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Diversity in chemical composition from two ecotypes of (*Mentha Longifolia* L.) and (*Mentha spicata* L.) in Iran climatic conditions

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Abstract

The genus Mentha, which belongs to the Lamiaceae family, subfamily Nepetoideae. Chemical composition from two ecotypes of (*Mentha Longifolia* L.) and (*Mentha spicata* L.) grown of Iran in Chelgard (Chaharmahal and Bakhtiari province) and Baghe-Bahadoran (Isfahan province) were investigated. The essential oil was extracted by a Clevenger approach and analyzed using GC/MS. Results indicated of aerial parts (*M. Longifolia*) 21 and 27, compounds were identified in Chelgard and Bagh-e Bahadoran province, respectively. The major constituents of the essential oil from the aerial of (*M. Longifolia*) in Chelgard province were; 1,8-cineole (37.16%), piperitenone oxide (18.97%), sabinene (13.93%), α-pinene (8.92%) and pulegone (6.14%). The main compositions in Baghe-Bahadoran province were; 1,8-cineole (34.26%), pulegone (27.97%), sabinene (7.89%), α-pinene (4.64%) and isopulegone (4.52%). Results indicated of aerial parts (*M. spicata*) 20 and 16, compounds were identified in Chelgard and Bagh-e Bahadoran province, respectively. The major constituents of the essential oil from the aerial of (*M. spicata*) in Chelgard province were; carvone (42.74%), trans dihydrocarvone (21.58%), 1,8-cineole (8.41%), pulegone (6.83%), Limonene (6.1%) and β-Caryophyllene (3.05%). The main compositions in Baghe-Bahadoran province were; carvone (54.34%), 1,8-cineole (21.78%), Linalool (5.82%), Limonene (5.2%) and trans dihydrocarvone (3.18%). The composition of the essential oil two ecotypes of (*Mentha Longifolia* L.) and (*Mentha spicata* L.) depends on many factors of genetic, environmental and their interaction effects, such as plant part, harvest-time, extraction-method, ecotype and geographic origin (climate, edaphic, elevation and topography.

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Introduction

Lamiaceae is subdivided in to two major groupings; the Lamioideae and Nepetoideae. The genus Mentha, which belongs to the Lamiaceae family, 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 metabolism of Lamiaceae includes various terpenoids and phenolic compounds (Hegnauer, 1989). The genus Mentha includes 25 to 30 species that grow in the temperate regions of Eurasia, Australia and South Africa (Dorman *et al.,* 2003). The species of section Mentha typically have chromosome number 2n=2x=12, but the other species vary widely, with (*M. spicata*) and (*M. longifolia*) have 2n=2x=48 and 2n=2x=24, respectively (Lawrence, 2007). The spearmint, *M. spicata*, is a hybrid of *M. longifolia* and *M. rotundifolia*, morphological, cytological and biochemical data have shown that the tetraploid species of *M. spicata* 2n=48 (Lawrence, 2007) originated by chromosomal doubling of hybrids between the two closely related and inter-fertile diploids, *M. longifolia* and *M. suaveolens* (Harley and Brighten, 1977). 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).

Oil from an individual of the polymorphic species *M. spicata* may have any (but only one) of the three ketone groups. The chemical constituents in the oil of *M. spicata* were carvone 58%, limonene 8%, dipentene 10%, dihydrocarveol7%. The very musty odor of *M. longifolia* (L.) Hudsis that of pure piperitone oxide, its principal ketone. This species has smaller amounts of the related ketone, piperitenone oxide. *M. longifolia* has piperitone oxide 56%, pipertenone 20% (Murray, 1960).

In studies (Saeidi *et al.,* 2012) the major compounds *Mentha longifolia* (L.) Hudson grown wild in Iran were 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%). In studies (Raluca Andro *et al.,* 2011) the major compounds *M. longifolia* were 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%). In studies (Golparvar *et al.,* 2013) the chemical composition of three ecotypes of spearmint (*Mentha spicata* L.) in Isfahan province were carvone, 1,8-cineole, limonene and piperitenone oxide. In studies (Padalia *et al.,* 2013) carvone (51.3-65.1%), limonene (15.1-25.2%), β-pinene (1.3-3.2%) and 1,8-cineole (≤ 0.1-3.6%) were the major constituents in the essential oils from five cultivars of *M. spicata,* while in one cultivar (Ganga) of *M. spicata* the major constituents were piperitenone oxide (76.7%), α- terpineol (4.9%), and limonene (4.7%). In studies (Boukhebti *et al.,* 2011) the major compounds of *Mentha spicata* (L.) were carvone (59.40%), limonene (6.12%), germacrene- D (4.66%), β-caryophyllene (2.969 %), β-bourbonene (2.796 %), α-terpineol (1.986 %), Terpinene-4-ol (1.120 %). It is well known that yield and yield components of plants are determined by a series of factors including plant genetic (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 determine the variation of chemical composition of the essential oils from the aerial parts of two ecotypes of (*Mentha Longifolia* L.) and (*Mentha spicata* L.) grown of Iran.

Material and methods

Plant material

The aerial parts aerial parts of two ecotypes of (*Mentha Longifolia* L.) and two ecotypes (*Mentha spicata* L.) were collected at Chelgard (Chaharmahal and Bakhtiari province) and Baghe-Bahadoran (Isfahan province) in Southwest Iran, during spring 2014. Chelgard is a city in and the capital of Kuhrang County, (Chaharmahal and Bakhtiari) province (32°, 28 N and 50°, 07 E). Baghe-Bahadoran, is a city in and the capital of Lenjan County, (Isfahan) province (32°, 22 N and 51°, 11 E).

Essential oil extraction

The fresh aerial of two ecotypes of (*Mentha Longifolia* L.) and (*Mentha spicata* L.) were dried inside for six days at room temperature (25 ± 5 °C), and the ground to fine a powder 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 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 ofretention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system) (Adams, 2007).

Results and discussion

Chemical composition

The chemical constituents identified by GC-MS, are presented in Table 1 and 2. Results indicated of aerial parts (*M. Longifolia*) 21 and 27, compounds were identified in Chelgard and Bagh-e Bahadoran province, respectively. The major constituents of the essential oil from the aerial of (*M. Longifolia*) in Chelgard province were; 1,8-cineole (37.16%), piperitenone oxide (18.97%), sabinene (13.93%), α-pinene (8.92%) and pulegone (6.14%). The main compositions in Baghe-Bahadoran province were; 1,8-cineole (34.26%), pulegone (27.97%), sabinene (7.89%), α-pinene (4.64%) and isopulegone (4.52%) (Table 1).

Results indicated of aerial parts (*M. spicata*) 20 and 16, compounds were identified in Chelgard and Bagh-e Bahadoran province, respectively. The major constituents of the essential oil from the aerial of (*M. spicata*) in Chelgard province were; carvone (42.74%), trans dihydrocarvone (21.58%), 1,8-cineole (8.41%), pulegone (6.83%), Limonene (6.1%) and β-Caryophyllene (3.05%). The main compositions in Baghe-Bahadoran province were; carvone (54.34%), 1,8-cineole (21.78%), Linalool (5.82%), Limonene (5.2%) and trans dihydrocarvone (3.18%) (Table 2).

In the present work, the 1,8-cineole, pulegone, piperitenone oxide, sabinene and α-pinene were the major components of two ecotypes *M. Longifolia* L. (Table 1). Also the carvone, 1,8-cineole, pulegone, limonene, trans dihydrocarvone and β-caryophyllene were the major components of two ecotypes *M. spicata* L. (Table 2). 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 and Mohammadi, 2013).

The results of this study showed that the 1,8-cineole and Piperitenone oxide content in (M. Longifolia) of Chelgard province was higher in comparison with Baghe-Bahadoran province, whereas pulegone content in Baghe-Bahadoran province was higher in comparison with Chelgard province (Table 1). For example, according to (Golparvar *et al.,* 2013) the major components two ecotypes *Mentha Longifolia* L. collected at Isfahan and Lorestan province, in Isfahan 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 (10.531%), 2,6-Dimethyl-2,4,6-octatriene (10.132%), Sabinene (6.98%), β-Caryophyllene (6.971%), Piperitone oxide (6.77%) and Pulegone (6.60%). 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-pipeitol (9.34%). In studies (Jaymand and Rezaei, 2002) indicated the major constituents obtained from of *Mentha longifolia* (L.) Huds. var. asiatica (Boriss.) Rech. f. of the leaf oil were; piperitone (67.6%), isomenthone (6.6%) and cis-piperitol (4.2%), while the flower oil contained piperitone (55.7%), carvone (16.2%) and pulegone (4.1%). An earlier report by (Jamzad *et al.,* 2013) indicated the major components aerial parts of *Mentha longifolia* (L.) Hudson var. *chlorodictya* Rech. f. collected from two different locations in (Gilan and Mazandaran Provinces) 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%). In studies (Jaymand *et al.,* 2002) indicated the major constituents obtained from *Mentha longifolia* (L.) Hudson var. kermanansis in flower oil were piperitenone oxide (44.3%), piperitone (25.3%) and piperitenone (10.6%) and in leaf oil were; piperitenone oxide (45.7%), piperitone (30.6%), piperitenone (5.6%), and 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 leaf oil were; piperitone (64%) and 1,8-cineole (28.4%). In studies (Mazandarani and Rezaei, 2003) indicated the major constituents for *Mentha longifolia* (L). Hudson var. Chlorodictya Rech. F. samples collected from two different habitats. Sample-1 were; p-menth-1-en- 9-01 (62.1 %), α-caryophyllene (6.3%) and carvacrol (4.8%) and for sample-2 were; p-menth-1-en-9-ol (36.1 %), 1,8-cineole (14.4%), piperitone (9.7%), carvacrol (9.3%) and germacrene D (901%).

Table 1. Chemical compositions of essential oils of two ecotypes *Mentha Longifolia* L.

Row	Compound[a]	RI	% GC peak area	
			Chelgard	**Baghe- Bahadoran**
	Monoterpene hydrocarbons			
1	α-Thujene	926	-	0.19
2	α-Pinene	935	8.92	4.64
3	Camphene	950	1.27	0.56
4	Sabinene	975	13.93	7.89
5	β-Myrcene	994	0.89	1.01
	Oxygenated monoterpenes			
6	1,8-Cineole	1035	37.16	34.26
7	(*Z*)-β-Ocimene	1045	0.64	1.78
8	γ-Terpinene	1063	-	0.37
9	Terpinolene	1087	0.65	0.41
10	Linalool	1103	0.29	0.31
11	3-Octanol, acetate	1127	0.34	-
12	1,3-Benzenediol, 4-ethyl	1138	2.86	-
13	trans-Pinocarveol	1144	-	0.72
14	Menthone	1155	-	2.07

Row	Compound[a]	RI	% GC peak area	
			Chelgard	Baghe- Bahadoran
15	(-)-Pinocarvone	1160	0.18	-
16	Menthol	1176	0.89	2.06
17	Isopulegone	1185	0.75	4.52
18	*cis*-Dihydrocarveol	1190	-	0.86
19	Myrtanol	1192	-	0.18
20	α-Terpineol	1195	-	0.69
21	*trans*-Carveol	1219	2.67	4.36
22	*cis*-Carveol	1230	-	0.56
23	Pulegone	1235	6.14	27.97
24	Carvone	1244	0.49	0.48
25	Piperitone	1254	0.41	0.42
26	Pulespenone	1345	0.63	-
27	α-Terpinolene	1349	-	1.93
28	Piperitenone oxide	1363	18.97	0.56
	Sesquiterpene hydrocarbons			
29	β-Bourbonene	1385	0.32	0.25
30	Germacrene D	1575	1.56	0.35
31	Caryophyllene oxide	1583	-	0.45
	Total		99.96	99.85

RI (Retention index on DB-5 fused silica capillary column).

Table 2. Chemical compositions of essential oils of two ecotypes *Mentha spicata L.*

Row	Compounds	RI	% GC peak area	
			Chelgard	Baghe- Bahadoran
	Monoterpene hydrocarbons			
1	α-Pinene	935	0.95	1.68
2	Camphene	950	-	0.28
3	Sabinene	975	0.39	0.71
4	β-Pinene	978	1.2	1.63
	Oxygenated monoterpenes			
5	Limonene	1032	6.11	5.2
6	1,8-Cineole	1035	8.41	21.78
7	(*Z*)-β-Ocimene	1045	-	0.34
8	Linalool	1103	0.85	5.82
9	α-Terpineol	1195	-	1.48
10	trans dihydrocarvone	1207	21.58	3.18
11	2(1H)-Pyridinethione, 1,5-dimethyl	1211	1.29	0.34
11	*trans*-Carveol	1219	1.26	-
12	*cis*-Carveol	1230	0.24	-
13	Pulegone	1235	6.83	-
14	Carvone	1244	42.74	54.34
15	Piperitone	1254	0.22	0.51

Row	Compounds	RI	% GC peak area	
			Chelgard	Baghe- Bahadoran
16	Pulespenone	1345	1.16	-
17	α-Terpinolene	1349	1.37	0.65
18	Piperitenone oxide	1363	0.28	-
	Sesquiterpene hydrocarbons			
19	β. Bourbonene	1385	1.31	0.34
20	β-Caryophyllene	1420	3.05	1.65
21	β-copaene	1434	0.28	-
22	γ-cadinene	1511	0.22	-
	Total		99.74	99.93

RI (Retention index on DB-5 fused silica capillary column).

The results of this study showed that the carvone and 1,8-cineole content in (*M. spicata*) of Baghe-Bahadoran province was higher in comparison with Chelgard province, whereas trans dihydrocarvone and pulegone content in Chelgard province was higher in comparison with Baghe-Bahadoran province (Table 2).

In studies (Golparvar and Adelpoor, 2013) indicated the major components three ecotypes of Mentha spicata L. from Kohgiluyeh va Boyer-Ahmad Province in Iran were carvone (74.57%), 1,8-cineole (10.28%), limonene (8.41%) for Yasouj Province, whereas C-Sakht Province had piperitenone oxide (53.19%), 1,8-cineole (27.47%), *trans*-caryophyllene (3.55%), and the main components of Bahram-Beigi Province were 1,8-cineole (8.79%), carvone (79.6%), and lmonene (3.53%). Results (Kokkini and Voko, 1989) indicated the major compounds of *Mentha spicata* (L.) grown wild in Greece, were linalool, piperitenone oxide, carvone-dihydrocarvone and pulegone-menthone-isomenthone. (Chauhan *et al.,* 2008) reported that the *Mentha spicata* L. (spearmint) collected from different sub-tropical and temperate zones of North-West Himalayan region of India were carvone between 49.62–76.65%, second major component was limonene between 9.57–22.31%, 1,8-cineole varied between 1.32–2.62%, whereas *trans*-carveol varied between 0.3- 1.52%. The compounds essential oil from aerial parts of *Mentha spicata* L. collected from "Tazouka" (Errachidia-Morocco) were carvone (29.00%) and trans carveol (14.00%) (Znini *et al.,* 2011). (Mustafa and Bader, 2005) reported that the difference among species could be related to the variants in the alleles numbers between *Mentha* species, and it may be more obvious in the asexual plants *M. longifolia*. The genetic variability, found among the species, could be due to out-breeding and the wide dispersal of seeds and pollen grains. Divergence between *M. longifolia* and *M. spicata* could be a reflection of the impact of environmental variation among the samples of *Mentha* species. (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.

Conclusion

The results of this study provide data on variation of phytochemical characteristics of the essential oils from two ecotypes of (*Mentha Longifolia* L.) and (*Mentha spicata* L.). The present study indicates the essential oil components of two ecotypes of (*M. Longifolia*) and (*M. spicata*) vary with genotype and chemotypes. Results of current study indicate that 1,8-cineole, pulegone, piperitenone oxide, sabinene and α-pinene for *M. Longifolia* and carvone, 1,8-cineole, pulegone, limonene, trans dihydrocarvone and β-caryophyllene for *M. spicata* are the main constituents of the essential oils. The composition of the essential oil two ecotypes of (*M. Longifolia* L.)

and (*M. spicata* L.) depends on many factors of genetic, environmental and their interaction effects, such as plant part, harvest-time, extraction-method, ecotype and geographic origin (climate, edaphic, elevation and topography.

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