

## Antimicrobial activity of berries extracts of four *Ribes* species, their phenolic content and anthocyanin composition

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### ABSTRACT

Phenolic compounds are widespread in berries and determine their antimicrobial activity. The aim of our study was to establish the amounts of phenolic compounds and the anthocyanin composition in berries of four *Ribes* species, and to evaluate the effect of berry extracts on the growth of common Gram-positive and Gram-negative bacteria, and also yeasts isolated from food processing plants. The phenolic content and anthocyanin composition were estimated spectrometrically and by HPLC, respectively. The highest amount of phenolic compounds, and also anthocyanins, was found in extracts of *R. aureum* 'Corona'. The anthocyanin content was the lowest in berries of *R. aureum* Au Gs-5, with equal amounts of delphinidins and cyanidins. Delphinidins were predominant (68.6%) in berries of *R. nigrum* 'Ben Tirran', while cyanidins dominated in *R. uva-crispa*. The berry extracts of *R. aureum* Au Gs-5 and *R. uva-crispa* 'Lūšiai' had the largest growth-suppressing effect on yeasts and most of the bacteria tested. All of the berry extracts suppressed the growth of pathogenic and conditionally pathogenic bacteria. The industrially important *Lactococcus lactis* was the most resistant to the *Ribes* berry extracts. There was no correlation between the amount of anthocyanins in the extracts and their antimicrobial properties. Extracts with a lower anthocyanin-to-phenolics ratio more effectively inhibited the growth of bacteria.

Key words: anthocyanin, bacteria, cyanidins, delphinidins, phenolics, *Ribes*

### INTRODUCTION

The *Ribes* species grow naturally in the Northern hemisphere, and more than 150 species have been described. Most commercial production of *Ribes* berry fruits takes place in Europe (Hummer and Dale, 2010). *Ribes nigrum* has the highest industrial importance; *Ribes rubrum* and *Ribes uva-crispa*

are also grown commercially and as a hobby. The berries are used for jam, juice, wine, and tea production, and as food colorants. The berries are consumed as a source of vitamins and sugars, and also due to their good organoleptic features. Polyphenols are an important component for *Ribes* berry quality (Nour et al., 2011).

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Phenolic compounds are widespread in berries and determine their antimicrobial activity (Xia et al., 2010). Anthocyanins comprise a large part of phenolic compounds in various berries, and a major part of phenolics in berries of the *Vaccinium*, *Ribes*, *Prunus* and *Sambucus* species (Anisimovienė et al., 2009; Ozgen et al., 2010). Anthocyanins are flavonoid pigments. They are related to plant disease resistance and exhibit antioxidant activity (Havsteen, 2002; Kong et al., 2003). Anthocyanins are water soluble pigments; therefore, they are widely used as natural colorants in various foods.

The largest concentration of anthocyanins is found in the peel of berries (Szajdek and Borowska 2008). The composition, biological activity and stability of anthocyanins in the *Ribes* genus depend on the plant species, environment, climatic conditions, cultivar and berry ripening (Blando et al., 2004; Manach et al., 2004; Hjalmarsson and Wallace, 2007; Lee et al., 2007). The berries of blackcurrant are the richest source of anthocyanins in the *Ribes* genus, with a concentration of 300–670 mg 100 g<sup>-1</sup> (Moyer et al., 2002; Horbowicz et al., 2008; Mattila et al., 2016). Red currant berries contain 12–20 mg 100 g<sup>-1</sup> anthocyanins (Nour et al., 2011), golden currant 170–308 mg 100 g<sup>-1</sup> (Maatta et al., 2001; Moyer et al., 2002), and gooseberries 3–26 mg 100 g<sup>-1</sup> (Jordheim et al., 2007), depending on the colour.

Flavonoids and related polyphenols are of major interest in human nutrition studies. Their presence in the diet is related to the protective effects against various diseases (Rodriguez-Mateos et al., 2014). Different concentrations and compositions of anthocyanins are found in berries of the *Ribes* species. Therefore, their usage in food production and impact on human health may vary. Anthocyanins are important due to their various biological effects. Anthocyanins are a type of flavonoids that are most easily available in the diet when berries are consumed. These compounds do not undergo intensive metabolization in the human body (Del Rio et al., 2013). They have been shown to be involved in antioxidant activity, radical scavenging, inhibition of platelet aggregation and lipoprotein oxidation, anti-inflammatory effects, decreasing capillary permeability and fragility, and found to prevent obesity, reduce the risk of liver ischemia, certain types of cancer and diabetes, and to improve vision (Gatto et al., 2002; Kim et al., 2005; Liobikas et al., 2009; da Silva Pinto et al., 2010; He and Guisti, 2010; Bishayee et al., 2011). The presence of phenolic compounds in a long

-term diet is associated with reduced incidence of chronic diseases and generally increased longevity (Rodriguez-Mateos et al., 2014). In food, anthocyanins inhibit lipid and protein oxidation (Cooke et al., 2005; Shipp and Abdel-Aal, 2010).

Berries rich in phenolics exhibit antimicrobial action against pathogenic bacteria (Heinonen, 2007). The antimicrobial effect of polyphenolic compounds has been demonstrated in several studies (Gatto et al., 2002; de Pascual-Teresa and Sanchez-Ballesta, 2008). These compounds prevent the actions of microorganisms, but do not kill them. Proanthocyanins from berries inhibit microorganism adhesion (Howell, 2002). Sour cherry extracts have been found to suppress the growth of both Gram-positive and Gram-negative bacteria (Liegiūtė et al., 2009). Cranberry juice suppresses the adhesion behaviour of *Escherichia coli* (Liu et al., 2006) and also inhibits the adhesive properties of oral *Streptococci*, thus weakening biofilm formation and mouth colonisation (Yamanaka et al., 2004). The ability of *Helicobacter pylori* to colonise mucous is also inhibited by cranberry extracts (Burger et al., 2000).

The aim of our study was to establish the amounts of phenolic compounds and the anthocyanin composition in berries of four *Ribes* species, and to evaluate the effects of berry extracts on the growth of common Gram-positive and Gram-negative bacteria, and also yeasts isolated from food processing plants. The results could be useful for the natural product-oriented food industry.

## MATERIAL AND METHODS

### *Plant material*

Four *Ribes* species were studied: *R. nigrum* L. 'Ben Tirran' with black berries (bred by the Scottish Crop Research Institute), *R. aureum* Au Gs-5 with yellow berries (bred by the Lithuanian Institute of Horticulture) and 'Corona' with black berries (bred in Germany), *R. petraeum* 'Jonkher van Tets' with red berries (bred in the Netherlands), and *R. uva-crispa* 'Lūšiai' with red berries (bred by the Lithuanian Institute of Horticulture) and 'Čiornyj negus' with black berries (bred by Michurinsk State Agrarian University). The plants were grown in the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, in a field with a close to neutral (pH 6.6–6.9) *Epicalcari-Endohypogleyic Cambisol* soil with 2.3% humus, 290 mg kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 180 mg kg<sup>-1</sup> K<sub>2</sub>O. The plants were grown in the temperate climate zone.

Berry ripening time is June-August and the average temperature ranges from 17 to 18°C, and precipitation ranges from 50 to 76 mm. Plants were grown at a spacing of 1 m × 3 m. Berries at the technical maturity stage were collected from the middle part of four-year-old plants. Samples were collected in 2011. Pooled samples from five plants were used to represent each variety after a minimum of 20 berries were collected from one bush. Berries were immediately frozen at -70°C before analysis.

#### **Isolation of anthocyanins and phenolics**

Phenolic compounds were extracted from frozen berries ground to a fine powder, using 90% aqueous methanol, 0.02% HCl at a ratio of 1:20 g mL<sup>-1</sup> and stored for 16 h at 4°C in the dark (Anisimovienė et al., 2009). The material was shaken twice for 30 min. at 4°C in the dark during the extraction procedure. Extracts were concentrated using a rotary evaporator (IKA RV-10 Germany) at 40°C until dryness. Same extracts were used for evaluating phenolic content, composition of anthocyanins and antimicrobial activity. Residues were dissolved in acidified water (pH 2.4). Total anthocyanin content was determined using the spectrophotometric differential pH method (Horbowicz et al., 2008). Absorbance of the extracts was measured at 520 and 700 nm, using a UV-VIS spectrophotometer Specord 210 PLUS (Analytik Jena AG, Jena, Germany). Equilibration time was 30 min., at 25°C in darkness. Anthocyanin content was calculated using the molar extinction coefficient of cyanidin-3-glucoside chloride. Results were expressed as cyanidin-3-glucoside chloride equivalents in mg 100 g<sup>-1</sup> fresh weight (FW).

Total phenolic content in crude methanol extracts was determined by the Folin-Ciocalteu method of Slinkard and Sigleton (1977), as referred to by Pantelidis et al. (Pantelidis et al., 2007). Absorbance of samples was measured at 765 nm, using a spectrophotometer (Specord 210 PLUS, Germany). Gallic acid (Sigma-Aldrich, CHEMIE GmbH, Germany) was used as a standard. Phenolic compounds content was expressed as mg 100 g<sup>-1</sup> FW.

#### **Anthocyanin composition**

The composition of anthocyanins was established using HPLC (Liobikas et al., 2009; Durst and Wrolstad, 2001). Anthocyanin standards (Extrasynthese, France; Polyphenols Laboratories AS, Norway) were used for identification. Separation and quantification was performed on an Agilent 1200 HPLC equipped with a DAD detector, on an XBridge Shield RP18 analytical column

(3.0 × 150 mm, particle size 3.5 μm). The mobile phase consisted of 10% acetic acid and 1% phosphoric acid (solvent A), and 100% acetonitrile (solvent B). The elution conditions were as follows: isocratic elution 0% B, 0-12 min.; linear gradient from 0% B to 7% B, 12-15 min.; 7% to 45% B, until 17 min.; 45% to 100% of B, 20 min., at a flow rate of 0.7 mL min<sup>-1</sup>. Injection volume of 10 μL was used, prior injection samples were diluted at 1:20 ratio. Detector wavelength of 520 nm was used. Analyses were performed in triplicate. Standards of cyanidin 3-O-glucoside (c3g), cyanidin 3-O-rutinoside (c3r), delphinidin 3-O-rutinoside (d3r), delphinidin 3-O-glucoside (d3g), pelargonidin 3-O-glucoside (pel3g), peonidin 3-O-rutinoside (peo3r) and malvidin 3-O-glucoside (m3g) (Extrasynthese, France; Polyphenols Laboratories AS, Norway) were used for identification and quantification of anthocyanins in samples.

#### **Antimicrobial activity of extracts**

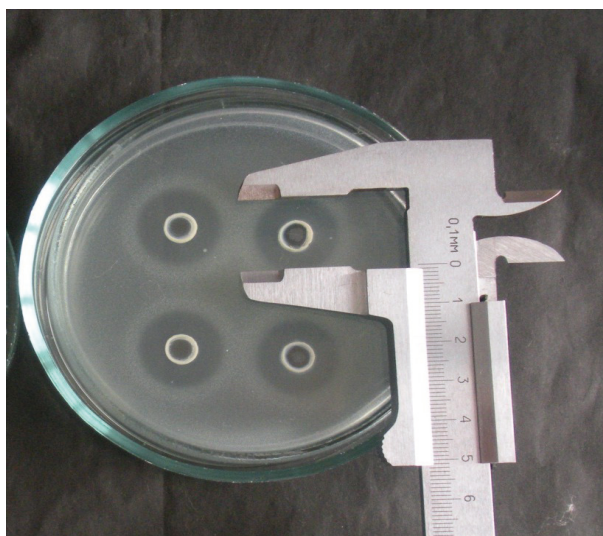
Seven bacterial cultures were used in the study. Reference cultures of *Listeria monocytogenes* (ATCC 19117), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 10876) and *Micrococcus luteus* (ATCC 9341) were grown on plate count agar nutrient medium, *Salmonella typhimurium* (ATCC14028) were grown on plate count agar (Liofilchem), *Lactococcus lactis subsp. lactis* were cultivated on M17 agar. Yeasts *Saccharomyces cerevisiae* and *Rhodotorula rubra* were isolated in food producing plants and cultivated on Sabouraud dextrose agar (Liofilchem).

Antibacterial activity was evaluated by agar diffusion test. Bacterial cultures were cultivated on agar at 37°C for 18 h, washed and diluted according to McFarland standard No. 0.5. 1 ml of suspension was added into 100 ml of solid medium, cooled to 47°C.

Yeast cultures were grown at 25°C for 24 h and then washed with sterile physiological solution, and the suspension, prepared according to McFarland standard No. 1, was added into dissolved and cooled potato-glucose agar and thoroughly mixed.

7 mL of cell suspension with nutrient medium was transferred into 90 mm Petri dishes. Filter paper disk (6 mm diameter) moistened with 5 μL of diluted berry extract was placed on the surface of the medium. 0.1%, 0.5% and 1.0% extracts were tested. Antimicrobial activity against bacteria was evaluated after 24 h cultivation, and after





**Figure 1.** Example of measurement of antimicrobial activity. The dish contains *R. aureum* 'Corona' extracts. The bacterial cultures used were those of *Listeria monocytogenes* ATCC 19117

24-48 h for yeast, by measuring the diameter of the inhibition zone (Fig. 1). 1% solutions of delphinidin-3-O-rutinozide chloride and cyanidin-3-O-rutinozide chloride were used as reference.

The experiments were repeated 3 times and the average and standard deviation is presented in tables. A spreadsheet application was used for the calculations. The concentration of different anthocyanins was estimated using the peak area calculated with Agilent ChemStation for LC software.

## RESULTS AND DISCUSSION

The amounts of phenolic (soluble) compounds varied considerably between the different *Ribes* berry extracts. However, this variation of phenolic compounds was smaller ( $171.6 \pm 13.8$ - $671.8 \pm 41.6$

mg 100 g<sup>-1</sup> FW) than the variation of anthocyanins ( $15.9 \pm 1.5$ - $615.5 \pm 15.9$  mg 100 g<sup>-1</sup> FW) (Tab. 1).

The highest amounts of phenolic compounds, and also anthocyanins, were found in extracts from the golden currant 'Corona' with black berries, blackcurrant 'Ben Tirran' and gooseberry 'Čiornyj negus' with black berries (Tab. 1). The amounts of phenolic compounds in the berries of gooseberry 'Lūšiai', golden currant Au Gs-5 and red currant 'Jonkher van Tets' were less than half the amount in the berries of blackcurrant 'Ben Tirran'. Generally, higher amounts of phenols were found in the *Ribes* species with dark berries in comparison with the species with red, green or yellow berries. Dark berries also had a high anthocyanin content. The anthocyanins-to-phenolics ratio in golden currant 'Corona' was 0.92, which implies that most of the phenolics in these berries belong to the anthocyanin class. The ratio of anthocyanins to phenolics in the other dark-coloured berries was close to 0.8. The ratio of anthocyanins to phenolics in the varieties with red, yellow or green berries was much lower: 0.39, 0.17 and 0.07 for red currant 'Jonkher van Tets', gooseberry 'Lūšiai' and golden currant Au Gs-5, respectively. This may be due to the presence of other phenolic compounds, including tannins, proanthocyanidins and phenolic acids that predominate in species with red, green or yellow berries (Maatta et al., 2001). The lowest amount of phenolics was measured in red currant 'Jonkher van Tets' berries, though their anthocyanin content was 1.5 times higher than in the berries of gooseberry 'Lūšiai' and four times higher than in the yellow berries of golden currant Au Gs-5.

Our results are in agreement with other studies showing that higher levels of phenolics are characteristic of blackcurrant berries in comparison with gooseberries and red currants (Moyer et al., 2002; Horbowicz et al., 2008; da Silva Pinto et

**Table 1.** Total phenolics, anthocyanins and composition of anthocyanins in the studied *Ribes* species

Cultivar	Anthocyanins to phenolics ratio	Phenolics (mg 100 g <sup>-1</sup> FW)	Anthocyanins (mg 100 g <sup>-1</sup> FW)	Composition of anthocyanins* (%)					
				D	C	Peo	Pel	M	X
Ben Tirran	0.77	621.4 ± 44.8	478.6 ± 14.8	68.6	29.0	1.4	0.6	0.3	0.0
Lūšiai	0.17	267.4 ± 26.2	44.4 ± 4.3	3.1	90.8	3.4	1.4	1.4	0.0
Čiornyj negus	0.76	500.4 ± 48.1	379.2 ± 35.3	6.9	75.8	0.7	0.0	0.4	16.2
Corona'	0.92	671.8 ± 41.6	615.5 ± 15.9	5.2	92.7	0.6	0.2	0.1	1.1
Au Gs-5	0.07	241.1 ± 23.1	15.9 ± 1.5	54.6	45.4	0.0	0.0	0.0	0.0
Jonkher van Tets	0.39	171.6 ± 13.8	66.7 ± 5.4	0.5	92.2	0.0	0.0	0.3	7.8

\*D – delphinidin; C – cyanidin; Peo – peonidin; Pel – pelargonidin; M – malvidin; X – unidentified anthocyanins. Mean and ± SD of 3 repetitions is presented

al., 2010). According to the data obtained from 33 cultivars and hybrids of blackcurrant grown in the USA, the amount of phenolics ranges from 498 to 1342 mg 100 g<sup>-1</sup> FW (Moyer et al., 2002). The amount of phenolics in red and black currants grown in Hungary was found to be 192 and 533 mg 100 g<sup>-1</sup> FW, respectively (Lugasi et al., 2011). In a recent study, the amount of phenolics in 11 red currant cultivars ranged from 67 to 153 mg 100 g<sup>-1</sup> FW (Djordjević et al., 2010). By contrast, in another study, the level of phenolics in gooseberries was found to be highly variable: from 3.2 mg 100 g<sup>-1</sup> FW (da Silva Pinto et al. 2010) to 1257-1321 mg 100 g<sup>-1</sup> DW (Pantelidis et al., 2007).

The *Ribes* species also varied in the total amount of anthocyanins and their composition. Delphinidins were predominant in the berries of blackcurrant 'Ben Tirran', constituting 68.6% of total anthocyanins (478.6 mg 100 g<sup>-1</sup> FW) (Tab. 1). Cyanidins dominated in gooseberry berries, regardless of the berry colour and the amount of anthocyanins, constituting 75.8-90.8% of total anthocyanins. However, 16.2% of anthocyanins could not be identified in the black berries of gooseberry 'Čiornyj negus'. Cyanidins (92.7%) were predominant in the dark berries of golden currant 'Corona', while the ratio of cyanidins to delphinidins was close to 1:1 in the yellow berries of *R. aureum* Au Gs-5. In red currant berries, 92.2% of anthocyanins were cyanidins; the remaining 7.8%

were unidentified. Peonidins, pelargonidins and malvidins were present in minor amounts in all the *Ribes* species analyzed; the highest percentage of peonidins (3.4%) was in gooseberry 'Lūšiai'.

We studied the yeasts *S. cerevisiae* and *R. rubra*, both of which can be found growing on the surface of berries. Differences between the yeasts in their susceptibility to anthocyanin-rich berry extracts were observed. Extracts prepared from the berries of *R. nigrum* 'Ben Tirran', *R. uva-crispa* 'Čiornyj negus' and *R. aureum* Au Gs-5 did not suppress the growth of *S. cerevisiae*, and neither did the pure anthocyanins D-3-R and C-3-R. However, *R. aureum* 'Corona' and *R. uva-crispa* 'Lūšiai' extracts did have an effect. The strongest suppression of *S. cerevisiae* growth was observed in *R. petraeum* 'Jonkher van Tets' extracts (Tab. 2).

In contrast, the growth of *R. rubra*, which had been isolated in a food producing company, was inhibited by all the berry extracts except *R. aureum* 'Corona' extracts and the pure anthocyanins D-3-R and C-3-R (Tab. 2). However, the inhibitory effect was relatively mild. Only the use of higher concentration (1%) extracts of 'Ben Tirran' and 'Čiornyj negus' led to the inhibition of *R. rubra* growth.

Next, we studied the effects of berry extracts on six pathogenic bacteria that may cause contamination in food processing. Additionally, we studied *L. lactis*, which is used in cheese and

**Table 2.** Growth inhibition of yeasts by berry extracts

Species, cultivar	Concentration of extract (%)	Inhibition zone size (mm)	
		<i>R. rubra</i>	<i>S. cerevisiae</i>
<i>R. nigrum</i> 'Ben Tirran'	0.1	0.0	0.0
	0.5	0.0	0.0
	1	10.3 ± 0.7	0.0
<i>R. uva-crispa</i> 'Lūšiai'	0.1	24.3 ± 1.4	9.0 ± 0.6
	0.5	0.0	0.0
<i>R. uva-crispa</i> 'Čiornyj negus'	0.1	0.0	0.0
	0.5	0.0	0.0
	1	9.3 ± 0.7	0.0
<i>R. aureum</i> 'Au Gs-5'	0.1	10.3 ± 0.7	0.0
<i>R. aureum</i> 'Corona'	0.1	0.0	0.0
	0.5	0.0	8.0 ± 0.6
	1	0.0	12.0 ± 1.1
<i>R. petraeum</i> 'Jonkher van Tets'	0.1	0.0	13.7 ± 0.7
	0.5	9.0 ± 0.6	20.0 ± 1.1
Delphinidin-3-O-rutinoside	1	0.0	0.0
Cyanidin-3-O-rutinoside	1	0.0	0.0

Mean and ± SD of 3 repetitions is presented

**Table 3.** Growth inhibition of Gram-positive cocci by berry extracts

Species, cultivar	Concentration of extract (%)	Inhibition zone size (mm)		
		<i>L. lactis</i>	<i>M. luteus</i>	<i>S. aureus</i>
<i>R. nigrum</i> 'Ben Tirran'	0.1	0.0	13.0 ± 1.1	21.0 ± 1.1
	0.5	0.0	15.3 ± 1.3	31.0 ± 2.3
	1	15.3 ± 0.7	22.3 ± 1.3	39.3 ± 3.3
<i>R. uva-crispa</i> 'Lūšiai'	0.1	15.0 ± 1.1	26.7 ± 2.6	36.0 ± 2.3
<i>R. uva-crispa</i> 'Čiornyj negus'	0.1	0.0	14.0 ± 1.1	16.0 ± 0.6
	0.5	8.0 ± 0.6	16.0 ± 2.3	34.7 ± 1.3
	1	14.0 ± 5.9	16.3 ± 0.7	42.0 ± 2.3
<i>R. aureum</i> Au Gs-5	0.1	17.7 ± 1.3	28.0 ± 2.3	46.0 ± 2.3
<i>R. aureum</i> 'Corona'	0.1	8.0 ± 0.6	7.0 ± 0.6	9.0 ± 0.6
	0.5	9.0 ± 0.6	19.0 ± 2.3	29.3 ± 1.3
	1	11.7 ± 1.3	20.6 ± 2.6	31.3 ± 3.3
<i>R. petraeum</i> 'Jonkher van Tets'	0.1	9.0 ± 0.6	19.3 ± 1.3	26.0 ± 2.3
	0.5	9.0 ± 0.6	23.3 ± 5.2	47.3 ± 3.3
Delphinidin-3-O-rutinoside	1	0.0	0.0	0.0
Cyanidin-3-O-rutinoside	1	0.0	0.0	0.0

Mean and ± SD of 3 repetitions is presented

buttermilk production. The species included in the study were: Gram-positive cocci (*L. lactis*, *M. luteus* and *S. aureus*), Gram-positive bacilli (*L. monocytogenes* and *B. cereus*) and Gram-negative bacilli (*S. typhimurium* and *E. coli*).

Different effects of *Ribes* berry extracts on the growth of *L. lactis* subsp. *lactis* were observed. The largest inhibition zone (18 mm) was obtained with 0.1% *R. aureum* Au Gs-5 berry extract (Tab. 3). Gooseberry extracts suppressed the growth of *L. lactis* subsp. *lactis*. Surprisingly, the extracts of 'Lūšiai' with red berries had a stronger effect than the extracts of 'Čiornyj Negus' with black berries and an 8 times higher amount of anthocyanins. Only the 1% extract of blackcurrant 'Ben Tirran' successfully suppressed the growth of *L. lactis* subsp. *lactis*. The effect of *R. petraeum* 'Jonkher van Tets' 0.1% and 0.5% extracts was moderate. In summary, extracts with medium or low total phenolics and low anthocyanin content had the highest suppressor effect on *L. lactis* subsp. *lactis* (Tab. 3). Remarkably, *L. lactis* was the most resistant to the *Ribes* berry extracts. The relatively mild effect of the berry extracts on the growth of *L. lactis* suggests that *Ribes* extracts could be used as natural anti-microbial additives in the production of dairy products.

The pathogenic and conditionally pathogenic Gram-positive bacteria *S. aureus* and *M. luteus* were found to be susceptible to all the berry extracts

(Tab. 3). Our results suggest that the action of berry active compounds is related to the bacterial cell wall. The most susceptible was *S. aureus* with 32 mm diameter inhibition zones, consistent with a previous study which had found *S. aureus* to be also one of the most susceptible bacteria to black mulberry (*Morus nigra*) juice (Khalid et al., 2011). Extracts of gooseberry 'Lūšiai', red currant 'Jonkher van Tets' and golden currant Au Gs-5 showed the largest suppressor effects among all the *Ribes* species studied. Extracts with medium or low total phenolics and low anthocyanin content were the strongest suppressors of *S. aureus* (Tab. 3).

*B. cereus* cultures were also susceptible to the *Ribes* extracts; the diameter of inhibition zones was 26 mm on average (Tab. 4). The pattern of growth suppression by the *Ribes* extracts against *B. cereus* was similar to that for *S. aureus*, suggesting the mechanisms of suppression in these bacteria may be similar. While *L. monocytogenes* was susceptible to the *Ribes* extracts, the effect of gooseberries 'Čiornyj negus' and 'Lūšiai', red currant 'Jonkher van Tets' and golden currant 'Corona' extracts was lower. The inhibition mechanism of *L. monocytogenes* is possibly different from that of *B. cereus* and *S. aureus*. Gram-negative *E. coli* and *S. typhimurium* bacteria were less susceptible to the extracts, and their suppression character was similar (Tab. 4).

Previous studies had shown that Gram-positive bacteria were more susceptible to anthocyanin-

**Table 4.** Inhibition of the growth of Gram-positive and Gram-negative bacilli by berry extracts

Species, cultivar	Concentration of extract (%)	Inhibition zone size (mm)			
		<i>L. monocytogenes</i>	<i>B. cereus</i>	<i>S. typhimurium</i>	<i>E. coli</i>
<i>R. nigrum</i> 'Ben Tirran'	0.1	29.3 ± 1.3	11.0 ± 0.6	9.0 ± 0.6	11.0 ± 0.6
	0.5	31.0 ± 1.3	28.7 ± 0.7	21.7 ± 1.3	21.2 ± 0.3
	1	33.0 ± 2.3	37.3 ± 1.3	27.3 ± 3.3	24.3 ± 0.7
<i>R. uva-crispa</i> 'Lūšiai'	0.1	14.0 ± 0.6	28.0 ± 1.1	22.0 ± 1.1	25.5 ± 0.7
<i>R. uva-crispa</i> 'Čiornyj negus'	0.1	16.3 ± 0.7	12.0 ± 0.6	10.0 ± 0.6	13.0 ± 0.6
	0.5	30.0 ± 1.1	30.7 ± 2.6	23.3 ± 0.7	21.3 ± 1.3
	1	39.3 ± 3.3	35.0 ± 2.3	25.3 ± 0.7	28.3 ± 0.7
<i>R. aureum</i> Au Gs-5	0.1	30.7 ± 0.7	42.7 ± 1.3	31.0 ± 1.1	30.0 ± 1.1
<i>R. aureum</i> 'Corona'	0.1	11.1 ± 0.3	9.0 ± 0.6	7.0 ± 0.6	9.0 ± 0.6
	0.5	24.3 ± 1.3	21.0 ± 1.1	19.7 ± 2.7	14.3 ± 0.7
	1	27.0 ± 1.1	25.3 ± 1.3	23.0 ± 2.3	22.0 ± 0.7
<i>R. petraeum</i> 'Jonkher van Tets'	0.1	13.3 ± 0.7	18.0 ± 0.6	13.0 ± 0.6	18.0 ± 1.1
	0.5	27.7 ± 0.7	39.3 ± 3.3	30.7 ± 1.3	29.0 ± 2.3
Delphinidin-3-O-rutinoside (D-3- R)	1	0	0	0	0
Cyanidin-3-O-rutinoside (C-3-R)	1	0	0	0	0

Mean and ± SD of 3 repetitions is presented

rich extracts than Gram-negative ones (Cisowska et al., 2011). In our study, the most susceptible microorganisms were the Gram-positive *S. aureus*, *B. cereus* and *L. monocytogenes*, whereas the Gram-negative *E. coli* and *S. typhimurium* were less susceptible. Similar results had been obtained in a study of the antimicrobial effect of mulberry juice (Khalid et al., 2011). However, the Gram-positive *L. lactis* was the most resistant to *Ribes* berry extracts.

*S. aureus* can grow in processed food and its toxins contaminate food even after the removal of the bacteria. The anthocyanin-rich *Ribes* berry extracts inhibited the growth of *S. aureus*, which makes them a promising natural additive for the food production industry. Also, pharmaceutical and scientific communities encourage studies on the potential antimicrobial activity of plant-derived substances due to the increasing incidence of drug-resistant pathogens (Savoia, 2012). Further, all the studied *Ribes* extracts suppressed the pathogenic Gram-negative *S. typhimurium*, which is difficult to eliminate from food because of the lipopolysaccharides present in their outer membrane (Nohynek et al., 2006).

There was no correlation between the amount of anthocyanins in extracts and their antimicrobial properties. Instead, the highest antimicrobial effect was identified in extracts with a low anthocyanins-to-phenolics ratio. The most efficient

as an antimicrobial agent was the extract from Au GS-5, which had the lowest ratio of anthocyanins to total phenolics. The extract from the variety 'Corona', which had the highest anthocyanins-to-total phenolics ratio, was effective as an inhibitor of bacterial growth only in higher concentrations and was the least promising as a food preservative. This may be due to the antimicrobial properties of other phenolics such as myricetin, quercetin, tannins, ferulates, and others (Puupponen-Pimia et al., 2001; Daglia, 2012; Aldulaimi, 2017).

## CONCLUSIONS

The highest amounts of phenolic compounds, and also anthocyanins, were found in extracts of golden currant 'Corona' with black berries, blackcurrant 'Ben Tirran', and gooseberry 'Čiornyj negus' with black berries. The lowest amount of phenolics, among the studied *Ribes* species, was measured in red currant 'Jonkher van Tets' berries. While delphinidins were predominant (68.6%) in the berries of blackcurrant 'Ben Tirran', cyanidins dominated in gooseberry berries, regardless of the berry colour and total amount of anthocyanins, constituting 75.8-90.8% of total anthocyanins. Cyanidins also dominated in red currant 'Jonkher van Tets'.

Berry extracts of *Ribes* exhibit antimicrobial activity. However, the degree of microbial growth suppression differs between species. The berry



extracts of *R. uva-crispa* 'Lūšiai' and *R. aureum* Au Gs-5 had the largest growth-suppressing effect on the yeasts and most of the bacteria tested. *R. aureum* 'Corona' berry extracts had the weakest antimicrobial effect. The antimicrobial effects of extracts from berries of the other *Ribes* species were moderate. The antimicrobial effect of berry extracts was more pronounced on Gram-positive bacteria than on Gram-negative ones. However, the Gram-positive *L. lactis* was the most resistant to the *Ribes* berry extracts. The highest antimicrobial effect was identified in extracts with lower anthocyanins-to-phenolics ratios. This might be due to the antimicrobial activity of other flavonoids, or more generally other phenolic compounds.

Overall, the extracts from *R. aureum* Au Gs-5 and *R. uva-crispa* 'Lūšiai' showed the best antibacterial characteristics. Both extracts had the lowest anthocyanins-to-phenolics ratio. The use of these extracts as natural additives in the food industry might be beneficial.

## FUNDING

This work was supported by the Research Council of Lithuania under grant No. SVE-01/2011.

## AUTHOR CONTRIBUTIONS

T.Š., A.Š., N.A., V.B., Š.M.-H., V.S – designed the experiments, analysed the data and contributed to manuscript writing; N.A. – performed the analysis of phenolic content; V.B – analysed anthocyanin composition; D.J – performed measurements of bacterial growth.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

## REFERENCES

- ALDULAIMI O., 2017. General overview of phenolics from plant to laboratory, good antibacterials or not. *Pharmacogn. Rev.* 11, 123-127.
- ANISIMOVĖNĖ N., RUBINSKIENĖ M., VIŠKELIS P., STACKEVIČIENĖ E., STANYS V., ŠIKŠNIANAS T. ET AL., 2009. Anthocyanins in currants, cherries, blueberries, and antioxidative activity of berry extracts. *Zemdirbyste* 96, 158-167.
- BISHAYEE A., MBIMBA T., THOPPIL R.J., HÁZNAGY-RADNAI E., SIPOS P., DARVESH A.S. ET AL., 2011. Anthocyanin-rich black currant (*Ribes nigrum* L.) extract affords chemoprevention against diethylnitrosamine-induced hepatocellular carcinogenesis in rats. *J. Nutr. Biochem.* 22, 1035-1046.
- BLANDO F., GERARDI C., NICOLETTI J., 2004. Sour cherry (*Prunus cerasus* L.) anthocyanins as ingredients for functional foods. *J. Biomed. Biotechnol.* 5, 253-258.
- BURGER O., OFEK I., TABAK M., WEISS E.I., SHARON N., NEEMAN, I., 2000. A high molecular mass constituent of cranberry juice inhibits *Helicobacter pylori* adhesion to human gastric mucus. *FEMS Immunol. Med. Microbiol.* 29, 295-301.
- CISOWSKA A., WOJNICZ D., HENDRICH A.B., 2011. Anthocyanins as antimicrobial agents of natural plant origin. *Nat. Prod. Commun.* 6, 149-156.
- COOKE D., STEWARD W.P., GESCHER A.J., MARCZYLO T., 2005. Anthocyanins from fruits and vegetables – Does bright colour signal cancer chemopreventive activity. *Eur. J. Cancer.* 41, 1931-1940.
- DA SILVA PINTO A.D.S., KWON Y.I., APOSTOLIDIS E., LAJOLO F.M., GENOVESE M.I., SHETTY K., 2010. Evolution of red currants (*Ribes rubrum* L.), black currants (*Ribes nigrum* L.), red and green gooseberries (*Ribes uva-crispa*) for potential management of type 2 diabetes and hypertension using *in vitro* models. *J. Food Biochem.* 34, 639-660.
- DAGLIA M., 2012. Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol.* 23, 174-181.
- DE PASCUAL-TERESA S., SANCHEZ-BALLESTA M.T., 2008. Anthocyanins: from plant to health. *Phytochem. Rev.* 7, 281-299.
- DEL RIO D., RODRIGUEZ-MATEOS A., SPENCER J.P.E., TOGNOLINI M., BORGES G., CROZIER A., 2013. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antiox. Redox Signal.* 18, 1818-1892.
- DJORDJEVIĆ B., SAVIKIN K., ZDUNIĆ G., JANKOVIĆ T., VULIĆ T., OPARNICA C. ET AL., 2010. Biochemical properties of red currant varieties in relation to storage. *Plant Foods Hum. Nutr.* 65, 326-332.
- DURST R.W., WROLSTAD R., 2001. Separation and characterization of anthocyanins by HPLC. In: *Current Protocols in Food Analytical Chemistry*. R.E. Wrolstad (Ed.), Wiley, New York, USA, F1.3.1-F1.3.13.
- GATTO, M.T., FALCOCCHIO, S., GRIPPA, E., MAZZANTI, G., BATTINELLI, L., NICOLOSI, G. ET AL., 2002. Antimicrobial and anti-lipase activity of quercetin and its C2-C163-O-acyl-esters. *Bioorg. Med. Chem.* 10, 269-272.
- HAVSTEEN B.H., 2002. The biochemistry and medical significance of the flavonoids. *Pharmacol. Ther.* 96, 67-202.
- HE J., GUISTI M.M., 2010. Anthocyanins: natural colorants with health-promoting properties. *Ann. Rev. Food Sci. Technol.* 1, 163-187.
- HEINONEN M., 2007. Antioxidant activity and antimicrobial effect of berry phenolics – a Finnish perspective. *Mol. Nutr. Food Res.* 51, 684-691.
- HJALMARSSON I., WALLACE B., 2007. Gooseberry and currant in Sweden: History and cultivar development.



- In: Plant Breeding Reviews Vol. 29. J. Janick (Ed.), John Wiley and Sons Inc., New Jersey, USA, 145-175.
- HORBOWICZ M., KOSSON R., GRZESIUK A., DĘBSKI H., 2008. Anthocyanins of fruit and vegetables – their occurrence, analysis and role in human nutrition. *Veg. Crop. Res. Bull.* 68, 5-22.
- HOWELL A.B., 2002. Cranberry proanthocyanidins and the maintenance of urinary tract health. *Crit. Rev. Food Sci. Nutr.* 42, 273-278.
- HUMMER K.E., DALE A., 2010. Horticulture of *Ribes*. *Forest Pathol.* 40, 251-263.
- JORDHEIM M., MÅGE F., ANDERSEN Ø.M., 2007. Anthocyanins in berries of *Ribes* including gooseberry cultivars with a high content of acylated pigments. *J. Agric. Food Chem.* 55, 5529-5535.
- KHALID N., FAWAD S.A., AHMED I., 2011. Antimicrobial activity, phytochemical profile and trace minerals of black mulberry (*Morus nigra* L.) fresh juice. *Pak. J. Bot.* 43, 91-96.
- KIM D.-O., HEO H.J., KIM, Y.J., YANG H.S., LEE C.Y., 2005. Sweet and sour cherry phenolics and their protective effects on neuronal cells. *J. Agric. Food Chem.* 53, 9921-9927.
- KONG J.-M., CHIA L.-S., GOH N.-K., CHIA T.-F., BROUILLARD R., 2003. Analysis and biological activities of anthocyanins. *Phytochemistry* 64, 923-933.
- LEE B.-B., CHA M.-R., KIM S.-Y., PARK E., PARK H.-R., LEE S.-C., 2007. Antioxidative and anticancer activity of extracts of cherry (*Prunus serrulata* var. *spontanea*) blossoms. *Plant Foods Hum. Nutr.* 62, 79-84.
- LIEGIŪTĖ S., MAJIENĖ D., TRUMBECKAITĖ S., LIOBIKAS J., BENDOKAS V., STANYS V. ET AL., 2009. Anthocyanin composition and antimicrobial activity of sour cherry (*Prunus cerasus* L.) fruit extracts. *Zemdirbyste* 96, 141-148.
- LIOBIKAS J., TRUMBECKAITĖ S., BENDOKAS V., BANIULIS D., MAJIENĖ D., KOPUSTINSKIENĖ D.M. ET AL., 2009. Pro-apoptotic effect of black currant (*Ribes nigrum* L.) berry extracts on rat heart mitochondria, *Zemdirbyste* 96, 149-157.
- LIU Y., BLACK M.A., CARON L., CAMESANO T.A., 2006. Role of cranberry juice on molecular-scale surface characteristics and adhesion behavior of *Escherichia coli*. *Biotechnol. Bioeng.* 93, 297-305.
- LUGASI A., HÓVÁRI J., KÁDÁR G., DENES F., 2011. Phenolics in raspberry, blackberry and currant cultivars grown in Hungary. *Acta Alimentaria* 40, 52-64.
- MAATTA K., KAMAL-ELDIN A., TÖRRÖNEN R., 2001. Phenolics compounds in berries of black, red, green and white currants (*Ribes* sp.). *Antiox. Redox Signal.* 3, 981-993.
- MANACH C., SCALBERT A., MORAND C., REMESY C., JIMENEZ L., 2004. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 79, 727-747.
- MATTILA P.H., HELLSTRÖM J., KARHU S., PIHLAVA J.-M. VETELÄINEN M., 2016. High variability in flavonoid contents and composition between different North-European currant (*Ribes* spp.) varieties. *Food Chem.* 204, 14-20.
- MOYER R.A., HUMMER K.E., FINN C.E., FREI B., WROLSTAD R.E., 2002. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus*, and *Ribes*. *J. Agric. Food Chem.* 50, 519-525.
- NOHYNEK L.J., ALAKOMI H.-L., KAHKONEN M.P., HEINONEN M., HELANDER I.M., OKSMAN-CALDENTY K.-M. ET AL., 2006. Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens. *Nutr. Cancer* 54, 18-32.
- NOUR V., TRANDAFIR I., IONICA M.E., 2011. Ascorbic acid, anthocyanins, organic acids and mineral content of some black and red currant cultivars. *Fruits* 66, 353-362.
- OZGEN M., SCHEERENS J.C., REESE R.N., MILLER R.A., 2010. Total phenolic, anthocyanin contents and antioxidant capacity of selected elderberry (*Sambucus canadensis* L.) accessions. *Pharmacogn. Mag.* 6, 198-203.
- PANTELIDIS G.E., VASILAKAKIS M., MANGANARIS G.A., DIAMANTIDIS G., 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chem.* 102, 777-783.
- PUUPPONEN-PIMIA R., NOHYNEK L., MEIER C., KAHKONEN M., HEINONEN M., HOPIA A. ET AL., 2001. Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol.* 90, 494-507.
- RODRIGUEZ-MATEOS A., VAUZOUR D., KRUEGER C.G., SHANMUGANAYAGAM D., REED J., CALANI L. ET AL., 2014. Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. *Arch. Toxicol.* 88, 1803-1853.
- SAVOIA D., 2012. Plant-derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiol.* 7, 979-990.
- SHIPP J., ABDEL-AAL E.-S.M., 2010. Food application and physiological effects of anthocyanins as functional food ingredients. *Open Food Sci. J.* 4, 7-22.
- SLINKARD K., SINGLETON V.L., 1977. Total phenol analysis: automation and comparison with manual methods. *Am. J. Enol. Vitic.* 28, 49-55.
- SZAJDEK A., BOROWSKA E. J., 2008. Bioactive compounds and health-promoting properties of berry fruits: A review. *Plant Foods Hum. Nutr.* 63, 147-156.
- XIA E.Q., DENG G.F., GUO Y.J., LI H.B., 2010. Biological activities of polyphenols from grapes. *Int. J. Mol. Sci.* 11, 622-646.
- YAMANAKA A., KIMIZUKA R., KATO T., OKUDA K., 2004. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. *Oral Microbiol. Immunol.* 19, 150-154.