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Chemical composition, antioxidant and antimicrobial effect of *Lavandula* essential oil used as a natural antioxidant for cold-pressed oils

Sanja Kostadinović Veličkovska*, Dejan Pljevljakušić, Emilija Arsov, Saša Mitrev, Ljupco Mihajlov, Daniela Dimovska and Fidanka Ilieva

*Contact e-mail: sanja.kostadinovik@ugd.edu.mk

Faculty of Agriculture, University "Goce Delčev", Krste Misirkov 10-A, 2000 Štip, North Macedonia

Introduction

Pure lavender infused with sunflower oil become a very popular new product for treating skin and hair. The base of this product is usually sunflower oil, extremely rich in Vitamin E, known to be a very efficient skin antioxidant (Badr et al., 2021). Lavender is highly aromatic due to the presence of essential oil that is of important economic value for the perfume, cosmetic, flavoring, and pharmaceutical industries. Several lavender essential oils are largely used in aromatherapy as antioxidant, antimicrobial, carminative, spasmolytic, sedative, antiseptic, anti-inflammatory, analgesic properties, antioxidant activity tonic, and anti-depressive agents. The cold-pressed sunflower or flaxseed oils are excellent mediums for Lavandula essential oils due to the high level of mono and polyunsaturated fatty acids and Vitamin-E-active compounds such as tocopherols and tocotrienols. This mixture is a powerful antioxidant agent for treating dry skin and hair and can be used for many cosmetic purposes (P., 2022).

Materials and Methods

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 sulfate 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. The chemical composition of the essential oils was analyzed 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.

Lavender infusion in cold-pressed oils

The base of the popular skin and hair product is usually cold-pressed sunflower oil reach with monounsaturated oleic and polyunsaturated linoleic acid. According to the findings of the research group of Kostadinović Veličkovska, cold-pressed sunflower oil is the richest source of α-tocopherol with an amount of over 20 mg/100g, while polyphenols as powerful bioactive compounds were presented at over 60 mg/kg oil (Kostadinović Veličkovska et al., 2015). Phytosterols, primarily β-sitosterol, campesterol, and stigmasterol are membrane constituents of plants that exhibit strong UV protection. Furthermore, their anti-inflammatory effects make them an ideal ingredient for use in products intended for the treatment of atopic eczema and the protection of baby skin. A combination of monoterpenes, sesquiterpenes, esters, and other valuable components from Lavandula essential oil and unsaturated fatty acids, vitamin-E-active compounds, polyphenols, and phytosterols from cold-pressed edible oils can be a promising mixture for dry skin.

Table 1. 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
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	t-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

n.i., stands for not identified compound with molecular weight stated in brackets

* tentative identification

Results and discussion

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 smallest 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%). 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%. 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%) (Table 1). The results from total phenolic content and antioxidant activity measured by two radicals (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 2). 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 higher amount of terpinene-4-oil and the highest amount of linalool in comparison to the other two samples (Table 3).

Table 2. 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

Table 3. Antimicrobial activity of essential oils from three *Lavandula* cultivars compared with commercial antibiotics as a positive control

Samples	Dosage	Staphylococcus aureus	Escherichia coli	Candida albicans
		(ATCC 25923)	(ATCC 25922)	(ATCC 1023)
	[μg]	[mm]	[mm]	[mm]
Cultivars				
Grosso	5 μL	92±3 a	74±2 a	53±1 b
Budrovka	5 μL	97±2 a	77±5 a	57±4 a
Hemus	5 μL	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

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 region of North Macedonia 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).

Reference

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