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ESSENTIAL OIL AND MICROELEMENT COMPOSITION OF *THYMUS CITRIODORUS* L. AND *LIPPIA CITRIODORA* H.B.K.

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Abstract. Lemon verbena (*Lippia citriodora* H.B.K., *Verbenaceae* family) is indigenous to South America and cultivated as an aromatic plant in various parts of world. Lemon thyme (*Thymus citriodorus* L.), *Lamiaceae* family, is a perennial medicinal plant native to southern Europe and is cultivated in the Mediterranean region. These species are cultivated mainly for the lemon-like aroma emitted from their leaves due to the presence of dimethyl-2,6-octadienal, also known as lemonal or citral, which is used in food and perfumery for its citrus effect. The aim of this study was to determine the mineral content and essential oil components of *L. citriodora* and *T. citriodorus* plants grown under semi-arid climatic conditions in Turkey. The aerial parts of lemon thyme and lemon verbena plants were extracted using hydro-distillation. The essential oil composition was analyzed by gas chromatography-mass spectrometry (GC-MS) and the microelement contents of the herbs were examined via inductively coupled plasma-optical emission spectrometry (ICP-OES). The microelement contents were 0.249, 1.630, 16.41, 0.106, and 13.1-36.2 mg kg^{-1} for cadmium (Cd), copper (Cu), iron (Fe), and manganese (Mn), respectively, in lemon thyme, and 0.275, 4.584, 248.1, 15.71, and 1.803 mg kg^{-1} for Cd, Cu, Fe, Mn, and zinc (Zn), respectively, in lemon verbena. Fifty compounds were identified in lemon verbena essential oil, including limonene (30.33%), *trans*-citral (17%), *cis*-citral (12.77%), caryophyllene oxide (5.71%), and geraniol acetate (4.02%) that together constituted 99.86% of the oil composition. We also identified 22 compounds constituting approximately 85.11% of lemon thyme essential oil, including *trans*-geraniol (30.07%), *trans*-citral (15.06%), *cis*-citral (11.71%), *cis*-geraniol (7.65%), and 3-octanol (6.18%).

Keywords: lemon tyme; lemon verbena; lemonal; citral; oil composition.

INTRODUCTION

Lemon verbena (*Lippia citriodora* H.B.K.) is native to South America and grows wild in Chile, Peru, and Argentina. The *Lippia*

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genus (*Verbenaceae* family) contains more than 200 species and *Lippia* spp. exhibit a wide range of genetic diversity for essential oil composition in various locations throughout the world (Argyropoulou *et al.*, 2007). Most *Lippia* species have been used mainly for gastrointestinal and respiratory diseases in traditional medicine. Some species of the herb are used for their antimalarial, antiviral, and cytostatic properties. The therapeutic effects of these herbs are conferred by their essential oils and phenolic compounds (flavonoids) (Catalan and Lampasona, 2002; Argyropoulou *et al.*, 2007; Malekirad *et al.*, 2011). The major constituents of *Lippia* essential oil were previously reported to be 1,8-cineole (12.4%), geranial (9.9%), 6-methyl-5-hepten-2-one (7.4%), and neral (6.9%) (Bellakhdar *et al.,* 1994), while 1,8-cineole was also reported to be a major component of the oil in another study (Malekirad *et al.*, 2011).

Lemon thyme (*Thymus citriodorus* L.) is a perennial and medicinal plant indigenous to southern Europe and is a member of the *Lamiaceae* family. Lemon thyme plants are cultivated in Mediterranean countries and grows to a height of approximately 20-40 cm and has hairy leaves and stems, opposite leaves, and half-grained white flowers that are small and pink in colour. Lemon thyme is a lemon-scented herb used in herbal teas. The essential oil is rich in geraniol (60%); other constituents include geranyl acetate (1.0%), geranyl butyrate (0.8%), nerol (2.8%), and citronellol (0.3%). The lemon fragrance is conferred by geranial and neral, which contribute 8.2 and 5.5%, respectively, to the total oil composition. Thymol is found in small (0.5%) quantities in the oil (Stahl-Biskup and Holthuijzen, 1995).

The essential oils of medicinal and spice plants are used in industry for their flavour and fragrance chemistry and for flavouring foods, drinks, and many other products. Essential oils can be used as alternative treatments for various diseases. Lemon verbena and lemon thyme essential oils contain citral and its isomers, geranial and neral, and are cultivated mainly because of the lemon-like aroma of their leaves. This lemon-like scent is caused by the presence of dimethyl-2,6-octadienal, also known as lemonal or citral, which is used in several chemical industries. It also has strong antimicrobial activity and pheromonal effects on insects (Hapke *et al.,* 2001). In addition, medicinal and spice plants contain essential trace elements, which may be beneficial to human health through consumption of the herbs. Recent scientific studies have demonstrated the importance of trace elements to human health and related studies on the mineral compositions of herbs, spices, and other crops are increasing worldwide.

The aim of this study was to determine the essential oil and microelement composition of *Thymus citriodorus* and *Lippia citriodora* plants, cultivated in the Southeast Anatolia, region of Turkey, and to compare the results with those of studies conducted under different ecological conditions.

MATERIALS AND METHODS

Plant materials

Lemon verbena and lemon thyme seedlings were obtained from the Ataturk Central Horticultural Research Institute, Yalova, Turkey. Cuttings of *L. citriodora* were rooted in a sandbox and then transferred to the collection garden (Department of Field Crops, Agriculture Faculty, Dicle University, Diyarbakir, Turkey) in April 2009. Lemon verbena and lemon thyme were harvested at flowering in July and September, respectively, of 2010.

Essential oil extraction

Essential oil was extracted from 20 g dry herbage samples by hydrodistillation for 3 h, under continuous steam using a Clevenger-type apparatus (v/w). The isolated oils were stored in tightly closed vials at 4°C until analysis.

Mineral content

The mineral contents of the samples were determined *via* inductively coupled plasma-optical emission spectrometry (ICP-OES), using an Optima 2100 DV system (PerkinElmer, Inc. Shelton, CT, USA)

Identification of essential oils

Lemon verbena and lemon thyme essential oils were analysed via gas chromatography-mass spectroscopy (GC-MS) using a Clarus 600 C gas chromatograph in tandem with a Clarus 600 mass spectrometer (PerkinElmer), equipped with an auto sampler. Sample volumes of 1 μL were injected using the split method. Chromatographic separations were accomplished using an Elite 5-MS capillary column (5% diphenyl-dimethylpolysiloxane; 0.25 mm i.d.×30 m; film thickness, 0.25 μm) with injections in the split mode and a split ratio of 20. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The column temperature was maintained initially at 60°C for 3 min, gradually increased to 130°C at a rate of 4°C/min, maintained at 130°C for 2 min, and finally increased to 240°C at a rate of 20°C/min. The injection port temperature was 240°C and an ionization voltage of 70 eV was applied with a mass range m/z of 20-550 amu. The separated components were tentatively identified by matching the data with electron ionisation-mass spectroscopy (EI-MS) results of the National Institute of Standards and Technology (NIST), and the National Bureau of Standards (NBS) mass spectral library data. Quantitative determination was based on peak area integration.

RESULTS AND DISCUSSION

Mineral content of herbs

The mineral contents of lemon verbena and lemon thyme are presented in *Table 1*. In lemon verbena, the contents of cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), phosphorus (P), and selenium (Se) were 0.249, 1.498, 1.630, 16.41, 0.106, 0.734, and 5.241 mg kg^{-1}, respectively. In lemon thyme, the contents of calcium (Ca), Cd, Cr, Cu, Fe, magnesium (Mg), Mn, P, Se, and zinc (Zn) were 8924, 0.275, 7.603, 4.584, 248.1, 2685, 15.17, 593.7, 0.219, and 1.803 mg kg^{-1}, respectively. dos Reis *et al.* (2010)

reported that, in *L. alba*, the mean values of Ca, Mg, and potassium (K) contents were 1,950, 2,856, and 2.335 mg 100 g^{-1} , respectively, and that the contents of the microelements barium (Ba), Zn, Cu, Fe, Mn, and nickel (Ni) were 2.16, 2.95, 0.94, 11.2, 4.25, and 0.09 mg 100 g^{-1}, respectively.

Table 1 - Microelement content in *T. citriodorus* and *L. citriodora* (mg kg^{-1})

Microelement	*T. citriodorus*	*L. citriodora*
Ca	-	8924
Cd	0.249	0.275
Co	-	-
Cr	1.498	7.603
Cu	1.630	4.584
Fe	16.41	248.1
Mg	-	2685
Mn	0.106	15.17
P	0.734	593.7
Se	5.241	0.219
Zn	-	1.803

The metals Mn, Fe, Cu, and Zn, and the non-metal Se are considered to be trace elements because of their essential, but very limited, presence in human body. Deficiencies in any of these microelements lead to health problems that can be avoided by maintaining sufficient levels. Lower limits for these elements are 27.4 mg kg^{-1} for Zn, 3.00 mg kg^{-1} for Cu, 2 mg kg^{-1} for Mn, and 20 mg kg^{-1} for Fe according to the permissible limits set by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (Ghani *et al*., 2012). Shaw *et al*. (2004) reported that the normal Cu content in plants was 4-15 mg kg^{-1}. Sharat Singh *et al*. (2010) reported that the daily microelement intake requirements for Fe, Zn, Mn, Cu, Cr, and Se were 10-15, 15, 2.5-5.0, 2-3, 0.05-0.2, and 0.05-0.2 mg per day, respectively. These microelement requirements can be met by consuming herbal teas.

Composition of essential oils

The chemical composition of lemon verbena essential oil is summarized in *Table 2*. The essential oil content of lemon verbena plants was 0.58%. Nazari *et al*. (2009) reported that the essential oil content of lemon verbena plants grown in Iran was 0.8%, and Rode (2000) reported that the essential oil content of dried leaves was season-and harvest-dependent and varied from 0.81 to 1.19%. We identified 34 compounds, representing 97.64% of the total oil composition of lemon verbena. Limonene (30.33%), *trans*-citral (17%), *cis*-citral (12.77%), caryophyllene oxide (5.71%) and geraniol acetate (4.02%) were the major components of *L. citriodora* essential oil. Nazari *et al*. (2009) identified 40 compounds in lemon verbena essential oil, accounting for 96.17% of the oil. Of these compounds, caryophyllene oxide (13.6%), 1,8-cineole (12.5%), and nerol (5.54%) were the major components.

Table 2 - Essential oil composition of *L. citriodora*

Component	RT*	Content (%)
1-Octen-3-ol	7.254	0.50
6-Methyl-5-heptene-2-one	7.393	3.48
β-Myrcene	7.467	0.40
p-Cymene	8.604	0.86
D-Limonene	8.824	30.33
Terpin	8.963	0.79
cis-Ocimene	9.323	1.97
Linalool	11.200	1.37
4-Ethyl-3-isopropyl-4-methylcyclohexanol	11.340	0.33
Limonene oxide, *cis*-	12.315	0.38
Limonene oxide	12.469	0.76
1-Cyclohexene-1-acetaldehyde, α,2-dimethyl-	12.829	0.44
Citronellal	12.968	0.54
Cyclopentene, 1,4-dimethyl-5-(1-ethylethyl)-	13.599	1.17
Pinane, 2,3-epoxy-	13.936	0.33
Terpineol	14.465	0.40
cis-Carveol	15.308	0.56
cis-Citral	16.020	12.77
(S)-(+)-Carvone	16.152	0.62
Bergamiol	16.335	0.50
3-Carvomenthenone	16.489	0.69
trans-Citral	17.039	17.00
Thymol	17.736	1.70
Carvacrol	17.993	0.33
Neryl acetate	19.900	0.57
Copaene	20.355	0.42
Geraniol acetate	20.546	4.02
Caryophyllene	21.859	2.65
Humulene	23.252	1.07
α-Curcumene	24.235	1.78
Spathulenol	25.996	2.06
Caryophyllene oxide	26.069	5.71
tau-Cadinol	26.744	0.84
α-Cadinol	26.883	0.30
Total		**97.64**
Essential oil yield (%)		**0.58**

*retention time

The essential oil composition of lemon verbena plants cultivated in Iran included geranial, 1,8-cineole, and limonene (Meshkatalsadat *et al*., 2010). Agah and Najafian (2012) used alternative drying methods in a study of *L. citriodora* plants and determined that the main oil constituents were limonene (4.0%), 1,8-cineole (1.92%), *trans*-ocimene (3.2%), neral (23.5%), geranial (34.2%), (E)-caryophyllene (3.2%), germacrene-D (5.5%), bicyclogermacrene (4.1%), (E)-nerolidol (1.8%) and caryophyllene oxide (2.4%). Khani *et al*. (2012) determined that lemon verbena essential oil constituents included citral (11.3%), limonene (10.6%), neral (7.9%), 4-phenyl undecan-4-ol (7.7%), α-curcumene (6.5%), α-cedrol (4.5%) and caryophyllene oxide (4.5%).

Argyropoulou *et al*. (2007) reported that lemon verbena grown in Greece contained mainly geranial, neral, and limonene, and that the oil composition differed at two developmental stages of the plants. In May, the main constituents were geranial (38.7%), neral (24.5%) and limonene (5.8%), which together constituted 69% of the total essential oil composition. At harvest in September, the major constituents were limonene (17.7%), geranial (26.8%) and neral (21.8%), representing 66.3% of the oil.

Velasco-Negueruela *et al*. (1993) showed that the main components of essential oils were α-thujone (28.29%), carvone (7.40%), β-caryophyllene (5.39%), germacrene-D (5.16%), bicyclogermacrene (4.53%), spathulenol (6.06%) and caryophyllene oxide (7.03%) for *Lippia turbinate*; α-thujone (68.94%) and carvone (10.34%) for *Lippia polystachya*; myrcenone (15.48%), myrcene (7.92%), limonene (9%), camphor (10.55%), (E)-tagetenone (6.30%), *trans*-dihydrocarvone (5.85%) and *cis*-dihydrocarvone (16.65%) for *Lippia junelliana*; camphor (16.20%), africanene isomers (5.04%), lippifoli-1(6)-en-5-one (16.70%) and undetermined compounds (14.89%) for *Lippia integrifolia*. Similarly, Malekirad *et al*. (2011) also reported that limonene, neral, geraniol, 1-octane-3-ol and curcumene were predominant components in lemon verbena plants, grown in Iran.

The differences between the results of our study and those of other studies highlight the impact that geographical and ecological factors can have on the qualitative and quantitative characteristics of the essential oils produced. In addition, other factors, such as the developmental stage of the plant and growing conditions, can influence the essential oil composition (Argyropoulou *et al*., 2007).

We determined that the essential oil content of lemon thyme was 0.9% and we identified 22 components within lemon thyme essential oil that together accounted for 85.11% of the total oil composition (*Table 3*). The major constituents included *trans*-geraniol (30.07%), *trans*-citral (15.06%), *cis*-citral (11.71%), and *cis*-

geraniol (7.65%) (*Table 3*). Omidbaigi *et al*. (2009) studied the effect of various harvest times on the quality and quantity of lemon thyme essential oil and showed that the highest oil yield (2.21%) was obtained at the beginning of flowering, with geraniol being the predominant (54.2-72.5%) component. The highest geraniol content (72.5%) in the essential oil was measured before the flowering stage and the lowest content (54.2 %) was measured at the fruit-set stage. The most abundant components in lemon verbena oil were previously reported to be geraniol (54.4%), geranial (13.9%), neral (10.1%), nerol (5.2%), 3-octanone (3.3%) and borneol (3.2%) (Omidbaigi *et al*., 2005). Stahl-Biskup and Holthuijzen (1995) also reported that the main component of lemon thyme oil was geraniol (more than 60%), with a high level maintained from July to October; they also detected geranyl acetate (1.0%), geranyl butyrate (0.8%), nerol (2.8%) and citronellol (0.3%).

Table 3 - Essential oil composition of *T. citriodorus*

Component	RT*	Content (%)
3-Thujene	5.626	0.25
Pinene	5.831	0.12
Camphene	6.293	1.65
1-Octen-3-ol	7.254	0.57
3-Octanone	7.459	1.90
β-Myrcene	7.467	0.17
3-Octanol	7.782	6.18
(+)-4-Carene	8.340	0.26
p-Cymene	8.626	1.18
D-Limonene	8.765	0.45
Borneol	13.665	1.89
3-Pinanone	13.834	0.26
Isogeraniol	13.951	0.94
cis-Geraniol	15.653	7.65
cis-Citral	16.078	11.71
trans-Geraniol	16.717	30.07
trans-Citral	17.127	15.06
Borneol acetate	17.523	0.62
Thymol	18.074	1.45
Caryophyllene	21.873	1.32
β-Bisabolene	24.881	0.71
Caryophyllene oxide	26.054	0.70
Total		**85.11**
Essential oil yield (%)		**0.90**

*retention time

The lemon-scented compounds geranial and neral contributed 8.2% and 5.5%, respectively, to the total oil composition, and thymol (0.5%) was found in remarkable quantities. The oil of lemon thyme plants cultivated in Hungary exhibited a geraniol and carvacrol chemotype (Horváth *et al*., 2006). The essential oil compositions observed in these other studies were similar to our results.

CONCLUSION

Lemon verbena and lemon thyme are lemon-scented herbs whose leaves are used as herbal medicines and as flavouring herbs in the food industry. Our results showed that lemon verbena has a limonene chemotype, while lemon thyme has a geraniol chemotype, and that the mineral contents of these herbs fall below WHO permissible levels and likely do not constitute a health hazard for consumers. The data obtained in this study provide a basis for further studies to enhance the selection of limonene and geraniol-chemotype plants with higher yields and suitable essential oil composition.

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