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COMPOSITION OF ESSENTIAL OIL OF TWO VARIETIES WILD MINT (*MENTHA LONGIFOLIA* SUBSP. *TYPHOIDES* VAR. *CALLIANTHA, MENTHA LONGIFOLIA* SUBSP. *TYPHOIDES* VAR. *TYPHOIDES*)

AHMET YENIKALAYCI[1*], SEDAT BOZARI[2] AND MURAT UNAL[3]

[1]*Department of Plant Production and Technologies, Faculty of Applied Sciences, Mus Alparslan University, 49250 Mus, Turkey*
[2]*Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Mus Alparslan University, 49250 Mus, Turkey*
[3]*Department of Biology, Faculty of Education, Van Yuzuncu Yıl University, 65080 Van, Turkey*
**Corresponding author's email: a.yenikalayci@alparslan.edu.tr*

Abstract

Two wild mint taxa *Mentha longifolia* L. Hudson subsp. *typhoides* (Briq) Harley var. *calliantha* (Stapf) Briq. and *Mentha longifolia* (L.) Hudson subsp. *typhoides* (Briq) Harley var. *typhoides* naturally growing in Mus province of Türkiye were cultivated in the experimental field of Mus Alparslan University in 2020 and 2022 growing seasons to determine essential oil content and compositions. According to the GC-MS analyses, 28 essential oil components were determined in the var. *calliantha* variety, with the pulegone (29.06%), caryophyllene oxide (14.28%), piperitone oxide (7.85%), thymol (5.98%), piperitenone (5.24%), β-linalool (5.23%), and linalyl acetate (4.28%) as main component. In the var. *typhoides*, 28 essential oil components were determined, and the main components were piperitenone oxide (31.57%), carvacrol (12.97%), linalool (11.63%), cis piperitone oxide (9.98%), α-pinene (5.51%) and borneol (3.44%).

Key words: Essential oil components, *Mentha longifolia*, variety *calliantha*, variety *typhoides,* Wild mint.

Introduction

Mentha sensu lato, an important genus of the *Lamiaceae* family, is an essential oil-bearing plant with precious essential oil used as a spice worldwide. The genus has 31 species and center of origin in Central Europa and Asia (Tucker & Naczi, 2007). Türkiye has 7 mint species (*M. spicata* L., *M. pulegium* L., *M. arvensis* L., *M. piperita* L., *M. suaveolens* Ehrh., *M. aquatica* L., *M. longifolia* L.) (Davis *et al*., 1982) and 15 infra-specific taxa belonging to these species (Başer *et al*., 2012). The most cultivated mint species in the world are *M. arvensis* (Japanese mint), *M. piperita* (English peppermint), and *M. spicata* (garden mint). Apart from these three important mint species, lemon mint (*M. citrata*), water mint (*M. aquatica*), apple mint (*M. gentiles*), and pineapple mint (*Mentha x gentilis* var. *variegata*) are also cultivated (Anon., 2020). The genus shows a wide diversity due to its susceptibility to genetic polymorphism and hybridization (Arslan, 2018).

According to 2019 data, 15,853 tons of mint was produced in an area of 1,2391 ha in Türkiye, and the export of mint and its derivatives was 479.6 tons for 1,390,324 USD in 2020 (Anon., 2020). *M. spicata* is the widely cultivated mint species in Türkiye. Most of the cultivated mint is exported as spice, and domestic mint essential oil production is limited (Anon., 2020).

M. longifolia has a broad range of uses in modern and traditional medicine. It has been reported that the species has antioxidant antibacterial, antimicrobial, (Yanmis *et al*., 2012; Al-Okbi *et al*., 2015; Farzaei *et al*., 2017; Sevindik *et al*., 2017; Elansary *et al*., 2020; Anon., 2022), carminative, antispasmodic (Hajlaoui *et al*., 2009; Abdulselam, 2011; Bayramoğlu & Candan, 2019), soothing against nervous disorders, (Bozari *et al*., 2021; Anon., 2022), and anticancer (Bayramoğlu & Candan, 2019; Elansary *et al*., 2020) activities. Majority of the essential oil composition of the species consist of pulegone (%0,3-54,41) (Gulluce *et al*., 2007; Mkaddem *et al*., 2009; Sharopov *et al*., 2012), piperitone oxide (Ghoulami *et al*., 2001; Ahmed *et al*., 2017), piperitenone oxide (%1,5-21,2) (Başer *et al*., 2012), 1,8 cineol (%4,37-11,54) (Al-Okbi *et al*., 2015; Anon., 2022), cis-piperitone epoxide (%7,8-77,6) (Sharopov *et al*., 2012), piperitenon (Verma *et al*., 2015), trans-piperitone oxide (Başer *et al*., 2012), trans-piperitone epoxide (%48,7), α-humulene (%1,5) (Verma *et al*., 2015), menthone (%0-19,31) (Okut *et al*., 2017), isopulegol (Başer *et al*., 2012; Verma *et al*., 2015), piperitone (%1,6-11,05) germacrene-D (%3,38-9,8), caryophyllene (%0,9-3,19) (Verma *et al*., 2015; Okut *et al*., 2017), borneol (%6,85) (Mkaddem *et al*., 2009; Anon., 2022), d-candinene (%3,53) α-pinen (%3,57), α-terpineol (%3,17) (Hajlaoui *et al*., 2009), thymol (%1,5-4,2), carvakrol (%0-2,7) (Sharopov *et al*., 2012), izomentone (%12,02) (Mkaddem *et al*., 2009; Anon., 2022), and linalool (Duali, 2010).

It is also known that *M. longifolia* is used in traditional treatments for various diseases such as respiratory disorders, gastrointestinal, inflammatory diseases, infectious, and menstrual irregularities. The species has various pharmacological activities in modern medicine such as anti-parasitic, antimicrobial, insect repellent, anti-inflammatory, antimutagenic, antioxidant, antinociceptive, hepatoprotective. It has been stated that *M. longifolia* has hepatoprotective, antidiarrheal and spasmolytic effects, and it is also used in irritable bowel syndrome, amenorrhea, oligomenorrhea, and oxidative stress-related diseases. It has been suggested that a wide range of natural components (such as phenolic acids, cinnamates, flavonoids, ceramides, terpenes, sesquiterpenes, and terpenoids) found in the species can be used in pharmacology. It has been reported that a wide variety of natural components such as flavonoids, phenolic acids, cinnamates, ceramides, sesquiterpenes, terpenes, and terpenoids found in the species are effective for pharmacological use (Gormez *et al*., 2015). Due to these properties, it has been stated that *M. longifolia* can be used in the development of new drugs, and more phytochemical and pharmacological studies should be done on it (Farzaei *et al*., 2017).

It is also used as an herbal tea for stomach pain. It is used as a carminative and to relieve nervous disorders. *M. longifolia* is also famous as an antiseptic and antitussive (cough suppressant). It is also used against headaches and cholera in Java (Akalin, 1952; Başer *et al*., 2012).

M. longifolia is a popular folk remedy that is naturally grown in Mediterranean regions, some part of Europe, Australia, and North Africa. It has been reported that some parts of this plant are used in folk medicine in some other countries, especially in Iran. It is also known to be used as antiseptic, anticarcinogenic, expectorant, sedative, diuretic, and also used in nausea, food, medicine, cosmetics, and hygiene industries (Mimica-Dukic *et al*., 1999; Mikaili *et al*., 2013). In a study on the total antioxidant capacity and radical scavenging activity of *M. longifolia* subsp. *typhoides* var. *typhoides* ethanol extract. Bayramoğlu & Candan (2019) reported that the plant could be a potential natural antioxidant source, and it could have a high effect as therapeutic agents in the prevention or treatment of many diseases that may occur due to oxidative stress and it has also a great potential in the development of new drugs and the treatment of many diseases (Farzaei *et al*., 2017).

Peppermint oil has a significant trade volume in world markets. Mint and its preparations are one of the most researched and interested plants in terms of medicinal aspects. Numerous studies have been conducted on its antiallergic, antibacterial, antifungal, antiviral, antitumor, analgesic, antiemetic, and spasmolytic effects. It is crucial in the pharmaceutical industry and is used in almost all countries due to its antimicrobial, antispasmodic, choleretic, carminative effects, odor-correcting, and aromatic properties. According to a study conducted in Germany, mint and its preparations are the third most common herbal medicine among 131 medicinal plants (Silva, 2020). It also plays a vital role in aromatherapy. Peppermint is also used in veterinary medicine. Mint essential oils come second after citrus essential oil in world's essential oil trade. Although mint is grown in Türkiye, essential oil is not obtained commercially; the oil and menthol that the pharmaceutical and cosmetic industry needs are imported (Arslan, 2018).

In a study conducted with 11 *Mentha* taxa, 3-oxo-1,2-epoxy type compounds (cis-and trans-piperitone oxide and piperitenone oxide) were found as the major components in 56% of the essential oil samples, while the remaining 25% contained 3-oxo compounds (pulegone, menthone, menthofuran, isomenthone, piperitenone, piperitone, and isopulegol), and 10% of the samples contained 2-oxo compounds (carvone, cis- and trans-dihydrocarvone) (Başer *et al*., 2012). It was also reported that 2% of the samples contained 1,8-cineole, and 1% trans-sabinene hydrate as the main components. The essential oil components obtained from the leaves of *M. longifolia* subsp. *longifolia* plants grown in the Bahcesaray region of Van Province of Türkiye contained menthone (19.31%), pulegone (12.42%), piperitone (11.05%), dihydrocarvone (8.32%), limonene (6.1%), 3-terpinolene (5.66%), 1,8-cineole (4.37%), germacrene d (3.38%), and caryophyllene (3.19%) (Okut *et al*., 2017). *M. longifolia* is used in Europe in the food industry as a preservative and flavoring agent due to its polyphenolic and terpenoid content. Phenolic antioxidants have been detected in its composition. It has been proven to be effective against more than 20 microbes as an antimicrobial agent (Patonay & Németh-Zámboriné, 2021). While studying the volatile oils of different *Mentha* species, different essential oil components were obtained. For example, in *M. longifolia* subsp. *typhoides* var. *typhoides* linalool (90.4%), iso-iso-pulegol (48.8%), and pulegone (30.4%) were found to be the main components. Linalool (93.2%) and trans-piperitone oxide (76%) were the main components in *M. x villosa-nervata*. Piperitone (93%) was the main component in *M. pulegium*, piperitenone oxide (73.6%) in *M. suaveolens*, carvone (63.6%) in *M. spicata* subsp. *spicata*, and trans-piperitone oxide (60.5%) in *M. spicata* subsp. *Tomentosa* (Duali, 2010).

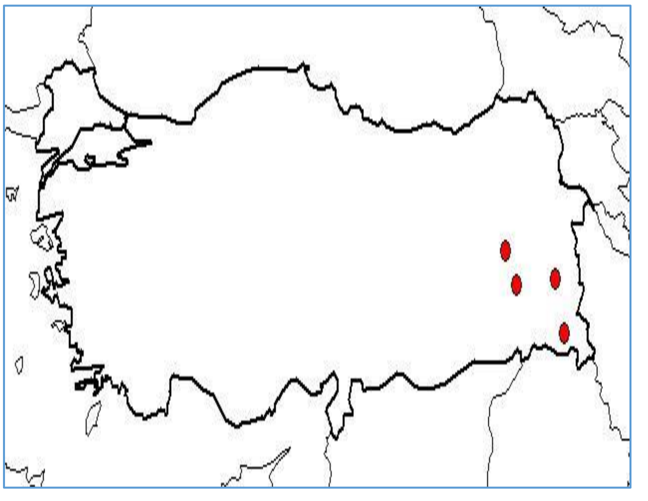

Fig. 1. The provinces of *calliantha* variety in Türkiye (Bakış *et al*., 2011).

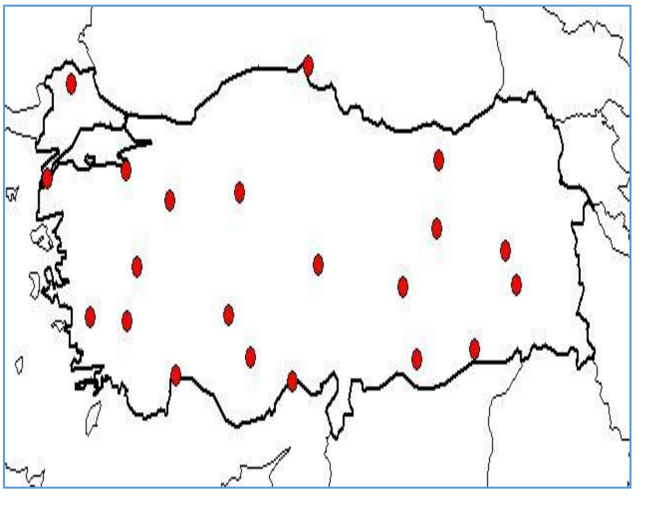

Fig. 2. The provinces of *typhoides* variety in Türkiye (Bakış *et al*., 2011).

The present study of two varieties of pennyroyal *calliantha* and *typhoides,* that grow naturally in the Mus province were evaluated for essential oil composition. These taxa are perennial herbaceous plants and are mostly found in swamps, rivers, and stream banks in Türkiye. *Mentha longifolia* subsp. *typhoides* var. *calliantha* is found in Northwest Iran and Eastern Anatolia of Türkiye at elevations of 1500-3000 meters. It blooms from June to September (Fig. 1). On the other hand, the var. *typhoides*, is found in a wide geographical range in Türkiye, Cyprus, Lebanon, Northwest Iran, and Northern Iraq (Fig. 2). This variety is found at elevations of 900-2135 meters and blooms from July to October (Bakış *et al*., 2011).

Table 1. The essential oil contents of var. *calliantha* and var. *typhoides*.

No.	RI	Component	var. *calliantha*	var. *typhoides*
1.	934	α-Pinene	-	5.51
2.	942	Methane, sulfinylbis	2.37	0.13
3.	967	Methanesulphinic acid methyl ester	-	0.62
4.	1022	1,8-Cineole	1.75	1.27
5.	1082	β-Linalool	5.23	-
6.	1086	Linalool	-	11.63
7.	1125	Camphor	2.73	2.72
8.	1153	Borneol	2.14	3.44
9.	1127	Cyclohexene	2.11	-
10.	1175	α-Terpineol	-	1.90
11.	1183	Cyclobutane	2.24	-
12.	1191	6-Allyl-o-creso	-	0.59
13.	1217	Nerol	0.38	-
14.	1222	Pulegone	29.06	-
15.	1253	Linalyl acetate	4.28	-
16.	1256	Piperitone oxide	7.85	31.57
17.	1258	Cis Piperitone oxide	-	9.98
18.	1265	Thyme camphor	0.82	-
19.	1271	Thymol	5.98	2.01
20.	1283	Carvacrol	-	12.97
21.	1318	Piperitenone	5.24	-
22.	1322	2-Cyclohexen	-	1.88
23.	1336	Arsonic acid	0.71	-
24.	1344	Neryl acetate	-	0.76
25.	1372	Durohydroquinone	-	1.36
26.	1419	Caryophyllene	1.21	3.31
27.	1449	Humulene	0.56	0.80
28.	1453	Carvenone	-	0.25
29.	1475	Germacrene-D	-	0.50
30.	1480	1,6-Cyclodecadiene	-	0.35
31.	1522	3-Ethynyl-4-(trimethylsilyl) furan	0.31	-
32.	1566	Spathulenol	4.32	1.02
33.	1570	Caryophyllene oxide	14.28	3.92
34.	1589	Junipene	0.51	-
35.	1620	Vulgarol	0.80	-
36.	1635	Cyclopentane	1.47	-
37.	1668	α-bisabolol	0.42	0.31
38.	1674	3-Cyclohexen	-	0.28
39.	1680	2-Pentadecanone	0.75	0.39
40.	1687	Nonadien	0.88	-
41.	1693	Aromadendrene oxide	0.42	-
42.	1764	Silane, chlorodimethyl	-	0.20
43.	1836	α-Picolinyl hydrazide	0.24	-
44.	1925	Epi manool	-	0.32
		Total	**99.06**	**99.99**

The purpose of this study is to determine the volatile oil content of two different mint varieties, compare these varieties, investigate their cultivation potential, and identify plant materials with high levels of active substances that meet the standard requirements of the pharmaceutical industry.

Material and Method

Plant materials: Var. *calliantha* was collected in June 2019 at an altitude of 2083 m and coordinates of 38061'69"N, 41065'63"E in the district of Hasköy, Mus. Var. *typhoides* was collected in July 2019 at an altitude of 1510 m and coordinates of 38076'43"N 410 88'41"E in the district of Korkut, Mus.

The identification of the species was carried out by Prof. Dr. Murat UNAL. The collected plants were transplanted into nursery plots at the Faculty of Applied Sciences of Mus Alparslan University. The soil of nursery plots consisted of the mixture of river sand and burnt manure in a 1:1:1 ratio. Size of the plots were assigned as 4 m^2. For nursery plots 3 replicates were done for each variety. In June 2019, when the varieties were planted, rainfall was observed for about a week and the monthly average rainfall was measured as 28.5 mm. During the summer, the parcels where cultivation takes place were irrigated once a week until the harvest season. The average temperature was measured as 20°C. No additional artificial fertilizer was applied. The plants were watered as needed during the summer months. In June and July 2020, during full flowering, the plants were harvested at a height of 10 cm and dried in the shade. The chemical composition of the volatile of dried herb samples was determined.

The extraction and GS-MS analysis of essential oils: The Essential oils were obtained from plant samples using the steam distillation method (Clevenger apparatus). Plant samples collected from the cultivation areas were dried in the shade for one week for hydro distillation. After grinding 40 grams of the samples in a blender (Waring), 300 ml of distilled water was added. It was subjected to distillation in Clevenger apparatus for 6 hours. The obtained samples were stored in light-proof bottles at +4 C in the refrigerator. While the oil yield of var. *callintha* was 3.007 μL/gr, it was 6.66 μL/gr in var. *typhoides*. The oils of dry plant samples taken from each plot were determined volumetrically (ml/g). The components of the volatiles were determined using a Gas Chromatography/Mass Spectrometry (GC/MS) instrument (Agilent 7890A) with a mass detector (Agilent 5975C). The working conditions of GC/MS were as follows: Capillary column: CP-Wax 52 CB (50 m x 0.32 mm, 0.25 μm), Oven temperature program: Started at 60°C and increased by 10°C per minute until it reached 220°C, then held at 220°C for 10 minutes. Total runtime: 60 minutes, Injector temperature: 240°C, Detector temperature: 250°C, Carrier gas: Helium (20 ml/min). The results were interpreted using the Wiley and NIST libraries.

Results and Discussion

The analysis results of the volatile oils obtained from the dry herbal samples of two types of wild mint harvested in 2020, which were propagated in nursery plots of the Applied Sciences Faculty Experimental Fields of Mus Alparslan University are presented in (Table 1).

As seen in Table 1, 28 components were identified in the essential oil of *Mentha longifolia* subsp. *typhoides* var. *calliantha*. The major components were pulegone (29.06%), caryophyllene oxide (14.28%), piperitone oxide (7.85%), thymol (5.98%), piperitenone (5.24%), β-linalool (5.24%), and linalyl acetate (4.28%). In var. *typhoides*, 28 components were identified. The main components of the essential oil of var. *calliantha* were piperitone oxide (31.57%), cis piperitone oxide (9.98%), carvacrol (12.97%), linalool (11.63%), α-Pinene (5.51%), Borneol (3.44%), caryophyllene oxide (3.92%) and caryophyllene (3.31%). The main components of the two varieties were found to be different from each other. The main component of var. *calliantha* was pulegone, which was not found in var. *typhoides* variety. The main component identified in var. *typhoides* was piperitone oxide (31.57%), while in var. *calliantha* it was 7.85%. While there has been almost no research on the volatile oil compositions of var. *calliantha,* some studies have been conducted on *typhoides* variety (Aksit *et al.,*2013).

When comparing the essential oil component of *M. longifolia* species with the *calliantha* variety, it was found that the values of the main components, pulegone, and piperitone oxide, were consistent with the literature (Ghoulami *et al*., 2001; Gulluce *et al*., 2007; Hajlaoui *et al*., 2009; Mkaddem *et al*., 2009; Abdulselam, 2011; Başer *et al*., 2012; Sharopov *et al*., 2012; Anon., 2022). However, the thymol content was slightly higher than previous studies (Sharopov *et al*., 2012). In the var. *typhoides*, pulegone was not detected, but the content of piperitone oxide, α-Pinene, and carvacrol were found to be high similar to previous research (Ghoulami *et al*., 2001; Gulluce *et al*., 2007; Başer *et al*., 2012; Sharopov *et al*., 2012; Yanmis *et al*., 2012). It was also indicated by Patonay & Németh-Zámboriné (2021) that the essential oil composition of *M. longifolia* varies widely.

Conclusion

Two cultivated subspecies of var. *calliantha* and var. *typhoides* that naturally growing in the province of Mus, were analyzed for their essential oil component. The main components of var. *calliantha* were found to be pulegone, caryophyllene oxide, thymol, piperitenone, β-linalool, piperitone oxide, and linalyl acetate, while var. *typhoides* had piperitone oxide, carvacrol, linalool, cis piperitone oxide, α-pinene, borneol, and caryophyllene oxide. The number of components identified was 28 for borth var. *calliantha* and var. *typhoides*. Pulegone and piperitone oxide were the main components of var. *calliantha* and var. *typhoides*, respectively. No significant problems were encountered in the cultivating of these two species under the continental climate conditions. Both species have great potential for developing new drugs and the treatment of many diseases. Therefore, these varieties can be cultivated and used effectively in areas where they are required.

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