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Phytochemical Analysis, Antioxidant and Antibacterial Activities of Four Lamiaceae Species Cultivated in Barnyard Manure

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ABSTRACT

The present study was conducted to determine essential oil yields, essential oil compositions, total phenolics, antioxidant and antibacterial activities of organic manure-treated medicinal plants of *Salvia officinalis* L. (sage), *Lavandula angustifolia* L. (lavender), *Melissa officinalis* L. (lemon balm) and *Origanum vulgare* ssp. *hirtum* (origano). Essential oil yields of investigated medicinal plants varied between 0.06±0.01%-3.43±0.06%. The 1,8-cineol (15.285±0.003%), viridiflorol (12.095±0.003%) and cis-thujone (12.200±0.003%) were the major essential oil components in *S. officinalis* L. Linalool (22.400±0.003%), 1,8-cineol (8.215±0.003%), linalyl acetate (7.900±0.003%) and lavadulyl acetate (7.690±0.003%) were the major components in *L. angustifolia* L. Citronellal (14.515±0.003%), geranial (13.050±0.003%) and β-caryophyllene (12.385±0.003%) were the major components in *M. officinalis* L. and carvacrol (65.080±0.003%) was the major component in *O. vulgare* ssp. *hirtum*. The highest total phenolics content and antioxidant activity were observed in *M. officinalis*. The best antibacterial activity against *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 35218 bacteria was observed in *O. vulgare* ssp. *hirtum*.

Keywords: *Salvia officinalis* L.; *Lavandula angustifolia* L.; *Melissa officinalis* L.; *Origanum vulgare* ssp. *hirtum*; Medicinal and aromatic plants

Ahır Gübresinde Yetiştirilen Dört Lamiaceae Türünün Fitokimyasal Analizleri, Antioksidant ve Antibakteriyel Aktiviteleri

ESER BİLGİSİ

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ÖZET

Bu çalışmada organik (ahır gübresinde) yetiştirilen *Salvia officinalis* L. (Tıbbi adaçayı), *Lavandula angustifolia* L. (İngiliz lavantası), *Melissa officinalis* L. (Oğul otu) ve *Origanum vulgare* ssp. *hirtum* (İstanbul kekiği) tıbbi bitkilerinin uçucu

yağ oranları, yağ bileşenleri, toplam fenolik içerikleri, antioksidant ve antibakteriyel aktiviteleri araştırılmıştır. Çalışma sonucunda incelenen bitkilerde uçucu yağ oranının % 0.06±0.01-% 3.43±0.06 arasında değişim gösterdiği belirlenmiştir. Uçucu yağ bileşenleri olarak: *S. officinalis* L.'te 1,8-cineol (% 15.285±0.003), viridiflorol (% 12.095±0.003) ve cistujone (% 12.200±0.003); *L. angustifolia* L.'de linalool (% 22.400±0.003), 1,8-cineol (% 8.215±0.003), linalyl acetate (% 7.900±0.003) ve lavadulyl acetate (% 7.690±0.003); *M. officinalis* L.'te citronellal (% 14.515±0.003), geranial (% 13.050±0.003) ve β-caryophyllene (% 12.385±0.003); *O. vulgare* ssp. *hirtum*'de carvacrol (% 65.080±0.003) tespit edilmiştir. En yüksek toplam fenolik içerik ve antioksidant aktivite *Melissa officinalis* L.'te görülmüştür. Kullanılan *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 ve *Escherichia coli* ATCC 35218 bakterilerine karşı en iyi antibakteriyel aktiviteyi *Origanum vulgare* ssp. *hirtum*'un gösterdiği belirlenmiştir.

Anahtar Kelimeler: *Salvia officinalis* L.; *Lavandula angustifolia* L.; *Melissa officinalis* L.; *Origanum vulgare* ssp. *hirtum*; Tıbbi ve aromatik bitkiler

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1. Introduction

Increasing infection risks stemming from antibiotic-resistant microorganism have made the discovery of new and natural antimicrobial substances the focus of various researches. What is more, various synthetic food additives for preservative purposes created serious concerns on sensitive and conscious consumers. Such concerns have brought about the concepts of organic food or organic agriculture. The expectations of conscious consumers have encouraged and even forced the producers and service providers to use natural preservatives. Then, a need has arisen for researchers to investigate and test efficiency of various plants against microorganisms. Antibacterial impacts of various plant extracts microorganisms and especially on food pathogens have been supported by several researchers.

As it is well known, reactive oxygen species, singlet oxygen, superoxide radicals, hydrogen peroxide, hydroxyl radicals and nitric oxide are unstable and extremely reactive compounds. Oxidative stress-induced reactive oxygen species are blamed to be the indicators of development and progress of various cardiovascular diseases. Antioxidants prevent negative impacts of free radicals and reactive oxygen species and protect the body. Today, BHT, BHA, propyl gallate and tert butyl hydroquinone are the most common synthetic antioxidants. However, reliability of these synthetic

antioxidants are argued because of their toxic and carcinogenic effects and resultant liver injury. Therefore, discovery of new, reliable and unharmed antioxidants from natural resources have become the most common research topic (Birman 2012).

Lamiaceae species are now cultivated worldwide, mainly to be used as culinary and medicinal herbs and they are widely studied as natural antioxidant sources since they are relatively rich in polyphenols (Cuvelier et al 1994). The Lamiaceae species of *Salvia officinalis* L. (sage), *Lavandula angustifolia* L. (lavender), *Melissa officinalis* L. (lemon balm), *Origanum vulgare* ssp. *hirtum* (origano) cultured in this study are popular herbal teas and essential-oil containing drugs. Their therapeutic actions are assigned to biologically active polyphenol components, such as flavonoids and phenolic acids, which possess antioxidant activities. They are naturally grown in Turkey and commonly used by local people in treatments of various diseases. However, culture of these plants is scarcely any. Therefore, they are commonly collected from their natural habitats and marketed then. Collection usually starts with the fresh shoots through the early development stages and such a collection results in excessive damage to plants. Damaged plants are then not able to develop efficient seeds and ultimately they experience various problems for their survival. Thus, for the preservation of natural plant cover and plant genetic sources, culture environments should be created for these plants under such pressures.

A common standardization also plays a significant role for the trade of these plants. Standardized production will then be possible only with the culture and breeding of species.

The primary objectives of the present study are to prevent genetic erosion in country flora; to grow high yield and quality medicinal plants and to raise an awareness on fertilizer utilization which has not been fully comprehended by Turkish farmers and to improve organic fertilizer (manure) use over agricultural fields. In sustainable agriculture, organic fertilizers not only supply plant nutrients but also improve soil organic matter contents. Thus, the objective is to encourage the use of organic fertilizers over cultivated lands. Another objective of the present study is to determine the essential oil yields, essential oil compositions, total phenolics, antioxidant and antibacterial activities of four medicinal and aromatic plants of Lamiaceae family *S. officinalis* L., *L. angustifolia* L., *M. officinalis* L., *O. vulgare* ssp. *hirtum* cultivated with organic barnyard manure.

2. Material and Methods

The seedlings supplied from Field Crops Central Research Institute of the General Directorate of Agricultural Researches and Policies constituted the primary materials of the present study. Experiments were conducted in randomized block design with three replications over 1500 m² area in Kürtün town of Gümüşhane Province. Average climatic data were recorded for years 2010-2013 as follows: 10.4 °C temperature; 39.51 mm precipitation; 64.0% relative humidity (Anonymous 2015). Experimental fields have sandy-clay-loam soil texture with slightly alkaline characteristics (pH 7.20). Soils were classified as unsaline (0.8%) and found to be sufficient in phosphorus (with available phosphorus content of 84.91 kg ha⁻¹).

While selecting plant species, the significant plants for regional development, the ones suitable for regional ecology and with high value-added were taken into consideration. A month before plantation of seedlings, 15 ton ha⁻¹ decomposed manure were applied. Maintenance and care practices were

regularly implemented based on climate conditions and 15 ton ha⁻¹ manure was also applied in autumn of every year (in November). No chemicals were used in experiments.

S. officinalis L., *L. angustifolia* L., *M. officinalis* L. and *O. vulgare* ssp. *hirtum* were harvested at full bloom stage in a sunny day at noon time of the year 2013. Plants were dried at shade and made ready for laboratory analyses.

2.1. Isolation of the essential oil (essential oil preparation)

Essential oil analyses were carried out in accordance with TS 8882 method. About 20 g sample was taken from dried plants of each species and placed into glass Clevenger flasks. About 200 mL (about ten times of sample weight) distilled water was added and samples were then subjected to hydro-distillation for about 3 hours. The essential oil accumulated on top and separated from the rest of the sample. The amount was recorded in ml from the graduated section of the flask and weights were then used to calculate percent essential oil yields.

2.2. Gas chromatography-mass spectrometry/flame ionization detector (GC-MS/FID)

The essential oil composition of samples was analyzed by gas chromatography (Agilent 7890A) coupled with flame ionization detector and mass spectrometry (Agilent 5975C) with capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 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⁻¹. The oven temperature was programmed as follow; 60 °C for 10 minutes, increased at 20 °C minute⁻¹ to 250 °C, and held at 250 °C for 8 minutes. MS spectra were monitored between 35-450 amu and the ionization mode used was electronic impact at 70 eV. The relative percentage of the components was calculated from GC-FID peak areas, and components were identified by WILEY, NIST and FLAVOR libraries.

2.3. Extraction of samples

Dry samples were extracted by methanol at three steps according to Cai et al (2004). Briefly, approximately 5 g of the arils were extracted twice with 10 mL of pure methanol for 1 hour, 10 mL for 30 minutes, and then with 5 mL for 30 minutes in an ultrasonic bath at room temperature.

2.4. Determination of total phenolic content

Spectrometric method defined by Spanos & Wrolstad (1990) was employed to determine total phenolic substance. About 100 µL sample were into a tube and 900 µL distilled water was added. Then, 5 mL 0.2 N Folin-Ciocalteu solution (10 times diluted with distilled water) and 4 mL saturated sodium carbonate solution (75 g L⁻¹) were added into samples and tubes were completely vortexed and left in dark for 2 hours. The extracts were combined and phenolic content of these extracts were measured at 765 nm by using UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). The results were expressed as gallic acid equivalent (mg GAE g⁻¹) by using standard calibration curve of this phenolic compound.

2.5. Determination of antioxidant activity

Antioxidant activities (AA) of the samples were determined by DPPH method (Lafka et al 2007). Antioxidant capacity of these extracts (same as total phenolic matter extraction procedure) was measured at 515 nm by UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Results were calculated as inhibition capacity (IC₅₀). % inhibition values (swiping effects of samples on DPPH radical) were calculated by using the Equation 1.

$$\% \text{ Inhibition} = [(A_{\text{DPPH}} - A_{\text{extract}}) / A_{\text{DPPH}}] \times 100 \quad (1)$$

Where; A_{DPPH}, absorbance of the control reaction; A_{extract}, absorbance in the presence of tested extracts; A_{DPPH}, absorbance value of 0.1 mL methanol+3.9 mL DPPH solution; A_{extract}, absorbance value of samples after 30 minutes; reset solution, pure methanol.

Percent inhibition values obtained from samples at different concentrations and concentration

values were inserted into graphs and effective concentration inhibiting DPPH effects by 50% (EC₅₀) was calculated for each sample (Lafka et al 2007).

2.6. Determination of antibacterial activity

The test organisms included gram-positive *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and gram-negative *Echerichia coli* ATCC 29213. All ATCC bacterial strains were obtained from BATEM Microbial Culture Collection, Antalya, Turkey. The bacteria were grown in the Müller Hinton Agar (MHA) at 37 °C and maintained on Müller Hinton Agar plate at 4 °C. In vitro antibacterial activity was examined for essential oil obtained from *S. officinalis* L., *L. angustifolia* L., *M. officinalis* L., *O. vulgare* ssp. *hirtum* traditionally used as medicinal plants. Antibacterial activities of these essential oils were evaluated by disc diffusion method (CLSI 2006). For all the bacterial strains, overnight cultures were grown in MHA and they were adjusted to an inoculation size of 0.5 McFarland 10⁸ CFU mL⁻¹ for inoculation of the agar plates. 100 µL of bacterial culture suspension was spread on MHA. Then the bacteria were spread over MHA with a sterile swab. Then, sterile filter paper disc was soaked into 10 µL of essential oil and blank disc (for sterilization control) and antibiotic disc were placed on it. Based on sensitivity characteristics of each bacterium, standard antibiotic discs selected from CLSI were used as positive control treatments. Bacteria were incubated at 37 °C overnight. After an incubation period of 24 h at 37 °C, antibacterial activity was evaluated by inhibition zones of bacterial growth. Three replications of each test were performed. The results are presented as average zone of inhibition of all the bacterial strains of ATCC.

2.7. Statistical analysis

The Kolmogorov-Smirnov and Levene's tests were applied to test normality and homogeneity of variance, respectively. Data sets were analyzed with one-way ANOVA and means were compared

with Tukey's post-hoc test. The Tukey test results were displayed in the form of letters. Parameters were displayed as mean±standard error of the mean (SEM). The alpha level was set at 5%. The statistical analysis was performed using Minitab 17 statistical software.

3. Results and Discussion

Descriptive statistics for essential oils and essential oil components of *S. officinalis* L., *L. angustifolia* L., *M. officinalis* L. and *O. vulgare* ssp. *hirtum* plants are provided in Tables 1-5. The descriptive statistics and results of Tukey's post-hoc test at $P<0.05$ for common components (essential oil, total phenolic content, antioxidant activity, antibacterial activity) are provided in Tables 6-8. Results of ANOVA carried out to compare the major components are provided in Table 9.

3.1. Essential oils and chemical composition of essential oils

Essential oils contents of *L. angustifolia* L., *M. officinalis* L., *O. vulgare* ssp. *hirtum*, and *S. officinalis* L. were respectively observed as $0.73\pm 0.04\%$, $0.06\pm 0.01\%$, $3.43\pm 0.06\%$ and $1.40\pm 0.04\%$ (Table 5). The differences between essential oil yields of the plants were found to be significant ($P<0.05$). Tukey's test revealed the highest essential oil yields in *O. vulgare* ssp. *hirtum* and the lowest in *M. officinalis* L. ($P<0.05$).

Bouaziz et al (2009) reported the essential oil yields of *S. officinalis* L. as 0.72%, and Ben Taarit et al (2010) as 0.66%. Mirjalili et al (2006) reported the essential oil yields of cultured *S. officinalis* L. in Iran as between 0.20-0.90%. Current finding for the essential oil yield of *S. officinalis* L. ($1.40\pm 0.04\%$) were higher than those reported in earlier studies. Environmental and agronomic practices may result in variations in essential oil yields (Chope & Terry 2009). Seidler-Lozykowska et al (2013) reported the essential oil yield of *M. officinalis* L. as 0.05%, (Padova) as 0.44% and (Warsaw), Carnat et al (1998) as between 0.02-0.3%. The current findings for essential oil yield of *M. officinalis* L. were

similar to those presented in earlier studies. Porto et al (2009) used the HD method of extraction and reported the essential oil yield of *L. angustifolia* L. as between 0.5-1.02% and Yazdani et al (2013) as between 0.25-2.0%. Milos et al (2000) reported the essential yield of *O. vulgare* ssp. *hirtum* as 2.9%.

De Martino et al (2009) reported the essential yield of *O. vulgare* ssp. *hirtum* collected from three different regions as between 2.35-3.15%. Current essential oil yields of *O. vulgare* ssp. *hirtum* were similar with those earlier ones. In the present study, 32 major components were identified in essential oils of *S. officinalis* L. (Table 1). The major components of the essential oil were identified as 1,8-cineol ($15.285\pm 0.003\%$), viridiflorol ($12.095\pm 0.003\%$), cis-thujone ($12.200\pm 0.003\%$), β -pinene ($9.410\pm 0.003\%$), α -pinene ($6.310\pm 0.003\%$). In essential oil of *L. angustifolia* L., 40 components were identified (Table 2). Linalool ($22.400\pm 0.003\%$), 1,8-cineol ($8.215\pm 0.003\%$), linalyl acetate ($7.900\pm 0.003\%$), lavadulyl acetate ($7.690\pm 0.003\%$) were identified as the major components. In essential oil of *M. officinalis* L., 15 components were identified (Table 3) and citronellal ($14.515\pm 0.003\%$), geranial ($13.050\pm 0.003\%$), β -caryophyllene ($12.385\pm 0.003\%$) were the major components. In essential oil of *O. vulgare* ssp. *hirtum*, 21 components were identified (Table 4). The major component was carvacrol ($65.080\pm 0.003\%$) and it was followed by thymol ($10.490\pm 0.003\%$), γ -terpinene ($7.340\pm 0.003\%$), para-cymene ($5.315\pm 0.003\%$). In previous studies, carvacrol (64.06%) was identified as the major component of essential oil of *O. vulgare* (Stupar et al 2014). Karamanos & Sotiropoulou (2013) reported the carvacrol content of essential oil of *O. vulgare* ssp. *hirtum* as between 56.46-84.88% based on plant organs, seasons and treatments and carvacrol was followed by π -cymene (4.19-21.4%) and α -pinene (0.11-1.88%). The results of the present study agree with the results of previous works. In a previous report, Stupar et al (2014) indicated the major components of *L. angustifolia* as linalool (37.61%) and linalool acetate (34.86%). Oh (2013) reported linalool and linalyl acetate contents

Table 1- The essential oil composition of *S. officinalis* L. (%)Çizelge 1- *S. officinalis* L. 'in uçucu yağ bileşenleri (%)

Parameters	Retention time	Mean±SEM	Standard deviation	Min-Max
cis-salvene	10.16	0.210±0.000	0.000	0.210-0.210
α-pinene	13.03	6.310±0.006	0.010	6.300-6.320
α-thujene	13.18	0.180±0.000	0.000	0.180-0.180
Camphene	14.90	3.100±0.000	0.000	3.100-3.100
β-pinene	16.74	9.410±0.012	0.020	9.390-9.430
Myrcene	19.14	0.685±0.003	0.005	0.680-0.690
α-terpinene	19.96	0.260±0.000	0.000	0.260-0.260
Limonene	20.81	1.120±0.000	0.000	1.120-1.120
1,8-cineol	21.27	15.285±0.009	0.015	15.27-15.30
cis-β-ocimene	22.28	0.650±0.000	0.000	0.650-0.650
γ-terpinene	22.85	0.510±0.000	0.000	0.510-0.510
para-cymene	23.95	0.230±0.000	0.000	0.230-0.230
α-terpinolene	24.43	0.185±0.003	0.005	0.180-0.190
cis-thujone	30.02	12.20±0.006	0.010	12.19-12.21
trans-thujone	30.69	4.200±0.000	0.000	4.200-4.200
cis-Sabinene hydrate	31.13	0.210±0.000	0.000	0.210-0.210
α-copaene	32.42	0.175±0.003	0.005	0.170-0.180
Camphor	33.40	3.265±0.003	0.005	3.260-3.270
Linalool	33.70	0.445±0.003	0.005	0.440-0.450
bornyl acetate	35.26	0.590±0.017	0.030	0.560-0.620
terpinen-4-ol	35.79	0.400±0.006	0.010	0.390-0.410
β-caryophyllene	35.97	5.700±0.000	0.000	5.700-5.700
delta-terpineol	37.82	0.260±0.006	0.010	0.250-0.270
α-humulene	38.19	5.125±0.003	0.005	5.120-5.130
α-terpineol	38.54	0.480±0.006	0.010	0.470-0.490
γ-murolene	38.60	0.405±0.003	0.005	0.400-0.410
Borneol	38.77	7.225±0.020	0.035	7.190-7.260
delta-cadinene	40.51	0.380±0.000	0.000	0.380-0.380
Caryophyllene oxide	46.91	0.365±0.003	0.005	0.360-0.370
humulene epoxide II	48.30	0.510±0.000	0.000	0.510-0.510
Viridiflorol	49.10	12.095±0.003	0.005	12.09-12.10
Manool	52.83	7.835±0.032	0.055	7.780-7.890

of *L. angustifolia* species respectively as between 30.3-38.7% and between 48.0-53.7%. Current major components of the essential oil of *L. angustifolia* L. were a bit lower than the earlier ones. The observed differences may probably be due to use of different parts of plant for analysis, different environmental and genetic factors, different chemotypes and the nutritional status of the plants as well as other factors that can influence the oil composition (Ahmadvand

et al 2013). Argyropoulos & Muller (2014) reported the major components of *M. officinalis* L. essential oil as citro-nellal (17.9±1.8%), neral (12.4±2.2%), geranial (16.1±2.7%). Seidler-Łożkowska et al (2013) indicated citral (neral+geranial) as the major component of the essential oil obtained from *M. officinalis* L. leaves and reported the citral contents as between 10.13% (Bonn)-35.83% (Bratislava). Present findings comply with these

earlier values. Bouaziz et al (2009) indicated the major components in essential oils of *S. officinalis* L. as b-thujone (17.76%), 1,8-cineole (eucalyptol) (16.29%), camphor (14.19%), α -thujone (7.41%), transcaryophyllene (5.45%), viridiflorol (4.63%). Ben Taarit et al (2010) reported the major components

in essential oils of the control plants of *S. officinalis* L. as athujone (23.43%), camphor (17.60%), 1,8-cineole (13.83%), viridiflorol (9.36%). The current findings were similar with the findings of the other researchers.

Table 2- The essential oil composition of *L. angustifolia* L. (%)

Çizelge 2- L. angustifolia L. uçucu yağ bileşenleri (%)

Parameters	Retention time	Mean±SEM	Standard deviation	Min-Max
α -pinene	13.03	0.585±0.003	0.005	0.580-0.590
Camphene	14.90	0.730±0.000	0.000	0.730-0.730
β -pinene	16.73	1.085±0.003	0.005	1.080-1.09
Myrcene	19.14	0.540±0.000	0.000	0.540-0.540
Limonene	20.81	3.350±0.000	0.000	3.350-3.350
1,8-cineol	21.25	8.215±0.003	0.005	8.210-8.220
cis- β -ocimene	22.28	0.380±0.000	0.000	0.380-0.380
trans- β -ocimene	23.03	0.495±0.003	0.005	0.490-0.500
3-octanone	23.20	0.715±0.003	0.005	0.710-0.720
meta-cymene	23.83	0.675±0.003	0.005	0.670-0.680
para-cymene	23.93	1.405±0.003	0.005	1.400-1.410
1-Octen-3-ol acetate	28.02	1.895±0.003	0.005	1.890-1.900
cis-Linalool oxide	30.46	1.760±0.012	0.020	1.740-1.780
trans-Linalool oxide	31.45	1.250±0.000	0.000	1.250-1.250
Camphor	33.40	5.125±0.003	0.005	5.120-5.130
Linalool	33.71	22.40±0.000	0.000	22.40-22.40
linalyl acetate	34.20	7.900±0.000	0.000	7.900-7.900
bornyl acetate	35.27	0.995±0.014	0.025	0.970-1.020
lavandulyl acetate	35.75	7.690±0.023	0.040	7.650-7.730
terpinen-4-ol	35.79	0.685±0.003	0.005	0.680-0.690
Lavandulol	37.78	0.610±0.006	0.010	0.600-0.620
Cyrtone	38.27	3.690±0.012	0.020	3.670-3.710
α -terpineol	38.54	2.020±0.000	0.000	2.020-2.020
Borneol	38.76	4.830±0.023	0.040	4.790-4.870
Eucarvone	39.11	0.740±0.006	0.010	0.730-0.750
neryl acetate	39.32	0.980±0.000	0.000	0.980-0.980
Carvone	39.99	0.660±0.000	0.000	0.660-0.660
geranyl acetate	40.16	2.435±0.003	0.005	2.430-2.440
γ -cadinene	40.69	0.985±0.003	0.005	0.980-0.990
Nerol	41.28	0.450±0.000	0.000	0.450-0.450
cumin aldehyde	41.35	1.965±0.003	0.005	1.960-1.970
Geraniol	42.46	1.050±0.006	0.010	1.040-1.060
meta-cymen-8-ol	42.54	0.725±0.003	0.005	0.720-0.730
para-cymen-8-ol	42.72	0.540±0.000	0.000	0.540-0.540
Caryophyllene oxide	46.91	3.735±0.003	0.005	3.730-3.740
1,10-di-epi-Cubenol	48.44	0.435±0.003	0.005	0.430-0.440
para-cymen-7-ol	49.16	0.500±0.000	0.000	0.500-0.500
epi- α -cadinol	51.01	5.360±0.006	0.010	5.350-5.370
Unidentified		0.425±0.061	0.105	0.320-0.530

3.2. Total phenolic content

Total phenolic contents of the present study are provided in Table 6. Total phenolic contents varied between 16.480 ± 0.087 - 76.110 ± 1.030 mg GAE g⁻¹ dw with the highest value in *M. officinalis* L. and the lowest value in *L. officinalis* L. ($P < 0.05$). In previous literatures, total phenolic compound of *L. officinalis* L. extract was reported as 76.8 mg GAE g⁻¹ dw (Rabiei et al 2014). Lin et al (2012) reported the total polyphenols of *M. officinalis* L. as 175.15 ± 11.02 mg g⁻¹ dw in frozen dry sample extracts and as 164.13 ± 12.02 mg g⁻¹ dw in hot air dry sample extracts. Barros et al (2013) reported the total phenolic contents of *M. officinalis* L. grown under field conditions and in vitro conditions respectively as 59.59 mg g⁻¹ and 30.21 mg g⁻¹ of infusion. Ben Farhat et al (2013) estimated the total phenolic contents spectrophotometrically and reported that the values ranged from (67.67-72.02 mg GAE g⁻¹ dw) for *S. argentea* extracts to (112.93-161.37 mg GAE g⁻¹ dw) for *S. officinalis* samples. Salem et al (2013) reported the phenolic contents of non-treated sage leaves as 36.5 ± 2.35 mg GAE g⁻¹ fw. Chun et al (2005) reported the total phenolic content in water extracts of the clonal oregano as

52.8 mg g⁻¹ dw compared to 39.4 mg g⁻¹ dw in the commercial sample. Martins et al (2014) indicated that decoction presented the highest concentration of flavonoids (75.25 mg g⁻¹ decoction) and total phenolic compounds (98.05 mg g⁻¹ decoction) for *O. vulgare* L. and it was followed by infusion and hydroalcoholic extracts, respectively. Total phenolic contents obtained from *M. officinalis* L., *O. vulgare* ssp *hirtum*, *S. officinalis* L. and *L. angustifolia* L. of the present study were slightly different from those earlier reported ones. The differences between the current and previous findings were probably because of differences in harvest times, climate, cultural practices and/or plant genetics. Plant genetics and cultural practices may significantly affect phenolic contents and thus they play significant roles in nutritional values of the food stuff (Yang et al 2007; Ozgen et al 2008).

3.3. Antioxidant activity

In the present study, antioxidant activities were determined by using DPPH method and the values varied between 0.930 ± 0.023 - 6.140 ± 0.058 g⁻¹ DPPH (Table 7). *M. officinalis* L. had the highest antioxidant activity (0.930 ± 0.023 g⁻¹ DPPH) and

Table 3- The essential oil composition of *M. officinalis* L. (%)

Çizelge 3- M. officinalis L. uçucu yağ bileşenleri (%)

Parameters	Retention time	Mean±SEM	Standard deviation	Min-Max
1-octen-3-ol	30.41	0.760±0.000	0.000	0.760-0.760
β-caryophyllene	33.35	12.385±0.032	0.055	12.33-12.44
Citronellal	31.78	14.515±0.026	0.045	14.47-14.56
α-humulene	38.18	1.210±0.006	0.010	1.200-1.220
α-copaene	32.41	1.015±0.003	0.005	1.010-1.020
β-bourbonene	33.35	0.890±0.000	0.000	0.890-0.890
Methyl citronellate	34.43	2.090±0.000	0.000	2.090-2.090
β-copaene	39.32	3.645±0.026	0.045	3.600-3.690
Geranial	39.69	13.05±0.023	0.040	13.01-13.09
δ-cadinene	40.51	1.110±0.017	0.030	1.080-1.140
humulene epoxide II	48.30	1.685±0.003	0.005	1.680-1.690
Fokienol	50.45	1.160±0.029	0.050	1.110-1.210
epi- α-cadinol	51.02	0.955±0.014	0.025	0.930-0.980
α-cadinol	52.59	1.615±0.009	0.015	1.600-1.630
Unidentified		4.230±0.000	0.000	4.230-4.230

it was respectively followed by *O. vulgare* ssp. *hirtum* (1.895 ± 0.006 g g⁻¹ DPPH), *S. officinalis* L. (1.895 ± 0.020 g g⁻¹ DPPH) and *L. angustifolia* L. (6.140 ± 0.058 g g⁻¹ DPPH) ($P < 0.05$). Sahin et al (2004) employed the free radical scavenging activity and lipid oxidation inhibition in *O. vulgare* ssp. *vulgare* extracts and studied the essential oils in vitro. The researchers reported the order of diphenylpicrylhydrazine with IC₅₀ as 9.9 ± 0.5 and 19.8 ± 0.5 µg mL⁻¹, respectively. Skotti et al (2014) investigated the antioxidant activity of some medicinal and aromatic plants by using DPPH method and reported the antioxidant activities as between 1.31 ± 0.01 - 3.16 ± 0.06 mol trolox mL⁻¹ for *O. vulgare*, as between 3.03 ± 0.09 - 6.34 ± 0.05 mol trolox mL⁻¹ for *M. officinalis* L. and as between 0.34 ± 0.01 - 1.64 ± 0.01 mol trolox mL⁻¹ for *S. officinalis*. Kaliora et al (2014) used DPPH method and reported that the infusion of dittany had highest antioxidant activity against the sage. In previous literatures investigating the essential

oils of *Origanum* species, thymol and carvacrol were reported to have high antioxidant activity (Barrata et al 1998; Milos et al 2000; Ruberto & Barrata 2000; Puertes-Mejia et al 2002). Although present findings are somehow similar to results of those earlier studies, differences in extraction and antioxidant activity methods, climate, soil, environmental factors, diseases and pesticide treatments, harvest time, drying and storage methods and plant parts used in analyses may significantly affect the antioxidant activity of plants (Bergonzi et al 2001; Wang & Zheng 2001). There are several studies indicating the relationships between antioxidant activity and phenolic contents of the plants (Ruberto & Barrata 2000; Dorman et al 2004; Cai et al 2006; Canadanović-Brunet et al 2008). In the current study, positive correlation was also found between total phenolic content and antioxidant activity in all plant extracts (Table 9).

Table 4- The essential oil composition of *O. vulgare* ssp. *hirtum* (%)

Çizelge 4- *O. vulgare* ssp. *hirtum* uçucu yağ bileşenleri (%)

Parameters	Retention time	Mean±SEM	Standard deviation	Min-Max
1-octen-3-ol	30.41	0.590±0.000	0.000	0.590-0.590
β-caryophyllene	35.96	3.105±0.003	0.005	3.100-3.110
α-humulene	38.18	0.230±0.000	0.000	0.230-0.230
α-pinene	13.03	0.560±0.000	0.000	0.560-0.560
α-thujene	13.18	0.910±0.000	0.000	0.910-0.910
Myrcene	19.14	1.540±0.000	0.000	1.540-1.540
α-phellandrene	19.31	0.180±0.000	0.000	0.180-0.180
α-terpinene	19.96	1.175±0.003	0.005	1.170-1.180
Limonene	20.81	0.210±0.000	0.000	0.210-0.210
β-phellandrene	21.27	0.200±0.000	0.000	0.200-0.200
γ-terpinene	22.86	7.340±0.006	0.010	7.330-7.350
3-octanone	23.20	0.113±0.038	0.053	0.075-0.150
para-cymene	23.96	5.315±0.003	0.005	5.310-5.320
cis-Sabinene hydrate	31.12	0.380±0.000	0.000	0.380-0.380
trans-Sabinene hydrate	33.95	0.150±0.000	0.000	0.150-0.150
carvacrol methyl ether	35.81	1.075±0.003	0.005	1.070-1.080
Borneol	38.76	0.250±0.000	0.000	0.250-0.250
Thymol	50.92	10.490±0.012	0.020	10.47-10.51
Carvacrol	51.74	65.080±0.017	0.030	65.05-65.11

Table 5- The essential oil yield of the plants (%)

Çizelge 5- Bitkilerin uçucu yağ verimi (%)

Species	Mean±SEM	Standard deviation	Min-Max	Tukey*
<i>Lavandula angustifolia</i> L.	0.73±0.04	0.07	0.67-0.80	C
<i>Melissa officinalis</i> L.	0.06±0.01	0.01	0.05-0.07	D
<i>Origanum vulgare</i> ssp. <i>hirtum</i>	3.43±0.06	0.10	3.33-3.53	A
<i>Salvia officinalis</i> L.	1.40±0.04	0.07	1.33-1.47	B

*, different letters represent groups with significant differences (P<0.05)

Table 6- The total phenolic content of the plant extracts (mg GAE g⁻¹)Çizelge 6- Bitki ekstraktlarının toplam fenolik içeriği (mg GAE g⁻¹)

Species	Mean±SEM	Standard deviation	Min-Max	Tukey*
<i>Lavandula angustifolia</i> L.	16.480±0.087	0.15	6.33-16.63	D
<i>Melissa officinalis</i> L.	76.110±1.030	1.78	74.33-77.89	A
<i>Origanum vulgare</i> ssp. <i>hirtum</i>	70.690±1.090	1.88	68.81-72.57	B
<i>Salvia officinalis</i> L.	63.275±0.915	1.59	61.69-64.86	C

*, different letters represent the groups with significant differences (P<0.05)

Table 7- The antioxidant activity of the plant extracts (IC₅₀ (g g⁻¹ DPPH))Çizelge 7- Bitki ekstraktlarının antioksidant aktivitesi (IC₅₀ (g g⁻¹ DPPH))

Species	Mean±SEM	Standard deviation	Min-Max	Tukey*
<i>Lavandula angustifolia</i> L.	6.140±0.058	0.10	6.04-6.24	A
<i>Melissa officinalis</i> L.	0.930±0.023	0.04	0.89-0.97	C
<i>Origanum vulgare</i> ssp. <i>hirtum</i>	1.890±0.006	0.01	1.88-1.90	B
<i>Salvia officinalis</i> L.	1.895±0.020	0.04	1.86-1.93	B

*, different letters represent the groups with significant difference (P<0.05)

3.4. Antibacterial activity

Antibacterial activity against *S. aureus* ATCC 43300, *S. aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212 and *E. coli* ATCC 35218 bacteria varied between 8.00±0.00-52.00±0.58 mm (Table 8). The highest antibacterial activity against the entire bacteria was observed in *O. vulgare* ssp. *hirtum* (P<0.05). Entire plants also exhibited relatively high antibacterial activity against *S. epidermidis* ATCC 12228 bacteria (P<0.05). Stagos et al (2012) using agar well-diffusion assay, tested the ability of Lamiaceae species to inhibit the growth of *S. aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) bacteria. The results showed that five out of seventeen extracts had

antibacterial activity against *S. aureus* but none against *P. aeruginosa*. Voda et al (2003) indicated that antifungal activity of oxygenated essential oil components vary based on the type and position over aromatic chain. Moon et al (2006) investigated the efficiency of five *Lavandula* species against various microorganism strains and reported that essential oils of these plants had antibacterial effects against *S. aureus*, metisiline-resistant *S. aureus* and *E. coli*, but hydrosol and water-extracts of these species were not able to exhibit antibacterial effects against the tested strains. Lin et al (2004) carried out in vitro studies and indicated distinctive antibacterial effects of water-extracts of *Origanum vulgare* against *L. monocytogenes*. Friedman et al (2002) carried out an antibacterial activity study with 96

Table 8- The antibacterial activity of the plant extracts (mm)

Çizelge 8- Bitki ekstraktlarının antibakteriyel aktivitesi (mm)

Bacteria	Species	Mean±SEM	Standard deviation	Min-Max	Tukey*
<i>Staphylococcus aureus</i> ATCC 43300	<i>Lavandula angustifolia</i> L.	18.525±0.303	0.53	18.00-19.05	B
	<i>Melissa officinalis</i> L.	16.875±0.072	0.13	16.75-17.00	C
	<i>Origanum vulgare</i> ssp. <i>hirtum</i>	41.025±0.563	0.98	40.05-42.00	A
	<i>Salvia officinalis</i> L.	13.500±0.289	0.50	13.00-14.00	D
<i>Staphylococcus aureus</i> ATCC 29213	<i>Lavandula angustifolia</i> L.	13.500±0.289	0.50	13.00-14.00	C
	<i>Melissa officinalis</i> L.	17.000±0.000	0.00	17.00-17.00	B
	<i>Origanum vulgare</i> ssp. <i>hirtum</i>	32.500±0.289	0.50	32.00-33.00	A
	<i>Salvia officinalis</i> L.	11.000±0.000	0.00	11.00-11.00	D
<i>Staphylococcus epidermidis</i> ATCC 12228	<i>Lavandula angustifolia</i> L.	38.000±0.577	1.00	37.00-39.00	B
	<i>Melissa officinalis</i> L.	15.000±0.000	0.00	15.00-15.00	D
	<i>Origanum vulgare</i> ssp. <i>hirtum</i>	52.000±0.577	1.00	51.00-53.00	A
	<i>Salvia officinalis</i> L.	25.000±0.577	1.00	24.00-26.00	C
<i>Enterococcus faecalis</i> ATCC 29212	<i>Lavandula angustifolia</i> L.	16.025±0.014	0.03	16.00-16.05	B
	<i>Melissa officinalis</i> L.	11.000±0.000	0.00	11.00-11.00	D
	<i>Origanum vulgare</i> ssp. <i>hirtum</i>	24.250±0.144	0.25	24.00-24.50	A
	<i>Salvia officinalis</i> L.	21.500±0.289	0.50	21.00-22.00	C
<i>Escherichia coli</i> ^a ATCC 35218	<i>Lavandula angustifolia</i> L.	10.000±0.000	0.00	10.00-10.00	
	<i>Melissa officinalis</i> L.	13.000±0.000	0.00	13.00-13.00	
	<i>Melissa officinalis</i> L.	29.000±0.000	0.00	29.00-29.00	
	<i>Salvia officinalis</i> L.	8.000±0.000	0.00	8.00-8.00	

*, different letters represent the groups with significant difference (P<0.05); ^a, statistical analyses were not performed since the replications are not different

Table 9- Total phenolics, essential oil, antioxidant activity and antibacterial activity

Çizelge 9- Toplam fenolik, uçucu yağ, antioksidant ve antibakteriyel aktivite

Parameters	Source	DF	SS	MS	F	Significance
Essential oil (%)	Between groups	3	19.130	6.3768	1348.4	0.000***
	Within groups	8	0.0378	0.0047		
	Total	11	19.168			
Total phenolic (mg GAE g ⁻¹ dw)	Between groups	3	6700.0	2233.3	967.07	0.000***
	Within groups	8	18.480	2.3100		
	Total	11	6718.5			
Antioxidant activity (IC50 (g g ⁻¹ DPPH))	Between groups	3	48.810	16.270	5035.2	0.000***
	Within groups	8	0.0259	0.0032		
	Total	11	48.836			
ATCC 43300	Between groups	3	1414.9	471.62	1264.5	0.000***
	Within groups	8	2.9800	0.3700		
	Total	11	1417.8			
ATCC 29213	Between groups	3	838.50	279.50	2236.0	0.000***
	Within groups	8	1.0000	0.1300		
	Total	11	839.50			
ATCC 12228	Between groups	3	2319.0	773.00	1030.7	0.000***
	Within groups	8	6.0000	0.7500		
	Total	11	2325.0			
ATCC 29212	Between groups	3	312.19	104.06	1329.4	0.000***
	Within groups	8	0.6300	0.0800		
	Total	11	312.82			

***, significant according to ANOVA (P<0.001)

essential 23 oil components and reported efficient activity of cinnamaldehyde, thymol, carvacrol and eugenol against *E. coli*, *Salmonella enterica* and *L. Monocytogenes*. Researchers also indicated relatively higher antimicrobial activity of Oreganol against gram-positive and negative bacterial pathogens. Current findings comply with the results of those earlier studies. Considering those earlier studies, it can be stated herein that essential oils of medicinal plants had higher antibacterial activity than the other extracts like water, methanol, ethanol and hexane (Ahmad et al 1998; Eloff 1998).

4. Conclusions

Together with widespread utilization of natural additives in food industry, the interest in natural antioxidants of the plants also increased day by day. Therefore, investigation of natural antioxidants has become a popular research topic, recently. Current findings revealed that essential oil plants of *S. officinalis* L., *L. angustifolia* L., *M. officinalis* L., *O. vulgare* ssp. *hirtum* cultured with organic manure could reliably be accepted as natural antioxidant sources and these plants could also reliably be used in pharmaceutical and food industries to prevent the effects of reactive oxygen species and to reduce the risks of cardiovascular diseases. It was observed in this study that essential oils of these plants exhibited antibacterial effects against *S. aureus*, *S. epidermidis*, *E. faecalis* and *E. coli* bacteria. Thus, they can be used in treatment of infectious diseases caused by resistant microbes. In addition, the data in the present study are supporting the use of these plants as tea or additive in foods, and traditional remedies for the treatment of infectious diseases.

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