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Volatile Compounds of the Leaves and Flowers of *Lavandula dhofarensis* A.G. Miller

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ABSTRACT: The leaves and flowers of *Lavandula dhofarensis* were collected from the Dhofar region of Oman and hydro-distilled to give low boiling volatiles, which did not condense at 10 °C. The dichloromethane extract of the hydrosol was analyzed by GC/FID and GC/MS. Sixty four compounds were identified in the volatiles of the leaves, accounting for 78.7% of the total. The major components were caryophyllene oxide (8.0%), germacrene (7.9%), spathulenol (7.8%), and β-caryophyllene (6.6%). Eighty six compounds were also identified in the volatiles of the leaves plus flowers, comprising 94.5% of the total. The major compounds were camphor (12.9%), viridiflorol (10.5%), α-terpinyl acetate (7.5%), valerenal (7.2%), α-gurjunene (5.6%), and spathulenol (5.5%). Compounds such as linalool, linalyl acetate, 1,8-cineole, and β-ocimene, which are usually found as the major components of lavender oils, were either absent or detected at low levels (<0.1%) in the hydrosol of *L. dhofarensis*. This investigation showed that the fragrance essence of *L. dhofarensis* is different from the other *Lavandula* species. *L. dhofarensis*is is regionally endemic to wetter areas of Oman.

Keywords: *Lavandula dhofarensis*; Lamiaceae; volatile composition; valerenal; caryophyllene oxide; viridiflorol; α-terpinyl acetate; spathulenol; leaves and flowers.

المواد المتطايرة من أوراق وزهور نبات *Lavandula dhofarensis*

جون وليم، ماجيك فاتوب، سلمى الكندي، فخر الدين سليمان و سالم السعيدي

ملخص: تم استخلاص المواد المتطايرة بواسطة التقطير من أوراق وزهور Lavandula dhofarensis من منطقة ظفار في سلطنة عمان. كما تم تحليل مستخلص ثاني كلوريد الميثان من هذه المواد المتطايرة منخفضة الغليان باستخدام الكروماتوغرافيا وطيف الكتلة. تم تحديد اربعة وستون مركبا في هذه المواد وهذه تمثل 78.7% من اجمالي المركبات المتطايرة في الأوراق. المركبات الرئيسة في الاوراق هي: Caryophyllene Oxide (8.0%)، Germacrene (7.9%)، Spathulenol (7.8%)، (6.6%) β-caryophyllene. كما تم تحديد ستة وثمانون من المركبات المتطايرة في الاوراق والزهور وهذه تمثل (94.5%) من اجمالي المركبات وتحتوي على الكافور (Camphor) (12.9%)، Viridiflorol (10.5%)، α-terpinyl acetate (7.5%)، (7.2%) Valerenal، α-gurjunene (5.6%) و Spathulenol (5.5%). المركبات التي غالباً ما تتواجد في زيوت اللافندر وهي: linalool، linalyl acetate، 1,8-cineole و β-ocimene غابت عن زيوت هذا النبات أو تواجدت بكميات ضئيلة أقل من (0.1%) . هذه الدراسة أظهرت أن عطرية هذا النبات تختلف عن أنواع الافندر الأخرى. كما وان *L. dhofarensis*is تتواجد فقط في المناطق الرطبة من سلطنة عمان.

مفتاح الكلمات : *Lavandula dhofarensis*; Lamiaceae; volatile composition; valerenal; caryophyllene oxide; viridiflorol; α-terpinyl acetate; spathulenol; أوراق وزهور.

1. Introduction

Lavandula dhofarensis A.G. Miller (Lamiaceae) (Figure) is a leafy wild-growing perennial herb, producing an aromatic smell of lavender or faintly of lemons (Miller and Morris, 1988). It grows in clumps; the stems are hairy and much-branched and the leaves are ovate with up to 5 pairs of segments. The leaves are 7.5 to 50 mm long and 3 to 20 mm wide. The flowers are lilac in color and 15 to 70 mm long. It is endemic to the Dhofar region of Oman where it is commonly known as 'heryen ekúlún' and 'hilbēn' in Jabbali vernacular (Miller and Morris, 1988). *L. dhofarensis* is not found in drier areas of Oman.

The genus *Lavandula* comprises about 30 species which are found in Mediterranean countries (Miller and Morris, 1988). Lavender oils have a delightful smell, and neurological, antimicrobial and insect repellant properties (Miller and Morris, 1988; Cavanaugh and Wilkinson, 2002; Lis-Balchin and Hart, 1999). The pleasant smell of the flowers of lavenders are used in linen chests to perfume clothes or deter clothes moth (Miller and Morris, 1988). It is believed that the smell of lavenders clears the head and lifts the spirits (Rich, 1997). The lavenders are useful medicinal plants (Cavanaugh and Wilkinson, 2002; Harborne and Williams, 2002; Chamberlain and Bollen, 2011) which accumulate volatile compounds in the leaves and flowers and their oils have several applications in animal health management, flavoring, and the cosmetic and perfume industries (Pirali-Kheirabadi and Teixeira da Silva, 2010; Guillen *et al*., 1996).

Figure. *Lavandula dhofarensis* A.G. Millller.

The chemical composition of the essential oil of the *Lavandula* species (*L. augustifolia* Miller (= *L. officinalis* Chaix = *L vera* De candolle), *L. viridis* L'Her, *L. pubescens* Dec, *L. dentate* L., *L. lanata* L., *L. canariensis* Miller, *L. latifolia* M., *L. stoechas* L. and *L. mutifida* L.) have been extensively investigated by different extraction methods and GC/MS analyses (Guillen *et al.*, 1996; Kim and Lee, 2002; Paul *et al*., 2004; Chorgrani *et al*., 2010; Porto *et al*., 2009; Pallado *et al*., 1997; An *et al*., 2001; Cong *et al*., 2008; Shellie *et al*., 2002; Da Porto and Decorti, 2010). Guillen *et al*. (1996) analyzed the components of the oil of the aerial parts of *L. latifolia* cultivated in North eastern Spain and found 57 compounds of which linalool (36.9%), 1,8-cineole (31.3%), and camphor (13.6%) were major components. Pallado *et al*. (1997) identified up to 38 compounds in the oil of *L. officinalis*. The group also observed that the chemical composition of oil varied with extraction methods. With supercritical fluid extraction, the major constituents were linalyl acetate (21.2%), camphor (14.2%), and linalool (13.9%). In contrast, Soxhlet extraction yielded camphor (19.7%), eucalyptol (17.2%), and eugenol (8.4%) as the major components; and steam distillation produced oil containing camphor (26.6%), linalool (20.1%), and eucalyptol (18.7%). An and Hatfield (2001) analyzed the fragrance of living *L. angustifolia* flowers by solid-phase micro-extraction coupled to GC and ion-trap MS. They identified 42 compounds, the major components being linalool, linalyl acetate, terpin-4-ol, (*E*)-caryophyllene, (*Z*)-and (*E*)-β-ocimene. Cong *et al.* (2008) identified 17 compounds in the hydro-distilled oil of *L. angustifolia* growing in China and found linalool (44.5%), geraniol (11.0%), lavandul acetate (10.8%), 3,7-dimethyl-2,6-octadien-1-ol (10.4%) and isoterpineol (6.8%) as major components.

Based on botanical features, the lavenders fall into four categories: *L. latifolia, L. angustifolia*, L. *stoechas*, sometimes known as French lavender, and *Lavandula x intermedia*, a sterile breed between *L. latifolia* and *L. angustifolia*. From a literature review, the most frequently identified volatile components of the aerial parts of the lavenders irrespective of group or analysis method are linalool, linalyl acetate 1,8-cineole, camphor, terpinen-4-ol, and β-ocimene (Porto *et al*., 2009). Broadly, the lavenders may be grouped as linalool or camphor chemotypes, based on the most abundant component of the floral oil. The percentage composition of each of the major components varies from one species to the other and the relative levels of each component determine the market value, smell and medicinal application of their oils. However, no references to the components of the oil or volatiles produced by *L. dhofarensis* were found in the literature.

As part of our investigation of the composition and bioactivity of the essential oil from fragrant endemic plants in Oman, we report here, for the first time, the chemical composition of steam-distilled volatiles of *L. dhofarensis* subspecies *dhofarensis.*

2. Experimental

2.1 Chemicals

Pure standards of linalool, α-terpineol, 1,4-cineol, terpinolene, camphor, cedrol, fenchol, anethole, and C_8-C_{20} alkane standard solutions were purchased from Fluka; eugenol, borneol, β-caryophyllene, caryophyllene oxide from Aldrich; *p*-cymene, γ -terpiene, α-terpineol, and veratrole from Acros Organics; phellandrene from Cica Reagent, and *p*-menth-1-en-4-ol from Merck.

2.2. Plant material

The fresh leaves and flowers of *L. dhofarensis* subspecies *dhofarensis* were collected from the Dhofar region of Oman, 4.4 km from the Ma'amura roundabout on the Salalah-Marbat road at an altitude of 40 m in September 2002 and identified by Dr. Shahina Ghazanfar. A voucher specimen was deposited in the Herbarium of the Botanical Garden at Sultan Qaboos University under the code name NP020.

Approximately 600 g of fresh plant material (either leaves only or combined leaves and flowers) were subjected to hydro-distillation using a 10-Liter Stove Still™ apparatus (Essential Oil University, New Albany, IN, USA) for 3 h. No condensed oil was visible in the arm of the modified Clevenger-type apparatus used but the condensed steam had a distinct and pleasant odor. The hydrosol was extracted twice with 2.0 ml of dichloromethane and dried over anhydrous sodium sulfate. The organic solution was transferred to GC-MS auto-sampler vials, ready for analysis.

2.3 Analysis of the volatile extract

GC/FID analyses were performed on a Focus GC gas chromatograph (Thermo Electron Corporation, Italy) equipped with FID detector, and a DB-1 column, 30 m x 0.25 mm, 0.25 µm film thickness (J and W Scientific, Folsom, CA, USA). Analyses were conducted under the following conditions: the carrier gas was He; flow rate 2.7 ml/min; injection port temperature, 250 ^{o}C; oven temperature, programmed from 35 - 250 °C at 5 °C /min up to 200 °C and 20 °C/min and then held at the upper limit of 250 °C. Split/split less injection: solutions of oil in dichloromethane were injected in split mode at a ratio of 1:20.

GC/MS analyses were performed on a Shimadzu (Kyoto, Japan) GCMS-QP5050A using a 30 m × 0.250 mm × 0.25 µm DB-1 column from J and W Scientific (Folsom, CA, USA). The carrier gas was helium at a flow rate of 2.7 ml/min and the split mode had a ratio 1:20. The injector and detector temperatures were 275 °C. After injection, the oven temperature was kept at 35 °C for 2 minutes, and then programmed at a rate of 2 °C/min to a temperature of 200 °C for 5 minutes followed by an increase to the final temperature of 240 °C at 5 °C/min. For the mass spectra, the electron impact ionization was at 70 eV, and the acquisition scan was from m/z 40 to 500 (1000 amu/sec at 0.5 sec intervals).

Qualitative data were obtained electronically from area percent data. Some compounds were identified by comparison of GC retention times with those of standards on a GC-FID instrument, by computer matching of mass spectral fragmentation patterns using digital library (Wiley spectral library of 229,000 spectra) of the GC-MS instrument or by comparison of their calculated retention indices relative to C_8-C_{20} standard n-alkane with literature values (Shellie *et al.*, 2002; Da Porto and Decorti 2010; Migel *et al*., 2004; Baratta *et al*., 1998; Gancel *et al*., 2003).

Table 1. Composition of *L. dhofarensis* subspecies *dhofarensis* volatiles for leaves only and combined leaves and flowers.

No.	Compound[a,e]	RI[b] (calc)		RI (Lit)[16-20]	Area (%)[c]	
		Leaves	Leaves + flowers		Leaves	Leaves + flowers
1	α-pinene	921		939, 930, 927	1.5	
2	verbenene	936			0.1	
3	sabinene	956		976, 958, 963	0.1	
4	1-octen-3-ol		961			0.2
5	3-octanol		978			0.2
6	*m*-cymene	1003	1003	1026 1006	0.1	0.3
7	thujol		1005			0.1
8	octyl formate		1055			0.6
9	terpinolene [d]		1071	1088,1064, 1075		0.7

No.	Compound[a,e]	RI[b] (calc)		RI (Lit)[16-20]	Area (%)[c]	
		Leaves	Leaves + flowers		Leaves	Leaves + flowers
10	nonanal		1080	1102, 1083		0.2
11	linalool [d]	1081	1082	1098, 1074 1098	0.1	0.6
12	fenchyl alcohol [d]		1088	1088		1.1
13	α-campholene aldehyde	1094	1094		0.2	0.2
14	camphor [d]		1095	1095		12.9
15	octenyl acetate		1097			1.2
16	*trans*-pinocarveol	1110		1106	0.2	
17	3-octanyl acetate		1111			0.7
18	*trans*-verbenol	1118	1118	1114	1.2	0.7
19	pinocarvone	1124	1124		0.2	0.2
20	nonenal		1130			0.1
21	*p*-mentha-1,5-dien-8-ol	1136	1136	1167	0.3	0.3
22	terpine-4-ol	1149	1149	1177	0.1	0.3
23	*p*-cymen-8-ol	1151	1151	1183	0.3	0.4
24	myrtenal	1153	1154		0.2	0.1
25	α-terpineol [d]	1161	1161	1189 1148	0.1	0.9
26	verbenone	1164			0.3	
27	berbenone		1164			0.2
28	decanal		1180			0.1
29	*trans*-carveol	1188			0.3	
30	octyl acetate		1194	1137		1.4
31	cumin aldehyde		1197	1200		1.5
32	carvone	1200	1201	1242	0.4	0.2
33	neral		1205			0.1
34	nerol		1232	1206		0.2
35	citral		1236	1245		0.2
36	linalyl acetate		1238			0.1
37	2-caren-10-al		1241			0.3
38	*trans*-anethole		1250			0.2
39	2-undecanone	1271		1291	2.3	0.1
40	carvacrol		1278	1298		0.3
41	*cis*-octahydro-8a-methyl-2(1H)-naphthalenone		1306			2.1
42	*cis*-octahydro-4a-methyl-2(1H)-naphthalenone		1308			1.2
43	eugenol [d]	1319	1319	1356 1327	0.1	0.1
44	bicycloelemene	1321			0.6	
45	α-terpinyl acetate	1324	1326		0.1	7.5
46	α-cubebene	1336		1345,1332	0.3	
47	2-heptadecanone	1343			0.5	
48	*E*-damascenone		1351	1380		0.1
49	α-copaene	1360	1360	1380, 1375,1374	0.3	0.5
50	β-bourbonene	1366	1366	1384, 1362,1379	2.3	2.2
51	methyleugenol		1368	1384, 1362,1379	2.3	1.3
52	β-elemene	1375	1375	1391	2.9	0.2
53	dehydroaromadendrene	1379			0.2	
54	2,4-dihydroxyeicosane		1385			0.1

No.	Compound[a,e]	RI[b] (calc)		RI (Lit)[16-20]	Area (%)[c]	
		Leaves	Leaves + flowers		Leaves	Leaves + flowers
55	β-caryophyllene [d]	1398	1397	1418 1391	6.6	0.3
56	aristole		1399			0.3
57	calarene		1410	1432		0.3
58	β–gurjunene		1419	1432, 1400		2.1
59	α-bergamotene	1421		1414	2.2	
60	dehydroaromadendrene		1425			1.8
61	α-humulene	1428		1454, 1447	2.0	
62	aromadendrene	1435	1435	1461, 1419	2.2	0.5
63	α-gurjunene		1439			5.6
64	β-ionone	1449			0.3	
65	1,1,3,6,8-pentamethyl-1,2-dihydronaphthalene		1449			0.2
66	α-amorphene		1452			0.2
67	germacrene	1455		1457, 1474	7.9	1.9
68	widdrene		1457	1429		0.3
69	β-selinene	1458		1485, 1476	0.4	
70	2,3,5,8-tetramethyl decane		1461			0.3
71	β-cubebene	1462	1408		1.0	0.1
72	β-cedrenoxide		1466			0.1
73	bicyclogermacrene	1468		1480, 1500	1.5	
74	α-muurolene	1475			0.6	
75	*retro*-ionone	1480			0.1	
76	β-bisabolene		1487	1509		0.5
77	calamenene	1488			0.8	
78	isogeraniol	1492			0.1	
79	δ-cadinene	1496	1495	1524	3.3	0.6
80	β-sesquiphellandrene		1498			0.1
81	bisabolol oxide	1499			0.1	
82	α-Calacorene		1504			0.4
83	1,3,5,5,6,6-hexamethyl-1,3-cyclohexadiene		1508			1.8
84	*trans, trans*-2,4-dodecadienal	1508			0.6	
85	elemol	1514			0.4	
86	farnesyl acetone	1530			2.1	
87	viridiflorol	1531		1590		10.5
88	spathulenol	1541	1540	1560, 1569	8.0	2.0
89	caryophyllene oxide [d]	1544	1543	1581	8.0	2.0
90	globulol		1548	1583		0.6
91	salvial-4(14)-en-1-one	1552	1552		0.5	0.2
92	dihydro-neoclovene	1556	1556		0.8	0.3
93	α-ionone		1559			0.2
94	2,4-dimethyl-1-decene		1563			0.2
95	humulene oxide	1568			2.6	0.5
96	1,2-methylenedioxy-5,6-dimethoxy-4-allylbenzene	1576			1.1	
97	carotol		1581			0.1
98	dihydro-*cis*-carveol	1589			0.4	
99	diepi-α-cedrene	1595			1.4	
100	widdrol	1600			1.2	

No.	Compound[a,e]	RI[b] (calc) Leaves	RI[b] (calc) Leaves + flowers	RI (Lit)[16-20]	Area (%)[c] Leaves	Area (%)[c] Leaves + flowers
101	isospathulenol		1600			0.5
102	γ-cadinol		1603			0.8
103	β-eudesmol		1605	1649		0.3
104	α-cadinol		1609	1653		0.3
105	torreyol	1610		1645	3.0	0.7
106	valerenal	1615			0.5	7.2
107	1,4-*cis*-1,7-*trans*-acorenone		1617			0.3
108	9-aristolen-1.α-ol	1618			0.7	
109	β-damascone	1620			0.9	
110	δ-tetrahydrocostunoli		1623			2.7
111	nerolidol epoxyacetate	1625			1.1	
112	thujyl alcohol		1625			0.5
113	3-butyl-3-octen-2-one		1630			0.3
114	(*Z*)-valerenyl acetate		1632			0.5
115	4*E*,6*E*-diisopropenyl-1*E*,2-cyclohexane	1636			0.1	
116	*p*-nonylphenol	1646			0.2	
117	tridecanal		1646			0.1
118	campherenone	1651			0.3	
119	α-cedrol	1661			0.4	
120	γ-1-cadinene aldehyde	1663			0.2	
121	citronellal		1671			0.6

[a] List of compounds in elution order from DB-1 column.
[b] RI relative to C_8-C_{20} *n* alkanes on DB-1 column.
[c] GC Peak area %
[d] Identification by standard
[e] Identification by MS/RI

3. Results and Discussion

A total of 64 and 86 components were identified in the volatiles of the leaves and leaves plus flowers respectively of *L. dhofarensis* subspecies *dhofarensis* (Table 1). These compounds accounted for 78.7% of the leaf volatiles and 94.5% of the leaf plus flower volatiles. For the leaves, 37.3% were sesquiterpene hydrocarbons and 30.1% were oxysesquiterpene derivatives (Table 2). For the combined leaves and flowers, 18.2% were sesquiterpene hydrocarbons and 34.0% were oxysesquiterpenes (Table 2). From Table 3, the major components in the leaf volatiles were caryophyllene oxide (8.0%), germacrene (7.9%), spathulenol (7.8%), and β-caryophyllene (6.6%).

Table 2. Compound distribution in the analyzed volatiles of *L. dhofarensis* subspecies *dhofarensis*.

Compound class	Amount present in volatiles (%) Leaves	Amount present in volatiles (%) Leaves and flowers
Monoterpene hydrocarbons	1.8	1.0
Oxymonoterpenes	6.5	30.0
Sesquiterpene hydrocarbons	37.3	18.2
Oxysesquiterpenes	30.1	34.0
Others	3.0	11.3

The major components in the leaf and flower volatiles were camphor (12.9%), viridiflorol (10.5%), α-terpinyl acetate (7.5%), valerenal (7.2%), α-gurjunene (5.6%), and spathulenol (5.5%). Compared to other *Lavandula* species (Paul *et al*., 2004; Chorgrani *et al*., 2010; Porto *et al.*, 2009; Pallado *et al*., 1997; An *et al*., 2001; Cong *et al*., 2008; Shellie *et al*., 2002; Da Porto and Decorti, 2010) the percentages for these compounds are high with the exception of camphor. Qualitative studies of lavender have also shown variable composition of the major components: linalool (35-37%), linalyl acetate (21-34%), 1,8-cineole (4-11%) and camphor (5-12%) (Da Porto and Decorti, 2010). Surprisingly, linalool, linalyl acetate, the two major components of several lavender flower oils, and 1,8-cineole, a major component of lavender herb oil, were found only at low levels (≤ 0.6%) in the volatiles of *L. dhofarensis* (Table 1). One possible reason for the difference observed is that other workers used mostly flower heads whereas in this study, the flowers were steam-distilled with the leaves and the amount of flowers compared to leaves was low. The flowers were not collected, extracted and analyzed separately because *L. dhofarensis* grows naturally as a small crop in Oman; the ecosystem could be harmed if, for example, 600 g of flower heads were taken from the wild. From Table 3, camphor, viridiflorol, α-terpinyl acetate, valerenal, and α-gurjunene were the major components of the flowers. Surprisingly, germacrene, torreyol, humulene oxide, 2-undecanone, and γ-elemene were present in the leaf volatiles at higher levels (Table 3) suggesting their absence or presence at trace levels in the flower essence. *L. stoechas* and *L. lanata* have high camphor levels in flowers while *L. augustifolia*, *L. dentate* and *L. pinnata* are low in camphor (< 2%). *L. dhofarensis* flowers have low levels of linalool and linalyl acetate (< 1%), and high levels of camphor (12.9%) and valerenal (7.2%). There is thus some similarity between *L. dhofarensis, L. lanata* and *L. stoechas*. The low levels of linalool and linalyl acetate, (Table 1) and the high level of camphor (12.9%) in the floral oil volatiles of *L. dhofarensis* (Table 1 and Table 3) distinctly support the grouping *L. dhofarensis* as a camphor chemotype.

Table 3. Major components of the volatiles of *L. dhofarensis* subspecies *dhofarensis.*

Constituent	Amount present in volatiles (%)	
	Leaves	Leaves and flowers
camphor		12.9
viridiflorol		10.5
caryophyllene oxide	8.0	2.0
germacrene	7.9	1.9
spathulenol	7.8	5.5
β-caryophyllene	6.6	0.3
α-terpinyl acetate	0.1	7.5
α-gurjunene		5.6
δ-cadinene	3.3	0.6
torreyol	3.0	0.7
valerenal	0.5	7.2
δ-tetrahydrocostunolide		2.7
humulene oxide	2.6	0.5
2-undecanone	2.3	0.1
β-bourbonene	2.3	2.2
β-elemene	2.9	0.2
β-gurjunene		2.1
1,3,5,5,6,6-hexamethyl-1,3-cyclohexadiene		1.8
dehydroaromadendrene		1.8
octyl acetate		1.4

4. Conclusion

The results taken together, this investigation showed that caryophyllene oxide, germacrene, spathulenol, viridiflorol, valerenal, camphor, and α-terpinyl acetate are the major components of the hydrosol of *L. dhofarensis*. *L. dhofarensis* is thus different from other lavenders and the presence of camphor could lower the market value and applications of the fragrance essence of *L. dhofarensis* in aromatherapy.

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6. References

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