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Original Article

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

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ABSTRACT

Stachys laxa, Boiss. & Buhse 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 pof *S. laxa* were evaluated in this study. The 16 populations of *S. 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.

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 *S.* 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]. *S. 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

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(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 *S. laxa* Boiss. & Buhse, which is necessary for exact evaluation of its biological and pharmacological effects in future studies.

MATERIAL AND METHODS

Plant materials

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

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

100-200 g 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 (30m×0.25 mm, film thickness 0.25µm) and N_2 (1ml/min) as a carrier gas was applied. The program for oven temperature was adjusted at 60 °C for 3min, then rising to 250 °C with a 6 °C/min rate and finally held constant at 250 °C for 5min. GC/MS analysis. TRACE MS GC/MS system coupled with a DB column (60m × 0.25 mm, film thickness 0.25µm) was performed for GC/MS analyses.

Table 1 Geographical Location of collected populations of *S. 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.

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 10min. 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 was 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 was 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 (Fig. 2a.). 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. 2a.). 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) (Fig. 2a.).

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 (Fig. 2a.), and PCA (Fig. 2b.).

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 more significant relationships with the amount of precipitation (Table 5). Finally, the lowest and highest content of

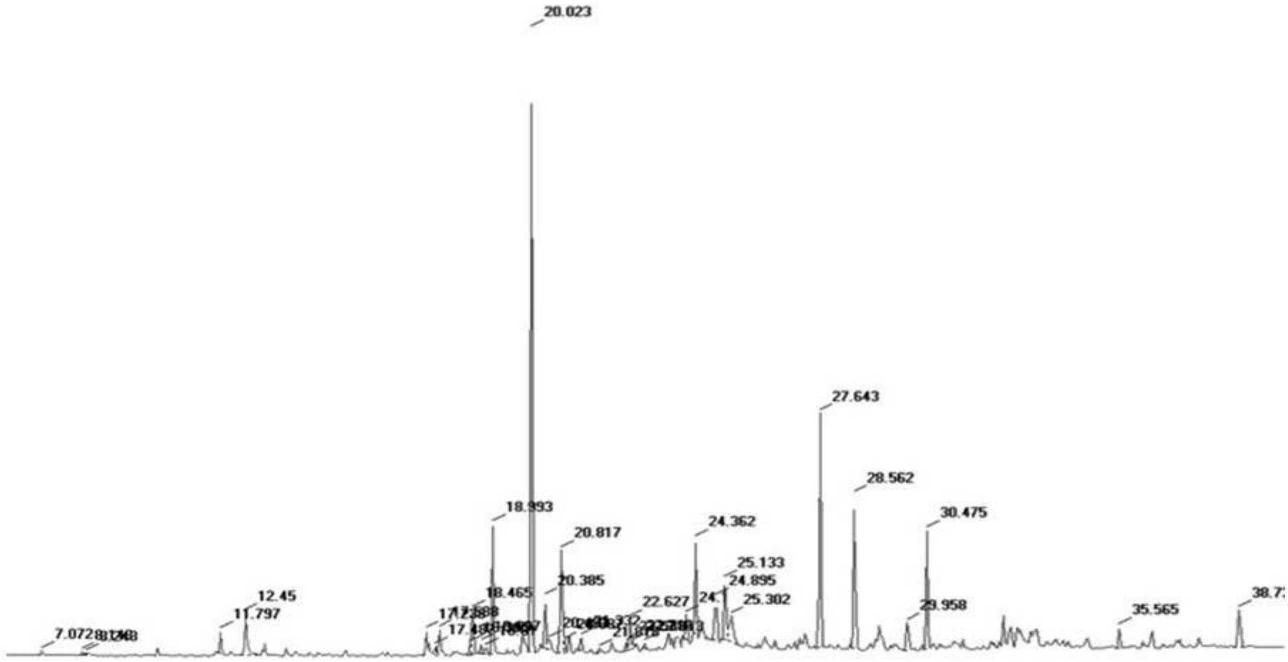

Fig. 1. Gas chromatography–flame ionization detector (GC–FID) chromatograms of essential oil from samples of *S. laxa*. Compounds are eluted using a DB-5 column.

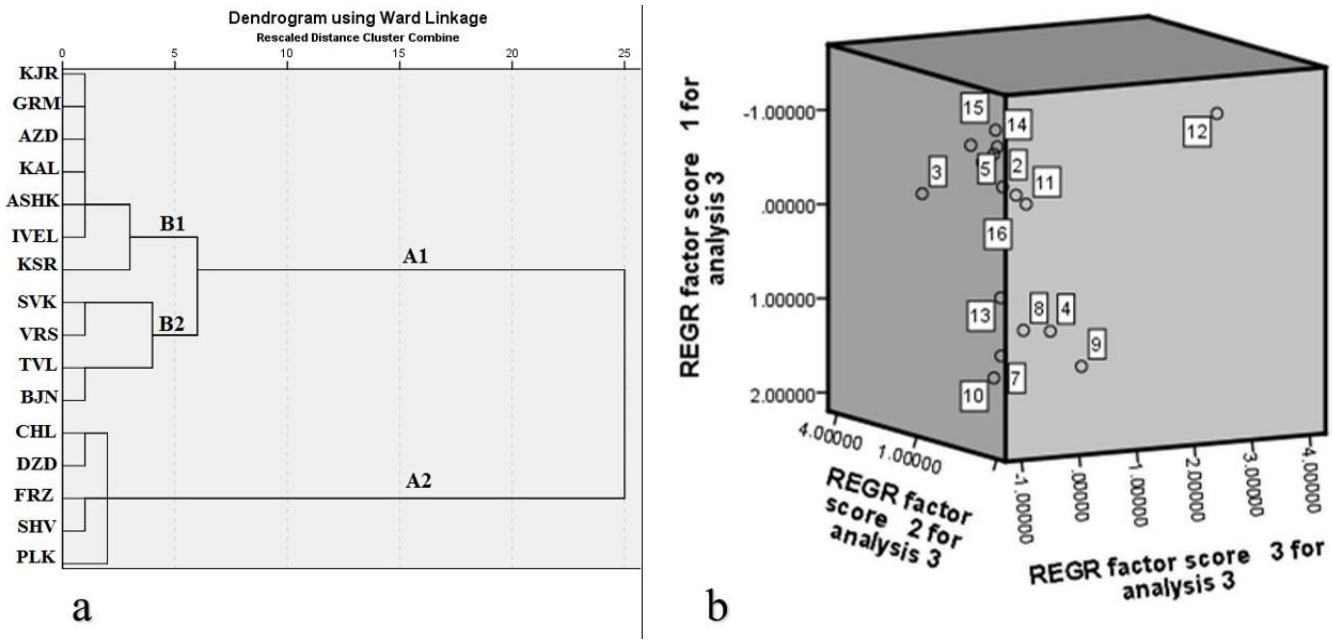

Fig. 2 a. Dendogram achieved by HCA of the distribution of the essential oil compounds. B. Principal component analysis of essential oils components of *S. laxa*. The numbers are referring to the sample numbers P of the populations listed in Table 1

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

S. 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 analyses 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 essential oils from full flowering and initial fruiting stages of *S. lavandulifolia* Vahl. were germacren D. Furthermore Hajdari *et al*. represented that the leaves and flowers of *S. 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 *S. 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 *S.* 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.

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

Number	Compound Name	RI	KAL	AZD	TYL	KSR	IVEL	SVK	VRS	BJN	DZD	PLK	KJR	FRZ	SHV	ASHK	GRM
1	α-Pinene	932	1.73	-	0.43	1.82	0.10	0.60	0.06	-	3.70	-	4.00	-	-	-	0.30
2	Limonene	1024	1.97	0.16	0.42	1.17	0.06	3.48	0.10	-	1.33	0.16	5.12	-	0.49	0.70	1.13
3	18-Cineole	1026	1.62	-	-	1.01	0.14	-	-	-	-	-	0.48	-	-	0.06	0.16
4	Linalool	1095	0.28	-	-	17.97	0.73	1.41	-	-	-	-	0.31	-	-	0.07	0.06
5	Myrtenal	1195	1.16	0.14	-	-	-	-	1.68	-	-	-	1.14	-	3.55	0.18	0.69
6	Myrtenol	1194	0.06	0.16	2.86	-	-	-	0.20	1.01	-	0.89	0.82	-	1.26	0.58	1.39
7	α-Copaene	1374	0.27	1.47	3.31	0.90	1.84	1.54	0.38	0.30	1.98	0.86	1.23	0.89	3.08	1.45	1.22
8	β-Bourbonene	1387	0.73	0.38	0.43	0.31	-	0.84	0.11	0.14	0.39	0.36	1.06	0.19	0.41	0.28	1.17
9	β-Elemene	1389	18.70	11.98	10.24	0.45	4.39	1.12	0.74	6.31	2.25	1.17	8.32	1.69	2.39	8.82	8.40
10	*Trans*-Caryophyllene	1408	6.10	4.60	0.99	2.76	1.93	12.68	13.22	0	3.16	2.09	0.64	2.15	4.57	3.94	1.36
11	Germacrene D	1484	8.23	2.66	4.25	1.10	10.99	13.81	12.48	4.02	40.59	28.49	7.00	42.85	32.31	11.16	1.98
12	Germacrene A	1508	2.00	1.56	-	-	2.25	-	-	-	-	-	2.01	-	-	1.69	2.30
13	β-Bisabolene	1505	-	-	0.72	-	-	0.31	1.09	1.81	0.64	0.81	-	0.98	1.26	-	-
14	δ-Cadinene	1522	1.16	4.31	3.59	-	-	3.70	-	1.35	2.91	5.28	5.50	4.33	4.62	4.78	5.13
15	Germacrene D-4-ol	1574	1.17	0.80	-	-	-	-	-	-	-	-	0.94	-	-	1.76	0.67
16	Spathulenol	1577	5.03	8.04	1.14	-	3.82	1.07	0.58	1.75	0.64	0.35	2.72	0.30	0.19	2.64	3.59
17	Caryophyllene oxide	1582	0.35	1.39	4.63	3.74	3.34	7.32	10.61	7.92	0.86	1.66	0.95	9.00	7.48	0.93	4.09
19	β-Sinensal	1699	-	-	17.98	-	-	1.94	-	7.42	0.83	2.71	-	0.71	-	-	-
20	Bisabolol<epi-alpha->	1683	3.97	3.55	-	-	1.34	-	-	-	-	-	2.99	-	-	14.02	7.45
21	α-Bisabolol	1685	-	-	0.98	-	-	5.93	2.34	9.37	1.96	2.50	-	8.50	-	-	-
22	Hexahydrofarnesyl acetone	1846	0.48	1.76	1.09	0.36	2.16	2.45	2.33	1.67	0.56	10.74	0.33	2.72	2.62	0.67	1.01
23	Farnesol<2Z6Z->	1698	2.01	7.40	-	-	-	-	-	-	-	-	4.20	-	-	6.12	0.56
24	Manool	2056	1.01	1.19	-	-	1.23	-	-	-	-	-	0.33	-	-	0.38	1.11
25	Aromadendrene oxide-(2)	1678	-	-	1.92	-	-	0.79	-	0.93	0.56	1.68	-	2.50	-	-	-
26	α-Cadinol	1652	-	-	1.46	0.56	3.80	0.85	-	1.44	0.91	4.46	-	2.38	-	-	-
27	α-Santalol	1674	1.47	6.23	-	-	-	-	-	-	-	-	2.47	-	-	2.19	3.13
28	Valeranone	1674	0.65	3.59	-	-	-	-	-	-	-	-	1.89	-	-	1.03	1.94
29	Cubebol<10-epi->	1533	-	-	5.68	-	-	2.96	16.29	7.79	-	-	-	1.80	7.40	-	-
30	E-β-Bisabolene	1529	-	-	0.30	-	-	1.04	0.22	0.72	0.43	0.81	-	0.35	0.46	-	-
31	Ledol	1602	1.79	1.69	-	0.96	0.30	-	-	-	-	-	0.87	-	-	3.02	2.64
32	Sesquisabinene	1457	-	-	0.12	-	1.77	0.31	1.55	-	0.16	0.39	-	0.09	0.87	-	-
33	(E)-β-Farnesene	1454	-	-	-	-	-	1.60	6.96	-	1.43	5.65	-	3.56	-	-	-
34	Cubebol<epi->	1493	-	-	10.25	-	-	2.19	0.74	1.82	0.83	2.45	-	3.96	6.01	-	-
35	Selinene<7-epi-alpha->	1520	0.29	2.57	-	-	4.53	-	-	-	-	-	1.97	-	-	2.46	1.52
36	4-Terpineol	1174	0.43	0.85	-	0.89	0.50	-	-	-	-	-	1.39	-	-	0.91	0.56
37	α-Terpineol	1186	0.67	0.65	-	5.14	0.88	-	-	-	-	-	0.82	-	-	0.71	0.71
	The number of components in each population		26	23	23	17	21	24	20	19	21	22	26	21	19	27	26

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* Boiss. & Buhse

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		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* Boiss. & Buhse

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.

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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