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Chemical composition and antimicrobial activity of essential oils from six lavender (*Lavandula angustifolia* Mill.) cultivars

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Abstract: The present study aimed to determine the chemical composition and *in vitro* antimicrobial potential for the first time of essential oils (EOs) from six cultivars (Druzhba, Raya, Hebar, Hemus, Yubileina, Sevtapolis) of lavender (*Lavandula angustifolia* Mill.) cultivated in Türkiye (Tekirdağ) against a panel of pathogenic and non-pathogenic microorganisms. The chemical composition of EOs, analysed using gas chromatography/mass spectrometry (GC/MS), revealed 34 different components in the six cultivars. The results indicate that the main major constituents of all EOs were monoterpenoid linalool (47.60–64.13%) and linalyl acetate (12.92–26.08%). Based on principal component analysis (PCA) cvs. Druzhba, Raya and Hebar were situated in the centre right quadrant of the plot and were characterised by linalool and linalyl acetate in subgroup one. The results of antimicrobial assays revealed that lavender EOs were active against all strains of bacteria tested. For bacteria, the strongest activity of cv. Hemus was observed against *Enterococcus faecalis* (IZ = 19 ± 0.10 mm, MIC = MMC = 6.25 (μg/mL), while the weakest potency was seen against the gram-negative *Salmonella enterica* (IZ = 21 ± 1.00 mm, MIC = MMC = 31.25 (μg/mL). Based on PCA, the first subgroup of cvs. Hebar and Raya was characterised by *Lactobacillus rhamnosus*, *E. faecalis* and *Lactobacillus pentosus* and was situated in the bottom right quadrant of the plot.

Keywords: aromatic plant; hydrodistillation; bioactive compound; antibacterial capacity; minimum inhibitory concentration

Microbial spoilage is a common source of food spoilage, which occurs due to the action of microorganisms, which include moulds, yeasts, and bacteria (Amit et al. 2017). Preservation of food materials from degradation during production, storage and marketing is an important issue in the food industry (Tenore et al. 2011). Chemical food additives and preservatives are mostly considered safe, but several of them have negative and potentially life-threatening side effects (Gyawali and Ibrahim 2014, Caleja et al. 2016, Amit et al. 2017, Kalem et al. 2017, Sambu et al. 2022). Therefore, consumers have several concerns about the safety of foods due to the potential hazards of the preservatives (Williams et al. 2009, Antolak and Kregiel 2017) such as sodium benzoate, potassium sorbate (Maden 2000, Hoang and Vu 2016, Beya et al. 2021, Sambu et al. 2022). In recent years, the use of preservatives with plant origin has gained importance to control microbial spoilage (Ju et al. 2017) and their effectiveness as a preservative for foods was reported in many studies (Dima and Dima 2015, Donsi and Ferrari 2016, Ju et al. 2019, Beya et al. 2021). Preservatives of plant origin are generally considered GRAS (generally recognised as safe) and contain bioactive compounds that play a crucial role in preserving foods against viruses, bacteria and fungi (Antolak and Kregiel 2017). Plant extracts consist of various quantities and profiles of phenolic compounds, essential oils and various bioactive components depending on the type of plant and technology applied (Jiang and Xiong 2016, Beya et al. 2021).

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Essential oils (EOs) are aromatic oily liquids obtained from plant materials such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots (Burt 2004, Łyczko et al. 2023). There are several extraction methods such as using the solvents, pressing and sublimation (Zhang et al. 2018). The steam distillation method is the most commonly used technique for extracting EOs from these materials (Hanif et al. 2019). EOs are widely used in medicine, cosmetics, aromatherapy and perfumes, as well as food preservation. They are rich in compounds related to antioxidant and antimicrobial properties (Nieto et al. 2018), such as menthol, eugenol, carvacrol, and thymol (Brewer 2011, Beya et al. 2021). EOs are composed of a mixture of more than 50 components with quite different concentrations, and their constituents play a key role in antimicrobial activity (Bhavaniramya et al. 2019). The anti-carcinogenic, anti-inflammatory, anti-mutagenic, antibacterial, antifungal and antiviral properties of EOs were proven by many researchers (Aloui and Khwaldia 2016, Wińska et al. 2019, Maddala and Singh 2023). The composition of EOs is influenced by genotype, environment, agronomy and processing technique (Salamon 2006, Mancini et al. 2015, Guo and Wang 2020).

Lavender is a perennial aromatic plant with a semi-shrub form belonging to the Lamiaceae family. The plant is native to the Mediterranean area and is widely cultivated for EO production (Basch et al. 2004). Lavender is one of the most important EO plants, and there are three important lavender species with high commercial value worldwide. These species are lavender (*Lavandula angustifolia* Mill. = *L. officinalis* L. = *L. vera* DC), lavender (*Lavandula intermedia* Emeric ex Loisel. = *L. hybrida* L.) and spike lavender (*Lavandula spica* = *L. latifolia* Medik.) (Kara and Baydar 2012). Lavender species EOs are commonly used in aromatherapy as antimicrobial agents (Soulaimani et al. 2019, Yıldırım et al. 2020, Giuliani et al. 2023, Speranza et al. 2023). The antimicrobial activity and other properties, such as antimutagenic and cytotoxic effects, have been referred to the abundance in many lavender essential oils of several monoterpenoids, including terpinen-4-ol, linalool, linalyl acetate and 1,8-cineole (Woronuk et al. 2011, Soulaimani et al. 2019). The required quantity and quality of the EO of lavender species are included in pharmacopoeias. In Ph. Eur. VII., min. 1.3 mL/100 g EO content is required for *Lavandulae flos* (*Lavandula angustifolia* Mill.), while the following limits are included for EO composition: max 1% limonene, max. 2.5% 1,8-cineole, max. 1.2% camphor, 20–45% linalool, 25–47% linalyl acetate, 0.1–8% terpinen-4-ol, 0.1–8% lavandulyl acetate, and max. 2% α-terpineol in the European Pharmacopoeia (Détár et al. 2020). *L. angustifolia* Mill. is the species with the most significant industrial importance because of the EO derived from it (Adaszyńska-Skwirzyńska and Dzięciol 2017). The main EO components of *L. angustifolia* are linalool (25–38%) and linalyl acetate (25–45%) (Lis-Balchin 2002).

Significant antimicrobial activity of lavender EO was reported against various microorganisms, including gram-positive bacteria such as *Bacillus cereus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, gram-negative bacteria such as *Pseudomonas aeruginosa*, *Salmonella enterica* serovar Typhimurium, *Escherichia coli*, *Klebsiella pneumoniae*, yeasts and moulds (Hammer et al. 1999, Moon et al. 2004, Rota et al. 2004, Hui et al. 2010, Soković et al. 2010, Blazekovic et al. 2011, Stanojević et al. 2011, Şerban et al. 2011, Zheljazkov et al. 2012, Danh et al. 2013, De Rapper et al. 2016, Blazekovic et al. 2018, El Hamdaoi et al. 2018, Najar et al. 2022, Slimani et al. 2022, Xylia et al. 2023).

To our knowledge, no previous study has investigated the different cultivars of lavender EOs cultivated in Türkiye (Tekirdağ) and their biological properties. The objective of the work was to evaluate the composition of EOs from six cultivars of lavender (*Lavandula angustifolia* Mill.), their antimicrobial properties against four pathogenic bacteria: *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 43300), *Bacillus subtilis* (NRRL NRS-744) as gram-positive bacteria; *Escherichia coli* (ATCC 25922) and *Salmonella enterica* serovar Typhimurium (ATCC 14028) as gram-negative bacteria and pathogenic *Candida*: *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 90030), and lactic acid bacteria: *Lactobacillus plantarum* (ELB75), *L. pentosus* (ELB41), *L. rhamnosus* (ELB99) and *L. acidophilus* (ATCC 11975). In conclusion, the present study aimed to determine the chemical composition and *in vitro* antimicrobial potential of EOs for the first time from six cultivars of lavender against a panel of pathogenic and non-pathogenic microorganisms. This knowledge will contribute to evaluating the applications of EO in the food preservation, pharmaceutical and cosmetic industries.

MATERIAL AND METHODS

Plant material. In the study, Druzhba (Dru), Raya (Ra), Hebar (Heb), Hemus (He), Yubileina (Yu), Sevtapolis

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(Sev) lavender cultivars belonging to *Lavandula angustifolia* Mill. were used as plant material.

Growth area. The experimental area is located in the herbarium of medicinal plants farmlands of Tekirdağ Namık Kemal University, Department of Field Crops, Faculty of Agriculture, Tekirdağ. Tekirdağ is located at latitude 40°99'04" and longitude 27°58'07" with an altitude of 10 m a.s.l. The climate is warm and temperate in Tekirdağ. There is much more rainfall in winter than in summer. The annual average temperature and average annual precipitation are 14.5 °C and 601 mm, respectively.

Sampling. Samplings from three-year-old lavender cultivars were carried out during the full blooming period in June 2021. Approximately 20 cm long stalks were cut in the full bloom stage from randomly selected individuals in the plantations (Détár et al. 2020). All the samples were dried and stored at room temperature until the time of EO extraction.

Essential oil extraction. Dried stemless flowers from lavender samples (300 g) were hydro-distilled for 3 h with 900 mL of deionised water using a Clevenger-type apparatus, according to Détár et al. (2020). The hydro-distilled oils were collected and stored in amber airtight vials at 4 ± 1 °C until analysis.

Gas chromatography-mass spectrometry. Gas chromatography/mass spectrometry (GC/MS) analyses of EOs were performed using GC-MS (Hewlett Packard Model 6890, Hewlett Packard Corporation, Palo Alto, USA) with an HP-5 (HP-5MS, crosslined 5% PH ME Siloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness; HP Part No. 19091s-(Hewlett Packard Corporation, Palo Alto, USA) coated with 5%-phenyl-methyl polysiloxane) bioactive compound, programmed as follows: initial temperature 60 °C (10 min), from 60 °C to 220 °C at a rate of 4 °C/min; from 220 °C to 240 °C at a rate of 1 °C/min. Helium was used as carrier gas at a constant flow rate of 0.8 mL/min. The injector temperature was 250 °C. The samples were injected with a splitting ratio of 10:1. Injected quantity was 1 µL. The GC/MS interface temperatures were maintained at 250 °C. The ion source and the detector temperatures were maintained at 250 °C and 150 °C, respectively. Ionisation energy was 70 eV. The mass spectra (MS) were recorded in full scan mode (mass range m/z 30 to 450). The compounds were identified by comparison of the retention index (RI) obtained on a nonpolar HP-5MS column with the RI in the literature by matching their recorded mass spectra with those in mass spectral library references (NIST 08, National Institute of Standards and Technology, Maryland, Wiley). The individual constituents were identified by retention indices and compared with constituents known from the literature (Adams 2001).

Antimicrobial activity

Microorganism strains. *L. angustifolia* EOs were evaluated for antimicrobial activity against a set of microorganisms, including four pathogenic bacteria: *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 43300), *Bacillus subtilis* (NRRL NRS-744) as gram-positive bacteria; *Escherichia coli* (ATCC 25922) and *Salmonella enterica* serovar Typhimurium (ATCC 14028) as gram-negative bacteria, pathogenic *Candida*: *Candida albicans* (ATCC 10231), *C. glabrata* (ATCC 90030), and lactic acid bacteria: *Lactobacillus plantarum* (ELB75), *L. pentosus* (ELB41), *L. rhamnosus* (ELB99) and *L. acidophilus* (ATCC 11975).

Disc diffusion assay. The agar disc diffusion method described by the Clinical and Laboratory Standards Institute (CLSI 2015) was used to test the antimicrobial activity of *L. angustifolia* EOs. For this, 0.1 mL of a cell suspension of 10^8 CFU (colony forming unit)/mL for pathogenic and lactic acid bacteria and 1–5 × 10^6 for yeast were respectively spread on the surface of Mueller Hinton, MRS (Merck Millipore Co., Darmstadt, Germany) and Sabouraud 2% dextrose agar (Merck Millipore Co., Darmstadt, Germany) plates. Sterile filter paper discs (diameter 6 mm) were individually impregnated with 10 µL of the EO and placed on the previously inoculated agar plates. The inoculated plates were kept at 4 °C for 2 h to allow the diffusion of the EOs and then incubated at 37 ± 1 °C for 18–24 h and 28 ± 1 °C for 48 h for bacteria and yeast, respectively. Antimicrobial activities of extracts were detected by measuring the diameters of inhibition zones in mm. Vancomycin (VCT, 5 µg/disc) was used as a negative control. Each test was performed in triplicate.

Determination of the minimum inhibitory concentration and minimum microbicidal concentration. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) according to the Clinical and Laboratory Standards Institute guidelines for bacteria and yeasts (CLSI 2015). EO dilutions of different lavender samples were performed in Mueller Hinton Broth (Merck Millipore Co., Darmstadt, Germany) for bacteria using dimethyl sulphoxide (DMSO, Sigma Aldrich

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Co., Missouri, USA). In the log phase, fresh overnight cultures of the tested microorganisms were used to prepare cell suspensions at 10^6 CFU/mL for bacteria. Then 100 µL of EO dilution was mixed with the same volume of cell suspension and incubated at 37 ± 1 °C for 18–24 h. The MIC was defined as the lowest EO concentration inhibiting the tested strains' macroscopic bacterial growth.

The minimum microbicidal concentration (MMC) was detected by taking the broth from each microwell, spreading it on Mueller Hinton Agar, and incubating it at 37 ± 1 °C for 18–24 h (CLSI 2015). The MMC was defined as the lowest concentration of EO at which 99.99% of the incubated microorganism was killed. Vancomycin was used as a standard antibacterial agent.

Statistical analysis. Relative area percentage composition and peak area percentage composition (%) values are expressed as means ± standard deviations (SD). The results were analysed with IBM SPSS Statistics 19 (Chicago, USA), using one-way ANOVA to compare the composition of EOs of each cultivar, where the significance level was set to $P < 0.05$. Duncan's multiple range test was applied to compare the means of main constituents in EOs. Post hoc methods were applied to check the normality and homogeneity of SD. Principal component analysis (PCA) was carried out with the help of the statistical software JMP Pro (version 16.0.0, Chicago, USA).

RESULTS AND DISCUSSION

Chemical composition of the EO. The oil extracted by hydro-distillation from the aerial parts of six different lavender cultivars was dark yellow to light yellow liquid. The essential oil yields of cvs. Druzhba, Raya, Hebar, Hemus, Yubileina and Sevtapolis were 0.043, 0.026, 0.043, 0.038, 0.035 and 0.032 mL/g, respectively. The GC/MS analysis of EOs from six different cultivars of lavender revealed the presence of 19, 16, 16, 17, 20 and 22 compounds, representing 100% of total oil compositions for cvs. Hemus, Raya, Yubileina, Sevtapolis, Hebar and Druzhba, respectively (Table 1). The predominant constituent was linalool, of which cvs. Raya and Yubileina had the highest linalool proportions (64.13% and 63.84%), while cvs. Hebar and Sevtapolis had the lowest percentages (47.60% and 51.37%) (Table 1).

To our knowledge, this study is the first report concerning the chemical characterisation of EOs from six different lavender cultivars simultaneously in the same region. The predominant constituents (≥ 10) were detected as linalool and linalyl acetate. At the same time, the minor compounds (ranging from 1% to 10% of EOs) included linalyl propionate, neryl acetate, α-terpineol, geranyl acetate, *trans*-caryophyllene, lavandulol, terpinen-4-ol. Concerning total ester percentages of the EOs of the cultivars, cv. Sevtapolis had the highest ratio of total ester percentage (36.09%) among the lavender cultivars (Table 1). Détár et al. (2020) reported that the most important major compounds were linalool, 1,8-cineole, *cis* β-ocimene, camphor, and isoborneol, while the minor compounds (in the range of 1–10%) included limonene, *trans*-β-ocimene, lavandulol, terpinen-4-ol, α-terpineol, linalyl acetate, lavandulyl acetate, ß-caryophyllene, *cis* β-farnesene and epi-α-bisabolol. The variance between the studies could be explained by the difference among the cultivars. Landmann et al. (2007) stated that the EO composition is primarily determined by plant genotype; however, it might also be affected by ontogenetic and environmental factors, as well as by morphogenetic features (Boeckelmann 2008, Guitton et al. 2010, Détár et al. 2020) and even the harvest time. The linalool, limonene, 1,8-cineole, isoborneol and terpinen-4-ol values of *L.* × *intermedia* showed decreases, when compared with the previous year (Détár et al. 2020). Considering the results available from previous studies about the EO composition of lavender cultivars, similar main components were observed in our study compared with the earlier reports (Table 2). As can be seen in Table 2, the major components reported in the literature are linalool and linalyl acetate. In contrast to the reports, Jianu et al. (2013) observed no linalool and linalyl acetate in the lavender cultivar from Romania. In addition to this, caryophyllene (24.12%), β-phellandrene (16%) and eucalyptol (1,8-cineole, 15.69%) were reported as the major components of *L. angustifolia* Miller. Stanojević et al. (2011) declared several environmental variances, such as climatic, seasonal or, geographical and genetic differences, could be the main reason. As stated in the literature, many factors such as geographical origin, genetic factors, morphological and genetic variability of the plant material and the season in which the plants were collected might be responsible for the variance in the chemical composition of EO. However, antagonistic and synergistic effects may occur with other minor compounds for the antimicrobial activity of essential oils against gram-positive, gram-negative and lactic acid bacteria. Therefore, the minor components may be responsible for the marked antimicrobial activity of EOs as much as the major constituents.

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Table 1. Essential oil composition of six different cultivars of lavender

	Component	Relative abundance (%)					
		He	Ra	Yu	Sev	Heb	Dru
1	Linalool	62.95	64.13	63.84	51.37	47.60	54.87
2	Linalyl acetate	13.53	18.77	12.92	19.36	26.08	13.95
3	Linalyl propionate	7.40	3.79	nd	5.75	nd	5.89
4	α-Terpineol	nd	nd	5.56	nd	5.43	nd
5	Neryl acetate	4.34	4.24	4.80	5.77	2.80	4.87
6	Geranyl acetate	2.35	1.20	1.53	2.48	2.03	1.75
7	Terpinen-4-ol	1.95	nd	2.97	nd	nd	2.56
8	Eucalyptol	0.97	nd	0.55	0.68	0.30	1.29
9	Lavandulol	1.25	nd	1.79	1.25	0.99	1.67
10	9-Octadecenoic acid	nd	nd	nd	nd	nd	0.71
11	1-Acetylpiperidine	0.20	nd	nd	nd	nd	nd
12	Thujanol	nd	nd	nd	nd	0.18	nd
13	β-Ocimene	nd	0.22	nd	nd	nd	1.22
14	Allocimene	nd	nd	nd	nd	0.24	0.35
15	Isopulegol	0.16	nd	nd	0.75	nd	nd
16	Linalool oxide	0.18	nd	nd	1.88	7.72	1.63
17	β-terpineol	nd	0.34	nd	nd	nd	nd
18	Linalyl isobutyrate	nd	0.32	nd	nd	nd	nd
19	Octen-1-ol acetate	0.70	1.19	0.60	2.20	0.65	0.46
20	3-Octyl acetate	nd	nd	0.17	0.25	nd	nd
21	Camphor	0.08	0.26	0.12	0.34	0.44	0.26
22	Borneol	0.85	0.70	1.13	1.46	1.93	0.94
23	Geraniol	nd	0.98	nd	nd	nd	nd
24	Cryptone	nd	nd	nd	nd	0.41	0.88
25	Nerol	0.43	nd	0.33	nd	0.29	0.36
26	Benzaldehyde	nd	nd	nd	nd	nd	0.34
27	Fenchyl acetate	nd	nd	nd	0.28	nd	nd
28	Limonene	0.34	nd	nd	nd	0.22	nd
29	1-Hydroxylinalool	nd	nd	nd	nd	0.18	nd
30	*trans*-Caryophyllene	1.65	1.80	1.37	2.09	0.72	2.01
31	β-Farnesene	nd	0.91	1.35	1.26	0.62	2.13
32	Germacrene D	nd	0.29	nd	nd	nd	nd
33	Caryophyllene oxide	0.45	0.60	0.76	2.82	0.93	0.54
34	T-Cadinol	0.20	nd	nd	nd	nd	0.37
Total esters		28.32	29.51	20.02	36.09	31.56	26.92

nd – not detected; Lavender cultivars: He – Hemus; Ra – Raya; Yu – Yubileina; Sev – Sevtapolis; Heb – Hebar; Dru – Druzhba

Antimicrobial activity.

The antimicrobial and antifungal activities of lavender EO were evaluated using agar disc-diffusion and broth microdilution methods. All the cultivars were noted to be strongly active against all the bacterial strains (Table 3). However, the antibacterial activity of EOs exhibited some variation among the six different lavender cultivars and bacterial strains. The antibacterial activity of most of the EOs was higher than that of the standard antibiotics tested. The inhibition zone diameters of the EO

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Table 2. Percentage composition (%) of essential oil from different cultivars: various authors in comparison with our study

	Cultivar/ Province	Harvest time	Linalool	Linalyl acetate	α-Terpineol	Geranyl acetate	Neryl acetate	Terpinen-4-ol
Détár et al. (2020)*	A	2018	40.9	17.7	3.4	1.2	nd	5.2
	Be		50.1	18.7	2.9	0.8	nd	5.5
	Bu		31.3	27.2	6.0	2.2	nd	0.4
	Hib		25.7	42.1	4.2	1.4	nd	0.9
	Mai		55.4	25.3	3.3	1.3	nd	0.1
	Mu		29.5	25.2	4.2	1.8	nd	7.3
Küçük et al. (2019)	Burdur-Yubileina	2017	38.9	16.3	3.6	1.8	1.0	8.3
	Burdur-Sevtapolis		43.0	15.4	3.3	1.7	1.0	3.0
Karık et al. (2017)	Munstead	2015–2016	21.34	52.84	1.02	0.94	0.78	4.89
	Hidcote		20.70	54.58	0.96	0.73	0.63	4.08
Pistelli et al. (2017)	Maliette_14		48.4	26.0	5.7	2.7	nd	0.1
	Maliette_15		45.5	26.2	6.6	3.4	nd	0.2
Zagorcheva et al. (2016)	min**	2015	12.16	27.25	0.01	1.09	0.76	0.01
	max**		31.67	51.44	0.33	2.06	1.23	7.82
Kara and Baydar (2010)	Raya	2009	28.5	25.7	3.61	8.80	2.97	3.96
	Raya	2010	37.3	19.7	3.46	1.85	6.5	4.94
	Munstead	2009	42.5	7.45	0.97	3.22	1.14	19.4
	Munstead	2010	37.8	8.98	1.09	1.13	4.36	19.5
	Vera	2009	38.1	11.4	2.44	3.11	0.81	3.27
	Vera	2010	43.9	5.38	1.17	nd	2.27	5.86
	A silver	2009	41.8	14.4	1.83	nd	0.67	1.64
	A silver	2010	43.6	11.2	1.62	0.47	1.58	2.56
Renaud et al. (2001)	min***	1999	2.8	5.1	nd	nd	nd	nd
	max***		38.3	16.8	nd	nd	nd	nd

nd – not detected; *Cultivars: A silver – Aromatico silver; Be – Beate; Bu – Budakalaszi; HİB – Hidcote Blue; Mai – Mailette; Mu – Munstead. **Minimum and maximum amount of six Bulgarian lavender cultivars. ***Minimum and maximum of six cultivars of lavender

extracts ranged from 14.5 ± 2.10 to 36.5 ± 1.20 mm, while MIC and MMC ranged from 6.25 to 50 µg/mL and 6.25 to 31.25 µg/mL, respectively. For bacteria, the strongest activity of cv. Hemus was observed against *E. faecalis* (IZ = 19 ± 0.10 mm, MIC = MMC = 6.25 (µg/mL), while the weakest potency was seen against the gram-negative *S. enterica* (IZ = 21 ± 1.00 mm, MIC = MMC = 31.25 (µg/mL) (Tables 3 and 4). The strongest activity against *B. subtilis* was detected for the essential oils from cvs. Hebar and Raya with MIC = MMC of 6.25 µg/mL. The highest minimal inhibitory concentration was detected for cv. Druzhba of 50 µg/mL against *E. coli*, *E. faecalis*, *S. aureus* and *S. enterica*. Similar to the results, it was reported that the lavender EO has strong antimicrobial activity against gram-positive bacteria such as *B. subtilis*, *S. aureus* and gram-negative bacteria including *E. coli* (Saeed et al. 2023). Regarding *Lactobacillus* strains, it was observed that EO from cv. Hemus showed the same effect with IZ ranging from 19 ± 0.01 mm to 20.5 ± 0.70 mm and MIC = MMC = 6.25 µg/mL, except for *L. pentosus* and *L. rhamnosus* which exhibited a comparatively higher MMC value (MMC = 25 µg/mL). However, it was detected that all lavender extracts had the same effect with IZ ranging from 14.5 ± 2.10 mm to 30 ± 0.01 mm for *L. acidophilus* and *L. plantarum* and MIC = MMC = 6.25 µg/mL. The results show that MIC values were often equivalent to MMC, indicating the microbicidal action of the studied EO extracts. According to Najar et al. (2022), the antimicrobial activity detected in the studied EO extracts might

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Table 3. Antimicrobial activity (zone of inhibition, mm) against different bacteria and yeasts of different extracts from six cultivars of lavender

Microorganism	Essential oil						Antibiotic	
	He	Ra	Yu	Sev	Heb	Dru	Vancotek (5 µg)	Gentamisin (5 µg)
Bacillus subtilis	37 ± 0.01	29 ± 0.10	16 ± 1.41	17.5 ± 2.10	29 ± 0.01	23.5 ± 1.00	nd	25 ± 0.01
Escherichia coli	22 ± 0.99	28 ± 0.10	25 ± 2.83	24 ± 0.01	24 ± 1.00	26.5 ± 1.73	30 ± 0.01	nd
E. faecalis	19 ± 0.10	23 ± 0.01	24 ± 0.01	27 ± 2.80	28 ± 1.40	30 ± 0.01	nd	32 ± 0.01
Lactobacillus acidophilus	20.5 ± 0.70	24.5 ± 0.70	15 ± 0.01	17 ± 0.01	30 ± 0.01	30 ± 0.01	15 ± 0.01	15 ± 0.02
L. plantarum	19 ± 0.01	23.3 ± 0.60	14.5 ± 2.10	18 ± 0.01	19 ± 1.00	19 ± 1.50	10.5 ± 0.60	nd
L. pentosus	25 ± 1.00	20.5 ± 0.70	25.5 ± 0.70	27 ± 0.01	8.5 ± 0.70	36.5 ± 1.20	13.5 ± 0.90	15 ± 0.01
L. rhamnosus	25.6 ± 0.60	22.3 ± 1.20	34 ± 0.01	21.7 ± 1.50	30 ± 0.60	33 ± 1.00	13.5 ± 0.60	14 ± 0.01
Staphylococcus aureus	26 ± 1.41	33 ± 1.40	35 ± 1.41	30 ± 0.01	24 ± 2.00	22 ± 2.12	nd	33 ± 0.01
S. enterica	21 ± 1.00	16 ± 0.01	20 ± 0.01	19 ± 1.40	14.5 ± 2.10	20 ± 1.41	16 ± 0.01	nd
Candida albicans	nd	nd	nd	nd	nd	nd	–	–
C. glabrata	nd	nd	nd	nd	nd	nd	–	–

nd – not detected. Lavender cultivars: He – Hemus; Ra – Raya; Yu – Yubileina; Sev – Sevtapolis; Heb – Hebar; Dru – Druzhba

be explained by the presence of linalool and linalyl acetate (monoterpenes) at high concentrations. The antimicrobial efficiency of monoterpenes was well documented in many studies (Carson and Riley 1995, Sellam et al. 2013, Moumni et al. 2020, Soulaimani et al. 2021). Linalyl acetate is reported to have antimicrobial activity, especially against gram-negative and gram-positive bacteria such as *E. coli* (MIC = 5 µg/mL) nd *S. aureus* (MIC = 1.25 mg/mL), respectively (Trombetta et al. 2005). Linalool is also known for its marked antibacterial activity against gram-negative bacteria such as *E. coli* (Randrianarivelo et al. 2009, Khalil et al. 2018). Antibacterial activity of linalool was also proven against *E. coli* O157:H7 (Zengin and Baysal 2014) and *S. aureus* (Silva et al. 2015). Gismondi et al. (2021) showed that the *Lavandula angustifolia* essential oil inhibited *Staphylococcus* species, including *S. aureus*, in the hospital ward. In addition to this, Tardugno et al. (2019) reported that the highest antimicrobial activity on *Listeria monocytogenes* and *Salmonella enterica* were detected as the samples rich in the specific constituents as linalool (38.17% and 61.98%). Among the cultivars, cv. Sevtapolis was more potent, with MIC

Table 4. Minimal inhibitory concentrations (MIC) and minimal microbicidal concentrations (MMC) of different lavender cultivars essential oil (µg/mL)

Microorganism	MIC						MMC					
	He	Ra	Yu	Sev	Heb	Dru	He	Ra	Yu	Sev	Heb	Dru
Bacillus subtilis	18.75	6.25	31.25	12.5	6.25	43.75	18.75	6.25	31.25	12.5	6.25	18.75
Escherichia coli	18.75	37.5	31.25	6.25	31.25	50	18.75	37.5	31.25	6.25	31.25	18.75
E. faecalis	6.25	31.25	6.25	6.25	18.75	50	6.25	31.25	6.25	6.25	18.75	6.25
Lactobacillus. acidophilus	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
L. plantarum	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
L. pentosus	25	37.5	6.25	6.25	37.5	6.25	25	37.5	6.25	6.25	37.5	25
L. rhamnosus	25	31.25	6.25	6.25	37.5	50	25	31.25	6.25	6.25	37.5	25
Staphylococcus aureus	12.5	6.25	6.25	6.25	6.25	50	12.5	6.25	6.25	6.25	6.25	12.5
S. enterica	31.25	12.5	6.25	6.25	6.25	50	31.25	12.5	6.25	6.25	6.25	31.25

Lavender cultivars: He – Hemus; Ra – Raya; Yu – Yubileina; Sev – Sevtapolis; Heb – Hebar; Dru – Druzhba

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values ranging from 6.25 to 12.50 µg/mL, while cv. Druzhba MIC values ranged from 6.25 to 50.00 µg/mL. These results could be explained by the difference in the mean percentages of some constituents, such as oxygenated or hydrogenated monoterpene, in these EOs. The antimicrobial activity of monoterpenes may result from increasing membrane fluidity and alteration of bacterial membrane permeability (Moo et al. 2021). Additionally, previous studies reported that water solubility and hydrogen bonding capacity are the main factors influencing MIC values and antibacterial mechanisms of terpenoids (Griffin et al. 1999, Zengin and Baysal 2014, Van de Vel et al. 2019, Moumni et al. 2020). However, it is a fact that the antimicrobial activity of EOs cannot be the result of only the major components but also all components of their biological activity (Moumni et al. 2020). EOs are a complex compound mixture, usually ranging from 20 to 60, at different concentrations (Chouhan et al. 2017, Guimarães et al. 2019). The minor constituents, such as oxygenated monoterpenes like eucalyptol (1,8-cineole) and camphor, or other monoterpenoids like terpineol, ocimene, and caryophyllene which are present in the studied EOs at different concentrations could provide significant contributions to the antimicrobial activity as was previously reported (Dorman and Deans 2000, Viljoen et al. 2003, Randrianarivelo et al. 2009, Moumni et al. 2020). Previous studies reported that minor constituents like 1,8-cineole and limonene had antimicrobial activity against bacteria such as *B. subtilis*, *Salmonella enteritidis*, *S. aureus* and *E. coli* (Soković et al. 2010, Li et al. 2014, Miladinović et al. 2015, Khalil et al. 2018, Moo et al. 2021).

Furthermore, previous results clearly demonstrated that EOs show stronger antimicrobial activity than that of their major components or their mixtures which propounds the synergistic effects of minor constituents (Griffin et al. 1999, Mourey and Canillac 2002, Moumni et al. 2020, Soulaimani et al. 2021). It should also be remembered that although these compounds' antimicrobial activity was highlighted in many previous works, their minimal microbicidal concentration varies based on which plant the extracts were derived from or which compounds exist together.

Principal components analysis. EO components from the six different cultivars of lavender were analysed using principal component analysis (PCA) (Figure 1). The first principal component (PC1) has the maximum variance. Thus, it accounts for the most significant variance in the data. Two principal components explained 98.29% of the overall variance (92.3% and 5.99% for PC1 and PC2, respectively). PC1 had positive correlations with linalool, linalyl acetate and total esters. The other average EO components obtained were collected in the centre of the coordinate. The biplot generated from PC1 and PC2 indicates that EO components from different cultivars were collected under three subgroups. Cvs. He

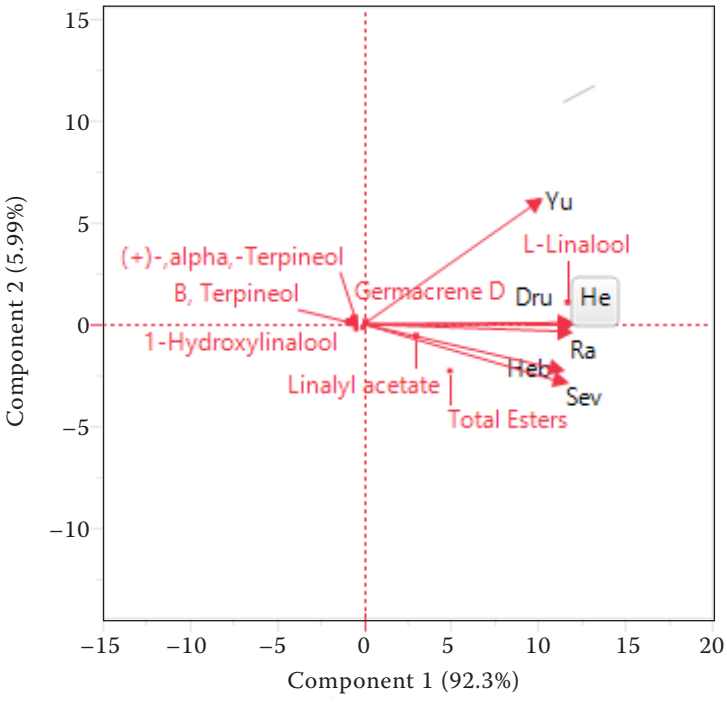

Figure 1. Principal component analysis of essential oil components from six different cultivars of lavender. Lavender cultivars: He – Hemus; Ra – Raya; Yu – Yubileina; Sev – Sevtapolis; Heb – Hebar; Dru – Druzhba

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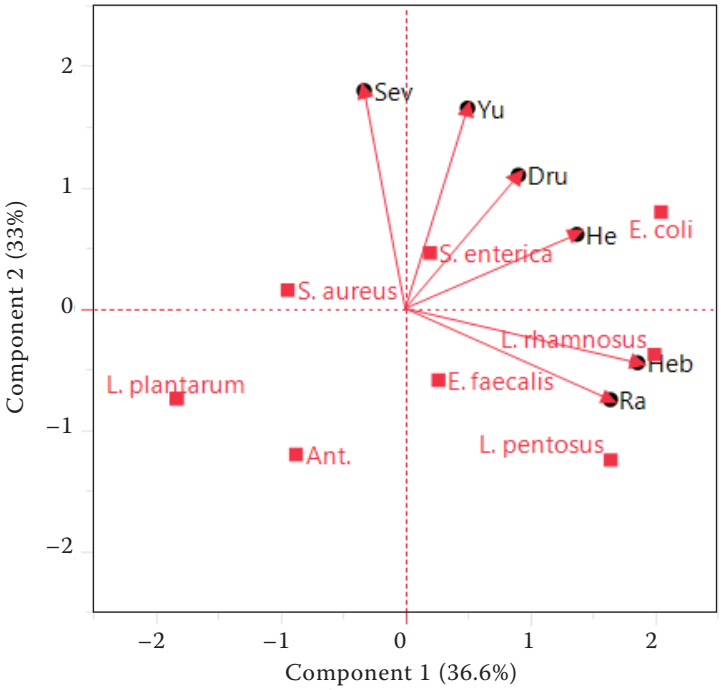

Figure 2. Principal component analysis of minimal inhibitory concentrations) for six different cultivars of lavender. Lavender cultivars: He – Hemus; Ra – Raya; Yu – Yubileina; Sev – Sevtapolis; Heb – Hebar; Dru – Druzhba

and Sev were situated in the lower right quadrant of the plot. The second subgroup, cvs. Dru, Ra and He, were situated in the centre right quadrant of the plot. The third subgroup, cv. Yu, was situated in the high right quadrant of the plot.

MIC for EOs from six different cultivars of lavender were analysed using principal component analysis (PCA) (Figure 2). The first two principal components (PC1 36.6% and PC2 33%) represented 69.6% of the variation. The biplot generated from PC1 and PC2 indicated that MICs of different cultivars of lavender EOs were collected under three subgroups. The first subgroups of cvs. Heb and Ra were characterised by *L. rhamnosus*, *E. faecalis* and *L. pentosus* and were

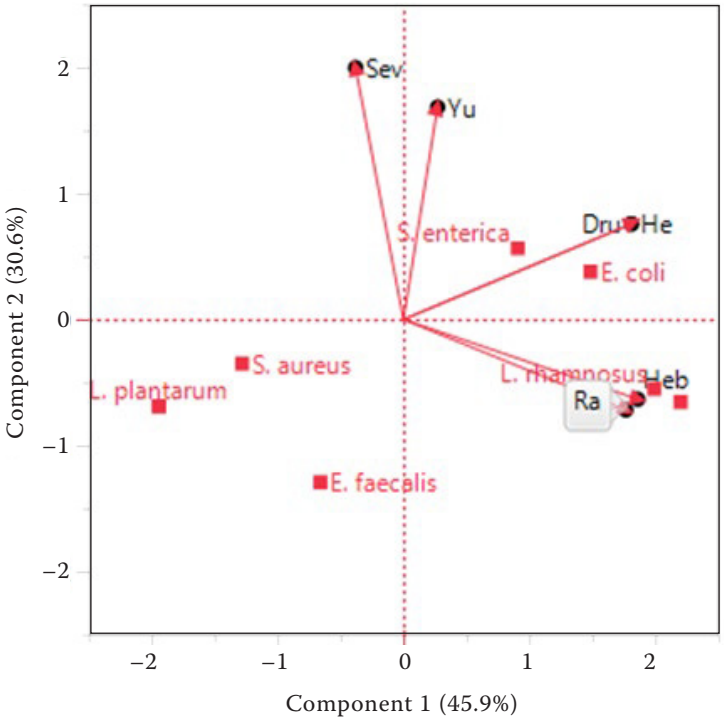

Figure 3. Principal component analysis of minimal microbicidal concentrations for six different cultivars of lavender. Lavender cultivars: He – Hemus; Ra – Raya; Yu – Yubileina; Sev – Sevtapolis; Heb – Hebar; Dru – Druzhba

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situated in the lower right quadrant of the plot. The second subgroup of cvs. Yu, Dru and He were characterised by *E. coli* and *S. enterica* and situated in the plot's top right quadrant. The third subgroup of cv. Sev was characterised by *S. aureus* and was situated in the top left quadrant of the plot.

MMC of EO from six different cultivars of lavender were analysed using principal component analysis (PCA) (Figure 3). The first two principal components (PC1 45.9% and PC2 30.6%) represented 76.5% of the variation. The biplot generated indicated that MMC for EO from different cultivars of lavender were collected under three subgroups. The first subgroup of cvs. He and Ra was characterised by *L. rhamnosus*, and was situated in the lower right quadrant of the plot. The second subgroup of cvs. Dru and He was characterised by *E. coli* and *S. enterica* and was situated in the top right quadrant of the plot. The third subgroup of cvs. Yu and Sev was situated in the top left quadrant of the plot.

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