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Chemotaxonomy of four varieties of *Mentha longifolia* L. using essential oil composition markers

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Abstract

Mentha longifolia L. is one of the most important medicinal plant of *Mentha* genus. Studies were carried out to evaluate the chemotaxonomic relationships of four varieties of this specious. The evaluate oils were isolated and analytical gas chromatography was done. After determining 51 components, cluster analysis were used to determine the chemotaxonomy relationships between varieties. Based on 14 major essential oil components two main clades were shown. *M. longifolia* var. *chlorodyctia* had the highest distance from other varieties. It seems that environmental factors such as soil, nutrition and weather have the most effective influence on chemical composition and *M. longifolia* var. *chlorodyctia* introduced the best chemotype for this species.

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Introduction

Mentha longifolia L. is a cosmopolitan genus of over specious, belonging to *Mentha* tribe, Lamiaceae family(Mozaffarian, 1999). This genus comprises about 25specious in Iran which 7 of them endemic (Mozaffarian, 1999). *Mentha longifolia* L. Huds. Is one of the specious of this genus that grows as wild in various regions of Iran (Lange and Croteau, 1999). This species is not only used in food processing technology as flavors or specious but also in hygiene and pharmaceutical products because of it`s antimicrobial properties. It is used in Iranian traditional medicines as a stomach pain- relieving agent, antispasmodic, digestive and carminative (Zargari, 1990). This specious show a large phenotypic plasticity and most varieties of it are capable of hybridization with each other. It has six varieties in Iran (Mozaffarian, 1999). The composition of volatile compounds is known for this species. (Abbaszadeh *et al*., 2013, Hajlaoue *et al*., 2010, Saeidi *et al*., 2012, Sharpov *et al*., 2011), and some data are found in chemotaxo-nomy studies of this species. Chemotaxonomy of mints of genus *Mentha* by applying Raman spectroscopy (Rosch *et al*.,2002). Essential oil of Five *Mentha* species using as-liquid partition chromatography was studied (Handa *et al*.,2006). Isopiperitenone and terpenoids were detected the main constituents between other components and correlation of the chemical data illustrates their value in plant classification. Also the chemotypes of *M. spicata* was studied in Greece and their chemotypes were characterized (Kokkini *et al*.,1989).

M. longifolia exhibits great morphological and chemical variability. Therefore, the present study aims to investigate the volatile compounds of four varieties of this species, and evaluate the chemotaxonomic relationships of these four varieties of this specious.

Material and methods

Plant material

Five specious from four varieties of *M. longifolia* were collected during the flowering period from Tehran province and Northera parts of country (table 1).

Table 1. Varieties of *M. longifolia* and their localities.

Variety	Locality
M. longifolia var. *callianta*	Firoozkohroad to Damavand, 2166m, Mazooji
M. longifolia var. *chlorodictya*	Gilan: Asalem to Khalkhal, 1963m, Mazooji
M. *longifolia* var. *longifolia*	Tehran: Firoozkohroad,Chapdare,2134m,Mazooji
M. longifolia var. *aciatica*	Mazandaran: Haraz road to lar,2270m,Mazooji
M. longifolia var. *chlorodictya*	Gilan: Asalem to Khalkhal, 1963m, Mazooji

The taxonomic description of these taxa, follows flora Iranica.

Essential oil extraction

For isolation of the volatile oils, the aerial parts of the plants were dried at room temperature and hydrodistilled for 5h anhydrous sodium thiosulfate and kept at 4°c in sealed required. Analytical gas chromatography was capillary column DB-5 (30 m. 0.25 mmid, 0.25 µm film thickness); Carrier gas, He; Split ratio, 1: 25°, and using a flame ionization detector. The column temperature was programmed at 50°c for 1 min, and then heated to 265°c at a rate of 2.5°c/min, then kept constant at 265°c for 20 min. GC-MS was performed on a thermoquest 2000 with quadruple detector, on capillary column DB-5(GC), carried gas, He; Flow rate, 1.5 ml/min. The column was held at 50°c for 1 min, and programmed up to 265°c for 20 min. Quantitative data were obtained from the electronic integration of the FID peak areas.

Cluster analysis

Hierachial clustering analysis of main components between species was performed using SPSS software

with Average Linkage method and the resulting dendrogram was illustrated.

Result and discussion

The classification of aromatic lants considered to belong to the *Mentha*- an important source of basic raw materials for the food, drug and cosetic industries- has always proved a difficult taske to the botanists and geneticist. Based on our results,Fifty one compounds were detected in the oil of four varieties representing the 96-74 to 98-52% of the total oil. The major constituents were Carvon, 1,8-cineol, cis-piperitone oxide, Pulegone, Menthone and iso-Menthone. The most chemical components showed two main clades (Fig 1).

One main clade consists of *M. longifolia* var. *chlorodictya*[2] as a separate clade with highest distance from the others varieties. The second main clade consist of four other varieties. As shown in Fig 1, in this clade, *M. longifolia* var. *aciatica* formed one subclade in 21 taxonomic level and three other specimens are placed in another subclade. There are very similarities between two varieties consist of *M. longifolia* var. *chlorodictya*[2]. The PCA confirmed the cluster analysis results (Fig 2).

Carvone –Pulegone ratios, were shown to distinguish readily two similar varieties consist of *M. longifolia* var. *longifolia* and *M. longifolia* var. *chlorodyctia*[2]. These two varities placed in one subclade near each other (Fig 1). The occurence of Menthon-Pulegone in *M. longifolia* var.*asiatica* *M.longifolia* var. *callicantha*. But in *M. longifolia* var. *chlorodictia* [1], major constituent are Iso-Menthone and Pulegone and can distict this variety from others.

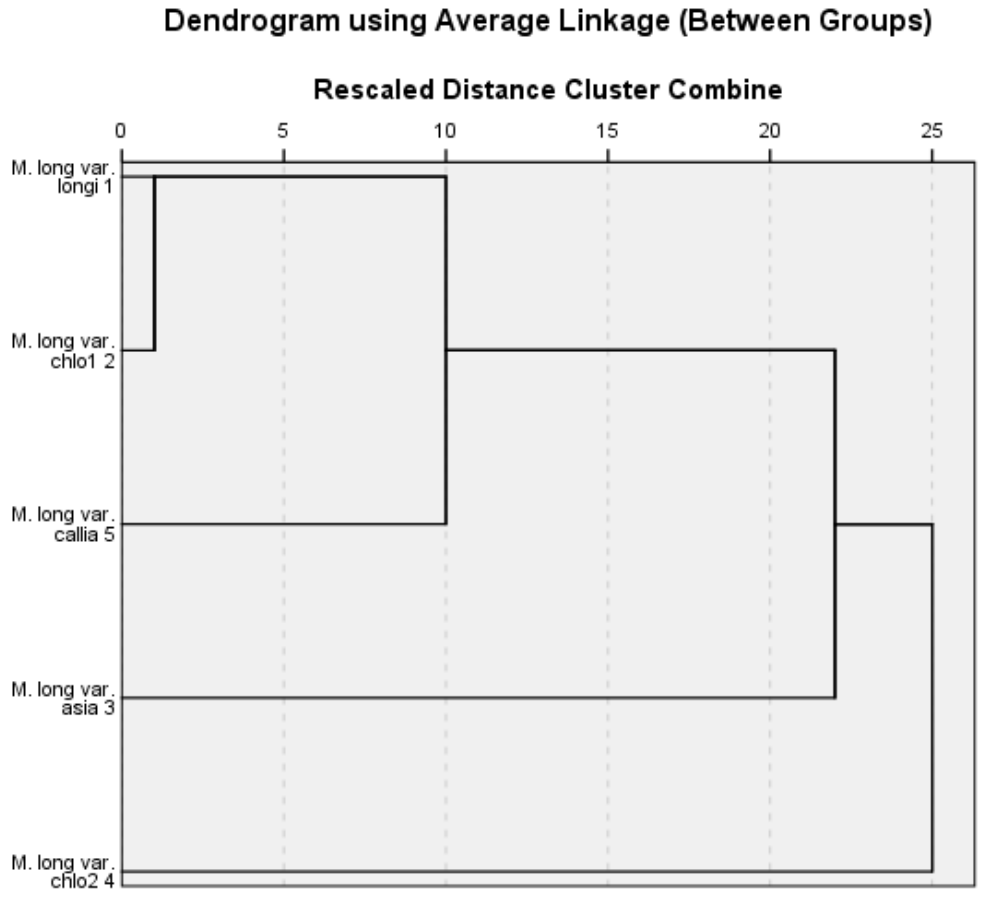

Fig. 1. Cluster analysis of four varieties resulted from analysis of chemical components data (Abbreviation: 1- *M. longifolia* var. *longifolia* , 2- *M. longifolia* var. *chlorodictya*[1], 3- *M. longifolia* var.*asiatica*, 4- *M.longifolia* var. *chlorodictya*[2], 5- *M. longifolia* var. *callianta*).

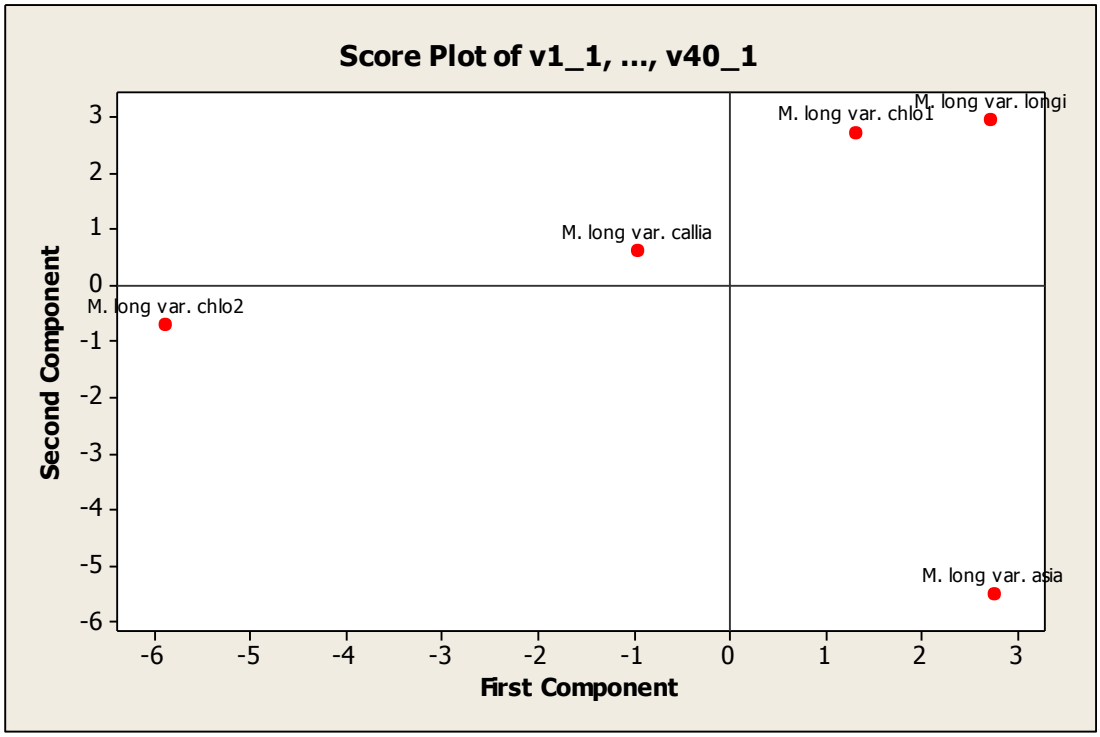

Fig. 2. PCA plot of four varieties of *M. longifolia*. *M. longifolia* var. *calyantha*[2], *M. longifolia* var. *calliantha, M. longifolia* var. *asiatica*, *M. longifolia* var. *longifolia*, *M. longifolia* var. *chlorodictya*[1].

These results showed that the environmental factors such as pH, soil structure and weather play an important role to change the content and kind of chemical components in one variety from different places. Also it seems that investigation with the help of a hierarchical cluster analysis, can able to characterize the different varieties using chemical components in different environmental conditions. The essential oil composition of *M. longifolia* were studied from other geographical locations, extensively and have produced a number of chemotypes (Mozaffarian, 1999; Abbaszadeh, *et al.*, 2013; Saeidi *et al.*, 2012, Sharpov *et al.*, 2011; Neugebaverova and Kaffkova, 2012; Gulluce *et al.*, 2007). Differences in the results can be explained by differences in the environmental conditions regions under study which affect on essential oil content of *M. longifolia* leaves (Saeidi *et al.*, 2012). Based on our results, genetic variation, growth stages, parts of plant utilized and maturity variation are important factors that determine the composition and yield of the essential oil obtained. These results correlated with (Hussain *et al.*, 2010 and Anwar *et al.*, 2009 Anwar *et al.* 2009; Hussain *et al.*, 2010). Based on results in Table 2, monoterpenes and sesquiterpenes are the most chemical compounds in this species. For example, Thymol, Cis-piperitone and 1,8-cineol are the most oxygenated monoterpenes, Sabenen and Limonene are monoterpene hydrocarbons; trans-caryophyllene and Germacreene D are the most sesquiterpene-hydroides. Also, Caryophyllene oxide is the main chemical composition from oxygenated sesquieterpene. This study showed that Piperitone oxide is the main component in this species but did not found in *M. longifolia* var. *aciatica*. This result shows that changing the climate and height affect on kind of the essential oils content. Cluster analysis based on 14 major essential oil components showed two main clades (Fig. 1). *M. longifolia* var. *chlorodictya* made a separate clade with the highest distance from other specimens. The other main clade consists of two subclades: *M.longifolia* var. *aciatica* made a one group and three other specimens placed in one subclade. There are very similarities between two varieties of *M. longifolia* from Tehran and Gilan provinces. It showed that environmental factors such

as soil, nutrition and weather play a large role in chemical similarities of these two specimens. Based on our results, many chemotypes were introduced in this research and *M. longifolia* var. *chlorodictya* is reported as the best variety of this species.

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