ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 17(2) 2018, 39–48
www.acta.media.pl ISSN 1644-0692 DOI: 10.24326/asphc.2018.2.4

ORIGINAL PAPER

Accepted: 19.09.2017

EFFECTS OF DIFFERENT HARVEST PERIODS ON ESSENTIAL OIL COMPONENTS OF *Lippia citriodora* KUNTH UNDER SEMI-ARID CLIMATIC CONDITIONS AND BIOLOGICAL ACTIVITIES OF ITS ESSENTIAL OIL

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ABSTRACT

Lemon verbena is cultivated mainly due to the lemon-like aroma emitted from its leaves that are utilized for most purposes. The chemical composition of the essential oil of lemon verbena was analyzed by GC/MS in July at pre flowering, in September at full flowering and in end of October at post flowering period. The *in vitro* antimicrobial activity of the essential oil, extracted from *Lippia citriodora* was tested against laboratory control strains belonging to the American Type Culture Collection (Maryland, USA) four Gram (+) and Gram (-) bacteria using the disc diffusion test. Antioxidant activity of the sample was determined by 1,1-diphenil-2--picrly-hydrazil (DPPH) assay. Well-known antioxidant compounds such as ascorbic acid and α-tocopherol were used as standard. Results showed, among different harvest periods high fresh and dry herbage and dry leaf yields per plant were obtained from full flowering stage. Harvest periods were found to have a significant effect on the content of essential oil and the highest amounts of limonene, neral and geranial and measured as 31.15, 11.92 and 15.53%, respectively at full flowering stage. In all samples, the main constituents were limonene, neral and geranial constituting 46.03%–58.59% of the total essential oil yield depend on development stages. Lemon verbena essential oil was found to have antibacterial activity especially against Gram (+) microorganisms. In DPPH system, the moderate radical scavenging activity was exhibited. Therefore, *L. citriodora* has potential to be used as a natural antioxidant and antimicrobial agent in food processing.

Key words: lemon verbena, development stage, volatile oil, limonene, antimicrobial activity, DPPH

INTRODUCTION

Lippia citriodora Kunth, is a medicinal and aromatic plant belonging to Verbenaceae family. Its leaves have a strong flavor and the typical smell lemon [Duke 1985]. The plant is indigenous to South America, and is cultivated mainly due to the lemon-like aroma emitted from its narrow leaves that are utilized for the preparation of herbal tea. Lemon verbena has a long history of folk uses in treating asthma, spasms, cold, fever, flatulence, colic, diarrhea, indigestion, insomnia and anxiety [Carnat et al.

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1999, Argyropolou et al. 2007]. In the United States, lemon verbena is listed in Generally Regarded As Safe (GRAS) for human consumption in alcoholic beverages [Gomes et al. 2006]. It is considered as a new source of essential oil with relatively high yield used in perfumery with its strong citral content. Lemon verbena essential oils have been studied by several researchers. The essential oils are popular among the herbalists because the oil contains citral and limonene.

Aromatic and medicinal plants have natural antimicrobial and antioxidant compounds. They constitute a constant source of active reagents against pathogen germs. Among these products, essential oils produced by aromatic plants as secondary metabolites, have gained a net interest by many investigators [Bensabah et al. 2013]. Although it was not possible to assign antimicrobial activity to a component in particular, oxygenated monoterpens such as citral which were present as a major component in Lippia oil, have inhibitory effect against Gram-negative and Gram-positive microorganisms [Parodi et al. 2013].

The oil content and its components of lemon verbena changed depend on harvest period. Shahhoseini et al. [2013] reported that oil yield of the plant was changed in different period and the highest content recorded at full flowering period. The main compounds in three stages were geranial and neral.

Many researchers determined the major components in essential oil are limonene, neral and geranial [Sangwan et al. 2001, Argyropoulou et al. 2007]. Argyropoulou et al. [2007] reported that the most important components of essential oil from lemon verbena at two developmental stages were geranial, neral and limonene that constituting 66.3% of the total essential oil content in May and increasing to 69% in September. These components rate changed between 21.8–24.55 and 26.8–38.7% depend on development stage.

Essential oil yield and composition depend on climatic conditions and developmental stage of plants. Harvesting time is a critical operation in medicinal plants. It is important accurately determine harvesting time for best yield and essential oil quality. Early harvesting probably leads to lower active substance and on the other hand late harvesting can leads to shattering of leaves, flowers and fruits [White et al. 1987, Rohloff et al. 2005].

The purpose of this paper is to analyse the composition of essential oil of lemon verbena cultivated in semi-arid climatic conditions of Diyarbakir, Turkey, at different development stages, using GC/MS and also to determine the antimicrobial and antioxidant activities of essential oil.

MATERIALS AND METHODS

Plant material and field experiment. *Lippia citriodora* plants were provided from Atatürk Horticultural Central Research Institute, Yalova, Turkey. Seedlings had propagated by cutting (10–15 cm) method in the greenhouse. Field experiment was conducted at the department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir (latitude 37°53'N, longitude 40°16'E, altitude 680 m), Turkey in 2016 year.

The experimental area soil had pH of 7.45 and with 1.16% organic matter. Meteorological data for the growing seasons showed long-term mean temperature of 23.2°C, humidity 40.2%, and total precipitation of 22.4 mm between April and October. The average temperature, humidity ratio and the amount of total precipitation values for the April––October period 2016 were 24.1°C; 36.1% and 15.4 mm, respectively. During harvest stages, at July mean temperature recorded as 31.6°C, at September 24.1°C and October 18.8°C [State Meteorology Institute, Diyarbakir, Turkey]. At the end of plant life, especially third harvest stage, temperature has decreased significantly.

The field experiment was established in Randomized Complete Block design, in three replications. The area of one plot was 6.3 m^2 (2.1 × 3 m^2). The soil of each experimental plot was watered soon after planting of cuttings to avoid wilting of the transplanted material. Field trial area were watered by sprinkler irrigation, hand weeded and when needed during experiment. Aerial parts of lemon verbena were collected in July (the beginning of flowering), in September (full flowering) and in October (post flowering) in the year of 2016. The plants were cut at 10 cm above the ground. The samples of each harvest

were dried in a shady and well ventilated place at a room temperature.

Essential oil extraction. Essential oil of 20 g dried leaf samples extracted by hydro-distillation for 3 hours using a Clevenger-type apparatus (v/w). The isolated oils were stored in tightly closed vials at 4°C until analysis. Essential oil of three development stages were mixed and used for biological activity studies.

Gas chromatography-Mass spectrometry (GC-MS) analysis. GC-MS analyses were performed with Agilent GC-6890 II series coupled with Agilent 5975C Mass Spectrometer. Column: HP-88, 100 m × 250 mm × 0.20 µm film thickness. Temperature programmed: from 70°C (1 min) to 230°C (20 min) at 10°C/min. The injection temperature: 250°C. Injection volume: 1.0 mL. Carrier gas: He. Injection mode: split (20 : 1). MS interface temp.: 250°C; MS mode: EI; detector voltage: 70 eV; mass range: 35–400 m/z; scan speed (amu/s). The components of the oil were identified by mass spectra with those of pure authentic samples and NIST08, Willey7n.1 and HPCH1607 libraries reference compounds. The ratios of compounds were evaluated according to FID results. Retention indices were computed from gas chromatograms by logarithmic interpolation between n-alkanes. The homologous series of n-alkanes C7-C40, Supelco, USA were used as standard. Retention indices calculated as HP-88 capillary column 18. All samples were repeated three times for GC-MS analysis. GC-MS total ion current chromatogram of lemon verbena volatile components in full flowering period was given in Figure 1.

Determination of antibacterial activity. Antimicrobial activity was assayed by the disc diffusion susceptibility test according to the recommendation of the National Committee for Clinical Laboratory Standards (NCCLS) [Appelbaum et al. 1998]. The disc diffusion test was performed on Muller-Hinton agar plates. Plates were dried at 35 to 36°C for about 30 min in an incubator before inoculation. Three to five freshly grown colonies of bacterial strains were inoculated into 50 mL of Muller-Hinton broth medium in a shaking water bath for 4 to 6 h until a turbidity of 0.5 McFarland (1 × 108 CFU/mL) was reached. The final inocula were adjusted to 5 × 105 CFU/mL using a spectrophotometer [Saleh et al. 2010]. The inoculum (100 µL) from the final inocula

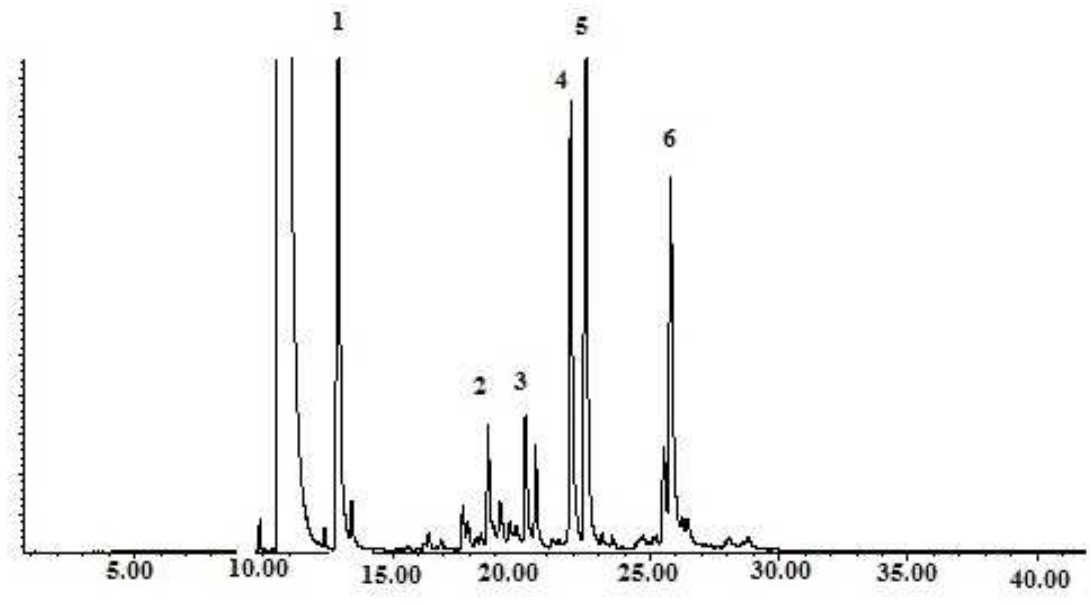

Fig. 1. GC/MS chromatogram of lemon verbena at full flowering period (September); the main components are given as 1 – limonene, 2 – E-caryophyllene, 3 – curcumene <Ar->, 4 – neral, 5 – geranial, 6 – germacrene D

was applied to each agar plate and uniformly spread with a sterilized cotton spreader over the surface. Sterile filter-paper disks (Oxoid, England, 6 mm in diameter) were impregnated with 10 µL essential oil extract (*L. citriodora*) each discs (6 mm). Plates were incubated at 37°C for 24 h. Standard disks of amoxycillin (A/30) was individually used as positive controls. The diameters of the inhibition zones were measured in mm (millimetres) using an inhibition zone ruler and the photos of plates were taken using Bio-rad screening system.

Scavenging activity of DPPH radical. Antioxidant activity of essential oil extract (*L. citriodora*) was measured by DPPH (1,1-diphenil-2-picrly-hydrazil) method at 517 nm absorbance [Saleh et al. 2010]. The assay was carry out by mixing 1.5 mL of various concentrations of essential oil (final concentrations were 5, 10 and 40 mg/mL) in ethanol with 2 mL 0.1 mM DPPH radical. Then, the mixture was incubated in dark for 40 minutes at room temperature. Ascorbic acid and α-tocopherol were used as controls, and antioxidant activity was calculated by the following equation as percent inhibition: % inhibition = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$.

RESULTS AND DISCUSSION

Field studies. Some agronomical results for lemon verbena are given in Table 1. For all investigated agronomical characteristics, including essential oil yield, the highest values were obtained from full flowering period (September harvest), in contrast of this observation the lowest values obtained from post flowering period (October). Plant height results of lemon verbena at different development stages were changed between 99.3 and 102.6 cm. The highest value was obtained from post flowering stage, October harvest.

Fresh herbage yield per plant harvested at September was the maximum (276.0 g plant^{-1}), while the lowest one was obtained from October (152.6 g plant^{-1}). For dry herbage yield, the lowest value was recorded from October harvest period as 65.2 g plant^{-1}, while the maximum value obtained from September harvest period as 125.0 g plant^{-1}. Dry leaf yield per plant changed between 30.3 and 73.3 g plant^{-1}. The highest value obtained from September harvest stage. When take into consideration of dry herbage yield, leaf/stem ratio was varied between 75.9 and 86.8%, depend on harvest stage (tab. 1). The harvest during September was favourable compared with July and October harvests according to the highest values of the fresh & dry weight and dry leaf yield per plant.

Semi – arid climatic conditions limited plant growing due to high temperature and insufficient irrigation supplement. Lipiec et al. [2013] pointed that water stress affects the stomatal conductance, the CO_2 assimilation and, consequently, introduces limits to the productivity and growth of plants. Therefore, herbage yield of plants might be decreased under stress conditions.

Essential oil content of lemon verbena was changed according to development stage. Through the end of vegetation, oil content decreased. Essential oil content of July, September and October harvest stages were determined as 0.83%, 0.88% and 0.63%, respectively (tab. 1). Results showed essential oil content at the beginning of vegetation increased and then through late vegetation was decreased. Essential oil content is also changed depend on different parts of plant. Vogel at al. [1999] reported that the highest

Table 1. Some agronomic properties of lemon verbena at semi-arid climatic conditions (mean ± standard deviation)

Harvest period	Plant height (cm)	Fresh herbage (g plant^{-1})	Dry herbage (g plant^{-1})	Dry leaf (g plant^{-1})	Essential oil content (ml 100 g^{-1})
July	99.3 ±3.2*	169.0 ±71.7	95.7 ±51.5	41.3 ±19.3	0.83 ±0.03
September	100.6 ±4.2	276.0 ±113.2	125.0 ±63.2	73.3 ±20.9	0.88 ±0.04
October	102.6 ±4.5	152.6 ±56.5	65.2 ±22.4	30.3 ±12.1	0.63 ±0.23

*n: 3

Table 2. Essential oil compositions of lemon verbena at different development times (mean ± standard deviation)

No	Components	RI	RT	July	September	October
1	Myrcene	1217	12.36	0.18 ±0.10	0.24 ±0.01	0.18 ±0.01
2	Limonene	1268	12.88	22.83 ±7.44	31.15 ±1.65	28.44 ±0.14
3	β-ocimene	1318	13.42	0.74 ±0.25	1.19 ±0.10	1.08 ±0.02
4	α-copaene	1557	16.22	0.18 ±0.01	0.18 ±0.02	0.22 ±0.01
5	Hepten-2-one<6-methyl-5->	1572	16.41	0.26 ±0.03	0.55 ±0.13	0.33 ±0.00
6	β-bourbonene	1611	16.88	0.42 ±0.01	0.29 ±0.04	0.33 ±0.00
7	α-terpinolene	1682	17.72	1.12 ±0.06	1.03 ±0.01	0.99 ±0.01
8	Trans-limonene oxide	1698	17.92	0.74 ±0.05	0.75 ±0.02	0.67 ±0.00
9	Citronellal	1728	18.25	0.34 ±0.01	0.33 ±0.01	0.27 ±0.00
10	γ-amorphene	1744	18.43	0.45 ±0.02	0.45 ±0.02	0.42 ±0.01
11	E caryophyllene	1769	18.71	4.35 ±0.78	3.43 ±0.36	4.35 ±0.25
12	Pulegone	1809	19.18	tr	1.75 ±0.09	1.88 ±0.01
13	Trans-chrysanthemal	1844	19.54	0.68 ±0.15	0.75 ±0.10	0.54 ±0.01
14	γ-cadinene	1868	19.81	1.1 ±0.05	0.99 ±0.08	1.09 ±0.02
15	Curcumene	1900	20.16	4.26 ±0.01	3.79 ±0.29	4.75 ±0.01
16	Geranyl isobutanoate	1944	20.55	2.35 ±0.24	2.68 ±0.03	2.81 ±0.08
17	Geranyl propanoate	2017	21.21	0.33 ±0.09	0.33 ±0.02	0.36 ±0.01
18	Trans-carveol	2042	21.43	0.39 ±0.05	0.34 ±0.04	0.46 ±0.01
19	Neral	2095	21.91	9.80 ±0.32	11.92 ±0.06	11.58 ±0.08
20	Geranial	2164	22.48	13.40 ±0.99	15.52 ±0.35	15.32 ±0.12
21	Piperitone	2243	23.12	0.53 ±0.09	0.45 ±0.03	0.41 ±0.02
22	Cis-Muurola-3.5-diene	2292	23.52	0.59 ±0.11	0.35 ±0.02	0.35 ±0.03
23	Junipene	2339	23.90	0.19 ±0.04	0.09 ±0.02	0.07 ±0.00
24	Cubenol	2403	24.42	0.25 ±0.07	0.10 ±0.02	0.12 ±0.02
25	Aromadendrene	2436	24.70	0.77 ±0.48	0.39 ±0.33	0.80 ±0.00
26	γ-gurjunene	2466	24.96	0.33 ±0.07	0.19 ±0.02	0.22 ±0.04
27	Germacrene D	2533	25.53	4.41 ±0.78	2.96 ±0.06	2.76 ±0.04
28	Caryophyllene oxide	2565	25.79	17.09 ±3.15	11.75 ±0.15	13.53 ±0.03
29	Ar-turmerol	2616	26.24	1.43 ±0.32	0.84 ±0.04	1.05 ±0.04
30	Longifolol	2638	26.45	2.17 ±0.53	1.22 ±0.11	1.28 ±0.17
31	Helifolenol B	2729	27.34	0.27 ±0.05	0.12 ±0.04	0.15 ±0.00
32	Cariophylladienol	2801	28.05	1.02 ±0.17	0.52 ±0.01	0.68 ±0.03
Total				92.97	96.64	97.49
Grouped Components						
Monoterpene hydrocarbons				24.69	33.37	30.51
Oxygen-containing monoterpenes				30.91	36.28	35.76
Sesquiterpene hydrocarbons				12.46	9.82	12.33
Oxygen-containing sesquiterpenes				24.20	16.17	18.14
Others				0.71	1.00	0.75

essential oil was found in young leaves of lemon verbena as 0.95%, and decreased to December and April. Our results are in accordance with Simon et al. 1992, Vogel et al. 1999, Sangwan et al. 2001, Argyropouloua et al. 2007].

Closer harvesting intervals (after two months) produced less herbage yield and oil yield while longer harvesting intervals produced more herbage yield mid oil yield [Ibrahim et al. 2014]. In this study, harvesting intervals between July and September improved herbage and essential oil yield, otherwise intervals between September and October is very short, thus herbage and oil yield decreased. Shahhoseini et al. [2013] reported that lemon verbena essential oil varied depends on phenological stage and the highest oil content was determined at full flowering stage as 0.90% and oil content at pre flowering stage recorded as 0.48% and at fruit set stage as 0.25%. These results are in agreement with results obtained from this study.

Essential oil components. Essential oil components of lemon verbena were classified in to monoterpenes (monoterpene hydrocarbons and oxygenated monoterpenes) and sesquiterpenes (sesquiterpene hydrocarbons and oxygenated sesquiterpenes). Also, the percentages of different classes of essential oil under all harvests were presented in Table 2. The results showed that oxygenated monoterpenes had the highest percentage in essential oil between rate of 30.91 and 36.28%. The others groups were fallowed as monoterpene hydrocarbons (24.69–33.57%), oxygenated sesquiterpenes (16.17–24.20%) and sesquiterpenes hydrocarbons (9.82–12.46%), respectively.

Thirty-two compounds were identified in the essential oils in lemon verbena at different development stages, representing between 92.97 and 97.49% of the total oil. The identified constituents with their retention times and retention index are summarized in Table 2. The main constituents of the oils at July harvest were limonene (22.83%), caryophyllene oxide (17.09%), geranial (13.40%), germacrene D (4.41%), curcumene and geranyl isobutanoate (2.35%); at September harvest (full flowering stage) limonene (31.15%), caryophyllene oxide (11.75%), geranial (15.52%), germacrene D (2.96%), curcumene (3.79%) and geranyl isobutanoate (2.68%) (fig. 1) and at October harvest limonene (28.44%), caryophyllene oxide (13.53%), geranial (15.32%), germacrene D (2.76%), curcumene 4.75 and geranyl isobutanoate (2.81%), respectively.

Results showed that maximum limonene, neral and geranial percentage was obtained in full flowering stage, and minimum limonene, neral and geranial percentage was shown in pre flowering period (tab. 3). Short interval and decrease of temperature between harvests could affect essential oil constituents.

Moreover, the peripheral factors are effective on the quality and quantity of active substances of pharmaceuticals such as agricultural factors (such as fertilization planting density and sowing date, drying method etc.) [Letchamo et al. 2004, Yadegari et al. 2013]. Moreover, biosynthesis of active substances of lemon verbena is also dependent on the light regimes, temperature and plant respiration [Letchamo et al. 2004]. Khalid [2006] reported that at water-stress conditions essential oil percentage and the main constituents of essential oil increased in different *Ocimum* species. These stress conditions might be positive effect on some essential oil content plant species, like lemon verbena to obtain high oil yield.

Table 3. Major essential oil components of *Lippia citriodora* harvested three different periods

Main compounds	Harvest periods		
	July	September	October
Limonene	22.83	31.15	28.44
Geranial	13.40 b	15.52 a*	15.32 a
Neral	9.80 b	11.92 a	11.58 a
Caryophyllene oxide	17.09 a	11.75 b	13.53 ab
LSD (0.05)	limonene: ns, geranial: 1.02 (0.01), neral: 0.74 (0.01), caryophyllene oxide: 4.20		

* Values followed by the same letter in a line do not differ significantly according to LSD at 0.05; ns: no significant

Major problem in semi arid climatic conditions to grow medicinal plants especially shrub ones is water deficit. Solinas and Deiana [1996] reported that secondary products of plants can be altered by environmental factors and water stress is a major factor affecting the synthesis of natural products. In another study, Simon et al. [1992] reported that fresh and dry weights of *Ocimum basilicum* L. were decreased as plant water deficit increased, however, the linalool and methyl chavicol contents increased as water stress increased.

The chemical composition and amount of essential oils are influenced by the time of harvest, and may vary according to the developmental stages/periods of the plants. The biosynthesis of essential oil components is low in the vegetative stage of plants. The enzymes necessary to the biosynthesis of some components are not active during the vegetative stage. Thus, the harvest stage is one of the most important factors affecting essential oil quality [Nurzyńska-Wierdak 2013, Rodrigues et al. 2013]. Kizil and Toncer [2016] reported that lemon verbena harvested at September was contain major component as limonene and total amount of limonene and citral (neral and geranial) determined as 60.1%. According to the literature, limonene is the component found in higher quantities in essential oils of the genus Lippia together with neral and geranial [Pascual et al. 2001, Shahhoseini et al. 2014, Kizil and Toncer 2016]. In this study, limonene and citral (neral + geranial) were identified at high percentages and in agreement with Argyropouloua [2007], Shahhoseini et al. [2013] and Nematian et al. [2014].

Antibacterial and antioxidant activities. Antimicrobial activity of essential oil was determined using disc diffusion assay and the results were compared to amoxicillin. The inhibition zone values determined for the essential oil and standard antibiotic are listed in Table 4.

Essential oil extract has noticeable activity on all organisms. It inhibited Gram (+) bacteria (*B. subtilis* and *S. aureus*) caused zone of 20 and 16 mm diameter, respectively. On the other hand, it has mild activity against Gram (-) bacteria. When it was compared with standard antibiotic, essential oil has antimicrobial activity as well as amoxycillin (tab. 4). It is well known that essential oils are more active against Gram-positive than Gram-negative bacteria [Tongnuanchan and Benjakul 2014, Dhifi et al. 2016].

The high antibacterial effect of lemon verbena essential oil against both antibiotic susceptible and resistant Gram-positive microorganisms is due to the high content of volatile oil. In earlier studies, it was found that the extracts and isolated volatile oil from lemon verbena show a strong antimicrobial activity against different types of bacteria and fungi [Mothana et al. 2010]. Generally, essential oils characterized by a high level of phenolic compounds, such as carvacrol, eugenol, and thymol, have important antibacterial activities [Dhifi et al. 2016], moreover, it is reported that the use of the whole essential oil provides an effect which is greater than that of the major components used together. This suggests that minor components are essential for activity and may have a synergistic effect [Dorman and Deans 2000, Dhifi et al. 2016].

Table 4. Antimicrobial activity of lemon verbena essential oil and standard antibiotic (amoxycillin)

Samples	Zone of inhibition (mm)			
	Bacillus subtilis ATCC 11774	*Escherichia coli* ATCC 25922	*Staphylococcus aureus* ATCC 25923	*Pseudomonas aeruginosa* ATCC 27853
L. citriodora	20	10	16	8
A/30	20	16	14	10

A/30: amoxycillin, 30 μg

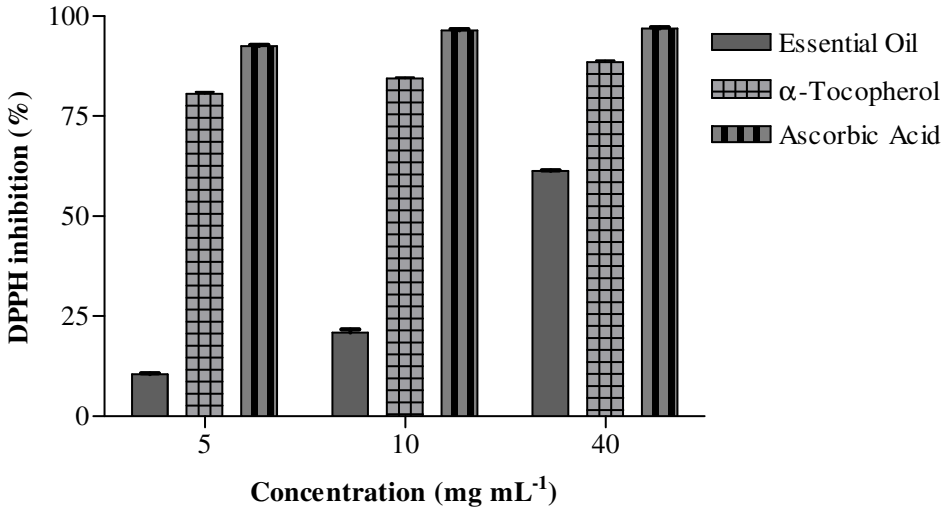

Fig. 2. Radical scavenging effect of lemon verbena essential oil extract, ascorbic acid and α-tocopherol on DPPH radicals. Each value is expressed as mean ± standard deviation (n = 3)

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To prolong the storage stability of foods and to reduce the damage to macromolecules, in industrial processing synthetic antioxidants are used. However, it has been documented that synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have side effects. Antioxidant activity of lemon verbena essential oil was tested using by DPPH radical scavenging assay. Essential oil of lemon verbena exerted moderate antioxidant effect, compared to the antioxidant activity of the standard compound such as ascorbic acid and alpha-tocopherol. At 40 mg mL^{-1} essential oil extract showed statistically significant increase compared with 5 and 10 mg mL^{-1} concentration. However, these increase is not comparable with ascorbic acid and alpha-tocopherol (fig. 2). Most of the essential oils are dominated by oxygenated monoterpenes such as alcohols, aldehydes, ketones, and esters. Essential oil components of lemon verbena shows that oxygenated monoterpenes are found more abundant compared to other groups (tab. 2). Hence, it can be suggested that he primary components such as the limonene, neral and geranial are mainly may be responsible for the antioxidant activity. On the other hand, it is also possible that the activity of the main components is modulated by other minor molecules

[Franzios et al. 1997]. Moreover, it is likely that several components of the essential oils play a role in defining the fragrance, the density, the texture, the colour and above all, cell penetration [Cal 2006], lipophilic or hydrophilic attraction and fixation on cell walls and membranes, and cellular distribution.

Choupani et al. [2014] reported lemon verbena leaves has a good source of natural antioxidants and its essential oil and components showed high antioxidant activity although it was lower that of BHT.

CONCLUSIONS

It has observed with this study, lemon verbena is not tolerant to severe winter condition such as semi-arid climatic conditions. Diyarbakir, located in the South-eastern Anatolia, Turkey, has been frost during winters. Thus, it can be cultivated, as annual plant, due to severe winter environmental conditions or in greenhouse conditions.

Lemon verbena and its essential oil components are very important from commercial point of view. The results of experiment describe suitable harvest stage for *L. citriodora.* It was confirmed September harvest stage of lemon verbena contain more herbage and essential oil yield than others stages. Moreover, it was further confirmed that lemon verbena could be cultured profitably for commercial production of oil components, limonene and citral (neral + geranial) under semi-arid conditions.

Essential oil of lemon verbena show strong antimicrobial activity against different types of bacteria and moderate antioxidant effect, compared to synthetic antioxidants ascorbic acid and α-tocopherol. The results presented can provide evidence that the essential oil of lemon verbena could be used in food industry and other fields as natural antioxidant.

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