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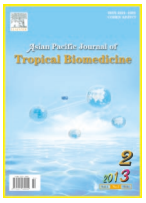

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Phytochemical composition of the essential oil of different populations of *Stachys lavandulifolia* Vahl

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Comments
This is a good study in which the authors evaluated the diversity in phytochemical composition of essential oils of wild various populations of *S. lavandulifolia* flowers. This paper showed secondary metabolites in the essential oil of *S. lavandulifolia* like α-pinene that it s really an important natural compound. In finally, this study suggests that populations having α-pinene can selective as natural products for food and pharmaceutical industries.
(Details on Page 127)

ABSTRACT

Objective: To examine the chemical variability in inflorescences of wild populations of *Stachys lavandulifolia* Vahl (*S. lavandulifolia*) collected throughout two provinces (Isfahan and Chaharmahal va Bakhtiary), Southwest Iran. **Methods:** The essential oils of *S. lavandulifolia* Vahl from seven locations were obtained by hydro-distillation and analysed by gas chromatography and gas chromatography-mass spectrometry. **Results:** The results revealed that distinct differences in the content of compounds depending on region of sample collection. The main constituents of the essential oils were α-thujone (0.3%-32.3%), α-pinene (trace to 37.3%), myrcene (0.5%-15.9%), β-phellandrene (1.1%-37.9%), germacrene D (0.4%-11.3%), Δ-cadinene (trace to 11.6%) and 1, 4-methano-1 H-indene (trace to 10.1%). **Conclusions:** The results of the present study indicated that essential oil components of *S. lavandulifolia* Vahl can be varied with genetic (ecotype), environmental conditions and geographic origin. In general, the essential oils of various populations of *S. lavandulifolia* Vahl were rich in monoterpenoids and sesquiterpenoids.

KEYWORDS
Stachys lavandulifolia Vahl, Lamiaceae, Essential oil, α-thujene, α-pinene, β-phellandrene, Chemotype

1. Introduction

The genus *Stachys*, which belongs to the Lamiaceae family, consists of about 300 species, and is justifiably considered as one of the largest genera of Lamiaceae that widespread throughout the world[1]. The genus is distributed mainly in warm temperate regions of the Mediterranean and Southwest Asia, Southern Africa, North and South America[2,3]. Assessed by the number and distribution of the species, two main centers of diversity for the genus in the old world area exist. One is confined to South and East Anatolia, Caucasia, North and West Iran and North Iraq, and the other to the Balkan Peninsula. The majority of species prefers alpine and subalpine habitats and grows under various ecological conditions in habitats like rocky places, mountain steppes, and stream banks or sometimes in forests[4]. In Iran, 34 species of this genus are present, among which, 13 are endemic[5]. *Stachys lavandulifolia* Vahl (*S. lavandulifolia*) is a native plant, which is known as Chay-e-kohi in Persian and Betony in English[6]. In Iranian traditional medicine, it is used as the herbal tea in

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gastrointestinal disorders, inflammatory diseases, anxiolytic, cough, sedative, antispasmodic, diuretic, ulcers, fevers and diarrhoea[6]. The decoction of the leaves and flowers is being used by the tribal people of Chaharmahal va Bakhtiari for treatment of skin infection, menorrhagia and anti-bacterial[7]. Also, the aerial part of *S. lavandulifolia* Vahl has been used by tribal people of Ilam Province West Iran, as carminative, sedative and cardio tonic, and for treatment of rheumatism, indigestion and headache. The aqueous extract of the aerial parts of *S. lavandulifolia* Vahl shows potent anti-inflammatory and wound healing activities in rat[8]. The previous experimental studies have demonstrated the anxiolytic and anti-diarrheal effects in mice[9,10]. Anti-inflammatory, antioxidant, antibacterial and anti-leishmania activities and effect reduced primary dysmenorrheal of *S. lavandulifolia* Vahl extract and essential oil have been shown in several pharmacological studies[11-13]. Previous investigations revealed that the main iridoid components of *Stachys* species, such as aucubin, harpagide and harpagoside, exert anti-inflammatory activities, as demonstrated in cellular systems of arachidonic acid metabolism[14,15]. Even though *Stachys* is one of the largest members of the Lamiaceae, the essential oil chemistry of the genus has not been studied in much detail. The composition of the volatiles is known only for a few species; for example, studies proved that the volatile oil of members of *S. lavandulifolia* Vahl (sect. Zietenia) consists mainly of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, diterpenoids and terpene esters[16-23]. The result of new study showed that the essential oil of *Stachys mialhesi* de Noé has antioxidant, antinociceptive and anti-inflammatory effects in laboratory animals. It can also be a natural source of isoscutellarein 7-O-(2″-O-6‴-O-acetyl-β-D-allopyranosyl-β-D-glucopyranoside. Germacrene-D, the dominant compound of the essential oil of *Stachys inflate* Benth, was reported as the major components of the volatile oils of *S. lavandulifolia* Vahl, *Stachys laxa* Boiss and *Stachys obtusicrena* Boiss[17,20,24]. In a different report, the major components in *Stachys tibetica* essential oil were aciphyllene (66.415%), fenchyl alcohol (8.897%), α-pinene (8.188%) and caryophyllene oxide[25]. In a study, lavandulifolioside B and 5-O-β-allopyranosyloxy-aucubin in *S. lavandulifolia* Vahl were identified comprehensively by extensive 1D and 2D NMR analyses[26]. There are some differences amongst *Stachys* taxa, indicating the existence of a chemical polymorphism. Chemical polymorphisms or chemotypes have been reported for many medicinal plants[27,28]. To our knowledge, there are no published reports on the chemical composition of the essential oils of the populations of *S. lavandulifolia* Vahl in Iran. For this reason, the chemical composition of these oils was analysed.

2. Materials and methods

2.1. Locations

Seven specimens of wild *S. lavandulifolia* Vahl were collected in different localities of two provinces in Iran. The monthly precipitation was obtained from variation weather stations located within the study area and the surrounding zones. The number of years registered at the weather stations ranged from 10-15. Soil physical and chemical characteristics such as pH, electricity conductivity, texture, organic carbon (%), contents of N, P, K, *etc.* were taken from a soil-sampling from different locations of Isfahan and Chaharmahal va Bakhtiari provinces. The slope and elevation information were obtained from the Digital Elevation Model using two well-known GIS software packages ILWIS(3.0 Academic). This array was geo-referenced using a metric UTM coordinate system and the geometric correction was carried out in the GIS ILWIS.

2.2. Plants

The inflorescences (0.05-0.10 kg) of wild populations of *S. lavandulifolia* Vahl were collected at the early flowering stage on 1-20 June 2010 (During 20 day's period). The samples of the plants were identified by regional floras and authors with floristic and taxonomic references[3], and voucher specimens were deposited at the Herbarium of Agriculture and Natural Resources Researches Centre of Chaharhal va Bakhtiari province, Iran (No.: SHK 2164) (Figure 1). Harvested inflorescence was shade dried at room temperature for one week and ground into a powder. Air-dried and ground (50 mesh) plant material was subjected to hydro-distillation (1 000 mL distillated water) for 2 h using a Clevenger-type apparatus according to the method recommended in British pharmacopoeia[29]. Samples were dried with anhydrous sodium sulphate and kept in amber vials at 4 °C until chromatographic analysis.

Figure 1. Aerial plant of *S. lavandulifolia* Vahl (Lamiaceae).

2.3. Identification of the oil components

Ghromatography (GC) and gas ghromatography-mass spectrometry (GC-MS) analysis methods were carried out to determine the composition of the essential oils. GC analysis was carried out on a Younglin Acme 6000 gas chromatograph equipped with Flame Ionization Detector (FID) and a HP-5MS 5% capillary column (30.00 m×0.25 mm, 0.25 μm film thicknesses). Carrier gas was helium at flow of 0.8 mL/min. GC oven temperature was kept at 50 °C for 5 min and programmed to 240 °C at a rate of 3 °C/min, and then programmed to 300 °C at a rate of 15 °C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 290 °C. The purity of Helium gas was 99.999% and 0.5 μL samples

were injected manually in the split mode. GC-MS analysis was performed on above mentioned Agilent Technologies 5973 Mass system. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 50-550. Retention indices were calculated for all components using a homologous series of n-alkanes (C_5-C_{24}) injected in conditions equal to samples ones. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY /ChemStation data system)[30]. Area percent was obtained electronically from the GC-FID response without the use of an internal standard or correction factors.

2.4. Chemical variability

Analytical data for cluster analysis were treated by means of the statistical package Minitab release 13.

3. Results

The seven specimens of *S. lavandulifolia* Vahl collected in different localities in Iran are as shown in Table 1.

The chemical constituents identified by GC and GC-MS and the results concerning the qualitative and quantitative analysis of the essential oils are presented in Table 2. The twenty seven compounds consisting up to 80% of the

Table 1

Environment condition of natural habitats of seven populations belonging to *S. lavandulifolia* (Lamiaceae).

Region	Province	Altitude (m ASL*)	Latitude	Longitude	P	pH	EC	OC	TN	N	K	TNV	Clay	Silt	Sand
Sheyda	Chaharmahal va Bakhtiari	2822	32° 37′ 22″ N	50° 34′ 47″ E	382	7.67	0.48	0.36	9.9	0.041	103	2.5	30	35	35
Broujen	Chaharmahal va Bakhtiari	2300	33° 00′ 40″ N	52° 18′ 44″ E	251	7.69	0.62	0.71	12.9	0.078	556	49.1	14	52	34
Naghan	Chaharmahal va Bakhtiari	1951	31° 56′ 11″ N	50° 43′ 27″ E	593	7.78	0.56	0.94	29.9	0.087	485	36.0	50	26	24
Frasan	Chaharmahal va Bakhtiari	2200	32° 19′ 01″ N	50° 32′ 11″ E	507	7.60	0.63	1.07	44.2	0.098	846	5.5	34	52	14
Gal-e-sang	Chaharmahal va Bakhtiari	2544	32° 27′ 59″ N	50° 17′ 18″ E	1415	7.46	0.61	2.32	40.2	0.206	819	9.0	32	56	12
Kelishadrokh	Isfahan	2090	32° 21″ 33″ N	51° 04′ 03″ E	324	7.76	0.74	0.16	17.5	0.026	503	24.0	26	38	36
Faraydan	Isfahan	2300	32° 58′ 59″ N	50° 24′ 22″ E	348	7.78	0.48	0.82	18.1	0.085	538	17.0	45	30	25

ASL: Above sea level, P: Annual precipitation (mm), EC: Electrical conductivity (dS/m), OC: Organic carbon (%), TN: Total nitrogen (%), N: Available P (mg/kg), K: Available K (mg/kg), TNV: Total neutralising value (%), Clay (%), Silt (%) and sand (%).

Table 2

Essential oil composition (%) of seven populations of *S. lavandulifolia*.

Compound	RI	Broujen (%)	Gal-e-sang (%)	Naghan (%)	Sheyda (%)	Frasan (%)	Faraydan (%)	Kelishadrokh (%)
α-thujene	930	16.29	23.52	13.39	23.76	0.28	26.17	32.34
α-pinene	935	0.19	0.71	0.23	0.70	37.29	0.68	tr
Benzaldehyde	959	–	–	–	6.28	2.91	0.18	–
Sabinene	974	–	3.13	4.10	–	–	4.71	2.22
β-pinene	978	0.78	1.11	3.47	–	1.60	2.01	1.95
Myrcene	990	3.86	0.91	15.87	4.41	6.14	6.72	0.52
α-phellandrene	1003	0.03	–	7.26	–	0.32	2.81	13.99
β-phellandrene	1027	14.44	28.27	1.10	37.93	12.37	17.89	2.11
β-Ocimene <(Z)>	1037	2.26	0.67	0.11	2.16	2.23	0.36	0.21
γ-terpinene	1061	0.29	0.18	0.21	0.15	0.35	0.16	0.34
Trans-sabinene hydrate	1092	0.03	0.12	0.03	0.08	0.09	0.51	0.08
Linalool	1099	0.09	tr	0.19	0.02	0.05	0.49	0.06
Allo ocimene	1121	tr	tr	0.11	0.05	0.18	0.01	0.17
α-Copaene	1372	1.10	0.05	1.03	0.04	–	tr	–
β-bourbonene	1383	1.18	0.13	–	–	–	1.64	0.54
β-cubebene	1393	–	0.16	–	0.09	0.21	0.36	–
β-elemene	1396	–	0.11	–	–	–	0.07	0.21
Naphthalene (1-methoxy)	1440	0.45	0.68	0.34	0.34	–	0.22	0.14
β- farnesene <(Z)>	1447	0.21	0.09	0.25	0.06	0.09	0.11	2.31
α-amorphene	1475	–	2.96	5.57	–	0.03	–	0.04
Germacrene D	1479	11.26	2.66	5.85	2.51	6.58	6.75	0.37
Bicyclogermacrene	1495	–	–	4.02	3.98	–	–	10.67
Δ-cadinene	1524	11.61	7.67	0.51	0.17	1.28	0.18	0.12
Cis-α-bisabolene	1536	0.08	0.04	0.23	0.15	0.09	0.04	0.76
Spathulenol	1570	0.07	–	–	0.07	0.13	0.07	0.09
1,4-methano-1 H-indene	1959	8.43	3.22	10.07	0.17	–	3.62	–
Isospathulenol	2259	0.70	0.07	0.47	0.03	0.31	0.21	–
Oil yield (%)	–	0.32	0.35	0.33	0.31	0.32	0.34	0.35

RI: Retention index, tr: trace (<0.01%).

essential oil were identified by GC and GC-MS analysis, the major components of *S. lavandulifolia* Vahl oil were: α-thujone (0.3%-32.3%), α-pinene (trace to 37.3%), myrcene (0.5%-15.9%),β-phellandrene (1.1%-37.9%), germacrene D (0.4%-11.3%), Δ-cadinene (trace to 11.6%) and 1,4-methano-1 H-indene (trace to 10.1%).

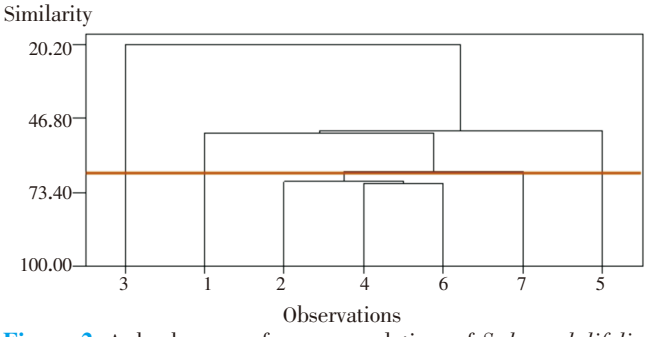

Figure 2. A dendrogram of seven populations of *S. lavandulifolia* using cluster analysis.
1: Kelishadrokh, 2: Faraydan, 3: Farsan, 4: Sheyda, 5: Naghan, 6: Gal-e-sang, 7: Broujen.

The dendrogram (Figure 2) represents graphically the relationships among the populations and the groups, based on their essential oil composition. Four groups were formed by the Average Linkage cluster analysis. The first cluster encompassed *S. lavandulifolia* Vahl accessions of Feryadan, Ghalehsang, Sheyda and Broujen, and exhibited high β-phellandrene (28.3%, 37.9%, 17.9% and 14.4%, respectively) and α-thujene (23.5%, 23.8%, 26.2% and 16.3%, respectively) contents. The second cluster enclosed accession of Kelishadrokh, and displayed high α-thujene and α-phellandrene contents (32.3% and 14.0%, respectively). The third cluster enclosed accession of Naghan, and displayed high myrcene and α-thujene contents (15.9% and 13.4%, respectively). The fourth cluster enclosed accession of Farsan, and displayed high α-pinene and β-phellandrene contents (37.3% and 12.4%, respectively).

4. Discussion

The yellow oil of samples of seven populations of *S. lavandulifolia* Vahl was obtained by hydro-distillation in the yield of 0.30%-0.35% (w/w). As previously reported[16-18], the yield (w/w) of the obtained essential oil of *S. lavandulifolia* Vahl ranged from 0.25% to 0.8%, based on dry weight. Also, the yield of the oils extracted from other species was: 0.18% from *Stachys setifera* ssp. Iranica[31], 0.18% from *Stachy chrysantha*[15], and 0.12% from *Stachy candida*[14]. In other study, Radulovic *et al.* reported that the yield of the obtained essential oils from Balkan Peninsula endemics species (*Stachys germanica* ssp. *heldreichii, Stachys iva, Stachys plumosa* and *Stachys scardica*) ranged from 0.024% to 0.037%, based on dry weight[32]. The essential oils yield obtained from twenty-two different *Stachys* species from turkey ranged from 0.1% to 0.25%[33].

The results of this study showed that monoterpenes and sesquiterpenes were the main constituent groups. Our results were in agreement with report by Ebrahimabad *et al.* on chemical components of *Stachys inflata* Benth essential oil, linalool and terpinol were major components[21]. The result of chemical analysis of *Stachys serotina* (Host) Fritsch essential oil by Jerkovic *et al.* showed that sesquiterpene hydrocarbons were the most abundant class of isolated volatiles of β-caryophyllene, δ-cadinene and α-humulene, germacrene D[34]. In previous study[33], the essential oils from twenty-two different *Stachys* species have been identified thirty-nine compounds. Germacrene-D (2.9%-45.3%), β-caryophyllene (2.3%-62.3%), caryophyllene oxide (trace to 7.8%), spathulenol (trace to 7.8%) and α-cadinene (1.4%-8.5%) have been identified as the main components of the essential oils[33]. Mirza and Baher reported that the oil of *Stachys lanata* Jacq collected from the National Botanical Garden in Tehran, Iran, was rich in α-thujone (25.9%), α-humulene (24.9%), β-caryophyllene (12.6%) and viridiflorol (10.5%)[35]. In previous studies, the main components of *S. lavandulifolia* Vahl oil were reported to be germacrene-D (13.2%), β-phellandrene (12.7%), β-pinene (10.2%), myrcene (9.4%), α-pinene (8.4%) and Z-β-ocimene (5.8%) for Tehran population in Central Iran[17], myrcene (20.9%), α-pinene (16.3%), α-terpinene (20%) and bicyclogermacrene (8.7%) for Lorestan population in West Iran[16], α-pinene (7.9%), 4-hydroxy-4-methyl-2-pentanone (9.3%) and hexadecanoic acid (5.9%) for Mazandaran population in North Iran[18] and β-caryophyllene and 1,8-cineole for *S. lavandulisolia* in Turkey[36]. In 2004, Sajjadi and Somae reported germacrene D, bicyclogermacrene, α-pinene, β-phellandrene, bicycloelemene, β-pinene and spathulenol as the major components of the oil of *Stachys inflata* Benth growing in Iran[37]. Feizbaksh *et al.* found α-pinene (20.1%), β-pinene (12.1%), spathulenol (7.2%) and germacrene D (5.3%) as the major components of oil of *S. lavandulifolia* Vahl collected from Ab-ali (Tehran province, Iran)[19]; this oil was rich in monoterpenoids (51.8%). In present study, a high α-thujene (32.34%) chemotype in Kelishadrokh from the Southwest of Isfahan province, Iran has previously been proposed in the oil (25.9%) of *Stachys lanata* collected from the National Botanical Garden in Tehran, Iran[35], and we now confirm this chemotype in *S. lavandulifolia* Vahl and report that it is also present in an area study. Also, we confirm high β-phellandrene (37.9%) in Sheyda population with high altitude (2 822 meter above sea level) in an area study. Javidnia *et al.* reported that β-phellandrene is as a main component of the oil of *S. lavandulifolia* Vahl collected from Fasham area, Tehran[17]. A high α-pinene (37.3%) in Farsan population has previously been proposed in the oil (20.1%) of *S. lavandulifolia* Vahl collected from Tehran, Iran[19]. In the essential oil of Naghah population, a main constituent was found to be myrcene (15.87%), which we now confirm this chemotype in *S. lavandulifolia* Vahl. Polymorphism chemical or chemotypes have been reported for many medicinal plants. The composition of the essential oil of *S. lavandulifolia* Vahl depends on many factors of genetic, environmental and their interaction effects, such as plant part, harvest-time, extraction-method, ecotype and geographic origin (climate, edaphic, elevation and

topography)[36]. Genetic differences cannot be directly deduced from the varying amounts of a secondary plant product[37]. Plants growing in different environments grow ordinarily at different rates; they differ in size and developmental stage[38]. The result of study by Sadrmomtaz *et al.* found microwave-assisted hydrodistillation method can achieve comparable results with those by hydrodistillation for determination of essential oils in fresh materials[39]. They reported that the major components by two methods were carvacrol (1.43%, 2.63%) and thymol (10.80%, 8.14%).

In conclusion, *S. lavandulifolia* Vahl with different chemical compositions have been reported. It is known that many factors influence the chemical constitution of *S. lavandulifolia* Vahl oils. We have shown that the volatile oil composition of *S. lavandulifolia* Vahl, in Iran is extremely variable. We propose four chemotypes with intriguing patterns in their geographic distribution. These chemotypes are distinguished by different levels of classes of natural products: monoterpenes and sesquiterpenes. The differences in the quantity or quality of the oils composition of the present and previous studies may be because of the chemotypes, phenological stage, drying conditions, mode of distillation and geographic and climatic factors[16-19,22,36].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Monoterpenes are the most representative metabolites constituting 90% of the essential oils in medicinal plants. Some researchers, however, have reported that levels of sesquiterpenes were higher in vegetative organs in most species compared with monoterpenes. Moreover, some sesquiterpenes have different medicinal functions as well as monoterpenes. It is interesting to note that there is significant diversity of chemical compositions of the essential oil for wild populations of *S. lavandulifolia* in different geographic regions and altitudes.

Research frontiers

The cutting-edge in the field of the research in this paper Human intervention in plant breeding has always aimed to increase production, improve quality, and protect plants against pests. Negative ecological impacts resulting from the use of chemicals and cultivation limited the number of genotypes. Knowledge of diversity in crop species and their wild relatives is of critical importance for crop improvement. For traditional Iranian medicinal plants, the major constraints in achieving higher yield and consistency are lack of genetic variability, absence of suitable genotypes for different planting systems, poor harvest index, and susceptibility to diseases. Research on most endemic medicinal plants has lagged behind that of crops; therefore, improvements depend on the utilization of the available genetic diversity.

Related reports

Results and discussion are based on related and topics researches about this study.

Innovations and breakthroughs

The innovations in the paper, according to my knowledge, there are no published articles on the diversity in phytochemical of essential oils of wild various populations of *S, lavandulifolia* flowers collected from Iran.

Applications

This study suggested that populations have the highest in α-pinene can selective as natural products for food and pharmaceutical industries.

Peer review

This is a good study in which the authors evaluated the diversity in phytochemical composition of essential oils of wild various populations of *S. lavandulifolia* flowers. This paper showed secondary metabolites in the essential oil of *S. lavandulifolia* like α-pinene that is really an important natural compound. In finally, this study suggests that populations having α-pinene can selective as natural products for food and pharmaceutical industries.

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