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Research article

COMPARISON OF *SALVIA MIRZAYANII* VOLATILE COMPOUNDS EXTRACTED BY HEADSPACE EXTRACTION AND HYDRODISTILLATION METHODS

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ABSTRACT: GC-MS analysis identified forty seven and fourteen components in oils extracted using HD and in the volatile fractions extracted using HSE, respectively. eudesm-7(11) en-4-ol (28.46%), α-terpinyl acetate (12%), germacrene D-4-ol(7.3%), 1,8-cineole (6.22%), δ-cadinene (6.49%), bicyclogermacrene (5.20%) and α-cadinol (5.06%) were recorded as the main components of the essential oil and The major components of the volatile emitted by headspace were linalyl acetate (52.29%), 1,8-cineole (19.77%), α-terpinyl acetate (7.56%) and trans-β-ocimene (4.50 %). Exploiting hydro-distillation, sesquiterpenes were identified more than monoterpenes however by using HSE just monoterpenes were recorded by GC/MS analysis. This study proved that HD and HSE methods could be complimentary extraction techniques in order to obtain the complete characterization of plant volatiles.

Keywords: *Salvia mirzayanii,* Headspace, Hydrodistillation, Gas chromatography-mass spectrometry, Essential oil, volatile component.

INTRODUCTION

Salvia, one of the largest genus of the Lamiaceae family, includes nearly 900 species which are spread throughout the world. In the Flora Iranica, this genus is represented by 60 species and 17 species of them are endemic to Iran [1]. *Salvia mirzayanii* (local name: Moor Talkh) growing in the southern parts of Iran [2], is used in folk medicine for the treatment of diarrhea, stomach ache, headache, hyper cholesterolemia, diabetes, and also for wound healing [3]. Spathulenol, δ-cadinene, linalool, α-terpinyl acetate, α-cadinol, β-eudesmol, cubenol and linalyl acetate were reported as major components of the *S. mirzayanii* essential oil [4] Ziaei *et al.* (2011) informed the presence of spathulenol, a sesquiterpene with immunomodulatory effects in this plant [5]. Literature search revealed that the chemical compositions depend on many parameters such as harvesting time, extraction technique, the geographical origin of the plant, and the part of the plant analyzed [6-8]. The essential oils of aromatic herbs are traditionally obtained using hydrodistillation. Because the emitted volatile fraction plays a fundamental role in a plant's life, various novel techniques have been developed for its extraction from plants. Among these, headspace allows for the rapid fingerprinting of a plant's volatiles [9-12]. It is a simple, rapid, solventless sample-pre-treatment technique that can automatically perform sampling, clean-up, concentration, derivatization [13]. The aim of this study was to investigate the applicability of HSE for the analysis of *Salvia mirzayanii* volatiles and compare qualitatively the HSE-obtained composition of volatile compounds emitted from the aerial parts with the composition of an essential oil obtained using the hydrodistillation method.

MATERIAL AND METHODS

Plant Material

The aerial parts of *S. mirzayanii* were collected in May 2011 from plants growing wild in Post-e-Chenar, in Fars province (South of Iran). Botanical authentication was done by the herbarium of Fars Research Center for Agriculture and Natural Resources (Shiraz, Iran), where a herbarium specimen No-8391 was deposited.

Hydrodistillation essential oil isolation

The aerial parts were air-dried at ambient temperature in the shade. The dried samples of *Salvia mirzayanii* were subjected to hydro-distillation using an all glass Clevenger-type apparatus for 3 hours, to extract essential oils, according to the method outlined by the European Pharmacopoeia [14]. The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4°C) before gas chromatography-mass spectrometric (GC/MS) analysis.

Headspace Extraction

Up to 3 gr of each *Salvia mirzayanii* dried sample was crashed and placed in 20 ml headspace vial, and immediately sealed with silicone rubber septa and aluminum caps. The vials were then transferred to the headspace tray. The headspace proceeded on the CombiPAL System which was provided with headspace auto-sampler, heater and agitator. The vial was heated up to 80° C and retained for 20 minutes while being agitated; the temperature of the sampling needle and transmission lines temperature was 85°C.

Identification of the oil components by GC/MS

The Essential oils were analyzed using an Agilent model 7890-A series gas chromatography and Agilent model 5975-C mass spectrometry. The HP-5 MS capillary column (phenyl methyl siloxane,30m × 0.25 mm i.d× 25µm) was used with Helium at 1ml/min as carrier gas. GC oven temperature was programmed from 60 ºC to 210 ºC at rate of 3 ºC /min and then was increased from 210 ºC to 240 ºC at rate of 20 ºC /min and kept costant at 240 ºC for 8.5 min. The split ratio was adjusted to 1:50 and the injection volume was 1000µl. The injector temperature was 280 ºC. The quadrupole mass spectrometer was scanned over 40-550 amu with an ionizing voltage of 70 eV. Retention indices were determined using retention times of n-alkanes (C8-C25) that were injected after the essential oil under the same chromatographic conditions. The retention indices for all components were determined according to the method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library data published mass spectra data [15-17].

RESULTS

GC–MS analyses of the *S. mirzayanii* essential oils isolated by hydrodistillation (HD) led to the identification of forty seven constituents, representing 99.79% of the total oil composition. The identified constituents with their respective percentages and RIa are summarized in (Table 1). eudesm-7(11) en-4-ol (28.46%), α-terpinyl acetate (12%), germacrene D-4-ol (7.3%), 1,8-cineole (6.22%), δ-cadinene (6.49%), bicyclogermacrene (5.20%) and α - cadinol (5.06%) were the main components (Table 1). In the aroma extracted by headspace extraction (HSE) fourteen compounds were detected and linalyl acetate (52.29%), 1,8-cineole (19.77%), α- terpinyl acetate (7.56%) and trans- β- ocimene (4.50 %) were the main ones (Table 2). Exploiting hydro-distillation, sesquiterpenes were identified more than monoterpenes however by using HSE just monoterpenes were discerned by GC/MS analysis.

DISCUSSION

In two former researches over this plant [4, 18], eudesm-7(11) en-4-ol, germacrene D-4-ol and bicyclogermacrene (our first, third and sixth major components) were not identified as main oil components. α-terpinyl acetate was the second major component in our research while Yamini *et al*. (2008) reported this monoterpene as the first major component of their study, also in another study [4], it was the 4th one. As this study, δ-cadinene and α-cadinol were reported as major components in the research of Javidnia *et al*. (2002), besides, 1,8-cineol was referred as main essential oil constituent by Yamini *et al*. (2008). As it is shown in the Tables (1 and 2), all of the fourteen components identified in the aroma were monoterpenes but the greater part of the essential oil composition representing 62.62% of the oil (16 chemical constituents) was sesquiterpenes. The second major component of aroma (1,8-cineol) was the fourth major component of oil and the third aroma major component (α-terpinyl acetate) was distinguished as the second major constituent of the essential oil. Eudesm-7(11) en-4-ol, germacrene D-4-ol, δ-cadinene, bicyclogermacrene and α- cadinol were not detected by GC/MS analysis over aroma obtained by HSE technique and exploiting HD technique, linalyl acetate and trans-β-ocimene were not discerned by GC/MS analysis. Many of the volatile compounds cannot always be recovered and often evaporate and also during the plants processing, different chemical reactions take place, so that its aroma no longer seems that of the actual plant and the product is different in composition that rarely happens in headspace technique [19].

Table 1. Essential Oil Chemical Compositions (%) of *Salvia mirzayanii*

No.	Compounds	RI	HD/GC-MS
1	α-thujene	845	0.14
2	α –pinene	897	0.83
3	Camphene	903	0.01
4	Sabinene	946	1.12
5	β-pinene	948	1.43
6	6-methyl-5-hepten-2-one	953	0.004
7	Myrcene	991	0.74
8	α –terpinene	1036	0.05
9	p-Cymene	1039	0.06
10	Limonene	1066	0.61
11	1,8-cineol	1096	6.23
12	cis-β-ocimene	1105	0.01
13	trans-β-ocimene	1158	0.03
14	γ-terpinene	1162	0.07
15	Terpinolene	1168	0.11
16	Linalool	1237	0.2
17	1,3,8-p-menthatrien	1242	0.02
18	δ-terpineol	1349	0.42
19	terpinene-4-ol	1360	0.17
20	α –terpineol	1392	0.93
21	Myrtenol	1410	0.05
22	trans-carveol	1413	0.06
23	δ-elemene	1416	0.9
24	α -terpinyl acetate	1429	12
25	β-Elemene	1435	1.4
26	Longifolene	1447	0.8
27	α –gurjunene	1448	1.2
28	α –guaiene	1459	0.55
29	allo-aromadendrene	1458	0.32
30	Bicyclogermacrene	1463	5.2
31	α –muurolene	1497	0.45
32	γ-cadinene	1510	0.33
33	cis-dihyroagarofuran	1518	4.71
34	δ-cadinene	1525	6.5
35	Elemol	1546	0.5
36	germacrene D-4-ol	1575	7.3
37	Spathulenol	1577	2.4
38	Globulol	1581	0.56
39	1-epi-cubenol	1624	0.41
40	α -epi-cadinol	1639	2.8
41	α –muurolol	1643	0.3
42	β-eudesmol	1647	2.2
43	α –cadinol	1653	5.06
44	eudesm-7(11)en-4-ol	1697	28.5
45	d-14-hydroxy-cadinene	1803	0.33
46	manoyl oxide	2010	0.47
47	Kauerene	2033	0.25
Total			99.79

RI, retention indices

Table 2. Headspace (HSE) volatile chemical Compositions (%) of *Salvia mirzayanii*.

No.	Compounds	RI	HD/GC-MS
1	α –pinene	932	0.9
2	Sabinene	971	1.8
3	β-pinene	975	1.3
4	Myrcene	988	1.3
5	Limonene	1026	0.9
6	1,8-cineol	1029	19.78
7	cis-β-ocimene	1034	0.4
8	trans- β –ocimene	1044	4.5
9	linalyl acetate	1253	52.3
10	α -terpinyl acetate	1346	7.6
11	β- elemene	1389	1.0
12	Longifolene	1406	1.28
13	α-gurjunene	1416	3.3
14	Bicyclogermacrene	1493	3.6
Total			99.96%

RI, retention indices

HSE is currently a well-established and widely used sampling technique to study the composition of the volatile fraction of medicinal and aromatic plants for which it has now become an important complement to essential oil analysis. For those applications where the essential oil composition is not officially required, HSE sampling is successfully used as an alternative to hydro- or steamdistillation to characterize the volatile fraction of a plant because its reliability is comparable but it is faster and easy to automate [20]. It could be concluded that HD and HSE methods could be complimentary extraction techniques in order to obtain the complete characterization of plant volatiles.

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