Botanica SERBICA 37 (2): (2013) 97-104

Original Scientific Paper

Variations of essential oil characteristics of *Clinopodium pulegium* (Lamiaceae) depending on phenological stage

Violeta Slavkovska[1*], Bojan Zlatković[2], Christian Bräuchler[3], Danilo Stojanović[1], Olga Tzakou[4] and Maria Couladis[4]

1 Department of Botany, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia

2 Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

3 Department Biologie I, Biodiversitätsforschung, LMU, Geschwister-Scholl-Platz 1 80539 Munich, Germany

4 Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, Panepistimiopouli Zographou 157 71, University of Athens, Athens, Greece

ABSTRACT: The variability of essential oil characteristics in different phenological stages of *Clinopodium pulegium* from its natural habitat (Svrljiški Timok gorge, Serbia) and from cultivated plants (Niš, Serbia) was determined.

The essential oils were obtained from aerial parts of the plants by hydrodistillation and analyzed by GC-MS. These are the first data on the essential oil characteristics of plants of the population from the Svrljiški Timok gorge.

Samples originating from both natural and cultivated populations were characterized by high amounts of essential oils in all stages of development (0.8% - 1.4%). Twenty-one compounds were identified, representing 95.3-99.6% of the total oils.

Differences in the essential oil composition were more quantitative than qualitative. The quality of the essential oil was stable and did not vary with differences in environmental conditions.

Dominance of the main components was modified by phenological stage. Pulegone was dominant in the vegetative (76.1% wild population, 62.7% cultivated population) and the flowering (49.5%, 64.6%) stages, while menthone (48.5%, 65.3%) displaced pulegone (34.7%, 18.4%) at the fruiting stage.

Cultivated plants in the vegetative and flowering stages can be considered a significant source of pulegone, and in the fruiting stage a significant source of menthone. Careful selection of the developmental stage of the plant is a potential tool which could be employed to obtain the preferred chemical composition of *C. pulegium* for commercial use.

Key words: *Clinopodium pulegium,* essential oil, pulegone, menthone, phenological stage

Received: 22 July 2013 Revision accepted: 15. August 2013

UDK 665.528.292.94

INTRODUCTION

Clinopodium pulegium [syn: *Micromeria pulegium* (Rochel) Benth.] is a species recently transferred from the genus *Micromeria* sect. *Pseudomelissa* (Bräuchler *et al.* 2006). Numerous *Pseudomelissa* taxa of genus *Micromeria* have been transferred to genus *Clinopodium*, after morphological and molecular studies (Bräuchler *et al.* 2008).

C. pulegium is an endemic S. Carpathian species distributed in S.W. Romania and E. Serbia, inhabiting

*correspondence: violetas@pharmacy.bg.ac.rs

rocky ground, mainly in gorges, at 1000 to 1200 m above sea level. This species is a perennial herb with erect straight stems and ovate, obtuse to acute, more or less crenate-dentate leaves. The leaves are densely punctate on the abaxial side. The bracts are linear. The verticillasters have 10-40 flowers. The calyx is 13-veined, shortly pubescent, sparsely hairy in the throat, the teeth are 1/3-1/2 the length of the tube, linear-lanceolate, equal. The corolla is white or lilac in color (Chater & Guinea 1972).

Previous studies on essential oil characteristics of the related species (Vladimir-Knezevic *et al.* 2001; Medine *et al.* 2004; Duru *et al.* 2004; Slavkovska *et. al.* 2005; Telci & Ceylan 2007; Šavikin *et al.* 2010; Radulović & Blagojević 2012; Karousou *et al.* 2012) have determined that they are rich in essential oil (over 0.5%) dominated by oxygenated monoterpenes of the menthane class. Pulegone, isomenthone and menthone are the most frequently present essential oil components.

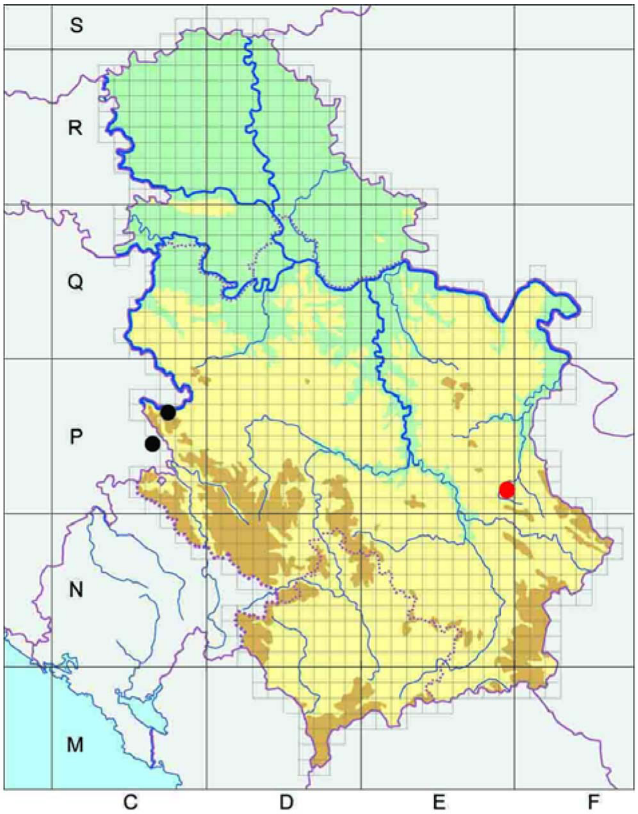
Several *Clinopodium* species have considerable antimicrobial (Medine *et al.* 2004; Duru *et al.* 2004; Castilho *et al.* 2007; Šavikin *et al.* 2010; Arslan & Dervis, 2010) and antioxidant (Öztürk *et al.* 2011; Nur Herken *et al.* 2012) activities.

The variability of the amount and composition of the essential oils depending on the season and phenological stages has been studied in many aromatic species of the Lamiaceae family (Santos-Gomes & Fernandes-Ferreira 2001; Milos *et al.* 2001, Mastelic & Jerkovic 2003; Chorianopulos *et al.* 2006, Marić *et al.* 2006; Lakušić *et al.* 2011; Nurzyńska-Wierdak *et al.* 2012; Rodrigues *et al.* 2013; Lakušić *et al.* 2013). There are few publications related to the essential oils of *Clinopodium* species (Putievsky *et al.* 1995; Dudai *et al.* 2001; Vladimir-Knežević *et al.* 2001). Pulegone was found to be the major component in oils with only small seasonal and phenological variation in composition.

In this paper we report the content and chemical composition of essential oil of *C. pulegium* collected from the recently recorded population from Svrljiški Timok gorge within the territory of Eastern Serbia (Bogosavljević *et al.* 2007). The variability of essential oil characteristics in different phenological stages of both plants growing in their natural habitat and cultivated plants of *C. pulegium* was also studied.

MATERIAL AND METHODS

Plant material: The material was collected from the Svrljiški Timok gorge, Serbia (Map 1) A representative specimen of the sampled population was deposited at the Herbarium of the Institute of Botany, Faculty of Pharmacy, University of Belgrade (HFF, specimen number: 3203). The plant material was collected during the vegetative



Map 1. Distribution of *Clinopodium pulegium* in Serbia [black spots – data from literature (Šilić, 1979; Bogosavljević et al., 2007); red spot – population from Svrljiški Timok gorge]

(June, 2009), flowering (July, 2009) and fruiting stage (September, 2009).

The plants were transferred from their natural habitat into a private garden in Niš. Voucher specimen has been deposited in the Herbarium of the University of Belgrade (BEOU, specimen number: 16404). The aerial parts of these cultivated plants were collected at different developmental stages: vegetative (May, 2010), flowering (August, 2010) and fruiting (beginning of October, 2010).

The material was dried at room temperature.

Isolation of the Essential Oil. The essential oils from the aerial parts of the plants were obtained by hydrodistillation for 3 h in a Clevenger-type apparatus (Eur. Phar. 6, 2007). The essential oils were dissolved using *n*-hexane and dried over anhydrous sodium sulphate and kept at -40°C until they were analyzed. The oil yield was expressed as ml/100g of dry weight of the plant material.

GC-MS Analysis: GC-MS analysis was carried out using a Hewlett Packard 5973-6890 GC-MS system operating in EI mode at 70 eV, equipped with a HP 5MS capillary column (30 m x 0.25 mm; film thickness 0.25 mm). The initial temperature of the column was 60°C and was heated to 280°C with a rate of 3°C/min. Carrier gas He; flow rate, 1 ml/min; split ratio, 1:10. Based on the estimated obtained volume of essential oil, the corresponding amount of capillary GC grade pentane was added to afford an appropriate concentration of 10 μl/ml. The injection volume was 1μl. Relative percentage amounts were calculated from total ion chromatograms (TICs).

Identification of the compounds was based on comparison of their retention indices (RI), their retention times (RT) and mass spectra with those obtained from authentic samples and/or the MS library (Adams 2001).

RESULTS AND DISCUSSION

Oil content. All samples were characterised by a high essential oil content (0.8% - 1.4%).

The plants from the natural habitat yielded the least amount of oil in the vegetative stage, 1% (v/w), the amount was higher in flowering stage, 1.4% (v/w), while it was slightly lower in the fruiting stage, 1.2% (v/w) (Fig. 1).

The cultivated plants in the vegetative stage contained 1.2% (v/w) of oil. The highest oil content was determined in the flowering stage, 1.3% (v/w), and the lowest in the full fruiting stage 0.8% (v/w).

Some studies have shown that the essential oil content depends on environmental factors (Kokkini *et al.* 2004; Lakušić *et al.* 2011). However, in our studies, the oil content was approximately equal in plants from natural habitats and cultivated plants. The highest amount of essential oil at the flowering stage could be associated with the biological role of essential oils. It is known that the essential oils of many *Clinopodium* species have antimicrobial and antifungal activities (Medine *et al.* 2004; Duru *et al.* 2004; Castilho *et al.* 2007; Šavikin *et al.* 2010; Arslan & Dervis 2010), and consequently protect the plant in this very important stage of development from pathogen attacks.

Composition of essential oil. Twenty-one compounds were identified, representing 95.3-99.6% of the total oils (Table 1). Differences in the essential oil composition were more quantitative than qualitative.

Monoterpenoids dominated in the oils (in concentrations higher than even 94%). Among monoterpenoids, oxygenated monoterpenes of the menthane type were present in a very high percentage (90.6%-94.7%). Sesquiterpene components were found in only small quantities (0.5%-2.5%).

Monoterpenoids also dominated in the essential oils of related *Clinopodium* species according to previous reports (Stojanović *et al.* 1999; Vladimir-Knezević *et al.* 2001; Duru *et al.* 2004; Slavkovska *et al.* 2005; Castilho *et al.* 2007; Radulović & Blagojević 2012).

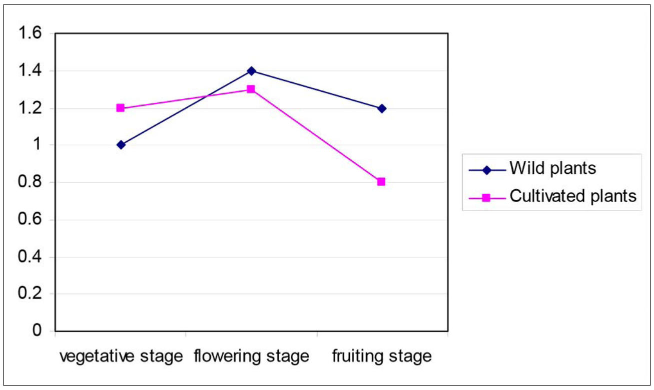


Fig. 1. Oil content (%, v/w) of *Clinopodium pulegium*

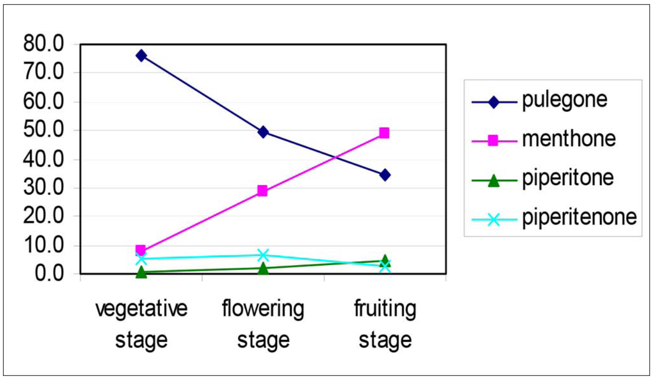


Fig. 2. The dominant constituents (%) of essential oils of wild *Clinopodium pulegium*

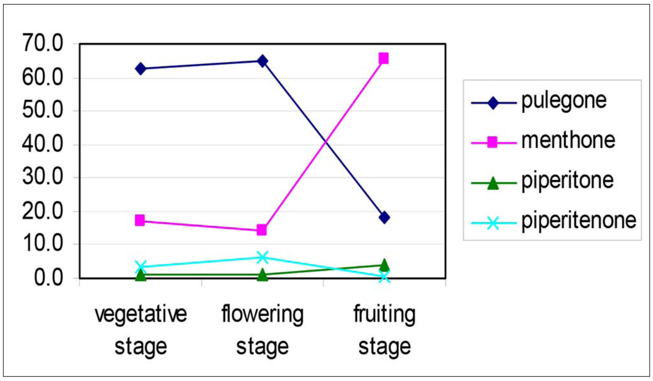


Fig. 3. The dominant constituents (%) of essential oils of cultivated *Clinopodium pulegium*

Based on our results we can conclude that the monoterpene and sesquiterpene ratio is a stable character in the oil of *C. pulegium* and largely independent of environmental conditions and phenological phases.

Dominant compounds in the essential oils. The oils were characterised by a large quantity of C-3 monoterpene

Table 1. Content and chemical composition of the essential oil of *Clinopodium pulegium*

			Wild plants			**Cultivated plants**		
			June	July	September	May	August	October
			vegetative stage	flowering stage	fruiting stage	vegetative stage	flowering stage	fruiting stage
	Oil content (%, v/w)		1.0	1.4	1.2	1.2	1.3	0.8
	Constituents	**RI**						
1.	α-pinene	937	0.3	0.9	tr	tr	tr	0.4
2.	sabinene	974	0.2	0.4	tr	tr	tr	0.3
3.	β-pinene	977	0.7	1.2	tr	tr	tr	1.0
4.	myrcene	988	0.1	0.5	tr	tr	tr	0.3
5.	3-octanol	993	0.2	-	-	-	-	-
6.	limonene	1027	1.7	4.1	3.3	4.2	3.5	2.4
7.	(E)- β-ocimene	1041	-	tr	tr	0.1	0.1	tr
8.	**menthone**	1148	7.8	**28.4**	**48.5**	**16.9**	**14.5**	**65.3**
9.	iso-menthone	1157	0.6	1.7	2.8	0.9	0.9	4.1
10.	isopulegone*	1176	0.9	2.1	1.6	3.5	3.5	1.0
11.	α-terpineol	1181	-	tr	tr	tr	0.3	tr
12.	**pulegone**	1232	**76.1**	**49.5**	**34.7**	**62.7**	**64.6**	**18.4**
13.	**piperitone**	1247	0.5	2.1	4.5	0.9	1.2	3.8
14.	**piperitenone**	1337	**5.4**	**6.3**	2.4	3.3	**6.2**	0.6
15.	piperitenone oxide	1360	0.3	0.5	0.2	3.9	2.3	0.2
16.	β-bourbonene	1380	-	tr	0.3	tr	tr	0.3
17.	(E)-caryophyllene	1412	0.1	0.4	0.5	0.5	0.4	0.5
18.	germacrene D	1479	-	1.1	0.3	0.8	1.4	0.4
19.	bicyclogermacrene	1492		0.4	tr	0.1	0.3	tr
20.	spathulenol	1571	0.2	tr	0.4	1.1	0.2	0.3
21.	caryophyllene oxide	1583	0.2	-	-	-	-	-
	Monoterpenoids		**94.6**	**97.7**	**98.0**	**96.4**	**97.1**	**97.8**
	Hydrocarbons		3.0	7.1	3.3	4.3	3.6	4.4
	Oxygenated monoterpenes		**91.6**	**90.6**	**94.7**	**92.1**	**93.5**	**93.4**
	Sesquiterpenoids		0.5	1.9	1.5	2.5	2.3	1.5
	Hydrocarbons		0.1	1.9	1.1	1.4	2.1	1.2
	Oxygenated sesquiterpens		0.4	-	0.4	1.1	0.2	0.3
	Other compounds		0.2	-	-	-	-	-
	Total		95.3	99.6	99.5	98.9	99.4	99.3

* Correct isomer not identified; RI, Retention Indices relative to C_9-C_{24} *n*-alkanes on HP-5MS; %, Relative percentage obtained from peak area; tr, trace (< 0.1%)

ketones such as: pulegone (18.4%-76.1%), menthone (7.8%-65.3%), piperitone (0.5%-4.5%), and piperitenone (0.6%-6.3%).

The main compounds of the oils from wild plants and cultivated plants were pulegone and menthone.

The concentration of pulegone in plants from the natural population (Fig. 2) was the highest (76.1%) in the vegetative stage, and declined over the flowering (49.5%) to the fruiting stage (34.7%). In contrast, the concentration of menthone was lowest in the vegetative stage (7.8%) and then linearly increased over the flowering (28.7%) to the fruiting stage (48.5%).

Quantitatively the oils of cultivated plants were slightly different (Fig. 3). Pulegone was consistently high in both the vegetative (62.7%) and flowering stage (64.6%), while its concentration decreased in the fruiting stage (18.4%). Menthone exhibited a completely opposite tendency. Its concentration was low in the vegetative (16.9%) and flowering stage (14.5%) and high in the fruiting stage (65.3%).

The contents of dominant components in the oils of wild and cultivated plants were characterised by a gradual decrease of pulegone and increase of menthone during plant development under natural conditions (Fig. 2). In the oil of cultivated plants an acute decrease of pulegone and increase of menthone from the flowering to the fruiting phase we observed (Fig. 3). The differences may have resulted from the fact that the wild and cultivated plants were gathered in different months.

The concentration of piperitone in the oil of the wild plants increased from the vegetative stage (0.5 %), through the flowering stage (2.1%) to the fruiting stage (4.5%). Cultivated plants also exhibited an upward trend in the concentration of piperitone from the vegetative stage, through the flowering to the fruiting stage (0.9%, 1.2%, 3.8%, respectively).

The oil of wild and cultivated plants in the flowering stage exhibited a significant presence of piperitenone (6.3% and 6.2%, respectively). This compound appeared in lower concentrations in the oil of other phenological stages (from 0.6% to 5.4%).

Very often, pulegone also appears in high concentrations in other species of this genus, for example: *C. thymifolium* (Scop.) Kuntze (Marinković *et al*. 2002; Slavkovska *et al*. 2005; Šavikin *et al*. 2010), *C. dalmaticum* (Benth.) Bräuchler & Heubel (Kostadinova *et al*. 2006; Šavikin *et al*. 2010; Radulović & Blagojević 2012; Karousou *et al*. 2012), *C. fruticosum* (L.) Kuntze (Dudai *et al*. 2001; Başer 2002), *C. cilicicum* (Hausskn. ex P.H.Davis) Bräuchler & Heubel (Duru *et al*. 2004). Also, pulegone can be found as a dominant component in the essential oils of other species of the Lamiaceae family, for example in the species of genus *Mentha* (Rodrigues *et al*. 2013) *Ziziphora* and *Cyclotrichium* (Baser 2002).

High concentrations of pulegone in the oil of plants in the vegetative and flowering stage can be explained by its biological role. Pulegone in the essential oils may act as part of the plant's protective biochemical response to the actions of other organisms, plants, insects, herbivores, parasites or pathogens (Franzios *et al*. 1997; Pavlidou *et al*. 2004; Koul *et al*. 2008). For example, pulegone is shown to be effective against *Musca domestica, Diabrotica virgifera, Peridroma saucia, Spodoptera litura*. Research done on *Spodoptera eridania* has shown that the larvae treated with pulegone develop into adults with lowered fecundity. Pulegone has also been observed to be toxic against the European corn borer, *Ostrinia nubilalis*. Pulegone is known as an effective fumigant against the rice weevil, *Sitophilus oryzae* (Koul et al. 2008). Moreover, research has shown that pure pulegone has a higher inhibitory activity on the growth of bacteria than the essential oil in total (Stojanović *et al*. 2000).

Pulegone has a significant role in allelopathic relations. The inhibitory activity of pulegone on seed germination and sprout growth of *Raphanus sativus* in *in vitro* conditions has been shown to be four times stronger than that of HCl (Jančić 1995).

The essential oil of *Clinopodium* species and its main component pulegone have also been shown to prevent seed germination and plant development of other species (Dudai *et al*. 1993; Dudai *et al*. 1999; Dudai *et al*. 2000).

Putievsky *et al*. (1995), Dudai *et al*. (2001) and Rodrigues *et al*. (2013) have shown that the concentration of some essential oil components increases or decreases at the expense of other components during the plant life cycle. The amounts of pulegone and menthone in the essential oil of *Mentha pulegium* L. (Rodrigues *et al*. 2013), and *C. pulegium* studied here follow the same pattern.

Franzios *et al*. (1997) demonstrated that the synergistic/antagonistic relationships between essential oil components can alter the toxicity of the entire essential oil. Pulegone was found to be significantly more effective as an insecticide, while menthone was less effective, when used in their authentic forms. A mixture of authentic pulegone and menthone, in relative quantities resembling their content in the oil of *M. pulegium* showed that the strong toxicity of pulegone was suppressed in the presence of menthone. Thus, we conclude that the essential oil obtained in the fruiting stage in our experiment would be less toxic, with a lower insecticidal activity than oils obtained in the vegetative and flowering stages. The use of *C. pulegium* essential oil as a bio-pesticide should be possible only if the essential oil is isolated in the vegetative or flowering stage of development.

CONCLUSION

C. pulegium originating from both natural and cultivated populations was characterized by high amounts of essential oils in all stages of development. However, this species had the highest yield in the flowering stage.

The quality of the essential oil was stable and did not vary with the change of environmental conditions.

The dominance of the main components was modified with phenological stages. Pulegone was dominant in the vegetative and the flowering stage, while menthone displaced pulegone in the fruiting stage.

Cultivated plants in the vegetative and flowering stage could be considered a significant source of pulegone, and in the fruiting stage a significant source of menthone. Careful selection of the developmental stage of the plant is a potential tool which could be employed to obtain the preferred chemical composition of *C. pulegium* for commercial use.

Aknowledgements — *T*he authors are grateful to the Serbian Ministry of Science and Technological Development (Project No. 173021 and 173030) for financial support.

REFERENCES:

Adams RP. 2001. Identification of Essential Oil Components by Gas Chromatography/ Quadruple Mass Spectrometry. Allured, Carol Stream, Illinois, USA.

Arslan M & Dervis S. 2010. Antifungal activity of essential oils against three vegetative compatibility groups of *Verticillium dahliae*. *World J. Microbiol. Biotechnol.* **26**:1813–1821.

Basȩr CHK. 2002. Aromatic biodiversity among the flowering plant taxa of Turkey. *Pure Appl. Chem.* **74**: 527–545.

Bogosavljević S, Zlatković B & Ranđelović V. 2007. Flora klisure Svrljiškog Timoka. 9th Symposium on the flora of Southeastern Serbia and neighbouring regions, Niš, Proceedings, 41-54.

Bräuchler C, Meimberg H & Heubl G. 2006. New names in Old World *Clinopodium* – the transfer of the species of *Micromeria* sect. *Pseudomelissa* to *Clinopodium*. *Taxon* **55:** 977-981.

Bräuchler C, Ryding O & Heubl G. 2008. The genus Micromeria (Lamiaceae), a synoptical update. *Willdenowia* **38**: 363-410.

Castilho P, Liu K, Rodrigues A, Feio S, Tomi F & Casanova J. 2007. Composition and antimicrobial activity of the essential oil of *Clinopodium ascendens* (Jordan) Sampaio from Madeira. *Flavour Fragr. J.* **22**: 139–144.

Chater OA & Guinea E. 1972. *Micromeria* Bentham. In: Tutin GT, Heywood HV, Burges AN, Moore MD, Valentine HD, Walters MS & Webb AD. (eds.), Flora Europaea, **3**, pp. 167-170. Cambridge University Press, London.

Chorianopulos N, Evergetis E, Mallouchos A, Kalpoutzakis E, Nychas G & Haroutounian AS. 2006. Characterization of the essential oil volatiles of *Satureja thymbra* and *Satureja parnassica*: Influence of harvesting time and antimicrobial activity. *J. Agric. Food Chem.* **54**: 3139-3145.

Dudai N, Poljakoff-Mayber A, Lerner HR, Putievsky E, Ravid U & Katzir I. 1993. Inhibition of germination and growth by volatile substances from *Micromeria fruticosa* L. *Acta Hort.* **344**: 123-131.

Dudai N, Mayer AM, Poljakoff-Mayber A, Putievsky E & Lerner HR. 1999. Essential oils as allelochemicals and their potential use as bio-herbicides. *J. Chem. Ecol.* **25**: 1079-1089.

Dudai N, Larkov O, Putievsky E, Lerner HR, Ravid U, Lewinsohn E & Mayer AM. 2000. Biotransformation of constituents of essential oils by germinating wheat seed. *Phytochem*. **55**: 375-382.

Dudai N, Larkov O, Ravid U, Putievsky E &Lewinsohn. 2001. Developmental control of monoterpene content and composition in *Micromeria fruticosa* (L.) Druce. *Ann. Bot.* **8:** 349-54.

Duru M, Ozturk M, Ugur A & eylan O. 2004. The constituents of essential oil and in vitro antimicrobial activity of *Micromeria cilicica* from Turkey. *J. Ethnopharmacol.* **94**: 43–48.

The European Pharmacopoeia 2007. 6th Edition. Council of Europe, Strasbourg, France.

Franzios G, Mirotsou M, Hatziapostolou E, Kral J, Scouras ZG & Mavragani-Tsipidou P. 1997. Insecticidal and genotoxic activities of mint essential oils. *J. Agr. Food Chem.* **45**: 2690-2694.

Jančić R. 1995. Biološka uloga etarskih ulja. In: Jančić R, Stošić D, Mimica-Dukić N & Lakušić B., Aromatične biljke Srbije, pp. 95-117. Dečije novine, Beograd – Gornji Milanovac.

Karousou R, Hanlidou E, & Lazari D. 2012. Essential oils of *Micromeria dalmatica* Benth., a Balkan endemic species of section *Pseudomelissa*. *Chem. & Biodiv.* **9**: 2775-2783.

Kokkini S, Hanlidou E & Karousou R. 2004. Clinal variation of *Mentha pulegium* essential oils along the climatic gradient of Greece. *J. Essent. Oil Res.* **16**: 588–593.

Kostadinova E, Alipieva K, Stefova M, Stafilov T, Antonova D, Evstatieva Lj, Matevski V, Kulevanova S, Stefkov G. & Bankova V. 2007. Chemical composition of the essential oils of three *Micromeria* species growing in Macedonia and Bulgaria. *Maced. J. Chem. Chem. Eng.* **26**: 3-7.

Koul O, Walia S & Dhaliwal GS. 2008. Essential oils as green pesticides: Potential and constraints. *Biopestic. Int.* **4**: 63–84.

Lakušić B, Ristić M, Slavkovska V, Milenković M & Lakušić D. 2011. Environmental and seasonal impacts on the chemical composition of *Satureja horvatii* Šilić (Lamiaceae) essential oils. *Chem. & Biodiv.* **8**: 483-493.

Lakušić D, Ristić M, Slavkovska V & Lakušić B. 2013. Seasonal variations in the composition of the essential oils of rosemary (*Rosmarinus officinalis*, Lamiaceae). *Nat. Prod. Commun.* **8**: 131-134.

Marić S, Maksimović M & Miloš M. 2006. The impact of the locality altitudes and stages of development on the volatile constituents of *Salvia officinalis* L. from Bosnia and Herzegovina. *J. Essent. Oil Res.* **18**: 178-180.

Marinković B, Marin DP, Knežević-Vukčević J, Soković DM & Brkić D. 2002. Activity of Essential Oils of Three Micromeria Species (Lamiaceae) Against Micromycetes and Bacteria. *Phytother. Res.* **16**: 336–339.

Mastelic J & Jerkovic I. 2003. Gas chromatography–mass spectrometry analysis of free and glycoconjugated aroma compounds of seasonally collected *Satureja montana* L. *Food Chem.* **80**: 135–140.

Medine G, Münevver S, Fikrettin S, Atalay S, Ahmet A & Hakan O. 2004. Biological activities of the essential oil and methanolic extract of *Micromeria fruticosa* (L) Druce ssp *serpyllifolia* (Bieb) PH Davis plants from the eastern Anatolia region of Turkey. *J. Sci. Food Agric*. **84**: 735–741.

Milos M, Radonic A, Bezic N & Dunkic V. 2001. Localities and seasonal variations in the chemical composition of essential oils of *Satureja montana* L. and *S. cuneifolia* Ten. *Flavour Fragr. J.* **16**: 157–160.

Nur Herken E, Celik A, Aslan M & Aydinlik N. 2012. The constituents of essential oil: Antimicrobial and antioxidant activity of *Micromeria congesta* Boiss. & Hausskn. ex Boiss. from East Anatolia. *J. Med. Food.* **15**: 835-839.

Nurzyńska-Wierdak R, Bogucka-Kocka A, Kowalski R & Borowski B. 2012. Changes in the chemical composition of the essential oil of sweet basil (*Ocimum basilicum* L.) depending on the plant growth stage. *Chemija* **23**: 216-222.

Öztürk M, Kolak U, Topçu G, Öksüz S & Choudhary MI. 2011. Antioxidant and anticholinesterase active constituents from *Micromeria cilicica* by radical-scavenging activity-guided fractionation. *Food Chem.* **126**: 31–38.

Pavlidou V, Karpouhtsis I, Franzios G, Zambetaki A, Scouras Z & Mavragani-Tsipidou P. 2004. Insecticidal and genotoxic effects of essential oils of Greek sage, *Salvia fruticosa*, and mint, *Mentha pulegium,* on *Drosophila melanogaster* and *Bactrocera oleae* (Diptera: Tephritidae) 1. *J. Agric. Urban Entomol.* **21**: 39–49.

Putievsky E, Dudai N, Ravid U, Katzir E, Michaelovich Y, Zuabi E & Saadi D. 1995. Morphology, phenology and essential oil of *Micromeria fruticosa* (L). Druce in different seasons and plant parts. *J. Herbs Spices Med. Plants* **3**: 27-34.

Radulovic SN & Blagojevic DP. 2012. Volatile secondary metabolites of *Micromeria dalmatica* Benth. (Lamiaceae): Biosynthetical and chemotaxonomical aspects. *Chem. & Biodiv.* **9**: 1303-1319.

Rodrigues L, Póvoa O, Teixeira G, Figueiredo AC, Moldão M & Monteiro A. 2013. Trichomes micromorphology and essential oil variation at different developmental stages of cultivated and wild growing *Mentha pulegium* L. populations from Portugal. *Ind. Crop. Prod.* **43**: 692– 700.

Santos-Gomes PC & Fernandes-Ferreira M. 2001. Organ- and season-dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. *J. Agric. Food Chem.* **49**: 2908-2916.

Slavkovska V, Couladis M, Bojovic S, Tzakou O, Pavlović M & Lakušić B. 2005. Essential oil and its systematic significance in species of *Micromeria* Bentham from Serbia & Montenegro. *Plant Syst. Evol.* **255**: 1-15.

Stojanović G, Palić I, Ursić-Janković J, Vajs V & Đoković D. 1999. Chemical composition of the essential oil of *Micromeria albanica* (Griseb. ex K. Maly) Silic. *J. Essent. Oil Res.* **11**: 785-787.

Stojanović G, Palić I, Vajs V, Nasković T & Ursić-Janković J. 2000. Uporedno proučavanje antimikrobne aktivnosti etarskog ulja *Micromeria albanica* i njegovih najzastupljenijih komponenti. *Arhiv za farmaciju* **3-4**: 316-317.

Šavikin PK, Menković RN, Zdunić MG, Tasić RS, Ristić SM, Stević RT & Dajić-Stevanović PZ. 2010. Chemical composition and antimicrobial activity of the essential oils of *Micromeria thymifolia* (Scop.) Fritsch., *M. dalmatica* Benth., and *Satureja cuneifolia* Ten. and its secretory elements. *J. Essent. Oil Res.* **22**: 91-96.

Šilić Č. 1979. Monografija rodova *Satureja* L., *Calamintha* Miller, *Micromeria* Bentham, *Acinos* Miller i *Clinopodium* L. u flori Jugoslavije, Zemaljski muzej BiH, Sarajevo. pp. 172-262.

Telci I & Ceylan M. 2007. Essential oil composition of *Micromeria fruticosa* Druce from Turkey. *Chem. Nat. Comp.* **43**: 629-631.

Vladimir-Knežević S, Blažević N & Kalođera Z. 2001. Seasonal variation in the content and composition of the essential oil of *Micromeria thymifolia* (Scop.) Fritsch. *Acta Pharm.* **51**: 147-151.

Botanica SERBICA

REZIME

Promenljivost osobina etarskog ulja vrste *Clinopodium pulegium* (Lamiaceae) u zavisnosi od fenološke faze

Violeta Slavkovska, Bojan Zlatković, Christian Bräuchler, Danilo Stojanović, Olga Tzakou, Maria Couladis

U radu je praćena promenljivost količine i sastava etarkog ulja *Clinopodium pulegium* kroz različite stadijume razvoja biljke (vegetativni, stadijum cvetanja i plodonošenja).
Materijal je sakupljan iz samonikle (klisura Svrljiškog Timoka) i gajene (Niš) populacije. Etarsko ulje je izolovano iz nadzemnih delova biljaka destilacijom vodenom parom i analizirano GC-MS metodom. Ovo su prvi podaci o osobinama etarskog ulja biljaka populacije iz klisure Svrljiškog Timoka.
Svi uzorci kako iz samonikle tako i iz gajene populacije su se odlikovali velikim sadržajem ulja (0.8% - 1.4%) u svim stadijumima razvoja. Dvadeset jedna komponenta je identifikovana što predstavlja 95.3-99.6% ukupnog etarskog ulja.
Razlike u sastavu ulja su bile više kvantitativne nego kvalitativne. Kvalitet etarskog ulja je bio stabilan a variranja uslovljena promenom sredine relativno mala.
Dominacija glavnih komponenata je bila uslovljena promenama fenoloških faza. Pulegon je bio dominantan u vegetativnoj (76.1% u ulju biljaka samonikle populacije, 62.7% u ulju biljaka gajene populacije) i fazi cvetanja (49.5%, 64.6%), dok je menton bio dominantan (48.5%, 65.3%) u fazi plodonošenja.
Rezultati su pokazali da se biljake u vegetativnoj i fazi cvetanja mogu smatrati značajnim izvorom pulegona, a u fazi plodonošenja značajnim izvorom mentona. Pažljivim odabirom razvojnog stadijuma biljke moguće je dobiti etarsko ulje željenog sastava koje bi se moglo upotrebiti u komercijalne svrhe.

Ključne reči: *Clinopodium pulegium*, etarsko ulje, pulegon, menton, fenološke faze