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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF BERRY FRUITS

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The main chemical composition, contents of total phenolic (TPh), total flavonoid (TF), and total monomeric anthocyianin (TMA), as well as the antioxidant activity of two raspberry cultivars (Meeker and Willamette), two blackberry cultivars (Čačanska bestrna and Thornfree) and wild bilberry were studied. The raspberry cultivars had the highest total solids among fruits investigated. Bilberry fruits had the highest sugar-to-acid ratio. Blackberry fruits were richer in crude fibers (cellulose) in comparison to raspberry and bilberry fruits. The content of pectic substances was highest in the bilberry. Also, bilberry had a highest content of TPh (808.12 mg GAE/100 g FW), TF (716.31 mg RE/100 g FW) and TMA (447.83 mg CGE/100 g FW). The antioxidant activity was evaluated spectrophotometrically, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay. The DPPH free radical scavenging activity, expressed as the EC_{50} value (in mg of fresh weight of berry fruit per ml of the reaction mixture), of bilberry (0.3157 ± 0.0145 mg/ml) was the highest. These results also showed that the antioxidant value of 100 g FW bilberry, raspberry - Willamette, raspberry - Meeker, blackberry - Čačanska bestrna and blackberry - Thornfree is equivalent to 576.50 mg, 282.74 mg, 191.58 mg, 222.28 mg and 272.01 mg of vitamin C, respectively. There was a significant positive correlation between the antioxidant activities and content of total phenolics (R_{TPh}^2=0.9627), flavonoids (R_{TF}^2=0.9598) and anthocyanins (R_{TMA}^2=0.9496) in berry fruits.

KEY WORDS: berry fruits, main chemical composition, phenolic compounds, antioxidant activity

INTRODUCTION

Free radicals and other reactive species can cause oxidation and biomolecular damages when the oxidative species exceed the antioxidative defense of the organism, resulting in oxidative stress. This is associated to aging and to the development of pathologies such as cancer, cardiovascular disease, neurodegenerative disorders, diabetes, and inflam-

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mation (1-3). To prevent or slow down the oxidative stress induced by free radicals, sufficient amounts of antioxidants need to be consumed. Fruit and vegetables contain a wide variety of antioxidant compounds (phytochemicals) such as phenolics that may help protect cellular systems from oxidative damage and lower the risk of chronic diseases (4).

Recently, an increasing number of studies have investigated the diverse protective effects of berry fruits (5-7). Protective effects of berry fruits have been attributed to various classes of phenolic compounds, mostly flavonoids and anthocyanins (8-10).

The raspberry fruit is rich in phenolic compounds such as phenolic acids (ellagic acid and hydrolysable tannins - ellagic acid derivatives), flavonoids (flavan-3-ols and their oligomers - mainly dimmers, quercetin) and anthocyanins (cyanidin-3-sophoroside, cyanidin-3-(2-glucosylrutinoside), cyanidin-3-glucoside, pelargonidin-3-sophoroside, cyanidin-3-rutinoside, pelargonidin-3-(2-glucosylrutinoside), pelargonidin-3-glucoside, pelargonidin-3-rutinoside) (11-15). Anthocyanins constitute the main group of phenolic compounds in raspberry. In this fruit, the content of ellagic acid is reported to be high, but not higher than of antocyanins (14).

Bilberries contain high quantities of anthocyanins (in which five antocyanidins – delphinidin, cyanidin, petunidin, peonidin, and malvidin are combined with three types of sugars - galactose, glucose, arabinose), flavanols (catechin, epicatechin), flavonols (quercetin, myricetin, rutin), phenolic acids (chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid, ellagic acid, gallic acid) and stilbene (*trans*-resveratrol) (16, 17).

It has been reported that ellagitannins and cyanidin-3-glucoside are the major phenolic compounds in blackberries. The anthocyanins (cyanidin-3-rutinoside and cyanidin-3-malonyl glucoside), flavonols (quercetin and kaempferol glycosides) and flavan-3-ol (epicatechin) were also identified in blackberries. Hydroxycinnamic acids are minor compounds, and they are found as ferulic, caffeic and *p*-coumaric acid esters (18).

The berries are not only available fresh, but are generally consumable frozen and processed into juice, wine, jam, syrup, soft spreads and tea. Also, berries are interesting as ingredients for use in ice cream and cake icing. Besides, they can be used in the development of functional foods with the objective of enhancing health conditions (19).

The berries have special significance for our country because they represent an important export product. According to raspberry and blackberry production, Serbia is among the leading world countries, whereas bilberries are traditionally collected in woods. Between 90 and 95% of cultivated raspberries in our country are North American Willamette cultivar, which is characterized by the excellent taste and a dark red colour. Besides the Willamette cultivar, in raspberry commercial plantings, Meeker is also cultivated to a minor extent. The predominant cultivar of blackberries in Serbia is Čačanska bestrna (50% of total production), followed by Thornfree and Black saten (20-22).

The phenolic compounds in berries have been reported to have antioxidant, anticancer, antiinflammatory, and antineurodegenerative biological properties (23-24). Because of the biological properties associated with berry fruits, the identification of their antioxidant activity is necessary for the evaluation of berry consumption on human health. The data on chemical composition and antioxidant activity of berry fruits, examined in this work, are poor concerning the growing region (25, 26). For these reasons, the aim of this study was to investigate the main chemical composition, total phenolics, flavonoid and anthocyianin content, as well as the antioxidant activity of two raspberry cultivars

(Meeker and Willamette), two blackberry cultivars (Čačanka and Thornfree) and wild bilberry. Also, the correlation between the total phenolics/flavonoids/anthocyanins and antioxidant activity of berry fruits was investigated.

EXPERIMENTAL

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, ascorbic acid, gallic acid and rutin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). These chemicals were of analytical reagent grade. The other chemicals and solvents used were of the highest analytical grade, obtained from “Zorka” Šabac (Serbia).

Plant material

Two raspberry (Willamette and Meeker) and two blackberry (Čačanska bestrna and Thornfree) cultivars were purchased from Alfa RS, Lipolist, Serbia. Wild bilberry from the region of Kopaonik mountain, were purchased from ITN, Belgrade, Serbia. Fresh undamaged berries were freozen and stored at -20°C for two months. The fruits were defrosted and mashed before chemical analyses.

Main chemical composition

Contents of total solids, total ash, sugar (total sugars, reducing sugars, sucrose) were assessed according to the Regulation on methods of sampling and chemical and physical analyses of fruit and vegetable producst, 29/83 (27) Total solids were determined by drying the samples at 105 °C to constant weight total ash was measured gravimetrically by incenerating the samples at 525 ± 25 °C to constant weight; cellulose (as crude fibers) was determined by the Kirschner-Ganakova method; sugar content was assessed by the method of Luff-Schoorl; pectin content was measured colorimetrically by carbasole method; acidity was determined by titration with NaOH standard solution, and protein content was evaluated by Kjeldahl's method (28).

Total monomeric anthocyanin content

Total monomeric anthocyanins (TMA) in raspberry, blackberry and bilberry fruits was determined according to Giusti and Wrolstad method (29) based on the pH-differential method previously described by Fuleki and Francis (30). Anthocyanin content was expressed as mg of cyanidin 3-glucoside equivalents per 100 g of fresh weight of berry fruit (mg CGE/100 g FW).

Extraction for measurement of phenolics, flavonoids and antioxidant activity

The weighed sample of berry fruit (20 g) was extracted at room temperature using an homogenizer, Ultraturax DIAX 900 (Heidolph Instruments GmbH, Kelheim, Germany).

The extraction was performed using 80% of methanol aqueous solution with 0.05% acetic acid with two portions of the solvent: 160 ml for 60 min, and 80 ml for 30 min. The obtained extracts were combined and evaporated to dryness under reduced pressure. The yields of the bilberry (B), raspberry - Meeker (M), raspberry - Willamette (W), blackberry - Čačanska bestrna (Č) and blackberry - Thornfree (T) extracts were: Y_B = 15.74 ± 0.71%, Y_M = 12.59 ± 0.48 %, Y_W = 12.18 ± 0.52%, $Y_Č$ = 7.52 ± 0.33% and Y_T = 9.65 ± 0.41%, respectively.

Total phenolic content

The amount of total phenolics (TPh) in the berry fruit extracts was determined spectrophotometrically (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan) by the Folin-Ciocalteu method (31). The total phenolic content was determined from the regression equation of the gallic acid calibration curve, and expressed as mg of gallic acid equivalents per 100 g fresh weight of berry fruit (mg GAE/100 g FW).

Total flavonoid content

Total flavonoids (TF) in the berry fruit extracts were measured by the aluminum chloride spectrophotometric assay (32). Total flavonoid content was determined from the regression equation of the rutin calibration curve, and expressed as mg of rutin equivalents per 100 g fresh weight of berry fruit (mg RE/100 g FW).

DPPH radical scavenging activity

The DPPH radical scavenging activity (SA) of berry fruits was determined spectrophotometrically using the modified DPPH method of Chen et al. (33). Briefly, 1 ml of extract solution in distilled water or 1 ml of distilled water (blank) was mixed with 2 ml of DPPH solution (2 mg of DPPH was dissolved in 50 ml of methanol). The range of the investigated extract concentrations was 0.002 - 0.5 mg/ml. The mixture was shaken vigorously and left at room temperature for 30 min, then the absorbance was read at 517 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The capability to scavenge the DPPH radicals (DPPH radical scavenging activity) was calculated using the following equation:

$$SA\ (\%) = 100 \times (A_{blank} - A_{sample})/A_{blank}$$

where A_{blank} is the absorbance of the blank, and A_{sample} is the absorbance of the sample. Ascorbic acid was used as a reference compound.

Statistical analysis

All measurements were carried out in triplicate, and presented as mean ± SD. The correlation and linear regression analyses were performed using Microsoft Office Excel 2003.

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RESULTS AND DISCUSSION

Results of the determination of the main chemical composition of raspberry, blackberry and bilberry are shown in Table 1.

Table 1. Main chemical composition of raspberry, blackberry and bilberry

Compound (g/100 g FW*)	Raspberry		Blackberry		Bilberry
	Meeker	Willamette	Čačanska bestrna	Thornfree	
Total solids	19.36 ± 0.19	16.56 ± 0.2	11.96 ± 0.38	15.57 ± 0.52	14.75 ± 0.29
Ash	0.54 ± 0.03	0.44 ± 0.005	0.29 ± 0.01	0.41 ± 0.01	0.25 ± 0.004
Cellulose	1.64 ± 0.01	1.50 ± 0.04	2.2 ± 0.10	2.97 ± 0.03	0.91 ± 0.02
Pectin	0.18 ± 0.04	0.16 ± 0.03	0.29 ± 0.01	0.30 ± 0.01	0.1 ± 0.00
Pectic acid	0.14 ± 0.02	0.070 ± 0.01	0.1 ± 0.00	0.10 ± 0.00	0.38 ± 0.01
Protopectin	0.35 ± 0.03	0.18 ± 0.05	0.15 ± 0.00	0.17 ± 0.00	0.25 ± 0.01
Acidity	1.28 ± 0.04	1.65 ± 0.00	1.36 ± 0.02	1.39 ± 0.02	0.52 ± 0.05
Total sugars	10.64 ± 0.12	7.85 ± 0.15	5.36 ± 0.03	5.98 ± 0.08	7.84 ± 0.08
Reducing sugars	9.81 ± 0.10	7.19 ± 0.07	1.46 ± 0.16	1.32 ± 0.03	6.69 ± 0.11
Sucrose	0.79	0.63	3.71	4.43	0.94
Proteins	1.58 ± 0.07	1.44 ± 0.06	1.4 ± 0.05	1.49 ± 0.03	1.01± 0.09

*FW – fresh weight of berry fruits

Numerous parameters, like variety, growing conditions, harvesting, maturity stage, transport and handling conditions affect the chemical composition of fruit. Besides, the methods of sample preparation (freezing, storage temperature, time of storage, etc.), and chemical analyses also influence the obtained results. For these reasons, it can be a quite difficult job to interprete and compare the results obtained by different researchers. From the results presented in Table 1, it is obvious that raspberry cultivars had the highest total solids amnog the investigated fruits. The highest the total solids, the more convenient and desirable is the fruit for processing. Generally, the highest share in total solids of fruit is contributed by carbohydrates, i.e. sugars. The content of sugar depends on all above mentioned factors. Organic acids, together with sugars, play an important role in the sensory characteristics of fruit. The dominant acid in berry fruit is citric acid. The results show that among the investigated fruits a highest sugar-to-acid ratio had bilberry.

From the results shown in Table 1 it can be seen that blackberry fruits were richer in crude fibers (cellulose) compared to raspberries and blueberries. On the other hand, bilberry had a highest content of pectic substances. Raspberry - Meeker had the highest share of water insoluble fraction (protopectin), which could be related to the fruit firmness, as softening of the fruit is accompanied with solubilization of pectins due to the action of different enzymes (34).

The feature that makes berry fruit very popular among consumers is their contents of anthocyanins, phenolics and flavonoids, as they exhibit antioxidant activity (35-38) in biological systems. It is observed that the contents of total phenolics, flavonoids and anthocyanins in bilberry are higher than in raspberry cultivars and bilberry (Table 2).

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Table 2. Contents of total phenolics, flavonoids and anthocyanins in berry fruits

Berry fruit	**TPh** (mg GAE/100 g FW)	**TF** (mg RE/100 g FW)	**TMA** (mg CGE/100 g FW)
Bilberry	808.12 ± 32.56	716.31 ± 30.82	447.83 ± 4.16
Raspberry - W	303.90 ± 12.40	169.51 ± 6.24	43,29 ± 2.05
Raspberry - M	265.38 ± 10.75	152.31 ± 5.35	47,34 ± 1.85
Blackberry - Č	235.09 ± 10.72	143.33 ± 5.38	50.95 ± 3.41
Blackberry - T	270.22 ± 12.45	172.95 ± 6.86	102.31 ± 4.04

Our results of phenolic content in examined Meeker (3.04 mg/g) and Willamette (2.65 mg/g) raspberry were higher than those in the study of Milivojević et al. (26), who reported for Meeker and Willamette raspberry phenolic content of 2.22 and 1.02 mg/g, respectively. Lugasi et al. (39) reported phenolic content of 244 mg/100 g for red raspberry. In the study of De Ancos et al. (40) it was shown that the amount of total phenolics in the fresh raspberries depends on the seasonal period of harvesting; late cultivars, Zeva (1776.02 mg of GAE kg^{-1}) and Rubi (1556.67 mg of GAE kg^{-1}), showed the greater phenolic content, and the early cultivars, Autumn Bliss (1212.42 mg of GAE kg^{-1}) and Heritage (1137.25 mg of GAE kg^{-1}), the lowest ones.

Jovančević et al. (41) reported for wild bilberry (*Vaccinium myrtillus* L.) collected in the summer of 2009, from 11 different localities in the mountain region of Montenegro that the total phenolic content in all analyzed samples ranged from 3.92 to 5.24 mg GAE/g FW, while the amounts of total anthocyanins varied between 0.27 to 0.46%. Može et al. (16) showed that the total phenolic contents of bilberries (*Vaccinium myrtillus* L.) sampled from seven different locations in Slovenia ranged from 1027 to 1629 mg/100 g FW. In the samples, 15 anthocyanins were identified by LC-MS/MS. Their contents were 1210.3 ± 111.5 mg CGE/100 g FW.

Sellappan et al. (42) showed that the average total anthocyanin and polyphenolic contents in blackberries were 116.59 ± 8.58 mg/100 g berry and 486.53 ± 97.13 mg/100 g berry. Šamec and Piljac-Žegarac (43) reported for blackberries the total phenol, flavonoid and anthocyanin content of 364.24 ± 9.09 mg GAE/100 g FW, 66.13 ± 3.76 mg CE/100 g FW and 121.82 ± 2.30 mg CGE/100 g FW, respectively.

The differences in the reported results could be due to the environmental conditions, period of harvesting, cultivar variability, or fruit maturity (40).

Several methods have been developed to determine the antioxidant activity of fruits, vegetables as well as herbs. Two major mechanisms, namely hydrogen atom transfer (HAT) and single electron transfer (SET), are well known in the evaluation of the antioxidant activity against free radicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is one of the methods that utilizes both the HAT and SET mechanism. It is considered to be predominantly based on the electron transfer reaction, whereas hydrogen-atom transfer reaction is only a marginal pathway (44). The antioxidant molecules can quench DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless/bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decre-

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ase in the absorbance at 517 nm (45, 46). Fig. 1 shows the dose response for the DPPH radical scavenging activity of raspberry, blackberry and bilberry extracts. The DPPH free radical scavenging activity of the extracts increased with increasing concentration.

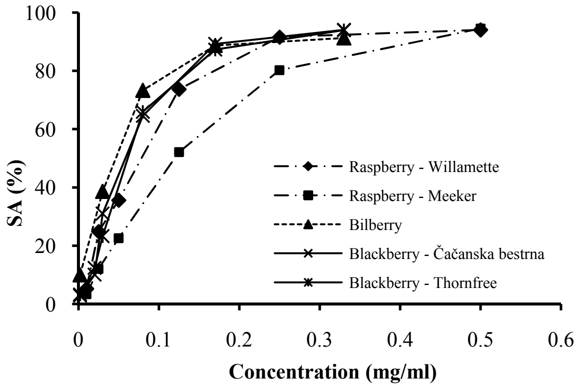

Figure 1. DPPH radical scavenging activity (SA) of the berry fruit extracts

The EC_{50} value, defined as the concentration of sample required for 50% scavenging of DPPH radicals under experimental conditions employed, is a parameter widely used to measure the free radical scavenging activity (47); a smaller EC_{50} value corresponds to a higher antioxidant activity. The EC_{50} values of berry fruits (in mg of extracts or fresh weight of berry fruit per ml of the reaction mixture) and ascorbic acid are shown in Table 3. Bilberry showed a higher DPPH free radical scavenging activity, expressed as EC_{50} value (0.3157 ± 0.0145 mg FW/ml), than the raspberry - Willamette (0.6437 ± 0.0290 mg FW/ml), raspberry - Meeker (0.9500 ± 0.0425 mg FW/ml), blackberry - Čačanska bestrna (0.8188 ± 0.0385 mg FW/ml) and blackberry - Thornfree (0.6691 ± 0.0324 mg FW/ml). Ascorbic acid (vitamin C), because of its antioxidant activity (EC_{50} = 1.82 ± 0.07 µg/ml) was used as a reference compound. These results show that the antioxidant value of 100 g bilberry, raspberry - Willamette, raspberry - Meeker, blackberry - Čačanska bestrna and blackberry - Thornfree is equivalent to 576.50 mg, 282.74 mg, 191.58 mg, 222.28 mg and 272.01 mg of vitamin C, respectively.

Table 3. EC_{50} values of different berry fruit extracts/berry fruits (FW) and ascorbic acid

Berry fruit / reference compound	**EC_{50} (mg extract or ascorbic acid/ml)**	**EC_{50} (mg FW/ml)**
Bilberry	0.0497 ± 0.0023	0.3157 ± 0.0145
Raspberry - W	0.0784 ± 0.0035	0.6437 ± 0.0290
Raspberry - M	0.1196 ± 0.0054	0.9500 ± 0.0425
Blackberry - Č	0.0616 ± 0.0029	0.8188 ± 0.0385
Blackberry - T	0.0646 ± 0.0031	0.6691 ± 0.0324
Ascorbic acid	0.0018 ± 0.0001	-

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It is interesting to consider the correlation between the content of total phenolics/ flavonoids/anthocyanins and antioxidant activity of berry fruits, as phenolic compounds contribute directly to the antioxidant activity (48). The $1/EC_{50}$ is representative of the antioxidant activity because the higher is this value, the more efficient is the berry fruit. The high correlation coefficients (R^2>0.90), calculated from the regression analysis, indicates that there is a significant positive correlation between the content of total phenolics/flavonoids/anthocyanins in berry fruits and DPPH radical scavenging activity (Figure 2).

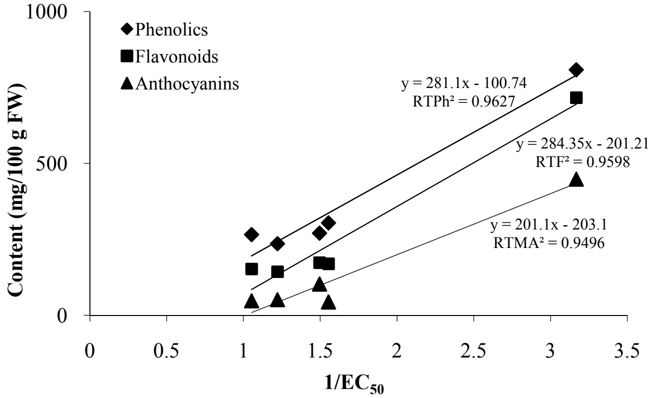

Figure 2. Correlation between content of total phenolics/ flavonoids/anthocyanins in berry fruits and $1/EC_{50}$ value

The linear relations shown in Fig. 2 demonstrate a high positive correlation between the antioxidant activities of berry fruits, determined by DPPH method and the content of total phenolics (R_{TPh}^2=0.9627), flavonoids (R_{TF}^2=0.9598) and anthocyanins (R_{TMA}^2 = 0.9496). This conclusion is in agreement with previous findings, obtained on blueberries (9, 10, 49-51). Also, these results suggest that some other phenolic compounds (probably some phenolic acids), in addition to anthocyanins and other flavonoids, are also responsible for the antioxidant activity of berry fruits.

CONCLUSION

In this work, the main chemical composition of the wild bilberry, two raspberry cultivars (Willamette and Meeker), two blackberry cultivars (Čačanska bestrna and Thornfree) was determined. The raspberry cultivars had the highest total solids among fruits investigated. The highest sugar-to-acid ratio was found in bilberry. The blackberry fruits were richer in crude fibers (cellulose) in comparison to the raspberry and bilberry fruits. The content of pectic substances were highest in the bilberry. The contents of total phenolics, flavonoids and anthocyanins in bilberry were higher than in the raspberry and blackberry cultivars. A highest DPPH free radical scavenging activity showed the bilberry. The antioxidant value of 100 g FW bilberry, raspberry-Willamette, raspberry-Meeker,

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blackberry - Čačanska bestrna and blackberry - Thornfree is equivalent to 576.50 mg, 282.74 mg, 191.58 mg, 222.28 mg and 272.01 mg of vitamin C, respectively. A high positive correlation between the antioxidant activities of berry fruits and the content of total phenolics, flavonoids and anthocyanins (R^2>0.90) indicates that some other phenolic compounds (probably some phenolic acids), in addition to anthocyanins and other flavonoids, were also responsible for antioxidant activity of berry fruits. The high antioxidant activity and significant positive correlation between the concentration of phenolics/flavonoids/anthocyanins and DPPH radical scavenging activity indicate that all investigated berry fruits can be considered as a good source of natural antioxidants that may have potential health effects.

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ХЕМИЈСКИ САСТАВ И АНТИОКСИДАТИВНА АКТИВНОСТ БОБИЧАСТОГ ВОЋА

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У овом раду испитани су основни хемијски састав, садржај укупних полифенолних једињења, флавоноида и антоцијана, као и антиоксидативна активност малине (сорти Meeker и Willamette), купине (сорти Чачанска бестрна и Thornfree) и дивље боровнице. Од испитаног воћа највећи садржај суве материје утврђен је код обе сорте малине. Највећи однос шећера и киселина одређен је за боровницу. У односу на малине и боровницу, у купинама је одређен већи садржај целулозе. Највећи садржај пектинских материја одређен је за боровницу. Боровница је имала највећи садржај укупних фенолних једињења, флавоноида и антоцијана (808,12 mg GAE/100 g FW, 716,31 mg RE/100 g FW, односно 447,83 mg CGE/100 g FW). Антиоксидативна активност на стабилне 2,2-дифенил-1-пикрилхидразил (DPPH) радикале испитана је спектрофотометријском методом. Од испитаног бобичастог воћа највећу скевинџер активност на DPPH радикале, изражену као EC_{50} вредност (mg свежег бобочастог воћа/ml реакционе смеше), показала је боровница (0,3157 mg/ml). Такође, утврђено је да је антиоксидативна вредност 100 g свеже боровнице, малине - Willamette, малине - Meeker, купине - Чачанска бестрна и купине - Thornfree једнака антиоксидативној вредности 576,50 mg, 282,74 mg, 191,58 mg, 222,28 mg, односно 272,01 mg витамина Ц. Утврђена је значајна позитивна корелација између антиоксидативне активности и садржаја укупних фенолних једињења (R_{TPh}^2= 0,9627), флавоноида (R_{TF}^2=0,9598) и антоцијана (R_{TMA}^2=0,9496) у бобичастом воћу.

Кључне речи: бобичасто воће, основни хемијски састав, антиоксидативна активност, фенолна једињења

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