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CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *MENTHA LONGIFOLIA* L. SUBSP. *LONGIFOLIA* GROWING WILD

NESE OKUT^{1*}, MEHMET YAGMUR², NILUFER SELCUK³ AND BUNYAMIN YILDIRIM⁴

¹Department of Field Crops, Faculty of Agriculture, University of Yuzuncu Yil Van 65080, Turkey

²Department of Field Crops, Faculty of Agriculture University of Ahi Evran Kirsehir 40100, Turkey

³Department of Biology Sciences, Faculty of Science, University of Yuzuncu Yil Van 65080, Turkey

⁴Department of Field Crops, Faculty of Agriculture University of Igdır Igdır 76000, Turkey

*Corresponding author's email: neseokut@gmail.com

Abstract

The essential oil of *Mentha longifolia* L., is very important to some culinary usage and antimicrobial activity. The essential oil of *Mentha longifolia* subsp. *longifolia* growing in the Bahçesaray area (Van Province, Turkey) was studied. This study designed for determine of essential oil constituent *Mentha longifolia* subsp. *longifolia* that collected from wild area. Mint leaves sample essential oils obtained by hydro distillation and essential oil components were determined using GC-MS. The main component of wild grown *Mentha longifolia* subsp. *longifolia* was Menthone (19.31%). Second one and others were Pulegone (12.42%), Piperitone (11.05%), Dihydrocarvon (8.32%), Limonene (6.1%), 3-Terpinolone (5.66%), 1,8-Cineole (4.37%), Germacrene D (3.38%) and Caryophyllene (3.19%), respectively.

Key words: *Mentha*, *Mentha longifolia* subsp. *longifolia*, Essential oils, Lamiaceae, Menthone, Plugone.

Introduction

The family Lamiaceae is subdivided into two subfamilies–Lamioideae and Nepetoideae. The genus *Mentha* belongs to the subfamily Nepetoideae (Bremer *et al.*, 1998). Lamiaceae (Labiatae) is an important plant family that has been investigated for its medicinal properties due to its large quantity of phenolic acids, flavonoids and essential oil (Hussain *et al.*, 2011; Shinwari *et al.*, 2011). The genus *Mentha*, one of the main members of the Lamiaceae family consists of about 25-30 species (Dorman *et al.*, 2003; Shinwari & Chaudheri, 1992). They are fast growing and generally tolerate a wide range of agro-climatic conditions with spreading distribution across Europe, Africa, Asia, Australia, and North America (Brickell & Zuk, 1997). *M. spicata*, *M. canadensis*, *M. piperita* etc., have economic importance due to their high-valued oil and good taste (Telci *et al.*, 2011, Bhat *et al.*, 2002). The essential oils of these species are used as perfumes, food flavors and pharmaceuticals. The interest in essential oils and their components is growing due to their friendly environment nature and human health, their broad acceptance by consumers, and their consumption for potential multi-purpose functional use (Ormancey *et al.*, 2001; Jabeen *et al.*, 2012).

Leaves, flowers and the stem of *Mentha* spp., are commonly used in herbal tea or as additives in marketable spice mixtures for many foods to present aroma and flavor (Kothari & Singh, 1995; Moreno *et al.*, 2002). In addition, they have anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatarrhal activities. Hence, *Mentha* spp. has been used as a folk medicine for treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver ailments (Cowan, 1999; Iscan *et al.*, 2002; Hussain *et al.*, 2015) and (Moreno *et al.*, 2002). Furthermore, it is well-documented that the essential oils or extracts from some *Mentha* species including *M. spicata*, *M. piperita*, *M. arvensis*, *M. rotundifolia*, *M. suaveolens* and

M. pulegium possess antimicrobial and antioxidant properties (Daferera *et al.*, 2003; Economou *et al.*, 1991) and (Kaur & Kapoor, 2002).

Genus *Mentha* contain fifteen taxa belonging to eight species in the flora of Turkey (Duman, 2000). *Mentha longifolia* L. is one of wild mint species in Turkey and includes three subspecies of two varieties. *Mentha longifolia* subsp. *longifolia* is a more intensive taxon than other taxa in the Eastern region of Turkey.

Mentha longifolia L. (common name: wild mint or horse mint) is a perennial herb. It can reach up to 1.5 m high in favorable environment. *M. longifolia* is an extremely variable species with an extensive distribution in Iraq, Mediterranean region, Europe and eastwards into Asia. In Turkey and Iraqi folk medicine, the leaves are used for relief of minor sore throat and minor mouth or throat irritation. It is also used in treatments for minor aches and sprains, and in nasal decongestants.

Mentha longifolia L. is believed to be beneficial in building the immune system and fighting secondary infections. Moreover this plant is used for the remedy of coughs, colds and influenza. Externally, wild mint is used to treat wounds and swollen glands as well (Van Wyk *et al.*, 1997). The leaves of *M. longifolia* have a wide range of culinary usage in South Africa and, because of their color, aroma and flavor; they are used in food preparation to develop taste and appearance. When the leaves are rubbed onto the body and beddings, the strong smell keeps mosquitoes' away (Hutchings & Van Staden, 1994). It has also been spread in granaries to keep rodents off the grain (Phillips & Foy, 1990). Carvone-scent mints (*Mentha spicata*, *M. longifolia* L. Hudson, etc.) are often cultivated for spice in limited areas in counties such as Turkey. For example *Mentha longifolia* is cultivated because of its commonly usage to making herbal cheese in Eastern Anatolia at Van City, Turkey (Ozcelik, 1994).

Chemical composition of the essential oil of wild mint herb is quite variable depending on the habitat and climate where the species grow. Forty five constituents were

identified in the essential oil of *M. longifolia* ssp. *longifolia* from Northeastern Turkey. The the essential oil composition of *M. longifolia* ssp. *longifolia* is characterized mainly by the presence of C-3 substituted compounds, including cis-piperitone epoxide (18.4%), pulegone (15.5%), piperitenone oxide (14.7%), menthone (7.9%), menthone (6.6%), trans-piperitone epoxide (4.1%) and secondly by the C-2substituted compounds, including carvone (4.9%). cis-epoxy piperitone, pulegone and piperitenone oxide as main components (Gulluce *et al.*, 2007). Hence, the identification and using local clues are important. Traditional information of different cultures and folklores about the type *Mentha* species, the place and time of the crop, consumption and method of consumption and etc are the most important parameters (Kumar, 2011).

Therefore, the objectives of this study were to analyze chemical composition of hydro distilled essential oils of *M. longifolia* ssp. *longifolia* plants collected from the eastern Anatolia region of Turkey (Van province) and by a GC-MS system to determine the essential oils components to investigate of the plant value.

Material and Methods

Plant material: Samples of *M. longifolia* subsp. *longifolia*, Lamiaceae, were collected at the flowering stage from Bahcesaray area (Van Province, Turkey) in June 2010 at an altitude of 2359 m. Bahcesaray is a county in Van province (38°7'43"N, 42°48'27"E) and it has been selected as one of the important plant areas of Turkey (Ozhatay *et al.*, 2005), because of different environmental and climate characteristics, this region has different chemotypes. The collected plants were identified by Dr. N. Selcuk from the University of Yuzuncu Yil, Department of Biology Sciences (Van, Turkey). An authenticated specimen of the plant was deposited in the herbarium of the Yuzuncu Yil University, Faculty of Science, Department of Biology, Van, Turkey. The wild population of plants was dried in the shade at the room temperature. The leaves of plant were separated from the stem and ground into small particles.

Isolation of essential: The essential oils from the air-dried leaves were isolated by the hydrodistillation for 3.5 h using an all-glass Clevenger-type apparatus as recommended by European Pharmacopoeia (Anon., 1996). The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored in an amber vial at low temperature (4°C) in the dark prior to analysis.

GC-MS analyses: Gas chromatography-mass spectrometry (GC/MS) was used for the identification of components in *M. longifolia* subsp. *longifolia* essential oil. GC/MS instrument (GC/MS-QP 2010 Plus Shimadzu, Japan) fitted with a TRB Wax capillary column (30m x 0.25 mm i.d., 0.25µm film thickness) was used for qualitative determination. Essential oils were diluted 1/10 in n-hexane (v/v) prior to analysis. Auto sampler was built for oil injection. Injector temperature was 240°C. Column temperature was programmed from 60°C to 240°C, temperatures held for 60°C for 2 min, then gradually increased to 240°C at 10°C /min kept there for 5 minutes. Helium was used as carrier gas and mass spectra were

recorded in the scan mode. The carrier gas was helium, at a flow rate of 1 mL/min. The ionization voltage was 70 eV. Split ratio was 1:20. Ion source temperature was 200°C, Interface temperature was 240°C. Solvent cut time was 3 min. The sample of 0.1µl was used for the analysis. The oil constituents were identified on the basis of their retention times (Rt) obtained with reference. Mass spectra with those of authentic samples, composition of their mass spectra and fragmentation patters reported in literature (Adam, 2001) and computer identical with MS-data bank (Wiley & Nist Library). The essential oil components have been identified as a preliminary study of previously collected plant species. After these studies, the best temperature program of the GC-MS was determined for analysis of the essential oils and the same program was applied for all samples. Each analysis in this experiment was repeated twice.

Identification of components: The formulation was analyzed for detection of chemical components by GC-MS technique. Identification of the constituents was based on the retention time (Rt) and on computer matching against the spectra library Wiley 7 and Nist 05. The relative quantity of compounds was estimated by integrating the total ion content of the individual peaks. Mass spectra with those of authentic samples, composition of their mass spectra and fragmentation patters reported in literature (Adam, 2001) and computer identical with MS-data bank (Wiley and Nist Library). The identity of the spectra above 95% was needed for the identification of compounds. The quantity of oil components was compared using peak area measurements.

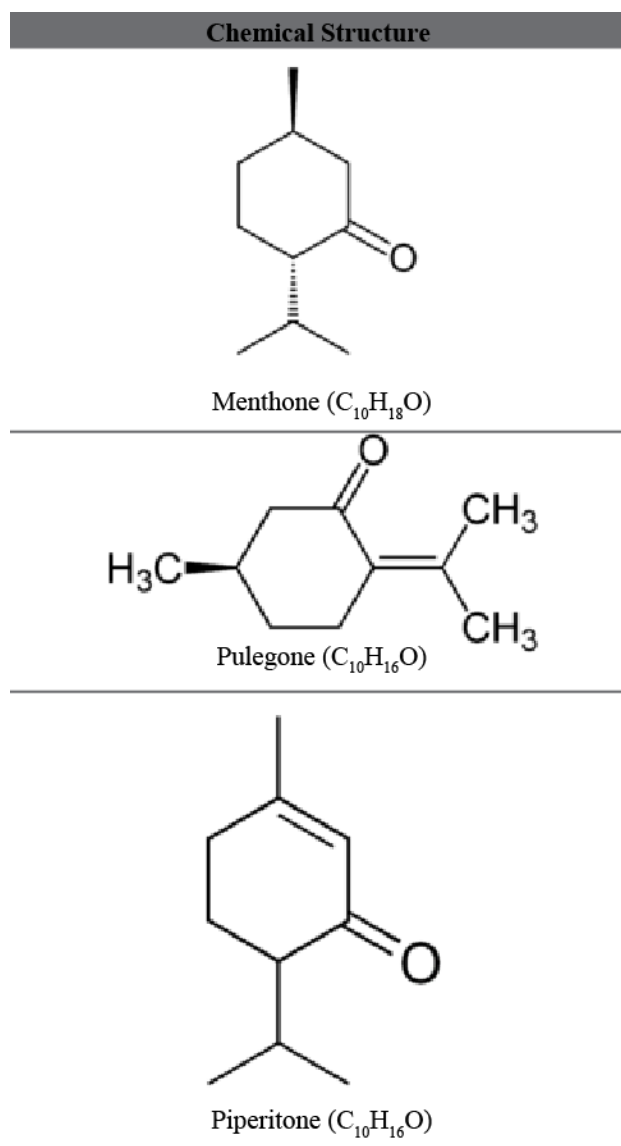
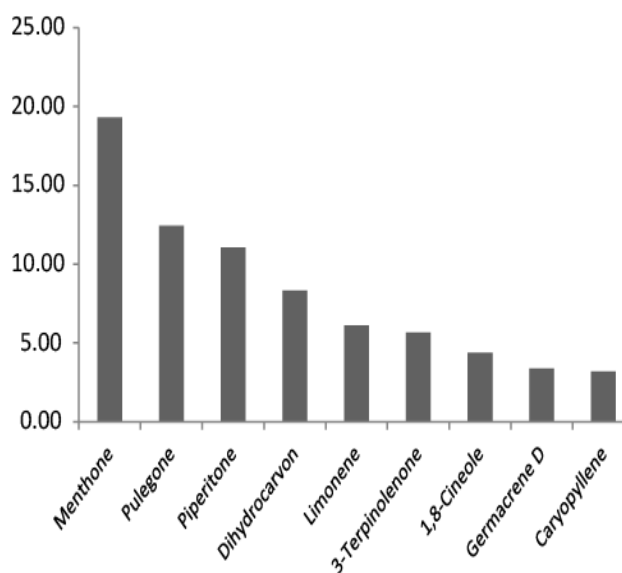
Results and Discussion

The edible wild plant grows naturally in the wild. It has different characteristic flavor from vegetable. So our objection was to determine essential oil composition of *Mentha longifolia* L. subsp. *longifolia* leaves from wild area. For this study, the chemical components were analyzed by GC-MS technique. Total forty compounds were identified representing 100% of the oil (Table 1), that they have been extracted from essential oil of *Mentha longifolia* subsp. *longifolia* leaves. Their retention times and percentage compositions are given in the Table 1. The main components were Menhone (19.31%), Pulegone (12.42%), Piperitone (11.05%), Dihydrocarvon (8.32%), Limonene (6.1%), 3-Terpinolenone (5.66%), 1,8-Cineole (4.37%), Germacrene D (3.38%), Caryophyllene (3.19%), respectively (Table 1 and Figs. 1 and 2).

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). Factors that determine the composition and yield of the essential oil obtained are numerous. In some instances it is difficult to segregate these factors from each other, since many are interdependent and influence one another. These variables may include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and postharvest drying and storage (Hussain *et al.*, 2010b; Anwar *et al.*, 2009).

Table 1. Chemical composition of the *Mentha longifolia* subsp. *longifolia* leaves essential oil.

Peak number	R _i (min) ^a	% Rate	Components ^s
1	3.040	0.66	α-Pinene
2	3.502	0.48	Camphane
3	3.995	1.22	β-Pinene
4	4.155	1.08	Sabinene
5	4.708	1.02	β-Myrcene
6	5.260	6.10	Limonene
7	5.396	4.37	1,8-Cineole
8	5.749	1.81	<i>cis</i> -Ocimene
9	8.091	1.68	β-trans-Ocimene
10	9.754	19.31	Menthone
11	10.016	0.83	β-Bourbonene
12	10.824	0.67	Menthyl acetate
13	10.892	0.60	Isopulegone
14	11.000	0.79	β-Elementene
15	11.149	3.19	Caryophyllene
16	11.520	8.32	Dihydrocarvone
17	11.592	0.71	Menthol
18	11.814	12.42	Pulegone
19	11.890	1.07	Trans-β-Farnesene
20	12.044	0.81	α-Humulene
21	12.309	0.63	α-Terpineol
22	12.402	1.25	Borneol
23	12.587	3.38	Germacrene D
24	12.678	0.92	<i>Cis</i> -Pulegone Oxide
25	12.831	11.05	Piperitone
26	12.982	0.91	Neryl acetate
27	13.075	0.40	delta-Cadinene
28	13.349	0.99	Neodihydrocarveol
29	13.648	1.62	Dihydrocarveole
30	13.733	0.41	Limonene oxide
31	14.060	0.77	Geraniol
32	14.117	0.45	p-menth-1,8-dien-3-one
33	15.118	5.66	3-Terpinolenone
34	15.781	1.26	Caryophyllene oxide
35	16.395	0.44	Germacrene D-4-ol
36	17.175	0.64	Spathulenol
37	17.604	0.55	Eugenol
38	17.701	0.60	Thymol
39	18.263	0.44	α-Cadinol
40	21.887	0.49	Phytol
Total		100	

^aRetention time (as minutes)^bCompounds listed in order of elution from a TRB Wax capillary columnFig. 1. Chemical structures of some active constituents of *Mentha longifolia* subsp. *longifolia* leaves essential oil.Fig. 2. The main components rates obtained in *Mentha longifolia* subsp. *longifolia* leaves essential oil.

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 & Mohammadi, 2013). For example, according to Gulluce *et al.* (2007) the major components of the essential oil composition of *M. longifolia* ssp. *longifolia* were collected from North eastern Turkey is characterized mainly by the presence of substituted compounds cis-piperitone epoxide (18.4%), pulegone (15.5%), piperitenone oxide (14.7%), menthone (7.9%), isomenthone (6.6%), trans-piperitone epoxide (4.1%) and carvone (4.9%). Hafedh *et al.* (2010) reported that analysis on the volatile oil obtained from *M. longifolia* spp. *longifolia* such as menthol (32.51%), menthone (20.71%), pulegone (17.76%), 1,8-cineole (5.61%), terpineol-4 (4.87%) and piperitone (2.16%) from Tunisia. In contrast some of these results, the amount of menthol in the essential oil in our research was less (0.71%). However, the amount of menthone, pulegone and piperitone were higher and these results were in agreement with Gulluce *et al.* (2007) and Hafedh *et al.* (2010).

Baser *et al.* (1999) collected eighteen samples of *M. longifolia* spp. *longifolia* from black sea area (Turkey) and found that cis and trans- piperitone oxides were the main constituents in seven samples. Four samples contained linalool and three samples had contained carvone. Another previous study with *M. longifolia* spp. *longifolia* from Izmir province (Turkey) had reported by Bayraktar (1977). Sample essential oil had menthyl acetate (0-47%), menthone (6-46%), menthol (16-30) and menthofuran (0-7%) as the main constituent. Those research has some similarities with main essential oil constituents of our *M. longifolia* spp. *longifolia* leaves samples.

In Greece, piperitone oxide-rich chemotypes and in Italy, piperitenone oxide-rich chemotypes were reported (Lawrence, 1981a; 1981b; Kokkini, 1988). In the oils of two *M. longifolia* spp. *longifolia* Israeli samples 1,8-cineole (29%), piperitone (14%) and cis-piperitone oxide (15%) was found as the main constituent in one, and pulegone (73%) in the other (Fleisher & Fleisher 1991). We have attained similar findings in our study for the rate of pulegone and piperitone in essential oil.

Essential oils rich components of known antimicrobial activities such as menthone, piperitone oxide, carvone and linalool are widely reported to occupy high levels of antimicrobial activity. Menthone, piperitenone oxide and carvone also showed substantial antimicrobial activities (Hussein *et al.*, 2010a). Pulegone is a monoterpene ketone present in essential oils from many mint species (Grundschober, 1979). Pulegone is a second major constituent of the essential oil obtained from our material. Pulegone has been used as a flavoring agent in foods and beverages, as well as a component in fragrance products and flea repellents (Hall & Oser, 1965; Tyler, 1993).

Our results about main chemical component of *Mentha longifolia* ssp. *longifolia* showed some similarities with previous studies (Gulluce *et al.*, 2007; Asekun *et al.*, 2007; Snoussi *et al.*, 2008). Furthermore Gulluce *et al.* (2007) has concluded similar results about essential oil constituent and suggested that, the essential oil of *M. longifolia* ssp.

longifolia may be used as natural preservatives in foods against the well known causal agents of foodborne diseases and food spoilage such as *Escherichia coli*, *Enterobacter* spp., *Bacillus* spp., *Salmonella* spp., *Staphylococcus aureus*, *Candida* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp. isolates

Conclusion

No study has been previously reported on *Mentha longifolia* ssp. *longifolia* essential oil from Bahceray-Van province (East Turkey). It is important to know essential oils and their components because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use. The main components obtained were Menthone (19.31%), Pulegone (12.42%), Piperitone (11.05%), Dihydrocarvon (8.32%), Limonene (6.1%), 3-Terpinolenone (5.66%), 1,8-Cineole (4.37%), Germacrene D (3.38%), Caryophyllene (3.19%). However, plants growing in different locations under different geographical and weather conditions show the differences in the quantities of major components.

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