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İ Ç İ N D E K İ L E R / C O N T E N T S

Effect of Ontogenetic Variability On Essential Oil Content and Its Composition in Lemon Balm (*Melissa officinalis* L.)*

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This study was carried out at Ankara ecological conditions in 2012. In experiment, *Melissa officinalis* ssp. *officinalis* was used as plant material and leaf and herb samples were taken at different three growth stages (before flowering, beginning of flowering and full flowering) with three replications. After drying samples in the shade, the essential oil were extracted by hydro-distillation and analyzed by GC/MS. The results indicated that essential oil content and its composition were significantly influenced by growth stages. Essential oil content was varied from 0.06% to 0.13% and from 0.03 to 0.08% in leaf and herb, respectively and the highest essential oil content was recorded at before flowering stage in leaf and herb. So, before flowering stage was determined as the most suitable harvesting time for maximum essential oil content in lemon balm. On the other hand, caryophyllene oxide was detected as the main component in both leaf and herb at all growth stages, excepting leaf at before flowering. At before flowering stage, citral was the main component and followed by caryophyllene oxide.

Keywords: *Melissa officinalis,* essential oil, ontogenetic variability, harvesting stage

*This research was derived from master thesis

Oğulotu (M*elissa officinalis* L.)'nda Uçucu Yağ Miktarı ve Bileşenleri Üzerine Ontogenetik Varyabilitenin Etkisi

Bu çalışma Ankara ekolojik koşullarında 2012 yılında yürütülmüştür. Denemede Melissa officinalis türünün ssp. officinalis alttürü kullanılmış olup, üç farklı gelişme döneminde (çiçeklenme öncesi, çiçeklenme başlangıcı, tam çiçeklenme) bitkilerden üç tekerrürlü olarak yaprak ve herba örnekleri alınmıştır. Örnekler gölgede kurutulduktan sonra su distilasyonu yöntemiyle uçucu yağları elde edilmiş ve GS/MS yardımıyla uçucu yağların bileşenleri belirlenmiştir. Elde edilen sonuçlara göre, uçucu yağ miktarı ve bileşenleri farklı gelişme dönemlerinden istatistiki olarak önemli oranda etkilenmiştir. Nitekim yaprak ve herbada uçucu yağ miktarları sırasıyla %0.06-0.13 ve %0.03-0.08 arasında değişmiş ve en yüksek uçucu yağ miktarı her iki örnekte çiçeklenme öncesi dönemde elde edilmiştir. Dolayısıyla, oğulotunda maximum uçucu yağ miktarı bakımından çiçeklenme öncesi dönem en uygun hasat zamanı olarak tespit edilmiştir. Diğer taraftan, caryophyllene oxide çiçeklenme öncesi dönem dışında tüm gelişme dönemlerinde ana bileşen olarak tespit edilmiştir. Çiçeklenme öncesi dönemde ise citral ana bileşen olup, bunu ikinci bileşen olarak caryophyllene oxide takip etmiştir.

Anahtar Kelimeler: *Melissa officinalis,* uçucu yağ, ontogenetik varyabilite, hasat dönemi

Introduction

Lemon balm (*Melissa officinalis* L.), a member of Lamiaceae, is a perennial herb growing up to 100 cm. It has been used for a long time to attract honeybee swarms to hives and the word *Melissa* means bee in Greek (Sari and Ceylan, 2002). It has known by ancient Greeks and Romans and its all wild forms are available in all Europen countries (Ceylan, 1987). Today, it's widely cultivated in Europe and United States for its therapeutic properties (Patora et al., 2003; Moradkhani et al., 2010).

According to latest study, there are two subspecies of *Melissa officinalis* (*Melissa officinalis* ssp. *officinalis* and *Melissa officinalis* ssp. *inodora*) and only *Melissa officinalis* ssp. *officinalis* has medicinal value and the characteristic lemony odor. Because of its essential oil content 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; Ceylan, 1987; Sari and Ceylan, 2002; Gunel, 2012).

Lemon balm is one of the oldest and still most popular medicinal plant. Today, it is used for several purposes such as medicine, perfume, cosmetic, food in a lot of countries. Also, it has a

lot of pharmacological properties. The most commonly known pharmacological properties are sedative, carminative, antispasmodic, antibacterial, anti-microbial, antiviral, antitumor (Said-Al Ahl et al., 2009). Most of these pharmacological properties have been attributed to essential oil components. But, these essential oil components varies depending on many factors (Tittle et al., 1982; Adzet et al., 1992; Hose et al., 1997; Sari and Ceylan, 2002; Patora et al., 2003; Saglam et al., 2004). Thus, the aim of this study is to determine essential oil content and its components at different growth stages in *Melissa officinalis*.

Material and Method

Definition Experiment Area and Plant Material

Experiment was conducted at Ankara conditions in 2012. *Melissa officinalis* ssp. *officinalis*, stayed in experiment field of Department of Field Crops, Faculty of Agriculture of Ankara University, was used as plant material in this research.

The soil was loamy with a pH of 8.3, has no salt problems and poor in organic matter (%1.53). The climatic datas during the months of the study were given Table 1.

Long-term average of annual total precipitation is 416.0 mm, mean temperature is 12.0 ^{o}C and relative humidity is 60.7%. In 2012, total precipitation was 345.2 mm, mean temperature was 14.1 ^{o}C and relative humidity was 58.4%.

To determine effects of ontogenetic variability on essential oil content and its components, leaf and herb samples from *Melissa officinalis* ssp. *officinalis* were taken randomly at three different growth stages (before flowering-10 May 2012, beginning of flowering-20 June 2012 and full flowering-13 July 2012) with three replications and air-dried in a closed room for essential oil isolation.

Table 1. Climatic data for the year 2012*

Months	Rainfall (mm)		Mean Temperature (^{o}C)		Relative Humidity (%)	
	Long Term	2012	Long Term	2012	Long Term	2012
January	39.2	93.3	0.3	-0.8	76.3	87.3
February	33.4	47.7	2.1	-1.9	71.1	83.1
March	36.7	43.0	6.2	3.7	63.4	69.3
April	50.0	24.8	11.3	14.7	59.8	51.9
May	50.3	65.1	16.1	17.2	56.9	60.1
June	50.3	1.2	20.2	23.7	52.0	41.8
July	15.5	4.6	23.6	26.6	46.0	37.4
August	12.0	7.4	23.3	23.7	45.8	40.3
September	17.5	3.6	18.7	22.1	49.8	36.4
October	33.2	18.6	13.0	16.8	60.9	56.8
November	35.4	35.9	6.7	9.1	70.4	78.5
December	42.5	-	2.3	-	76.5	-
Total Rainfall (mm)	416.0	345.2				
Mean Temperature (^{o}C)			12.0	14.1		
Relative Humidity (%)					60.7	58.4

*www.mgm.gov.tr

Essential oil content obtained from this study was subjected to analysis of variance according to completely randomized design by using TARIST statistical program and significance level of differences between means was controlled by LSD test.

Extraction of Essential oil

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

Chromatography (GC/MS) 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. 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. 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) 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 rate, held for 10 min and finally raised to 250 ◦C/min. 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. Relative percentages of the separated compounds were calculated from FID chromatograms.

Results and Discussion

The results of variance analysis and the average values of essential oil content in leaf and herb as affected by growth stages of *Melissa officinalis* were given in Table 2 and Table 3, respectively. According to Table 2, effect of growth stages on essential oil content in leaf and herb was significant at P<0.01 level. According to Table 3, significant differences were determined in leaf and herb in terms of essential oil content. It varied from 0.06-0.13% and 0.03-0.08% in leaf and herb, respectively. While the highest essential oil content in leaf (0.13%) was obtained at before flowering stage. The maximum essential oil content in herb (0.08%) was also determined at before flowering stage, but there was no statistical difference between before flowering stage and beginning of flowering stage and these growth stages were in same statistical group. The minimum essential oil content was determined in both samples at full flowering stage.

Table 2. The variance analysis results of essential oil content in leaf and herb of *Melissa officinalis* at different growth stages

Sources of Variation	Leaf			Herb		
	D.F.	Average of Squares	F	D.F.	Average of Squares	F
Total	8	0.000886		8	0.000669	
Growth Stages	2	0.003377	60.303**	2	0.002411	27.089**
Error	6	0.000057		6	0.000089	

** Significiant at P<0.01 level

Table 3. Essential oil content identified in leaf and herb of *Melissa officinalis* at different growth stages

Growth Stages	Essential Oil Content (%)	
	Leaf	Herb
Before flowering	0.13[a]	0.08[a]
Beginning of flowering	0.10[b]	0.07[a]
Full flowering	0.06[c]	0.03[b]
Mean	0.098	0.062
$LSD_{0.01}$	0.021	0.029

Essential oil, has common usage in many areas such as food, cosmetic, medicine, is one of the most important secondary metabolites. However, amount of these secondary metabolites synthesized by medicinal and aromatic plant varies depending on many factors. Harvesting stage is one of the most important factors effecting essential oil content (Moradkhani, 2010; Saeb and Gholamrezaee, 2012). Thus, it's important to know the period which has the highest essential oil content. Baytop (1984) and Ceylan (1987) reported that essential oil content in *Melissa officinalis* should be not less than 0.05%. Essential oil content we found in leaf and herb are within the limits of these researcher's said. So, as a result of this study, it can be said that the most convenient harvesting time as concerns maximum essential oil content in *Melissa officinalis* is vegetative stage in Ankara conditions. Because of obtaining the lowest essential oil content at full flowering, the harvesting shouldn't be delayed this time.

When compared the other studies in *Melissa officinalis*, there were differences in terms of growth stage obtained maximum essential oil content. In Bornova conditions Arabaci (1989) and in Diyarbakir conditions Kizil (2009) determined the highest essential oil content at full flowering stage. Otherwise, Holla et al. (2000) reported that maximum essential oil content was obtained at beginning of flowering. Results found from our study are within limits defined in literature; but there are differences according to researcher's findings. These differences may be due to ecological conditions and material used in study. Besides, results determined in this study are agreement with Saeb and Gholamrezaee (2012) and Ayanoğlu et al. (2005) who stated the highest essential oil content at before flowering stage.

As seen from the Table 4, thirteen, fourteen and fifteen components, representing 87.2%, 96.7% and 84.6%, were detected in leaf essential oil at before flowering, beginning of flowering and full flowering, respectively. In leaf essential oil, there were differences in terms of main components according to growth stages. So, citral (25.2%) was the main component and it was followed by caryophyllene oxide (21.9%) and Z-citral (19.1%) at before flowering. Caryophyllene oxide was the main component at beginning of flowering (24.6%) and full flowering (26.5%) stage, followed by citral (18.4%) and *β*-caryophyllene (13.0%).

When considered the herb, caryophyllene oxide (29.3%, 27.5% and 24.1%, respectively) was the main component at all growth stages (Table 4). But, there were some differences, varying from citral to estragole as concern second and third components. At the before flowering, second and third components were citral (15.2%) and *β*-caryophyllene (12.1%), respectively. *β*-caryophyllene (14.7%) and germacrene D (8.5%) at the beginning of flowering and estragole (16.8%) and t-cadinol (9.5%) at the full flowering were determined as second and third components, respectively. At full flowering stage, estragole was also determined in both leaf and herb. But, it was higher in herb than leaf. This may be due to essential oil components of stem, containing trace amounts of essential oil.

When compared other study in related to essential oil components at different growth stages, different results were obtained. In a study conducted by Pino et al. (1999) in Kuba conditions, neral (29.90%) and geraniol (41.%) were determined as main components in *M. officinalis*. In other study by Tinmaz et al. (2001) in Canakkale conditions, citronellal (39%), citral (33%) and geraniol (2%) were the main components. These components have been identified in our study, but their levels were lower. At before flowering and beginning of flowering stage, decadienal (29.38% and 28.04%) and geraniol (25.30% and 24.97%); at full flowering stage carvacrol (37.62%) and methyl

citronallate (32.34%) were determined as the main components by Saeb and Gholamrezaee (2012). Caryophyllene oxide, the main component except leaf in vegetative stage in our study, was determined as fourth component by Saeb and Gholamrezaee (2012).Essential oil components in leaf and herb of *Melissa officinalis* as affected by growth stages were given Table 4.

Table 4. Essential oil compositions identified in leaf and herb of *Melissa officinalis* as affected by growth stages

Components	RI	Leaf			Herb		
		Before Flowering	Beginning of Flowering	Full Flowering	Before Flowering	Beginning of Flowering	Full Flowering
α-pinene	912	-	-	-	-	0.7	-
6-methyl-5-hepten-2-one	975	0.5	-	-	-	-	-
P-cymene	1016	-	-	-	-	0.5	-
Linalool	1093	3.5	3.9	2.3	3.6	2.8	8.8
Camphor	1136	-	-	-	-	0.8	-
Citronellal	1146	5.9	11.4	7.0	4.8	2.7	-
Z-citral	1234	19.1	13.5	7.2	10.4	3.6	-
Geraniol	1248	0.9	-	0.8	0.7	-	-
Citral	1264	25.2	18.4	11.3	15.2	5.6	-
Estragole	1277	-	-	0.8	-	-	16.8
α-copaene	1368	-	0.8	0.8	0.6	0.7	1.2
β-bourbonene	1377	1.1	1.1	1.4	-	-	-
β-caryophyllene	1411	5.1	10.0	13.0	12.1	14.7	8.4
α-humulene	1445	0.6	1.7	1.7	1.1	2.1	1.5
Germacrene D	1473	-	1.6	3.8	3.4	8.5	7.9
β-cubebene	1506	-	-	-	-	-	1.7
δ-cadinene	1515	-	0.9	1.6	1.0	2.3	2.3
Caryophyllene oxide	1578	21.9	24.6	26.5	29.3	27.5	24.1
3-cyclohexanone-1-carboxaldehyde	1601	1.3	1.7	-	2.0	2.0	-
Naphthalene	1637	1.1	2.3	1.7	2.9	3.1	6.6
T-cadinol	1651	1.0	2.5	4.7	5.0	7.8	9.5
Total		87.2	96.7	84.6	92.1	85.4	88.8

Conclusion

In conclusion, it was shown that essential oil content and its composition were affected significantly by growth stages. According to this study, before flowering stage was determined as the most suitable harvesting time of *Melissa officinalis* for maximum essential oil content in Ankara conditions, but there is a need for more comprehensive studies on this subject. Also, there are significant differences in essential oil components. Except in leaf at before flowering, caryophyllene oxide was the main component in all samples at all growth stages.

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