Avicenna Journal of Phytomedicine (AJP)

Original Research Paper

Mineral elements and essential oil contents of *Scutellaria luteo-caerulea Bornm. & Snit*

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Article history:
Received: Jul 29, 2013
Received in revised form: Sep 1, 2013
Accepted: Sep 16, 2013
Vol. 4, No. 3, May-Jun 2014, 182-190

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keywords:
Atomic absorption
Essential oils
Gas chromatography mass
Mineral elements
Spectrometry
Spectrophotometry

Abstract

Objective: *Scutellaria luteo-caerulea Bornm. & Snit.* is one of the species of genus *Scutellaria*, within the family of the *Lamiaceae*, that is used for immune system stimulation and antibacterial effects in traditional medicine in Iran. The aims of this study were to analyze essential oils and mineral element contents of leaves of *S. luteo-caerulea* in flowering stage of development.

Materials and Methods: The essential oils were obtained by hydrodistillation of the leaves of *S. luteo-caerulea and* were analyzed by gas chromatography mass spectrometry (GC/MS). Moreover, microwave digestion with atomic absorption spectrophotometry were used for the mineral elements assay.

Results: Ninety-seven constituents were detected. Between them, the major components were trans-caryophyllene (25.4%), D-germacrene (7.9%), and linalool (7.4%). Determination of mineral elements showed that the highest minerals were Ca^{2+} (65.14±1.95 µg/ml) and K^{+} (64.67±3.10 µg/ml).

Conclusion: Presence of different essential oils and rich sources of Ca^{2+} and K^{+} candidate this plant as an auxiliary medication in different diseases, but more complementary researches are needed about its potency and side effects.

Please cite this paper as:
Nikbin M, Kazemipour N, Maghsoodlou MT, Valizadeh J, Sepehrimanesh M, Davarimanesh A. Mineral elements and essential oil contents of *Scutellaria luteo-caerulea Bornm. & Snit*. Avicenna J Phytomed, 2014; 4 (3): 182-190.

Introduction

In recent years, medicinal plants have been widely used in the treatment and prevention of diseases because they have lower cost and fewer adverse effects in the body. The genus *Scutellaria* is a diverse and widespread genus within the family of the Lamiaceae (the mint family). They have over 350 species, commonly called skullcaps, and are found worldwide from Siberia to the tropics of South and North America, on the islands of Japan, and throughout a large part of Europe and Asia (Cole et al., 2007). This is a very distinctive genus in several

morphological characters; perhaps the most obvious is the little crest (scutellum, literally a little shield) across the top of the calyx, the origin of the generic name (Michigan Flora Online,http://michiganflora.net/genus.aspx?id=Scutellaria).

The extracts of this genus possess antitumor (Dai et al., 2011; Fang et al., 2012; Yin et al., 2004; Yu et al., 2007), hepatoprotective (Lin and Shieh, 1996), antioxidant (Ye and Huang, 2012; Yuan et al., 2011), anti-inflammatory (Jung et al., 2012; Zhang et al., 2012), anticonvulsant (Liu et al., 2012), antibacterial (Lu et al., 2012; Pant et al., 2012), and antiviral (Tayarani-Najaran et al., 2012; Zandi et al., 2012) effects.

S. luteo-caerulea Bornm. & Sint. is found in the most region of Iranian plateau, such as Turkmenistan, and Iran. This species is very similar to *S. multicaulis*, but distinct according to the color and size of corolla and shape of the leaves (Bor, 1970). In Iran, it is grown in the eastern provinces including Northern, Razavi, and Southern Khorasan and Sistan and Baluchistan and locally named Boshghabi Eshghabadi (Bor, 1970). Geographical distribution and shape of this Persian plant are shown in Figure 1.

Essential oils, also known as ethereal oils, are aromatic and largely volatile compounds. They are commonly extracted by steam distillation or solvent extraction and are usually devoid of long-term genotoxic risks (Bakkali et al., 2008; Benchaar et al., 2008). The essential oils of only a few species of *Scutellaria* such as *S. albida* ssp. *albida*, *S. sieberi*, *S. rupestris* ssp. *adenotricha*, *S. barbata*, *S. lateriflora*, *S. galericulata*, *S. parvula*, *S. baicalensis*, and *S. rubicunda subsp. Linnaeana* have been investigated (Shang et al., 2010).

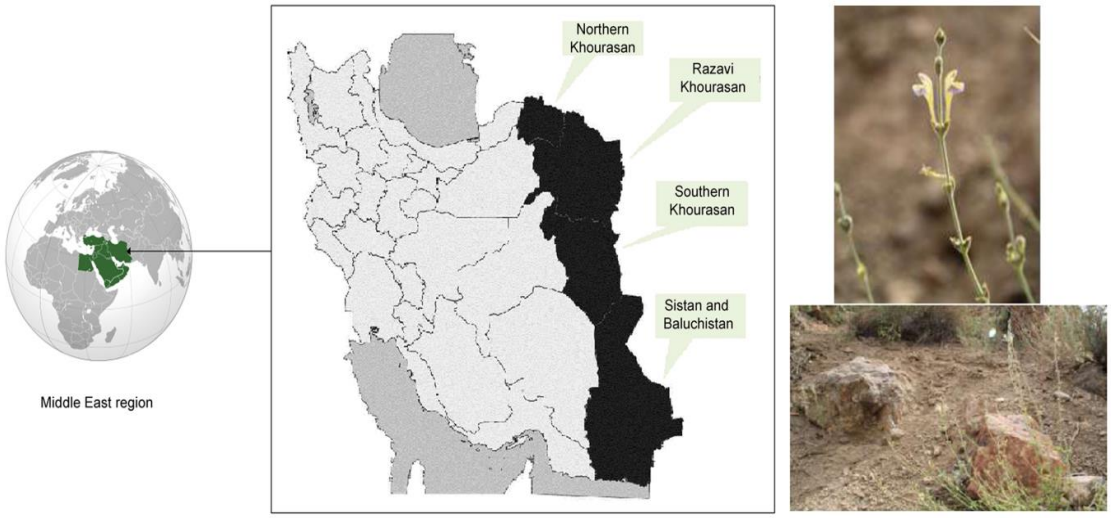

Figure 1. Geographical distribution of *S. luteo-caerulea* in Iran. This plant has a yellow corolla with blue-violet edges.

On the other hand, mineral elements play important roles in biological reactions and have structural functions. Therefore, scientists attempt to determine their concentration levels with different methods such as atomic absorption spectrometry (Hicsonmez et al., 2009). Nevertheless, no data exist concerning the essential oil composition and mineral elements contents of *luteo-caerulea Bornm. & Snit.*

Beacause of the most beneficial effects of the therapeutic plants are due to mineral contents or essential oils and lack of any data about this plant species, the purpose of the

present investigation is to evaluate the essential oils and mineral elements of the leaves of *S. luteo-caerulea Bornm. & Snit.*

Experimental procedure

Plant material

S. luteo-caerulea Bornm. & Snit. was collected from Taftan of Sistan and Baluchistan province in Iran (GPS coordinates: 61.20816, 28.22529) during the spring season (May, 2010). All plants were in the flowering stage of developing and the taxonomic identification of each plant was confirmed by the Biology Department of University of Sistan and Baluchistan, Zahedan, Iran. The plant also matched the digital herbarium of Botanical Garden and Botanical Museum Berlin-Dahlem, Freie University, Berlin (http://ww2.bgbm.org/herbarium/(Barcode:B 100241801/ImageId:278532).

Extraction of essential oil

Bulked plant's leaves were used as the crude source, dried in the shade and powdered by the grinder. One hundred twenty gram of dried powder was exposed to hydrodistillation for 3 h using a Clevenger-type apparatus. The obtained essential oil was collected and anhydrous sodium sulfate was used to absorb the small amount of water containing essential oil. The essential oil was then stored at 4 °C until use.

Essential oil analysis

The essential oil was analyzed by GC/MS. GC analyses were performed using an Agilent 6890 GC, equipped with a HP-5 capillary column, 30 m length, 0.25 mm I.D. and 0.25 µm stationary phase film thickness, and an Agilent 5973 mass selective detector. For GC-MS detection, an ESI system with the ionization energy of 70 eV was used. Helium (99.999%) was used as the carrier gas, at the flow rate of 1 ml/min. The injection port temperature was set at 250 °C, column temperature was initially kept at 40 °C for 1 min, and then gradually increased to 240 °C at the rate of 3 °C /min. The components were identified by comparing their mass spectra with those in the GC/MS library and literature (Adams, 2001) and by comparing their relative retention times with those of authentic samples on the HP-5 MS capillary column (Table 1).

Flame atomic absorption spectroscopy (FAAS) with microwave digestion

In order to measure the concentration of mineral elements such as Ca^{2+}, K^{+}, Na^{+}, Mg^{2+}, Mn^{2+}, Cr^{3+}, and Fe^{2+}, method described by Rechcigl and Payne (1990) was used. Briefly, 0.5 g of dried powdered sample was digested with 10 ml of concentrated nitric acid and then was placed inside a domestic microwave oven. The sample was irradiated at a 900 W power and 250 °C temperature for 10 min. Then, 5 ml of concentrated HCl was added and irradiation was continued for another 5 min. After digestion, the vessel was cool and then 10 ml of double distilled water was added and the mixture was filtered by Whatman No. 42 filter paper and diluted with double distilled water to a final volume of 100 ml. The solution was used for elemental analysis by atomic absorption spectrometer PU 9100X (Philips Scientific).

Statistical analysis

The results of the three replicates of mineral element contents were pooled and expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey were carried out using SPSS version 16. Significance was accepted at $p<0.05$.

Results

Hydrodistillation of the dried leaves of *S. luteo-caerulea* yielded 0.33% (v/w) of a

yellowish essential oil. Ninety-seven compounds, representing 89.04% of the oil, were identified. Quantitative and qualitative analytical results are shown in Table 2. The essential oil consisted mainly of sesquiterpene hydrocarbons and oxygenated monoterpene. Trans-caryophyllene (24.8%), germacrene-D (7.9%), α-humulene (4.9%), and patchoulene(4.7%) were the main sesquiterpene hydrocarbons, whereas linalool (7.4%) and pulegone (1.1%) were the main oxygenated monoterpenes.

Table 1. Authentic samples on the HP-5 MS capillary column that used for calculation of retention indices

No.	Compounds	Formula	RI	t_R (min)
1	n-Hexane	C_6H_{14}	600	3.166
2	n-Heptane	C_7H_{16}	700	4.617
3	n-Octane	C_8H_{18}	800	7.329
4	n-Nonane	C_9H_{20}	900	11.373
5	n-Decane	$C_{10}H_{22}$	1000	16.249
6	n-Undecane	$C_{11}H_{24}$	1100	21.39
7	n-Dodecane	$C_{12}H_{26}$	1200	26.456
8	n-Tridecane	$C_{13}H_{28}$	1300	29.830
9	n-Tetradecane	$C_{14}H_{30}$	1400	35.925
10	n-Pentadecane	$C_{15}H_{32}$	1500	39.321
11	n-Hexadecane	$C_{16}H_{34}$	1600	44.442
12	n-Heptadecane	$C_{17}H_{36}$	1700	47.021
13	n-Octadecane	$C_{18}H_{38}$	1800	51.321
14	n-Nonadecane	$C_{19}H_{40}$	1900	55.621
15	n-Eicosane	$C_{20}H_{42}$	2000	59.921
16	n-Heneicosane	$C_{21}H_{44}$	2100	64.221
17	n-Docosane	$C_{22}H_{46}$	2200	68.521
18	n-Tricosane	$C_{23}H_{48}$	2300	72.821
19	n-Tetracosane	$C_{24}H_{50}$	2400	77.121
20	n-Pentacosane	$C_{25}H_{52}$	2500	81.421
21	n-Hexacosane	$C_{26}H_{54}$	2600	85.721
22	n-Heptacosane	$C_{27}H_{56}$	2700	90.021

RI: retention inex, t_R: retention time.

Table 2. Chemical composition and percentage of essential oil of *Scutellaria luteo-caerulea*.*

No.	Compounds	%	RI	t_R (min)
1	Hexanal	< 0.1	702	4.670
2	Octane	< 0.1	722	5.212
3	Cyclohexene oxide	< 0.1	746	5.855
4	2-Hexenal	0.5	755	6.112
5	3-Hexen-1-ol	0.3	775	6.663
6	2-Hexen-1-ol	< 0.1	791	7.072
7	1-Hexanol	0.2	794	7.176
8	Styrene	0.2	806	7.566
9	Heptanal	< 0.1	812	7.817
10	Tricyclene	< 0.1	845	9.149
11	p-Methylbenzyl alcohol	< 0.1	850	9.368
12	α-Pinene	0.1	857	9.636
13	Benzaldehyde	0.4	860	9.774
14	Camphene	0.1	869	10.127
15	Sabinene	< 0.1	895	11.169
16	β-Pinene	0.1	898	11.279
17	3-Octanone	< 0.1	901	11.409
18	1-Octen-3-ol	1.8	910	11.830
19	β-Myrcene	< 0.1	915	12.108
20	3-Octanol	0.8	923	12.456
21	δ-3-Carene	< 0.1	932	12.883
22	α-Terpinene	0.2	937	13.138
23	p-Cimene	0.2	941	13.317
24	1,8-Cineole	0.6	948	13.650
25	Limonene	0.8	950	13.771
26	cis-Ocimene	< 0.1	958	14.160
27	Acetophenone	0.5	965	14.485
28	Trans-β-Ocimene	0.4	969	14.666
29	γ-Terpinene	0.3	977	15.045
30	α-Terpinolene	0.1	1002	16.372
31	Nonanal	0.1	1010	16.782
32	Linalool	7.4	1026	17.567
33	Benzene (1,3-dimethyl-2-butenyl)-	0.3	1039	18.250
34	Camphor	0.8	1042	18.421
35	Phenoprene	0.8	1054	19.034
36	6-[(Z)-1-Butenyl]-1,4-cycloheptadiene	0.1	1059	19.280
37	2-Methylnorbornene	< 0.1	1070	19.857
38	4-Terpineol	0.4	1081	20.407

39	α-Terpineol	0.7	1094	21.063
40	Cyclooctene, 4-methylene-6-(1-propenylidene)	1.1	1112	21.979
41	Pulegone	1.1	1128	22.807
42	Geraniol	0.6	1156	24.229
43	Borneol, acetate	0.1	1174	25.155
44	bicyclogermacrene	0.6	1243	27.912
45	Cadina-1,4-diene	1.2	1261	28.528
46	(-)-Cycloisosativene	0.6	1285	29.308
47	α-Copaene	3.1	1297	29.732
48	α-Longipinene	5.2	1302	29.948
49	trans-Caryophyllene	25.4	1332	31.806
50	α-Gurjunene	0.4	1343	32.459
51	Aromadendrene	< 0.1	1345	32.593
52	Valencene	0.3	1346	32.659
53	α-Humulene	5.1	1356	33.228
54	α -Cubebene	0.4	1357	33.327
55	β-Cubebene	0.2	1359	33.448
56	Epizonarene	< 0.1	1365	33.798
57	D-Germacrene	7.9	1374	34.323
58	α-Ylangene	0.4	1376	34.472
59	Patchoulene	4.8	1381	34.792
60	δ-Cadinene	0.3	1385	35.004
61	γ-Cadinene	0.9	1389	35.270
62	Pentadecane	0.5	1392	35.452
63	β-Himachalene	2.6	1397	35.757
64	Cadina-1,4-Diene	0.4	1401	35.951
65	α-cadinene	0.3	1406	36.140
66	(-)Dehydroaromadendrane	0.5	1414	36.396
67	3-Hexen-1-ol, benzoate	0.9	1432	37.027
68	β-Bisabolene	0.2	1439	37.238
69	Caryophyllene oxide	3.8	1449	37.590
70	β -Humulene	0.3	1455	37.781
71	Ledene	0.5	1462	38.034
72	endo-2-Methylbicyclo [3.3.1]nonane	0.6	1474	38.441
73	Naphthalene, 1,2,3,4,6,8a-hexahydro-1-isopropyl-4,7-dimethyl	0.3	1500	39.308
74	Aromadendrene	0.6	1504	39.529
75	tau-Cadinol	1.5	1511	39.869
76	t-Muurolol	1.2	1520	40.328
77	α -Muurolene	0.3	1531	40.920
78	Heptadecane	0.6	1567	42.750
79	Mintsulfide	0.1	1569	42.829
80	Octadecane	< 0.1	1666	46.135
81	Neophytadiene	< 0.1	1725	48.110
82	Nonadecane	< 0.1	1757	49.463
83	Hexadecanoic acid, methyl ester	< 0.1	1764	49.788
84	Dibutyl phthalate	< 0.1	1769	49.988
85	n-Hexadecanoic acid	0.5	1809	51.703
86	Eicosane	< 0.1	1831	52.640
87	Linolenic acid, methyl ester	< 0.1	1883	54.884
88	Phytol	0.3	1903	55.757
89	Linoleic acid	< 0.1	1925	56.703
90	(E)-9-Octadecenoic acid	0.7	1930	56.923
91	Octadecanoic acid	< 0.1	1944	57.508
92	n- Heneicosane	< 0.1	1969	58.589
93	α-Farnesene	0.1	1998	59.831
94	Docosane	< 0.1	2034	61.387
95	Tricosane	< 0.1	2096	64.069
96	Tetracosane	< 0.1	2157	66.660
97	Squalene	< 0.1	2338	74.457

∗ (The compounds are listed in order of their elution on HP-5).

Table 3. Mineral elements of leaves of *Scutellaria luteo-caerulea Bornm. & Sint*

Elements	**Concentration (µg/ml)**
Ca^{2+}	65.14 ± 1.95^{a}
K^{+}	64.67 ± 3.10^{a}
Mg^{2+}	7.07 ± 1.02^{b}
Mn^{2+}	4.38 ± 1.68^{b}
Fe^{2+}	2.79 ± 0.25^{c}
Cr^{3+}	2.21 ± 0.23^{c}
Na^{+}	0.27 ± 0.02^{d}

Different superscript letters indicate significant differences ($p<0.05$).

The values of several oxygenated sesquiterpene, such as caryophyllene oxide (3.8%), tau-cadinol (1.4%), and t-muurolol (1.2%) were also significant. The GC-MS chromatogram of essential oils of *S. luteo-caerulea* was shown in Figure 2. In this figure, only components that had higher than 0.1 % value were numbered. Mineral elements of leaves of *S. luteo-caerulea* were shown in Table 3. According to our founding, Ca^{2+} (65.14±1.95 µg/ml) and K^{+} (64.67±3.10 µg/ml) had the highest concentrations followed by Mg^{2+} (7.07±1.02).

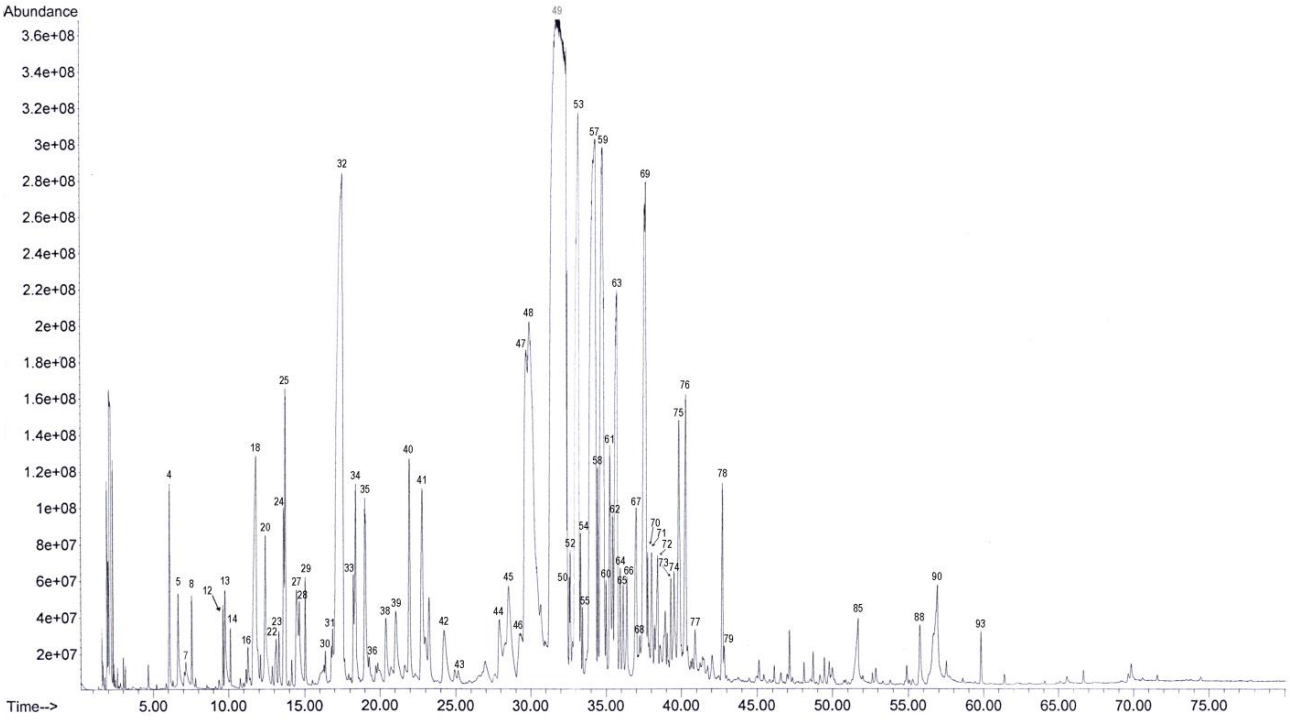

Figure 2. The GC-MS chromatogram of essential oils of *S. luteo-caerulea*. Only components that had higher than 0.1 % value were numbered. Component number according to Table 2.

Discussion

In this study, the therapeutic valency of the *S. luteo-caerulea Bornm. & Snit.* was measured by analysis of the essential oils and mineral element contents of leave part of this plant. Essential oils of *S. luteo-caerulea Bornm. & Snit.* had not been evaluated previously, but several studies were conducted for other species and subspecies. Skaltsa and their colleagues reported that linalool was the main compound of the oil of *S. albida* ssp. *albida* (Skaltsa et al., 2000a) and found it in high amounts in other species including *S. sieberi* and *S. rupestris* ssp. *adenotricha* (Skaltsa et al., 2005b). The essential oil contents of *Scutellaria pinnatifida* was evaluated by Ghannadi and Mehregan (2003). Their results demonstrated that germacrene-D and beta-caryophyllene were the most essential oils that found in the aerial parts of this widespread Iranian Skullcaps.

In another study by Yu et al. (2004), the essential oils of leaves of *S. barbata* from

China was evaluated. They reported that the main components of the oil was hexahydrofarnesyl acetone followed by 3,7,11,15-tetramethyl-2-hexadecen-1-ol, menthol and 1-octen-3-ol. Yaghmai (1988) described that β-cadinene and calamenene were the major components of the oils of *S. lateriflora* along with β-elemene, α-cubebene, and α-humulene. Caryophyllenes were the main compounds of *S. rubicund*a subsp. *linnaean*a reported by Rosselli et al. (2007).

In another part of this study, sample preparation with microwave digestion was used for mineralization of this plant. This method was faster and easier than the old digestion method such as wet and dry ashing. Totally, seven different minerals were existed in this plant as different levels. Analytical results of all the analyzed elements in this plant were given in Table 3. The results showed high concentrations of Ca^{2+} and K^{+} and low concentration of Na^{+}, Cr^{3+}, and Fe^{2+} in the plant. Calcium is an essential element that is found in high concentrations in plants (Hicsonmez et al., 2009) and plays different roles such as structural, in the cell wall and membranes, a counter-cation for inorganic and organic anions, in the vacuole, and an intracellular messenger in the cytosol (Marschner, 1995). Another main element in *S. luteo-caerulea Bornm. & Snit.* is K^{+}. It is needed for activation of some enzymes, protein synthesis in ribosomes, turgor provision, and water homeostasis and also plays roles in photosynthesis (Maathuis, 2009).

The content of nine mineral elements in the root, stem, and leaf of *S. baica*lensis was evaluated by Zhu et al. (2011). Their results showed that the main mineral elements in all three parts were K^{+}, Ca^{2+}, Mg^{2+}, P^{4-}, Al^{3+}, and Fe^{2+}. In another study, the contents of six elements, Ca^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, and K^{+}, in five parts of planted *S. baicalensis* were determined by FAAS. They reported that Ca^{2+} in the flowers, seeds, and roots and Fe^{2+} in the stems and leaves were the main elements (Sheng et al., 2009). Our findings are in concordance with the results of these two studies that demonstrated that Ca^{2+} and K^{+} are found in high concentration in this genus. In summary, our study demonstrated that *S. luteo-caerulea Bornm. & Sint* was the rich sources of Ca^{2+} and K^{+} and had many different essential oils specially trans-caryophyllene, D- germacrene, and linalool. Further studies are required for analysis of its *in vivo* effects and to develop new drugs and therapeutic agents from essential oils of this plant.

Acknowledgments

Authors are specifically grateful to different staff and sections of the Department of Biology and Chemistry of University of Sistan and Baluchistan, Iran, for assisting in this research. Financial support was provided by University of Sistan and Baluchistan, Iran.

Conflict of interest

There is not any conflict of interest in this study.

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