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PRELIMINARY PHYTOCHEMICAL SCREENING FOR SECONDARY METABOLITES IN LEAVES AND BARK OF 25 BROADLEAF DECIDUOUS SPECIES

Preliminarna fitohemijska analiza sekundarnih metabolita u listovima i kori 25 lišćarskih vrsta

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Abstract

Plants produce a numerous and diverse secondary metabolites, organic compounds which are not essential and do not participate directly in the growth and development, but may have very important role in their adaptation and adjustment to particular environmental conditions. For humans, secondary metabolites are very important in medicine, pharmacology, food and cosmetics industries. The presence of eight types of secondary metabolites (anthocyanins, coumarins, emodins, fatty acids, phenols, saponins, steroids and tannins) in water extracts of leaves and barks of 25 broadleaf deciduous species from 15 families was qualitatively investigated by rapid phytochemical screening methods. According to literature data, in this study for the first time is determined the presence of six types of secondary metabolites in analyzed dendro species: anthocyanins in one species; both coumarins and phenols in five species; emodins in six species; saponins in eight species; and tannins in four species. Particular attention in further research should be given to Fagus sylvatica L., Populus tremula L., Quercus petraea (Matt.) Liebl., Robinia pseudoacacia L. and Sorbus aria (L.) Crantz. Since preliminary results of this study are promising it would be desirable both to identify active compounds and assess their potential antimicrobial and antioxidant activities.

Key words: anthocyanins, Bosnia and Herzegovina, coumarins, emodins, phenols, saponins, tannins.

INTRODUCTION – Uvod

People constantly have been explored their environment with special emphasis on plants and their properties. This has resulted in the use of a large number of plants, especially in healthcare which medicinal values lay in their phytochemical composition and physiological activities. Namely, plants produce a huge and diverse range of secondary metabolites (SMs), bioactive organic compounds which do not participate directly in their growth and development, but may have very important role in the adaptation and adjustment to particular environmental conditions. Secondary metabolites are

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distributed in a limited number of taxonomic plant groups, belonging to specific groups of chemical compounds and synthesized under specific conditions and, in many cases, with unclear role and contribution to the plant organism. Their concentrations vary between species, individuals and tissues but also from season to season (GOTTLIEB 1990; WINK 1990, 1999; WINK AND WATERMAN 1999; MIKA ET AL. 2005; KOCHE 2010; HARINI ET AL. 2014, TIWARI AND RANA 2015;²).

According to FORESTRY COMMISION (2001), only 25% of felled tree is transformed into timber, and remaining plant material (usually rich with SMs and plant fibres) is relatively unexplored and unexploited. In fact, that large quantity of tree residues can present source for searching, isolation and identification of some new active compounds, and for massive production of some SMs since it is considered that, in the realm of wood processing, everything that is not a structural polysaccharide or lignin is secondary metabolite.

In order to "add values" to some tree species the presence of eight types of SMs in 25 broadleaf deciduous species was investigated by rapid phytochemical screening methods in this study.

MATERIAL AND METHODS – Materijal i metode

Identification and sampling of plant material

Plant material of 25 broadleaf deciduous species from 15 families was collected in July 2013. in natural populations near Sarajevo (B&H), in Vlakovo (690 m ASL, silicates, 43°53'15" N, 18°14'01" E) and on Mt. Bjelašnica (1392 m ASL, limestone-dolomite, 43°43'43" N, 18°15'14" E). Taxonomic identification of the plants was carried out during field work. Leaves and barks (free from diseases) were separated immediately into paper bags. Then, the plant material was washed thoroughly with running tap water and rinsed with distilled water, and was drying during 10 days in the overshadowed at room temperature in a well ventilated room. Thereafter, dried material is grounded in mixer and powder is packed in plastic bags and stored to the implementation of the extraction process.

Chemicals

All used chemicals were of analytical grade.

Preparation of extracts

To obtain the aqueous extracts shredded materials were transferred to glass beakers, with adding 50 mL of distilled water, boiled 30 minutes at 50 °C in a water bath, and filtered through Whatman No.1 filter paper. Then, filtrate was centrifuged at 2,500 rpm for 15 minutes and stored in sterile glass

² https://secure.fera.defra.gov.uk/treechemicals/review/index.cfm, accessed: 02.09.2016.

bottles at 5 °C to perform phytochemical screening (modified SAVITHRAMMA ET AL. 2011, modified SUBHASHINI DEVI ET AL. 2014).

Phytochemical screening

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Preliminary qualitative phytochemical screenings of water extracts were carried out with the following methods:

Anthocyanins: 2 mL of 2N hydrochloric acid and 2 mL of ammonia were added to 2 mL of aqueous extract. Discoloration of red-pink colour in the blue-violet indicates the presence of anthocyanins (PARIS AND MOYSE 1969).

Coumarins: 3 mL of 10% sodium hydroxide were added to 2 mL of aqueous extract. The presence of coumarins is indicated by formation of yellow colour (RIZK 1982).

Emodins: 2 mL of ammonium hydroxide and 3 mL of benzene were mixed with 2 mL of aqueous extract. Emodins are indicated in the case of appearance of red colour (RIZK 1982).

Fatty acids: 5 mL of ether were added to 0.5 mL of water extract. Solution pours out on filter paper and allows evaporating and drying. Transparency on filter paper indicates the presence of fatty acids (AYOOLA 2008).

Phenols: 2 mL of 2% solution of iron (III) chloride mixed with 2 mL of aqueous extract. Appearance of blue-green or black coloration indicates the presence of phenols (SUBHASHINI DEVI ET AL. 2014).

Saponins: 5 mL of water extract was diluted with 20 mL of distilled water and agitated for 15 minutes. The presence of saponins is indicated by foam formation (KUMAR ET AL. 2009).

Steroids: 1 mL of the aqueous extract was dissolved in 10 mL of chloroform followed by addition, by sides of the test tube, 10 mL of concentrated sulphuric acid. The presence of steroids is indicated when the upper (chloroform) layer turns red and lower (sulphuric acid) layer showed yellow with green fluorescence (GIBBS 1974).

Tannins: a few drops of 1% lead acetate were added to 2 mL of water extract. Appearance of yellowish precipitate indicates the presence of tannins (TREARE AND EVANS 1985).

RESULTS AND DISCUSSION – Rezultati i diskusija

Using various phytochemical screening tests it is possible to detect bioactive principles, and facilitate quantitative estimation and qualitative separation of active compounds (VARADARAJAN ET AL. 2008). The phytochemical screening and qualitative estimation of 25 analyzed broadleaf deciduous species showed that their leaves were rich in phenols and fatty acids followed by saponins, tannins and emodins, with no steroids (Table 1). To our knowledge, emodins were observed for the first time in six species. Barks were rich in tannins, phenols, fatty acids and saponins, but without anthocyanins, emodins and steroids (Table 1). Of the 25 analyzed species in this study, according to available literature data, for some of them the

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presence of SMs either has not been investigated at all (Acer obtusatum Waldst & Kit. ex Willd., Quercus petraea L.) or just for one or two SMs (Crataegus monogyna Jacq., Euonymus europaeus L., Prunus avium (L.) L., Pyrus pyraster (L.) Burgsd., Robinia pseudoacacia L., Sorbus aria (L.) Crantz, S. domestica L. and S. torminalis (L.) Crantz) until today. From 117 obtained positive tests, 69 of them were observed in bark extracts. The largest number of newly found SMs, to our knowledge, was present in R. pseudoacacia (4), followed by Fagus sylvatica L., Populus tremula L., Q. petraea and Sorbus aucuparia L. (3), and A. obtusatum, A. tataricum L., C. monogyna, Daphne mezereum L., Fraxinus ornus L. and P. pyraster (2).

Anthocyanins, one of the classes of flavonoids and nonphotosynthetic pigments, are important plant pigments responsible for the red, pink, purple, and blue colours in plants. They have different functional roles in plants, as: antioxidants and sunscreens, mediators of reactive oxygen species-induced signalling cascades, chelating agents for metals and/or metalloids and delayers of leaf senescence (LANDI ET AL. 2015). Many studies have shown that these pigments as strong antioxidants prevent the development of heart disease and cancers have anti-inflammatory activity, preventing bacterial infection etc. (SEERAM 2008, and references in the article). Anthocyanins are found in leaves extracts in four species and, for the first time, in *D. mezereum*. The data for presence of anthocyanins in analyzed species found in the literature have shown a certain variation in comparison to the results obtained during this study (LINDEBERG 1971, MARKHAM 1989, JEPPSSON 1995, WU ET AL. 2002, HEINRICH 2005, KIM ET AL. 2005, MAMDOH 2010).

Coumarins and their derivatives are widely distributed in nature, with very important roles in plant defence mechanisms against herbivores and microorganisms. Humans use them as anticoagulant, antioxidant, antimicrobial, anticancer, anti-diabetic, analgesic and anti-inflammatory agents, and as vitamin K antagonist (MIRUNALINI AND KRISHNAVENI 2011, MATOS 2015). In this study, coumarins were observed in seven species of which at six, to our knowledge, for the first time: *C. monogyna, E. europaeus, F. sylvatica, P. tremula, Q. petraea* and *R. pseudoacacia* (Table 1). However, in this study has not confirmed the presence of coumarins in other analyzed species, although there are reports in the literature (MARINOVA ET AL. 1994, PEARCE 2000, LUTSENKO ET AL. 2006, STOILOVA ET AL. 2007, YANG ET AL. 2009, MAMDOH 2010, MARKOVIĆ ET AL. 2010).

Emodins have multiple ecological functions in higher plants (protect against herbivores, pathogens, competitors and extrinsic abiotic factors) and represent a very useful group of SMs for humans, as laxative compounds and anti-inflammatory, antimicroorganism and antifeedant agents (IZHAKI 2002, SUBHASHINI DEVI ET AL. 2014). They were present in the bark of five analyzed species: *C. monogyna*, *F. ornus*, *P. tremula*, *P. avium* and *S. aria*, and in the leaves of *Rhamnus fallax* Boiss. (Table 1). Since in the literature are not found

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data for presence of emodins in analyzed broadleaf deciduous species in this study, these data are the first published for those species.

Fatty acids have important functions in plants, especially as an important source of reserve energy and essential components of membrane lipids, as well as in defence against pathogens (KACHROO AND KACHROO 2009). In humans, they participate in regulation of membrane structure and function; regulation of intracellular signalling pathways, transcription factor activity, and gene expression; and regulation of the production of bioactive lipid mediators (CALDER 2015). The presence of fatty acids, in this study, was observed in 15 species (Table 1), and the results were completely consistent with the literature data (LINDEBERG 1971, LEVITT 1980, MARKHAM 1989, AYERS ET AL. 1996, PUKACKA AND CZUBAK 1998, AI-Taweel 2000, Kostova 2001, STRANSKY ET AL. 2001, LUTSENKO ET AL. 2006, CODREANU ET AL. 2007, BRINDZA ET AL. 2009, MARKOVIĆ 2010, ČEKSTERYTĖ 2012, ŽEPIČ ET AL. 2013).

Phenols, the most important group of SMs in plants, are included in many physiological processes in plants and represent adaptive features developed during plant evolution. Also, they are of human interest because of their wide range of functions performed as: a disinfectant and raw materials for the production of medicaments, herbicides and cosmetics (LATTANZIO ET AL. 2006). Phenols were observed in 16 analyzed species, where 14 of them possessed phenolic compounds in leaves, and 13 species in the bark (Table 1). Although phenols are one of the most researched SMs in plants (PETRYNIAK ET AL. 1981, MARKHAM 1989, HAMILTON ET AL. 2004, HEINRICH 2005, LUTSENKO ET AL. 2006, OLIVIER ET AL. 2006, RIMPAPA ET AL. 2007, CONFORTI ET AL. 2008, HEGAB-GHAREIB 2010, MALEŠ ET AL. 2010, ZOOBI-MOHD 2010, KYLLI 2011, MANOJLOVIC ET AL. 2012, OLSZEWSKA 2012), in this study for the first time phenols are determined in five species: *A. tataricum, F. sylvatica, P. tremula, Q. petraea* and *S. aria* (Table 1).

Saponins are stored in plant cells as inactive precursors which are very easily converted to the biologically active antibodies in response to pathogen attack, playing a very important role in allelopathic defence against microorganisms, fungi and herbivores. For humans they have significant commercial values, and are used as medicines, detergents, sweeteners, food additives and cosmetics (MERT-TÜRK 2006, KOUL 2008). In this study, saponins are observed in 14 species, of which the newly discovered in eight: *A. obtusatum, A. tataricum, D. mezereum, F. ornus, R. pseudoacacia, Sambucus nigra* L., *S. aria* and *S. aucuparia* (Table 1). It is noticed, based on data from the available literature, that in this study is not confirmed the presence of saponins in three species (AYERS ET AL. 1996, AL-TAWEEL 2000, KOSTOVA 2001, PFEIFFER ET AL. 2003, LUTSENKO ET AL. 2006, OLIVIER ET AL. 2006, ALEXANDER 2009, BRINDZA ET AL. 2009, AHMET 2011).

Plant steroids are a class of lipids with different functions, which play an important role in a number of biological processes: from growth and development to resistance to stress (BISHOP AND KONCZ 2002). The

examination on the presence of steroids in the leaves and bark of analyzed species were negative. This is in agreement with many literatures reporting of steroids absence in plants, except for *P. avium* which possess steroids in leaves (EL-TAHAWI 1983).

Tannins, phenolic compounds of high molecular weight, are widely distributed SMs in plant flora, mainly located in a bark. Plants produce them as protective substances against herbivores, insects and other pests. Humans use tannin rich plants as antioxidant, antimicrobial, anti-inflammatory and healing agents in a number of diseases, and as astringent (BHANDARY ET AL. 2012, DOUGHARI 2012, SAVITHRAMMA ET AL. 2011, SUBHASINI DEVI ET AL. 2014, GUETAFF ET AL. 2016). Tannins presence was confirmed in 16 analyzed species, of which for the first time in four: *A. obtusatum, P. pyraster, Q. petraea* and *S. domestica* (Table 1). In this study has not confirmed the presence of tannins in four species, although it is noted in some articles (LINDEBERG 1971, ROWE AND CONNER 1979, MARKHAM 1989, PALTA 1992, AYERS ET AL. 1996, KOSTOVA 2001, KIM ET AL. 2005, ANDRÉ ET AL. 2006, OLIVIER ET AL. 2006, BRINDZA ET AL. 2009, MALEŠ ET AL. 2010, MARKOVIĆ 2010, AHMET 2011, GUZIK 2012).

The obtaining of diverse results in comparison to literature data probably lie in the use of different both qualitative methods for determining of SMs and solvent of extraction, and plant individuals' exposure to different ecological conditions. Also, according to WINK (1999), accumulation and concentration of SMs is not uniform for different plant parts, individuals and species, and it is result of very complex interactions between biosynthesis, transport, storage and degradation of certain SM.

The results of this study, at the same time, represent valuable data source for further more detailed investigations of some here analyzed broadleaf deciduous species, and increase potential of commercial values of certain broadleaf deciduous species.

CONCLUSIONS – Zaključci

The aim of present study was to examine qualitative composition of SMs in leaves and bark of 25 broadleaf deciduous species from 15 families. Preliminary phytochemical screening reveals the presence of various SMs in analyzed species, mainly distributed in bark. The results suggest that this approach of tree species research should continue and expand with use of other qualitative and some quantitative methods, which are more sophisticated and precise. Also, this preliminary study draws attention to the need for further studies of isolation and identification of active compounds in species of interest, and for the assessing of their potential antimicrobial and antioxidant activities.

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SAŽETAK

Biljke proizvode brojne i raznolike sekundarne metabolite, organska jedinjenja koja nisu esencijalna i direktno ne sudjeluju u rastu i razviću, ali koja mogu imati vrlo važnu ulogu u adaptaciji i prilagodbi biljaka određenim okolišnim uvjetima. Za ljude su sekundarni metaboliti vrlo važni u medicini, farmakologiji te industriji hrane i kozmetike. Prisutnost osam vrsta sekundarnih metabolita (antocijanini, emodini, fenoli, kumarini, masne kiseline, saponini, steroidi i tanini) u vodenim ekstraktima listova i kore 25 lišćarskih drvenastih vrsta, u okviru 15 porodica, je kvalitativno istraživano brzim fitohemijskim metodama. Prema literaturnim podacima, u ovoj studiji je po prvi put utvrđeno prisustvo šest tipova sekundarnih metabolita u analiziranim drvenastim vrstama: antocijanini u jednoj vrsti; kumarini i fenoli u po pet vrsta; emodini u šest vrsta; saponini u osam vrsta; te tanini u četiri vrste. Posebnu pažnju u daljnjem istraživanju treba posvetiti Fagus sylvatica L., Populus tremula L., Quercus petraea (Matt.) Liebl., Robinia pseudoacacia L. i Sorbus aria (L.) Crantz. Budući da su preliminarni rezultati ovog istraživanja obećavajući, bilo bi poželjno identificirati aktivna jedinjenja i procijeniti njihovu potencijalnu antimikrobnu i antioksidativnu aktivnost.

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ANNEX

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Table 1. The results of phytochemical analyses of secondary metabolites presence by rapid screening methods for analyzed dendro species. The bolded numbers mark presence of SM recorded for the first time to our knowledge.

Tabela 1. Rezultati fitohemijske analize prisustva sekundarnih metabolita pomoću brzih metoda za analizirane drvenaste vrste. Podebljani brojevi označavaju prisustvo uočenog sekundarnog metabolita po prvi put, prema našim saznanjima.

Secondary metabolite/ Sekundarni metabolit	Plant part/ <i>Dio</i>	Anthocyanins/ Antocijanini		Coumarins/ <i>Kumarini</i>		Emodins/ <i>Emodini</i>		Fatty acids/ Masne kiseline		Phenols/ <i>Fenoli</i>		Saponins/ <i>Saponini</i>		Steroids/ Steroidi		Tannins/ <i>Tanini</i>	
Species and family/ <i>Vrsta i porodica</i>	biljke	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD
Acer campestre L. Aceraceae	L	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0
	В	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1
Acer obtusatum Waldst & Kit. ex Willd. Aceraceae	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	В	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
<i>Acer pseudoplatanus</i> L.	L	0	0	0	1	0	0	1	1	1	1	0	0	0	0	0	1
Aceraceae	В	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1
Acer tataricum L.	L	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	1
neeraeeae	В	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
<i>Carpinus betulus</i> L. Betulaceae	L	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1
	В	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Clematis vitalba</i> L.	L	0	0	0	1	0	0	1	1	1	1	1	1	0	0	0	0
Ranunculaceae	В	0	0	1	1	0	0	1	1	1	1	0	0	0	0	0	0
Cornus mas L.	L	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	1
	В	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crataegus monogyna Jacq.	L	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0
Rosaceae	В	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	1
Daphne mezereum L.	L	1	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0
Thymelaeaceae	В	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
Euonymus europaeus L.	L	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
Celastraceae	В	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
Fagus sylvatica L.	L	0	1	0	0	0	0	1	1	1	0	0	1	0	0	0	0
1 agaecae	В	0	0	1	0	0	0	1	1	1	0	1	1	0	0	1	1
Fraxinus ornus L.	L	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	1
Oleaceae	В	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
<i>Hedera helix</i> L. Araliaceae	L	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
	В	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0

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Populus tremula L. Salicaccae L 0 1 0 0 1 1 1 0 0 1 1 1 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1 1 1 1 0 0 0 1 1 1 0 0 0 1 1 0 0 0 1 1 0 0 0 1 1 0 0 0 1 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 0 1 0 0 0 0 0 0 0 0 0																		
B 0 0 1 0 0 0 0 0 0 0 0 0 0 1 1 Prunus avium (L.) L. Rosaceae L 0 0 0 0 0 0 0 0 0 1 0 0 0 1 1 Prunus avium (L.) L. B L 0 1 0 0 1 0 1 0 </td <td>Populus tremula L.</td> <td>L</td> <td>0</td> <td>1</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>1</td> <td>1</td> <td>1</td> <td>0</td> <td>0</td> <td>1</td> <td>0</td> <td>0</td> <td>1</td> <td>1</td>	Populus tremula L.	L	0	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1
Prunus avium (L.) L. L 0 0 0 0 0 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0 0 1 1 0 0 0 0 1 1 1 0 0 0 0 1 1 0 0 0 0 1 1 0		В	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1
B 0 1 0 0 1 1 0 0 0 0 0 1 1 Pyrus pyraster (L.) Burgsd. Rosaceae L 0 1 0 1 1 0 0 0 1 1	Prunus avium (L.) L.	L	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Pyrus pyraster (L.) Burgsd. Rosaceae L 0 1 0	Rosueede	В	0	1	0	0	1	0	1	1	0	0	0	0	0	0	1	1
Rosaceae B 0<	<i>Pyrus pyraster</i> (L.) Burgsd.	L	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Quercus petraea (Matt.) Liebl. Fagaceae L 0 0 1 0 <	Rosaceae	В	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Fagaceae B 0 1 1 1 1 1 1 0 0 0 0 1 1 1 1 1 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1<	Quercus petraea (Matt.) Liebl.	L	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Rhamnus fallax Boiss. L 0 0 0 0 0 0 0 1 1 1 1 1 1 0 0 0 1 Rhamnaceae B 0 0 0 0 0 0 0 0 0 1 1 1 1 1 0 0 0 1 Rhamnaceae B 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 1 Robinia pseudoacacia L. Fabaceae L 0 0 1 0 0 1 1 0 0 0 1 1 Fabaceae B 0 0 1 0 0 1 1 1 1 0 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0	Fagaceae	В	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Rhamnaceae B 0 0 0 0 0 0 0 1 1 1 0 0 0 1 Robinia pseudoacacia L. Fabaceae L 0 0 1 0 0 0 0 1 1 1 0 0 0 1 B 0 0 1 0 0 0 0 1 1 0 0 0 1 1 Salix caprea L. Salicaceae L 0 0 0 0 0 0 0 0 1 1 0	<i>Rhamnus fallax</i> Boiss.	L	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	1
Robinia pseudoacacia L. Fabaceae L 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 1 1 0 0 1 0 0 1 1 1 0 0 1 1 1 0 0 1 1 1 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 1 1 0 <td>Rhamnaceae</td> <td>В</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>1</td> <td>1</td> <td>1</td> <td>0</td> <td>0</td> <td>0</td> <td>1</td>	Rhamnaceae	В	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1
Fabaceae B 0 0 1 0 0 1 1 0 0 1 0<	Robinia pseudoacacia L.	L	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1	1
Salix caprea L. Salicaceae L 0 0 0 0 0 0 1 1 1 1 0 1 0 0 1 1 B 0 0 0 0 0 0 1 1 1 1 0 1 0 1 1 1 Sambucus nigra L. Adoxaceae L 0 1 0 1 0 1 0 0 0 0 0 0 0 1 1 B 0 1 0 1 0 0 1 1 1 1 0 0 0 1 1 B 0 1 0 1 0 0 1 1 1 0	Fabaceae	В	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0
B 0 0 0 0 0 0 1 1 1 1 0 1 0 0 1 1 Sambucus nigra L. Adoxaceae L 0 1 0 1 0 1 0 0 0 0 0 0 1 1 1 0 1 0 0 0 1 1 B 0 1 0 1 0 0 1 1 1 0 1 1 1	<i>Salix caprea</i> L.	L	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1	1
Sambucus nigra L. L 0 1 0 1 1 0 1 0	Sancaceae	В	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1	1
B 0 1 0 1 0 0 1 1 1 0 0 0 0 0 0 0	Sambucus nigra L. Adoxaceae	L	0	1	0	1	0	0	1	1	0	1	1	0	0	0	0	0
		В	0	1	0	1	0	0	1	1	1	1	0	0	0	0	0	0

Preliminary phytochemical screening for secondary metabolites in leaves and bark of 25 broadleaf deciduous species

Sorbus aria (L.) Crantz	L	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
	В	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Sorbus aucuparia L.	L	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1
	В	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	1
<i>Sorbus domestica</i> L. Rosaceae	L B	0	1	0	0 0	0	0 0	0	0 0	1	1	0	0 0	0	0 0	0	0
<i>Sorbus torminalis</i> (L.) Crantz Rosaceae	L	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1
	В	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0

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Note : L-leaf; B-bark; 0-lacking; 1-present; OD-our data; LD-literature data. *Napomena*: L-list; B-kora; 0-negativan rezultat; 1- pozitivan rezultat; OD-naši podaci; LD-literaturni podaci.