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Comparison of phenolics, antioxidant capacity and total phenol bioaccessibility of *Ribes* spp. grown in Turkey

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Abstract

In this study, anthocyanins (13), flavanols (6), phenolic acids (10), flavonol glycosides (17), antioxidant capacity, and bioaccessible phenolic content of *Ribes* spp., grown in Turkey were investigated. Individual phenolic compounds were identified and quantified with LC-QTOF/MS in red and black currants and hybrid Jostaberry. Significant variations in the individual phenolic compounds could be observed between the different cultivars. In all black currant cultivars, cyanidin 3,5-di-O-glucoside was the predominant anthocyanin compounds ($p \leq 0.01$). Cyanidin 3-O-sambubioside and cyanidin 3-O-rutinoside were detected in red currants. In all *Ribes* cultivars quercetin 3-O-rutinoside was the major flavonol glycoside and epigallocatechin found as the dominant flavonol compound. Rosenthal had the highest amount of total phenols, antioxidant capacity levels by DPPH and CUPRAC. Boskoop Giant characterized with the highest amount of total anthocyanin and bioaccessibility of phenolic compounds (84.27%).

Keywords: anthocyanins; flavanols; flavonol; glycosides; jostaberry.

Practical Application: Comparison of antioxidative properties of Ripes spp. fruits for human health.

1 Introduction

Berries have remarked for their nutritional and bioactive properties based on good source of polyphenolic compounds (Anttonen & Karjalainen, 2006). Many research announced that polyphenolic compounds, mostly flavonoids, and anthocyanins have amazing health-promoting properties (Kim et al., 2005). Also, epidemiological studies indicate that the consumption of anthocyanins prevents diabetes, oxidative stress, and the risk of cardiovascular disease (Zafra-Stone et al., 2007). Total phenols and total anthocyanins have been reported as the main contributors to the antioxidant effect of many berries (Tabart et al., 2006; Djordjević et al., 2010). In literature, berry flavonols such as myricetin, quercetin, kaempferol, and their derivatives concentrations show an alteration which could be explained by cultivars, their growth conditions, maturation, environmental condition and the methodological procedures applied (Mikulic-Petkovsek et al., 2012).

In the world berry production, *Ribes* spp., are significant currants second rate in consumer preferences immediately after strawberries (Djordjević et al., 2010). The most important *Ribes* species are; *Ribes nigrum* L., (black currants) and *Ribes rubrum* L., (red currant) cultivars (Djordjević et al., 2014). The main phenolic compounds reported in *Ribes* spp. include anthocyanins (Slimestad & Solheim, 2002; Anttonen & Karjalainen, 2006; Borges et al., 2010), flavonols, flavanols glycosides, and phenolic acids. These various compounds may work synergistically to promote human health.

Despite the enormous research on the total phenolic content of *Ribes* spp., studies investigating their bioaccessibility are scarce (Bordonaba & Terry, 2008; Contessa et al., 2013; Nour et al., 2013). Determination of total phenolic content bioavailability is important because only gastrointestinal sustainable phenolic can be bioaccessible for absorption (Chiang et al., 2013). Thus, the health effects of polyphenols depend on the amount consumed and on their bioavailability which appears to differ greatly. For this reason, knowledge of bioavailability is necessary for exploring the health effects of polyphenols in the fruit. Most polyphenols exist in the form of glycosides, esters, or polymers which cannot be absorbed in their native form in foods. Therefore, some polyphenols can be absorbed less efficiently and bioavailability than others, even though they are present large amounts. However, bioavailability research has practical, technical and ethical difficulties. Therefore, there is a need to develop and use *in vitro* models that mimic the physiological processes that occur in human digestion. (Minekus et al., 2014). Because of this, the impact of bioaccessibility on the stability of phenolic compounds has been one of the more widely investigated topics during the last decade.

This research aimed to investigate certain cultivars of *Ribes* species grown in Turkey for their content and composition of individual phenolic compounds including anthocyanins, flavonols, phenolic acids and flavonol glycosides, antioxidant capacities, total anthocyanin content, total polyphenolic content, and their bioaccessibility. For the detection of bioaccessibility, samples were processed by an *in vitro* digestive enzymatic extraction that mimics the conditions in the gastrointestinal tract. The polyphenol composition of the berry fruits has been characterized utilizing

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quadrupole time of flight mass spectrometry (LC QTOF/MS) and for other bioactive characteristics, spectrophotometric techniques were used.

2 Materials and methods

2.1 Plant material

Black currant (*R. Nigrum* L. cv. Boskoop Giant, Rosenthal, Goliath); red currant (*R. Rubrum* L. cv. Red Lake) and a cross between black currant and gooseberry (*R. Nidigrolaria* L. cv. Jostaberry) fruits were harvested from Bursa, Turkey. For this study, we selected 5 fields in the same region and nearby. 25 kg healty fruit were harvested at commercial ripening from different parts of the field for each cultivar. The fruits were transferred to the laboratory and the moisture content of all cultivars were immediately analyzed. The samples were frozen (-80 °C) and stored for analyses.

2.2 Reagents, standards and solutions

HPLC-grade methanol, ethanol, Folin–Ciocalteu phenol reagent, formic acid, sodium chloride, sodium carbonate, sodium acetate, ammonium acetate, and copper (II) chloride were purchased from Merck (Darmstadt, Germany). HPLC-grade acetonitrile, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), neocuproine, gallic acid and potassium chloride, sodium hydroxide, pepsin, bile extract, pancreatin, concentrated hydrochloric acid (37% w/v) and concentrated sulfuric acid (95-98%) were purchased from Sigma (St. Louis, MO, USA). The standards were used for the quantification of phenolic compounds were purchased from Extrasynthese (Genay Cedex, Francea) and Sigma-Aldrich Chemie (St. Louis, MO, USA).

2.3 Extraction of extractable, hydrolyzable and bioaccessible fractions of phenols

Extractable, hydrolysable, and bioaccessible fraction were extracted according to Vitali et al. (2009) with slight modifications. The extractable and hydrolyzable fraction was used for total phenolic content, antioxidant capacity, total anthocyanins, and individual phenolic compounds analysis (Vitali et al., 2009). For the determination of bioaccessible fractions, investigated *Ribes* varieties were processed by an *in vitro* digestive enzymatic extraction that mimics the conditions in the gastrointestinal tract.

2.4 Determination of total phenolic content and antioxidant capacity

The extractable, hydrolyzable and bioaccessible fraction of total phenolic content of fruits was determined using Folin-Ciocalteu method (Singleton et al., 1999). Gallic acid was used as a standard and the results were expressed as mg GAE/g dw.

Extractable and hydrolyzable extracts of investigated samples using 1,1-Diphenyl-2-picrylhydrazy Radical Scavenging, (DPPH) and Cupric Ion Reducing Antioxidant Capacity (CUPRAC) methods. DPPH method was determined by the method outlined by Brand-Williams et al. (1995) and CUPRAC method was carried out as described by Apak et al. (2004). A calibration curve was prepared, Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and the results were expressed as μmol TE/g dw for each method. TPC, CUPRAC and DPPH results were calculated as the sum of extractable and hydrolyzable fraction extracts.

2.5 Total anthocyanin

Anthocyanin quantification was performed by the pH-differential method (Giusti & Wrolstad, 2001). Calculation of the anthocyanins concentration was based on a cyanidin-3-glucoside molar extinction coefficient 26,900 and a molecular mass of 449.2 g/mol. Results were expressed as milligrams of cyanidin-3-glucoside equivalents per 100 g of dry weight basis.

2.6 Individual phenolic compounds

Identification and quantification of anthocyanins and flavonols, phenolic acids and flavonol glycosides in *Ribes* fruits were carried out with an Agilent 6550 LC-QTOF/MS-ESI (Agilent Technologies, USA) system equipped with a Poroshell 120 EC-C 18 column (4.6 × 100 mm, 2.7 μm film thickness). For elution of these compounds, the mobile phase consisted of two solvents: formic acid (1%, v/v) in water (A) and formic acid (1%, v/v) in acetonitrile (B). All phenolic compounds were identified using an LC-QTOF mass spectrometer with electrospray ionization (ESI) operating in the negative (non-anthocyanin phenolic compounds) and positive (for anthocyanins) ion mode. Identification of the components was carried out by comparing the retention times and MS2 fragmentation in currant samples. For all compound, MS identification was confirmed with authentic standards. The MS data are presented in Table 1. Concentrations were expressed in mg/kg dw.

For non-anthocyanin and anthocyanin compounds of samples, the total run time was 20, 40 min, with 8, 5 min of equilibration treatment performed before each analysis, the analysis was carried out using full-scan data-dependent MS2 scanning from m/z 50 to 3000, 100 to 1600, flow rate was 0.6, 0.3 mL/min, column temperature was set at 40 °C, 25 °C, nitrogen was used as nebulizing and drying gas at a pressure of 35, 45 psi and the flow rate was adjusted to 14, 12 L/min. respectively.

2.7 Statistical analysis

All results were expressed as mean values ± standard deviation (SD) for six replicates. Statistical analyses were performed with JMP software, v.9.0.2 (SAS, USA). Fisher's LSD test was used to compare the means among treatments with significant differences ($p \leq 0.01$).

3 Results and discussion

3.1 Total phenolic content

The content of extractable, hydrolyzable and bioaccessible phenolics of *Ribes* spp. cultivars are presented in Figure 1. Significant differences in extractable fraction, hydrolyzable fraction and total phenolic content among the different species were recorded ($p < 0.01$). Generally, total phenolic content was in fruits vary according to numerous genetic, environmental, and technologic factors (Manach et al., 2004).

The highest total phenolic content (123.22 mg GAE/g dw) detected in Rosenthal cultivar, followed by Boskoop giant (112.01 mg GAE/g dw). Mikulic-Petkovsek et al. (2013) recorded that total phenolic concentrations 3519.2 and 3774.1 mg GAE/kg fw for Goliath and Rosenthal, respectively. Our finding higher than these results for both varieties, but Mikulic-Petkovsek et al. (2015) 4525.26-6803.41 mg GAE/100 g fw of total phenolic content detected in Rosenthal varieties, which is higher than our findings.

Red Lake cultivar showed the highest content of hydrolyzable phenols, while the lowest was recorded in hybrid Jostaberry

Table 1. Tentative identification of phenolic compounds in *Ribes* spp. using LC-QTOF/MS-ESI.

Compound Name	m/z	Detected Mass	RT	Deviation (ppm)	Mass	MS/MS
Cyanidin 3-O-glucoside	449.1084	449.1060	9.70	5.34	484.0772	287.0631/139.0392
Cyanidin 3-O-sambubioside	581.1502	581.1524	9.54	-3.79	616.1190	287.0621/118.0894
Cyanidin 3-O-rutinoside	595.1663	595.1647	10.1	2.69	630.1351	449.1126/287.0644
Cyanidin 3-O-galactoside	449.1084	449.1068	9.50	3.56	484.0772	287.0641/137.0269
Cyanidin 3,5-di-O-glucoside	611.1612	611.1618	8.,29	-0.98	646.1301	449.1206/287.0639
Peonidin 3-O-rutinoside	609.1820	609.1841	11.36	-3.45	644.1508	301.0787/121.0334
Peonidin 3-O-glucoside	463.1241	463.1258	11.49	-3.67	498.0928	301.0775/89.0622
Pelargonidin 3,5-di-O-glucoside	596.1670					287.0617/111.3086
Pelargonidin 3-O-rutinoside	579.1741	579.1741	10.34	0.00	579.171	433.1217/271.0684
Petunidin 3-O-glucoside	479.1241	479.1241	10.84	0.00	479.1190	317.0741/137.0277
Delphinidin 3-O-rutinoside	611.1613	611.1623	9.99	-1.64	646.1301	287.0643/137.0263
Delphinidin 3-O-glucoside	465.1077	465.1077	8.89	0.00	465.1027	303.0597/273.0463
Malvidin 3-O-glucoside	493.1346	493.1361	11.93	-3.04	528.1031	331.0914/85.0316
Catechin	289.0711	289.0712	4.49	-0.35	290.079	289.0712/245.0819/205.0506
Epigallocatechin	305.0660	305.0665	3.83	-1.64	306.0739	125.0247/160.8413/197.9165
Epicatechin	289.0711	289.0713	5.07	-0.69	290.079	245.08140/205.0549/125.0244
Epigallocatechin gallate	457.0770	457.0764	5.09	1.31	458.0849	125.0244/169.0142/161.0244
Epicatechin gallate	441.0820	441.0816	5.60	0.91	442.0899	169.0147/125.0252/289.0719
Catechin	289.0711	289.0712	4.49	-0.35	290.079	289.0712/245.0819/205.0506
Chlorogenic acid	353.0871	353.0869	4.34	0.57	354.0950	191.0561/353.0878/161.0244
2-Hydroxybenzoic acid	137.0237	137.0242	6.64	-3.65	138.0316	93.0368/65.0421/75.0240
Gentisic acid	153.0187	153.0181	4.86	3.92	154.0266	108.0216/53.0396
4-hydroxy benzoic acid	137.0237	137.0240	4.83	-2.19	138.0316	137.0237/108.0219
Ellagic acid	300.9983	300.9985	5.51	-0.66	302.0062	257.0087/229.2126/185.0235
Caffeic acid	179.0343	179.0351	5.13	-4.47	180.0422	134.0373/89.0396/135.0451
Vanillic acid	167.0343	167.0345	5.14	-1.20	168.0422	135.0460/108.0228
Gallic acid	169.0136	169.0143	2.32	-4.14	170.0215	125.0253/147.8918/107.0149
Protocatechuic acid	153.0187	153.0191	3.53	-2.61	154.0266	109.0297/141.8689/118.0304
P-Cumaric acid	163.0394	163.0394	5.68	0.00	164.0473	119.0513/145.0298
Myricetin 3-O-glucoside	479.0821	479.0818	5.20	0.63	480.0900	317.6521/137.0267
Myricetin 3-O-galactoside	479.0821	479.0818	5.16	0.63	480.0900	317.6521/137.0268
Myricetin 3-O-rhamnoside	463.0875	463.0872	5.44	0.65	464.0954	463.0872/317.0598
Quercetin 3-glucoside	463.0875	463.0869	5.46	1.30	464.0954	463.0869/301.9782
Quercetin 3-D-galactoside	463.0875	463.0868	5.43	1.51	464.0954	463.0868/301.9782
Quercetin	301.0298	301.0338	8.50	-13.29	301.0298	151.0045/107.0150/ 178.9991
Quercetin 3-O-rhamnoside	301.0347	301.0351	6.53	-1.33	302.0426	301.0351
Quercetin 3-D-xyloside	433.0771	433.0761	5.58	2.31	434.0850	433.0761/301.347
Quercetin 3-O-rutinoside hyd.	609.1451	609.1448	5.31	0.49	610.1530	300.0290/463.0881/343.0463
Isorhamnetin	315.0503	315.0513	7.06	-3.17	316.0582	300.0277/151.0033/ 107.0142
Isorhamnetin 3-O-rutinoside	623.1611	623.1626	7.70	-2.41	624.1690	477.1087/315.2321
Isorhamnetin 3-O-glucoside	477.1031	477.1025	5.69	1.26	478.1110	477.1025/315.0904
Syringetin 3 glucoside	507.1138	507.1131	5.67	1.38	508.1217	507.1131/345.0301
Kaempferol	285.0398	285.0404	7.05	-2.10	286.0477	152.0687/67.1025
Kaempferol 3-B-D glucoside	447.0931	447.0952	11.10	-4.70	448.1010	447.0938/ 284.0336
Kaempferol 3-O-D-galactoside	447.0931	447.0938	13.15	-1.57	448.1010	284.0336/169.0142
Kaempferol 3-B-D rutinoside	593.1501	593.1482	19.46	3.20	594.1580	285.0412/447.0897/ 327.0504

m/z: mass-to-charge ratio; RT: retention time; MS: mass spectrum.

cultivar (Table 2). In terms of total phenolic content, Red lake cultivar contained the lowest amount among all species. In literatüre, total phenolic content was found that 0.418 g GAE 100 g^{-1} fw (Plessi et al., 2007) and 1115-1193 mg GAE 100/g (Pantelidis et al., 2007) in red lake cultivar; and 18.94-35.85 mg GAE/g dw (Woznicki et al., 2015) and 251.9 mg GAE/100 g fw (Djordjević et al., 2014) in black currant cultivar. In comparison with those researches, the results obtained by our study of total phenolic content in both red and black currants are much higher than other results.

In Jostaberry relatively high amount of total phenolic content was detected (1809.24 mg GAE/kg fw), the amount of this cultivar was following by the report of Mikulic-Petkovsek et al. (2015) results. Bioaccessibility of total phenolic content in investigated *Ribes* spp. cultivars ranged from 69.91% to 89.07%. Data on bioaccessibility of total phenolic content from *Ribes* spp. are quite limited.

3.2 Antioxidant capacity

The antioxidant capacities of the samples were determined by DPPH and CUPRAC methods. In the comparison of the levels of DPPH and CUPRAC antioxidant capacities among *Ribes* varieties, differences were observed ($p < 0.01$) (Table 2). According to our results, *Ribes nigrum* (black currant) varieties had the highest antioxidant capacity among all *Ribes* species. High antioxidant potential of black currant has been reported previously (Ehala et al., 2005) and this property correlated with its high content of phenolic compounds.

DPPH values of *Ribes* species ranged from 68.61 to 181.38 µmol TE/g dw. The highest DPPH antioxidant capacity was detected in Boskoop giant followed by Rosenthal cultivar, whereas the lowest value was detected in Red lake cultivar.

These levels are comparable with Mikulic-Petkovsek et al. (2015) reported that DPPH level ranged from 161.65-365.50 mg AAE 100/g fw for Rosenthal, 59.98-103.81 mg AAE 100/g fw for red currant and 33.93-103.02 mg AAE 100/g fw for Jostaberry cultivars, in three maturity stages.

The values of CUPRAC were in the range of 299.64 µmol TE/g dw (Red lake) to 914.31 µmol TE/g dw (Rosenthal) in *Ribes* species. Following the Rosenthal, the highest level of CUPRAC was detected in Boskoop giant and Goliath cultivar, respectively.

3.3 Total anthocyanin content

Significant variations ($p < 0.01$) in the total anthocyanin content could be observed between the different currant varieties (Table 2). These variations could be from the cultivars, maturation and agricultural treatment, as well as environmental and weather conditions.

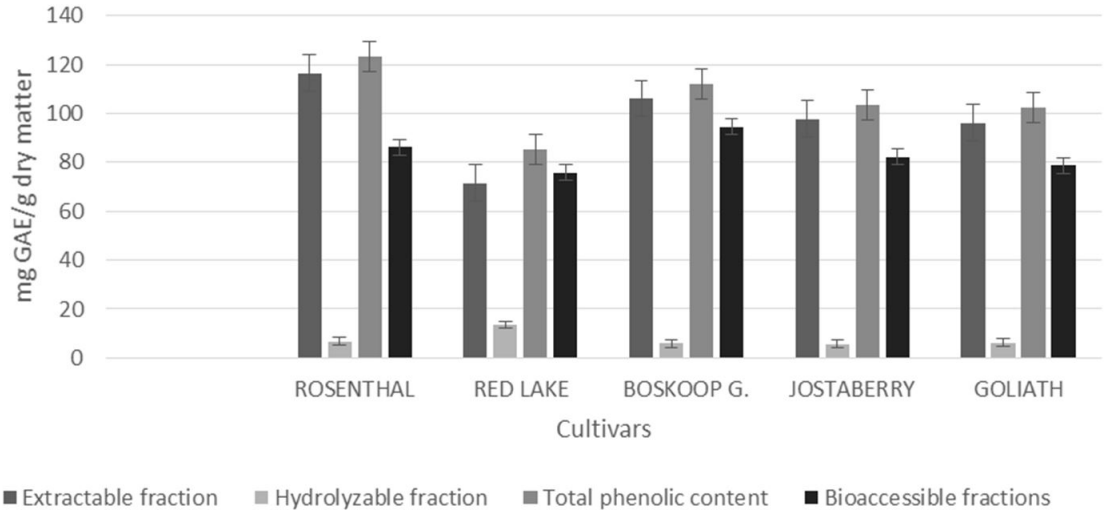

Figure 1. The content of extractable, hydrolysable and bioaccessible fraction of phenolic content of *Ribes* spp. Data are presented as Mean ± SD (n = 6).

Table 2. Total anthocyanin and antioxidant capacity of *Ribes* spp.

	Rosenthal	Red Lake	Boskoop G.	Jostaberry	Goliath
Total anthocyanin (mg cy-3 glu/100 g)	1586.76 ± 60.07^{b}	127.13 ± 6.83^{e}	1777.73 ± 90.07^{a}	349.89 ± 32.54^{d}	1101.07 ± 60.07^{c}
Antioxidant capacity (µmol TE/g)					
DPPH	75.79 ± 6.51^{cd}	181.38 ± 4.11^{a}	68.61 ± 4.85^{d}	165.35 ± 4.88^{b}	84.15 ± 6.27^{c}
CUPRAC	914.31 ± 62.31^{a}	299.64 ± 7.85^{c}	847.17 ± 67.12^{a}	369.33 ± 29.99^{c}	638.15 ± 59.90^{b}

DPPH: 1,1-diphenyl-2- picrylhydrazy radical scavenging method; CUPRAC: Cupric ion reducing antioxidant capacity method. Results are expressed on a dry weight basis in mg/kg and are given as Mean ± SD (n = 6) with different letter. (a-d) in the same line are significantly different ($p \leq 0.01$).

Boskoop giant cultivar had the highest total anthocyanin content (1777.73 mg cy-3 glu/100 g dw), while the lowest content (127.13 mg cy-3 glu/100 g dw) was recorded in Red lake cultivar. Following the Boskoop giant, Rosenthal and Goliath varieties contained a relatively high amount of total anthocyanin content. The similar result was found by Anttonen & Karjalainen (2006), Plessi et al. (2007), and Mikulic-Petkovsek et al. (2015) however, our results of total anthocyanin content for black currant were higher than Slimestad & Solheim (2002) (250 mg cy-3 glu/100 g fw), Bordonaba & Terry (2008) (83-199 mg/100 g fw), Contessa et al. (2013) (224.79 mg cy-3 glu/100 g fw), Nour et al. (2013) (116.7 to 287.78 mg/100 g), Woznicki et al. (2015) (7.23-12.65 mg cy-3 glu/100 g dw) and Paunović et al. (2017) (207.5 to 372.9 mg cy-3 glu/100 g) results. In literature, Plessi et al. (2007), Pantelidis et al. (2007), Borges et al. (2010), Djordjević et al. (2014) and Mattila et al. (2016) reported that the amounts of total anthocyanins in red currants average 2 mg cy-3 glu/100 g fw, 7.5-7.8 mg cy-3 glu/100 g fw, 14.7 mg cy-3 glu/100 g fw, 11.9 mg/100 g fw and 138-462 mg/100 g dw, respectively. In addition, according to Mikulic-Petkovsek et al. (2015) research Jostaberry fruits contained between 265.38-734.72 mg/kg fw total anthocyanin in three maturity stages. Our results for the same varieties were between the authors' findings.

3.4 Phenolic profile

Phenolic compounds of *Ribes* species include anthocyanins, phenolic acids and flavonols being the dominant group (Anttonen & Karjalainen, 2006). Significant variations ($p \leq 0.01$) in the individual phenolic compounds could be observed among the different *Ribes* species cultivars (Table 3 and 4). These differences among fruits for the quantity and the composition of phenolic compounds might be explained by several factors such as the genotype, phenotypic differences, species, cultivar properties, and growing condition (Strack, 1997).

3.5 Anthocyanin compounds

Significant differences in individual anthocyanin content among the different *Ribes* species were recorded ($p \leq 0.01$). Anthocyanins belonging to the group of flavonoids were the major phenolic group in black currant as they accounted 94.48-97.96% of total phenolic compounds analyzed, whereas the quite low proportion of anthocyanins were recorded in red currant (22.13%) (Table 3). According to our results, cyanidin 3,5 di-glucoside was the major anthocyanin in all black currant varieties (3339.19-5447.18 mg/kg dw), followed by delphinidin 3-rutinoside, cyanidin 3-rutinoside and delphinidin 3-glucoside and Boskoop giant cultivar had the highest amount. In the comparison of order, our findings were similar with Slimestad & Solheim (2002), Rubinskiene et al. (2005), Anttonen & Karjalainen (2006), Borges et al. (2010), Gavrilova et al. (2011), Mikulic-Petkovsek et al. (2016), except of cyanidin 3,5 di-glucoside which is determined as the predominant anthocyanin compound of black currants.

Red lake cultivar contained only cyanidin glycosides (119.66 mg/kg dw of cyanidin 3-O-sambubioside and 125.96 mg/kg dw of cyanidin 3-O-rutinoside) with the lowest content of total amount of individual anthocyanin (245.62 mg/kg dw). Especially, cyanidin 3 O-sambubioside is determined only in the Red lake cultivar, appear to be characteristic for red currant and probably contribute to their color. In literatüre, cyanidin 3-O-(2"-xyl) rutinoside, cyanidin 3-sambubioside and cyanidin 3-rutinoside were determined as the major anthocyanin compounds in red currants, respectively (Borges et al., 2010), while other authors reported cyanidin 3-rutinoside as the dominant anthocyanin in red currant cultivars (Mikulic-Petkovsek et al., 2015).

Although Jostaberry had the same anthocyanin compounds as black currant, the concentrations of these anthocyanins were

Table 3. Characterization of anthocyanin and phenolic acids determined in *Ribes* spp.

Compound Name	Rosenthal	Red Lake	Boskoop G.	Jostaberry	Goliath
Anthociyanins (mg/kg)					
Cyanidin 3-O-glucoside	25.87 ± 1.63^{c}	Nd	165.38 ± 14.07^{b}	46.04 ± 3.29^{c}	402.17 ± 28.79^{a}
Cyanidin 3-O-sambubioside	Nd	119.66 ± 3.09^{a}	Nd	Nd	Nd
Cyanidin 3-O-rutinoside	2177.97 ± 82.10^{c}	125.96 ± 9.71^{e}	3333.56 ± 63.93^{a}	1380.81 ± 62.06^{d}	2627.47 ± 52.79^{b}
Cyanidin 3-O-galactoside	172.08 ± 16.77^{b}	Nd	115.83 ± 8.67^{c}	81.13 ± 8.44^{d}	303.0 ± 21.05^{a}
Cyanidin 3,5-di-O-glucoside	4038.12 ± 98.51^{b}	Nd	5447.18 ± 87.71^{a}	665.35 ± 28.83^{d}	3339.19 ± 157.33^{c}
Delphinidin 3-O-rutinoside	3518.01 ± 88.97^{b}	Nd	4652.73 ± 90.46^{a}	602.78 ± 12.39^{d}	2913.42 ± 96.84^{c}
Delphinidin 3-O-glucoside	357.48 ± 20.59^{c}	Nd	789.44 ± 22.75^{b}	83.06 ± 12.41^{d}	987.86 ± 74.95^{a}
Phenolic acids (mg/kg)					
Chlorogenic acid	Nd	1.79 ± 0.11^{c}	4.45 ± 0.14^{a}	1.15 ± 0.04^{d}	4.17 ± 0.28^{b}
2-Hydroxybenzoic acid	2.47 ± 0.38^{c}	14.36 ± 1.70^{b}	11.12 ± 1.19^{b}	14.72 ± 2.45^{b}	42.94 ± 5.19^{a}
Gentisic acid	Nd	3.60 ± 0.21^{a}	0.69 ± 0.22^{d}	2.69 ± 0.11^{b}	1.42 ± 0.13^{c}
4-hydroxy benzoic acid	0.15 ± 0.00^{b}	0.11 ± 0.01^{c}	0.06 ± 0.01^{d}	0.20 ± 0.03^{a}	0.17 ± 0.02^{ab}
Ellagic acid	Nd	0.83 ± 0.12^{b}	0.65 ± 0.05^{c}	1.78 ± 0.17^{a}	Nd
Caffeic acid	2.35 ± 0.13^{d}	7.92 ± 0.25^{a}	2.80 ± 0.17^{c}	8.02 ± 0.19^{a}	6.14 ± 0.35^{b}
Vanillic acid	52.28 ± 1.89^{a}	16.57 ± 1.17^{c}	17.19 ± 1.47^{c}	17.30 ± 0.79^{c}	34.87 ± 3.48^{b}
Gallic acid	0.07 ± 0.00^{e}	0.27 ± 0.03^{a}	0.16 ± 0.02^{c}	0.12 ± 0.02^{d}	0.21 ± 0.03^{b}
Protocatechuic acid	5.72 ± 0.20^{d}	17.49 ± 1.10^{b}	1.16 ± 0.31^{e}	14.03 ± 1.64^{c}	21.65 ± 3.33^{a}
P-Coumaric acid	0.58 ± 0.09^{d}	3.01 ± 0.07^{a}	1.38 ± 0.08^{c}	1.51 ± 0.06^{c}	2.82 ± 0.13^{b}

Results are expressed on a dry weight basis in mg/kg and are given as mean ± standard deviations (n = 6) with different letter (a-e) in the same line are significantly different ($p \leq 0.01$). Nd: not detected.

Table 4. Individual quantities of flavanol and flavonol glycosides determined in *Ribes* fruits.

Compound Name	Rosenthal	Red Lake	Boskoop G.	Jostaberry	Goliath
Flavanol compounds (mg/kg)					
Catechin	25.14 ± 2.68^{b}	45.58 ± 2.03^{a}	26.36 ± 3.49^{b}	12.93 ± 0.56^{d}	22.53 ± 0.51^{c}
Epigallocatechin	11.26 ± 0.97^{d}	256.13 ± 17.34^{a}	158.11 ± 14.24^{c}	195.93 ± 15.34^{b}	150.11 ± 7.44^{c}
Epicatechin	4.18 ± 0.09^{d}	18.48 ± 0.60^{a}	14.05 ± 2.00^{bc}	16.01 ± 1.30^{b}	12.38 ± 1.40^{c}
Epigallocatechin gallate	1.27 ± 0.16^{b}	0.34 ± 0.03^{d}	1.66 ± 0.09^{a}	0.19 ± 0.03^{e}	0.85 ± 0.04^{c}
Epicatechin gallate	0.05 ± 0.00^{b}	0.12 ± 0.00^{a}	0.13 ± 0.03^{a}	0.11 ± 0.00^{a}	0.13 ± 0.04^{a}
Flavonol glycosides (mg/kg)					
Myricetin 3-O-glucoside	0.85 ± 0.05^{e}	153.99 ± 3.52^{a}	131.04 ± 0.23^{c}	16.40 ± 0.90^{d}	140.26 ± 4.22^{b}
Myricetin 3-O-rhamnoside	0.60 ± 0.03^{e}	30.78 ± 0.46^{a}	9.50 ± 0.18^{c}	3.07 ± 0.07^{d}	25.39 ± 1.08^{b}
Quercetin 3 O-rhamnoside	3.54 ± 0.08^{b}	Nd	3.66 ± 0.09^{b}	6.69 ± 0.35^{a}	Nd
Quercetin 3 D-xyloside	0.93 ± 0.07^{c}	0.64 ± 0.05^{c}	1.23 ± 0.17^{c}	5.81 ± 0.49^{b}	11.86 ± 1.23^{a}
Quercetin 3-O-rutinoside	105.64 ± 5.68^{e}	257.42 ± 6.56^{a}	180.26 ± 3.62^{b}	160.82 ± 6.63^{c}	129.63 ± 2.42^{d}
Isorhamnetin	0.01 ± 0.00^{e}	0.78 ± 0.03^{a}	0.19 ± 0.03^{c}	0.61 ± 0.05^{b}	0.12 ± 0.01^{d}
Isorhamnetin 3-O-rutinoside	Nd	4.0 ± 0.21^{b}	2.29 ± 0.07^{c}	7.57 ± 0.36^{a}	1.40 ± 0.17^{d}
Isorhamnetin 3-O-glucoside	Nd	1.67 ± 0.06^{a}	1.49 ± 0.09^{a}	0.69 ± 0.02^{c}	0.95 ± 0.21^{b}
Syringetin 3 glucoside	0.41 ± 0.03^{c}	0.89 ± 0.07^{a}	0.57 ± 0.05^{b}	Nd	0.37 ± 0.02^{c}
Kaempferol	0.26 ± 0.01^{d}	3.18 ± 0.12^{b}	4.48 ± 0.14^{a}	0.19 ± 0.01^{d}	0.44 ± 0.03^{c}
Kaempferol 3-O-D-galactoside	0.46 ± 0.01^{d}	4.66 ± 0.14^{b}	5.50 ± 0.04^{a}	0.15 ± 0.01^{e}	1.37 ± 0.05^{c}
Kaempferol 3-B-D rutinoside	6.07 ± 0.24^{d}	19.41 ± 1.02^{b}	15.18 ± 0.50^{c}	31.13 ± 3.05^{a}	4.65 ± 0.35^{d}

Results are expressed on a dry weight basis in mg/kg and are given as mean ± standard deviations (n = 6) with different letter (a-e) in the same line are significantly different ($p \leq 0.01$). Nd: not detected.

low. In Jostaberry the prevailing anthocyanins had cyanidin glycosides yielding dark blue or almost black fruit color. Cyanidin 3-O-rutinoside was found as the major anthocyanin compound, followed by cyanidin 3,5-di-O-glucoside and delphinidin 3-O-rutinoside.

3.6 Phenolic acid compounds

Individual phenolic acid compounds levels changed significantly in tested samples (Table 3). In genaral, vanillic acid, 2-hydroxybenzoic acid and protocatechuic acid were abundant in all *Ribes* species. The highest concentration of 2-hydroxybenzoic acid and protocatechuic acid were detected in Goliath cultivar (42.94 mg/kg dw and 21.65 mg/kg dw, respectively) and vanillic acid in Rosenthal cultivar (52.28 mg/kg dw). In total, the highest total individual phenolic acid was found in Goliath cultivar, while the lowest content was determined in Boskoop giant cultivar. Ellagic acid, 4-hydroxybenzoic acid and gallic acid were determined as the lowest phenolic acid compounds in all cultivar.

In literature, it was found that the differences for concentration of phenolic acid compounds among the *Ribes* species (Gavrilova et al., 2011; Mikulic-Petkovsek et al., 2015). It might be that cultivar, origin of the fruit, maturation period, agricultural teratment and growing condition caused these differences.

3.7 Flavanol compounds

The mean (± standard deviation) and range of the concentrations of individual flavanol compounds in *Ribes* species are given in Table 4. In statistical evaluation, it was found that the differences were important ($p \leq 0.01$) among cultivars. Red lake cultivar was the richest species in terms of the total flavonol content (320.65 mg/kg dw), followed by Jostaberry cultivar (225.16 mg/kg dw). Red lake, Jostaberry, Boskoop giant and Goliath cultivars contained a high amount of epigallocathechin. After epigallocatechin, catechin, and epicatechin were the abundant flavonol compounds in all cultivars. The common flavonols present in different parts of black currant plants are epigallocatechin, gallocatechin, catechin, epicatechin and epigallocatechin gallate (Tabart et al., 2011) and epigallocatechin concentration in black currant were in the range of 5.86-5.95 mg/100 g fw, 27.9 mg/kg fw in Goliath and 36.3 mg/kg fw in Rosenthal cultivar (Gavrilova et al., 2011; Mikulic-Petkovsek et al., 2015; Mikulic-Petkovsek et al., 2016). When these flavanol levels were compared with the results obtained in this study, epigallocatechin concentration in black currant was different; however different growing condition, maturation, and cultivar were examined.

3.8 Flavonol glycoside compounds

Six (6) glycosides from the group of quercetin derivatives, three (3) glycosides from the group of myricetin, three (3) glycosides from the group of isorhamnetin, four (4) glycosides from the group of kaempferol and one (1) glycoside from the group of syringetin have been investigated in our study. Significant variations in flavonol glycoside compounds could be observed between the different *Ribes* species (Table 4). Myricetin 3-O-galactoside, quercetin 3-glucoside, quercetin 3-D-galactoside, quercetin, kaempferol 3-B-D glucoside compounds were not detected any *Ribes* species. The amount of isorhamnetin, isorhamnetin 3-O-glucoside, and syringetin-3-glucoside were quite low in all species.

Quercetin 3-O-rutinoside concentration ranged from 105.64 to 257.42 mg/kg dw was the major flavonol glucoside compound in all *Ribes* species, except Goliath cultivar. Quercetin

glycosides represented the main portion of total flavonols for Jostaberry (60.69%), Red lake (54.05%), Rosenthal (92.72%) and Boskoop Giant (52.10%) cultivars, whereas myricetin glycosides represented the main portion of total flavonols for Goliath (52.35%) cultivar. In previous studies, quercetin glycosides have been reported to be the dominant flavonol in black currants varieties (Mikulic-Petkovsek et al., 2016), while Mattila et al. (2016) reported myricetin glycosides as the main flavonol. These difference may be due to the difficulty in quantifying myricetin, as this flavonol is unstable and sensitive to interference from other compounds (Justesen et al., 1998). Gavrilova et al. (2011) reported that quercetin 3-O-rutinoside level found between 0.47-1.89 mg/100 g fw in red currants and 4.24-4.58 mg/100 g fw in black currants, these findings were in line with our results. Myricetin has been quantified in all *Ribes* cultivars analyzed; however, levels of these compound in red currant were significantly higher than black currant cultivars. The third prevailing flavonol compounds belonged to the group of kaempferol derivatives (6.46-31.47 mg/kg dw) in all species. In our research, the highest levels of total kaempferol compounds have been measured in the Jostaberry (31.47 mg/kg dw), followed by Red lake (27.25 mg/kg dw), on the contrary, Paunović et al. (2017) reported the amount of kaempferol was very low in all cultivars.

Red lake cultivar had the highest amount of total individual flavonol glycoside and especially quercetin (258.06 mg/kg dw) and myricetin (184.77 mg/kg dw) derivatives were present in high amount and followed by kaempferol derivatives (27.25 mg/kg dw) in this cultivar. For Jostaberry, total individual flavonol glycoside concentration was found 233.13 mg/kg dw. Quercetin 3-O-rutinoside level was the most abundant among the flavonol glycoside quantified, while syringetin 3-glucoside was not determined. Mikulic-Petkovsek et al. (2015) reported that flavonols were only present in small amounts in *Ribes* species ranging from 5% to 11% of total analyzed phenolic compounds and flavonols ranged from 36.12 to 53.94 mg/kg fw in Jostaberry.

It is difficult to make a direct comparison of bioactive compounds found in our research and those reported by other authors in *Ribes* species, since growing conditions, genotype, species, cultivar, fruit maturity, agro techniques, climatic factors, geographic region, and different extraction methods may affect the composition and concentration of phenolic compounds in these fruits (Strack, 1997; Tabart et al., 2006; Rubinskiene et al., 2005; Kellogg et al., 2010; Vagiri et al., 2013; Mikulic-Petkovsek et al., 2013). The data obtained from fruits, all in the same field and therefore cultivated in the same conditions, have confirmed that the genome and cultivar is the principal factor that determines the contents and compositions of phenolic compounds (Plessi et al., 2007).

The results of our study indicate that *Ribes* species, especially black currants are an exceptionally rich source of phenolic compounds and presents a nutritionally rich and healthy fruit. In all black currant cultivars cyanidin 3,5-di-O-glucoside, delphinidin 3-O-rutinoside, and cyanidin 3-O-rutinoside were the predominant anthocyanin compounds, respectively and in red currants only cyanidin 3-O-sambubioside and cyanidin 3-O-rutinoside were detected. In almost all *Ribes* species fruits quercetin 3-O-rutinoside was the major flavonol glycoside and epigallocatechin found as the dominant flavanol compound. It was found that black currants had the highest bioactive properties. Among the black currants, Rosenthal cultivar had the highest amounts of total phenols and antioxidant capacity levels, whereas Boskoop Giant cultivar characterized with the best content of anthocyanins and bioaccessible total phenolic content.

The wide cultivar of the phenolic compounds and values of antioxidant capacities found in the studied *Ribes* species fruit and implies their potential beneficial effects for human health. The obtained data can be used to encourage people to consume more these healthy fruits and can be also used further studies aimed at introducing promising varieties for cultivation.

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