

The Essential Oil Components of five *Micromeria* Species grown in Anatolia

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Abstract

In this study, the essential oil of five *Micromeria* species, six plant samples grown in Anatolia; *M. juliana*, two different locality of *M. myrtifolia*, *M. cristata* subsp. *cristata*, *M. cristata* subsp. *phrygia* and *M. cristata* subsp. *orientalis* were evaluated. The oils obtained by hydrodistillation and analyzed by GC/MS. Totally forty-seven compounds were detected in the oil of studied *Micromeria* species representing 91.2- 98.5%. **β -Caryophyllene** (1.4- 58.8%), **Caryophyllene oxide** (6.4- 33.9%) were found all studied species in high ratio. While two compounds were identified as the main components of the essential oils of *M. juliana*, two different locality of *M. myrtifolia*, **Borneol** (23.2- 35.3%) was main components of the essential oils of *M. cristata* subsp. *cristata*, *M. cristata* subsp. *phrygia* and *M. cristata* subsp. *orientalis*.

Keywords: Essential oil, *Micromeria*, β -Caryophyllene, Caryophyllene oxide, Borneol.

Anadolu'da Yetişen Beş *Micromeria* Türünün Uçucu Yağ Bileşenleri

Özet

Bu çalışmada, Anadolu'da yetişen beş *Micromeria* türüne ait altı bitki örneğinin; *M. juliana*, *M. myrtifolia*'nın iki farklı lokalitesi, *M. cristata* subsp. *cristata*, *M. cristata* subsp. *phrygia* ve *M. cristata* subsp. *orientalis*, uçucu yağları incelenmiştir. Uçucu yağlar hidrodestilasyon yoluyla elde edilmiş ve GC/MS ile analiz edilmiştir. Toplamda *Micromeria* türünün uçucu yağının % 91.2- % 98.5 ini oluşturan kırkyedi bileşik tespit edilmiştir. **β -Caryophyllene** (%1.4- %58.8), **Caryophyllene oxide** (%6.4- 33.9) çalışılan tüm türlerde yüksek oranlarda belirlenmiştir. Bu iki bileşik *M. juliana*, *M. myrtifolia*'nın iki farklı lokalitesinin uçucu yağlarının ana bileşini olarak belirlenirken, **Borneol** (23.2- 35.3%) *M. cristata* subsp. *cristata*, *M. cristata* subsp. *phrygia* and *M. cristata* subsp. *orientalis*'in uçucu yağlarının ana bileşeni olarak belirlenmiştir.

Anahtar Kelimeler: Uçucu yağ, *Micromeria*, β -Caryophyllene, Caryophyllene oxide, Borneol.

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1. Introduction

The genus *Micromeria* Benth., which is a member of Labiatae (Lamiaceae) family, is widely found in the eastern part of Mediterranean area [1] and represented in Turkey by 8 species and 13 taxa, 7 of them being endemic [2]. In general, *Micromeria* species are used as a herbal tea and substituted for mint. They are used as remedies against colds, heart disorders, chest pains, headache, stomachache, skin infections, inflamed eyes, kidney stone, and topical anesthetic in toothache and wounds [3-6]. *Micromeria* species are also reported to have many pharmacological activities such as antirheumatic, antiseptic, anesthetic and the extracts of some species reported that they showed antioxidant, anticholinesterase antibacterial, antifungal, insecticidal activities [7]. Several essential oil studies on *Micromeria* species have been reported from Italy [8], Serbia and/or Montenegro [9-11], Croatia [12], Greece [13], Hungary [14,15], Macedonia and Bulgaria [16], Yugoslavia [17], Canary Islands [18], Saudi Arabia [19,20], Iran [21], Israel [22] and Lebanon [23]. Most of them reported that the species were rich in Borneol, Pulegon, piperitone oxide, piperitone, spathulenol and limonene. In point of *Micromeria* species which grown in Turkey, same results have been reported. Borneol is the most abundant compound of the essential oils of *M. cristata* (Hampe) Griseb. subsp. *phrygia* P.H. Davis [3, 24], *M. cristata* (Hampe) Griseb. subsp. *xylorrhiza* (Boiss. & Heldr.) P.H. Davis, *M. cristata* (Hampe) Griseb. subsp. *carminea* (P.H. Davis) P.H. Davis, *M. cristata* (Hampe) Griseb. subsp. *orientalis* P.H. Davis [24]. The major components of other studied species as in follows; β -Caryophyllene is *M. juliana* (L.) Benth. ex Reichb. [24] and *M. myrtifolia* Boiss. & Hohen [25]; Germacrene-D is *M. cremnophilla* ssp. *amana* [26].

In this study we report the essential oils components of five species six plant samples of *Micromeria*; *M. juliana*, two different locality of *M. myrtifolia*, *M. cristata* subsp. *cristata*, *M. cristata* subsp. *phrygia* and *M. cristata* subsp. *orientalis*. *M. cristata* subsp. *phrygia* and *M. cristata* subsp. *orientalis* are endemic species for Turkey.

2. Materials and Methods

2.1. Plant material

Locality, altitude, collection time and Herbarium number of six samples of *Micromeria* are as follows;

1: *Micromeria juliana*; Balıkesir-Edremit, Mount Ida - Atkayası ; 1000 m; 20.06.2005; TD 3083, **2:** *M. myrtifolia*; Antalya, 19th km of between Manavgat and Serik; 30 m; 13.06.2005; TD3080, **3:** *M. myrtifolia*; Tekirdağ, Mount Ganos , Western parts of Uçmakdere village; 50 m; 17.07.2005; TA 2115, **4:** *M. cristata* subsp. *cristata*; Amasya: 10th km of between Amasya and Taşova; 873 m; 30.06.2007; TD3611, **5:** *M. cristata* ssp. *phrygia*; Kütahya; 10 km from parting of the Kütahya-Gediz; 1025 m; 12.09.2008; TD3665, **6:** *M. cristata* ssp. *orientalis*; Malatya-Akçadağ, East part of Develi village; rocky side; 25.07.2008; TA2695.

The voucher specimens were deposited in the Herbarium and Department of Biology Education, Necatibey Education Faculty, Balıkesir University.

2.2. Isolation of essential oils

40-150 g of dried aerial parts of the plants were cut in small pieces and then subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The essential oils were collected and stored in sealed vials protected from the light at -18 °C before analysis. The essential oils of species were diluted by dichlorometane (1:3, v/v) before the GC run. The yields of essential oils are as follow: (The name of species; amount of dried plant (g): amount of essential oils(g): percentage of yields) **1:** *Micromeria juliana*; 66: 0.010: 0.015, **2:** *M. myrtifolia*; 72: 0.073: 0.101, **3:** *M. myrtifolia* 70 : 0.065 : 0.093, **4:** *M. cristata* subsp. *cristata*; 124 : 0.010 : 0.008, **5:** *M. cristata* ssp. *phrygia* ;133 : 0.012 : 0.010, **6:** *M. cristata* ssp. *orientalis*; 40 : 0.043 : 0.110.

2.3. GC/MS conditions

GC/MS analyses were performed on Thermo Electron Trace 2000 GC model gas chromatography and Thermo Electron DSQ quadropole mass spectrometry. A nonpolar Phenomenex DB5 fused silica column (30 m x 0.32mm, Ø with 0.25 µm film thickness) was used with helium at 1mL/min (20 psi) as a carrier gas. The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min and then kept constant at 220°C for 15 min. The split ratio was adjusted to 1:20 the injection volume was 0.1 µL and EI/MS was recorded at 70eV ionization energy. Mass range was *m/z* 35-500 amu. A homologous series of *n*-alkanes were used as reference in the calculation of Kovats Indices (KI). Identification of the compounds was based on the comparison of their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as the literature data.

3. Results and Discussion

3.1. Composition of the essential oils

The chemical compositions of the essential oils of five *Micromeria* species, six plant samples were evaluated. Forty-seven compounds, which accounted for 91.2- 98.5% of the total composition of oils are reported in Table 1.

The essential oil of *Micromeria juliana*, which accounted for 98.5 % of the total composition of the oil, was characterized by its high proportion of sesquiterpenes (81.5%) followed oxygenated monoterpenes hydrocarbons (14.0%), other hydrocarbons (2.7%) and monoterpenes hydrocarbons (0.3%). Of the 34 components detected, the most abundant were β -Caryophyllene (58.8%), Caryophyllene oxide (9.4%), and Pulegone (8.6%).

In *M. myrtifolia* (TD 3080) oil, 29 components were determined representing 91.2 % of the total oil. The oil consisted mainly of sesquiterpenes (77.7%), other hydrocarbons (9.5%), oxygenated monoterpenes hydrocarbons (5.4%), and monoterpenes hydrocarbons (0.6%). β -Caryophyllene (40.5%), Caryophyllene oxide (18.6%), and α -Copaene (6.0%) were the major components.

In different locality of *M. myrtifolia* (TA 2115) oil, 34 components were identified representing 96.7 % of the total oil, which consisted of sesquiterpenes (81.6%), oxygenated monoterpenes (9.9%), monoterpene hydrocarbons (0.5%) and other hydrocarbons (4.7%). The major components of the oil were Caryophyllene oxide (33.9%), β -Caryophyllene (32.0%), and γ -muurulene (8.4%), respectively.

Table 1. Essential oils composition of the *Micromeria* species^a

RI	Compound	1	2	3	4	5	6
935	Thujene	t	t	t	t	t	t
941	α -Pinene	0.1	0.1	0.1	t	t	t
954	Camphene	-	-	-	t	t	t
976	Sabinene	0.1	0.1	0.1	-	-	-
979	1-Octen-3-ol	t	t	t	t	t	t
981	β -pinene	0.1	t	0.3	t	t	t
990	Myrcene	t	0.2	t	-	-	-
1011	Δ^3 - Carene	t	t	t	t	t	t
1025	p-Cymene	-	-	-	0.7	0.3	0.9
1028	Limonene	t	0.2	t	-	-	-
1032	1,8-Cineole	t	t	t	4.0	4.6	3.5
1062	γ -Terpinene	t	-	t	t	t	t
RI	Compound	1	2	3	4	5	6
1082	α -Terpinene	t	t	t	-	-	-
1123	trans-p-Mentha-2,8-dienol	t	-	t	t	t	t
1141	Verbenol	-	-	-	5.6	5.0	4.5
1146	Camphor	-	-	-	12.4	13.5	9.8
1155	p- Menthone	5.3	0.1	5.3	-	-	-
1164	Isomenthone	t	0.1	t	t	t	t
1169	Borneol	-	-	-	23.2	35.3	33.7
1176	Terpinen-4-ol	t	-	t	1.5	1.1	1.6
1206	Verbenone	t	t	0.8	0.6	0.8	0.7
1218	Carveol	0.1	-	t	-	-	-
1236	Pulegone	8.6	3.3	4.6	-	-	-
1247	Piperitone oxide	t	3.5	t	-	-	-
1253	Piperitone	t	1.6	t	-	-	-
1289	Bornyl acetate	-	-	-	4.0	4.6	4.2
1292	Thymol	t	0.3	t	-	-	-
1327	Menthyl acetate	t	-	t	-	-	-
1341	Neoisomenthyl acetate	t	t	-	-	-	-
1344	Piperitenone	1.6	4.6	2.4	-	-	-
1365	Piperitenone oxide	1.1	0.9	1.5	-	-	-
1377	α -Copaene	t	6.0	t	-	-	-
1387	B-Bourbonene	t	2.2	t	-	0.5	1.4
1394	β -Elemene	0.1	t	t	-	-	-
1421	β -Caryophyllene	58.8	40.5	32.0	1.4	1.5	2.6
1455	α -Humulene	3.2	3.3	2.0	-	-	-
1480	γ -Muurulene	t	0.7	8.4	-	-	-
1482	Germacrene-D	t	-	t	0.6	t	t
1524	δ -Cadinene	t	6.4	3.5	t	t	t
1546	α -Calacorene	-	-	-	1.1	1.0	1.3
1574	Germacrane D-4-ol	-	-	1.8	-	-	-
1578	Spathulenol	-	-	-	11.3	10.7	8.1
1584	Caryophyllene oxide	19.4	18.6	33.9	6.4	9.5	6.8
1607	Epoxy-ionone	-	0.5	-	-	-	-
1639	α -Cadinol	-	-	-	4.7	5.0	13.0
1641	Aromadendrene	-	-	-	3.2	3.5	t
1642	γ -Muurolol	-	-	-	17.2	1.2	6.1
	TOTAL	98.5	91.2	96.7	97.9	98.1	98.2

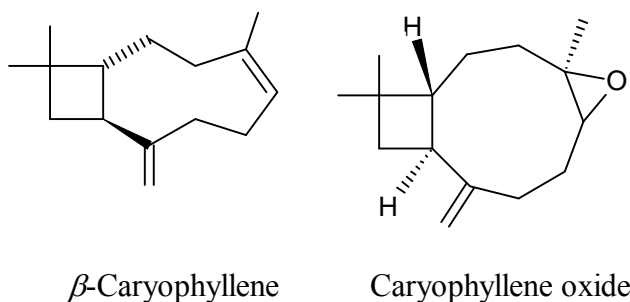
^aGC-MS analyses were replicated three times (Mean RSD % value is 0.1); t:trace (<0.1%)

1-M. juliana (TD 3083) 2 - M. myrtifolia (TD3080). 3- M. myrtifolia (TA 2115), 4-M. cristata subsp. cristata (TD3611), 5-M. cristata ssp. phyrigia (TD3665), 6-M. cristata ssp. orientalis (TA2695)

In *M. cristata* subsp. *cristata* oil, 26 components were identified representing 97.9% of the oil. The oil consisted mainly of oxygenated monoterpenes (46.7%), sesquiterpenes (44.8%), monoterpene hydrocarbons (4.7%) and other hydrocarbons (1.7%). Borneol (23.2%), γ -Murolool (17.2%), and Camphor (12.4%) were determined as the major components of the oil.

In the essential oil of *M. cristata* subsp. *phyrigia*, totally 27 components were found and the oil contained oxygenated monoterpenes (59.5%), sesquiterpenes (31.9%), monoterpene hydrocarbons (4.9%) and other hydrocarbons (1.8%). The major components were Borneol (35.3%), Camphor (13.5%), and Sapathulenol (10.7%).

In *M. cristata* subsp. *orientalis* oil, 27 components were identified, representing 98.2 % of the total oil. The oil consisted mainly of oxygenated monoterpenes (53.8%), sesquiterpenes (38.0%), other hydrocarbons (2.0%) and monoterpene hydrocarbons (4.4%). Borneol (33.7%), α -Cadinol (13.0%) and Camphor (9.8%) were the major components.



Scheme 1. β -Caryophyllene and Caryophyllene oxide

The essential oil of *M. juliana* and two different localities of *M. myrtifolia* were consisted of sesquiterpenes while the essential oil of other three subspecies of *M. cristata*; *M. cristata* subsp. *cristata*, *M. cristata* ssp. *phyrigia*, *M. cristata* ssp. *orientalis* were consisted of oxygenated monoterpenes.

3.2. Discussions

The principal aim of this study was to analyze (GC/MS) the essential oil of five *Micromeria* species, six plant samples, grown in Anatolia two of them, *M. cristata* subsp. *phyrigia* and *M. cristata* subsp. *orientalis*, are endemic species for Turkey. The GC/MS analyses the essential oil obtained by hydrodistillation from dry aerial parts of six samples showed that **β -Caryophyllene** (1.4- 58.8%), **Caryophyllene oxide** (6.4-33.9%) (Scheme 1) were found the major essential oil components of the studied plants. Furthermore, while **Pulegone** (3.3-8.6%), ***p*-Menthone** (0.1-5.3 %), **Piperitone oxide** (1.6-4.6%), **α -Humulene** (2.0-3.3%) and **γ -Muurulune** (trace to 8.4%) were found to be rich in the oil of *M. juliana* and two different localities of *M. myrtifolia*, **Borneol** (23.2-35.3%), **Camphor** (9.8-13.5%), **Sapathulenol** (8.1-11.3%), **Bornyl acetate** (4.0-4.6%) and **α -Cadinole** (4.7-13.0%) were found in the oil of *M. cristata* subsp. *cristata*, *M. cristata* ssp. *phyrigia*, *M. cristata* ssp. *orientalis*. In addition these components, **Verbenone** (trace to 0.8%) and **δ -Cadinene** (trace to 6.4%) were determined all studied species. The results are in accordance with previous studies [3, 24-25].

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