figure 4).

When the experimental groups were compared with the control groups (FOS, inulin and glucose), it was found that the media grown in the extract gave significantly higher results. The highest free radical inhibition value was determined in *S. huberi* water extract in both *L. fermentum* and *L. plantarum* compared to the control groups (Figure 4). A study evaluated the antioxidant capacity of the *L. plantarum* strain from the plant *Annona muricata* L. It was observed that the antioxidant capacity of the bacteria increased at the end of fermentation (Putri *et al.*, 2022). This study and the data obtained, as in our study, support the existence of a synergistic effect between probiotic bacteria and plants, but determining which probiotic bacterial strain is effective in which plant species is important because different combinations can produce different results. Therefore, clinical studies are important to understand these mechanisms.

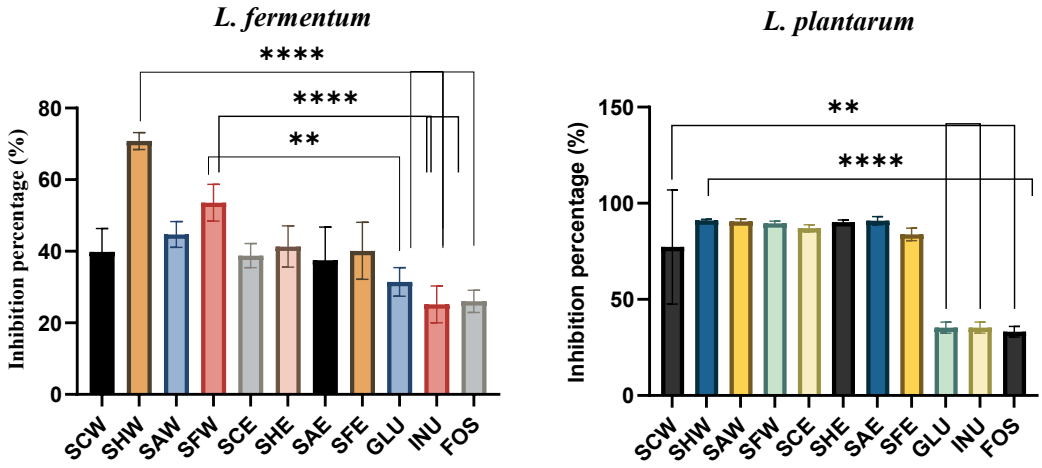


Figure 4. *DPPH free radical inhibition percentages. Values are presented as mean ± SD (n = 3). Statistical significance was determined by one-way ANOVA followed by Duncan's multiple range test. $^{**}p = 0.0013$; $^{****}p < 0.0001$. S. sclarea water (SCW), S. huberi water (SHW), S. aethiopis water (SAW), S. fruticosa water (SFW), S. sclarea ethanol (SCE), S. huberi ethanol (SHE), S. aethiopis ethanol (SAE), S. fruticosa ethanol (SFE), Glucose (GLU), Inulin (INU), Fructooligosaccharide (FOS).*

Assessment of Phenolic Substance Utilization of Probiotic Bacteria During Fermentation

To evaluate whether lactic acid bacteria utilized total phenolic compounds during fermentation, total phenolic content was determined before and after fermentation in media containing the tested plant extracts. Previous studies have demonstrated that phenolic compounds can significantly modulate gut microbial composition by inhibiting pathogenic bacteria while stimulating beneficial microorganisms (Rani & Anthony, 2014). In addition, phenolic compounds have been reported to exert prebiotic-like effects, increasing the population of beneficial bacteria, including probiotics, indicating a reciprocal interaction between phenolics and probiotic microorganisms (Succi *et al.*, 2017) (see Figure 5).

In the present study, the most pronounced decrease in total phenolic content was observed in the *S. sclarea* water extract (SCW) fermented with *L. plantarum*, whereas the changes observed for *L. fermentum* were not statistically significant at the predefined significance level of $p <$

0.01 (Figure 5). These results suggest strain-dependent differences in the interaction between lactic acid bacteria and phenolic compounds during fermentation.

Similar trends have been reported in the literature. Valero-Cases *et al.* (2017) demonstrated that certain lactic acid bacteria strains fermented with pomegranate juice utilized phenolic compounds during growth, while simultaneously contributing to changes in phenolic profiles through microbial metabolism. Likewise, Udeh *et al*. (2025) reported that *L. fermentum* was able to maintain total polyphenol content and antioxidant activity in finger millet–based beverages during fermentation, emphasizing the role of fermentation time and microbial strain in determining phenolic stability.

In the present study, increases in total phenolic content observed in some experimental groups may be attributed to enzymatic transformations, such as hydrolysis of bound phenolics, release of conjugated compounds from the plant matrix, or microbial bioconversion during fermentation. Conversely, decreases in phenolic content may result from microbial utilization of phenolics as carbon sources or their degradation through enzymatic activities of fermenting microorganisms. Overall, these findings suggest that lactic acid bacteria may contribute to the transformation and release of phenolic compounds during fermentation rather than directly producing them. This interpretation agrees with previous reports describing qualitative and quantitative changes in phenolic profiles following fermentation processes (Jan *et al*., 2022).

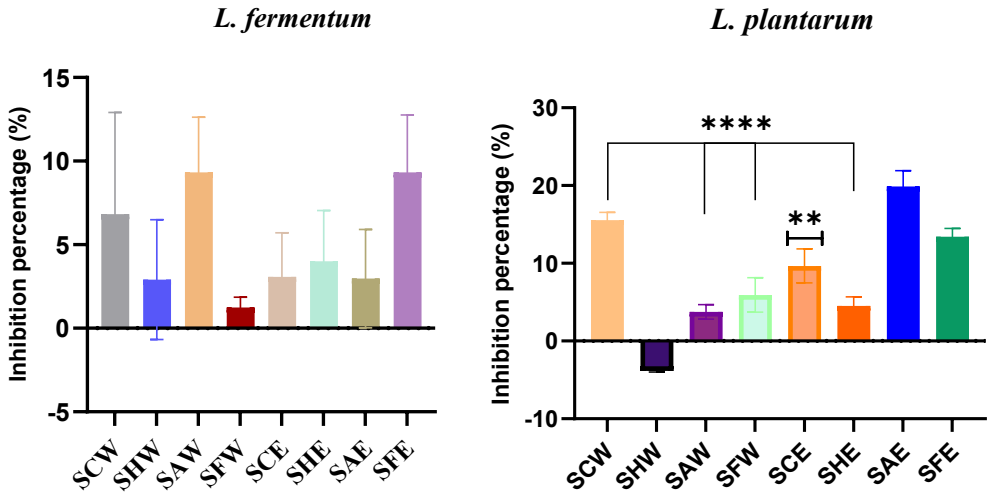


Figure 5. *Phenolic substance utilization of probiotic bacteria inhibition percentages.* Statistical significance was determined by one-way ANOVA followed by Duncan's multiple range test. $^{**}p$ = 0.0040; $^{****}p < 0.0001$. *S. sclarea water (SCW), S. huberi water (SHW), S. aethiopis water (SAW), S. fruticosa water (SFW), S sclarea ethanol (SCE), S. huberi ethanol (SHE), S. aethiopis ethanol (SAE), S. fruticosa ethanol (SFE). Values are presented as mean ± SD (n = 3). No mark indicates no statistically significant difference at p < 0.01.*

CONCLUSION

In vitro evidence from the present study suggests that certain *Salvia* extracts exhibit potential prebiotic activity by selectively enhancing the growth and metabolic activity of *Lactobacillus fermentum* and *Lactobacillus plantarum*. Among the tested samples, ethanol extracts of *Salvia huberi* and *Salvia sclarea* showed higher prebiotic activity under the tested conditions compared to conventional prebiotics such as inulin and fructooligosaccharides (FOS), particularly in *L. fermentum*. In the case of *L. plantarum*, the ethanol extract of *S. huberi* demonstrated pronounced prebiotic activity, whereas the ethanol extract of *S. sclarea* was also notably effective in supporting bacterial growth.

In addition, the *Salvia* extracts improved cell-surface hydrophobicity, which is essential for effective bacterial adhesion and colonization in the gut. Hydrophobicity results showed that different *Salvia* extracts (SCE, SHW, SAE) were effective on both bacterial strains. The extracts

also exhibited antioxidant properties, with *S. huberi* water extract showing the highest antioxidant activity in both bacterial strains, helping to neutralize free radicals and reduce oxidative stress. In addition, the utilization of phenolic compounds was observed during fermentation, suggesting that these bioactive compounds play an important role in modulating the gut microbiota. Notably, the highest phenolic content after fermentation was observed in the *S. sclarea* water extract fermented with *L. plantarum*, suggesting that water-soluble phenolics may play a role in probiotic–phenolic interactions distinct from those associated with ethanol extracts.

These findings highlight the potential of *Salvia* extracts as effective prebiotics with promising applications in improving gut health and supporting the proliferation of beneficial probiotic strains. Future studies should focus on identifying the specific bioactive constituents responsible for these effects and further validating their functional impact through in vivo models and clinical studies targeting gastrointestinal health. Overall, these findings suggest that *Salvia* extracts represent promising candidates that warrant further validation in vivo and in clinical settings to better elucidate their prebiotic efficacy and potential applications in gut health.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent via email upon request.

Funding

The funding was not received for conducting this study

Authorship Contribution Statement

M.N.D.: Experimented, methodology, analyzed the respective data, and writing the original draft preparation. **B.A.:** experimented, methodology, analyzed the respective data, and writing. **M.K.:** collecting and analyzing plant species. All authors have read and agreed to the latest version of the manuscript.

Orcid

Mevlüde Nur Dilgen https://orcid.org/0000-0002-9720-8662
Betül Aydın https://orcid.org/0000-0002-9092-1350
Murat Koç https://orcid.org/0000-0002-0829-4571

REFERENCES

Ab Azid, S.M, Wichienchot, S, Ishak, W.R.W, & Safuan, S. (2024). Prebiotic activity of plants from cucurbitaceae family and in vitro fermentation by Gut Microbiota. *Jurnal Gizi dan Pangan*, *19*(Supp. 2), 342-351. https://doi.org/10.25182/jgp.2024.19.Supp.2.342-351

Al-Habsi, N., Al-Khalili, M., Haque, S.A., Elias, M., Olqi, N.A., & Al Uraimi, T. (2024). Health benefits of prebiotics, probiotics, synbiotics, and postbiotics. *Nutrients*, *16*(22), 3955. https://doi.org/10.3390/nu16223955

Aydın, B. (2024). Exploring the multifaceted health benefits of *Tripolium pannonicum*: Antimicrobial, antioxidant, and prebiotic properties with phytochemical insights. *Chemistry & Biodiversity*, *22*(2), e202402588. https://doi.org/10.1002/cbdv.202402588

Aziz, T., Xingyu, H., Sarwar, A., Naveed, M., Shabbir, M.A., Khan, A.A., & Jalal, R.S. (2023). Assessing the probiotic potential, antioxidant, and antibacterial activities of oat and soy milk fermented with *Lactiplantibacillus plantarum* strains isolated from Tibetan Kefir. *Frontiers Microbiology*, *14*, 1265188. https://doi.org/10.3389/fmicb.2023.1265188

Bayram, O.Y., Kinik, O., & Büyükkileci, C. (2024) Antimicrobial activity and sensory acceptability of probiotic-strained (Torba) yogurt with medicinal and aromatic plants. *South African Journal of Botany*, *174*, 218-227. https://doi.org/10.1016/j.sajb.2024.08.053

Braca, A., De Tommasi, N., Di Bari, L., Pizza, C., Politi, M., & Morelli, I. (2001) Antioxidant principles from Bauhinia tarapotensis. *Journal of Natural Products*, *64*(7), 892-895. https://doi.org/10.1021/np0100845

Castorena-Alba, M. M., Vázquez-Rodríguez, J. A., López-Cabanillas Lomelí, M., & González-Martínez, B. E. (2018). Cholesterol assimilation, acid and bile survival of probiotic bacteria isolated from food and reference strains. *CyTA-Journal of Food*, *16*(1), 36-41. https://doi.org/10.1080/19476337.2017.1335347

Chae, Y.R., Lee, H.B., Lee, Y.R., Yoo, G., Lee, E., Park, M., & Park, H.Y. (2024) Ameliorating effects of Orostachys japonica against high-fat diet-induced obesity and gut dysbiosis. *Journal Ethnopharmacology, 333*, 118443.

Cui, H., Zhang, X., Zhou, H., Zhao, C., & Lin, L. (2015) Antimicrobial activity and mechanisms of *Salvia sclarea* essential oil. *Botanical Studies, 56*, 1-8. https://doi.org/10.1186/s40529-015-0096-4

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S., Berenjian, A, & Ghasemi, Y. (2019). Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. *Foods, 8*. https://doi.org/10.3390/foods8030092

Delgado, S., Guadamuro, L., Flórez, A.B., Vázquez, L., & Mayo, B. (2019). Fermentation of commercial soy beverages with lactobacilli and bifidobacteria strains featuring high β-glucosidase activity. *IFSET, 51*, 148-155. https://doi.org/10.1016/j.ifset.2018.03.018

Dilgen, M.N., Aydın, B., Vural, N., & Koç, M. (2025). Analysis of phytochemical profiles of different extracts of *Salvia* (*S. sclarea, S. huberi, S. fruticosa* and *S. aethiopis*) by HPLC-PDA, evaluation of their antioxidant capacity and antimicrobial activity. *Chemistry & Biodiversity*, e03218. https://doi.org/10.1002/cbdv.202403218

Dimitrellou, D., Moschakis, T., & Kandylis, P. (2025). Impact of apple pulp on textural characteristics, microstructure, volatile profile, and sensory acceptance of yogurts. *Foods*, *14*(14), 2453. https://doi.org/10.3390/foods14142453

Farid, W., Masud, T., Sohail, A., Ahmad, Naqvi, N.S.S.S., Khan, A.A., Khalifa, S.A., Hussain, A., & Ali, S. (2021). Gastrointestinal transit tolerance, cell surface hydrophobicity, and functional attributes of Lactobacillus Acidophilus strains isolated from Indigenous Dahi. *Food Science Nutrition*. *9*, 5092-5102. https://doi.org/10.1002/fsn3.2468

Feng, T., & Wang, J. (2020). Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut microbes*, *12*(1), 1801944. https://doi.org/10.1080/19490976.2020.1801944

Figueroa-Gonzalez, I., Rodriguez-Serrano, G., Gomez-Ruiz, L., Garcia-Garibay, M., & Cruz-Guerrero, A. (2019). Prebiotic effect of commercial saccharides on probiotic bacteria isolated from commercial products. *Food Science Technology International*, *39*(3), 747-753. https://doi.org/10.1590/fst.07318

Gibson, G., & Roberfroid, M. (1995). Dietary modulation of the human colonic microbiota introducing the concept of prebiotics. *Journal Nutrition*, *125*(6), 1401-12. https://doi.org/10.1093/JN/125.6.1401

Gul, S., & Durante-Mangoni, E. (2024). Unraveling the Puzzle: Health Benefits of Probiotics A Comprehensive Review. *Journal Clinical Medicine, 13(*5), 1436. https://doi.org/10.3390/jcm13051436

Haritash, A.K., & Kaushik, C.P. (2009). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. *Journal of Hazardous Materials*, *169*(1), 1-15. https://doi.org/10.1016/j.jhazmat.2009.03.137

Holkem, A.T., Silva, M.P.D., Favaro-Trindade, C.S., (2023). Probiotics and plant extracts: A promising synergy and delivery systems. *Critical Reviews in Food Science and Nutrition*, *63*(28), 9561-9579. https://doi.org/10.1080/10408398.2022.2066623

Huebner, J., Wehling, R.L., Hutkins, R.W., (2007). Functional activity of commercial prebiotics. *International Dairy Journal*, *17*(7), 770-775. https://doi.org/10.1016/j.idairyj.2006.10.006

Jankute, M., Nataraj, V., Lee, O.Y.C., Wu, H.H., Ridell, M., Garton, N.J., Besra, G.S. (2017). The role of hydrophobicity in tuberculosis evolution and pathogenicity. *Scientific Reports*, *7*(1), 1315.

Jan, S.K., & Kumar, A.N. (2022) Effect of Diverse Fermentation Treatments on Nutritional Composition, Bioactive Components, and Anti- Nutritional Factors of Finger Millet (Eleusine coracana L.). *Journal Biotechnology*, *10*, 46–52. https://doi.org/10.7324/JABB.2022.10s107

Kim, S., Lee, J.Y., Jeong, Y., Kang, C.H. (2022). Antioxidant activity and probiotic properties of lactic acid bacteria. *Fermentation*. *8*, 29. https://doi.org/10.3390/fermentation8010029

Latif, A., Shehzad, A., Niazi, S., Zahid, A., Ashraf, W., Iqbal, M.W., Korma, S.A. (2023). Probiotics: Mechanism of action, health benefits and their application in food industries. *Front Microbiology, 14,* 1216674. https://doi.org/10.3389/fmicb.2023.1216674

Lau, L.Y.J., & Quek, S.Y. (2024). Probiotics: Health benefits, food application, and colonization in the human gastrointestinal tract. *Food Biotechnology*, *3*(1), 41-64. https://doi.org/10.1002/fbe2.12078

Lau, L.Y.J., Huang, K., & Quek, S.Y. (2025). Impact of phenolic-rich feijoa (Acca sellowiana'Kakariki') peel extract on the mucin-adhesive properties of selected probiotic strains. *Food Bioscience,* 106320. https://doi.org/10.1016/j.fbio.2025.106320

Maftei, N.M., Raileanu, C.R., Balta, A.A., Ambrose, L., Boev Marin, D.B., & Lisa, E.L. (2024). The Potential Impact of Probiotics on Human Health: An Update on Their Health-Promoting Properties. *Microorganisms, 12*(2), 234. https://doi.org/10.3390/microorganisms12020234

Mashudin, M., Hasham, R., Cheng, K.K., Leong, H.Y., Othman, N.Z., Shadan, N.H., & Hamzah, M.A.A. (2024). Prebiotic effects of extracts from pineapple and Curcuma xanthorrhiza rhizomes towards the growth of Lacticaseibacillus paracasei. *Food Bioscience, 59*, 104045. https://doi.org/10.1016/j.fbio.2024.104045

Milutinović, M., Dimitrijević-Branković, S., & Rajilić-Stojanović, M. (2021). Plant extracts rich in polyphenols as potent modulators in the growth of probiotic and pathogenic intestinal microorganisms. *Front Nutrition, 8*, 688843. https://doi.org/10.3389/fnut.2021.688843

Morais, S.G.G., Borges, G.D.S.C, dos Santos Lima, M., Martín-Belloso, O., & Magnani, M. (2019). Effects of probiotics on the content and bioaccessibility of phenolic compounds in red pitaya pulp. *International Food Resistance*, *126*, 108681. https://doi.org/10.1016/j.foodres.2019.108681

Murugesan, V., Palanivel, P., Ramesh, G., Ganesh, D., Michael, H.S.R., Bandhumy, Lingam., S., & Sivaraman, R.K. (2025). Exploring the antibacterial potential of Clidemia hirta leaf extract against the pathogenicity of Pseudomonas aeruginosa: in vitro and in silico approaches. *Front Pharmacology*, *16*,1555542. https://doi.org/10.3389/fphar.2025.1555542

Putri, D.N., Widyarti, S., & Jatmiko, Y.D. (2022). Increasing antioxidant activity of soursop (Annona muricata L.) and noni (Morinda citrifolia L.) leaves fermented by Lactobacillus plantarum BP102. *Berk Penelit Hayati 27*, 73-77. https://doi.org/10.23869/bphjbr.27.2.20223.

Quldchikh, S., TirTouil, A., & Meddah, B. (2020). Zizyphus lotus (L.) extracts as prebiotics in the aggregation and adhesion of probiotic and inhibition of pathologic bacteria from patients with colorectal cancer. *Journal Food Studies, 9*(1). https://doi.org/10.7455/ijfs/9.1.2020.a3

Raddatz, G.C., Fonseca, V.R., Cichoski, A.J., Zepka, L.Q., Jacob-Lopes, E., Campagnol, P.C.B., & de Menezes, C.R. (2022). Viability and stability evaluation of Lactobacillus casei LC03 co-encapsulated with red onion (Allium cepa L.) peel extract. *LWT, 153*,112434. https://doi.org/10.1016/j.lwt.2021.112434

Rani, S., & Anthony, U. (2014). Effect of Germination and Fermentation on Polyphenols in Finger Millet (Eleusine coracana). *International Journal of Food and Nutritional Sciences*, *3*, 65–68.

Rastall, R., & Gibson, G. (2015). Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Current Opinion in Biotechnology, 32*, 42-46. https://doi.org/10.1016/j.copbio.2014.11.002

Russo, A., Formisano, C., & Rigano, D. (2013). Chemical composition and anticancer activity of essential oils of Mediterranean sage (*Salvia officinalis* L.) grown in different environmental conditions. *Food Chemical Toxicology*, *55*, 42–47. https://doi.org/10.1016/j.fct.2012.12.036

Rodríguez-Sojo, M.J., Ruiz-Malagón, A.J., Hidalgo-García, L., Molina-Tijeras, J.A., Diez-Echave, P., López-Escanez, L., & Rodríguez-Nogales, A. (2023). The Prebiotic Effects of an Extract with Antioxidant Properties from Morus alba L. Contribute to Ameliorate High-Fat Diet-Induced Obesity in Mice. *Antioxidants, 12*(4), 978. https://doi.org/10.3390/antiox12040978

Sadeghi, M., Panahi, B., Mazlumi, A., Hejazi, M.A., Komi, D.E.A., & Nami, Y. (2022). Screening of potential probiotic lactic acid bacteria with antimicrobial properties and selection of superior bacteria for application as biocontrol using machine learning models. *Lebensm Wiss Technolology*, *162*, 113471. https://doi.org/10.1016/j.lwt.2022.113471

Singleton, V.L., & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Botany*, *16*(3), 144-158. https://doi.org/10.5344/ajev.1965.16.3.144

Shargh, M.S., Dastar, B., Zerehdaran, S., Khomeiri, M., & Moradi, A. (2012). Effects of using plant extracts and a probiotic on performance, intestinal morphology, and microflora population in broilers. *Journal Applied Poultry*, *21*(2), 201-208. https://doi.org/10.3382/japr.2010-00145

Shehata, M.G., Abd-Rabou, H.S., & El-Sohaimy, S.A. (2019). Plant extracts in probiotic encapsulation: evaluation of their effects on strains survivability in juice and drinkable yogurt during storage and an in-vitro gastrointestinal model. *Journal of Pure and Applied Microbiology*, *13*, 1 https://dx.doi.org/10.22207/JPAM.13.1.70

Skenderidis, P., Mitsagga, C., Lampakis, D., Petrotos, K., & Giavasis, I. (2019). The effect of encapsulated powder of goji berry (Lycium barbarum) on growth and survival of probiotic bacteria. *Microorganisms*, *8*(1), 57. https://doi.org/10.3390/microorganisms8010057

Stasiak-Różańska, L., Berthold-Pluta, A., Pluta, A.S., Dasiewicz, K., & Garbowska, M. (2021). Effect of simulated gastrointestinal tract conditions on survivability of probiotic bacteria present in commercial preparations. *International Journal of Environmental Research and Public Health*, *18*(3), 1108. https://doi.org/10.3390/ijerph18031108

Succi, M., Tremonte, P., Pannella, G., Tipaldi, L., Cozzolino, A., Coppola, R., & Sorrentino, E. (2017). Survival of commercial probiotic strains in dark chocolate with high cocoa and phenols content during the storage and in a static in vitro digestion model. *Journal of Function Foods*, *35*, 60–67. https://doi.org/10.1016/j.jff.2017.05.019.ü

Sutherland, J., Miles, M., Hedderley, D., Li, J., Devoy, S., Sutton, K., & Lauren, D. (2009). In vitro effects of food extracts on selected probiotic and pathogenic bacteria. *International Journal Food Science Nutritional*, *60*(8), 717-727. https://doi.org/10.3109/09637480802165650.

Thuaytong, W., & Anprung, P. (2011). Bioactive compounds and prebiotic activity in Thailand-grown red and white guava fruit (Psidium guajava L.). *Food Science Technology International*, *17*(3), 205-212. https://doi.org/10.1177/1082013210382066

Udeh, H.O., Duodu, K.G., & Jideani, A.I.O. (2025). Effect of Fermentation Time on the Physicochemical Properties and Phenolic Composition of Malted Finger Millet Beverages. *Food Science Nutrition*, *13*(3), e4659. https://doi.org/10.1002/fsn3.4659

Valero-Cases, E., Nuncio-Jáuregui, N., & Frutos, M.J. (2017). Influence of fermentation with different lactic acid bacteria and in vitro digestion on the biotransformation of phenolic compounds in fermented pomegranate juices. *Journal Agricultural Food Chemistry*, *65*(31), 6488-6496. https://doi.org/10.1021/acs.jafc.6b04854

Van Engeland, C., Haut, B., & Debaste, F. (2025). A closer look at the potential mechanisms of action of protective agents used in the drying of microorganisms: A review. *Microorganisms*, *13*(2), 435. https://doi.org/10.3390/microorganisms13020435