Macedonian Journal of Chemistry and Chemical Engineering, Vol. **26**, No. 1, pp. 3–7 (2007)
GHTMDD – 494 ISSN 1857 – 5552
Received: November 6, 2006 UDC: 582.929.4.063.7–113.55
Accepted: March 20, 2007
Original scientific paper

CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF THREE *MICROMERIA* SPECIES GROWING IN MACEDONIA AND BULGARIA

Emanuela Kostadinova[1], Kalina Alipieva[1], Marina Stefova[2], Trajče Stafilov[2], Daniela Antonova[1], Ljuba Evstatieva[3], Vlado Matevski[4], Svetlana Kulevanova[5], Gjoshe Stefkov[5], Vassya Bankova[1]

[1]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Skopje, Republic of Macedonia*
[3]*Institute of Botany, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4]*Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje, Republic of Macedonia*
[5]*Institute of Pharmacognosy, Faculty of Pharmacy, Skopje, Republic of Macedonia*
bankova@orgchm.bas.bg

The essential oils from three species of genus *Micromeria* Bentham (fam. *Lamiaceae*) growing wild in Bulgaria (*M. dalmatica* and *M. cristata*) and Macedonia (*M. cristata* and *M. juliana*) have been analyzed and discussed in context with the results obtained for the corresponding *Micromeria* essential oils from the region. The obtained essential oils were analyzed by GC-MS and their components were identified according to their mass spectra and linear retention indices.

M. dalmatica was characterized by high quantities of essential oil, with oxygenated monoterpenes with *p*-menthane skeleton being the main constituents. The two samples of *M. cristata* from Bulgaria showed significant differences in their chemical composition. Monoterpenic alcohols prevailed in the sample from Bosnek, whereas sesquiterpenes dominated the oil from plants of the Ilindentsi population. Very similar content of total monoterpenes, oxygenated monoterpenes and sesquiterpenes was found in the essential oil of *M. cristata* from Alshar, Macedonia, and from Ilindentsi, Bulgaria. However, these two samples differed substantially in the variety of identified sesquiterpene compounds (20 compared to 5 and 4 in the other *M. cristata* oil samples). High content of sesquiterpenes was also found in the essential oil of *M. juliana* from Macedonia, with a distribution pattern in agreement with the data found for other samples of *M. juliana* from the region.

Key words: *Micromeria; Micromeria dalmatica*; *Micromeria cristata*; *Micromeria juliana*; *Lamiaceae*; essential oil composition; pulegone; piperitenone; menthone; α-bisabolol; verbenol

ХЕМИСКИ СОСТАВ НА ЕТЕРИЧНИТЕ МАСЛА ОД ТРИ ВИДА *MICROMERIA* КОИ РАСТАТ ВО МАКЕДОНИЈА И БУГАРИЈА

Анализирани се етеричните масла од три вида од родот *Micromeria* Bentham (фам. *Lamiaceae*) кои растат во Бугарија (*M. dalmatica* и *M. cristata*) и Македонија (*M. cristata* и *M. juliana*) и резултатите се дискутирани во контекст со резултатите добиени за етерични масла од соодветните видови *Micromeria* од регионот. Добиените етерични масла со гасна хроматографија – масена спектрометрија и идентификацијата на компонентите е извршена според нивните масени спектри и линеарни ретенциони индекси.

M. dalmatica се карактеризираше со големо количество етерично масло, во кое кислородните монотерпени со ментански скелет се доминантни компоненти. Двата примерока од *M. cristata* од Бугарија значително се разликуваа во хемискиот состав (монотерпенските алкохоли доминираа во примерокот од Боснек, додека сесквитерпените беа доминантни компоненти во примерокот од Илинденци). Слична содржина на вкупни монотерпени, кислородни монотерпени и сесквитерпени беше најдена и во етеричното масло од *M. cristata* од Алшар, Македонија, и во она од Илинденци, Бугарија. Сепак, овие два примерока значително се разликуваа по бројот на идентификувани сескви-

терпенски соединенија (20 споредено со 5 и 4 соединенија во другите две етерични масла од *M. cristata*). Големо количество од сесквитерпени беше исто така најдено и во етеричното масло од *M. juliana* од Македонија, а распределбата на сесквитерпенските компоненти беше во согласност со онаа за аналогни примероци од регионот.

Клучни зборови: *Micromeria; Micromeria dalmatica*; *Micromeria cristata*; *Micromeria juliana*; *Lamiaceae*; етерично масло; пулегон; пиперитенон; ментон; α-bisabolol; verbenol

INTRODUCTION

The species of genus *Micromeria* Bentham (fam. *Lamiaceae*) are perrenial herbs or dwarf shrubs. *Micromeria dalmatica* Benth is endemic for the Balkan peninsula (from southern Bulgaria, Dalmatia, Montenegro to northern and eastern Greece) [1, 2]. *Micromeria cristata* (Hampe) Griseb. is distributed in south-east Europe (northern and central part of the Balkan Peninsula) and south-west Asia, whereas *Micromeria juliana* (L.) Benth.et Reichenb. is found in the Mediterranean region, westwards to south-east France, north-west Africa and south-west Asia [1–3]. Plants of the genus *Micromeria* are used as remedies for a number of health problems: kidney stones [4], cough [5], and different infections and inflammations [6].

A considerable research has been directed towards chemical characterization of this genus because often the chemical analysis can be helpful in taxonomic differentiation between species with very similar morphology and for correlation with biological activity. Previous studies of the essential oils of different *Micromeria* species growing in Serbia and Montenegro have revealed their significant variability [7]. The composition of the essential oils of *M. cristata*, *M. juliana*, *M. dalmatica*, *M. albanica* and *M. thymifolia* from Serbia and Montenegro and their antimicrobial activity has also been reported [8, 9] as well as the analysis of the aromatic compounds of *M. juliana* from Croatia [4]. This work is a contribution in the research of the composition of the essential oils of the genus *Micromeria* from the Balkan Peninsula. Here, the results of a study on the essential oils of three species of *Micromeria* growing spontaneously in Bulgaria (*M. dalmatica* and *M. cristata*) and Macedonia (*M. cristata* and *M. juliana*) are reported and discussed in context with the results obtained for other *Micromeria* essential oils from the region.

EXPERIMENTAL

Plant material. Aerial parts of *M. dalmatica* were collected at flowering stage in July 2005 from: Rhodopi Mtn. near Trigrad, Bulgaria. Aerial parts of *M. cristata* were collected at flowering stage in July 2005 from two location in Bulgaria: Pirin Mountain, near the village of Ilindentsi, and Vitosha Mountain, Bosnek, and from one location in Macedonia, Alshar. Aerial parts of *M. juliana* were collected at flowering stage in July 2005 from a locations near Prilep in Macedonia. Voucher specimens are deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences, and the Herbarium of the Faculty of Pharmacy, Skopje.

Analysis of the oils. Oils were prepared by hydrodistillation of the air-dried plant material in a Clevenger-type apparatus for 2 h. GC-MS analyses were performed on a GC Hewlett Packard 6890 + MS 5973 (Hewlett Packard, Palo Alto, California, USA). A HP5-MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness, Agilent Technologies, Wilmington, Delaware, USA) was used. One μl of the analyzed essential oils was injected using a split ratio of 1:20. The ion source was set at 250 °C and the ionisation voltage was 70 eV. The temperature was programmed from 40 °C to 280 °C at 6 °C·min^{-1}, the inlet temperature was set to 260 °C, and the transfer line was kept at 280 °C. Helium was used as a carrier gas, at flow rate 0.8 ml·min^{-1}. Alkanes series C8-C24 (Retention Index Standard for GC, Sigma Aldrich) were used as reference points for calculation of linear retention indices (LRI) as described for linear temperature programming regime [10]. Compounds were identified using computer searches on commercial libraries and comparison of linear retention indices with literature data [11]. Relative percentage amounts of the separated compounds were calculated from Total Ion Chromatograms by computerized integration.

RESULTS AND DISCUSSION

The obtained essential oils were analyzed by GC-MS and identified according to their mass spectra and linear retention indices. The results for the five analyzed essential oils are represented in Table 1.

M. dalmatica was characterized by high quantities of essential oil. In the studied population, oxygenated monoterpenes with menthane skeleton were the main constituents, similarly to data reported for this species in Serbia [7]. Monoterpenic ketones were the main constituents: pulegone 35.8 %, piperitenone 18.6 %, menthone 15.8 %, and piperitone 8.0 %, which is consistent with the data published for the Serbian populations [7, 9].

Table 1

The composition (%) of the essential oils from Micromeria species from Bulgaria (BG) and Macedonia (MK)

Compound	RT/min	LRI*	*M. dalmatica* Trigrad, BG	*M. cristata* Bosnek, BG	*M. cristata* Ilindentsi, BG	*M. cristata* Alshar, MK	*M. juliana* Prilep, MK
MONOTERPENES			**85.3**	**40.8**	**4.7**	**5.8**	**9.3**
Monoterpene hydrocarbons			**5.8**	**0.1**	**0.2**	**0.8**	**0.9**
alpha-pinene	8.1	935	0.1				
sabinene	9.1	973	0.8				
beta-pinene	9.2	977	1.0				
beta-myrcene	9.6	992	0.2				
limonene	10.5	1027	3.5	0,1	0.2	0.8	0.9
(Z)-beta-ocimene	10.8	1038	0.1				
(E)-beta-ocimene	11.1	1050	0.1				
Oxygenated monoterpenes			**79.5**	**40.7**	**4.5**	**4.2**	**7.7**
eucaliptole	10.6	1030	0.5				
eucarvone	12.1	1088		2.1		0.3	
p-mentha-2,8-dienol	13.0	1124	0.1				
alpha-campholenal	13.1	1128		3.5		0.3	
trans-pinocarveol	13.4	1141		2.8		0.4	1.5
trans-verbenol	13.5	1145		16.6		0.2	
(E)-2-caren-4-ol	13.6	1149					3.8
trans-p-menthane-3-one	13.8	1157	15.8				
pinocarvone	14.0	1165		1.1			
borneol	14.1	1168		6.1		2.2	2.3
terpinen-4-ol	14.4	1181			0.3		
cis-isopulegon	14.5	1185	0.6				
gamma-terpineol	14.8	1197	0.1				
myrtenal	14.8	1196		2.5			
myrtenol	14.9	1201				0.3	
verbenone	15.2	1214		1.5			0.1
pulegone	16.0	1248	35.8	0.5	4.2	0.5	
piperitone	16.3	1261	8.0				
bornyl acetate	17.0	1291		4.0			
piperitenone	18.3	1350	18.6				

Compound	RT/min	LRI*	*M. dalmatica* Trigrad, BG	*M. cristata* Bosnek, BG	*M. cristata* Ilindentsi, BG	*M. cristata* Alshar, MK	*M. juliana* Prilep, MK
SESQUITERPENES			**10.1**	**13.9**	**59.1**	**63.3**	**40.5**
Sesquiterpene hydrocarbons			**9.7**	**6.3**	**4.1**	**8.1**	**14**
alpha-copaene	19.1	1386	0.2			0.4	
(E)-caryophyllene	20.1	1433			0.3	1.4	4.5
9-epi-(E)-caryophyllene	20.6	1457	0.1			0.3	
alpha-gurjunene	20.7	1461		2.8			
allo-aromadendrene	20.8	1466		1.2		0.2	0.9
germacrene D	21.3	1490	6.9		2.7	1.6	6.1
bicyclogermacrene	21.5	1500	2.2		1.1		
germacrene A	21.6	1505					2.5
beta-bisabolene	21.8	1515				0.4	
gamma-cadinene	21.9	1520		2.3		0.9	
delta-cadinene	22.0	1525	0.2			1.9	
trans-cadina-1,4-diene	22.3	1540				1.0	
Oxygenated sesquiterpenes			**0.4**	**7.6**	**55**	**55.2**	**26.5**
beta-atlantol	23.7	1612				9.9	
1-epi-cubenol	23.9	1623				2.8	
unidentified, MS: 222(58), 207(59), 151(67), 110(100), 95(42),83(58), 69(73)	22.5	1551					6.9
(E)-nerolidol	22.9	1571				1.2	
spatulenol	23.2	1587	0.4		1.5		5.6
caryophyllene oxide	23.4	1597			14.3	2.1	11.2
davanol	23.7	1613				0.5	2.1
gamma-eudesmol	24.2	1639				1.2	
alpha-cadinol	24.4	1650		7.4	0.7	8.8	
beta-eudesmol	24.7	1666				1.8	
alpha-bisabolol	25.3	1696			38.5	7.7	0.7
germacrone	25.4	1703				18.5	
beta-cyperone	26.4	1760		0.2		0.7	
OTHERS			**0.2**	**0.0**	**1.2**	**2.1**	**2.4**
isopropyl myristate	27.6	1828			0.2	0.3	1.2
octadecane	27.1	1799				0.3	
4-phenyl-2-butanone	15.5	1227			0.2		
benzyl benzoate	26.6	1771				0.7	
4-methylbenzoylacetone	26.7	1776					1.2
octen-3-ol	9.3	981	0.2				
2-octanone	9.8	1000				0.1	
hexahydrofarnesyl acetone	27.9	1845			0.8	0.7	

*Alkanes series C8-C24 were used as reference points for calculation of linear retention indices (LRI) as described for linear temperature programming regime [10].

The two samples of *M. cristata* from Bulgaria showed significant differences in their chemical composition. In the sample from Bosnek monoterpenic alcohols prevailed: verbenol 16.6 % and borneol 6.1 %. This composition was similar to those reported for *M. cristata* from Serbia [8] and Turkey [12]. In the oil from plants of the Ilindentsi population sesquiterpenes dominated; the major components were α-bisabolol (38.5 %) and caryophyllene oxide (14.3 %). In Turkish samples [12], caryophyllene oxide was found to be among the important constituents but in the Serbian ones it was absent [7] or present at low concentration [8]. The low percentage of monoterpenes in the Ilindentsi population demonstrates that considerable changes in the oil composition of *M. cristata* are possible under different ecological conditions. This variability has already been demonstrated by the results published for *M. cristata* from Serbia and Montenegro, where the ratio of monoterpenes/sesquiterpenes found was 30 %/58 % [7] and 64 %/23 % [8].

The content of total monoterpenes, oxygenated monoterpenes, and sesquiterpenes found in the essential oil of *M. cristata* from Alshar, Macedonia, is almost identical with the sample from Ilindentsi, Bulgaria, and the Serbian one [8]. However, the Macedonian sample significantly differs in the variety of compounds identified, especially sesquiterpenes (20 in this sample compared to 4 and 5 sesquiterpenes in the other two studied *M. cristata* oils). The most abundant sesquiterpenes in this sample were found to be germacrone 18.5 %, beta-atlantol 9.9 %, delta-cadinol 8.8 %, and alpha-bisabolol 7.7 %.

High content of sesquiterpenes (40.5 %) was also found in the essential oil of *M. juliana* from the region of Prilep in Macedonia. Germacrene D (6.1 %) and caryophyllene (4.5 %) were the most abundant sesquiterpene hydrocarbons, whereas caryophyllene oxide (11.2 %) and spathulenol (5.6 %) were most abundant oxygenated sesquiterpenes. This sesquiterpene distribution pattern is in agreement with the data found for samples of *M. juliana* from Montenegro [7], and partly with the results for other samples from Montenegro [8] and Croatia [4].

The relative stability of the qualitative composition of the essential oils within each of the studied taxons compared to literature data implies that the genetic factor, but also the ecological conditions play an important role in the content and composition of the essential oils. Consequently, the characterization of the essential oils can be used as additional attributes in defining the species of the genus *Micromeria.*

Acknowledgement: The authors are grateful for the financial support provided by the Ministry of Education and Science of the Republic of Macedonia and by the Bulgarian Ministry of Education and Science in the frame of bilateral cooperation in the period of 2005-2006 (contracts 17-2035/4 and BM-5, respectively).

REFERENCES

[1] A. O. Chater, E. Guinea, Genus *Micromeria* Bentham. In: Tutin T. G., Heywood V. H., Burges N. A., Moore, D. M., Valentine D. H., Walters S. M., Webb D. A., *Flora Europaea*, Vol. 3, pp. 167–170. Cambridge, University Press (1972).

[2] M. Anchev, *Micromeria* Benth. In: Velchev, V. (ed.), *Fl. Reipubl. Popularis Bulgaricae*. Vol. **9**, pp. 356–362. In: *Aedibus Acad. Sci. Bulgaricae, Serdicae* (1989) (in Bulgarian).

[3] K. Micevski, The Genus *Micromeria* in the Flora of Republic of Macedonia. Macedonian Academy of Sciences and Arts, *Contributions*, Section of Biological and Medical sciences, **23**, 1–2, 11–23 (2002) (in Macedonian).

[4] J. Mastelić, I. Jerković, D. Kuštrak, Aromatic compounds of *Micromeria juliana* (L.) Bentham ex Reichenb. from Croatia. *J. Essent. Oil Res.* **17**, 516–518 (2005).

[5] Y. Diab, L. Auezova, H. Chebib, Chemical composition of the essential oil of *Micromeria libanotica* Boiss., an endemic species of Lebanon. *J. Essent. Oil Res.* **17**, 449–450 (2005).

[6] L. Viegi, A. Pieroni, P. M. Guarrera, R. Vangelisti, A review of plants used in folk veterinary medicine in Italy as basis for a databank. *J. Ethnopharmacol.* **89**, 221–244 (2003).

[7] V. Slavkovska, M. Couladis, O. Bojović, M. Pavlović, B. Lakušić, Essential oil and its systematic significance in species of *Micromeria* Bentham from Serbia & Montenegro. *Plant Syst. Evol.* **255**, 1–15 (2005).

[8] G. Stojanović, I. Palić, J. Ursić-Janković, Composition and antimicrobial activity of the essential oil of *Micromeria cristata* and *Micromeria juliana*. *Flavour Fragr. J.* **21**, 77–79 (2006).

[9] B. Marinković, P. D. Marin, J. Knežević-Vukčević, M. D. Soković, D. Brkić, Acitivity of essential oils of three *Micromeria* species (Lamiaceae) against micromycetes and bacteria, *Phytother. Res.* **16**, 336–339 (2002).

[10] I. G. Zenkevich, Kovats Retention Index System, *Encyclopedia of Chromatography*, Marcel Dekker, New York, 2003.

[11] R. P. Adams, *Identification of essential oil components by gas chromatography/quadrupole mass spectrometry*, Allured Publishing Corporation, 2001.

[12] N. Tabanka, N. Kirimer, B. Demirci, F. Demirci, K. H. Kan Baser, Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *Phrygia* and the enantiomeric distribution of borneol, *J. Agric. Food Chem.* **49**, 4300–4303 (2001).