

Research Article GC Analyses of Salvia Seeds as Valuable Essential Oil Source

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The essential oils of seeds of *Salvia verbenaca, Salvia officinalis*, and *Salvia sclarea* were obtained by hydrodistillation and analyzed by gas chromatography (GC) and GC-mass spectrometry. The oil yields (w/w) were 0.050, 0.047, and 0.045% in *S. verbenaca, S. sclarea*, and *S. officinalis*, respectively. Seventy-five compounds were identified. The essential oil composition of *S. verbenaca* seeds showed that over 57% of the detected compounds were oxygenated monoterpenes followed by sesquiterpenes (24.04%) and labdane type diterpenes (5.61%). The main essential oil constituents were camphor (38.94%), caryophyllene oxide (7.28%), and 13-*epi*-manool (5.61%), while those of essential oil of *S. officinalis* were α -thujone (14.77%), camphor (13.08%), and 1,8-cineole (6.66%). In samples of *S. sclarea*, essential oil consists mainly of linalool (24.25%), α -thujene (7.48%), linalyl acetate (6.90%), germacrene-D (5.88%), bicyclogermacrene (4.29%), and α -copaene (4.08%). This variability leads to a large range of naturally occurring volatile compounds with valuable industrial and pharmaceutical outlets.

1. Introduction

The genus Salvia (Lamiaceae) comprises nearly 900 species widely spread throughout the world, which display marked morphological and genetic variations according to their geographical origin [1]. Several Salvia species, namely, Salvia officinalis, Salvia sclarea, and Salvia verbenaca, are widely used in folk medicine [2]. Potential therapeutic activities of these Salvia species are due to their essential oils [3], since these species are known to possess antioxidant, antimicrobial, antifungal, and aromatic properties [4]. Chemical composition of essential oils reveals differences among these Salvia species [5-7]. Numerous investigations on Salvia officinalis show that 1,8-cineole, α -thujone, β -thujone, and camphor are the main compounds of the essential oil [8-10]. Linalool, linalyl acetate, and germacrene-D characterize S. sclarea plants [11]. Salvia species also display great intraspecific essential oil variations according to geographical origin, since sabinene, cadinene, terpinen-4-ol, and pinene are shown to be typical compounds of S. verbenaca essential oil originated from Saudi Arabia [4], while β -phellandrene and (*E*)-caryophyllene prevail in essential oil from Greece [7].

In Tunisia, *S. verbenaca* essential oil shows variations of composition according to the region origin [12, 13] and in respect to the studied plant part [14].

These numerous studies are focused on aerial parts of these species, while works interested in seeds are scanty in spite of their interest. In fact, *Salvia* seeds provide dietary and healthy oil rich in essential fatty acids (linolenic and linoleic acids) [12, 15, 16] that promote decrease in coronary heart diseases [17]. Besides, the seeds of *Salvia* species often produce mucilage on wetting [18]. This mucilage is used for lacquerware [19]. In eastern countries, the mucilage is used for the treatment of eye diseases [20].

In our continuing research on essential oil with pharmacological potential and food industry applications, we report in this paper chemical analysis of *S. verbenaca*, *S. sclarea*, and *S. officinalis* seeds as a new valuable essential oil source.

2. Materials and Methods

2.1. Plant Material. The seeds of Salvia verbenaca (accession PI 420430) were originated from Spain; Salvia officinalis (accession W6 20659) and Salvia sclarea (accession W6

20660) which are from Italy were kindly supplied by the US National Plant Germplasm System (NPGS).

2.2. Essential Oil Isolation. The seeds of each species were ground in a mortar before essential oil isolation [12] and were subjected to conventional hydrodistillation for 90 min followed by a liquid-liquid extraction using diethyl ether and *n*-pentane mixture (v/v) as solvent. The concentration step was carried out at 35° C using a Vigreux column and the essential oils obtained were dried over anhydrous sodium sulphate and stored in amber vials at -18° C until they were analyzed.

3. Chromatographic Analysis

3.1. Gas Chromatography (GC-FID). The essential oils were analysed by gas chromatography using a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A polar HP Innowax (PEG) column and an apolar HP-5 column (30 m × 0.25 mm, 0.25 μ m film thicknesses) were used. The carrier gas was N₂ with a flow rate of 1.6 mL/min; split ratio was 60 :1. The analysis was performed using the following temperature program: oven temps isotherm at 35°C for 10 min, from 35 to 205°C, at the rate of 3°C/min, and isotherm at 205°C during 10 min. Injector and detector temperatures were held, respectively, at 250 and 300°C. The volume injected was 1 μ L.

3.2. Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analysis was performed on a gas chromatograph HP 5890 (II) interfaced with a HP 5972 mass spectrometer with electron impact ionization (70 eV). A HP-5MS capillary column (30 m × 0.25 mm, 0.25 μ m film thickness) was used. The column temperature was programmed from 50°C to rise to 240°C at a rate of 5°C/min. The carrier gas was helium with a flow rate of 1.2 mL/min; split ratio was 60 : 1. Scan time and mass range were 1 s and 40–300 m/z, respectively.

3.3. Compounds Identification. The identification of the essential oil constituents was based on the comparison of their retention indexes relative to (C_8-C_{22}) *n*-alkanes with those of literature or with those of authentic compounds available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC-MS data system and other published mass spectra [21].

3.4. Statistical Analyses. Data were subjected to statistical analysis using "Statistica" statistical program package [22]. The percentages of volatile compounds are means of three experiments; the one-way analysis of variance (ANOVA) followed by Duncan multiple range test was employed and the differences between individual means were deemed to be significant at P < 0.05.

4. Results

Hydrodistillation of full ripened seeds of *S. verbenaca*, *S. sclarea*, and *S. officinalis* offered essential oils with average yields of 0.050, 0.047, and 0.045% (w/w on the dry weight basis), respectively.

Essential oil constituents of *Salvia* seeds were presented in Table 1. The results of analysis of essential oil of *S. verbenaca* seeds by GC and GC-MS techniques revealed the occurrence of thirty-two compounds. The essential oil composition showed that over 57% of the detected compounds were oxygenated monoterpenes followed by sesquiterpenes (24.04%) and labdane type diterpenes (5.61%). Apart from camphor, the main essential oil constituents of this sample were caryophyllene oxide (7.28%), 13-*epi*-manool (5.61%), δ elemene (3.97%), and β -eudesmol (3.76%).

The essential oil of *S. officinalis* seeds showed a higher percentage of monoterpenes (56.59%) than sesquiterpenes (17.32%). Oxygenated derivatives were major among the monoterpenes (50.14%), while they represented only 5.93% of sesquiterpenes. Seeds essential oils were characterised by the predominance of α -thujone (14.77%), camphor (13.08%), and 1,8-cineole (6.66%). Viridiflorol (2.66%) and α -humulene (3.71%) were also detected in seeds essential oil of sage. Furthermore, other minor compounds were found, especially the labdane type diterpene 13-*epi*-manool.

Essential oil compounds of *S. sclarea* seeds were representing 80.79% of total essential oil components. The essential oil is predominated by monoterpenes accounting for 47.98%; their oxygenated derivatives (38.38%) prevailed on hydrocarbon ones (9.60%). The sesquiterpenes pool is less numerous (29.39%). In addition, one diterpene compound, 13-*epi*-manool, is detected at a level of 0.59% and some phenols (2.03%) such as thymol and carvacrol were produced in small amounts (0.1% and 1.93%, resp.). The oxygenated monoterpenes were characterised by linalool (24.25%), geraniol (2.79%), and their ester derivates (linalyl acetate (6.90%) and geranyl acetate (1.94%)). The sesquiterpenes characteristics of seeds were germacrene-D (5.88%), bicyclogermacrene (4.29%), and α -copaene (4.08%).

5. Discussion

As for *S. verbenaca*, the oil yield was higher than that offered by seeds originated from Tunisia [12]. Compared with leaves of *S. verbenaca* from Tunisia [14], seeds herein studied appeared as moderately rich in volatile oil. Recovered essential oil from *S. sclarea* seeds appeared to be near to literature data which showed that inflorescences oil yield of a cultivated strain developed in India [23]. In *S. officinalis*, the fruits of sage cultivated in Tunisia are distinguished by oil yields of 0.39% [24]. Thereby seeds appeared as oil-moderate organs contrary to the different other parts of the species owing to the genus *Salvia* its aromatic reputation amongst Lamiaceae family.

As regards essential oil composition of *S. verbenaca*, it is worthy to note that tricyclene and camphor are also common to the seeds sample from Tunisia [12] so we may suggest that these two compounds are significant markers of

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TABLE 1: Essential oil composition (%w/w) of Salvia verbenaca (Sv), Salvia officinalis (So), and Salvia sclarea (Ss) seeds.

Number	Compounds*	RI ^a	RI^{b}	Sv	So	Ss	Identification
1	Hexanal	800	1093	0.42	—	—	GC-MS, Co-GC
2	(E)-2-Hexenal	852	1232	_	—	0.03	GC-MS, Co-GC
3	1-Hexanol	878	1360	_	1.29	—	GC-MS, Co-GC
4	Tricyclene	927	1014	0.96 ^a	0.23 ^b	0.08 ^c	GC-MS
5	α-Thujene	930	1035	_	3.08 ^b	7.48 ^a	GC-MS
6	α-Pinene	939	1032	0.44^{b}	1.26 ^a	0.27 ^c	GC-MS
7	δ-3-Carene	1012	1159	0.50^{a}	0.03 ^b	_	GC-MS
8	p-Cymene	1022	1280	0.37^{b}	1.52 ^a	0.12 ^c	GC-MS
9	Limonene	1030	1203	_	_	0.17	GC-MS, Co-GC
10	β -Phellandrene	1030	1218	_	_	0.03	GC-MS
11	1,8-Cineole	1033	1213	_	6.66 ^a	0.12^{b}	GC-MS, Co-GC
12	cis-Sabinene hydrate	1068	1556	_	0.19	_	GC-MS
13	cis-Linalool oxide	1072	1478	_	0.13	_	GC-MS
14	α-Fenchone	1087	1406	0.26	_	_	GC-MS
15	trans-Linalool oxide	1087	1450	1.05	_	_	GC-MS
16	Linalool	1098	1553	0.84^{b}	0.68^{b}	24.25 ^a	GC-MS, Co-GC
17	<i>n</i> -Undecane	1100	1100	2.65 ^a	0.48 ^c	0.75 ^b	GC-MS, Co-GC
18	α -Thujone	1102	1429	0.52 ^b	14.77 ^a	_	GC-MS
19	cis-allo-Ocimene	1113	1382	1.55 ^a	0.14 ^b	1.46 ^b	GC-MS
20	β -Thujone	1114	1451	_	4.30	_	GC-MS
21	Geijerene	1140	1338	_	1.01	_	GC-MS
22	Camphor	1143	1532	38.94 ^a	13.08 ^b	_	GC-MS
23	Borneol	1165	1719	_	3.54	_	GC-MS
24	Terpinen-4-ol	1177	1611	0.88 ^a	0.09 ^c	0.37 ^b	GC-MS
25	p-Cymen-8-ol	1185	1864	_	0.17		GC-MS
26	δ-Terpineol	1187	1682	0.49^{b}	2.42 ^a	0.20 ^c	GC-MS
27	α-Terpineol	1189	1706	2.03 ^a	0.91 ^b	0.20 ^c	GC-MS
28	Myrtenal	1190	1648	_	0.55	_	GC-MS
29	Myrtenol	1194	1804	_	0.28	_	GC-MS
30	cis-Sabinol	1210	1800	_	0.18	_	GC-MS
31	Nerol	1228	1797	_	_	0.98	GC-MS, Co-GC
32	Linalyl acetate	1239	1565	2.53 ^b	0.08 ^c	6.90 ^a	GC-MS
33	Geraniol	1254	1857	_	0.33 ^b	2.79 ^a	GC-MS, Co-GC
34	Bornyl acetate	1285	1597	0.79 ^a	0.16 ^b	_	GC-MS
35	Thymol	1293	2198	_	0.37 ^a	0.10^{b}	GC-MS
36	Carvacrol	1296	nd	_	0.83 ^b	1.93 ^a	GC-MS
37	δ-Elemene	1337	1479	3.97 ^a	0.07 ^c	0.85 ^b	GC-MS
38	α-Cubebene	1348	1456	_	_	2.86	GC-MS
39	α -Terpinyl acetate	1353	1709	4.77 ^a	1.81 ^b	0.29 ^c	GC-MS
40	Eugenol	1355	2192		0.83		GC-MS, Co-GC
41	Neryl acetate	1366	1733	2.40^{a}	_	0.36 ^b	GC-MS, Co-GC
42	α-Ylangene	1372	1493		0.04^{b}	0.24 ^a	GC-MS
43	α-Copaene	1376	1497	_	0.01 ^b	4.08 ^a	GC-MS
44	β -Damascenone	1381	1838	_	0.06		GC-MS
45	Geranyl acetate	1383	1765	_		1.94	GC-MS, Co-GC
45	β -Bourbonene	1383	1533	1.73 ^a	_	0.35 ^b	GC-MS
40	β -Elemene	1384	1600		0.16		GC-MS
48	Methyl eugenol	1389	2030		0.10		GC-MS, Co-GC
48	β -Caryophyllene	1402	1612	0.27 ^a	0.19 ^b	0.24 ^a	GC-MS, CO-GC
49 50	β -Cubebene	1413	1549	1.50 ^a	0.19	0.24 0.72 ^b	GC-MS GC-MS
50	Aromadendrene	1419	1628	0.66 ^a	0.18 ^b	0.72	GC-MS

TABLE 1: Continued.

Number	Compounds*	RI ^a	RI ^b	Sv	So	Ss	Identification
52	(Z) - β -Farnesene	1441	1668	1.76	—	—	GC-MS
53	α-Humulene	1454	1687	0.67 ^b	3.71 ^a	0.08^{c}	GC-MS, Co-GC
54	allo-Aromadendrene	1460	1661	1.29 ^b	1.43 ^a	0.06 ^c	GC-MS
55	α-Amorphene	1474	1680	_	0.47	_	GC-MS
56	γ-Muurolene	1477	1704	0.05	_	_	GC-MS
57	Germacrene-D	1479	1726	_	1.18 ^b	5.88 ^a	GC-MS
58	epi-Cubebol	1491	1900	_	0.34 ^a	0.24^{b}	GC-MS
59	Bicyclogermacrene	1494	1755	_	1.29 ^b	4.29 ^a	GC-MS
60	(<i>E</i> , <i>E</i>)-α-Farnesene	1506	1758	—	0.24	_	GC-MS
61	β -Bisabolene	1508	1741	1.10 ^a	0.72 ^b	_	GC-MS
62	γ-Cadinene	1513	1776	_	0.08	_	GC-MS
63	δ-Cadinene	1517	1773	_	0.53 ^a	0.24^{b}	GC-MS
64	α-Calacorene	1542	1942	_	_	0.10	GC-MS
65	Germacrene-B	1558	1854	_	0.08^{b}	1.29 ^a	GC-MS
66	(E)-Nerolidol	1563	2050	_	1.41	_	GC-MS
67	Spathulenol	1575	2144	_	0.08^{a}	0.03 ^b	GC-MS
68	Caryophyllene oxide	1579	2008	7.28 ^a	0.16 ^c	3.18 ^b	GC-MS
69	Viridiflorol	1592	2104	_	2.66	_	GC-MS
70	Humulene epoxide I	1596	2045	_	_	1.55	GC-MS
71	Humulene epoxide II	1606	2071	_	0.25	_	GC-MS
72	T-Cadinol	1642	2187	_	0.15 ^b	0.52 ^a	GC-MS
73	α -Cadinol	1643	nd	_	0.41^{b}	0.88^{a}	GC-MS
74	β -Eudesmol	1649	nd	3.76 ^a	0.47 ^c	1.72 ^b	GC-MS
75	13-epi-Manool	Nd	nd	5.61 ^a	2.22 ^b	0.59 ^c	GC-MS
Co	npound classes						
	Alcohols			2.46 ^a	0.42^{b}	0.03 ^c	
Alip	Aliphatic hydrocarbons		anes	_	1.29	_	
	Aldehydes			0.84^{a}	0.48 ^c	0.75 ^b	
	Monoterpene hydrocar		3.82 ^c	6.45 ^b	9.60 ^a		
	Oxygenated monoterp		57.30 ^a	50.14 ^b	38.38 ^c		
	Sesquiterpene hydrocar		13.00 ^b	11.39 ^b	21.27 ^a		
	Norisoprenoids with 13 c		_	0.06	_		
	Phenylpropanes		_	1.01	_		
	Phenols		_	1.10^{b}	2.03 ^a		
	Oxygenated sesquiterp		11.04 ^a	5.93 ^c	8.12 ^b		
	Labdane type diterper		5.61 ^a	2.22 ^b	0.59 ^c		
	Total		92.03 ^a	80.17 ^c	80.79 ^b		

* Components are listed according to their elution on apolar column (HP-5). RI: retention indices relative to $C_8 - C_{22}$ *n*-alkanes on the ^aHP-5 and ^bHP-Innowax columns; nd: not detected; GC/MS: identification based on comparison of mass spectra; Co-GC: identification based on retention time comparison to authentic compounds. Values (means of three replicates) in the same lines with different letters (a-c) are significantly different at *P* < 0.05.

essential oil compounds of *S. verbenaca* seeds whatever the sample origin is. Interestingly, the essential oil of the studied seeds could be employed as antimicrobial agent since their high percentage of camphor is associated with an efficient antimicrobial activity according to Magiatis et al. [25] and Bougatsos et al. [26].

In *S. officinalis*, the predominance of α -thujone, camphor, and 1,8-cineole endowed to the essential oil an antimicrobial activity [27]. Viridiflorol and α -humulene identified in several *S. officinalis* essential oils showed antiacetyl-cholinesterase activity used in the treatment of Alzheimer's

disease [28], antifungal property [29], and cytotoxic activity against some tumor cell lines [30]. Furthermore, the labdane type diterpene 13-*epi*-manool displays *in vitro* a cytotoxic activity against human leukemic cell lines [31]. We noted that seeds had a similar qualitative composition to aerial parts with the predominance of α -thujone, β -thujone, camphor, and 1,8-cineole. These monoterpenes are taken as significant parameter to differentiate *S. officinalis* from other species [32]. Their amount is lower than that in leaves but matches the ranges of the standard ISO 9909 for official sage oil except for the toxic ketone α -thujone which had a lower amount (14.77%). These findings promote the use of the seeds essential oil in food industry.

Similar to the studied S. sclarea essential oil, the sesquiterpenes were mainly composed of germacrene-D, α -copaene, and bicyclogermacrene in plant inflorescence according to Carrubba et al. [11] and Lorenzo et al. [33]. In our sample, the essential oil of S. sclarea flowering shoots raised in experimental plots in India was characterised by linalool (36.6-41.9%) and linalyl acetate (13.2-19.2%) as main compounds [23]. The wild-growing S. sclarea collected at flowering stage from central Greece [7] and Spain [6] showed a close similar composition regarding linalool and linalyl acetate (30.43%, 32.97% and 19.75%, 16.85%, resp.). Generally, S. sclarea essential oil is extracted from flowering shoots which are found to be rich in linalool and linalyl acetate. According to Carrubba et al. [11] high amounts of linalool and linalyl acetate are typical of good quality oil suitable for flavouring purposes. Furthermore, linalool plays a major role as antiinflammatory suggesting that linalool-producing species are potentially anti-inflammatory agents [34]. Moreover, linalool has an ecological role since it constitutes one of the common components of floral scent that can attract a large variety of insects that convey pollen [35]. The present study showed that essential oil derived from seeds had a similar composition to the flowering parts. Thus, seeds seemed to display the same enzymatic patterns of the essential oil biosynthesis as the flowers, while the essential oil composition of S. sclarea vegetative organs displayed different qualitative trends from reproductive parts.

6. Conclusions

Overall, it emerges that tricyclene and camphor were biochemical markers of the essential oil of *S. verbenaca* seeds. Being rich in camphor, seeds could be used as antimicrobial agent. Another point that should be highlighted is that *S. officinalis* seeds had the same α -thujone chemotype as leaves, whereas these two organs showed some quantitative differences leading to the safe use of seeds essential oil in food industry. From a qualitative standpoint, seeds of *S. sclarea* seemed to have the same enzymatic trend as flowers characterized by the prevalence of linalool. It is noteworthy to mention that linalool-producing seeds as *S. sclarea* were suitable for flavouring purposes and constitute potential antiinflammatory agents.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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