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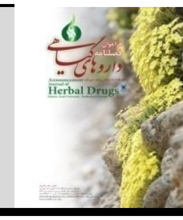

Phytochemical of essential oil of *Stachys lavandulifolia* Vahl. collected from a natural habitat in western Isfahan, Iran

Fatemeh Soleimani Meimand[1*], Mohammad Reza Vahabi[1], Mohammad Fazilati[2], Vahid Karimiyan[3]

[1] *Department of Range and Watershed Management, College of Natural Resources, Isfahan, Iran;*

[*] **Email:** *mail.fsoleimani@gmail.com*

[2] *Department of Biochemical, Faculty of Basic sciences, Tehran, Iran;*

[3] *Young Researchers' Club, Islamic Azad University, Yasooj Branch, Yasooj, Iran;*

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ABSTRACT

Background & Aim: The Lamiaceae family is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide. The genus *Stachys* is one of the largest representative genera of the Lamiaceae family and includes about 300 species, in the subtropical and tropical regions of both hemispheres. In Iran this genus is represented by 34 species. Isfahan province has different medical plants such as species *Stachys lavandulifolia* Vahl. This study was aimed at investigating of phytochemical of *S. lavandulifolia* in the west of Isfahan province.

Experimental: The aerial parts of *S. lavandulifolia* were air-dried. The essential oil of the plant was isolated by hydro-distillation with a yield of 0.25% (v/w). The chemical composition of volatile oil was analyzed by capillary GC and GC/ MS.

Results & Discussion: Result indicated that main components were germacrene-D (15.96%), thymol (14.64%), γ-cadinene (13.33%), α-pinene (7.80%), and *trans*-caryophyllene (6.91%).

Industrial and practical recommendations: *Stachy lavandulifolia* Vahl is a medical plant that can be a potential source of monoterpenes and sesquiterpenes.

1. Introduction

Stachys L. (Lamiaceae) is among the largest genera of Lamiaceae. The *Stachys* genus counts between 275 (Bhattacharjee, 1980), and 300 (Mabberley, 1997; Willis & Airy-Shaw, 1973) species worldwide. The genus has a sub cosmopolitan distribution range with centers of diversity in the warm temperate region of the Mediterranean and Southwestern Asia, Southern Africa, North and South America. The highest number of taxa is found in the Asiatic center, which mainly embraces two phyto-geographical regions, namely the Mediterranean and Irano-Turanian area (Bhattacharjee, 1980). Plants of this genus have been used in folk medicine for centuries to treat genital tumors, sclerosis of the spleen, inflammatory diseases, cough, ulcers, and infected wounds (Hartwell, 1982). In phytotherapy, tea made from the whole plant or leaves is used, on account of its sedative, antispasmodic, diuretic and emmenagogue activity (Lewis & Elvin-Lewis, 1977; Duke, 1986). As most plants used for medicinal purposes, *Stachys* taxa has been submitted to several investigations in order to determine the content of biologically active compounds. These investigations have reported the presence of flavonoids, phenolic acids, iridoids, and terpenoids (Bankova *et al*., 1999; Bilusic Vundac *et al*., 2005; Ghasemi Pirbalouti and Mohammadi, 2013). Many representatives of the family Lamiaceae, *Stachys* species also produce essential oils (Halim *et al.*, 1991; Ghannadi & Alemdoost, 1996; Sajjadi, 1996; Harmandar *et al.*, 1997; Mariotti *et al.*, 1997; Duru *et al.*, 1999; Pe´lissier *et al*., 1999; Chalchat *et al*., 2000, 2001; Javidnia *et al.*, 2003; Sajjadi & Mehregan, 2003; Sefidkon *et al.*, 2003; Duman *et al.*, 2005; Grujic-Jovanovic *et al.*, 2004; Javidnia *et al*., 2004; Tirillini *et al*., 2004; Flamini *et al.*, 2005; Petrovic *et al*., 2006), but in spite of the large size of this genus, the composition of volatile compounds is known only in a small number of species. *S. lavandulifolia* grows in many parts of Iran, Iraq, and Anatolia (Rechinger, 1982). The plant is known as Chaye-kuhi in Iran and its' English name is Betony (Amin, 1991). Phytochemical analysis has shown that the plant contained flavonoids, saponins, and bitter compounds (Alamdoost, 1996). Boiled extracts obtained from the aerial parts of this plant is used in Iranian folk medicine in painful and inflammatory disorders (Zargari, 1990). The methanolic extract of *S. sieboldii* has anti-anoxia action in mice (Yamahara *et al.*, 1990). Ramezani et al. (2002) reported that spathulenol and caryophyllene oxide as the main constituents of *S. lavandulifolia*. In the present study a sample of *S. lavandulifolia* with different chemical composition has been reported.

2. Materials and methods

2.1. Plant material

The studied areas included five isolated geographical sites, located at Isfahan province (central parts of Iran), were named as Hurestaneh (Golpayegan), Siahdarreh (Fereydan1), Fereydunshahr, Afus (Fereydan 2), and Golestankuh (Khansar) (Table 1). The aerial parts of *S. lavandulifolia* were collected at full flowering from these five natural geographical habitats during June 2011. The air-dried materials (80 g) were finely ground, then subjected to hydro-distillation for 4 hours with water as solvent, using a Clevenger apparatus according to the standard procedures. The materials gave yellow oil in a yield of 0.25% v/w. The oils were stored at 4 ºC for further analyses.

2.2. Phytochemical composition

To analyze the phytochemical composition of *S. lavandulifolia*, gas chromatography (GC) analyses were performed on a GC/MS apparatus (Agilent Technologies 7890 A) equipped with a Flame Ionization Detector (FID) and a HP-5MS fused- silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness) containing 5% poly phenyl methyl siloxane. The column temperature (60 ºC), with 5 min initial hold, was gradually increased by 270 ºC at a rate of 3ºC/min, using Helium as carrier gas with a flow rate of 1.0 mL/min and ionization voltage of 70 ev. Injector, detector, and ion source temperatures were 260, 260, and 240ºC, respectively. The injection mode was 1 µL of a 1:1000 n-hexane solution. Relative concentrations of the components were achieved by peak area normalization.

The chemical compounds of each essential oil were identified by calculating their retention indices (R_i) under temperature programmed conditions for *n*-alkanes (C_9-C_{30}) and the oil on DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their Mass spectra with those of the internal reference Mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds in literature (Adams, 2001).

Fig 1. *Stachys lavandulifolia* Vahl.

3. Results and discussion

A total of 49 different compounds, belonging to six main chemical groups (terpenes, hydrocarbons, alcohols, acids, esters, phenols) were identified in *S.*

lavandulifolia essence (Table 2 and Fig. 2). We found a wide variation among plants obtained from different geographical region in terms of the relative frequency of all identified compounds. For example, the plants collected from the Khansar region contained a relatively high amount of terpenes (36.11%) versus relatively low hydrocarbon content (19.44%). In contrast, the plants collected from the Fereydan region contained low terpene (19.51%), but high hydrocarbon content (39.02%). The same variations were also observed in relative content of other identified compounds. Altogether, terpenes and hydrocarbons constituted the majority of *S. lavandulifolia* identified compounds in all studied geographical regions ranging from 50% in the Fereydunshahr region to 58.82% in the Golpaygan region. Acids, in contrast were among the less abundant compounds in *S. lavandulifolia* ranging from 2.77% in the Khansar population to 10% in the Fereydunshahr population.

The yield of the oil obtained from *S. lavandulifoila* was 0.25% (v/w). The yield of the oils extracted from other species was, 0.18% from *S. setifera* ssp. *iranica* 0.18% from *S. chrysantha,* and 0.12% from *S. candida* (Javidnia *et al.*, 2003). From 49 compounds, the major components were germacrene-d (15.96%), thymol (14.64%), γ-cadinene (13.33%), α-pinene (7.80%), and *trans*-caryophyllene (6.91%). Another study the main compound of the oil reported germacrene-D (13.2%) this finding was like our study but they are different in terms of their amount (Javidnia *et al.*, 2003).

Table 1. Geographical coordinates of the five studied sites

Site	Longitude	Latitude	Altitude (a.s.l)	Slope (%)
Hurestaneh	50°26'19"N,	32°45'09"E	2137	22
Fereydan1	50°34'03"N,	32°58'24"E	2425	6
Fereydunshahr	50°34'27"N,	33°12'04"E	2630	10
Fereydan 2	50°04'22"N,	33°00'28"E	2636	16
Khansar	50°24'12"N,	33°09'29"E	2729	23

In a study that was done in Iran (Khoram Abad) 14 compounds were investigated and main compounds were α-terpinene (20%), α-pinene (16.3%), myrcene (20.9%). These findings were different in terms of number and kind of compounds with our study (Amiri *et al.*, 2009). The major component of the *S. oblique* oil was germacrene-D, which was also the main component of the *S. lavandulifolia* oil (Harmandar *et al.*, 1997; Ghasemi & Mohammadi, 2013).

Table 2. Composition of the essential oil of *Stachys lavandulifolia*

Peak	Compounds	RI	%
1	**α – Pinene**	**937**	**7.80**
2	β- Pinene	982	0.6
3	1,8- Cineole	1031	0.53
4	3 – Octanol	993	0.26
5	Formic acid, octyl ester	984	0.25
6	Linalool	1098	0.60
7	*trans*-verbenol	1140	0.84
8	Terpinen-4-ol	1175	0.49
9	α –Terpineol	1187	0.53
10	Geraniol	1255	1.61
11	**Thymol**	**1290**	**14.64**
12	1,5,5- Trimethyl-6 methylene-cyclohexene	1346	0.20
13	Bicyclogermacrene	1494	0.14
14	α-Copaene	1378	2.40
15	α-Cubebene	1348	1.02
16	β -Phellandrene	1382	1.91
17	*cis*-3- methyl-cyclopentene	1157	0.2
18	Bicyclo[3.1.0]hexane, 6-isopropylidene-1-methyl	964	0.1
19	Cyclohexene, 5-methyl-3-(1-methylethyl)	1001	0.3
20	***trans*-Caryophyllene**	**1418**	**6.91**
21	**Germacrene-D**	**1480**	**15.96**
22	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene	1087	5.40
23	**γ-Cadinene**	**1513**	**13.33**
24	α-bisabolol	1685	2.14
25	1-Naphthalenamine,4-boromo	1467	2
26	Isospathulenol	1352	0.6
27	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	985	0.1
28	Aromadendrene	1438	0.2
29	Dibenzothiophene (CAS)	1235	0.3
30	Alloaromadendrene	1458	0.2
31	2-Pentadecanone,6,10,14-trimethyl	1437	2.29
32	1,2-Benzenedicarboxylic acid,bis(2-methylpropyl)ester	1449	2.29
33	1-Pentadecene	1489	0.4
34	Octadecanol	2082	0.45
35	Farnesyl acetone	1914	0.2
36	5,9,13-Pentadecatrien-2-one, 6,10,14- trimethyl	1124	0.28
37	Dibutyl phthalate	991	1.36
38	Hexacosane	2600	0.3
39	Octadecane	1799	0.3
40	**Phytol**	**2116**	**6.21**
41	Tricosane	2300	0.3
42	Heptacosane	2696	0.22
43	Pentacosane	2500	0.2
44	Heptadecane	1700	0.36
45	1,2-Benzenedicarboxylic acid, diisooctyl ester	1291	0.4
46	Bis(2-ethylhexyl)phthalate	1264	0.29
47	Docosane	2197	0.23
48	Octacosane	2795	0.1
49	Tetracosane	2399	0.36

The main components of the oils of *S. aegiptica* (α-pinene) and *S. glutinosa* (α-pinene and β-phellandrene) were presented as the major components of the *S. lavandulifolia* oil (Halim *et al.*, 1991; Mariotti *et al*, 1997). β-Pinene was one of the main components of *S. recta* and *S. balansae*oils

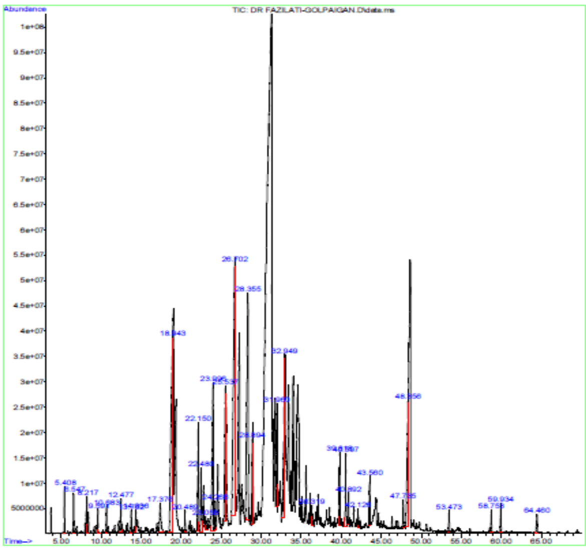

Fig 2. GC of the essential oil of *Stachys lavandulifolia*.

4. Conclusion

The main constituents of the investigated essential oils of *Stachys lavandulifolia* are as follows: germacrene-D (15.96%), thymol (14.64%), γ-cadinene (13.33%), α-pinene (7.80%), and *trans*-caryophyllene (6.91%). Growth and development of plants and quantity, quality of secondary metabolites in different ecosystems and natural habitat is effected by different environmental factors like climate conditions, altitude, soil, etc. This study revealed there are common components between different studies and also some differences between them because of geographical conditions.

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