Chemical composition and biological activity of lavandin and lavender essential oils

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Received: May 23, 2022
Accepted: July 20, 2022

The main object of our study was an investigation of the chemical composition, antioxidant, and antimicrobial activity of three *Lavandula* essential oils (two lavandin oils cv. Grosso and Budrovka, and one lavender cv. Hemus). The oils were produced by hydrodistillation from plant material organically planted in the region of North Macedonia. The gas chromatography-mass spectrometry technique was applied for the identification and quantification of 93 compounds. The main components in all three varieties were linalool and linalyl acetate. The highest amount of linalool was quantified in essential oils in lavandin cv. Budrovka (35.55%), while the smallest amount was measured in lavender cv. Hemus (21.20%). The highest amount of linalyl acetate was detected in the sample of lavandin cv. Grosso (30.49%). Although there is no relationship between antioxidant activity of lavandin and lavender essential oils measured by DPPH and ABTS radicals, results from antimicrobial activity showed statistically higher antibacterial activity of lavandin essential oils against *z aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). The strongest antifungal activity against *Candida albicans* (ATCC 1023) was measured by lavandin essential oil cv. Budrovka.

Keywords: *Lavandula* essential oil, lavandin, lavender, gas chromatography, chemical composition, antioxidant activity, total phenolic content (TPC), antimicrobial activity.

1. INTRODUCTION

The genus *Lavandula*, commonly known as lavender, is one of the economically important members of the Lamiaceae (*Labiatea*) family, comprising about 39 species and 400 varieties [1-3]. It is stated in some sources that etymologically the name “lavender” comes from the Latin verb “lavare” which means “to wash” or “to bathe”, as lavender was used for cleaning and disinfecting externally in addition to its internal use for disease treatment for many years [4]. This genus includes spontaneous and cultivated forms, widely distributed across the Mediterranean Basin, Canary Islands, Cape Verde Islands, North Africa, Southwest Asia, Arabian Peninsula, and tropical NE Africa and India [5-7]. Within this genus, many species are highly aromatic due to the presence of essential oils that are of important economic value for the perfume, cosmetic, flavouring, and pharmaceutical industries [8, 9]. Several lavender EOs are largely used in aromatherapy as antioxidant, antimicrobial, carminative, spasmolytic, sedative, antiseptic, anti-inflammatory, analgesic properties, antioxidant activity tonic, and anti-depressive agents [10-13]. Three Lavandula species are principally cultivated to produce essential oils: *L. angustifolia* (fine lavender), *L. latifolia* (spike lavender), and the sterile hybrid *L. intermedia* (lavandin). In the food industry *L. angustifolia*, well known as “true lavender” or “English lavender” is a small shrub that produces essential oil with a highly fragrant, refreshing, sweet, balsamic herbaceous odour imparting a sense of “clean” and woody undertones, which is frequently used as a flavouring agent for beverages, ice cream, candy, baked goods, chewing gum, etc [14-17]. In

general, the lavender essential oil is well known and the literature data state that the oil yield is up to 3.3%, but these data vary depending on the plant parts involved in the distillation process [18, 19]. Lavender doesn't contain only essential oils but also anthocyanins, phytosterols, sugars, minerals, coumaric acid, glycolic acid, valeric acid, and its esters, ursolic acid, herniarin, coumarin, and tannins [20].

The chemical composition of lavender oils depends largely on the species from which it was obtained. Several studies concerning the chemical composition of the essential oils of *L. stoechas* from some Mediterranean regions (e.g., Morocco, Corsica, Greece, and Turkey) highlight that the most common chemotype of the species is camphor–fenchone [24-27]. Some authors also report a fenchone–1,8-cineol chemotype and a pulegone chemotype [28-35]. The species *L. stoechas* presents a chemical composition quite different from other species like *L. dentata, L. angustifolia, L. latifolia* and *L. hybrida* [36-38]. The composition of essential oils is an important parameter for the qualitative evaluation of aromatic species. Essential oils are significantly influenced by abiotic (climatic, soil, topographic, agronomic, and post-harvest techniques) and biotic factors (plant age, stage of development, genetic characteristics) [39-42]. The main compounds in the *Lavandula* essential oils include a considerable number of bioactive constituents, such as monoterpenes, sesquiterpenes, diterpenes, triterpenes, polyphenols, and coumarins [42-46]. Cytotoxic activities have been attributed to the pre-eminence in many lavender oils of some monoterpenoids, including linalool, linalyl acetate, 1,8-cineole, β-ocimene, terpinen-4-ol, and camphor. According to some authors, the antimicrobial and antifungal activity of Lavandula essential oils is caused by the content of linalool. They have a strong antibacterial effect against *S. aureus*, *E. coli,* and *P. aeruginosa* [46-50].

The aim of this study was to estimate differences in biological activity between lavender and lavandin essential oils and to mark the most important components in the oils which contribute to the biological activity. Therefore, the determination of the chemical composition, antioxidant, and antimicrobial potential of *Lavandula* essential oils obtained from three cultivars was performed. The essential oils from two cultivars "Grosso" and "Budrovka" belong to the lavandin varieties and "Hemus" cultivar belongs to the lavender variety. The herbs were organically planted in the region of North Macedonia and this is the first report on the chemical composition, antioxidant and antimicrobial potential of *Lavandula* species growth in the region of North Macedonia.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

Flowering stalks of lavandin and lavender were obtained from harvests conducted on 15th June 2021 at an organically cultivated trial located near Štip, Republic of North Macedonia (41°46'15.01"N, 22°5'41."E). In this experimental year, 175 L/m^2 rainfall amount was recorded. The samples were taken from three different *Lavandula* cultivars namely *L.* × *intermedia* cv. Grosso, *L. intermedia* cv. Budrovka and *L. angustifolia* cv. Hemus in the full flowering stage. At the time samples were taken the trial was in the fourth year of vegetation. The plant specimens were identified and authenticated by Dr. Ljupčo Mihajlov, from the Faculty of Agriculture, University "Goce Delčev"-Štip. The plant specimen vouchers of three observed cultivars are deposited in the herbarium of the Institute for Medicinal Plants Research "Dr. Josif Pančić" (Belgrade, Serbia) under numbers IPLB#210615_23-32.

2.2. SAMPLE PREPARATION

The collected plant samples were air-dried and grounded. The amount of 50 g of each variety separately was placed in the round-bottom flask (1 L) and subjected to hydrodistillation for 2.5h in the Clevenger-type apparatus according to the European Pharmacopoeia [51]. After the distillation process oil samples were dried through anhydrous sodium sulphate and collected for further analysis. For GC and GCMS analyses 20 µL of oil were dissolved in 2 mL of EtOH, while for the antioxidant and antimicrobial activities the essential oils were dissolved in hexane for DPPH analysis and other appropriate solvents for TPC and TEAC assay as described in sections 2.4. and 2.5.

2.3. GC AND GCMS ANALYSES

The chemical composition of the essential oils was analysed using the GC technique coupled with GCMS. Analyses were performed on a Shimadzu GCMS-QP2010 ultra mass spectrometer fitted with a flame ionic detector and coupled with a GC2010 gas chromatograph. The InertCap5 capillary column (60.0 m × 0.25 mm × 0.25 µm) was used for separation. Helium (He), at a split ratio of 1:5 and a linear velocity of 35.2 cm/s was used as carrier gas. Initially, the oven temperature was 60°C, which was held for 4 min, then increased to 280°C at a rate of 4°C/min, and held for 10 min. The injector and detector temperatures were 250°C and 300°C, respectively. The ion source temperature was 200°C. The identification of the constituents was performed by comparing their mass spectra and retention indices (RIs) with those obtained from authentic samples (homologous series of *n*-alkanes C8-C32) and/or listed in the NIST/Wiley mass-spectra libraries, using different types of searches (PBM/NIST/AMDIS) and available and available literature data [52, 53].

2.4. TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY

The total phenolic content (TPC) of essential oils was determined with Folin–Ciocalteau reagent. For each

sample, 10 µL of diluted (1:100) oil were added to 750 µL water and 50 µL of Folin–Ciocalteu reagent. The solution with a total volume of 850 µL was incubated in the dark for 5 min. Then, 150 µL of 20% sodium carbonate solution was added and samples were incubated in the dark for 1 h. The reference solution was prepared with distilled water instead essential oils and treated with the Folin–Ciocalteu reagent in the same way as the assayed samples. The samples turned to a blue colour with different degrees, depending on the content of phenolic compounds in the samples. The absorbance at 750 nm was recorded against the absorbance of the reference solution. The measurements were performed in duplicate. The content of total phenolic compounds was calculated using a calibration curve of gallic acid (the linearity range: 5-50 mg/100 µL).

The Trolox equivalent antioxidant assay (TEAC) employed in this study gives a measure of the antioxidant activity. The chromophore ABTS (2,2'-azino-bis (3- ethylbenzothiazoline-6-sulphonic acid)) was dissolved in distilled water to 6.8 mM concentration and left the mixture to stand in dark at room temperature for 14 h before use. ABTS radical cation (ABTS•+) was produced by reacting with 5.2 mM potassium persulphate solution. After forming of ABTS radical cation, the solution was diluted with water in a 1:8 v/v ratio to 0.6 mM. The concentration of the resulting blue green ABTS radical solution was adjusted to an absorbance of 0.80 ± 0.020 at 735 nm. A 328 µL volume of reagent is pipetted into a quartz cuvette with the subsequent addition of 10 µL of essential oil. The decrease in absorbance at 735 nm was measured after 30 min and incubation at 37°C. The estimation of the antiradical activity with TEAC assay was calculated using a calibration curve of Trolox with different concentrations (1 - 20 mg/L) dissolved in methanol and was used as a standard for the preparation of the calibration curve. Trolox equivalent antiradical capacity (TEAC) was expressed as a percentage of decolorization of ABTS radical cation (ABTS•+).

For the DPPH assay, the antioxidant activities of the essential oils were expressed as a percentage of decolorisation of a solution of the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical) at 517 nm. DPPH reagent was dissolved in hexane and 0.25 mL of the stock solution with a concentration of 0.5M has used for the determination of the antioxidant activity. BHA (butylated hydroxyanisole) with different concentrations (10–100 mg/L) was dissolved in hexane and was used as a standard for the preparation of the calibration curve. The measurements for the oils were performed by direct incorporation of 5 mL of pure essential oil to 495 mL of DPPH reagent.

2.5. ANTIMICROBIAL ASSAYS

The samples of essential oils were investigated for their "*in vitro*" antibacterial and antifungal properties using a disk-diffusion method in Petri dishes. The essential oils were tested for antibacterial activity against one Gram-positive bacterial strain *S. aureus* (ATCC 25923), against one Gram-negative bacterial strain *E. coli* (ATCC 25922), and for antifungal activity using *C. albicans* (ATCC 1023). For this purpose, 5 µL of each essential oil or 4.42×10^3 µg (calculated by relative density 0.885 g/cm^3) was tested and compared by antimicrobial activity of commercial antibiotics.

In brief, each suspension of microorganisms was suspended in Mueller Hinton (MH) broth. Furthermore, the suspension of microorganisms is diluted by using the McFarland scale. An inoculum equivalent to the no. 1 of the McFarland scale was prepared and diluted approximately to 10^6 colonies forming unit (cfu)/mL. They were "flood-inoculated" onto the surface of MH agar and MH Dextrose Agar (MDA) and then dried. Six-millimetre diameter wells were cut from the agar using a sterile cork-borer, and 60 µL of each sample of *Lavandula* essential oils were delivered into the wells. The plates were incubated at 37 °C and the diameters of the growth inhibition zones were measured after 24 h. Gentamicin (70 µg/well), nalidixic acid (80 µg/well), ciprofloxacin (15 µg/well), and erythromycin (30 µg/well) were used as a positive control. The controls were performed with only sterile broth and with only overnight culture and 10 µL of 70% ethanol.

The antibacterial activity is ranked from no activity (-, inhibition diameter < 10 mm), low (+, inhibition diameter between 10 and 15 mm), moderate (++, inhibition diameter between 15 and 20 mm), and high activity (+++, diameter inhibition ≥ 20 mm). All tests were performed in triplicate and clear halos greater than 10 mm were considered positive results. The antibacterial and antifungal activity tests of Lavandula essential oils from three varieties are shown in Table III.

2.6. STATISTICAL ANALYSES

All observations were done in triplicate. Differences among observed chemical composition, antioxidant, and antimicrobial activity of three essential oils (two lavandin oils and one lavender oil), were estimated by one-way ANOVA, followed by *post hoc* Tukey's test at $P < 0.05$ level of significance. Statistically significant differences between mean values were denoted by different row-wise letters (a-c). All observations of biological activities were done in triplicate. Statistical analysis was conducted using the R CRAN software package.

3. RESULTS AND DISCUSSION

3.1. CHEMICAL COMPOSITION OF *LAVANDULA ESSENTIAL* OILS

The two major compounds in all three samples (two samples of lavandin and one sample of lavender) were linalool and linalyl acetate. According to the results in Table I, the highest amount of linalool was quantified in lavandin cv. Budrovka (35.55%), while the small-

Table I - Chemical composition of the essential oils of three *Lavandula* cultivars

			L. x intermedia		*L. angustifolia*
			cv. Grosso [b]	**cv. Budrovka**	**cv. Hemus**
#	Compound	RI [a]	%m/m	%m/m	%m/m
1	3-*cis*-hexenol	858.0	0.01±0.00a	-	-
2	*n*-hexanol	867.5	0.06±0.01b	0.33±0.03a	-
3	tricyclene	919.2	0.02±0.00b	0.03±0.00b	0.07±0.01a
4	α-thujene	922.2	0.11±0.03a	0.03±0.01b	0.18±0.02a
5	α-pinene	930.2	0.81±0.04a	0.40±0.04b	0.77±0.05a
6	camphene	947.2	0.41±0.02b	0.35±0.01b	0.76±0.02a
7	thuja-2,4(10)-diene	952.3	-	0.02±0.01a	-
8	sabinene	972.3	0.32±0.05a	0.08±0.01c	0.12±0.02b
9	1-octen-3-ol	975.7	-	0.36±0.07a	0.04±0.01b
10	β-pinene	977.6	1.14±0.04a	0.25±0.03b	0.29±0.04b
11	3-octanone	980.8	0.03±0.01c	0.08±0.02b	0.32±0.06a
12	myrcene	986.6	0.68±0.10a	0.13±0.02b	0.78±0.12a
13	butyl butanoate*	988.8	0.05±0.01b	0.07±0.02b	0.12±0.01a
14	dehydro-1,8-cineole	989.4	-	0.03±0.00a	-
15	3-octanol	991.4	0.02±0.00a	0.01±0.00b	-
16	α-phellandrene	1001.6	0.02±0.00b	0.01±0.00b	0.09±0.01a
17	hexyl acetate	1004.4	0.18±0.04b	0.12±0.02b	0.37±0.08a
18	δ-3-carene	1007.3	0.08±0.01b	0.03±0.00b	1.02±0.05a
19	α-terpinene	1012.4	0.01±0.00b	-	0.04±0.01a
20	*p*-cymene	1016.2	0.01±0.00b	0.03±0.01b	0.14±0.05a
21	*o*-cymene	1019.0	0.22±0.01b	0.44±0.02a	0.40±0.03a
22	limonene	1023.1	0.64±0.13b	0.60±0.13b	0.93±0.12a
23	1,8-cineole + *trans*-β-ocimene	1030.8	12.48±0.02b	12.11±0.07b	15.59±0.09a
24	*cis*-β-ocimene	1037.8	0.12±0.03b	0.04±0.01c	3.34±0.07a
25	γ-terpinene	1051.6	0.04±0.00b	0.02±0.00b	0.14±0.03a
26	*cis*-sabinene hydrate (IPP vs OH)	1061.4	0.19±0.02b	0.35±0.08a	0.06±0.00c
27	*cis*-linalool oxide (furanoid)	1065.6	0.12±0.02b	4.96±0.08a	0.12±0.01b
28	*p*-mentha-2,4(8)-diene	1079.2	-	-	0.07±0.00a
29	terpinolene	1081.9	0.21±0.04a	-	0.15±0.03b
30	*trans*-linalool oxide (furanoid)	1084.0	-	4.34±0.07a	0.05±0.00b
31	linalool	1102.0	30.19±2.04b	35.60±1.06a	21.11±3.5c
32	1-octen-3-yl acetate	1099.2	-	0.08±0.03b	0.55±0.11a
33	*trans*-*p*-mentha-2,8-dien-1-ol	1118.0	-	0.03±0.00a	-
34	*allo*-ocimene	1120.0	0.02±0.00c	0.08±0.01a	0.04±0.00b
35	α-campholenal	1123.5	0.04±0.00b	0.05±0.01b	0.20±0.04a
36	*cis*-*p*-mentha-2,8-dien-1-ol	1133.3	-	0.02±0.00b	0.04±0.01a
37	hexyl isobutanoate*	1136.6	0.14±0.02b	0.30±0.03a	0.08±0.01c
38	*trans*-pinocarveol	1137.5	-	-	0.03±0.00a
39	camphor	1146.2	5.64±1.12b	8.82±1.03a	0.59±0.09c
40	camphene hydrate	1149.4	0.02±0.00b	0.06±0.01a	-
41	isoborneol	1156.7	0.01±0.00b	0.05±0.01a	-
42	lavandulol	1159.8	0.39±0.10c	0.48±0.07b	0.52±1.12a
43	borneol	1164.8	-	-	1.81±0.03a
44	terpinen-4-ol	1180.1	3.03±0.09b	10.90±1.14a	3.90±0.05b
45	hexyl butanoate*	1181.8	2.80±0.09b	2.58±0.02b	0.33±0.03a
46	cryptone	1184.6	0.38±0.06b	1.02±0.03a	0.18±0.08c
47	*p*-cymen-8-ol	1191.5	0.03±0.00a	0.59±0.03a	-
48	α-terpineol	1191.5	0.71±0.11a	0.22±0.02b	0.95±0.08a
49	myrtenal	1194.8	-	0.11±0.02a	-
50	hexyl 2-methyl butanoate*	1229.1	0.01±0.00b	0.13±0.02a	-
51	nerol	1224.7	0.01±0.00b	0.04±0.00b	0.11±0.02a
52	isobornyl formate	1231.1	0.12±0.03b	0.44±0.08a	0.13±0.03b
53	hexyl isovalerate*	1233.6	0.11±0.08b	0.18±0.07a	-
54	cumin aldehyde	1242.4	0.02±0.01c	0.19±0.91a	0.06±0.01b
55	carvone	1245.6	-	0.12±0.02a	0.03±0.01b
56	linalool acetate	1253.4	30.97±2.34a	10.23±1.18c	26.62±4.52b
57	carvenone	1258.4	0.02±0.00b	0.07±0.02a	0.07±0.01a
58	lavandulyl acetate	1284.8	2.55±0.08b	1.09±0.08c	5.79±1.12a
59	bornyl acetate	1289.0	0.03±0.01c	0.11±0.01b	0.25±0.06a

Table I continue

			L. x intermedia		*L. angustifolia*
			cv. Grosso	**cv. Budrovka**	**cv. Hemus**
60	*p*-cymen-7-ol	1291.3	0.01±0.00b	-	0.03±0.00a
61	3-thujanol acetate	1293.1	0.01±0.00b	0.04±0.01a	0.03±0.00a
62	carvacrol	1295.3	-	0.04±0.01a	-
63	hexyl tiglate	1314.2	0.14±0.03a	0.02±0.00b	0.04±0.00b
64	*cis*-piperitol acetate	1334.9	0.03±0.00b	0.14±0.03a	-
65	linalool propanoate	1337.2	-	0.02±0.00a	0.03±0.00a
66	neryl acetate	1345.3	0.11±0.04b	0.04±0.01c	0.22±0.05a
67	geranyl acetate	1362.9	0.14±0.04b	0.03±0.00c	0.28±0.06a
68	hexyl hexanoate*	1364.8	-	0.14±0.02a	-
69	daucene	1373.2	0.09±0.01a	0.01±0.00b	-
70	2-epi-α-funebrene	1377.0	0.08±0.01a	0.02±0.00b	-
71	β-bourbonene	1380.8	0.04±0.00a	-	-
72	β-elemene	1383.0	0.01±0.00c	0.04±0.00b	0.08±0.02a
73	7-*epi*-sesquithujene	1390.3	0.08±0.01b	0.03±0.00a	-
74	sesquithujene	1402.3	0.06±0.00a	0.02±0.00b	0.07±0.02a
75	α-santalene	1408.1	0.16±0.41b	0.02±0.00c	0.48±0.12a
76	*trans*-caryophyllene	1414.8	1.18±0.06b	0.06±0.00c	4.23±0.09a
77	α-*trans*-bergamotene	1421.4	0.12±0.02b	0.11±0.01b	0.20±0.05a
78	β-copaene	1428.5	0.05±0.01a	0.03±0.00c	0.04±0.01b
79	*cis*-β-farnesene	1438.7	1.15±0.09b	0.02±0.00c	3.37±0.11a
80	*trans*-β-farnesene	1435.8	0.09±0.01b	0.15±0.03a	-
81	α-humulene	1452.9	0.01±0.00c	0.04±0.00b	0.09±0.01a
82	sesquisabinene	1457.6	0.04±0.01a	-	0.04±0.00a
83	*cis*-muurola-4(14),5-diene	1462.1	0.02±0.00b	-	0.13±0.03a
84	dauca-5,8-diene	1468.6	0.02±0.00b	-	0.04±0.00a
85	germacrene D	1483.8	0.40±0.09a	-	0.21±0.04b
86	neryl isobutanoate	1501.3	0.09±0.01b	0.12±0.04a	0.03±0.00c
87	bicyclogermacrene	1501.5	0.05±0.01a	0.01±0.00b	0.04±0.00a
88	γ-cadinene	1514.4	0.17±0.02b	-	0.39±0.10a
89	β-sesquiphellandrene	1516.9	0.08±0.01a	-	-
90	*trans*-calamenene	1521.4	0.01±0.00b	0.02±0.00a	-
91	caryophyllene oxide	1579.1	0.07±0.01b	0.04±0.00b	0.33±0.04a
92	τ-cadinol	1635.1	0.08±0.01a	-	-
93	*epi*-α-bisabolol	1674.1	0.15±0.04a	0.02±0.00b	0.18±0.07a
	Monoterpenes		54.67±4.04	72.53±3.80	54.01±5.73
	Sesquiterpenes		3.90±0.76	0.60±0.04	9.41±0.59
	Esters		37.48±2.80	15.80±1.64	34.32±5.97
	Alcohols		2.99±0.02	4.54±0.09	0.77±0.07
	Aldehydes		0.06±0.01	0.34±0.12	0.25±0.05
	Ketones		0.44±0.08	1.15±0.05	0.54±0.03
	Oxygenated compounds		0.40±0.02	4.60±0.07	0.63±0.08
	SUM of identified		99.94±7.73	99.56±11.8	99.93±12.52

[a] RI, retention indices as determined on HP-5 column using homologous series of C8-C30 alkanes
[b] Different letters next to mean values indicate statistical differences according to the post hoc Tukey's test at the level of P < 0.05 row-wise
* tentative identification

est amount was measured in lavender cv. Hemus (21.20%). On the other hand, the essential oil from cv. Budrovka had the lowest amount of linalyl acetate (10.31%), while the highest amount of this monoterpenoid was detected in the sample of lavandin cv. Grosso (30.49%). Different varieties of narrow-leaved lavender contained the same main compounds (linalool 15.9-23.9%, linalyl acetate 1.2-4.7%, *cis*-ocimene 1.1-2.4%, and lavandulol 3.4-4.6%), however, the compounds found in low concentrations were different, which could affect their biological properties [21-23]. Due to the same examined lavender cultivar, our results presented in Table I had very good similarity with the chemical composition of essential oil of *L. angustifolia* cultivated in Poland [27, 29]. According to their published results, the same components were quantified in the amounts of 30.6% and 14.2% respectively [27]. A statistically significant difference in amounts of 1,8-cineole and β-ocimene was detected in the essential oil of lavender cv. HEMUS (15.62%) while the other two samples of *Lavandula* oils had a similar amount of those compounds. The most important marker for lavandin essential oils was camphor which was quantified in the amounts of 5.70 and 8.77% in "Grosso" and "Budrovka" cultivars, respectively.

The highest abundance of total monoterpenes was identified and quantified for lavandin essential oil from "Budrovka" cultivar (70.24%), while lavandin essential oil from "Grosso" cultivar had the highest percentage of total esters (37.02%). The lavender essential oil from "Hemus" cultivar was the richest source of sesquiterpenes (9.53%) and alcohols (2.49%). Aldehydes, ketones, and other oxygenated compounds were presented in amounts of less than 2%. According to the ISO 3515:2002 standard as quality criteria [21], lavender essential oils should contain linalool (25-38%), linalyl acetate (25-45%), and camphor (0.5-1.0%), while lavandin essential oils should contain linalool (24-35%), linalyl acetate (28-38%), and camphor (6-8%) according to the ISO 8902:2009 [22].

In general, the chemical composition of essential oils from *Lavandula* varieties is affected by fertilizers and environmental and climatic conditions which vary during the harvesting year [4, 8, 12, 17, 24, 25, 31]. In addition, the fenchone chemotype of Sicilian biotypes of *L. stoechas* L. spp. *stoechas* had variations in the chemical composition of essential oils based on the areas of origin of the biotypes (major Sicily Island and minor Pantelleria island) [19]. Furthermore, the extraction techniques significantly influenced the yield and chemical composition of *Lavandula* essential oils. Some studies revealed that the distillation process should be continued for up to 2 hours to obtain optimum oil yields with better quality [24].

The sums of total alcohol and oxygenated compounds were significantly higher for the essential oil of lavandin cv. Budrovka since this oil had the highest amount of terpinene-4-ol and -*cis* and -*trans* linalool oxide (10.85%, 4.95%, and 4.35%, respectively). Although some authors referred to γ-terpinene as a major monoterpene in some varieties of *Lavandula* essential oils (26.8%), our study indicated a significant amount only in lavender cv. "Hemus" (0.14%) [1]. A research group by Wagner reported eucalyptol as a major terpene (34.33%) in the essential oil from *L. dentata* [2]. The main lactone in the essential oil of the aerial parts of *L. atriplicifolia* Benth was C-10 massoia lactone (46.45%) [7]. However, camphor was the main component (26.9%) in the *L. tenuisecta* essential oil [9]. Chemical compositions of essential oils obtained from our lavandin samples (cv. Budrovka and cv. Grosso) were in line with the results published by Rai et al. and Śmigielski et al. [12, 18]. According to their findings, the major component of essential oil from *L. angustifolia* was linalyl acetate and linalool with an amount of over 20% and 14.2%, respectively, and this oil provided a significant improvement in psoriatic conditions in experimental rats [12]. Other oxygenated compounds such as carvacrol were the most dominant in essential oils from wild and cultivated *L. mairei* [16]. One of the best reviews of the chemical composition of *Lavandula* essential oil was published by Eldeghedy et al., [33]. According to their findings, the main constituents in the different species were linalool (39.5%), linalyl acetate (26.7%), eucalyptol (43.08%), cadinol (28.63%), and linalyl acetate (46.41%) for lavender, Fren. lavender, lavandin, *L. angistifolia*, *L. latifolia*, and *L. asp*, respectively [33]. Results from gas chromatography analysis published by different authors showed that there is significant variation between species in the quality and quantity of *Lavandula* essential oil composition. In the work of Adaszynska-Skwirzynska et al., the main components of the essential oils of *L. angustifolia* 'Blue River' and 'Ellagance Purple' obtained from flowers were linalool, linalyl acetate, lavandulol acetate and α-terpineol [34]. Essential oils from leafy stalks contained mainly: borneol, *epi*-bicyclosesquiphellandrene, caryophyllene, eucalyptol, and linalool [34]. The working group of Kucukyumuk studied the effect of nitrogen fertiliser on the lavandin yield [35]. The conclusion was that nitrogen fertiliser has a significant effect, not only on plant growth but, also on lavandin yield. In addition, nitrogen fertilisation increased yields and some quality parameters such as plant height, branch height, length of flower, essential oil content, and affected essential oil components (such as linalool, linalyl acetate, and camphor) [35]. Our results presented in Table I were in good agreement with the chemical profile of *L*. x *intermedia* essential oil published by de Elguea-Culebras et al. [36]. Their results showed that the major compounds of *L*. x *intermedia* essential oil were linalool (38.5%), linalyl acetate (26.2%), and camphor (15.2%) [36, 37]. These three components were selected for a few studies because linalool and linalyl acetate contents are two major constituents, and the low content of camphor is very important because it gives lavender oil an undesirable odour. Usually, linalyl acetate is high in lavender oil while linalool is higher in lavandin oil [38]. Lavandulol and lavandulyl acetate are considered marker compounds for lavender essential oil [39]. In our samples, the lavandulyl acetate quantities were between 0.39 and 0.48% for lavandin and 0.52% for lavender essential oil. The highest amount of lavandulyl acetate was determined in lavender oil from "Hemus" cultivar (5.79%) while the other two examined lavandin essential oils from "Grosso" and "Budrovka" varieties had less than half of the amount (2.55 and 1.09 respectively). However, It is necessary to note that all three examined cultivars did meet the requirements of the Ph. Eur. for the lavandulol content (min. 0.1%).

3.2. ANTIOXIDANT ACTIVITY OF LAVANDULA ESSENTIAL OILS

Considering that essential oils are mixtures of organic compounds, it is difficult to distinguish compounds that play a crucial role in the antioxidant system. The ability of DPPH to neutralise a free radical is frequently consistent with the high level of a diverse group of phenolic compounds. The results from total phenolic content and antioxidant activity measured by two rad-

icals (ABTS and DPPH) indicated significantly higher antioxidant activity of essential oil from lavandin cv. *Budrovka* in comparison to the antioxidant activity of the other two samples (Table II). Statistical analysis from the total polyphenol content indicated no significant difference between the antioxidant activity of essential oils from lavandin cv. Grosso and lavender cv. Hemus (Table II). In the work of Badr et al., examined lavender oils displayed a remarkable antioxidant potential followed in descending order by α-terpinyl acetate, camphene, and α-terpinyl acetate [5]. Relatively low values for total phenolic compounds in our examined essential oils can be explained by the fact that phenolic compounds are concentrated mainly in the plant residue material [28]. New phenolic compounds such as lavandunat, lavandufurandiol, lavandufluoren, lavandupyrones A and B, lavandudiphenyls A and B were isolated by Yadikar et al. [40]. The amount of each of these constituents varies in different species and depends on the genotype, geographical origin, climatic conditions, growing conditions, harvest time, and extraction method. [41]. In the work of Lilia et al., the examined samples of essential oils of *L. stoechas* were very powerful in the case of the reference ascorbic acid which is a strong antioxidant [42]. Nurzyńska-Wierdak and Zawiślak explained high variability of the chemical composition of the examined lavender leaves, flower buds and flowers originate from ontogenetic variability which was related to the complicated transformations of these compounds, where the contents of phenolic compounds were less dependent upon developmental factors [43]. Interesting dependencies also result from the performed analysis of the correlation between the contents of bioactive substances and antioxidant activity against DPPH radical, indicating the significant share of phenolic compounds and essential oil in the antioxidant potential of lavender. A strong negative correlation between flavonoid compounds and antioxidant activity in turn most probably originates from the specific structure of these compounds, and mainly the position of OH radicles. It is well known that *Lavandula* essential oils are rich in oxygenated terpenoids mainly monoterpenes, which have a significant ability to neutralise free radicals. The antioxidant activity of examined raw material (lavender leaves and flowers) may result from the high content of linalool and linalyl acetate in the oil [43].

Table II - The total phenolic content and antioxidant activity results of essential oils from three *Lavandula* cultivars

Cultivars	Total phenolic content[a]	Antioxidant ABTS	Antioxidant DPPH
	[mg GAE/100 g DW]	[mg/L Trolox]	[mg/L BHA]
Grosso	45.2±1.5 b	15.2±1.1 b	50.9±2.3 b
Budrovka	58.9±2.8 a	19.1±2.0 a	59.8±4.9 a
Hemus	39.2±1.9 c	16.8±1.4 b	47.4±1.8 b

[a] Means followed by different letters differ significantly according to Tukey's *post hoc* test at P < 0.05 level

3.3. ANTIMICROBIAL ACTIVITY OF LAVANDULA ESSENTIAL OILS

Although results from antioxidant activity measured by two radicals (DPPH and ABTS) were not statistically linked by lavender and lavandin varieties, the antimicrobial activity was strongly determined. Both samples of essential oils of lavandin had significantly higher antibacterial activity against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) (Table III). Only antifungal activity against *C. albicans* (ATCC 1023) was the highest for lavandin variety cv. Budrovka. This might be explained by the fact that this oil had three times a higher amount of terpinene-4-oil and the highest amount of linalool in comparison to the other two samples. The Working group of D'Auria discovered that lavender oil (2%) killed 100% of the *C. albicans* ATCC 3153 cells within 15 min; linalool (0.5%) killed 100% of the cells within 30 s. According to their findings, essential oil inhibited germ tube formation as did the main components linalool and linalyl acetate. Both the essential oil and its main components inhibited hyphal elongation of *C. albicans* ATCC 3153 (about

Table III - Antimicrobial activity of essential oils from three *Lavandula* cultivars compared with commercial antibiotics as a positive control

Samples	Dosage	*Staphylococcus aureus* (ATCC 25923)	*Escherichia coli* (ATCC 25922)	*Candida albicans* (ATCC 1023)
	[µg]	[mm]	[mm]	[mm]
Cultivars				
Grosso	4.42x10^3 µg	92±3 a	74±2 a	53±1 b
Budrovka	4.42x10^3 µg	97±2 a	77±5 a	57±4 a
Hemus	4.42x10^3 µg	84±4 b	51±3 b	49±5 b
Positive control				
Gentamycin	70 µg	55	48	18
Nalidixic acid	80 µg	49	51	15
Ciprofloxacin	15 µg	32	32	22
Erythromycin	30 µg	79	17	13

[a] Means followed by different letters differ significantly according to Tukey's *post hoc* test at P < 0.05 level
[b] Conversion of 5 µL of oil in µg by their specific gravity (conversion factor 0.885 g/cm^3 at 25ºC)

50% inhibition at 0.016% with each substance). They conclude that linalool is responsible for the fungicidal activity, whereas linalyl acetate appears to be somewhat inhibiting germ tube formation and hyphal elongation [44]. Although our results showed that examined essential oils from *Lavandula* had lower antifungal activity against *C. albicans* (ATCC 1023), the working group of Zuzarte published impressive antifungal activity of essential oil from *L. luisieri* against three *C. albicans* strains (ATCC 10231, D5 and M1) [15]. The same variety of *L. intermedia Grosso* was studied by Moon et al., and they concluded that there is a strong correlation between linalool and linalyl acetate content and its antifungal activity. According to their findings, the lack of correlation between the major oil components and antifungal activity suggests that the different susceptibilities of the fungi may be related to either the minor components of the oil or differences in the cell wall/cell membrane of the fungi themselves [20]. Similar biological activity with Gentamicin as a positive control was obtained from the essential oil of *L. pubescens* [27]. Some authors stated that the vapor of lavender essential oil can exert an antibiotic activity in hospital contexts, especially against *S.* species and methicillin-resistant microorganisms [3, 13]. The latest research study indicated that nanosized *Lavandula* oil droplets of nanoemulsions can facilitate the interaction of the active components with the bacterial membranes and increase their antimicrobial efficacy [6]. The essential oil from *L. angustifolia* showed a significant effect against Gram-negative at concentrations of 5, 10, and 20 mg/mL, and against Gram-positive strains at concentrations of 10 and 20 mg/mL which was attributed to the chemical composition of L. *angustifolia* [14]. According to the finding of the research group of Rashed, the variation in inhibition may be attributed to the cell membrane constituents of bacteria. The cell membrane of Gram-positive bacteria contains an outer peptidoglycan layer which is an ineffective permeability barrier, while the cell membrane of Gram-negative bacteria contains a more effective permeability barrier in the outer membrane consisting of lipopolysaccharides and that is the reason why Gram-negative strains were more resistant than Gram-positive strains [14]. Some authors believe that essential oils affect the cell membrane of microorganisms [30]. That can be explained by the hydrophobicity of essential oils and their components which allows them to accumulate in cell membranes and disturb the structures. This causes impairment of microbial enzyme systems, due to a leakage of intracellular constituents, caused by the increase of membrane permeability [30]. It is interesting to notice that borneol exhibited antimicrobial activity against *B. cereus, E. coli* , and *S. aureus* (MIC ranging from 0.03 to 0.25 mg/mL) and *S. typhimurium* (MIC 0.12 to 800 mg/mL). Almost identical antibacterial activity was found for linalool (MIC 0.25 mg/mL), while 1,8-cineole was inactive against all tested strains [42]. Our results indicated a significant amount of borneol only in lavender essential oils from "Hemus" variety (1.81±0.03). The effect of the foliar application of 24-epibrassinolide (24-eBL), a brassinosteroid analogue, on the growth and secondary metabolite production of lavandin (*L.* x *intermedia* var. Super) was examined [46]. The published results unequivocally confirmed that 24-eBL may be a promising compound for use in lavandin cultivation because of its positive effects on plant growth, phenolic content, essential oil content, and oil quality [47]. The newest findings stated that *L. angustifolia* could be a promising choice for antidiabetic medication by inhibiting the carbohydrate metabolizing enzyme α-glucosidase [48]. Results initially showed promising *in vivo* toxicity of the essential oil on the tissue to achieve an anti-MRSA pharmaceutical use [48]. The latest research in natural insecticides proved that essential oils of some variety of *Lavandula* exhibited a strong fumigant and contact toxicity against *R. dominica* and *S. oryzae* adults. Published results from this field proved that the *Lavandula* essential oils flowering tops could be potentially exploited for the development of new antibacterial, antifungal as well as bio-insecticide products [49]. Promising results from the study of the working group of Miastkowska proved that lavender oils had a strong potential to enhance the local, tissue-derived proinflammatory and pro-regenerative response, while simultaneously limiting the inflammatory stimulation of the immune system cells, with in-house preparation performing significantly better in the in vitro cell models [50].

CONCLUSION

The results from our study showed that the chemical composition of three *Lavandula* essential oils (two lavandin oils cv. Grosso and Budrovka and one lavender cv. Hemus) organically planted in the North Macedonia region is strongly linked to the antioxidant and antimicrobial potential of the oils. Linalool and linalyl acetate were the most abundant compounds among the 93 identified. The difference in the chemical composition can be linked to the statistically higher antibacterial activity of lavandin essential oils against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). Camphor, β-pinene, and *cis*-linalool oxide are components that are predominant in lavandin oils and might be responsible for stronger antibacterial activity. Esters such hexyl hexanoate, hexyl isovalerate, neryl isobutanoate, terpenes such *trans*-β-farnesene and cumin aldehyde were the most abundant in the lavandin essential oil cv. Budrovka, in combination with linalool, might have a synergetic antifungal effect against *C. albicans* (ATCC 1023).

Acknowledgements

This work was supported by the bilateral Austri-

an-North Macedonian project called “Oxidomics-guided development of cold pressed oils rich in antioxidants by improving manufacturing processes” (2022-2023).

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