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Identification of the essential oils composition from four ecotypes of *Mentha longifolia* (L.) Huds. growing wild in Isfahan province, Iran

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ABSTRACT
Mentha longifolia L., commonly known as wild mint, belongs to family Lamiaceae. The aim of this study was to identify the chemical composition of essential oil extracted from four ecotypes of *Mentha longifolia* L. grown wild (Shahreza, Chadegan, Isfahan, and Falavarjan) in Isfahan province (Central Iran). The essential oil was extracted by a Clevenger approach and analyzed using GC/MS. In the aerial parts of the plant were identified 26, 30, 22 and 25 compounds for Shahreza, Chadegan, Isfahan and Falavarjan ecotypes, respectively. The major constituents of the essential oil from the aerial parts of *M. longifolia* in Shahreza province were piperitenone oxide (26.71%), 1,8-cineole (20.72%), α-pinene (14.28%), pulegone (7.81%), sabinene (7.06%) and trans-caryophyllene (4.23%). The main compositions in Chadegan province were piperitenone oxide (29.13%), 1,8-cineole (28.84%), sabinene (9.05%), pulegone (8.97%) and α-pinene (6.31%). The main compositions in Isfahan province were pulegone (44.75%), 1,8-cineole (13.82%), 2-cyclohexen-1-ol, 1-methyl (8.49%), isopulegone (8.07%) and menthone (4.37%). In Falavarjan province the constituents were pulegone (33.39%), 1,8-cineole (29.79%), sabinene (11.23%) and isopulegone (7.28%).

Key words: *Mentha longifolia*, chemical constitutes, piperitenone oxide, 1,8-cineole

Introduction

Lamiaceae is subdivided into two major groups – Lamioideae and Nepetoideae. The genus *Mentha* belongs to the subfamily Nepetoideae (Bremer et al., 1998). Lamiaceae is one of the large plant families used as a framework to evaluate the occurrence of some typical secondary metabolites (Wink, 2003). The typical secondary metabolites of Lamiaceae includes various terpenoids and phenolic compounds (Hegnauer, 1989). Genus *Mentha* includes 25 to 30 species that grow in the temperate regions of Eurasia, Australia and South Africa (Dorman et al. 2003).

Mentha longifolia (L.) Huds. is an aromatic perennial rhizomatous herb with erect to straggling stems, square in cross section, finely pubescent and up to 1.5 m long, leaves simple, opposite, up to 90 mm long and 22 mm wide, flowers small (corolla 3-5mm long) that grows mostly in semi-shady places on moist soils. Its leaves or fresh shoots are mostly used as peppermint-scent and for flavoring in salads and cooked foods (Shinwari et al., 2011). The essential oils of some *Mentha* species are potential candidates for exhibiting antimicrobial, antioxidant, antispasmodic, carminative, radical-scavenging and cytotoxic activities (Gulluce et al., 2007).

Piperitone, piperitenone oxide and piperitone epoxides were reported as the major oil constituents of *M. longifolia* (Kharkwal et al., 1994). Saeidi et al. (2012) found that the major compounds of *Mentha longifolia* wild grown in Iran are piperitenone oxide (7.41 to 59.67%), pulegone (3.61 to 49.43%), 1,8–cineole (7.25 to 24.66%), α-terpineol (2 to 6%) and β-pinene (1.32 to 4.19%). Raluca Andro et al. (2011) reported piperitone-oxide (36.74%), limonene (17.61%), β-cubebene (8.05%), β-mircene (7.38%), trans-β-ocimene (5.64%) and β-cariophyllene (3.20%) as major compounds of *M. longifolia*. It is well known that the yield and the components of plants are determined by a series of factors including plant genetics (Shafie et al., 2009), climate, edaphic, elevation, topography and also an interaction of various factors (Rahimmalek et al., 2009). Therefore, the main goal of this study was identification of the chemical composition of essential oil isolated from four ecotypes of *Mentha longifolia* grown wild in Isfahan province, Iran.

Materials and Methods

Plant materials

The aerial parts from the four ecotypes of *Mentha longifolia* were collected at Isfahan province (Shahreza, Chadegan, Isfahan, and Falavarjan) in central Iran, during 2014.

Shahreza is a county in Isfahan province (32°, 00 N and 51°, 52 E). Chadegan is also a county in Isfahan province, (32°, 46 N and 50°, 38 E). Isfahan is the capital of the Isfahan province (32°, 38 N and 51°, 39 E). Falavarjan is another county in Isfahan province (32°, 28 N and 51°, 28 E).

Essential oil extraction

Fresh aerial parts from the four ecotypes of *Mentha longifolia* were dried for six days at room temperature (25±5°C) and powdered using Moulinex food processor. The essential oil was extracted from 50 g of ground tissue in 1 L of water contained in a 2 L flask and heated by heating jacket at 100°C for 3 h in a Clevenger–type apparatus, according to producers outlined in the British Pharmacopoeia. The collected essential oil was dried over anhydrous sodium sulphate and stored at 4°C until analyzed.

Identification of the oil components

Compositions of the essential oils were determined by GC–MS. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. HP-5MS column (30 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas with flow rate of 1.0 mL/min. The oven temperature was kept 20°C at 50°C for 4 min and programmed to 280°C at a rate of 5°C /min, and kept 20°C constant at 280°C for 5 min, at split mode. The injector temperature was at 20°C at 280°C. Transfer 20 line temperatures 280°C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 450.

Identification of the essential oil components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (Adams, 2007).

Results

The chemical constituents identified by GC-MS are presented in Table 1. Results indicated that in the aerial parts of *M. longifolia*, 26, 30, 22 and 25 compounds were identified in Shahreza, Chadegan, Isfahan and Falavarjan province, respectively. The major constituents of the essential oil from the aerial parts of *M. longifolia* in Shahreza province were piperitenone oxide (26.71%), 1,8-cineole (20.72%), α-pinene (14.28%), pulegone (7.81%), sabinene (7.06%) and trans-caryophyllene (4.23%). In Chadegan province we found piperitenone oxide (29.13%), 1,8-cineole (28.84%), sabinene (9.05%), pulegone (8.97%) and α-pinene (6.31%). The main compounds in the Isfahan ecotype were pulegone (44.75%), 1,8-Cineole (13.82%), 2-cyclohexen-1-ol, 1-methyl (8.49%), isopulegone (8.07%) and menthone (4.37%). The main compositions of the essential oil in Falavarjan province were pulegone (33.39%), 1,8-cineole (29.79%), sabinene (11.23%) and isopulegone (7.28%).

Discussion

In the present work, 1,8-cineole, pulegone, piperitenone oxide, sabinene and α-pinene were the major components of the four ecotypes of *M. longifolia*. The results of this study showed that the pulegone content in *M. longifolia* of Isfahan and Falavarjan province was higher in comparison with the other areas, whereas the piperitenone oxide content of Shahreza and Chadegan province was higher in comparison with the other provinces (Table 1). Recent findings indicated that some of the medicinal plant characteristics can be affected by genetic and ecological factors, including precipitation, temperature and plant competition. Since essential oils are the product of a predominantly biological process further studies are needed to evaluate if the reported characteristics of each population are maintained at the level of individual plants and along the breeding and selection program when grown under climatic conditions (Ghasemi Pirbalouti & Mohammadi, 2013). For example, according to Golparvar et al. (2013a) the major components of two ecotypes *Mentha longifolia.* collected at Isfahan province, were 1,8-cineole (15.58%), piperitenone oxide (15.05%), pulegone (9.58%), sabinene (9.52%) and the major components in Lorestan province were p-mentha-3,8-diene (10.53%), 2,6-dimethyl-2,4,6-octatriene (10.13%), sabinene (6.98%), β-caryophyllene (6.971%), piperitone oxide (6.77%) and pulegone (6.60%).

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An earlier report by Rezaei et al., (2000) indicated the volatile constituents of *Mentha longifolia* (L.) Hudson var. chlorodictya Rech. F. from three different locations. The major constituents of the sample 1 were piperitenone oxide (33.91%), isopiperitenone (11.98%) and piperitone (8.40%); sample 2 – isopiperitenone (57.96%), piperitone oxide (19.99%) and 1,8-cineole (5.49%); and sample 3 – piperiton (43.96%), 1,8-cineole (13.73%), trans-piperitol (12.92%) and cis-piperitol (9.34%).

Table 1. *Chemical compositions of essential oils of four ecotypes Mentha longifolia (L) Hudson.*

No	Compound	RI	Shahreza	Chadegan	Isfahan	Falavarjan
	Monoterpene hydrocarbons					
1	α-Thujene	926	-	0.21	-	0.21
2	α-Pinene	935	14.28	6.31	0.84	3.81
3	Camphene	950	1.86	0.29	1.27	0.69
4	Sabinene	975	7.06	9.05	3.21	11.23
5	β-Myrcene	994	3.98	1.11	1.12	1.08
	Oxygenated monoterpenes					
6	1,8-Cineole	1035	20.72	28.84	13.82	29.79
7	(*Z*)-β-Ocimene	1045	0.56	0.61	4.11	1.38
8	γ-Terpinene	1063	-	-	0.64	0.26
9	Terpinolene	1087	0.23	0.81	-	0.39
10	Linalool	1103	0.54	0.36	0.31	-
11	3-Octanol, acetate	1113	0.23	-	-	-
12	2-Octanol, acetate	1124	0.46	1.12	-	-
13	1,3-Benzenediol, 4-ethyl	1138	0.29	-	-	-
14	trans-Pinocarveol	1144	0.16	0.68	0.51	0.24
15	Menthone	1154	0.39	0.71	4.37	1.11
16	Mnthofuran	1168	-	0.15	0.78	0.62
17	Borneol	1170	2.65	1.12	-	
18	Isopulegone	1185	1.17	1.03	8.07	7.28
19	Myrtanol	1192	-	0.17	-	0.17
20	α-Terpineol	1195	0.23	0.24	1.41	0.74
21	2-Cyclohexen-1-ol, 1-methyl	1220	3.01	1.92	8.49	4.78
22	2(1H)-Pyrimidinone, 4-amino-5-methyl-	1231	-	-	0.97	0.33
23	Pulegone	1235	7.81	8.97	44.75	33.39
24	Carvone	1244	0.65	0.53	0.83	0.81
25	Piperitone	1273	0.26	0.21	-	-
26	Naphthalene, 1-isocyano-	1294	0.59	0.61	1.79	0.51
27	2-Cyclopenten-1-one, 3-methyl-2-(trimethyl silyl)	1308	-	0.19	0.23	0.23
28	Pulespenone	1345	-	0.31	-	-
29	α-Terpinolene	1349	1.21	2.45	-	0.24
30	Piperitenone oxide	1363	26.71	29.13	1.49	0.19
	Sesquiterpene hydrocarbons					
31	trans-Caryophyllene	1425	4.23	2.25	0.64	0.25
32	α-Humulene	1458	0.25	0.21	-	-
33	Germacrene-D	1575	0.13	0.17	-	-
34	Caryophyllene oxide	1583	-	0.16	0.31	0.19
	Total		**99.66**	**99.92**	**99.96**	**99.92**

[a] Compounds listed in order of elution, RI (Retention Indices)

Jaymand & Rezaei (2002) obtained piperitone (67.6%), isomenthone (6.6%) and cis-piperitol (4.2%) as major constituents in *Mentha longifolia* (L.) Huds. var. *asiatica* (Boriss.) Rech. f. of the leaf oil, while the flower oil contained piperitone (55.7%), carvone (16.2%) and pulegone (4.1%).

An earlier report by Jamzad et al. (2013) indicated that major components in the aerial parts of *Mentha longifolia* (L.) Hudson var. *chlorodictya* Rech. f. collected from two different locations (Gilan and Mazandaran Provinces) in Iran were cis-piperitone oxide (36.4 and 40.5%), piperitenone oxide (22.5 and 37.3%) and caryophyllene oxide (13.65 and 7.43%). Jaymand et al. (2002) reported that the major constituents obtained from *Mentha longifolia* (L.) Hudson var. *kermanansis* in flower oil are piperitenone oxide (44.3%), piperitone (25.3%) and piperitenone (10.6%) and in leaf oil – piperitenone oxide (45.7%), piperitone (30.6%), piperitenone (5.6%). For *Mentha longifolia* (L.) Hudson var. *kotschiana* in flower oil were piperitone (58.2%), 1,8-cineole (26.7%) and piperitenone oxide (4.6%) and in the leaf oil – piperitone (64%) and 1,8-cineole (28.4%). In 2003, Mazandarani & Rezaei investigated the major constituents of *Mentha longifolia* (L). Hudson var. *chlorodictya* Rech. F. samples collected from two different habitats. In Sample-1 they found p-menth-1-en- 9-o1 (62.1%), α-caryophyllene (6.3%) and carvacrol (4.8%) and for sample-2 – p-menth-1-en-9-ol (36.1%), 1,8-cineole (14.4%), piperitone (9.7%), carvacrol (9.3%) and germacrene D (9.01%). Analyzing the chemical composition of three ecotypes of spearmint (*Mentha spicata* L.) in Isfahan province, Golparvar et al. (2013b) detected carvone, 1,8-cineole, limonene and piperitenone oxide. In another report by Rezaei et al. (2000) the volatile constituents of *Mentha longifolia* (L.) Hudson var. *chlorodictya* Rech. F. from three different locations, were identified: sample 1 – piperitenone oxide (33.91%), isopiperitenone (11.98%) and piperitone (8.40%); sample 2 – isopiperitenone (57.96%), piperitone oxide (19.99%) and 1,8-cineole (5.49%); and sample 3 – piperiton (43.96%), 1,8-cineole (13.73%), trans-piperitol (12.92%) and cis-pipeitol (9.34%).

The percentage of chemical composition in oil of *M. longifolia* grown in different countries is different: e.g. Piedmont valley (Italy) – rich in piperitenone oxide (77.43%); Southern Africa – rich in piperitenone oxide (15-66%) (Viljoen et al., 2006); Gabes (Tunisia) – pulegone (54.41%), isomenthone (12.02%), 1,8-cineole (7.41%), borneol (6.85%), and piperitone oxide (3.19%) (Mkaddem et al. 2009); Iran – carvone (61.8%) and limonene (19.4%) (Monfared et al., 2002) and Serbia – trans- and cis-dihydrocarvone (23.64% and 15.68%), piperitone (17.33%), 1,8-cineole (8.18%), and neoisodihydrocarveol (7.87%) (Dzamic et al., 2010). The variations in chemical composition of the essential oils with respect to season might have been due to the influence of the phenological status and environmental conditions. They can influence the regulation of the biosynthesis of essential oil (Masotti et al., 2003). Another report on oil of *M. longifolia* from Himalayan region showed carvone (61.12-78.70%), dihydrocarveol (0.40-9.45%), *cis*-carvyl acetate (0.16-6.43%) and germacrene D (1.25-5.73%) as major constituents (Mathela et al., 2005).

Talebi Kouyokhi et al. (2008) reported that phytochemical variations were not only found among samples of different regions, but also among samples of the same region with different altitude reflecting the effect of environment on essential oil components.

In conclusion, the results obtained in our study indicated that the major components of the essential oil from the four ecotypes of *M. longifolia* collected from Isfahan province are 1,8-cineole, pulegone, piperitenone oxide, sabinene and α-pinene. The differences of our results with the other reports could be attributed to genetic factors (genus, species, and ecotype), chemotype, distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases, and extraction and quantification methods.

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