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Chemical Composition and Antioxidant Activity of *Fraxinus ornus* L. and *Fraxinus excelsior* L.

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Abstract

In this work, the total content of phenolics, flavonoids, and phenolic acids in the leaves and bark extracts of *Fraxinus ornus* L. and *Fraxinus excelsior* L. was determined. In addition, the identification and quantification of phenolic acids was performed using the HPLC-DAD technique. Samples were tested for antioxidant activity using the ABTS and DPPH methods. Eight extract samples were prepared by Soxhlet and ultrasound extraction using 70 % ethanol as solvent. The content of phenolic compounds ranged from 7.59 for *F. ornus* to 88.93 mg GAE/g for *F. excelsior* in the bark extracts obtained by Soxhlet extraction. The highest content of total flavonoids in *F. ornus* and *F. excelsior* was found in the leaves extracts obtained by ultrasound extraction, which was 5.68 and 1.74 mg GAE/g extract, respectively. The results also showed that the highest content of total phenolic acids was found in *F. ornus* (105.33 mg CAE/g) and *F. excelsior* (97.97 mg CAE/g) in the bark extract obtained by Soxhlet extraction. The highest content of gallic acid (112.96 ± 1.32 mg g^{-1} extracts) and chlorogenic acid (246.94 ± 0.82 mg g^{-1} extracts) was found in the *F. ornus* bark extract obtained by ultrasound extraction. As for antioxidant activity, the bark extract obtained by Soxhlet extraction of *F. ornus* showed the best antioxidant activity by ABTS method with IC_{50} value of 0.062 mg ml^{-1}. The results of the DPPH method show that the leaves extract obtained by Soxhlet extraction had the best antioxidant activity for the *F. ornus* sample, with an IC_{50} value of 0.23 mg ml^{-1}.

Keywords

Fraxinus ornus, Fraxinus excelsior, phenolic compounds, flavonoids, phenolic acids, ABTS, DPPH

1 Introduction

Fraxinus, commonly called ash, is a genus of flowering plants in the olive and lilac family Oleaceae. It contains 45–65 species of usually medium to large trees that are mostly deciduous, though a number of subtropical species are evergreen. The genus is widespread in most of Europe, Asia, and North America.[1] It is a valuable broadleaved tree due to its ecological characteristics, excellent wood properties, and high economic value.[2] Chemical analysis of *Fraxinus* species revealed the presence of many compounds that mainly belong to the groups of hydroxycoumarins, secoiridoid glucosides, phenylethanoids, and flavonoids, along with carbohydrate and trace elements.[3]

The determination of chemical compounds in *Fraxinus* species is related to various extracts of different parts of the plant such as leaves, bark or seeds using different extraction methods and solvents.

Chemical analysis of the leaves and bark extract of *Fraxinus ornus* showed the presence of coumarins such as esculin, esculetin, esculetin 7-O glucoside, fraxin, and fraxetin.[4,5] *Tonguç*[6] also studied the chemical composition of *F. ornus* seeds and found that the extract contained phenols, sugars, flavonoids, carotene, and xanthophyll. Chemical composition of *Fraxinus excelsior* leaves and stems extract showed the presence of phenolic acids, flavonoids and polyphenols.[7] Salidroside, nuzhenide, excelside A, excelside B, oleoside dimethyl ester, and coumarins were found in the extracts of the *F. excelsior* seeds.[8–10]

Fraxinus extracts have been found to possess a variety of biological activities.[11–15] The bark of *F. ornus* possesses anti-inflammatory, antiprotozoal, dermatological, antioxidant, and many other effects.[16] It was used in traditional medicine for wound healing and treatment of inflammation, arthritis, and dysentery. The bark has also been used as an antimicrobial, antiparasitic, and insect repellent.[9,17] The extract from *F. excelsior* leaves from showed various biological activities such as antioxidant[18–20] and anti-inflammatory,[21,22] while the extract from the seeds had diuretic and antihypertensive effects.[23]

The aim of this work was to determine the content of total phenols, flavonoids, and phenolic acids, identification and quantification of selected phenolic acids, as well as the antioxidant activity of the *F. ornus* and *F. excelsior* extracts.

Experimental

2.1 Chemicals and reagents

All used reagents, solvents, and standards were of the highest purity grade, and were purchased from Sigma-Aldrich Co (Germany). The content of total phenols, total flavonoids, total phenolic acids, and antioxidant activity of the extracts were determined using UV/Vis spectrophotometer (Perkin-Elmer Lambda 25). Identification and quantification of phenolic acids were performed using an HPLC sys-

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tem (Agilent Technologies 1290 Infinity) with DAD detector (G4212).

2.2 Plant material and isolation of extracts

Plant material (leaves and bark) of *F. ornus* and *F. excelsior* was collected in the Sarajevo region of Bosnia and Herzegovina, at two different localities, altitude 550 m, in June after blooming. The plant material was air dried at ambient temperature in a ventilated room to a constant weight. After drying, the samples were stored in paper bags in a dry place until use. The identification of the plant material was confirmed by the plant taxonomist, Bašić Neđad, and the specimens were kept in the herbarium of the Department of Forest Ecology at the Faculty of Forestry, University of Sarajevo.

Isolation of secondary metabolites from the leaves and barks of *F. ornus* and *F. excelsior* was performed by two methods. All samples were subjected to Soxhlet (S) and ultrasound (US) extraction using ethanol (70 %) as solvent. Mass of samples was similar for both methods of extraction, and results for yield of extract were expressed as %.

After extraction, the solvent was evaporated using a rotary vacuum evaporator, and dry extracts were stored in a freezer until use.

2.3 Total phenolic compounds

The total phenolic compound content (TPC) of the extracts was determined by the slightly modified Folin-Ciocalteu method.[24] A certain volume of the standard/extract solution was added to a measuring vessel of 10 ml, after which 5 mL of distilled water and 500 µl of Folin-Ciocalteu reagents were added. Previously, the Folin-Ciocalteu reagent was diluted 1 : 2 with water. After 3 min, 1.5 ml of 20 % sodium carbonate was added, and the solution was diluted to 10 ml and left at room temperature for 45 min. The absorbance was measured at 725 nm. Ethanol was used as a blank. Gallic acid was used as a standard to obtain a calibration curve. The concentration of phenolic compounds was measured in triplicate.

All results were expressed as mean ± standard deviation of three independent measurements. The total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of extracts (mg g^{-1}).

2.4 Total flavonoids

The total flavonoid content (TFC) was determined by a modified method using $AlCl_3$.[25] Reaction mixtures were prepared by mixing 0.5 ml of extract solution and 0.5 ml of 2 % aluminium chloride. After incubation at room temperature for 10 min, the absorbance was measured at 415 nm.

A blank sample contained 0.5 ml of $AlCl_3$ and 0.5 ml of ethanol. The total flavonoid content in the extracts was calculated using the regression equation of the calibration curve of quercetin (3–25 µg ml^{-1}), and the results were expressed as mg quercetin equivalents per gram of extract (mg QE/g).

2.5 Total phenolic acids

The quantification of total phenolic acids content (TPAC) was determined using the Arnow method.[26] The method is based on the reaction with Arnow reagent (sodium molybdate and sodium nitrite), forming a pink complex. Samples for the determination of total phenolic acids were prepared by mixing 200 µl of extract solution, 200 µl of 0.5 M HCl, 200 µl of Arnow reagent, 200 µl of 1 M NaOH, and 1.2 ml of distilled water. After 10 min, the absorbance of the formed pink coloured complex was measured spectrophotometrically at 495 nm. A calibration curve was prepared using caffeic acid standard. The results are expressed as milligrams of caffeic acid equivalents per gram of plant extract (mg CAE/g).

2.6 ABTS assay

The free radical scavenging capacity of plant extracts to scavenge free radicals was determined using the ABTS radical cation decolourization assay.[27] The ABTS radical cation ($ABTS^{+}$•) was prepared by the reaction between 7 mM ABTS solution in water and 2.45 mM potassium persulfate. The mixture was allowed to stand in the dark at room temperature for 12–16 h before use. To determine the antioxidant activity of the extracts, the $ABTS^{+}$• solution was diluted to an absorbance of 0.70–0.90 at 734 nm. Solutions of all extracts and standards were prepared in ethanol. After addition of 0.1 ml of extract solution to 1.0 ml of diluted $ABTS^{+}$• solution, absorbance was measured 10 min after initial mixing at room temperature. All determinations were performed in triplicate, for each concentration of standard and samples. Percent inhibition was calculated and plotted as a function of concentration of standards and samples. Results are expressed as IC_{50} value (the mass concentration in mg ml^{-1} of plant extract required to reduce the ABTS radical signal by 50 %). Gallic acid, caffeic acid, chlorogenic acid, and quercetin were used as standards.

The percentage of antioxidant activity (%*AA*) was calculated according to Eq. (1), where A_0 represents absorbance of ABTS before adding a sample and A_t absorbance of ABTS with antioxidant after 10 min.

$$\%AA = (A_0 - A_t)/A_0 \cdot 100 \quad (1)$$

2.7 DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extract was determined based on the scavenging activity of the stable free radical DPPH.[28] The DPPH solution in ethanol was freshly prepared, and 1 mL of this solution was mixed with 100 µl ethanolic solutions of plant extracts. The mixture was allowed to stand in the dark at room temperature for 30 min, and then the de-

crease in absorbance at 517 nm was measured. Ethanol was used as a blank. Gallic acid, caffeic acid, chlorogenic acid, and quercetin served as positive controls. Solutions of all extracts and standards were prepared in ethanol. All measurements were performed in triplicate. The percentage of antioxidant activity for the DPPH radical scavenging by the extract components was calculated according to Eq. (1).

The sample concentration causing 50 % inhibition (IC_{50}) was calculated from the plot of percent inhibition versus extract concentration. The results were expressed in mg ml^{-1} of plant extract required to reduce the DPPH radical signal by 50 %.

2.8 HPLC-DAD analysis

Qualitative and quantitative analysis of selected phenolic acids was performed using HPLC system with diode array detector.

A mixture of methanol, HPLC water, acetonitrile, and glacial acetic acid in a volume ratio of 1 : 4 : 1; 0.004 was used as the mobile phase using modified method.[29] The flow rate was 0.4 ml min^{-1}, and the injected volume was 5 µl. The temperature at which the separation was performed was 25 °C. All extracts and standards were dissolved in ethanol and diluted to appropriate concentration. The compounds detected in the samples were identified by comparing the retention times and UV spectra of the compounds in the samples with the retention times and UV spectra of the standards. The samples were also spiked with standards. Quantitative determination was done by comparing peak area of standard and corresponding identified compound.

3 Results and discussion

3.1 Extraction

The highest yield of extracts was obtained from the leaves extract of *F. ornus* (35.53 %) by Soxhlet extraction, while the lowest yield was observed from the extract of *F. excelsior*, (6.40 %), obtained by ultrasound extraction. Soxhlet extraction proved to be a more efficient extraction method compared to ultrasound extraction. The yield of leaves extracts obtained by Soxhlet extraction of *F. ornus* (35.53 %) and *F. excelsior* (24.21 %) were higher than the yield of their bark extracts, 15.75 % and 17.87 %, respectively. The yield of leaves extracts of *F. ornus* and *F. excelsior* were higher than the yield of bark extracts.

3.2 Total phenolic compounds

Table 1 shows that both the lowest and highest TPC were found in the bark extracts obtained by Soxhlet extraction, and ranged from 7.59 for *F. ornus* to 88.93 mg GAE/g_{DM} (DM = dry extract) for *F. excelsior*.

Table 1 – Total phenolic compounds, total flavonoids, and total phenolic acids content in *Fraxinus* extracts

Sample		TPC / mg GAE/g_{DM}	TFC / mg QE/g_{DM}	TPCA / mg CAE/g_{DM}
F. ornus	Leaves-S	**48.84 ± 0.54**	4.31 ± 0.22	50.32 ± 3.17
	Bark-S	7.59 ± 0.08	0.14 ± 0.02	**105.33 ± 3.32**
	Leaves-US	45.50 ± 0.34	**5.68 ± 0.15**	37.91 ± 0.88
	Bark-US	38.98 ± 0.88	0.64 ± 0.01	56.52 ± 4.74
F. excelsior	Leaves-S	39.34 ± 0.49	1.56 ± 0.22	96.01 ± 2.16
	Bark-S	**88.93 ± 6.41**	0.48 ± 0.06	**97.97 ± 3.63**
	Leaves-US	26.90 ± 0.74	**1.74 ± 0.11**	70.45 ± 2.99
	Bark-US	29.19 ± 1.53	not detected	73.18 ± 6.87

Soxhlet extraction proved to be a more efficient method for the isolation of phenolic compounds in leaves and bark extracts, except for the bark extract of *F. ornus* in which TPC was 5 times lower compared to results of the extract obtained by ultrasound extraction. The highest content of total phenolic compounds for *F. ornus* (48.84 mg GAE/g dry extract) was detected in the leaves extract obtained by Soxhlet extraction, while that of *F. excelsior* (88.93 mg GAE/g extract) was in the bark extract obtained by the same type of extraction.

The results showed that TFC for both *Fraxinus* species, *F. ornus* and *F. excelsior*, was found in the leaves extracts obtained by ultrasound extraction, and were 5.68 and 1.74 mg GAE/g extract, respectively. In both bark extract samples, the content of total flavonoids was lower compared to the leaves extract, while in the bark extract of *F. excelsior* was not detected at all in the bark sample obtained by ultrasonic extraction.

The results also showed that the highest TPAC was detected for *F. ornus* (105.33 mg CAE/g dry extract) and *F. excelsior* (97.97 mg CAE/g dry extract) in the bark extract obtained by Soxhlet extraction. The lowest content of these compounds was detected in the extracts obtained by ultrasound extraction. Both extracts of *F. excelsior*, leaves and bark, obtained by Soxhlet extraction had similar content of total phenolic acids, while both extracts obtained by ultrasonic extraction had a lower and very similar value for the content of total phenolic acids.

Extraction had a significant influence on the content of phenolic acids in the studied sample. For both biological species, the content of total phenolic acids in the extracts obtained by Soxhlet extraction was higher than in those obtained by ultrasound extraction.

Tahirovic et al.[30,31] studied the total phenolic compounds of various *Fraxinus* species, including *F. ornus* and *F. excelsior* from Bosnia, as well as antioxidant activity. In the studies of *Tahirović et al.*[31] for *F. ornus*, it was confirmed that the leaves extracts had a higher content of total flavonoids, while the content of total phenolic acids was higher in the bark extracts. This is in agreement with our studies.

The content of total phenolic compounds was higher in the bark extract, in contrast to our studies. Referring to the research results of *Tahirovic et al.*[30] for *F. excelsior*, higher content of total phenols and flavonoids was found in leaves extracts, while the content of total phenolic acids was higher in bark extracts. Comparing the research results with those presented here, only the composition of total phenols differs, because in our study, the phenolic content in bark extracts is higher than in leaves extracts for both extraction methods.

Vicaş et al.[7] also investigated chemical composition and antioxidant activity of *F. excelsior* extracts. Total phenolic content in aqueous extract of the leaves and stem was higher than in ethanol extracts, while the content of total phenolic acids was five times higher than total flavonoids in the mixture of leaves and stems. Also, *Aydoğan*[32] found that the content of total phenolic compounds in the *F. excelsior* leaves extract obtained by ultrasound-assisted extraction using methanol/water was higher than in the extract obtained by infusion extraction with water. The results cannot be compared with our research due to different extraction methods, solvents, as well as the way of expressing the results.

3.3 HPLC analysis

The extracts were subjected to HPLC-DAD analysis. An analysis of phenolic acids, namely gallic acid, chlorogenic acid, and caffeic acid was performed. Caffeic acid was not detected in any extract of *F. ornus* and *F. excelsior.*

Table 2 shows that the highest content of gallic acid (112.96 ± 1.32 mg g^{-1} dry extract) and chlorogenic acid (246.94 ± 0.82 mg g^{-1} extract) was found in the *F. ornus* bark extract obtained by ultrasound extraction.

Table 2 – HPLC analysis of gallic and chlorogenic acid content in *Fraxinus* extracts

Sample		Gallic acid / mg/g_{DM} extract	Chlorogenic acid / mg/g_{DM} extract
F. ornus	Leaves-S	28.30 ± 0.35	26.31 ± 0.49
	Bark-S	83.25 ± 2.38	135.32 ± 1.31
	Leaves-US	28.13 ± 0.07	22.53 ± 0.06
	Bark-US	**112.96 ± 1.32**	**246.94 ± 0.82**
F. excelsior	Leaves-S	10.12 ± 0.61	not detected
	Bark-S	**28.04 ± 0.92**	**32.26 ± 1.00**
	Leaves-US	8.01 ± 0.16	not detected
	Bark-US	19.36 ± 0.08	19.25 ± 0.21

The lowest content of both identified acids for *F. ornus* extracts was in the leaves extract obtained by ultrasound extraction. It can also be noted that the values of the content of these acids in the leaves extract obtained by ultrasonic extraction were close to the values of the leaves extracts obtained by Soxhlet extraction. In general, the content of these acids in the studied *F. ornus* extracts was higher in the bark extracts than in the leaves extracts.

Table 2 also shows that the content of gallic acid (28.04 ± 0.92) and chlorogenic acid (32.26 ± 1.00 mg g^{-1} dry extracts) was highest in the bark extract of *F. excelsior* obtained by Soxhlet extraction. It is interesting to note that no chlorogenic acid was detected in the leaves extract of *F. excelsior* obtained by both types of extraction. According to the results, all the extracts of *F. ornus* were much richer in the content of chlorogenic acid than those of *F. excelsior*.

Vicas et al.[7] investigated the presence of some organic acids in leaves and stem extracts of *F. excelsior* using the HPLC method. Gallic acid was not detected in the mentioned extracts, while chlorogenic acid was detected in the leaf extracts. *Kostova*[17] and *Kostova and Iossifova*[11] also reported that caffeic acid, gallic acid, and *p*-coumaric acid were detected in the extract of *F. ornus*. *Aydoğan et al.*[32] investigated the influence of the infusion extraction method using only water as the extracting agent and the ultrasound-assisted extraction technique on the chemical composition and the extracts. The results showed that the extract from *F. excelsior* leaves obtained by the ultrasound-assisted extraction did not contain gallic acid, and contained more than ten times chlorogenic acid than the extract obtained by the infusion method. Low content of gallic acid was found in this extract. The results of the phenolic acid content of the other mentioned authors cannot be compared with our results due to the difference in presentation of the results.

3.4 Antioxidant activity

ABTS and DPPH methods were used to test the antioxidant activity of the isolated extracts. To compare the antioxidant activity, the following standards were used for both methods: gallic acid, chlorogenic acid, caffeic acid, and quercetin (Table 3). The results of antioxidant activity for the standards show that gallic acid exhibited the best antioxidant activity by both methods, ABTS and DPPH.

Table 3 – Antioxidant activity of *Fraxinus* extracts

Sample		ABTS IC_{50} / mg ml^{-1}	DPPH IC_{50} / mg ml^{-1}
F. ornus	Leaves-S	0.088 ± 0.006	**0.231 ± 0.005**
	Bark-S	**0.062 ± 0.002**	0.490 ± 0.010
	Leaves-US	0.079 ± 0.003	0.279 ± 0.004
	Bark-US	0.352 ± 0.022	0.610 ± 0.030
F. excelsior	Leaves-S	0.120 ± 0.003	**0.300 ± 0.010**
	Bark-S	**0.096 ± 0.002**	0.460 ± 0.010
	Leaves-US	**0.096 ± 0.007**	0.470 ± 0.010
	Bark-US	0.123 ± 0.004	0.450 ± 0.010
Standards	Gallic acid	**0.0047 ± 0.0001**	**0.0123 ± 0.0005**
	Caffeic acid	0.0248 ± 0.0007	0.0223 ± 0.0007
	Chlorogenic acid	0.0381 ± 0.0006	0.0514 ± 0.0005
	Quercetine	0.0146 ± 0.0006	0.0209 ± 0.0003

As for antioxidant activity, the bark extract obtained by Soxhlet extraction from of *F. ornus* showed the best antioxidant activity by the ABTS method (IC_{50} 0.062 mg ml^{-1}). This extract has TPCA as well as a high gallic acid content.

The results of the DPPH method for both *Fraxinus* species show that the leaf extract obtained by Soxhlet extraction had the best antioxidant activity for the *F. ornus* sample, with an IC_{50} value of 0.23 mg ml^{-1}. Comparing the results of antioxidant activity of *F. excelsior* extracts, the best antioxidant activity was obtained for the same type of extract with an IC_{50} value of 0.30 mg ml^{-1}.

Considering all the obtained results, it can be concluded that the bark extracts obtained by Soxhlet extraction for both samples had a high content of total phenolic acids, as well as the best antioxidant activity according to the ABTS method. On the other hand, *F. ornus* leaf extracts contained a high content of total phenolic compounds and showed the highest antioxidant activity according to the DPPH method.

Phenolic compounds are known to influence antioxidant activity. The composition of extracts is very heterogeneous, so there is a possibility of synergistic or antagonistic action of individual components.

In general, the IC_{50} values of the ABTS method are lower than are those of the DPPH method, although they are based on the same reaction mechanism. One of the possible reasons for this is a decrease in the stability of DPPH radicals due to steric effects.[33] In addition, *Brand-Williams et al*.[28] confirmed that the interaction of a potential antioxidant with DPPH depends on its structural conformation. *Bernatoniene et al*.[34] pointed out that different substances in the extracts might be involved in the inactivation of free radicals in the DPPH and ABTS assays, resulting in different antioxidant activity of the extracts.

Tahirovic et al. also investigated the antioxidant activity of extracts from *F. excelsior* and *F. ornus* using DPPH and FRAP methods.[30,31] The results showed that *F. ornus* leaf extract showed better antioxidant activity by both methods, while the *F. excelsior* bark extract showed better antioxidant activity.

4 Conclusion

The results show that the examined samples have high contents of total phenolic compounds and total phenolic acids. The extracts showed good antioxidant activity. The results also show that there is no direct relationship between the content of total phenolic compounds, total flavonoids with the type of plant material for extraction, as well as the type of extraction. It is planned to perform a more detailed chemical analysis of the composition of the tested extracts in order to determine which compounds contribute to antioxidant activity and other biological activities, such as coumarins.

List of abbreviations and symbols

TPC – total phenolic compound content
TFC – total flavonoids content
TPAC – total phenolic acids content
HPLC-DAD – High-performance liquid chromatography with photodiode-array detection
ABTS – 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
DPPH – 2,2-diphenyl-1-picrylhydrazyl
IC_{50} – sample concentration required to inhibit 50 % of radicals
CAE – caffeic acid equivalents
GAE – gallic acid equivalents FRAP-Ferric Reducing Antioxidant Power

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SAŽETAK

Kemijski sastav i antioksidativna aktivnost *Fraxinus ornus* L. i *Fraxinus excelsior* L.

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U ovom radu određen je ukupni sadržaj fenola, flavonoida i fenolnih kiselina u ekstraktima lišća i kore *Fraxinus ornus* L. i *Fraxinus excelsior* L. Dodatno, identifikacija i kvantifikacija fenolnih kiselina provedena je pomoću tehnike HPLC-DAD. Uzorci su ispitani na antioksidativno djelovanje metodama ABTS i DPPH. Osam uzoraka ekstrakta, pripremljeno je Soxhlet i ultrazvučnom ekstrakcijom uporabom 70 % etanola kao otapala. Sadržaj fenolnih spojeva kretao se od 7,59 za *F. ornus* do 88,93 mg GAE/g za *F. excelsior* u ekstraktima kore dobivenim Soxhlet ekstrakcijom. Najveći sadržaj ukupnih flavonoida u uzorcima *F. ornus* i *F. excelsior* određen je u ekstraktima listova dobivenih ultrazvučnom ekstrakcijom i iznosio je 5,68 odnosno 1,74 mg GAE/g ekstrakta. Rezultati su također pokazali da je najveći sadržaj ukupnih fenolnih kiselina utvrđen u *F. ornus* (105,33 mg CAE/g) i *F. excelsior* (97,97 mg CAE/g) u ekstraktu kore dobivenom Soxhlet ekstrakcijom. Najveći sadržaj galne kiseline (112,96 ± 1,32 mg g^{-1} ekstrakata) i klorogenske kiseline (246,94 ± 0,82 mg g^{-1} ekstrakata) detektiran je u ekstraktu kore *F. ornus* dobivenom ultrazvučnom ekstrakcijom. Što se tiče antioksidativnog djelovanja, ekstrakt kore uzorka *F. ornus* dobiven Soxhlet ekstrakcijom pokazao je najbolje antioksidativno djelovanje metodom ABTS s IC_{50} vrijednošću od 0,062 mg ml^{-1}. Rezultati metode DPPH pokazali su da je ekstrakt lišća dobiven Soxhlet ekstrakcijom imao najbolje antioksidativno djelovanje za uzorak *F. ornus* s IC_{50} vrijednošću od 0,23 mg ml^{-1}.

Ključne riječi
Fraxinus ornus, Fraxinus excelsior, fenolni spojevi, flavonoidi, fenolne kiseline, ABTS, DPPH

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