The antioxidative and antimicrobial properties of the blue honeysuckle (*Lonicera caerulea* L.), Siberian rhubarb (*Rheum rhaponticum* L.) and some other plants, compared to ascorbic acid and sodium nitrite

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**A B S T R A C T**

The aim of the present study was to evaluate the antioxidative and antimicrobial effects of the ethanol and buffered water infusions of six different plants grown in Estonia, namely Siberian rhubarb (*Rheum rhaponticum* L.), blue honeysuckle (*Lonicera caerulea* L.), tomato (*Lycopersicon esculentum* Mill.), bilberry (*Vaccinium myrtillus* L.), sea-buckthorn (*Hippophae rhamnoides* L.) and black currant (*Ribes nigrum* L.), compared to the food additives ascorbic acid (E300) and sodium nitrite (NaNO2, E250). Additionally, the content of vitamin C and the content of anthocyanins, flavonols and total polyphenols in the studied samples were estimated using High performance liquid chromatography (HPLC) method.

Of the bacterial species used in present study, gram-positive bacteria were represented by *Listeria monocytogenes*, *Kocuria rhizophila* and *Bacillus subtilis*. Gram-negative foodborne pathogenic bacteria were represented by *Escherichia coli* and *Campylobacter jejuni*. Probiotic bacterial species, often used in dairy products, were represented by *Bifidobacterium bifidum* and *Lactobacillus acidophilus*.

The studied plant infusions had both antioxidative and antimicrobial properties. The highest antioxidative effect in the buffered water infusion was found with the berries of blue honeysuckle. However, in the 30% ethanol infusions the antioxidative effect was the highest with the petioles of the Siberian rhubarb, exceeded only by the ascorbic acid solution with the concentration of 10 mg/ml. Among tested plant infusions, the roots of the Siberian rhubarb exhibited the highest antibacterial effect against all bacterial species assayed.

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**1. Introduction**

Food-borne illnesses, the spread of antibiotic-resistant pathogens and concerns regarding safety of synthetic antimicrobial agents have increased consumer demand for the use of plant extracts as natural antimicrobials and antioxidants in foods (Al-Zoreky, 2009; Roasto et al., 2007; Staszewski, Pilosof, & Jagus, 2011; Tiwari et al., 2009). Nature offers many different types of antimicrobial compounds that play an important role in the natural defence of all kinds of living organisms (Rauh et al., 2000; Rodriguez Vaquero, Alberto, & Manca de Nadra, 2007). Plant extracts containing flavonols, other phenolic compounds and organic acids are potent antioxidants and some of them have shown additionally good antimicrobial activity, which makes their possible use in food systems reasonable (Chaovalanikit, Thompson, & Wrolstad, 2004; Choi et al., 2006; Kalogerosopoulos, Konteles, Troullidou, Mourtzinos, & Karathanos, 2009).

Probiotic bacteria are widely used in functional foods. Therefore, there is an interest in probiotic bacteria such as *Lactobacillus acidophilus* as well as *Bifidobacteria* together with plant derived compounds, especially in their application in food technology, for their positive impact on human health (Chaovalanikit et al., 2004; Pascal-Teresa, Moreno, & García-Viguera, 2010; Sanders, 2000). Thus, it is important to study the antimicrobial effect of plant
derived compounds against probiotic bacteria, whereas applicable plant extracts should not have strong inhibition towards beneficial probiotic bacteria in foods. The plant material used in foods has to be safe also regarding the pharmacological activity. All the plant materials in the present study are the long term consumed components of the human diet, with no concern towards safety when consumed in normal amounts, except of the root of the Rheum rhaponticum, which has not been a part of a human diet so far. According to our previous compositional studies (Püssa, Raudsepp, Kuzina, & Raal, 2009), the R. rhaponticum root could be an attractive candidate for the use as a natural antioxidant, antimicrobial or functional additive in foods due to its high content of polyphenols. A special extract of the roots of R. rhaponticum (ERr 731) has been used as a medication to treat menopausal symptoms since 1993 with the brand name Phytoestrol® since 2006 PhytoStrol® (Kaszkin-Bettag, Richardson, Rettenberger, & Heger, 2008). The safety studies of the extract of the R. rhaponticum root in the concentrations of 100, 300, and 1000 mg of ERr 731/kg body weight (bw)/day in the long term toxicity studies in the beagle dogs of the concentrations of 100, 300, and 1000 mg of ERr 731/kg body weight (bw)/day in the long term toxicity studies in the beagle dogs of the study (Rauha et al., 2000). The reservoir of the solutions of tested substances and generally the material activity of natural substances and plant extracts (Al-Zoreky, Koo, & Chun, 2011). Ascorbic acid was chosen in the current study to be a reference, because it is often used in food industries as an antioxidant in food matrices. Further treated by two different methods: A) the decoction method centrifuged once more and diluted for the measurements up to the 1:80 (w/v). The second preparation method B) dry plant material was macerated in 10 fold excess (w/v) of 30% ethanol at 45°C and freeze dried. The obtained material was used for the preparation of the plant samples was macerated in 10 fold excess (w/v) of 30% ethanol at the room temperature for 24 h with periodical shaking (18 r/min) on a rotating shaker (Multi RS-60, BIOSAN, Latvia). The obtained infusion was centrifuged at 3220 g for 10 min on the Eppendorf 510R cooling centrifuge (Hamburg, Germany). The obtained supernatant was centrifuged once more and diluted for the measurements up to the ratio 1:80 (w/v). For HPLC analysis 1:20 (w/v) dilutions, for antioxidant efficiency 1:40 (w/v) dilutions and for antimicrobial activity analyses 1:20, 1:40 and 1:80 (w/v) dilutions together with the initially obtained infusion (A) or infusion (B) were used.

2.2. Bacterial species and strains

Both gram-negative and gram-positive foodborne pathogenic bacteria together with well-known probiotics and bacteria commonly used in sensitivity testing were selected in our study. The antimicrobial activity was tested against selected bacteria such as B. subtilis, K. rhizophila (ATCC 9341), L. acidophilus (ATCC 4356), B. bifidum (Bb12), L. monocyctogenes (ATCC 19115), E. coli (NCCB 100282) and C. jejuni (ATCC 33291). Bacterial strains were obtained from the strain collections of the Estonian Veterinary and Food Laboratory and Department of Food Hygiene of Estonian University of Life Sciences.

2.3. Chemicals and standards

DPPH (2,2-diphenyl-1-pirclylhydrazyl) and ascorbic acid were purchased from Sigma, sodium nitrite from Merck, phosphate buffer with pH = 7, was made with di-sodium hydrogen phosphate dihydrate, HNa2O4P2H2O (Fluka) 98%, M = 177 g/mol and potassium dihydrogen phosphate (H2KOP) M = 136.09 g/mol, 99.5% (Merck). Cyanidin chloride, quinic acid, quercetin, quercitrin, chlorogenic acid, epigallocatechin, catechin, kaempferol, myricetin, procyanidin (B1, B2) and quercetin 3-glucoside were purchased from Sigma, Sigma–Aldrich or Fluka. Chloramphenicol was obtained from LAB M. All the used standards were of HPLC grade purity. All the used solvents were of HPLC grade and purchased from Romil (Cambridge, UK) and formic acid of MS grade was from Fluka.

The solutions were made using ultrapure water (EASypureRF 1051, Barnstead Thermolyne Co. USA).

2.4. Preparation of plant materials and infusions

Grounded and freeze dried plant material, except the petioles of the Siberian rhubarb, which were thermally dried at 45 °C, was further treated by two different methods: A) the decoction method (Bisset & Wichtl, 1994), similar to the home made tea preparation: to the dry material of 500 mg the aqueous phosphate buffer (pH = 7) was added in the ratio 1:10 (w/v) and the mixture was heated at 95 °C for 10 min. The obtained infusion was cooled down, centrifuged at 3220 g for 10 min on the Eppendorf 510R cooling centrifuge (Hamburg, Germany). The obtained supernatant was centrifuged once more and diluted for the measurements up to the ratio 1:80 (w/v). The second preparation method B) dry plant material was macerated in 10 fold excess (w/v) of 30% ethanol at the room temperature for 24 h with periodical shaking (18 r/min) on a rotating shaker (Multi RS-60, BIOSAN, Latvia). The obtained infusion was centrifuged at 3220 g on the Eppendorf 510R cooling centrifuge (Hamburg, Germany). The obtained supernatant was centrifuged once more and diluted for the measurements up to 1:80 (w/v). For HPLC analysis 1:20 (w/v) dilutions, for antioxidant efficiency 1:40 (w/v) dilutions and for antimicrobial activity analyses 1:20, 1:40 and 1:80 (w/v) dilutions together with the initially obtained infusion (A) or infusion (B) were used.

2.5. The free radical scavenging activity

The free radical scavenging activity was determined using the stable free radical DPPH decolourization assay at an absorption
maximum 515 nm using Analyticjena Spectord 200 spectrophotometer (Analyticjena AG, Germany) with WinASPECT Software package. DPPH methanol solution (6.02 × 10⁻⁵ M) was made and kept covered from light in a refrigerator.

Hundred microlitres of tested infusion (A) or infusion (B) was mixed with 3900 µl DPPH solution in a spectrophotometric cuvette and the absorbance was recorded immediately after mixing and after every 10 min during 60 min period until a steady state of the reaction was registered. The reference cuvette (blank), contained aqueous phosphate buffer or 30% ethanol, respectively. The used method was a modification of the methods previously described by Huang, Ou, and Prior (2005) and Helmja, Vaher, Püssa, Raussepp, and Kaljurand (2008). All the antioxidative efficiency assays were performed in duplicate and as a reference of an antioxidant ascorbic acid in buffered water or in 30% ethanol was used in the concentration of 1 mg/ml and 10 mg/ml on both occasions.

2.6. LC–MS/MS analysis

Samples were analysed using liquid chromatography–electrospray ionization tandem mass spectrometry (LC–DAD–ESI/MS²) in the negative ion mode on an 1100 Series LC/MSD Trap XCT (Agilent Technologies, Santa Cruz, CA, USA). The ion trap was connected to an Agilent 1100 Series HPLC instrument consisting of an autosampler, solvent membrane degasser, binary pump and column thermostat. The HPLC 2D ChemStation software with the ChemStation Spectral SW module was used both for process control and the dehydroascorbic acid ([M−H]⁻ = 175) and the ascorbic acid ([M−H]⁻ = 173) concentrations were summarized and quantified using the ascorbic acid calibration curve, obtained in the same conditions and the calibration curve of the mixture containing 10 standard substances (quinic acid, quercitin, quercitrin, chlorogenic acid, epigallocatechin, catechin, kaempferol, myricetin, procyanidin [B1, B2], quercitin-3-glucoside), all in concentration of 100, 50, 25, and 12.5 mg/ml was used as reference at the same wavelength. To quantify flavonoids in the analysed samples the UV chromatograms at 370 nm were integrated (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Test material</th>
<th>AOX (%)</th>
<th>Vitamin C (mg/ml)</th>
<th>Vitamin C (mg/ml)</th>
<th>Anthocyanins (mg/ml)</th>
<th>Anthocyanins (mg/ml)</th>
<th>Flavonols (mg/ml)</th>
<th>Flavonols (mg/ml)</th>
<th>Polyphenols (mg/ml)</th>
<th>Polyphenols (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopersicon esculentum Mill.</td>
<td>37</td>
<td>49</td>
<td>0.02</td>
<td>0.03</td>
<td>0.00</td>
<td>0.00</td>
<td>0.05</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Vaccinium myrtillus L.</td>
<td>37</td>
<td>91</td>
<td>0.07</td>
<td>0.15</td>
<td>0.20</td>
<td>0.18</td>
<td>0.24</td>
<td>0.28</td>
<td>1.3</td>
</tr>
<tr>
<td>Hippophae rhamnoides L.</td>
<td>74</td>
<td>74</td>
<td>0.13</td>
<td>0.32</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.11</td>
<td>0.2</td>
</tr>
<tr>
<td>Ribes nigrum L.</td>
<td>84</td>
<td>95</td>
<td>0.12</td>
<td>0.21</td>
<td>0.13</td>
<td>0.11</td>
<td>0.16</td>
<td>0.18</td>
<td>0.8</td>
</tr>
<tr>
<td>Rheum rhaponticum L. root</td>
<td>21</td>
<td>87</td>
<td>0.02</td>
<td>0.03</td>
<td>0.00</td>
<td>0.00</td>
<td>0.26</td>
<td>0.17</td>
<td>7.7</td>
</tr>
<tr>
<td>Rheum rhaponticum L. petiole</td>
<td>48</td>
<td>98</td>
<td>0.10</td>
<td>0.13</td>
<td>0.01</td>
<td>0.01</td>
<td>0.10</td>
<td>0.13</td>
<td>0.6</td>
</tr>
<tr>
<td>Lonicera caerulea L.</td>
<td>86</td>
<td>84</td>
<td>0.14</td>
<td>0.19</td>
<td>0.35</td>
<td>0.36</td>
<td>0.42</td>
<td>0.49</td>
<td>2.4</td>
</tr>
<tr>
<td>Ascorbic acid 1 mg/ml</td>
<td>25</td>
<td>77</td>
<td>1.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0</td>
</tr>
</tbody>
</table>

AOX – antioxidative efficiency, showing the reduced amount of DPPH.

a Infusion, made into phosphate buffer and heated at 95 °C for 10 min.

b Infusion, obtained from infusing plant material in 30% ethanol for 24 h at room temperature, with continuous shaking.

2.7. Antimicrobial activity test

Slightly modified agar well-diffusion method similar to Al-Zoreky (2009), Kalogeropoulos et al. (2009) and Rodriguez Vaquero et al. (2007) was used. To obtain freshly grown cultures each bacterial species was grown in an appropriate agar medium at the optimal growing conditions. Before experimental use, cultures from the solid medium were subcultivated in liquid media. For L. monocytogenes, E. coli, C. jejuni, B. bifidum, L. acidophilus 1 µl loopful of bacterial mass was subcultivated in 10 ml of Mueller-Hinton broth (Oxoid) for L. monocytogenes, E. coli, C. jejuni or MRS broth (Oxoid) for L. acidophilus, B. bifidum and by following incubated at 37 °C for 20 h. Certain amount of incubated bacterial suspension was mixed with 400 ml sterilized 45 °C Mueller-Hinton agar (Oxoid) to obtain final density of 10⁵ cfu/ml and then poured into Petri dishes for the solidification at the room temperature. Test-agar pH 7 (Merck) and Test-agar pH 8 (Merck) was used for testing B. subtilis and K. rhizophila, respectively. The control of the purity of the bacterial suspensions was carried out and the density of the bacterial suspensions was controlled. Wells were made into agar gel (6 mm in diameter) using sterilized stainless steel borer and finally filled with 30 µl of six plant infusions with different dilutions as 1:10, 1:20, 1:40, 1:80 (w/v). Most of the plates were incubated at 37 °C with the exception of C. jejuni, B. subtilis and Kocuria luteus. C. jejuni was incubated at 42 °C. B. subtilis and K. rhizophila at 30 °C. 1000 mg/l chloramphenicol (LAB M) was used as a positive control (Control (+), Table 2). Ethanol and phosphate buffer (pH = 7) were used as negative controls (Control (−), Table 2), respectively. After 24 h of incubation, the radius of the clear inhibition zone from the edge of the agar well was measured using a ruler to an accuracy of 0.5 mm and the antibacterial effect was calculated as a mean of duplicate tests. A total of 392 ethanol and water infusions of the six plants were prepared 1:10, 1:20, 1:40 and 1:80 (w/v), respectively and analysed for antimicrobial activity against seven different bacterial species (Table 2).

2.8. Statistical analyses

Principal component analysis (PCA), Spearman's rank correlation and t-tests were carried out using R statistical software (version 2.14.1). Calibration curves of pure standards were done using linear regression method in Microsoft Excel.

3. Results and discussion

3.1. Antioxidative effect

All the studied plant infusions, except the infusion of the root of Siberian rhubarb, showed higher antioxidative capacity than the
Table 2
Antimicrobial activity* of studied plant water (A) and ethanol (B) infusions against selected bacteria.

<table>
<thead>
<tr>
<th>Test material</th>
<th>Conc. (w/v)</th>
<th>B. subtilis</th>
<th>K. rhizophila</th>
<th>L. monocytogenes</th>
<th>E. coli</th>
<th>B. bifidum</th>
<th>L. acidophilus</th>
<th>C. jejuni</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>L. esculentum Mill.</td>
<td>1:10</td>
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<td>1:20</td>
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<td>1:40</td>
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<td>1:80</td>
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<td>–</td>
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<tr>
<td>V. myrtillus L.</td>
<td>1:10</td>
<td>–</td>
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<td>1:80</td>
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<tr>
<td>H. rhamnoides L.</td>
<td>1:10</td>
<td>1.5 ± 0.7</td>
<td>3</td>
<td>2</td>
<td>1.5 ± 0.7</td>
<td>–</td>
<td>–</td>
<td>2</td>
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<tr>
<td></td>
<td>1:20</td>
<td>0.5</td>
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<td>–</td>
<td>0.5</td>
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<td>1:40</td>
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<tr>
<td>R. nigrum L.</td>
<td>1:10</td>
<td>1.5 ± 0.7</td>
<td>3</td>
<td>1.5 ± 0.7</td>
<td>–</td>
<td>1</td>
<td>1.5 ± 0.7</td>
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<tr>
<td></td>
<td>1:20</td>
<td>0.5</td>
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<tr>
<td>R. rhaponticum L. root</td>
<td>1:10</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>1</td>
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<tr>
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<td>6</td>
<td>3</td>
<td>2</td>
<td>3</td>
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<td>–</td>
<td>0.5</td>
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<tr>
<td></td>
<td>1:40</td>
<td>1.5 ± 0.7</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>1.5 ± 0.7</td>
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<td>1:80</td>
<td>0.5</td>
<td>–</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>–</td>
<td>0.75 ± 0.35</td>
</tr>
<tr>
<td>R. rhaponticum L. petiole</td>
<td>1:10</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>3.5 ± 0.7</td>
<td>0.75 ± 0.35</td>
<td>0.75 ± 0.35</td>
<td>3.5 ± 0.7</td>
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<td>2</td>
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<td>0.5</td>
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</tr>
<tr>
<td>L. caerulea L.</td>
<td>1:10</td>
<td>2</td>
<td>2.5 ± 0.7</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
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<td>1</td>
<td>0.5</td>
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<tr>
<td></td>
<td>1:40</td>
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<td></td>
<td>1:80</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control (++)</td>
<td>15</td>
<td>14</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>8</td>
<td>5</td>
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<tr>
<td>Control (−)</td>
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<td>–</td>
<td>–</td>
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</tr>
</tbody>
</table>

– no visible growth inhibition detected.
A – infusion, made into phosphate buffer and heated at 95 °C for 10 min.
B – infusion, obtained from infusing plant material in 30% ethanol for 24 h at room temperature, with continuous shaking.
* Values are mean of the inhibition zones ± SD (standard deviation) obtained from analyses in duplicate, zero SD values are not shown.
solution of ascorbic acid with the concentration of 1 mg/ml, prepared in the buffered water (A). The content of ascorbic acid in all plant infusions (A) and 30% ethanol infusions (B) was measured. It was found that sample heating prior to analysis resulted in remarkably lower ascorbic acid content compared to the solutions, prepared in 30% ethanol at the room temperature (Table 1). This is in accordance with the results of the previous studies, indicating high susceptibility of ascorbic acid to the temperature rise (Zhang & Hamauzu, 2004). It may be one of the reasons why the antioxidative properties of the buffered water infusions were weaker than in the ethanol infusions (Table 1). The other reason could be that ethanol infusions had better antioxidant properties due to the antioxidative effect of the lipophilic compounds present in ethanol solutions but not in the water infusions. In the plant material the antioxidative properties may depend on the presence of the water soluble ascorbic acid as well as the semi polar polyphenolic compounds or unpolar compounds, such as carotenoids (Zhang & Hamauzu, 2004). Regarding antioxidative properties, the order of the studied plant infusions in buffered water (A), starting from the highest antioxidative effect, was: blue honeysuckle, black currant, ascorbic acid (10 mg/ml), sea buckthorn, the petioles of the Siberian rhubarb, bilberry/tomato, ascorbic acid (1 mg/ml) and roots of the Siberian rhubarb (Table 1). Bilberry had the highest antioxidative properties as tomato (A). Above mentioned results are applicable to the heated (buffered) water solutions, where the unpolar compounds of the studied plant matrices, which may also have antioxidative properties, have not diffused into the solution. In 30% ethanol infusion (B), however, the order of antioxidative properties of the studied plant materials was different. Starting with the highest antioxidative effect the antioxidativities decreased in the order: ascorbic acid (10 mg/ml), rhubarb petiole, black currant, bilberry, rhubarb root, blue honeysuckle, ascorbic acid (1 mg/ml), and sea buckthorn, tomato (Table 1). The antioxidative effect of the blue-coloured berries was rather difficult to estimate as the DPPH absorbs light at the same wavelength (515 nm) as the anthocyanins (Chaovanalikit et al., 2004). More accurate method to estimate the antioxidative effect of the blue-coloured berries could be the ABTS assay where the oxidation reaction is monitored at 734 nm (Floegel et al., 2011) or HPLC/MS<sup>2</sup> method, by measuring the loss of DPPH by its molecular weight. Nevertheless, at the dilution rate of 1:40 (w/v) the tested plant material did not interfere with the colour loss of DPPH during the reaction, the results of our study are presented in Table 1. Out of the studied plant parts, the berries of the blue honeysuckle displayed the highest antioxidative properties in the water infusion, whereas in ethanol infusion the best antioxidative properties were found for rhubarb petiole, followed by black currant, bilberry and the blue honeysuckle. The results showed that antioxidant properties of the plants depended on the solvent. The antioxidative activity was positively correlated with the ascorbic acid content in the water infusion ($r$ = 0.93, $p < 0.001$), but no statistically significant correlations were found between the studied parameters in the ethanol infusion. Previous studies have reported that antioxidative properties of the plants are dependent on the content of the polyphenols (Villaño, Fernández-Pachon, Moyá, Troncoso, & García-Parrilla, 2007), especially on the anthocyanins (Chaovanalikit et al., 2004; Paško et al., 2009; Viljanen, Kylli, Hubbermann, Schwarz, & Heinonen, 2005). The highest content of anthocyanins in the current study was found in the blue honeysuckle. The profile of anthocyanins in the blue honeysuckle is well characterized in the study of Chaovanalikit et al. (2004). The highest content of polyphenols in our study was found in the Siberian rhubarb root, which regardless, had the lowest antioxidative capacity in the water infusion. The result could be explained by the finding, that main constituents of the Siberian rhubarb root infusion are hydroxystilbenes (Püssa et al., 2009), mostly characterized by modest antioxidativities (Helmja et al., 2008), and also by the possible matrix—solvent interactions (Tsimgiannis & Oreopoulou, 2004). At the same time, the berries with higher anthocyanin content had also higher antioxidative properties, which are in accordance with the previous studies of Paško et al. (2009), and Viljanen et al. (2005).

### 3.2. Antimicrobial effect

Our findings confirmed the results of the study by Sebiomio, Awofodu, Awosanya, Awotona, and Ajayi (2011) that ethanol infusions have higher antibacterial effect than the water infusions. Among the seven tested microorganisms, <i>L. acidophilus</i>, <i>K. rhizophila</i>, <i>E. coli</i> and <i>B. subtilis</i> were more susceptible to the plant ethanol infusions, whereas <i>B. bifidum</i>, <i>L. monocytogenes</i> and <i>C. jejuni</i> were less susceptible. Plant water infusions had strongest growth inhibition effect against <i>K. rhizophila</i>, <i>B. subtilis</i> and <i>C. jejuni</i>, whereas <i>L. monocytogenes</i>, <i>B. bifidum</i>, <i>L. acidophilus</i> and <i>E. coli</i> were less sensitive. In our study, Siberian rhubarb root, blue honeysuckle and black currant water infusions were most effective against both gram-positive bacteria like <i>B. subtilis</i> and <i>K. rhizophila</i> as well as against gram-negative bacteria such as <i>C. jejuni</i>, and the inhibition zones ranged between 0.5 and 10 mm, being bigger at the highest concentration of the plant materials 1:10 (w/v) (Table 2).

Our study showed that some plant infusions, e.g. of blue honeysuckle and Siberian rhubarb petiole can elicit high antibacterial activity against food–borne bacteria such as <i>L. monocytogenes</i>, <i>E. coli</i> or <i>C. jejuni</i> without strong inhibition towards probiotics, especially <i>B. bifidum</i>. Therefore, selected plant infusions in combination with the tested probiotic bacteria can be used in food processing as potential antioxidants, antimicrobials, and functional ingredients (Table 2).

Kosikowska, Smolarz, and Malm (2010) have found that all of the <i>Rheum</i> spp. (rhubarb) infusions had higher antimicrobial activity against strains of gram-positive bacteria (Staphylococcus spp.) than against those of gram-negative bacteria (<i>E. coli</i>). In our study it was shown that Siberian rhubarb root water and ethanol infusions had the strongest effect towards both gram–negative (<i>C. jejuni</i>) and gram–positive bacteria (<i>B. subtilis</i>). <i>L. monocytogenes</i> is one of the most important gram–positive bacteria causing food production problems; therefore it was chosen for our susceptibility study. In a recent study by Xi, Sullivan, Jackson, Zhou, and Sebranek (2012), it was found that 3% cranberry powder in naturally-cured frankfurter sausages significantly reduced <i>L. monocytogenes</i> growth and could be used instead of sodium nitrite in natural and organic processed meats. In our study <i>L. monocytogenes</i> was found not to be susceptible to most of the tested plant infusions with the exception of the blue honeysuckle and root/petiole of Siberian rhubarb. Additionally, <i>E. coli</i> and <i>C. jejuni</i> were selected in our study. <i>E. coli</i> is a well–known component of human gut microbiota, and some enterohemorrhagic strains of <i>E. coli</i> have recently caused serious food–borne poisoning cases in the European Union (EU). Among the studied plants, the blue honeysuckle and sea buckthorn ethanol infusions had the highest antibacterial activity against <i>E. coli</i>. The strong or moderate antimicrobial activity was measured for Siberian rhubarb root, blue honeysuckle and black currant infusions. Campylobacter spp. is the most important gastroenteritis causing bacteria in EU associated mainly with the consumption of contaminated chicken meat (Meremäe et al., 2010). In our study it was found that <i>C. jejuni</i> was the most susceptible bacterium, showing the widest inhibition zones with rhubarb root, whereas the same results were reported both for water and ethanol infusions with inhibition zones from 4 to 10 mm for the dilutions of 1:20 and 1:10 (w/v) (Table 2). The strong antibacterial effect of
Siberian rhubarb root infusions should be further studied with respect to broiler chicken meat which is very often contaminated with \textit{Campylobacter} spp.

There was very limited antibacterial effect of tomato infusions in our study. Contrary, \textit{Vcake et al. (2010)} showed that tomato seeds in different extracts had effect against gram-positive gastrointestinal bacteria but all tested gram-negative bacteria were resistant to different extracts which was explained by different structure of cell wall of these bacteria. We did not study tomato seeds separately that is one of the possible explanations to the differences compared to the results of \textit{Taveira et al. (2010)}.

There were no clear differences on susceptibility patterns of gram-negative and gram-positive bacteria in our study.

Sodium nitrite is used in cured meats because of colour, flavour, antioxidant effects and as an effective antimicrobial agent to control the growth of certain food-borne pathogenic bacteria, especially \textit{Clostridium botulinum} \textit{(Shahidi & Pegg, 1992)}. Additionally, it is known that by far not all bacteria are inhibited by NaN\textsubscript{3} and for some of food-borne pathogenic bacteria; sodium nitrite only slows the bacterial growth \textit{(Xi, Sullivan, Jackson, Zhou, & Sebranek, 2011)}. In our study sodium nitrite 2.5\% and 5\% buffered water solutions did not show any antimicrobial activity against tested bacteria even at different pH ranges from 4.0 to 6.5. Our results may be explained by bacterial species selection or by the use of agar-well method instead of nutrient broth media which probably can more properly imitate the action of nitrite in acidified food products. Generally, the antibacterial effect of tested plants was dependent on the solvent (A or B), dilution rates (1:10, 1:20, 1:40 or 1:80) and tested bacterial strains. Compared to plant water infusions, the antimicrobial properties of ethanol infusions of bilberry, sea buckthorn, black currant, Siberian rhubarb and blue honeysuckle were more effective against tested bacteria. Compared to other dilutions, the strongest dilution of 1:10 both for water and ethanol infusions was found to be the most effective.

Among all the tested plant ethanol and water infusions, the root of the Siberian rhubarb showed the best antimicrobial properties against tested bacteria. The rhubarb root indicated antimicrobial activity against \textit{B. subtilis}, \textit{K. rhizophila}, \textit{L. monocytogenes}, \textit{E. coli}, \textit{L. acidophilus}, \textit{B. bifidum} and \textit{C. jejuni} with the exception of rhubarb root water infusion which had no antimicrobial activity against \textit{L. acidophilus}. The maximum inhibitory zone was determined for \textit{C. jejuni} (10 mm of inhibition zone) followed by \textit{B. subtilis} (4–9 mm) at the dilution of 1:10 (w/v). The smallest measured inhibition zones (0.5 mm) were detected against \textit{B. subtilis} and \textit{L. monocytogenes} at the dilution of 1:80 (w/v). Compared to the root of the Siberian rhubarb, the Siberian rhubarb petiole water and ethanol infusions showed antibacterial activity in lesser amount, against \textit{K. rhizophila} (inhibition zones 1–3.5 mm), \textit{E. coli} (0.5–0.75 mm) and \textit{L. acidophilus} (2–4 mm) at the dilutions 1:10 and 1:20. Fig. 1 is illustrating the influence of the rhubarb root infusions (1:10, 1:20, 1:40, 1:80) to the growth of \textit{B. subtilis}.

4. Conclusions

Among the studied plants, the blue honeysuckle had the highest content of anthocyanins and showed continuously good antioxidative effect in water and ethanol solution as well as the antibacterial activity, whereas in the water infusion the antibacterial activity against probiotic bacteria was not detected, which makes the blue honeysuckle a good functional ingredient candidate to use in probiotic foods. The results showed that antioxidant properties of the plants depended on the used solvent and on the content of vitamin C and anthocyanins.

Among all the tested plant infusions, the ethanol infusion of the root of Siberian rhubarb showed the highest antimicrobial activity against all the tested bacteria including probiotic bacteria in the ethanol infusion. The ethanol infusion of the berries of the sea buckthorn showed also high antimicrobial activity against \textit{L. acidophilus}, therefore it could be used in the probiotic functional foods in the form of water infusion. All the more, the antioxidative properties of the sea buckthorn were similar, both in the water and ethanol infusions. The infusions of tomato and sodium nitrate showed minimum or no antimicrobial activity against tested bacteria. In the light of the results of the current study it can be concluded that the roots and petioles of Siberian rhubarb and the berries of the blue honeysuckle, bilberry, black currant and sea buckthorn may have the potential use in the food industry as natural antioxidants and/or antimicrobials and functional ingredients in foods. However, studies with different food matrices which prove that chemical, physical and sensory attributes are reliable, have yet to be performed.

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References


