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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THE ESSENTIAL OIL AND SOLVENT EXTRACTS OF *MENTHA PULEGIUM* L.

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Abstract. *We report the total phenolic (TPC; expressed as gallic acid equivalents, GAE, per milligram of dry extract weight) and the total flavonoid contents (TFC; expressed as quercetin equivalents, QE, per milligram of dry extract weight) and antimicrobial and antioxidant activities of the essential oil and hexane, diethyl ether, ethyl acetate and methanol extracts of* Mentha pulegium *L. (Lamiaceae) collected in Serbia. The total phenolic content was in the range of 129.43-388.29 μg GAE/mg, while TFC ranged from 57.81 to 160.94 QE/mg; the highest TPC and TFC were found in the methanol extract. The antimicrobial activity (against five bacteria and two fungi species) of the essential oil and solvent extracts was assessed using disc-diffusion method. However, the studied samples demonstrated a poor antimicrobial potential. The antioxidant activity was screened using five different tests: 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical cation decolorization assay (ABTS), total reducing power (TRP), ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity assay (CUPRAC); the methanol extract showed the strongest antioxidant potential. The results of the different antioxidant assays were correlated mutually and with the total flavonoid and total phenolic contents (regression analysis and agglomerative hierarchical clustering).*

Key words: Mentha pulegium *L., antioxidant activity, antimicrobial activity*

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1. INTRODUCTION

Mentha pulegium L. (syn. *Pulegium vulgure* Miller), commonly known as pennyroyal, is a plant species native to Europe, North Africa, Anatolia and Near East but its present distribution range covers all parts of the world, except for South America and Antarctica (Fadhel and Boussaïd, 2004; Chalchat et al., 2000; Mahboubi and Haghi, 2008). This is a short-lived perennial aromatic plant (Mahboubi and Haghi, 2008), with leaves that have a strong mint-like odor (Díaz-Maroto et al., 2007). According to the previous literature data, the dominant volatile metabolite of *M. pulegium* is pulegone (this alone can comprise up to 90% of the pennyroyal essential oil), followed by other biosynthetically related *p*-menthane type monoterpenoids (Díaz-Maroto et al., 2007; Mahboubi and Haghi, 2008; Chalchat et al., 2000; Reis-Vasco et al., 1999; Teixeira et al., 2012).

Volatile secondary metabolites, often isolated from the plant material in the form of essential oil, are extensively studied for their flavoring, preserving, antimicrobial, antioxidant and other useful properties (Atrea et al., 2009; Burt, 2004; Kostaki et al., 2009; Sökmen et al., 2004; Teixeira et al., 2012). According to the literature data, pennyroyal essential oil has antifungal, insecticidal and antioxidant properties (Sivropoulou et al., 1995; Ait-Ouazzou et al., 2012; Mahboubi and Haghi, 2008). Similarly as in the case of other representatives of the same plant genus–*Mentha* (Lamiaceae) species are used as traditional remedies for gastrointestinal disorders, as mouth fresheners, astringents, tonics, laxatives, antibacterials and culinary herbs (Agnihotri et al., 2005)– infusions and decoctions of aerial parts of pennyroyal are used as carminative, digestive, diuretic, antispasmodic, anti-inflammatory, antiseptic, expectorant remedies (Mahboubi and Haghi, 2008; Gruenwald et al. 1998; Teixeira et al., 2012) and for the treatment of colds, headaches, hepatic injuries, asthma and tuberculosis (Larousse, 2001; Zargari, 1990).

The chemical profile of a plant species can be influenced by both genetic and environmental factors and there are often several different chemotypes of the single plant taxa (Radulović et al., 2013). Thus, in order to assess a potential pharmacological value (biological activity) and safety (toxicity) of a botanical drug, one has to have as much chemical/activity data as possible on different populations of the studied plant species. In that sense, we decided to study the total phenolic (TPC) and the total flavonoid contents (TFC) and to evaluate antioxidant and antimicrobial activity of the essential oil and several solvent extracts of *M. pulegium* population from Serbia (no data available in the literature). We opted for the extracts prepared with the commonly used solvents of different polarity (methanol, diethyl ether, ethyl acetate and hexane) in order to get insight into the antioxidant potential of different types of *M. pulegium* constituents (different chemical/physical properties).

Moreover, we decided to screen antioxidant activity of each of the extracts using five different tests–2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical cation decolorization assay (ABTS), total reducing power (TRP), ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity assay (CUPRAC)–and to correlate them with TPC and TFC. Thus, this research complements the existing knowledge about the activity of *Mentha pulegium* L. extracts and the essential oil.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Folin-Ciocalteu's phenol reagent, ABTS, DPPH, ascorbic acid, gallic acid and neocuproine were purchased from Sigma Co. (St. Louis, Missouri, USA). Trolox was purchased from Acros Organics (Morris Plains, New Jersey, USA). Trichloroacetic acid, $K_3[Fe(CN)_6]$, quercetin, $K_2S_2O_8$, acetate buffers (pH 3.6 and pH 7), TPTZ (tripyridyltriazine) and phosphate buffer (pH 6.6) were purchased from Merck (Darmstadt, Germany). All the chemicals and reagents were of analytical purity.

2.2. Plant material; preparation of solvent extracts

Mentha pulegium aerial parts were collected in July 2013, from the wild-growing population (the village of Berilovac, Pirot, Serbia), during the flowering stage. Plant material was dried at room temperature, milled, macerated with hexane, diethyl ether, ethyl acetate or methanol (40 g of plant material in 400 mL of solvent) and then kept for 72 hours in the dark, at room temperature, with occasional shaking. The resulting extracts were filtered and concentrated on a rotary vacuum evaporator to dryness.

2.3. Isolation of the essential oil

Dry plant material (100 g) was subjected to hydrodistillation for 2.5 h using the original *Clevenger*-type apparatus. The obtained oil was separated by extraction with diethyl ether. Oil solutions were dried over anhydrous magnesium sulfate. The solvent was evaporated on vacuum rotary evaporator. The yield of *M. pulegium* essential oil was 0.4 % (v/w) based on dry plant weight.

2.4. Total phenolic and total flavonoid contents and antioxidant activity of *M. pulegium* solvent extracts

Total phenolic and total flavonoid contents and antioxidant activity of *M. pulegium* solvent extracts were assayed using solutions prepared by dissolving dry extracts in methanol (1:1, w/v). All spectrophotometric measurements of antioxidative potential of *M. pulegium* L. extracts were performed on a UV-visible spectrophotometer Perkin Elmer lambda 15 (Massachusetts, USA). All assays were performed in three independent repetitions. Mean values of the measurements are given in Table 1.

2.4.1. Total phenolic content (TPC)

Total phenolic contents of the extracts were determined according to the method of Singleton et al. (1999). An aliquot (0.25 mL) of the methanol solution of the extract was mixed with 0.625 mL of Folin–Ciocalteu reagent and 2.5 mL of sodium carbonate solution and the reaction mixture was diluted with distilled water to a total volume of 10 ml. The mixture was shaken and stored in the dark for 30 minutes. After that, the coloration of the sample was measured at a wavelength of 760 nm. Gallic acid was used as the standard for the calibration curve. Total phenolic content of *M. pulegium* was expressed as micrograms of gallic acid equivalents (GAE) per milligram of dry extract weight (μg GAE/mg dw).

2.4.2. Total flavonoid content (TFC)

Total flavonoid content was determined using the method described by Zhishen et al. (1999). An aliquot (0.25 mL) of extracts' solution in methanol or standard solution of quercetin (0.2, 0.4, 0.6, 0.8 or 1mL) was poured in to 10 mL volumetric flask containing distilled water. Sodium nitrite (0.3 ml) was added to the flask. After 5 minutes, 1.5 ml aluminium chloride was added. At 6th min, 1 mL sodium hydroxide was added and the total volume was made up to 10 mL with distilled water. The solution was mixed and the absorbance was measured against prepared reagent blank at 520 nm. Total flavonoid content was quantified as quercetin equivalent (QE) per milligram of dry extract weight (μgQE/mg dw).

2.4.3. 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH)

Stable DPPH radical was used for determination of free radical scavenging activity according to the method of Hatano et al. (1988). The appropriate amounts of extracts were mixed with 2.5 ml of DPPH solution and methanol was added to a total volume of 10 ml. The mixtures were stirred and left to stand in the dark for 1hour. The absorbance of the solution was measured at 515 nm. The free radical capacity toward DPPH radical was determined based on the Trolox calibration curve and the results were expressed as Trolox equivalents (TE) per milligram of dry extract weight (μg TE/mg dw).

2.4.4. ABTS radical cation decolorization assay (ABTS)

The $ABTS^{\bullet+}$ decolorization assay was employed as described in Re et al. (1999). A stock solution of $ABTS^{\bullet+}$ was prepared by mixing ABTS solution and potassium persulfate (1:1). The mixture was maintained in the dark at room temperature for 12 hours before use. The working $ABTS^{\bullet+}$ solution was prepared by dilution the stock solution in methanol to achieve an absorbance value of 0.7 ±0.02 at 734 nm. An aliquot of extract solution in methanol was poured into $ABTS^{\bullet+}$ working solution (1.8 mL) and methanol was added to the volume of 5 ml. Absorbance was measured at 734 nm immediately after mixing extract and working solutions; measurement of absorbance was repeated 6 times during following 6 minutes (after 60 seconds long intervals). Methanol was used as a referent solution, and for the standard calibration curve Trolox solution was utilized. The radical scavenging capacity of extracts was quantified as Trolox equivalents per milligram of dry extract weight (μg TE/mg dw).

2.4.5. Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay was based on the methodology of Benzie and Strain (1996). FRAP reagent was prepared by combining TPTZ (tripyridyltriazine) with ferric chloride and acetate buffer (1:1:10). The samples were mixed with FRAP reagent (1.0 mL) and water was added to a total volume of 5 mL. Solutions were kept at 37°C for 5 minutes and the absorbance determined at 595 nm, using methanol as a referent solution. Ferrous ion solution was used as a standard for the calibration curve and FRAP values were expressed as micromoles Fe^{2+} per mg of dry extract weight (μmol Fe/mg dw).

2.4.6. Total reducing power (TRP)

The capacity of extracts to reduce iron (III) to iron (II) was determined according to the method of Oyaizu (1986). The sample (1 mL of extract solution in methanol) was mixed with 2.5 mL of potassium hexacyanoferrate III and 2.5 mL of phosphate buffer (pH 6.6). After 30 minutes of incubation at 50°C in the dark, 2.5 mL trichloroacetic acid and 1.5 mL ferric chloride were added. The total volume with the addition of water was 10 ml. The absorbance was measured at 700nm. Negative (methanol) and positive (ascorbic acid) control reactions were performed. The results were quantified as ascorbic acid equivalent (AAE) per milligram of dry extract weight (mg AAE/mgdw).

2.4.7. Cupric reducing antioxidant capacity assay (CUPRAC)

CUPRAC assay was performed by following method of Apak et al. (2004). To an aliquot of the extract's solution in methanol, 1 ml of neocuproine solution, 1 ml of ammonium acetate buffer (pH 7) and1 ml of copper II chloride solution were added and a total volume set to 5 ml with distilled water. In this way prepared solution was kept at room temperature for 30 minutes. After that, the absorbance was measured at 450 nm. In the same manner, absorbances of a series of Trolox solutions were determined. The results of CUPRAC method were expressed as Trolox equivalent (mg TE/mg dw).

2.5. Antimicrobial activity

The antimicrobial activity of *M. pulegium* extracts was evaluated against two gram-positive (*Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538)) and three gram-negative (*Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Salmonella abony* (NCTC 6017)) bacteria. The antifungal activity was tested against *Aspergillus brasiliensis* (ATCC 16404) and *Candida albicans* (ATCC 10231). Microbial strains belonged to the American Type Culture Collection (ATCC; Gaithersburg, Maryland, USA) except *S. abony*, belonging to National Collection of Type Cultures (NCTC, Public Health England, London, United Kingdom).

A disc-diffusion method was used for the determination of the antimicrobial activity of the extracts, according to National Committee for Clinical Laboratory Standards (NCCLS, 1997). Inoculates of the bacterial and fungal strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. A volume of 100 µl of the suspension containing 1.0 x 10^8 CFU/ml of bacteria or 1.0 x 10^4 CFU/ml of fungal spores was spread on Mueller-Hinton agar (Torlak, Serbia) or sabouraud dextrose agar (Torlak) respectively, in sterilized Petri dishes (90 mm in diameter) making the 4 mm thick layer. The discs (12.7 mm in diameter, "Antibiotica Test Blattchen"-Schleicher and Schull, Dassel, Germany) were impregnated with 50 µl of extracts' solutions (conc. 100 mg/ml) and placed on the inoculated agar. Negative controls were prepared using the methanol. Chloramphenicol (30 µg, Torlak), cefalexin (30 µg, Torlak) and Nystatin (30 µg, Torlak) were used as positive standards. The inoculated plates were kept at 4°C for 2 hours and incubated at 37°C (24 h) for bacterial strains and at 28°C (48 h) for fungal strains. The antimicrobial activity was evaluated by measuring the zone (in millimetres) of inhibition against the test microorganisms using appliance "Fisher-Lilly Antibiotic Zone Reder" (Fisher Scientific Co. USA). All microorganisms were completely insusceptible to the control discs imbued with methanol (negative control). Antimicrobial assay was performed in triplicate and the mean values are reported.

2.6. Statistical analysis

In order to examine the interrelationships between the investigated samples, their results were mutually correlated using cluster and linear regression analyses. Cluster Analyses (CA) was carried out with the data of total phenolic content and antioxidant activity to identify various groups. The CA produced a dendrogram by means of the Ward´s method of hierarchical clustering, based on the Euclidean distance between assays. All computations were done using the Statistica 8 software (StatSoft, Tulsa, Oklahoma, USA).

3. RESULTS AND DISCUSSION

3.1. Total phenolic and total flavonoid contents of *M. pulegium* solvent extracts and their antioxidant potential

Total phenol and flavonoid contents of *M. pulegium* solvent extracts were determined by Folin-Ciocalteu method; the results are summarized in Table 1. The highest TPC (expressed as gallic acid equivalents, GAE, per milligram of dry extract weight) and TFC (expressed as quercetin equivalents, QE, per milligram of dry extract weight) were found in methanol (388.29 μg GAE/mg and 150.00 μg QE/mg, respectively) and diethyl ether (201.33 μg GAE/mg and 160.94 μg QE/mg, respectively) extracts. As expected, the lowest content of polyphenols and flavonoids (129.43 μg GAE/mg and 57.81 μg QE/mg, respectively) was in hexane extract (non polar solvent). Comparison of the present with the results of the corresponding previous studies suggests that TPC and TFC content in *M. pulegium* may significantly vary (Karray-Bouraoui et al., 2010; Hajlaoui et al., 2009; Khaled-Khodja et al., 2014; Stagos et al., 2012; Žugić et al., 2014). This could be due to the influence of environmental factors to the biosynthesis of phenolic/flavonoid compounds. Nevertheless, it suggests there could be several different chemotypes of this plant species.

Table 1 Total phenolic (TPC) and total flavonoid (TFC) contents and antioxidant activity (DPPH, ABTS, TRP, FRAP and CUPRAC assays) of *M. pulegium* solvent extracts

Extract	TPC (μg GAE/mg)	TFC (μg QE/mg)	DPPH (μg TE/mg)	ABTS (μg TE/mg)	TRP (mg AAE/mg)	FRAP (μmol Fe/mg)	CUPRAC (mg TE/mg)
Hexane	129.43	57.81	12.72	7.51	37.64	42.11	50.93
Diethyl ether	201.33	160.94	24.66	39.33	40.62	78.32	57.20
Ethyl acetate	151.00	139.06	24.86	47.56	43.59	83.37	63.24
Methanol	388.29	150.00	25.07	106.60	61.42	336.00	108.34

The antioxidant activity of *M. pulegium* solvent extracts was screened using five different tests: 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical cation decolorization assay (ABTS), total reducing power (TRP), ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity assay (CUPRAC). The results of these experiments are reported in Table 1. The reducing capacity of extracts is regarded as a significant

indicator of their potential antioxidant activity. The reducing ability is generally associated with the presence of compounds that can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Jayanthi and Lalitha, 2011). Combination of different methods should provide better insight into antioxidant potential of plant extract (complex mixture of natural products with diverse chemical structure). For example, the ABTS method gives a measure of the antioxidant activity of the range of antioxidant determined by the decolorization of the $ABTS^{\bullet+}$. FRAP, CUPRAC and TRP assays use different chromogenic redox reagents with different standard potentials. FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and produce a colored complex of ferrous tripyridyltriazine (Fe^{2+}-TPTZ). CUPRAC method is based on the absorbance measurement of the CUPRAC chromophore, Cu(I)-neocuproine (Nc) chelate, formed as a result of the redox reaction of antioxidants with the CUPRAC reagent, Cu(II)-neocuproine; CUPRAC reagent is stable, easily accessible, low-cost (its advantage over other chromogenic reagents, e.g., ABTS, DPPH), and it is sensitive toward thiol-type antioxidants and is performed in at nearly physiological pH (its advantage over FRAP).

As it could be seen from the data summarized in Table 1, methanol extract had the highest antioxidant potential (all applied assays); contrary to that, the lowest values were obtained for hexane extract. For example, according to the ABTS test, antioxidant activity of 1 mg of *M. pulegium* methanol and hexane extracts was equivalent to that of 106.60 and 7.51 μg of Trolox, respectively. The high scavenging properties of methanol extract are not surprising, as among herein applied solvents, methanol was the most suitable for the extraction of polar compounds with functional groups/structure that have radical scavenging/reducing properties (e.g. phenols). Antioxidant activity of *M. pulegium* extract was previously studied on several occasions, and by using different antioxidant assays (Ahmad et al., 2012; Alpsoy et al., 2011; Khaled-Khodja et al., 2014; Stagos et al., 2012; Sarikurkcu et al., 2012; Žugić et al., 2014). Differences between the results of different authors once again confirm there is high variability in the chemical profile of *M. pulegium*.

Table 2 Correlation coefficients between TPC, TFC and the results of antioxidant assays (DPPH, ABTS, TRP, FRAP, CUPRAC)

	TPC	TFC	DPPH	ABTS	TRP	FRAP	CUPRAC
TPC	1.00	0.52	0.52	0.94	0.98	0.96	0.96
TFC		1.00	0.98	0.66	0.45	0.48	0.46
DPPH			1.00	0.71	0.48	0.53	0.51
ABTS				1.00	0.96	0.97	0.97
TRP					1.00	0.99	1.00
FRAP						1.00	1.00

Highlighted correlations are significant at $p<0.05$

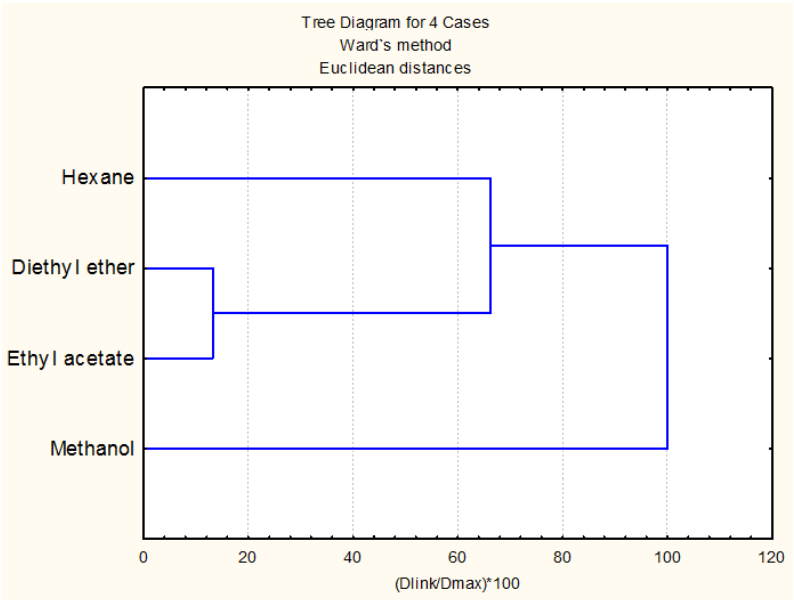

Fig. 1 Dendrogram obtained by agglomerative hierarchical clustering of antioxidant potential (determined by 5 different methods: DPPH, ABTS, TRP, FRAP and CUPRAC) of 4 *M. pulegium* solvent extracts.

Flavonoids and (other) phenolic compounds are known to have antioxidant properties, but we wanted to test if *M. pulegium* solvent extracts contained other types of compounds that also have reducing properties. Thus, we decided to test if the results of DPPH, ABTS, TRP, FRAP and CUPRAC assays correlate both mutually and with TPC and TFC. As mentioned above, different herein applied antioxidant assays might give insights into different chemical properties of the constituents of the analyzed samples. Correlation coefficients for DPPH, ABTS, TRP, FRAP, CUPRAC, TPC and TFC are given in Table 2. There was a strong positive correlation between the results of some of the assays (e.g. r=1 for CUPRAC/FRAP and CUPRAC/TRP; r=0.97 for ABTS/CUPRAC; r=0.96 for CUPRAC/TPC and ABTS/FRAP; in all cases $p<0.05$). Low correlation was found between TFC/FRAP (r=0.45), TFC/CUPRAC (r=0.46) and TFC/TRP (r=0.48), which indicates that flavonoids are not the main class of compounds that contributes to the antioxidant activity measured in this way.

We also wanted to explore mutual relationships (similarity) between different *M. pulegium* extracts. We did this by means of AHC (agglomerative hierarchical cluster analysis), and by using the results of TPC, TFC, DPPH, ABTS, FRAP, TRP and CUPRAC assays as variables. The dendrogram of the AHC analysis, with three statistically different classes, is given in Fig. 1. According to this, diethyl ether and ethyl acetate extracts (the same cluster) are mutually similar while hexane and methanol extract form separate classes. Such a result suggests that the chemical profiles of diethyl ether and ethyl acetate extracts were comparable (at least when speaking about compounds with reducing properties). This is not surprising as these solvents have similar polarities.

Table 3 Results of the antimicrobial activity testing, inhibition zones presented in millimeters

Sample	*Gram-negative bacteria*			*Gram-positive bacteria*		*Fungi*	
	E. coli	*S. abony*	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. brasiliensis*
Hexane ex.	/	/	/	/	/	17	/
Diethyl ether ex.	17	/	/	17	/	/	/
Ethyl acetate ex.	/	/	/	18	13	14	/
Methanol ex.	/	/	/	/	/	/	/
Essential oil	/	/	/	/	/	16	/
Methanol	/	/	/	/	/	/	/
Cephalexin	19	18	/	36	39	nt	nt
Chloramphenicol	22	25	12	30	29	nt	nt
Nystatin	nt	nt	nt	nt	nt	18	17

/ - not active, nt- not tested

3.2. Antimicrobial activity of *M. pulegium* essential oil and solvent extracts

Antimicrobial activity of the essential oil and solvent extracts against five bacteria and two fungi species was assessed using disc-diffusion method. The results are summarized in Table 3. The studied samples had poor antimicrobial potential: methanol extract was inactive against all tested strains and none of the extracts showed activity against *A. brasiliensis*, *S. abony* or *P. aeruginosa*. The growth of *S. aureus* was inhibited by ethyl acetate and diethyl ether extracts (inhibition zones were 18 and 17 mm, respectively); these extracts showed some activity against *E. coli* (diethyl ether extract; 17 mm) and *B. subtilis* (ethyl acetate extract; 13 mm) as well. Hexane and ethyl acetate extracts and the essential oil were active against *C. albicans* (inhibition zones were 17, 14 and 16 mm, respectively). The antibacterial activity (disc diffusion method) of *M. pulegium* essential oil/extracts against a number of different microorganisms (e.g. *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*) was previously studied by several research groups (Teixeira et al., 2012; Sivropoulou et al., 1995; Mahboubi and Haghi, 2008; Ait-Ouazzou et al., 2012; Hajlaoui et al., 2009; Khaled-Khodja et al., 2014; Stagos et al., 2012). Similarly as in the case of TPC, TFC and antioxidant potential of *M. pulegium* extracts, there was a significant difference between some of the previous results: in some cases (e.g. present results) tested samples were poor antimicrobials, while in others, they significantly inhibited the growth of microorganisms (Hajlaoui et al., 2009). Nonetheless, it appears that gram-positive bacteria strains are generally more susceptible to pennyroyal essential oil than gram-negative ones (Delaquis et al., 2002). The reason for this might be sought in the fact that Gram-negative organisms possess an outer membrane which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Vaara, 1992).

4. CONCLUSIONS

Herein, we reported the total phenolic (TPC) and the total flavonoid contents (TFC) and antimicrobial and antioxidant activities of the essential oil and hexane, diethyl ether, ethyl acetate and methanol extracts of *Mentha pulegium* L. collected in Serbia. The differences in the antimicrobial and antioxidative activities, and TPC and TFC of herein analyzed samples show these have different chemical profiles. Similarly, the differences

between the herein presented and previous results (literature data) suggest that M. *pulegium* metabolic profile might be strongly influenced by both genetic and environmental factors.

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ANTIOKSIDANTNA I ANTIMIKROBNA AKTIVNOST ETARSKOG ULJA I EKSTRAKATA BILJNE VRSTE *MENTHA PULEGIUM* L.

U ovom radu je određen sadržaj ukupnih fenola (TPC; izražen u ekvivalentima galne kiseline, GAE, po miligramu suvog biljnog ekstrakta) i ukupnih flavonoida (TPC; izražen u ekvivalentima kvarcetina, QE, po miligramu suvog biljnog ekstrakta), kao i antimikrobna i antioksidantna aktivnost etarskog ulja i heksanskog, dietil-etarskog, etil-acetatnog i metanolnog ekstrakta biljne vrste Mentha pulegium *L. (Lamiaceae; populacija iz Srbije). Sadržaj ukupnih fenola analiziranih uzoraka se kretao u opsegu od 129,43 do 388,29 μg GAE/mg, dok je TFC bio u interval od 57,81 do 160,94 QE/mg; najviše vrednosti za TPC i TFC nađene su za metanolni ekstrakt. Antimikrobna aktivnost (prema pet bakterijskih i dva soja gljivica) etarskog ulja i ekstrakata je određena disk-difuzionom metodom. Proučavani uzorci su imali slabu antimikrobnu aktivnost. Antioksidantna aktivnost je ispitivana pomoću DPPH (2,2-difenil-1-pikrilhidrazil radikal), ABTS (2,2'-azino-bis(3-etilbenzotijazolin-6-sulfonska kiselina radikal katjon), TRP (ukupna redukciona sposobnost), FRAP (sposobnost redukcije feri-jona) i CUPRAC (sposobnost redukcije Cu(II)-jona) metodama; najviši antioksidantni potencijal je imao metanolni ekstrakt. Rezultati različitih metoda za određivanje antioksidantnog potencijala su korelisani međusobno, kao i sa sadržajem ukupnih fenola i flavonoida (regresiona analiza i aglomerativna hijerarhijska klaster analiza).*

Ključne reči: Mentha pulegium *L., antioksidantna aktivnost, antimikrobna aktivnost*