World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

BİOCHEMİCAL MODULATION OF *ALLIUM AMPELOPRASUM* (LEEK) EXTRACTS TREATED WITH PROBIOTIC MICROORGANISMS

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World Journal of Biology Pharmacy and Health Sciences, 2026, 28(02), 116–126

Publication history: Received on 29 September 2026; revised on 05 November 2026; accepted on 07 November 2026

Article DOI: https://doi.org/10.30574/wjbphs.2026.28.2.0544

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ABSTRACT

In this study, extracts of *Allium ampeloprasum* (A) an inulin containing plant commonly consumed as food were treated with *Lactobacillus bulgaricus* (Lb), *Lactobacillus plantarum* (Lp), *Lactobacillus lactis* (Ll), and *Enterococcus faecium* (Ef). The fatty acid, vitamin, phytosterol, flavonoid, and resveratrol contents, as well as the antimicrobial activities of the extracts, were determined. Extracts prepared using plant-probiotic combinations (A+Lb, A+Lp, A+Ll, and A+Ef) were evaluated comparatively. The results showed that the total fatty acid level increased the most in the A+Lp and A+Lb groups ($p<0.0001$), followed by the A+Ll and A+Ef groups ($p<0.001$). Significant increases in vitamin and phytosterol contents were observed in the A+Ll groups ($p<0.0001$), and overall, the results varied across the groups. Conversely, flavonoid contents were found to decrease at varying rates across the groups. The study concluded that the groups treated with A+ (Lb, Lp, Ll, and Ef) exhibited significantly higher levels of antimicrobial compared to the control. In summary, it was determined that leek serves as an energy source and positively influences the growth of the bacteria used in the study (which are considered potential probiotics); furthermore, these bacteria, while growing in the leek extracts, exerted varying effects on beneficial plant-derived compounds.

Keywords: *L. bulgaricus, L. lactis, L. plantarum, E. Faecium,* Prebiotic, Probiotic, Leek.

1 INTRODUCTION

Probiotics are known as food components containing live microorganisms and when consumed in sufficient quantities as part of a healthy lifestyle, exert beneficial effects on the host, particularly by helping to maintain or improve the balance of the gut microbiota [1]. The most widely recognised benefits of probiotic microorganisms for human health include the alleviation of symptoms of lactose intolerance and constipation, the prevention and treatment of various types of diarrhoea, the stimulation of the immune system, and their anti-tumour and anti-carcinogenic effects. Probiotics prepare the immune system for action within the gastrointestinal tract (GIT), and it has been proven that without them, the immune system cannot function normally [2]. The beneficial effects of probiotics have been well

documented, particularly in relation to disorders of the intestinal system [3,4]. Probiotics assist in the detoxification (elimination from the body) of toxic substances ingested through food and in alleviating constipation. In addressing the problem of bad breath, they help to increase the body's resistance to many diseases by replacing pathogenic bacteria in the small and large intestines, bringing them under control and strengthening the immune system. Probiotics trigger numerous mechanisms that are effective in alleviating intestinal disorders by preventing the colonisation of bacteria particularly pathogenic bacteria in the human gut. Among the best-known and most extensively studied types of microorganisms within the category of probiotics, lactic acid bacteria in particular, as well as species belonging to the genera *Bifidobacterium*, *Bacillus* and *Saccharomyces*, are widely used as probiotics and are known to influence microbial diversity [5,6,7]. It is known that their effects on the gut microbiota are not solely attributable to microorganisms, but are also linked to the nutritional components and prebiotics present in the gut environment. Prebiotics can selectively support the activity of beneficial microorganisms and enhance the beneficial effects of probiotics in the gut environment. In this way, the gut microbiota can play a regulatory role in gut functions by establishing a probiotic-prebiotic relationship. In particular, plants such as chicory root, *Jerusalem artichoke*, onion, garlic, A. and asparagus which are rich in prebiotics such as inulin and similar fructans can be metabolised by specific microorganisms in the gut microbiota, thereby supporting the growth and metabolic activity of these microorganisms [8]. As the effects on the gut microbiota are not solely attributable to microorganisms, but also to the nutrients in the gut environment, this study is of great importance in that further research into prebiotics and probiotics could pave the way for scientific progress and advancements in many fields and areas, particularly in medicine.

2 MATERIAL AND METHOD

The A samples used in the study were collected from the province of Elazig and stored at –20 °C.

2.1 Lipid Extraction

Methanol extracts of the A samples were prepared and centrifuged at 4 °C. The cell pellets, whose wet weights had been determined previously, were homogenised in hexane-isopropanol. The resulting supernatants were centrifuged again at 4 °C and subsequently used in fatty acid and ADEK vitamin analyses [9]. The weight measurements were used to determine total fatty acid content.

2.2 Analysis of Fatty Acid Methyl Esters

Supernatant samples were prepared by extraction with a methanol-sulphuric acid solution. Following vortexing and the incubation period, a solution of NaCl and $KHCO_3$ was added. The hexane phase was separated and evaporated under a nitrogen stream. The fatty acids were then analysed using a SHIMADZU GC 17 instrument [10,11].

2.3 Phytosterol Analysis

The samples were homogenised with a mixture of hexane and isopropyl alcohol and hydrolysed with KOH. Analyses were carried out using an HPLC system [12].

2.4 HPLC Analysis of ADEK Vitamins and Sterols

Supernatant samples were vortexed with a KOH solution and incubated. The resulting extracts were extracted using a mixture of acetonitrile and methanol [13,14].

2.5 Determination of Resveratrol and Flavonoid Content

All analyses were carried out at 25 °C. Flavonoid and resveratrol determinations were carried out using an HPLC system [15].

2.6 Treatment and Cultivation of Extract A with Certain Probiotic Bacteria

Probiotic bacteria were inoculated into minimal medium [16]. Once bacterial growth had ceased, the samples were centrifuged and the resulting pellets were [17]. The antimicrobial activities of these pellets, as well as their fatty acid, vitamin, flavonoid and resveratrol contents, were assessed. For the control groups, the same procedures were applied to selected probiotic bacteria grown in minimal medium and to Sample A, and the results were compared [17].

~~2.7~~ Antimicrobial Activity

The probiotic bacteria cultures were provided from the culture collection of Anadolu University, Faculty of Arts and Sciences, Department of Biology, Microbiology Laboratory. The microorganisms used to evaluate the antimicrobial activity of the extracts, including *Staphylococcus aureus* COWAN1, *Bacillus megaterium* DSM 32 (Bm), *Klebsiella pneumoniae* FMC 5 (Kp), and *Escherichia coli* ATCC 25922 (Ec), as well as the yeasts *Candida albicans* FMC 17 (Ca) and *Candida glabrata* ATCC 66032 (Cg), and the dermatophyte species *Trichophyton* sp. (T) and *Epidermophyton* sp. (E),

were also obtained from the same culture collection. The microorganisms were supplied by Microbiology Laboratory Department of Biology, Faculty of Science, Fırat University. Bacterial strains were inoculated onto Müller–Hinton agar, yeasts onto yeast malt extract agar and dermatophyte fungi onto Sabouraud dextrose agar. Inoculum concentrations were adjusted to 10^6 for bacteria/mL, 10^4 for yeasts/mL and 10^4 for fungi/mL. After thorough mixing, 15 mL of each inoculated medium was transferred to sterile Petri dishes with a diameter of 9 cm and spread evenly Once he agar had solidified, wells with a diameter of 6 mm were made. 10 µL of A+ probiotic bacterial extracts were pipetted directly into the wells in turn. The Petri dishes were initially incubated at 4 °C for 1.5–2 hours. They were then incubated, and the mean inhibition zone diameter (mm) was used to assess antimicrobial activity [18].

2.8 Statistical Analysis

SPSS 15.0 software was used for statistical analysis. Analysis of variance (ANOVA) and the LSD test were used to assess differences between groups. p values less or equal to 0.05 were considered to be statistically significant [19].

3 RESULTS AND DISCUSSION

3.1 Fatty Acid Levels of Certain Probiotic Bacteria Extracted with A

It was found that the amount of palmitic acid (16:0) increased significantly in the A+Lb and A+Ll groups ($p<0.0001$), and that this increase was pronounced in the A+Lp and A+Ef groups ($p<0.001$). It was observed that the level of stearic acid (18:0) increased significantly in the A+Lb and A+Lp groups ($p<0.0001$), and that this increase was also pronounced in the A+Ll and A+Ef groups ($p<0.001$). It was observed that the level of oleic acid (18:1) increased across the groups, with this increase being more pronounced in the A+Lb and A+Lp groups ($p<0.0001$). It was found that linoleic acid (18:2) increased significantly in the A+Lp and A+Lb groups ($p<0.0001$), while this increase was only partial in the A+Ll and A+Ef groups ($p<0.001$). It was determined that the amount of linolenic acid (18:3) increased in the A+Lb, A+Lp and A+Ll groups ($p<0.0001$), while this increase was only partial in the A+Ef group ($p<0.001$). It was found that the total fatty acid level increased most significantly in the A+Lp and A+Lb groups ($p<0.0001$), followed by the A+Ll and A+Ef groups ($p<0.001$). (Table 1). Research into fatty acid analyses has shown that probiotically adapted Lp cultures exhibit a higher degree of variability compared to standard cultures [20]. This finding supports the possibility that the fatty acid composition of A+Lp in our study may be variable. It has been reported that the addition of lactic acid bacteria leads to the production of free fatty acids [21]. It has been stated that lactic acid bacteria can produce linoleic acid and linolenic acid, which are beneficial lipids [22,23,24]. A study on Ll also identified fatty acid levels, noting that the 16:00 level was particularly prominent; consequently, it was stated that culture media can significantly alter the fatty acid profile of the bacteria [25]. In our study, it was found that the levels of 16:0 and 18:0 fatty acids in A+Ll were significantly increased ($p<0.0001$); in a study examining Ll under different culture conditions, it was reported that environmental conditions alter the bacterium's membrane fatty acid composition, and in particular, the increase in 18:0 levels is consistent with our findings. Furthermore, it has been reported that fatty acids such as 16:0, 18:0 and 18:1 are among the major components of Ll's membrane lipids [26]. When these findings are considered together, it can be surmised that extract A may influence the bacterium's membrane lipid profile and fatty acid metabolism by altering the culture medium in which Ll is present. A significant increase in fatty acid levels was observed in A+Lb; it has been reported that the fatty acid profiles of lactic acid bacteria can vary depending on environmental conditions and that these levels are present in cellular lipids [27]. As there are few studies that directly examine the fatty acid profile of the A+Lb group, it is not possible to make a definitive interpretation regarding the mechanism behind the changes observed in our study. It is therefore predicted that the increases in fatty acids identified in the A+Lb group may stem from possible interactions between the components of the A. extract and the bacterium's lipid metabolism; however, further studies are required to confirm this relationship at the metabolic level. It was determined that the presence of 16:0, 18:0 and 18:1 in the fatty acid profile of the A+EF group was consistent with findings from similar studies [28]. Another study reported that environmental factors led to increases and decreases in the saturated and unsaturated fatty acid profiles, respectively [29]. The observation of increased fatty acid levels across all groups generally suggests that there is an interaction between plant and bacterial lipid metabolism. The fact that these increases vary depending on the bacterium is thought to be due not only to the presence of fatty acids in the extract but also to the microorganism's specific metabolic activity. The lack of sufficient research demonstrating the effects of interactions between A + probiotic bacteria on the fatty acid profile indicates that our study offers a novel approach to elucidate his plant–microorganism interaction in terms of lipid metabolism.

Table 1: Fatty Acid Levels of Selected Probiotic Bacteria Treated with A. and Their Comparison (µg/1 g).

Sam.	16:00	18:00	18:1	18:2	18:3	Total µg/1g
A+Lb	153.98±27.07^{cd}	136.16±20.68^{cd}	125.10±49.10^{cd}	383.78±246.94^{cd}	168.55±31.95^{cd}	967.58±216.81^{cd}
A+Lp	94.66±16.67^{d}	126.06±21.77^{cd}	94.96±52.03^{cd}	551.45±350.43^{cd}	140.05±16.25^{cd}	1007.20±414.03^{cd}
A+Ll	104.93±17.21^{cd}	78.91±8.16^{d}	51.53±2.92^{d}	176.58±15.75^{d}	146.16±58.84^{cd}	558.13±36.52^{d}
A+Ef	87.08±8.93^{d}	62.40±15.61^{d}	24.13±1.44^{c}	134.26±19.80^{d}	86.06±17.94^{d}	393.95±63.21^{d}
A	45.21±0.01	16.90±0	-	22.03±0.03	-	84.14±0.04
Lb	33.76±3.26^{a}	14.20±1.90^{a}	51.10±5.10	49.43±0.31^{a}	15.66±1.56	164.46±1.16
Lp	20.21±0.68^{a}	26.76±8.13^{a}	32.53±0.23	59.43±3.26^{a}	38.33±0.33	177.28±11.51
Ll	36.30±2.10^{a}	12.91±0.31^{a}	31.11±17.26	36.30±5.50^{a}	18.91±0.08	135.55±20.90
Ef	42.55±1.55^{a}	28.86±2.06^{a}	68.15±8.85	85.55±0.95^{a}	11.66±0.33	236.78±6.51

(A+Lb: A.+ *L. bulgaricus*, A+Lp: A.+ *L. plantarum*, A+Ll: A.+ *L. lactis*, A+Ef: A.+ *E. faecium*, A: A-Control, Lb: *L. bulgaricus*, Lp: *L. plantarum*, Ll: *L. lactis*, Ef: *E. faecium*, 16:00 = palmitic acid, 18:0 = stearic, 18:1 = oleic, 18:2 = linoleic, 18:3 = linolenic acid), (a: $p > 0.05$, c: $p<0.01$, cd: $p<0.0001$, d: $p<0.001$).

3.2 Lipophilic Vitamin and Phytosterol Levels in Certain Probiotic Bacteria Extracted with A.

It was determined that the δ-tocopherol content increased in the A+Lp group compared with the control ($p<0.0001$), that this increase was moderate in the A+Ll group ($p<0.001$), and that it was slight in the A+Lb group ($p<0.05$). It was found that vitamin D levels increased significantly in the A+Ll group compared with the control ($p<0.0001$), were markedly elevated in the A+Lb and A+Ef groups ($p<0.001$), while the increase in the A+Lp group was only partial ($p<0.01$). It was observed that α-tocopherol levels increased significantly in the A+Ll and A+Lb groups compared with the control group ($p<0.0001$), remained at a marked level in the A+Lp group ($p<0.001$), and increased to a partial extent in the A+Ef group ($p<0.01$). It was found that vitamin K_1 levels increased significantly in the A+Ll group ($p<0.0001$), increased to a partial extent in the A+Lb group ($p<0.001$), and that this increase was also observed in the A+Lp group ($p<0.01$) It was found that vitamin K_2 levels increased markedly in the A+Lb and A+Lp groups ($p<0.0001$), while the increase was very slight in the A+Ll and A+Ef groups ($p<0.05$). It was observed that ergosterol levels were highest in the A+Ll group ($p<0.0001$), while they increased significantly in the A+Lb, A+Lp and A+Ef groups ($p<0.001$). It was found that stigmasterol levels were higher in the A+Ll and A+Lb groups compared with the control group ($p<0.0001$), and that this increase was marked in the A+Lp and A+Ef groups ($p<0.001$). It was determined that β-sitosterol levels were highest in the A+Lp group compared with the control ($p<0.0001$), followed by the A+Ll and A+Lb groups ($p<0.001$) and the A+Ef group ($p<0.01$). It was observed that retinol levels increased most significantly in the A+Lb group compared with the control group ($p<0.0001$); this increase was marked in the A+Ll group ($p<0.001$), partial in the A+Lp group ($p<0.01$), and to a lesser extent in the A+Ef group ($p<0.05$). It was found that the retinol ester content increased significantly in the A+Ll group compared with the control ($p<0.0001$); this increase was pronounced in the A+Ef group ($p<0.001$), partial in the A+Lb group ($p<0.01$) and minimal in the A+Lp group ($p<0.05$). (Table 2). In our study, variations in the levels of lipophilic vitamins and sterols were observed in the A. groups. A study has indicated that A. contains lipophilic compounds and is particularly rich in tocopherol [30]. Furthermore, it has been reported that the growth of lactic acid bacteria during A. fermentation alters the metabolite profile [31-32]. In our study, the increase in vitamin K_2 levels, particularly in the A+Lb and A+Lp groups, was noteworthy. One study indicated that lactic acid bacteria influence vitamin K_2 production. It was reported that the Ll and Ef strains are capable of producing long-chain menaquinones, which are derivatives of vitamin K_2 [33,34]. Furthermore, a metabolic pathway associated with menaquinone biosynthesis has been identified in Lp [35]. These findings suggest that the increases in vitamin K_2 observed in our study may be related to bacterial menaquinone metabolism. The increase in retinol levels, particularly in the A+Lb group, is also noteworthy. It has been demonstrated that gut bacteria can participate in vitamin A metabolism and influence the formation of retinol and other retinoids [36]. It has been reported that probiotics have an effect on vitamin D absorption and metabolism [37,38]. Consequently, the increases in retinol and vitamin D observed in our study are associated with changes resulting from bacterial interactions. Upon evaluation of the sterol results, the increase in ergosterol, stigmasterol and β-sitosterol levels in the probiotic groups was noteworthy. In particular, the fact that β-sitosterol reached its highest level in the A+Lp group is consistent with studies showing that Lp fermentation can alter the phytosterol profile. Indeed, increases in stigmasterol glycoside and β-sitosterol glycoside levels have been reported as a result of Lp fermentation [39]. However, as ergosterol is primarily a sterol of fungal origin, it cannot be said that the increase observed in our study stems directly from bacterial synthesis. These changes may be related to

the release of extract components during fermentation or to changes in their extractability. Overall, the combined use of A. extract and probiotic bacteria has altered the profile of lipophilic vitamins and sterols. Our findings are consistent with the effects of bacterial metabolism and fermentation reported in the literature, particularly with regard to K_2, retinol and β-sitosterol. However, further studies are required to determine whether the observed changes stem directly from bacterial synthesis or from alterations in the bioavailability and extraction properties of components.

Table 2: Lipophilic Vitamin and Phytosterol Levels of Selected Probiotic Bacteria Treated with A. and Their Comparison (µg/1 g).

Sam.	A+ Lb	A+ Lp	A+Ll	A+Ef	A	Lb	Lp	Ll	Ef
δ-t	0.0008±0^{b}	0.0352 ±0^{cd}	0.0045 ±0^{d}	-	-	0.0003±0	0.0011±0^{cd}	0.0001±0	0.0002±0
D	0.0318±0.005^{d}	0.0175 ±0^{c}	0.0752 ±0^{cd}	0.3±0^{d}	-	0.0009±0	0.0010±0	-	0.0003±0
α-t	0.2902±0^{cd}	0.1309 ±0^{d}	0.4224 ±0^{cd}	0.0162 ±0^{c}	0.0001±0	0.0074±0^{d}	0.0056±0	0.0102±0.003^{cd}	0.0058±0.002
K_1	0.0362±0^{d}	0.0159 ±0^{c}	0.0790 ±0^{cd}	0.0019 ±0	0.0020±0.001	0.0118±0.004^{d}	0.0024±0	0.0002±0	0.0006±0
K_2	0.0236±0^{cd}	0.0352 ±0^{cd}	0.0002 ±0^{b}	0.0001 ±0^{b}	-	0.0002±0	0.0003±0	-	-
Er.	0.0747±0^{d}	0.0508 ±0^{d}	0.1137 ±0^{cd}	0.0325 ±0^{d}	0.0020±0.001	0.0004±0	0.0620±0.026^{cd}	0.0030±0.001	0.0069±0
S	0.1127±0^{cd}	0.0108 ±0^{d}	0.1308 ±0^{cd}	0.0148 ±0^{d}	0.0060±0	0.0018±0	0.0153±0.005	0.0023±0.001	0.0040±0.002
β-s	0.0627±0^{d}	0.2240 ±0^{cd}	0.0984 ±0^{d}	0.0239 ±0^{c}	0.118±0	0.0035±0.001	0.0177±0.006	0.0040±0	0.0038±0.001
R	0.0238±0^{cd}	0.0017 ±0^{c}	0.0057 ±0^{d}	0.0006 ±0^{b}	-	-	-	-	0.0003±0^{cd}
Ra	0.0095±0^{c}	0.0036 ±0^{b}	0.0569 ±0^{cd}	0.0209 ±0^{d}	-	-	-	-	-

(A+Lb: A.+ *L. bulgaricus*, A+Lp: A. + *L. plantarum*, A+Ll: A. + *L. lactis*, A+Ef: A. + *E. faecium*, A: (A Control), Lb: *L. bulgaricus*, Lp: *L. plantarum*, Ll: *L. lactis*, Ef: *E. faecium*, δ-tocopherol: δ-t:, D: vitamin D, α-tocopherol: α-t:, vitamin K_1: K_1:, vitamin K_2: K_2, ergosterol: Er, stigmasterol: S, β-sitosterol: β-s, retinol: R, retinol ester: Ra), (cd: p<0.0001, d: p<0.001, c: p<0.01, b: p<0.05).

3.3 Flavonoid and Resveratrol Contents of Some Probiotic Bacterial Extracts Obtained with A.

No significant difference in rutin levels was observed between the groups and the control group (p>0.05). On average, the rutin level was found to be lower in the A+Lb and A+Lp groups compared to the control, while there was neither an increase nor a decrease in the A+Ef group; conversely, in the A+Ll group, the rutin value was observed to have increased, albeit to a small extent, compared to the control (p<0.05). When comparisons were made between the groups, it was found that the myricetin level had decreased significantly compared with the control (p<0.0001). It was observed that morin levels were not present at a significant level in A. While quercetin levels were found to have decreased significantly in the A+Lb and A+Lp groups (p<0.0001), they were found to have increased in the A+Ll and A+Ef groups (p<0.0001). It was found that the A+Ll and A+Ef groups broke down the flavonoid quercetin more effectively, thereby increasing the quercetin level in the medium. It was observed that there were generally significant differences in kaempferol levels between the groups (p<0.001), and that these levels were even higher in all groups compared with the control; this is thought to be due to the bacteria breaking down kaempferol more effectively. While it was observed that the average catechin level decreased in the A+Ll group compared with the control (p<0.001), it was found to have increased in the A+Lb, A+Lp and A+Ef groups (p<0.001). No significant difference was found in naringin and naringenin levels across all plant groups compared with the control group (p>0.05); however, a slight decrease was observed compared with the control (p<0.001). It was found that resveratrol levels decreased in all groups compared with the control, with this decrease being more pronounced in the A+Lb and A+Lp groups (p<0.001). Consequently, it was found that the flavonoid levels present in A. were consumed by certain bacterial species, leading to a reduction in these levels in the medium. Conversely, it is thought that some species do not consume these compounds but, on the contrary, break them down more effectively and release them into the medium, thereby contributing to high levels of these compounds (Figure 1). In this study, it was determined that different probiotic bacteria applied to A. extract caused changes in the

flavonoid and resveratrol profiles in various ways. While no significant difference was observed between the groups in terms of rutin levels, an increase was observed in the A+Ll group compared with the control group. It is thought that this is due to the rutin flavonoid in the medium being broken down and extracted by Ll, thereby causing the level to rise further. In other words, based on this result, it was observed that Ll extracts the rutin flavonoid from the plant more effectively than methanol (the methanol used in preparing the control group) and releases it into the medium. Conversely, while quercetin levels decreased in the A+Lb and A+Lp groups, they increased in the A+Ll and A+Ef groups; furthermore, the rise in kaempferol levels across all bacterial groups suggests that the bacteria's effects on phenolic compounds may depend on species or strain characteristics. It has been reported in the literature that lactic acid bacteria can hydrolyse flavonoid glycosides, particularly via glycosidase enzymes, converting them into lower molecular weight compounds and free aglycones [40]. Indeed, studies conducted with Lp have shown that rutin and quercetin derivatives can undergo bacterial biotransformation, and that isoquercetin and quercetin can be formed as a result of rutin metabolism [41,42]. These findings suggest that the increase in quercetin observed in our study, particularly in the A+Ll and A+Ef groups, may be related to the release of bound or glycosylated flavonoids present in A. as a result of bacterial enzymatic activity. The fact that catechin levels increased in the A+Lb, A+Lp and A+Ef groups while decreasing in the A+Ll group, and that myricetin levels decreased significantly in all groups, also supports the notion that bacteria may exhibit different metabolic characteristics towards the same phenolic compound. Similarly, it has been reported that lactic acid bacteria not only hydrolyse phenolic compounds but can also convert them into different phenolic metabolites through further metabolic reactions [40]. Therefore, in our study, it is thought that the increases observed in certain compounds may be related to release or biotransformation, while the decreases may be linked to advanced microbial metabolism or interaction with bacterial cells. The decrease in resveratrol levels across all bacterial groups particularly in the A+Lb and A+Lp groups suggests that this compound's interaction with bacteria may differ from that of flavonoids. Overall, the results indicate that probiotic bacteria do not unilaterally alter the levels of A. derived phenolic compounds; rather, the effect depends on both the chemical structure of the compound and the metabolic characteristics of the bacteria used. However, in order to definitively identify the specific biotransformation pathways underlying the observed changes, the relevant parent compounds and metabolites must be analysed in conjunction with analytical methods such as LC-MS/MS.

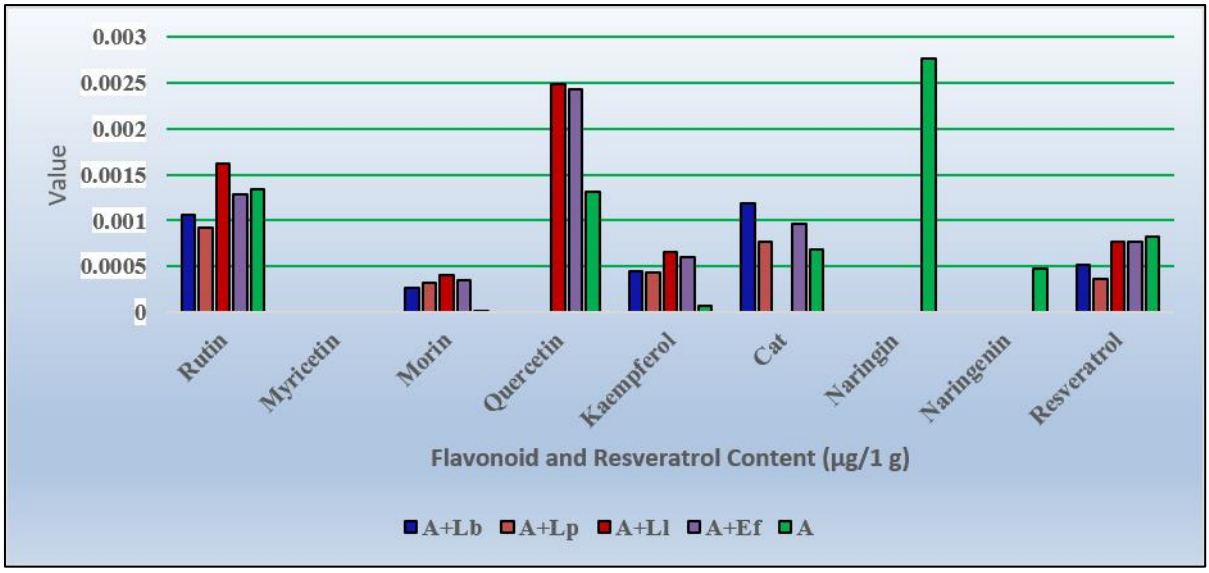

Figure 1: Flavonoid and Resveratrol Contents of Selected Probiotic Bacterial Extracts Extracted with A.

(b: $p<0.05$, d: $p<0.001$, cd: $p<0.0001$).

3.4 Antimicrobial Activity of A–Probiotic Samples Prepared for Fatty Acid, Vitamin and Flavonoid Analysis

A general assessment of the activity of the A. groups for fatty acid revealed that their effect on dermatophyte fungi and pathogenic yeasts was generally high. While the A+Lb and A+Lp groups were found to be most effective against Cg (23 mm) and least effective against Ca (7 mm), the A+Ll group was found to be effective against Cg and E (27 mm) and least effective against Bm (11 mm). It was determined that the A+Ef group was the most effective against dermatophyte fungi and yeasts, with this effect observed on Cg (29 mm), Ec and T (27 mm), E (21 mm), Ca (17 mm), Sa (11 mm) and Bm (10 mm). It was observed that A from the control group also exhibited a high level of activity against dermatophyte fungi and pathogenic yeasts, with the highest activity observed against Cg (21 mm.) (Figure 2).

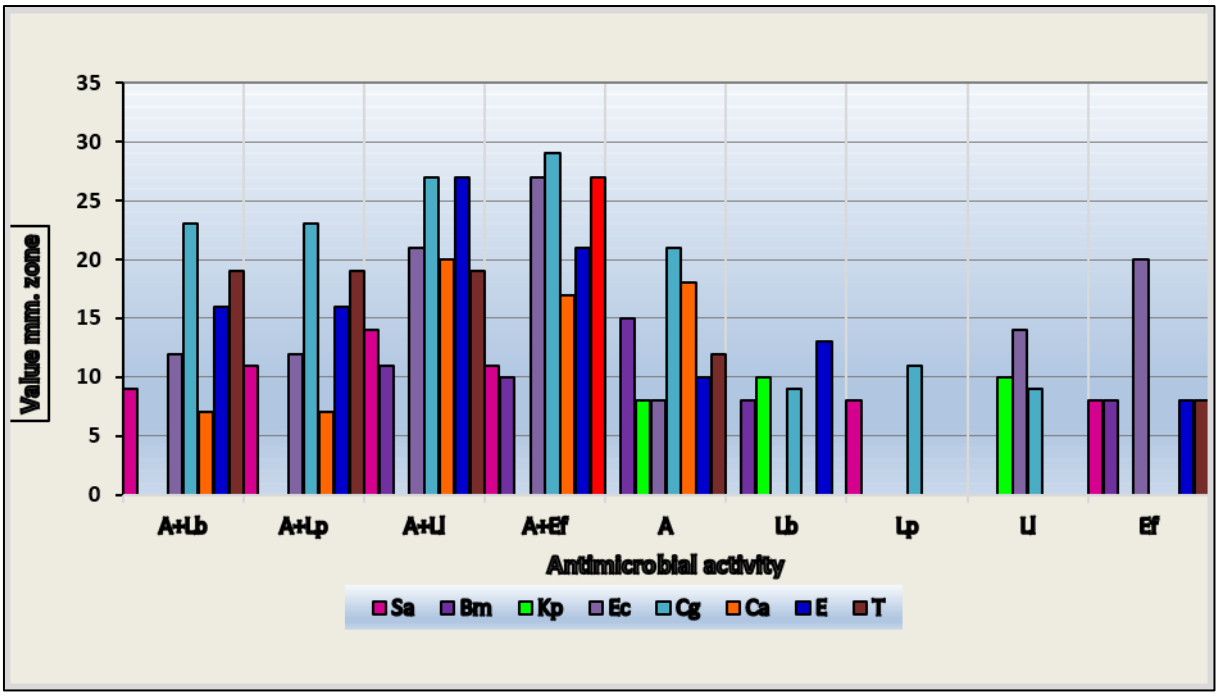

Figure 2: Antimicrobial Activity of A–Probiotic Samples Prepared for Fatty Acid Analysis

It has been reported that free fatty acids destabilise the microbial cell membrane, thereby increasing membrane permeability, disrupting electron transport and oxidative phosphorylation, and potentially affecting cellular energy metabolism [43,44]. However, it has been suggested that the antimicrobial effect of probiotic extracts may be due not only to fatty acids but also to the influence of organic acids, bacteriocins and other metabolites. It is therefore thought that the differences in inhibition zones observed in our study may be attributable to variations in the quantity and composition of the components present in the extracts.

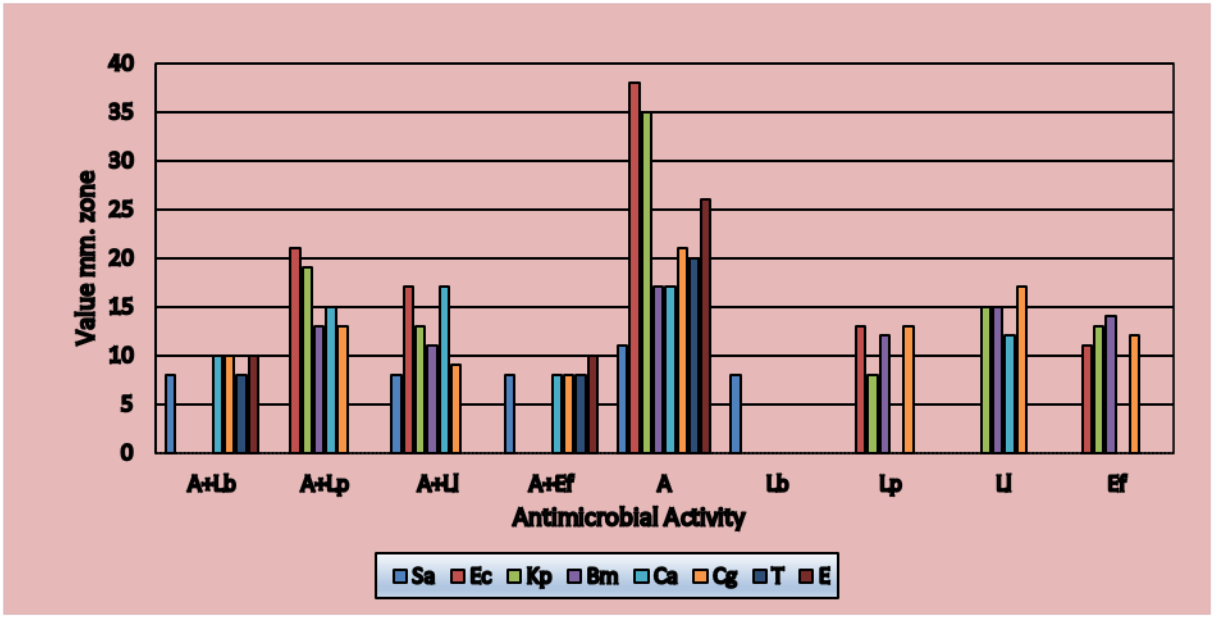

Figure 3: Antimicrobial Activity of A–Probiotic Samples Prepared for Vitamin Analysis

When the activity of the A groups vitamin was examined in general, it was found that A+Lb had an effect on dermatophyte fungi and yeasts; this effect was greatest on Ca, Cg and E (10 mm.), while the least effect was observed on Sa and T (8 mm.). It was found that while A+Lp produced a 13 mm inhibition zone in the Cg and Bm groups, it exhibited the highest effect on Ec (21 mm). It was observed that A+Ll produced 17 mm inhibition zone on Ca and Ec, while A+Ef was effective on E (10 mm) and on the Ca, Cg, T and Sa groups (8 mm in diameter). Among the activity of the A groups in general, it was found that A+Lb had an effect on dermatophyte fungi and yeasts; this effect was greatest on Ca, Cg and E (10 mm), while the least effect was observed on Sa and T (8 mm). It was found that while A+Lp produced

a 13 mm zone in the Cg and Bm groups, it exhibited the greatest effect on Ec (21 mm). It was observed that A+Ll produced a 17 mm inhibition zone on Ca and Ec, while A+Ef was effective on E (10 mm) and on the Ca, Cg, T and Sa groups (8 mm in diameter). It was found that the control group, A, formed a zone of 38 mm in diameter on Ec the highest value and a zone of 11 mm in diameter on Sa the lowest value (Figure 3).

It has been reported that phytosterols can exhibit bacteriostatic and antifungal properties, and that this effect may arise in conjunction with other components of the extract [45]. Furthermore, it has been reported that extracts derived from probiotic bacteria may contain antimicrobial compounds in the form of organic acids, fatty acids and proteins [46]. These findings suggest that the antimicrobial activity of vitamin-phytosterol extracts may be related not only to their vitamin content but also to the potential synergistic effects of phytosterols and other accompanying bioactive components.

A general assessment of the activity of the A. groups flavonoid revealed that they had an effect on dermatophyte fungi and pathogenic yeasts. It was found that A+Lb produced an 11 mm inhibition zone on Ca and T, while this zone measured 10 mm in the Cg and E groups. It was observed that A+Lp produced inhibition zones in the Cg and E groups, while the zone measured 12 mm on T and 9 mm on Ca. It was found that A+Ll produced an 8 mm inhibition zone in all groups except the Bm and Ec groups; that A+Ef was effective in all groups except Sa; and that A produced an inhibition zone of 13 mm in Cg, 9 mm in E and Bm, and 10 mm in Ca (Figure 4).

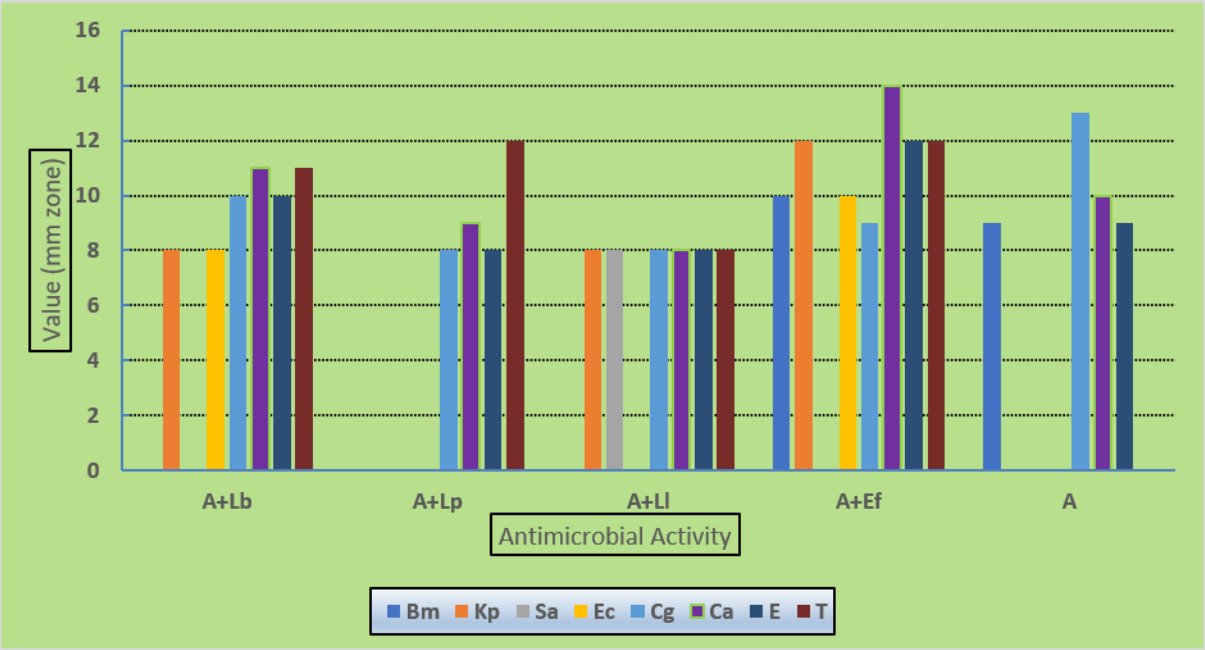

Figure 4: Antimicrobial Activity of A–Probiotic Samples Prepared for Flavonoid Analysis

While antimicrobial activity in flavonoid-phytosterol extracts is generally lower, it has been observed to vary depending on the pathogen and the probiotic strain. The activity of the A+Ef group, particularly on Ca (14 mm), E (12 mm), T (12 mm), Kp (12 mm) and Bm (10 mm), was found to be more pronounced than that of the other groups. It has been reported that flavonoids can disrupt the integrity and permeability of the cell membrane, affect nucleic acid synthesis and energy metabolism, and inhibit biofilm formation [47,48]. However, the antimicrobial activity of flavonoids can vary significantly depending on their chemical structure and the position of their hydroxyl groups. The pathogen specific inhibitions observed in the flavonoid phytosterol extracts in our study also suggest that the quantity and structural characteristics of these components may influence antimicrobial activity.

When assessed overall, it is observed that extracts intended for fatty acid analysis exhibit more pronounced inhibitory effects, particularly against fungi and yeasts; that vitamin-phytosterol extracts demonstrate high activity against certain bacterial pathogens; and that flavonoid phytosterol extracts, while exhibiting more limited activity, are active against a variety of microorganisms. These differences may stem from the chemical composition of the extracts and the co-presence of fatty acids, flavonoids, phytosterols, organic acids and other probiotic metabolites. It has previously been reported that the antimicrobial activity of probiotic bacteria may arise from the combined effect of numerous metabolites. The present results indicate that the prepared probiotic bacterial extracts possess pathogen-specific antimicrobial potential and that this activity may be related to the chemical composition of the extracts. Overall, these findings suggest that extracts derived from probiotic bacteria may serve as natural antimicrobial sources and represent a promising line of research for the development of safer and more sustainable antimicrobial strategies aimed at

controlling pathogenic microorganisms. Consequently, the present study could provide a meaningful scientific contribution by offering potential solutions to antimicrobial resistance, which is one of the increasingly significant problem worldwide.

4 CONCLUSION

In conclusion, this study demonstrates that the combined evaluation of leeks and probiotic bacterial strains represents an important approach for investigating plant-derived bioactive compounds and antimicrobial properties. It is believed that the data obtained may contribute to a better understanding of the interactions between probiotic microorganisms and plant-derived bioactive compounds, as well as to research into functional foods.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

This study was supported by the Scientific Research Projects Coordination Unit of Fırat University (FÜBAP), under Project No. 1861. The authors would like to thank Prof. Dr. Ökkeş Yılmaz and Prof. Dr. Merih Kıvanç for their technical and material support throughout the study.

Funding Source

This study was funded by the Scientific Research Projects Coordination Unit of Fırat University (FÜBAP), under Project No. 1861.

Disclosure of conflict of interest

All authors declared no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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