

The Effect of Temperature on the Essential Oil Components of *Salvia potentillifolia* Obtained by Various Methods

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The chemical compositions of the essential oils of *Salvia potentillifolia* (Lamiaceae) were analyzed by GC and GC-MS. The oils were obtained by four different methods (hydrodistillation, steam distillation, and two extraction methods) to investigate the effect of temperature on the volatile compounds. In total, 121 components were detected in the various oils. The major compounds characterized were α -pinene (30.2, 31.2, 10.6, and 14.8 %) and β -pinene (15.0, 14.6, 7.6, and 11.4%), respectively. Surprisingly, the percentage concentrations of α -pinene and β -pinene in the hydrodistilled and steam distilled oils were high, even though prepared at high temperature, whereas the contents of both compounds were lower in the essential oils obtained by the extraction methods prepared without added heat. The percentage concentration of 8,13-epoxy-labda-14-en-2-one, a diterpenoid, in the extracted essential oil was 22 times higher than in the hydrodistilled and steam distilled essential oils. However, the concentrations of *cis-p*-menth-2-en-1-ol was four times, and β -bourbonene and β -caryophyllene two times superior than their percentage concentrations in the hydrodistilled and steam distilled essential oils.

Keywords: *Salvia potentillifolia*, essential oil, hydrodistillation, steam distillation, solvent extraction, GC-MS.

Salvia species are used as traditional medicines all around the world, possessing antibacterial, antioxidant, antidiabetic, and antitumor properties, [1a-1c,2a], as well as antihypersensitivity [2b] and platelet antiaggregating [2c] activities. Some members of this genus have economic importance as flavouring agents in perfumery and cosmetics. *Salvia* species known as “Adaçayı” or “Şalba” in Muğla Turkey have been used for folkloric medicinal purposes, being consumed as a tea [2d].

S. potentillifolia Boiss. & Heldr. ex Benth (Lamiaceae) is an endemic species and grows in open habitats in the Eastern Mediterranean [2e]. The essential oil obtained from this species collected from Antalya, Turkey has been reported [3a], but has not been investigated in detail. It was observed that the percentages of the essential oil components differed depending on the method of extraction [3b-3d]. In this present study, we aimed to investigate the constituents of the essential oils obtained by four different methods, namely, hydrodistillation, steam-distillation, solvent extraction, and solvent extraction-steam distillation to understand the effects of temperature on the essential oil constituents.

Yields of essential oils obtained by hydrodistillation, steam distillation, solvent extraction, and solvent extraction-steam distillation were found to be 0.88, 0.55, 0.72, and 0.01%, respectively, based on the dry weight of the aerial parts of the plant. One hundred and twenty-one components were detected in the various oils obtained from *S. potentillifolia* using the four methods, 109 of which were identified using GC and GC-MS analytical methods, and literature comparisons. Of the total essential oils obtained by hydrodistillation, steam distillation, extraction, and extraction-steam distillation 99.2%, 99.1%, 98.4%, and 94.1%, respectively were identified. The major components of the essential oils were α -pinene (30.2, 31.2, 10.6, and 14.8%), β -pinene (15.0, 14.6, 7.6, and 11.1%), 1,8-cineol (7.4, 7.3, 9.0, and 10.6%), terpinen-4-ol (3.5, 1.8, 2.1, and 3.1%), β -myrcene (2.8, 3.1, 1.5, and 2.2%), limonene (2.6, 2.8, 1.8, and 3.0%), sabinene (2.5, 2.8, 1.7, and 1.1%), caryophyllene oxide (2.4, 2.5, 8.9, and 1.9%) and camphor (2.3, 1.7, 3.6, and 4.5%) (Table 1). Other components formed less than 2.2% of the total yield.

Most of the essential oil consisted of monoterpene hydrocarbons, represented by 62.1% in the essential

Table 1: The percentage concentrations of the constituents of the essential oils of *Salvia potentillifolia* obtained by different methods.^a

Peak No	Compounds	Kovats Index (RI) *	Hydro-distillation method (%)	Steam distillation method (%)	<i>n</i> -Hexane extraction method (%)	<i>n</i> -Hexane extract-steam distillation method (%)	Identification methods**
1	(<i>E</i>)-2-Hexenal	771	0.06	0.03	tr	-	a, b, c
2	(<i>Z</i>)-3-Hexen-1-ol	787	0.08	tr	tr	-	a, b, c
3	1-Heptenal	806	0.03	tr	tr	-	a, b, c
4	Santolina triene	888	0.1	0.1	0.05	-	a, b
5	Tricylene	895	0.08	0.09	tr	-	a, b, c
6	α -Thujene	901	1.5	1.6	0.5	0.8	a, b
7	α-Pinene	914	30.2	31.2	10.6	14.8	a, b
8	2-Methylpropyl benzene	917	0.1	0.2	tr	-	b
9	Camphene	925	1.9	1.9	0.8	1.3	a, b
10	Unidentified	929	0.1	0.2	0.05	0.06	b
11	1-Octen-3-ol	942	0.04	0.02	tr	0.2	a, b, c
12	Sabinene	954	2.5	2.8	1.7	1.1	a, b, c
13	β-Pinene	960	15.0	14.6	7.6	11.1	a, b, c
14	2,3-Dehydro-1,8-cineol	969	0.06	0.03	0.08	-	a, b, c
15	(<i>E,E</i>)-2,4-Nonadienal	974	0.02	tr	tr	-	a, b, c
16	β -Myrcene	977	2.8	3.1	1.5	2.2	a, b, c
17	(<i>Z</i>)-4-Hexen-1-ol acetate	982	0.03	0.02	0.03	-	b
18	Unidentified	987	0.04	0.04	tr	0.05	b
19	α -Phellandrene	989	0.04	0.03	tr	-	a, b, c
20	Benzene acetaldehyde	1000	0.06	0.02	0.04	-	a, b, c
21	α -Terpinene	1003	0.8	0.6	0.1	0.1	a, b, c
22	<i>p</i> -Cymene	1007	2.1	2.2	1.9	4.6	a, b, c
23	<i>trans</i> -2-Caren-4-ol	1009	0.1	0.07	0.4	tr	a, b, c
24	1,8-Cineol	1015	7.4	7.3	9.0	10.6	a, b, c
25	Limonene	1018	2.6	2.8	1.8	3.0	a, b, c
26	<i>cis</i> - β -Ocimene	1026	0.1	0.1	0.1	-	a, b, c
27	Thujol	1029	0.07	0.04	0.1	0.08	a, b, c
28	<i>trans</i> - β -Ocimene	1037	0.08	0.08	0.07	-	a, b, c
29	γ -Terpinene	1047	1.9	1.5	0.7	0.8	a, b, c
30	<i>cis-p</i> - Menth-2-en-1-ol	1049	0.6	1.1	4.1	2.0	a, b, c
31	<i>cis</i> -Linalool oxide	1055	0.03	0.02	0.04	-	a, b, c
32	C ₁₁ H ₂₂	1059	0.03	0.02	0.04	0.1	b
33	Eucarvone	1060	0.05	0.04	0.1	-	a, b, c
34	Fenchone	1064	0.08	0.07	0.09	-	a, b, c
35	C ₁₀ H ₁₆ O	1068	0.06	0.03	0.04	0.09	b
36	Unidentified	1073	0.03	0.04	0.1	0.09	b
37	Terpinolene	1074	0.4	0.3	0.1	-	a, b, c
38	<i>cis</i> - Sabinene hydrate	1076	0.3	0.4	1.8	0.3	a, b, c
39	C ₁₀ H ₁₆ O	1078	0.05	0.04	0.09	-	b
40	<i>cis-p</i> -Mentha-2,8-dien-1-ol	1080	0.2	0.1	0.2	-	a, b, c
41	Linalool	1082	0.4	0.3	0.5	0.9	a, b, c
42	<i>cis</i> -Thujone	1088	0.10	0.9	0.2	-	a, b, c
43	<i>trans</i> -Thujone	1090	0.07	0.6	0.1	0.6	a, b, c
44	α-Campholenal	1094	0.8	0.6	1.2	0.6	b
45	<i>trans</i> -Sabinene hydrate	1098	0.2	0.1	0.2	tr	a, b, c
46	Camphor	1106	2.3	1.7	3.6	4.5	a, b, c
47	<i>cis</i> - Pinocamphone	1110	0.8	0.6	2.1	2.1	a, b
48	<i>cis</i> -Verbenol	1112	0.5	0.2	0.6	0.8	a, b, c
49	<i>trans</i> -Verbenol	1116	0.96	0.6	2.2	2.2	a, b, c
50	Pinocarvone	1122	0.4	0.3	1.0	1.1	a, b
51	Unidentified	1128	0.1	0.10	0.2	0.5	b
52	Borneol	1132	2.3	1.3	2.9	4.8	a, b, c
53	<i>trans</i> -Pinocamphone	1133	0.09	0.05	0.1	0.1	b, c
54	Terpinen-4-ol	1142	3.5	1.8	2.1	3.1	a, b, c
55	Myrtenal	1145	0.4	0.3	0.9	1.2	a, b, c
56	α-Terpineol	1150	1.7	1.0	2.1	1.9	a, b, c
57	Verbenone	1153	0.2	0.1	0.4	1.5	a, b, c
58	Myrtenol	1154	0.6	0.3	1.3	1.0	a, b, c
59	<i>trans</i> -Piperitol	1162	0.08	0.4	0.05	-	a, b
60	<i>trans</i> -Carveol	1168	0.2	0.1	0.5	0.5	a, b
61	Cuminaldehyde	1175	0.06	0.07	0.3	-	a, b
62	Carvone	1177	0.08	0.06	0.2	0.2	a, b, c
63	Phenylethyl acetate	1186	0.06	0.06	0.2	tr	b, c
64	<i>cis</i> -Verbenyl acetate	1188	0.04	Tr	tr	-	a, b, c
65	<i>p</i> -Mentha-1,3-diene-7-al	1194	0.05	0.04	0.1	-	b, c
66	Cuminol	1208	0.03	Tr	0.05	-	b, c
67	Bornyl acetate	1211	1.2	1.1	2.0	2.3	a, b, c
68	Sabinyl acetate	1214	0.1	0.07	0.3	tr	a, b, c
69	Carvacrol	1216	tr	tr	0.2	-	a, b, c
70	<i>trans</i> -Carvyl acetate	1218	0.04	0.2	0.7	0.04	a, b, c
71	Thymol acetate	1222	tr	tr	tr	-	a, b, c
72	<i>cis</i> -Carvyl acetate	1229	0.03	0.02	0.16	tr	a, b, c
73	Eugenol	1241	0.07	0.06	0.21	tr	a, b, c
74	α -Terpinenyl acetate	1244	0.51	0.57	0.93	0.45	a, b, c
75	α -Cubebene	1250	0.1	0.19	0.18	0.36	a, b, c
76	Geranyl acetate	1251	0.04	0.03	0.04	-	a, b, c
77	<i>trans</i> -Jasmone	1253	tr	tr	tr	-	b, c
78	<i>cis</i> -Jasmone	1257	0.03	0.03	0.07	-	b, c
79	α -Copaene	1261	0.11	0.2	0.23	0.39	a, b, c
80	β-Bourbonene	1263	0.59	0.93	1.54	0.16	a, b, c
81	α-Bourbonene	1264	0.04	0.06	0.11	0.13	a, b, c

Table 1 (Contd.)

82	Isolatedene	1265	tr	tr	tr	-	b, c
83	β -Elemene	1267	0.05	0.09	0.08	-	a, b, c
84	Alloaromadendrene	1270	-	-	-	0.22	a, b, c
85	β -Caryophyllene	1277	1.66	2.86	3.99	2.03	a, b, c
86	C ₁₅ H ₂₄	1281	0.07	0.12	0.16	0.11	b
87	β -Gurjunene	1282	0.22	0.46	0.69	0.94	a, b, c
88	α -Cadinene	1286	0.07	0.09	0.13	0.35	a, b, c
89	α -Amorphene	1287	-	-	-	0.19	a, b, c
90	α -Humulene	1289	0.32	0.56	0.47	0.66	a, b, c
91	β -Cadinene	1292	0.06	0.2	0.04	0.34	a, b, c
92	Germacrene D	1299	0.85	1.75	1.41	1.72	a, b, c
93	Bicyclogermacrene	1301	0.05	0.07	tr	-	a, b
94	Unidentified	1303	0.12	0.17	0.22	0.99	b
95	γ -Elemene	1304	0.14	0.08	0.05	-	a, b, c
96	τ -Cadinene	1306	0.03	0.03	tr	0.06	a, b, c
97	Calamene	1309	0.36	0.65	1.53	0.59	a, b, c
98	δ -Cadinene	1313	0.32	0.29	0.11	0.26	a, b, c
99	Elemol	1319	0.1	0.09	0.17	tr	a, b, c
100	Spathulenol	1326	0.17	0.14	0.64	0.26	a, b, c
101	Caryophyllene oxide	1328	2.44	2.47	8.87	1.93	a, b, c
102	Aristolene epoxyde	1330	0.1	0.1	0.15	0.09	b, c
103	8-Hydroxy-endo-cycloisolongifolene	1332	0.03	0.03	tr	-	b, c
104	γ -Gurjunen epoxide-I	1335	0.29	0.31	0.56	0.23	b, c
105	Selina-4,11-dien-2-ol	1336	0.12	0.1	0.28	-	b
106	Cubenol	1342	0.15	0.12	0.27	-	a, b, c
107	τ -Cadinol	1344	0.13	0.1	0.22	-	a, b, c
108	α -Eudesmol	1346	0.16	0.12	0.35	tr	a, b, c
109	7,8-Dehydro-8 α -hydroxy isolongifolene	1347	0.14	0.12	0.38	-	b
110	α -Cadinol	1348	0.07	0.06	0.14	-	a, b, c
111	Alloaromadendrene oxide-I	1349	0.03	0.02	0.07	tr	b
112	Ledene oxide-II	1354	0.21	0.15	0.6	tr	a, b, c
113	Patchouli alcohol	1356	0.08	0.09	0.25	-	b, c
114	Dibutyl phthalate	1370	tr	0.03	0.19	-	b
115	C ₁₉ H ₃₃	1405	0.07	0.08	0.3	-	b
116	C ₁₉ H ₃₁	1408	0.06	0.07	0.24	-	b
117	C ₁₉ H ₃₃	1411	0.03	0.04	0.14	-	b
118	Kaur-16-ene	1415	tr	tr	0.05	tr	b
119	Manoyl oxide	1417	0.03	0.04	0.15	tr	a, b
120	Dehydroabietol	1422	0.03	0.03	0.13	tr	a, b
121	8,13-Epoxy-labda-14-en-2-one	1445	0.14	0.14	3.11	tr	b
Total identified (%)		99.2	99.1	98.4	94.1		
Monoterpene hydrocarbons		62.1	63.0	27.5	40.0		
Oxygen-containing monoterpenes		27.3	23.1	43.4	43.0		
Sesquiterpene hydrocarbons		5.0	8.5	10.6	8.4		
Oxygen-containing sesquiterpenes		4.3	4.0	13.0	2.5		
Diterpenoids		0.2	0.2	3.4	0		
Others		0.4	0.2	0.5	0.2		

^aValues expressed are means of three parallel measurements ($p < 0.01$); tr = trace; Kovats index on DB-1 fused silica capillary column.

^{**} identification methods: [¹³C]-GC (Co- injection with authentic compounds); ^bGC/MS (Gas chromatography –Mass spectrometry); [^cLiterature comparison].

oil obtained by hydrodistillation, 63.0% by steam distillation, 27.5% by extraction, and 40.0% by extraction-steam distillation. All oils also contained oxygenated monoterpenoids (27.3, 23.1, 43.4, and 43.0%, respectively), sesquiterpene hydrocarbons (5.0, 8.5, 10.6, and 8.4%) and oxygenated sesquiterpenoids (4.3, 4.0, 13.0, and 2.5%). All the oils contained diterpenoids (0.2, 0.2, 3.4, and trace %) (Table 1). While the components of the oils obtained by hydrodistillation and steam distillation were qualitatively similar, there were a small number of quantitative differences. The result of the study was surprising in that the percentage concentrations of α - and β -pinene in the hydrodistilled, steam distilled, and extraction-steam distilled oils, which were prepared at raised temperature, were high, while the levels of both of these compounds were quite low in the solvent extracted oils, for which no heat was used. The percentage concentration of 8,13-epoxy-labda-14-en-2-one, a diterpenoid, in the extracted essential oil was eight times higher than its

percentage concentration in the other essential oils. However, *cis-p*-meth-2-en-1-ol was four times, and β -bourbonene and β -caryophyllene two times superior than their percentage concentrations in the hydrodistilled and steam distilled essential oils. Previously, thermal degradation of *cis*-sabinene hydrate acetate had been reported. The degradation products were found to be terpinen-4-ol, α -terpinene, γ -terpinene and terpinolene [3c].

When the high and low temperature methods are compared, the percentage concentrations of monoterpene hydrocarbons were higher than the oxygenated monoterpenoids in the hydrodistilled essential oil. However, the percentage concentrations of monoterpene hydrocarbons were found to be less than those of oxygenated monoterpenoids in the extracted essential oil. Therefore, it can be said that temperature is of great importance in the isolation of the desired compounds to be extracted from the plant.

Experimental

Plant material: *Salvia potentillifolia* Boiss. & Heldr. ex Bentham (Lamiaceae) was collected from the Gölhisar-Altınyayla region (Burdur), Turkey in July, 2006. The voucher specimen (No: S-1003), identified by Dr Tuncay DIRMENCI, has been deposited in the Herbarium of the Muğla University, Turkey.

Isolation of the essential oils and preparation of the n-hexane extract:

1. **Hydrodistilled essential oil.** This was obtained from the dried aerial parts of *S. potentillifolia* by hydrodistillation using a Clevenger type apparatus for 4 h [4a].
2. **Steam distilled oil and n-hexane extracted oils.** These were obtained according to the method of Linskens and Jackson [4b].
3. **Solvent extraction-steam distilled oil.** Plant material (970 g) was extracted with *n*-hexane. After removal of the solvent, the dry extract was steam distilled.

All oils were dried over anhydrous sodium sulfate and stored under nitrogen in a sealed vial until required.

Gas chromatography (GC): GC analyses of the essential oils were performed using a Shimadzu GC-17 AAF, V3, 230V series gas chromatogram equipped with a FID and a DB-1 fused silica capillary column (30 m x 0.25 id., film thickness 0.25 µm); the initial oven temperature was held at 60°C for 5 min, then increased to 220°C with 2°C/min increments and held at this temperature for 10 min; injector temperature and detector temperature were 250 and 270°C, respectively; carrier gas was He at a flow rate of 1.4 mL/min; sample size, 1.0 µL; split

ratio, 1:50. The percentage composition of the essential oils was determined with a Class-GC computer program. The relative percentages of the oil constituents were expressed as percentages by peak area normalization.

Gas chromatography-mass spectrometry (GC-MS):

The analysis of the essential oil was performed using a Varian Saturn 2100, (Quadrupole, EI-mode, 70 eV) equipped with a DB-1 MS fused silica capillary column (30 m x 0.25 mm ID, film thickness 0.25 µm). Carrier gas was helium at a flow rate of 1.7 mL/min. The oven temperature was held at 60°C for 5 min, then increased up to 220°C with 2°C/min increments and held at this temperature for 10 min. Injector and MS transfer line temperatures were set at 220°C and 290°C, respectively. Ion source temperature was 200°C. The injection volume was 0.5 µL with a split ratio of 1:30. Mass range was from *m/z* 28 to 650 amu. Scan time 0.5 sec with 0.1 interscan delays. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µL were injected manually in the splitless mode. Identification of components of the essential oils was based on GC retention indices and computer matching with the Wiley, NIST-2005 and TRLIB Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature and, when ever possible, by co-injection with authentic compounds.

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