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DETERMINATION OF PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY O*F Fraxinus excelsior* L. AND *Fraxinus angustifolia* VAHL. LEAVES AND BARK EXTRACTS

Određivanje sadržaja fenolnih jedinjenja i antioksidacijskog kapaciteta ekstrakata lišća i kore *Fraxinus excelsior* L. i *Fraxinus anfustifolia* Vahl.

Azra Tahirović[1], Nađad Bašić[1]

Abstract

In this work, *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl. leaves and branches bark extracts have been estimated for their phenolic content and antioxidant capacity. The antioxidant capacity was examined by DPPH and FRAP methods.

Higher contents of total phenols (23.94- 46.98 mg GAE g^{-1}) and flavonoids (6.30 – 9.14 mg RE g^{-1} and 3.67 – 5.34 mg QE g^{-1}) have been determined in leaves than in the bark for both species. The bark contained higher amounts of total phenolic acids (17.79 – 36.67 mg CAE g^{-1}), coumarins (27.91 – 70.98 mg CE g^{-1}) than the leaves. *F. excelsior* bark had higher content of proanthocyanidins (4.14 – 7.95 mg LCE g^{-1}) while *F. angustifolia* leaves were richer in proanthocyanidins (5.76 – 11.16 mg LCE g^{-1}). Generally, higher amounts of bioactive compounds and better antioxidant capacity was found for *F. angustifolia*. Also, extracts of *F. excelsior* bark and *F. angustifolia* leaves displayed higher antioxidant activities. Established correlations between phenols (r^2 = 0.8381 – 0.9228), phenolic acids (r^2 = 0.8799 – 0.9843), coumarins (r^2 = 0.9223 – 0.9716) and antioxidant capacity determined by DPPH and FRAP shown these compounds are the main contributors to the antioxidant capacity in leaves and bark of investigated species.

Key words: *Fraxinus, F. excelsior, F. angustifolia, phenols, DPPH, FRAP*

INTRODUCTION – *Uvod*

The genus *Fraxinus* of Oleaceae includes approximately 70 woody species widely distributed in Europa, Asia, North and Central America (WALLANDER, 2008, WEI AND GREEN, 1996). Two widespread trees in Europe including Bosnia and Herzegovina are *Fraxinus excelsior* L. (common ash) and *Fraxinus angustifolia* Vahl. (narrow-leaved ash) (FERNADEZ-MANJARRES ET AL., 2006). In Central and Northern Europe, common ash is gradually replaced towards the Mediterranean basin by the narrow-leaved ash. Although *F. excelsior* can be found in entire Bosnia in different forest communications,

[1] Faculty of Forestry, University of Sarajevo, Zagrebačka 20, 71000 Sarajevo, BiH

it is mainly abundant in central part of the country (STEVANOVİĆ, ET AL., 1983). *Fraxinus excelsior* is valuable timber tree in forestry because of toughness and elasticity of its wood, and it is used in reforestation programs in some parts of Europe (RAQUİN ET AL., 2002). The bark of *F. excelsior* and *F. angustifolia* was used for tanning and as a colorant. Beside that, both species are used in traditional and folk medicine in different parts of the world (EUROPEAN MEDİCİNES AGENCY, 2012).

The bark and the leaves of *F. excelsior* were used in folk medicine for wound healing, diarrhea and dysentery (KOSTOVA AND İOSSİFOVA, 2007) and as antipyretic (LUST, 1974). The alcoholic extracts of *F. excelsior* bark posses an anti-inflammatory properties (MAYER ET AL., 1995). The bark of *F. angustifolia* is best known for its anti-inflammatory use, but it is also used as antioxidant, diuretic, digestive and astringent , and it is effective against hemorrhoids and fever (ATMANİ ET AL., 2009; WRİGHT, ET AL., 2007). Decoctions and infusions prepared from leaves and bark *of F. angustifolia* are used in treatman of rheumatism, hemorrhoids and fever in Algeria (BABA-AİSSA, 1999). *F. excelsior* and *F. angustifolia* Vahl leaves are included in European pharmacopoeia. Studies on chemical composition revealed presence of hydroxycinammic derivates, simple coumarins, iridoids, flavonoids, tannins, mannitol, lignanas, mucilages (EUROPEAN MEDİCİNES AGENCY, 2012 AND REFERENCES THEREİN). Generally, presence of coumarins, secoiridoids and phenylethanoids is the characteristic of all *Fraxinus* species (KOSTOVA AND IOSSIFOVA, 2007).

The aim of this work was to determine total amounts of phenols, flavonoids, phenolic acids, coumarins and proanthocyanidins in leaves and bark of two *Fraxinus* species from Sarajevo region. Antioxidant capacity of the extracts was determined by DPPH and FRAPS assays and their correlations to content of active compounds in leaves and bark extracts. According to the literature data, natural populations of *F. excelsior* in Bosnia are investigated in terms of genetic characteristics (BALLIAN, ET AL., 2007). However, there is no data available concerning chemical composition and antioxidant capacity of *F. excelsior* and *F. angustifolia*. Obtained results are important from the aspect of potential medical and industrial use of the investigated species.

MATERIAL AND METHODS – *Materijal i metode*

Plant material – *Biljni materijal*

Leaf and bark from up to four years old branches *of F. excelsior* and *F. angustifolia* samples were collected in May and June in 2014 at two different localities in Sarajevo region. Three samples per species were collected and identified at the Department of Forest Ecology by Prof Bašić. The plant material was air-dried and grounded with electric mill before

analysis. Also, voucher specimens were deposited at the Herbarium of the Department of Ecology at Faculty of Forestry.

Chemicals and reagents – *Hemikalije i reagensi*

Quercetin, rutin, gallic acid, caffeic acid, ascorbic acid, coumarin, aluminum chloride, ferric chloride, Folin-Ciocalteu's reagent, sodium carbonate 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 the same suppliers.

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*

Ultrasonic extraction with some modifications was used for preparation of leaf and bark extracts (HAGERMAN, 2000B). Shortly, 0.5 g of leaf or bark was extracted twice with 12 mL of 80% aqueous methanol. Each extraction was performed for 30 minute at 40^{o}C. Obtained supernatants were combined and the volume was adjusted to 25 mL with extraction solvent. The extracts were kept at -20^{o}C until analysis.

Determination of total phenols - *Određivanje ukupnih fenola*

The Folin-Ciocalteu method (SINGLETON ET AL., 1974) was used for the determination of total phenols (TP). The absorbance of the colored product was measured at 765 nm after incubation at 40^{o}C for 30 minutes. A calibration curve was prepared with gallic acid as a 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 methods based on aluminum chloride described by ORDONEZ ET AL. (2006) and QUETTIER ET AL. (2000) were used for the determination of total flavonoids. Two standards, quercetin and rutin were used for the expression of the results. After one hour of incubation at room temperature, absorbance of the samples was measured at 420 nm and 415 nm. For each procedure, sample blanks were also included. 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 (TPA) were quantified with Arnov method (GAWLIC-DZIKI, 2012) with some modifications. One milliliter 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 distilled water), 1 mL of NaOH (1M) and the volume was made up to 10 mL

with distilled water. Calibration curve was established with standard solutions of caffeic acid and absorbance 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 total coumarins – *Određivanje ukupnih kumarina*

Coumarin contents (TCM) were estimated following method described by OSORİO AND MARTİNS (2004). 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. After shaking thoroughly, 7 mL of distilled water was added and mixing well, 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).

Determination of total proanthocyanidins – *Određivanje ukupnih proantocijanidina*

Total proanthocyanidins (TPA) were determined with butanol/HCl assay (HAGERMAN, 2000B). Absorbance of the sample was read at 550 nm before and after heating of the samples in boiling water-bath for 60 minutes. Butanol/HCl mixture containing solvent instead extract was used as a blank. The results were expressed as mg of leucocyanidin equivalents (LCE) per gram of dry sample taking into account that the specific absorbance of leucocyanidin was 460.

Determination of antioxidant capacity – *Određivanje antioksidacijskog kapaciteta*

DPPH assay - DPPH esej

Measurement of antiradical activity was adapted from SANCHEZ-MORENO ET AL. (1998) as described previously (TAHIROVIĆ, ET AL., 2015). In brief, 0.1 ml of diluted extract was added to 1.9 ml of freshly prepared 2,2-diphenyl-1picrylhydrazyl radical (DPPH) solution dissolved in methanol ($7.116x10^{-5}$ $moldm^{-3}$). After 30 min of incubation in dark, the absorbance was read at 517 nm. Results were expressed as mg vitamin C equivalents per gram of dry sample (mg AAE g^{-1} DW).

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. 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. Absorbance of the colored 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 ferrous sulphate and the results were expressed as μmol Fe(II) equivalents per gram of dry sample (μmol Fe(II) g^{-1}).

Statistical analysis- *Statistička analiza*

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

RESULTS AND DISCUSSION- *Rezultati i diskusija*

Methanolic extracts of *F. excelsior* and *F. angustifolia* leaf and bark were investigated for the contents of phenols, flavonoids, phenolic acids, coumarins and proanthocyanidins. Antioxidant activities of the extracts were also investigated by two methods: DPPH assay with ascorbic acid as a standard, and FRAP assay with ferrous sulphate as a standard. Results for contents of bioactive compounds are presented in Table 1.

Table 1. Content of investigated polyphenolic compounds in extracts of *F. excelsior* and *F. angustifolia leaves and bark*
Tabela 1. Sadržaj ispitivanih polifenolnih jedinjenja u ekstraktima F. excelsior i F. angustifolia 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. excelsior			
Leaf(1)	30.51±0.01	7.62±0.004	4.16±0.03	10.09±0.005	26.67±0.15	3.74±0.10
Leaf(2)	23.94±0.01	6.83±0.07	3.67±0.01	12.49±0.036	20.18±0.04	2.90±0.09
Leaf (3)	30.75±0.001	6.30±0.005	4.42±0.06	9.85±0.03	23.88±0.01	4.79±0.05
Average	**28.40**	**6.92**	**4.08**	10.81	23.58	3.81
Bark(1)	31.47±0.01	1.29±0.004	0.74±0.003	22.95±2.12	46.09±0.02	4.14±0.11
Bark(2)	22.77±0.02	1.37±0.01	0.83±0.001	17.79±0.02	27.91±0.04	7.95±0.06
Bark (3)	30.06±0.07	0.91±0.002	0.64±0.02	20.68±0.03	39.68±0.06	7.69±0.05
Average	28.09	1.19	0.73	**22.24**	**37.89**	**6.59**
			F. angustifolia			
Leaf(1)	39.72±0.01	8.19±0.07	4.79±0.01	18.52±0.04	32.04±0.05	7.06±0.05
Leaf(2)	46.98±0.03	9.14±0.02	5.34±0.01	24.94±0.05	41.72±0.06	5.76±0.03
Leaf (3)	38.48±0.012	8.41±0.03	4.89±0.01	17.43±0.05	35.67±0.1	11.16±0.03
Average	**41.72**	**8.58**	**5.01**	20.30	36.47	**7.99**
Bark(1)	30.35±0.01	1.08±0.02	0.64±0.004	27.55±0.07	52.84±0.12	5.62±0.05
Bark(2)	39.85±0.01	1.71±0.01	0.89±0.002	36.67±0.06	70.98±0.20	5.53±0.1
Bark (3)	31.48±0.01	1.74±0.01	0.95±0.002	27.06±0.03	57.48±0.19	3.8±0.01
Average	33.89	1.51	0.83	**30.43**	**60.43**	4.99

From the results it can be seen that the most abundant compounds were phenols, coumarins, and phenolic acids in *F. excelsior* leaf and bark. The average contents in leaves were: phenols 28.40 mg GAE g^{-1} DW, coumarins 23.58 mg CE g^{-1} DW, and phenolic acids 10.81 mg CAE g^{-1} DW. In bark, average values were for phenols 28.09 mg GAE g^{-1} DW, coumarins 37.89 mg CE g^{-1} DW, and phenolic acids 22.24 mg CAE g^{-1} DW. The average contents of flavonoids in leaves were 6.92 mg RE g^{-1} DW and 4.08 mg QE g^{-1} DW, while bark contains in average much less of flavonoids (1.19 mg RE g^{-1} DW and 0.73 mg QE g^{-1} DW). On the other hand, bark had higher average contents of proanthocyanidins (6.59 mg LCE g^{-1} DW) than leaves (3.81 mg LCE g^{-1} DW).

Investigations of *F. angustifolia* leaf and bark extracts reveled that similar to *F. excelsior,* the most abundant compounds were phenols, coumarins and phenolic acids. In leaves, investigated compounds were abundant in following average values: phenols 41.72 mg GAE g^{-1} DW, coumarins 36.47 mg CE g^{-1} DW, and phenolic acids 20.30 mg CAE g^{-1} DW. In bark, average values were for phenols 33.89 mg GAE g^{-1} DW, coumarins 60.43 mg CE g^{-1} DW, and phenolic acids 30.43 mg CAE g^{-1} DW. Leaves were richer in the contents of flavonoids (8.58 mg RE g^{-1} DW and 5.01 mg QE g^{-1} DW) and proanthocyanidins (7.99 mg LCE g^{-1} DW) than bark. Total flavonoids in bark were 1.51 mg RE g^{-1} DW and 0.83 mg QE g^{-1} DW, and total proanthocyanidins were 4.99 mg LCE g^{-1} DW.

In comparison with other investigators, our results for total phenols and flavonoids in leaves are higher than the results reported BY IORDAKE ET AL. (2013). They investigated active compounds in *Fraxini folium* (*F. excelsior* or *F.angustifolia*) and obtained polyphenols in range 1.78 – 2.50% expressed as gallic acid and flavonoids 0.135 – 0.259% expressed as rutin. However, our results for total phenolic acids were lower from reported 2.7 – 3.2% caffeic acid. These differences can be generally explained by different ecological factors. According to GADCKE ET AL. (1993) the amounts of flavonoids in *F. excelsior* and *F. angustifolia* can vary between 0.6-2.2% from which 0.1-0.9% can be rutin. This is in agreemeent with our findings in this work. Also, contents of coumarins in leaves can be variable. For example, some researches stataed low levels of coumarins in leaves which were in range 0.01 - 0.05%. but the authors sugessted reevaluation of the data (CARNAT ET AL., 1990). The levels of coumarins *in F. ornus* leaves were from 0.3% up to 4.6% depending on the sampling season. (KOSTOVA, 2001). It is reported that contents of tannins ranging from 0.6 to 4% in *Fraxini folium* (GAEDCKE ET AL., 1993).

Phytochemical investigations on *Fraxinus* species bark revealed the presence of different compounds such as: coumarins, simple phenols, flavonoids, secoiridoids and phenylethanoids (KOSTOVA AND IOSSIFOVA, 2007). Literature data concerning total amounts investigated compounds in *F.*

excelsior bark are quite limited, but some results are given for *F. angustifolia* bark. Several researches investigated contents of phenols, flavonoids and tannins in *F. angustifolia* bark extracts but the results are expressed in grams of extracts and different standards are used so they are not comparable with our results (ATMANİ ET AL., 2009; BERBOUCKA ET AL., 2010; AYOUNİ ET AL., 2016). One of the main compounds in *Fraxini cortex* are coumarins (WU ET AL. 2007). They are usually found in free or as glucosides in all *Fraxinus* species (MURRAY, ET AL., 1982). Detail list of coumarins is given in detailed review by KOSTOVA AND IOSSİFOVA (2007). Following values for coumarin contents in different F. species were reported: *F. rhynchophylla* 3.4%; *F. stylosa* 3.5%, *F.chinesis* 3.9% (TANG AND EİNSENBRAND, 1992); *F.ornus* 7.8-9% (NYKOLOV, ET AL., 1993). on the basis of given results we can see that *F. excelsior* and *F. angustifolia* bark are rich source of coumarins (Table 1). Comparing contents of investigated compounds between two species we can conclude that *F. angustifolia* samples are richer in the content of all investigated compounds except proanthocyanidins which are higher in *F. excelsior* bark. Also, both species are rich in coumarin content. Coumarins are known as compounds with antioxidant, anti-inflammatory, anticancer, anticoagulant activities with excellent pharmaceutical potential (KOSTOVA, ET AL., 2011).

An approach involving at least two different assays is applied in order to evaluate antioxidant properties of plant extracts (PRİOR, ET AL., 2005). Radical scavenging activities of the extracts were analysed with DPPH method while reducing capacity was investigated with FRAP method. In DPPH assay, ascorbic acid was used as a standard while in FRAP assay, ferrous sulphate was employed for estimation of antioxidant capacity. In all cases, obtained higher values point to greater antioxidant capacity of the extracts. Obtained results are presented in Table 2.

Table 2. Antioxidant capacities of *F. excelsior* and *F. angustifolia* leaf and bark extracts

Tabela 2. Antioksidacijski kapaciteti ekstrakata lista i kore F. excelsior i F. angustifolia

	F. excelsior		*F. angustifolia*	
	DPPH (mg AAE g^{-1})	FRAP (µmol Fe(II) g^{-1})	DPPH (mg AAE g^{-1})	FRAP (µmol Fe(II) g^{-1})
Leaf (1)	18.84±1.14	874.00±46.68	29.30±1.68	1101.39±22.19
Leaf (2)	16.49±0.28	854.87±44.98	42.18±0.78	1870.61±16.21
Leaf (3)	17.68±0.65	781.10±25.04	32.11±1.72	1325.29±3.36
Average	17.67	836.65	**34.53**	**1432.43**
Bark(1)	23.99±1.47	1087.56±6.77	28.64±0.83	1259.78±10.53
Bark (2)	16.65±0.05	713.94±4.24	41.99±0.48	1743.00±19.20
Bark (3)	20.65±0.38	824.89±11.70	31.42±0.59	1255.27±15.53
Average	**20.43**	**875.46**	34.02	1419.35

Average values of antioxidant capacity for *F. excelsior* leaf and bark samples in DPPH assay were 17.67 and 20.34 mg AAE g^{-1} DW respectively. These results were consistent with FRAP assay were we obtained 836.65 µmol Fe(II) g^{-1} DW for leaves and 875.46 µmol Fe(II) g^{-1} DW for bark. Also, we can conclude that *F. excelsior* bark had higher antioxidant capacity than the leaves. Average values for *F. angustifolia* leaves and bark in DPPH assay were 34.53 and 34.02 mg AAE g^{-1} DW respectively. In FRAP assay, we obtained values of 1432.43 µmol Fe (II) g^{-1} DW for leaves and 1.419.35 µmol Fe(II) g^{-1} DW for bark. From these results, it can be noticed that *F. angustifolia* leaves have higher antioxidant capacity than the bark. Also, these results show that *F. angustifolia* leaf and bark have higher antioxidant capacity than *F. excelsior* species.

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

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

	Correlation coefficient (r^2)			
	leaves		**bark**	
	DPPH	**FRAP**	**DPPH**	**FRAP**
Phenols	**0.9228**	**0.8482**	**0.8381**	**0.8293**
Flavonoids (R)	**0.8593**	**0.8146**	0.3904	0.3437
Flavonoids(Q)	**0.8586**	**0.7882**	0.2155	0.1619
Phenolic acids	**0.9301**	**0.8799**	**0.9843**	**0.9842**
Coumarins	**0.9716**	**0.9233**	**0.9598**	**0.9567**
Proanthocyanidins	0.3295	0.217	0.2962	0.3323

Very high correlations were observed between DPPH and FRAP and the total phenols, phenolic acids, and coumarins contents in leaf and bark. Correlation coefficients between DPPH assay and bioactive compounds in leaves and bark were in in range for phenols (r^2 = 0.8381-0.9228), phenolic acids (r^2 = 0.9301-0.9843), coumarins (r^2 = 0.9598-0.9716). Also high correlations between FRAP and phenols (0.8293-0.8482), coumarins (0.9233-0.9567) and phenolic acids (0.8799-0.9842) in leaf and bark were obtained. Phenolic compounds are recognized as important antioxidants in different plants (LI, ET AL. 2008). Correlations between total phenolics *in F. angustifolia* bark and DPPH/FRAP were proven BY AHMANI ET AL. (2009). In fact, *Fraxinus* species have been used as anti-inflammatory drugs probably due to presence of several phenolic acids and coumarins (KOSTOVA, ET AL., 2011). WU, ET AL.

(2007) found for Chinese *Cortex fraxini* that phenolic - coumarin reach fraction had the best free radical-scavenging activity and reducing power which supports high correlation of both antioxidant capacity with coumarin contents in this work.

Both methods shown good correlations with flavonoids in leaves while for bark it was week (r^2 = 0.1619-0.3437). This may be the result of increased flavonoid concentrations in leaves than in bark. Similar observation that flavonoids in leaves of *F. angustifolia* (kaemferol, rutin, quercetin, isoquercetin) contribute significantly to the antioxidant capacity was noticed by AYOUNY ET AL. (2016). According to the same authors, phenylethanoids are the most important antioxidant in the bark. Also, week correlations were observed between DPPH or FRAP values of the extracts and proantocyanidins contents in leaf (r^2 = 0.3295 and r^2 = 0.217) and bark (r^2 = 0.2962 and r^2 = 0.3323). These results indicate that proanthocyanidins in leaves and bark contribute to the antioxidant capacity at very low level.

CONCLUSION – *Zaključak*

The most abundant compounds in leaf and bark extracts of *F. excelsior* and *F. angustifolia* were phenols, phenolic acids and coumarins.

F. excelsior leaves is richer in the contents of phenols and flavonoids than bark, while *F. angustifolia* leaves are richer in the contents of phenols, flavonoids and proanthyocyanidins than bark.

Bark of both species had higher contents of coumarins and phenolic acids than leaves. *F. excelsior* bark had higher content of proanthyocyanidins while with *F. angustifolia* bark was the opposite situation.

F. angustifolia leaves had higher contents of all investigated compounds than *F. excelsior.* Also, *F. angustifolia* bark was richer in contents of all investigated compounds expect proanthocyanidins.

Leaves of *F.angustifolia* had higher antioxidant activity than the bark while *F. excelsior* bark had higher capacity than the leaves. Generally, *F angustifolia* had higher antioxidant activity than *F. excelsior* leaves and bark.

Our results indicate that phenols including phenolic acids and coumarins are the major contributors to the antioxidant properties of leaves and bark extracts. Also, high correlation coefficients were obtained between DPPH/FRAP method and content of phenols, phenolic acids and coumarins.

It can be concluded that leaf and bark of both species can be considered as a potential source of antioxidant compounds.

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SAŽETAK

Metanolni ekstrakti uzoraka lista i kore grana *F. excelsior* L. i *F. angustifolia Vahl.* ispitivani su na sadržaj ukupnih fenola, flavonoida, fenolnih kiselina, kumarina i proantocijanidina kao i antioksidacijsku aktivnost. Spektofotometrijske metode su korištene za određivanje sadržaja bioaktivnih jedinjenja i antioksidacijske aktivnosti ekstrakata lista i kore. Ukupni fenoli su određeni Folin-Ciocalteu metodom a određivanje ukupnih flavonoida prema rutinu i kvercitinu kao standardima rađeno je $AlCl_3$ metodom. Kiselinsko-butanolna metoda je upotrebljena za kvantifikaciju ukupnih proantocijanidina. Ukupne fenolne kiseline su određene Arnovom metodom a ukupni kumarini metodom Osoria i Martineza (2004). Za mjerenje antioksidacijske aktivnosti korištene su DPPH i FRAP metoda uz primjenu askorbinske kiseline i Fe(II) sulfata kao standarda.

Sadržaj ukupnih fenola po gramu suhog uzorka (s.u) za uzorke listova varira od 23.94 – 46.98 mg GAE g^{-1} s.u; ukupnih flavonoida 6.30 – 9.14 mg RE g^{-1} s.u. i 3.67 – 5.34 mg QE g^{-1} s.u; fenolnih kiselina 9.85 – 24.94 mg CAE g^{-1}; sadržaj ukupnih kumarina iznosili su 20.18 – 41.72 mg CE g^{-1} s.u. i proantocijanidina 2.90 -11.16 mg LCE g^{-1} s.u.

U uzorcima kore grana sadržaj ispitivanih jedinjenja iznosio je: ukupni fenoli 22.77 – 39.85 mg GAE g^{-1} s.u; ukupni flavonoidi 0.91 – 1.74 mg RE g^{-1} s.u i 0.64 – 0.95 mg QE g^{-1} s.u; ukupne fenolne kiseline su bile u granicama 17.79 – 36.67 mg CAE g^{-1}, a ukupni kumarini 27.91 – 70.98 mg CE g^{-1} s.u; i ukupni proantocijanidini 3.8 – 7.95 mg LCE g^{-1} s.u.

Poređenjem prosječnih sadržaja aktivnih jedinjenja u listovima i kori dvije vrste može se zaključiti da *F. angustifolia* ima veći prosječni sadržaj svih

ispitivanih jedinjenja u odnosu na *F. excelsior*, izuzev proantocijanidina koji su veći u kori *F. excelsior.*

Antioksidacijski kapaciteti za *F. excelsior* kretali su se u području: 16.49 – 18.84 i mg AAE g^{-1} s.u (za list) i 16.65 – 23.99 mg AAE g^{-1} s.u. (za koru) dok su vrijednosti antioksidacijskog kapaciteta bili u području 29.30 – 42.18 mg AAE g^{-1} s.u za listove *F. angustifolia* i za koru 28.64 – 41.99 mg AAE g^{-1} s.u.

Vrijednosti antioksidacijskog kapaciteta (FRAP) iznosili su za listove i koru *F. excelsior* 781.10 – 874 µmolFe(II) g^{-1} s.u i 713.94 – 1087.56 µmolFe(II) g^{-1} s.u . Za list i koru *F. angustifolia* vrijednosti FRAPA su bile u granicama 1101- 1870 µmolFe(II) g^{-1} s.u i 1255.27 – 1743 µmolFe(II) g^{-1} s.u.

Na osnovu dobivenih podataka za prosječne vrijednosti antioksidacijskog kapaciteta, može se zaključiti da listovi i kora *F. angustifolia* imaju bolja antioksidacijska svojstva.

Linearnom regresijom između sadržaja aktivnih komponenti i antioksidacijskog kapaciteta određeni su koeficijenti korelacije (r^2). Utvrđeno je postojanje visoke korelacije između ukupnih fenola, fenolnih kiselina i kumarina i antioksidacijskog kapaciteta za list i koru.

Na osnovu dobivenih rezultata može se zaključiti da list i kora grana običnog i poljskog jasena predstavljaju značajan potencijalni izvor prirodnih supstanci antioksidativnog karaktera.

Corresponding author:Azra Tahirović; Faculty of Forestry University of Sarajevo, Zagrebačka 20, 71000 Sarajevo, Bosnia&Herzegovina; e-mail:. a.tahirovic@sfsa.unsa.ba