Journal of Herbal Drug
journal homepage: www.jhd.iaushk.ac.ir

Comparative analysis of chemical composition of *Mentha longifolia* (L.) Huds

Ahmad Reza Golparvar[1*], Amin Hadipanah[2], Mohammad Mehdi Gheisari[3], Saeed Salehi[4], Reza Khaliliazar[1], Omid Ghasemi[1]

[1]*Department of Agronomy and plant Breeding, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran;*
Email:** ***dragolparvar@gmail.com

[2]*Department of Horticultural, Science and Research Branch, Islamic Azad University, Tehran, Iran;*

[3]*Department of Chemistry, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran;*

[4]*Young Researchers and Elite Club, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *February 19th 2016*
Accepted *April 15th 2016*

Key words:
- ✓ *Mentha longifolia (L.) Huds*
- ✓ *Chemical* composition
- ✓ *Essential oil*

ABSTRACT

Background & Aim: *Mentha longifolia* (L.) Huds is an aromatic perennial herb that belongs to the family Lamiaceae. The aim of this study was to identify chemical composition of aerial parts (including leaf, stem and flowers) of *M. longifolia* collected from (Kamu mountain) Isfahan province.
Experimental: The essential oils were extracted using the hydrodistillation method and analysed by GC-MS.
Results: The essential oil yields obtained from the aerial parts of *M. Longifolia* were 1.34, 0.76 and 0.97 ml/100 g dry matter in leaf, stem and flowers, respectively. Results indicated significant differences ($p < 0.01$) among the aerial parts for the main constituents in the essential oil. The major constituents of the leaf oil were 1,8-cineole (37.16%), piperitenone oxide (18.97%), sabinene (13.94%), α-pinene (8.92%) and pulegone (6.14%). The major constituents of the stem oil were 1,8-cineole (36.81%), pulegone (18.61%), piperitenone oxide (12.21%), sabinene (7.05%) and the major constituents of the flower oil were piperitenone oxide (37.67%), 1,8-cineole (23.02%), sabinene (13.56%) and α-pinene (10.45%).
Recommended applications/industries: Differences in the volatile composition of the plant material could be attributed to the growth and cultivation conditions of the plant, to the methods of extraction and to the harvesting time.

1. Introduction

Mentha longifolia (L.) Huds belongs to the mint family (Lamiaceae), subfamily Nepetoideae, tribe Mentheae (Bremer *et al.*, 1998). The genus Mentha includes 25 to 30 species that grow in the temperate regions of Eurasia, Australia and South Africa (Dorman *et al.*, 2003). *M. longifolia* 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 3-5 mm 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 (Ghahreman, 1984; Shinwari *et al.*, 2011). The leaves, flowers and stems of the *Mentha* species have been used as carminative, antispasmodic, antiemetic, stimulant, analgesic. Their leaves have been also consumed as herbal tea and spice (Zargari, 1990; Gulluce *et al.*, 2007). Various researchers reported that chemical components in medicinal and aromatic plants in general is primarily related to their genetic (Shafie *et al.*, 2009), climate, edaphic, elevation and topography (Loziene and Venskutonis, 2005). Many plant species constitutively produce large quantities of terpenoid-rich resins and essential oils within specialized glandular tissues, such as glandular trichomes, secretory cavities, and secretory ducts. The glandular cells of these secretory tissues are of interest for their remarkable ability to rapidly generate substantial amounts of specific terpenoid products. Terpenoids are known to have many important biological and physiological functions (Fahn, 2000).

Developmental and environmental factors are known to greatly influence the yield and composition of *M. longifolia* oil. For example, in studies (Jaymand and Rezaei, 2002) it was reported that major constituents obtained from leaf oil of *Mentha longifolia* (L.) Huds. var. asiatica (Boriss.) Rech. f. 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%). Jaymand *et al.* (2002) indicated the major constituents in flower oil obtained from *Mentha longifolia* (L.) Hudson var. kermanansis 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%). An earlier report by (Golparvar *et al.*, 2013) indicated the major components of aerial parts of *Mentha longifolia* (L.) collected from two different locations in Iran (Isfahan and Lorestan Provinces) were piperitone oxide (6.7 and 15.05%) and pulegone (6.6 and 9.58%). An earlier report by (Abedi *et al.*, 2015) indicated the major components aerial parts of *Mentha longifolia* (L.) Hudson collected from four different locations in Iran (Shahreza, Chadegan, Isfahan and Falavarjan Provinces) were 1,8-cineole (13.8 to 29.7%) and pulegone (7.8 to 44.75%). The main goal of this study was comparative analysis of chemical composition of *Mentha longifolia* (L.) Huds in Iran.

2. Materials and Methods

2.1. Plant material

The aerial parts from (leaf, stem and flower) of *Mentha longifolia* were collected at (Kamu mountain) Isfahan province in central Iran (33°, 36' N and 51°, 14' E), during 2014. Kamu is a city in Qamsar district, Kashan County, Isfahan province. The samples of the plants were indentified by regional floras and authors with floristic and taxonomic references, and voucher specimens were deposited at the Herbarium of Agticulture Researches Islamic Azad University, Isfahan (Khorasgan), Iran.

2.2. Essential oil extraction

Harvested fresh aerial parts from (leaves, stem and flowers) were dried at room temperature (25 ± 5 °C). Dried plant material was powered (100 g, and subjected to hydro–distillation (1000 ml distillated water) for 3 h 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.

2.3. GC/MS analysis

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 (C_5-C_{24}) 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).

2.4. Statistical analysis

The data was statistically analyzed based on completely randomized design (CRD) model using $SPSS_{19}$ software. Means of the main constituents of the essential oils were compared by Duncan's multiple range test at $p \leq 0.05$ probability level.

3. Results and discussion

The essential oils extracted from the aerial parts of leaf, stem and flower produced a clear, yellow liquid. A significant difference ($p < 0.05$) in oil yields was obtained from the aerial parts of leaf, stem and flower. The essential oil yields were obtained from the aerial of *M. Longifolia* 1.34, 0.76 and 0.97 ml / 100 g dry matter identified in leaf, stem and flower, respectively (Table 1).

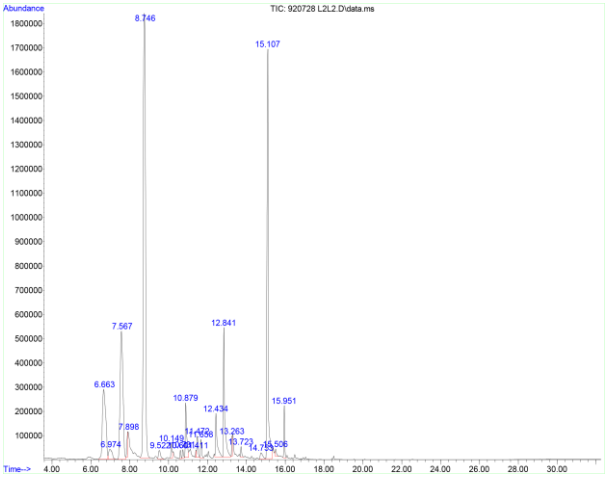

Fig 1. The chromatograms found in of the leaf oil of *M. longifolia*.

Results indicated significant differences ($p < 0.01$) among the aerial parts for the main constituents in the essential oil (Table 1). The chemical constituents identified by GC-MS, are presented in (Table 1). GC–MS analyses resulted in *M. Longifolia* essential oil, 24, 27 and 25 compounds were identified in leaf, stem and flower, respectively.

The oil of leaf components corresponding to 99.97% and consisted mainly of oxygenated monoterpenes (68.29%) and monoterpene hydrocarbons (29.81%) with a small amount of sesquiterpene hydrocarbons (1.87%). The major constituents of the leaf oil were 1,8-cineole (37.16±2.4%), piperitenone oxide (18.97±1.2%), sabinene (13.94±0.4%), α-pinene (8.92±0.2%) and pulegone (6.14±0.6%) (Figure 1).

The oil of stem components corresponding to 99.89% and consisted mainly of oxygenated monoterpenes (84.57%) with a small amount of monoterpene hydrocarbons (11.57%) and sesquiterpene hydrocarbons (3.48%). The major constituents of the stem oil were 1,8-cineole (36.81±2.1%), pulegone (18.61±1.5%), piperitenone oxide (12.21±0.6%) and sabinene (7.05±0.2%) (Figure 2).

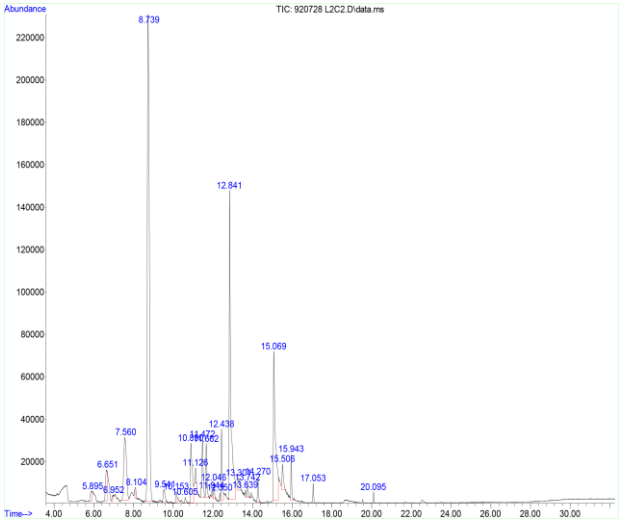

Fig 2. The chromatograms found in of the stem oil of *M. longifolia*.

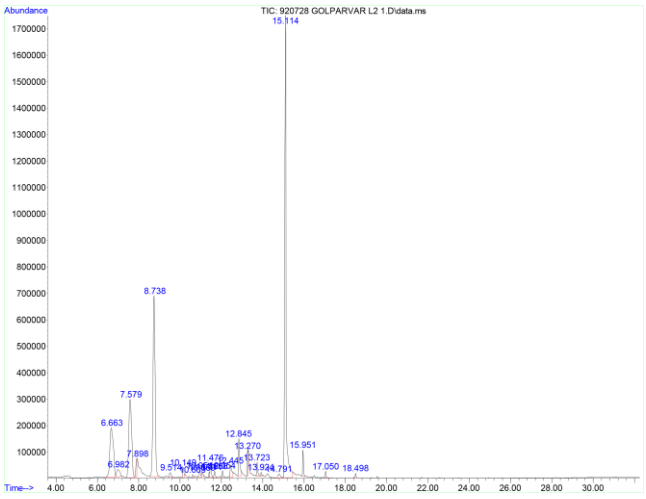

Fig 3. The chromatograms found in of the flower oil of *M. longifolia*.

Table 1. Chemical compositions of essential oils of leaf, stem and flower of *Mentha Longifolia* L.

Row	Compounds	RI	GC peak Area (%)			ANOVA
			Leaf	Stem	Flower	
1	α-Thujene	926	-	0.59	-	
2	α-Pinene	935	8.92±0.2^{ab}	1.86±0.1^{b}	10.45±0.5^{a}	$p < 0.01$
3	Camphene	950	1.27±0.1^{a}	0.21±0.1^{b}	1.36±0.1^{a}	$p < 0.01$
4	Sabinene	975	13.94±0.4^{a}	7.05±0.2^{b}	13.56±0.5^{a}	$p < 0.01$
5	β-Myrcene	994	0.89	0.42	0.96	
6	1,8-Cineole	1035	37.16±2.4^{a}	36.81±2.1^{a}	23.02±1.1^{b}	$p < 0.01$
7	(*Z*)-β-Ocimene	1045	-	-	0.53	
8	γ-Terpinene	1063	0.65	0.91	-	
9	Terpinolene	1087	0.64	0.53	0.73	
10	Linalool	1103	0.29	0.24	-	
11	3-Octanol, acetate	1113	-	-	0.19	
12	2-Octanol, acetate	1127	0.34	-	-	
13	cis-Allo-ocimene	1130	2.87	-	-	
14	1,3-Benzenediol, 4-ethyl	1138	-	4.34	-	
15	trans-Pinocarveol	1144	-	-	0.54	
16	Menthone	1155	-	-	0.32	
17	(-)-Pinocarvone	1160	-	0.46	-	
18	Menthofuran	1168	0.19	-	0.25	
19	Borneol	1170	0.88±0.1^{b}	2.98±0.4^{a}	1.01±0.2^{ab}	$p < 0.01$
20	Isopulegone	1185	0.75±0.1^{ab}	2.32±0.2^{a}	0.01±0.0^{b}	$p < 0.01$
21	Myrtanol	1192	-	0.23	0.49	
22	α-Terpineol	1195	-	0.63	0.35	
23	*trans*-Carveol	1219	-	0.24	-	
24	*cis*-Carveol	1230	2.67	2.87	1.19	
25	Pulegone	1235	6.14±0.6^{ab}	18.61±1.5^{a}	2.65±0.4^{b}	$p < 0.01$
26	Carvone	1244	0.49	-	1.09	
27	Piperitone	1254	-	0.79	-	
28	Isobornyl acetate	1273	-	0.27	-	
29	Naphthalene, 1-isocyano-	1294	0.41	0.64	0.87	
30	Pulespenone	1345	-	0.93	0.18	
31	α-Terpinolene	1349	0.63	-	0.59	
32	Piperitenone oxide	1363	18.97±1.2^{ab}	12.21±0.6^{b}	37.67±2.5^{a}	$p < 0.01$
33	β-Bourbonene	1415	0.32	1.41	-	
34	β-Caryophyllene	1425	1.55	1.53	1.32	
35	α-Humulene	1458	-	0.54	0.36	
36	Germacrene-D	1575	-	-	0.24	
37	Caryophyllene oxide	1583	-	0.27	-	
	Monoterpene hydrocarbons		29.81	11.57	28.18	
	Oxygenated monoterpenes		68.29	84.57	69.83	
	Sesquiterpene hydrocarbons		1.87	3.48	1.92	
	Oxygenated sesquiterpenes		-	0.27	-	
	Total		99.97	99.89	99.93	
	Essential oil yield (%)		1.34±0.4^{a}	0.76±0.2^{b}	0.97±0.3^{ab}	$p < 0.05$

RI: Retention indices determined on HP-5MS capillary column.

The oil of flower components corresponding to 99.93% and consisted mainly of oxygenated monoterpenes (69.83%) and monoterpene hydrocarbons (28.18%) with a small amount of sesquiterpene hydrocarbons (1.92%). The major constituents of the flower oil were piperitenone oxide (37.67±2.5%), 1,8-cineole (23.02±1.1%), sabinene (13.56±0.5%) and α-pinene (10.45±0.5%) (Figure 3).

Monoterpenes are a large and diverse class of volatile C_{10} 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). Monoterpenes are the major essential oil constituents of members of the mint (Lamiaceae) family, including *M. longifolia*, which has been developed as a model system for the study of monoterpene metabolism. 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).

The biosynthesis of secondary metabolites, although controlled genetically, is strongly affected by the environmental influences of a particular growing region, and also by the agronomic conditions, harvesting time and the type of processing. In addition, for maximum oil production, long days and high light intensities are required during the maturation period (Thompson, 2003). Developmental and environmental factors are known to greatly influence the yield and composition of peppermint oil. For example, oil yield and menthol content increase with leaf (and thus oil gland) maturity, and a range of stress conditions (related to light, temperature and moisture status) tend to promote the accumulation of pulegone and menthofuran (Mahmoud and Croteau, 2003).

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 (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%). Raluca-Andro *et al.*, (2011) reported that 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%). An earlier report by Golparvar *et al.*, (2015) indicated the major components of aerial parts of *Mentha longifolia* (L.) Hudson collected from two different locations in Iran (Chelgard and Baghe-Bahadoran) were 1,8-cineole (37.16 and 34.26%) pulegone (6.14 and 27.97%) and sabinene (13.93 and 7.89%), respectively. The percentage of chemical composition in oil of *M. longifolia* grown in different countries, 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); Serbica 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).

A comparison of our results with the previous report by (Raluca-Andro *et al.*, 2011; Saeidi *et al.*, 2012; Golparvar *et al.*, 2015) suggests few differences in the volatile composition of the plant material could be attributed to the growth and cultivation conditions of the plant, to the methods of extraction and to the harvesting time.

4. Conclusion

In conclusion, the results of this study provide data on variation of phytochemical characteristics of the essential oils from leaf, stem and flower of *Mentha longifolia* (L.) Huds. Results of current study indicate that 1,8-cineole, pulegone, piperitenone oxide are the main constituents of the leaf, stem and flower essential oils. Over all, morphological characteristics can vary under different agroclimatic conditions, interactions between genotype and environment.

5. Acknowledgment

This research project has been supported by Islamic Azad University, Isfahan (Khorasgan) branch, Isfahan, Iran. This support is highly appreciated.

6. References

Abedi, R., Golparvar, A.R. and Hadipanah, A. 2015. Identification of the essential oils composition from four ecotypes of *Mentha longifolia* (L.) Huds growing wild in Isfahan province, Iran. *Journal of BioScience and Biotechnology,* 4(2): 117-121.

Adams, R.P. 2007. Identification of essential oil components by gas chromatography/mass spectrometery, 4th Ed., p. 456, Allured Publishing Corporation, Carol Stream, IL.

Bremer, K., Chase, M.W. and Stevens, P.F. 1998. An ordinal classification for the families of flowering plants. *Annals of Missouri Botanical Garden*, 83: 531–553.

British Pharmacopoeia. 1988. British pharmacopoeia, vol. 2. HMSO, London 137–138.

Chen, X.W., Serag, E.S., Sneed, K.B. and Zhou, S.F. 2011. Herbal bioactivation, molecular targets and the toxicity relevance. *Chemical Biology Interact*, 192(3): 161-176.

Dorman, J.J., Kosar, M., Kahlos, K., Holm,Y. and Hiltunen, R. 2003. Antioxidant prosperities and composition of aqueous extracts from Mentha species, hybrids, varieties and cultivars. *Journal of Agriculture and Food Chemical*, 51: 4563–4569.

Dzamic, A.M., Sokovic, M.D., Ristic, M. and Novakovic, M. 2010. Antifungal and antioxidant activity of *Mentha longifolia* (L.) Hudson (Lamiaceae) essential oil. *Botanica Serbica*, 34(1): 57-61.

Fahn, A. 2000. Structure and function of secretory cells. *Adv Botanical Research*, 31: 38–75.

Ghahreman, A. 1984. Color Atlas of Iranian Plants. Institute of Forestries and Grasslands, Botany Division, No. 5: 512 pp.

Golparvar, A.R., Hadipanah, A. and Gheisari, M.M. 2013. Chemical analysis and Identification of the components of two ecotypes of (*Mentha Longifolia* L.) in Iran province. *International Journal of Agriculture and Crop Sciences*, 5(17): 1946-1950.

Golparvar, A.R., Hadipanah, A. and Mehrabi, A.M. 2015. Diversity in chemical composition from two ecotypes of (*Mentha Longifolia* L.) and (*Mentha spicata* L.) in Iran climatic conditions. *Journal of Biodiversity and Environmental Sciences*, 6(4): 26-33.

Gulluce, M., Shain, F., Sokmen, M., Ozezer, H., Daferera, D. and Sokmen, A. 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. spp. *longifolia*. *Food Chemical*, 103: 1449–1456.

Hallahan, D.L. 2000. Monoterpenoid biosynthesis in glandular trichomes of labiate plants. *Advance Botanical Research*, 31: 77-120.

Jamzad, M., Jamzad, Z., Mokhber, F., Ziareh, S. and Yari, M. 2013. Variation in essential oil composition of *Mentha longifolia* var. *chlorodichtya* Rech.f. and *Ziziphora clinopodiodes* Lam. growing in different habitats. *Journal of Medicinal Research*, 7(22): 1618-1623.

Jaymand, K., Mirza, M., Jamzad, Z. and Bahernik, Z. 2002. Investigation of essential oil of *Mentha longifolia* (L.) Huds. Var. Kermanansis and *Mentha longifolia* (L.) Huds. Var. Kotschiana. *Iranian journal of Medicinal and Aromatic plants*, 18: 1-9.

Jaymand, K. and Rezaei, M.B. 2002. Chemical constituents of essential oil from *Mentha longifolia* (L.) Hudson var. Asiatica (Boriss) Rech. F. from Iran. *Journal of Essential Oil Research*, 14(2): 107-108.

Li, F., Lu, J. and Ma, X. 2011. Profiling the reactive metabolites of xenobiotics using metabolomic technologies. *Chemical Research Toxicol*, 24(5): 744-51.

Loziene, K. and Venskutonis, P.R. 2005. Influence of environmental and genetic factors on the stability of essential oil composition of *Thymus pulegioides*. *Biochemical Syst Ecology*, 33: 517–525.

Mahmoud, S.S. and Croteau, R.B. 2003. Menthofuran regulates essential oil biosynthesis in peppermint by controlling a downstream monoterpene reductase. Plant Biology 100(24), 14481-14486.

Mkaddem, M., Bouajlla, J., Ennajar, M. and Lebrihi, A. 2009. Chemical composition and antimicrobial and antioxidant activities of *Mentha longifolia L*. essential oils. *Journal of Food Science*, 74(7): 358-363.

Raluca-Andro, A., Atofani, D., Boz, I., Magdalena-Zamfirache, M., Burzo, I. and Toma, C. 2011. Studies concerning the histo-anatomy and biochemistry of *Mentha longifolia* (L.) Huds. *During vegetative phenophase*, 25-29.

Saeidi, Z., Babaahmadi, H., Allah Saeidi, K., Salehi, A., Saleh Jouneghani, R. and Amirshekari, H. 2012.

Essential oil content and composition of *Mentha longifolia* (L.) Hudson grown wild in Iran. *Journal of Medicinal plants Research*, 6(29): 4522-4525.

Shafie, M.S.B., Zain Hasan, S.M. and Shah, M.S. 2009. Study of genetic variability of Wormwood capillary (*Artemisia capillaris*) using inter simple sequence repeat (ISSR) in Pahang region, Malaysia. *Plant Omics*, 2: 127-134.

Thomassen, D., Slattery, J.T. and Nelson, S.D. 1990. Menthofuran-dependent and independent aspects of pulegone hepatotoxicity: roles of glutathione. *Journal of Pharmacol Exp Ther*, 253: 567-572.

Thompson, J.D., Chalchat, J.C. and Michet, A. 2003. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *Journal of Chemical Ecology*, 29(4): 858-880.

Viljoen, A.M., Petkar, S., Van Vuuren, S.F. and Cristina Figueiredo, A. 2006. Chemo-Geographical variation in essential oil composition and the antimicrobial properties of "Wild Mint" - *Mentha longifolia* subsp. *Polyadena* (Lamiaceae) in Southern Africa. *Journal of Essential Oil Research*, 18: 60-65.

Zargari, A. 1990. Medicinal plants: Tehran University Press. 4: 28–42.