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;
\(^2\)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;

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**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%), sabine (7.05%) and the major constituents of the flower oil were piperitenone oxide (37.67%), 1,8-cineole (23.02%), sabine (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; Gullice 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 (Lozine 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 (4.1%), 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. kermananis 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 (Shahrreza, Chahadan, 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 Agriculture 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 distilled 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 (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).

2.4. Statistical analysis

The data was statistically analyzed based on completely randomized design (CRD) model using SPSS\textsuperscript{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 	extit{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 	extit{M. longifolia}.](image)

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 	extit{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 monoterpen 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%), a-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 monoterpen 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 	extit{M. longifolia}.](image)

The oil of flower components corresponding to 99.97% and consisted mainly of oxygenated monoterpenes (84.57%) and monoterpen hydrocarbons (29.81%) with a small amount of sesquiterpene hydrocarbons (1.87%). The major constituents of the flower oil were 1,8-cineole (37.16±2.4%), piperitenone oxide (18.97±1.2%), sabinene (13.94±0.4%), a-pinene (8.92±0.2%) and pulegone (6.14±0.6%) (Figure 3).

![Fig 3. The chromatograms found in of the flower oil of 	extit{M. longifolia}.](image)
The oil of flower components corresponding to 99.93% and consisted mainly of oxygenated monoterpenes (69.83%) and monoterpen 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

\[ \text{RI: Retention indices determined on HP-5MS capillary column.} \]
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 Mentha longifolia, which has been developed as a model system for the study of monoterpane 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 monoterpane 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 involves 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 piperiton-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 sabine (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.

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6. References


