Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2014

43

29-34

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: _____

Original Scientific Paper

Seasonal Variation in Content and Chemical Composition of Essential Oils from Leaves of *Mentha longifolia* Huds. (*Lamiaceae*)

Nikšić H.[a*], Kovač-Bešović E.[a], Durić K.[a], Korać N.[b], Omeragić E.[a], Muratović S.[a]

[a]*University of Sarajevo, Faculty of Pharmacy, Zmaja od Bosne 8, Sarajevo, BiH*

[b]*Public Institution Occupational Medicine of Sarajevo Canton, Sarajevo, BiH*

Article info
Received: 03/11/2014
Accepted: 08/12/2014

Keywords:
essential oil,
Menthalongifolia,
phenophase,
piperitone oxide

***Corresponding author:**
Haris Nikšić
E-mail: harisniksic@gmail.com
Phone: +387 61 219 444
Fax:

Abstract: The aim of the present study was to appraise variation in content and the chemical composition of essential oil from the leaves of *Mentha longifolia* as affected by harvesting season. Quantities of the essential oils were determined according to the European Pharmacopoeia 4th Ed. and chemical profiles by using a gas chromatography – mass spectrometry. The highest content of the essential oil was found plant during the flowering stage (1.9%). The main constituents of the essential oil through all the three phenophases were oxygened monoterpenes piperitone oxide (1.9-63.6%) and 1,8-cineole (5-12%), and sesquiterpenes trans-caryophyllene (3-9.3%) and germacrene D (0.16-10%). In general, the composition of the essential oil in all the three investigated phenophases was generally similar with quantitative differences. Oxygenated monoterpenes were dominant during flowering stage and after flowering stage, followed by sesquiterpene hydrocarbons. Presence of β-ocimene, γ-terpinene, and carvone were noticed only before flowering; cederole, β-cubebene and α-caryophyllene during the flowering stage; and 3-carene after flowering. Analysis of the qualitative and quantitative composition of main constituents of the essential oil in all the three investigated phenophases led to the conclusion that the piperitone oxide, as the major compound could be used as the stable chemotype marker for the taxonomy of *Mentha longifolia*.

INTRODUCTION

The genus *Mentha* L., member of the family *Lamiaceae*, consists of approximately 14-25 species, grows widely throughout the temperate regions of the world (Harley, 1972; Gobert et al., 2002). *Mentha longifolia*, member of the family Lamiaceae is a perennial bushy plant and upright, reaches height of about 1 m. Strongly aromatic, the leaves are formed in pairs opposite to each other along the square-shaped stem. *Mentha longifolia*is is used in herbal medicine and is native to the Mediterranean region and Middle East. It is mainly used for the treatment of respiratory ailments, but many other uses have been recorded. Leaves are used the most , usually for preparation of tea against coughs, colds, stomach cramps, asthma, flatulence, indigestion and headaches (Gulluce et al., 2007).

In the Lamiaceae family, essential oils are mainly produced in secretary structures known as glandular trachomes, of which there are two main kinds, peltate and capitates. The amount of essential oils produced is directly connected with the number and physiology of these structures. Essential oils are very complicated mixtures of

natural compounds at quite different concentrations (Bozin et al., 2006). Factors that determine the composition and yield of the essential oil obtained are numerous. In some instances it is difficult to segregate these factors from each other, since many are interdependent and influence each other. These variables may include seasonal and maturity variation, geographical and climatic conditions, genetic variation, growth stages, part of plant utilized and postharvest drying, storage and mode of distillation. The chemical composition of the essential oils from plants is thus subject to quantitative and qualitative variations. Biological activity which is dependent on the chemical composition is similarly subject to variation. Plant material collected at different times of the year may contain different novel compounds with other bioactivities. Examination of the published literature on the oil composition of *Mentha longifolia* reveals that it can exist in a myriad of chemical forms, as can be seen from the main constituents found in these oils (Dzamić et al., 2010). The main constituents in essential oil were piperitone oxide (13.90-50.50%), 1,8-cineole (8.18-17.80%), carvone (0.5-21.5%), beta caryophyllene (2.0-22.0%) and menthol (0.0-32.50%). Literature revealed that essential oil contents depend not only on temperature, relative humidity, but also on duration of sunshine, air movement and rainfall (Viljoen et al., 2006).

The aim of the present study was to appraise variation in content and the chemical composition of essential oil from the leaves of *Mentha longifolia* Huds. (Lamiaceae) as affected by harvesting season (from May to September). The present study describes the qualitative and quantitative composition, together with the content of essential oil of *Mentha longifolia*, native to Herzegovina, during three different phenophases (before flowering, in the flowering stage and after flowering).

EXPERIMENTAL

Chemicals and Reagents: (-)–α Thujone (≥ 96% GC) analytical standard (SigmaGermany)(No. 89231); (-)-β Pinene 99+% (Sigma Germany); R -/+) – Limonene analytical standard (Fluka Germany) (No.62118); Eucalyptol analytical standard (Fluka Germany) (No. 29210); Linalool analytical standard (Fluka Germany) (No. 51782); (+)-Carvone analytical standard (Fluka Germany) (No. 22070); Thimol standard (Fluka Germany) (No.50409); (−)-trans-Caryophyllene ≥98.5% (sum of enantiomers, GC) (Sigma Germany) (No. 22075).

Plant Material

Aerial parts of wild growing flowering plants of *Mentha longifolia* Huds. (Lamiaceae) were collected on the banks of the Jablaničko lake (Bosnia and Herzegovina) during three phenophases (before flowering, flowering and after flowering) in 2011. Voucher specimens of collected plants No. 1060/1 before flowering, 1060/2 flowering stage, 1060/3 after identity conformation by an independent expert were deposited at the Herbarium of the Department of Biology, Faculty of Sciences, University of Sarajevo.

Isolation of the Essential Oil

The leaves of *Mentha longifolia* were shade dried (15 days) at room temperature. Air-dried plants of *Menth alongifolia* were submitted to hydrodistillation according to European Pharmacopoeia 4th Ed., using Clevenger apparatus (Klaus Hofmann GmbH, Germany). The essential oil samples of each phenophase were dried over anhydrous sodium sulfate. The quantity of predistilled essential oils was determined volumetrically (Council of Europe, 2002).

Essential Oil Analysis

Qualitative and quantitative analyses of the essential oils were carried out by using gas chromatography/mass spectrometry system (GC-MS, Agilent Tecnologies series 6890N/5975B, United States of America) at electron energy of 70eV, equipped with a split-splitless injector (200°C) and a flame ionization detector (FID) (250°C). helium (1 mL/min) was used as a carrier gas. The capillary columns (HP 5MS 30m x 0.25mm; film thickness 0.25µm Agilent Tecnologies) were used. The temperature programmes were 50°C to 280°C at a rate of 10°C/min until 130°C and 130-280°C at a rate of 12°C/min, respectively with split ratio, 1:10. Co-elution and MS analysis based on the identification of the individual compounds, and the comparison of their relative retention times (RI) with those of the reference samples were performed. For the components, mostly sesquiterpenes and aliphatic compounds, for which reference substances were not available, the identification was performed by matching their retention times and mass spectra with those obtained from the authentic samples and/or The National Institute of Standards and Technology, known as the National Bureau of Standards (NIST/NBS), Wiley libraries spectra as well as with literature data (Adams, 2007).

RESULTS AND DISCUSSION

The mean oil content in the plants of *Mentha longifolia* collected in flowering stage amounted to 1.9% v/w (volume of essential oil/weight dry leaf) in dry matter. In plants collected after flowering stage the essential oil content was 1.45% v/w, while in the plants collected before flowering it was 0.49 % v/w (Table 1.).The essential oil content was in accordance with the earlier published data (Mkaddem et al., 2009; Kofidis et al. 2006).

Table 1. Essential oil content during phenophases

Phenophase	before flowering	flowering stage	after flowering
Essential oil content % v/w	0.49	1.9	1.45

The percentage composition of the essential oils during three investigated phenophases is presented in Table 2. A total of the 33, 36 and 26 chemical constituents, representing 98.42, 98.17 and 98.84% of the total content, were identified in the essential oil before flowering, in the flowering stage and after flowering, respectively. In the oil obtained from the plants collected in flowering stage the oxygenated monoterpenes were found to be the major class of substances (87.1%), followed by the sesquiterpene hydrocarbons (6.8%) and oxygenated sesquiterpenes (5.57%) (Figure 2). The oil extracted from the leaves of *Mentha longifolia* in the period before floweringcontained oxygenated monoterpenes (63.57%) and higher amount of sesquiterpene hydrocarbons (23.16%). The essential oil from the plants collected after flowering was composed mainly of monoterpenic fraction 89% (oxygenated monoterpenes 78.51% and monoterpene hydrocarbons 10.39%) (Table 2, Figure 2). In the essential oil of *Mentha longifolia* during investgated phenophases, three compounds were dominant: piperitone oxide, 1.8-cineole andgermacrene D.

Table 2. Chemical composition of *Mentha longifolia* L. essential oil during investigated phenophases

Peak No	Components	R.I.[a]	before flowering %	flowering stage %	after flowering %
	Monoterpene hydrocarbons		**10,42**	**3,06**	**10,39**
1	α-thujene	**932**	0,13		
2	α-pinene	**938**	1,06	0,78	5,89
3	sabinene	**974**	0,43	0,47	0,68
4	β-pinene	**978**	0,92	0,99	2,57
5	β-mircene	**992**	0,77	0,69	0,48
6	terpinolene	**1008**		0,07	0,35
7	limonene	**1035**		0,06	0,11
8	δ-carene	**1031**			0,31
9	*E*-β-ocimene	**1050**	6,97		
10	γ-terpinene	**1063**	0,14		
	Oxygenated monoterpenes		**63,57**	**87,1**	**78,51**
11	1,8-cineole	**1036**	5	12,03	9,3
12	*trans*-sabinene hydrate	**1098**		0,68	
13	linalool	**1099**	1,94		
14	*cis*-sabinol	**1143**		0,16	0,27
15	borneole	**1167**	0,58	0,52	0,52
16	piperitone oxide	**1170**	1,91	63,58	59,99
17	terpinen-4-ol	**1178**	0,7	0,1	
18	1-α-terpineole	**1188**	0,13	0,91	0,34
19	carvone	**1243**	52,26		
20	bornyil acetate	**1288**	0,24		0,86
21	thymol	**1291**		1,69	1,2
22	*trans*-carvyl acetate	**1342**	0,23		
23	piperitenone	**1343**		1,98	2,43
24	piperitenone oxide	**1369**	0,13	4,81	3,35
25	*cis*-jasmone	**1395**	0,45	0,64	0,25
	Sesquiterpene hydrocarbons		**23,16**	**6,79**	**8,25**
26	α-copaene	**1375**	0,26	0,19	0,12
27	β-burbonene	**1383**	1,25	0,54	0,21
28	β-cubebene	**1390**		0,48	
29	β-elemene	**1391**	0,61	0,18	0,21
30	*cis*-caryophyllene	**1405**		0,82	
31	*trans*-caryophyllene	**1419**	9,27	2,98	4,2
32	α-humulene	**1452**	0,89	0,44	
33	*allo*-aromadendrene	**1462**		0,23	
34	α-amorfene	**1485**		0,26	
35	germacrene D	**1490**	9,94	0,16	3,17
36	α-murolene	**1500**	0,14	0,11	
37	biciclogermacrene	**1501**	0,29		
38	γ-cadinene	**1514**		0,31	
39	δ-cadinene	**1523**	0,51	0,09	0,34
	Oxygenated sesquiterpenes		**2,1**	**5,57**	**0,42**
40	spathulenol	**1578**	0,14		
41	caryophyllene oxide	**1582**	0,85	4,33	0,34
42	cedrole	**1601**		0,51	
43	τ-murolol	**1651**	0,47	0,2	0,08
44	α-cadinole	**1654**	0,64	0,53	
	Aliphatic compounds		**1,27**	**1,22**	**1,27**
45	3-octanole	**991**	1,27	1,16	1,27
46	*n*-udecanole	**1370**		0,06	
	Total identified (%)		**98,42**	**98,17**	**98,84**

[a]Retention indices relative to C9-C24 *n*-alkanes on the HP-5MS column

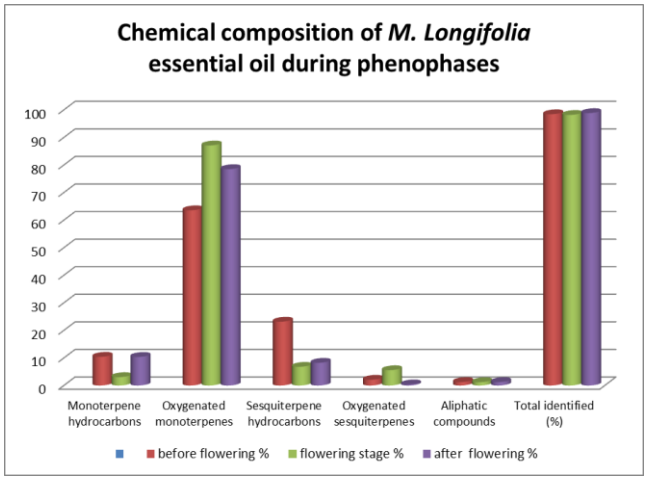

Figure 1. Major classes of substances in the investigated essential oil during three phenophases (%)

The essential oil from the plants in the flowering stage and after flowering stage revealed the high piperitone oxide content, which is in accordance with the earlier published data. The content of piperitone oxide in before flowering stage was significantly lower (1.91%), but high amount of carvone was recorded. In opposite, in samples collected in flowering stage and after flowering stage, piperitone oxide was dominant compound (63.58% and 59.99%). The following major compounds of the essential oils in all the three investigated phenophases were 1.8-cineole (5-12%), germacrene D (0.16-9.94%), trans-caryophyllene (3-9.3%), and α-pinene (0.78-5.89%). Apart from the general similarity in the main compounds, there were significant differences with respect to their quantity (Table 2). Furthermore, some qualitative differences in the chemical composition of the essential oil during investigated phenophases were also observed. In the essential oil obtained from the plants collected before flowering stage the presence of notable amount of carvone 52.26% and β-ocimene 7% was recorded. Vijoen et al. (2006) reported piperitenone oxide (15-66%) as the main compounds identified in the essential oils of piperitenone oxide chemotype of *Mentha longifolia*. Gulluce et al. (2007) reported *cis* piperitone epoxide, pulegone, piperitenone oxide, as the main components of essential oil from *Mentha longifolia*, growing in Turkey. Also Stanisavljević et al (2014) reported that dominate components in essential oils from Serbia were: piperitone (50-71%), carvone (2.9-20%), menthone (up to 17%), trans-caryophylene (4.3-5.4%), 1,8-cineole (0.8-1.3%). Samples of essential oils from Iran were characterized by a high amount of three oxygenated monoterpenescarvone (1–26%) and pulegone (7.5–31%) and piperitone oxide (4–14%) (Giti et al., 2014). Earlier data pertaining to *Mentha longifolia* essential oil point out the persistence of four chemotypes: piperitone oxide, piperitone oxide/piperitenone oxide, p-menthone/piperitone oxide and trans-dihydrocarvonechemotype (Aksita et al., 2013). The investigated essential oils, obtained from the plant material in flowering stage and after flowering stage could be categorized as piperitone oxide chemotype, but plant material collected in period before flowering stage has a specific chemical composition and could not be categorized in one of the four previously described chemotypes. The minor variations in the chemical compositions of *Mentha longifolia* essential oil across countries might be attributed to the varied agro-climatic (climatical, seasonal, geographical) conditions of the regions, isolation regimes and adaptive metabolism of plants.

CONCLUSIONS

In general, harvesting season affected the chemical composition of *Mentha longifolia* essential oils. The variation in the content of the essential oils investigated in the present study, with respect to species and harvesting season, was quantitatively significant (0.49- 1.9%). In essential oil from *Mentha longifolia*, the major variation observed was in piperitenone oxide (1.9–63.6%), 1.8 cineole (5–12%), *trans*-caryophyllene (3-9.27%) and germacrene D (0.16–9.94%). The qualitative and quantitative composition of main constituents of the essential oil in all the three investigated phenophases led to the suggestion that the piperitone oxide, as the major compound could be used as the stable chemotype marker in the *Mentha longifolia* taxonomy. Also the information observed on seasonal variation may be useful in selecting the best season for optimal yield.

REFERENCES

Adams, R., Adams, R. (2007). Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. (1st Ed.) Carol Stream, Ill.: Allured Pub. Corp. Council of Europe. (2002). *European Pharmacopeia*. (4th Ed.), Strasbourg Cedex, France, (p.p. 183–184).

Aksita, H., Demirtas, I., Telci, I., Tarimcilar, G. Chemical diversity in essential oil composition of Mentha longifolia (L.) Hudson subsp typhoides (Briq.) Harley var. typhoides from Turkey. *Journal of essential oil research, 25(5):430-437*

Bozin, B., Mimica-Dukić, N., Simin, N., Anackov, G. (2006). Characterization of the volatile composition of essential oils of some *Lamiaceae* species and the antimicrobial and antioxidant activities of the entire oils. *Journal of Agricultural and Food Chemistry, 54:1822-1828.*

Džamić, A. M., Soković, M. D., Ristić, M. S., Novaković, M., Grujić-Jovanović, S., Tešević, V. (2010). Antifungal and antioxidant activity of *Mentha longifolia* (L.) Hudson (Lamiaceae) essential oil. *Botanica Serbica, 34:57-61*

Giti, B., Ali, M., Salimpour, F.(2014). Essential oil composition of four varieties of Mentha longifolia L. From northern parts of Iran. *International Journal of Plant, Animal and Environmental Sciences, 4(2): 639-643.*

Gobert, V., Moja, S., Colson, M., Taberlet, P. (2002). Hybridizationin the section Mentha (Lamiaceae) inferred from AFLP markers. *American Journal of Botany, 89(12):2017-2023.*

Gulluce, M., Shain, F., Sokmen, M., Ozer, H., Daferera, D., Sokmen, A. (2007). Antimicrobial and antioxidant properties of the essential oils andmethanol extract from *Mentha longifolia* L. spp. longifolia. *Food Chemistry, 103:1449-1456*

Harley, R.M. (1972). Mentha L. in: Flora Europaea III. Tutin, T.G, Heywood, V.H, Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (eds.) Cambridge University Press, Cambridge, (p.p.183-186).

Kofidis, G., Bosabalidis, A., Kokkini, S. (2006). Seasonal variations of essential oils ina linalool-rich chemotype of Mentha spicata grown wild in Greece. *Journal of Essential Oil Research, 16, 469-472.*

Mkaddem, M., Bouajila, J., Ennajar, M., Lebrihi, A., Mathieu, F., Romdhane, M. (2009). Chemical composition and antimicrobialand antioxidant activities of *Mentha longifolia* L. and viridis essential oils. *Journal of Food Science, 74 (7), 358-363.*

Stanisavljević, D., Đordević, S., Milenković, M., Lazić, M., Veličković, D., Randelovic, N., Zlatković, B. (2014). Antimicrobial and Antioxidant Activity of the Essential Oils Obtained from *Mentha longifolia* L. Hudson, Dried by Three Different Techniques. *Recordes of natural products, 8 (1): 61-65.*

Viljoen, A.M., Petkar, S., Van-Vuuren, S.F., Figueiredo, A.C., Pedro, L.G., Barroso, J.G. (2006). The chemo geographical variation in essential oilcomposition and the antimicrobial properties of wild mint, Menthalongifolia subsp. polyadena (Lamiaceae) in Southern Africa. *Journal of Essential Oil Research, 18, 60–65.*

Summary/Sažetak

Cilj ovog istraživanja je utvrditi razlike u sadržaju i hemijskom sastavu eteričnog ulja iz lista vrste *Mentha longifolia* tokom vegetacijskih stadija biljke tokom sezone branja. Tokom tri fenofaze poređen je odnos sadržaja i hemijskog sastava izoliranih eteričnih ulja. Sadržaj eteričnih ulja u drogama određen je prema četvrtoj Evropskoj farmakopeji, a hemijski sastav eteričnog ulja analiziran je tehnikom gasna hromatografija – masena spectrometrija (GC-MS). Najveći sadržaj eteričnog ulja pronađen je u fazi cvjetanja biljke od 1,9%. Glavni sastojci eteričnog ulja tokom sve tri fenofaze su bili oksidovani monoterpeni piperiton oksid (1.9 – 63.6%) i 1,8-cineol (5-12%),te seskviterpeni β-kariofilen (3-9,3%) i germakren D (0,16-10%). Premda je sastav eteričnog ulja u sve tri ispitivane fenofaze kvalitativno sličan, utvrđene su kvantitativne razlike u sastavu. Oksidirani monoterpeni bili su dominantna frakcija u fazama cvjetanja i nakon cvjetanja nakon koje slijedi frakcija seskviterpenski ugljikovodonika. Prisutnost β-ocimena, γ-terpinena i karvona utvrđena je samo u fazi prije cvjetanja, dok je prisustvo cederola, β-kubebena i α-kariofilena zabilježeno sami u fazi cvjetanja, a komponenta 3-karen bila je prisutna samo nakon nakon cvjetanja. Analiza kvalitativnog i kvantitativnog sastava glavnih sastojaka eteričnog ulja u sve tri istraživane fenofaze dovelo je do zaključka da piperiton oksid, kao glavna komponenta se može se koristiti kao pouzdan hemotipski marker u taksonomiji vrste *Mentha longifolia*.