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ANTIOXIDANT CAPACITY AND PHENOLIC CONTENT OF FRAXINUS ORNUS L. AND FRAXINUS PENNSYLVANICA MARSCH. LEAVES AND BARK EXTRACTS

Antioksidacijski kapacitet i sadržaj fenola ekstrakata lišća i kore *Fraxinus ornus L.* i *Fraxinus pennsylvanica* Marsch.

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Abstract

Total contents of phenols, flavonoids, phenolic acids and proanthocyanidins have been determined in methanolic extracts of *Fraxinus ornus* L. and *Fraxinus pennsylvanica* Marsch. leaves and branches bark. DPPH and FRAP assay were used in the determination of antioxidant capacity. *F. ornus* leaves had higher contents of flavonoids (13.08 mg RE g^{-1} DW) and proanthocyanidins (7.29 mg LCE g^{-1} DW) while the bark had higher contents of phenols (56.47 mg GAE g^{-1} DW), phenolic acids (14.32 mg CAE g^{-1} DW) and coumarins (94.81 mg CE g^{-1} DW). *F. pennsylvanica* leaves were richer in contents of phenols (25.73 mg GQE g^{-1} DW), flavonoids (2.87 mg CE g^{-1} DW and 5.13 mg RE g^{-1} DW), phenolic acids (14.60 mg CAE g^{-1} DW) and coumarins (20.01 mg CE g^{-1} DW) while the bark had more proanthocyanidins (6.88 mg CE g^{-1} DW*). F. ornus* leaves had lower contents of phenolic acids (11.09 mg CAE g^{-1} DW) than *F. pennsylvanica* leaves. Also*, F. pennsylvanica* bark had higher contents of flavonoids (1.25 mg RE g^{-1} DW) and proanthocyanidins (6.88 mg CE g^{-1} DW) than *F. ornus* bark. Antioxidant capacity for both species was higher for the leaves than the bark. Generally, *F. ornus* had better antioxidant capacity than *F. pennsylvanica*. Very high correlations were found between antioxidant capacity and phenols (r^2= 0.9361-0.9805), flavonoids (r^2= 0.9358-0.9876) and coumarins (r^2= 0.9661-0.9938) in leaves. In bark, correlations were found for phenols (r^2= 0.9744-0.9796) and coumarins (r^2= 0.9757-0.9911).

Key words: *F. ornus*, *F. pennsylvanica*, *phenols, DPPH, FRAP*

INTRODUCTION – *Uvod*

Manna ash (*Fraxinus ornus* L.) and green ash (*Fraxinus pennsylvanica* Marsch.) belong to *Oleaceae* family, genus *Fraxinus,* which includes approximately 70 species distributed naturally in Northern hemisphere (KOSTOVA AND IOSSIFOVA, 2007; LEE ET AL., 2012). Manna ash grows in southern-western Europe including France, Italy, the Mediterranean isles, the Balkan Peninsula, up to western Turkey. In

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forestry, the timber wood is used in production of small tool handles and household items, and in production of firewood. Some varieties are used as ornamental trees in urban areas and in afforestation of degraded sites (CAUDULLO AND DE RIGO, 2016). In folk medicine, especially in Bulgaria and Poland, *F. ornus* leaves and bark is used in treatment of diarrhoea, dysentery, arthritis and inflammation. Bark is also used in industrial production of esculin (KOSTOVA, 2001, KOSTOVA AND IOSSIFOVA, 2007). Leaves, flowers and bark of manna ash contain hydroxycoumarins, flavonoids, phenylethanoids and secoiridoids. As the main coumarins in bark esculin, esculetin and fraxetin are found. Bark also contains caffeic acid esters and tannins. Antioxidant, antimicrobic, antiinflammatory, immunomodulatory and antiviral properties of bark extracts are reported (IOSSIFOVA ET AL., 1994; MARINOVA ET AL., 1994; STEFANOVA ET AL., 1995; KOSTOVA, 2001).

The green or red ash (*Fraxinus pennsylvanica* Marsch.) is mainly distributed in eastern and central North America as native species. It is introduced in Europe and can be found from Spain to Russia (WESTWOOD ET AL., 2017). The timber wood is used in production of baseball bats, tool handles due to its high resistance, strongest and durability. *F. pennsylvanica* is also frequently used as ornamental trees in urban areas (ROYER, ET AL., 2012). Similar to other *Fraxinus* species, coumarins, flavonoids, phenolic acids, secoiridoids are generally found in *F. pennsylvanica*. Esculin, esculetin and fraxin are mainly present in bark. (KOSTOVA AND IOSSIFOVA, 2007). Antimicrobial activity of *F. pennsylvanica* leaf extracts (VANDAL ET AL., 2015) and bark extracts (OMAR ET AL., 2000) has been reported so far.

The aim of this work was to investigate contents of selected bioactive compounds in methanolic extracts of *F. ornus* and *F. pennsylvanica* leaf and young branches bark. Antioxidant capacity of the extracts was also investigated with DPPH and FRAP methods. Correlations between antioxidant capacity and bioactive compounds were observed. Obtained data presents significant contribution to knowledge of antioxidant capacity and contents of bioactive compounds of the investigated species.

MATERIAL AND METHODS – *Materijal i metode*

Plant material – *Biljni materijal*

F. ornus and *F. pennsylvanica* leaves and bark were collected from young branches in May and June in 2014. at two different localities in Kanton Sarajevo. It was taken three samples per species and identified at the Department of Forest Ecology. Before analysis, the plant material was dried in a ventilated room and grounded with an electric mill. Voucher specimens were deposited at the Herbarium of the Department of Ecology at Faculty of Forestry.

Chemicals and reagents – *Hemikalije i reagensi*

Folin-Ciocalteu's reagent, sodium carbonate, aluminium chloride, ferrum(III) chloride, coumarin, quercetin, rutin, gallic and caffeic acid, ascorbic acid, and absolute

methanol were purchased from Sigma Chemicals (Germany) and Aldrich (Germany). 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$), 2,4,6-tripyridil-S-triazine (TPTZ) were obtained from Sigma Chemicals (Germany) and Aldrich (Germany).

Potassium chloride and ferrous ammonium sulphate were obtained from Kemika Zagreb (Croatia) and butanol from Merck Chemical Suppliers (Germany). All other chemicals and solvents were of analytical grade.

Sample extracts preparation - *Priprema ekstrakata uzoraka*

Leaf and bark extracts were prepared by ultrasound extraction. Leaf or bark (0.5 g per sample) was extracted twice with 12 ml of 80% aqueous methanol for 30 minute at controlled temperature (40^{o}C). Obtained supernatants from each step were combined and the final volume was adjusted to 25 ml with extraction solvent. The extracts were kept at -20^{o}C until use.

Determination of total phenols - *Određivanje ukupnih fenola*

Determination of total phenols (TP) was done with Folin-Ciocalteu reagent with some modifications (SINGLETON ET AL., 1974). After incubation for 30 minutes (40^{o}C), absorbance of the blue product was measured at 765 nm. Gallic acid was used as a standard in preparation of a calibration curve. 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***

Total flavonoids were determined by methods described by ORDONEZ ET AL. (2006) and QUETTIER ET AL. (2000) with aluminium chloride. Quercetin and rutin were used as standards for the expression of the results. Absorbance of the samples was measured at 420 nm (quercetin) and 415 nm (rutin) one hour after incubation at room temperature. Sample blanks were also included in each procedure in order to correct absorbance of the sample. 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***

Arnow`s method (GAWLIC-DZIKI, 2012) with some modifications was used for determination of total phenolic acids (TPHA). Briefly, one milliliter of previously diluted sample was mixed with 5 mL of water, 1 mL HCl (0.5 M), 1 mL of Arnow`s reagent (10 g Na_2MoO_4 and 10 g $NaNO_2$ dissolved in 100 mL of distillated water) and 1 mL of NaOH (1M). Final volume was made up to 10 mL with distillated water. Caffeic acid was used as a standard to prepare calibration curve and absorbance was measured at 490 nm. The results are expressed as caffeic acid equivalents per gram of dry sample (mg CAE g^{-1}).

Determination of total coumarins – *Određivanje ukupnih kumarina*

Method described by OSORIO AND MARTINS (2004) was used to determine contents of coumarins in leaf and bark samples. To 0.5 ml of diluted plant extract, 2 ml distilled water and 0.5 ml of lead acetate (5%, w/v) solution were added in a test tube and after shaking volume was made up to 10 ml with distilled water. 2 ml of this solution was taken in another test tube and 8 ml of 0.1 M (v/v) hydrochloric acid solution was added. The solution was kept for 30 minutes at room temperature and absorbance was recorded at 320 nm. The total coumarin content was expressed as mg of coumarin equivalents per gram of dry sample (mg CE g^{-1}).

Determination of total proanthocyanidins – *Određivanje ukupnih proantocijanidina*

Butanol/HCl assay described by HAGERMAN (2000B) was used for determination of total proanthocyanidins (TPA). Before and after heating of the samples in boiling water-bath (60 minutes), absorbance of the sample was read at 550 nm. The results were expressed as mg of leucocyanidin equivalents per gram of dry sample (LCE g^{-1}) assuming that the specific absorbance of leucocyanidin was 460.

Determination of antioxidant capacity – *Određivanje antioksidacijskog kapaciteta*

DPPH assay - DPPH esej

Measurement of antiradical capacity was done according to SANCHEZ-MORENO ET AL. (1998). Previously diluted extract (0.1 ml) was added to 1.9 ml of freshly prepared 2,2-diphenyl-1picrylhydrazyl radical (DPPH) solution dissolved in methanol. Absorbance was read at 517 nm after incubation in the dark for 30 minutes. Results were expressed as vitamin C equivalents according to obtained equation:

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

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 mean values of results were expressed as vitamin C equivalents per gram of dry sample (AAE g^{-1}).

FRAP assay – FRAP esej

BENZIE AND STRAIN (1996) method was used in the determination of ferric reducing antioxidant power (FRAP). The method is based on reduction of ferric tripyridiyltriazine (Fe(III)-TPTZ) to blue coloured ferrous trypyridyltriazine (Fe(II)-TPTZ) which is monitored spectrophotometrically at 593 nm. Acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in 40 mM HCl acid) and $FeCl_3$ (20 mM) were mixed in the ratio 10:1:1 to prepare FRAP reagent and wormed at 37°C before use. The reagent (1.9 mL) was mixed with 0.1 mL of the extracts and leaved in the dark for 30 minute before measurements. Absorbance of the coloured product was measured at 593 nm against the blank which contained 0.1 ml of water instead of the extract. A standard

curve was made with Fe(II) sulphate and the results were expressed as mmol Fe(II) equivalents per gram of dry sample (mmol Fe(II) g^{-1}).

Statistical analysis- ***Statistička analiza***

All measurements were carried out in triplicate and obtained results are expressed as means. Regression analysis was used in order to investigate correlation between analysed active compounds and antioxidant capacity.

RESULTS AND DISCUSSION- *Rezultati i diskusija*

Contents of phenols, flavonoids, phenolic acids, coumarins and proanthocyanidins were investigated in methanolic extracts of *F. ornus* and *F. pennsylvanica* leaf and bark samples. Antioxidant capacity of the extracts was also determined by DPPH and FRAP assay. Ascorbic acid and ferrous sulphate were used as standards, respectively. Obtained results for contents of bioactive compounds are presented in Table 1.

Table 1. Contents of investigated polyphenolic compounds in extracts of *F.ornus* and *F. pennsylvanica leaves and bark*
Tabela 1. Sadržaji ispitivanih polifenolnih jedinjenja u ekstraktima F. ornus i F. pennsylvanica lista i kore

Samples	**TP** (mg $GAEg^{-1}$)	**TFr** (mg QEg^{-1})	**TFq** (mg REg^{-1})	**TPA** (mg $CAEg^{-1}$)	**TCM** (mg CEg^{-1})	**TPC** (mg LCE g^{-1})
			F. ornus			
Leaf(1)	46.69±0.18	6.34±0.05	13.20±0.01	6.80±0.14	50.96±0.10	6.89±0.02
Leaf(2)	56.20±0	6.16±0.05	13.33±0.01	14.96±0.02	69.17±0.15	6.42±0.06
Leaf (3)	46.81±0.06	6.09±0.003	12.74±0.01	11.51±0.10	54.69±0.20	8.56±0.06
Average	49.90	**6.20**	**13.08**	11.09	58.27	**7.29**
Bark(1)	47.60±0.02	0.85±0.003	1.45±0.006	15.00±0.05	78.42±0.25	4.16±0.04
Bark(2)	63.46±0.06	0.64±0.002	0.95±0.02	16.38±0.05	109.96±0.11	5.80±0.08
Bark (3)	58.36±0.16	0.61±0.002	0.87±0.004	11.58±0.09	96.04±0.38	4.59±0.15
Average	**56.47**	0.70	1.09	**14.32**	**94.81**	4.85
			F. pennsylvanica			
Leaf(1)	23.99±0	3.36±0.02	6.09±0.02	16.50±0.01	22.70±0.05	4.81±0.92
Leaf(2)	25.88±1.20	2.22±0.01	3.92±0.02	11.49±0.05	17.16±0.05	4.0±0.04
Leaf (3)	27.33±0.06	3.04±0.003	5.37±0.02	15.81±0.09	20.18±0.04	4.14±0.04
Average	**25.73**	**2.87**	**5.13**	**14.60**	**20.01**	4.32
Bark(1)	24.57±0.01	0.76±0.006	1.34±0.002	10.51±0.02	14.95±0.02	6.64±0.26
Bark(2)	24.79±0.01	0.61±0.002	1.09±0.003	11.15±0.03	14.28±0.06	7.33±0.02
Bark (3)	26.72±0.01	0.71±0.002	1.33±0.004	7.91±0.06	16.40±0.21	6.67±0.09
Average	25.36	0.69	1.25	9.86	15.21	**6.88**

Generally, the most abundant compounds in *F. ornus* leaves are coumarins, phenols and flavonoids and in the bark coumarins, phenols and phenolic acids. The average contents of coumarins, phenols and flavonoids in leaves are 58.27 mg CE g^{-1} DW, 49.90 mg GAE g^{-1} DW, 13.08 mg RE g^{-1} DW respectively. In the bark, average values for coumarins, phenols, phenolic acids are 94.81 mg CE g^{-1} DW, 56.47 mg GAE g^{-1} DW, and 14.32 mg CAE g^{-1} DW, respectively. The average contents of flavonoids and proanthocyanidins in the leaves are higher (6.20 mg QE g^{-1} DW, 13.08 mg RE g^{-1} DW, 7.29 mg LCE g^{-1} DW), than their average contents in the bark (0.70 mg QE g^{-1} DW, 1.09 mg RE g^{-1} DW, 4.85 mg LCE g^{-1} DW). Also, average phenolic acid contents in the bark are higher than in the leaves (Table 1).

The most abundant compounds in *F. pennsylvanica* leaves and bark were phenols, coumarins and phenolic acids. In leaves, their average contents were for phenols 25.73 mg GAE g^{-1} DW, coumarins 20.01 mg CE g^{-1} DW, and phenolic acids 14.60 mg CAE g^{-1} DW. In the bark, average values were for phenols 25.36 mg GAE g^{-1} DW, coumarins 15.21 mg CE g^{-1} DW, and phenolic acids 9.86 mg CAE g^{-1} DW. Leaves are richer in the contents of all investigated compounds, except proanthocyanidins with their average content of 4.32 mg LCE g^{-1} DW in leaves compering to the bark with the value of 6.88 mg LCE g^{-1} DW. We can conclude that *F. ornus* leaves had higher average contents of phenols, flavonoids, coumarins and proanthocyanidins than *F. pennsylvanica* leaves. *F. ornus* bark is richer in the contents of phenols, phenolic acids and coumarins while *F. pennsylvanica* bark had higher contents of flavonoids and proanthocyanidins.

Literature data concerning quantitative determination of the investigated compounds in *F. ornus* and *F. pennsylvanica* leaves and bark are quite limited. It is reported that different *Fraxinus* species contains phenols, phenolic acids, flavonoids, coumarins, sterols, triterpenes (KOSTOVA AND IOSSIFOVA, 2007 AND REFERENCE THEREIN). Obtained average results for total phenols of *F. ornus* and *F. pennsylvanica* leaves were higher compared to the results for *F. excelsior* and *F. angustifolia* 1.78-2.505% (IORDAKE ET AL., 2013). According to our previous investigations on *F. excelsior* and *F. angustifolia* leaves and bark extracts, we can conclude that *F. ornus* leaves is a reach source of phenols. Also, *F. pennsylvanica* had the highest average content of total flavonoids in leaves compared to that for *F. excelsior* (4.08 mg RE g^{-1} DW) and *F. angustifolia (*5.01 mg RE g^{-1} DW). We found that *F. angustifolia* leaves were the richest in the contents of phenolic acids (20.30 mg CAE g^{-1} DW) and proanthocyanidins (7.99 mg LCE g^{-1} DW). Since the average contents of coumarins for *F. excelsior* and *F. angustifola* leaves were 23.58 mg CE g^{-1} DW and 36.47 mg CE g^{-1} DW, we can conclude that *F. ornus* leaves had highest total coumarins content. (TAHIROVIĆ AND BAŠIĆ, 2016). According to KOSTOVA (2011), the levels of coumarins *in F. ornus* leaves varied from 0.3% up to 4.6% depending on the sampling season. Results obtained in this work for coumarin content in *F. ornus* leaves are generally higher. NYKOLOV ET AL. (1993) reported that *F. ornus* bark taken from five different regions in Bulgaria had total coumarin contents in the range of 7.8-9% which is lower than the results obtained in this work for *F. ornus* bark. These results are also higher in

comparison with the results obtained for *F. excelsior* (37.89 mg CE g^{-1} DW) and *F. angustifolia* bark (60.43 mg CE g^{-1} DW) (TAHIROVIĆ AND BAŠIĆ, 2016). Also, F. *pennsylvanica* had the highest content of proanthocyanidins and *F. ornus* highest content of total phenols compared to other investigated species (TAHIROVIĆ AND BAŠIĆ, 2016).

Two different assays were used to evaluate antioxidant capacity of investigated extracts. DPPH method was used to estimate radical scavenging activities with ascorbic acid as a standard while reducing capacity was estimated with FRAP method and ferrous sulphate used as a standard. Generally, greater antioxidant capacity of the extracts is related to higher values. Results obtained for antioxidant capacity are given in Table 2.

Table 2. Antioxidant capacity of *F. ornus* and *F. pennsylvanica* leaf and bark extracts
Tabela 2. Antioksidacijski kapacitet ekstrakata lista i kore F. ornus i F. pennsylvanica

	F. ornus		*F. pennsylvanica*	
	DPPH (mg AAE g^{-1})	FRAP (μmol Fe(II) g^{-1})	DPPH (mg AAE g^{-1})	FRAP (μmol Fe(II) g^{-1})
Leaf (1)	40.08±0.79	1577.09±8.98	25.39±0.06	972.11±4.19
Leaf (2)	43.84±5.85	1836.27±3.37	19.45±0.89	865.06±127.40
Leaf (3)	40.07±0.98	1624.33±8.82	23.54±0.87	959.21±59.82
Average	**41.33**	**1679.23**	**22.79**	**932.13**
Bark(1)	24.02±0.35	906.42±19.38	17.10±1.26	733.06±5.63
Bark (2)	27.31±1.92	1028.30±11.65	17.34±0.33	745.44±11.10
Bark (3)	24.75±1.78	935.25±4.12	17.65±0.75	757.56±56.12
Average	25.36	956.65	17.36	745.35

Average values of antioxidant capacity obtained with DPPH assay for *F. ornus* leaves and bark were 41.33 and 25.36 mg AAE g^{-1} DW respectively. With FRAP assay, average results were 1679.23 μmol Fe(II) g^{-1} DW for the leaves and 956.65 μmol Fe(II) g^{-1} DW for the bark. It can be noticed that *F. ornus* leaves had higher antioxidant capacity than the bark. Average values for *F. pennsylvanica* leaves and bark in DPPH assay were 22.79 and 17.36 mg AAE g^{-1} DW respectively. In FRAP assay, we obtained values of 932.13 μmol Fe(II) g^{-1} DW for the leaves and 745.35 μmol Fe(II) g^{-1} DW for the bark. It can be concluded that *F. ornus* leaves and bark have higher antioxidant capacity than *F. pennsylvanica*. Obtained average results for DPPH and FRAP assay for *F. ornus* are higher than previously reported results for *F. excelsior* leaves (17.67 mg AAE g^{-1} DW and 836.65 μmol Fe(II) g^{-1} DW) and *F. angustifolia* leaves (34.53 mg AAE g^{-1} DW and 1432.43 μmol Fe(II) g^{-1} DW) respectively. However, *F. ornus* bark had higher antioxidant capacity compared to reported results for *F. excelsior* bark (20.43 mg AAE g^{-1} DW and 875.46 μmol Fe(II) g^{-1} DW) but lower compared to the results for *F. angustifolia* bark (34.02 mg AAE g^{-1} DW and 1419.35 μmol Fe(II) g^{-1} DW (TAHIROVIĆ AND BAŠIĆ, 2016).)

Correlations between contents of bioactive compounds in leaves and bark and their antioxidant capacity were investigated with linear regression and correlation coefficients are given in Table 3.

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

	Correlation coefficient (r^2)			
	leaves		**bark**	
	DPPH	**FRAP**	**DPPH**	**FRAP**
Phenols	**0.9361**	**0.9805**	**0.9796**	**0.9744**
Flavonoids (R)	**0.9876**	**0.9594**	0.2242	0.268
Flavonoids(Q)	**0.9778**	**0.9358**	0.0086	0.0217
Phenolic acids	0.1295	0.1146	0.6756	0.668
Coumarins	**0.9661**	**0.9938**	**0.9911**	**0.9757**
Proanthocyanidins	0.7581	0.4776	0.5668	0.7196

Very high correlations between antioxidant capacity and the total phenols, flavonoids and coumarins contents in leaves were obtained. Correlation coefficients between DPPH assay and bioactive compounds in leaves were for phenols (r^2 = 0.9361), flavonoids (r^2 = 0.9876 and 0.9778), coumarins (r^2 = 0.9661). Coefficients obtained for FRAP values were r^2 = 0.9805 (phenols), r^2 = 0.9594 and 0.9358 (flavonoids); r^2 = 0.9938 (coumarins) respectively. Week correlations were found for both methods with phenolic acid contents in leaves (r^2 = 0.1295 and r^2 =0.1146). In bark, both methods correlate with phenols (r^2 = 0.9744-0.9796) and coumarin contents (r^2 = 0.9757-0.9911) at high level while moderate correlation was noticed with phenolic acid (r^2 = 0.668-0.6756) and proanthocyanidin contents (r^2 = 0.5668-0.7196). Week correlations were observed between antioxidant capacity and flavonoid contents in bark for both methods (r^2 = 0, 2242-0.268 and r^2 = 0.0086-0.0217). Similarly to our previous investigations, flavonoids in bark do not correlate with antioxidant capacity which can be explained by their lower contents in the bark compared to the leaves (TAHIROVIĆ AND BAŠIĆ, 2016). Correlations between antioxidant capacity and different bioactive compounds of *Fraxinus* species have been investigated (ATMANI ET AL., 2009; KOSTOVA, ET AL., 2011; WU, ET AL., 2007; AYOUNY ET AL., 2016; TAHIROVIĆ AND BAŠIĆ, 2016). All investigations support significant contribution of phenols, flavonoids and coumarins to the antioxidant capacity.

CONCLUSION – *Zaključak*

The most abundant compounds in *F. ornus* leaves are phenols, coumarins and flavonoids and in the bark phenols, phenolic acids and coumarins.

The most abundant compounds in *F. pennsylvanica* leaves and bark are phenols, coumarins and phenolic acids.

F. ornus leaves are richer in the contents of flavonoids and proantocyanidins than the bark, while *F. ornus* bark has higher content of phenols, phenolic acids and coumarins.

F. pennsylvanica leaves are richer in the contents of all investigated compounds than the bark, except content of proanthocyanidins.

F. ornus leves are richer in contents of all investigated compounds compared *to F. pennsylvanica* except phenolic acids content.

F. ornus bark has higher contents of phenols, phenolic acids, coumarins than *F. pennsylvanica* bark.

F.ornus and *F. pennsylvanica* leaves have higher antioxidant capacity than their bark.

Generally, *F ornus* leaves and bark have higher antioxidant capacity than *F. pennsylvanica.*

According to our results, phenols, flavonoids and coumarins are the main contributors to the antioxidant capacity of leaves. On the other hand, phenols and coumarins contribute to the antioxidant activity of the bark.

It can be concluded that further investigations on chemical composition and antioxidant capacity of selected species are required.

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SAŽETAK

Sadržaj ukupnih fenola, flavonoida, fenolnih kiselina, kumarina i proantocijanidina kao i antioksidacijski kapacitet određivani su u metanolnim ekstraktima uzoraka lista i kore grana vrsta *F. ornus L.* i *F. pennsylvanica* Marsch. Za određivanje sadržaja bioaktivnih jedinjenja i antioksidacijskog kapaciteta ekstrakata korištene su spektrofotometrijske metode analize. Folin-Ciocalteu metoda je korištena u određivanju ukupnih fenola a $AlCl_3$ metoda za određivanje ukupnih flavonoida uz rutin i kvercitin kao standarde. Kvantifikacija ukupnih proantocijanidina izvršena je primjenom kiselinsko-butanolne metode. Arnowa metoda je korištena za određivanje ukupnih fenolskih kiselina a metoda Osoria i Martineza za ukupne kumarine. DPPH, i FRAP metoda, uz askorbinsku kiselinu i Fe(II) sulfata kao standarde, korištene su za određivanje antioksidacijskog kapaciteta uzoraka.

Sadržaji ukupnih fenola po gramu suhog uzorka (s.u) za uzorke listova su u području 23.99–56.20 mg GAE g^{-1} s.u; ukupnih flavonoida 2.22–6.34 mg RE g^{-1} s.u. i 3.92–13.33 mg QE g^{-1} s.u; fenolnih kiselina 6.80–16.50 mg CAE g^{-1} s.u.; sadržaji ukupnih kumarini iznose 17.16–69.17 mg CE g^{-1} s.u. i proantocijanidina 4.00–8.56 mg LCE g^{-1} s.u.

U uzorcima kore grana, sadržaji ispitivanih jedinjenja iznose: ukupni fenoli 24.57–56.20 mg GAE g^{-1} s.u; ukupni flavonoidi 0.61–0.85 mg RE g^{-1} s.u i 0.87–1.45 mg QE g^{-1} s.u; ukupne fenolne kiseline su u granicama 7.91–16.38 mg CAE g^{-1} a ukupni kumarini 14.28–109.96 mg CE g^{-1} s.u; i ukupni proantocijanidini 4.16–7.33 mg LCE g^{-1} s.u.

F. ornus list ima veći sadržaj ukupnih fenola, flavonoida, kumarina i proantocijanidina dok *F. pennsylvanica* list ima veći sadržaj ukupnih fenolnih kiselina. *F. ornus* kora ima veći sadržaj ukupnih fenola, fenolnih kiselina i kumarina dok *F. pennsylvanica* kora ima veći sadržaj flavonoida (ekvivalenti rutina) i proantocijanidina.

Prosječne vrijednosti antioksidacijskog kapaciteta dobivenog DPPH i FRAP metodom za *F. ornus* list iznose 41.33 mg AAE g^{-1} s.u. i 1679.23 µmolFe(II) g^{-1} s.u. i za *F. ornus* koru 25.36 mg AAE g^{-1} s.u. i 956.65 µmolFe(II) g^{-1} s.u.
Prosječne vrijednosti antioksidacijskog kapaciteta dobivenog DPPH i FRAP metodom za *F. pennsylvanica* list iznose 22.79 mg AAE g^{-1} s.u i 932.13 µmolFe(II) g^{-1} s.u i za *F. pennsylvanica* koru 17.36 mg AAE g^{-1} s.u i 743.35 µmolFe(II) g^{-1} s.u.
Dobiveni rezultati su pokazali da *F. ornus* list i kora imaju veći antioksidacijski kapacitet.
Linearnom regresijom utvrđena je visoka korelacija između antioksidacijskog kapaciteta i sadržaja ukupnih fenola, flavonoida i kumarina za list kao i sadržaja fenola i ukupnih kumarina za koru.

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