Antibacterial and antioxidative properties of different parts of garden rhubarb, black currant, chokeberry and blue honeysuckle.

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Abstract

BACKGROUND: It is important to find plant materials that can inhibit the growth of *Listeria monocytogenes* and other food spoiling bacteria both *in vitro* and *in situ*. The aim of the study was to compare antibacterial and antioxidative activity of selected plant-ethanol infusions: leaves and berries of black currant (*Ribes nigrum* L.), berries of chokeberry (*Aronia melanocarpa* (Michx.) Elliott) and blue honeysuckle (*Lonicera caerulea* L. *var. edulis*); petioles and dark and light roots of garden rhubarb (*Rheum rhaponticum* L.), in the perspective to use them further in food matrices as antibacterial and antioxidative additives.

RESULTS: The strongest bacterial growth inhibition was observed in 96% ethanol infusions of the dark roots of rhubarbs. In 96% ethanol, nine out of ten studied plant infusions had antibacterial effect against *L. monocytogenes*, but in 20% ethanol, only the infusions of dark rhubarb roots had similar effect. Chokeberry and other berries had the highest antioxidative activity, both in 20% and 96% ethanol infusions.

CONCLUSION: Combination of dark rhubarb roots and berries of black chokeberry or some other anthocyanin-rich berries would have good perspective as both antibacterial and antioxidative additives in food.

Keywords: antibacterial activity, antioxidative activity, *Aronia, Lonicera*, *Rheum, Ribes*

INTRODUCTION

Due to the raising customer awareness, there is an increasing trend to seek for new natural food additives that can be used as antibacterial (AB) and/or antioxidative (AO) agents in foods. It is particularly important to find plant materials that can inhibit the growth of *Listeria monocytogenes*, resistant to many environmental stress factors. In addition, *Campylobacter jejuni* is highly prevalent bacterium in broiler chicken meat of Baltic origin and the most often reported bacterial cause of human intestinal infections.[1]

Synergistic effect of different polyphenolic compounds is mainly responsible for antimicrobial,[2] antioxidative,[3, 4] health beneficial[5, 6, 7] and plant protective properties[8] of a plant material. There are studies where polyphenolic composition of rhubarb roots[9], black currant leaves and berries[10, 11], edible honeysuckle berries[12, 13] and chokeberry berries[14, 15] have been sufficiently described. Kosikowska et al.[16] and Raudsepp et al.[17] have shown very strong AB effect of garden rhubarb roots. Hasper et al.[18] have established that only minimal toxicity concerns exist regarding the use of garden rhubarb root preparations for human internal consumption.

According to Zheng et al.[19] and Vagiri et al.[11], polyphenolic composition of a plant product depends on variety, maturity and part of the plant, weather and processing technology. Raudsepp et al.[10] and Vagiri et al.[20] have ascertained that European black currant varieties may have two- to three-fold differences in the anthocyanin content, even if grown at the same conditions. Differences in total polyphenolic and total anthocyanin content may result in significantly different AB, AO and other properties of the plant products. Therefore, it is important to conduct the selection among the cultivars and plant parts to choose the ones with the highest beneficial properties[21].

The aim of this study was to gain comparable information about *in vitro* AB and radical scavenging activities of different plant species and their different parts. The more successive

aim was to select plant materials for further use as antimicrobials or AO compounds in foods. Results of the preceding studies were reviewed, the plant species and their varieties with multiple beneficial capacities and high horticultural relevance in the Northern Europe were selected. In particular, the highest anthocyanin content of the cultivated berries and high AB or AO activity of other plant parts were taken into account. The 20% and 96% ethanol concentrations in the infusions were chosen to compare the summary effects of hydrophilic and more hydrophobic polyphenol complexes.

According to our knowledge, this is the first study, where ethanol infusions of abovementioned plants and their different parts were comparatively analysed for AB and AO activities.

EXPERIMENTAL

The plant material

The planting material of rhubarb varieties was obtained from the collection of Pūre Horticultural Research Centre, Latvia. All studied plants were grown in the plantation of Polli Horticultural Research Centre, Estonia (58°06′N°25°32′E). Two dark-rooted rhubarbs (′Victoria′ and seedling 303) and one light-rooted (′Ogres′) rhubarb were selected among 16 different cultivars or seedlings, according to the content of hydroxyanthraquinones. Berries of chokeberry (selected among three seedlings), blue honeysuckle (haskap berry) ′Tomitška′ (selected among five cultivars) and black currant ′Ben Alder′ (selected among 37 cultivars); leaves of black currant ′Pamyati Vavilova′ and petioles of abovementioned garden rhubarbs were freeze-dried with VirTis AdVantage 2.0 EL freeze-dryer (SP Industries, Warminster, USA) and kept at the temperature -40°C until powdering. The roots of garden rhubarb cultivars and seedling were washed, diced and dried at 50°C in a drying oven (Binder FED101, Binder GmbH, Tuttlingen, Germany) and kept at room temperature.

87

88 **Sample preparation and chemical analysis**

89 All dried plant materials were powdered with a blender (Stollar/Kinetix® Control) to the
90 particle size diameter ≤3 mm, the necessary fraction was obtained with the analytical sieve
91 shaker AS300 control (Retsch GmbH, Germany). For the infusions, 1 g of each powder in
92 duplicate were mixed with 20 mL of 20% and 96% of aqueous ethanol. The mixtures were
93 rotated on Multi RS-60 Multirotator (Biosan, Riga, Latvia) at 40 rpm for 24 h at the room
94 temperature, followed by centrifugation at 2594g for 10 min on Sigma 4-16KS (Sigma
95 Laborzentrifugen GmbH, Germany) centrifuge. The supernatants were collected and further
96 diluted by two, four and eight times for the estimation of AB and AO properties, and for
97 quantitation of total polyphenols content (TPC) and total anthocyanins. In addition, *trans*-
98 rhapontin (Merck), rutin (Sigma), *trans*-resveratrol (Sigma) and emodin (Sigma) as single
99 phenolic compounds were included into the AB and AO studies at four concentrations: 0.125;
100 0.25; 0.5 and 1 $g \cdot L^{-1}$. TPC and anthocyanin content of plant infusions were estimated by areas
101 under HPLC-UV chromatographic curves at 280 and 520 nm, respectively[22], using UHPLC-
102 MS Shimadzu Nexera X2 system (Shimadzu Scientific Instruments, Kyoto, Japan). For the
103 estimation of TPC and anthocyanin content, chlorogenic acid (Aldrich) and cyanidin 3-*O*-
104 glucoside chloride (kuromanin chloride, Sigma) calibration curves were used, respectively. The
105 qualitative analyse of plant extracts and the content of ascorbic acid (AA) and citric acid (CA)
106 were analysed with 1100 Series LC/MSD Trap-XCT (Agilent Technologies, Santa Cruz, CA,
107 USA) using AA (Sigma) and CA (Sigma) as calibration standards[17]. Total acidity and total
108 sugar content were estimated with the FTIR spectrometer Bruker ALPHA ATR Platinum
109 system (Bruker Optics GmbH, Germany). The AO of the infusions were measured using DPPH
110 radical scavenging method[23], AO activities were expressed in rutin equivalents ($g \cdot L^{-1}$).
111 Additionally, pH values of the 20% ethanol infusions were measured.

The bacterial strains

AB effect of plant infusions was determined against Gram-negative *Campylobacter jejuni* ATCC 33291, *Salmonella* Enteritidis ATCC 13076, *Escherichia coli* NCCB 100282, *Yersinia ruckeri* NCIM 13282 and Gram-positive *Listeria monocytogenes* ATCC 13929, *Bacillus cereus* ATCC 11778, *Kocuria rhizophila* ATCC 9341, *Bacillus subtilis* BGA and *Bacillus pumilus* CV 607 bacteria, obtained from the collections of the Estonian Veterinary and Food Laboratory and the Chair of Food Hygiene and Veterinary Public Health of Estonian University of Life Sciences.

The antibacterial activity (AB) test

AB activity testing was performed by modified agar well-diffusion method as previously described by Raudsepp et al.[17]. In case of *C. jejuni*, *L. monocytogenes*, *S.* Enteritidis and *E. coli*, the suspensions with final density of 10^5–10^6 per mL were prepared and, using sterile swabs, transferred uniformly onto the agar surface, for *C. jejuni* a sterile spatula was used. In case of *B. cereus*, *Y. ruckeri*, *K. rhizophila*, *B. subtilis* and *B. pumilus*, definite amount of incubated bacterial suspension was mixed with 400 mL of sterilized and thereafter cooled down to 45 °C Mueller-Hinton agar (Oxoid), to obtain final density of 10^5–10^6 cfu·mL^{-1} and then poured onto Petri dishes for the solidification at the room temperature. Thereafter, the wells (5 mm in diameter) were made into agar gel using sterile tools. Subsequently, the wells were filled with 30 µL of plant ethanol infusion in four different dilutions: 1:20 (w/v), 1:40, 1:80 and 1:160. Plates were incubated under conditions described in Table 1, the diameter of inhibition zone in millimetres was measured and the AB effect of a plant ethanol infusion was calculated as a mean of duplicate tests. As negative controls, 20% and 96% ethanol were used, and as a positive control, chloramphenicol (LAB M; 1000 mg·L^{-1}) was used.

137

138 [Insert table 1 here]

139

140 **Statistical analysis**

141 MS Excel 2013 software was used to evaluate the correlations between different chemical
142 properties and AB activities of the infusions. Correlation was considered strong, if r was equal
143 or higher than ±0.65, moderate if r ≥±0.41 to ±0.64 or weak if the r value was in the interval 0
144 to ±0.4.

145

146 **RESULTS AND DISCUSSION**

147 **Chemical composition of plant infusions**

148 The total polyphenol content (TPC) of the plant infusions (1:20, w/v) varied from 0.18 to 5.21
149 $g \cdot L^{-1}$ in 20% ethanol infusions and from 0.06 to 7.03 $g \cdot L^{-1}$ in 96% ethanol infusions, rhubarb
150 petioles being the lowest and rhubarb 'Victoria' roots the highest in TPC (Fig. 1). The
151 anthocyanin content varied from 0 to 0.83 $g \cdot L^{-1}$ and from 0 to 1.87 $g \cdot L^{-1}$ in 20% and 96% ethanol
152 infusions respectively, chokeberry having the highest anthocyanin content in 20% ethanol and
153 blue honeysuckle in 96% ethanol. The ascorbic acid content varied from 0.31 to 2.14 $g \cdot L^{-1}$ in
154 20% ethanol and from 0.16 to 2.4 $g \cdot L^{-1}$ in 96% ethanol, chokeberry having the lowest and blue
155 honeysuckle the highest in 20% ethanol and black currant berries the highest AA content in
156 96% ethanol. The pH of studied infusions varied from 3.15 (black currant berries) to 3.8
157 (petioles of rhubarb 'Victoria'). The infusions of the berries and the rhubarb petioles contained
158 anthocyanins, rhubarb roots and black currant leaves did not (Fig. 1). It was noted that the dark-
159 rooted rhubarb cultivars had more anthocyanins in their petioles than the light-rooted cultivars

(Fig. 1). The total acidity was highest in the petioles of rhubarb 'Ogres' (8.7 $g \cdot L^{-1}$) and the sugar acid ratio was the highest in chokeberry berries (6.3), followed by blue honeysuckle berries (4.4). Blue honeysuckle had the highest content of total sugars (24.3 $g \cdot L^{-1}$), which exceeded black currant and chokeberry berries approximately by 6 $g \cdot L^{-1}$. The qualitative analyse of the plant extracts revealed that the polyphenolic composition of the plants differed notably, containing polyphenols with different properties (Table 2, Fig. 2), hence some differences in the AO and AB properties.

[Insert Figure 1 and 2 and table 2. here]

Antibacterial (AB) effect

The *in vitro* AB activities, in the form of the diameters of growth inhibition zones of selected plant infusions were determined against both Gram-positive (Table 3) and Gram-negative (Table 4) bacteria. The results indicated remarkable *in vitro* AB effect of several plant infusions (Fig. 3). In case of 20% ethanol-plant infusions, the Gram-negative bacteria were less susceptible than Gram-positive bacteria. This is in agreement with Goňi et al.[24], who reported Gram-negative bacteria being generally less susceptible to different antibacterial agents due to the outer lipopolysaccharide membrane, which restricts the diffusion of hydrophilic compounds into the bacterial cell. However, Taguri et al.[25] have concluded that the result of Gram-staining does not correlate with AB effect, and susceptibility of bacteria, growing in Mueller-Hinton medium depends mostly on the particular bacterial species. In the present study it was found that the strongest AB activity of tested plant 96% ethanol infusions were against *C. jejuni*, which is a Gram-negative micro-aerobic bacterium, also against *B. cereus*, which is a Gram-positive aerobic bacterium, with inhibition zones 18 mm and 15.5 mm, respectively.

[Insert Figure 3 here]

Against Gram-negative bacteria *C. jejuni*, *S.* Enteritidis and *E. coli*, the most effective at all tested dilutions were 96% ethanol infusions of the roots and petioles of the dark-rooted rhubarb 303 and the roots of ′Victoria′ with inhibition zone diameters 7–18 mm (Fig. 3). Weaker AB effects of the same plant infusions against the abovementioned bacteria were established in 20% ethanol (Fig. 3). Regarding *Y. ruckeri*, the infusions of the petioles of dark rhubarb no 303 in 96% and 20% ethanol were equally effective (Table 4).

The most effective against all studied Gram-positive bacteria was 96% ethanol infusion of the roots of dark rhubarb seedling 303, inhibition zone diameters were in the range of 8–15.5 mm at all dilutions. Among Gram-positive bacteria, *B. cereus* was the most susceptible to all ten tested plant infusions in 20% as well as in 96% ethanol (Fig. 2, Table 3). It is notable that *L. monocytogenes*, which is known as a relatively resistant bacteria to different environmental factors, was susceptible to nine out of ten tested 96% ethanol infusions. Generally, more concentrated infusions (w/v) 1:20 or 1:40 had stronger AB or bacteriostatic effects against tested bacteria (Table 3).

It has been shown that solubility in water is a significant factor determining the extent to which hydrophobic compounds can be accumulated up to damaging lethal levels in bacterial cell phospholipid membranes[24, 26]. In the current study, plant ethanol infusions were used, therefore, the mode of action of antibacterial agents cannot be explained only by cell membrane damage. *Trans*-resveratrol and emodin, both constituents of rhubarb roots, showed AB activity against gram-negative *C. jejuni*, *S.* Enteritidis, and *E. coli*, and against gram-positive *L. monocytogenes* at their highest used concentration (1 $g \cdot L^{-1}$). Li et al.[27] have estimated by cell membrane permeability and flow cytometry assays ability of hydrophobic emodin (the octanol-water

partition coefficient log K_{ow} +4.01; ECHA[28]) to destroy cell membrane integrity and increase membrane permeability; fluorescence spectroscopy assay had indicated ability of emodin to influence conformation of membrane proteins in case of Gram-positive *Haemophilus parasuis*. These mechanisms can possibly be used also for explanation of AB effect against Gram-negative bacteria of rhubarb root 96% ethanol infusion that, in addition to emodin, contains several other relatively hydrophobic hydroxyanthraquinones[9].

Important finding of the present study was that Gram-positive foodborne pathogenic bacteria *L. monocytogenes* and *B. cereus* as well as Gram-negative pathogens *C. jejuni*, *S.* Enteritidis and *E. coli* were inhibited by the ethanol infusions of the roots and petioles of rhubarb (both seedling 303 and 'Victoria'), which makes rhubarb a promising candidate for the use as the source of natural antibacterials in food. In rhubarb, presumably hydroxyanthraquinones are the major active components having many biological and pharmacological properties including AB activity[29, 30]. In the study of Lu et al.[2], the minimum inhibitory concentration (MIC) of crude extracts of rhubarb was positively related to the hydroxyanthraquinones' content, and similarly to the results of the current study it was found that rhubarb may have the potential use as an antibacterial agent for control of some pathogenic bacteria.

[Insert Table 3 and Table 4 here]

The free radical scavenging ability

The highest antioxidativity (AO), expressed by the DPPH free radical scavenging activity, had chokeberry berries with the highest content and variability of anthocyanins, both in 20% and 96% aqueous ethanol infusions, that is in correspondence with the results of Tian et al.[21] Chokeberry was followed by 20% aqueous ethanol infusion of black currant berries with the

lowest total polyphenols and total anthocyanins among the berries. Obviously, AO properties of black currant berries are primarily dependent on the hydrophilic compounds such as ascorbic acid[20], and subsequently on semi-polar anthocyanins[3, 13] and flavon-3-ols, particularly rutin[31], all of which are good antioxidants and better extractable with a more hydrophilic solvent (20% ethanol). Honeysuckle with the highest total polyphenol content among berries and dark rhubarb roots (Fig. 1, b) with the absolutely highest TPC, were also efficient. The AO properties of chokeberry, black currant berries and blue honeysuckle berries were however very similar (Fig 1). In the case of rhubarb roots, AO is obviously more dependent on relatively hydrophobic constituents like hydroxyanthraquinones emodin, aloe emodin, and chrysophanol as well as resveratrol di- and trimers[9, 32], which are extractable from the plant matrix with a more nonpolar solvent such as 96% ethanol. The content of anthocyanins was positively correlated with the AO of both 20% (r=0.65) and 96% (r=0.47) ethanol infusions of the plants (Fig. 4), that is in the correspondence with the results of Heinonen et al.[3] and Shih et al.[33] Also, content of citric and ascorbic acids, both outstanding transition metal chelators, had moderate positive correlation with free radical scavenging activities of the plant infusions (Fig. 4). It has been stated that organic acids, including citric acid, generally enhance the DPPH radical scavenging activity of ascorbic acid at the steady rate, whereas citric acid slows it down during the first minute of the reaction[34]. In the current study, the TPC in the plant infusions was weakly positively correlated with AO properties (Fig. 4) that may be caused by the high content of hydroxyanthraquinones and stilbenes in rhubarb root that have very strong AB[2,16], but weaker radical scavenging capacity[32]. In berries the bulk of the TPC were anthocyanins - strongly antioxidative molecules[3, 33], but not equally good antibacterial compounds.

The AO properties of the studied polyphenol standard compounds were in the descending order rutin > *trans*-resveratrol > *trans*-rhapontin > emodin, which is in agreement with Villaño et al.[35].

[Insert Figure 4 here]

CONCLUSIONS

The roots and petioles of rhubarb showed the highest AB activity against studied bacteria that highlights rhubarb as a promising candidate for the use as a source of natural antibacterials in food, if possible contamination by the soil microflora has been reduced to minimum. The highest *in vitro* AB activities were measured for dark roots of rhubarb infusions in 96% ethanol. Also, *trans*-resveratrol and emodin, as single compounds, both present in rhubarb roots, revealed remarkable AB activity against studied bacteria.

AB activity was more strongly correlated with total polyphenolic content of the plant infusions than with the total content of anthocyanins On the other hand, the highest AO activity was determined for plant materials containing anthocyanins The AB and AO of the studied plant infusions were not unambiguously correlated indicating that different compounds may be involved in antioxidative properties compared to antibacterial properties.

Combination of powders of dark rhubarb roots and petioles with berries of black chokeberry, black currant or some other anthocyanin-rich berries would have outstanding perspective to use as functional ingredients in food matrices.

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Figure 1. Chemical content and properties ($g \cdot L^{-1}$) of the plant infusions (1:20, w/v): total polyphenols (TPC), total anthocyanins (Anth.), ascorbic acid (AA), and antioxidativity (AO) in rutin equivalents ($g \cdot L^{-1}$) of 20% ethanol infusions (a) and of 96% ethanol infusions (b). Bars are listed in the descending order of AO.

Figure 2. The base peak chromatograms of the studied plant extracts in 20% ethanol. The peak numbers are described in the table 2.

Figure 3. The bacterial growth inhibition zone diameters (mm) of the plant infusions (1:20, w/v) in 20% and 96% ethanol, against each bacteria, listed in the descending order of summarized AB activities.

Figure 4. Correlations between characteristics of plant infusions in 20% and 96% ethanol solutions. TPC-Anth.-Total polyphenol content minus anthocyanins' content, AB-antibacterial activity ●–strong positive correlation, $r \geq 0.65$; ○-strong negative correlation, $r \leq -0.65$; ◑-weak correlation, r~0 to ±0.4; ◕- moderate positive correlation, r=0.41 to 0.64; ◔-moderate negative correlation, r=-0.64 to -0.41

407 **Table 1.** Used media and incubation conditions for different bacteria

Bacterial culture	Agar-media	Incubation conditions
Gram negative		
Campylobacter jejuni ATCC 33291	Columbia blood agar (Oxoid) + 5% lysed horse blood (Oxoid)	42 °C, 48 h, micro aerobic
Salmonella Enteritidis ATCC 13076	Mueller Hinton agar (Oxoid)	37 °C, 48 h, aerobic
Escherichia coli NCCB 100282	Mueller Hinton agar (Oxoid)	37 °C, 48 h, aerobic
Yersinia ruckeri NCIM 13282	Plate-count agar (Difco), pH 6.5	30 °C, 24-26 h, aerobic
Gram positive		
Listeria monocytogenes ATCC 13929	Mueller Hinton agar (Oxoid)	37 °C, 48 h, aerobic
Bacillus cereus ATCC 11778	Iso-Sensitest Agar (Oxoid), pH 6 + 625 µg/l CAP	30 °C, 24-26 h, aerobic
Kocuria rhizophila	Iso-Sensitest Agar (Oxoid), pH 8	37 °C, 24-26 h, aerobic
Bacillus subtilis BGA	Plate-count agar (Difco), pH 8	37 °C, 24-26 h, aerobic
Bacillus pumilus CV 607	DST-agar (Oxoid), pH 7	37 °C, 24-26 h, aerobic

408

409 Table 2. The qualitative composition of the studied plants' 20% ethanol extracts, analysed
410 with negative and/or positive ion mode. The most potent antioxidative compounds
411 (unpublished data) are marked in **bold**. The peak numbers are referring to the Fig. 2. *-the
412 compound is better extractable with 96% ethanol compared to 20% ethanol.

Peak no.	The most abundant compounds in Ribes nigrum leaves	[M-H]-/fragments
1	Catechin gallate	305/179;219;261;137
2	Chlorogenic acid I	353/191;179
3	Dihydro ferulic acid rhamnoside	341/195;163;129
4	Chlorogenic acid II	353/191
5	Ferulic acid derivative	399/193;301
6	Coumaryl quinic acid	337/191
7	Coumaroylquinic acid pentoside	675/337;191
8	Myricetin-glucoside	479/317;179;151
9	Quercetin-3-rutinoside syn. Rutin	609/301
10	Quercetin glucoside	463/301
11	Quercetin acetylglucoside	505/301
12	Kaempferol rutinoside	593/285
13	Kaempferol-3-O-glucoside	447/285
14	Kaempferol acetylglucoside	489/285
15	Isorhamnetin acetylglucoside	519/315
16	Chrysophanol glucoside	415/373;355
17	Oxylipin	327/311;211;171
18	Oxylipin 9S,12S,13S-trihydroxy-10E-octadecenoic acid (9,12,13-TriHOME)	329/311;211;171
Peak no.	The most abundant compounds in Rheum rhaponticum roots	[M-H]-/fragments
1	Procyanidin B1	577/407;289
2	Catechin	289/245
3	Epicatechin	289/245
4	Piceatannol-O-glucoside 1	405/243
5	Resveratrol-O-glucoside	389/227
6	Piceatannol-O-glucoside 2	405/243
7	Piceid	389/227
8	Piceatannol	243/225
9	Rhapontigenin-O-glucoside 1	419/257
10	Rhapontigenin-O-glucoside 2	419/257
11	Rhapontigenin-O-glucoside 3	419/257
12*	Aloe-emodin-O-glucoside	431/269
13	Rhapontigenin	257/241
14*	Torachryson-O-glucoside	407/245
15*	Emodin-O-glucoside	431/269
16	Deoxyrhapontigenin-O-galloylglucosde	555/313;169
17*	Torachrysone-O-acetylglucoside	449/245
18*	Chrysophanol-O-glucoside	415/253
19*	Rhein-O-glucoside	445/283
20*	Chrysophanol-O-acetylglucoside	457/253
21	Deoxyrhapontigenin	241/226
22	Resveratrol dimer	453/453

413

414

415 Table 2. continued.

Peak no.	The most abundant compounds in Rheum rhaponticum petioles	[M-H]-/fragments	[M+H]+/fragments
1	Citric acid	191/111;173	
2	Gallic acid	331/169	
3	Catechin	289/245	
4	Paracoumaric acid-glucoside	325/145	
5	Ferulic acid glucoside	355/193	
6	Epicatechin	289/245	
7	Myricetin glucuronide	493/317;179	
8	Cyanidin-3-O-glucoside		449/287
9	Cyanidin-3-O-rutinoside		595/287
10	Myricetin rutinoside	625/317	
11	Taxifolin glucoside	465/303;151	
12	Epigallocatechin gallate or gallocatechin gallate	441/289	
13	Myricetin-rhamnoside	463/317	
14	Rutin	609/301	
15	Quercetin glucuronide	477/301	
16	Quercetin rhamnoside	447/301	
17	Kaempferol rutinoside	593/285	
18	Phloridzin	435/273	
19	Myricetin glucoside glucuronide	479/316	
20	Deoxyrhapontin	403/241	
21	Quercetin glucoside	463/301	
22	9S,12S,13S-trihydroxy-10E-octadecenoic acid (9,12,13-TriHOME)	329/171;229	
Peak no.	The most abundant compounds in Ribes nigrum berries	[M-H]-/fragments	[M+H]+/fragments
1	Chlorogenic acid	353/191;179	
2	Caffeic acid-O-glucoside	345	
3	Coumaryl quinic acid	(341)/179;161	
4	Delphinidin-3-O-glucoside	337/191	465/303
5	Delphinidin-3-O-rutinoside		611/465;303
6	Cyanidin-3-O-glucoside		449/287
7	Cyanidin-3-O-rutinoside		595/287
8	Isorhamnetin-3-O-rutinoside		625/317
9	Myricetin-O-glucoside		481/319
10	Rutin	609/301	611/303
Peak no.	The most abundant compounds in Aronia melanocarpa berries	[M-H]-/fragments	[M+H]+/fragments
1	Chlorogenic acid I	353/191;179	355/163
2	Cyanidin3,5-di-O-glucoside		611/287
3	Chlorogenic acid II	353/191;179	355/163
4	Cyanidin-3-O-glucoside		449/287
5	Cyanidin-3-O-**α**-arabinopyranoside		419/287
6	Cyanidin-3-O-**α**-arabinopyranoside		419/287
7	Delphinidin-3-O-(2“-O-**β**-xylopyranosyl)-**β**-glycopyranoside		596/303
8	Eriodictyol-7-O-β-glycuronide		465/289
9	Rutin	609/301	611/303
10	Delphinidin-3-O-glucopyranoside		465/303
Peak	The most abundant compounds in Lonicera caerulea berries	[M-H]-/fragments	[M+H]+/fragments
1	Cyanidin3,5-di-O-glucoside		611/449;287
2	Cyanidin3,5-di-O-glucoside isomer		611/449;287
3	Chlorogenic acid	353/191;179	355/163
4	Cyanidin-3-O-glucoside		449/287
5	Cyanidin 3-O-rutinoside		595/287
6	Peonidin 3-O-glucoside		463/301
7	Quercetin O-rhamnoside-O-glucoside		609/463

416

Table 3. Antibacterial activity of plant infusions against Gram-positive bacteria (inhibition zones (mm) ± standard deviation)

Plant infusions	Conc. (w/v)	*B. cereus*		*B. pumilus*		*B. subtilis*		*K. rhizophila*		*L. monocytogenes*	
		A	B	A	B	A	B	A	B	A	B
The dark roots of rhubarb 303	1:20	16	15.5±2.1	11*	13.5±0.7	11	14	11	13.5±0.7	9	11.5±0.7
	1:40	15	12.5±0.7	8*	11.5±0.7	-	12±3	10	11±2	7	10
	1:80	11	11.5±0.7	8*	11.5±2.1	-	9±1.4	-	9±2	-	8.5±0.7
	1:160	10	9	-	9	-	8	-	9*	-	8
Petioles of rhubarb 303	1:20	11.5±0.71	12	-	9*	-	10	9	10	-	12
	1:40	10	9.5±0.7	-	8*	-	10*	-	9*	-	10
	1:80	10	10	-	7*	-	-	-	-	-	8.5±0.7
	1:160	8	8	-	-	-	-	-	-	-	6
The pale roots of rhubarb ′Ogres′	1:20	10	10	-	10	-	-	-	-	-	12
	1:40	8	9	-	8	-	-	-	-	-	10
	1:80	7	8	-	-	-	-	-	-	-	8
	1:160	0	-	-	-	-	-	-	-	-	-
Petioles of light rhubarb ′Ogres′	1:20	10	11	-	-	-	-	-	8	-	8
	1:40	8.5±0.7	10	-	-	-	-	-	-	-	7
	1:80	7.5±0.7	9	-	-	-	-	-	-	-	6
	1:160	-	7	-	-	-	-	-	-	-	0
Roots of rhubarb ′Victoria′	1:20	13	14	9	11	9	13*	10*	12*	-	12
	1:40	12	12	8	9	9*	11*	8*	9*±2	-	8
	1:80	8	11	-	7	-	-	-	-	-	7
	1:160	-	10	-	-	-	-	-	-	-	6
Petioles of rhubarb ′Victoria′	1:20	12	10	-	11*	-	-	-	-	-	11±2
	1:40	9	9	-	11*	-	-	-	-	-	9
	1:80	7	7	-	-	-	-	-	-	-	8
	1:160	-	-	-	-	-	-	-	-	-	7
Berries of black currant ′Ben Alder′	1:20	12	10*	-	10±2	-	9	8±2*	9*	-	11±2
	1:40	10	9*	-	-	-	7	-	-	-	7
	1:80	7	-	-	-	-	-	-	-	-	-
	1:160	-	-	-	-	-	-	-	-	-	-
Leaves of black currant ′Pamjati Vavilova′	1:20	14	10.5±0.7	8*	-	-	8*	-	-	-	10
	1:40	13	9	7*	-	-	-	-	-	-	9
	1:80	10	8	6*	-	-	-	-	-	-	9
	1:160	8	7	-	-	-	-	-	-	-	8
Berries of black chokeberry	1:20	10	12	-	10*	-	-	-	12*	-	10.5±0.7
	1:40	8	10	-	10*	-	-	-	11*	-	8
	1:80	7	9	-	-	-	-	-	-	-	7
	1:160	-	7	-	-	-	-	-	-	-	6
Berries of blue honeysuckle ′Tomitška′	1:20	10*	10	-	9*	-	-	-	-	-	-
	1:40	-	8	-	8*	-	-	-	-	-	-
	1:80	-	7	-	7*	-	-	-	-	-	-
	1:160	-	-	-	7*	-	-	-	-	-	-
Control (-)		-	-	-	-	-	-	-	-	-	-
Control (+)		29±2		28.5±3		33±2		37.5±0.7		26.5±2	

- No visible growth detected; *bacteriostatic effect was detected; A –20% ethanol-plant infusion; B –96% ethanol-plant infusion

Table 4. Antibacterial activity of plant infusions against Gram-negative bacteria (inhibition zones (mm) ± standard deviation)

Plant infusions	Conc. (w/v)	*C. jejuni*		*S.* Enteritidis		*E. coli*		*Y. ruckeri*	
		A	B	A	B	A	B	A	B
The dark roots of rhubarb 303	1:20	-	18±3	10*	11.5±0.7	10*	11±1.4	8	9*
	1:40	-	13.5±0.7	-	10	-	9	7	8*
	1:80	-	12	-	8	-	8	-	-
	1:160	-	10	-	7	-	7	-	-
Petioles of rhubarb 303	1:20	-	12	-	11±2	-	11.5±0.7	17	16±2
	1:40	-	11	-	9.5±0.7	-	9	14	12
	1:80	-	10	-	9	-	8	9	9
	1:160	-	8	-	8	-	7.5±0.7	-	-
The pale roots of rhubarb ′Ogres′	1:20	-	16	-	12	-	12	-	12
	1:40	-	15	-	10	-	10	-	10
	1:80	-	14	-	-	-	-	-	8
	1:160	-	10	-	-	-	-	-	-
Petioles of rhubarb ′Ogres′	1:20	-	12	-	12*	-	10	10*	14
	1:40	-	11	-	9*	-	9	8*	9
	1:80	-	-	-	8*	-	7	-	-
	1:160	-	-	-	-	-	-	-	-
Roots of rhubarb ′Victoria′	1:20	-	18±2	8	11.5±0.7	8	11.5±0.7	14	9
	1:40	-	15	6	10	-	10±2	10	8*
	1:80	-	13	-	9	-	9	-	-
	1:160	-	10	-	8	-	8	-	-
Petioles of rhubarb ′Victoria′	1:20	-	8	-	9	-	9	13±3	8
	1:40	-	7	-	8	-	8	-	-
	1:80	-	-	-	7	-	7	-	-
	1:160	-	-	-	7	-	7	-	-
Berries of black currant ′Ben Alder′	1:20	-	15±2	-	10	-	10	10	12±2
	1:40	-	13±2	-	9	-	9	8*	9
	1:80	-	12	-	-	-	-	-	-
	1:160	-	12	-	-	-	-	-	-
Leaves of black currant ′Pamjati Vavilova′	1:20	-	13	-	9.5±0.7	-	11	12*	10*
	1:40	-	11	-	9	-	10	8*	8*
	1:80	-	10	-	8	-	9	-	-
	1:160	-	9	-	8	-	8	-	-
Berries of black chokeberry	1:20	10	15	-	9±2	-	11	-	8*
	1:40	8	12	-	7	-	11	-	-
	1:80	-	10	-	7	-	11	-	-
	1:160	-	8	-	6	-	8	-	-
Berries of blue honeysuckle ′Tomitška′	1:20	15	14±2	-	9	-	8.5±0.5	9*	10
	1:40	10	13	-	7	-	7	7*	7
	1:80	8	8	-	-	-	-	-	-
	1:160	-	-	-	-	-	-	-	-
Control (-)		-	-	-	-	-	-	-	-
Control (+)		40±2		31.5±2.1		26±2		34±4	

-No visible growth detected; *bacteriostatic effect was detected; A –20% ethanol-plant infusion; B –96% ethanol-plant infusion