Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2017, 9(1):77-83. DOI: 10.15835/nsb9110035
Notulae Scientia Biologicae
Original Article

Total Phenol Content and Antimicrobial Activity of Lingonberry (*Vaccinium vitis-idaea* L.) from Several Areas in the Eastern Carpathians

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Abstract

This study evaluated the antimicrobial activity and the total phenol content of *Vaccinium vitis idaea* L. berry fruit from five different localities with distinct growth sites in the Eastern Carpathians. The antibacterial effect of lingonberry was studied on nine selected Gram-positive and negative, foodborne, and illness causing and spoilage bacteria. The total phenol content was estimated by the Folin-Ciocalteu method. The present results showed stronger antibacterial effect of lingonberry on Gram-negative bacteria, especially on *Pseudomonas fluorescens* and *Pseudomonas aeruginosa.* The total phenol content varied between 3.72 and 2.1 mM GAE/ml. As data suggested, *Vaccinium vitis-idaea* fruits originating from different geographic regions and environment, differ from each other in terms of bioactive compound quantity and activity. In the selection of new perspective cultivars of lingonberry, the geographical origin of fruits must be considered. Two Step Cluster analysis detected relatively well supported relationship between samples provided from similar growth sites. Correlation analysis showed no correlation between altitude, phenol content and antimicrobial activity.

Keywords: antibacterial effect, lingonberry, total phenolics, *Vaccinium vitis-idaea*

Introduction

Lingonberry fruit (*Vaccinium vitis idaea* L.) is an important berry crop harvested from the wild throughout its distribution in northern regions of the world. Throughout history, it has been used as a supplier of energy and vitamins, confering health benefits. It's fruit differs from most of the other wild berry species in long term storage potential. This storage ability depends primarily on their benzoic acid content, with up to 65 mg of benzoic acid per 100 g of berries (Hjalmarsson and Ortiz, 2001). Several studies have shown that it is a rich source of bioactive compounds, and the interest with regard to its composition has been intensified due to the increased awareness of possible positive health benefits (Gündüz, 2013). The chemical constituents have been well documented. It is rich in fibre, vitamins, minerals, in various phenolic compounds and organic acids. Lingonberry provides significant health benefits due to it's bioactive composition. Phenolic compounds, which are secondary metabolites present in all higher plants, act as defence compounds against plant pathogens (Burdulis *et al*., 2009) and their production is often induced as a response to various stress conditions. The chemical substituents of *Vaccinium vitis idaea* L. show antioxidative, antibacterial, anti-inflammatory, antitumor, antiviral, vasoprotective and antifungal activities (Negi, 2012; Su, 2012). Flavonoids, phenolic acids, lignans and complex phenolic polymers (polymeric tannins) are the typical substituents, which are rich sources of flavonoids, such as flavonols. Kylli *et al.* (2011) considered, that the main phenolic compounds in lingonberry are proanthocyanidins, comprising 63-71% of the total phenolic content. According to the results of Makkar *et al.* (2009) the most important secondary metabolites of lingonberry are: 63.2 g/kg dry matter crude protein, 33.3 g/kg dry matter ether extract, 476.1 g/kg dry matter neutral detergent fibre, 354.1 g/kg dry matter acid detergent fibre, 243.3 g/kg dry matter total phenols, 149.2 g/kg dry matter total tannins, 174.5 g/kg dry matter condensed tannins, 96.3 g/kg dry matter tannin activity; percents increase in gas on addition of polyethylene glycol. In fact, wild berries contain more flavonols than many vegetables and fruits commonly used (Puupponen-Pimiä *et al*., 2005). Anthocyanins (anthocyanidin glycosides) are the predominating group of flavonoids (up to 2,000-5,000 mg/kg fresh weight) (Määttä *et al*., 2001). More simple phenolic acids, such as hydroxycinnamic acids and hydroxybenzoic acids, are also common (Herrmann, 1989). Chlorogenic acid, which is an ester

Received: 18 Feb 2017. Received in revised form: 08 Mar 2017. Accepted: 12 Mar 2017. Published online: 30 Mar 2017.

between caffeic acid and quinic acid, is a commonly occurring compound. Lingonberry also contains condensed tannins, called proanthocyanidins (Chung *et al*., 1998). It is rich in diphenolic compounds called lignans (10-15 mg/kg dry weight (Mazur *et al*., 2000), whereas more than 116 anthocyanins and flavonoids compounds have been isolated and identified primarily from the fruits or leaves (Kalt *et al*., 2008; Su, 2012). Rimando *et al*. (2004) detected pterostilbene, piceatannol and resveratrol in an amount of 5,884 ng/g dry sample. Ek *et al*. (2006) identified a total of 28 phenolic compounds, including flavonols, anthocyanidins, catechins and their glycosides. The leaf volatiles of lingonberry have been analyzed by Radulovic *et al*. (2010), who identified 187 terpenoids, the major contributors being α-terpineol (17.0%), pentacosane (6.4%), (E, E)-α-farnesene (4.9%), linalool (4.7%) and (Z)-hex-3-en-1-ol (4.4%). Fatty acid derived compounds (34.1%) were also identified, while Liu *et al*. (2014) found arbutin as the major phenolic compound. Organic acid content of *Vaccinium vitis-idaea* was described by Jensen *et al*. (2002) with a high level of hydrophilic carboxylic acids of 2.27-3.05% and iridoid glucosides. Szakiel *et al*. (2012) managed to extract oleanolic and ursolic acid from fruits and leaves of lingonberry. Häkkinen *et al*. (1999) detected p-coumaric, caffeic, ferulic, p-hydroxybenzoic, gallic and ellagic acids. The analyses of Radulovic *et al*. (2010) demonstrated the presence of hexanoic, octanoic, nonanoic and dodecanoic acid and different fatty acid esters. Ek *et al*. (2006) identified different caffeoyl and ferulic acid conjugates and Kylli *et al*. (2011) found hydroxycinnamic and hydroxybenzoic acids. The two isomeric acids, oleanolic and ursolic, can be identified as the main triterpenoid constituents in *V. vitis-idaea*. Bere (2007) noticed, that n-6 fatty acids and n-3 fatty acids were present in lingonberry, in 0.14 respectively 0.18 g/100g concentration. According to Viljakainen *et al*. (2003), the main acids of the northern region wild-berry juices are citric and malic acids, while juice of lingonberry contain benzoic acid in amount of 0.1-0.7 g/l. In case of lingonberry, it is the benzoic acid concentration which is especially high (up to 1.3 g/l free benzoic acid) and the pH is very low (pH 2.6-2.9). Thus, evaluating the antimicrobial efficacy of phenolic compounds either in food matrices or in human body, pH was a very important parameter to be considered (Puupponen-Pimiä, 2005). It was demonstrated that lingonberry may affect the activity of several human pathogenic and food spoilage bacteria (Caillet *et al*., 2012). Several studies have demonstrated that this berry fruit possesses a high antimicrobial activity (Lehtonen *et al*., 2013). Geoghegan *et al*. (2010) demonstrated the inhibitory effect of quercetin from lingonberry against *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. According to the results of Türi *et al*. (1999), aqueous extracts from the fruit contain substances enhancing the aggregation of *E. coli*. *V. vitis-idaea* extracts can cause 50% decrease in *E. coli* viability (Samoilova *et al*., 2014) with a high growth-inhibitory effect (Wojnicz *et al*., 2012). Vamanu *et al*. (2013) showed that *V. vitis idaea's* tincture was effective against potentially pathogenic strains, such as *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida strains*, *Listeria strains* and *Bacillus cereus*. Phenolic acids potentially act as metabolic inhibitors of proline in *Listeria monocytogenes* (Lacombe, 2010).

In the current study, the first specific objective was to investigate the antimicrobial activity of lingonberry fruits against some Gram-positive and Gram-negative foodborne, illness causing and spoilage bacteria: *Escherichia coli, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Pseudomonas fluorescens, Listeria monocitogenes S1, Listeria monocitogenes S2, Serratia marcescens* and *Staphylococcus aureus*. The second main goal was to determine the total phenolic content of lingonberry samples using different growth site origin (5 locations in the Eastern Carpathians).

Materials and Methods

Study sites

Lingonberry samples from 5 locations in the Eastern Carpathians were collected between 18th and 23rd of August 2013. All these locations are within the natural range of the species and can be characterized as habitat types like peat bogs, pine forests and alpine meadows. The sample collection sites are illustrated in Fig. 1 and the geographic coordinates are summarized in Table 1.

Sampling design

Berries in consumption maturity were picked from five different locations of Eastern Carpathians. The sampling sites were chosen by the type of the habitat. The growth sites differed considerably in environmental conditions such as humidity, light exposure, surrounding vegetation, soil type and altitude. The environmental conditions of the peat bog in

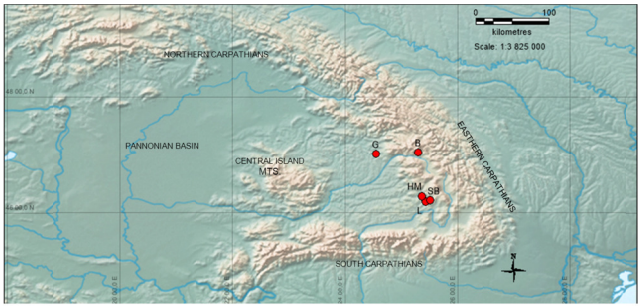

Fig. 1. The lingonberry sample collection sites: L- Tinovul Luci; HM – Harghita-Mădăras; SB – Sântimbru Băi; G – Gălăoaia; B – Bilbor

Table 1. List of *Vaccinium vitis idaea* samples populations from the Eastern Carpathians

No.	Code	Country	Residential area	Latitude (N)	Longitude (E)	Altitude (m)	Habitat type
1	L	RO	Tinovul Luci	46.18	25.43	1083	peat bog
2	HM	RO	Harghita-Mădăras	46.27	25.36	1681	alpine meadow
3	SB	RO	Sântimbru Băi	46.21	25.51	1250	alpine meadow
4	G	RO	Gălăoaia	47.01	24.54	1381	edge of pine forest
5	B	RO	Bilbor	47.03	25.29	1173	edge of pine forest

Note: L- Tinovul Luci; HM – Harghita-Mădăras; SB – Sântimbru Băi; G – Gălăoaia; B – Bilbor (the site origin in the Eastern Carpathians of lingonberry).

Tinovul Luci were the following: humid microclimate, dense and homogeneous woody crops, and middle shadow. On the alpine meadows at Sântimbru Băi and Harghita-Mădăras's alpine meadow there were sunny hillside, wind exposure and low humidity, whilst the residential area of the population in Gălăoaia and Bilbor can be characterized as a wind protected, sunny hillside with scattered woody vegetation. In Tinovul Luci's peat bog, samples were collected from 4 points with homogenous edaphic conditions, with a distance of about three kilometres between one from another: the first from the edge of the peat bog, the second from nearby the Kormos creek, the third from the edge of the *Betula nana* population situated in the peat bog, and the fourth sampling point was located east from the previous ones. From the manually collected lingonberries 200 g samples were weighted and stored in PE bags. Unlike the others, the samples from Harghita-Mădăras (HM) were stored in a water filled recipient. At the end of each collection day, samples were cooled at 5 °C. In the laboratory, all the samples were frozen and stored at –20 °C until the analysis.

Sample preparation

In parallel 2.2 grams of the defrosted berries were measured in two Eppendorf tube, homogenized in a blender, centrifuged at 14,000 rpm for 10 min. During the experiments, the resulted supernatant was used.

Antibacterial activity

To determine the antibacterial effect, a number of nine Gram-positive and Gram-negative foodborne, illness causing and spoilage bacterial strains were selected. These bacterial strains were*: Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Serratia marcescens, Bacillus subtilis, Pseudomonas fluorescens, Listeria monocytogenes S1* and *Listeria monocytogenes S2.* The bacterial cultures were grown 24 h at 28 °C and respectivelly 37 °C on Nutrient and TSB agar. 0.1 ml bacterial suspension with 0.5 turbidity in physiological solution was spread in case of each bacteria with surface streaking on Nutrient agar (meat extract 1 g, yeast extract 2 g, peptone 5 g, NaCl 5 g, agar 15 g, distilled water 1,000 ml) and TSB agar (casein 17 g, peptone from soybean 3 g, NaCl 5 g, dipotassium phosphate 2.5 g, glucose 2.5 g, agar 20 g, distilled water 1,000 ml) plates. In centre of all inoculated mediums a 8 mm diameter hole was cut with the help of a sterile test-tube. In the hole, 0.05 ml of lingonberry supernatant was dropped. The incubation was realized at 37 °C in the case of *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* at Petri dishes inoculated at 28 °C with *Bacillus cereus, Serratia marcescens, Bacillus subtilis, Pseudomonas fluorescens, Listeria monocytogenes S1, Listeria monocytogenes S2.* The inhibitory area dimension was measured. The antibacterial effect of lingonberry samples were expressed in accordance with the size of the inhibition zone. For *Listeria monocytogenes* species the TSB agar was used.

Determination of the total phenol content

The total phenol content of the lingonberry samples was determined using the gallic acid equivalence method (GAE). The principle of the method is that the reagent and the phenols hydroxid group form a blue coloured complex and the absorbance of the solution is proportional with the phenol content of the sample (Singleton *et al*., 1999). To 100 µl supernatant 5 ml ultrapure water, 500 µl Folin-Ciocalteau reagent and 1.5 ml of 20% sodium carbonate solution was added, with a parallel reagent blank preparation. The resulting extract was incubated at 40 °C for 30 minutes. After incubation, the absorbance on 765 nm was measured. The phenol content – gallic acid equivalence was determined using a calibration curve. The total phenol content of the samples was determined using a calibration line equation and expressed in mM gallic acid/ml.

Data analysis

Correlation-analysis was used to detect relationship between variables: altitude and phenol content and antimicrobial effect in light of phenol content. Two-Step Cluster analysis was performed to reveal natural groupings of samples within the dataset that would otherwise not be apparent.

Results

Antibacterial effect

The effects of the lingonberry extracts separately on Gram-positive and Gram-negative bacteria are shown in Tables 2 and 3.

The analyzed lingonberry samples showed bacteriostatic effect against the studied bacterial strains. On Gram-positive bacteria, the most inhibitory effect was detected at *Staphylococcus aureus,* with a large inhibition zone of 26.68 ±4.82 mm, using the L2 sample.

For the two *Bacillus* strains, the inhibition zone varied between 20.51 ± 1.28 -15.34 ± 0.45 (*B. cereus*) and 18.25 ± 0.74-12.41 ± 0.85 (*B. subtilis*). In both cases, the most effective samples were the SB and L1. A slight bacteriostatic effect was shown at HM samples. The two *Listeria monocytogenes* strains were slightly inhibited by the HM (*Listeria monocytogenes S2* inhibition zone 11.41 ± 0, *Listeria monocytogenes S1* 9.4 ± 0) and the most effective antibacterial effect was observed in the case of L2 sample of *Listeria monocytogenes S2* (18.86 ± 1.05) and SB sample of *Listeria monocytogenes S1* (14.84 ± 0.11).

The antibacterial effect against Gram-negative bacteria was the most promising. The largest inhibitory zones were observed on the two *Pseudomonas* species. On the studied *Pseudomonas fluorescens* the measured inhibition zones varied between 21.58 ± 0.46 mm (L2 lingonberry sample), -30.33 ± 0.1 mm (L4 lingonberry sample). The inhibition zone dimensions were 22.85 ± 0.88 mm (L2 sample) and 36.42 ± 4.18 mm (L4 sample). In both cases, the most effective activity was detected on L4 sample.

Inhibition zone diameter (mm) varied between 12.33 ± 0 (HM)-17.66 ± 0,17 MM (L2) on the assayed *Escherichia coli.* Regarding the food spoilage bacteria *Serratia marcescens,* the determined inhibition zone diameter varied between 9.84 ± 0 mm (HM) and 16.37 ± 1.95 mm (L2).

Total phenol content

The total phenol content varied between 3.72 and 2.1 mM/ml (Table 4). The highest values were found in the case of sample L2. The highest phenol content was determined on L2 lingonberry sample collected from Tinovul Luci, near the

Table 2. Antimicrobial effect of lingonberry samples on Gram-positive bacteria

Lingonberry sample code	*Bacillus cereus*	*Bacillus subtilis*	*Listeria monocytogenes* S2	*Listeria monocytogenes* S1	*Staphylococcus aureus*
	Inhibition zone diameter (mm) ± SD				
L1	18.41 ±0.56	18.25 ±0.74	13.88 ±2.85	12.14 ±0.58	20.61 ±4.75
L2	18.3 ±0.63	16.12 ±1.21	18.86 ±1.05	12.06 ±0.7	26.68 ±4.82
L3	17.06 ±1.54	16.59 ±1.04	12.61 ±0.39	11.76 ±1.28	19.73 ±4.89
L4	19.27 ±0.14	16.99 ±0.96	16.35 ± 0	10.9 ±0.87	18.9 ±1.71
G	18.25 ±0.1	15.54 ±0.41	13.08 ±2.83	12.95 ± 0	16.89 ±3.57
HM	15.34±0.45	12.41±0.85	11.41 ± 0	9.4 ± 0	16.6±2.21
B	18.72±0.86	16.89±0.27	14.08±2.3	13.83±0.54	18.02±3.64
SB	20.51±1.28	18.17±0.7	13.9±2.49	14.84±0.11	19.89±3.62

Note: L- Tinovul Luci; HM – Harghita-Mădăras; SB – Sântimbru Băi; G – Gălăoaia; B – Bilbor (the site origin in the Eastern Carpathians of lingonberry).

Table 3. Antimicrobial effect of *Vaccinium vitis idaea* L. samples on Gram-negative bacteria

Lingonberry sample code	*Serratia marcescens*	*Escherichia coli*	*Pseudomonas fluorescens*	*Pseudomonas aeruginosa*
	Inhibition zone diameter (mm) ± SD			
L1	13.1 ±0.53	13.97 ±2.53	30.09 ±0.23	34.06 ±1.72
L2	16.37 ±1.95	17.66 ±0.17	21.58 ±0.46	22.85 ±0.88
L3	11.17 ±2	15.06 ±1.06	27.05 ±0.84	33.78 ±1.09
L4	14.43 ±0.52	16.21 ± 0	30.33 ±0.1	36.42 ±4.18
G	13.79 ±0.69	15.79 ± 0	22.32 ±0.96	35.21 ±3.04
HM	9.84 ± 0	12.33 ± 0	24.26±6.2	33.98±0.27
B	13.32±1.8	12.83±1.16	22.55±1.47	33.27±1.64
SB	12.3±0.65	15.32±3.09	27±0.1	34.71±0.22

Note: L- Tinovul Luci; HM – Harghita-Mădăras; SB – Sântimbru Băi; G – Gălăoaia; B – Bilbor (the site origin in the Eastern Carpathians of lingonberry).

Table 4. Total phenol content of the studied lingonberry samples with Eastern Carpathian provenience and from different habitat types (L 1, 2, 3, 4-peat bog; HM, SB- alpine meadow; B, G pine forest)

Code	Absorbance at 765 nm ± SD	Total phenol content (mMgallic acid/ml)
B	0.126± 0.07274	2.8597
HM	0.10185±0.01005	2.3133
SB	0.14825±0.00015	3.3631
G	0.10815±0.00765	2.4559
L4	0.10865±0.00035	2.4672
L3	0.09245±0.00505	2.1007
L2	0.1641±0.0005	3.7217
L1	0.1411±0.0083	3.2013

Kormos creek, 3.7217 mM/ml. The minimum value was detected in the case of L3 sample, with 2.1007 mM/ml, which originated also from peat bog, on the edge of the *Betula nana* population.

Cluster analysis

A dendrogram obtained with IBM SPSS 20.0 Two Step Cluster analysis is presented in Fig. 2. Within the dendrogram using Ward Linkage, samples from Gălăoaia (G) and Bilbor (B) belonging to different sites but with similar type of habitat formed one subcluster and showed a relatively well supported relationship with the sample from Sântimbru băi (SB). The four samples from Tinovul Luci (L1, L2, L3, L4) provenience formed a well supported and separated cluster with a separate subcluster in case of L1 and L4 and in the same group with L3, yet L2 could be characterised by a completely distant position.

Correlation analysis

Using each variable and defining correlation as significant at the 0.01 and 0.05 levels, specific data were obtained as presented in Table 5.

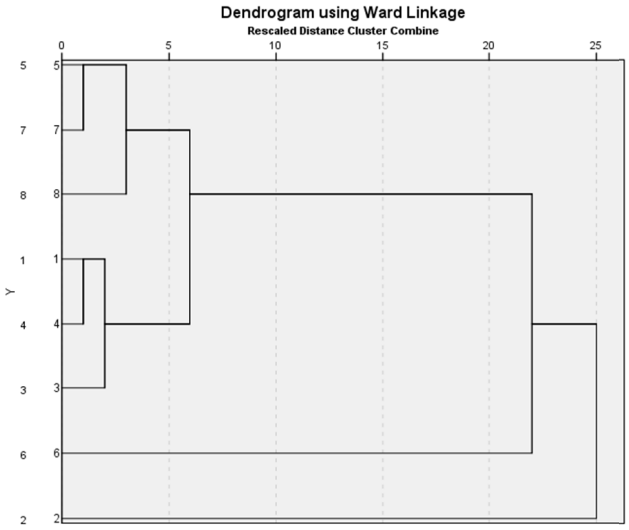

Fig. 2. Dendrogram generated with IBM SPSS 20.0 using Ward Linkage among eight *Vaccinium vitis idaea* L. samples with 5 different Eastern Carpathian provenience (1, 2, 3, 4-Tinovul Luci; 5-Gălăoaia; 6-Harghita-Mădăras; 7-Bilbor; 8-Sântimbru Băi; the site origin in the Eastern Carpathians of lingonberry)

The outcome of correlation analysis showed a strong correlation between antimicrobial effects of the samples against *Bacillus cereus* and *Bacillus subtilis*, *Listeria monocytogenes S1* and *Serratia marcescens, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Significant values have been found between the effect on *Listeria monocytogenes S2* and *Bacillus cereus, Listeria monocytogenes S1* and *E. coli, E. coli* and *Serratia marcescens.* The phenol content was not significantly correlative with altitude or antimicrobial activity.

Table 5. Correlation between each lingonberry sample obtained data: antimicrobial effect on different bacteria, phenol content and altitude; strong correlation is presented in bold, significant values in italics

Correlations

	B. cereus	*B. subtilis*	*E. coli*	*Listeria m. S1*	*Listeria m. S2*	*Pseud. aero.*	*Pseud. fluor.*	*Serratia m.*	*St. aureus*	Phenol content	Altitude
B. cereus	1	**,836****	,453	,468	*,781**	,075	,249	,543	,245	,545	-,573
B. subtilis	**,836****	1	,328	,351	,691	,079	,482	,410	,330	,463	*-,825**
E. coli	,453	,328	1	*,759**	,167	-,471	-,033	*,751**	,669	,377	-,524
Listeria S1	,468	,351	*,759**	1	,117	-,685	-,088	**,912****	*,819**	,657	-,618
Listeria S2	*,781**	,691	,167	,117	1	,009	-,158	,278	,116	,485	-,360
Pseud. aer	,075	,079	,471	-,685	,009	1	,543	-,560	**-,872****	-,643	,241
Pseud. fluor.	,249	,482	,033	-,088	-,158	,543	1	-,192	-,116	-,152	-,362
Serratia	,543	,410	,751*	**,912****	,278	-,560	-,192	1	,661	,605	-,602
St. aureus	,245	,330	,669	*,819**	,116	**-,872****	-,116	,661	1	*,750**	-,587
Phenol content	,545	,463	,377	,657	,485	-,643	-,152	,605	*,750**	1	-,360
Altitude	-,573	-,825*	,524	-,618	-,360	,241	-,362	-,602	-,587	-,360	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Discussion

Lingonberry fruits, due to their bioactive compounds, are reported to possess potential health-promoting effects and bactericide effects. The results showed that the assayed bacterial species exhibit different sensitivities. In addition, the same bacterial strains showed differences in sensitivity toward fruit samples with different origin. According to Paudel *et al*. (2014) in a brine shrimp toxicity test, lingonberry showed 4.7 ± 0.19 PH-IC50 (μg/mL), 8.5 ± 0,51 mm inhibition zone against *Staphylococcus aureus*. Polymeric proanthocyanidin extracts of lingonberries have strong antimicrobial effect against *Staphylococcus aureus* (Kylli *et al*., 2011). Myricetin inhibits the growth of all lactic acid bacteria derived from the human gastrointestinal tract flora (Puupponen-Pimiä *et al*., 2001). Some authors consider that tannins from *V. vitis-idaea* L. could potentially be used against *Actinobacillus actinomycetem-comitans, Porphyromonas gingivalis* and *Prevotella intermedia,* which are selected periodontal pathogens (Ho *et al*., 2010). The antimicrobial activity of lingonberry against two other oral pathogens, *Streptococcus mutans* and *Fusobacterium nucleatum* was evaluated by Riihinen *et al*. (2014), the antimicrobial activity against planctonic cells being associated with both the polymeric and A-type lingonberry procyanidins.

In contrast with the present results, lingonberry is considered to have slight antimicrobial activity against *Bacillus subtilis* and *Micrococcus luteus* (Rauha *et al*., 2000). According to Nohynek *et al*. (2006), lingonberry phenolics have strong antimicrobial activity against *Bacillus cereus, Staphylococcus epidermidis* as detected in this study too. Due to the results of Huttunen *et al*. (2013), 60% and 40% concentrations of the lingonberry honey have antimicrobial effect against *S. pneumoniae, S. pyogenes* ($p < 0.01$) and inhibit the growth of *S. aureus* ($p < 0.01$) with bacterial survival of 56%. According to Chen *et al*. (2012) the minimum inhibitory concentration of *Vaccinium vitis-idaea* extracts against *Staphylococcus aureus* is 18.75 mg/ml, *Escherichia coli* 18.75 mg/ml, *Enterobacter aerogenes* 37.50 mg/ml, *Salmonella typhi* 37.50 mg/ml, *Streptococcus hemolytic* 37.50 mg/ml, *Shigella* sp. 18.75 mg/ml, *Bacillus subtilis* 18.75 mg/ml. Antibacterial effect against *Helicobacter pilori* activity was observed in 10% concentration. Tannins from *Vaccinium vitis-idaea* have antibacterial effects against *Porpyromonas ginigivalis* and *Prevotella intermedia* (Hayashi *et al*., 2008), and inhibitory effect against *Listonella anguillarum* (serotypes O1 and O2), *Yersinia ruckeri*, *Photobacterium damselae* subsp. *piscicida* and *Lactococcus garvieae* (Bulfon *et al*., 2014). Tolmacheva *et al*. (2014) applied aqueous and ethanolic extracts from *Vaccinium vitis idaea* in a radial growth inhibition assay against wild-type *Chromobacterium violaceum* ATCC 31532 and reporter *Chromobacterium violaceum* NCTC 13274 strains observed a prominent growth-inhibition activity.

The present findings showed that the studied extracts have had stronger effect on Gram-negative bacteria, especially on *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, yet not as much on Gram-positive bacteria. According to Puupponen-Pimiä *et al*. (2001), these variations may reflect differences in the bacterial cell wall structure. The correlation results showed that the phenol content was not significantly correlative with the antimicrobial activity. Annuk *et al*. (1999) have studied the antimicrobial activity of aqueous extracts of lingonberry against the Gram-negative pathogen *Helicobacter pylori*, and concluded that tannic acid seemed to be the responsible component of the previously mentioned fact. Furthermore, Holopainen *et al*. (1988) related that the activity is known to be due to the arbutin and metylarbutin. Lingonberries are also rich in benzoic acid, a commonly used antimicrobial agent in foods. Häkkinen *et al*. (1999) showed that the antimicrobial activity of berry extracts against Gram-negative strains are not inhibited by pure phenolic compounds, whereas the inhibitory effects are due to complex phenolic polimers and other bioactive compounds by themselves or in combination with phenols. The antimicrobial effect might be due to the synergism of bioactive compounds and its amount is in correlation with the plant origin and environmental conditions. Several studies have reported the influence of genotype and climatic conditions on total phenolics of different cultivars of *Vaccinium* species (Connor *et al*., 2002; Jovancevic *et al*., 2011). According to those studies, the degree of anthocyanidin accumulation does not primarily depend on the light conditions, but on a favourable impact of low temperatures and a limiting effect of high temperatures. In the present research, the total phenol content was higher in the case of SB, B, L1 and L2 samples.

During fruit collection at Sântimbru Băi (SB), Harghita-Mădăras (HM) and Bilbor (B) it has been observed a sunny environment, also in case of the L1 sampling point, which was on the edge of a peatbog, with sparse canopy where ripening fruits receive greater amount of solar radiation. However, this study noted lower phenol content on the HM samples originating from quite a similar environment. The low content of phenols in the case of the HM sample can be explained by the different storage method and the late ripening period determined by the high altitude. As the results of the Cluster analysis show, dendrogram samples from Gălăoaia (G) and Bilbor (B), which belong to different populations but have a similar type of habitat, form one subcluster and show a relatively well supported relationship with the sample from Sântimbru Băi (SB). In the same way, three samples from Tinovul Luci (L1, L3, L4) form a well supported and separated group. As data suggests, *Vaccinium vitis-idaea* fruits originating from different geographic regions and environment differ from each other in terms of bioactive compounds, in quantity and activity.

In the selection of new perspective cultivars of lingonberry, the geographical origin of fruits must be considered. The L2 sample, which was gathered from the same peatbog as the L1, L3 and L4 samples, can be characterized by humid microclimate, dense canopy, penumbra and highly acidic soil. Unlike the previous ones, the L2 sample had a completely distant position in the dendrogram. However, taking into account also that the L2 sample was collected from near a creek, which can be a geographic barrier into the clonal spread of the species, this may indicate the presence of a different genotype in Tinovul Luci (L) population, which give reason to additional genetic analyses.

Conclusions

The results of this study showed stronger antibacterial effect of lingonberry samples on Gram-negative bacteria, especially on *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. The growth of the studied foodborne, illness causing and spoilage bacterial strains, was also affected by the analysed lingonberry samples. The results confirm that the *Vaccinium vitis idaea* berry fruits belonging to different growing sites differ from each other in terms of bioactive compounds in quantity and activity.

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