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Four Ecotypes of *Mentha pipertia* in Iran; Phytochemical study

Amin Hadipanah*, Aysan Ghahremani**, Kazem Aghaee*, Mahmoudreza Zolfaghar and Hamidreza Ardalani****

**Department of Horticultural Sciences, Science and Research Branch, Islamic Azad University, Tehran, IRAN*
***Department of Horticultural Sciences, Shirvan Branch, Islamic Azad University, Shirvan, IRAN*
****Department of Agronomy, Varamin-Pishva Branch, Islamic Azad University, Varamin-Pishva, IRAN*

(Corresponding author: Hamidreza Ardalani)
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ABSTRACT: Peppermint (*Mentha piperita* L.) belongs to the Lamiaceae family is an herb medicinal and aromatic perennial. The aim of this study was to identification of the phytochemical components of Peppermint cultivated in Iran climatic conditions. The study carried out in Isfahan, Chaharmahal and Bakhtiari provinces, Central and Southwest of Iran, on 2014. The essential oils of samples were obtained by hydro-distillation, and analysed by GC/MS. The major constituents of the essential oil Saman were menthol (47.36%), menthone (12.96%), menthofuran (7.26%), menthyl acetate (6.89%) and 1,8-cineole (6.86%). The major constituents of the oil Kuhrang were menthol (41.42%), menthone (15.25%), menthofuran (10.85%), 1,8-cineole (8.38%) and menthyl acetate (5.71%). The major constituents of the oil Kamu were menthol (38.67%), menthone (18.68%), menthofuran (8.24%), Isomenthone (6.82%) and menthyl acetate (5.24%). The major constituents of the oil Chadegan were menthol (44.68%), menthone (14.35%), menthofuran (6.83%) and 1,8-cineole (8.76%).

Keywords: (*Mentha piperita* L.), chemical composition, ecotypes, phytochemical study, peppermint

INTRODUCTION

Peppermint (*Mentha piperita* L. belongs the family Lamiaceae, is one of the most important medicinal and aromatic plants which used in food, sanitary and cosmetic industries. The leaves of peppermint are strongly scented due to the presence of essential oils. Peppermint is widely used for its medicinal properties such as anti-spasmodic, anti-sickness, anti-helminthic, carminative, and stomachic, etc. Peppermint cultivated in the temperate, Mediterranean and subtropical regions of the world (Nostro *et al.,* 2000; Ormancey *et al.,* 2001). Peppermint (*Mentha piperita* L.) which is tetraploid (2n = 72), is a sterile natural hybrid of *M. aquatica* L. (2n = 96) and *M. spicata* L. (2n = 48) (Tucker, 1992). The plant is a perennial with 50-60 cm tall. The square stems are usually reddish-purple and smooth. The leaves are short, oblong-ovate and serrate. The flowers are purple-pinkish and appear in the summer months. The plant has runners above and below ground (Yazdani *et al.,* 2003; Mozaffarian, 2008). The essential oil of peppermint is between 1 to 2.5% in the leaves dried which is mostly made up from menthol (50%), menthone (10 to 30%), menthyl esters (up to 10%) and further monoterpene derivatives (pulegone, piperitone, and menthofurane) (Murrray, 1995). Among the major components found in peppermint leaves are fatty acids such aslinoleic, linolenic, and palmitic acid. A variety of volatile compounds, mainly menthol, menthone and isomenthone have also been identified along with β-carotene, chlorophyll, α- and γ-tocopherols and ascorbic acid (Figueroa Perez *et al.,* 2014). Developmental and environmental factors are known to greatly influence the yield and composition of mentha species oil. For example, Gavahian *et al.,* (2015) reported the major constituents of essential oils in *M. piperita* were neoiso-menthol, iso-menthone and menthofuran. Golparvar and Hadipanah (2013) reported the essential oils composition of *Mentha piperita* L. collected from Isfahan province were camphane (14.01%), menthone (13.89%), menthol (12.37%) β-pinene (7.62%), pulegone (6.41%), β-cubebene (4.95%), α-pinene (4.743%), γ-terpinene (4.08%), delta-carane (3.81%) and piperiton (3.04%). Derwich *et al.,* (2010) reported 29 compounds identified in the leaves oil. In addition they reported the yield of essential oil of *Mentha piperita* was 1.02% and the major compound in were menthone (29.01%), followed by menthol (5.58%), menthyl acetate (3.34%), menthofuran (3.01%), 1,8-cineole (2.40%), isomenthone (2.12%), limonene (2.10%), α-pinene (1.56%), germacrene-D (1.50%), β-pinene (1.25%), sabinene (1.13%), and pulegone (1.12%). A report by Ka *et al.,* (2005) on the chemical composition of *M. piperita* essential oil indicated that the major constitutes were menthol (18 mg/g) and neo-menthol (0.72 mg/g), as well as menthol (28-42 %), menthone (19-27 %), and 1,8- cineole (4-5 %) (Iscan *et al.,* 2002).

Various researchers reported that essential oil yield and its components in medicinal and aromatic plants in general is primarily related to their genetic (Shafie *et al.* 2009), climate, edaphic, elevation and topography (Abdossi *et al.*, 2015; Abedi *et al.*, 2015, Ardalani *et al.*, 2014). The aim of this study was to chemical analysis and identification of the components of Peppermint (*Mentha piperita* L. cultivated in Iran province.

MATERIALS AND METHODS

A. Plant material

The aerial parts from of (*Mentha piperita* L.) were collected on 2014 from 4 localities in Isfahan, Chaharmahal and Bakhtiari Provinces, Central and Southwest of Iran (Table 1).

Table 1: Geographical and environmental conditions collected from *M. piperita* in geographic different.

Site no	Locality	Province	Latitude	Longitude	Altitude (m asl[1])
1	Saman	Chaharmahalva Bakhtiari	32°27'31.9" N	50°54'11.2" E	1871
2	Kuhrang	Chaharmahalva Bakhtiari	32°27'59.6"N	50°17'18.0" E	2128
3	Kamu	Isfahan	33°36'21.4" N	51°14'04.9" E	1984
4	Chadegan	Isfahan	32°46.11'.3"N	50°38'55.3" E	1867

B. Essential oil extraction

Fresh aerial parts were dried at room temperature (25 ± 5°C). Dried plant material was powered (100 gm, and subjected to hydro-distillation (1000 ml distillated water) for 3 hrs using a Clevenger-type apparatus according to the method recommended in BP (British Pharmacopoeia, 1988). Samples were dried with anhydrous sodium sulfate and kept in amber glass vials at 4°C ± 1°C until use.

C. 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 × 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. Retention indices were calculated for all components using a homologous series of n-alkanes (C5-C24) injected under conditions used with the oil samples. 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 (WILLEY/ChemStation data system) (Adams 2007).

RESULTS AND DISCUSSION

A. Essential oil yield

The essential oils extracted from the aerial parts of *M. piperita* produced a clear, yellow liquid. The essential oil yields were obtained from the aerial of *M. piperita*, 1.87, 1.52, 1.48 and 1.32 ml / 100 g dry matter identified in Saman, Kuhrang, Kamu and Chadegan province, respectively (Table 2).

B. Chemical composition

The chemical constituents identified by GC-MS, are presented in Table 2. GC-MS analyses resulted in *M. piperita* essential oil, 25, 23, 27 and 30 compounds were identified in Saman, Kuhrang, Kamu and Chadegan province, respectively.

The oil of Saman components corresponding to 98.88% and consisted mainly of oxygenated monoterpenes (95.58%) with a small amount of monoterpene hydrocarbons (1.77%) and sesquiterpene hydrocarbons (1.28%). The major constituents of the oil Saman were menthol (47.36%), menthone (12.96%), menthofuran (7.26%), menthyl acetate (6.89%) and 1,8-cineole (6.86%). The oil of Kuhrang components corresponding to 99.82% and consisted mainly of oxygenated monoterpenes (93.59%) with a small amount of monoterpene hydrocarbons (3.81%) and sesquiterpene hydrocarbons (2.0%). The major constituents of the oil Kuhrang were menthol (41.42%), menthone (15.25%), menthofuran (10.85%), 1,8-cineole (8.38%) and menthyl acetate (5.71%).

The oil of Kamu components corresponding to 99.57% and consisted mainly of oxygenated monoterpenes (95.59%) with a small amount of monoterpene hydrocarbons (3.49%). The major constituents of the oil Kamu were menthol (38.67%), menthone (18.68%), menthofuran (8.24%), Isomenthone (6.82%) and menthyl acetate (5.24%). The oil of Chadegan components corresponding to 99.22% and consisted mainly of oxygenated monoterpenes (92.77%) with a small amount of monoterpene hydrocarbons (5.24%). The major constituents of the oil Chadegan were menthol (44.68%), menthone (14.35%), menthofuran (6.83%) and 1,8-cineole (8.76%).

Table 2 : Chemical composition of essential oils four ecotypes of *Mentha piperita* L.

No	Compounds	RI	% GC peak area			
			Saman	Kuhrang	Kamu	Chadegan
1	α-Thujene	926	tr	-	-	0.67
2	α-Pinene	935	1.24	0.78	0.85	2.42
3	Sabinene	975	-	-	0.26	tr
4	β-Pinene	985	0.13	1.74	-	1.25
5	β-Myrcene	994	0.35	0.98	tr	-
6	α-phellandrene	1005	-	-	0.19	tr
7	p-Cymene	1019	tr	-	0.57	tr
8	Limonene	1025	1.85	2.31	0.98	2.12
9	1,8-Cineole	1035	6.86	8.38	4.93	8.76
10	(*Z*)-β-Ocimene	1045	tr	-	0.73	0.19
11	γ-Terpinene	1063	-	0.31	tr	tr
12	trans-Sabinene hydrate	1074	-	-	-	tr
13	Terpinolene	1087	tr	-	0.87	0.64
14	Linalool	1103	0.21	0.15	0.57	0.43
15	cis-Allo-ocimene	1130	-	-	tr	-
16	Menthone	1155	12.96	15.25	18.68	14.35
17	Isomenthone	1160	4.27	3.65	6.82	4.68
18	Menthofuran	1166	7.25	10.85	8.24	6.83
19	Neomenthol	1169	2.65	0.97	3.41	2.02
20	Borneol	1170	tr	-	-	-
21	Menthol	1178	47.36	41.42	38.67	44.68
22	Terpinen-4-ol	1181	-	0.31	2.11	tr
23	Isomenthol	1185	3.17	tr	-	tr
24	α-Terpineol	1195	-	0.19	-	tr
25	Pulegone	1235	0.63	1.84	3.57	2.17
26	Carvone	1244	-	-	tr	-
27	Piperitone	1254	1.47	2.54	2.25	1.69
28	Menthyl acetate	1277	6.89	5.71	5.24	4.86
29	Pulespenone	1345	-	-	tr	-
30	α-Terpinolene	1349	tr	-	-	0.13
31	β-Bourbonene	1415	-	tr	-	-
32	β-Caryophyllene	1425	0.15	-	0.34	0.28
33	α-Humulene	1458	-	tr	tr	0.24
34	Germacrene-D	1565	1.12	1.97	-	tr
35	Caryophyllene oxide	1576	tr	tr	tr	0.14
36	Viridiflorol	1585	0.24	0.41	0.12	0.54
	Monoterpene hydrocarbons		1.77	3.81	3.49	5.24
	Oxygenated monoterpenes		95.58	93.59	95.59	92.77
	Sesquiterpene hydrocarbons		1.28	2	0.36	0.53
	Oxygenated sesquiterpenes		0.25	0.42	0.13	0.68
	Total		98.88	99.82	99.57	99.22
	Essential oil yield (%)		1.87	1.52	1.48	1.32

RI: Retention indices determined on HP-5MS capillary column.tr, trace (< 0.1%).

Monoterpenes are a large and diverse class of volatile C10 isoprenoids that are the major constituents of many plant essential oils and resins. These natural products play important chemoecological roles in the interactions of plants with their environments (Hallahan, 2000).In Mentha species, essential oil biosynthesis and storage is restricted to the peltate glandular trichomes (oil glands) on the aerial surfaces of the plant. Pulegone is a monoterpene ketone present in the leaves and flowering tops of several members of the mint family. The metabolism of pulegone is rather complex in terms of pathways and metabolites, but it could be classified into several major metabolic pathways. The pathway leading to the formation of menthofuran involving the 9-hydroxylation with a subsequent reduction of carbon-carbon double bond and furan ring formation. Reduction of pulegone to menthone and isomenthone followed by hydroxylation in ring or side chain and subsequent conjugation with glucuronic acid (Thomassen *et al.*, 1990; Chen *et al.*, 2011; Li *et al.*, 2011) (Fig. 1).

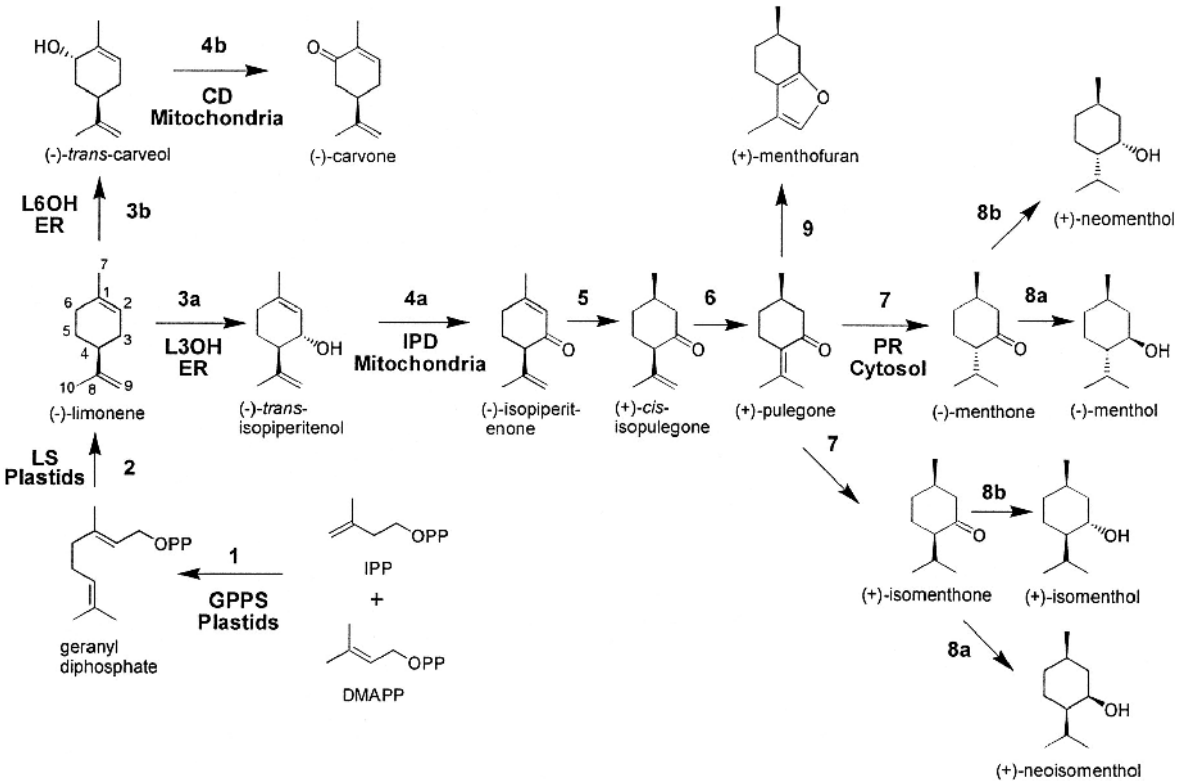

Fig. 1. The monoterpene biosynthesis pathways in Mentha.

Menthone and menthol and their isomers are the main constituents of the oil. Mahboubi and Kazempour (2014) reported the major constituents of essential oils in *M. piperita* were menthol (36.9%), menthone (28.8%) and methyl acetate (4.5%) were the main components of peppermint oil followed by carveone (3.8%), neomenthol (3.8%), 1,8-cineole (3.8%) and limonene (3.29%). Yazdani *et al.* (2003) reported the highest of menthol content in essential oil of *Mentha piperita* L. in different origin cultivated in Iran, was (56.4%) from Sari province. The highest of menthol content from the dried leaves was (1.49%) from Kerend-e Gharb, Kermanshah province. Jaimand *et al.*, (2001) reported the main components of the oils from two cultivars of *M. piperita* were neo-menthol (42.62%), 1,8-cineole (16.51%), and piperitone (12.25%) in Karaj, and L-menthol (37.55%), L-menthone (19.13%), 1,8-cineole (11.48%), and menthofuran (4.45%) in Kamu village. Soltani *et al.*, (2009) reported the main constituents of the oil were menthone, menthol, menthofuran, pulegone, 1,8-cineole, and menthyl acetate for first harvested and whereas the main constituents of the oil at second harvest were menthol, menthone, neo-menthone, 1,8-cineole and menthyl acetate. Mirza *et al.*, (2011) reported the major constituents of essential oils in *M. piperita* at early, full and after flowering stages were menthol (27.7%, 26.9% and 27%), menthon (37%, 21.9% and 17.2%) and menthofuran (16%, 22% and 25.3%) respectively.

The menthol content in peppermint of Chinese origin was lower whereas limonene was higher than USA origin (Aflatuni *et al.*, 2000). The major components of *M. piperita* essential oil analyzed in Serbia were menthol (37.4%), menthyl acetate (17.4%) and menthone (12.7%) (Iscan *et al.*, 2002). Menthol and menthone were the main components of *M. piperita* (Sokovic *et al.*, 2009). Menthol (64.0%), menthyl acetate (9.2%) and menthofuran were dominant in *M. piperita* collected from Italy (Ashok *et al.*, 1999). Menthanol (36.24%) and menthone (32.42%) also were the major compounds of the *M. piperita* essential oil collected from Karaj (Iran) province (Behnam *et al.*, 2006). Similarly, menthon (44.1%), menthol (29.5%), menthylacetate (3.8%) and menthofuron (0.9%) were the major compounds of *M. piperita* from Turkey (Arldogan *et al.*, 2002). However, the leaves of *M. piperita* grown in Korea had linalyl acetate (28.2%), menthol (33.4%), 1,8-cineole (46.1%), limonene (64.5 to 94.2%), and p-menth-2-en-ol (34.5%) (Seun-Ah *et al.*, 2010). The main components of the oils of *M. piperita* from east- Azerbaijan (Iran) province were α-terpinene (20.11%), pipertitinone oxide (17.10%), and trans-carveol (19.48%) (Eteghad *et al.*, 2009). The chemical composition of *M. piperita* L essential oil from Tehran (Iran) province, contained α-terpinene (19.7%), isomenthone (10.3%), trans-carveol (14.5%), pipertitinone oxide (19.3%), and β-caryophyllene (7.6%) as the major compounds (Yadegarinia *et al.*, 2006).

Intensive research on the chemical characteristics has been conducted on this species (Carla and Decorti, 2009). Golparvar *et al.*, (2015) reported that phytochemical variations were not only found among (Ardalani *et al.*, 2014) 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

A comparison of our results with different reports, differences in the volatile composition of the plants could be attributed to genetic (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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