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Phytochemical screening, quantitative determination of phenolic compounds, and antioxidative activity of *Ostrya carpinifolia*

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Abstract: *Ostrya carpinifolia* is an interesting and suitable species for reforestation and landscaping. For the first time ever, phenolic profile, antioxidative and antimicrobial activity of *O. carpinifolia* was done in this study. Aqueous and methanol extracts of the aerial parts were analyzed using either fast screening methods of secondary metabolites, and UV/VIS spectrophotometry for determination of polyphenolic contents and antioxidant activity (DPPH). Antimicrobial activity of methanol extracts was investigated using the disc diffusion method against a selected nine microorganisms. Phytochemical tests confirm the presence of cardiac glycosides, coumarins, emodins, flavonoids, tannins, terpenes, terpenoids and steroids, while anthocyanins, fatty acids and saponins were absent in all aqueous extracts. Leucoanthocyanins were observed only in the stem extract. Methanol extracts of leaves contain the highest level of total phenolics and flavonoids (35.574 and 30.908 mg CE g^{-1} DW, respectively), while the inflorescences extracts were the richest with total proanthocyanidins and phenolic acids (19.165 mg CE and 9.342 mg CAE g^{-1} DW, respectively). All methanol extracts showed very strong antioxidative activity, where the lowest activity was recorded for inflorescences (IC_{50}: 0.242 mg mL^{-1}) and the highest for stem (IC_{50}: 0.107 mg mL^{-1}). Analyzed extracts showed no antimicrobial activity against the test organisms. ANOVA indicated the presence of significant differences between the total phenolics and flavonoids and DPPH ($p<0.05$). Duncan's test confirmed the presence of statistically significant and very high positive correlation ($R=0.989$) between total phenolics and phenolic acids contents. Obtained results indicate the necessity of further research of European hop-hornbeam.

INTRODUCTION

Plants have an enormous ability to synthesize different bioactive organic compounds (i.e. secondary metabolites, SMs) that are not directly involved in their growth, development and reproduction but in absence they may be harmful or even fatal for plant's survivability. Secondary metabolites have essential roles in stabilizing cellular structure, plant adjustment and defense reacting under biotic and abiotic stresses. They are included in different physiology processes: pigmentation, pollination, seed germination, signaling, and resistance mechanisms under suboptimal conditions. The main characteristics of SMs are large chemical diversity, complexity and structure heterogeneity, as also the presence of diverse mechanisms and pathways of their biosynthesis (Bennet and Wallsgrove, 1994; Croteau, Kutchan and Lewis, 2000; Boudet, 2007; Edreva, Velikova, Tsonev, *et al*., 2008; Acquaviva, Menichini, Ragusa, *et al*., 2012; Bartwal, Mall, Lohani, *et al*., 2013; Lattanzio, 2013; Murkovic, 2016).

According to available data, more than 200,000 different SMs have been reported, and this number is still continually increasing (Verpoorte, van der Heijden, ten Hoopen, *et al*., 1999; Hadacek, 2002; Edreva *et al*., 2008; Springob and Kutchan, 2009; Bartwal *et al*., 2013). Among them, the most abundant group of SMs with wide array of roles in plants are phenolic compounds including phenolic acids, tannins, lignins and most diverse flavonoids (Bartwal *et al*., 2013) with increasing biological interest of proanthocyanidins as the most widespread bioflavonoids (Škerget, Kotnik,

Hadolin, *et al*., 2005). Namely, in recent years, phenolics and phenolic-related compounds gained huge importance and attention in medicine, pharmacology, food processing and cosmetics industry, due to their antioxidant, antimicrobial and anti-inflammatory properties (Oliviera, Sousa, Morais, *et al*., 2008; Abou-Zeid, Bidak and Gohar, 2014; Ashraf, Sarfraz, Rashid, *et al.*, 2015; Hofmann, Nebehaj and Albert, 2016).

Ostrya carpinifolia Scop. (European hop-hornbeam), the only native species of genus in Europe and endemic to temperate West Eurasia, is a small to medium-sized broadleaved deciduous tree (Pasta, de Rigo and Caudullo, 2016). In terms of forestry, it is very interesting and suitable species for the reforestation of many degraded sites and landscaping (Weber, 2011; Ivetić, Devetaković, Davorija, *et al*., 2015; Pasta *et al*., 2016). Although, to date, chemical composition and biological activities of this species have been scarcely studied (Wollenweber, 1975), according to published data about other *Ostrya* species, *O. carpinifolia* can also be a source of natural phytochemicals with beneficial effects in broad arrays (Matsuki and Koike, 2006; Barbehenn, Weir and Salminen, 2008; Park, Kim, Ko, *et al*., 2010; Kim, Park and Lim, 2010).

Based on the lack of information and keeping the above facts in view, the aims of this study are: 1) qualitative and quantitative analysis of aqueous and methanol extracts of different aerial parts of *Ostrya carpinifolia* and 2) evaluation of antioxidant and antimicrobial activities of methanol extracts of European hop-hornbeam.

MATERIAL AND METHODS

Plant material

Plant material (leaves, stems, inflorescences) was collected from healthy trees of *O. carpinifolia* in its natural population near Sarajevo (43˚51'19'' N, 18˚27'27'' E, 595 m ASL, S-SW), in May 2015. Different plant parts were separated immediately and packed into paper bags. In the laboratory, the plant materials were washed with running tap water, rinsed with distilled water and naturally air dried during 10 days at room temperature in a dry, shaded and well-aerated place. Then, dried materials were grounded into powder using mixer and stored in plastic bags, at room temperature, until extraction processes.

Chemicals and reagents

Folin-Ciocalteu's reagent, catechin, caffeic acid, DPPH (1,1-diphenyl-2-picryl-hydrazyl), sodium carbonate, absolute methanol, and aluminium chloride were purchased from Sigma-Aldrich (Steinheim, Germany), while Amoxicillin, Müeller Hinton and Sabouraud dextrose agars were purchased from HiMedia Chemicals Ltd. (Mumbai, India). All other used chemicals and solvents were of analytical grade.

Tested microorganisms

Antimicrobial activity was tested against a panel of microorganisms: Gram-positive bacteria (*Staphylococcus epidermididis* ATCC 8739TM, *Staphylococcus aureus* subsp. *aureus* ATCC 6538 TM, *Bacillus subtilis* subsp. *spizizenii* ATCC 6633™, *Enterococcus faecalis* ATCC 19433, and *Bacillus vulgatus* ATCC 8482), Gram-negative bacteria (*Salmonella abony* NCTC 6017TM, *Escherichia coli* ATCC 8739TM, and *Pseudomonas aeruginosa* ATCC 9027) and fungus *Candida albicans* (ATCC 10231). The bacterial strains were cultured in Müeller Hinton agar while fungus was cultured on Sabouraud dextrose agar.

Aqueous extracts preparation

Per 5 g of ground plant materials were placed in to the glass beakers and 50 mL of distilled water was added in each. After 30 min of incubation at 55°C in water bath the extracts were cooled, centrifuged for 20 min at 1800 rpm (Centric 322 B, Techtnica), and filtered through Whatman No.1 filter papers.

Preliminary phytochemicals screening

Preliminary fast phytochemical standard tests for the screening and qualitative identification of bioactive chemical constituents of plant aqueous extracts of *O. carpinifolia* were carried out by modified methods of Trease and Evans (2002), Savithramma, Linga Rao and Suhrulatha (2011), and Subhashini Devi, Satyanarayana and Tarakeswara Naidu (2014). Leaves, stem and inflorescences extracts were screened for 12 phytochemical constituents: anthocyanins, cardiac glycosides, coumarins, emodins, fatty acids, flavonoids, leucoanthocyanins, saponins, steroids, tannins, terpenes, and terpenoids.

Methanol extracts preparation

Grinded plant materials (0.5 g) were extracted with 25 mL of 80% methanol. The extraction process was carried out in two replicates (twice with 12 mL and adjusted to 25 mL) using both times an ultrasonic bath (Elma sonic S 60 H) for 30 min. Extracts were then centrifuged at 1800 rpm for 10 min. The obtained supernatants were stored in plastic tubes at 5°C for further analysis.

The Shimadzu UV-mini 1240 spectrophotometer was used for determination of polyphenolic contents and antioxidant activity of all methanol extracts. All samples were analyzed in triplicates.

Determination of total phenolic content

Total phenolic content of the methanol extracts was determined using modified Folin-Ciocalteu method (Wolfe, Wu and Liu, 2003). An aliquot of the methanol extract (20μL) was mixed with distilled water followed by addition of 100 μL of Folin-Ciocalteu's reagent and 300 μL of freshly prepared sodium carbonate solution (7.5%). After mixing, the tubes were incubated for 30 min at 45°C in water bath until blue color development. The absorbance of the resulting solvents was measured at 765 nm. Total phenolic content was expressed as mg of catechin equivalents per gram of dry plant sample (mg CE g^{-1}).

Determination of total flavonoids

Total flavonoid content was determined by the modified method of Ordoñez, Gomez, Vattuone, *et al*. (2006). The methanol extract aliquots of both leaves (20 μL) and stem and inflorescences (60 μL) were mixed with 25 μL

of 10% aluminum chloride water solution followed by the addition of 25 μL of 1M sodium acetate water solution. The mixture was left for homogenization and incubation at room temperature (24°C) for 20 min. The absorbance was measured at 415 nm. Total flavonoid content was expressed as mg of catechin equivalent per gram of dry sample (mg CE g^{-1}).

Determination of total proanthocyanidins

Total proanthocyanidins content was measured by modified method of Wettstein, Jende-Strid, Ahrenst-Larsen, *et al*. (1977). An aliquot of methanol extract (50 μL) was mixed with 750 μL of 4% vanillin-methanol solution and 375 μL of hydrochloric acid. The tubes were vortexed and the absorbance was immediately measured at 500 nm. Total proanthocyanidins content was expressed as mg of catechin equivalent per gram of dry weight (mg CE g^{-1}).

Determination of total phenolic acids

Quantification of total phenolic acids was carried out using slightly modified Arnow method (Haydrich and Goślińska, 2004). Namely, all the analyzed samples are diluted with methanol (1:1, v/v) because of reading of phenolic acids excessive values. The tubes were vividly shaken and after 20 min of incubation at room temperature, absorbance was measured at 490 nm. The results were expressed as caffeic acid equivalents per gram of dry sample (mg CAE g^{-1}).

DPPH Radical Scavenging Activity Assay

The antioxidant activity of the methanol extracts was assessed by DPPH free radical scavenging modified method by Meda, Lamien, Romito, *et al*. (2005). Aliquots of diluted methanol extracts (100, 80, 60, 40, and 20 μL) were mixed with freshly prepared ethanol DPPH solution and incubated for 30 min at room temperature in the dark. The absorbances of the resulting solutions were measured at 515 nm against 96% ethanol as a blank. The DPPH scavenging ability in percentage (AA %) was calculated as follows:

$$AA\% = \frac{(A_a - A_b) \times 100}{A_a}$$

where:

A_a– the absorbance of DPPH blank
A_b– the absorbance of DPPH with sample in different concentrations.

The antioxidant activity was expressed as half maximal inhibition concentration (IC_{50}) defined as the concentration of sample required for inhibition of 50% of initial amount of DPPH radicals. The IC_{50} values were calculated graphically based on the calibration curves for each sample. The lower IC_{50} values indicated the higher antioxidative activity in the methanol extracts.

Estimation of antimicrobial activity

Standard serial dilution assay with disc diffusion method (Bauer, Kirby, Sherris, *et al*., 1966) was used to test the antimicrobial activity of methanol extracts against selected microorganisms. Inoculum was prepared by diluting overnight grown microbial culture with 0.9% NaCl and adjusting the concentration of the medium to match the 0.5% McFarland standard. Then, 1 mL of bacterial suspension was spread on sterile Müeller Hinton agar plate, and Sabouraud dextrosa agar was inoculated with 1 mL of fungal suspension. Sterile discs impregnated with 25 μL of methanol extracts were placed on the inoculated agars. Standard disc of Amoxicillin (Amx10mcg) was used as control in bacterial plates, and 80% methanol was used as blank in bacterial and fungal plates. The inoculated plates with discs were incubated at 37°C for 24 hours to allow maximum growth of the organisms.

Statistical Analysis

Data was analyzed by Statistica 7 for Windows using a one-way analysis of variance (ANOVA). To determine the significant differences between groups, after analysis of variance, Duncan's *post-hoc* test was used at $p<0.05$.

RESULTS AND DISCUSSION

According to Boudet (2007) and Bartwal, *et al*. (2013), variations among plant species functionalities are result of their chemical features and content, where the key place takes the polyphenolic compounds, the most abundant and heterogeneous group of secondary metabolites. Besides important roles in the plant kingdom (color, taste, technical properties, mechanical support, protection, pollination, etc.) these compounds are primarily responsible for plant utilization in medicine, pharmacology, agriculture, cosmetic, aromatherapy and food industry, due to their positive effect on human health. With the respect to significance of natural products and concerns about unsafety and carcinogenic potential of widely used synthetic antioxidants and chemical preservatives, the science efforts are directed towards exploiting new antioxidants and antimicrobial compounds from natural sources and analyzing the phenols potential in these activities.

Scarcity of data on phenolic content, antioxidative and antimicrobial activity of *Ostrya carpinifolia* indicates a lack of exploration of this species. Exceptions are data of the Wollenweber (1975) who reported that *Ostrya* species possess polyphenols with dominant role of flavonoids. Therefore, this paper presents, for the first time, phenolic profile, antioxidative and antimicrobial activity of *Ostrya carpinifolia*.

***Polyphenolic compounds of* Ostrya carpinifolia**

Phenolic compounds are generally present in many plants and can be concentrated in different plant parts. Among phenolic compounds, tannins, coumarins, phenolic acids and flavonoids, stand out with their antioxidant potential. Preliminary phytochemical tests confirm the presence of cardiac glycosides, coumarins, emodins, flavonoids, tannins, terpenes, terpenoids and steroids in all aqueous extracts of *Ostrya carpinifolia* (Table 1).

Table 1: Obtained results of phytochemical analysis of secondary metabolites presence in aqueous extracts of *Ostrya carpinifolia* by rapid screening methods ("–"indicates absence and "+" presence).

Secondary metabolites	Leaf	Stem	Inflorescences
Anthocyanins	–	–	–
Cardiac glycosides	+	+	+
Coumarins	+	+	+
Emodins	+	+	+
Fatty acids	–	–	–
Flavonoids	+	+	+
Leucoanthocyanins	–	+	–
Saponins	–	–	–
Steroids	+	+	+
Tannins	+	+	+
Terpenes	+	+	+
Terpenoids	+	+	+

Leucoanthocyanins were observed in the stem extract but not in the leaves and inflorescence extracts. Anthocyanins, fatty acids and saponins were absent in all studied extracts. Previous chemical investigation of *Ostrya japonica* Sarg. and *O. virginiana* (Mill.) K.Koch indicated the presence of polyphenolic compounds (Matsuki and Koike, 2006; Barbehenn, *et al.*, 2008; Kim, *et al.*, 2010; Park, *et al.*, 2010). In the family Betulaceae, for the genera *Alnus*, *Betula*, *Carpinus* and *Corylus* have been shown to produce wide variety of polyphenolic compounds (Wollenweber, 1975; Matsuki and Koike, 2006; Barbehenn, *et al*., 2008; Kim, *et al*., 2010; Park, *et al*., 2010; Mushkina, Gurina, Konopleva, *et al.*, 2013; Costea, Vlase, Viorel, *et al.*, 2016; Hofmann, *et al.*, 2016; Orodan, Vodnar, Toiu, *et al.*, 2016; Riethmüller, Könczölb, Szakálc, *et al*., 2016).

Although it is known that microsite conditions and the position of the leaves can influence phenolic content and its seasonal changes (Zhang, Gao, Zhang, *et al*., 2010; Vagiri, Conner, Stewart, *et al*., 2015; Hofmann, *et al*., 2016), it is obvious that this family represents a valuable source of polyphenolic compounds and should be more investigated in the future, since our results indicate the presence of antraquinone emodin, coumarins and steroids in *O. carpinifolia*.

From Figure 1 and Table 2 it is visible that leaves, in comparison to other plant parts, possessed very high concentrations of all analyzed phenolic compounds, except phenolic acids. In addition, in all plant parts the total proanthocyanidins had relatively high and more or less uniform values, whereas the total phenolic acids were at least present. The total flavonoids content was the highest in the leaves and the lowest in the stem.

Previous studies of phenolic compounds in Betulaceae members were, mainly, done on different type of leaves extracts. Analyses showed that these species were rich with phenolics, flavonoids and phenolic acids (Wollenweber, 1975; Barbehenn, *et al*., 2008; Park, *et al*., 2010; Mushkina, *et al*., 2013; Costea, *et al*., 2016; Hofmann, *et al*., 2016; Orodan, *et al*., 2016; Riethmüller, *et al*., 2016). This data is in accordance with our results, except for phenolic acids which were present in the lower values in *O. carpinifolia* (Table 2). The presence of total proanthocyanidins in family Betulaceae is, for the first time, determined in this study. Namely, from obtained results is visible that all aerial parts of *O. carpinifolia* are relatively rich with proanthocyanidins.

It is known, among other things, that phenolic compounds are generally included in plant defense against pathogens and predators, in processes of pigmentation, UV protection and etc., and mostly included in plant-microbe interactions and initiation of mycorrhizal symbioses as signaling molecules (Winkel-Shirley, 2001; Crozier, Clifford and Ashihara, 2006; Mandal, Chakraborty and Dey, 2010; Khodammi, Wilkes and Roberts, 2013). Accordingly, it is possible to assume that the presence of relative high concentrations of phenolics, flavonoids and proanthocyanidins enable this species to survive on thermophilic habitats and to be used as a pioneer species in the reforestation and landscaping.

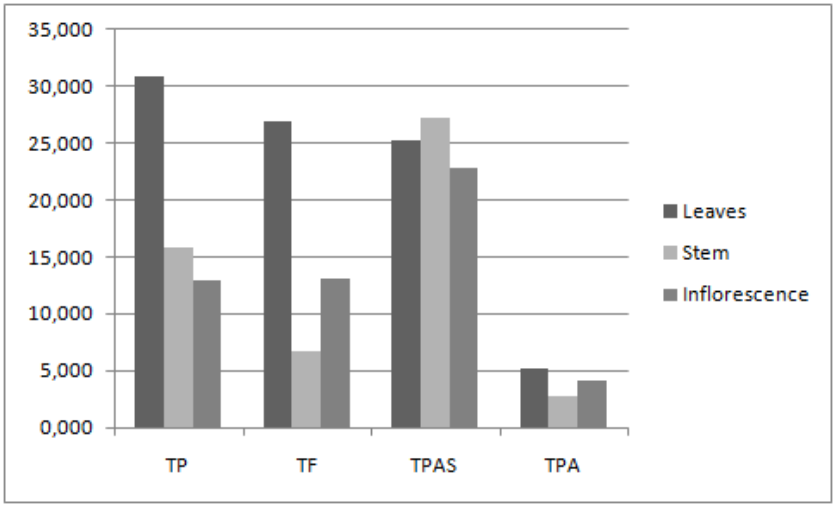

Figure 1: Representations of mean values of the analyzed polyphenolic compounds in different parts of *Ostrya carpinifolia.* TP – total phenolics, TF – total flavonoids, TPAS – total proanthocyanidins (all expressed in mg CE g^{-1} DW), TPA – total phenolic acids (mg CAE g^{-1} DW).

Table 2: Basic descriptive parameters of analyzed secondary metabolites and antioxidant activity of *Ostrya carpinifolia* (PP – plant part, *X* – average, SD – standard deviation, CV – coefficient variation, TP – total phenolics, TF – total flavonoids, TPAS – total proanthocyanidins, TPA – total phenolic acids, IC_{50} – half maximal inhibition concentration).

PP	Parameter	TP (mg CE[a] g^{-1})	TF (mg CE g^{-1})	TPAS (mg CE g^{-1})	TPA (mg CAE[b] g^{-1})	IC_{50} (mg mL^{-1})
Leaves	**Min**	27.982	21.975	17.422	3.467	0.164
	Max	35.574	30.908	32.469	6.860	0.213
	X	30.831	26.903	25.313	5.224	0.184
	SD	3.192	3.092	4.744	1.127	0.016
	CV (%)	10.353	11.494	18.743	21.580	8.459
Stem	**Min**	11.223	5.772	17.036	1.368	0.086
	Max	21.953	7.543	35.849	4.337	0.125
	X	15.895	6.628	27.299	2.666	0.107
	SD	4.121	0.753	6.651	1.176	0.013
	CV (%)	25.925	11.358	24.363	44.133	11.860
Inflorescence	**Min**	8.518	9.060	10.032	1.845	0.153
	Max	21.338	17.455	49.165	9.342	0.356
	X	12.908	14.171	22.861	4.050	0.242
	SD	5.739	4.486	18.263	3.574	0.084
	CV (%)	44.462	31.656	79.887	88.250	34.975

[a] – catechin equivalent
[b] – caffeic acid equivalent

Table 3: Duncan test ($p<0.05$) for the analyzed secondary metabolites (TP – total phenolics, TF – total flavonoids, TPAS – total proanthocyanidins, TPA – total phenolic acids) and antioxidant activity (IC_{50} – half maximal inhibition concentration) of methanol extracts of *O. carpinifolia*. Bold text indicates statistically significant correlations.

	TP	TF	TPAS	TPA	IC_{50}
TP	1.00000	-0.468653	0.884935	**0.988785**	-0.626422
TF	-0.468653	1.000000	-0.825824	-0.553269	0.703915
TPAS	0.884935	-0.825824	1.000000	0.924325	-0.781791
TP	**0.988785**	-0.553269	0.924325	1.000000	-0.735095
IC_{50}	-0.626422	0.703915	-0.781791	-0.735095	1.000000

***Antioxidative and antimicrobial activity of* Ostrya carpinifolia**

Antioxidant potential of phenolic compounds, especially flavonoids, is connected to their ability of metal chelating and scavenging harmful free radicals and reactive oxygen species which attack lied to cell damages and various disorders and diseases (Michalak, 2006; Siatka and Kašparová, 2010; Bartwal, *et al.*, 2013; Radić, Vujčić, Glogoški, *et al.*, 2016; Rawat, Jugran, Bahukhandi, *et al*., 2016).

The antioxidant activity of methanol extracts of *O. carpinifolia* was evaluated on the basis of their ability to scavenge free DPPH radicals. This is the first report about antioxidant activity of *O. carpinifolia* while the antioxidant activity of *O. japonica* was previously reported (Kim, *et al*., 2010; Park, *et al*., 2010).

All examined methanol extracts showed very high free radical scavenging activity (Table 2). Very low concentration of stem extracts exhibited the highest (IC_{50}: 0.107 mg mL^{-1}) activity, followed by leaves (IC50: 0.184 mg mL^{-1}), while inflorescences exhibited the smallest antioxidant activity (IC_{50}: 0.242 mg mL^{-1}).

According to available reports about family Betulaceae, strong antioxidant activity was obtained in *Carpinus betulus* L., *Betula megrelica* Sosn., *Corylus colurna* L. and *Alnus sibirica* (Spach) Turcz. ex Kom. (Kim, *et al*., 2010; Zardiashvili, Jokhadze, Kuchukhidze, *et al.* 2014; Hofmann, *et al*., 2016; Riethmüller, *et al*., 2016), and mostly moderate to low activity in *Alnus glutinosa* (L.) Gaertn, *Alnus incana* (L.) Moench, *Betula pendula* Roth, and *Corylus avellana* L. (Acquaviva, *et al*., 2012; Mushkina, *et al*., 2013; Costea, *et al*., 2016; Orodan, *et al*., 2016; Riethmüller, *et al*., 2016).

Very low concentrations of all samples showed strong antioxidant activity, where the lowest activity was recorded for inflorescences (IC_{50}: 0.242 mg mL^{-1}) and the highest was exhibited by stem's extracts (IC_{50}: 0.107 mg mL^{-1}), that had the lowest total flavonoids values in comparison to other samples.

Analysis of variance indicated the presence of significant differences between the DPPH radical scavenging and contents of total phenolics and flavonoids ($p<0.05$). Duncan's test (Table 3) confirmed the presence of statistically significant and very high positive correlation ($R=0.989$) between total phenolics and phenolic acids contents.

Considering no correlation was found between DPPH IC_{50} values and phenolic components it can be presumed that antioxidant activity is not simply related to the total phenolics and flavonoids content and that highest antioxidative activity of stem extracts is not the result of high concentration of any of analyzed phenolic compounds. This can be explained, as we assume, with: strong influence of unanalyzed constituents (e.g. tannins), presence of antioxidants with different chemical structures, and different antagonistic,

synergistic and additive reaction among present components. Similar results were previously registered at *Carpinus betulus* and *Corylus* species (Hofmann, *et al*., 2016; Riethmüller, *et al*., 2016).

Since the methanol extracts of *O. carpinifolia* are potential natural antioxidants, it is necessary to analyze and compare *O. carpinifolia* extracts in different solvents in order to isolate and identify their chemical constituents and potential bioactive compounds.

Although the analyzed phenolic compounds in methanol extracts of *O. carpinifolia* were present in significant amounts and antioxidant activity was strong, no antibacterial and antifungal activities against the selected nine microorganisms were observed.

According to relevant literature, extracts of different parts of some related species to *O. carpinifolia* had shown antimicrobial activity, where Gram-positive bacteria (at the most *Staphylococcus aureus*, *Staphylococcus epidermididis*, *Bacillus subtilis*) were more sensitive than Gram-negative bacteria (Oliviera, *et al*., 2008; Acquaviva, *et al*., 2012; Orodan, *et al*., 2016). Antimicrobial activity of extracts of *Betula pendula* and *Corylus avellana* against *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* was very low or not identified at all (Oliviera, *et al*., 2008; Orodan, *et al*., 2016), which coincides with our results.

Obviously, further investigation of antimicrobial activity of *O. carpinifolia* extracts on unanalyzed bacteria and fungi strains in this study should be considered.

CONCLUSIONS

Ostrya carpinifolia represents a valuable source of polyphenolic compounds and should be more investigated in the future, since our results indicate the presence of nine groups of secondary metabolites. This study, also, showed that the methanol extracts of *O. carpinifolia* are potential natural strong antioxidants, which can be explained with intense influence of unanalyzed compounds, presence of antioxidants with different chemical structures, and different antagonistic and synergistic reactions among present constituents. According to obtained results, it is possible to assume that the presence of relative high concentrations of phenolics, flavonoids and proanthocyanidins and a strong antioxidative activity enable this species to survive on thermophilic habitats and to be used as a pioneer species in the reforestation and landscaping. From all the above it can be suggested the necessity for further research of *Ostrya carpinifolia* extracts in different solvents in order to identify and isolate their chemical constituents and potential bioactive compounds as a potential natural source of antioxidant activities.

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Summary/Sažetak

Ostrya carpinifolia je zanimljiva i pogodna vrsta za pošumljavanje i uređenje okoliša. U ovoj studiji je, po prvi put, određen fenolni profil te antioksidativna i antimikrobna aktivnost *O. carpinifolia*. Vodeni i metanolni ekstrakti nadzemnih dijelova crnog graba analizirani su pomoću kvalitativnih metoda za brzo ispitivanje sekundarnih metabolita te UV/VIS spektrofotometrijom za određivanje polifenolnog sadržaja i antioksidativne aktivnosti (DPPH). Antimikrobna aktivnost metanolnih ekstrakata je analizirana disk difuzijskom metodom protiv devet odabranih mikroorganizama. Fitohemijski testovi su potvrdili prisutnost emodina, flavonoida, kardijačnih glikozida, kumarina, tanina, terpena, terpenoida i steroida. Prisustvo antocijanina, masnih kiselina i saponina nije uočeno niti u jednom vodenom ekstraktu, dok su leukoantocijanini uočeni samo u ekstraktu stabla. Metanolni ekstrakti listova su imali najveću količinu ukupnih fenola i flavonoida (35,574 i 30,908 mg CE g^{-1} SM), dok su ekstrakti cvjetova bili najbogatiji s ukupnim proantocijanidinima i fenolnim kiselinama (19,165 mg CE i 9,342 mg CAE g^{-1} SM). Svi metanolni ekstrakti pokazali su vrlo jaku antioksidativnu aktivnost, pri čemu je najniža aktivnost zabilježena za cvjetove (IC_{50}: 0,242 mg mL^{-1}) a najviša za stabljiku (IC_{50}: 0,107 mg mL^{-1}). Analizirani metanolni ekstrakti nisu pokazali antimikrobnu aktivnost protiv testnih mikroorganizama. ANOVA je pokazala prisutnost značajnih razlika između ukupnih fenola i flavonoida i DPPH ($p < 0,05$). Duncanov test potvrdio je prisutnost statistički značajne i vrlo visoke pozitivne korelacije (R=0,989) između ukupnih sadržaja fenola i fenolnih kiselina. Dobiveni rezultati ukazuju na potrebu daljnjeg istraživanja *Ostrya carpinifolia*.