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The Chemical Composition of Essential Oils of *Melissa officinalis* subsp. *altissima* from Turkey

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Abstract: *Melissa officinalis* is a perennial herb and it is a member of the *Lamiaceae* family. In this study, the determining of chemical contents of *M. officinalis* subsp. *altissima* originated from the wild flora of Turkey was aimed. The seeds collected from the wild flora of Antalya, were grown in greenhouse conditions and experimental field. Then dried leaves were hydrodistillated with clevenger apparatus and samples were analysed with a gas chromatography. In total, twenty-three constituents were identified, representing 92.5–94.7% of the oils. The oils of all samples were rich in sesquiterpenes such as caryophyllene oxide (44.5-33.3%), β-copaene (10.00-8.72%) and caryophyllene (5.57-8.26%). Caryophyllene oxide was the major component in all three samples (44.5-33.4-38.58%, respectively).

Key words: *Melissa officinalis* subsp. *altissima,* essential oil composition, GC-MS analysis, sesquiterpenes, caryophyllene oxide

Türkiye'den *Melissa officinalis* subsp. *altissima*'nın Esansiyel Yağının Kimyasal Kompozisyonu

Özet: *Melissa officinalis* çok yıllık bir bitkidir ve *Lamiaceae* ailesinin bir üyesidir. Bu çalışmada, Türkiye doğal florası orjinli *M. officinalis* subsp *altissima*'nın kimyasal içeriğinin belirlenmesi amaçlanmıştır. Antalya doğal florasından toplanan tohumlar, sera koşulları ve deneme arazilerinde büyütülmüştür. Daha sonra kurutulan yapraklar Clevenger aparatı ile hidrodistile edildi ve numuneler gaz kromatografi ile analiz edildi. Toplamda % 92,5-94,7 oranında 23 bileşen belirlenmiştir. Tüm örneklerdeki yağlar karyofilen oksit (% 44,5-33,3), β-copaene (%10,00-8,72) ve karyofilen (%5,57-8,26) gibi seskiterpenlerce zengindi. Karyofilen oksit her üç örnekte (sırasıyla % 44.5-33.4-38.58) 'de önemli bir bileşen olmuştur.

Anahtar kelimeler: *Melissa officinalis* subsp. *altissima*, uçucu yağ kompozisyonu, GC-MS analizi, sesquiterpenler, kariyofilen oksit

Introduction

Melissa officinalis is a member of the *Lamiaceae* (mint) family. Three subspecies, namely *M. officinalis* subsp. *officinalis*, *M. officinalis* subsp. *altissima* and *M. officinalis* subsp. *inodora* are known. There are many reports on *M. officinalis* subsp. *officinalis* but there are only a few reports on *M. officinalis* subsp. *altissima*. It is native to the Mediterranean region and the dry leaves of *M. officinalis* are used as a herbal tea for their properties in folk medicine (Meftahizade et al., 2010). *Melissa officinalis* is known as lemon balm for its fragrance and it has commercial value due to characteristic scent (Craker and Simon, 1992). Lemon scented herbs are used for remedies in most of disorders such as antispasmodic, carminative and sedative.

Lemon balm is used against rheumatism, hypersensitivities treatment and indigestion (Petersen and Simmonds, 2003; Weitzel and Petersen, 2011). Nearly 100 chemicals have been identified in *M. officinalis* and essential oil of lemon balm has 39% citronellal, 33% citral and 2% geranial as main components (Hohmann et al., 1999; Tagashira and Ohtake, 1998). In addition to the main components it has other phytochemicals such as linalool and β-caryophyllene-oxide (Adzet et al., 1992; Shalaby et al., 1995). It is rich in flavonoids (cynaroside, cosmosin, rhamnocitrin and isoquercitrin), which is included terpenes (ursolic and oleanolic acid), caffeic acids derivatives (rosmarinic acid), and phenolic acids (carnosic acid) (Bolkent et al., 2005; Geuenich et al., 2008; Ribiero et al., 2001). Its essential oil has light yellow color (Saeb and Gholamrezaee, 2012) and it has antimicrobial activity (Moradkhani et al., 2010) and antioxidative activity (Hohmann et al., 1999; Moradkhani et al., 2010). Especially triterpene acids and phenolic acids are well known antioxidants (Schultze et al., 1995). Some of literature reported on *Melissa* essential oils show difference in terms of content due to various ecological conditions (Lawrence, 2008) such as geographical features, climatic variations or soil properties. The differences may result from genetic variability (Adzet et al., 1992), diurnal changes or ontogenetic differences (Van den Berg et al., 1997).

There are three subspecies, *M. officinalis* subsp. *officinalis*, *M. officinalis* subsp. *altissima* and *M. officinalis* subsp. *inodora*. Especially *M. officinalis* subsp. *officinalis* is commonly used as herbal tea in most of region and our knowledge is more about subsp. *officinalis* than the other subspecies (Dawson et al., 1998; Van den Berg et al., 1997). *M. officinalis* subsp. *altissima* has bad smell and it is described as "scentless to fetid" (Tucker and Debaggio, 2000). There are many reports on *M. officinalis* subsp. *officinalis* but there are only a few reports on *M. officinalis* subsp. *inodora* and *M. officinalis* subsp. *altissima*. The aim of this study was to determine the essential oil composition of *M. officinalis* subsp. *altissima*, according to our knowledge, have not yet been performed this plant in Turkey.

Materials and Methods

Plant material

The seeds of *M. officinalis subsp. altissima* were collected from the wild flora of Antalya (Serik-Akbas, altitude 220 m). Seedlings were grown in greenhouse condition and then they were transferred to the experimental field of the Faculty of Agriculture, Akdeniz University located in Mediterranean Region of Turkey (33 m above sea level and 36° 53' N; 30° 38' E). This region was characterized by a Mediterranean climate with 1068 mm of total rain fall, 18.7 °C mean air temperature, 13.6 °C minimum air temperature and 24.2 °C maximum air temperature. Soil characteristics of the experimental field were clay loam, high in lime (15.1%), low in salt (0.022%), and alkaline (pH 7.9). The layer of 0-30 cm soil had low concentrations of organic material (1.55%) and sufficient amount of nitrogen (0.118%). Available phosphorus content of the soil was low (5.1 kg/da) and useful potassium content was high (124.8 kg/da).

In order to determine the essential oil variation within the population, three different plant samples (Sample A, Sample B and Sample C) were analysed. Aerial parts of *M. officinalis* subsp. *altissima* were collected in second year of the plants during flowering stage. The fresh leaves were dried under shadow at room temperature.

Essential oil isolation

Dried leaves were hydrodistillated in Clevenger apparatus. For this study, 500 mL deionised water was added to 50 g of leaf samples and they were heated at 100 °C for 3 h. As a result, percentage of essential was measured by the volumetric method (v/w) for each sample.

Gas Chromatography-Mass Spectrometry

Samples were diluted 1:50 with hexane for analyses. GC-MS analyses were performed on a gas chromatography (Agilent 7890A)-mass detector (Agilent 5975C) GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (250 °C). Temperature

was programmed from 60°C (10 min.) to 250 °C (10.5 min.) and analysis was carried out in a total time of 60 minutes. Helium was used as carrier gas at the flow rate of 0.8 ml/min. The capillary column used was HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm. Samples were injected 1 µL with 50:1split rate. The identification of the essential oil components of *M. officinalis* subsp. *altissima* was confirmed by comparison of their relative retention times and mass spectra with OIL ADAMS, NIST and Wiley libraries.

Results and Discussion

In this study composition of the essential oil from leaves of three *M. officinalis* subsp. *altissima* plants were analyzed and chemical components of samples with retention time are shown in Table 1 and Figure 1. Oil yield was less than 0.5 ml oil/kg herb. When compared with other herb which belonging to the Lamiaceae family, essential oil rate is quite low. In total, twenty-two constituents were identified, representing 92.31–92.54% of the oils. The oils of three samples did not display significant differences and major constituents were similar. Caryophyllene oxide was determined as major component in all three samples (44.5-33.4-38.58%, respectively). Basta et al. (2005) also found caryophyllene oxide as main component in *M. officinalis* subsp. *altissima* samples from Greece, but their rates (15.8-24.4%) were much lower than Turkish samples. Also, other major constituents apart from caryophyllene oxide were found to be different in both genotypes; β-copaene, tyranton, caryophyllene and aromadendrene epoxide were in Turkish samples, but caryophyllene, germacrene D, β-pinene and sabinene were in Greek samples. On the other hand, Van den Berg et al. (1997) identified the main components of the cultivated *M. officinalis* subsp. *altissima* as germacrene D (34.79-51.50%) and β-caryophyllene (7.27–12.66%). This could be a different chemotype with high percentage of germacrene D which was not found in our samples.

Table 1. Composition of the essential oil of *M. officinalis* subsp. *altissima.*
Tablo 1. . M. officinalis subsp. *Altissima'nın uçucu yağ kompozisyonu*

Components Bileşenler	RT	Sample A Örnek A	Sample B Örnek B	Sample C Örnek C
β-pinene	11.740	1.23	3.78	1.24
Sabinene	12.114	0.85	3.53	0.96
γ-terpinene	14.757	-	-	1.02
Tyranton	16.505	5.04	9.98	2.03
β-bourbonene	18.120	0.76	1.61	1.64
Bornyl acetate	18.592	-	-	1.31
Terpinen-4-ol	18.698	1.81	1.92	3.29
Caryophyllene	18.779	5.57	8.26	7.94
α-humulene	19.308	0.75	-	0.95
β-copaene	19.553	6.62	10.08	8.72
Myrtenol	19.925	1.07	2.79	0.51
Nerolidol	21.229	-	-	0.80
Caryophyllene oxide	21.405	44.50	33.34	38.58
Humulene epoxide	21.755	2.59	2.28	2.36
Hexahydrofarnesyl acetone	21.866	1.94	-	1.20
Fokienol	22.001	-	-	0.53
Spathulenol	22.076	1.10	-	0.47
α-cadinol	22.813	-	-	1.03
Caryophylla-4(12),8(13)-dien-5-β-ol	23.389	3.20	2.47	3.06
Caryophylla-3,8(13)-dien-5-β –ol	23.644	3.11	2.97	2.03
Aromadendrene epoxide	23.983	7.93	5.99	7.88
Manool oxide <13-epi->	24.171	4.47	3.31	4.96
Total identified (%)		92.54	92.31	92.51

RT : Retention time in minutes from GC

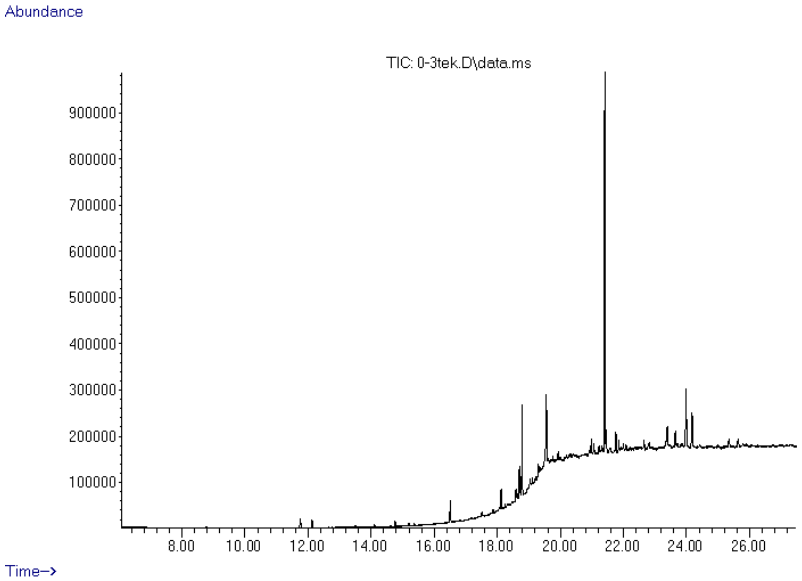

Figure 1. Gas chromatogram of essential oil of *M. officinalis* subsp. *altissima* (sample C)
Şekil 1. M. officinalis subsp. *altissima (Örnek C)'ın uçucu yağının gas kromotogramı*

The oils of all samples were rich in sesquiterpenes such as caryophyllene oxide (44.5-33.3%), β-copaene (10.00-8.72%) and caryophyllene (5.57-8.26%). However, Basta et al. (2005) found all the samples were rich in monoterpenes (34.0–72.8%) with β-pinene and sabinene but two samples were characterized by the presence of a significant sesquiterpene fraction (53.8-47.8%, respectively) with caryophyllene oxide and (*E*)-caryophyllene being the dominant component.

Three samples revealed some variation in terms of the essential oil components. In sample A caryophyllene oxide (44.5%), aromadendrene epoxide (7.93%), β-copaene (6.62%), caryophyllene (5.57%) and tyranton (5.04%); in sample B, caryophyllene oxide (33.34%), β-copaene (10.08%), tyranton (9.98%), caryophyllene (8.26%) and aromadendrene epoxide (5.99%); in sample C, caryophyllene oxide (38.58%), β-copaene (8.72%), caryophyllene (7.94%) and aromadendrene epoxide (7.88%) were the main components (Table 1.). Also, *M. officinalis* subsp. *altissima* exhibited a different chemical profile from *M. officinalis* subsp. *officinalis* which contains significant amounts of citral and citronellal (Basta et al., 2005).

M. officinalis subsp. *altissima* which is native to Turkey and Greece has not been studied intensively despite of the fact that its essential oil constituents could be interesting and its cultivation is straightforward. This study showed that *M. officinalis* subsp. *altissima* originated from the wild flora of Turkey was appeared to be from Greek genotypes in terms of chemical contents and was rich in caryophyllene oxide which could be used as antifungal agent.

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