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Phytochemical and antimicrobial profile of black currant berries and leaves

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A B S T R A C T

The purpose of study was to identify polyphenolic compounds and antimicrobial properties in berries and leaves of black currant (*Ribes nigrum* L.). Black currant berries and leaves showed different characteristics. Berries had a higher levels of the studied parameters compared to leaves. Berry extracts contained 2.90 to 5.90 times more total phenolics, flavonoids, condensed tannins and gallotannins compared to leaf extracts, and total antioxidant activity was 5.82 times higher in berries than in leaves. The main flavonol found in our sample of black currant berries and leaves was quercetin, followed by myricetin, while kaempferol was present in very small amounts. The most abundant phenolic acid in berry extract was caffeic acid, while leaf extract was dominated by ferulic acid. Microbial properties of extracts were examined using eight selected indicator strains. The tested extracts showed strong antimicrobial activity, ranging from 55.82 to 199.21 μg mL^{-1}. The results suggest that berries and leaves of black currant are a good source of polyphenolic compounds and have strong antimicrobial activity.

Keywords: black currant, berry, leaf, polyphenolic compounds, antimicrobial activity.

И З В О Д

Циљ истраживања је био да се идентификују полифенолна једињења и антимикробна својства у бобицама и листовима црне рибизле (*Ribes nigrum* L.). Бобице и листови црне рибизле су показали различите карактеристике. Бобице су се одликовале већим садржајем испитиваних параметара у поређењу са листовима. Екстракти бобица су садржали од 2,90 до 5,90 пута више укупних фенола, флавоноида, кондензованих танина и галотанина у односу на екстракте листова, док је антиоксидативна активност у бобицама била 5,82 пута већа у поређењу са листовима. Главни флавонол утврђен у узорку бобица и листова црне рибизле био је кверцетин, следио је мирицетин, док је камферол био присутан у веома малим количинама. Најзаступљенија фенолна киселина у бобицама била је кафеинска киселина, док је у листовима доминирала ферулна киселина. Микробна својства испитивана су помоћу осам одабраних сојева индикатора. Испитивани екстракти су показали снажну антимикробну активност, у распону од 55,82 до 199,21 μg mL^{-1}. Резултати указују да су бобице и листови црне рибизле добар извор полифенолних једињења и имају снажну антимикробну активност.

Кључне речи: црна рибизла, бобица, лист, полифенолна једињења, антимикробна активност.

1. Introduction

Black currants (*Ribes nigrum* L.) has drawn much attention due to its unique biological and nutritional properties. As their berries are rich in numerous beneficial phytonutrients and antioxidants, interest in their consumption has increased in recent years. Berries stand out for their higher content of anthocyanins, phenolic acid derivatives and flavonols (myricetin and quercetin derivatives) compared to other small fruits (Moyer et al., 2002; Benvenuti et al., 2004; Anttonen and Karjalainen, 2006). Also, black currant berries are a good source of condensed tannins (Gu et al., 2004). Condensed tannins are an important group of phenolic compounds, whichmakes berries interesting for health promoting products. Numerous studies have reported that the consumption of berries on a regular basis offers potential health benefits against a number of diseases (cancer, diabetes, arthritis, cardiovascular and neurodegenerative diseases, etc.) (Mikkonen et al., 2001; Konczak and Zhang, 2004). Black currant leaves also contain significant amounts of total phenols, flavonoids and phenolic acids (Raudsepp et al., 2010), and have been used for the treatment of rheumatism, arthritis and diuretic purposes (Stević et al., 2010; Tabart et al., 2012). By virtue of their very strong biological activity, black currant berries and leaves contribute to the antioxidant, anti-inflammatory, antimicrobial and anticancer activities (Puupponen-Pimiä et al., 2005; Mazza, 2007).

With this in mind, the purpose of the investigation was to identify phytochemical and antimicrobial activity in black currant berries and leaves.

2. Materials and methods

2.1. Plant Material

Plants of black currant cv. 'Titania' were grown at the Fruit Research Institute, Čačak, Republic of Serbia (latitude 43°54' N, longitude 20°21' E, altitude 242 m) during three consecutive seasons (2017–2019). The orchard was planted in 2011 at a 3 m × 1 m spacing. During the experiment, the orchard was subjected to standard cultural practices (pruning, fertilisation and drip irrigation).

Berries were sampled at the June, in the stage of full ripeness, while leaf samples were collected in July, at the stage of full development. Berries and leaves were picked from the interior and exterior of each of the 5 bushes. A total of 100 g berries and 50 leaves were sampled from per three replications. Samples were extracted immediately after harvesting.

2.2. Sample preparation

Approximately 10 g of berries were extracted by 96% ethanol (100.0 mL) using an ultrasonic bath (model B-220, Branson Instruments, Smith-Kline Co., USA). After extraction, the solvent was removed by a rotary evaporator (Devarot, Slovenia) under vacuum and was dried at 30°C to constant weight.

Leaf samples were extracted by ultrasound-assisted extraction (UAE) in an ultrasonic water bath (B-220, Branson and Smith Kline Company, Danbury, CT, USA). Extraction solvent was removed by evaporation using a rotary evaporator (Devarot, Elektromedicina, Ljubljana, Slovenia), and dried at 60°C. The dried extracts were stored in glass bottles at 4°C until analysis.

The chemical analysis of the berries and leaves included the following parameters: 1. The total phenolic content of extracts was assessed using the Folin–Ciocalteu phenol reagent method (Singleton et al., 1999). The absorbance was detected at 765 nm. Results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GA g^{-1} DW). 2. For the determination of total flavonoid content, the samples were measured using a colorimetric assay (Markham, 1989). The absorbance was read at 415 nm. Results were expressed as milligrams of rutin equivalents per gram of dry extract (mg RU g^{-1} DW). 3. The method of precipitation of proanthocyanidins with formaldehyde was used to determine condensed tannins (Verrmeris and Nicholson, 2006). The concentration of condensed tannins was calculated as the residuum of the total phenolic and unprecipitated phenolic concentrations. Results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GA g^{-1} DW). 4. Gallotannins were determined by the potassium iodate assay (Verrmeris and Nicholson, 2006), based on the reaction of KIO_3 with galloyl esters, using gallic acid equivalents. Absorbance was measured spectrophotometrically at 550 nm. Results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GA g^{-1} DW). 5. Total antioxidant activity was assayed by the phosphomolybdenum method (Prieto et al., 1999), using ascorbic acid (AA) as a standard. The absorbance was read at 695 nm. Results were expressed as micrograms of AA per gram of dry extract (mg AA g^{-1} DW). 6. The extraction of phenolic compounds was performed as described by Mišan et al. (2011). Phenolic compounds were analysed by reversed phase HPLC analysis. The spectra of the compounds were recorded between 210 and 400 nm. Results were expressed as milligrams per 100 grams of dry extract (mg 100 g^{-1} DW). 7. Antimicrobial activity was evaluated by the microdilution method (Satyajit et al., 2007), using eight selected indicator strains *(Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Proteus mirabilis, Bacillus subtilis,* and fungi: *Candida albicans* and *Aspergillus niger)*.

2.3. Statistical analysis

The experimental data were subjected to the statistical analysis of variance (ANOVA) using Statistica 7 (StatSoft, Inc., Tulsa, OK, USA). Differences between means during the three years of the experiment were compared by LSD test at $P \leq 0.05$ significance levels.

3. Results and discussion

3.1. Polyphenolic compounds

Polyphenolic compounds are responsible for many of the positive, health-supporting effects of black currants, and contribute to the characteristic sensory properties of black currant berries (Laaksonen et al., 2013). Total phenolics, flavonoids, condensed tannins, gallotannins and total antioxidant capacity in black currant berries and leaves are presented in Figure 1.

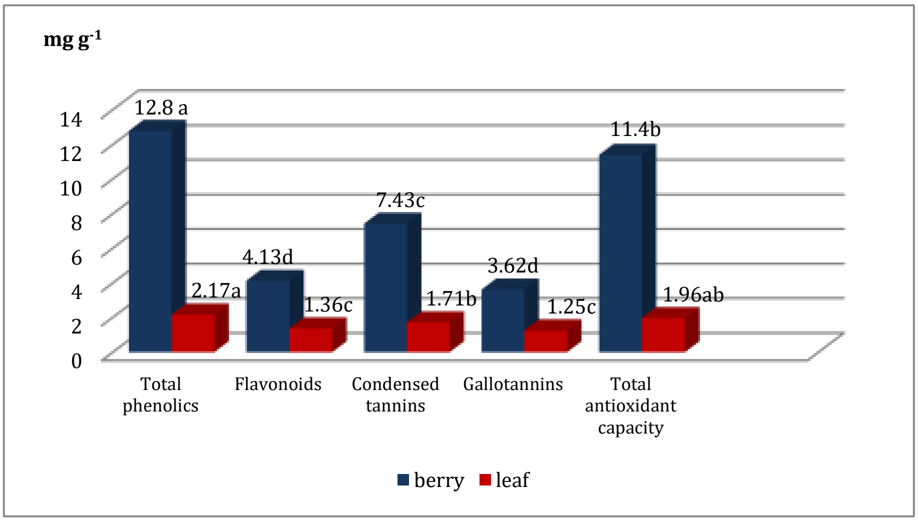

Figure 1. Total phenolics, flavonoids, condensed tannins, gallotannins and total antioxidant capacity in black currant berries and leaves

The levels of polyphenolic compounds in the analyzed extracts were calculated by the spectrophotometric methods described above. High amounts of phenols and flavonoids were present in both berry and leaf extracts. Actually, berry extracts contained 2.90 to 5.90 times more total phenolics, flavonoids, condensed tannins and gallotannins compared to leaf extracts, and total antioxidant capacity was 5.82 times higher in berries than in leaves. Our results showed that flavonoids constituted 32.3% of the total phenols in berries and 62.7% in leaves. As found by Haminiuk et al. (2012), flavonoids, mostly present as glycosides, are the main bioactive compounds with the highest antioxidant activity by virtue of their chemical structure. As part of the chemical composition analysis, total amounts of condensed tannins and gallotannins in the extracts were also calculated. The concentration of condensed tannins was 7.43 mg GA g^{-1} in berry and 1.71 mg GA g^{-1} in leaf, while the measured concentration of gallotannins was 3.62 mg GA g^{-1} in berry and 1.25 mg GA g^{-1} in leaf. Also, results demonstrated that berry extracts had higher contents of antioxidative activity (11.4 mg AA g^{-1}) in comparison to leaf extracts (1.96 mg AA g^{-1}), which is in correlation with the higher concentrations of total phenolics, total flavonoids, condensed tannins and gallotannins in berry extracts. The present results are in agreement with the findings of Rubinskiene et al. (2006), who reported high antioxidant activity of black currant fruit. By contrast, Tabart et al. (2006) found higher contents of phenolics and antioxidants in the leaf than in the berry. The results obtained indicate the potential use of black currant berries and leaves as polyphenol-rich products.

Phenolic components in berries and leaves were identified by the HPLC-DAD analysis, as presented in Table 1.

Table 1.
Phenolic components in black currant berries and leaves

Phenolic components (mg 100 g^{-1})	Berries	Leaves
ellagic acid	4.143±0.46 d	0.120 ±0.01 f
ferulic acid	3.895±0.38 d	0.957±0.07 a
caffeic acid	5.422±0.57 c	0.523±0.04 c
p-coumaric acid	2.511±0.32 e	0.270±0.02 d
quercetin	10.35±0.73 a	0.712±0.05 b
myricetin	5.931±0.59 b	0.241±0.02 e
kaempferol	3.993±0.39 d	0.116±0.01 f

Berries had higher contents of flavonols and phenolic acids compared to leaves. Quercetin was the dominant flavonol in both berry (10.35 mg 100 g^{-1}) and leaf (0.712 mg 100 g^{-1}) extracts, followed by myricetin (5.931 and 0.241 mg 100 g^{-1}, respectively), whereas the levels of kaempferol (3.993 and 0.116 mg 100 g^{-1}, respectively) were very low. Berry extracts contained 15 times more quercetin than leaf extracts. Our results are in accordance with those of Mättää et al. (2003) and Milivojević et al. (2010), who showed that black currants contained high amounts of quercetin and a small amount of kaempferol. However, Mikkonen et al. (2001) and Tabart et al. (2012) found a higher level of myricetin than quercetin in black currant. Also, Tabart et al. (2011) reported that leaf extracts had a higher content of flavonols, with quercetin prevailing, compared to berry extracts, which was not confirmed in the present study. Many studies have suggested that quercetin is a potent antioxidant that has important biological, pharmacological and medicinal properties, and potential anticancer properties (Xue et al., 2002; Seeram et al., 2003). In the present study, the content of phenolic acids ranged from 2.511 to 5.422 mg 100 g^{-1} in berries, and from 0.120 to 0.957 mg 100 g^{-1} in leaves, with caffeic acid dominating the berry and ferulic acid prevailing in the leaf. As emphasized by Kähkönen et al. (2001) and Mättää et al. (2003), caffeic and coumaric acids contribute to the high antioxidant activity.

3.2. Antimicrobial activity

Antimicrobial activity has a role in controlling the invasion and growth of plant pathogens. It has been investigated in order to characterize and develop new healthy food ingredients, medical compounds, and pharmaceuticals. Many studies have confirmed the great antimicrobial potential of plant extracts (Tekwu et al. 2012; Vieira et al. 2014).

Table 2.
Antimicrobial activity in black currant berries and leaves

Microbial strains	MIC values (μg mL^{-1})			
	UE		A	B
	Berries	Leaves		
Staphylococcus aureus ATC 25923	125.05	199.21	0.97	/
Klebsiella pneumoniae ATCC 13883	122.98	170.24	0.49	/
Escherichia coli ATCC 25922	55.82	125.49	0.97	/
Proteus vulgaris ATCC 13315	120.70	128.04	0.49	/
Proteus mirabilis ATCC 14153	130.21	158.96	0.49	/
Bacillus subtilis ATCC 6633	133.83	160.03	0.24	/
Candida albicans ATCC 10231	75.65	80.02	/	1.95
Aspergillus niger ATCC 16404	70.59	113.67	/	0.97

A Amaricin
B Nystatin

The obtained results of antimicrobial activity are shown in Table 2, as IC_{50} values. All extracts showed strong antimicrobial activity, in the ranging from 55.82 to 199.21 μg mL^{-1}. Berries had higher antibacterial activity against the bacterium *Escherichia coli* (55.82 μg mL^{-1}), while leaves showed the highest antifungal activity against *Candida albicans* (80. 02 μg mL^{-1}). The other Gram-positive and Gram-negative bacteria showed certain resistance to the tested berries and leaves. Raspberry and black currants display good antibacterial activity against various bacteria, such as *Escherichia*, *Mycobacterium*, *Salmonella* and *Staphylococcus* species, and inhibited the growth of a wide range of human pathogenic bacteria, both Gram-negative and Gram-positive ones (Cavanagh et al., 2003). The high antimicrobial activity of extracts may be associated with the high phenol concentration (Rauha et al., 2000; Pereira et al., 2006).

4. Conclusions

Berry and leaf extracts of black currant are an exceptionally rich source of phenolic compounds. High amounts of quercetin and ferulic acid in the berry and leaf of black currant are directly associated with the high antioxidant activity of the tested extracts. As rich sources of phenolic compounds exhibiting high antimicrobial activity, berries and leaves of black currant have good potential to be used as natural antimicrobial agents in the pharmaceutical and food industries.

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Declaration of competing interest

The authors declare there are no competing interests.

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