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PHYSICOCHEMICAL, CHEMICAL AND ANTIBACTERIAL CHARACTERIZATION OF *LAVANDULA ANGUSTIFOLIA* ESSENTIAL OILS CULTIVATED IN ÇANKIRI, TÜRKİYE

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ABSTRACT

The present study investigates the physicochemical, chemical, and antibacterial properties of essential oils obtained from the flower (LF-EO) and stem-leaf (LSL-EO) fractions of *Lavandula angustifolia* cultivated under the semi-arid continental conditions of Çankırı, Türkiye. The essential oils were extracted by hydrodistillation using a Neo-Clevenger apparatus and characterized in accordance with ISO 3515:2002 and related standards. The physicochemical parameters of LF-EO, including density (0.8937 g mL^{-1}), refractive index (1.4566), optical rotation (-9.50°), and ester value (55.04 mg KOH g^{-1}), were consistent with the ISO specifications for high-quality lavender oil. GC-MS analysis identified 98.11 % (LF-EO) and 98.42 % (LSL-EO) of total volatile constituents, revealing β-linalool (44.77 % and 34.55 %) and linalyl acetate (12.94 % and 6.60 %) as the major compounds, alongside camphor, borneol, and 1,8-cineole. The compositional profile of LF-EO corresponded to the French Maillette chemotype defined by ISO 3515:2002.

Both oils exhibited notable antibacterial activity against Gram-positive and Gram-negative bacteria. LF-EO demonstrated strong inhibition against *Bacillus cereus* and *Listeria monocytogenes* (30 mm), while LSL-EO showed moderate activity against *Escherichia coli* (20 mm) and *Salmonella typhimurium* (17 mm). The enhanced antimicrobial performance of LF-EO was attributed to its high content of oxygenated monoterpenes and esters, which contribute to membrane permeability and destabilization effects.

Overall, the results confirm that *Lavabdula angustifolia* cultivated in Çankırı produces a French-type lavender oil of superior physicochemical and biological quality, suitable for pharmaceutical, cosmetic, and aromatherapeutic applications.

1. INTRODUCTION

The use of antibiotics in both veterinary and human medicine is of vital importance, enabling a wide range of essential therapeutic and surgical interventions [1]. However, the increasing emergence of multidrug-resistant bacteria has become one of the most critical global health challenges, leading to higher morbidity and mortality rates and reducing the efficacy of conventional antibiotic therapies [2], [3]. This growing resistance crisis has created an urgent need to explore alternative antimicrobial agents derived from natural sources [4].

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Among natural bioactive compounds, essential oils have received increasing attention due to their broad-spectrum antimicrobial, antioxidant, and anti-inflammatory activities, combined with low toxicity and biodegradability. Lavandula angustifolia (lavender), a perennial evergreen shrub native to the Mediterranean basin, is one of the most valuable aromatic and medicinal plants traditionally used to treat pain, parasitic infections, burns, and inflammatory conditions [5]. The essential oil of lavender has been shown to possess sedative, anti-inflammatory, analgesic, and antimicrobial properties, primarily attributed to its rich content of linalool and linalyl acetate [6].

Nevertheless, the chemical composition and biological activity of lavender essential oil are highly influenced by factors such as geographic origin, cultivation conditions, and distillation parameters. To date, no detailed report has been published on the antimicrobial properties of L. angustifolia cultivated in the Çankırı region of Türkiye. Therefore, the present study aims to characterize the essential oils obtained from local lavender samples and to evaluate their antimicrobial activities against selected bacterial strains.

2. MATERIALS AND METHODS

Fresh aerial parts of *Lavandula angustifolia Mill.* (lavender) were collected during the flowering stage (July-August) from the cultivation and application fields of Çankırı Şehit Mehmet Ata Agricultural Vocational and Technical Anatolian High School (Çankırı, Türkiye).

2.1. Preparation of Plant Extracts

Aerial parts of *L. angustifolia* were separated into flower (LF, 1 kg) and stem-leaf (LSL, 1 kg) fractions. Each fraction was dried under controlled laboratory conditions (25 ± 2 °C, dark environment, good air circulation) and ground into fine powder under liquid nitrogen to prevent loss of volatile constituents. Hydrodistillation of each fraction was carried out separately using a Neo-Clevenger apparatus at 120 °C for approximately 3 h. The distillate collected in the receiver arm was allowed to separate into aqueous and oil phases, and the essential oils were recovered as flower essential oil (LF-EO) and stem-leaf essential oil (LSL-EO), respectively [7,8]. Due to the very low yield of the stem-leaf oil (LSL-EO), physicochemical characterization according to ISO 3515:2002 [9] was performed only for the flower essential oil (LF-EO). Both LF-EO and LSL-EO were subjected to GC-MS analysis and antimicrobial tests.

2.2. Chemical Analysis of Essential Oils

The chemical composition of LF-EO and LSL-EO was analyzed using a gas chromatograph coupled to a mass spectrometer (Agilent 7890A GC / 5975C MSD; Agilent Technologies, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; 5 % phenyl methylpolysiloxane). The injector temperature was 280 °C and operated in split mode with a split ratio of 30:1. Helium (99.999 % purity) was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}.

The oven program was: 50 °C (10 min), increased at 2 °C min^{-1} to 72 °C (2 min), then 1 °C min^{-1} to 82 °C (2 min), 2 °C min^{-1} to 120 °C (10 min), 5 °C min^{-1} to 200 °C (10 min), and 10 °C min^{-1} to 250 °C (10 min), with a total run time of 105 min.

One microliter of each diluted sample (1:100 in n-hexane) was injected automatically (10 μL syringe). The transfer line temperature was 280 °C; ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. The MS was operated in electron-impact (EI) mode at 70 eV, scanning m/z 50-650 with a solvent delay of 3 min. Compound identification was based on NIST-05 spectral library matches and comparison of calculated retention indices with literature data. Relative component percentages were obtained by area normalization without correction factors.

2.3. Antimicrobial Activity Assay

The antimicrobial activities of LF-EO and LSL-EO were tested against selected bacterial strains of clinical relevance to human and animal health. The following standard strains were used: *Staphylococcus aureus* NCTC 10788, *Bacillus cereus* NCTC 7464, *Salmonella typhi* NCTC 11994, *Listeria monocytogenes* ATCC 11994, and *Escherichia coli* NCTC 2001.

Fresh cultures (24-48 h) were grown on solid media under aerobic or microaerophilic conditions. The bacterial inocula were standardized to 0.5 McFarland (≈ 0.5 × 10^8 CFU mL^{-1}) in nutrient broth (CM0001B, Thermo Fisher Scientific). Twenty microliters of each essential oil were impregnated onto sterile blank antibiotic discs and air-dried in sterile environment at room temperature for 5-10 min.

Then 100 µL of the standardized bacterial suspension was spread evenly onto Mueller-Hinton (MH) agar (CM0337B, Thermo Fisher Scientific) using sterile spreaders. The LF-EO and LSL-EO-impregnated discs were placed on the inoculated agar surfaces, and the plates were incubated under conditions specific to each bacterial strain [10]. After incubation, inhibition zones were measured in millimeters and compared to the control discs.

3. RESULTS

3.1. Physicochemical Characterization of LF-EO and General Characteristics of LSL-EO

The physicochemical parameters specified by ISO 3515:2002 [9] and related standards were determined for the flower essential oil (LF-EO). Due to the low yield of the stem-leaf essential oil (LSL-EO), quantitative ISO-based analyses could not be reliably performed; however, its general physical properties such as appearance, color, and odor were recorded for comparison.

The results obtained for LF-EO are summarized in Table 1. Both LF-EO and LSL-EO were transparent, oily liquids with a characteristic lavender aroma. LF-EO exhibited a more floral and fresh scent, while LSL-EO showed a sharper, more camphoraceous tone. Both oils were light yellow in color, typical of Lavandula angustifolia essential oil.

For LF-EO, the relative and absolute densities at 20 °C were 0.8937 and 0.8907 g mL^{-1}, respectively, within the ISO range of 0.880-0.890 g mL^{-1}. The refractive index (1.4566 at 20 °C) and optical rotation (-9.50°) were also consistent with the ISO specifications (1.455-1.460 and -12.5° to -9.5°, respectively).

The miscibility test (1 mL oil + 3 mL 70 % ethanol at 20 °C) resulted in a clear, homogeneous solution. The acid value was 0.94 mg KOH g^{-1}, and the ester value calculated was 55.04 mg KOH g^{-1}, placing LF-EO at the upper boundary of the ISO specification range (45.5-56). The evaporation residue at 105 °C was 0.63 %, indicating negligible non-volatile matter.

Table 1. Physicochemical parameters of LF-EO determined according to ISO 3515:2002 and related standards

Parameter	Test Method	Result (LF-EO)	Unit	ISO Specification (France "Maillette")
Appearance	ISO 3515:2002	Transparent, oily liquid	-	Transparent, fluid liquid
Color	ISO 3515:2002	Light yellow	-	Light yellow
Odor	ISO 3515:2002	Characteristic, intense, fresh, plant-derived lavender scent	-	Characteristic, fresh, lavender-like
Relative density (20 °C/20 °C)	ISO 279:1988	0.8937	-	0.880-0.890
Absolute density (20 °C/20 °C)	TS EN ISO 2811-1:2016	0.8907	g mL^{-1}	-
Refractive index (20 °C)	ISO 280:1998	1.4566	-	1.455-1.460
Optical rotation (20 °C)	ISO 592:1998	-9.50	°	-12.5 to -9.5
Miscibility with 70 % ethanol (20 °C)	ISO 875:1999	1 mL oil + 3 mL Ethanol → homogeneous mixture	v/v	1 part oil + 3 parts Ethanol → homogeneous mixture
Acid value	ISO 1242:2023	0.94	mg KOH g^{-1}	< 1.0
Saponification Value	ISO 709:2001	56	mg KOH g^{-1}	-
Ester value	ISO 709:2001	55.04	mg KOH g^{-1}	45.5-56
Evaporation residue (105 °C)	In-house method	0.63	%	-

3.2. Chemical Characterization of Essential Oils

The total-ion chromatograms (TICs) of *Lavandula angustifolia* flower (LF) and stem-leaf (LSL) essential oils (EO) are presented in Figure 1. The chromatographic patterns reveal complex yet distinct volatile profiles for the two organs, characterized by well-resolved peaks between 10 and 50 min under HP-5MS column conditions. The flower oil (LF-EO) displayed a dominant peak at 19.9 min, corresponding to β-linalool, while the stem-leaf oil (LSL-EO) showed a broader mid-range pattern with pronounced signals for camphor (22.4 min) and endo-borneol (24.5 min). GC-MS analysis led to the identification of 98.42 % of the total volatile fraction in LSL-EO and 98.11 % in LF-EO (Table 2). In both oils, β-linalool was the major component, accounting for 34.55 % in LSL-EO and 44.77 % in LF-EO. Linalyl acetate, the primary ester responsible for the characteristic floral aroma of lavender, was also more abundant in LF-EO (12.94 %) than in LSL-EO (6.60 %).

Other important oxygenated monoterpenes (camphor 15.49 %, endo-borneol 10.26 %) were considerably higher in the stem-leaf oil compared with their respective proportions in the flower oil (7.16 %, 4.48 %). Minor constituents such as neryl acetate, trans-geranyl acetate, and lavandulyl acetate appeared exclusively or at higher levels in LF-EO, contributing to the complex floral-sweet tonality typical of authentic lavender oil.

The distribution of chemical classes confirmed the dominance of oxygenated monoterpenes, representing 88.75 % of the total composition in LSL-EO and 86.16 % in LF-EO. Sesquiterpene hydrocarbons constituted 5.79 % and 1.49 %, while monoterpene hydrocarbons were detected at 0.72 % in LSL-EO and 7.23 % in LF-EO. Oxygenated sesquiterpenes were present only in the stem-leaf fraction (1.74 %) and were not detected in the flower oil. Unidentified or trace compounds accounted for less than 3 % of the total composition in both samples.

These chromatographic and compositional findings demonstrate that both oils are dominated by oxygenated terpenoids, yet differ markedly in the relative abundance of alcohols, esters, and ketones, reflecting their distinct tissue origins.

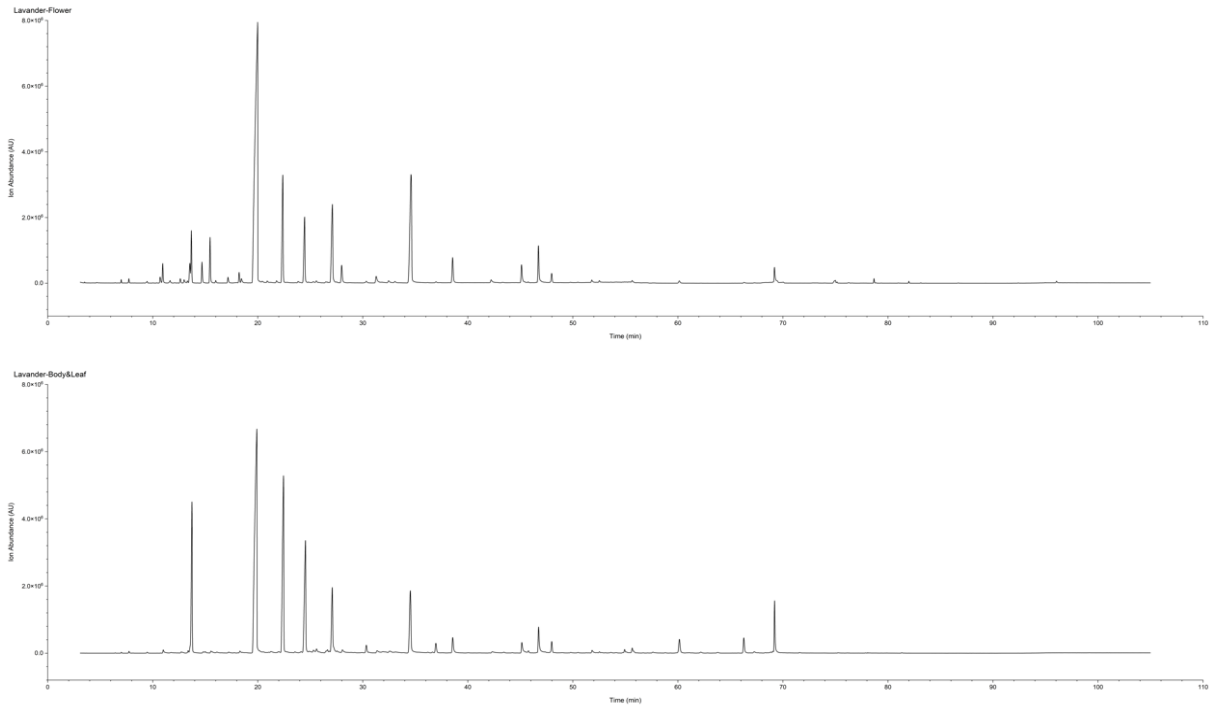


Figure 1. Total-ion chromatograms (TICs) of Lavandula angustifolia essential oils: LF-EO (top) and LSL-EO (bottom)

Table 2. The GC-MS compositional analysis results of essential oils obtained from different parts (stem-leaf and flower) of the Lavandula angustifolia plant.

RT	RI	RI[a]	Compounds	%Area		Formula
				LSL-EO	LF-EO	
7.00	920	929	α-Pinene	-	0.13	$C_{10}H_{16}$
7.73	938	943	Camphene	-	0.19	$C_{10}H_{16}$
11.00	970	964	β-Pinene	0.56	1.06	$C_{10}H_{16}$
11.65	993	990	3-Octanone	-	0.36	$C_8H_{16}O$
12.01	1007	1004	α-Phellandrene	-	0.19	$C_{10}H_{16}$
12.63	1012	1009	n-Hexyl acetate	-	0.26	$C_8H_{16}O_2$
12.99	1019	1017	α-Terpinene	-	0.25	$C_{10}H_{16}$
13.37	1021	1022	*p*-Cymene	0.16	-	$C_{10}H_{14}$
13.54	1019	1026	D-Limonene	-	1.22	$C_{10}H_{16}$
13.73	1026	1028	Eucalyptol	10.40	2.82	$C_{10}H_{18}O$
14.69	1030	1032	trans-β-Ocimene	-	1.15	$C_{10}H_{16}$
15.46	1041	1040	cis-β-Ocimene	-	2.42	$C_{10}H_{16}$
16.83	1082	1088	Terpinolene	-	0.62	$C_{10}H_{16}$
18.36	1083	1088	Linalool oxide	-	0.34	$C_{10}H_{18}O_2$
18.45	1084	1090	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	-	0.38	$C_{13}H_{22}O_4$
19.90	1105	1098	β-Linalool	34.55	44.77	$C_{10}H_{18}O$
22.44	1140	1146	Camphor	15.49	7.16	$C_{10}H_{16}O$
24.54	1171	1173	endo-Borneol	10.26	4.48	$C_{10}H_{18}O$
25.60	1185	1177	4-Terpinenol	0.37	-	$C_{10}H_{18}O$
26.63	1195	1187	Cryptone	0.33		$C_9H_{14}O$
27.09	1196	1190	α-Terpineol	5.84	6.91	$C_{10}H_{18}O$
28.06	1224	1226	Isobornyl formate	0.57	-	$C_{11}H_{18}O_2$
30.34	1230	1226	cis-Geraniol	-	0.67	$C_{10}H_{18}O$
34.53	1266	1257	Linalyl acetate	6.60	12.94	$C_{12}H_{20}O_2$
36.96	1287	1290	Bornyl acetate	0.84	-	$C_{12}H_{20}O_2$
38.56	1302	1293	Lavandulyl acetate	1.42	1.93	$C_{12}H_{20}O_2$
39.47	1336	1336	Hexyl (E)-2-methylbut-2-enoate	-	0.30	$C_{11}H_{20}O_2$
42.23	1352	1356	n-Hexyl butanoate	0.30	1.32	$C_{10}H_{20}O_2$
45.12	1370	1368	Neryl acetate	-	4.12	$C_{12}H_{20}O_2$
46.74	1390	1383	trans-Geranyl acetate	3.20	0.63	$C_{12}H_{20}O_2$
47.99	1412	1415	Caryophyllene	0.78	0.20	$C_{15}H_{24}$
50.16	1445	1448	α-Himachalene	-	0.13	$C_{15}H_{24}$
51.83	1449	1456	cis-β-Farnesene	0.24	1.16	$C_{15}H_{24}$
54.93	1508	1507	β-Bisabolene	3.17	-	$C_{15}H_{24}$
55.65	1509	1513	γ-Cadinene	0.16	-	$C_{15}H_{24}$
60.15	1577	1582	Neryl (S)-2-methylbutanoate	0.47	-	$C_{15}H_{26}O_2$
66.28	1583	1583	Caryophyllene oxide	1.44	-	$C_{15}H_{24}O$
69.86	1669	1667	τ-Cadinol	1.27	-	$C_{15}H_{26}O$
			Total identified	**98.42**	**98.11**	
			Monoterpene hydrocarbons	**0.72**	**7.23**	
			Oxygenated monoterpenes	**88.75**	**86.16**	
			Sesquiterpene hydrocarbons	**5.79**	**1.49**	
			Oxygenated sesquiterpenes	**1.74**	**0.00**	
			Others	**1.42**	**3.23**	

RT: Retention time (min),
RI: Retention Indices on HP-5MS column
RI[a]: Retention Indices on literature

3.3. Antibacterial Activity

The antibacterial activity of *Lavandula angustifolia* flower and stem-leaf essential oils (LF-EO and LSL-EO) was evaluated against five clinically relevant bacterial strains, and the inhibition zone diameters are

presented in Table 3. Both essential oils exhibited broad-spectrum antibacterial effects, with inhibition zones ranging from 17 mm to 30 mm.

Among the tested strains, *Bacillus cereus* and *Listeria monocytogenes* showed the highest sensitivity, displaying 30 mm inhibition zones for LF-EO and 19-20 mm for LSL-EO. *Staphylococcus aureus* was equally inhibited by both oils (20 mm), while *Escherichia coli* and *Salmonella typhimurium* exhibited slightly variable responses. LF-EO produced 19 mm and 20 mm inhibition zones against *E. coli* and *S. typhimurium*, respectively, whereas LSL-EO caused 20 mm and 17 mm inhibition zones.

Overall, LF-EO demonstrated stronger inhibitory effects against Gram-positive bacteria, consistent with its higher linalool and ester content, whereas LSL-EO exhibited a slightly broader effect on Gram-negative species, likely due to its higher camphor and eucalyptol levels, which are known to disrupt bacterial membrane integrity. These results indicate that both essential oils possess relevant antibacterial potential, with compositional differences influencing their relative spectrum and potency.

Table 3. Inhibition zone diameters (mm) showing the antibacterial activity of Lavandula angustifolia flower essential oil (LF-EO) and stem-leaf essential oil (LSL-EO) against clinically relevant Gram-positive and Gram-negative bacteria. Values represent mean diameters of inhibition zones (mm)

Bacterial strain	LF-EO (mm)	LSL-EO (mm)
Escherichia coli[a]	19	20
Salmonella typhimurium[a]	20	17
Bacillus cereus[b]	30	19
Staphylococcus aureus[b]	20	20
Listeria monocytogenes[b]	30	20

[a] *Gram-negative bacteria;* [b] *Gram-positive bacteria.*

4. DISCUSSION

The physical and chemical characteristics of LF-EO demonstrate full compliance with the quality parameters defined by ISO 3515:2002 [9] for *Lavandula angustifolia* oil. Its transparent, oily appearance and light-yellow color confirm the freshness and authenticity of the essential oil. The distinct odor difference between the two fractions (LF-EO (floral, soft) and LSL-EO (sharp, camphoraceous)) reflects their divergent chemical profiles, where LF-EO is dominated by linalool and linalyl acetate, whereas LSL-EO contains higher proportions of camphor and borneol.

The slightly higher density of LF-EO (0.8937) compared with the ISO upper limit (0.880-0.890) can be attributed to its elevated oxygenated-monoterpene content (> 85 %), particularly linalool and borneol, as confirmed by GC-MS analysis. These compounds contribute not only to the oil's density but also to its viscosity and solvency characteristics. The refractive index (1.4566) and optical rotation (-9.50°) indicate an optically pure, chemically stable composition typical of genuine lavender oil. The optical rotation, positioned near the upper limit of the ISO range (-12.5° to -9.5°), suggests a slight predominance of dextrorotatory isomers of linalool and linalyl acetate, both responsible for the sweet-floral aroma associated with high-quality lavender oils.

The complete miscibility of LF-EO with 70 % ethanol reflects low sesquiterpene content and the absence of resinous impurities, confirming proper distillation and minimal degradation. The acid value (0.94 mg KOH g^{-1}) indicates negligible free fatty acids, while the ester value (55.04 mg KOH g^{-1}) reflects a high esterification degree. These findings, consistent with the measured linalyl acetate (12.94 %) and lavandulyl acetate (1.93 %) contents, reveal a well-preserved ester profile characteristic of freshly distilled oils. The inverse relationship between acid and ester values further underscores the stability of LF-EO, whereas the camphor-rich LSL-EO likely exhibits a lower ester-to-acid ratio, typical of more oxidized or leaf-derived fractions. The low evaporation residue (0.63 %) confirms the absence of non-volatile impurities such as waxes or pigments, verifying the production of high-purity essential oils suitable for pharmaceutical, cosmetic, and aromatherapeutic use.

Overall, the composition of LF-EO corresponds to the French Maillette chemotype defined by ISO 3515:2002 [9], confirming that *L. angustifolia* cultivated in Çankırı yields a high-quality French-type lavender oil characterized by high linalool and linalyl acetate and low camphor content.

According to the GC-MS data, linalool was quantified at 44.77 % in LF-EO and 34.55 % in LSL-EO. Comparable levels of linalool (35.3-36.0 %) have been reported for fresh flower oils of *L. angustifolia* of Italian origin [11]. Similarly, Perović et al. [12] reported 40-50 % linalool in French and Italian ecotypes, consistent with our LF-EO profile, which falls within the "high-linalool" chemotype group associated with the Maillette series. Linalyl acetate reached 12.94 % in LF-EO and 6.60 % in LSL-EO, confirming a higher degree of esterification in the floral fraction. Caprari et al. [11] reported lower levels of this ester (2.75-3.77 %) for *L. angustifolia*; therefore, the higher concentrations found here are indicative of enhanced aromatic richness and perfumery quality.

Camphor contents of 7.16 % in LF-EO and 15.49 % in LSL-EO align with literature values reporting 6-7 % camphor in floral parts and up to 20-25 % in stem-leaf fractions. The higher camphor concentration in vegetative tissues corresponds to increased biosynthesis of oxygenated monoterpenes producing more herbaceous and medicinal odor tones. Likewise, eucalyptol (1,8-cineole) levels (2.82 % in LF-EO and 10.40 % in LSL-EO) match the 9-11 % range reported by Caprari et al. [11]. Borneol, quantified at 4.48 % in LF-EO and 10.26 % in LSL-EO, was slightly below the 15.6-19.4 % reported for some European floral samples but showed the same pattern of greater accumulation in vegetative organs.

These results are consistent with the observations of Betlej et al. [13], who found that flower oils of Polish *L. angustifolia* ecotypes were dominated by linalool and linalyl acetate, whereas leaf oils contained mainly oxygenated monoterpenes such as camphor and cineole. In agreement, our LF-EO sample exhibited a pronounced linalool-ester axis, while LSL-EO was characterized by camphor- and borneol-rich oxygenated profiles. Under the environmental conditions of Çankırı, characterized by high solar exposure and a semi-arid continental climate, the elevation of the linalool-ester axis in LF-EO likely results from enhanced enzymatic esterification and photo-oxidative conversion during floral development. Such conditions are known to favor ester accumulation over ketone formation, yielding a more aromatic and perfumery-type essential-oil profile.

The essential oils obtained from the flower (LF-EO) and stem-leaf (LSL-EO) fractions of *Lavandula angustifolia* exhibited notable antibacterial activity against both Gram-positive and Gram-negative bacterial strains (Table 3). The strong inhibitory effects observed against *Bacillus cereus* and *Listeria monocytogenes* (30 mm for LF-EO) are consistent with previous reports indicating the high susceptibility of these Gram-positive species to lavender-derived monoterpenes [14]. These results highlight the contribution of linalool, linalyl acetate, and borneol-oxygenated monoterpenes known to penetrate and destabilize the peptidoglycan-rich membranes of Gram-positive bacteria. The greater efficacy of LF-EO compared with LSL-EO against *B. cereus* and *L. monocytogenes* can be directly linked to its higher ester content (55.04 mg KOH g^{-1}) and elevated linalool concentration (44.77 %), which together enhance lipophilicity and membrane permeability. Previous studies have also shown comparable inhibition zones ranging from 18 to 30 mm for lavender oils against Listeria species, confirming that the oil obtained from flowers conforms to the high-activity chemotype described for French Maillette cultivars [14].

The activity against *Staphylococcus aureus* (20 mm for both oils) also agrees with earlier reports on lavender essential oils showing inhibition zones between 8 and 30 mm against methicillin-sensitive and -resistant strains [15,16]. Given that *S. aureus* is a frequent opportunistic pathogen in humans and animals, these results suggest that lavender oils, particularly LF-EO, could offer supplementary therapeutic potential where antibiotic resistance complicates conventional treatment [17].

In contrast, the moderate inhibition observed against the Gram-negative strains *Escherichia coli* (19-20 mm) and *Salmonella typhimurium* (17-20 mm) reflects the well-known structural barrier of their outer lipopolysaccharide membrane, which restricts penetration of hydrophobic molecules. Nonetheless, the comparable inhibition zones observed in this study align with previous findings demonstrating measurable sensitivity of *E. coli* and *S. typhimurium* to lavender and bergamot essential oils in disk diffusion and broth dilution assays [18]. The ability of LSL-EO to inhibit *E. coli* slightly more than *S. typhimurium* may derive from its higher camphor (15.49 %) and eucalyptol (10.40 %) content-two

oxygenated monoterpenes reported to disrupt bacterial cell membranes and induce leakage of intracellular materials [19,20].

Although a standard antibiotic was not included as a positive control in the present study, the antibacterial activity of *Lavandula angustifolia* essential oil can be evaluated in the context of previously published studies employing comparable agar diffusion methodologies. For instance, Soković et al. [21] reported inhibition zone diameters ranging from 14 to 28 mm for *Lavandula angustifolia* essential oil against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus*, values that are comparable to those observed in the present study. Similarly, Hussain et al. [22] demonstrated that lavender essential oil produced inhibition zones of 16–25 mm against both Gram-positive and Gram-negative bacteria, highlighting its broad-spectrum antibacterial potential. The pronounced activity observed here against Gram-positive strains, particularly *Bacillus cereus* and *Listeria monocytogenes* (up to 30 mm), is consistent with previous reports indicating a higher susceptibility of Gram-positive bacteria to terpene-rich essential oils due to differences in cell wall structure [23].

Furthermore, the detectable inhibition of *Escherichia.coli* and *Salmonella typhimurium* is consistent with the moderate to strong antibacterial effects of lavender essential oil against *Escherichia coli* and *Staphylococcus aureus* reported by Gök and Erdoğdu [24].

Taken together, these comparisons suggest that the inhibition zone diameters reported in the present study reflect a substantial antibacterial effect, supporting the biological relevance of the findings despite the absence of a conventional antibiotic control.

Overall, both essential oils displayed a broad antimicrobial spectrum, but with LF-EO exhibiting stronger activity against Gram-positive bacteria and LSL-EO showing moderate effects against Gram-negative species. This differential behavior corresponds to their compositional divergence: the linalool-linalyl acetate-rich LF-EO possesses high membrane affinity and ester-driven diffusion capacity, while the camphor-cineole-borneol-dominant LSL-EO exerts a more oxidative or irritant-type antibacterial mechanism. These complementary actions suggest that the chemical variability between floral and vegetative fractions of *L. angustifolia* contributes to their overall antimicrobial potential. Future in vivo and cytotoxicity studies are recommended to further evaluate the therapeutic applicability of these oils in veterinary and clinical contexts.

5. CONCLUSIONS

This study presents a comprehensive characterization of *L. angustifolia* essential oils obtained from flower (LF-EO) and stem-leaf (LSL-EO) fractions cultivated in Çankırı, Türkiye. The physicochemical properties of LF-EO fully met ISO 3515:2002 [9] standards, confirming its purity and stability. GC-MS profiling identified a French Maillette-type composition rich in linalool (44.77 %) and linalyl acetate (12.94 %), with moderate camphor, borneol, and 1,8-cineole levels. Both oils showed broad antibacterial activity, with LF-EO producing the strongest inhibition against *Bacillus cereus* and *Listeria monocytogenes* (30 mm). These results indicate that *L. angustifolia* grown under Çankırı's semi-arid continental conditions yields a high-quality oil of pharmaceutical and cosmetic value. Further investigations focusing on cytotoxicity, stability, and in vivo efficacy are recommended to validate its therapeutic applications in human and veterinary medicine.

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Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of any artificial intelligence (AI) tools. All content, including text, data analysis, and figures, was solely generated by the authors.

Contributions of the Authors

AKT: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing-original draft, writing-review and editing, verification, supervision,

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ART: conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing-review and editing,

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