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Chemical Diversity in Essential Oil Compositions of Leaf, Herb and Flower in Lemon Balm (*Melissa officinalis* L.)

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Abstract

This research was conducted to determine chemical variability in terms of essential oil compositions in leaves, herb and flowers of lemon balm. In experiment, *Melissa officinalis* ssp. *officinalis* was used as the material. Leaf and herb samples were taken before flowering stage while flower samples were taken at full flowering stage. The essential oils were isolated by hydro-distillation and analyzed by GC/MS. According to results, significant variations were observed in leaves, herb and flowers as concerns essential oil components. Thirteen, fifteen and fourteen components were identified in leaves, herb and flowers, respectively. In leaves and flower, main component was citral (25.22% and 21.20%), followed by caryophyllene oxide (21.95% and 18.44%) and z-citral (19.08% and 16.03%). Compared to leaf and flower, herb showed differences in terms of major components. While, caryophyllene oxide (29.25%) was the main component, citral (15.20%) and β-caryophyllene (12.14%) were the second and third components in herb, respectively.

Keywords: Lemon balm, *Melissa officinalis,* essential oil compositions, caryophyllene oxide

Oğulotu (*Melissa officinalis* L.)'nda Yaprak, Herba ve Çiçekte Uçucu Yağ Bileşenlerindeki Kimyasal Değişim

Özet

Bu çalışma oğulotu (*Melissa officinalis* L.)'nun yaprak, herba ve çiçeğindeki uçucu yağ bileşenleri açısından değişimi belirlemek için yapılmıştır. Denemede materyal olarak *Melissa officinalis* ssp. *officinalis* kullanılmıştır. Yaprak ve herba örnekleri çiçeklenme öncesi dönemde alınırken, çiçek örnekleri tam çiçeklenme döneminde alınmıştır. Uçucu yağlar su distilasyonu yöntemiyle elde edilmiş olup, GC/MS cihazıyla bileşen analizi yapılmıştır. Araştırma sonuçlarına göre, yaprak, herba ve çiçekte uçucu yağ bileşenleri bakımından önemli değişimler tespit edilmiştir. Yaprak, herba ve çiçekte sırasıyla 13, 15 ve 14 uçucu yağ bileşeni belirlenmiştir. Yaprak ve çiçekte ana bileşen citral (%25.22 ve %21.20) olurken, bunu caryophyllene oxide (%21.95 ve %18.44) ve z-citral (%19.08 ve %16.03) izlemiştir. Yaprak ve çiçekle kıyaslandığında, ana bileşenler bakımından herbada farklılıklar görülmüştür. Nitekim herbada caryophyllene oxide (%29.25) ana bileşen olurken, citral (%15.20) ve β-caryophyllene (%12.14) sırasıyla ikinci ve üçüncü bileşen olmuştur.

Anahtar kelimeler: Oğulotu, *Melissa officinalis,* uçucu yağ bileşenleri, caryophyllene oxide

Introduction

Lemon balm (*Melissa officinalis* L.), belonging to Lamiaceae family and one of the important medicinal plant, is a herbaceous perennial plant and is reaching up to 100 cm. This species originates from Southern Europe, Asia Minor and southern parts of North America and distributed in all Mediterranean countries including the coastal regions of Turkey (Baytop, 1984; Ceylan, 1987).

There are three subspecies (ssp. *officinalis*, ssp. *inodora* and ssp. *altissima*) of *M. officinalis* and only ssp. *officinalis* has medicinal value and the characteristic lemony odor. Because the essential oil rate of lemon balm is quite low, the production cost and price of the oil are very high. Therefore, lemon balm oil is sometimes adulterated with *Cymbopogon* spp. or Citrus pile oil (Baytop, 1984; Cosge et al., 2009).

Lemon balm is used for several purposes such as additive in food, ingredients in cosmetics, and herb tea. Especially, it is used in traditional medicine from ancient times. English writer John Evely (1620-1706) described this plant as "rule of brain, strengthening to mental and removing from melancholia". Also, its essential oil was named "leader of the oils" in Hebrew (Bahtiyarca Bağdat, 2006). Besides, the leaves of the plant are used as herbal tea for colds, gastrointestinal disorders, headache, insomnia and calming nerves in folk medicine. The leaves and young flowering shoots of lemon balm have antibacterial, antiviral, antispasmodic, carminative, sedative, digestive and tonic effects (Ayanoglu et al., 2005). Most of its pharmacological properties have been attributed to essential components. There are many studies determining essential oil composition of *M. officinalis* under different conditions (El-Gergaihi et al., 1985; Adzet et al., 1992; Koller et al., 1999; Tinmaz et al., 2001; Saglam et al., 2004; Simionatto et al., 2009; Vaverkova et al., 2012). However, information about comparative essential oil compositions of leaf, herb and flower in lemon balm is limited. Especially, the study on chemical composition of flower oil in lemon balm is quite limited. Therefore, the aim of this study was to determine the chemical composition of leaf, herb and flower essential oil in *M. officinalis* ssp. *offcinalis.*

Materials and Methods

Plant material

This study was carried out in Ankara conditions in 2012. *Melissa officinalis* ssp. *officinalis*, studied previously and existed in experiment field of Department of Field Crops, Faculty of Agriculture of Ankara University (39° 57' N, 32° 52' E), was used as plant material in this research.

Method

To determine diversity in essential composition of *Melissa officinalis*, leaf, herb and flower samples were taken with three replications from the plant. While leaf and herb samples were taken at before flowering stages (10 May 2012), flower samples were taken at full flowering stage (13 July 2012) and all samples were dried at the shade for essential oil isolation.

Isolation of essential oil

The essential oils have been extracted from (50 g) air-dried leaves, herbs and flowers by hydrodistillation for 3 h, using Clevenger-type apparatus. The amount of essential oil was determined according to volumetric method.

Chromatography (gc ms^{-1}) analysis

All gas chromatography (GC) analyses were carried out on a Hewlett Packard 6890 N GC instrument, fitted with a HP 5MS 30 m×0.25 mm×0.25 µm film thickness capillary column and FID detector. The column temperature was programmed from 50°C to 150°C at an initial rate of 3°C min^{-1}. The injector and detector temperatures were programmed at 220°C and 290°C, respectively. Helium was used as the carrier gas at a flow rate 1 mL min^{-1}. The gas chromatography-mass spectrometry (GC/MS) analyses were performed using a Hewlett Packard 5973 (mass selective detector)-6890 GC/MS system operating in the electron ionization system with ionization energy of 70 eV (equipped with a HP 5MS 30 m × 0.25 mm × 0.25 µm film thickness capillary column), using He (1 mL min^{-1}) as the carrier gas. The initial temperature of the column was 50°C and then heated gradually to 150°C with a 3°C min^{-1} rate, held for 10 min and finally raised to 250 ◦C min^{-1}. Diluted samples (1/100 in acetone, v/v) of 1.0 µL were injected automatically and in the splitless mode. The identification of chemical compounds obtained from our study was performed by matching their retention indices and mass spectra with those obtained from the Flavor2.L, Wiley7n.1 and NIST98.L spectral and literature data. Essential oil compounds were determined according to their relative percentages from FID chromatograms.

Results and Discussion

Essential oil content and its components identified in leaf, herb and flower of *Melissa officinalis* are listed in Table 1. According to Table 1, essential oil content of plant parts was found as different amounts and essential oil rates of leaf and flower were 0.13%, 0.08% and 0.04% (v w^{-1}), respectively. As seen, while the high essential oil content was determined in leaf, essential oil amount decreased in herb.

Essential oil components were varied depending on different plant parts (Table 1). Thirteen, fifteen and fourteen components, representing 87.42%, 91.78% and 93.90% of total oil, were identified in leaf, herb and flower of *Melissa officinalis*, respectively. Caryophyllene

oxide, citral, z-citral, β-caryophyllene, citronellal and 2-pentadecanone were determined in diffrerent parts as major components. While the main component was citral (25.22% and 21.20%), it was followed by caryophyllene oxide (21.95% and 18.44%) and z-citral (19.08% and 16.03) in leaf and flower, respectively. The main component of the herb was caryophyllene oxide (29.25%), followed by citral (15.20%) and β-caryophyllene (12,14%).

There are many studies identified essential oil components of *M. officinalis* (Ceylan, 1987; Ilisulu, 1992; Pino et al., 1999; Tinmaz et al., 2001; Asgari and Sefidkon, 2004). Ceylan (1987) reported that *M. officinalis* was rich in citral and citronellal and according to Ilisulu (1992), characteristic odor of the plant is due to these components. In our study, citral and citronellal were determined in all plant parts of *M. officinalis.* In many studies related to *Melissa officinalis*, different components were determined as main components of essential oil. In Cuba conditions, Pino et al. (1999) found neral (29,9%) and gerenial (41%) as the main components.

According to Tinmaz et al. (2001), citronellal (39%), citral (33%) and gerenial (2%) were the main components of *M. officinali*s. Sari and Ceylan (2002) determined differences according to locations in terms of essential oil components and found citral, citronellal, gereniol, linalool as major components of *M. officinali*s in İzmir conditions. *β*-pinene (6.4–18.2%), sabinene (6.9–17.4%), (*E*)-caryophyllene (7.2–15.3%) and caryophyllene oxide (12.6–24.4%) were determined as main components of *M. officinalis* by Basta et al. (2005). Cosge et al. (2009) found citronellal (36.62-43.72%) and citral (10.10-17.43%) as the main components. Meftahizade et al. (2010) reported that neral (43.8%) and β-caryophyllene (13.5%) were the main components of *M. officinalis*.

As seen, many studies determined essential oil components of *M. officinali*s reported that citral and citronellal were the main components. While citral was higher than the other studies, citronellal was lower rate in our study. Unlike the other studies, caryophyllene oxide was the main component of herb in our study. This component has not been determined as the main component and it was found to be lower in other studies. This difference may be due to many factors, i.e. genetic factors, climatic factors and other factors. Thus, according to Moradkhani et al. (2010), essential oil composition is strongly affected by several factors such as plant genotype, light intensity, nutrient, temperature, harvesting time etc.

According to literature, there are limited study conducted to determine essential oil content and composition of flower in *M. officinalis*. Adinee et al., (2008) determined trans-carveol (28.89%), citronellol (25.24%), δ-3-carene (5.26%), citronellal (4.90%), geraniol (2.20%), 1-octene-3-ol (2.03%), spathulenol (2.06%) in essential oil of *M. officinalis* flower. In the other study, Norouzi et al. (2012) reported that α-pinene (25.61%), α-bisabolene (13.06%), caryophyllene oxide (10.74%) and δ-cadinene (7.89%) were the main components in flower of *M. officinalis*.

Table 1. Essential oil rate and its components of leaf, herb and flower in *Melissa officinalis**

Components	RI	Leaf	Herb	Flower
6-methyl-5-hepten-2-one	975	0.54	-	-
p-cymene	1016	-	-	3.35
gamma-terpinene	1051	-	-	3.56
linalool	1093	3.54	3.64	3.04
citronellal	1146	5.87	4.75	4.93
z-citral	1234	19.08	10.39	16.03
geraniol	1248	0.90	0.74	-
citral	1264	25.22	15.20	21.20
α-copaene	1368	-	0.59	-
β-bourbonene	1377	1.14	-	1.01
β-caryophyllene	1411	5.10	12.14	4.28
α-humulene	1445	0.56	1.13	-
germacrene D	1473	-	3.36	1.62
δ-cadinene	1515	-	1.04	-
caryophyllene oxide	1578	21.95	29.25	18.44
3-cyclohexanone-1carboxaldehyde	1600	1.34	1.97	1.16
naphthalene	1637	1.14	2.90	0.99
copaene	1643	-	0.59	-
t-cadinol	1651	1.04	4.09	0.87
2-pentadecanone	1837	-	-	12.9
Total (%)		87.42	91.78	93.90
Essential Oil Rate (%)		0.13	0.08	0.04

*Greater than 0,5% of components were given

Compared our results, there are significant differences as concerns essential oil components of flower. Our study, while citral was the main component, it could not be found in other studies. Besides, caryophyllene oxide was higher than the other study and 2-pentadecanone, one of the major components our study, could not be detected in other studies.

As consequently, significant differences in terms of essential oil content and its composition were determined in leaf, herb and flower of *Melissa officinalis*. While the highest essential oil content was obtained in leaf, it was followed by herb and flower, respectively. Besides, citral was the main component in leaf and flower whereas caryophyllene oxide was the main component in herb.

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