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Essential Oil Diversity among *Stachys laxa* Boiss. & Buhse Populations in North of Iran

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Abstract

Stachys laxa, a member of the Lamiaceae family, is a medicinal and aromatic that found in semi-cold and Mediterranean regions of northern Iran. The essential oil composition of Iranian populations of *Stachys laxa* were evaluated in this study. The 16 populations of *Stachys laxa* were collected from North of Iran. The essential oils were extracted by hydrodistillation using Clevenger type and were analyzed by GC and GC/MS. Based on the essential oils analysis, 37 different chemical compounds were identified in this study within the sixteen populations of *S. laxa*. The results revealed that distinct differences in the content of compounds depending on region of sample collection. The main constituents of the identified essential oils were β-Elemene (1.1-18.7%), trans-Caryophyllene (0-13.2%), Germacrene D (1.1-46.6%), and Caryophyllene oxide from (0.3-32.3%). According to the GC/MS results, some components such as Germacrene A, Spathulenol, Germacrene D, and α-cadinol were the effective components to separate different populations of *S. laxa*. In general, the lowest and highest content of germacrene D was founded from 1.1 to 46.6% in the population of Kiasar (KSR), and Chalus (CHL), respectively. The *S. laxa* populations had high diversity in term of essential oil components, which could be considered in future studies.

Keywords: Germacrene D, population, Lamiaceae, GC/MS analysis

Introduction

Lamiaceae family is one of the largest families distributed throughout the world. Due to the essential oils in most of the genera from this family, they have a pleasant to sharp smell [1]. There are 34 species of *Stachys* in Iran, among them, 12 species are endemic [2]. Phytochemical studies on different species of *Stachys* show phenolic acids, tannin, flavonoids and phenylethanoid glycoside as main components [3, 4]. Total phenol and antioxidant activity of nine species of *Stachys* displayed a direct correlation which showed that polyphenols are the main antioxidants [5]. Moreover, the antibacterial effects of essential oils from different species have been studied and *S. candida* showed appropriate antibacterial effects [6]. In another study, essential oils from *S. cardiac*, *S. candida*, *S. euboica*, *S. recta* and *S. menthifolia* have also shown antibacterial and antifungal properties [7]. It is known that environmental conditions such as altitude, light intensity, temperature, relative humidity affect the quantity and quality of plant essential oils and therefore their biological properties [8]. `In addition, the differences in the quantity or quality of oil composition between the *Stachys* spp. may be because of the collection time, chemotypes, drying conditions, method of extraction [9 , 10].

One of the endemic species of *Stachys* is *S. laxa* Boiss. & Buhse, spreading in the northeast of Iran [2, 11, 12]. *Stachys laxa* is a perennial plant with the thin stem and 30-60 cm height. It is covered with densely hairs. Basal and cauline leaves are similar to each other (oblong to elliptic), Floral leaves are elliptic to lanceolate. It has pink to purple corolla. Its nutlets are obovids (Salmaki, Zarre et al. 2012). Although there are several studies about essential oil components of different species of *Stachys*, their components and their percentages are relatively different. Maybe there is a difference among different genotype of one species base on their pharmaceutical compounds that can affect the final quality of the drug [13]. The objective of the present study was to evaluate the phytochemical diversity of Iranian wild populations of *Stachys laxa* Boiss. & Buhse, which is necessary for exact evaluation of its biological and pharmacological effects in future studies.

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Material and Methods

Plant materials

Aerial parts (leaves and flowers) of *Stachys laxa* populations were collected during May and June (flowering period) from North of Iran based on Flora Iranica (Rechinger 1982). Sixteen populations was selected with 20km distance from each other and five plants collected for each area randomly. Voucher specimens for each populations were deposited at Islamic Azad University Herbarium (IAUH) (Table 1).

Table 1 Geographical Location of collected populations of *Stachys laxa*

Population code	Latitude (N)	Longitude (E)	Altitude (m)	Voucher No.
CHL	36˚ 8.418’	51˚ 11.3604’	1950	IAUH-15136
DZD	36˚ 13.897’	51˚ 18.874’	2170	IAUH-15137
PLK	36˚ 18.589’	51˚ 13.49’	1040	IAUH-15138
KJR	36˚ 23.509’	51˚ 28.205’	1260	IAUH-15139
FRZ	35˚ 45.846’	52˚ 52.897’	2110	IAUH-15140
SHV	37˚ 30.501’	57˚ 30.891’	970	IAUH-15141
ASHK	37˚ 10.339’	56˚ 48.72’	1010	IAUH-15142
GRM	37˚ 17.972’	56˚ 60.401	1100	IAUH-15143
KAL	37˚ 21.563’	56˚ 0.271’	880	IAUH-15144
AZD	36˚ 54.588’	55˚ 29.018’	1050	IAUH-15145
TYL	36˚ 57.987’	55˚ 19.924’	620	IAUH-15146
KSR	36˚ 14.366’	53˚ 32.836’	1340	IAUH-15147
IVEL	36˚ 14.868’	53˚ 41.932’	1820	IAUH-15148
SVK	36˚ 20.868’	53˚ 41.932’	790	IAUH-15149
VRS	35˚ 54.273’	52˚ 59.187’	1500	IAUH-15150
BJN	37˚ 28.783’	57˚ 26.072’	1020	IAUH-15151

Pop. shows different populations; CHL: Chalus, DZD: Dozdebon, PLK: PooladKooh, KJR: Kajoor, FRZ: Firoozkooh, SHV: Shirvan, ASHK: Ashkhaneh, GRM: Garmeh, KAL: Kalaleh, AZD: Azadshahr, TYL: Tylabad, KSR: Kiasar, IVEL: Ivel, SVK: Savadkooh, VRS: Veresk, BJN: Bojnord.

Climatic data

Climatic information such as elevation, maximum annual temperature, minimum annual temperature, mean annual temperature and mean annual precipitation were collected for the sixteen studied populations from the website: www.en.climate-data.org[14].

Determination of essential oils constituents

100-200g of dried arial parts (leaves and flowers) without branches were extracted using Clevenger for about 3-4 hours with Clevenger. In order to separation and determination of essential oils components, Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC/MS) were used for three times.

GC analysis. TRACE GC gas chromatography (Thermoquest-Finnigan Co.), equipped with a flame ionization detector with DB-5 capillary (30 m × 0.25 mm, film thickness 0.25 µm) and N_2 (1 ml/min) as a carrier gas was applied. The program for oven temperature was adjusted at 60 °C for 3 min, then rising to 250 °C with a 6 °C/min rate and finally held constant at 250 °C for 5 min.

GC/MS analysis. TRACE MS GC/MS system coupled with a DB column (60 m × 0.25 mm, film thickness 0.25 µm) was performed for GC/MS analyses. The temperature program for oven was adjusted at 60 °C, then rising to 250 °C with a 4 °C/min rate and finally held constant at 250 °C for 10 min. Helium (1 ml/min) was used as a carrier gas with 70 eV ionization energy.

Statistical analyses

SPSS v. 22 software (IBM Inc, Chicago, IL) was applied in this study for statistical analysis. To compare the means, one-way ANOVA test was performed. Hierarchical cluster analysis (HCA) was performed based on average-linkage method using standard Euclidean coefficient. Factor analysis based on principal component analysis (PCA) was carried out to determine the most variable characters.

Results

GC–FID chromatograms of the essential oils from dried aerial part of *S. laxa* is shown in Figure 1. The variation of essential oils components were listed in Table 2. According to the GC/MS, 37 different chemical compounds were identified in this study within the sixteen populations of *S. laxa*. The results revealed that distinct differences in the content of compounds depending on region of sample collection. The main constituents of the identified essential oils were *β*-Elemene from 1.12 (SVK; Savadkooh) to 18.70% (KAL; Kalaleh), *trans*-Caryophyllene from trace (BJN; Bojnord) to

13.22% (VRS; Veresk), Germacrene D from 1.10 (KSR; Kiasar) to 46.61% (CHL; Chalus), and Caryophyllene oxide from 0.3 (KAL; Kalaleh) to 32.3% (BJN; Bojnord).

Factor analysis was used based on principal components to provide a reduced dimension model indicating differences measured among groups. PCA allows to evaluate multi-collinear data and to determine the traits most suitable for classification. PCA revealed that the first four factors (PC_1-PC_4) comprise almost 71.27% of the total variation for *S. laxa* populations in Iran (Table 3). In the first principal component (PC_1) with about 29.77% of the total variation, some characters such as content of germacrene A, spathulenol, and germacrene D, possessed the highest variance and correlation, respectively. In the second principal component (PC_2) with about 14.7% of the total variation, content of α-cadinol, and hexa hydrofarnesyl showed the maximum variance. Third principal component (PC_3) indicated about 13.6% of the total variation by content of 1.8-Cineole. While, the highest variance were observed for α-pinene content in PC_4 (Tables 3). In general, germacrene A was the best phytochemical traits for auditing and identifying populations of *S. laxa* in Iran.

Hierarchical cluster analysis (HCA) was performed to classify *S. laxa* populations, based on the average-linkage method using standard squared Euclidean distances of components. The hierarchical structuring of the investigated populations was shown in a dendrogram (Figure 2. a.). Cluster analysis divided the sixteen *S. laxa* populations into two main cluster groups (A_1, and A_2) at a similarity coefficient of 10 (as cut-off line) in average distance value (ADV) of 25 with high diversity in the dendrogram (Fig. 2.a.). Groups were clearly distinguished in cluster analyses from each other. The first main group (A_1) was divided into 11 populations. The first group (A1) consisted of populations from Kajoor (KJR), Garmeh (GRM), Azadshahr (AZD), Kalaleh (KAL), Ashkhaneh (ASHK), Ivel (IVEL), Kiasar (KSR), Savadkooh (SVK), Veresk (VRS), Tylabad (TYL), and Bojnord (BJN) with similar phytochemical traits. The second main group (A_2) was divided into 5 populations. The second group (A_2) was comprised of Chalus (CHL), Dozdebon (DZD), Firoozkooh (FRZ), Shirvan (SHV), PooladKooh (PLK) populations which made it distinct from the other population (A1) (Figure 2. a.).

Figure (2. b.) illustrated the graph obtained from the principal component analysis (PCA) of essential oils components. The scatter plot of the principal components (PC_1-PC_3) showed that the distribution structure of populations was consistent with cluster analysis groups. The high similarity between the expression patterns of these groups were shown by cluster analysis (Figure 2. a.), and PCA (Figure 2. b.).

Simple correlation coefficient analysis showed the existence of significant positive and negative correlations among ecological data and essential oils components of *S. laxa* (Table 5). Five ecological factors such as altitude, maximum annual temperature, minimum annual temperature, average annual temperature, and average annual precipitation for different locations of this study listed in Table 4. Based on the results, germacrene D showed the most correlations with altitude, and average precipitation, respectively. Conversely, *α*-pinene, (*E*)-*β*-farnesene and guaiadiene showed a more significant relationships with the amount of precipitation (Table 5). Finally, the lowest and highest content of germacrene D was founded from 1.10 to 46.61% in the population of Kiasar (KSR), and Chalus (CHL), respectively (Table 3). Excessive precipitation and day/night temperature differences in the highlands increase the production of essential oils content and germacrene D as an indicator of population auditing.

Discussion

Stachys laxa extended in cold semi-arid regions in Golestan and Mazandaran provinces in Iran. Phytochemical analyses for 16 populations of *S. laxa* were performed. The results of GC/MS showed 37 different chemical compounds within the sixteen populations of *S. laxa*. According to FA results, identified Germacrene D and Germacrene A as the best phytochemical traits in *S. laxa* populations. HCA and PCA showed the similar results and the results of both analysis divided *S. laxa* populations into three main groups. There were correlations with essential oils and ecological factors that in research Germacrene D showed correlation with altitude and average precipitation. Vokou et al. [15] showed the higher the altitude, the higher the content of essential oil measured in *Origanum vulgare* ssp. *Hirtum*. Edaphic factors including the physicochemical characters of the soil have been also found to influence the oil components. Similar to our results, Germacrene D showed positive correlation with altitude. Based on the results, DZD, CHL and FRZ showed the highest amount of Germacrene D (Table 5). Ghelichnia showed topographic factors such as downhill and elevation can directly change the qualitative properties of essential oils [16]. According to the GC/MS results, 37 different components were identified while Sajjadi and Mehregan identified 33 different components and the major components includes germacrene D, β-caryophyllene, β-phellandrene, caryophyllene oxide [11]. The main and the highest amount components of the essentioal oils were germacrene D (1.98- 46.61%), trans-Caryophyllene (0- 13.22%), δ-Cadinene (0- 5.50%), Caryophyllene oxide (0.35-10.61%). Although the highest content of germacrene D (46.61) and trans-caryophyllene (9.03) were observed from Chalus and the lowest amount of germacrene D (1.10) showed from Kiasar. Kiashi et al. were reported germacrene D, α- Pinene and hexadecanoic as the main component of essential oil of *S.laxa* [17]. Nejadhabibvash [18] showed that the highest amount of essentioal oils from full flowering and initial fruiting stages of *Stachys lavandulifolia* Vahl. were germacren D. Furthermore Hajdari et al. represented that the leaves and flowers of *Stachys sylvatica* L. were characterized by α-Pinene, β-pinene and germacrene D [19]. Essential oils from *S. sylvatica* in Turkey showed high proportions of germacren D, α-Pinene, β-caryophyllene [20].Analysis of essential oils from *S. recta* in Serbia

showed germacrene D and E-caryophyllene [21]. In the several research germacren D, β-caryophyllene, α-cadinene, caryophyllene oxide and spathulenol were identified as constituents of essential oils of *Stachys* [22, 23, 24].

The amount of essential oils can be controlled by edaphic and genetic factors. In other words, different populations of plants produce different amount of essential oils in different ecological conditions[25]. Among different essential oils components from this study, β-Elemene and Spathulenol showed positive correlation with minimum temperature. Then in regions with lower minimum temperature, the amount of these components would be increased. Furthermore, the most of components (such as Germacrene A and Germacrene D-4-ol) show negative correlations with stem height. In other words, in populations with lower stem height, the amount of some components such as Germacrene A and Germacrene D-4-ol would be higher. Jerkovic *et al.* results of *Stachys serotina* (Host) Fritsch showed that sesquiterpene hydrocarbons were the most abundant class of isolated volatiles of β-caryophyllene, δ-cadinene and α-humulene, germacrene D[26]. According to Gorena study, thirty-nine essential oils from different *Stachys* species have been identified and included: Germacrene-D, β-caryophyllene, caryophyllene oxide, spathulenol and α-cadinene have been identified as the main components of the essential oils similarly to our results this components separated different population [27].

Conclusions

The current study indicated differences in phytochemical characteristics of *S. laxa* populations collected from 16 locations (especially Golestan and Mazandaran Provinces) in Iran. Based on GC/MS results, some components such as Germacrene A, Spathulenol, Germacrene D, and *α*-cadinol were the effective components to separate different populations of *S. laxa*. The highest amount of germacrene D showed in the Chalus population. In general, the results of the present study indicated that there was a high phytochemical diversity in the populations of *S. laxa* in Iran. This feature leads us to think about how to facilitate the management of genetic resources. The essential oil components of *S. laxa* can be varied with environmental conditions and geographic origin. The essential oils of various populations of *S. laxa* were rich in germacrene D. The variation of essential oil compounds in *S. laxa* and the array of environments in which it was found indicates that selection in these different environments could lead to differentiation among populations and chemotypes of *S. laxa*. The variability in essential oils observed in *S. laxa* populations may explain the survival and adaptability to prevailing environmental conditions and production practices. The present studies increase the knowledge concerning the variation in biology and compositions between differing plants of the same species and help in understanding diversity which could be offered scope in management strategy.

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Table 2 Compositions of the essential oils obtained after fourteen investigated populations of *S. laxa*

Number	Compound Name	RI	CHL	DZD	PLK	KJR	FRZ	SHV	ASHK	GRM
1	α-Pinene	932	5.89	3.70	-	4.00	-	-	-	0.30
2	Limonene	1024	1.344	1.33	0.16	5.12	-	0.49	0.70	1.13
3	1,8-Cineole	1026	-	-	-	0.48	-	-	0.06	0.16
4	Linalool	1095	-	-	-	0.31	-	-	0.07	0.06
5	Myrtenal	1195	-	-	-	1.14	-	3.55	0.18	0.69
6	Myrtenol	1194	-	-	0.89	0.82	-	1.26	0.58	1.39
7	α-Copaene	1374	1.34	1.98	0.86	1.23	0.89	3.08	1.45	1.22
8	β-Bourbonene	1387	1.16	0.39	0.36	1.06	0.19	0.41	0.28	1.17
9	β-Elemene	1389	2.64	2.25	1.17	8.32	1.69	2.39	8.82	8.40
10	trans-Caryophyllene	1408	9.03	3.16	2.09	0.64	2.15	4.57	3.94	1.36
11	Germacrene D	1484	46.61	40.59	28.49	7.00	42.85	32.31	11.16	1.98
12	Germacrene A	1508	-	-	-	2.01	-	-	1.69	2.30
13	β-Bisabolene	1505	1.53	0.64	0.81	-	0.98	1.26	-	-
14	δ-Cadinene	1522	2.58	2.91	5.28	5.50	4.33	4.62	4.78	5.13
15	Germacrene D-4-ol	1574	-	-	-	0.94	-	-	1.76	0.67
16	Spathulenol	1577	0.73	0.64	0.35	2.72	0.30	0.19	2.64	3.59
17	Caryophyllene oxide	1582	1.13	0.86	1.66	0.95	9.00	7.48	0.93	4.09
19	β-Sinensal	1699	1.45	0.83	2.71	-	0.71	-	-	-
20	Bisabolol<epi-alpha->	1683	-	-	-	2.99	-	-	14.02	7.45
21	α-Bisabolol	1685	1.69	1.96	2.50	-	8.50	-	-	-
22	Hexahydrofarnesyl acetone	1846	0.94	0.56	10.74	0.33	2.72	2.62	0.67	1.01
23	Farnesol<2Z6Z->	1698	-	-	-	4.20	-	-	6.12	0.56
24	Manool	2056	-	-	-	0.33	-	-	0.38	1.11

25	Aromadendrene oxide II		0.44	0.56	1.68	-	2.50	-	-	-
26	α-Cadinol	1652	0.64	0.91	4.46	-	2.38	-	-	-
27	α-Santalol	1674	-	-	-	2.47	-	-	2.19	3.13
28	Valeranone	1674	-	-	-	1.89	-	-	1.03	1.94
29	Cubebol<10-epi->	1533	-	-	-	-	1.80	7.40	-	-
30	E-β-Bisabolene	1529	0.47	0.43	0.81	-	0.35	0.46	-	-
31	Ledol	1602	-	-	-	0.87	-	-	3.02	2.64
32	Sesquisabinene	1457	1.01	0.16	0.39	-	0.09	0.87	-	-
33	(E)-β-Farnesene	1454	7.71	1.43	5.65	-	3.56	-	-	-
34	Cubebol<epi->	1493	2.16	0.83	2.45	-	3.96	6.01	-	-
35	Selinene<7-epi-alpha->	1520	-	-	-	1.97	-	-	2.46	1.52
36	terpinene-4-ol	1174	-	-	-	1.39	-	-	0.91	0.56
37	α-Terpineol	1186	-	-	-	0.82	-	-	0.71	0.71
	The number of components in each population		22	21	22	26	21	19	27	26

Table 2. continued

Number	**Compound Name**	**RI**	**KAL**	**AZD**	**TYL**	**KSR**	**IVEL**	**SVK**	**VRS**	**BJN**
1	α-Pinene	932	1.73	-	0.43	1.82	0.10	0.60	0.06	-
2	Limonene	1024	1.97	0.16	0.42	1.17	0.06	3.48	0.10	-
3	18-Cineole	1026	1.62	-	-	1.01	0.14	-	-	-
4	Linalool	1095	0.28	-	-	17.97	0.73	1.41	-	-
5	Myrtenal	1195	1.16	0.14	-	-	-	-	1.68	-
6	Myrtenol	1194	0.06	0.16	2.86	-	-	-	0.20	1.01
7	α-Copaene	1374	0.27	1.47	3.31	0.90	1.84	1.54	0.38	0.30
8	β-Bourbonene	1387	0.73	0.38	0.43	0.31	-	0.84	0.11	0.14
9	β-Elemene	1389	18.70	11.98	10.24	0.45	4.39	1.12	0.74	6.31
10	trans-Caryophyllene	1408	6.10	4.60	0.99	2.76	1.93	12.68	13.22	0
11	Germacrene D	1484	8.23	2.66	4.25	1.10	10.99	13.81	12.48	4.02
12	Germacrene A	1508	2.00	1.56	-	-	2.25	-	-	-
13	β-Bisabolene	1505	-	-	0.72	-	-	0.31	1.09	1.81
14	δ-Cadinene	1522	1.16	4.31	3.59	-	-	3.70	-	1.35
15	Germacrene D-4-ol	1574	1.17	0.80	-	-	-	-	-	-
16	Spathulenol	1577	5.03	8.04	1.14	-	3.82	1.07	0.58	1.75
17	Caryophyllene oxide	1582	0.35	1.39	4.63	3.74	3.34	7.32	10.61	7.92
19	β-Sinensal	1699	-	-	17.98	-	-	1.94	-	7.42
20	Bisabolol<epi-alpha->	1683	3.97	3.55	-	-	1.34	-	-	-
21	α-Bisabolol	1685	-	-	0.98	-	-	5.93	2.34	9.37
22	Hexahydrofarnesyl acetone	1846	0.48	1.76	1.09	0.36	2.16	2.45	2.33	1.67
23	Farnesol<2Z6Z->	1698	2.01	7.40	-	-	-	-	-	-
24	Manool	2056	1.01	1.19	-	-	1.23	-	-	-
25	Aromadendrene oxide-(2)	1678	-	-	1.92	-	-	0.79	-	0.93
26	α-Cadinol	1652	-	-	1.46	0.56	3.80	0.85	-	1.44
27	α-Santalol	1674	1.47	6.23	-	-	-	-	-	-
28	Valeranone	1674	0.65	3.59	-	-	-	-	-	-
29	Cubebol<10-epi->	1533	-	-	5.68	-	-	2.96	16.29	7.79
30	E-β-Bisabolene	1529	-	-	0.30	-	-	1.04	0.22	0.72
31	Ledol	1602	1.79	1.69	-	0.96	0.30	-	-	-
32	Sesquisabinene	1457	-	-	0.12	-	1.77	0.31	1.55	-
33	(E)-β-Farnesene	1454	-	-	-	-	-	1.60	6.96	-
34	Cubebol<epi->	1493	-	-	10.25	-	-	2.19	0.74	1.82
35	Selinene<7-epi-alpha->	1520	0.29	2.57	-	-	4.53	-	-	-
36	4-Terpineol	1174	0.43	0.85	-	0.89	0.50	-	-	-
37	α-Terpineol	1186	0.67	0.65	-	5.14	0.88	-	-	-
	The number of components in each population		26	23	23	17	21	24	20	19

Populations abbreviations used in this table listed in Table 1. RI: retention index takes from Adams.

Table 3 Factor analysis of essential oils components of *S. laxa*

Rotated Component Matrix[a]				
Essential oils components	**Component**			
	1	**2**	**3**	**4**
Germacrene A	**0.911**	-0.127	0.068	-0.045
Spathulenol	**0.856**	-0.172	-0.062	-0.154
Germacrene D4	**0.814**	-0.272	-7	0.075
1.8-Cineole	0.302	-0.207	**0.709**	0.176
Pinene	-0.082	-0.246	0.102	0.912
Linalool	-0.292	-0.094	-0.025	0.043
Caryophyllene Oxide	-0.643	-0.151	-0.214	-0.650
Trans Carophyllene	-0.365	-0.429	-0.348	0.042
α-Cadinol	-0.142	**0.905**	-0.016	-0.075
Hexa Hydrofarnesyl	-0.171	0.858	-0.213	-0.127
Initial Eigenvalues % of variance	29.77	14.70	13.62	13.17
Eigenvalues Cumulative[%]	29.77	44.48	58.1	71.27

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

Table 4 Climatic characters for different studied localities

Pop.	**Altitude (m)**	**Average annual temperature (°C)**	**Minimum annual temperature (°C)**	**Maximum annual temperature (°C)**	**Average annual precipitation (mm)**
CHL	1950	13.85	10.83	20.68	90.08
DZD	2172	13.98	7.9	20.12	32.83
PLK	1035	13.73	7.57	19.94	30.41
KJR	1256	10.85	4.13	17.66	17.41
FRZ	2106	12.89	6.05	19.77	21.58
SHV	970	12.67	4.8	19.67	23.25
ASHK	1010	14.86	8.25	21.51	20.25
GRM	1100	14.32	7.65	21.02	19.08
KAL	876	17.37	11.3	23.5	26.25
AZD	1050	17.65	11.8	23.55	31.5
TYL	615	14.73	8.45	21.07	20.33
KSR	1340	14.66	8.45	20.9	20.41
IVEL	1815	12.81	6.23	19.44	14.91
SVK	790	13.8	7.14	20.55	17.08
VRS	1497	16.25	10.51	22.03	38.75
BJN	1020	13.25	6.38	20.11	21.41

The numbers are referring to the sample numbers P of the populations listed in Table 1.

Table 5 Correlation between ecological data and essential oils components of *S. laxa*

Number	**Compound Name**	**Altitude**	**Average temperature**	**Maximum Temperature**	**Minimum Temperature**	**Average Precipitation**
1	α-Pinene	0.444	-0.222	0.123	-0.235	**0.645****
2	18-Cineole	-0.181	0.312	0.243	0.308	-0.152
3	Myrtenol	**-0.578***	-0.151	-0.251	-0.137	-0.257
4	β-Elemene	-0.468	0.473	0.351	**0.513***	-0.173
5	trans-Caryophyllene	0.018	0.380	0.469	0.383	0.426
6	Germacrene D	**0.648***	-0.311	-0.101	-0.301	**0.557***
7	Germacrene A	-0.134	0.111	0.024	0.134	-0.301
8	Spathulenol	-0.249	**0.503***	0.395	**0.522***	-0.147
9	(E)-β-Farnesene	0.430	0.035	0.318	--2	**0.736****
10	Guaiadiene<69->	0.202	-0.202	--5	-0.208	**0.542***

Note: *Significant difference in α = 5% ** Significant difference in α = 1% minus sign shows the negative correlation between data's and plus sign shows positive correlation.

References

1. Ghahreman, A., *Plant Systematics*. Tehran University Publication Center, 1994.
2. Rechinger, K., et al., *Flora Iranica, Akademische Druck-U.* Verlagsanstalt, Graz, 1982. 150(2): p. 108-216.
3. Tundis, R., L. Peruzzi, and F. Menichini, *Phytochemical and biological studies of Stachys species in relation to chemotaxonomy: a review*. Phytochemistry, 2014. 102: p. 7-39.
4. Lashgargahi, Z. and A. Shafaghat, *Volatile Constituents of Essential Oils Isolated from Fresh and Dried Stachys lavandulifolia Vahl. and Stachys byzantina C. Koch. Two Lamiaceae from North-West Iran*. Journal of Essential Oil Bearing Plants, 2017. 20(5): p. 1302-1309.
5. Khanavi, M., et al., *Comparison of the antioxidant activity and total phenolic contents in some Stachys species*. African Journal of Biotechnology, 2009. 8(6).
6. Skaltsa, H.D., et al., *Essential oil analysis and antimicrobial activity of eight Stachys species from Greece*. Phytochemistry, 2003. 64(3): p. 743-752.
7. Skaltsa, H.D., et al., *Composition and antibacterial activity of the essential oils of Stachys candida and S. chrysantha from southern Greece*. Planta medica, 1999. 65(03): p. 255-256.
8. Mahzooni-Kachapi, S., et al., *The effect of altitude on chemical compositions and function of essential oils in Stachys lavandulifolia Vahl.(Iran).* Int. J. Med. Arom. Plants, 2014. 4: p. 107-116.
9. Salimi F., Shafaghat, A., Sahebalzamani H. and Habibzadeh, H. (2011). Analysis and Comparison of Chemical Composition of Essential Oils from Stachy byzantina C. Koch. Wet and Dried, Archives of Applied Science Research, 3(5): 381-383.
10. Semnani, K.M., Akbarzadeh, M. and Changizi, S. (2006). Essential Oils composition of Stachys byzantina, S. inflate, S. Lavandulifolia an S. laxa from Iran, Flavour and Fragrance Journal 21: 300-303.
11. Se, S. and I. Mehregan, *Composition of the essential oil of Stachys laxa Boiss. & Buhse.* Iranian Journal of Pharmaceutical Research, 2010: p. 57-58.
12. Salmaki, Y., et al., *A taxonomic revision of the genus Stachys (Lamiaceae: Lamioideae) in Iran.* Botanical Journal of the Linnean Society, 2012. 170(4): p. 573-617.
13. Omidbaigi, R., *Production and processing of medicinal plants. Vol. 2*. Razavi Ghods Astan Publication, 2009. 347.
14. https://en.climate-data.org. 2019.
15. Vokou, D., S. Kokkini, and J.-M. Bessiere, *Geographic variation of Greek oregano (Origanum vulgare ssp. hirtum) essential oils*. Biochemical Systematics and Ecology, 1993. 21(2): p. 287-295.
16. H., G., *Ecological identification of Stachys and Nepeta species in Mazandaran (with emphasis on medicinal properties).* The Journal of Plant Research, 2008.
17. Fatemeh Kiashi, Abbas Hadjiakhoondi, Zahra Tofighi1, Mahnaz Khanavi, Yousef Ajani, Sheyda Ahmadi Koulaei, Narguess Yassa. 2021. Compositions of Essential Oils and Some Biological Properties of *Stachys laxa* Boiss. & Buhse and *S. byzantina* K. Koch, Research Journal of Pharmacognosy (RJP) 8(2), 2021: 5–15.
18. Nejadhabibvash, F., et al., *Effect of Harvesting Time on Content and Chemical Composition of Essential Oil from Stachys lavandulifolia Vahl (Lamiaceae).* Journal of Medicinal plants and By-product, 2018. 7(2): p. 181-187.
19. Hajdari, A., et al., *Essential oil composition and antioxidant activity of Stachys sylvatica L.(Lamiaceae) from different wild populations in Kosovo*. Natural product research, 2012. 26(18): p. 1676-1681.
20. Renda, G., et al., *Volatile Constituents of three Stachys L. species from Turkey*. Marmara Pharmaceutical Journal, 2017. 21(2): p. 278-285.
21. Grujic-Jovanovic, S., et al., *Composition and antibacterial activity of the essential oil of six Stachys species from Serbia*. Flavour and Fragrance Journal, 2004. 19(2): p. 139-144.
22. Bahadori MB, Zengin G, Dinparast L, Eskandani M. The health benefits of three hedgenettle herbal teas (*Stachys byzantina*, *Stachys inflata*, and *Stachys lavandulifolia*)-profiling phenolic and antioxidant activities. *Eur J Integ Med*. 2020; 36(10): 1–7.
23. Alizadeh F, Ramezani M, Piravar Z. Effects of *Stachys sylvatica* hydroalcoholic extract on the ovary and hypophysis-gonadal axis in a rat with polycystic ovary syndrome. *Middle East Fertil Soci J*. 2020; 25(1): 1–7.
24. Aghaei Y, Hossein Mirjalili M, Nazeri V. Chemical diversity among the essential oils of wild populations of *Stachys lavandulifolia* VAHL (Lamiaceae) from Iran. *Chem Biodivers*. 2013; 10(2): 262–273.
25. Mann, C. and E.J. Staba, *Commercial Formulations of Chamomile*. Herbs, spices, and medicinal plants: recent advances in botany, horticulture, and pharmacology, 1992. 1: p. 235.
26. Jerkovic, I., et al., *Chemical composition of the essential oil from Stachys serotina.* Chemistry of natural compounds, 2012. 48(3): . 508-509.
27. Goren, A.C., et al., *Essential oil composition of twenty-two Stachys species (mountain tea) and their biological activities.* Phytochemistry Letters, 2011. 4(4): p. 448-453.