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Anmicrobial Activity and Composition of Different Cultivars of Honeysuckle Berry *Lonicera caerulea* L. †

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Abstract: The aim of this work was comparative study of composition and antimicrobial properties in eleven cultivars of honeysuckle berries. Using spectrophotometric methods, we compare the content of total phenolic compounds (TPC) and anthocyanis, chromatic characteristics of berries, which were grown in collection of Vytautas Magnus University Botanical Garden and collected at maturation stage. Also, content of ascorbic acid and saccharides were evaluated by HPLC using diode ray and light scattering detectors. Antimicrobial activity of ethanolic and water extracts of honeysuckle berry was evaluated by the agar well diffusion method. Bacterial tests have identified antimicrobial properties of honeysuckle berries against undesirable in food products bacteria but without affecting *Candida* and *Saccharomyces cerevisiae* yeast. The cultivar 'Morena' had the highest anthocyanins (781 mg/100 g) and total phenolic compounds (799 mg/100 g), the lowest anthocyanins (282 mg/100 g) and TPC (300 mg/100 g) content was detected in 'Vostorg' cultivar. Cultivars 'Pavlovskaja' and 'Pereselenka' had high content of ascorbic acid. The maximum glucose and fructose content were detected in 'Leningradskaja' cultivar.

Keywords: Honeysuckle Berry; chemical composition; antimicrobial activity

1. Introduction

For healthy diet it is recommended to consume at least 400 g of vegetables and fruit per day. Also according World Health Organisation recommendations consumption of sugar should be lowered and increased amount of dietary fiber. Berries are good source of dietary fibre, polyphenols, ascorbic acid and other bioactive compounds [1]. The edible blue honeysuckle comes from Russia and in the recent years has been considerably planted in some European countries, Lithuania is among them. Its interesting characteristics are high resistance to cold, different soil acidities, pests and various diseases [2]. The berries are rich in an ascorbic acid and phenolic contents, which have nutritional and health promoting properties for humans. In Japan honeysuckle berries are used in traditional medicine for slowing the aging process, preventing heart diseases and gastrointestinal dysfunction [3]. Anthoyanins, plant pigments, responsible for red to blue colour of fruits, are the biggest contributors to total phenolics in blue honeysuckle berries. High content of cyanidin-3-glucoside suggest their good antioxidant, anti-inflammatory, antimicrobial, cardioprotective and hepatoprotective activities [4,5]. In recent years increased consumer demand for the use of plant extracts as natural antimicrobial agents in food instead synthetic food additives. Some studies have shown antimicrobial properties of honeysuckle berries. Phenolic acids present in honeysuckle berries can act as natural antimicrobial agents to control *Candida parapsilosis*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus epidermidis*, or *Streptococcus mutant* [6]. Antibacterial effects of ethanol and buffered water infusions obtained from freeze-dried fruits of *L. caerulea* were studied on gram-positive bacteria (*Listeria monocytogenes*, *Kocuria rhizophila*, *Bacillus subtilis*), gram-negative foodborne pathogenic bacteria (*Escherichia coli*, *Campylobacter jejuni*) and gram-positive bacteria selected as probiotic bacterial species often used in dairy products (*Bifidobacterium bifidum*, *Lactobacillus acidophilus*). It has

been found that some blue honeysuckle infusions can elicit high antimicrobial activity against foodborne pathogens without strong inhibition towards probiotics. Consequently, tested probiotic bacteria with honeyberry extract can be used in the food processing industry as potential antimicrobials and functional components [7].

The studies on bioactivity of honeysuckle berries started only recently, the data are limit. It is well known that composition of bioactive compounds depends on genotype, climatic conditions, agronomic practices. This is preliminary report on honeysuckle berries planted in Lithuania. The aim of this study was comparative study of composition and antimicrobial properties in eleven cultivars of honeysuckle berries grown in Lithuania.

2. Materials and Methods

Berries of eleven *Lonicera caerulea* L. cultivars were grown in collection of Vytautas Magnus University Botanical Garden and collected at maturation stage. Fresh berries were homogenized by blender (Bosch MSM16500) and stored at 4 °C temperature in the refrigerator.

The dry matter content was determined by drying crushed berries in moisture analyzer MB 64M, pH of crushed berries was determined directly by pH-meter (Denver Instrument Company, USA). The soluble solids were determined by refractometer Atago RX-5000CX (Japan). Colour CIE L*a*b* characteristics was evaluated by chroma meter CR-410 (Konica Minolta).

Ethanolic extract: 2 g of crushed berries were extracted with 15 mL of 95% (*v*/*v*) food grade ethanol acidified with 0.1 N HCl in an ultrasonic bath (Ultrasonix cleaner proclean 3.ODSP) for 20 min. The obtained extract was decanted and a new 15 mL portion of solvent was used. Extraction was repeated three times, all three extracts combined and volume adjusted to 50 mL with acidified ethanol. This extract was used for total phenolics and total anthocyanins content evaluation.

Total anthocyanin content: the absorption of 1:10 diluted ethanolic extract was measured on a spectrophotometer Genesys-5 (Thermo Spectronic, Rochester, NY, USA) at 535 nm. The concentration of anthocyanins was determined from the calibration curve, which was constructed by measuring the absorption of cyanidin-3-glucoside (MW 449.4, ε = 26.900) reference solution.

Total phenolic content (TPC). The TPC was measured with Folin–Ciocalteu reagent [8]. 1 mL of sample were mixed with 5 mL of 10-fold diluted (*v*/*v*) Folin–Ciocalteu reagent and 4 mL of 7.5% Na_2CO_3. After incubation for 30 min at room temperature in the dark, the absorbance was measured at 765 nm. The results were expressed in mg of gallic acid equivalents.

Vitamin C content. Extract for vitamin C determination was prepared according slightly modified method Auzanneaau et al. [1]. 5 g of berry paste were extracted with 10 mL of oxalic acid solution (10 g/L) in an ultrasonic bath for 20 min. After centrifugation for 10 min at 5300 rpm (2700 g), the obtained supernatant was filtered through a paper filter and then through a 0.22 μm pore size membrane filter. Shimadzu Prominence series (Shimadzu Corp., Kyoto, Japan) HPLC system with Atlantis dC18 5 m 4.6 × 150 mm column (Waters, Ireland) was used for separation and quantification of vitamin C. Mobile phase A—0.1% TFA in H_2O, B—0.1% TFA in ACN. Time program: B Conc. 0% → 3% (0.0–5.0 min) → 15% (6.0 min) → 20 % (10.0 min) → 100% (12.0 min) → 100% (25.0 min). Flow rate 1.4 mL/min. Column temperature 30 °C, injection volume 20 μL. Vitamin C was recorded at 210 nm using a SPD-M20A diode array detector (Shimadzu Corp., Kyoto, Japan). Quantification of vitamin content was done using calibration curve of standard solutions.

Determination of saccharides. An aqueous extract was prepared from 2 g of berry paste [9]. Samples were extracted with 60 mL of water in a water bath at 60 °C for 30 minutes (GFL No-1092, Germany), then clarified with Carrez I and Carrez II solution, diluted to 100 mL with water and filtered through a paper filter followed by a 0.22 μm pore size membrane filter. Obtained filtrate was used for HPLC analysis. Separation conditions were as follows: the eluent was a mixture of 75 parts by volume of ACN and 25 parts by volume water, flow rate was 1.2 mL/min, 20 μL was injected. The YMC-Pack Polyamine II 250 × 4.6 mm, 5 μm (YMC Co., Ltd., Japan) column was used with a temperature of 28 °C. Detection was performed using an Evaporative Light Scattering Detector ELSD-LTII (Shimadzu Corp., Japan). Calibration curves of fructose, glucose and sucrose were used for the quantification.

Antimicrobial activity was evaluated by the agar well diffusion method. Different extracts were prepared from homogenized berries to analysis. 2 g of berry paste were extracted with 8 mL of water in an ultrasonic bath (Ultrasonix cleaner proclean 3.ODSP) for 30 min. It was then centrifuged for 10 min at 5300 rpm (Labofuge 200 Heraeus Thermo Scientific. Rotor 3760, 2700 g). Ethanolic extract was prepared from 3 g of berry cakes and 10 mL of ethanol. Extraction and centrifugation conditions were the same. Obtained supernatants were used for evaluation of antimicrobial activity. Undesirable in food products the yeasts and bacteria were used in the test cultures. Bacteria were grown in peptone-soy bouillon. Yeasts were grown on a slant potato dextrose agar. Eight-millimeter diameter wells were pushed in the agar and filled with 50 μL of sample. The plates were incubated overnight at 37 °C. After incubation the inhibition zones were measured and the effect was calculated as a mean of three replicate tests.

3. Results

3.1. pH, Soluble Solids and Dry Matter Amount of Honeysuckle Berries

Eleven cultivars of *Lonicera caerulea* L. were investigated in this study. Berries were grown in the Vytautas Magnus University Botanical Garden collection. Only fruits at maturity stage, based on the colour and texture were collected. After crushing the berries pH, total soluble solids and dry matter amount was evaluated (Table 1).

Table 1. pH, TTS and dry matter content in fresh honeysuckle berries. Dry matter content and TSS are expressed as means of triplicate analysis, pH was measured in one uniform sample.

Cultivar	pH	TSS, °Brix	Dry Matter, %
'Eisbar'	3.13	12.10 ± 0.021	15.41 ± 0.332
'Leningradskaja'	3.37	13.01 ± 0.021	14.35 ± 0.262
'Čelnočnaja'	3.04	11.03 ± 0.007	11.70 ± 1.414
'Vostorg'	3.17	11.03 ± 0.017	17.36 ± 1.252
'Morena'	2.99	12.45 ± 0.044	16.34 ± 0.184
'Obilnaja'	3.04	12.10 ± 0.014	13.93 ± 0.078
'Pavlovskaja'	3.02	10.57 ± 0.028	15.03 ± 0.987
'Pereselenka'	3.01	10.32 ± 0.028	13.40 ± 0.658
'Nimfa'	3.14	10.32 ± 0.106	18.49 ± 1.853
'Kalinka'	2.99	10.07 ± 0.402	15.72 ± 1.181
'Balalaika'	3.37	16.09 ± 0.078	18.41 ± 0.262

pH measured in fresh berries ranged from 2.99 ('Morena' and 'Kalinka' cultivars) to 3.37 ('Leningradskaja' and 'Balalaika' cultivars).

Dry matter content was around 15%. The highest values of total soluble solids (16.09 °Brix) and dry matter content (18.41%) were obtained for 'Balalaika' cultivar. The lowest dry matter content (11.70%) was detected in 'Čelnočnaja'.

On average TSS was 11.74 °Brix. Other authors reported that TSS values in the same studied cultivars depended on year: for 2016 it was 10.8 °Brix, for 2015 and 2014, i.e., 14.8 and 14.6, respectively [1].

3.2. Qualitative and Quantitative Composition of Saccharides

Fructose and glucose were predominant free sugars in honeysuckle beries. Only in 'Čelnočnaja', 'Pavlovskaja' and 'Pereselenka' cultivars was found sucrose (Figure 1).

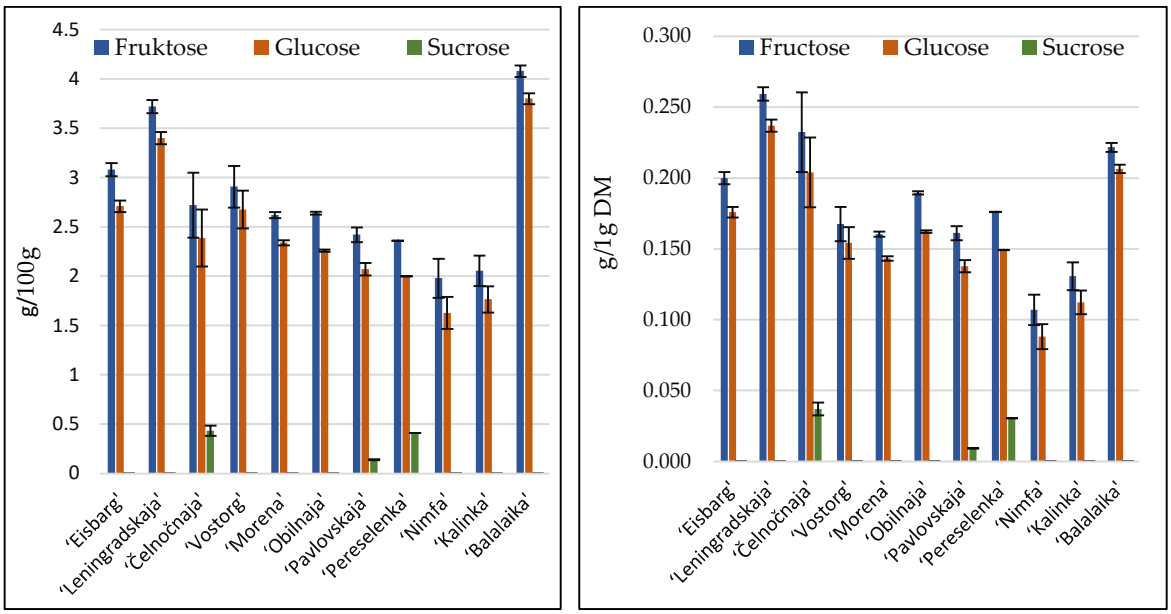

Figure 1. Glucose, fructose and sucrose content in eleven cultivars of honeysuckle beries: (**a**) g/100 g; (**b**) g/g DM. Data are mean of triplicate analysis with standard deviation.

The differences in sacharides content between cultivars were observed. Glucose content in samples ranged from 3.80 g/100 g ('Balalaika') to 1.62 g/100 g ('Nimfa'), and from 0.24 g/g DM ('Leningradskaja') to 0.09 g/g DM ('Nimfa'). Fructose content was higher and ranged from 4.08 g/100 g ('Balalaika') to 1.98 g/100 g ('Nimfa'), and 0.26 g/g DM ('Leningradskaja') to 0.11 g/g DM ('Nimfa'). 'Nimfa' seems to be the cultivar with the lowest content of sacharides. The highest content of sucrose (0.43 g/100 g) was found in 'Čelnočnaja' cultivar, less was found in 'Pereselenka' (0.41 g/100 g) and 'Pavlovskaja' (0.14 g/100 g) cultivars. Glucose content in berries grown in Switzeland in the 3 years period was between 80.0 and 327 mg/g DM, fructose content was in the range of 140–337 mg/g DM [1]. Content of sacharides is an important parameter of berries, because determines the taste and has great influence on consumer acceptibility.

3.3. Ascorbic Acid Content

Another important parameter in the composition of berries is the amount of ascorbic acid. It was in average 3.3 mg/g DM. Unusually large amnount of vitamin C was found in 'Pereselenka' (10.5 mg/g DM) and 'Pavlovskaja' (7.6 mg/g DM) cultivars (Figure 2). If calculate the average value after excluding maximum values it would be 34.3 g/100 g and 2.28 mg/g DM. Vitamin C content was the lowest in 'Nimfa' cultivar—13.46 g/100 g and 0.73 g/g DM. Ascorbic acid content reported for honeysuckle beries grown in Switzeland were 1.78–4.21 mg/g DM [1], grown in Slovenia—17.75–25.77 mg/100 g [2]. The differences coud be result of different extraction techniques and growing conditions.

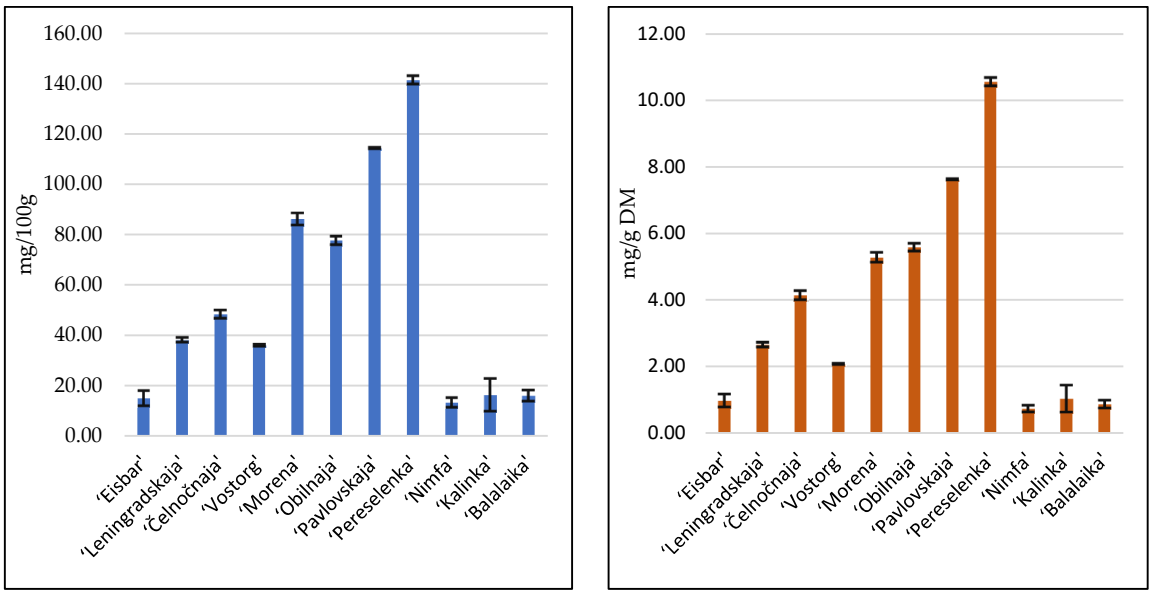

Figure 2. Content of ascorbic acid in eleven cultivars of honeysuckle beries: (**a**) g/100 g; (**b**) g/g DM. Data are mean of dublicate analysis with standard deviation.

3.4. Chromatic Properties of Honeysuckle Berries

Cromatic properties of honeysuckle berries were presented in Table 2. The L* values of the *Lonicera caerulea* L. cultivars were not variable and ranged from 23.34 to 26.44. The cultivar 'Pereselenka' was characterized by a high a* (13.15) and b* (5.33) values, also the chroma C (14.18) and hue angle h° (0.38) for this cultivar was the highes. The lowest values of C and h° were determined in 'Balalaika' cultivar, 6.90 and 0.18 respectively.

Table 2. Colour characteristics of eleven cultivars fresh honeysuckle berries. Results are expressed as means of triplicate analysis and standard deviation.

Cultivar	L*	a*	b*	C	h°
'Eisbar'	25.68 ± 0.11	10.23 ± 0.33	2.83 ± 0.09	10.61 ± 0.34	0.27 ± 0.01
'Leningradskaja'	25.53 ± 0.13	6.85 ± 0.04	1.36 ± 0.06	6.98 ± 0.05	0.20 ± 0.01
'Čelnočnaja'	25.76 ± 0.09	10.28 ± 0.03	3.06 ± 0.01	10.73 ± 0.03	0.29 ± 0.01
'Vostorg'	24.73 ± 0.60	9.09 ± 0.06	2.34 ± 0.08	9.38 ± 0.04	0.25 ± 0.01
'Morena'	23.99 ± 0.09	6.91 ± 0.02	1.39 ± 0.01	7.04 ± 0.02	0.20 ± 0.01
'Obilnaja'	23.61 ± 0.76	8.30 ± 0.12	1.81 ± 0.01	8.49 ± 0,12	0.21 ± 0.01
'Pavlovskaja'	25.91 ± 0.58	10.22 ± 0.36	3.15 ± 0.16	10.69 ± 0.39	0.30 ± 0.01
'Pereselenka'	28.22 ± 1.01	13.15 ± 0,39	5.33 ± 0.16	14.18 ± 0.42	0.38 ± 0.00
'Nimfa'	23.34 ± 1.87	8.58 ± 1.14	2.19 ± 0.29	8.85 ± 1.18	0.25 ± 0.01
'Kalinka'	26.44 ± 0.13	8.30 ± 0.05	2.23 ± 0.06	8.59 ± 0.06	0.26 ± 0.01
'Balalaika'	24.41 ± 0.16	6.79 ± 0.04	1.23 ± 0.06	6.90 ± 0.03	0.18 ± 0.01

3.5. Total Phenolic Compounds and Anthocyanins Content

Total phenolic compounds and anthocyanins content presented in Figure 3. The average content of total phenolic compounds expressed in mg of gallic acid equivalents was 492.1 mg/100 g and 31.6 mg/g DM, total anthocyanins—451.5 mg of cyanidin-3-glucoside equivalents/100 g and 29.2 mg of cyanidin-3-glucoside equivalents /g DM. Literature refers to values between 8.4 and 65 mg cyanidin-3-glucoside equivalents/g DM and from 7.0 to 57.1 mg gallic acid equivalents/g DM [1].

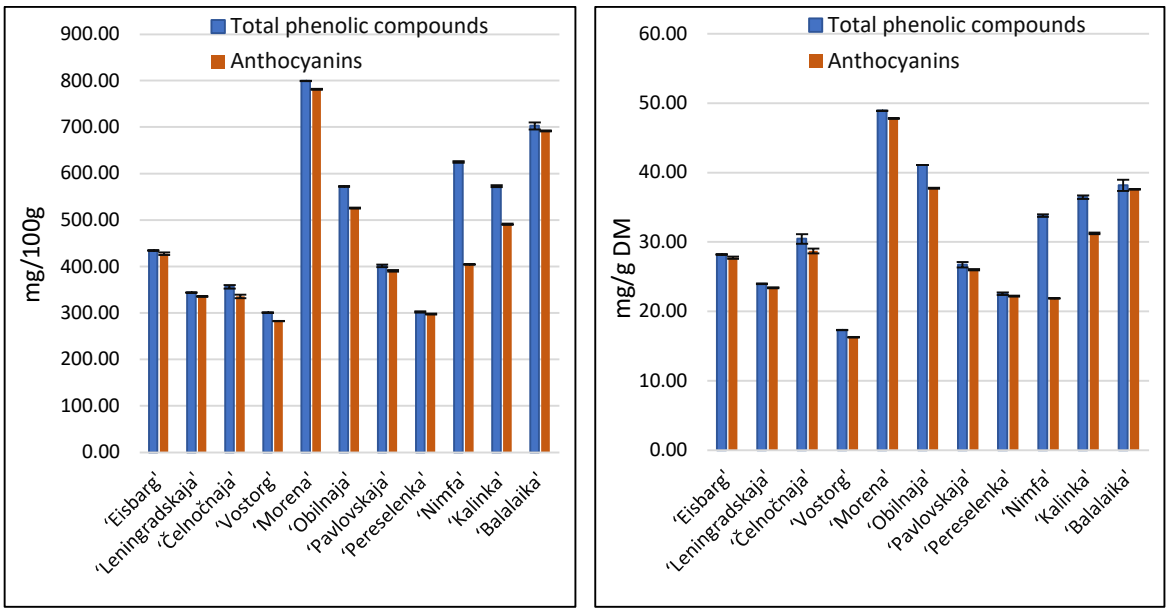

Figure 3. Content of total phenolic compounds and anthocyanins in eleven cultivars of honeysuckle berries: (**a**) g/100 g; (**b**) g/g DM. Data are mean of triplicate analysis with standard deviation.

The cultivar 'Morena' had the highest anthocyanins (781 mg/100 g) and total phenolic compounds (799 mg/100 g), the lowest anthocyanins (282 mg/100 g) and TPC (300 mg/100 g) content was detected in 'Vostorg' cultivar. The same tendency was observed after conversion to mg/g DM.

3.6. Antimicrobial Activity

Investigation of antimicrobial activity showed that aqueous extracts of honeysuckle berries weakly inhibit the growth of gram-negative and gram-positive test cultures (Table 3).

B. subtillis were the most sensitive, zones of inhibition were 9.5–11.0 mm. *S. typhimurium* showed largest resistance to aqueous berry extracts, only 'Kalinka' and 'Balalaika' cultivars showed 9.0 mm and 8.5 mm zones of inhibition. No inhibition zone was observed against yeast *C. albicans* and *S. cerevisea.*

Berry cakes, by-product of juice extraction, are of particular interest because they could be used as a cheap raw material for functional ingredients production. Investigation of antimicrobial activity of ethanolic extracts of berry cakes showed greater inhibition effect compare to aqueous berry extracts on test cultures (Table 4). Only *E. coli* were resistant to 'Eisbar' and 'Leningradskaja' cultivars. Again no inhibition zone was observed against yeast *C. albicans* and *S. cerevisea*. Pure ethanol used as control wasn't show any inhibition effect on test cultures.

Table 3. The antimicrobial influence of aqueous berry extracts on test cultures. Inhibition zone in mm, including the 8 mm hole. If inhibition zone wasn't observed, results is presented as 0.

Cultivar	*C. freundii*	*E. coli*	*E. feacalis*	*S. typhimurium*	*L. monocytogenes*	*S. aureus*	*P. aeruginosa*	*B. subtillis*
'Eisbar'	0	8.50 ± 0.00	9.00 ± 0.00	0	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	10.00 ± 0.00
'Leningradskaja'	0	8.50 ± 0.00	8.75 ± 0.35	0	9.00 ± 0.00	9.00 ± 0.00	8.75 ± 0.35	10.50 ± 0.71
'Čelnočnaja'	9.00 ± 0.00	8.50 ± 0.00	8.75 ± 0.36	0	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	10.50 ± 0.01
'Vostorg'	0	8.50 ± 0.00	8.5 ± 0.00	0	9.00 ± 0.00	8.50 ± 0.71	8.75 ± 0.35	10.00 ± 0.00
'Morena'	9.00 ± 0.00	8.50 ± 0.00	8.5 ± 0.00	0	9.00 ± 0.00	8.25 ± 0.35	9.00 ± 0.00	11.00 ± 1.41
'Obilnaja'	9.00 ± 0.00	8.75 ± 0.35	8.75 ± 0.35	0	9.00 ± 0.00	0	8.75 ± 0.35	10.50 ± 0.71
'Pavlovskaja'	8.50 ± 0.00	8.50 ± 0.00	8.50 ± 0.00	0	9.00 ± 0.00	8.50 ± 0.71	8.75 ± 0.35	9.50 ± 0.71
'Pereselenka'	9.00 ± 0.00	8.25 ± 0.35	8.25 ± 0.35	0	9.00 ± 0.00	8.00 ± 0.00	9.00 ± 0.00	11.00 ± 1.14
'Nimfa'	9.00 ± 0.00	8.75 ± 0.35	8.75 ± 0.35	0	10.00 ± 0.00	9.00 ± 0.00	8.50 ± 0.71	11.00 ± 0.00
'Kalinka'	8.50 ± 0.00	8.50 ± 0.00	8.50 ± 0.00	9.00 ± 0.00	8.50 ± 0.71	8.00 ± 0.00	8.25 ± 0.35	10.50 ± 2.12
'Balalaika'	8.50 ± 0.00	0	8.50 ± 0.00	8.50 ± 0.00	9.00 ± 0.00	8.25 ± 0.35	8.75 ± 0.35	10.00 ± .0.00

Table 4. The antimicrobial influence of ethanolic berry cakes extracts on test cultures. Inhibition zone in mm, including the 8 mm hole. If inhibition zone wasn't observed, results is presented as 0.

Cultivar	*C. freundii*	*E. coli*	*E. feacalis*	*S. typhimurium*	*L. monocytogenes*	*S. aureus*	*P. aeruginosa*	*B. subtillis*
'Eisbar'	14.00 ± 0.00	0	13.67 ± 0.58	14.00 ± 0.00	11.00 ± 0.00	14.33 ± 0.57	10.00 ± 0.000	10.00 ± 0.000
'Leningradskaja'	15.00 ± 1.00	0	14.00 ± 0.00	12.67 ± 0.58	13.00 ± 0.00	13.33 ± 0.57	10.00 ± 0.00	11.33 ± 0.58
'Čelnočnaja'	15.33 ± 0.58	13.67 ± 0.57	15.00 ± 0.00	15.00 ± 0.00	14.00 ± 0.00	15.00 ± 0.00	9.00 ± 0.00	13.00 ± 0.00
'Vostorg'	13.67 ± 0.58	11.67 ± 0.58	13.33 ± 0.58	14.00 ± 0.00	14.00 ± 1.00	12.67 ± 1.53	9.33 ± 0.58	11.33 ± 0.58
'Morena'	13.00 ± 0.00	10.33 ± 0.58	14.33 ± 0.58	15.67 ± 0.58	9.33 ± 0.58	13.00 ± 0.00	9.00 ± 0.00	10.00 ± 0.00
'Obilnaja'	17.00 ± 0.00	13.00 ± 0.00	15.00 ± 0.00	14.67 ± 0.58	13.67 ± 0.58	15.33 ± 1.16	10.33 ± 0.58	11.00 ± 0.00
'Pavlovskaja'	15.67 ± 0.58	14.00 ± 0.00	12.33 ± 0.58	16.67 ± 1.16	12.67 ± 0.58	14.33 ± 0.58	10.67 ± 0.58	9.00 ± 0.00
'Pereselenka'	16.00 ± 0.00	9.33 ± 0.58	13.00 ± 0.00	14.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	9.33 ± 0.58	10.67 ± 0.58
'Nimfa'	9.33 ± 0.58	9.00 ± 0.00	13.00 ± 0.00	14.33 ± 0.58	11.33 ± 1.15	14.67 ± 0.58	9.33 ± 0.58	11.00 ± 0.00
'Kalinka'	12.33 ± 1.53	13.67 ± 1.53	13.00 ± 0.00	16.00 ± 0.00	12.00 ± 0.00	16.00 ± 0.00	10.00 ± 1.00	11.00 ± 0.00
'Balalaika'	16.33 ± 1.16	9.00 ± 0.00	13.00 ± 0.00	15.00 ± 0.00	9.33 ± 0.58	14.00 ± 0.00	9.00 ± 0.00	9.33 ± 0.58
Ethanol	0	0	0	0	0	0	0	0

4. Conclusions

Investigation of the antimicrobial properties showed that European cranberry extracts inhibited the growth of wide range of human pathogenic bacteria, both gram-negative (*Escherichia coli* and *Salmonella typhimurium*) and gram-positive (*Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus subtilis*).

Author Contributions: I.J. conceived and designed the experiments, performed HPLC analysis, analyzed the data, and wrote the paper. A.P. performed preparation of sample, spectrophotometric asay and other measurements. A.Š. performed antimicrobial activity experiment, analyzed the data. L.Č. provided berry material and also did a great contribution in preparing the manuscript. All authors have read and agreed to the published version of the manuscript.

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

Abbreviations

The following abbreviations are used in this manuscript:

HPLC	High-performance liquid chromatography
TPC	Total Phenolic Compounds
TFA	Trifluoroacetic acid
ACN	Acetonitrile
DM	Dry matter

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