



Study of extraction and chemical compounds of *Scrophularia striata* Boiss. and *Scrophularia deserti* Delile using HS-SPME and GC-MS

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Abstract

In this study, from June 2019 to May 2019 aerial parts of *Scrophularia striata* Boiss. were collected from Ilam city and those of *Scrophularia deserti* Delile from Dehloran city, Ilam province in western Iran. The aerial parts of the medicinal plants were pulverized. The essential and volatile oils were extracted by headspace solid-phase microextraction (HS-SPME) and their chemical compounds analyzed by Gas chromatography-mass spectrometry (GC-MS). Using HS-SPME, 68 and 49 chemical compounds were identified from *S. striata* and *S. deserti*, respectively. Spathulenol (18.41%) was the highest chemical compound of the essential oil of *S. striata*, followed by caryophyllene oxide (15.38%), linalol (11.85%), alpha-terpineol (8.34%), trans-caryophyllene (4.44%) and geraniol (3.10%). The most important identified compounds from *S. deserti* were caryophyllene oxide (15.38%), linalool (11.85%), alpha-terpineol (8.34%), trans-caryophyllene (4.44%) and geraniol (3.10%). The most important chemical compounds of *S. deserti* essential oil included alpha-pinene (25.54%), alpha-phellandrene (19.60%), beta-myrcene (11.29%) and trans-caryophyllene (6.78%). Results show major constituents of this two plant include with a high amount of Spathulenol and caryophyllene oxide has high pharmaceutical and healthful value.

Citation:

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Introduction

The Scrophulariaceae family is composed of 220 genera. The genus is one of the major genera of the Scrophulariaceae family. These genera such as *Scrophularia farinosa* Boiss and *Scrophularia amplexicaulis* Benth mainly distribute in mountainous areas and are rarely found in deserts, such as *Scrophularia deserti* Delile. This genus has 60 species in Iran and can be used as heart stimulants, circulatory stimulants and diuretics [1]. *Scrophularia deserti* belonging to the Scrophulariaceae family, occurs mostly as grassy or shrubby and rarely as tree, its leaves are alternate, crossed, simple and without ear-rings, its flowers are five feathers, zygomorphic, its flower cup has lobes and its fruit is usually like capsule with multiple seeds [2]. *S. deserti* occurs in Persian Gulf countries and also in Iran, especially in western and southern parts of the country, such as Ilam, Kermanshah, Hamadan, Khuzestan including Ahvaz, Lorestan, Bushehr, Hormozgan and Yazd [3]. *S. deserti* has antimicrobial, antifungal, wound healing, anti-cancer, wound cleansing, anti-diabetic, and anti-inflammatory effects. The most im-

portant constituents of the plant include harpagoside B and scopolioside-D2 belonging to iroid glycosides [4, 5]. *S. deserti* occurs mostly as grassy or shrubby and rarely as tree, its leaves are alternate, crossed, simple and without ear-rings, its flowers are five feathers, zygomorphic, its flower cup has lobes and its fruit is usually like capsule with multiple seeds [6]. Studies show that the plant is gastric tonic, and has traditionally been used to treat diseases such as scrofula, scabies, tumors, eczema, psoriasis and inflammatory infections, and is somehow effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [7, 8].

Therefore, due to the popular use of *S. deserti* and *S. striata* in traditional medicine of Ilam, as part of continuous phytochemical investigations of chemical compounds, the essential oils of the two plants' aerial parts were analyzed using GC-MS and HS-SPME.

Materials and methods

Plant preparation

S. striata samples were collected from Ilam in June 2019 and *S. deserti* samples from Dehloran in May 2019. The plant was identified and confirmed using morphological keys of Ilam Province Plant Flora Book at Ilam University of Medical Sciences Biotechnology and Medicinal Research Center. The collected plants were cleansed and shade dried in the open air.

The dried plant was pulverized by a plant mixer and analyzed by HS-SPME for chemical composition analysis. The characteristics of the medicinal plants studied are listed in Table 1.

Table 1. The details of *Scrophularia striata* and *Scrophularia deserti*

Plant name	Scientific name	Herbal family	Collecting area	Geographical coordinates
Organic snapper	<i>Scrophularia striata</i>	Scrophulariaceae	Ilam	32° 41' 28" North, 47° 15' 58" East
Snapdragon desert	<i>Scrophularia deserti</i>	Scrophulariaceae	Dehloran	33° 38' 14.64" North, 46° 25' 21.72" East



Scrophularia striata



Scrophularia deserti

Figure 1. Pictures of medicinal plants of *Scrophularia striata* and *Scrophularia deserti*

Identification of compounds using HS-SPME

In this experiment, the essential oil of the plant was extracted by HS-SPME technique. In this technique, about 2 grams of dried plant and its powder were placed in the vial and the vial temperature was set at 60-70 °C. These optimum temperature conditions will saturate the vapor content of the substances in the plant essential oil in the headspace of the solid surface. The SPME syringe with a lid on it was then placed in the headspace of the container and the material in the vapor was absorbed by the silica phase in the instrument needle. After the silica fiber was allowed to sufficiently saturate with volatile components, the fiber was directly placed into the GC/MS input section and materials present in the fiber were adsorbed due to the temperature of the input and then entered into the GC/MS apparatus for identification [9].

HS-SPME Method

2 g of each plant extract was used for analysis. The device condition was as follows: Gas chromatograph (Agilent6890N) was coupled to Agilent 5973 Mass detector; Column: HP - 5. (30 m length × 0.25 mm (ID) × 0.25 μm (stationary phase thickness); Injector type: split/splitless and column temperature program: 50oC, hold time 0.00 min and rate of -oC/min; temperature

200oC, hold time, 0.00 min and rate of 5oC/min and temperature 240°C, hold time 0.00 min and rate of 10oC/min. Carrier gas: He (99.999%); Injection type: splitless; Library: Wiley 7n; Injector temperature: 250°C and flow rate: 0.9 mL/min. Extraction mode: (HSSPME); SMPE fiber: PDMS 100 μm thickness (SUPELCO); sample weight: 0.5 g; extraction temperature: 60oC; extraction time: 20 min; sonication time: 10 min (Euronda sonication instrument, Italy) and desorption time in GC-MS injector port: 3 min [10].

Results

2 g of each plant extract was used for analysis. The device condition was as follows: Gas chromatograph (Agilent6890N) was coupled to Agilent 5973 Mass detector; Column: HP - 5. (30 m length × 0.25 mm (ID) × 0.25 μm (stationary phase thickness); Injector type: split/splitless and column temperature program: 50oC, hold time 0.00 min and rate of -oC/min; temperature 200oC, hold time, 0.00 min and rate of 5oC/min and temperature 240°C, hold time 0.00 min and rate of 10oC/min. Carrier gas: He (99.999%); Injection type: splitless; Library: Wiley 7n; Injector temperature: 250°C and flow rate: 0.9 mL/min. Extraction mode: (HSSPME); SMPE fiber: PDMS 100 μm thickness (SUPELCO); sample weight: 0.5 g; extraction temperature: 60oC; extraction time: 20 min; sonication time: 10 min (Euronda sonication instrument, Italy) and desorption time in GC-MS injector port: 3 min [10].

Table 2. Identified constituents from essential oil of *Scrophularia striata* aerial parts using headspace solid-phase microextraction (GC-MC)

No.	Retention time	compound	%
1	4.49	2-Hexenal	0.01
2	6.15	Alpha-thujene	0.03
3	6.33	Alpha.-pinene	0.39
4	6.65	Sbinene	0.02

No.	Retention time	compound	%	No.	Retention time	compound	%
5	6.74	Camphene	0.35	50	25.54	Valencene	0.42
6	7.97	2-.Beta.-pinene	0.31	51	25.78	bicyclogermacrene	1.70
7	8.25	3-Octanol	0.06	52	26.09	Beta-bisabolene	0.64
8	8.39	Delta.3-Carene	0.05	53	26.79	beta.-Bisabolene	0.15
9	8.77	alpha.-Terpinene	0.64	54	26.92	CIS-.Alpha.-Bisabolene	0.46
10	9.06	p-Cymene	0.30	55	27.29	Trans-geraniol	2.45
11	9.24	1,8-Cineole	1.66	56	27.50	Spathulenol	18.41
12	9.36	2-Dodecanol, 2-methyl-	0.37	57	27.66	farnesol	1.90
13	9.80	beta.-trans-Ocimene	0.50	58	27.83	Caryophyllene oxide	15.83
14	10.14	gamma.-Terpinene	1.22	59	28.08	Alpha-santalol	2.46
15	10.56	trans-Sabinene hydrate	1.04	60	28.23	Beta-selinene	0.91
16	10.68	CIS-Linaloloxide	1.22	61	28.86	Dihydrocarveol	1.40
17	11.12	Alpha.-Terpinolene	0.29	62	29.00	Gamma-cadinene	0.52
18	11.20	Trans-linalool oxide	0.63	63	29.14	t-Muurolol	1.35
19	11.43	alpha.-Naginatene	0.12	64	29.43	Caryophyllenol	1.48
20	11.79	Linalool	11.85	65	30.34	virtenal	2.35
23	12.68	5-Hepten-1-ol, 2,6-dimethyl-	0.39	66	30.97	beta.-Citronellal	0.15
24	12.90	Isopulegol	0.08	67	32.75	geranyl ethyl ether	0.09
25	13.09	Camphor	1.57	68	34.22	Eicosane	0.15
27	13.78	alpha.-Thujone	0.23				
28	13.97	Borneol L	1.03				
30	14.92	Alpha-Terpineol	8.34				
31	15.37	Alpha-fenchene	0.70				
34	17.20	Geraniol	3.10				
36	17.89	Borneol, acetate	0.59				
37	18.33	Nerol	0.20				
38	18.76	Thymol	0.69				
39	19.07	Carvacrol	0.57				
40	19.60	Cuminyal alcohol	0.23				
41	20.27	cis-2,6-Dimethyl-2,6-octadi-ene	0.75				
42	20.96	alpha.-Amorphene	0.03				
45	22.54	alpha.-Gurjunene	0.22				
46	23.08	trans-Caryophyllene	4.44				
48	23.90	Aromadendrene	1.04				
49	25.35	Germacrene-D	1.92				

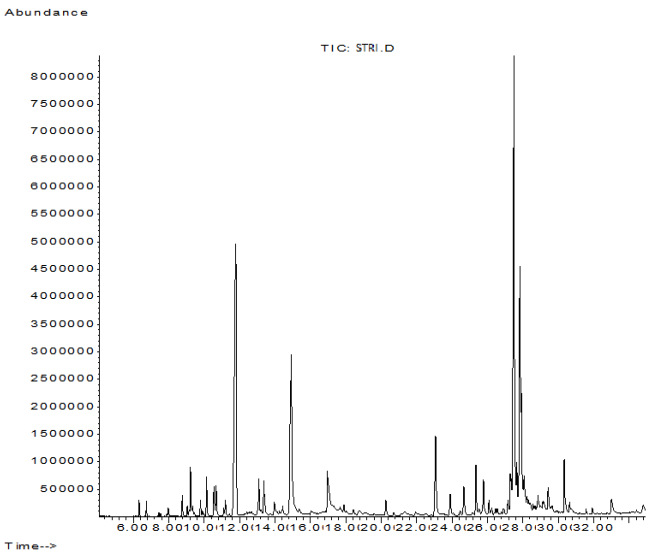


Figure 1. Chromatogram of essential oil of *Scrophularia striata* aerial parts using headspace solid-phase microextraction (GC-MC)

According to our results, the most important chemical compounds of *S. deserti* essential oil include alpha-pinene (25.54%), alpha-phellandrene (19.60%), beta-myrcene (11.29%) and trans-caryophyllene (6.78%).

Table 3. Identified constituents from essential oil of *Scrophularia deserti* aerial parts using headspace solid-phase microextraction (GC-MC)

No.	Retention time	compound	%
1	4.49	2-Hexenal	0.01
2	6.15	Alpha-thujene	0.03
3	6.33	Alpha.-pinene	0.39
4	6.65	Sbinene	0.02
5	6.74	Camphene	0.35
6	7.97	2-.Beta.-pinene	0.31
7	8.25	3-Octanol	0.06
8	8.39	Delta.3-Carene	0.05
9	8.77	alpha.-Terpinene	0.64
10	9.06	p-Cymene	0.30
11	9.24	1,8-Cineole	1.66
12	9.36	2-Dodecanol, 2-methyl-	0.37
13	9.80	beta.-trans-Ocimene	0.50
14	10.14	gamma.-Terpinene	1.22
15	10.56	trans-Sabinene hydrate	1.04
16	10.68	CIS-Linaloloxide	1.22
17	11.12	Alpha.-Terpinolene	0.29
18	11.20	Trans-linalool oxide	0.63
19	11.43	alpha.-Naginatene	0.12
20	11.79	Linalool	11.85
23	12.68	5-Hepten-1-ol, 2,6-dimethyl-	0.39
24	12.90	Isopulegol	0.08
25	13.09	Camphor	1.57
27	13.78	alpha.-Thujone	0.23
28	13.97	Borneol L	1.03
30	14.92	Alpha-Terpineol	8.34
31	15.37	Alpha-fenchene	0.70
34	17.20	Geraniol	3.10
36	17.89	Borneol, acetate	0.59
37	18.33	Nerol	0.20
38	18.76	Thymol	0.69
39	19.07	Carvacrol	0.57

No.	Retention time	compound	%
40	19.60	Cuminyl alcohol	0.23
41	20.27	cis-2,6-Dimethyl-2,6-octadiene	0.75
42	20.96	alpha.-Amorphene	0.03
45	22.54	alpha.-Gurjunene	0.22
46	23.08	trans-Caryophyllene	4.44
48	23.90	Aromadendrene	1.04
49	25.35	Germacrene-D	1.92
50	25.54	Valencene	0.42
51	25.78	bicyclogermacrene	1.70
52	26.09	Beta-bisabolene	0.64
53	26.79	beta.-Bisabolene	0.15
54	26.92	CIS-.Alpha.-Bisabolene	0.46
55	27.29	Trans-geraniol	2.45
56	27.50	Spathulenol	18.41
57	27.66	farnesol	1.90
58	27.83	Caryophyllene oxide	15.83
59	28.08	Alpha-santalol	2.46
60	28.23	Beta-selinene	0.91
61	28.86	Dihydrocarveol	1.40
62	29.00	Gamma-cadinene	0.52
63	29.14	t-Muurolol	1.35
64	29.43	Caryophyllenol	1.48
65	30.34	virtenal	2.35
66	30.97	beta.-Citronellal	0.15
67	32.75	geranyl ethyl ether	0.09
68	34.22	Eicosane	0.15

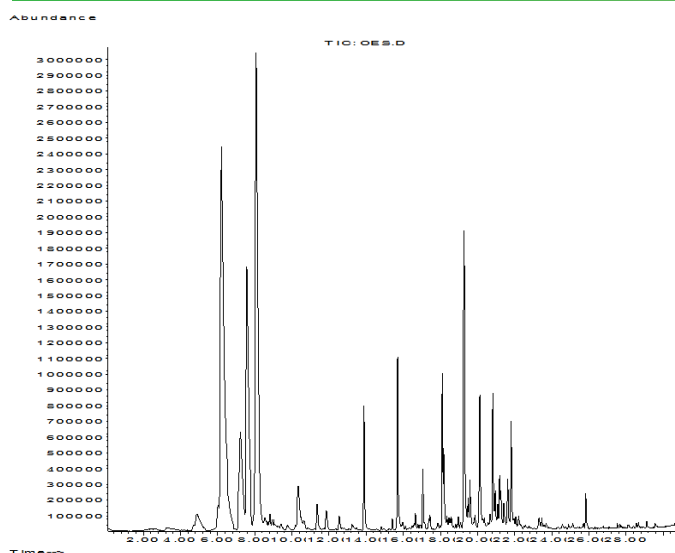


Figure 2. Chromatogram of essential oil of *Scrophularia deserti* aerial parts using headspace solid-phase microextraction (GC-MC)

Discussion

S. striata has a variety of uses in traditional medicine, especially in Ilam province, such as relief of pain and inflammation caused by eye and ear infections, relief of pain due to gastrointestinal disorders, treatment of colds, skin wounds and burns, and hemorrhoids [11]. Phytochemical studies have shown that *Scrophularia buddleia* is a rich source of iridoid glycosides [12]. Another study shows that certain compounds such as alkaloids, glycoside resins, iridoid, cryptophilic acid and flavonoids have been identified and isolated from the *Scrophularia* plants [13, 14]. A phytochemical study revealed that the active ingredients of *S. striata* included cinnamic acid and three flavonoids, namely quercetin, isorhamnetin, nepitrin, phenyl glycoside and propanoids [15, 16]. The results of Kerdar et al. (2018) study showed that the essential oil of *S. striata* aerial parts, using hydraulic (water) extraction, was composed of a total of 26 compounds such as n-hexane (16.3%), caryophyllene oxide (15.36%), spathulenol (13.1%), alpha-cadinol (12.35%), and docosane (6.33%) [17]. Based on these observations, there is a wide variation in the chemical composition of the essential oil from the same plant in different regions. Caryophyllene oxide, an oxygenated sesquiterpene, is commonly known as a preservative and antifungal agent against dermatophytes [18]. Spathulenol is used as a pesticide, antibacterial and antifungal toxin [19]. Alpha-cadinol has been identified as an antifungal and hepatoprotective agent, as well as a potential therapeutic agent for drug-resistant TB [20,21]. Pasharan et al. (2012) study showed that in the essential oil of *S. amplexicaulis*, there was high contents of phenolic compounds derivatives and oxygenated monoterpenes, such as eugenol (53.8%), eugenol acetate (24.5%), beta-caryophyllene (5.7%), and caryophyllene oxide (6.4%) [22]. The results of the phytochemical study of *S. oxysepala* essential oil revealed that this plant contains the main compounds including phytol (25.3%), methyl benzyl alcohol (9.3%), dihydrogenogenol (6.7%), methyl benzaldehyde (5.3%), 1) and eugenol (1.3%) [23]. The main constituents of *S. frigida* essential oil were palmitic acid (30.49%), phytol (12.9%), L-linalool (11.41%) and hexahydrofarnesyl acetone (6.65%) [24], indicating that these compounds vary depending on the location of the plant collection, which can be caused by factors such as environmental conditions and plant genotypes, explaining the differences in the chemical composition

of these plants. The major chemical compounds isolated and identified from *S. striata* are cinnamic acid, some flavonoids such as quercetin, isorhamnetin-3-O-rutinoside, nepitrin and a glycoside (octreotide 1). The plant has anti-inflammatory, antibacterial, antioxidant, anticancer, analgesic and neuroprotective as well as wound healing effects [25]. Spathulenol, caryophyllene oxide, linalool, alpha-terpineol, trans-caryophyllene and geraniol alpha-phellandrene, beta-myrcene and trans-caryophyllene can be active ingredients of these two plants that have the above-mentioned therapeutic effects [26-33]. Studies have shown that medicinal plants have both traditional and modern research with the use of stand-alone medicinal and therapeutic effects and their beneficial effects through their active ingredients.

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Authors' contributions

MB reviewed the literature and prepared the first draft of manuscript; NA, MB and MH reviewed the literature, helped in preparing first draft of manuscript, checked and corrected the grammar. All authors read and approved the final report.

Conflict of interests

All authors declare that no conflict of interest exist.

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