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PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF CRATAEGUS MONOGYNA JACQ. AND CRATAEGUS MACROCARPA HEGETSCHW. LEAVES AND FRUITS EXTRACTS

Sadržaj fenola i antioksidacijska aktivnost ekstrakata lišća i ploda *Crataegus monogyna* Jacq. i *Crataegus macrocarpa* Hegetschw.

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Abstract

Phenolic contents of methanolic extracts prepared from leaves and fruits of *Crataegus monogyna* Jacq. and *Crataegus macrocarpa* Hegetschw. were determined. The antioxidant capacity was assessed by DPPH, ABTS and FRAP assay. The results showed that leaves were richer in the content of phenols (59.23 - 91.91 GAE g^{-1}), flavonoids (2.38 - 4.08 mg QE g^{-1} and 5.24 - 8.9 mg RE g^{-1}) , phenolic acids (33.40 - 68.98 CAE g^{-1}) and proanthocyanidins (26.15 - 48.60 CE g^{-1}) while in fruits anthocyanins dominateted (0.43 - 0.80 CG g^{-1}). Leaves also had higher antioxidant capacity than fruits for both species. Generelly, *C. monogyna* fruits had higher content of anthocyanins. Total phenols, phenolic acids and proanthocyanidins were highly corelated with DPPH (r^2 = 0.8703 - 0.9618), ABTS (r^2 = 0.7833 - 0.9443) and FRAP (r^2 = 0.903 - 0.9695) assay. The results suggests that these compounds were the major contributors to the antioxidant capacity in leaves and fruits extracts of both species.

Higher contents of bioactive compounds and higher antioxidant capacity were determined for *C. x macrocarpa* samples. Therefore, *C. x macrocarpa* leaves and fruits are valuble source of antioxidant polyphenols with high potential for use in preparation of different natural health products.

Key words: *C. monogyna, C. x macrocarpa, phenols, antioxidant capacity*

INTRODUCTION – *Uvod*

Natural oxidants from fruits and vegetables have been studed for severel decedes in order to isolate compounds which can prevent or decrease different patological conditions assosiated with oxidative stress (cancer, heart deasese, atherosleroses, neuridegenerative desorders, diabiites, aging) (MANDEL ET AL., 2007; SEİFİRED ET AL., 2007; TEMPLE, 2000). Especially important group of natural antioxidants are phenols and studies showed their protective role against heart deases and cancer due to high antioxidant activity. The antioxidant activity of phenols is based on their redox properties and ability to scavenge wide range of reactive radical

species. According to investigations in recent years the most important plant antioxidants are flavonoids and phenolic acids (OSAWA, 1994, RİCE-EVANS ET AL., 1995).

The genus *Crataegus* belongs to Rosaceae family and it is represented by more than 200 species widespread in the Northen Hemisfere of Europe, Asia and America. Different plant parts (leaves, flowers, fruits) are used as cardiotonic, hypotensive, diuretic, antispasmodic and atherosclerotic agents (CHANG ET AL., 2002, ÖZCAN ET AL., 2005). Hawthorn leaves, flowers and fruits are rich source of different phenolic compounds with phenolic acids, flavones, flavonols and proanthocyanidins as the main active ingredients (CHANG ET AL., 2002; KIM ET. AL., 2000). It was found that hawthorn extracts possess antiradical activity (BERNATONIENE ET AL., 2008; BARROS ET AL., 2010; ZHANG ET AL., 2001).

Genus *Crataegu*s in Bosnia and Herzegovina have been studied by several researches (BECK, 1927; MALY, 1919, 1940; FUKAREK 1974; JANJIĆ, 1998; BAŠIĆ, 2004, CHRISTENSEN AND JANJIĆ, 2006). One of the most abundant species of genus *Crataegus* in Bosnia flora is *Crataegus monogyna* Jacq with very wide ecological amplitude (BAŠIĆ, 2004). Since interspecies breedings are common in *Crataegus* this study included investigations on *C. x macrocapa* a hybride between *C. laevigata x C. rhypidophylla*. Presence of this hybride is well documented in Europe for a long time, and today is known under name *C. x macrocapa* Hegetschw which was introduced for the first time by HRABĚTOVÁ-UHROVÁ (1969). This hybride specie is recently registreted in flora of Bosnia and Herzegovina (BAŠİĆ, 2004).

Investigations of *Crataegus* species in terms of chemical composition and antioxidant activity have been started recently by our research group and they were focused mainly on C. *monogyna* and C. *rhypidophylla* from native populations around Sarajevo. According to our best knowledge this is the first paper on phenolic content and antioxidant activity of *C. monogyna* and *C. x macrocarpa* from Zenica region.

In this work we investigated phenolic contents and antioxidant activity of C. *monogyna* and *C. x macrocarpa* leaf and fruit methanolic extracts. Obtained extracts were used in spectrophotometric determinations of total phenols, flavonoids, phenolic acids, monomeric anthocyanins and proanthocyanidins. Antioxidant activity for all extracts was investigated with three methods: DPPH, ABTS and FRAP using Trolox as a standard for expression of final results. Correlations between antioxidant capacities and different phenolic compounds were also investigated.

MATERIAL AND METHODS – *Materijal i metode*

Plant material – *Biljni materijal*

C. monogyna and *C. x macrocarpa* leaf and fruit samples were collected in October 2014. in Zenica region at locality of Smetovi. Two samples per species were collected from wider area and samples were identified by Prof Bašić, a plant taxonomist. Voucher specimens were deposited at the Herbarium of the Department of

Ecology at Faculty of Forestry. The plant material was air-dried at room temperature and powdered before analysis.

Chemicals and reagents – *Hemikalije i reagensi*

1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$), 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid diammonium salt (ABTS), 2,4,6-tripyridil-S-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as well as quercetin, rutin, gallic and caffeic 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 sourced from Kemika Zagreb (Croatia). All other chemicals and solvents were of analytical grade.

Sample extracts preparation - *Priprema ekstrakata uzoraka*

Leaf and fruit samples (0.5 g each) were extracted twice in the extraction solvent containing 80% methanol (12 mL) with ultrasound bath, (Elmecs, Italy). Each extraction step was performed at room temperate for 30 minutes. Obtained supernatants for each sample were combined and collected in a volumetric flask and volume adjusted to 25 mL with extraction solvent. The extracts were kept at -20^{o}C until analysis.

Determination of total phenols - *Određivanje ukupnih fenola*

Procedure with Folin-Ciocalteu method described by SINGLETON ET AL. (1974) was used for the determination of total phenols (TP). The absorbance of the colored product was measured at 765 nm. Appropriate calibration curve was prepared with gallic acid as standard, and final results are expressed as mg of gallic acid equivalents per gram of dry sample (mg GAE g^{-1}).

Determination of total flavonoids – *Određivanje ukupnih flavonoida*

Colorimetric method with $AlCl_3$ given by CHRIST AND MULLER (1960) and ABDENNACER ET AL. (2015) was used for the determination of total flavonoids. Briefly, sample aliquot (0.5 mL) was mixed with 1.5 mL methanol and 0.1 mL CH_3COONa (1M). Six minutes later, 0.1 mL $AlCl_3$ (10%) was added and dilution was made up to 5 mL with water. The solution was kept at room temperature for 30 minutes after that absorbance was measured at 430 nm against blank. Sample blanks were also included. Standard solutions of rutin and quercetin were used to prepare calibration curves. Final results for total flavonoids (TFq and TFr) are expressed as mg equivalents of quercetin /rutin per gram of dry sample (mg QE g^{-1} and mg RE g^{-1}).

Determination of total phenolic acids – *Određivanje ukupnih fenolnih kiselina*

Total phenolic acids (TPHA) were quantified with Arnov method described by GAWLIC-DZIKI (2012) with some modifications. One mililitre of appropriately

diluted sample was mixed with 5 mL of water, 1 mL HCl (0.5 M), 1 mL of Arnov`s reagent (10 g Na_2MoO_4 and 10 g $NaNO_2$ dissolved in 100 mL of distillated water), 1 mL of NaOH (1M) and the volume was made up to 10 mL with distillated water. Calibration curve was established with standard solutions of caffeic acid and apsorbance was measured at 490 nm. Solvent instead of extract was used as a blank. The results are expressed as caffeic acid equivalents per gram of dry sample (mg CAE g^{-1}).

Determination of monomeric anthocyanins – *Određivanje monomernih antocijanina*

In the determination of total monomeric anthocyanins (TMA), pH differential method by LEE ET AL. (2005) was used Extracts were diluted in the ratio 1:10 and apsorbances were measured at 520 and 700 nm at room temperature after 15 min. 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 $mol^{-1}cm^{-1}$ and molar weight (MW) (449.2 g mol^{-1}) were used for calculations.

Determination of total proanthocyanidins – *Određivanje ukupnih proantocijanidina*

Total proanthocyanidins (TPA) were determined with butanol/HCl assay (HAGERMAN, 2002). The method was based on their acid hydrolysis to anthocyanidins and color formation with the added reagent which is monitored spectrophotometrically. Absorbance of the sample was read at 550 nm before and after heating of the samples at 95°C for 40 minutes. Butanol/HCl mixture was used as a blank. The results were expressed as mg of cyanidin chloride equivalents (CE) per gram of dry fruit.

Determination of antioxidant capacity – *Određivanje antioksidacijskog kapaciteta*
DPPH assay - *DPPH esej*

DPPH assay was done according to BRAND-WILLIAMS ET AL. (1995) and THAIPONG ET AL. (2006). The method is based on the ability of standard and extracts to scavenge stable DPPH radical which leads to its decolonization and formation of yellow non-radical form. Stock solution of DPPH in methanol (0.094 M) was freshly prepared and diluted with methanol to absorbance of 1.1 ±0.02 at 515 nm. After that, 100 μL of previously diluted extracts was mixed with 1.9 mL of working DPPH solutions and kept in the dark for 30 minutes before measurements. Calibration curve was prepared with standard solutions of Trolox and the results are express in terms of Trolox equivalent antioxidant capacity (TEAC) as mmol Trolox equivalents per gram of dry sample weight.

ABTS assay - *ABTS esej*

ABTS assay was done according TO REE ET AL. (1999) with some modification given by THAIPONG ET AL. (2006). Basically it is a decolorisation assay which can be applied to lipophilic and hydrophilic antioxidants. In this assay radical

monocation of 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) is reduced with standard or extract. Stock solutions of ABTS (7 mM) and potassium persulphate (2.45 mM) were prepared in water and kept in the dark for 16 hours. Equal volumes of the stock solutions are mixed and diluted to absorbance of 1.1 ±0.02 at 734 nm to prepare ABTS radical cation ($ABTS^{\bullet+}$) solution. Freshly prepared solution was used for each assay. Working solution of $ABTS^{\bullet+}$ (1.9 mL) was mixed with 100 μL of previously diluted extracts and after 6 minutes the reduction in apsorbance was measured at 734 nm. Calibration curve was prepared with standard solutions of Trolox and the results are express in terms of Trolox equivalent antioxidant capacity (TEAC) as mmol Trolox equivalents per gram of dry sample weight.

FRAP assay – *FRAP esej*

Ferric reducing antioxidant power (FRAP) was measured according to BENZIE AND STRAIN (1996) method. The method is based on reduction of ferric tripyridiyltriazine (Fe(III)-TPTZ) to ferrous trypyridyltriazine (Fe(II)-TPTZ) by sample extracts. As a result of reagent reduction, a blue product is formed which can be monitored spectrophotometrically. Briefly, FRAP reagent was prepared by mixing 300 mM acetate buffer, pH 3.6; 10 mM TPTZ in 40 mM HCl acid and 20 mM $FeCl_3$ in the ratio 10:1:1. The fresh working solution was wormed at 37°C before using. This reagent (1.9 mL) was mixed with 0.1 mL of the extracts and leaved in the dark for 30 minute before measurements. Apsorbance of the colored product was measured at 593 nm against the blank which contained 0.1 ml of methanol instead of the extract. A standard curve was made with Trolox and the results were expressed as mmol Trolox equivalents per gram of dry sample weight.

Statistical analysis- *Statistička analiza*

All measurements were carried out in triplicate and obtained results are expressed as mean±SD. Correlation between investigated active compounds and antioxidant activity was established by regression analysis.

RESULTS AND DISCUSSION- *Rezultati i diskusija*

Phenolic compounds isolated from plant materials represent a reach source of natural antioxidants which receive much attention during last years. Flavonoids (flavonols, flavones and anthocyanins) show good antioxidant properties capable to scavenge free radicals (RICE-EVANS AND MULLER, 1997). Anthocyanins used as natural colorant becomes important due to their antioxidant and antibacterial properties (NAZ ET AL., 2007). Also, hydroxycinammic acids represent important class of phenolic compounds since they act as antioxidants in plant protection (CHEN AND HO, 1997). They are usually found at higher concentrations in plants (MANACH ET AL., 2004) and some of them such as caffeic acid can inhibite formation of mutagenic compounds in humans (OLTHOF ET AL., 2001).

In this study we investigated contents of a range of bioactive compounds (phenols, flavonoids, phenolic acids, anthocyanins and proanthocyanidins) of *C. monogyna* and *C. x macrocarpa* leaf and fruit methanolic extracts. Antioxidant capacity of the extracts was also examined with three methods: DPPH, ABTS and FRAP using Trolox as a standard. The results for quantitative contents of bioactive compounds for two investigated *Crataegus* species are presented in Table 1.

Table 1. Content of investigated polyphenolic compounds in extracts of *C. monogyna* and *C. x macrocarpa*
Tabela 1. Sadržaj ispitivanih polifenolnih jedinjenja u ekstraktima C. monogyna i C. x macrocarpa

Samples	**TP** (mg GAEg^{-1})	**TFq** (mg QEg^{-1})	**TFr** (mg REg^{-1})	**TPLA** (mg CAEg^{-1})	**TPA** (mg CEg^{-1})	**TMA** (mg CG g^{-1})
			C. monogyna			
Leaf(1)	59.23±0.13	3.52±0.24	7.70±0.53	33.40±0.02	26.15±0.69	0.12±0.01
Leaf(2)	64.75±0.16	2.38±0.02	5.24±0.04	39.41±0.03	35.34±0.02	0.09±0.004
Average	61.98	2.95	6.47	36.41	30.74	0.10
Fruit(1)	25.01±0.06	0.79±0.002	1.76±0.01	14.16±0.05	12.00±0.02	0.80±0.01
Fruit(2)	31.38±0.06	0.31±0.01	0.720±0.003	17.94±0.03	14.12±0.09	0.44±0.01
Average	28.19	0.55	1.24	16.05	13.06	**0.62**
			C. x macrocarpa			
Leaf(1)	91.91±0.08	3.24±0.01	7.10±0.01	68.98±0.14	48.60±0.19	0.27±0.04
Leaf(2)	72.98±0.36	4.08±0.01	8.90±0.02	46.23±0.06	37.61±0.15	0.15±0.01
Average	**82.44**	**3.66**	**7.99**	**57.60**	**43.11**	**0.21**
Fruit(1)	56.23±0.05	0.74±0.01	1.65±0.01	37.49±0.05	21.84±0.07	0.67±0.01
Fruit(2)	40.97±0.53	0.85±0.07	1.81±0.02	25.96±0.24	15.02±0.02	0.43±0.01
Average	**48.60**	**0.79**	**1.65**	**31.72**	**18.43**	0.55

Total phenolic content (TP), total flavonoids/rutin content (TFr), total flavonoids/quercetin content (TFq), total phenolic acid (TPLA), total proanthocyanidins (TPA), total monomeric anthocyanins (TMA)
Sadržaj ukupnih fenola (TP), ukupnih flavonoida/rutin (TFr), ukupnih flavonoida/kvercetin (TFq), ukupnih fenolnih kiselina (TPLA), ukupnih proantocijanidina (TPA), ukupnih monomernih antocijanina (TMA)

Leaf and fruit extracts of *C. monogyna* were rich in phenolics, phenolic acids and proanthocyanidins. The average values in leaves were: phenols 61.98 mg GAE g^{-1} DW, phenolic acids 36.41 mg CAE g^{-1} DW, and proanthocyanidins 30.74 mg CE g^{-1} DW. In fruits, avarage values were for phenols 28.19 mg GAE g^{-1} DW, phenolic acids

16.05 mg CAE g^{-1} DW, and proanthocyanidins 13.06 mg CE g^{-1} DW . Avarage flavonoid content was 2.95 QE g^{-1} and 6.47 RE g^{-1} DW for leaves and for fruits 0.55 QE g^{-1} and 1.24 RE g^{-1} DW (Table 1). According to the results of several studies given by EDWARDS ET AL. (2012) for *C. monogyna* fruits total phenols were in the range 9.1-17.8 mg g^{-1} and 16.42-57.07 mg g^{-1}; total flavonoids 4.46-147 mg g^{-1}, , and total proanthocyanidins 19.29. mg g^{-1}. Also, leaves contained bioactive compounds in the following order: proanthocyanidins 32.83-53.48 mg g^{-1} and flavonoids 24.95-28.60 mg g^{-1}. Results obtained in this work for flavonoids and proanthocyanidins in leaves and fruits are lower than the above mentioned which can be explained by different ecological conditions (climate, type of soil, exposore to the light) (BAHRİ-SAHLOUL ET AL., 2009 A; LİU ET AL., 2005; BAHORUN ET AL., 1994).

Similarly to the results for *C. monogyna*, the most abundant compounds in of *C. x macrocarpa* leaf and fruit were phenols, phenolic acids and proanthocyanidins. Average contents in leaves were for phenols 82.44 mg GAE g^{-1} DW, phenolic acids 57.60 CAE g^{-1} DW and proanthocyanidins 43.11 mg CE g^{-1} DW. In fruits, it was determined avarage content of phenols 48.60 mg GAE g^{-1} DW, phenolic acids 31.72 CAE g^{-1} DW and proanthocyanidins 18.43 mg CE g^{-1}DW (Table 1). However, we could not find literature data to compare our results with the results of other investigators.

Generally, leaf extracts of *C. monogyna* had higher contents of investigated compounds than fruits except monomeric anthocyanins content (0.10 mg CG g^{-1} DW in leaves and 0.62 mg CG g^{-1} DW in fruits).). This is in agreement with data given BY EDWARDS ET AL. (2012) for content of anthocyanins (0.15-0.58 mg g^{-1}). Similar observations are found for *C. x macrocarpa* leaf (0.21 mg CG g^{-1} DW) and fruit extracts (0.55 mg CG g^{-1} DW). Also, several studies concerning different plants confirmed that level of anthocyanins are higher in fruits than in leaves which is probably connected with coloration role of anthocyanins in fruits (ABDENNACER ET AL., 2015 AND REFERENCES THEREIN). Compering content of investigated compounds between two species we can conclude that *C. x macrocarpa* leaves are richer in the content of phenols, flavonoids, phenolic acids, proanthocyanidins and anthocyanins while fruits of *C. monogyna* have only higher content of monomeric anhtocyanins.

Three different assays for the estimation of antioxidant capacity (AC) of plant extracts were used in this work. Since different reaction mechanisms can be involved in evaluation of antioxidant capacity two or more reactions are usually applied. In all cases, Trolox was used as a standard, and the results are expressed as Trolox equivalent antioxidant capacity (TEAC). Generally, the higher DPPH, ABTS and FRAP values point to greater antioxidant activity of the sample. Antioxidant activity of polyphenols is due to redox properties acting as a reducing agents, hydrogen donors and singlet oxygen quenchers (HANRAFI AND HAMRANI, 2010). The results are given in Table 2.

Table 2. Antioxidant capacities of *C. monogyna* and *C. x macrocarpa* leaves and fruits extracts
Tabela 2. Antioksidacijski kapaciteti ekstrakata lišća i plodova C. monogyna i C. x macrocarpa

	DPPH (mmol Trolox g^{-1})	**ABTS** (mmol Trolox g^{-1})	**FRAP** (mmol Trolox g^{-1})
C. monogyna			
Leaf (1)	0.34±0.035	0.44±0.002	0.378±0.001
Leaf (2)	0.37±0.001	0.51±0.01	0.45±0.01
Average	0.36	0.47	0.41
Friut (1)	0.12±0.001	0.24±0.02	0.17±0.01
Fruit (2)	0.11±0.01	0.27±0.01	0.19±0.002
Average	0.11	0.25	0.18
C. x macrocarpa			
Leaf (1)	0.48±0.06	0.82±0.01	0.57±0.004
Leaf (2)	0.44±0.01	0.61±0.03	0.44±0.01
Average	**0.46**	**0.72**	**0.50**
Fruit (1)	0.29±0.03	0.531±0.0003	0.40±0.01
Fruit (2)	0.22±0.01	0.485±0.001	0.25±0.01
Average	**0.26**	**0.51**	**0.32**

Antioxidant capacities determined with DPPH, ABTS and FRAP were higher for *C. x macrocarpa* leaves and fruits samples than *C. monogyna* samples. Average values of antioxidant capacity found for *C. monogyna* leaves were DPPH =0.36 mmol Trolox g^{-1} DW, ABTS =0.47 mmol Trolox g^{-1} DW, and FRAP =0.41 mmol Trolox g^{-1} DW while for *C. x macrocarpa* leaves were DPPH =0.46 mmol Trolox g^{-1} DW, ABTS =0.72 mmol Trolox g^{-1} DW, and FRAP =0.50 mmol Trolox g^{-1} DW (Table 2). Fruits of both species had lower antioxidant capacity than leaves. Average values of antioxidant capacity found for *C. monogyna* fruits were DPPH = 0.11 mmol Trolox g^{-1} DW, ABTS =0.25 mmol Trolox g^{-1} DW, FRAP =0.18 mmol Trolox g^{-1} DW while higher value were determined in *C. x macrocarpa* fruits DPPH =0.26 mmol Trolox g^{-1} DW, ABTS =0.51 mmol Trolox g^{-1} DW, and FRAP =0.32 mmol Trolox g^{-1} DW (Table 2). This can be explained with higher contents of all investigated compounds in *C. x macrocarpa* leaves and fruits than *C. monogyna* samples. This is in agreement with several studies where it was found that antioxidant capacity of leaf extracts are higher than fruit extracts (ABDENNACER ET AL., 2015 AND REFERENCES THEREIN). We can also conclude that leaves and fruits of both *Crataegus* species have high antioxidant capacity. Values obtained for antioxidant capacity in this work for *C.*

monogyna fruits are much higher than the values reported by RUIZ-RODRIGEZ ET AL. (2014) (TEAC = 1.54 - 7.11 mmol Trolox $100g^{-1}$ fresh weight) and EGEA, ET AL. (2010) (TEAC $_{ABTS}$ = 8.43 μmol Trolox g^{-1} fresh weight). According to RUIZ-RODRIGEZ ET AL. (2014), AC for fresh fruits were in the range of 0.84 - 6.12 mmol Trolox100 g^{-1} for ABTS assay; 0.76 - 2.03 mmol Trolox100 g^{-1} for DPPH assy, and 3.28 - 10.99 mmol Trolox100 g^{-1} for FRAP assay. ÖZYÜREK ET AL. (2012) found that different variety of *C. monogyna* leaves in Turkey had $TEAC_{ABTS}$ in range 0.077-0.330 mmol Trolox g^{-1} and $TEAC_{FRAP}$ in range 0.064 - 0.141 mmol Trolox g^{-1} which is lower than values obtained in this work (Table 2). Also, very similar values of AC for DPPH i FRAP method were obtained for all investigated samples while AC values for ABTS were higher. They were in the following order: ABTS>FRAP>DPPH. Similar results were obtained for guava fruits extracts which is explained with differences in the ability of antioxidant compounds to reduce DPPH, ASBTS and FRAP reagents (THAIPONG ET AL., 2006). It is also reported that stereoselectivity of the reagents as well as solvent used for extractions can be important factors influencing on scavenging effect of plant extracts (YU ET AL., 2002). Lower results for FRAP assay can be the results of uncomplete reaction of the reagent with flavonoids and phenolic acids (BERKER ET AL., 2007).

Linear regression was used to establish correlation coefficients between contents of bioactive compounds and antioxidant capacities. The obtained results are presented in Table 3.

Table 3. Correlation coefficients between phenolic compounds and DPPH, ABTS and FRAP assay.
Tabela 3. Korelacijski koeficijenti između fenolnih jedinjenja i DPPH, ABTS i FRAP eseja

	Correlation coefficient (r^2)		
Phenolic Compounds	**DPPH**	**ABTS**	**FRAP**
Phenols	**0.9618**	**0.8949**	**0.9695**
Flavonoids(Q)	0.7302	0.4087	0.5477
Flavonoids (R)	0.7301	0.4066	0.5499
Phenolic acids	**0.8703**	**0.9443**	**0.9216**
Proanthocyanidins	**0.8953**	**0.7833**	**0.903**
Anthocyanins	0.4845	0.2368	0.3737

Very high correlations were noticed between DPPH, ABTS and FRAP and contents of phenols, phenolic acids, and proanthocyanidins. Correlation coefficients for phenolic content and DPPH, ABTS, FRAP assay were 0.9618; 0.8949, 0.9695 respectively. Strong correlations showed ABTS (r^2 = 0.9443), FRAP (r^2 =0.9216) and DPPH method (r^2 =0.8703) with phenolic acid content. Also, strong correlations were observed between proanthocyanidins and DPPH (r^2 =0.8953), FRAP (r^2 =0.903) and

ABTS (r^2 =0.7833). High correlations were also noticed between antioxidant capacity with DPPH and flavonoid content (r^2 =0.7302 and 0.7301) while other two methods showed insignificant correlations (0.4-0.55). Insignificant correlations were found between antioxidant capacity with all three methods and anthocyanins content.

These results suggest that phenols, phenolic acids and proanthocyanidins are major contributors to the antioxidant capacity as the most abundant compounds in leaves and fruits. Similarly, strong correlations were found for total phenols and proanthocyanidins with ABTS and FRAP in callus extracts (BAHORUN ET AL., 1994), phenols, proanthocyanidins, phenolic acids in fruit extracts (RUIZ-RODRIGUEZ ET AL., 2014), phenols and proanthocyanidins with DPPH and FRAP in fruit extracts (MRAIHI ET AL., 2013).

Interestingly, although flavonoid content expressed as quercetin was much lower compered to rutin equivalents, correlation coefficients for antioxidant capacities and flavonoid contents expressed as quercetin or rutin equivalents were very similar for the same method. This is in agreement with observation of JUNG ET AL. (2007) that quercetin (flavonol aglicon) has stronger antioxidant activity as a result of presence multiple hydroxyl groups.

CONCLUSION – *Zaključak*

Leaf and fruit extracts of *C. monogyna* and *C. x macrocarpa* were rich inphenolics, phenolic acids and proanthocyanidins.

Leaves of both species are richer in the content of phenols, flavonoids, phenolic acids and proanthocyanidins while fruits of both species are richer in content of monomeric anthocyanins.

C. x macrocarpa leaves had higher contents of all investigated compounds than *C. monogyna*. On the other hand, *C. monogyna* fruit had higher content of total monomeric anthocyanins than *C. x macrocarpa* fruit.

Leaves of both species had higher antioxidant activity than the fruits with all three methods. *C. x macrocarpa* leaves and fruits had higher antioxidant activity than *C. monogyna* leaves and fruits.

Our results indicate that phenols including phenolic acids and proanthocyanidins are most probably the major contributors to the antioxidant properties of leaves and fruits extracts. This is supported by high correlation coefficients obtained between different method for antioxidant capacity and content of phenols, phenolic acids and proanthocyanidins in this work.

It can be concluded that both species especially *C. x macrocarpa*, can be considered as a valuable source of antioxidant compounds.

REFERENCES – *Literatura*

ABDENNACER, B., KARIM, M., YASSINE, M. NESRINE, R., MOUNA, D., MOHAMED, B. (2015): Determination of phytochemicals and antioxidant activity of methanol extracts obtained from the fruits and leaves of Tuniisian *Lycium intricatum* Boiss. Food Chemistry, 174, 577-584.

BAHORUN, T., TROTIN, F., POMMERY, J., VASSER, J., PINKAS, M. (1994): Antioxidant activities of *Crataegus monogyna* Extracts. Planta Medica, 60: 323-328.

BAHRI-SAHLOUL, R., AMMAR, S., GREC, S., HARZALLAH-SKHIRI, F. (2009B): Chemical characterization of *Crataegus azarolus* L. fruit from 14 genotypes found in Tunisia. Journal of Horticulture Science of Biotechnology, 84, 23-28.

BARROS, L., CARVALHO, A.M., FERREIRA, I.C.F.R. (2010): Comparing the composition and bioactivity of *Crataegus monogyna* flowers and fruits used in folk medicine, Phytochemical Analysis. 22, 181–188.

BAŠIĆ, N. (2004): Morfološko-taksonomska istraživanja glogova (*Crataegus* L.) na području Bosne i Hercegovine. Magistarski rad. Sarajevo.

BECK, G. (1927): Flora Bosne, Hercegovine i oblasti Novoga Pazara. III. Choripetalae (Kaj): 169-172. Beograd-Sarajevo

BENZİE I.F. AND STRAİN J.J. (1999): The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry, 239(1) 70-6.

BERKER, K.I.; GÜÇLÜ, K.; TOR, I.; APAK, R. (2007): Comparative Evaluation of Fe(III) Reducing PowerBased Antioxidant Capacity Assays in the Presence of Phenanthroline, Batho-phenanthroline, Tripyridyltriazine (FRAP), and Ferricyanide Reagents, Talanta, 72, 1157-1165.

BERNATONIENE, J., MASTEIKOVA, R., MAJIENE D., SAVICKAS, A., KEVELAITIS, E., BERNATONIENE, R., DVORAČKOVA, K., CIVINSKIENE, G., LEKAS, R., VITKEVIČIUS, K., PEČIURA, R. (2008): Free radical-scavenging activities of *Crataegus monogyna* extracts. Medicina (Kanaus), 44(9).

BRAND-WİLLİAMS W, CUVELİER ME, BERSET C. (1995): Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technology, 28:25-30.

CHANG, Q., ZUO, Z., HARRISON, F., CHOW, M.S.S. (2002): Hawthorns: An overview of chemical, pharmacological and clinical studies. Journal of Clinical Pharmacology. 42, 605-612.

CHEN, J.H., AND HO, C.T. (1997): Antioxidant activity of caffeic acid and its related hydroxycinammic acid compounds. Journal of Agricultural Food Chemistry. 45, 2374-2378.

CHRIST, B. AND MULLER, K.H. (1960): Determination of the amount of flavonol derivates in drugs. Archiv der pharmazie, 293, 1033-1042.

CHRISTENSEN, K. I. AND JANJIĆ, N. (2006): Taxonomic notes on European taxa of *Crataegus* (Rosaceae). Nordic Journal of Botany, 24: 143-147.

EDWARDS, J.E., BROWN, P.N., TALENT, N., DİCKİNSON, T.A., SHİPLEY, P.R. (2012): A review of the chemistry of the genus *Crataegus.* Phytochemistry, 79, 5-26.

EGEA, I., SANCEZ-BEL, P., ROMOJARO, F., PRETEL, MT. (2010): Six edible wild fruits as potential antioxidant additives or nutritional supplements. Plant Foods Human Nutrition 65:121-129.

FUKAREK, P. (1974): Neke vrste drveća i grmlja koje su pogrešno navedene u Flori Bosne i Hercegovine i susjednih krajeva. ANU BiH-Radovi LIV, Odjeljenje prirodno-matematičkih nauka, 15: 45-60.

GAWLIC-DZIKI, U. (2012): Dietary spices as natural effectors of lipoxygenase, xanthine oxidase, peroxidase and antioxidant agents. LTW-Food Science and Technology, 47, 138-146.

HAGERMAN, A.E. (2002): The Tannin Handbook. Miami University Oxford.

HARNAFI, H. AND AMRANI, S. (2008). Spectrophotometric methods for determination of plant polyphenols content and their antioxidant activity assessment: An Overview, Pharmacognosy Reviews, 2(3), 20-22.

HRABĚTOVÁ-UHROVÁ A. (1969): Hloh (*Crataegus* L.) v Československu. Preslia, (Praha). 41: 162-182.

JANJIĆ, N. (1998): Neki zanimljivi dendrološki nalazi iz sarajevskog područja. Radovi Šumarskog Fakulteta Univerziteta u Sarajevu, 28(1): 85-103.

JUNG, S.J., KIM, D.H., HONG, Y.H. (2007): Flavonoids from the flower of *Rhododendron yedonese var. poukhanense* and their antioxidant activities. Archives of Pharmacal Research, 30(2), 146:50.

KIM, S.H., KANG, K.W., KIM, K. W., KIM, N.D. (2000): Procyanidins in *Crataegus* extract evoke endothelium-dependent vasorelaxation in rat aorta. Life Science, 67:121-131.

LEE, J., DURST, RW., WROLSTAD, E. (2005): Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by pH differential method: Collaborative study. Journal of AOAC International, 88(5), 1269-1278.

LIU, R.H., YU, B.Y., QIU, S.X., ZHENG, D. (2005): Comparative analysis of eight major polyphenolic compounds in leaves of *Crataegus* L. by HPLC. Chinese Journal of Natural Medicine, 3, 162-167.

MALÝ, K. (1919): Prilozi za floru Bosne i Hercegovine 5 i 6. Glasnik Zemaljskog Muzeja BiH, Sarajevo, 31: 61-92.

MALÝ, K. (1940): Notizen zur Flora von Bosnien-Herzegovina. Glasnik Zemaljskog Muzeja BiH, Sarajevo, 52: 21-46.

MANAH, C., SCALBERT, A, MORAND, C., REMESY, C., JIMENEZ, L. (2004): Polyphenols: food sources and bioavailability, The American Journal of Clinical Nutrition, 79:727-747.

MANDEL, S., AMIT, T., REZNICHENKO, L., WEINREB, O., YOUDIM, M-B. (2006): Green tea catechins as a brain permeable, natural iron chelators-antioxidants for the treatment of neurodegenerative disorders. Molecular Nutrition & Food Research, 50, 229-34

MRAIHI, F., JOURNI, M., CHERIF, JK. SOKMEN, M., SOKMEN, A., TRABELSI-AYADI, M. (2013): Phenolic content and antioxidant potential of *Crataegus* fruits grown in Tunisia as determined by DPPH, FRAP, and β-carotene/linoleic acid assay. Journal of Chemistry, volume 2013, 1-6.

NAZ, S., SIDDIQI, R., AHMAD, S., RASOOL, S.A., SAYEED, S.A., (2007): Antibacterial activity directed isolation of compounds from *Punica granatum*. Journal of Food Science, 72, 341-345.

OLTHOF, M.R., HOLMAN, P.C.H., KATAN, M.B. (2001): Chlorogenic acid and caffeic acid are absorbed in human. Journal of Nutrion, 131, 66-71.

OSAWA, T. (1994): Novel natural antioxidants for utilization in food and biological systems in Postharvest Biochemistry of Plant Food-Materials in the Tropics, I. Uritani, V.V. Garcia, and E.M. Mendoza, Eds., 241-251, Japan Scientific Societies Press.

ÖZCAN, M., HACISEFEROGULLARY, H., MARAKOGLU, T., ARSLAN, D. (2005). Hawthorn (*Crataegus spp*) fruit: Some physical and chemical properties. Journal of Food Engineering, 69, 409-413.

ÖZYÜREK, M., BENER, M., GÜÇLÜ, K., DÖNMEZ, A.A., SÜZGEÇ-SELÇUK, S., PIRILDAR, S., MERIÇLI, A.H., APAK, R. (2012): Evaluation of antioxidant activity of *Crataegus s*pecies collected from different regions of Turkey. Records of Natural Products, 6:3, 262-277.

RE R, PELLEGRİNİ N, PROTEGGENTE A, PANNALA A, YANG M, RİCE-EVANS C.(1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay.Free Radical Biology and Medicine, 26, (9-10), 1231-7.

RICE-EVANS, C.A., MILLER, N.J. (1997): Structure-antioxidant activity relationship of flavonoids and isoflavonoids. In: Ricde-Evans, C.A., packer, L. (Eds.), Flavonoids in Health and Disease. Marcel Dekker, New York.

RICE-EVANS, C.A., MILLER, N.J., BOLWELL, P.G., BRAMLEY, P.M., PRIDHAM, J.B. (1995): The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Research, 22 (4), 375-383.

RUIZ-RODRIGEZ, B., DE ANCOS, B. SANCEZ-MORENO, C., FERNANDEZ-RUIZ, V., SANCEZ-MATA, M., CAMARA, M., TARDIO, J. (2014). Wild blackthorn (*Prunus s*pinosa* L.) and hawthorn (*Crataegus monogyna* Jacq.) as valuable source of antioxidants. Fruits, 69, 61-73.

SEİFRİED, H. E., ANDERSON, D. E., FİSHER, E. I. AND MİLNER, J. A. (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. The Journal of Nutritional Biochemistry, 18 (9): 567-579.

SINGLETON, V.L., ORTHOFER, R., LAMUELA-RAVENTOS, R.M. (1974): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods of Enzymology, 229, 152-178.

TAMPLE, N.J. (2000): Antioxidants and disease: more questions than answers. Nutrition Research, 20,449-459.

THAIPONG, K., BOONPRAKOB, U., CROSBY, K., CISNEROS-ZEVALLOS, L., BYRNE, D.H. (2006): Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis, 19, 669-675.

YU, L., HALEY, S., PERRET, J., HARRIS, M., WILSON, J., QIAN, M. (2002): Free radical scavenging properties of wheat extracts. Journal of Agricultural Food Chemistry, 50:1619-1624.

ZHANG, Z., CHANG, Q., ZHU, M., HUANG, Y., HO, W.K.K., CHEN, Z.-Y. (2001): Characterization of antioxidants present in hawthorn fruits. Journal of Nutritional Biochemistry, 12, 144.

SAŽETAK

Metanolni ekstrakti uzoraka lista i ploda *C. monogyna* Jacq i *C. x macrocarpa* Hegetschw. analizirani su na sadržaj ukupnih fenola, flavonoida, fenolnih kiselina, monomernih antocijanina i proantocijanidina kao i antioksidacijsku aktivnost. U određivanju sadržaja bioaktivnih jedinjenja i antioksidacijske aktivnosti ekstrakata, korištene su spektrofotometrijske metode. Ukupni fenoli su određeni Folin-Ciocalteu metodom, a $AlCl_3$ metodom vršeno je određivanje ukupnih flavonoida prema rutinu i kvercitinu kao standardima. Određivanje monomernih antocijanina izvršeno je primjenom pH diferencijalne metode a kiselinsko-butanolna metoda je upotrebljena za kvantifikaciju ukupnih proantocijanidina. Ukupne fenolne kiseline su određene Arnovom metodom. Za mjerenje antioksidacijske aktivnosti korištene su tri metode: DPPH, ABTS i FRAP metoda a rezultati su izraženi u ekvivalentima Troloxa po gramu suhog uzorka.

Sadržaji ukupnih fenola po gramu suhog uzorka (s.u) za uzorke listova kretali su se u području 59.23 - 91.91 mg GAE g^{-1} s.u; ukupnih flavonoida 5.24 - 8.90 mg RE g^{-1} s.u. i 2.38 - 4.08 mg QE g^{-1} s.u; fenolnih kiselina 33.40 - 68.98 mg CAE g^{-1} ; sadržaji ukupnih monomernih antocijanina bili su u granicama 0.09 - 0.27 mg CGE g^{-1} s.u. i proantocijanidina 26.15 - 48.60 mg CE g^{-1} s.u.

U uzorcima plodova sadržaji ispitivanih jedinjenja kretali su se u intervalima: ukupni fenoli 25.01 - 56.23 mg GAE g^{-1} s.u; ukupni flavonoidi 0.72 - 1.81 mg RE g^{-1} s.u i 0.31 - 0.85 mg QE g^{-1} s.u; ukupne fenolne kiseline su bile u granicama 14.16 - 37.49 a

ukupni monomerni antocijanini 0.43 - 0.80 mg CGE g^{-1} s.u; i ukupni proantocijanidini 12.00 - 21.84 mg CE g^{-1} s.u.

Poređenjem prosječnog sadržaja aktivnih jedinjenja u listovima dvije vrste može se zaključiti *C. x macrocarpa* ima veći prosječni sadržaj svih ispitivanih jedinjenja u odnosu na *C. monogyna.* Samo plodovi *C. monogyna* imaju veći prosječni sadržaj monomernih antocijana u odnosu na plodove *C. x macrocarpa.*

Antioksidacijski kapacitet u listovima *C. monogyna* kretao se u području 0.34 - 0.51 mmol Troloxa g^{-1} s.u., dok je u listovima *C. x macrocarpa* bio u području 0.44 - 0.82 mmol Troloxa g^{-1} s.u. Vrijednosti antioksidacijskog kapaciteta za plodove su iznosili 0.11 - 0.27 mmol Troloxa g^{-1} s.u za *C. monogyna* i za *C. x macrocarpa* 0.22 - 0.531 mmol Troloxa g^{-1} s.u.

Generalno, na osnovu prosječnih vrijednosti kapaciteta za sve tri metode može se zaključiti da listovi i plodovi *C. x macrocarpa* imaju bolja antioksidacijska svojstva a što je u saglasnosti sa većim sadržajem aktivnih komponenti u ovoj vrsti.

Linearnom regresijom između sadržaja aktivnih komponenti i antioksidacijskog kapaciteta primjenom tri metode, određeni su koeficijenti korelacije (r^2). Utvrđeno je postojanje visoke korelacije između ukupnih fenola, fenolnih kiselina i proantocijanidina i antioksidacijskog kapaciteta (AC_{DPPH}, AC_{ABTS} i AC_{FRAP}).

Fenoli i proantocijanidini najbolje koreliraju sa FRAP metodom dok fenolne kiseline najbolje koreliraju sa ABTS metodom.

Na osnovu dobivenih rezultata se može zaključiti da je vrsta *C. x macrocarpa* bogata u sadržaju antioksidacijskih aktivnih komponenti i kao takva je interesantna za detaljnija ispitivanja hemijskog sastava.