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Antimicrobial Potential of Beverages Preparation Based on Fermented Milk Permeate and Berries/Vegetables

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Abstract: Nowadays, taking into consideration the current dynamics of drug resistance development, many researchers are working to develop new antimicrobial compound combinations for the food and beverage industry, which can overcome this problem. The aim of this study was to evaluate the antimicrobial properties of milk permeate fermented with *Lactobacillus plantarum* LUHS135, *Lactobacillus plantarum* LUHS122, and *Lactobacillus faraginis* LUHS206 strains in combination with berry/vegetable (B/V) pomace (gooseberries, chokeberries, cranberries, sea buckthorn, rhubarb) against a variety of pathogenic strains (methicillin-resistant *Staphylococcus aureus*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Acinetobacter baumanni*, *Proteus mirabilis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus mutans*, *Streptococcus epidermis*, *Staphylococcus haemolyticus*, *Pasteurella multocida*, and *Enterobacter cloacae*) as a potential antimicrobial combination for beverage preparation. The highest number of the tested pathogenic strains was inhibited by gooseberries, sea buckthorn, and rhubarb combinations with strain LUHS122 fermented beverages (13 pathogens out of 15 tested). Twelve out of 15 tested pathogens were inhibited by gooseberry combinations with LUHS135 and LUHS206 fermented milk permeate. Selected B/V in combination with fermented milk permeate are promising antimicrobial ingredients for beverage preparation, possessing antimicrobial activity almost against all the tested pathogenic strains.

Keywords: antimicrobial properties; fermentation; berries; vegetables

1. Introduction

Nowadays, taking into consideration the current dynamics of development of drug resistance many researchers are working to create new antimicrobial compound combinations for food and beverage industry, which can overcome the problem.

Lactic acid bacteria (LAB) strains are used as technological starters for food preparation because of their important characteristics, especially their antimicrobial properties. Also, LAB can improve sensory properties, as well as nutritional value of food products [1]. Most of the LAB strains' antimicrobial properties can be explained by their excretion of different compounds, such as organic acids, bacteriocin-like inhibitory substance (BLIS), and enzymes [2]. The incorporation of fermented foods and beverages to the main diet is a very important part of balanced nutrition, because fermented foods and beverages have many benefits for consumer health [3]. Functional beverages could be a healthy alternative in human nutrition. Also, for their preparation, food industry byproducts could be used. Our previous studies showed that fermented milk permeate beverage prototypes with selected LAB strains contained galactooligosaccharides (GOS) (from 8.7 to 26.8 $mg_{GOS}/100\ mL_{sample}$) [4]. The above-mentioned study proved a real possibility for milk permeate sustainable valorization to the fermented beverages of higher functional value.

Also, the nutritional value of fermented milk permeate beverages can be further improved by adding natural functional additives. Our previous studies revealed that berries and fruits possess a wide spectrum of antimicrobial properties against pathogenic and opportunistic bacterial strains [1].

In this study, gooseberries, chokeberries, cranberries, sea buckthorn, and rhubarb were selected as popular berries/vegetables (B/V) in the Nordic European diet.

Gooseberry, widely cultivated in Europe, belongs to the *Ribes* L. genus and the *Saxifrgaaceae* family [5]. The composition of this berry is very attractive, as it contains many nutrients, various sugars, organic acids, anthocyanins, inorganic micro- and macro-elements, and vitamins, as well as various amino acids [6]. For this reason, gooseberry is an important stock for the food industry. Gooseberry is rich in vitamin C, containing 200 mg/100 g [7]. Furthermore, these berries are rich in flavonoids, which have many desirable properties (antioxidant, diseases prevention, etc.) [7]. In addition, gooseberry is rich in iron and iodine, which are associated with a lowered risk of atherosclerosis. Gooseberry is used for the prevention of dysentery, foot pain, arthritis, bone dysplasia, and kidney diseases [7]. For above-mentioned reasons, gooseberry is a considered a medicinal plant [8].

Black chokeberry (*Aronia melanocarpa*) is a fruit with specific taste and dark color, known as a very good source of phenolics, which are associated with many health benefits [9]. The chemical composition of black chokeberries, as well as their desirable effects on human health, have been very popular subjects of investigation [9–12]. The main biological active compounds in black chokeberry are anthocyanins, proanthocyanidins, and hydroxycinnamic acids, whereas quercetin, quercetin glycosides, and epicatechin are minor components [9,10]. A previous study found that black chokeberry and its extracts possess cardioprotective, hepatoprotective, anticarcinogenic, antidiabetic, antimutagenic, and many other effects [9].

Cranberries have a unique flavor and very intense reddish-purple color [13]. Cranberries sensory properties are related to their composition, which is rich in anthocyanin pigments [14,15]. The procyanidins of cranberries possess protective effects against urinary tract infections and cardiovascular diseases [14,16].

Sea buckthorn plant (*Hippophae rhamnoides*) belongs to the Hippophae genus and to the Elaeagnaceae family, and extracts of the different botanical parts of sea buckthorn are very popular in pharmaceutical preparations. Sea buckthorn is a good source of lipids, vitamins, phenolics, carotenoids, phytosterols, and tocopherols [17]. Sea buckthorn seeds and oils are used for the prevention of gastric ulcers [18], cardiovascular diseases [19], atopic dermatitis [20], dry mouth in Sjogren Syndrome patients [21], and depression [22]. Additionally, this plant and its products possess a wide range of desirable effects, including the effective regeneration of skin and mucous membranes, improved immune functions, reduced oxidation, and a lowered risk of cardiovascular diseases [23].

Rhubarb (*Rheum rhabarbarum* L.) is characterized by strong antioxidant properties. However, this plant is cultivated only for its petiole [24,25], because its leaves have a toxic oxalic acid [26]. In Europe, it is cultivated mainly in Germany, France, and England [27]. Rhubarb stalks are used for food and drink preparation, as well as in traditional medicine for the treatment of gastrointestinal hemorrhage and constipation jaundice [28]. A previous study found that this plant has anticancer properties [29]. However, it should be mentioned that the consumption of rhubarb in large quantities can lead to adverse effects, which are associated with accumulation of calcium in the body [26].

Finally, we hypothesized that the combination of the selected B/V with fermented milk permeate beverages can increase antimicrobial properties of the end-product.

The aim of this study was to evaluate antimicrobial properties of milk permeate fermented with *Lactobacillus plantarum* LUHS135, *Lactobacillus plantarum* LUHS122, and *Lactobacillus faraginis* LUHS206 strains milk permeate in combination with B/V pomace (gooseberries, chokeberries, cranberries, sea buckthorn, rhubarb) against a variety of pathogenic strains (methicillin-resistant *Staphylococcus aureus*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Acinetobacter baumanni*, *Proteus mirabilis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus mutans*, *Streptococcus epidermis*, *Staphylococcus haemolyticus*, *Pasteurella multocida*, and *Enterobacter cloacae*) as a potential antimicrobial combination for beverage preparation.

2. Materials and Methods

2.1. Berries/Vegetables and Lactic Acid Bacteria Strains Used for the Development of Antimicrobial Combinations

The B/V used for the development of antimicrobial combinations included gooseberries, chokeberries, cranberries, sea buckthorn, and rhubarb. The B/V obtained from the local supermarket in Kaunas (Lithuania). All the used B/V were grown in Lithuania by local farmers. B/V were crushed with a blender, and the prepared pomace (from each B/V) was used for the development of antimicrobial beverage combinations. The *Lactobacillus plantarum* LUHS135, *Lactobacillus plantarum* LUHS122, and *Lactobacillus faraginis* LUHS206 strains were selected according to their carbohydrate fermentation, antimicrobial, and antifungal characteristics [30]. Pure LAB strains were stored at −80 °C in a Microbank system (Pro-Lab Diagnostics, Merseyside, UK) and grown in the Man, Rogosa and Sharpe (MRS) broth (CM 0359, Oxoid, Hampshire, UK) under anaerobic conditions, at 30 °C for 48 h, prior to use. Selected LAB strains were used for beverages based on milk permeate preparation, as described by Zokaityte et al. (2020) [4]. Our previous studies showed that the fermentation with different LABs, as well as addition of apple byproducts, improved the sensory perception of the final fermented beverages and increased the acceptability [4]. The viable LAB count in fermented milk permeate beverages was higher than 6.7 $\log_{10}$ CFU mL^{-1}. The *Lactobacillus plantarum* LUHS135, *Lactobacillus plantarum* LUHS122, and *Lactobacillus faraginis* LUHS206 were incubated and multiplied in MRS broth culture medium (Biolife, Milan, Italy) at 30 °C under anaerobic conditions. A total of 3% ($v_{\text{innoculum}}/v_{\text{milk permeate}}$) of LAB with a cell concentration of 9.2 $\log_{10}$ CFU mL^{-1} was inoculated in milk permeate, followed by anaerobic fermentation for 48 h at 30 °C. After fermentation, 15% ($v_{\text{B/V}}/v_{\text{milk permeate}}$) of B/V pomace in milk permeate-based beverage was added.

The parameters of the nonfermented milk permeate are shown in Supplementary Materials Tables S1–S3.

2.2. Evaluation of the Berries/Vegetables and Fermented Milk Permeate Combination Antimicrobial Activity

All B/V and their combinations with fermented milk permeate were assessed for their antimicrobial activities against a variety of 15 pathogenic and opportunistic bacterial field isolates, recently isolated from clinical material of different domestic animal species (methicillin-resistant *Staphylococcus aureus* M87fox, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Salmonella enterica* 24SPn06, *Bacillus cereus* 18-01, *Pseudomonas aeruginosa* 17-331, *Acinetobacter baumanni* 17-380, *Proteus mirabilis*, *Enterococcus faecalis*

86, *Enterococcus faecium* 103, *Streptococcus mutans*, *Streptococcus epidermis*, *Staphylococcus haemolyticus*, *Pasteurella multocida, Enterobacter cloacae*) by the agar well diffusion method. For the agar well diffusion assay, suspensions of 0.5 McFarland standard of each pathogenic bacteria strain were inoculated onto the surface of cooled Mueller–Hinton agar (Oxoid, Basingstoke, UK) using sterile cotton swabs. Wells of 6 mm in diameter were punched in the agar and filled with 50 µL of the B/V. The antimicrobial activities against the tested bacteria were established by measuring the inhibition zone diameters (mm). The experiments were repeated three times, and the average of the inhibition zones was calculated. The antimicrobial activities against the tested bacteria were established by measuring the diameters of inhibition zone diameter of inhibition zones (DIZ) in mm [1,30]. The experiment design is shown in Figure 1.

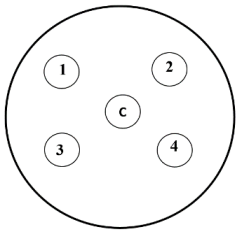


Figure 1. Scheme of the agar well diffusion method: C—control (physiological solution); 1—pure berries/vegetables (B/V) pomace; 2—with *Lactobacillus plantarum* LUHS135 fermented beverages + B/V; 3—with *Lactobacillus plantarum* LUHS122 fermented beverages + B/V; 3—with *Lactobacillus faraginis* LUHS206 fermented beverages + B/V.

The experiments were made in triplicate and the average and standard deviation of the DIZ were calculated.

2.3. Statistical Analysis

All analysis was performed at least in triplicate (n = 3) and were expressed as the average ± standard deviation. Results were analyzed via one-way analysis of variance (one-way ANOVA) using statistical package SPSS for Windows XP version 15.0 (SPSS Inc., Chicago, IL, USA, 2007). The results were recognized as statistically significant if $p \leq 0.05$.

3. Results and Discussion

The DIZ of gooseberry pomace and DIZ of gooseberry pomace with lactic acid bacteria (*Lactobacillus plantarum* LUHS135, *Lactobacillus plantarum* LUHS122, and *Lactobacillus faraginis* LUHS206) strains fermented milk permeate combinations against pathogenic opportunistic microorganisms are shown in Table 1. Comparing the DIZ of pure gooseberry pomace, it was found that gooseberries inhibited 9 out of 15 tested pathogenic and opportunistic strains, and the highest DIZ against *Pasteurella multocida* and *Streptococcus mutans* was found (25.6 mm and 23.0 mm, respectively). Comparing the DIZ of pure gooseberry pomace and DIZ of gooseberry pomace combination with LUHS135 permeate combination, it was found that addition of LUHS135 led to a broader spectrum of pathogen inhibition (inhibited 12 out of 15 tested pathogens), and the combination showed antimicrobial activity against *Enterococcus faecalis, Enterobacter cloacae*, and *Citrobacter freundii* (DIZ 11.6 mm, 12.6 mm, and 12.3 mm, respectively). However, compared to DIZ against pathogens which were inhibited by both samples (pure pomace and the gooseberry pomace combination with LUHS135 beverage), in most of the cases, the DIZ induced by the combination against pathogenic and opportunistic strains was similar or smaller. Comparing pure gooseberry pomace DIZ with gooseberry pomace combination with LUHS122 beverage, it was found that the addition of LUHS135 led to additional inhibition of *Enterococcus faecium, Bacillus cereus*, *Enterobacter cloacae,* and *Citrobacter freundii* (DIZ 12.6 mm, 11.3 mm, 12.4 mm, and 11.1 mm, respectively). However, in most of the cases, the gooseberry pomace combination with LUHS122 showed a lower DIZ, compared to the pure pomace (except against *Salmonella enterica* and *Streptococcus mutans*). Moreover,

the gooseberry pomace combination with LUHS206 showed a broader spectrum of pathogen inhibition (inhibited 12 out of 15 tested pathogens).

Many desirable properties of gooseberries fruit have been described. In addition, this fruit was reported to possess hypolipidemic, hypoglycemic and antimicrobial activities [31]. However, studies about the physical, chemical, and antimicrobial characteristics of different cultivars are very scarce [32]. Usually, this fruit is associated with the high concentration of vitamin C [33]. Gooseberries are also a good source of phytochemicals (polyphenols, tannins, emblicol, linoleic acid, corilagin, phyllemblin, and rutin) [34], which are related to antimicrobial activity of the fruit.

Pure chokeberries showed antimicrobial activity against 3 out of 15 tested pathogenic and opportunistic strains (*Bacillus cereus*, *Streptococcus mutans*, and *Pasteurella multocida*; Table 2). The combination of chokeberries with LUHS135, as well as with LUHS206 permeate, additionally showed antimicrobial properties against *Staphylococcus epidermis* and *Staphylococcus haemolyticus*. However, the combination of chokeberries with LUHS122, compared with the pure berry pomace, additionally inhibited only *Staphylococcus haemolyticus*. The highest DIZ of the chokeberry combination with LUHS206 against *Streptococcus mutans* was found to be 20.9 mm. For the pure berry pomace combination with LUHS135 and LUHS206 beverages against *Pasteurella multocida*, a DIZ higher than 20 mm was determined.

A previous study showed that chokeberry polyphenols differ in their biological activity, and only epicatechin and quercetin show antimicrobial activities against *Candida albicans*, but they do not inhibit *Staphylococcus aureus* and *Proteus vulgaris*. However, whole berries have many health benefits [35]. Tannin antimicrobial activity can be explained by the inhibition of extracellular microbial enzymes, direct action on microbial metabolism through the inhibition of oxidative phosphorylation, or the deprivation of the substrates required for microbial growth [36]. Taguri et al. [37] reported that different types of phenolics, as well as their oxidation products—proanthocyanidins and hydrolyzable tannins—possess antibacterial activities against food pathogens, and pathogen sensitivity to phenolics depends on bacteria species and bioactive compound structure.

Pure cranberry pomace and its combination with fermented milk permeate beverages inhibited the same number of the tested pathogenic and opportunistic strains (10 out of 15 tested pathogens) (Table 3). Also, in most of the cases, combinations with fermented milk permeate showed lower DIZ, compared to pure cranberry pomace, against *Pseudomonas aeruginosa* (except the combination with LUHS135), against *Bacillus cereus* (except the combination with LUHS135), against *Streptococcus mutans* (except the combination with LUHS122), and against *Streptococcus epidermis*.

Table 1. Inhibition zones of gooseberries and the ones with *Lactobacillus plantarum LUHS135*, *Lactobacillus plantarum LUHS122*, and *Lactobacillus faraginis LUHS206* fermented beverage combinations against pathogenic opportunistic microorganisms.

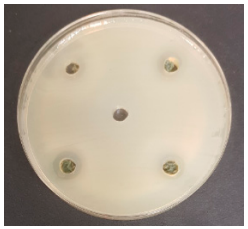
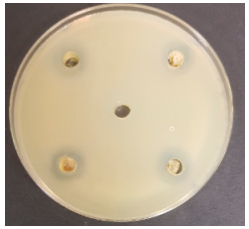
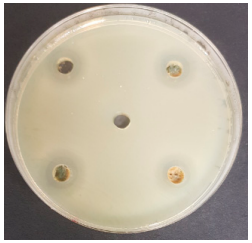
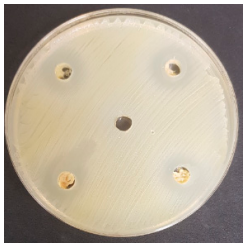
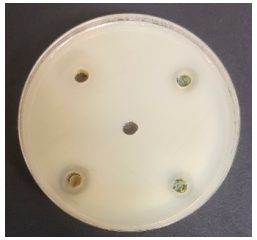
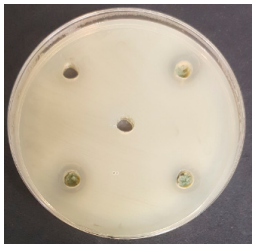
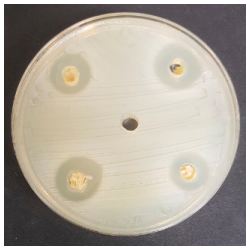
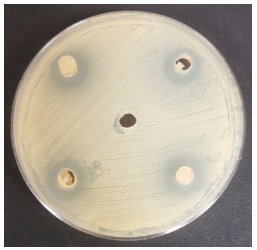
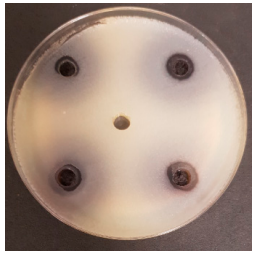
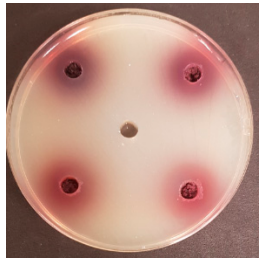
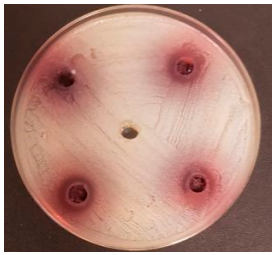
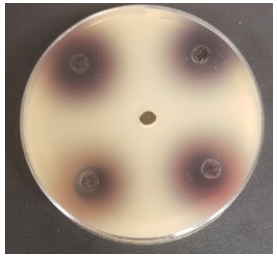
Inhibition Zones, mm															
Pathogenic and Opportunistic Bacteria Strains															
B/V and LAB	***Klebsiella pneumoniae***	***Salmonella enterica* 24 SPn06**	***Pseudomonas aeruginosa* 17-331**	***Acinetobacter baumannii* 17-380**	***Proteus mirabilis***	***MRSA* M87fox**	***Enterococcus faecalis* 86**	***Enterococcus faecium* 103**	***Bacillus cereus* 18 01**	***Streptococcus mutans***	***Enterobacter cloacae***	***Citrobacter freundii***	***Streptococcus epidermis***	***Staphylococcus haemolyticus***	***Pasteurella multocida***
Goo	nd	12.3 ± 0.2 ^{b}	14.2 ± 0.6 ^{a}	12.1 ± 0.3 ^{c}	13.3 ± 0.7 ^{a}	13.9 ± 0.2 ^{b}	nd	nd	nd	23.0 ± 0.4 ^{a}	nd	nd	19.3 ± 0.2 ^{b}	15.2 ± 0.3 ^{b}	25.6 ± 0.4 ^{c}
Goo 135	nd	11.1 ± 0.3 ^{a}	14.2 ± 0.4 ^{a}	11.3 ± 0.4 ^{b}	13.4 ± 0.1 ^{a}	14.2 ± 0.4 ^{b}	11.6 ± 0.6 ^{a}	nd	nd	23.2 ± 0.6 ^{a}	12.6 ± 0.6 ^{b}	12.3 ± 0.4 ^{a}	14.4 ± 0.3 ^{a}	15.3 ± 0.6 ^{b}	23.4 ± 0.5 ^{b}
Goo 122	nd	13.2 ± 0.1 ^{c}	14.4 ± 0.4 ^{a}	9.1 ± 0.2 ^{a}	12.3 ± 0.4 ^{a}	12.3 ± 0.8 ^{a}	nd	12.6 ± 0.3	11.3 ± 0.6	24.0 ± 0.3 ^{a}	12.4 ± 0.4 ^{b}	11.1 ± 0.3 ^{a}	17.5 ± 0.2 ^{b}	15.0 ± 0.4 ^{b}	24.6 ± 0.3 ^{b}
Goo 206	nd	11.1 ± 0.6 ^{a}	14.9 ± 0.7 ^{a}	9.2 ± 0.3 ^{a}	11.6 ± 0.3 ^{a}	14.3 ± 0.6 ^{b}	15.6 ± 0.4 ^{b}	nd	nd	24.3 ± 0.7 ^{a}	10.0 ± 0.6 ^{a}	11.6 ± 0.4 ^{a}	18.0 ± 0.6 ^{b}	13.3 ± 0.6 ^{a}	21.2 ± 0.3 ^{a}
Images of the Inhibition Zones															
															
Salmonella enterica 24 SPn06					·*Pseudomonas aeruginosa* 17-331							*Acinetobacter baumanni* 17-380			

Table 1. *Cont.*

Inhibition Zones, mm														
Pathogenic and Opportunistic Bacteria Strains														
B/V and LAB	***Klebsiella pneumoniae***	***Salmonella enterica* 24 SPn06**	***Pseudomonas aeruginosa* 17-331**	***Acinetobacter baumannii* 17-380**	***Proteus mirabilis***	***MRSA* M87fox**	***Enterococcus faecalis* 86**	***Enterococcus faecium* 103**	***Bacillus cereus* 18 01**	***Streptococcus mutans***	***Enterobacter cloacae***	***Citrobacter freundii***	***Streptococcus epidermis***	***Staphylococcus haemolyticus***	***Pasteurella multocida***
															
	Proteus mirabilis					*MRSA* M87fox						*Enterobacter cloacae*			
															
	Citrobacter freundii					*Staphylococcus epidermidis*						*Staphylococcus haemolyticus*			

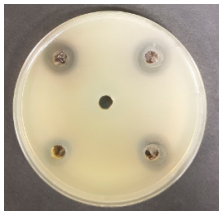
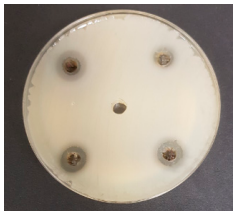
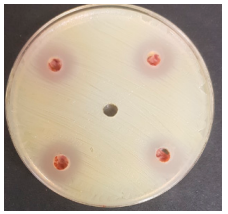
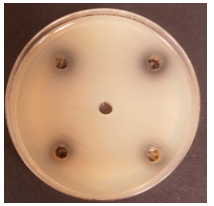
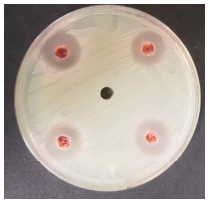
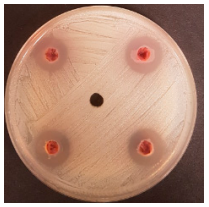
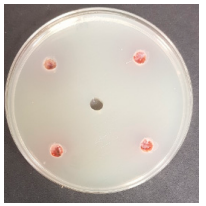
[a–c] Mean values with different letters are significantly different ($p \leq 0.05$); MRSA—Methicillin-resistant *Staphylococcus aureus*; 135—*Lactobacillu plantarum* LUHS135, 122—*Lactobacillu plantarum* LUHS122, 206—*Lactobacillu faraginis* LUHS206; Goo—gooseberries; nd—not determined.

Table 2. Inhibition zones (mm) of pure chokeberry pomace and beverages against pathogenic opportunistic microorganisms.

B/V and LAB	Inhibition Zones, mm																
	Pathogenic and Opportunistic Bacteria Strains																
	Enterococcus faecium 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecium* 103	*Enterococcus faecalis* 86	*Enterococcus faecium* 103	*Bacillus cereus* 18 01
Cho	nd	nd	nd	nd	nd	nd	nd	nd	10.6 ± 0.4 [a]	11.4 ± 0.4 [a]	nd	nd	nd	nd		20.3 ± 0.6 [b]	
Cho 135	nd	nd	nd	nd	nd	nd	nd	nd	11.3 ± 0.9 [a]	14.6 ± 0.9 [b]	nd	nd	11.3 ± 0.3 [a]	10.2 ± 0.5 [a]		20.4 ± 0.5 [b]	
Cho 122	nd	nd	nd	nd	nd	nd	nd	nd	10.4 ± 0.2 [a]	16.3 ± 0.4 [c]	nd	nd	nd	12.3 ± 0.4 [b]		18.8 ± 0.4 [a]	
Cho 206	nd	nd	nd	nd	nd	nd	nd	nd	11.3 ± 0.3 [a]	20.9 ± 0.7 [d]	nd	nd	14.2 ± 0.2 [b]	12.4 ± 0.7 [b]		20.0 ± 0.6 [b]	
Images of the Inhibition Zones																	
																	
	Bacillus cereus												*Streptococcus mutans*				
																	
	Staphylococcus haemolyticus												*Pasteurella multocida*				

[a–d] Mean values with different letters are significantly different ($p \leq 0.05$); MRSA—Methicillin-resistant *Staphylococcus aureus;* 135—*Lactobacillu plantarum* LUHS135, 122—*Lactobacillu plantarum* LUHS122, 206—*Lactobacillu faraginis* LUHS206; Cho—chokeberries; nd—not determined.

Table 3. Inhibition zones (mm) of pure cranberry pomace and beverages against pathogenic opportunistic microorganisms.

B/V and LAB	Inhibition Zones, mm														
	Pathogenic and Opportunistic Bacteria Strains														
	Klebsiella pneumoniae	*Salmonella enterica* 24 SPn06	*Pseudomonas aeruginosa* 17-331	*Acinetobacter baumannii* 17-380	*Proteus mirabilis*	*MRSA* M87fox	*Enterococcus faecalis* 86	*Enterococcus faecium* 103	*Bacillus cereus* 18 01	*Streptococcus mutans*	*Enterobacter cloacae*	*Citrobacter freundii*	*Streptococcus epidermis*	*Staphylococcus haemolyticus*	*Pasteurella multocida*
Cra	nd	nd	17.8 ± 0.7 ^{c}	10.6 ± 0.3 ^{a}	nd	15.0 ± 0.6 ^{c}	nd	nd	17.2 ± 0.4 ^{b}	25.6 ± 0.7 ^{b}	11.7 ± 0.7 ^{a}	11.2 ± 0.3 ^{a}	19.9 ± 0.8 ^{b}	16.6 ± 0.9 ^{a}	26.3 ± 0.5 ^{a}
Cra 135	nd	nd	17.9 ± 0.8 ^{c}	9.2 ± 0.2 ^{a}	nd	14.6 ± 0.5 ^{b}	nd	nd	16.6 ± 0.9 ^{b}	21.3 ± 0.3 ^{a}	12.0 ± 0.4 ^{a}	11.9 ± 0.7 ^{a}	16.8 ± 0.4 ^{a}	19.3 ± 0.4 ^{b}	27.4 ± 0.6 ^{a}
Cra 122	nd	nd	12.2 ± 0.4 ^{a}	11.3 ± 0.1 ^{b}	nd	15.3 ± 0.3 ^{c}	nd	nd	14.4 ± 0.8 ^{a}	25.5 ± 0.4 ^{b}	12.3 ± 0.3 ^{a}	11.0 ± 0.9 ^{a}	15.0 ± 0.9 ^{a}	17.1 ± 0.5 ^{a}	26.6 ± 0.4 ^{a}
Cho 206	nd	nd	15.1 ± 0.3 ^{b}	12.0 ± 0.4 ^{b}	nd	12.2 ± 0.4 ^{a}	nd	nd	14.5 ± 0.4 ^{a}	20.6 ± 0.7 ^{a}	11.1 ± 0.9 ^{a}	11.4 ± 0.4 ^{a}	15.3 ± 0.4 ^{a}	16.2 ± 0.6 ^{a}	26.9 ± 0.3 ^{a}
Images of the Inhibition Zones															
															
Pseudomonas aeruginosa 17-331			*Acinetobacter baumanni* 17-380			*MRSA* M87fox									
															
Bacillus cereus 18 01			*Enterobacter cloacae*			*Citrobacter freundii*									
															
Staphylococcus epidermidis			*Staphylococcus haemolyticus*			*Pasteurella multocida*									

[a–c] Mean values with different letters are significantly different ($p \leq 0.05$); MRSA—Methicillin-resistant *Staphylococcus aureus;* 135—*Lactobacillus plantarum* LUHS135, 122—*Lactobacillus plantarum* LUHS122, 206—*Lactobacillus faraginis* LUHS206; Cra—cranberries; nd—not determined.

Berries belonging to the Vaccinium species provide a very good source of compounds, which are associated with antimicrobial properties [38,39], e.g., the antimicrobial properties of cranberry concentrates against Staphylococcus aureus and E. coli O157:H7 are well known [40–42]. Česonienė et al. [43] showd that cranberry extracts inhibit a wide range of Gram-negative (*Escherichia coli* and *Salmonella typhimurium*) and Gram-positive (*Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus subtilis*) pathogens. A previous study found that *V. oxycoccus* juice showed binding activity with *Streptococcus agalactiae* and *Streptococcus pneumoniae* due to *S. pneumoniae* binding activity to low molecular size fractions of cranberry juices [38].

Sea buckthorn pomace and its combination with LUHS135 and LUHS206 beverages inhibited 12 out of 15 tested pathogenic and opportunistic strains, and the sea buckthorn combination with LUHS122 additionally showed antimicrobial activity against *Salmonella enterica* (Table 4). Also, the sea buckthorn combination with LUHS206 increased its antimicrobial activity against *Acinetobacter baumanni*. The sea buckthorn combination with LUHS135 and LUHS122 beverages showed increased antimicrobial activity against *Proteus mirabilis*. The sea buckthorn combinations with all the tested LAB strains showed increased antimicrobial activity against *Enterococcus faecalis*. The sea buckthorn combinations with LUHS135 and LUHS122 beverages showed increased antimicrobial activity against *Pasteurella multocida*. Opposite tendencies (lower DIZ, compared with pure pomace) of the sea buckthorn combinations with LUHS135 against *Bacillus cereus*, *Enterobacter cloacae, Citrobacter freundii, Streptococcus epidermis*; combinations with LUHS122 against *Enterobacter cloacae* and *Streptococcus epidermis*; and combinations with LUHS206 against *Bacillus cereus, Enterobacter cloacae,* and *Streptococcus epidermis* were established.

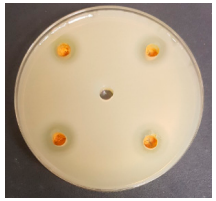
The antimicrobial properties of sea buckthorn have the capacity to inhibit *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus pneumoniae* [44]. The antibacterial properties of sea buckthorn are associated with lipophilic bioactive compounds such as fatty acids (FA) [23]. FAs have the capacity to kill bacteria or inhibit their growth, and many organisms defend themselves against parasitic or pathogenic bacteria using this mechanism [17]. A previous study found that long-chain FAs have stronger antimicrobial activities against Gram-positive than Gram-negative bacteria [45]. Traditionally, buckthorn is used in medicine because of its wide spectrum of biologically active FAs and other compounds, e.g., antibacterial and antioxidant [46].

The diameters of inhibition zones of rhubarb and fermented milk permeate beverages against pathogenic opportunistic microorganisms are shown in Table 5. Pure rhubarb pomace, as well as its combination with LUHS135 and LUHS206 beverages, inhibited 12 out of 15 tested pathogenic and opportunistic strains. The rhubarb pomace combination with LUHS122 additionally showed antimicrobial activity against *Salmonella enterica* (DIZ 13.1 mm). Moreover, the rhubarb combination with LUHS135 and LUHS122 increased its DIZ against *Proteus mirabilis*, *Enterococcus faecalis*, and *Pasteurella multocida*. The rhubarb combination with LUHS206 showed higher DIZ against *Acinetobacter baumanni*, *Enterococcus faecalis*, and *Pasteurella multocida*. Opposite tendencies of all LAB and rhubarb tested combinations against *Enterobacter cloacae*, *Streptococcus epidermis*, and *Bacillus cereus* (except combination with LUHS122) were also found. The above-mentioned combinations decreased DIZ against the mentioned pathogenic strains.

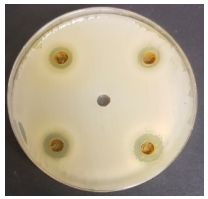
Table 4. Inhibition zones (mm) of pure sea buckthorn pomace and beverages against pathogenic opportunistic microorganisms.

B/V and LAB	Inhibition Zones, mm														
	Pathogenic and Opportunistic Bacteria Strains														
	Klebsiella pneumoniae	*Salmonella enterica* 24 SPn06	*Pseudomonas aeruginosa* 17-331	*Acinetobacter baumannii* 17-380	*Proteus mirabilis*	*MRSA* M87fox	*Enterococcus faecalis* 86	*Enterococcus faecium* 103	*Bacillus cereus* 18 01	*Streptococcus mutans*	*Enterobacter cloacae*	*Citrobacter freundii*	*Streptococcus epidermis*	*Staphylococcus haemolyticus*	*Pasteurella multocida*
SeB	nd	nd	12.0 ± 0.6 [a]	12.0 ± 0.4 [a]	15.9 ± 0.2 [a]	15.6 ± 0.4 [a]	11.3 ± 0.9 [a]	nd	18.3 ± 0.4 [b]	22.8 ± 0.6 [a]	12.0 ± 0.6 [b]	10.4 ± 0.4 [b]	20.3 ± 0.4 [b]	17.6 ± 0.3 [a]	25.2 ± 0.3 [a]
SeB 135	nd	nd	12.3 ± 0.7 [a]	11.0 ± 0.7 [a]	17.8 ± 0.3 [b]	15.3 ± 0.7 [a]	17.2 ± 0.7 [b]	nd	15.5 ± 0.6 [a]	23.7 ± 0.4 [a]	10.2 ± 0.2 [a]	9.5 ± 0.3 [a]	16.3 ± 0.3 [a]	18.4 ± 0.5 [b]	26.6 ± 0.3 [b]
SeB 122	nd	13.1 ± 0.4	13.3 ± 0.9 [a]	12.1 ± 0.6 [a]	17.9 ± 0.4 [b]	15.4 ± 0.8 [a]	17.1 ± 0.3 [b]	nd	18.9 ± 0.3 [b]	23.5 ± 0.5 [a]	9.6 ± 0.4 [a]	11.6 ± 0.6 [c]	16.6 ± 0.4 [a]	18.8 ± 0.6 [b]	26.1 ± 0.2 [b]
SeB 206	nd	nd	12.2 ± 0.3 [a]	15.5 ± 0.3 [b]	16.2 ± 0.6 [a]	14.3 ± 0.9 [a]	16.3 ± 0.5 [b]	nd	16.4 ± 0.2 [a]	23.6 ± 0.4 [a]	10.3 ± 0.4 [a]	10.3 ± 0.4 [b]	17.7 ± 0.4 [a]	17.0 ± 0.4 [a]	26.0 ± 0.4 [b]

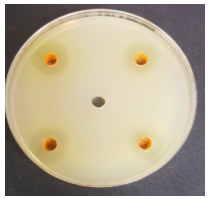
Images of the Inhibition Zones



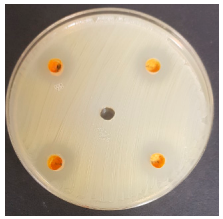
Pseudomonas aeruginosa 17-331



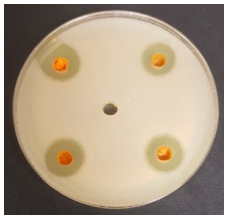
Acinetobacter baumanni 17-380



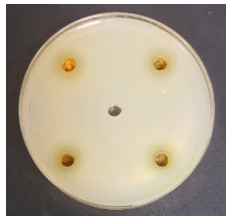
Proteus mirabilis



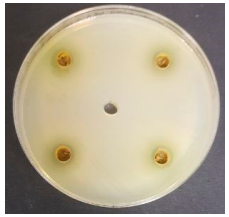
MRSA M87fox



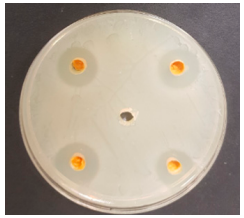
Bacillus cereus 18 01



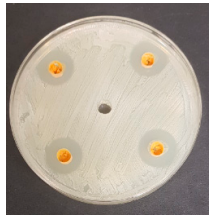
Enterobacter cloacae



Citrobacter freundii



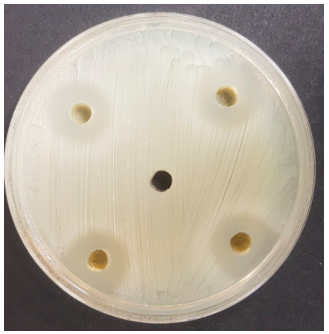
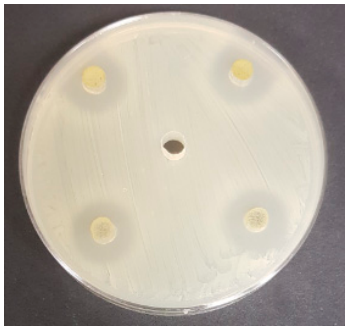
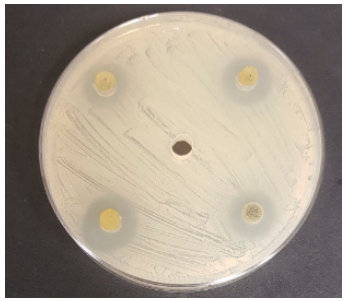
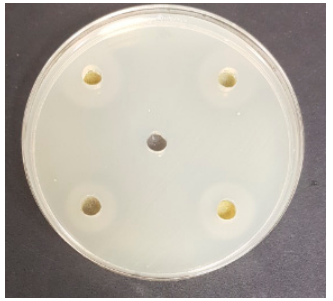
Staphylococcus epidermidis



Staphylococcus haemolyticus

[a–c] Mean values with different letters are significantly different ($p \leq 0.05$); MRSA—Methicillin-resistant *Staphylococcus aureus;* 135—*Lactobacillus plantarum* LUHS135, 122—*Lactobacillus plantarum* LUHS122, 206—*Lactobacillus faraginis* LUHS206; SeB—sea buckthorn; nd—not determined.

Table 5. Inhibition zones (mm) of pure rhubarb pomace and beverages against pathogenic opportunistic microorganisms.

B/V and LAB	Inhibition Zones, mm														
	Pathogenic and Opportunistic Bacteria Strains														
	Klebsiella pneumoniae	*Salmonella enterica* 24 SPn06	*Pseudomonas aeruginosa* 17-331	*Acinetobacter baumannii* 17-380	*Proteus mirabilis*	*MRSA* M87fox	*Enterococcus faecalis* 86	*Enterococcus faecium* 103	*Bacillus cereus* 18 01	*Streptococcus mutans*	*Enterobacter cloacae*	*Citrobacter freundii*	*Streptococcus epidermis*	*Staphylococcus haemolyticus*	*Pasteurella multocida*
Rhu	nd	nd	12.0 ± 0.6 ^{a}	12.0 ± 0.4 ^{a}	15.9 ± 0.2 ^{a}	15.6 ± 0.4 ^{a}	11.3 ± 0.9 ^{a}	nd	18.3 ± 0.4 ^{c}	22.8 ± 0.6 ^{a}	12.0 ± 0.6 ^{c}	10.4 ± 0.4 ^{a}	20.3 ± 0.4 ^{c}	17.6 ± 0.3 ^{a}	25.2 ± 0.3 ^{a}
Rhu 135	nd	nd	12.3 ± 0.7 ^{a}	11.0 ± 0.7 ^{a}	17.8 ± 0.3 ^{b}	15.3 ± 0.7 ^{a}	17.2 ± 0.7 ^{b}	nd	15.5 ± 0.6 ^{a}	23.7 ± 0.4 ^{a}	10.2 ± 0.2 ^{b}	9.5 ± 0.3 ^{a}	16.3 ± 0.3 ^{a}	18.4 ± 0.5 ^{b}	26.6 ± 0.3 ^{b}
Rhu 122	nd	13.1 ± 0.4	13.3 ± 0.9 ^{a}	12.1 ± 0.6 ^{a}	17.9 ± 0.4 ^{b}	15.4 ± 0.8 ^{a}	17.1 ± 0.3 ^{b}	nd	18.9 ± 0.3 ^{c}	23.5 ± 0.5 ^{a}	9.6 ± 0.4 ^{a}	11.6 ± 0.6 ^{b}	16.6 ± 0.4 ^{a}	18.8 ± 0.6 ^{b}	26.1 ± 0.2 ^{b}
Rhu 206	nd	nd	12.2 ± 0.3 ^{a}	15.5 ± 0.3 ^{b}	16.2 ± 0.6 ^{a}	14.3 ± 0.9 ^{a}	16.3 ± 0.5 ^{b}	nd	16.4 ± 0.2 ^{b}	23.6 ± 0.4 ^{a}	10.3 ± 0.4 ^{a}	10.3 ± 0.4 ^{a}	17.7 ± 0.4 ^{b}	17.0 ± 0.4 ^{a}	26.0 ± 0.4 ^{a}
Images of the Inhibition Zones															
															
	Streptococcus mutans									*Staphylococcus epidermidis*					
															
	Staphylococcus haemolyticus									*Pasteurella multocida*					

[a–c] Mean values with different letters are significantly different ($p \leq 0.05$); MRSA—Methicillin-resistant *Staphylococcus aureus;* 135—*Lactobacillus plantarum* LUHS135, 122—*Lactobacillus plantarum* LUHS122, 206—*Lactobacillus faraginis* LUHS206; Rhu—rhubarb; nd—not determined.

Usually, the combination of many compounds with different structures is responsible for inhibiting pathogens [47], antioxidative effects [48,49], health benefits [50,51] and plant protective properties [52] in plant materials. A previous study found that rhubarb root composition is rich in phenolic compounds [53]. Kosikowska et al. [54] and Raudsepp et al. [55] found that rhubarb roots possess very strong antimicrobial activity. Hasper et al. [56] showed that rhubarb root toxicity is very low, and they can be used for food preparation. The antimicrobial activity of the rhubarb is correlated with total polyphenolic concentration, but not with the total content of anthocyanins [57].

Finally, the highest number of the tested pathogenic and opportunistic strains was inhibited by gooseberries, sea buckthorn, and rhubarb combinations with LUHS122 (13 pathogens out of 15 tested). Twelve out of 15 tested pathogens were inhibited by gooseberries, sea buckthorn, and rhubarb combinations with LUHS135 and LUHS206, as well as with LUHS135 and LUHS206 fermented milk permeate (Figure 2). Other tested berries/vegetables and their combinations with LAB strains inhibited 10 strains and a lower number of the tested pathogenic and opportunistic strains.

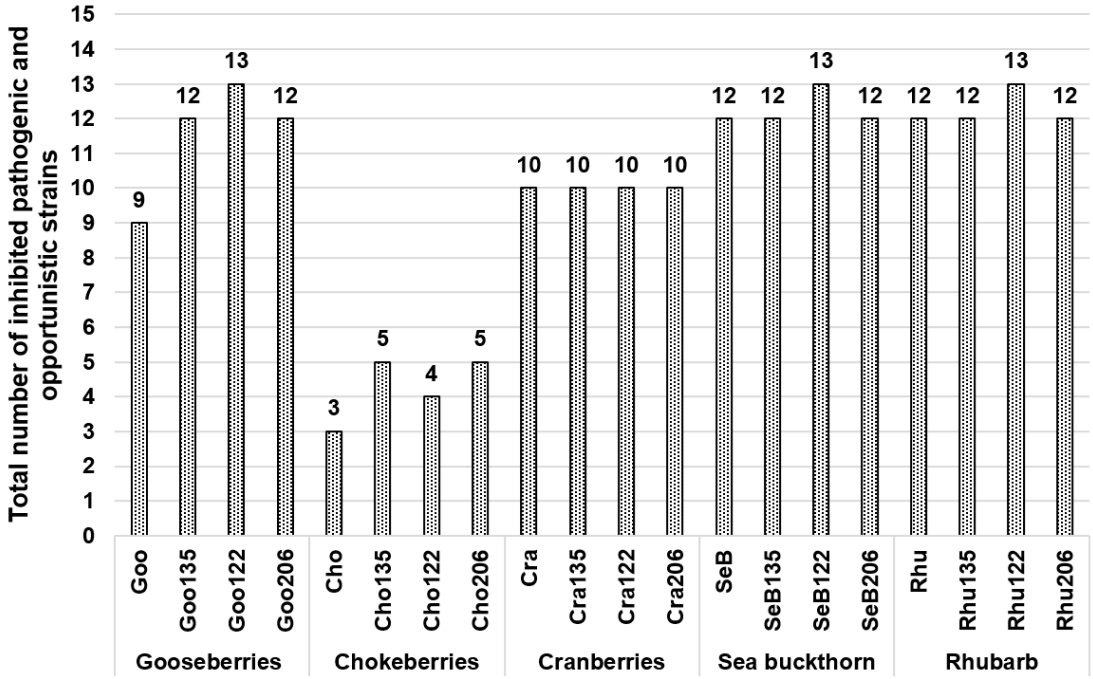


Figure 2. Total number of inhibited pathogenic and opportunistic strains, inhibited by the tested berries/vegetables (gooseberries, chokeberries, cranberries, sea buckthorn, rhubarb) and their combinations with milk permeate fermented with *Lactobacillu plantarum* LUHS135, *Lactobacillu plantarum* LUHS122, and *Lactobacillu faraginis* LUHS206. Goo—gooseberries; Cho—chokeberries; Cra—cranberries; SeB—sea buckthorn; Rhu—rhubarb; 135—*Lactobacillus plantarum* LUHS135; 122—*Lactobacillus plantarum* LUHS122; 206—*Lactobacillus faraginis* LUHS206.

4. Conclusions

The highest number of tested pathogenic and opportunistic strains was inhibited by gooseberries, sea buckthorn, and rhubarb combinations with LUHS122 (13 pathogens out of 15 tested). Twelve out of 15 tested pathogens were inhibited by gooseberry, sea buckthorn, and rhubarb combinations with LUHS135 and LUHS206, as well with LUHS135 and LUHS206 strains. Finally, selected B/V in combination with fermented milk permeate is a promising antimicrobial beverage, possessing antimicrobial activity almost against all the tested pathogenic strains. The results showed that further research should be directed toward the mechanisms of action of different origin compounds (microbial and plant-based) on their antimicrobial activity explanation.

Supplementary Materials: The following are available online at http://www.mdpi.com/2306-5710/6/4/65/s1, Table S1: Average values and standard deviations (Mean ± STDV) (n = 3) of the acidity parameters, pH and total titratable acidity (TTA), and total lactic acid bacteria (LAB) viable counts in the milk permeate (MP) samples throughout fermentation ($t_{sampling}$ = 0, 6, 12, 24, 48 h); Table S2: Average values and standard deviations (Mean ± STDV) (n = 3) of the diameter of inhibition zones (mm) of the nonfermented milk permeate (MP) against 15 pathogenic and opportunistic bacterial strains; Table S3: Average values and standard deviations (Mean ± STDV) (n = 3) of the antimicrobial activities of the nonfermented milk permeate (NFMP) against 15 pathogenic and opportunistic microbial bacterial in liquid medium (+ indicates pathogen growth; - indicates that pathogen growth was not established).

Author Contributions: Conceptualization, E.B.; methodology M.R.; software, E.M. (Erika Mozuriene); validation, R.R.; formal analysis, E.Z., V.L., V.S., P.Z., M.C., V.C., G.K., R.L., L.M., E.M. (Ema Monstaviciute), M.P., M.S., E.V., L.Z.; investigation, E.Z., V.L., V.S., P.Z., M.C., V.C., G.K., R.L., L.M., E.M. (Ema Monstaviciute), M.P., M.S., E.V., L.Z.; data curation, E.Z.; writing—original draft preparation, E.B., E.Z.; writing—review and editing, E.B.; visualization, E.M. (Erika Mozuriene); supervision, E.B. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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