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COMPARISON OF POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF EXTRACTS FROM FRUITS OF TWO *CRATAEGUS* SPECIES

Poređenje sadržaja polifenola i antioksidacijske aktivnosti ekstrakata plodova dvije vrste gloga

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Abstract

Methanolic and acidified methanolic extracts of *C. monogyna* and *C. rhipidophylla* dried fruits were used in estimation of total phenolic contents and antioxidant activities. The extracts of fruit were examined for their antioxidant activity by DPPH method. Antioxidant activity of the extracts varied from 17.65 to 22.05 mg ascorbic acid equivalents per g^{-1} dry weight. Total phenols, flavonoids, monomeric anthocyanins and proanthocyanidins in fruits ranged from 21.53 to 34.72 mg gallic acid equivalents per g^{-1} dry weight, 0.75 to 1.92 mg rutin equivalents and 0.13-0.93 mg quercetin equivalents per g^{-1} dry weight, 0.51 to 0.82 mg cyanidin-3- glucoside equivalents per g^{-1} dry weight, 11.27-18.77 mg cyanidin chloride equivalents per g^{-1} dry weight respectively. The higher values of total flavonoids generally are obtained with 80% methanol extracts for both species.

The amounts of all investigated compounds and antioxidant activity were significantly higher in *C. monogyna* fruits which were confirmed by one-way ANOVA analysis. Correlations between antioxidant activity and total phenols and proanthocyanidin contents were found as the main compounds influencing the antioxidant capacity of the samples. Obained results suggest that both species represent valuable source of antioxidant compounds.

Key words: *C. monogyna, C. rhipidophylla, phenols, DPPH*

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INTRODUCTION – *Uvod*

Wild fruit of different trees and shrubs have been used in human diet and folk medicine for a long time. Among them, fruits of *Crataegus monogyna* or *Crataegus leavigata* species are traditionally used in treatment of cardiovascular diseases, regulation of blood pressure, treatment of arteriosclerosis and angina pectoris (CHANG ET AL. 2002). In food industry, fruits are particularly used in production of herbal tea and juices (CHANG ET AL. 2002, BARROS ET AL. 2010). Investigations on chemical composition of hawthorn fruits revealed that they are rich source of different phenolic compounds. Generally, phenolic acids, flavones, flavonols and proanthocyanidins seems to be the main active ingredients abundant in leaves, flowers and fruits (THE COUNCIL OF EUROPE 2004, CHANG ET AL. 2002, KIM ET. AL. 2000).

Different investigators found that hawthorn extracts possess antiradical activity (BERNATONIENE ET AL. 2008, TADIĹ ET. AL. 2008, BARROS ET AL. 2010) and to inhibit LDL oxidation (ZHANG ET AL. 2001, QUETTIER ET AL. 2003). Plant polyphenols are well known antioxidants which can participate in antioxidant defense in organisms against free radical species (PIETTA 2000). Therefore, in last decades interest in finding new natural oxidants increased greatly (EGEA ET AL. 2010, RUIZ-RODRIGUEZ ET AL. 2014). These compounds are important since they can be used in food industry to substitute synthetic antioxidants and in production of different dietary supplements.

Biological characteristics of genus *Crataegu*s in Bosnia and Herzegovina have been studied by several researches (BECK 1927, MALY 1919, 1940; FUKAREK 1974; JANJIĹ 1998, 2002; BAĠIĹ 2004). Genus *Crataegus* is represented by four autochthonic species with *C. monogyna* Jacq. as the most abundant specie (CHRISTENSEN AND JANJIĹ 2006). *Crataegus monogyna* Jacq. also known as a common hawthorn is characterized by highly wide ecological amplitude. On the other hand, *C. rhipidophylla* Gand was recently included in Flora of Bosnia and Herzegovina (JANJIĹ 1998, 2002). Two other species (C. *microphylla* subs. *malyana* and *C. leavigata* (Poiret) DC) was not subject of this work. Investigations of *Crataegus* species in terms of chemical composition and antioxidant activity has been started recently by our research group and are still in progress. According to our best knowledge this is the first paper on phenolic content and antioxidant activity of *C. rhipidophylla* from our country.

In this work we investigated phenolic contents and antioxidant activity of C. *monogyna* and *C. rhipidophylla* fruits in extracts obtained with two extractants. Extracts were prepared with aqueous 80% methanol and acidified aqueous 80% methanol (1.5 moldm^{-3} in HCl acid). Obtained extracts were used in spectrophotometric determinations of total phenols, flavonoids, monomeric anthocyanins and proanthocyanidins. Antioxidant activity for all extracts was investigated with DPPH method and possible correlation of antioxidant capacity with phenolic compounds was investigated.

MATERIAL AND METHODS – *Materijal i metode*

Plant material – *Biljni materijal*

C. monogyna and *C. rhipidophylla* fruit samples were collected in September and October 2013. from different localities at TrebeviĺMountain in fully ripe stage. Three samples per species were collected and samples were identified by one of the author and voucher specimens were deposited at the Herbarium of the Department of Ecology at Faculty of Forestry. The samples were dried at room temperature and whole fruits were powdered. The samples and their locations are depicted in Table 1.

Table 1. Samples and sampling locations
Tabela 1. Uzorci i lokaliteti uzorkovanja

Samples Uzorci	Abbreviations Skraćenice	Locations Lokacije
C. monogyna 1	M1	Petroviĺi
C. monogyna 2	M2	Dobre vode
C. monogyna 3	M3	Vidikovac
C. rhipidophylla 1	R1	Petroviĺi
C. rhipidophylla 2	R2	Miljeviĺi
C. rhipidophylla 3	R3	Dobre vode

Chemicals and reagents – *Hemikalije i reagensi*

1,1-diphenyl-2-picrylhydrazyl radical (DPPHÅ), quercetin, rutin, ascorbic acid, aluminium chloride, Folin-Ciocalteu's reagent, sodium carbonate and absolute methanol were purchased from Sigma Chemicals (Germany) and Aldrich (Germany). Butanol was obtained from Merck Chemical Suppliers (Germany). Potassium chloride and ferrous ammonium sulfate were obtained from Kemika Zagreb (Croatia). All chemicals and solvents were of analytical grade.

Sample extracts preparation- *Priprema ekstrakata uzoraka*

Extraction of fruit samples were done according to modified procedure by MRAIHI ET AL. (2013). Briefly, 1 g of powdered fruit sample was extracted separately twice with 12 ml of 80% aqueous methanol (marked as A) or acidified 80% methanol prepared by mixing 80 ml of absolute methanol with 20 ml of 1.5 moldm^{-3} HCl (marked as B). Extractions were carried out in an ultrasound bath (Elmecs, Italy) for 30 minutes at room temperature. After the first step of extraction, the extracts (with solvent A and with solvent B) were centrifuged at 3000 rpm for 15 minutes, and obtained supernatants were decanted. Extraction was repeated with the same amount of the same solvent for each sample. Obtained supernatants were combined and the volume was adjusted to 25 ml in volumetric flask with appropriate solvent (A or B). Obtained extracts (with solvent A and solvent B) are stored at -20^{o}C until analysis.

Determination of total phenols- ***Određivanje ukupnih fenola***

Total phenols (TP) were determined spectrophotometrically with Folin-Ciocalteu method according to the procedure described by SINGLETON ET AL. (1974). Gallic acid standard was used for preparation of appropriate calibration curve. The absorbance of the colored product was measured at 765 nm. Final results are expressed as mg of gallic acid equivalents per gram of dry sample (mg GAE g^{-1}).

Determination of total flavonoids ï ***Određivanje ukupnih flavonoida***

Two procedures with different standards are used in the spectrophotometric determination of total flavonoids (TF) based on aluminum chloride method. Total flavonoids (TFq and TFr) were determined according to procedure described by ORDONEZ ET AL. (2006) using quercetin as a standard, and according to the procedure given by QUETTIER ET AL. (2000) with rutin as a standard. Apsorbances of the samples were measured at 420 nm and 415 nm respectively. For each procedure, sample blanks were also included. As a blank, appropriate volume of solvent A or B was used instead standard / sample. Final results are expressed as mg equivalents of quercetin (QE)/rutin (RE) per gram of dry sample.

Determination of monomeric anthocyanins – ***Određivanje monomernih antocijanina***

Total monomeric anthocyanins (TMA) were determined with pH differential method by LEE ET AL. (2005). Absorbances were measured at 520 and 700 nm at room temperature, 15 min after dilution of extracts (1:10). The content of total monomeric anthocyanins was expressed in mg of cyanidin-3-glucoside equivalents (CGE) per gram of dry fruits. A molar extinction coefficient of cyanidin-3-O-glucoside of 26900 l $mol^{-}1cm^{-1}$ and molar weight (MW) (449.2 g mol^{-1}) were used for calculations.

Determination of total proanthocyanidins – ***Određivanje ukupnih proantocijanidina***

The butanol/HCl assay was used to quantify the total proanthocyanidins (TPA) (PORTER 1986, MRAIHI ET AL. 2013). Proanthocyanidins were determined by VIS spectrophotometry method based on their acid hydrolysis and color formation with the reagent. Absorbance was read at 550 nm before and after heating of the samples at 95^{o}C for 40 minutes. As a blank, butanol/HCl mixture was used. The results were expressed as mg of cyanidin chloride equivalents (CE) per gram of dry fruit. A molar extinction coefficient of cyanidin of 17360 l $mol^{-}1cm^{-1}$ and molar weight (MW) (287 g mol^{-1}) were used for calculations.

DPPH radical scavenging activity – ***aktivnost "hvatanja" slobodnih radikala***

Measurements of antiradical activity were adapted from SANCHEZ-MORENO ET AL. (1998). In brief, 0.1 ml of diluted extract was added to 1.9 ml of freshly prepared 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·) solution dissolved in methanol (7.12 x

10^{-5} moldm^{-3}). After 30 min of incubation in the dark, the absorbance was read at 517 nm. Results were expressed as vitamin C equivalents using the following equation:

$$ = 17.787 \times + 1.2817$$

Where I (%) = $[(A_{DPPH}-A_{ext})/A_{DPPH}]$ x100 presents percentage inhibition of DPPH radical and c is a concentration of ascorbic acid standards expressed in mgdm^{-3}. All measurements were performed in triplicate. The results were expressed as vitamin C equivalents (AAE) per gram of dry fruit.

Statistical analysis- ***Statistička analiza***

All measurements were carried out in triplicate and obtained results are expressed as mean±SD. All statistical analysis was performed using Statgraphics Plus 5.0 software (Stat. Inc., USA). Obtained results were analysed using the one-way ANOVA. The correlations within variables were examined by Pearson correlation.

RESULTS AND DISCUSSION- *Rezultati i diskusija*

Polar solvents such as methanol, ethanol and water are frequently used in the extraction of phenolic compounds from plant materials. These solvents cause denaturation of plant cell membranes realising phenolic compounds from plant materials. Acidified methanol or ethanol can be applied for the same purposes, and usually acidification is done with weak organic acids or strong mineral acids at low concentrations (KODAMI ET AL. 2013). For some compounds, such as anthocyanins according to DAI AND MUMPER (2010) extraction efficiency and stability is better when acidified alcohol are used instead hydro-alcohol solutions. Also, in our previous work we found that extraction efficiency of hydro-alcohol solutions is better in comparison to pure solvents applied under same conditions (TAHIROVIĆ AND BAĠIĆ 2014).

In this study contents of different bioactive compounds (phenols, flavonoids, anthocyanins and proanthocyanidins) as well as antioxidant capacity for *C. monogyna* and *C. rhipidophylla* fruit samples are presented in Table 2.

Table 2. Total phenolic content and antioxidant activity of *C. monogyna* and *C. rhipidophylla* samples.
Tabela 2. Sadržaj ukupnih fenola i antioksidacijski kapacitet za C. monogyna i C. rhypidophylla uzorke.

	TP (mg GAE g^{-1})	TFr (mg RE g^{-1})	TFq (mg QE g^{-1})	TMA (mg CG g^{-1})	TPA (mg CE g^{-1})	AC_{DPPH} (mg AAE g^{-1})
			(A)			
M1	23.34±0.06	1.62±0.01	0.77±0.003	**0.82±0.01**	11.53±0.00	17.65±0.71
M2	27.40±0.06	1.53±0.03	0.72±0.003	0.60±0.01	13.75±0.03	19.92±0.52
M3	**34.72±0.06**	**1.92±0.01**	**0.93±0.003**	0.64±0.00	**17.28±0.09**	**21.22±0.58**
R1	**26.2±0.00**	**1.23±0.01**	**0.56±0.01**	**0.69±0.01**	**14.47±0.09**	**18.9±0.14**
R2	21.53±0.01	0.89±0.00	0.39±0.01	0.51±0.02	11.53±0.03	17.92±0.18
R3	23.27±0.15	0.84±0.01	0.36±0.01	0.54±0.01	12.67±0.06	18.76±0.57
			(B)			
M1	24.73±0.06	1.31±0.01	0.21±0.01	**0.79±0.00**	14.93±0.07	21.94±0.39
M2	26.83±0.12	1.37±0.01	0.22±0.00	0.63±0.01	12.65±0.1	21.1±0.15
M3	**34.25±0.06**	**1.92±0.01**	**0.29±0.00**	0.65±0.02	**18.77±0.12**	**22.05±0.39**
R1	**29.10±0,00**	**1.23±0.02**	**0.19±0.01**	**0.65±0.01**	**15.6±0.19**	20.89±0.11
R2	21.87±0,26	0.75±0.01	0.13±0.01	0.6±0.01	11.27±0.03	20.65±0.29
R3	25.87±0.06	0.91±0.01	0.14±0.01	0.64±0.02	13.86±0.03	**21.33±0.09**

total phenolic content (TP), total flavonoids/rutin content (TFr), total flavonoids/quercetin content (TFq), total monomeric anthocyanins (TMA), total proanthocyanidins (TPA) and antioxidant capacity (AC) of *C. monogyna* and *C. rhipidophylla* samples extracted with 80% methanol (A) and acidified 80% methanol (B)
sadržaj ukupnih fenola (TP), ukupnih flavonoida/rutin (TFr), ukupnih flavonoida/kvercetin (TFq), ukupnih monomernih antocijanina (TMA), ukupnih proantocijanidina (TPA) i antioksidacijski kapacitet (AC) za C. monogyna i C. rhipidophylla uzorke ekstrahovane sa 80% metanolom (A) i zakiseljenim 80% metanolom (B)
- data are expressed as mean±SD (n=3)
-podaci su izraženi kao srednja vrijednost±SD (n= 3)

From the obtained results it can be seen that amounts of phenolic compounds in *C. monogyna* were as follows: total phenols 23.34-34.72 mg GAE g^{-1} DW; total flavonoids 1.31-1.92 mg RE g^{-1} DW and 0.21 ï 0.93 mg QE g^{-1} DW, total monomeric anthocyanins 0.60 ï 0.82 mg CG g^{-1} DW, and total proanthocyanidins 11.53 ï 18.77 mg CE g^{-1} DW. Except for total monomeric anthocyanins, the highest contents of all other investigated compounds were found in sample M3 (Vidikovac) for both extraction systems. The highest total monomeric anthocyanin contents were found in M1 (Petroviĺ i) for both extraction system.

Phenolic compounds in *C. rhipidophylla* fruits were in the range: total phenols 21.53-29.10 mg GAE g^{-1} DW; total flavonoids 0.75-1.23 mg RE/g^{-1} DW and 0.13 -

0.56 mg QE g^{-1}DW; total monomeric anthocyanins 0.51-0.69 mg CG g^{-1} DW; total proanthocyanidins 11.27-15.60 mg CE g^{-1} DW. The highest contents of all investigated compounds were found in R1 (PetroviĆi) for both extraction systems.

Antioxidant activity determined with DPPH method for *C. monogyna* ranged from 17.65 to 22.05 mg AAE g^{-1} DW, and from 17.92 to 21.33 mg AAE g^{-1} DW for *C. rhipidophylla*. The highest antioxidant capacity was found for M3 and R1 respectively in 80% methanol extracts. The highest antioxidant capacity was found for M3 and R3 samples in acidified methanol. Generally, higher amounts of investigated compounds and higher antioxidant capacity had *C. monogyna* samples.

Phenolic content and content of flavonoids, proanthocyanidins of plant fruits is influenced by genotype, habitat conditions and ripeness of fruits (BARROS ET AL. 2010, BAHORUN ET AL. 1994; BAHRI-SAHLOUL ET AL. 2009B; FROECHLIER ET AL. 2009).

According to review by EDWARDS ET AL. (2012), content of total phenols in *C. monogyna* fruits determined by different investigators were in the range 9.1 - 17.8 mg g^{-1} and 16.426 - 57.07 mg g^{-1}; total flavonoids content was 4.46 - 147.3 mg $g^{-1,}$ total proanthocyanidins were 19.29 mg g^{-1} and total anthocyanins were in range 0.150 - 0.580. It can be seen that our results for total phenols and total anthocyanins are in agreement with presented data, while our results for total flavonoids and proanthocyanidins are below literature values.

Generally, in both extraction systems, higher contents of total phenols and proanthocyanidins were found for both species which is similar to investigations of MRAIHI ET AL. (2013.) obtained for *C.monogyna*. We can conclude that both species are rich in phenolic a compound which is important parameter for determination of pharmacological properties of the plants. Proanthocyanidins are considered to be important bioactive compounds in hawthorn fruits (CHANG ET AL. 2002). According to European pharmacopoeia the hawthorn fruits used in official medicine must not contain less than 1.0% procyanidins (THE COUNCEL OF EUROPE 2004). As it can be seen, total procyanidins in *C. ripidophylla* ranged from 1.13-1.56% and meets required criteria for total proanthocyanidin content. Generally, both species are rich in proantocyanidin content.

Flavonoids are important group of phenolic compounds with different roles in plant physiology (DEWICK 2009). The main flavonoids found in *Crataegus* fruits are flavonol-O-glycosides (hiperoside, rutin) and flavone-C-glycosides (vitexin). *Crataegus* species are also characterized by high variability in flavonoid content and therefore there is no strict concentration range for any of the mentioned compounds (FONG AND BAUMEN 2002). Studies by BAHORUN ET AL. (1994) showed that flavonoid content in *Crataegus* fruits decreases during ripening stage. This can explain relatively low contents of flavonoids in our work since we used fully ripened fruits. This is also in agreement with finding of BARROS ET AL. (2010).

In addition to rutin, we used quercetin as a second standard for the determination of total flavonoids. Total flavonoid contents were much higher with rutin as a standard for both species in both extraction systems.

According to the obtained results we can conclude that both species are valuable source of monomeric anthocyanins which are known for their antioxidants effects and prevention of various diseases associated with oxidative stress (ANDERSEN AND JORDHEM 2006). Generally, higher values of monomeric anthocyanins are obtained for *C. monogyna* compared with the values reported by EDWARDS ET AL. (2012) and MRAIHI ET AL. (2013) with a total value of 5.89 mg CE 100 g^{-1} DW.

One-way ANOVA was performed to analyze statistically significant differences for variable means between species, extracts and extracts per species. Average values for all variables are given in Table 3. and corresponding statistical data for one-way ANOVA are given in Table 4. In the case of antioxidant capacity AC_{DPPH}, statistically significant higher mean values are obtained with extracts B for both species (AC(B(M)) = 21.7; AC(B(R) = 20.96. These results could be connected to higher amount of proanthocyanidins in B extraction system which could be a main reason for higher correlation found between AC_{DPPH} and TPA (r = .631), table 5.

Table 3. Average values of phenolic content and antioxidant activity
Tabela 3. Prosječne vrijednosti sadržaja fenola i antioksidacijske aktivnosti.

	TP (mg GAE g^{-1})	**TFr (mg RE g^{-1})**	**TFq (mg QE g^{-1})**	**TMA (mg CG g^{-1})**	**TPA (mg CE g^{-1})**	**AC_{DPPH} (mg AAE g^{-1})**
S	26.59 ±4.20	1.29±0.39	0.41±0.26	0.65±0.09	14.03±2.28	20.19±1.52
M	28.54±4.54	1.61±0.25	0.52±0.30	0.69±0.09	14.82±2.60	20.65±1.61
R	24.64±2.76	0.98±0.19	0.29±0.16	0.60±0.07	13.23±1.61	19.74±1.33
A	26.08±4.46	1.34±0.40	0.62±0.21	0.63±0.11	13.54±2.05	19.06±1.32
A(M)	28.49±4.99	1.69±0.17	**0.81±0.09**	0.69±0.10	14.19±2.51	**19.60±1.65**
A(R)	23.66±2.05	0.99±0.18	**0.44±0.10**	0.58±0.09	12.89±1.29	**18.53±0.56**
B	27.11±3.98	1.25±0.38	0.20±0.06	0.66±0.06	14.51±2.45	21.33±0.58
B(M)	28.60±4.33	1.53±0.29	**0.24±0.04**	0.69±0.08	15.45±2.68	**21.70±0.53**
B(R)	25.61±3.14	0.96±0.21	**0.15±0.03**	0.63±0.03	13.58±1.89	**20.96±0.34**

total phenols (TP), total flavonoids/rutin (TFr), total flavonoids/quercetin (TFq), total monomeric anthocyanins (TMA), total proanthocyanidins (TPA) and antioxidant activity (AC_{DPPH}) in two extraction system (A and B)

ukupni fenoli (TP), ukupni flavonoidi/ rutin (TFr), ukupni flavonoidi/kvercetin (TFq), ukupni monomerni antocijanini (TMA), ukupni proantocijanidini (TPA) i antioksidacijska aktivnost (AC_{DPPH}) u ekstrakcioonom sistemu (A i B).

S- samples, M ï *C.monogyna*; R- *C. rhipidophylla*; A ï 80% methanol; B- acidified 80% methanol; A(M) and A(R)- *C. monogyna* and *C. rhipidophylla* extracted with A; B(M) and B(R)- *C. monogyna* and *C.rhipidophylla* extracted with B.

S- uzorci, M- *C.monogyna*; R- *C. rhipidophylla*; A ï 80% metanol; B- zakiseljeni 80% methanol; A(M) i A(R)- *C. monogyna* i *C. rhipidophylla* ekstrahovani sa A; B(M) i B(R)- *C. monogyna* i *C.rhipidophylla* ekstrahovani sa B.

From the results it can be seen that *C. monogyna* had higher values for all investigated variables compared to *C. rhipidophylla* (M and R, Table 3). Related to the species, significant differences are obtained for the content of all investigated bioactive compounds while mean difference for antioxidant capacity was not significant (AC (A(M))= 20.65 mg AAE g^{-1} DW and AC(B(R))= 19.74 mg AAE g^{-1} DW). Also, highly significant mean differences ($p<0.01$) are found for total flavonoids (TFq) and antioxidant activity in case of different extracts A and B as for whole sample so for both particular species. Extract A delivered statistically significant higher mean values of total flavonoids (TFq) for both species (TFq (A (M)) = 0.81 mg QE g^{-1} DW TFq (A(R) = 0.44 mg QE g^{-1} DW) which suggests that 80% methanol has better extraction efficiency in the determination of flavonoids when quercetin is to be used as a standard. However, the extraction efficiency of two extraction systems was very similar for other investigated compounds for both species with no significant difference (Table 4).

Table 4. ANOVA ï *p* values
Tabela 4. ANOVA – p vrijednosti

	TP	TFr	TFq	TMA	TPA	AC_{DPPH}
Species (n=36) Specije (n=36)	**.004**	**.000**	**.007**	**.003**	**.035**	.074
Extracts (n=36) Ekstrakti (n=36)	.469	.487	**.000**	.404	.204	**.000**
M (between A and B) M (izmeĽu A i B)	.960	.188	**.000**	.959	.317	**.002**
R(between A and B) R(izmeĽu A i B)	.138	.781	**.000**	.139	.383	**.000**

M ï *C.monogyna*; R- *C- rhipidophylla*; A -80% methanol, B- acidified 80% methanol.
M ï *C.monogyna*; R- *C- rhipidophylla*; A -80% metanol, B- zakiseljeni 80% metanol

Finally, correlation analysis is used to examine relations between measured variables. Significant correlations are bolded and presented in table 5.

The highest correlations are obtained between total phenols and total proanthocyanidins (.921), and in some less extend between phenols and total flavonoids (TFr) ((.799). Also it was found that total flavonoids (TFr) positively correlate with proanthocyanidins (.685), flavonoids (TFq) (.603), and in some smaller extend with monomeric anthocyanins (.434). This is confirmed with observation in the presented data. Antioxidant capacity was positively correlated with all variables except correlation between flavonoids (TFq) and AC_{DPPH} (-.397). These results suggest that phenols and proanthocyanidins are the major contributors to the antioxidant activity of fruits for both species.

Table 5. Correlations (*p values*)
Tabela 5. Korelacije (*p vrijednosti*)

	TFr	TFq	TMA	TPA	AC_{DPPH}
TP	**,799** (.000)**	0.307 (.069)	0.084 (0.628)	**,921** (.000)**	**,578** (.000)**
TFr	1	**,603** (.000)**	**,434** (.008)**	**,685** (.000)**	0.273 (.107)
TFq		1	0.1892 (.269)	0.133 (.438)	**-.397*(0.016)**
TMA			1	0.161 (.347)	0.104 (.545)
TPA				1	**,631** (.000)**

**. Correlation is significant at the 0.01 level (2-tailed).
*** Korelacija je značajna na 0.01 nivou (2-strana)*
*. Correlation is significant at the 0.05 level (2-tailed).
** Korelacija je značajna na 0.05 nivou (2-strana)*

Antioxidant activity of *C. monogyna* fruit extracts was investigated by several researches (EGEA, ET AL. 2010, BERNATONINE ET AL. 2008, RUIZ-RODRIGEZ ET AL. 2014, MRAIHI ET AL. 2013). Literature data are quite differing since different extraction methods, AC methods as well as different interpretations of the results are used. For example, MRAIHI ET AL. (2013) found high correlation between $TEAC_{DPPH}$ and total phenolic content and moderate correlation with proanthocyanidins content. RUIZ ï RODRIGEZ ET AL. (2014) reported high correlation between antioxidant capacity and vitamin C content and that phenolic compounds are the major contributors to the antioxidant capacity.

Total flavonoids and total monomeric anthocyanins did not significantly influence the antioxidant activity of the extracts which can be related to their lower concentrations comparing to phenolic and proanthocyanidins content.

CONCLUSIONS – *Zaključcı*

C. monogyna fruits were characterized by high total phenols content with average values from 28.49 to 28.6. Beside total phenols, total proanthocyanidins (14.19 - 15.95) and total flavonoids expressed as rutin equivalents (TFr = 1.53 - 1.69) were the major components. The lowest contents were measured for monomeric anthocyanidins TMA = 0.69 (A and B) and flavonoids expressed as quercetin equivalents with the values of TFq=0.81 - 0.24.

C. rhipidophylla fruits also showed high total phenols content, (TP) = 25.61-23.66. Similarly to *C. monogyna* fruits, dominant compounds were proanthocyanidins (TPA = 12.89 - 13.58) followed by flavonoids (rutin equivalents) with the values of TFr= 0.96 - 0.99. The lower contents were found for monomeric anthocyanidins TMA = 0.58- 0.63 and for flavonoids (quercetin equivalents) TFq = 0.15 ï 0.44. Statistically significant difference in extraction efficiency between 80% methanol and acidified

methanol was noticed in total flavonoid contents expressed as quercetin equivalents for both investigated species. The higher values of total flavonoids generally are obtained with 80% methanol extracts for both species.

The amounts of all investigated compounds were significantly higher in *C. monogyna* fruits. This is also related with higher antioxidant activity of *C. monogyna* fruits determined with DPPH method. Strong correlations were found between AC_{DPPH} and total proanthocyanidns and total phenols as the main compounds which contribute to antioxidant activity.

It can be concluded that both species can be considered as a valuable source of antioxidant compounds.

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SAŽETAK

Uzorci plodova dvije vrste gloga (*C.monogyna* i *C. rhipidophylla*) analizirani su na sadrġaj ukupnih fenola, flavonoida, monomernih antocijanina i proantocijanidina kao i antioksidacijsku aktivnost u vodeno - metanolnim i zakiseljenim vodeno - metanolnim ekstraktima. U odreĿivanju sadrġaja bioaktivnih jedinjenja i antioksidacijske aktivnosti ekstrakata, koriġtene su spektrofotometrijske metode. OdreĿivanje monomernih antocijanina izvrġeno je primjenom pH diferencijalne metode a kiselinsko-butanolna metoda je upotrebljena za kvantifikaciju ukupnih proantocijanidina. Ukupni fenoli su odreĿeni Folin-Ciocalteu metodom, a $AlCl_3$ metodom vrġeno je odreĿivanje ukupnih flavonoida prema rutinu i kvercetinu kao standardima. Za mjerenje antioksidacijske aktivnosti upotrebljena je DPPH metoda.

Sadrġaji ukupnih fenola po gramu suhog uzorka (s.u) za uzorke *C. monogyna* iznosili su 23.34 ï 34.72 mg GAE g^{-1} s.u; ukupnih flavonoida 1.31-1.93 mg RE g^{-1} s.u. i 0.21-

0.93 mg QE g^{-1} s.u; sadrġaji ukupnih monomernih antocijanina su se kretali u granicama 0.60-0.82 mg CGE g^{-1} s.u. i proantocijanidina 11.53-18.77 mg CE g^{-1} s.u. U uzorcima *C.rhipidophylla* sadrġaji ispitivanih jedinjenja iznosili su: ukupni fenoli 21.53-29.10 mg GAE g^{-1} s.u; ukupni flavonoidi 0.75-1.23 mg RE g^{-1} s.u i 0.13-0.56 mg QE g^{-1} s.u; ukupni monomerni antocijanini 0.51-0.69 mg CGE g^{-1} s.u; ukupni proantocijanidini 11.27-15.60 mg CE g^{-1} s.u.

Vrijednosti antioksidacijske aktivnosti za uzorke *C. monogyna* kretale su se od 17.65 do 22.05 mg AAE g^{-1} s.u., a za *C. rhipidophylla* od 17.92 do 21.33 mg AAE g^{-1} s.u.

Veću antioksidacijsku aktivnost su imali ekstrakti dobiveni sa zakiseljenim metanolom za obje vrste i to za *C. monogyna* AC = 21.70 mg AAE g^{-1} s.u., i za *C. rhipidophylla* AC = 20.96 mg AAE g^{-1} s.u.

Generalno, u oba ekstrakciona sistema veći sadrġaj ukupnih fenola, flavonoida, monomernih antocijanina i proantocijanidina je utvrĽen za *C. monogyna. Takođe* najveća antioksidacijska aktivnost, odreĽena DPPH metodom, izmjerena je u ekstraktima *C. monogyna*.

ANOVA testom, utvrĽene su statistiļki znaļajne razlike u sadrġaju flavonoida prema kvercetinu (TFq) i vrijednostima AC_{DPPH} za obje vrste u oba ekstrakciona sredstva. TakoĽe je utvrĽena i statistiļki znaļajna razlika u sadrġaju ispitivanih bioaktivnih jedinjenja izmeĽu vrsta.

Korelacijska analiza je pokazala postojanje znaļajnih korelacija izmeĽu sadrġaja ukupnih fenola (TP), proantocijanidina (TPA) i antioksidacijske aktivnosti (AC_{DPPH}). Rezultati dobiveni u ovom radu pokazuju da se plodovi ispitivanih vrsta glogova mogu smatrati prirodnim izvorom fenolnih jedinjenja sa dobrom antioksidacijskom aktivnoġću.