Essential oil composition of *Lavandula angustifolia* “Hidcote”: Comparison of hydrodistillation and supercritical fluid extraction methods

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We investigated the hydrodistillation (HD) and supercritical fluid extraction (SFE) methods for the extraction of essential oils with antibacterial actions from lavender for use in aromatherapy and cosmetics. Extracts were analyzed by flame ionization detector-gas chromatography and mass spectrometer-gas chromatography. The HD method extracted 66 compounds, while SFE extracted 46. The principal components of the oils extracted by HD were linalyl acetate (25.3%), terpinen-4-ol (16.4%), and linalool (13.0%). The principal components extracted by SFE were linalyl acetate (30.6%), terpinen-4-ol (14.1%), and lavandulyl acetate (8.4%). The extraction rate of essential oil for the SFE method was at least 10 times that for the HD method. We also clarified that SFE is suitable for obtaining compounds that are unstable at relatively high temperatures and that HD is suitable for compounds with high volatility.

Key words: *Lavandula angustifolia*, essential oil composition, hydrodistillation, supercritical fluid extraction, linalyl acetate, terpinen-4-ol

1. INTRODUCTION

Lavender, a member of the genus *Lavandula* of flowering plants in the mint family Lamiaceae, is a popular perennial shrub. Cultivated around the world, from countries along the Mediterranean coast of North Africa and Europe, to Turkey, India, Japan, North America and Australia, lavender is one of the most commonly used medicinal herbs^[1]. The volatile oil of lavender is used in cosmetics as a perfume and in aromatherapy as a soap and skin lotion. In addition, lavender has also been reported to possess antibacterial, antioxidant, and sedative activities, and pharmaceutical, insecticidal and analgesic applications have been suggested^[2-10].

Further, we previously reported the antimicrobial activity of isoprenoid compounds^[11]. Hydrodistillation (HD) is commonly used for the extraction of volatile oil. We previously reported the essential oil composition and antimicrobial activity of the essential oil and water extracts from Japanese wild *Rosa rugosa* via the HD method^[12]. However, new extraction methods have recently been developed, such as the supercritical fluid extraction (SFE) method^[13-22].

Recent reports have compared the extraction of volatile oil from commonly used medicinal plants by the HD and SFE methods^[14-15, 20]. In the SFE method, medium with properties of supercritical carbon dioxide, particularly in the

middle of the liquid, and gas are passed through medicinal plant cells to extract volatile oil. That is, a supercritical fluid is considered to be the ideal medium, equipped with the solubility of a liquid and diffusibility of a gas. We hypothesized that this new method might be more effective for using a supercritical fluid than the HD method. Here, we extracted the essential oils of lavender (*Lavandula angustifolia* “Hidcote”) via the HD and SFE methods and compared results.

2. Materials and methods

2.1 Plant material

Lavender (*Lavandula angustifolia* cv. Hidcote) was collected at Hirosaki in July 2011. The only plant materials collected were petals, which were air-dried at room temperature and subjected to extraction via the HD or SFE methods.

2.2 HD and SFE extraction

HD was conducted with 1168.6 g of lavender petals. Distillation time was 6 to 7 h, followed by extraction of the essential oil with 2 L of floral water (hydrosol). Floral water was then extracted with hexane, diethyl ether, and ethyl acetate solvent (i.e. stronger polar solvents were gradually added).

In the SFE method, extracts (0.32 g) of lavender petals (2.5 g) in the extraction cell obtained via carbon dioxide entered the

supercritical state. Extraction conditions were maintained at a pressure of 120 bar and column temperature of 40 °C. Carbon dioxide flow rate was set at 27 L/h. And the extraction was performed over 2 h.

The HD and SFE extracts were diluted with ether and analyzed by GC-MS. Mass spectrometer-gas chromatography (GC-MS) analysis was conducted on a JEOL Q1000GC-Mk II Mass Spectrometer (Japan Electron Optics Laboratory Co. Ltd., Tokyo, Japan), consisting of an HP-5 column (30 m × ϕ 0.32 mm × 0.25 μ m film thickness: coated with 5%-phenyl-poly [methylsiloxane]) with a 1 ml/min helium carrier flow rate. Temperature was programmed to deliver a 15 °C/min gradient initiated with a 4.7-min hold at 50 °C and terminated with a 2-min hold at 280 °C. Injector and GC-transfer line temperatures were both set at 200 °C.

HD and SFE extracts were identified using retention indices (RIs).

2.3 Analysis

Essential oils were analyzed via flame ionization detector-gas chromatography (GC-FID). GC-FID analysis was performed using a Hitachi Gas Chromatograph G-5000A (Hitachi, Tokyo, Japan). Nitrogen was passed through a poly (alkylene glycol) column (30 m × 0.25 mm i.d.) at a carrier flow rate of 60 ml/min using a 10 °C/min gradient initiated with a 5-min hold at 40 °C and terminated upon reaching 200 °C. Injector temperature was 150 °C and detector temperature was 250 °C.

Components were measured under non-isothermal conditions and identified by comparison with the RIs calculated from the Van den Dool-Kratz equation and the MS coincidence rate^[23]. The ratios of components were computed from the peak area ratio of GC-MS. Oil-drilling rate and extractability was calculated from the sample weight and extraction yield.

3. Results & Discussion

The HD method extracted 11.6 g of essential oil from 1168.6 g of lavender petals at a yield of 0.99% (w/w). The relative peak area to total peak area of GC for principal components of oil extracted by the HD method was as follows: linalyl acetate (25.3%), terpinen-4-ol (16.4%), linalool (13.0%), and ocimene (13.6%). The yield of ether extracted from floral water by the HD method was 0.26 g and 0.02% (w/w). The major component of the extract was linalool oxide. *Cis*-linalool oxide and *trans*-derivative were obtained at 27.6%, and epoxy-linalool at 8.6%. The basic components of the hexane extract from floral water by the HD method were terpinen-4-ol (46.6%), linalool (27.1%), and α -terpineol (7.7%).

Using the HD method, we identified 66 compounds from the essential oil of lavender. Details on essential oils and three types of solvent extraction are summarized in Table 1.

Although linalool hydrate was obtained from the floral water portion, this compound might be an artificial product, as linalool is present in the essential oil of the secondary product of steam distillation.

In contrast, the SFE method extracted 0.32 g (13.1% [w/w]) of essential oils from 2.5 g of lavender petals. Basic components consisted of linalyl acetate (30.6%), terpinen-4-ol (14.1%), lavandulyl acetate (8.4%), β -caryophyllene (7.3%), and β -farnesene (7.2%). As shown in Table 2, 46 compounds were identified. Although the principal component of the volatile oil naturally changed by neither of the methods, phytol and lavanduol which were not contained by hydrodistillation, were obtained.

The extraction rate of essential oils by the HD method was 0.99%, while that by the SFE method was 13%. The HD extract contained the principal components linalyl acetate and terpinen-4-ol, while the SFE extract contained lavandulyl acetate. Comparison of the HD and SFE methods showed that the former is suitable for the isolation of large quantities of essential oil, while the latter is suitable for determining the components of essential oil and the analysis of food. The method selected will therefore depend on the application. To compare the HD and SFE methods, an equivalent number of petals should be used. However, analysis of the oil extracted from a high number of petals is impossible for the SFE method due to cost. In contrast, the HD method requires more petals for analysis than the SFE method. However, additional compounds with high volatility might escape together with carbon dioxide. Although SFE equipment is generally considered to be specialized and relatively expensive to operate, this method is best suited for component analysis, including that of vegetables or other food items.

4. Conclusions

The SFE method is suitable for obtaining compounds that are unstable at relatively high temperatures. We also consider the HD method appropriate for highly volatile compounds. Therefore, both methods should be used according to their respective purposes. In conclusion, the HD method should be used to isolate large quantities of essential oil and the SFE method to determine the components of essential oil. The SFE method enabled control of carbon dioxide and thus provided optimal extraction of essential oil. Loss of essential oil was further reduced by the addition of a co-solvent to the SFE method. These findings will help contribute to the development of essential oil chemistry.

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Table 1. Components of essential oil and floral water extract from *L. angustifolia* "Hidcote" via HD method

NO.	RT	RI	MS(%)	compounds	peak area % (retention indexes (RI))			
					essential oil	extracted with		
						hexane	ether	ethyl acetate
1	5:37	938	98.2	α -pinene	0.57(938)	0.05(938)		
2	5:58	952	70	camphene	0.01(953)			
3	6:03	958	96	4,4-dimethyl-2-buten-4-olide			1.25(957)	7.51(967)
4	6:09	961	89.4	benzaldehyde			0.13(961)	0.11(972)
5	6:34	970	96	1-ethyl-4-methyl-benzene	0.1(983)			
6	6:40	978	96	β -pinene	0.1(979)			
7	6:42	984	93.2	1-octen-3-ol	0.16(985)	0.48(984)	0.02(983)	
8	6:51	990	91.5	3-octanone	0.05(991)	0.12(990)		
9	6:54	993	96.5	β -myrcene	2.51(993)	0.38(993)		
10	7:00	995	70.2	3-octanol		0.03(998)		
11	7:13	999	93.1	carene	0.03(1009)			0.1(1023)
12	7:13	1013	93.5	2-formylpyrrole			0.02(1011)	0.05(1018)
13	7:20	1020	93.1	α -terpinen	0.15(1018)			
14	7:28	1023	96.2	<i>p</i> -cymene	0.24(1026)	0.17(1025)		
15	7:34	1032	91.2	eucalyptol		0.07(1032)		
16	7:38	1037	97.5	benzyl alcohol			5.01(1034)	3.11(1041)
17	7:43	1042	90.4	4-methyl-4-vinyl-1,4-butanolide			2.39(1042)	2.68(1048)
18	7:44	1043	97.1	ocimene	13.63(1043)	1.33(1042)		
19	7:59	1052	72.5	<i>o</i> -cresol			0.1(1059)	
20	8:02	1060	97.6	γ -terpinen	0.83(1062)	0.11(1060)		
21	8:04	1063	77.9	2-acetylpyrrole			0.02(1064)	
22	8:04	1066	87.5	γ -caprolactone				0.13(1064)
23	8:10	1070	91.6	sabinen hydrate	0.14(1071)			
24	8:29	1071	96.3	terpinolene	0.3(1071)			
25	8:14	1075	96.5	linalool oxide		4.87(1074)	27.6(1074)	3.10(1077)
26	8:39	1101	96.1	linalool	13.0(1101)	27.1(1109)		
27	8:41	1110	86.9	hotrienol			0.33(1104)	0.15(1109)
28	8:50	1111	96.5	1-octen-3-yl acetate	1.68(1116)	0.77(1113)		
29	8:48	1114	98.8	phenylethyl alcohol			1.18(1113)	0.51(1119)
30	8:58	1143	70	isopulegol			0.05(1127)	
31	9:11	1142	96.3	<i>L-trans</i> -pinocarveol	0.1(1144)	0.23(1141)		
32	9:15	1148	90.3	(+)-camphor		0.34(1149)		
33	9:18	1151	90.4	hexyl isobutyrate	0.17(1153)			
34	9:08	1152	82.8	(+)-norinone			0.14(1140)	
35	9:31	1168	90.3	borneol	0.52(1171)	1.4(1169)		
36	9:36	1176	90.8	epoxylinalol		0.12(1177)	8.64(1177)	2.37(1175)
37	9:36	1177	71.1	(<i>E,E</i>)-1,3,5-undecatriene	0.12(1177)			
38	9:39	1182	93.7	terpinen-4-ol	16.41(1181)	46.6(1180)		
39	9:34	1186	91.3	<i>m</i> -cymen-8-ol			1.29(1184)	
40	9:43	1188	74.6	<i>p</i> -cymen-8-ol		0.39(1187)	0.85(1187)	
41	9:49	1192	88.8	α -terpineol	0.91(1195)	7.74(1193)		
42	9:46	1194	96.6	2,6-dimethyl-3,7-octadiene-2,6-diol			3.73(1191)	8.19(1193)
43	10:01	1206	82.8	<i>cis</i> -verbenone			0.14(1211)	
44	10:01	1212	92.1	eucarvone		0.1(1213)		
45	10:13	1230	94.4	nerol		0.48(1231)		
46	10:12	1232	97.8	linalool hydrate			8.57(1230)	32.4(1233)
47	10:25	1245	53.6	(+)-carvone		tr(1250)		
48	10:31	1259	98.2	linalyl acetate	25.3(1259)	2.17(1258)		
49	10:41	1274	87.8	geranial		0.03(1275)		
50	10:41	1277	96.6	2,6-dimethyl-1,7-octadiene-3,6-diol			0.99(1275)	1.06(1277)
51	10:53	1290	98.6	lavandulyl acetate	7.11(1294)	2.17(1292)		
52	11:25	1366	84.2	8-hydroxylinalool			0.64(1346)	0.43(1367)
53	11:36	1368	94.2	neryl acetate	0.82(1368)	0.3(1367)		
54	11:43	1381	70.6	3-hydroxy-2,4,4-trimethylpentyl-2-methylpropanoate		0.06(1381)		
55	11:47	1383	94.2	geranyl acetate	1.65(1388)	0.53(1386)		
56	12:12	1430	97.9	β -caryophyllene	6.45(1434)	0.77(1432)		
57	12:21	1430	90.8	coumarin		0.1(1451)	5.36(1447)	0.51(1449)
58	12:19	1436	91.4	α -bergamotene	0.14(1447)			
59	12:37	1454	93.1	humulene	0.26(1469)			
60	12:27	1459	95.2	β -farnesene	3.54(1462)	0.4(1462)		
61	12:44	1479	77.6	γ -muurolene		0.03(1494)		
62	12:56	1519	91	2,4-di- <i>t</i> -butylphenol				0.18(1518)
63	13:01	1530	97.7	γ -cadiene	0.67(1528)	0.05(1526)		
64	13:38	1589	96.3	caryophyllene oxide	0.26(1602)	tr(1596)		
65	14:03	1642	92.6	τ -cadinol	0.55(1657)			
66	14:41	1744	97.6	ayapanin			0.24(1741)	

HD, hydrodistillation; RT, retention time on polyalkylene glycol column (min); RI, retention indices as determined on column using series of *n*-hydrocarbon homologs; Peak (%), peak area relative to total peak area.

Table 2. Essential oil components extracted from lavender via SFE method.

No.	RT	RI	MS %	Compound	Peak %
1	6:41	984	97.9	1-octen-3-ol	tr
2	6:52	992	96.8	β -myrcene	0.6
3	7:27	1025	98.4	<i>p</i> -cymene	0.1
4	7:31	1032	92.9	Limonene	0.1
5	7:53	1052	99	ocimene	0.8
6	8:01	1061	98.7	γ -terpinen	tr
7	8:10	1070	97.7	<i>cis</i> -sabinene hydroxide	0.2
8	8:15	1076	94.9	<i>cis</i> -linalyl oxide	0.2
9	8:15	1091	95.2	<i>trans</i> -linalyl oxide	0.1
10	8:39	1101	97.1	Linalool	8.1
11	8:49	1115	96	1-octen-3-yl-acetate	0.4
12	8:56	1124	77.4	<i>trans</i> -3-carene-2-ol	tr
13	9:03	1133	93.1	(3 <i>E</i> ,5 <i>Z</i>)-2,6-dimethyl-1,3,5,7-octatetraene	tr
14	9:10	1142	76.9	<i>trans</i> -pinocarveol	tr
15	9:17	1152	94.2	<i>n</i> -hexyl isobutyrate	tr
16	9:31	1171	87.4	Lavandulol	0.8
17	9:36	1177	88.8	(3 <i>E</i> ,5 <i>Z</i>)-1,3,5-undecatriene	0.2
18	9:39	1181	95.4	Terpinen-4-ol	14.1
19	9:44	1188	82.4	<i>p</i> -cymen-8-ol	0.1
20	9:47	1192	88.9	2,6-imethyl- 3,7-octadiene-2,6-diol	0.3
21	10:00	1211	90.4	Carveol	0.1
22	10:02	1214	78.4	Berbenone	tr
23	10:31	1259	98.9	Linalyl acetate	30.6
24	10:41	1275	obs.	Verbenol acetate	0.7
25	10:52	1292	98.9	Lavandulyl acetate	8.4
26	11:16	1333	89.7	Cyclobutanecarboxylic acid, hexyl ester	0.3
27	11:29	1356	81.8	8-hydroxylinalool	0.9
28	11:36	1368	96.8	Neryl acetate	0.3
29	11:46	1386	97	Geranyl acetate	0.9
30	12:07	1425	81.5	β -bergamotene	0.1
31	12:12	1434	98.9	Caryophyllene	7.3
32	12:18	1445	85.6	α -bergamotene	0.2
33	12:20	1449	85.6	Coumarin	0.4
34	12:27	1462	97.9	β -farnesene	7.2
35	12:30	1468	94.7	Humulene	0.4
36	13:01	1528	7.3	γ -cadiene	0.5
37	13:04	1534	72	β -sesquiphellandrene	0.1
38	13:22	1570	99.8	Caryophyllene oxide	0.2
39	13:32	1590	88.4	Epiglobulol	0.1
40	13:51	1630	78.5	Cubenol	0.2
41	14:03	1657	92.1	τ -cadinol	0.9
42	14:11	1674	90.4	Alloarmadendrene oxide-(1)	0.2
43	14:42	1742	94	Ayapanin	0.4
44	14:59	1782	92.2	Benzyl benzoate	tr
45	16:29	1998	85.7	Ethyl hexadecanoate	tr
46	17:15	2119	90.2	Phytol	0.1

Unknown compounds, 58; area ratio of overall identified components, 86.6%; SFE, supercritical fluid extraction; RT, retention time on polyalkylene glycol column (min); RI, retention indices as determined on column using series of *n*-hydrocarbon homologs; Peak (%), peak area relative to total peak area

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