

Article

The Effect of Selected Herbal Extracts on Lactic Acid Bacteria Activity

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Abstract: This study aimed to investigate the effect of plant extracts (valerian *Valeriana officinalis* L., sage *Salvia officinalis* L., chamomile *Matricaria chamomilla* L., cistus *Cistus* L., linden blossom *Tilia* L., ribwort plantain *Plantago lanceolata* L., marshmallow *Althaea* L.) on the activity and growth of lactic acid bacteria (LAB) during the fermentation and passage of milk through a digestive system model. The tested extracts were also characterized in terms of their content of polyphenolic compounds and antioxidant activity. It was observed that the addition of the tested herbal extracts did not inhibit the growth of LAB in fermented milk drinks, such as yogurts. However, they can gradually inhibit fermentation when added at concentrations above 2% by weight, and hence should be used in limited amounts. The microflora of yogurts containing the tested herbal extracts did not die during digestion in model digestive juices, and no stimulating effect of the added plant extracts was noted either. Nevertheless, due to the antioxidant properties, a slight addition of the herbal extracts containing polyphenols to different kinds of food products can increase the nutritional quality, thus making them functional foods.

Keywords: lactic acid bacteria; plant extracts; milk fermentation; total phenolic content; antioxidant capacities



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1. Introduction

The benefits of fermented milk drinks seem to be recognized by consumers as the consumption of these products continues to increase, particularly in industrialized countries. In recent years, manufacturers have been outdoing each other in inventing novel products to attract customers. For instance, yogurts are prepared with various fruits, which are often quite exotic and rarely found in local markets. It has been found that plant extracts that were used in folk medicine and sometimes even in culinary applications may serve as interesting additives. However, the effect of their addition on the microflora of fermented milk beverages should be investigated.

Valerian (*Valeriana officinalis* L.) has been a known herb since ancient Greek and Roman times. The action of this herb was described by Dioscorides, Hippocrates, and Plinius Secundus. Valerian is one of the most popular herbs used in various medications, mainly for its calming and relaxing effects. In folk medicine, it was used as a sedative and as a relaxant

of the smooth muscles of the digestive tract, urinary tract, and blood vessels. The name of the herb is derived from the Latin word “valere”, which means “to be healthy” [1,2]. Sage (*Salvia officinalis* L.) contains compounds that inhibit lipid peroxidation, and also exhibit antibacterial, antiallergic, antiviral, and analgesic properties. In addition, sage is used in medicine to fight rheumatism and arthritis, and sometimes to prevent the general weakening of the body [3–5]. Chamomile (*Matricaria chamomilla* L.) has been used for centuries as a poultice for healing wounds and burns, and as an eyewash to treat conjunctivitis. This herb has not lost its relevance [6–8]. It is known to have anti-inflammatory, antibacterial, bacterial toxin-binding, disinfectant, antispasmodic, choleric, sedative, and laxative effects, and is hence used widely in medicine. Furthermore, it is used in the food industry for the production of liqueurs and in the cosmetic industry for the production of creams, lotions, soaps, bath liquids, and toothpastes [6–8]. Cistus (*Cistus* L.) acts as an antioxidant and removes free radicals and prevents the formation of new ones [9,10]; it also has antibacterial, anti-inflammatory, antiviral, and antifungal effects [9,10]. Linden blossom (*Tilia* L.) exhibits a high healing effect and was therefore used by our ancestors for generations. In traditional medicine, the linden flower is commonly used for nervous tension and excessive nervous excitability. In some cases, it is used as a prophylactic against atherosclerosis and hypertension [11–16]. Linden flowers are also characterized by anti-inflammatory, antipyretic, diaphoretic, diuretic, mildly astringent, and analgesic properties [11–16]. Since ancient times, infusions prepared from linden or sage have been used to treat respiratory diseases. Ribwort plantain (*Plantago lanceolata* L.) has also been used in folk medicine. Its leaves are used to treat cuts, bruises, bites, and burns, as well as for chronic gastrointestinal catarrh, acidity, and damage to the gastric and intestinal mucosa (e.g., by bacterial toxins or other chemical compounds) [17–20]. This herb has bacteriostatic, antimicrobial, anti-inflammatory, antispasmodic, and expectorant properties. It also improves blood clotting and has astringent and sealing effects on blood vessels [17–20]. Marshmallow (*Althaea* L.) was valued for its prohealth properties by Egyptians and Syrians in ancient times. The generic name *Althaea* comes from the Greek word “althe” which means “to heal”. Both the root and leaves of marshmallow are rich in mucus, which is the basic ingredient of this plant used in medicine [21–24]. Marshmallow has anti-inflammatory, protective, coating, and antitussive properties [21–24].

In food production, the use of herbs can stabilize the microflora of products, by preventing the development of harmful microorganisms and supporting the growth of desired bacteria. Only few scientific reports have shown that when selected plant extracts are used in appropriate portions, they can have a beneficial effect on lactic acid bacteria (LAB) [25,26]. Bifidobacteria and some lactobacilli can transform polyphenols into important metabolites that have important functions in the human body is known to be [25,26]. Moreover, observations of market trends suggest that plant extracts are used or can be used as food additives in the production of flavored fermented dairy products (including yoghurts or other fermented milks, sour cream, acid and rennet cheeses), and many products derived from them. Only limited studies have analyzed the influence of phenolic compounds on the growth and viability of other lactic acid bacteria such as *Streptococcus thermophilus* used in the production of yogurt.

The present study aimed to investigate the effect of selected herbal additives on the activity and growth of lactic acid bacteria (LAB) during fermentation and passage of milk through the digestive system model. Additionally, the tested extracts were characterized in terms of their content of polyphenolic compounds and antioxidant activity.

2. Materials and Methods

2.1. Materials

The following plant extracts were used in the research: valerian (*V. officinalis* L.), sage (*S. officinalis* L.), chamomile (*M. chamomilla* L.), cistus (*Cistus* L.), linden blossom (*Tilia* L.), ribwort plantain (*P. lanceolata* L.), and marshmallow (*Althaea* L.). They were purchased from GreenVit sp. z.o.o. (Zambrów, Poland). These were water extracts

obtained by percolation at elevated temperatures. Then, they were concentrated in a vacuum evaporator, and possibly dried further under vacuum. Marshmallow and plantain extracts were obtained in liquid form, while the rest were in powder form. Maltodextrin was used as the drying carrier.

2.2. Determination of the Effect of Plant Extracts on the Lactic Acid Fermentation of Milk

In the first stage of the work, the ability of LAB to ferment milk was checked in the presence of selected plant extracts. Briefly, the milk samples intended for lactic acid fermentation was prepared from UHT milk (containing 3.2% of fat) in a volume of 100 mL, and the plant extracts were added at amounts of 0.2, 0.6, 1.0, 1.4, 2.0, 3.0, 4.0, and 5.0%. Then, the samples were transferred to a water bath heated to 42 °C, and the starter culture was added (at an amount of 0.04%). The yogurt starter culture YC-X16 (received kindly from Chr. Hansen Poland) was used in the research. This freeze-dried culture is composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Fermentation of milk samples was carried out at 42 °C. For the next 4 h, the pH of the samples was measured every 30 min until the end of fermentation, using a standard laboratory stationary pH meter with three replications.

2.3. Determination of the Effect of Plant Extracts on the Populations of Lactic Acid Bacteria

The effect of plant extracts on the population of LAB cells was investigated immediately after the fermentation process and digestion of fermented milk in model digestive juices. At this stage, however, based on the results of the first stage, only the following portions of plant extracts were used: 0.2, 0.6, 1.0, and 1.4% extracts added to UHT milk. Fermentation was carried out as in the first stage of the research. After the end of the process, the number of LAB cells in the samples was determined. M17 (MERCK) and MRS agar (De Man Rogosa Sharpe Agar, MERCK) were used in the analysis. The inoculated Petri plates were incubated in an incubator at 37 °C under aerobic (M17 agar) or anaerobic (MRS agar) condition. The cell count was determined after 72 h, and the results are expressed in colony forming units in 1 mL of the sample (CFU/mL).

The next stage of the work consisted of several steps. The first step involved the digestion of the fermented milk samples added with plant extracts under gastric juice conditions, and the second step involved the digestion of the samples under intestinal juice conditions [27]. Gastric juice was prepared as described by Ziarno and Zareba [27]. Briefly, 4.8 g of NaCl, 1.56 g of NaHCO₃, 2.2 g of KCl, and 0.22 g of CaCl₂ were dissolved in 1000 mL of distilled water. The pH of the prepared solution was adjusted to 2.40 with 1 M HCl. Then, the solution was sterilized in an autoclave at 121 °C for 15 min. Immediately before the experiment, pepsin (Sigma-Aldrich) was added to the solution at an amount of 285 µL/100 mL gastric juice. The model intestinal juice was also prepared as described by Ziarno and Zareba [27]. Briefly, 5 g of NaCl, 0.6 g of KCl, 0.25 g of CaCl₂, and 8.5 g of beef bile were dissolved respectively in 1 M NaHCO₃. The pH of the prepared solution was adjusted to 7.0 with 1 M HCl. The whole mixture was successively sterilized in an autoclave at 121 °C for 15 min. Immediately before the experiment, one Kreon Travix 10,000 capsule (Abbott Laboratories) was added to 200 mL of model intestinal juice. The capsule contains a mixture of digestive enzymes, which at a dose of 150 mg shows the activity of 10,000 IU Ph. Eur lipase, 8000 units Ph. Eur amylase, and 600 Ph. Eur proteases. Digestion was carried out in a static system by mixing 35 mL of model gastric juice with the appropriate amount of pepsin and 35 mL of fermented milk sample. Gastric juice digestion was performed for 3 h at 37 °C, and then the mixture was transferred to the same amount of model intestinal juice for digestion which lasted for 5 h at 37 °C. Finally, the number of viable LAB cells was determined as described above.

2.4. Determination of TPC of Plant Extracts

Total phenolic content (TPC) was estimated in the plant extracts using the Folin-Ciocalteu method as described previously [28] with a slight modification. First, appro-

priately diluted plant extract (3 mg/mL) was mixed with deionized water (20 mL) and Folin–Ciocalteu reagent (0.5 mL). After 30 s, 5 mL of Na₂CO₃ (20%, *v/v*) was added to the solution. Then, the solution was incubated at 21 °C for 1 h, and its absorbance was measured at 765 nm using a UV/Vis spectrophotometer (Model 8500; Techcomp, Hong Kong). The results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract) using a standard gallic acid calibration curve. The analysis was performed in three independent replications.

2.5. Determination of Antioxidant Capacities of Plant Extracts

Before the analysis of antioxidant activity, each extract (3–6 mg) was diluted with distilled water (2–4 mL). The plant extract solutions were thus prepared in triplicate, and their average values of antioxidant capacity were determined as mmol Trolox equivalents per gram of extract (mmol TE/g extract).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was performed according to a procedure described by Yen and Chen [29] with minor modification. Briefly, 1 mL of 0.3 mmol/L freshly prepared DPPH methanol solution was mixed with 0.2 mL of the plant extract solution and 3.8 mL of methanol. The samples were vortex-mixed at high speed for 10 s and incubated for 10 min in the dark at room temperature. Then, their absorbance was measured at 517 nm using a UV/Vis spectrophotometer. A standard curve was obtained using the Trolox standard in the range of 8–40 µmol/L.

ABTS (2,2'-azinobis(3-ethylbenthiazoline-6-sulfonic acid)) radical scavenging activity of the extracts was determined according to the method described by Re et al. [30]. First, the ABTS•+ solution was prepared by mixing ABTS aqueous solution (14 mmol/L) with potassium persulfate aqueous solution (4.9 mmol/L). The prepared solution was kept for 12–16 h in the dark at room temperature. Before the analysis, the ABTS•+ solution was diluted with phosphate-buffered saline (0.01 mol/L, pH 7.4) to achieve an absorbance value of 0.7 ± 0.05 at 734 nm. Then, 40 µL of the plant extract solution or Trolox solution was mixed with 4 mL of ABTS•+ working solution. The reactive mixture was incubated at room temperature in the dark, and after exactly 6 min, its absorbance was recorded at 734 nm. A series of Trolox solutions (final concentrations 0–15 µM) were used for calibration.

FRAP (ferric reducing antioxidant power) assay was performed as described by Benzie and Strain [31] with some modification. Before the assay, the FRAP reagent was freshly prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 20 mmol/L FeCl₃ solution, and 10 mmol/L TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) in 40 mmol/L HCl in a 10:1:1 (*v/v/v*) proportion and stored away from light. Then, 100 µL of the appropriately diluted sample extract was mixed with 0.3 mL distilled water and 3 mL FRAP reagent. The absorbance of the reaction mixture was measured spectrophotometrically at 593 nm after incubation at 37 °C for 10 min. The blank solution was obtained by mixing 0.3 mL distilled water with 3 mL of FRAP reagent. A standard curve was prepared using Trolox in the range of 80–500 µmol/L. All determinations were carried out in triplicate.

2.6. ESI-QTOF Qualitative Analysis of Plant Extracts

High-resolution liquid chromatography (LC)–mass spectrometry (MS) analyses (exact masses, MS/MS fragmentation patterns, molecular formulas) were performed on a Thermo Scientific Ultimate 3000 RS chromatographic system coupled with a Bruker Impact II HD (Bruker, Billerica, MA, USA) quadrupole time-of-flight (QTOF) mass spectrometer. Chromatographic separations were carried out on a Waters BEH C18 column (2.1 × 150 mm, 1.7 µm; Milford, MA USA), equipped with precolumn. Mobile phase A used was 0.1% (*v/v*) formic acid, while mobile phase B was acetonitrile containing 0.1% (*v/v*) of formic acid. A gradient from 7 to 80% of phase B over 30 min was used for separation. The flow rate was set at 0.5 mL/min, and the column was held at a temperature of 60 °C. The injection volume was 5 µL. The light absorption patterns of the investigated sample components were obtained in the wavelength range of 190–600 nm using a photodiode array detector (Thermo Ultimate DAD-3000) with an analytical flow cell. The column's effluent was split

into 1:3 proportions between the two detectors operating in parallel, the mass spectrometer, and the charged aerosol detector (CAD), to identify the main constituents of the investigated samples. For identification, data were collected from the mass spectral analyses in both positive and negative ion modes with electrospray ionization (ESI). Linear (centroid) mass spectra were acquired over a mass range from m/z 50 to 2000 with the following MS parameters: positive ion capillary voltage, 4.5 kV; negative ion capillary voltage, 3.0 kV, dry gas (N₂) flow, 6 L/min; dry gas temperature, 200 °C; and nebulizer gas (N₂) pressure, 0.7 bar. Argon was used as the collision gas. The MS/MS collision energy and parent mass isolation width were automatically set between 2.5 and 35 eV and between 2 and 6 mass units, depending on the m/z of the fragmented ion. The parameters for ion transfer were optimized for m/z 50–2000, with collision cell transfer time at 80 μ s and prepulse storage at 10 μ s. The acquired data were calibrated internally with 10 mM sodium formate introduced to the ion source via a 20- μ L loop at the beginning of each separation process. Data processing was carried out using Bruker DataAnalysis 4.3 software. The main components of the sample were identified from the CAD peak areas, while the constituents were identified based on the light absorption properties, precise mass measurements (measurement error <5 ppm) of the primary ion m/z , which allowed for calculating the molecular formula, and software-aided analysis of the isotopic and MS/MS fragmentation patterns [32].

2.7. Statistical Analysis

Multifactor analysis of variance (ANOVA) is used in statistical analysis to determine the influence of significant factors in a multivariate model. This is a typical system used for experimental analysis which, in addition to checking the influence of one factor, allows checking the interaction of individual factors with each other. In this study, in conjunction with ANOVA, Tukey's test was used at a significance level of 0.05 to analyze the mutual influence of two factors on each other and find those that differ significantly from each other, which shows the pairs that are statistically significant.

3. Results

3.1. Determination of the Effect of Plant Extracts on Lactic Acid Fermentation

In the first stage of the research, changes in the pH of milk samples enriched with portions of plant extracts were analyzed. The obtained results are presented in Figure 1. The change in pH during the fermentation process indicated that none of the added plant extracts inhibited fermentation. Statistical analysis showed that only the milk samples added with the first three doses of the extracts (from 0 to 1.0%) constituted one homogeneous group ($p = 0.001$), where the pH values were higher by on average 0.2–0.3 than the samples added with higher doses of the tested plant extracts. The milk samples with 1.0% or higher portion of the plant extracts constituted separate homogeneous groups ($p = 0.001$) for each analyzed extract. It should be noted that the pH did not reduce below 4.5 for the addition of any extract above 2.0% concentration. For selected additives (sage and cistus), a pH of even 4.1 was achieved for the lowest dose, and 4.8 for 5.0% dose. For the remaining additives, the differences in pH observed between their lowest and the highest portion were approximately 0.4. This difference is significant and confirms that a large amount of additives can inhibit the biochemical activity (i.e., acidifying activity) of LAB present in the yogurt starter culture. In all cases of milk samples fermented in the presence of the tested plant extracts, the pH was no longer statistically significantly reduced after 3.5 h of fermentation.

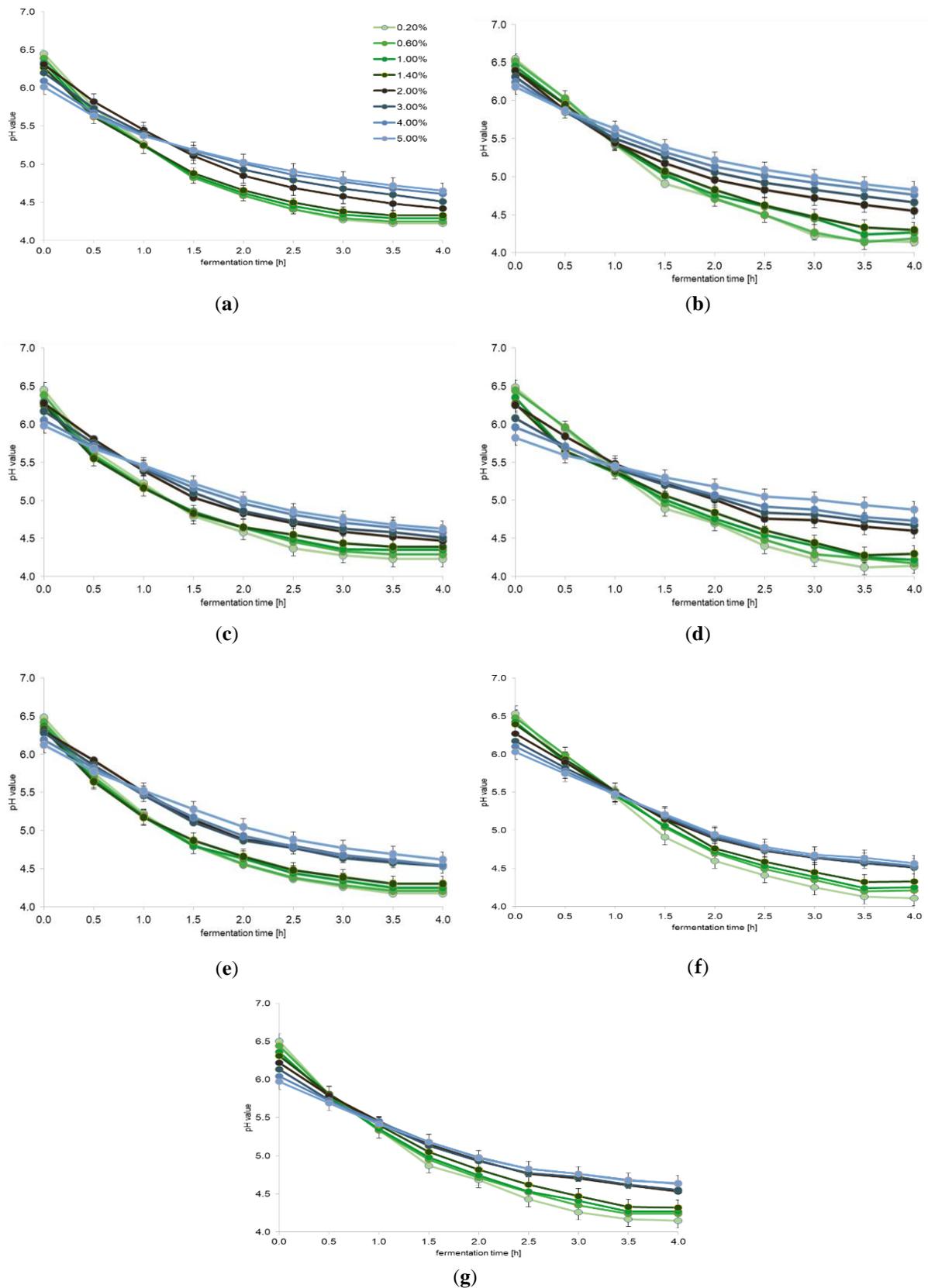


Figure 1. The effect of plant extracts on lactic acid fermentation (average values and standard deviations of three replicates): (a) valerian (*Valeriana officinalis* L.); (b) sage (*Salvia officinalis* L.); (c) chamomile (*Matricaria chamomilla* L.); (d) cistus (*Cistus* L.); (e) linden blossom (*Tilia* L.); (f) ribwort plantain (*Plantago lanceolata* L.); and (g) marshmallow (*Althaea* L.).

3.2. Determination of the Effect of Plant Extracts on the Populations of Lactic Acid Bacteria

The results of the determination of both tested LAB populations (Table 1) immediately after the end of the fermentation process confirmed that most of the tested plant extracts did not reduce the populations ($p < 0.05$). This means that the tested LAB cultures well tolerated the presence of the extracts at an amount of up to 3.0% in milk. Only with the addition of sage extract, we observed some slight, but statistically significant, reduction in the *Lactobacillus* cell population (Table 1).

Further analyses showed that the types of additives and the digestion process had an impact on the population of *S. thermophilus* cells ($p < 0.05$); however, no effect on the lactobacilli population was observed ($p > 0.05$). It should be noted that both bacterial species had different survival rates in the samples and reacted differently to digestion in the model digestive juices (Table 1). Statistical analysis carried out for individual bacterial species showed that only digestion influenced the number of *S. thermophilus* cells in most cases. The exceptions were the milk samples fermented with the addition of cistus extract and marshmallow extract, in which the digestion step did not statistically significantly influence the *S. thermophilus* cell population. This may suggest that these two extracts improved the survival of *S. thermophilus* cells under the conditions of the model digestive system. In the case of *Lactobacillus* bacteria, neither the type of plant extract or its dose nor digestion in the model digestive system had a statistically significant influence on the bacterial cell population (Table 1).

Table 1. The effect of plant extracts on the populations of lactic acid bacteria (average values and standard deviations of five replicates).

Plant Extracts	Sampling Time	Additive Level						Additive Level					
		0.2%	0.6%	1.0%	1.4%	2.0%	3.0%	0.2%	0.6%	1.0%	1.4%	2.0%	3.0%
		<i>Streptococcus thermophilus</i> Population [log CFU/mL]						<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> Population [log CFU/mL]					
Valerian (<i>Valeriana officinalis</i> L.)	after fermentation	7.9 ± 0.3 ^a	7.6 ± 0.5 ^a	7.8 ± 0.3 ^a	7.8 ± 0.3 ^a	7.6 ± 0.3 ^a	7.4 ± 0.3 ^a	7.2 ± 0.3 ^a	7.1 ± 0.5 ^a	7.0 ± 0.5 ^a	7.0 ± 0.3 ^a	6.6 ± 0.3 ^a	6.4 ± 0.3 ^a
	after digestion	6.6 ± 0.3 ^b	6.5 ± 0.3 ^b	7.0 ± 0.3 ^b	6.8 ± 0.3 ^b	6.5 ± 0.3 ^b	6.3 ± 0.3 ^b	6.7 ± 0.3 ^a	6.5 ± 0.3 ^a	6.8 ± 0.3 ^a	6.9 ± 0.4 ^a	6.5 ± 0.3 ^a	6.1 ± 0.3 ^a
Sage (<i>Salvia officinalis</i> L.)	after fermentation	7.8 ± 0.3 ^a	8.0 ± 0.3 ^a	7.7 ± 0.4 ^a	7.9 ± 0.2 ^a	7.7 ± 0.3 ^a	7.3 ± 0.4 ^{a,b}	7.2 ± 0.3 ^a	6.7 ± 0.3 ^{a,b}	6.4 ± 0.3 ^b	6.4 ± 0.3 ^b	6.4 ± 0.3 ^b	6.5 ± 0.3 ^{a,b}
	after digestion	6.9 ± 0.3 ^b	7.0 ± 0.2 ^b	6.3 ± 0.3 ^{b,c}	6.8 ± 0.3 ^{b,c}	6.6 ± 0.3 ^{b,c}	6.2 ± 0.3 ^c	6.8 ± 0.3 ^{a,b}	6.3 ± 0.4 ^b	6.2 ± 0.4 ^b	6.2 ± 0.4 ^b	6.2 ± 0.4 ^b	6.1 ± 0.3 ^b
Chamomile (<i>Matricaria chamomilla</i> L.)	after fermentation	7.9 ± 0.3 ^a	7.9 ± 0.3 ^a	8.0 ± 0.3 ^a	7.9 ± 0.3 ^a	7.7 ± 0.3 ^a	7.5 ± 0.3 ^a	7.2 ± 0.4 ^a	7.0 ± 0.5 ^a	6.8 ± 0.5 ^a	6.8 ± 0.3 ^a	6.5 ± 0.3 ^a	6.4 ± 0.3 ^a
	after digestion	6.8 ± 0.3 ^b	6.3 ± 0.3 ^b	6.9 ± 0.3 ^b	6.9 ± 0.3 ^b	6.5 ± 0.3 ^b	6.3 ± 0.3 ^b	6.6 ± 0.4 ^a	6.3 ± 0.4 ^a	6.6 ± 0.4 ^a	6.7 ± 0.4 ^a	6.5 ± 0.5 ^a	6.2 ± 0.4 ^a
Cistus (<i>Cistus</i> L.)	after fermentation	8.1 ± 0.3 ^a	8.0 ± 0.3 ^a	7.9 ± 0.3 ^a	8.0 ± 0.3 ^a	7.8 ± 0.3 ^{a,b}	7.4 ± 0.4 ^{a,b}	7.2 ± 0.5 ^a	7.2 ± 0.3 ^a	7.2 ± 0.3 ^a	7.6 ± 0.3 ^a	7.2 ± 0.3 ^a	6.8 ± 0.3 ^a
	after digestion	7.9 ± 0.4 ^a	7.8 ± 0.4 ^{a,b}	7.8 ± 0.4 ^{a,b}	7.5 ± 0.3 ^{a,b}	7.6 ± 0.3 ^{a,b}	7.1 ± 0.2 ^b	6.7 ± 0.4 ^a	7.0 ± 0.4 ^a	7.0 ± 0.4 ^a	7.5 ± 0.4 ^a	7.0 ± 0.4 ^a	6.6 ± 0.4 ^a
Linden blossom (<i>Tilia</i> L.)	after fermentation	8.2 ± 0.3 ^a	8.3 ± 0.3 ^a	8.3 ± 0.3 ^a	8.2 ± 0.3 ^a	7.9 ± 0.5 ^a	7.8 ± 0.5 ^a	7.5 ± 0.4 ^a	7.7 ± 0.3 ^a	7.2 ± 0.3 ^a	7.5 ± 0.3 ^a	7.3 ± 0.3 ^a	6.9 ± 0.3 ^{a,b}
	after digestion	6.9 ± 0.3 ^b	6.8 ± 0.3 ^b	6.9 ± 0.3 ^b	6.8 ± 0.3 ^b	6.6 ± 0.3 ^b	6.4 ± 0.3 ^b	7.0 ± 0.3 ^a	6.8 ± 0.3 ^{a,b}	7.0 ± 0.3 ^a	6.7 ± 0.3 ^{a,b}	6.7 ± 0.3 ^{a,b}	6.3 ± 0.3 ^b
Ribwort plantain (<i>Plantago lanceolata</i> L.)	after fermentation	8.1 ± 0.3 ^a	8.1 ± 0.4 ^a	8.3 ± 0.3 ^a	8.3 ± 0.3 ^a	7.9 ± 0.2 ^a	7.5 ± 0.5 ^{a,b}	7.3 ± 0.5 ^a	7.1 ± 0.3 ^a	7.1 ± 0.3 ^a	7.3 ± 0.3 ^a	7.2 ± 0.3 ^a	6.8 ± 0.3 ^a
	after digestion	7.1 ± 0.3 ^b	6.7 ± 0.3 ^{b,c}	6.6 ± 0.3 ^{b,c}	6.5 ± 0.3 ^{b,c}	6.5 ± 0.3 ^{b,c}	6.2 ± 0.3 ^c	7.0 ± 0.4 ^a	6.7 ± 0.3 ^a	7.0 ± 0.4 ^a	7.2 ± 0.3 ^a	6.8 ± 0.3 ^a	6.5 ± 0.3 ^a
Marshmallow (<i>Althaea</i> L.)	after fermentation	8.2 ± 0.3 ^a	8.1 ± 0.3 ^a	8.0 ± 0.3 ^a	8.0 ± 0.3 ^a	7.8 ± 0.3 ^a	7.4 ± 0.2 ^a	7.7 ± 0.5 ^a	7.4 ± 0.3 ^a	7.5 ± 0.4 ^a	7.8 ± 0.3 ^a	7.4 ± 0.3 ^a	7.0 ± 0.3 ^{a,b}
	after digestion	7.2 ± 0.3 ^{a,b}	7.2 ± 0.3 ^{a,b}	7.2 ± 0.3 ^{a,b}	7.3 ± 0.3 ^a	7.1 ± 0.3 ^{a,b}	6.8 ± 0.3 ^b	7.2 ± 0.3 ^a	7.1 ± 0.3 ^{a,b}	7.1 ± 0.3 ^{a,b}	7.3 ± 0.3 ^a	7.0 ± 0.3 ^{a,b}	6.6 ± 0.3 ^b

^{a,b,c} the same letter indices within a given bacterial species and for a given plant extract mean no statistically significant differences at the significance level of 0.05.

3.3. TPC and Antioxidant Capacities of Plant Extracts

The phenolic content determined in the tested plant extracts is presented in Table 2. A significant difference was observed in the phenolic content between cistus and linden blossom extracts and the rest of the tested plant extracts. As we mentioned in the materials and methods section marshmallow and plantain extracts were in liquid form while the rest were in powder form. As can be seen from the data presented in Table 2, liquid marshmallow and plantain extracts were not preparations with the lowest total phenolic content, as well as antioxidant capacity. Statistical analysis showed significant differences in the phenolic content, which resulted in the identification of seven homogenous groups (at a 5% significance level).

Table 2. The TPC and Trolox equivalent antioxidant capacity of plant extracts determined by DPPH, ABTS, and FRAP assays (average values and standard deviations of three replicates).

Plant Extracts	Total Phenolic Content [mg GAE/g of Extract]	Antioxidant Capacities [mmol TE/g Extract]		
		DPPH	ABTS	FRAP
Valerian (<i>Valeriana officinalis</i> L.)	30.97 ± 0.49 ^f	0.104 ± 0.003 ^b	0.159 ± 0.003 ^b	0.090 ± 0.004 ^a
Sage (<i>Salvia officinalis</i> L.)	61.42 ± 0.43 ^c	0.137 ± 0.002 ^c	0.186 ± 0.004 ^c	0.122 ± 0.004 ^{b,c}
Chamomile (<i>Matricaria chamomilla</i> L.)	32.56 ± 0.24 ^e	0.099 ± 0.003 ^b	0.120 ± 0.003 ^a	0.092 ± 0.004 ^a
Cistus (<i>Cistus</i> L.)	106.38 ± 0.01 ^a	0.154 ± 0.005 ^d	0.208 ± 0.004 ^d	0.136 ± 0.003 ^d
Linden blossom (<i>Tilia</i> L.)	104.72 ± 0.39 ^b	0.161 ± 0.007 ^d	0.210 ± 0.007 ^d	0.133 ± 0.004 ^{c,d}
Ribwort plantain (<i>Plantago lanceolata</i> L.)	41.84 ± 0.20 ^d	0.129 ± 0.004 ^c	0.150 ± 0.003 ^b	0.113 ± 0.006 ^b
Marshmallow (<i>Althaea</i> L.)	24.06 ± 0.34 ^g	0.084 ± 0.003 ^a	0.123 ± 0.005 ^a	0.098 ± 0.007 ^a

Different letters (^{a–g}) within the same column indicate significant difference at the significance level of 0.05.

The plant extracts were also screened for DPPH, ABTS, and FRAP radical scavenging activities. It was observed that cistus and linden blossom extracts exhibited the highest DPPH, ABTS, and FRAP radical scavenging activity, whereas the lowest DPPH, ABTS, and FRAP activity was exhibited by marshmallow, chamomile, and valerian extract, respectively. Correlation analysis between TPC and DPPH radical scavenging ability, TPC and ABTS radical scavenging ability, and TPC and FRAP radical scavenging ability showed a high degree of correlation ($r_2 = 0.930, 0.923, \text{ and } 0.931$, respectively). However, the r_2 values of 0.931, 0.936, and 0.883 determined for correlation between DPPH and ABTS radical scavenging activity, DPPH and FRAP radical scavenging activity, and ABTS and FRAP radical scavenging activity, respectively, indicated that mainly phenolic compounds contributed to the total antioxidant activity in the tested plant extracts.

3.4. Qualitative Analysis of Plant Extracts

The results of ultrahigh-performance LC (UHPLC)-QTOF-CAD analyses of plant extracts are shown in Tables 3–9. The identified compounds are presented according to their elution order. Compounds were tentatively identified based on their HRMS and HRMS2 spectra. The fragmentation patterns and molecular formulas of the compounds were compared with available literature data.

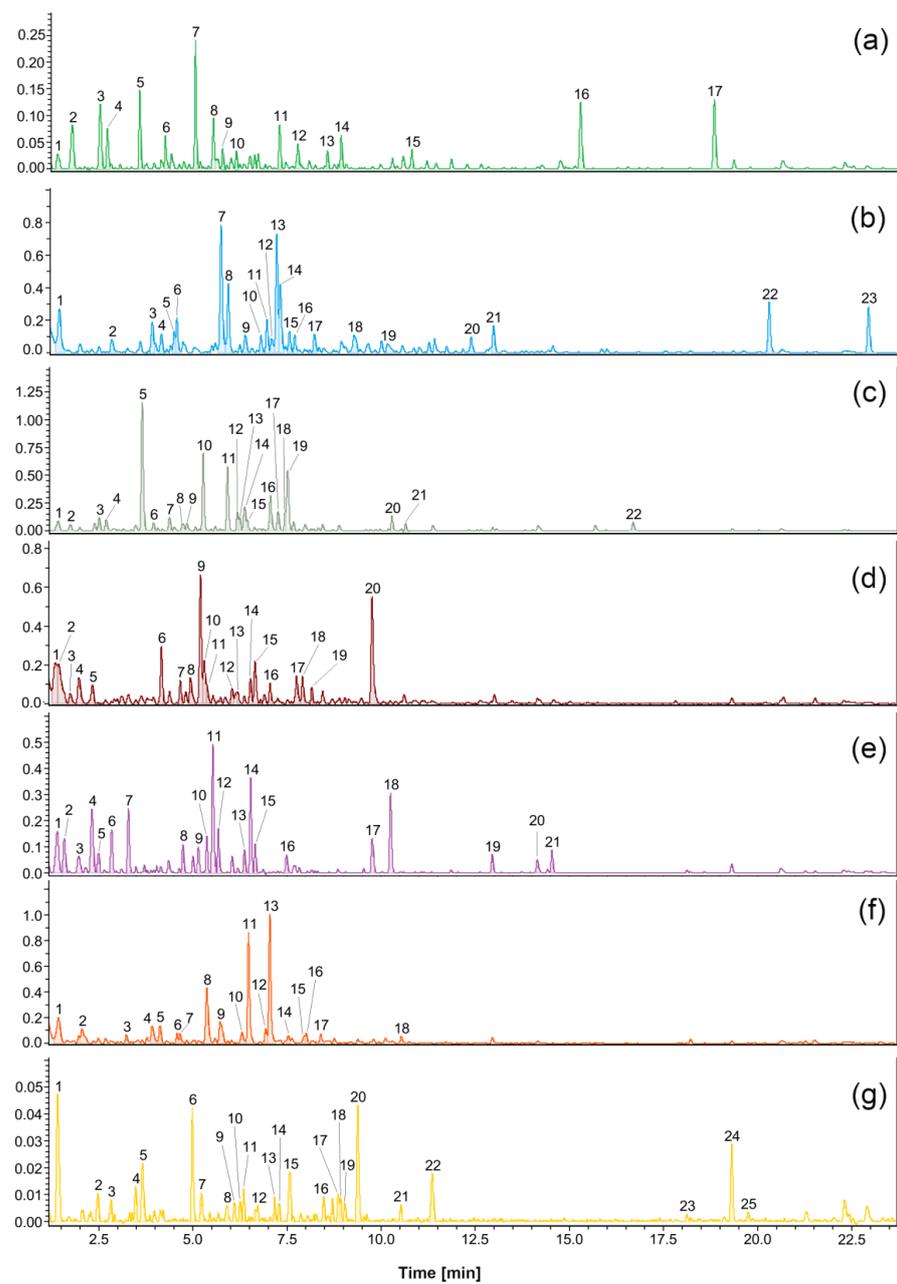


Figure 2. UHPLC-QTOF-CAD chromatograms of the studied plant extracts: (a) valerian (*Valeriana officinalis* L.); (b) sage (*Salvia officinalis* L.); (c) chamomile (*Matricaria chamomilla* L.); (d) cistus (*Cistus* L.); (e) linden blossom (*Tilia* L.); (f) ribwort plantain (*Plantago lanceolata* L.); and (g) marshmallow (*Althaea* L.).

Table 3. Compounds identified in the valerian (*Valeriana officinalis* L.) extract using UHPLC-QTOF-MS/MS. Compound numbers correspond to those indicated in Figure 2.

Peak	RT [min]	Molecular Ion [M-H] ⁻	Ion Mode	MS/MS Fragments	Formula	Error [ppm]	mSigma	Tentative Identification
1	1.4	255.0510	-	255(100), 193(10)	C ₁₁ H ₁₁ O ₇	0.2	13.2	piscidic acid
		449.1298	-	221(100)	C ₁₈ H ₂₅ O ₁₃	0.6	43.8	unidentified
2	1.8	361.1504	-	361(100), 199(5)	C ₁₆ H ₂₅ O ₉	-0.1	11.3	unidentified
3	2.5	351.0717	-	191(100), 215(11)	C ₁₆ H ₁₅ O ₉	1.3	20.1	caffeoylquinic acid (quinone form)
4	2.7	353.0873	-	173(100), 191(64), 179(45)	C ₁₆ H ₁₇ O ₉	1.4	11.0	neochlorogenic acid
5	3.5	553.1929	-	391(100), 195(21)	C ₂₆ H ₃₃ O ₁₃	-0.4	27.4	unidentified hexoside (lignan)
6	4.2	697.2346	-	373(100), 535(14), 181(11)	C ₃₂ H ₄₁ O ₁₇	0.4	15.4	hydroxy-pinorelinol di-hexoside
		553.1916	-	391(100), 195(62)	C ₂₆ H ₃₃ O ₁₃	1.9	118.8	unidentified hexoside (lignan)
7	5.0	681.2390	-	357(100)	C ₃₂ H ₄₁ O ₁₆	1.5	2.6	pinorelinol di-hexoside
8	5.5	637.2348	-	197(100), 221(52), 341(31)	C ₂₇ H ₄₁ O ₁₇	0.2	10.4	kanokoside C isomer
9	5.7	535.1814	-	373(100), 181(45)	C ₂₆ H ₃₁ O ₁₂	1.4	4.8	hydroxy-pinorelinol hexoside
10	6.1	493.2285	-	493(100), 331(47)	C ₂₂ H ₃₇ O ₁₂	1.1	16.2	rhodiolide isomer
11	7.3	519.1869	-	357(100), 151(8)	C ₂₆ H ₃₁ O ₁₁	0.6	7.1	pinorelinol hexoside
12	7.8	609.1822	-	301(100)	C ₂₈ H ₃₃ O ₁₅	0.5	22.0	hesperidin isomer
		457.1709	-	293(100)	C ₂₁ H ₂₉ O ₁₁	1.4	76.6	unidentified
13	8.5	345.1552	-	345(100)	C ₁₆ H ₂₅ O ₈	0.7	4.4	unidentified monoterpene hexoside (iridoid)
		347.1708	-	347(100)	C ₁₆ H ₂₇ O ₈	1.1	30.1	unidentified monoterpene hexoside (iridoid)
14	8.9	477.2339	-	477(100), 315(35)	C ₂₂ H ₃₇ O ₁₁	0.5	6.4	unidentified monoterpene diglycoside
15	10.8	573.2553	-	573(100), 231(4)	C ₂₇ H ₄₁ O ₁₃	-0.1	2.3	unidentified
16	15.3	249.1488	-	249(100), 163(4)	C ₁₅ H ₂₁ O ₃	3.4	0.7	valerenolic acid
17	18.8	291.1592	-	291(100), 249(19)	C ₁₇ H ₂₃ O ₄	3.4	3.1	acetylvalerenolic acid

Table 4. Compounds identified in the sage (*Salvia officinalis* L.) extract using UHPLC-QTOF-MS/MS. Compound numbers correspond to those indicated in Figure 2.

Peak	RT [min]	Molecular Ion [M-H] ⁻	Ion Mode	MS/MS Fragments	Formula	Error [ppm]	mSigma	Tentative Identification
1	1.4	197.0458	-	179(53), 135(24), 123(23), 195(6), 151(4)	C ₉ H ₉ O ₅	-1.3	10.2	danshensu isomer
2	2.8	325.0933	-	179(100), 135(14)	C ₁₅ H ₁₇ O ₈	-1.9	11.8	caffeic acid-deoxyhexoside
3	3.9	389.1789	+	227(100), 209(98), 191(56), 131(12)	C ₁₈ H ₂₉ O ₉	4.3	13.8	unidentified
4	4.1	355.1041	-	295(100), 265(51), 160(17), 193(16), 175(10)	C ₁₆ H ₁₉ O ₉	-1.9	19.8	ferulic acid-hexoside
		583.2047	-	373(100), 361(98), 298(58), 295(33), 313(27)	C ₂₇ H ₃₅ O ₁₄	-2.5	12.4	unidentified
5	4.5	493.0628	-	299(100), 271(50), 241(9)	C ₂₁ H ₁₇ O ₁₄	-0.9	12.2	unidentified

Table 4. Cont.

Peak	RT [min]	Molecular Ion [M-H] ⁻	Ion Mode	MS/MS Fragments	Formula	Error [ppm]	mSigma	Tentative Identification
6	4.6	227.1275	+	209(100), 191(87), 149(55), 167(43), 131(41)	C ₁₂ H ₁₉ O ₄	1.4	0.6	unidentified
7	5.7	461.0731	-	285(100), 255(33)	C ₂₁ H ₁₇ O ₁₂	-1.2	1.5	luteoline-oxyhexoside
8	5.9	593.1522 447.0938	- -	285(100), 339(12), 255(5) 284(100), 256(7)	C ₂₇ H ₂₉ O ₁₅ C ₂₁ H ₁₉ O ₁₁	-1.7 -1.1	6.5 2.4	luteoline-hexoside- deoxyhexoside luteoline-hexoside
9	6.4	551.1770	-	235(100), 533(88), 295(69), 160(64)	C ₂₆ H ₃₁ O ₁₃	0.1	20.5	unidentified
10	6.8	577.1200	-	269(100)	C ₂₆ H ₂₅ O ₁₅	-0.2	11.6	apigenin-hexoside- deoxyhexoside
11	7.0	445.0779	-	269(100)	C ₂₁ H ₁₇ O ₁₁	-0.6	9.0	apigenin-oxyhexoside
12	7.1	431.0984	-	268(100)	C ₂₁ H ₁₉ O ₁₀	0.0	3.3	apigenin-hexoside
13	7.2	359.0768	-	161(100), 197(77), 179(32)	C ₁₈ H ₁₅ O ₈	1.1	4.3	rosmarinic acid
14	7.3	555.1141	-	359(100), 161(22), 135(16), 193(14)	C ₂₇ H ₂₃ O ₁₃	0.7	7.9	salvianolic acid K isomer
15	7.5	475.0881	-	284(100), 299(61)	C ₂₂ H ₁₉ O ₁₂	0.2	24.7	hispidulin/diosmetin- oxyhexoside
16	7.7	463.1224 609.1771	+ +	301(100) 301(100), 463(4)	C ₂₂ H ₂₃ O ₁₁ C ₃₅ H ₂₉ O ₁₀	2.3 -2.7	8.8 44.0	kaempferide-hexoside kaempferide-hexoside- deoxyhexoside
17	8.2	769.1637	-	285(100), 255(26), 575(4)	C ₃₆ H ₃₃ O ₁₉	-2.1	22.5	luteolin-oxyhexoside- pentoside-ferulic acid
18	9.3	753.1679 621.1842	-	269(100), 486(7), 193(4) 313(100), 297(44)	C ₃₆ H ₃₃ O ₁₈ C ₂₉ H ₃₃ O ₁₅	-0.9 -2.7	18.8 14.2	apigenin-oxyhexoside- pentoside-ferulic acid unidentified
19	10.2	711.3968	-	503(100), 453(10)	C ₃₇ H ₅₉ O ₁₃	-1.0	19.4	unidentified
20	12.4	493.1140	-	359(100), 323(40), 135(21), 179(16), 295(14)	C ₂₆ H ₂₁ O ₁₀	0.0	13.0	unidentified
21	13.0	327.2178	-	327(100), 211(12), 229(5), 171(3)	C ₁₈ H ₃₁ O ₅	-0.3	6.2	unidentified
22	20.3	329.1760	-	285(100)	C ₂₀ H ₂₅ O ₄	-0.4	13.3	carnosol isomer
23	22.9	331.1926	-	287(100)	C ₂₀ H ₂₇ O ₄	-3.3	11.9	carnoic acid isomer

Table 5. Compounds identified in the chamomile (*Matricaria chamomilla* L.) extract using UHPLC-QTOF-MS/MS. Compound numbers correspond to those indicated in Figure 2.

Peak	RT [min]	Molecular Ion [M-H] ⁻	Ion Mode	MS/MS Fragments	Formula	Error [ppm]	mSigma	Tentative Identification
1	1.4	315.0719 329.0874	- -	315(100), 152(10) 167(100), 329(28)	C ₁₃ H ₁₅ O ₉ C ₁₄ H ₁₇ O ₉	0.7 1.2	3.8 18.3	protocatechuoyl- hexoside vanilic acid-hexoside
2	1.7	353.0877	-	191(100), 179(40), 135(30)	C ₁₆ H ₁₇ O ₉	0.2	1.6	chlorogenic acid
3	2.5	353.0875	-	191(100)	C ₁₆ H ₁₇ O ₉	1.0	7.7	caffeoylquinic acid
4	2.7	353.0874	-	191(100), 173(83)	C ₁₆ H ₁₇ O ₉	1.3	4.5	caffeoylquinic acid
5	3.7	355.1035 515.1201	- -	193(100), 149(44) 353(100), 191(59), 179(22), 135(12)	C ₁₆ H ₁₉ O ₉ C ₂₅ H ₂₃ O ₁₂	0.0 -1.2	5.5 34.5	ferulic acid hexoside dicafeoylquinic acid

Table 5. Cont.

Peak	RT [min]	Molecular Ion [M-H] ⁻	Ion Mode	MS/MS Fragments	Formula	Error [ppm]	mSigma	Tentative Identification
6	4.0	639.1564	-	313(100), 477(53), 270(36)	C ₂₈ H ₃₁ O ₁₇	0.4	11.4	isorhamnetin-di-hexoside
7	4.4	479.0834	-	317(100), 287(10), 165(6)	C ₂₁ H ₁₉ O ₁₃	-0.7	15.1	myricetin-oxyhexoside
		609.1464	-	284(100), 447(38)	C ₂₇ H ₂₉ O ₁₆	-0.4	28.5	luteolin-di-hexoside
8	4.7	367.1037	-	367(100), 173(19), 193(9), 134(7)	C ₁₇ H ₁₉ O ₉	-0.6	6.5	feruloylquinic acid
		463.0883	-	300(100), 227(8)	C ₂₁ H ₁₉ O ₁₂	-0.2	9.7	quercetin-hexoside
9	4.8	609.1469	-	301(100)	C ₂₇ H ₂₉ O ₁₆	-1.3	5.3	quercetin-hexoside-deoxyhexoside
10	5.3	355.1039	-	193(100), 149(27)	C ₁₆ H ₁₉ O ₉	-1.3	9.5	ferulic acid hexoside
11	5.9	593.1516	-	285(100)	C ₂₇ H ₂₉ O ₁₅	-0.6	5.0	kaempferol-hexoside-deoxyhexoside
		447.0937	-	284(100)	C ₂₁ H ₁₉ O ₁₁	-1.0	3.7	kaempferol-hexoside
12	6.2	493.1002	-	331(100), 287(83), 315(55)	C ₂₂ H ₂₁ O ₁₃	-2.9	6.4	petuletin-hexoside
13	6.2	467.1679	-	323(100), 305(66), 189(25)	C ₂₆ H ₂₇ O ₈	6.8	28.6	unidentified
14	6.4	623.1624	-	315(100), 300(88), 271(28)	C ₂₈ H ₃₁ O ₁₆	-1.1	29.1	isorhamnetin-hexoside-deoxyhexoside
		515.1201	-	353(100), 191(36), 179(26), 135(11)	C ₂₅ H ₂₃ O ₁₂	-1.3	5.4	dicafeoylquinic acid
15	6.5	681.1674	-	313(100), 270(45), 519(27), 477(24)	C ₃₀ H ₃₃ O ₁₈	-0.3	13.3	isorhamnetin-hexoside-acylhexoside
		515.1202	-	353(100), 191(71), 179(25)	C ₂₅ H ₂₃ O ₁₂	-1.3	25.4	dicafeoylquinic acid
16	7.1	431.0991	-	268(100)	C ₂₁ H ₁₉ O ₁₀	-1.6	7.7	apigenin-hexoside
17	7.3	515.1204	-	353(100), 179(18), 191(15), 135(6)	C ₂₅ H ₂₃ O ₁₂	-1.7	7.2	dicafeoylquinic acid
		445.1727	-	445(100), 243(44), 183(6), 139(3)	C ₂₀ H ₂₉ O ₁₁	-2.5	22.4	unidentified
18	7.5	479.1169	+	317(100)	C ₂₉ H ₁₉ O ₇	-9.1	14.9	isorhamnetin-hexoside
		609.1773	+	301(100), 463(4)	C ₃₅ H ₂₉ O ₁₀	-2.9	41.0	kaempferide-hexoside-deoxyhexoside
19	7.5	477.1045	-	477(100), 299(65), 271(63), 315(46)	C ₂₂ H ₂₁ O ₁₂	-1.4	10.3	isorhamnetin-hexoside
		711.2156	-	711(100), 549(20), 433(10), 271(66)	C ₃₂ H ₃₉ O ₁₈	-2.0	9.1	unidentified-hexoside-deoxyhexoside-pentoside (flavonoid)
20	10.3	475.1216	+	271(100)	C ₂₃ H ₂₃ O ₁₁	3.9	7.4	apigenin-acylhexoside
21	10.6	519.1141	-	271(72), 299(46), 313(16), 151(7)	C ₂₄ H ₂₃ O ₁₃	0.5	1.9	isorhamnetin-acylhexoside
22	16.7	447.2009	+	219(100), 181(65), 231(30), 411(25), 358(23)	C ₂₄ H ₃₁ O ₈	1.0	8.8	unidentified

Table 6. Compounds identified in the cistus (*Cistus* L.) extract using UHPLC-QTOF-MS/MS. Compound numbers correspond to those indicated in Figure 2.

Peak	RT [min]	Molecular Ion [M-H] ⁻	Ion Mode	MS/MS Fragments	Formula	Error [ppm]	mSigma	Tentative Identification
1	1.4	305.0667	-	305(100), 219(12), 165(8)	C ₁₅ H ₁₃ O ₇	-0.1	5.1	galocatechin isomer
2	1.4	469.0054	-	425(100), 299(24)	C ₂₁ H ₉ O ₁₃	-1.0	12.5	valoneic acid dilacton isomer
3	1.8	633.0743	-	301(100), 275(49), 229(23), 257(22)	C ₂₇ H ₂₁ O ₁₈	-1.5	1.1	strictinin isomer
4	2.0	305.0672 591.1370	- -	305(100), 219(13), 261(8), 179(8) 285(100), 305(12)	C ₁₅ H ₁₃ O ₇ C ₂₇ H ₂₇ O ₁₅	-1.9 -2.5	5.4 41.7	galocatechin isomer unidentified
5	2.3	289.0722 633.0742	- -	289(100), 245(17), 203(9) 301(100), 275(41), 257(22), 229(11)	C ₁₅ H ₁₃ O ₆ C ₂₇ H ₂₁ O ₁₈	-1.6 -1.4	1.1 35.6	epicatechin strictinin isomer
6	4.2	479.0846	-	316(100), 271(83)	C ₂₁ H ₁₉ O ₁₃	-3.2	13.7	myricetin-hexoside
7	4.7	327.1458	-	327(100), 165(10)	C ₁₆ H ₂₃ O ₇	-2.8	9.2	unidentified
8	5.0	449.0738	-	316(100), 271(68)	C ₂₀ H ₁₇ O ₁₂	-2.8	4.1	myricetin-pentoside
9	5.2	463.0897	-	316(100), 271(91), 179(2)	C ₂₁ H ₁₉ O ₁₂	-3.2	3.5	myricetin-deoxyhexoside
10	5.3	463.0898	-	271(100), 300(83)	C ₂₁ H ₁₉ O ₁₂	-3.6	0.8	quercetin-hexoside
11	5.4	609.1474	-	271(100), 300(76)	C ₂₇ H ₂₉ O ₁₆	-2.1	6.5	quercetin-hexoside-deoxyhexoside
12	6.1	433.0785	-	300(100), 271(86), 255(35), 243(18)	C ₂₀ H ₁₇ O ₁₁	-2.0	5.8	quercetin-pentoside
13	6.2	449.1075	+	197(100), 287(34), 179(11)	C ₂₁ H ₂₁ O ₁₁	0.9	11.9	unidentified
14	6.5	577.1573 447.0944	- -	283(100), 255(57), 285(55), 431(24) 255(100), 227(87), 284(49)	C ₂₇ H ₂₉ O ₁₄ C ₂₁ H ₁₉ O ₁₁	-1.8 -2.6	15.6 11.4	kaempferol-di-deoxyhexoside kaempferol-hexoside
15	6.7	773.1905	+	147(100), 319(18)	C ₃₆ H ₃₇ O ₁₉	2.4	44.2	myricetin-di-deoxyhexoside-hexoside
16	7.1	507.2219	+	219(100), 189(5)	C ₂₆ H ₃₅ O ₁₀	1.1	9.2	unidentified
17	7.8	523.2199	-	475(100), 327(17), 149(7)	C ₂₆ H ₃₅ O ₁₁	-2.7	3.3	unidentified
18	7.9	627.1315	+	147(100), 319(58)	C ₃₀ H ₂₇ O ₁₅	4.8	37.4	myricetin-hexoside-deoxyhexoside
19	8.2	551.2145	-	329(100), 269(36), 314(35)	C ₂₇ H ₃₅ O ₁₂	-1.9	7.2	unidentified
20	9.8	595.1432	+	147(100), 287(39)	C ₃₀ H ₂₇ O ₁₃	2.5	13.1	unidentified

Table 7. Compounds identified in the linden blossom (*Tilia* L.) extract using UHPLC-QTOF-MS/MS. Compound numbers correspond to those indicated in Figure 2.

Peak	RT [min]	Molecular Ion [M-H] ⁻	Ion Mode	MS/MS Fragments	Formula	Error [ppm]	mSigma	Tentative Identification
1	1.4	315.0715	-	315(100), 152(8)	C ₁₃ H ₁₅ O ₉	2.0	0.3	protocatechuoyl—hexoside
2	1.6	153.0187	-	153(100), 109(40)	C ₇ H ₅ O ₄	4.3	14.4	protocatechuic acid
3	2.0	577.1340	-	289(100), 407(72)	C ₃₀ H ₂₅ O ₁₂	2.1	4.2	procyanidin isomer
4	2.3	289.0712	-	289(100)	C ₁₅ H ₁₃ O ₆	1.8	2.3	catechin
5	2.5	353.0867 577.1341	- -	191(100), 353(6) 289(100), 407(71)	C ₁₆ H ₁₇ O ₉ C ₃₀ H ₂₅ O ₁₂	3.1 1.8	23.4 7.4	chlorogenic acid procyanidin isomer
6	2.9	577.1344	-	289(100), 407(72)	C ₃₀ H ₂₅ O ₁₂	1.2	13.7	procyanidin isomer
7	3.3	289.0714	-	289(100), 245(19), 203(9)	C ₁₅ H ₁₃ O ₆	1.3	5.2	catechin
8	4.8	609.1457	-	299(100), 271(63), 447(12)	C ₂₇ H ₂₉ O ₁₆	0.7	17.2	quercetin-hexoside-deoxyhexoside
9	5.2	593.1516	-	283(100), 285(43), 447(36)	C ₂₇ H ₂₉ O ₁₅	-0.7	23.8	kaempferol-hexoside-deoxyhexoside
10	5.4	609.1469	-	271(100), 300(68)	C ₂₇ H ₂₉ O ₁₆	-1.2	10.4	quercetin-hexoside-deoxyhexoside
11	5.5	465.1014 595.1634	+ +	303(100) 287(100)	C ₂₁ H ₂₁ O ₁₂ C ₂₇ H ₃₁ O ₁₅	2.8 3.9	4.9 7.6	quercetin-hexoside kaempferol-hexoside-deoxyhexoside
12	5.7	449.1066 595.1634	+ +	303(100) 303(100), 449(4)	C ₂₁ H ₂₁ O ₁₁ C ₂₇ H ₃₁ O ₁₅	2.7 2.8	3.8 2.4	quercetin-deoxyhexoside quercetin-di-deoxyhexoside
13	6.4	595.1640	+	287(100)	C ₂₇ H ₃₁ O ₁₅	3.0	5.3	kaempferol-hexoside-deoxyhexoside
14	6.6	449.1062 579.1690 711.2102	+ + +	287(100) 287(100), 433(4) 287(100), 433(4)	C ₂₁ H ₂₁ O ₁₁ C ₂₇ H ₃₁ O ₁₄ C ₃₂ H ₃₉ O ₁₈	3.6 3.2 4.1	4.9 6.0 11.5	kaempferol-hexoside kaempferol-di-deoxyhexoside kaempferol-di-deoxyhexoside-pentoside
15	6.7	447.0937	-	271(100), 300(73)	C ₂₁ H ₁₉ O ₁₁	-0.9	3.1	quercetin-deoxyhexoside
16	7.5	463.0886	-	301(100)	C ₂₁ H ₁₉ O ₁₂	-0.8	15.0	quercetin-hexoside
17	9.8	595.1429	+	147(100), 287(34)	C ₃₀ H ₂₇ O ₁₃	2.9	24.8	kaempferol-hexoside-deoxyhexoside-coumaric acid
18	10.3	593.1854	+	285(100), 447(6)	C ₂₈ H ₃₃ O ₁₄	1.8	9.2	unidentified-hexoside-deoxyhexoside (flavonoid)
19	13.0	327.2174	-	327(100), 211(9)	C ₁₈ H ₃₁ O ₅	1.0	1.5	unidentified
20	14.2	329.2327	-	329(100), 211(17)	C ₁₈ H ₃₃ O ₅	2.1	3.2	unidentified
21	14.6	289.2375	+	235(100), 253(74), 217(64), 135(19), 161(14)	C ₁₆ H ₃₃ O ₄	-0.7	8.0	unidentified

Table 8. Compounds identified in the ribwort plantain (*Plantago lanceolata* L.) extract using UHPLC-QTOF-MS/MS. Compound numbers correspond to those indicated in Figure 2.

Peak	RT [min]	Molecular Ion [M-H] ⁻	Ion Mode	MS/MS Fragments	Formula	Error [ppm]	mSigma	Tentative Identification
1	1.4	373.1142	-	373(100), 211(92), 123(24)	C ₁₆ H ₂₁ O ₁₀	-0.4	8.3	geniposidic acid
2	2.1	461.1672	-	461(100), 315(3)	C ₂₀ H ₂₉ O ₁₂	-1.7	7.5	decaffeoylacteoside
3	3.3	451.2192	-	405(100), 179(8), 243(8), 167(7)	C ₂₀ H ₃₅ O ₁₁	-1.7	4.6	caryoptoside isomer
4	3.9	813.1363	-	285(100), 637(23), 351(18), 461(15)	C ₃₃ H ₃₃ O ₂₄	0.6	7.9	luteolin-tri-oxyhexide isomer
5	4.1	637.1044	-	285(100), 351(89)	C ₂₇ H ₂₅ O ₁₈	0.4	20.9	luteolin-di-oxyhexide isomer
6	4.6	639.1941	-	639(100), 621(42), 161(28), 135(9), 447(7)	C ₂₉ H ₃₅ O ₁₆	-1.6	15.8	unidentified phenylethanoid
7	4.7	639.1933	-	639(100), 621(59), 161(28), 133(16), 475(11)	C ₂₉ H ₃₅ O ₁₆	-0.4	11.7	unidentified phenylethanoid
8	5.4	637.1046	-	285(100), 461(44)	C ₂₇ H ₂₅ O ₁₈	0.0	7.6	luteolin-di-oxyhexoside isomer
9	5.7	461.0721	-	285(100), 461(12), 813(8)	C ₂₁ H ₁₇ O ₁₂	1.1	6.8	luteolin-oxyhexoside isomer
		989.1849	-	639(100), 285(64), 161(41), 477(32), 461(21)	C ₄₃ H ₄₁ O ₂₇	-0.8	43.6	unidentified luteolin derivative (flavonoid)
		639.1935	-		C ₂₉ H ₃₅ O ₁₆	-0.7	8.1	unidentified phenylethanoid
10	6.3	545.2231	-	545(100), 337(14), 235(10), 193(8)	C ₂₅ H ₃₇ O ₁₃	1.5	10.4	unidentified calceolarioside A isomer forsythoside isomer
		477.1400	-	477(100), 161(22), 133(11), 315(9), 179(2)	C ₂₃ H ₂₅ O ₁₁	0.4	9.5	
		755.2407	-	755(100), 161(21), 179(10), 593(10), 135(8)	C ₃₄ H ₄₃ O ₁₉	-0.4	12.5	
11	6.5	623.1980	-	623(100), 161(26), 461(12)	C ₂₉ H ₃₅ O ₁₅	0.2	5.6	verbascoside isomer
		639.1930	-	639(100), 161(30), 477(13), 133(6)	C ₂₉ H ₃₅ O ₁₆	0.2	16.6	unidentified phenylethanoid
12	6.9	445.0779	-	269(100)	C ₂₁ H ₁₇ O ₁₁	-0.6	24.9	apigenin-oxyhexoside forsythoside isomer
		755.2401	-	755(100), 161(24), 593(11), 133(8)	C ₃₄ H ₄₃ O ₁₉	0.4	19.4	
13	7.1	623.1987	-	623(100), 161(17), 461(7), 133(5)	C ₂₉ H ₃₅ O ₁₅	-0.9	7.0	verbascoside isomer
14	7.5	475.0877	-	274(100), 299(73)	C ₂₂ H ₁₉ O ₁₂	1.0	7.5	kaempferide-oxyhexoside
15	8.0	637.2138	-	637(100), 461(59), 175(41)	C ₃₀ H ₃₇ O ₁₅	0.0	15.9	leucoseptoside A isomer
16	8.0	621.1826	-	321(100), 323(21), 179(18), 487(14)	C ₂₉ H ₃₃ O ₁₅	-0.2	4.8	unidentified
17	8.4	629.2674	-	583(100), 421(21), 451(13), 289(9)	C ₂₆ H ₄₅ O ₁₇	-1.9	5.9	unidentified
18	10.5	651.2304	-	651(100), 175(24), 160(12), 193(7), 475(5)	C ₃₁ H ₃₉ O ₁₅	-1.5	12.5	unidentified

Table 9. Compounds identified in the marshmallow (*Althaea* L.) extract using UHPLC-QTOF-MS/MS. Compound numbers correspond to those indicated in Figure 2.

Peak	RT [min]	Molecular Ion [M-H] ⁻	Ion Mode	MS/MS Fragments	Formula	Error [ppm]	mSigma	Tentative Identification
1	1.4	326.1246	-	164(100), 236(26), 147(20)	C ₁₅ H ₂₀ NO ₇	-0.2	16.1	phenylalanyl-hexoside
2	2.5	417.1043	-	417(100), 152(8)	C ₁₇ H ₂₁ O ₁₂	-1.0	4.6	gentisic acid-dipentoside
3	2.8	179.0357 361.0966	- -	179(100), 135(62) 361(100), 281(13), 171(7)	C ₉ H ₇ O ₄ C ₂₄ H ₁₃ N ₂ O ₂	-4.1 4.7	41.7 55.9	caffeic acid unidentified
4	3.5	227.0568	-	227(100), 139(22), 165(9)	C ₁₀ H ₁₁ O ₆	-3.1	9.2	unidentified
5	3.7	623.0037	-		C ₁₇ H ₁₉ O ₂₃ S	1.0	32.2	flavonoid disulfo-hexoside
6	5.0	193.0479	+	193(100), 134(36), 178(36), 191(10)	C ₁₀ H ₉ O ₄	8.3	9.7	scopoletin isomer
7	5.2	425.0562	-	425(100), 297(88), 315(86), 241(75), 327(73)	C ₁₄ H ₁₇ O ₁₅	2.5	38.1	unidentified
8	5.9	636.9843	-		C ₁₇ H ₁₆ O ₂₄ S	-1.7	12.3	flavonoid sulfo-glycoside
9	6.1	733.0950	-		C ₂₈ H ₂₉ O ₂₁ S	-3.1	37.4	flavonoid sulfo-glycoside
10	6.3	541.0317	-	254(100), 285(33), 175(9)	C ₂₁ H ₁₇ O ₁₅ S	-4.3	12.5	theograndin I isomer
11	6.4	433.1521	-	433(100), 403(86), 311(76), 299(58)	C ₂₂ H ₂₅ O ₉	-3.9	5.5	unidentified
12	6.7	527.0522 639.1223	- -	285(100), 527(4), 213(4), 447(4) 301(100), 371(25), 299(24), 459(22)	C ₂₁ H ₁₉ O ₁₄ S C ₂₇ H ₂₇ O ₁₈	-4.1 -3.1	40.7 62.4	flavonoid sulfo-glycoside unidentified-deoxyhexoside-hexoside (flavonoid)
13	7.2	524.2881	-	524(100), 362(50)	C ₂₇ H ₄₂ NO ₉	-3.1	25.3	unidentified
14	7.3							unidentified
15	7.6	557.0266	-	301(100), 254(77), 315(58), 271(42)	C ₂₁ H ₁₇ O ₁₆ S	-4.2	21.0	theograndin II isomer
16	8.5	555.0464	-	254(100), 284(30), 299(24), 175(7)	C ₂₂ H ₁₉ O ₁₅ S	-2.5	9.6	flavonoid sulfo-oxyhexoside
17	8.9	541.0672	-	299(100), 284(68), 461(6)	C ₂₂ H ₂₁ O ₁₄ S	-2.7	14.1	flavonoid sulfo-hexoside
18	8.9	541.0310	-	285(100), 254(67), 461(22)	C ₂₁ H ₁₇ O ₁₅ S	-3.1	34.2	flavonoid sulfo-oxyhexoside
19	9.1	571.0411	-	300(100), 254(79), 315(46), 491(23)	C ₂₂ H ₁₉ O ₁₆ S	-2.0	24.2	flavonoid sulfo-oxyhexoside
20	9.4	307.0731	-	233(100), 205(76), 263(75), 191(9)	C ₁₇ H ₁₁ N ₂ O ₄	-2.2	2.1	unidentified
21	10.5	475.0896	-		C ₂₂ H ₁₉ O ₁₂	-3.1	56.1	unidentified
22	11.4	555.0469	-	254(100), 284(80), 299(71), 475(35), 175(40)	C ₂₂ H ₁₉ O ₁₅ S	-3.4	1.8	flavonoid sulfo-oxyhexoside
23	18.1	311.2241	-		C ₁₈ H ₃₁ O ₄	-4.2	4.5	unidentified
24	19.3	459.2037	-		C ₂₅ H ₃₁ O ₈	-2.8	24.7	unidentified
25	19.8	459.2030	-		C ₂₅ H ₃₁ O ₈	-1.3	56.6	unidentified

4. Discussion

4.1. Antimicrobial Activity of Plant Extracts

Our experiments showed that with the increase in dose the tested plant extracts gradually limited the acidifying activity of the tested LAB, but did not limit the viability of these cells. This finding is interesting considering the necessity to keep the LAB cells alive and maintain them biologically active in fermented milk drinks throughout the declared shelf life. Perhaps, these plant extracts could be used in the dose range studied to stop the activity of starter bacteria and consequently prevent acidification of the fermented milk beverages with the cultures used in this study. It should be noted that there are no studies to date in this regard.

Various spices and herbal extract may affect activity or vitality of lactic acid bacteria, and this phenomenon depend on the genus, species and even sometimes strain of lactic bacteria, the species of herbs and the method of obtaining the extract from them. It is known from research that some LABs have the ability to degrade certain phenolic compounds found in food, including those with high antioxidant activity [33]. Polyphenol-resistant bacteria have the ability to metabolize polyphenol compounds, depending on their chemical structure (substitutions in the phenolic ring) and concentration. *Lactobacillus plantarum* has been described to exhibit several enzymatic activities, such as that of tannase, phenolic acid decarboxylase (PAD), and benzyl alcohol dehydrogenase which can degrade some phenolic compounds [33]. Only limited studies have analyzed the influence of phenolic compounds on the growth and viability of other LAB species [34,35].

We did not observe any negative or positive effect of the added extracts on the viability of lactobacilli cells during the fermentation process. This is in line with the results reported by Otaibi and Demerdash [36], who showed that appropriately selected portions of sage extracts resulted in better survival of yogurt bacterial cells, while El-Nawawy et al. [37] indicated a beneficial effect of extracts on the multiplication of bacteria in yogurt. An increase was observed in the numbers of *S. thermophilus* cells than *L. delbrueckii* subsp. *bulgaricus*. However, after digestion, the number of *Streptococcus* cells decreased by an average of 1 log cycle, while the population of *Lactobacillus* cells remained stable. This is also confirmed by the studies of Zareba et al. [38] and Ziarno and Margol [39], which proves that LAB poorly tolerate digestive juices, but their survival rate is highly dependent on the culture used. The results obtained in this study thus confirm that the viability of LAB in selected starter cultures can be maintained at a fairly high level, even under such drastic conditions as digestive juices.

There are studies on the antimicrobial effect of plant extracts available in the literature. The antimicrobial activity of plant extracts investigated in this work is often the subject of scientific research [40], but only a few concern the effect of extracts on the activity or population of selected LABs [36]. Due to the lack of comprehensive information on the effect of selected plant extracts on LABs, it is worth taking a brief look at the effect of other plant additives on the bacteria in question. Fortunately, a few reports are found in the literature on the beneficial or inhibitory effects of different plant additives on LABs [41–46].

Among the few publications dealing with the effects of valerian (*V. officinalis* L.) on bacteria, there are some reports on its antibacterial activity [47]. However, no data are available regarding the effect of purge on LABs. Sage (*S. officinalis* L.) oils have proven antibacterial and antifungal properties [40,48–50]. Their effect on yogurt bacteria has also been studied proving that the appropriate portions of these additives do not inhibit the growth of yogurt bacteria, and on the contrary, they may have a positive effect on their survival and increase their number during storage [36]. This was also confirmed by the results of our research. Moreover, El-Nawawy et al. [37] reported a beneficial effect of sage extracts on the population of *S. thermophilus* and *L. bulgaricus*. On the other hand, Hołderna-Kedzia and Kedzia [51] showed a negative effect of these extracts on *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus casei* ATCC 393, *Lactobacillus rhamnosus* Hansen 1968, *Bifidobacterium bifidum* ATCC 35914, *S. thermophilus* ATCC 14485, and *Saccharomyces boulardii* SB48 ATCC-MYA-796. The antibacterial effect of chamomile (*M. chamomilla* L.) extracts

has been described in many studies [49,51–53]. However, there are no scientific reports on the effect of chamomile extract on LABs. Several researchers have studied the activity of cistus (*Cistus* L.) extract against pathogenic fungi and bacteria [54–57]. Few reports even indicate that linden blossom has a slight antibacterial effect (*Tilia* L.) [58,59], but there are no data on its influence on LABs. Ethanol and methanol extracts of ribwort plantain (*P. lanceolata* L.) have already been tested against the strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Candida albicans*, and *Candida tropicalis* and found to be active against these bacteria [60–63].

For marshmallow (*Althaea* L.) extracts, it has been shown that the extracts inhibited the growth of bacteria such as *Pseudomonas fluorescens*, *P. aeruginosa*, *Bordetella bronchiseptica*, *S. aureus*, *S. epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis*, *B. subtilis*, *B. cereus*, *Aspergillus niger*, *C. albicans*, and *Saccharomyces cerevisiae* [64,65]. It was also showed that marshmallow extracts did not have any inhibitory effect on *E. coli*, *K. pneumoniae*, or *Serratia marcescens* [64]. However, the effect of these extracts on LABs has not yet been studied.

4.2. TPC and Antioxidant Capacities of Plant Extracts

It is reported that the metabolites present in plant extracts, including phenolic compounds, are responsible for their various biological activities, such as total phenolic content and antioxidant activity [20,22,66–68]. Therefore, it is worth analyzing the research proving the antioxidant activity of selected herbal extracts. Phenolic compounds are widespread in the world of plants. Based on the structure of the carbon skeleton, phenolic compounds can be divided into phenolic acids and flavonoids.

Wang et al. [69] and Şen and Mat [66] indicated that sesquiterpenes, iridoids, flavonoids, and alkaloids were isolated from valerian (*V. officinalis* L.) extracts. Katsarova et al. [67] showed the lowest antioxidant activity for valerian extracts among the eight tested plant extracts (*V. officinalis*, *Melissa officinalis*, *Crataegus monogyna*, *Hypericum perforatum*, *Serratula coronata*, and their two combinations): oxygen radical absorbing capacity— $820.5 \pm 21.9 \mu\text{mol TE/g}$; hydroxyl radical averting capacity— $381.6 \pm 14.0 \mu\text{mol GAE/g}$; and polyphenolic content— $43.36 \pm 1.3 \text{ mg/g}$. In our study, the valerian extract showed slightly less TPC, expressed as mg GAE/g extract, compared to the above value, despite the fact that its chemical composition included a large diversity of ingredients with antioxidant properties. Furthermore, compared to the other tested plant extracts, its antioxidant capacities were lower.

The crude extract of sage (*S. officinalis* L.) studied by Koşar et al. [70] contained hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, and diterpenoids, in addition to caffeic acid, carnosic acid, luteolin, luteolin-7-O-glucoside, and rosmarinic acid. However, the composition of the plant extract may vary depending on the method used for extraction [5,71–73]. The sage extract obtained by Houghton [5] also contained cyclic monoterpenes, such as 1,8-cineol, α -pinene, and camphor. The crude extract and sub-fractions demonstrated varying degrees of antioxidant capacity. Rosmarinic acid and abietane diterpenes were thought to be responsible for the potent scavenging properties of *Salvia* taxa [74,75]. Our research confirmed a good correlation between the high TPC in the sage extract and its high antioxidant capacities. Modern analytical methods based on semipreparative HPLC, high-resolution MS, nuclear magnetic resonance spectroscopy, infrared spectroscopy, and single-crystal X-ray diffraction were used to analyze the chemical composition of different sage extracts obtained from the areal parts. A substantial difference was found between the composition of sage flower CO₂ extract and that of sage flower resin extract [76]. In addition to the known compounds, novel compounds were identified in sage flowers. Among these, some were preidentified in our research, namely danshensu, caffeic acid, rosmarinic acid, luteolin, and apigenin.

The basic active substances of chamomile (*M. chamomilla* L.) include essential oils, flavonoids, coumarins, sesquiterpenes, polyacetylenes, spiroether, choline, mucus compounds, vitamin C, and mineral salts. Chamomile oil contains compounds that have

a specific healing effect, such as antiallergic chamazulene and bisabolol and its oxides. Many bioactive phenolic compounds, including herniarin and umbelliferone (coumarin), chlorogenic acid and caffeic acid (phenylpropanoids), apigenin, apigenin-7-O-glucoside, luteolin and luteolin-7-O-glucoside (flavones), quercetin and rutin (flavonols), and naringenin (flavanone), have been found in chamomile extract [77–79]. Among flavonoids, apigenin is the most promising due to its multiple therapeutic functions. It exists in the form of various glycosides or in very small quantities as free apigenin. The method of extraction not only determines the chemical composition of chamomile extract but also its antioxidant activity [72]. Flavonoids represent the major fraction of water-soluble components in chamomile [71]. Their values in chamomile aqueous extract were established as follows: quercetin equivalent per gram of extract— $27.65 \pm 0.007 \mu\text{g}$; GAE per gram of extract— $146.97 \pm 0.046 \mu\text{g}$, and tannic acid equivalent per gram of extract— $132.22 \pm 0.023 \mu\text{g}$. In contrast, analysis of chamomile extract containing many flavonoids and numerous organic acids and their derivatives in this study revealed the low TPC compared to other tested plant extracts tested, as well as one of the lowest antioxidant capacities.

Viapiana et al. [9] determined the content of phenolic acids and flavonoids in *Cistus* (*Cistus* L.) extracts. Their results revealed that aqueous *cistus* extracts were richer in phenolic compounds and showed strong antioxidant activities. The total amount of polyphenols in the leaf, stalk, and bud extracts of *Cistus incanus* determined by Dimcheva and Karshева [73] varied between 36.26 and 115.32 mg GAE/g dry weight (dw), depending on the time of extraction. After 180 min of *C. incanus* extraction, the phenolic content was slightly higher than that observed in our study. Such result has also been shown by other studies. Researchers showed that the place of origin is the main factor differentiating the antibacterial activities of *cistus* samples. Dimcheva et al. [10] found catechins, flavonoids, and gallic and vanillic acids in *Cistus* extracts. The compounds identified by Gori et al. [80] in crude ethanolic leaf extract of *C. incanus* included gallic acid derivatives, condensed tannins, and flavonol glycosides. In total, 19 compounds were identified based on the fragmentation of individual polyphenols and by comparing their retention times and UV/Vis spectra with authentic standards. As in our research, the presence of epicatechin, myricetin hexoside, and quercetin pentoside was found in the *C. incanus* extract by Gori et al. [80]. In our study, the *Cistus* extract had the highest TPC among the tested plant extracts, and thus showed the highest antioxidant capacities. Its composition included many organic acids and their derivatives, as well as flavonoids such as catechin, quercetin, kaempferol, and myricetin.

Several studies have been performed on linden blossom (*Tilia* L.) extracts. The results revealed the presence of terpenoids, quercetin, and kaempferol derivatives (such as tiliroside), phenolic compounds, esters, aliphatic acids, hydrocarbons, condensed tannins, and a coumarin scopoletin in the extracts [11,16,81,82]. Wissam et al. [83] stated that TPC and the antioxidant activity were determined in the ethanolic extracts of dried linden blossom leaves ($0.3303 \pm 0.0896 \text{ mg/mL}$ calculated as DPPH scavenging activity). In our study, the DPPH value of linden blossom extract was determined as 0.161 ± 0.007 , but expressed as mmol TE/g extract. This value was the second highest recorded in our experiments. TPC of the linden blossom extract was also one of the largest and resulted from the presence of quercetin, kaempferol, other flavonoids, and their derivatives, as well as many organic acids. In addition, 3,4-dihydroxybenzoic acid, myricetin, rutin, ferulic acid, and 3,4-dihydroxybenzaldehyde were found in abundance in the *Tilia tomentosa* flower [84]. In turn, the major phenolic compound observed in acetone and methanolic extracts of *Tilia argentea* was protocatechuic acid. The leaf samples of *Tilia* species were found to contain many compounds similar to those in flower samples, but each of these samples possessed a unique chemical profile including the percentage and type of flavonoid constituents [85].

Some scientists showed that phenolic compounds, mainly flavonoids and hydroxycinnamic acids, were the main components of hydrophilic ribwort plantain (*P. lanceolata* L.) extracts [19]. Galvez et al. [20] found that luteolin was biologically important among the flavonoids. According to [17], phenylethanoids, especially Aukubin, are responsible for

the antimicrobial effects of ribwort plantain extracts. It was reported that these extracts exhibited a strong antioxidant activity [19]. Lukova et al. [68] studied the antioxidant activity of the ethanol extracts of *P. lanceolata* leaves by DPPH scavenging test, CUPRAC (cupric reducing antioxidant capacity) assay, and FRAP assay and established the following values: DPPH— $59.04 \pm 0.09\%$; CUPRAC— $21.9 \pm 0.58 \mu\text{M TE/g dw}$; and FRAP— $51.85 \pm 1.54 \mu\text{M TE/g dw}$. The *Plantago* plantain leaf extract tested in our study was characterized by an average content of TPC expressed as mg GAE/g of extract, as well as average antioxidant capacities expressed by DPPH, ABTS, or FRAP scavenging ability.

In general, marshmallow (*Althaea* L.) ethanol extracts show high antioxidant activity, which is due to the presence of active compounds such as flavonoids and mucus polysaccharides [22]. However, this was not confirmed by the results of our research. The leaves of marshmallow contain the coumarin scopoletin, as well as many flavonoids (hypolaetin-8-glucoside, isoquercitrin, kaempferol, caffeic acid, p-coumaric acid, ferulic acid, p-hydroxybenzoic acid, salicylic acid, p-hydroxyphenylacetic acid, vanillic acid) [86,87]. Elmastas et al. [22] indicated the strong total antioxidant activity of ethanolic marshmallow extract. They reported that the marshmallow extract showed effective reducing power, free radical scavenging activity, superoxide anion radical scavenging activity, and metal chelating ability at the same concentration (50, 100, and 250 mg/mL, respectively). In comparison, the marshmallow extract tested in our study contained only a few antioxidant substances (some flavonoids and organic acids), which resulted in its lower antioxidant capacities.

5. Conclusions

This study showed that the addition of herbal extracts from valerian (*V. officinalis* L.), sage (*S. officinalis* L.), chamomile (*M. chamomilla* L.), cistus (*Cistus* L.), linden blossom (*Tilia* L.), ribwort plantain (*P. lanceolata* L.), and marshmallow (*Althaea* L.) did not inhibit the growth of LAB in fermented milk drinks such as yogurts. In light of the presented results, yogurts enriched with the plant extracts tested in this study can be of interest to customers. However, these herbal extracts should be added in limited amounts because they gradually inhibit the fermentation activity. Now, knowing in what dose range dairy products fermented with the addition of selected herbal extracts can be tested in the future, including storage research. When added at concentrations above 2% by weight, which probably can be used to prevent overacidification of fermented milk after the fermentation process is complete, herbal extracts from valerian, sage, chamomile, cistus, linden blossom, ribwort plantain, or marshmallow should be tested for the storage stability of fermented milk beverages such as yoghurts containing live lactic acid bacteria.

Nevertheless, due to the antioxidant properties, a slight addition of the herbal extracts containing polyphenols to different kinds of food products can increase the nutritional quality, thus making them functional foods. The microflora of yogurts containing the tested herbal extracts did not die during digestion in model digestive juices, and this amount of bacteria surviving digestion can benefit the health of consumers. Thus, the tested plant extracts had neither an inhibitory nor a stimulating effect on bacteria in the fermented milk samples.

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