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Determination of Antimicrobial and Antioxidant Activities of *Lavandula angustifolia* Volatile Oil*

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Abstract: This study aimed to evaluate the antimicrobial effect of the oil extracted from *Lavandula angustifolia* (Province of Denizli/Türkiye) on some gram-negative/positive pathogenic microorganisms and lactic acid bacteria (by agar well diffusion, MIC test), and antioxidant capacity (DPPH, metal chelating, FRAP). As a result of the analysis, it was determined by headspace/GC-MS analysis that eucalyptol was the highest with a value of 22.58%, α-linalool was the second with a value of 19.44% and linalyl acetate was 14.61%. Lavandulol, an essential component of Lavender, was determined by 1.39%. The highest antimicrobial effect of *L. angustifolia* oil was found on *Bacillus subtilus* and Methicillin-resistant *Staph. aureus* (MRSA) with zone diameters of 24 to 22 mm, respectively), followed by *Staph. aureus*. Antibacterial effects of *L. angustifolia* oil on *Staph. aureus*, *E. coli*, *Campliobacter jejuni*, *Salmonella poona*, *Lactobacillus reuteri* and *Lactobacillus plantarum* were detected as 17, 16, 14, 14, 12, 12, and 11 mm zone diameters, respectively. Therewithal, a significant antioxidative effect was determined by DPPH, FRAP, and metal chelating analyzes.

Keywords: *Lavandula angustifolia*, antimicrobial activity, headspace/GC-MS, antioxidant capacity

1. Introduction

Since ancient times, mankind has always used plants as a source formula. Until the 19th century, doctors mostly used natural resources to relieve their patients' pain. The World Health Organization estimates that 80% of people in developing countries have used traditional medicine to date (Moussii et al., 2020). Due to the use of medicinal plants in traditional medicine and their unique chemical composition, which provides numerous biochemical properties, volatile oils are widely used in many countries around the world for many purposes. Recently, volatile oils have become very important with the accelerated development of scientific research and technological revolution in various aspects. The importance gained by these natural derivatives is due not only to the content of biologically active volatile compounds but also to their use as additives in processed food and pharmaceutical production (Rashed et al., 2019, 2020; Özyazıcı and Kevseroğlu, 2019a).

Lavender (*Lavandula* spp.) is a perennial volatile oil plant from the Lamiaceae family in a semi-shrub form. There are about 39 Lavender species, mostly of Mediterranean origin, among them the most suitable and economical species produced especially for *Lavandula* section flowers and volatile oils (Kara and Baydar, 2011; Özyazıcı and Kevseroğlu, 2019b).

The chemical composition and pharmacological evaluation of *Lavandula angustifolia* have been the subject of many studies over the years. Many of these studies have focused on the extracts, fractions, and volatile oils of plant parts and flowers. In pharmacological and biological tests, extracts, fractions, and volatile oils of *L. angustifolia* have

*: This study, it was presented as an oral presentation at the 3rd International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2020) held in Ankara on 19-20 March 2020, and the summary text was published in the aforementioned congress proceedings book.

been reported to have antidepressant, anticonvulsive, sedative, spasmolytic, local anesthetic, antioxidant, antibacterial, and mast cell degranulation inhibitory effects (Hajhashemi et al., 2003; Rashed et al., 2019). As extraction, lavender oil is usually extracted from the inflorescences and leaves of the plant. Major compounds in *L. angustifolia* include linalool, linalyl acetate, 1,8 cineole, ocimene, terpene, and camphor. In addition, *L. angustifolia* is an important source of therapeutic properties and has other bioactivities such as anti-inflammatory. Besides antioxidant, antibacterial and antimicrobial activities, *L. angustifolia* has been reported to be a safe and effective herbicide (Xiaotian et al., 2020).

The need for alternative medical agents and interest in their use increased because of many related factors, such as the necessity and reduced sensitivity of antimicrobial compounds with safer and low toxicity profiles (Blažeković et al., 2018). Among these factors, the antibacterial activity of some medicinal plants, which has been proven by several studies, can be mentioned. Volatile oils have been shown in relevant studies to provide therapeutically more effective responses than antibiotics used in many pathogenic microorganism-induced conditions (de Rapper et al., 2013; Rashed et al., 2019; Van Vuuren et al., 2019). One or more essential ingredients of volatile oils may be responsible for this antimicrobial activity, which can inhibit the growth of some fungi and bacteria (Moussii et al., 2020).

The present study aimed to determine, the potential antioxidant capacity and antimicrobial effects of *L. angustifolia* volatile oil on some gram-negative/positive pathogenic microorganisms and lactic acid bacteria.

2. Materials and Methods

The *Lavandula angustifolia* plant was collected from the Denizli Province of Türkiye (Gözler Town, Pamukkale County, 38º 05' 42" N-29º 08' 33" E). The collected *L. angustifolia* was immediately taken to a -80 ºC cold chain until analysis was done.

2.1. Volatile oil extraction from *L. angustifolia*

100 grams of dried lavender samples were taken and placed in a 500 mL flask. By adding 300 mL distilled water, volatile oils were obtained after 180 minutes at 100 °C by Clevenger apparatus. The volatile oils obtained were collected by passing them through calcium (desiccant). Samples were stored in amber bottles at +4 °C conditions. These processes were repeated 6 times to obtain enough amount of volatile oil (Clevenger, 1928; Jaradat et al., 2016).

2.2. Headspace/GC-MS conditions

The analysis was carried out by Thermo Trace 1310 GC ISQ single quadrupole mass spectroscopy (Thermo Fisher Scientific, Austin, TX). Shade-dried lavender samples were ground with an IKA blender. 0.5 grams of ground sample was taken and placed in a 25 mL Chromacol 20-HSV vial, and loaded on the GC-MS device (THERMO, Triplus RSH Trace1310- ISQ LT). Headspace conditions: The vial was heated at 130 °C for 90 minutes in the Triplus RSH headspace oven. It was loaded into the GC-MS with a 2.5 mL injection volume from the heated headspace vial.

GC oven temperature program: After standing at 60 °C for 6 minutes, the temperature was increased to 230 °C by 2 °C per minute. The analysis was terminated by waiting for 30 minutes at 230 °C. *Flow rate:* Helium 1.2 mL min^{-1}. *Column information:* Thermo TG-WAXMS GC column (60 m x 0.25 mm ID x 0.25 µm). *MS conditions:* Ion source and detector temperature 250 °C, mass scanning range 55-550 (amu). National Institute of Standards and Technology (NIST), Wiley 9, Redlip, Mainlip, and WinRI libraries were used to identify the peaks obtained from the analysis.

2.3. Antimicrobial activity

The antimicrobial effect of *L. angustifolia* oil (extracted with Clevenger device) on indicator microorganisms was investigated by well diffusion agar and minimum inhibition concentration (MIC) assay method that was performed according to the methods designed by Bauer et al. (1966), and Ericsson and Sherris (1971). The effect of the lavender was evaluated for gram-positive bacteria *Staphylococcus aureus* ATCC6538, *Staphylococcus aureus* ATCC29213, *Bacillus subtilus* B354, and Methicillin-resistant *Staph. aureus* (MRSA), and gram-negative bacteria *Escherichia coli* RSSK09036, *E. coli* k-2, *Pseudomonas aeruginosa* ATCC27853, *Salmonella parathypi* A NCTC13, *Salmonella poona* RM2350, *Campliobacter jejuni* ATCC33560, and probiotic bacteria *Lactobacillus reuteri* ATCC55730, *Lactobacillus plantarum* FI8595. MRS Broth (*Lactobacillus* Broth acc. to De Man, Rogosa and Sharp: MRS, Merck 1.10661), MRS Agar (*Lactobacillus* Agar acc. to De Man, Rogosa and Sharpe) Merck 1.10660, Tryptic Soy Broth (TSB) Merck 1.05459 were used for the growth of lactic acid bacteria, and Mueller Hinton Agar Merck 1.05437 for agar test, Mueller Hinton Broth (Merck 1.10293) for MIC test.

2.3.1. Agar well diffusion test

Indicator microorganisms were stored at a temperature below 5 °C. They were removed and reactivated in tryptic soy broth at 37 °C for 18 hours. 12 mL of Mueller-Hinton agar (cooled 50 °C after autoclave) was poured into 90 mm Petri dishes and 1 ml fresh culture indicator bacteria was added with a density of 0.5 Mcfarland and left to dry at 37 °C for 30 minutes. 6 mm diameter wells were drilled in frozen agars. 100 µL of *L. angustifolia* oil was added to the wells and incubated overnight at +4 °C for diffusion (Ryan et al., 1970; Anonymous, 2006).

The Petri dishes were then incubated under optimum conditions. The diameters of the inhibitory zones were measured in millimeters. Mueller Hinton Agar standard method procedure developed by Bauer et al. (1966) and Stella and Marin (2009) was used to eliminate or reduce variability in this test method. The procedure was adopted by the Clinical and Laboratory Standards Authority (CLSI, former NCCLS) as a consensus standard (Anonymous, 2007).

2.3.2. Minimum inhibition concentration test

MICs obtained with the spectrophotometric microdilution method for *L. angustifolia* oil against indicator microorganisms were measured at 600 nm in a 96-well plate reader (Multiskan™ FC Microplate Photometer thermofisher.com), 150 µL of dual-strength Mueller Hinton Broth (MHB) and Dual-strength *L. angustifolia* oil were added to the first wells, dilutions (1:1 v/v) were then added to the other wells. Then, 30 µL of bacterial suspension of fresh culture indicator bacteria with a density of 0.5 Macfarland was mixed into prepared plates and incubated at optimum temperatures for 18-24 hours. Then, sample density was detected by plate reader and MIC was expressed as the highest dilution inhibiting growth (turbidity max in the tube is low). Among these MIC dilutions, those with positive detection of nonviable cells > 99% in the medium are considered minimum bactericidal concentration (MBC) (Lalitha, 2004).

2.4. Antioxidant analysis methods

1,1-diphenyl-2-picrylhydrazine (DPPH), ethanol, Throlox butylhydroxytoluene (BHT), $NaCH_3COO$, HCl, 2,4,6-tripyridyl-s-triazine (TPTZ), Ferrozin, and $FeCl_2$ were purchased from Sigma-Aldrich GmbH (Sternheim, Germany).

BHT and Throlox were used as standard in antioxidant analysis methods. The analysis was measured with a UV spectrophotometer (THERMO Scientific Evolution 201 UV). Since the oil is very concentrated, 1 mL of *L. angustifolia* oil is dissolved with Dimethyl Sulfoxide (DMSO) and completed to 25 mL.

2.4.1. DPPH (1,1-Diphenyl-2-picrylhydrazine) free radical scavenging activity

The DPPH· assay provides basic information on the antiradical activity of the extracts. The radical scavenging activity of the *L. angustifolia* oil was determined spectrophotometrically by monitoring the disappearance of DPPH·at 517 nm, according to the modified methodology of Blois (1958). The bleaching rate of a stable free radical, DPPH, is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH (10^{-3} M) was prepared in ethanol and 0.5 mL of this solution was added to 1.5 mL of *L. angustifolia* solution in ethanol at different concentrations (15-45 µg mL^{-1}). These solutions were vortexed thoroughly and incubated in the dark. 30 minutes later, the absorbance was measured at 517 nm against the blank samples. The lower absorbance of the reaction mixture indicates higher DPPH·free radical scavenging activity.

Standards (BHT and Throlox) and *L. angustifolia* oil were compared against the absorbance of blank and then calculated of % inhibition values.

2.4.2. Chelating activity of ferrous ions (Fe^{2+})

The chelating of ferrous ions by *L. angustifolia* oil and the standards were performed according to the method of Dinis et al. (1994). The reaction was performed in an aqueous medium. Briefly, 0.4 mL *L. angustifolia* oil (10 µg mL^{-1}) was added to a solution of 0.2 mL $FeCl_2$ (2 mM). The reaction was initiated by the addition of 0.4 mL ferrozine (5 mM) and the total volume was adjusted to 4 mL with ethanol. Then the mixture was shaken vigorously and left at room temperature for 10 min. Standards (BHT and Throlox) (µg mL^{-1}) and *L. angustifolia* oil were compared against the absorbance of blank (containing $FeCl_2$ and ferrozine) at 562 nm.

2.4.3. Ferric reducing antioxidant power (FRAP)

FRAP method (suitable to determine hydrophilic and lipophilic antioxidants) was introduced to determine the total amount of antioxidants by the reduction capacity of iron (III). The oxidant in the FRAP assay was prepared by mixing 2.5 mL 10 mM TPTZ dissolved in 40 mM HCl, 25 mL acetate buffer, 20 mM 2.5 mL $FeCl_3$ and water. This mixture is called as FRAP reagent. The final solution contained 1.67 mM Fe (III) and 0.83 mM TPTZ (Benzie and Strain, 1996; Prior et

al., 2005). *L. angustifolia* oil (10 µg mL^{-1}) and standards (BHT and Throlox) (10 µg mL^{-1}) were added to the final solution (1.67 mM Fe (III) and 0.83 mM TPTZ) and incubated in dark for 10 minutes at room temperature. Standards and *L. angustifolia* oil were compared against the absorbance of blank at 595 nm.

3. Results

3.1. Volatile oil components

Chromatography (Figure 1) and specific parameter information of the 15 components of volume fraction are presented in Table 1. As a result of the analysis, it was determined that the highest eucalyptol (24.72%), a-linalool (21.33%) and linalyl acetate (16.03%) were obtained in the headspace/GC-MS analysis. (ñ)-Lavandulol acetate which is an important component of lavender, was determined as 1.52%. Other essential components include á-myrcene (11.48%), á -cis-ocimene (7.90%), camphor, (1R,4R)-(+) (4,25), cyclohexane ethanamine N-à-dimethyl (2.78%), 1-borneol (2.56%), a-methyl-a-[4-methyl-3-pentenyl]oxirane methanol (2.46%), trans-linalool oxide (1.82%), 5-chloropentanoic acid,4-methyl pentyl ester (1.38%), 1-octen-3-yl acetate (1%), acetic acid, geraniol ester (0.52%) and butanoic acid, hexyl ester (0.49%) (Table 1).

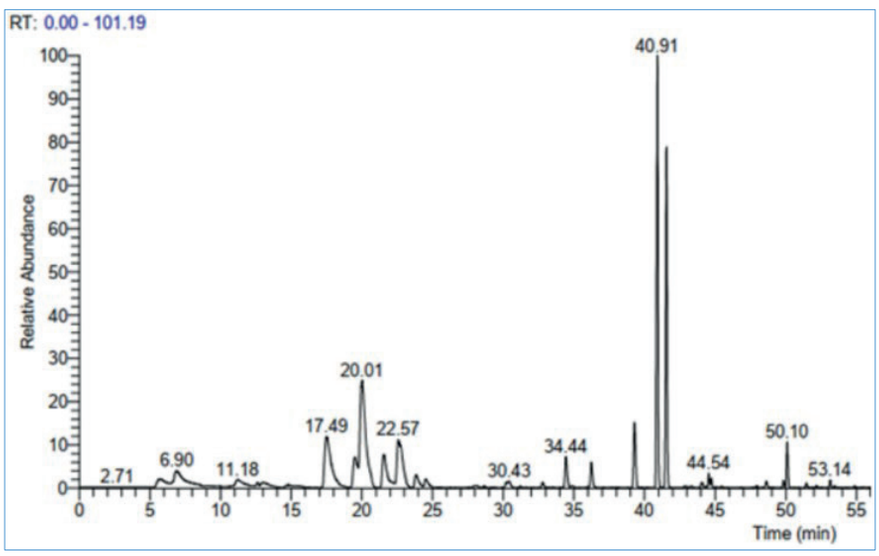

Figure 1. Chromatograms of compounds identified by headspace/GC-MS
RT: Retention Time

Table 1. Identification of chemical components of *L. angustifolia* oil

RT	RI	Area, %	Compound name
6.92	1213	2.78	Cyclohexaneethanamine. N-à-dimethyl-
17.49	958	11.48	á-Myrcene
20.01	1059	24.72	Eucalyptol
22.57	976	7.90	á-cis-Ocimene
23.85	1100	1.14	5-Chloropentanoic acid.4-methylpentylester
30.43	1109	1	1-octen-3-ylacetate
32.78	1183	0.49	Butanoic acid. hexyl ester
34.44	1164	2.46	à-Methyl-à-[4-methyl-3-pentenyl]oxiranemethano
36.23	1164	1.82	Trans-linalool oxide
39.29	1121	4.25	Camphor. (1R.4R)-(+)
40.91	1082	21.33	á-Linalool
41.56	1272	16.03	Linalylacetate
44.54	1270	1.52	(ñ)-Lavandulol. acetat
50.10	1138	2.56	1-Borneol
53.14	1352	0.52	Acetic acid. geraniol ester

RT: Retention Time, RI: Retention Index

3.2. Antimicrobial activity

3.2.1. Agar well diffusion test results

Lavandula angustifolia oil antibacterial effect was detected more on *Bacillus subtilus* and MRSA values were (24 to 22 mm zone diameter, respectively). Following that, *Staph. aureus*, *E. coli*, *Campliobacter jejuni*, *Salmonella poona*, *Lactobacillus reuteri* and *Lactobacillus plantarum* (17, 16, 14, 14, 12, 12, 11 mm zone diameter, respectively). *Lavandula angustifolia* oil did not show an antimicrobial effect only on *E. coli* k-2 (Table 2).

3.2.2. Minimum inhibition concentration results

The graph regarding the reproduction concentrations of *L. angustifolia* oil doses applied to bacteria, compared to the control group at 600 nm is given in Figure 2. The highest inhibition effects of *L. angustifolia* were determined for *Staphylococcus aureus* at a concentration of 7.09 mg mL^{-1} at a rate of 94.07%, for the other strain of *Staph. aureus* (ATCC 29213) at a concentration of 3.54 mg mL^{-1}, at a rate of 75.43%. For *Bacillus subtilis*, it was found 90.62% at a concentration of 7.09 mg mL^{-1}, and it was found the highest inhibitory effect. It was determined that the highest inhibition effects were 73.14% at a concentration of 10.63 mg mL^{-1} in *Salmonella* Paratyphi A and 74.59% at a concentration of 14.17 mg mL^{-1} in *Salmonella poona*. *Lavandula angustifolia* oil showed the highest inhibition effect on *Campliobacter jejuni* at a concentration of 3.54 mg mL^{-1}, at a rate of 71.43%, and for *E.coli* at a concentration of 3.54 mg mL^{-1}, at the rate of 88.31%. It was determined that *Lactobacillus* has 84.10% maximum inhibition effects at a concentration of 10.63 mg mL^{-1} in *L. reuteri* and 86.92% in *L. plantarum*. It was determined that the highest inhibitory effect was 75.43% for MRSA at a concentration of 3.54 mg mL^{-1} (Figure 2).

Table 2. Antimicrobial zone diameters of *L. angustifolia* oil on indicator microorganisms

	Microorganism	Diameter region (mm)	TE 30	N 30	DMSO control
Gram-positive	*Staph. aureus* ATCC 29213	17	14		0
	Bacillus subtilus B 354	24	22	12	0
	MRSA (Methicillin Resistant *Staph.*)	22	20		0
	Salmonella parathypi A NCTC13	12	21		0
Gram-negative	*Campliobacter jejuni* ATCC33560	14			0
	E. coli k-2	0	12	22	0
	Salmonella poona RM 2350	14	20		0
	E.coli RSSK 09036	16	18		
Lactic acid bacteria	*Lb. reuteri*	11	14		0
	Lb. Plantarum	14	15		0

Agar well diffusion assay, supplemental Tables CLSI (CLSI 2006-7) procedural standards were taken as reference in the results. Tetracycline (TE 30) and Neomycinmcg (N30) discs were used for antimicrobial susceptibility testing of bacterial cultures.

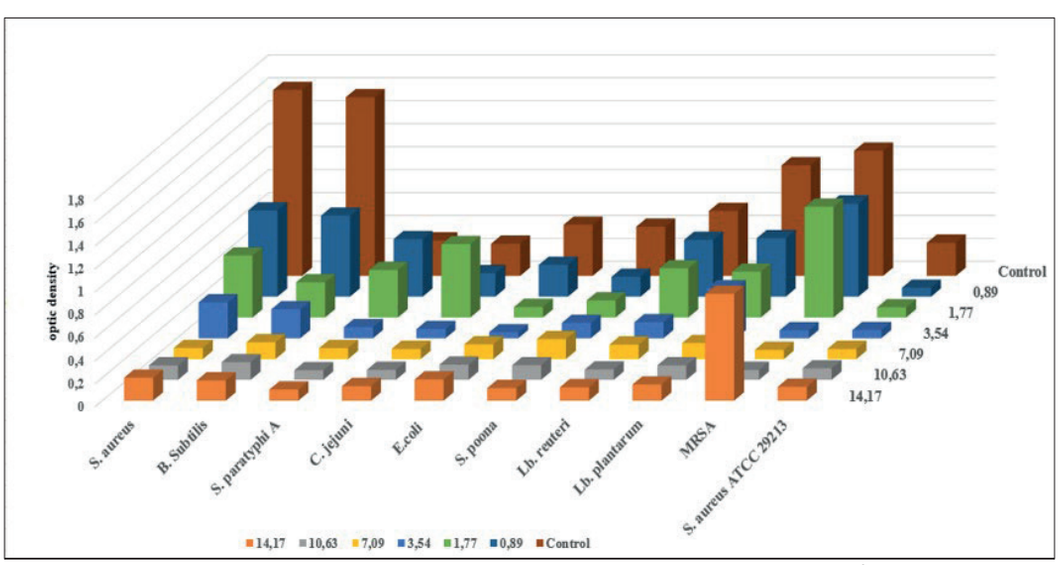

Figure 2. Growth concentrations of *L. angustifolia* volatile oil doses (14.17-0.89 mg L^{-1}) applied to indicator bacteria by spectrophotometric (at 600 nm) method compared to the control group

3.3. Antioxidant capacity

Results are given graphically in Figure 3. Analysis of data showed that all of the *L. angustifolia* values were lower than the values of standards (BHT and Throlox).

DPPH inhibition effect, FRAP analysis, and Chelating activity of ferrous ions (Fe^{2+}) values were determined as 15.54%, 0.495, and 13.77, respectively for antioxidant effects of *L. angustifolia* oil.

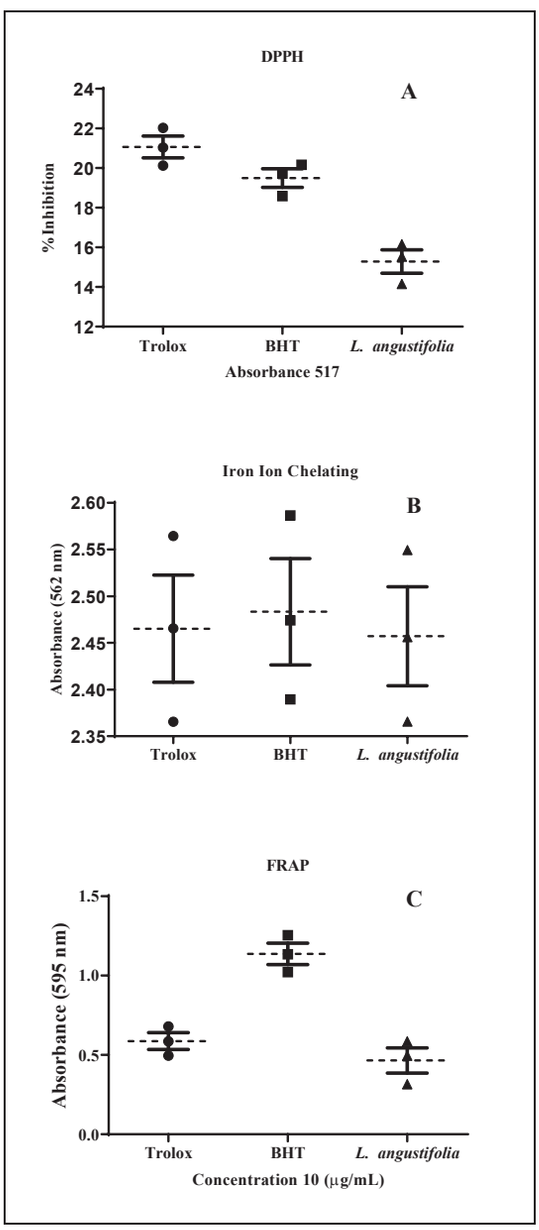

Figure 3. A. DPPH antioxidant capacity of LA (% inhibition), B. Chelating activity of ferrous ions (Fe^{2+}), C. FRAP Determination (Absorbance)
As controls, 1 mg mL^{-1} Throlox and BHT were used.

4. Discussion and Conclusion

Volatile oils of *Lavandula*, widely from *L. angustifolia*, *L. latifolia*, *L. stoechas* and *L. x intermedia*, have been used for centuries such as cosmetically and therapeutically. However, scientific or clinical evidence on the biological activities of these oils has been rather scant. Among the known and suggested effects of *L. angustifolia* oil is that it is effective for antibacterial, antifungal, carminative, soothing, anti-depressive, burns, and insect bites. Although scientific and clinical data supporting traditional lavender uses are still inconclusive and often controversial, methodological and oil identification problems have severely hampered the assessment of the therapeutic significance of most research on *Lavandula* (Cavanagh and Wilkinson, 2002).

Several pharmacological properties have been reported with *L. angustifolia* volatile oil, including anesthetic, sedative, analgesic, anticonvulsant, antispasmodic, cholinesterase inhibitor, antioxidant, antibacterial, antifungal effects, and inhibition of microbial resistance (Ali-Shtayeh et al., 2020).

In this research, it was aimed to characterize the chemical compositions of the volatile oil obtained from the inflorescences of *L. angustifolia* oil by the headspace/GC-MS method. The antimicrobial effects, maximum inhibition concentration, and antioxidant capacity of the oil obtained by Clevenger on gram-positive/negative bacteria were also determined. As a result of the analysis, it was determined that the main component rates were eucalyptol (22.58%), α-linalool (19.44%), and linalyl (14.61%) in analysis. Lavandulol, one of the most important components of *L. angustifolia*, was determined at a rate of 1.39%. Xiaotian et al. (2020) examined the chemical components of *L. angustifolia* with the GC-QTOF/MS device. They showed rates of eucalyptol (2.05%), linalool (24.30%), and linalyl acetate (28.89%). The eucalyptol level of *L. angustifolia* was lower, and the linalool and linalyl levels were higher than the present study values (Xiaotian et al., 2020). The reason for these differences was thought to be related to the location where the Lavender plant was collected and measurement methods.

Roller et al. (2009) reported that all four *L. angustifolia* oils inhibited the growth of Methicillin-Sensitive and -Resistant *Staph. aureus* (MSSA and MRSA). They found inhibition diameters of 8 to 30 mm on the *Staph. aureus* members of oil doses ranging from 1 to 20 microliters. While there was no difference in the application of the oils individually, regardless of the

chemical composition of the oils or the *Staph. aureus* strain used, a few double combination applications resulted in different inhibition effects on the bacteria. It was noted that *L. luisieri* oil (rich in necro) acts synergistically on *L. stoechas* (high in 1,8-cineol, fenchone, and camphor) and *L. angustifolia* (rich in linalool) and *Staph. aureus*. The results show that combinations of *L. angustifolia* oils need to be investigated further for possible use in antibacterial products.

The antibacterial properties of volatile oils were studied in various research about their direct effect on a number of microorganisms (Kalemba and Kunicka, 2003; Burt, 2004). Most studies have shown that gram-negative/positive indicator bacteria are susceptible to *L. angustifolia* (Bakkali et al., 2008). It was aimed to determine the minimal inhibition and minimal bactericidal concentrations of the tested volatile oils in the growth of a particular microorganism in solid media.

Adaszyńska-Skwirzyńska and Szczerbińska (2018) determined the antimicrobial activity test (MIC test) of *L. angustifolia* volatile oil on seven different commercial microorganisms (*C. albicans*, *E. coli*, *P. aeruginosa*, *S. enteritidis*, *S. typhimurium*, *S. pullorum*, *S. aureus*). The study is in parallel with present research and it is thought that the antimicrobial activity is caused by many volatile oils and terpenoid structures in *L. angustifolia* oil. The antibacterial activity of *L. angustifolia* volatile oil against thirty-eight tortoise-borne pathogenic bacterial strains belonging to seven indicator species by disc diffusion, MIC, and MBC tests were reported (Hossain et al., 2017). As a result, *L. angustifolia* had an antibacterial effect on *Aeromonas hydrophila*, *A. caviae*, *A. dhakensis*, *Citrobacter freundii*, *Proteus mirabilis*, *Salmonella enterica* except for *Pseudomonas aeruginosa*. The MIC and MBC values of *L. angustifolia* oil were reported as 0.5-1% (v/v) and 0.5-2% (v/v), respectively. This study demonstrated that *L. angustifolia* volatile oil has the potential to be used as an antibacterial agent against pathogenic bacteria isolated from pet turtles.

Puškárová et al. (2017) showed the antibacterial and antifungal effects of six different volatile oils, including *L. angustifolia*, on pathogenic, environmental bacteria. These volatile oils have been reported to show different fungistatic and fungicidal activities when tested in a direct application and in the vapor phase. This study demonstrated novel approaches to assess the antimicrobial potential of volatile oils in both direct contact and vapor phase as well as the valuable properties of phenol-free arborvitae oil. Along with these results, it has also shown that all volatile oils tested, which are stated to have no genotoxic effect, can be used as broad-spectrum antimicrobial agents. The antioxidant activity of volatile oils is a biological feature of great interest. Because oils have the ability to destroy free radicals, they can play an important role in preventing certain diseases that can result from oxidative stress damage caused by free radicals, such as the brain (Ali-Shtayeh et al., 2020). In another study, the antimicrobial and antioxidant capacities of *L. luisieri* oil were investigated by extracting *Lavandula luisieri*'s volatile oil with the Clevenger method. As a result of the research, an antimicrobial effect was observed on *L. monocytogenes*, *E. faecium* and *Staph. aureus* species similar to the present study. At the same time, an antioxidant effect was observed as a result of the analysis made with the DPPH method. Research results (parallel to the present study) were found to have a lower effect than standards (Throlox and rosmarinic acid) (Giménez-Rota et al., 2019).

The present study showed that *L. angustifolia* oil has an antimicrobial effect on gram-positive/negative microorganisms. Determination of important active ingredients of the volatile oil composition with headspace/GC-MS and its antioxidant effect can be an alternative and safer to industrial chemical and traditional preservatives due to their naturalness. At the same time, the importance of its use in complementary medicine and aromatherapy for preventive and symptomatic treatment of diseases. It is thought that the present research may shed light on *in vitro*/*in vivo* studies to be carried out with *L. angustifolia* oil.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

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Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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