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Chemical composition and antioxidant activity of three Lamiaceae species from Bosnia and Herzegovina

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Abstract: The components of essential oils of rosemary, sage and lavender were investigated by GC-MS and assayed for their antioxidant activities. The plants were collected after the end of the vegetative cycle. The principal components of sage essential oil were 1,8-cineole (28.03%), α-thujone (11.98%), veridiflorol (11.17%), and α-humulene (11.0%). Rosemary essential oil was mainly composed of α-pinene (14.02%), camphor (13.62%), 1,8-cineole (13.02%), borneol (12.45%), and berbenone (10.04%). Predominant compounds in lavender essential oil were 1,8-cineole (40.68%) and camphor (29.82%). Antioxidant activity was examined by two different methods: the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and determination of ferric reducing antioxidant power (FRAP). The results indicate that the tested essential oils have low antioxidant activity compared to synthetic antioxidant butylated hydroxytoluene (BHT). In relation to the other oils investigated, rosemary essential oil showed the highest antioxidant activity by both methods.

INTRODUCTION

Production and usage of the essential oils is the part of tradition, cultural and historical heritage of Bosnia and Herzegovina (B&H). Many plants, such as chamomile, sage and immortelle are already recognized at global market due to its unique composition and organic farming. B&H is a leading medicinal and aromatic plant exporting country in the region and has a high potential for development of the products based on geographical origin and organic farming.

Various beneficial properties of essential oils have been recognized since ancient times. Nowadays essential oils found application in pharmaceutical, cosmetics, sanitary and food industries owing to their odour and biological effects such as antiseptic, antibacterial, antioxidant activity etc. (Bakkali *et al.*, 2008). In B&H, there are 30 medical species of the family Lamiaceae. Although entire flora of B&H is dominated by species of family Asteraceae (Redžić, 2007), in medicinal flora the most abundant are species of family Lamiaceae (13%), with usage of its ground herbal organs, leaves and flowers. These are mostly ingredients for preparation of different kind of tea infusion which is common for treatment of respiratory system illnesses, stomach discomfort and nervous tension.

As a part of our research on plants from southern part of B&H, we carried out analysis of chemical composition and antioxidant activity of essential oils from three Lamiaceae species which are widely used in folk medicine, cosmetics, phytopharmacy, and the flavouring of food products. Essential oils of sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* L.) and rosemary (*Rosmarinus officinalis* L.) were analyzed. Samples were collected in October, in order to examine yield and antioxidant activity of essential oils in non-vegetative cycle.

EXPERIMENTAL

Plant material

Sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.) and lavender (*Lavandula angustifolia* L.) were collected near the city Mostar, central Herzegovina (43°20’30”N; 017°48’47”E), in October 2014. The

species were identified at the Department of Biology, Faculty of Science and Education, University of Mostar. The plant material was air-dried for 20 days and stored at ambient temperature (25±2°C) without exposure to direct sunlight.

Isolation of the essential oils

The air-dried samples of each plant were submitted to hydro-distillation for 1.5 hour using Clevenger type apparatus according to European Pharmacopeia. The collected *n*-pentane extracts were dried over anhydrous sodium sulphate and stored in sealed vials at -15°C until analysis. The essential oil yields were determined by the gravimetric method.

Gas Chromatography-Mass Spectrometry

Analysis of the oils were carried out using Shimadzu GC-MS QP2010 system equipped with an AOC-20i autosampler, using two fused silica capillary columns with different polarity. The non-polar column was Inert Cap (30 m × 0.25 mm × 0.25 μm) and the polar column was Rtx-Wax (30 m × 0.25 mm × 0.25 μm). Solutions of 2 μL of essential oil in pentane were injected in splitless mode at 250°C. Helium was the carrier gas. The operating conditions for non-polar column were as follows: flow rate of carrier gas: 1.15 mL min^{-1}; oven temperature program: 70°C (1.5 min), 70-120°C (5°C min^{-1}), 120-240°C (4°C min^{-1}), 240°C (2 min). For polar column operating conditions were as follows: flow rate of carrier gas: 1.21 mL min^{-1}; oven temperature program: 60°C (2 min), 60-240°C (3°C min^{-1}), 240°C (10 min). MS conditions: ionization voltage: 70 eV, ion source temperature: 250°C, mass range: m/z 40-400.

Identification

Identification of oil components was based on retention indices on a polar and non-polar column relative to a homologous series of *n*-alkanes (C_8-C_{40}) as well as on comparison of their mass spectra with the NIST and Wiley spectra library. Relative percentages of components were calculated based on GC peak areas without using correction factors.

Determination of antioxidant capacity of essential oils

Free Radical Scavenging Capacity Using the Stable Radical (DPPH)

Antioxidant activity of the essential oils were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, 2,2'-diphenyl-1-picrylhydrazyl (DPPH). In the DPPH assay, the ability of essential oils of interest to act as donors of hydrogen atoms or electrons in transformation of DPPH· into its reduced form DPPH-H was investigated (Brand-Williams *et al.*, 1995; Jukić *et al.*, 2006). The 50 μL methanolic solution of the essentials oils (concentrations of stock solutions were 5, 10, 15, 20, 30, 40 and 50 g L^{-1}) was placed in a cuvette, and 1 mL of 6 × 10^{-5} M methanolic solution of DPPH was added. The reaction progress absorbance of the mixture is monitored at 517 nm for 1 hour using an UV-Vis (double-beam) Shimadzu spectrophotometer. Synthetic antioxidant butylated hydroxytoluene (BHT) was used as a positive control (concentrations of methanolic stock solutions were 0.1, 0.5, 0.7, 1.0, 1.5, 2.0, 10.0 and 20.0 g L^{-1}). Methanol was used to zero the spectrophotometer. The absorbance of DPPH radical without antioxidant was measured daily. All determinations were performed in triplicate. Inhibition of DPPH expressed in percentage was calculated according to equation (1), were $A_{C(0)}$ is the absorbance of the control at t=0 min, and $A_{A(t)}$ is the absorbance of the antioxidant at t=1 h.

$$\text{Inhibition (\%)} = \frac{A_{c(o)} - A_{A(t)}}{A_{c(0)}} x100 \quad (1)$$

Determination of FRAP- Ferric Reducing/Antioxidant Power

The total antioxidant potential of essential oils and BHT were determined using the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain (1996) as a measure of antioxidant power. The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colour Fe^{2+} tripyridyltriazine compound from the colourless oxidized Fe^{3+} form by the action of electron donating antioxidants (Politeo *et al.*, 2007; Tomaino *et al.*, 2005). The FRAP reagent was prepared by mixing 10 parts of acetate buffer (300 mmol L^{-1}, pH 3.6) with 1 part of TPTZ (2,4,6-tripyridil-*s*-triazine, 10 mmol L^{-1} in 40 mmol L^{-1} hydrochloric acid) and with 1 volume of ferric chloride (20 mmol L^{-1}). All solutions were used on the day of preparation. A linear calibration graph for $FeSO_4 \times 7H_2O$ in the concentration range over 0.1-5.0 mmol L^{-1} was prepared. The corresponding regression calibration equation was: A=0.6152c+0.0606, were A is absorbance at 593 nm, c is concentration of $FeSO_4 \times 7H_2O$ in mmol L^{-1}, (R^2 = 0.9998). The procedure for preparation of calibration graph was as follows. The reaction mixture was consisted of 150 μL of deionized water, 1.5 mL of FRAP reagent and 50 μL solution of $FeSO_4 \times 7H_2O$ at different concentrations given above. The FRAP reagent (1.5 mL) was warmed to 37°C and a reagent blank reading was taken at 593 nm. The same procedure was used for spectrophotometric measurements with solutions of essentials oils. Instead of 50 μL of solution of Fe^{2+}, a 50 μL methanolic solution of the essential oil was added in reaction mixture in concentration range over 5-50 g L^{-1}. All reaction mixtures were incubated at 37°C throughout the monitoring period. The change in absorbance between the final reading (4-min reading) and blank reading was selected for the calculation of FRAP values. The butylated hydroxytoluene (BHT) was used as a positive control (concentrations of methanolic stock solutions were 0.1-20.0 g L^{-1}). In the FRAP assay, the antioxidant efficiency of the antioxidant tested was calculated with reference to the reaction signal given by a Fe^{2+} solution of known concentration. All determinations were performed in triplicate.

RESULTS AND DISCUSSION

The yields of essential oils obtained by hydro-distillation were 2.1% for sage, 1.1% for rosemary, and 1.1% for lavender. The chemical composition is presented in Table 1 according to the retention indices of compounds on a non-polar column. All oil extracts are characterized by a high concentration of monoterpenes (sage 65.5%, rosemary 88% and lavender 90.4%), including mostly oxygenated monoterpenes (Table 1). Concerning the content of sesquiterpenes, rosemary and lavender have similarly low content (5-8%) while sage oil contain total of 30.9% of sesquiterpenes.

Table 1: Chemical composition (%) of essential oils from Lamiaceae species

Name	K_i[a]	K_i[b]	*S. officinalis*	*R. officinalis*	*L. angustifolia*
α-Pinene	935	1095	4.1	14.0	2.5
Sabinene	974	1133	-	-	0.3
β-Pinene	978	1127	2.4	0.6	2.7
β-Myrcene	986	1166	0.6	1.4	-
δ–3-Carene	1009	1160	-	1.0	3.3
(*E*)-3-Caren-2-ol	1024	1508	-	0.4	0.2
Limonen	1025	1197	-	1.6	-
Cymol	1025	1261	0.3	-	-
1,8-Cineole	1033	1203	28.0	13.0	40.7
γ-Terpinene	1056	1244	0.2	0.4	0.2
Sabinene hydrate	1068	1454	0.1	0.1	0.1
α-Terpinolene	1085	1275	tr	0.9	0.6
Linalool	1100	1542	0.7	6.7	0.4
α-Thujone	1109	1408	12.0	-	-
β-Thujone	1119	1426	8.5	-	-
Chrysantenone	1126	1486	-	0.2	-
allo-Ocimene	1126	1365	-	-	0.3
cis-Verbenol	1144	1663	-	0.5	-
Camphor	1150	1495	3.3	13.6	29.8
3-Pinanone	1160	1522	0.1	-	-
Pinocarvone	1163	1637	-	0.7	-
Borneol	1174	1685	4.0	12.5	5.2
4-Terpineol	1180	1587	0.4	1.3	0.8
p-Cymen-8-ol	1186	1827	-	-	0.7
α-Terpineol	1195	1657	0.6	2.6	2.0
Berbenone	1207	1677	-	10.0	0.1
trans-Carveol	1217	1848	tr	0.1	tr
Myrtenal	1219	1602	-	-	0.2
β-Citronelol	1225	1758	-	0.3	-
cis-Myrtanol	1241	1848	-	0.8	-
Carvone	1242	1706	-	-	0.4
Geraniol	1249	1819	-	2.9	-
β-Citral	1267	1712	-	0.2	-
(+)-Isopiperitone	1268	1807	-	0.5	-
m-Thymol	1278	2192	tr	tr	-
Bornyl acetate	1283	1566	0.2	2.9	tr
Carvacrol	1296	2151	tr	tr	-
α-Terpinelyl acetate	1331	-	-	tr	-
Piperitenone	1336	1848	-	0.1	-
Eugenol	1349	2139	-	0.1	-
1-Acetomethyl-3-isopropenyl-2-methyl-cyclopentane	1361	2028	-	0.1	-
α-Ylangene	1368	1471	tr	-	-
Copene	1375	1480	0.2	0.2	tr
2-Ethylidene-6-methyl-3,5-heptadienal	1392	2028	-	1.1	-
Methyl eugenol	1397	1994	-	0.7	-
α-Gurjunene	1406	1517	-	-	0.3
trans-Caryophyllene	1419	1579	2.1	1.3	1.1

Cumarine	1430	2389	-	-	tr
α-Guaiene	1439	1671	0.2	-	-
Neryl acetone	1446	1840	tr	0.3	-
α-Humulene	1456	1650	11.0	0.2	tr
Aromadendrene	1460	1625	0.6	-	tr
γ-Muurolene	1473	1672	0.3	0.2	-
Alloaromadendrene	1487	1626	0.5	-	-
Ledene	1490	1679	1.2	-	-
α-Selinene	1494	1702	tr	tr	-
α-Amorphene	1496	1707	tr	tr	-
β-Bisabolene	1506	1714	-	0.1	-
γ-Cadinene	1511	1740	0.1	0.2	1.0
δ-Cadinene	1516	1741	0.3	0.3	0.7
Nerolidol D	1522	1912	-	-	0.3
Germacrene-D-4-ol	1574	2034	-	-	0.1
Caryophyllene oxide	1580	1966	0.1	1.0	1.0
Viridiflorol	1596	2061	11.2	-	-
β-Selinene	1598	1981	0.3	-	-
Ledol	1603	1999	0.1	-	tr
Humulene oxide	1608	2003	1.0	0.1	-
Cubenol	1612	2032	-	-	0.2
δ-Cadinol	1625	2161	-	tr	-
Aromadendrene oxide 1	1631	2254	1.2	-	-
Aromadendrene epoxide	1634	2266	-	0.2	0.1
tau-Cadinol	1639	2148	tr	0.3	0.1
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	1640	2147	-	-	2.4
α-Cadinol	1653	2203	0.2	0.7	0.4
Andrographolide	1667	-	tr	0.1	tr
Aromadendrene oxide 2	1678	2365	0.1	-	-
3-Methyl-5-propyl-4-butylidene-cyclohex-2-ene-1-one	1681	-	-	-	tr
α-Bisabolol	1683	2195	-	0.2	-
6-Isopropenyl-4,8a-dimethyldecahydro-1-naphthalenol	1691	-	-	-	0.1
Bifromene	1925	2338	0.1	-	-
Manool	2042	-	2.3	-	-
Monoterpene Hydrocarbons			7.6	19.9	9.9
Oxygenated Monoterpenes			57.7	68.0	80.6
Sesquiterpene Hydrocarbons			16.8	2.5	5.5
Oxygenated Sesquiterpenes			13.9	2.5	2.3
Diterpene Hydrocarbons			0.1	-	-
Oxigenated diterpenes			2.3	0.1	-
Others			0.2	3.7	-
Total			98.6	96.7	98.3

Note: A = Kovats index on apolar column Inert Cap; B = Kovats index on polar column Rtx-Wax; Percentage and order of elution are given on the apolar column; tr = trace < 0,1%

Forty-six components were indentified in the sage oil, representing 98.6% of the total oil. The major compound is 1,8-cineole with 28.0%, followed by α-thujone (12.0%), viridiflorol (11.2%) and α-humulene (11.0%). Other compounds present in noticeable amounts were β-thujone (8.5%), α-pinene (4.1%), borneol (4.0%), camphor (3.3%), β-pinene (2.4%), manool (2.3%) and *trans*-caryophyllene (2.1%). Analysed oil showed surprisingly high concentration of 1,8-cineole and low content of camphor with respect to the previously investigated samples from Herzegovina (Marić *et al.*, 2006). This variability might be attributed to different

developmental stages, type of soil and exposure to light. In order to obtain the same chemical composition, essential oils should be extracted under the same conditions from the same organ of the plant which has been growing on the same soil, under the same climate and picked in the same season (Bakkali *et al.*, 2008).

The predominant components in rosemary oil are α-pinene (14.0%), camphor (13.6%), 1,8-cineole (13.0%), borneol (12.5%) and berbenone (10.0%). Other main

components included linalool (6.7%), α-terpineol (2.6%) and geraniol (2.9%). In rosemary extract 52 components were determined representing 96.7% of the essential oil. Lakušić *et al.* (2012) found that essential oils of rosemary from Balkan Peninsula can be classified in two major types: 1,8-cineol and camphor-type, and two intermediate types: camphor/1,8-cineole/borneol type and 1,8-cineole/camphor type. Analysed sample belongs to intermediate type which contains approximately equal amounts of camphor, 1,8-cineol and borneol. Among Balkan Peninsula this chemotype of rosemary is distributed along south Adriatic coast (Albania) and Italian Adriatic coast (Pescara) (Arnold *et al.*, 1997).

Total of 41 compounds were identified in the oil from lavender, corresponding to 98.3% of the components in oil. Two main components are 1,8-cineole (40.7%) and camphor (29.8%), followed by borneol (5.2%), β-pinene (2.7%), α-pinene (2.5%), 2-isopropyl-5-methyl-9-methylenebicyclo[4.4.0]dec-1-ene (2.4%) and α-terpineol (2.0%). High concentration of 1,8-cineole in lavender is typical for old leaves, collected from August till June (Lakušić *et al.*, 2014).

There are 22 compounds that occur in all three oils. 1,8-Cineole is the main component of sage and lavender, but it is also one of major compound in rosemary. The main component in rosemary is α-pinene which is also present in other two oils in appreciable amounts. However, some of the components present in one of the oil as one of the major compounds were absent in the other oils. For example, significant constituents from sage oil like α-thujone, β-thujone, viridiflorol and manool were absent in rosemary and lavender oils.

The oils were evaluated for antioxidant activity using two different methods. Like in numerous studies, 2,2'-diphenyl-1-picrylhydrazyl radical scavenging method (DPPH) and ferric reducing antioxidant power (FRAP) can be cited as relatively simple methods that can be used to measure the antioxidant potential of essential oils (Pajero *et al.*, 2003; Politeo *et al.*, 2011; Tomaino *et al.*, 2005). The DPPH method is sensitive and requires little sample material. The FRAP method is fast, easy to handle, with highly reproducible results. The antioxidant activity of the tested essential oils of rosemary, sage and lavender has been evaluated as a series of mass concentrations of essential oils (5-50 g L^{-1}). Concentrations of essential oils for both antioxidant methods are given as the concentrations of stock solutions.

All examined essential oils were able to reduce the stable, purple-coloured radical DPPH into yellow-coloured DPPH–H, without reaching 50% of DPPH radical inhibition (Figure 1). Rosemary essential oil showed the greatest inhibition of DPPH radicals by 33.7% at a concentration of 50 g L^{-1}, then lavender with 13.3% inhibition at a concentration of 20 g L^{-1} and sage with 9.2% of inhibition at a concentration of 50 g L^{-1}. According to this method, all examined oils had a low antioxidant activity compared to synthetic antioxidant butylated hydroxytoluene (BHT) (IC_{50} = 1.5 g L^{-1}) (Figure 2).

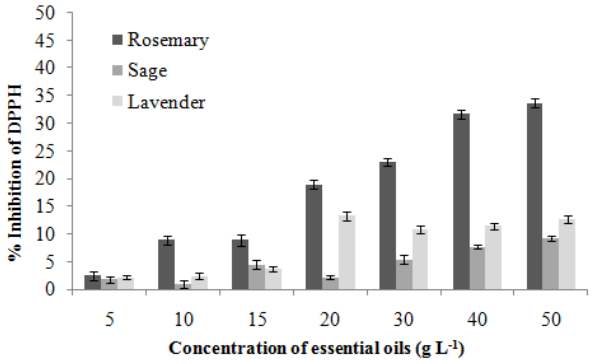

Figure 1. The inhibition percent of the DPPH radical in the presence of different concentrations of essential oils of rosemary, sage and lavender.

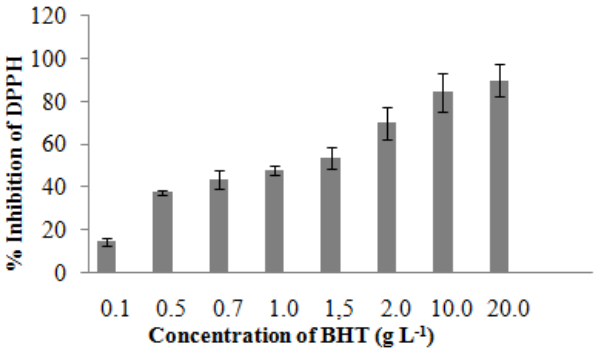

Figure 2. The inhibition percent of the DPPH radical in the presence of different concentrations of BHT.

The reducing power of the essential oils and BHT were determined by FRAP assay (Figure 3 and Figure 4). FRAP assay is based on redox-colorimetric reaction of the reduction of colourless Fe^{3+} compounds into blue colored Fe^{2+} tripiridyltriazine in the presence of the antioxidants (Politeo *et al.*, 2011). As in the previous method, the essential oil of rosemary showed the greatest reducing power. The essential oil of rosemary at the concentration of 30 g L^{-1} is equivalent to 1.6 mmol L^{-1} Fe^{2+} then sage at 20 g L^{-1} is equivalent to 0.9 mmol L^{-1} Fe^{2+} and lavender at 30 g L^{-1} is equivalent to 0.6 mmol L^{-1} Fe^{2+} in reducing power. The concentration of 2 g L^{-1} of synthetic antioxidant BHT is equivalent to 2.6 mmol L^{-1} Fe^{2+} in reducing power. High concentrations of all tested samples showed decrease in antioxidant activity.

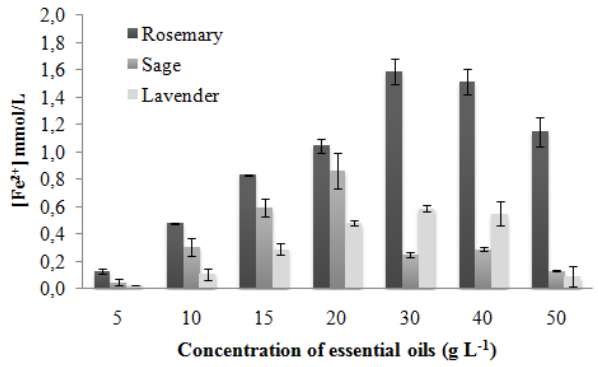

Figure 3. The antioxidant capacity in the presence different concentrations of essential oils of rosemary, sage and lavender measured by the FRAP assay.

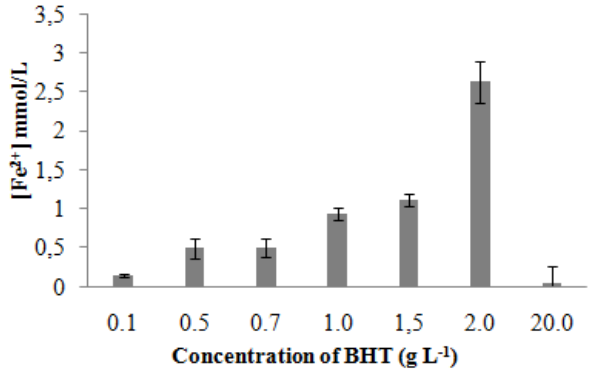

Figure 4. The antioxidant capacity in the presence different concentrations of BHT measured by the FRAP assay.

The results obtained from both methods show that the essential oils of rosemary, sage and lavender have low to moderate antioxidant activity. The major compound present in analyzed oils, 1,8-cineole, was previously tested and showed weak antioxidant activity using FRAP assay and almost none inhibition of DPPH radical (Zengin & Baysal, 2014). Low values of antioxidant activity obtained by both methods could be consequence of high content of other compounds such as camphor, borneol, β-pinene and α-pinene, none of which exhibited antioxidant activity (Tepe *et al.*, 2005). The lowest antioxidant activity is recorded for lavender oil where 1,8-cineole, camphor and α-pinene represent more than 75% of the total content. Somewhat greater antioxidant ability of rosemary oil could be attributed to other compounds present or to synergy effect between oil components. Dawidowicz and Olszowy (2014) showed that camphor, one of the main components in essential oil of sage (*Salvia hispanica*), has lower antioxidant ability than the essential oil. This finding suggests that antioxidant properties of sage oil are significantly affected not only by main components but also by other components. Earlier studies on 1,8-cineole/camphor/α-pinene type of rosemary oil (Rašković *et al.*, 2014) revealed notably stronger radical scavenging activity then it is found in our sample. Antioxidant properties are influenced by several factors, including the species, part of the plant, season of harvesting, geographical origin and extraction method, which also influence the chemical composition of plant essential oils (Teixeira *et al.*, 2013). Therefore, all effects of Lamiaceae essential oils should be carefully examined, considering the chemical composition of the investigated oil.

CONCLUSION

These results showed that studied oils consisted of various components among which most of them were present in all three species in different relative proportion. Nevertheless, some constituents were found only in one of the oils. The results obtained from both antioxidant methods showed low activity of sage and lavender and moderate activity for rosemary essential oil. It is difficult to make a direct relation among bioactivity and composition in such complex mixture like rosemary oil, but it can be suggested that synergism among components may increase antioxidant activity. Season of harvesting (October, non-flowering period) influences the chemical composition of essential oils (Teixeira *et al.*, 2013) and thereby affects the antioxidant activity. Although plants collected in October gave good yields on essential oil distillation, it was found that this chemical composition of all three species has low antioxidant activity.

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Summary/Sažetak

Ispitivane su komponente eteričnih ulja ružmarina, kadulje i lavande GC-MS-om kao i njihova antioksidacijska aktivnost. Ulja su destilirana u listopadu, nakon vegetativnog ciklusa biljke, kako bi se ispitala antioksidacijska aktivnost u ovom periodu. Glavne komponente eteričnog ulja kadulje su 1,8-cineol (28.03%), *a*-tujon (11.98%), veridiflorol (11.17%) i *a*-humulen (11.0%). Ružmarinovo eterično ulje čine najvećim dijelom *a*-pinen (14.02%), kamfor (13.62%), 1,8-cineol (13.02%), borneol (12.45%) i berbenon (10.04%). Glavni spojevi eteričnog ulja lavande su 1,8-cineol (40.68%) i kamfor (29.82%). Antioksidacijska aktivnost je ispitivana koristeći dvije metode: 2,2'-difenil-1-pikrilhidrazil (DPPH) metodu vezanja radikala i FRAP metoda određivanja antioksidacijskog kapaciteta. Rezultati pokazuju da analizirana eterična ulja kadulje i lavande imaju slabu antioksidacijsku aktivnost u usporedbi sa sintetskim antioksidansom butiliranim hidroksitoluenom (BHT), dok ružmarinovo eterično ulje ima umjerenu aktivnost.