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’dede yetiştirilen 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 çiçeklerinde uçucu yağlar Clevenger tipti aparey ile hidrodistilasyon yöntemine göre çıkarılmıştır. Uçucu yağların analizi gaz chromatografi (GC-FID) ve gaz chromatografi-kütle spektrometrisi (GC-MS) sistemleri kullanarak 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. Bitkin uçucu yağlardaki ana bileşenler limonen, neral ve geranial olarak bulunmuştur. Bu bileşenlerin oranı günün farklı zamanı ve alınmış bitkinin bileşimine göre değişmiştir. Limonen, neral ve geranial’in 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 varyabiliteli, diurnal varyabiliteli.
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 (López 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% (Slovic 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, nerol 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-xylulose-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.

| RRI* | Compounds                  | Leaf (Yaprak) | Flower (Çiçek) |
|------|---------------------------|---------------|---------------|
|      |                           | Lower (Alt)   | Middle (Orta) | Upper (Üst)   |
|      |                           | 10:00 am      | 4:00 pm       | 10:00 am      | 4:00 pm      |
| 1002 | α-pinene                  | -             | -             | 1.0          | 1.2          |
| 1104 | sabine                     | -             | -             | 2.2          | 1.8          |
| 1182 | limonene                   | 15.0          | 31.4          | 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           | 0.9          | 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-chrysanthenal        | 0.3           | -             | -            | -            | -            | -            | -            |
| 1517 | linalool                   | 0.4           | -             | 6.9          | -            | 3.6          | -            | 0.8          |
| 1532 | linalyl acetate            | -             | -             | 1.3          | -            | 4.3          | -            | 2.3          |
| 1551 | isocurral                  | 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-terpinol                 | -             | -             | 1.3          | -            | 0.9          | -            | 1.2          | 0.8          |
| 1679 | bornol                     | -             | -             | 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 indices (Nisbi tutulma indeksleri).
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 α-curcumene (1.5-5.6%) in the essential oils according to plant parts and day time.
REFERENCES

Abad, M., S. Sánchez, P. Bermejo, A. Villar, and L. Carrasco. 1995. Antiviral activity of some medicinal plants. Methods and Findings 17: 108-114.

Anonymous. 2011. TSE EN ISO 6571-Baharatlar, Çeşniler ve Tibbi Bitkiler-Uçucu Yağ Muhtevasının Tayini (hidrodistilasyon yöntemi). Türk Standartları Enstitüsü, Ankara.

Arabaci, O., H. E. Tokul, N. G. Öğretmen, and E. Bayram. 2015. The effect of diurnal variability on yield and quality in naturally grown Coridothymus capitatus L. genotypes. Ege Üniversitesi Ziraat Fakültesi Dergisi 52 (2): 141-150.

Argyropoulou, C., D. Daferera, P. A. Tarantilis, C. Fasseas, and M. Polissiou. 2007. Chemical composition of the essential oil from leaves of Lippia citriodora HBK (Verbenaceae) at two developmental stages. Biochemical Systematics and Ecology 35: 831-837.

Azizi, A., F. Yan, and B. Honermeier. 2009. Herbage yield, essential oil content and composition of three oregano (Origanum vulgare L.) populations as affected by soil moisture regimes and nitrogen supply. Industrial Crops and Products 29: 554-561.

Barillas, K. V. S. 1992. Estudio de la actividad antinfiamatoria de diversas especies de la flora de Guatemala. Facultad de Farmacia. PhD Thesis. Universidad Complutense de Madrid.

Belkamel, A., V. Janneot and Y. Dehbi. 2005. Verveine odorante Aloysia triphylla-Verbenaceae composition chimique et biosynthese, International Congress On Medicinal Plants, Errachidia, March 16-19, 2005, Morocco, pp. 113.

Bellakhdar, J., A. I. Idrissi, S. Canigueral, J. Iglesias, and R. Vila. 1994. Composition of lemon verbena (Aloysia triphylla (L’Herit.) Britton) oil of Moroccan origin. Journal of Essential Oil Research 6: 523-526.

Carnat, A., A. Carnat, D. Fraisse, and J. Lamaison. 1999. The aromatic and polyphenolic composition of lemon verbena tea. Fitoterapia 70: 44-49.

Castro, D., L. Ming, and M. Marques. 2000. Biomass production and chemical composition of Lippia alba (Mill.) NE Br. ex Britt & Wilson in leaves on different plant parts in different seasons, I Latin-American Symposium on the Production of Medicinal, Aromatic and Condiments Plants 569, pp. 111-115.

Ceylan, A. 1996. Tibbi Bitkiler İı (Uçucu Yağ Bitkileri), Ege Üniversitesi Ziraat Fakültesi Yayın No: 481, İzmir.

El-Hamidi, A., S. Ahmed, and F. Shaarawy. 1982. Lippia citriodora grown in Egypt. A new crop under development, III International Symposium on Spice and Medicinal Plants, XXI HIC 132, pp. 31-34.

Gasquet, M., F. Delmas, P. Timon-David, A. Keita, M. Guindo, N. Koita, D. Diallo, and O. Doumbou. 1993. Evaluation in vitro and in vivo of a traditional antimalarial, “Malarial 5”. Fitoterapia-Milano 64: 423-423.

Gezici, S., N. Şekeroğlu, and A. Kijjoa. 2017. In vitro anticanic activity and antioxidant properties of essential oils from Populius alba L. and Rosmarinus officinalis L. from South Eastern Anatolia of Turkey. Indian Journal of Pharmaceutical Education and Research 51: 498-5503.

Hassiotis, C. N., D. M., Lazari, and K. E. Vlachonasios. 2010. The effects of habitat type and diurnal harvest on essential oil yield and composition of Lavandula angustifolia Mill., Fresenius Environmental Bulletin 19 (8): 1491-1498.

Jalal, K., M. Rahmat, F. T. Mohamad, and N. H. M. 2009. Influence of drying methods, extraction time, and organ type on essential oil content of rosemary (Rosmarinus officinalis L.). Nature and Science 7: 42-44.

Kim, N. S., and D. S. Lee. 2004. Headspace solid-phase microextraction for characterization of fragrances of lemon verbena (Aloysia triphylla) by gas chromatography-mass spectrometry. Journal of separation science 27: 96-100.

Khueger, P., M. Daros, R. Silva, M. Farias, and T. De Lima. 1997. Neuropharmacological evaluation of crude and semipurified extracts from Lippia alba Will. NE Br. (Verbenaceae), Abstracts. International joint Symposium. Chemistry, Biological and Pharmacological Properties of Medicinal Plants from the Americas. Poster Session, p. B23.

Kulan, E. G. 2013. Eskişehir Köşullarında Yetiştirilen Leyhan (Ocimum basilicum L.) Bitkisinin Bazı Bitkisel Özelliklerin ve Diurnal Varyabilitesinin Belirlenmesi. Eskişehir Osmangazi Üniversitesi Fen Bilimleri Enstitüsü Tezi. 116 s.

Lopez, A. A., N. H. Rojas, and C. M. Jimenez. 1979. Plant extracts with cytostatic properties growing in Cuba. II Revista Cubana de Medicina Tropical 31: 105-111.

Montes, M., L. Valenzuela, T. Wilkomirsky, and M. Arrive. 1973. Sur la composition de l'essence d' Aloysia triphylla (cedron). Planta Medica 23: 449-124.

Nakamura, T., E. Okuyama, A. Tsukada, M. Yamazaki, M. Maruno, and H. Nishimoto. 1997. Acteoside as the analgesic principle of cedron (Lippia triphylla), a Peruvian medicinal plant. Chemical and Pharmaceutical Bulletin 45: 499-504.

Özék, T., N. Kirimer, K. Baser, and G. Tunem. 1996. Composition of the essential oil of Aloysia triphylla (L’Herit.) Britton grown in Turkey. Journal of Essential Oil Research 8: 581-583.
Pascual, M., K. Slowing, E. Carretero, D. S. Mata, and A. Villar. 2001. *Lippia*: traditional uses, chemistry and pharmacology: A Review. *Journal of Ethnopharmacology* 76: 201-214. doi.org/10.1016/S0378-8741(00)00234-3

Paşa, C. 2013. Kaz Dağları’nda yayılış gösteren bazı *Hypericum* türlerinde uçucu yağ oranı ve bileşenlerinin diurnal, ontogenetik ve morfogenetik varyasyonunun belirlenmesi üzerine bir araştırma. Namık Kemal Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Ana Bilim Dalı Doktora Tezi 222 s.

Rehman, R., M. A. Hanif, Z. Mustag, A. M. Al-Said. 2016. Biosynthesis of essential oils in aromatic plants: A review. *Food Reviews International* 32: 2 117-160.

Santos-Gomes, P. C., M. Fernandes-Ferreira, and A. M. Vicente. 2005. Composition of the essential oils from flowers and leaves of vervain *Aloysia triphylla* (L’Herit.) Britton grown in Portugal. *Journal of Essential Oil Research* 17: 73-78.

Sartoratto, A., A. L. M. Machado, C. Delarmelina, G. M. Figueira, M. C. T. Duarte, and V. L. G. Rehder. 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Brazilian Journal of Microbiology* 35: 275-280.

Sellami, I. H., E. Maamouri, T. Chahed, W. A. Wannes, M. E. Kchouk, and B. Marzouk. 2009. Effect of growth stage on the content and composition of the essential oil and phenolic fraction of sweet marjoram (*Origanum majorana* L.). *Industrial Crops and Products* 30 (3): 395-402.

Tan, U. 2016. Mayıs papatyası (*Matricaria recutita* L.)’nda farklı ekim zamanları ve çeşitlerin agronomik-teknolojik özellikleri etkisi. Adnan Menderes Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Ana Bilim Dalı Yüksek Lisans Tezi. 71 s.

Terblanché, F., G., and Kornelius. 1996. Essential oil constituents of the genus *Lippia* (Verbenaceae) a literature review. *Journal of Essential Oil Research* 8: 471-485.

Toncer, O., S. Karaman, S. Kızıl, and E. Diraz. 2009. Changes in essential oil composition of oregano (*Origanum onites* L.) due to diurnal variations at different development stages. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 37: 177-181.

Vogel, H., M. L., Silva, and I. Razmilic. 1999. Seasonal fluctuation of essential oil content in lemon verbena (*Aloysia triphylla*). II WOCMAP Congress Medicinal and Aromatic Plants, Part I: Biological Resources, Sustainable Use. Conservation and Ethnobotany. Acta Horticulturae 500: 75-80. Doi:10.17660/ActaHortic.1999.500.9

Yaldız, G., N. Sekeroğlu, M. Özgüven, and M. Kirpik. 2005. Seasonal and diurnal variability of essential oil and its components in *Origanum onites* L. grown in the ecological conditions of Çukurova, *Grasas & Aceites* 56 (4): 254-258.