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Morphological and Diurnal Variability of Essential Oil in Lemon Verbena (Lippia citriodora H.B.K.)

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ABSTRACT*: The purpose of this paper is to analyze the composition of the essential oil from leaves and flowers of lemon verbena (**Lippia citriodora** H.B.K.) cultivated in Menemen-İzmir/Turkey and determine the effect of morphological and diurnal variability on the composition of essential oil. The essential oils from leaves and flowers lemon verbena were isolated by hydrodistillation with clevenger type apparatus. The chemical compounds of essential oil were identified by using a gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) systems. The yields of essential oils in leaves was found to be highest and lowest value in the lower part of leaves, with 1.64% at 4:00 pm and 0.78% at 10:00 am. Essential oil yield in flowers was varied 1.17% at 4:00 pm to 0.86% at 10:00 am in the day time. Main components of the all essential oils were found as limonene, neral and geranial. The ratio of those components were changed according to day time and the part of plant. The minimum and maximum ratio of limonene, neral and geranial were obtained between 12.2-22.6%, 15.6-23.6%, 22.7-35.8% in the leaves and 16.3-28.5%, 20.2-16.2%, 30.3-23.8% in the flowers respectively.*

Keywords*: **Lippia citriodora**, lemon verbena, essential oil, morphological variability, diurnal variability.*

Limonotu (Lippia citriodora H.B.K.) Uçucu Yağının Morfolojik ve Gün İçindeki Değişimi

ÖZ: *Bu çalışmanın amacı, Menemen-İzmir/Türkiye'de kültürü yapılan limonotu (**Lippia citriodora** H.B.K.) bitkisinin yaprak ve çiçeklerindeki uçucu yağların bileşimini analiz etmek ve morfolojik ve gün içindeki değişiminin uçucu yağların bileşimine etkisini belirlemektir. Yaprak ve çiçeklerdeki uçucu yağlar Clevenger tipi aparey ile hidrodistilasyon yöntemine göre çıkarılmıştır. Uçucu yağların analizi gaz kromatografisi (GC-FID) ve gaz kromatografisi-kütle spektrometrisi (GC-MS) sistemleri kullanılarak belirlenmiştir. Yapraklardaki uçucu yağlar en yüksek %1,64 ile öğleden sonra saat 16.00'da, en düşük %0,78 ile sabah 10.00'da bitkinin dip kısımlarından alınan yapraklardan elde edilmiştir. Çiçeklerdeki uçucu yağlar 16.00'da alınan örneklerde %1,17, 10.00'da alınan örneklerde %0,86 olarak belirlenmiştir. Bütün uçucu yağlardaki ana bileşenler limonen, neral ve geranial olarak bulunmuştur. Bu bileşenlerin oranı günün farklı zamanı ve alındığı bitki bölümüne göre değişim göstermiştir. Limonen, neral ve geranial'ın en yüksek en düşük oranları yaprak uçucu yağ örneklerinde %12,2-22,6, %15,6-23,6, %22,7-35,8 ve çiçek uçucu yağ örneklerinde %16,3-28,5, %20,2-16,2, %30,3-23,8 arasında saptanmıştır.*

Anahtar Kelimeler: Lippia citriodora *H.B.K., limonotu, uçucu yağ, morfolojik varyabilite, diurnal varyabilite.*

INTRODUCTION

The genus *Lippia* L. (Verbenaceae) includes approximately 200 species of herbs, shrubs and small trees. The species are mainly distributed throughout the South and Central America countries, and Tropical Africa territories (Argyropoulou *et al*., 2007). The most of them are traditionally utilized as gastrointestinal and respiratory remedies. Some *Lippia* species have shown antimalarial (Gasquet *et al*., 1993), antiviral (Abad *et al*., 1995) and cytostatic activities (Lopez *et al*., 1979).

Besides, the leaves from the majority of these species are utilized as seasoning for food preparations. With regard to these culinary purposes, it is necessary highlight the importance of the species. In general, the genus appears to present a consistent profile of chemical composition, pharmacological activities and folk uses. In most cases, the leaves or aerial parts, and flowers are used. They are commonly prepared as an infusion or decoction, and administered orally. Its aromatic properties are due to essential oils found in concentrations from 0.4-1.2% (Slowing Barillas, 1992).

The lemon-like fragrance is attributed to the component citral (neral+geranial) found in lemon verbena oils in concentrations between 11-52% (Klueger *et al*., 1997). It is cultivated mainly due to the lemon-like aroma emitted from its leaves that are utilized for the preparation of herbal tea, which is reputed to have antispasmodic, antipyretic, sedative and digestive properties. Lemon verbena has a long history of folk uses in treating asthma, spasms, cold, fever, flatulence, colic, diarrhea, indigestion, insomnia and anxiety (Santos-Gomes *et al*., 2005).

This plant was cultivated and processed for mainly essential oil production and also production of herbal tea. The oil is characterized by high concentration of limonene, neral and geranial. Essential oil composition of the medicinal and aromatic plants could be affected from environmental conditions, cultural practices, harvesting time, storage conditions, drying, and distillation techniques (Vogel *et al*., 1997; Azizi *et al*., 2009; Jalal *et al*., 2009; Sellami *et al*., 2009; Toncer *et al*., 2009).

The purpose of this paper is to analyze the composition of the essential oil from the leaves and flowers of *Lippia citriodora* H.B.K. cultivated in Turkey, as well as any changes in the composition of essential oil at morphological and diurnal variability, using GC-FID and GC-MS.

MATERIALS AND METHODS

Plant materials: Plants were collected during the full blooming period from field experiment area of Aegean Agricultural Research Institute in 2018. Leaf and flower samples were collected to the same two years old plant. Leaf samples were gathered at 10:00 am and 4:00 pm in the lower, middle and upper side of the plant. Flower samples were gathered in the full blooming stage at the same time on leaves.

Isolation of the essential oils: The essential oils from air-dried plant materials were obtained by hydrodistillation for 3 h, using a Clevenger-type apparatus. The obtained oils were dried over anhydrous sodium sulphate and stored at +4°C in the dark until analyzes (Anonymous, 2011).

GC-MS analysis: The essential oil composition of samples was analyzed by gas chromatography (Agilent 5975C) coupled by flame ionization detector and mass spectrometry (Agilent 5975C) using capillary column (HP Innowax Capillary; 60.0 m×0.25 mm×0.25 μm). Essential oils were diluted 1:50 ratio with hexane. GC-MS/FID analysis was carried out at split mode of 50:1. Injection volume and temperature were adjusted as 1 μL and 250 °C, respectively. Helium (99.9%) was the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was programmed as follows: 60°C for 10 minutes, increased at 20°C/minute to 250°C, and held at 250°C for 8 minutes. MS spectra were monitored between 35 to 450 amu and the ionization mode used was electronic impact at 70eV. The relative percentage of the components was calculated from GC-FID peak areas, and components were identified by Wiley 7n, Nist 05 and Flavour and Fragrance Natural and Synthetic Compounds (ver. 1.3) Libraries.

RESULTS and DISCUSSION

The essential oils extracted from the leaf and flower gathered at the full blooming stage showed the highest essential oil concentration reported previous studies. The concentration of essential oils was found to be highest in the leaves located in lower part of plant with 1.64% at 4:00 pm. Concentration decreased and reached its lowest values same leaves with 0.78% at 10.00 am. Essential oil yield was varied 0.92% (10:00 am) 1.05% (4:00 pm) and 0.94% (10:00 am) 1.06% (4:00 pm) in the leaves located middle and upper part of plant respectively. The same essential oil concentration results were obtained from flower with 0.86% (10:00 am) and 1.17% (4:00 pm). When we summarized the essential oil results, we can say that oil concentrations were increased in the day time both leaf and flower samples. The differences in essential oil yield should be resulted from the harvesting time, ecological conditions, distillation process etc. (Vogel *et al*., 1997; Azizi *et al*., 2009; Jalal *et al*., 2009; Sellami *et al*., 2009; Toncer *et al*., 2009;). Vogel *et al*. (1997), reported that essential oil concentration of *Lippia citriodora* H.B.K. was increased 0.20% to 0.90% in the daytime in their study from Chile. The level of essential oils extracted from lemon verbena has already been shown to be 0.1% to 1.57% by a number of previous studies (El-Hamidi *et al*., 1982; Özek *et al*., 1996; Castro *et al*., 2000; Belkamel *et al*., 2005).

The water-distilled essential oils from leaf and flower of *Lippia citriodora* H. B. K. were characterized by GC-FID and GC-MS in this study. The chemical composition of the essential oil is summarized in Table 1. 15-21 compounds according to plant parts and collecting time were identified, representing 100% of the total oil. Geranial, neral and limonene were found to be the main components, followed by β-caryophyllene, caryophyllene oxide, geranyl acetate, spathulenol and *ar*-curcumene. All components were varied according to plant parts (leaf and flower) leaf located parts (lower, middle and upper) and daytime (10:00 am and 4:00 pm). The highest ratio of geranial (35.8%) was obtained from leaf located middle part of plant gathered at 10:00 am and the lowest (23.8%) was obtained from flowers gathered at 4:00 pm. It was clearly seen that geranial amount was decreased in the daytime all the samples of essential oils (Table 1.). It was changed in the leaf and flower samples belong to daytime 32.2-25.3%, 35.8-25.0%, 33.7-22.7% and 30.3-23.8% respectively. The other main component in the essential oil was neral and its amount was decreased in the daytime like geranial. It was changed in the leaf and flower samples belong to daytime 26.6-18.6%, 22.1-17.7%, 21.5-15.6% and 20.2-16.2% respectively. Limonene was the third most abundant component in the essential oil. This component was increased in the essential oil in the daytime contrarily geranial and neral. Limonene amount was chanced and increased in the leaf and flower samples belong to daytime 15.0-31.4%, 12.2-21.6%, 14.7-22.6% and 16.3-28.5% respectively. There were no significant changes determined from β-caryophyllene, caryophyllene oxide, geranyl acetate, spathulenol and *ar*-curcumene in the essential oils depend on plant parts and day time. The minimum and maximum level of β-caryophyllene 2.6-6.2%, caryophyllene oxide 3.0-6.0%, geranyl acetate 1.1-3.9%, spathulenol 2.5-4.9% and *ar*-curcumene 1.5-5.6% were obtained in the essential oils respectively (Table 1).

Deviations in effective substances within 24 hours are called ‘diurnal variability. Numerous studies have been conducted to determine such differences and their effects. At the end of these studies, it was determined at which hours the harvest should be done in order to obtain the best drug in some essential oil plants. Diurnal variability studies of chamomile, oregano, lavender, lemon balm and sage plants showed that the volatile oil ratios changed at different times of the day. With such studies, it is possible to determine the proportions of the essential oil and components during the day and in which ecological conditions these differences are effective (Ceylan, 1996; Yaldız *et al*., 2005; Toncer *et al*., 2009; Hassiotis *et al*., 2010). Morphogenetic and diurnal variability of essential oils in different plant species showed that the composition of essential oils varied according to plant parts and different times of day (Kulen, 2013; Paşa, 2013; Arabacı *et al*., 2015; Tan, 2016).

In aromatic plants species, biosynthesis of essential oils occurs through two complex natural biochemical pathways involving different enzymatic reactions. Isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are the universal precursors of essential oil biosynthesis and

are produced by the cytosolic enzymatic MVA (mevalonic acid) pathway or by plastidic and enzymatic 1-deoxy-d-xylolose-5-phosphate (DXP) pathway, also called the 2-C-methylerythritol-4-phosphate (MEP) pathway. In the particular plant cell part, prenyl diphosphate synthases condense isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) further to form prenyl diphosphates, which are used as substrates for geranyl diphosphate (GPP; C10) or for fernesyl diphosphate (FPP; C15). Essential oils are final terpenoid products and are formed by a huge group of enzymes known as terpene synthases (TPS) (Rehman *et al*., 2016).

These results are in agreement with previous reports (Montes *et al*., 1973; Bellakhdar *et al*., 1994; Nakamura *et al*., 1997; Carnat *et al*., 1999; Kim and Lee, 2004; Sartoratto *et al*., 2004; Santos-Gomes *et al*., 2005; Gezici *et al*., 2017). According to the literature, geranial, neral and limonene is the component found to occur in higher quantities in essential oils of the genus *Lippia*, followed by: *p*-cymene, *α*-pinene, camphor, *β*-caryophyllene, linalool and thymol in a decreasing order (Terblanché and Kornelius, 1996; Pascual *et al*., 2001). In our study, *β*-caryophyllene, caryophyllene oxide, geranyl acetate, spathulenol and *ar*-curcumene were also found.

The change of the main components of the essential oil in the leaves and flowers of the lemon verbena (*Lippia citriodora* H.B.K.) during the day is shown in Figure 1 and 2.

Table 1. The composition of the essential oil of *Lippia citriodora* H.B.K.
Çizelge 1. *Lippia citriodora* H.B.K.'nin uçucu yağ kompozisyonu.

RRI*	Compounds Bileşik	Leaf (Yaprak)						Flower (Çiçek)	
		Lower (Alt)		Middle (Orta)		Upper (Üst)			
		10:00 am	4:00 pm	10:00 am	4:00 pm	10:00 am	4:00 pm	10:00 am	4:00 pm
1002	*α*-pinene	-	-	-	-	-	-	1.0	1.2
1104	sabinene	-	-	-	-	-	-	2.2	1.8
1182	limonene	15.0	31.4	12.2	21.6	14.7	22.6	16.3	28.5
1192	1,8-cineole	-	-	-	-	-	-	5.4	4.3
1232	*β*-ocimene	0.7	0.7	0.4	0.9	0.7	1.0	1.3	1.0
1319	6-methyl-5-hepten-2-one	1.5	1.2	0.5	1.4	0.6	0.8	-	-
1493	*trans*-chrysanthemal	0.3	-	-	-	-	-	-	-
1506	camphor	-	-	-	0.8	-	-	-	-
1517	linalool	0.4	-	-	6.9	-	3.6	-	0.8
1532	linalyl acetate	-	-	-	1.3	-	4.3	-	2.3
1551	isocitral	0.6	0.7	-	-	-	-	-	-
1584	*β*-caryophyllene	4.3	4.7	5.1	3.7	6.2	5.5	2.7	2.6
1665	neral	26.6	18.6	22.1	17.7	21.5	15.6	20.2	16.2
1673	*α*-terpineol	-	-	-	1.3	-	0.9	1.2	0.8
1679	borneol	-	-	-	0.5	-	-	-	-
1696	germacrene	0.6	0.4	1.1	0.8	2.2	3.6	2.0	1.4
1698	neryl acetate	0.3	-	-	0.7	-	0.6	-	-
1714	geranial	32.2	25.3	35.8	25.0	33.7	22.7	30.3	23.8
1716	*α*-cedrene	-	-	-	-	0.8	0.6	0.6	0.5
1721	bicyclogermacrene	0.8	1.4	2.0	1.0	3.1	2.6	0.7	1.0
1728	geranyl acetate	3.2	3.0	3.5	3.9	3.1	3.4	1.1	1.2
1751	*ar*-curcumene	2.4	2.3	3.2	1.5	2.8	2.3	5.6	4.6
1812	geraniol	-	-	-	0.8	-	-	-	-
1980	caryophyllene oxide	4.8	4.2	6.0	3.5	4.4	3.9	3.1	3.0
2003	nerolidol	0.4	0.4	0.6		0.6	0.4	0.9	0.8
2104	spathulenol	3.4	3.4	4.9	2.5	3.4	3.1	3.8	3.4
2147	*T*-cadinol	1.1	1.1	1.5	1.0	1.5	1.4	-	-
2185	*α*-bisabolol	-	-	-	2.3	-	-	-	-
2201	isospathulenol	0.3	0.4	0.5	-	-	-	-	-
	Total (%)	98.9	99.2	99.4	99.1	99.3	98.9	98.4	99.2
	Essential Oil (%)	0.78	1.64	0.92	1.05	0.94	1.06	0.86	1.17

*RRI: Relative retention ındices (Nisbi tutulma indeksleri).

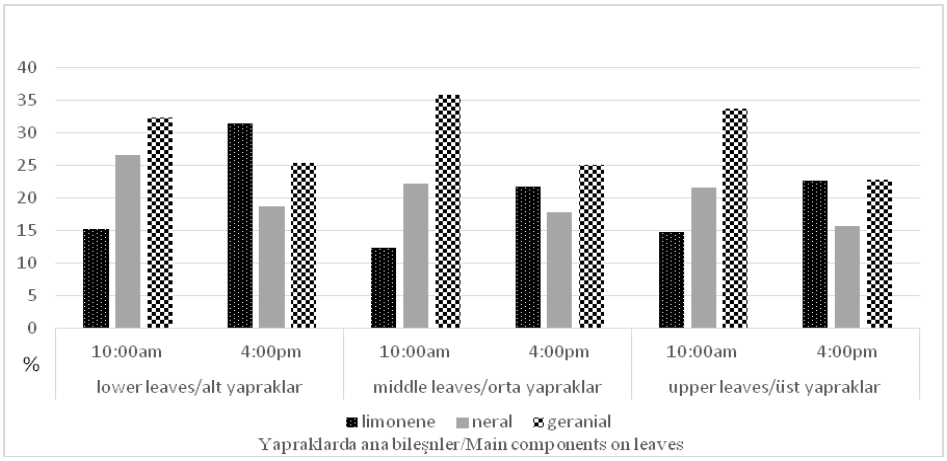

Figure 1. Main components and variation of lemon verbena leaves essential oil according to time.
Şekil 1. Limonotu yapraklarında uçucu yağın ana bileşenlerinin zamana göre değişimi.

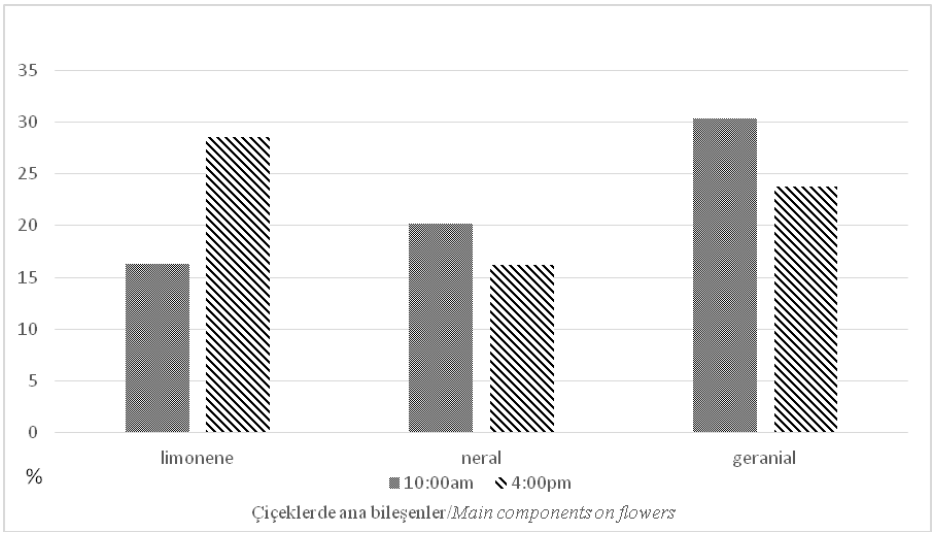

Figure 2. Essential oil composition and variation of lemon verbena flowers acording to time.
Şekil 2. Limonotu çiçeklerinde uçucu yağın ana bileşenlerinin zamana göre değişimi.

CONCLUSION

The aim of this study is to determine the change in the amount and chemical structure of the essential oil in leaves and flowers of the plant. Despite the fact that the lemon verbena (*Lippia citriodora* H. B. K.) has just started cultivation in our country, quite good yield values have been obtained. According to the results the maximum and minimum amount of major components were characterized as geranial (35.8-22.7%), neral (26.6-15.6%), and limonene (31.4-12.2%) and the other components were obtained as *β*-caryophyllene (2.6-6.2%), caryophyllene oxide (3.0-6.0%), geranyl acetate (1.1-3.9%), spathulenol (2.5-4.9%) and *ar*-curcumene (1.5-5.6%) in the essential oils according to plant parts and day time.

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