Works of the Faculty of Forestry
University of Sarajevo
No. 2, 2014 (29-40)

UDK 547.56:582.711.714
582.711.714:581.47

PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *CRATAEGUS MONOGYNA* L. FRUIT EXTRACTS

Sadržaj fenola i antioksidacijska aktivnost ekstrakata plodova *Crataegus monogyna* L.

Azra Tahirović[1], Nedžad Bašić[1]

Abstract
The aim of this work was to determine the content of total polyphenols, total flavonoids, total monomeric anthocyanins, total proanthocyanidins, and antioxidant activity of *Crataegus monogyna* L. fruit in water, hydroalcohol and alcohol extracts. Phenolic content and antioxidant assay of the different fruit extracts was determined using spectrophotometric methods. Obtained results indicated that the content of total polyphenols in the investigated extracts varied from 2.01 to 4.60 mg GAE g^{-1} of fresh hawthorn fruit. The content of flavonoids ranged from 0.254 to 0.595 mg RUE g^{-1} fresh fruit extracts. Total monomeric anthocyanins varied from 0.004 to 0.132 mg cyanidin-3-glucoside g^{-1} of fresh fruit and total proanthocyanidins varied from 0.187 to 1.168 mg cyanidin chloride g^{-1} fresh fruit. The best antioxidant activity was obtained for hawthorn extract with 80% methanol. A good correlation between antioxidant activity and total polyphenols (R^2 = 0.9473) and proanthocyanidins (R^2 = 0.7469) was observed.

Keywords: *Crataegus monogyna* L., natural phenolic compounds, antioxidant activity

INTRODUCTION- *Uvod*

Crataegus L. is a polymorphic genus from Rosacea family, native to northern temperature zones including North America, East Asia, Central Asia, and Europe. Hawthorns grow as large shrubs or small trees with pome red fruit and thorny branches. The genus *Crataegus*, known as Hawthorns is represented by four species in the flora of Bosnia and Herzegovina (BECK, 1927; MALY, 1919, 1940; FUKAREK, 1974; JANJIĆ, 1998, 2002; BAŠIĆ, 2004). In Bosnia and Herzegovina, one of the most abundant species with high ecological amplitude is *Crataegus monogyna* Jacq., known as a common hawthorn (CHRISTENSEN AND JANJIĆ, 2006).

Crataegus species have found special medicinal use for the treatment of mild heart diseases. In Europe, the fruits, leaves, and flowers were traditionally used in the

[1] University of Sarajevo, Faculty of Forestry

treatment of heart problems for their antispasmodic, cardiotonic, hypotensive, and antiaterosclerotic effects (CHANG ET AL., 2002). In China and Europe, hawthorn fruit is eaten and used to make commercial products such as wine, jam, and candy (CHANG ET AL., 2002; BERNATONIENE ET AL., 2008; TADIĆ ET. AL., 2008; BARROS ET AL., 2010). The European pharmacopoeia allows for the use *C. monogyna* Jacq. (Lindm) and *C. laevigata* (Poiret) D.C.; their hybrids, and some less common species in the preparations of phytomedicines. The hawthorn berries should contain at least 1% procyanidins, while the leaf and flower must contain minimum of 1.5% flavonoids expressed as hiperoside. Leaves and flowers contain flavonoids (0.1-2% including rutin, hyperoside, vitexin, vitexin-2''-O-rhamnoside and oligomeric proanthocyanidins (1-3%), phenolic acids (chlorogenic and caffeic acids), triterpene acids (oleanolic and ursolic acids), organic acids and sterols (THE COUNCIL OF EUROPE, 2004). The main group of active compounds in hawthorn extracts are flavonoids represented by procyanidins, flavones and flavonols (CHANG ET. AL., 2002; KIM ET. AL., 2000).

Special attention is paid to free radical scavenging and antioxidant properties of different polyphenols (SKERGET ET AL., 2005; KATALINIĆ ET AL., 2006; ZHOU AND YU ET AL., 2006). Plant polyphenols have attracted attention owing to their radical scavenging activities (PIETTA, 2000), and hawthorn extracts have also been shown to have antioxidant effects (BAHORUN ET AL., 1994; 2003; LJUBUNCIC ET AL., 2005) and to inhibit LDL oxidation (ZHANG ET AL., 2001; QUETTIER ET AL., 2003). One of the most important sources of polyphenols compounds is small red fruits (HALVORSEN ET AL., 2002). Because of the positive effects of fruits polyphenols on human health, the interest in consuming fruits and their products is growing. Therefore, it is important to determine distribution of total polyphenols and to investigate antioxidant activity of these fruits.

The purpose of the presented study was to evaluate content of phenols, flavonoids, monomeric anthocyanins, and proanthocyanidins in extracts of different polarity prepared from fresh fruits harvested from native trees of *C. monogyna* near Sarajevo. Estimation of the antioxidant activity of all extracts was performed. Antioxidant activity of hawthorn fruit was determined by using DPPH assay. The existence of possible correlation between investigated phenolic compounds and antioxidant activity of the fruit was examined as well.

MATERIALS AND METHODS - *Materijali i metode*

Plant material – *Biljni materijal*

Fully ripened fruits of *C. monogyna* were collected in October 2013. near Sarajevo. The voucher specimen of *C. monogyna* was confirmed and deposited in Herbarium at the Department of Forest Ecology, Faculty of Forestry, University of Sarajevo. Immediately after harvesting, fruits were frozen at -20^{o}C until analysis.

Chemicals and reagents - *Hemikalije i reagensi*

2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$), rutin, aluminium chloride, sodium carbonate, sodium acetate, gallic acid, ascorbic acid and Folin & Ciocalteu's reagent were purchased from Sigma-Aldrich Chemical Co. (USA). Butanol was obtained from Merck Chemical Suppliers (Germany). Potassium chloride and ferrous ammonium sulfate were obtained from Kemika Zagreb (Croatia). All other chemicals and solvents were of analytical grade.

Extraction procedure – *Procedura ekstrakcije*

The fresh fruit of hawthorn (*C. monogyna* L.), 2 g was mieed in a blender and extracted with the following solvents: absolute ethanol, absolute methanol, ethanol (80%), methanol (80%), ethanol (50%), methanol (50%) and water. Extraction was carried out in an ultrasonic bath for 30 minutes with 25 ml of the solvents. Obtained solutions were filtered in a 50 ml volumetric flask. The residue was extracted again in the same way. The extracts were combined and the solutions were diluted to volume with an appropriate solvent. Obtained extracts were stored in a refrigerator at 4^{o}C until analysis.

The content of total phenolics – *Sadržaj ukupnih fenola*

Determination of total phenolics was determined spectrophotometrically by using the Folin-Cioalteu's assay (SINGLETON ET AL., 1974) with some modification. Briefly, to appropriate volume of undiluted extracts 7.5 ml of water was added. The mixture was vortexed for 20 s and 500 µl of FC reagent was added. The mixture was vortexed for additional 20-30 s and 1.5 ml of filtered 20% sodium carbonate solution was added in time interval from 1 min to 8 min after addition of the FC reagent. The mixture was placed in a water bath at 40^{o}C for 30 min. The absorbance of the colored product was measured at 765 nm. Different concentrations of gallic acid were used to prepare a calibration curve, and the level of total phenolics was calculated. Results are expressed in mg of gallic acid equivalents per gram (mg GAE g^{-1}) of fresh fruit.

Determination of total flavonoids – *Određivanje ukupnih flavonoida*

Total flavonoids in plant extracts were determined using spectrophotometric method by QUETTIER ET AL. (2000). Briefly, equal volumes of plant extract and 2% aluminium chloride ($AlCl_3$) solution dissolved in methanol were mixed. The samples were incubated for an hour at room temperature, and after that absorbance was measured at 415 nm. Sample blank was used in the same procedure, but without addition of aluminum chloride. The same procedure was repeated for the standard solutions of rutin, and the calibration curve was constructed. Results are expressed in mg rutin equivalents per g of fresh fruit.

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

Total monomeric anthocyanins were determined by a pH-differential method (LEE ET AL., 2005). Two dilutions of each fruit extracts were prepared, one with potassium chloride buffer (pH 1.0), and the other with sodium acetate buffer (pH 4.5). Dilution factor was 1:10 (v/v). Absorbance was measured simultaneously at 520 and 700 nm at room temperature, 15 min after dilution of extracts. The content of total anthocyanins was expressed in mg of cyanidin-3-glucoside equivalents (CGE) per g of fresh fruits. A molar extinction coefficient of cyanidin-3-glucoside of 26900 l $mol^{-1}cm^{-1}$ and molar weight (MW) (449.2 g mol^{-1}) were used for calculations. Data are presented as mean value ±SD of three measurements.

Determination of total proanthocyanidins – *Određivanje ukupnih proantocijanidina*

The proanthocyanidins were determined by UV-VIS spectrophotometry method based on acid hydrolysis and color formation. The HCl/butanol assay was used to quantify the total proanthocyanidins (HAGERMAN, 2002). In a sealed tube appropriate volume of sample was diluted to 1 ml with methanol, and 6 ml of 95% butanol/concentrated HCl (95:5 v/v) followed by addition of 0.2 ml a solution of $NH_4Fe(SO_4)_2$ x12 H_2O in 2 M HCl (0.2% m/v). Absorbance was read at 550 nm before and after heating for 40 min at 95°C. As a blank, butanol/HCl mixture was used. The results are expressed in mg of cyanidin chloride equivalents per g of fresh fruit, and data are presented as a mean value ± SD of three measurements.

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

Measurement of antiradical activity was adapted from SANCHEZ-MORENO ET AL. (1998). In brief, 0.1 ml of diluted extract was added to 1.9 ml of freshly prepared 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) solution dissolved in methanol (7.116 x 10^{-5} $moldm^{-3}$). After 30 min of incubation in dark, the absorbance was read at 517 nm. Results are expressed as vitamin C equivalents using the following equation:

$$I = 17.787 \times c + 1.2817$$

Where I (%) = $[(A_{DPPH}-A_{ext})/A_{DPPH}]$ x 100 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 mean values of results are expressed as vitamin C equivalents per a fresh base of fruits ±SD.

Statistical analysis - *Statistička analiza*

Linear regression analysis was performed quoting the correlation coefficient between antioxidant activities and concentrations in phenolic classes. All results are expressed as mean value ±SD of three parallel measurements.

RESULTS AND DISCUSSION – *Rezultati i diskusija*

The extraction of bioactive compounds from plant crude material may be performed using various solvents as extractants, and the most frequently used are water, methanol, ethanol, acetone, ethyl acetate (CAI ET AL., 2006, MILIAUSKAS ET AL., 2005, BERNATONIENE ET AL., 2008). Especially, water and ethanol are used in manufacturing different pharmaceutical preparations from hawthorn fruits (BERNATONIENE ET AL., 2008). According to some investigators (PINELO ET AL., 2005, SPIGNO ET AL., 2007), alcohol/water solutions showed better influence on extractability of phenolic compounds in comparison to the mono-component solvents. In this work, we investigated content of selected classes of bioactive compounds in different extracts of *C. monogyna* fresh fruit, and their antioxidant activity with one of the most applied assay-DPPH method. Results obtained for total phenolics, flavonoids, monomeric anthocyanins and proanthocyanidins content are presented in Table 1.

It can be seen that total phenolics content ranged from 2.01 mg GAE g^{-1} FW to 4.60 mg GAE g^{-1} FW (fresh weight) in different extraction systems. The highest amount of phenolics was found in 80% methanol extract followed by 50 % methanol extracts (4.185 mg GAE g^{-1} FW), while the lowest amount of phenolics was in the absolute ethanol extract (2.010 mg GAE g^{-1} FW) followed by water extract (2.02 mg GAE g^{-1} FW). Methanol extracts contained higher amount of total phenolics compared to ethanol extracts which can be caused by higher polarity of the solvent. Also, alcohol/water mixture showed better efficiency in extraction than pure alcohols which is in accordance with other investigators mentioned above.

Table 1. Total phenolics content, total flavonoids content, total monomeric anthocyanidins content and total proanthocyanidins content of hawthorn fruits extracted with different extraction systems.
Tabela 1. Sadržaj ukupnih fenola, ukupnih flavonoida, ukupnih monomernih antocijanina, ukupnih proantocijanidina u plodovima gloga ekstrahovanih različitim ekstrakcionim sistemima

Solvent	Total phenolics[a]	Total flavonoids[b]	Total monomeric anthocyanins[c]	Total proanthocyanidins[d]
water	2.020±0.010	0.254±0.004	0.004±0	0.187±0.002
50% methanol	4.185±0.012	0.431±0.007	0.065±0.004	0.625±0.016
50% ethanol	3.962±0.011	0.454±0.001	0.078±0.002	0.961±0.007
80% methanol	4.603±0.059	0.595±0.005	0.131±0.003	1.168±0.009
80% ethanol	3.803±0.012	0.447±0.003	0.125±0.014	0.765±0.009
absolute methanol	3.016±0.010	0.586±0.004	0.132±0.010	0.838±0.034
absolute ethanol	2.010±0	0.392±0.005	0.070±0.008	0.510±0.011

Expressed as: a - mg equivalents of gallic acid g^{-1} FW; b - mg equivalents of rutin g^{-1} FW; c - mg equivalents of cyanidin-3-glucoside g^{-1} FW; d - mg equivalents of cyanidin chloride g^{-1} FW.

Phenolics 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). Phenolics content is very important parameter since it determines the pharmacological properties of plants. Concentration of phenols in medicinal plants is between 0.23-2.85 mg GAE g^{-1} while nutritive plants have phenols in concentration range 0.26-17.51 mg GAE g^{-1}. Our results showed that *C. monogyna* fruit ranks high among medicinal plants in terms of phenolics compound with the highest value of 4.603 mg GAE g^{-1}. According to extensive review given by Edwards et al. (2012), content of total phenolics in *C. monogyna* fruits can be found with the range 9.1-17.8 mg g^{-1}, and 16.42-57.07 mg g^{-1} determined by different investigators. On the other hand, it is difficult to compare our results with finding of other authors due to differences in extraction method applied, mode of expression of results (on dry or fresh basis of hawthorn). For instance, MRAIHI ET AL. (2013) used acidified 80% methanol to extract bioactive compounds from lyophilized *C. monogyna* fruits. They obtained for total phenolics 122.26 mg GAE/100 g for pulp and 123.35 mg GAE /100 g for peel. BERNATONIENE ET AL. (2008) used dried *C. monogyna* fruits and extraction was performed with water and 70% ethanol. They found that ethanolic hawthorn fruit extract contained 182 mg/100 ml phenolic compounds which was three times more than water extracts.

Flavonoids are an important group of phenolic compounds which is generally more abundant in hawthorn flowers and leaves than in fruits. Levels of total flavonoids measured in this work were rather low. Total flavonoids ranged between 0.254 mg RUE g^{-1} FW to 0.595 mg RUE g^{-1} FW in investigated extracts. Literature values for total flavonoids find in *C. monogyna* fruits were very variable and ranged 4.46-147 mg g^{-1} fruits (EDWARDS ET AL., 2012). The highest amount of flavonoids was in 80% methanol extract while the lowest amount was determined in water extract. Better results were obtained with methanol as a solvent than with ethanol. In general, concentration of total flavonoids increased with increasing methanol content in extracts.

Total monomeric anthocyanins in investigated extracts were in the range 0.004 – 0.132 mg CGE g^{-1} FW. Content of anthocyanins was the lowest compared with other investigated bioactive compounds. This is similar to investigations carried out by MRAIHI ET AL. (2013) which also found the lowest content of monomeric anthocyanins in methanolic extracts (peel – 5.58 mg/100 g DW, and pulp 0.31 mg/100 g DW) which is lower than our results for 80% methanol extract. FROHLICHER ET AL. (2009) reported that content of total anthocyanins for fresh and dried hawthorn fruits was in range 0.150-0.580 mg/g which is higher than our results. Higher amount of anthocyanins was found in 80% methanol and ethanol compared with other extraction systems. Again, the lowest amount of anthocyanins was measured in water extract.

Proanthocyanidins are considered to be important bioactive compounds in hawthorn fruits (CHANG ET AL., 2002). Amount of total proanthocyanidins was much higher compared with the amount of flavonoids and anthocyanidins in all extraction

systems. The amount of proanthocyanidins ranged between 0.187 mg CE g^{-1} FW to 1.168 mg CE g^{-1} FW. The lowest amount was found in water extract while the highest amount of proanthocyanidins was measured in 80% methanolic extract and 50 % ethanol. Concentrations of proanthocyanidins decreased with increasing amount of ethanol in extraction systems. Comparing with other investigations, much higher amount of proanthocyanidins is measured in dried fruits 19.29 mg g^{-1} (BAHORUN ET AL., 1994), 873 mg/100 g in peel, and 507.31 mg/100 g in pulp (MRAIHI ET AL., 2013).

Antioxidant activity of *C. monogyna* extracts obtained with solvents of different polarity was investigated by DPPH method, and obtained results are presented in Table 2. Results are expressed as percent of DPPH inhibition (I %) and ascorbic acid (AA) equivalents g^{-1} FW.

Table 2. Antioxidant activity of *C. monogyna* extracts
Tabela 2. Antioksidacijska aktivnost C. monogyna ekstrakata

Solvent	I (%)	AC[a]
Water	24.81±0.19	0.661±0.006
50% methanol	83.00±0.48	2.29±0.01
50% ethanol	84.71±1.71	2.344±0.05
80% methanol	91.15±0.37	2.524±0.010
80% ethanol	79.88±0.56	2.21±0.02
absolute methanol	65.01±0.87	1.79±0.02
absolute ethanol	37.64±0.25	1.021±0.007

a- expressed as mg equivalents of ascorbic acid g^{-1} FW.

The antioxidant activity of different extracts varied from 0.661 to 2.524 mg AA g^{-1} FW. The lowest inhibition showed water extract while the highest inhibition showed the extract obtained with 80% methanol. Except water (I = 24.81%) and absolute ethanol (I = 37.64%), all investigated extracts showed strong scavenging activity against DPPH radicals, ranging from 65.01% to 91.15 %. These results can be considered indicative of a good antioxidant capacity. The antioxidant capacities were observed to decrease with ethanol content increasing in extracts. It was also observed that antioxidant capacities increased with methanol increasing from 50% to 80%. (Table 2). Similar BERNANTIONE ET AL. (2008) obtained that ethanol extract had stronger activity in free radical scavenging than water extract which is probably in connection with higher content of phenolic compounds in ethanol extract.

Correlation between specific classes of bioactive compounds and antioxidant activity was also investigated. Obtained results are presented in Table 3. Correlation coefficients were obtained from graphs plotting amount of ascorbic acid equivalents against amount of appropriate class of bioactive compounds in different extraction

systems. Obtained results showed that there is higher correlation between total phenolics ($R^2 = 0.9473$) and total proanthocyanidins ($R^2 = 0.7469$) content while lower correlation was observed with total flavonoids ($R^2 = 0.5154$) and monomeric anthocyanins ($R^2 = 0.289$).

Table 3. Correlation of total phenolics, flavonoids, monomeric anthocyanins and proanthocyanidins with antioxidant activity of hawthorn (*C. monogyna*) fruit
Tabela 3. Korelacija ukupnih fenola, flavonoida, monomernih antocijanina i proantocijanidina sa antioksidacijskom aktivnošću plodova gloga (C. monogyna)

Phenolic compounds	**Correlation coefficients**
Total phenolics	$y = 0.6743x - 0.4378$ $R^2 = 0.9473$
Total flavonoids	$y = 4.4405x - 0.1695$ $R^2 = 0.5154$
Total monomeric anthocyanins	$y = 0.1798x + 1.1163$ $R^2 = 0.289$
Total proanthocyanidins	$y = 1.9538x + 0.4247$ $R^2 = 0.7469$

There is no general conclusion about the correlation between the content of phenolic compounds from plants and antioxidant activity, according to the literature. The reason could be different methods of extractions, different methods used for determination of antioxidant capacity as well as results interpretation (STRATIL ET AL., 2007). Results obtained in this work are in agreement with the work of WANG ET AL., (2003) who determined that phenolic compounds had a major contribution to antioxidant activity.

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 content and proanthocyanidins content. Also, according to JUNG ET AL. (2007), antioxidant activity of flavonol glycoside such as rutin, is much weaker than some flavonol aglycones which can be second reason for lower antioxidant activity of flavonoids.

CONCLUSIONS – *Zaključci*

It can be concluded that extracting solvent system affects significantly the polyphenol compounds content and the antioxidant activity measured.
All investigated extracts showed antioxidant activity but methanol and ethanol extracts had stronger antioxidant activity compared with water extract.
The performed study indicated that *C. monogyna* fresh fruit extracts are rich source of polyphenols and proanthocyanidins.

The best results regarding the most abundant bioactive compounds were obtained with 80% methanol.
Phenolic compounds and proanthocyanidins are the main bioactive compounds responsible for the antioxidant activity of fruits extracts but flavonoids and anthocyanins also contribute with some smaller extent.
Results obtained in this study indicate that investigated *C. monogyna* fruits can be considered as a natural source of phenolic compounds with good antioxidant activity.

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SAŽETAK

U ovome radu su prikazani rezultati određivanja ukupnih fenola, flavonoida, monomernih antocijanina i proantocijanidina kao i antioksidacijske aktivnosti u ekstraktima svježih plodova jednokoštuničavog gloga (*Crataegus monogyna* L). Za ekstrakciju su korišteni rastvarači različite polarnosti i to: voda, metanol, etanol kao i vodeno-alkoholne smjese u različitim omjerima, a ekstrakcija je vršena ultrazvučnom metodom. Određivanje ukupnih fenola je vršeno spektrofotometrijski Folin-Ciocalteu metodom a ukupnih flavonoida $AlCl_3$ metodom. Za određivanje ukupnih monomernih antocijanina primjenjena je pH diferencijalna metoda a ukupnih proantocijanidina kiselinsko-butanolna metoda. DPPH metoda je korištena za procjenu antioksidacijske aktivnosti različitih ekstrakata.

Dobiveni rezultati su pokazali da se ekstrakti razlikuju u sadržaju bioaktivnih jedinjenja kao i po antioksidacijskoj aktivnosti. Najveći sadržaj bioaktivnih jedinjenja je određen u ekstraktima sa 80% metanolom, dok je najmanji sadržaj određen u ekstraktu sa čistom vodom. Sadržaj ukupnih fenola se kretao od 2.01 do 4.60 mg GAE g^{-1} s.u. (svježeg uzorka), ukupnih flavonoida od 0.254 do 0.595 mg RUE g^{-1} s.u. Sadržaj monomernih antocijanina je iznosio od 0.004 do 0.132 mg cijanidin-3-glukozida g^{-1} s.u. a ukupnih proantocijanidina od 0.187 do 1.168 mg cijanidin hlorida g^{-1} s.u.

Antioksidacijska aktivnost ekstrakata je varirala od 0.661 do 2.524 mg AA g^{-1} s.u. Najmanja inhibicija DPPH radikala je određena za vodeni ekstrakt, dok je najveći procenat inhibicije DPPH određen za 80% metanolni ekstrakt gloga. Izuzev vodenog ekstrakta (I = 24.48%) i ekstrakta u apsolutnom etanolu (I = 37.64%), svi ostali ispitivani ekstrakti su pokazali visok stepen inhibicije koji se kretao od 65.01% do 91.15 %.

Metodom linearne regresije određena je visoka korelacija između antioksidacijske aktivnosti ekstrakata i sadržaja ukupnih fenola (R^2 = 0.9473), kao i sadržaja ukupnih proantocijanidina (R^2 = 0.7469). Ukupni flavonoidi i monomerni antocijanini nisu značajno utjecali na antioksidacijsku aktivnost, što se može povezati sa njihovim nižim sadržajem u ispitivanim ekstraktima.

Rezultati dobiveni u ovoj studiji indiciraju da se ispitivani plodovi *Crataegus monogyna* mogu smatrati prirodnim izvorom fenolnih jedinjenja sa dobrom antioksidacijskom aktivnošću.

Works of the Faculty of Forestry
University of Sarajevo
No. 2, 2014 (41-51)

UDK 581.19:547.56:582.477

PHENOLIC CONTENT IN THE NEEDLES OF *CRYPTOMERIA JAPONICA* (THUNB. EX L. F.) D. DON, *CUPRESSOCYPARIS* × *LEYLANDII* (A.B. JACKS. & DALLIM.) DALLIM. "CASTLEWELLAN GOLD" AND *SEQUOIADENDRON GIGANTEUM* (LINDL.) J. BUCHHOLZ

Sadržaj fenolnih jedinjenja u iglicama *Cryptomeria japonica* (Thunb. ex L. f.) D. Don, *Cupressocyparis* × *leylandii* (A.B. Jacks. & Dallim.) Dallim. "Castlewellan Gold" i *Sequoiadendron giganteum* (Lindl.) J. Buchholz

Fatima Pustahija[1], Mirel Subašić[1], Nađad Bašić[1]

Abstract

Various phenolic compounds can be found in a gymnosperms and have been related to their bioactive properties, esspecially as a allelochemicals. Total phenol, flavonoid (flavone and flavonol) and protoanthocyanindin content was estimated quantitatively by using spectrophotometric method in the needle methanol extracts of mature *Cryptomeria japonica*, *Cupressocyparis* × *leylandii* „Castewellan Gold" and *Sequoiadendron giganteum* individuals. Although there is a strong intraspecific variability on the basis of the studied group of compounds, Duncan's test showed that *C. japonica* is clearly distinguishable from the other two taxa analyzed, and in particular on the basis of total proanthocyanidins and phenolics content. On the other hand, *S. giganteum* and *C.* × *leylandii* only differ on the basis of the flavonoid content calculated in terms of quercetin equivalent. In all three taxa proanthocyanidins had the highest variability. The ratios and relatively high content of analyzed phenolic compounds for all three studied taxa indicate that they may be considerd as a potential both chemotaxonomic characters and valuable sources of antioxidants, which should be confirmed by further researchs.

***Key words**: Cupressaceae, giant sequoia, gymnosperm, Leyland cypress, phenolic compounds, spectrophotometry, sugi*

INTRODUCTION - *Uvod*

Phenolics, heterogeneous group of natural substances, poses at least one aromatic ring with one or more hydroxyl groups attached, and play a major role in the plant defence against herbivores, pathogens, microbial infections and UV radiation, as attractants for pollinators and seed-dispersing animals, in pigmentation, and affect

[1] University of Sarajevo, Faculty of Forestry