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The effects of ecological factors of site on quality and quantity of the essence from phlomis cancellata Bunge.In Mazandaran province (Iran).

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ABSTRACT: Identification of aromatic plants, their habitats, and phytochemical study of them has increased because of global attitudes to use the organic essences in different industries. Phlomis cancellata is one of the aromatic plants which belong to Labiatae family. The species uses in traditional medicine and its essence can find suitable role in different industries as it is unknown species to trade. In order to investigating of ecological effects on quality and quantity of the species essence, five associations of Phlomis cancellata have been studied. Vegetative organs of the species were collected from different natural habitat in Mazandaran province (Iran) from five altitudes such as 2000, 2200, 2400, 2600, and 2800 m a.s.l and after shrivelling in vitro thermal position, extracting of essence was done by Hydrodistilation. The compositions of the essential oil were identified and analysed using GC-FID and GC/MS and by measuring the Retention Index and Mass spectrums. Pearson correlation coefficient was used to assess the relationship between edaphic factors and the essence suing SPSS v.22 software. As result, the efficiency rate was the most in the altitude 2400 m and the component amount in the first altitude level (2000 m) was the highest rates. Although the quality composition of five altitudes has differed, six elements were generally observed in all altitudes that were α-Pinene, β-Bourbonene, β-Elemene, Germacrene-D, Germacrene B and t-Muurolol as the main compounds. Sesquiterpene proportion, however, was the most elements in all location. Therefore, regarding the high efficiency rate, number of components, and the valuable rate of some components, the extracted essence of first altitude level (2000 m) is the most favourable quality to use in different industries, especially medicinal industries.
Keywords: Phlomis cancellata Bunge, essence, ecological factor, Mazandaran, Iran.

INTRODUCTION

The taking advantage of herbs as medicines predates written human history (Bellamy and Pfister, 1992). Although many of the herbs used by humans to season food also yield useful medicinal compounds (Tapsell et al., 2006; Lai and Roy, 2004), in case of human diseases,it may also protect against oxidative stress and inflammation, both of which are risk factors for cancer initiation andpromotion as well as other pathological conditions (Surh, 1999; Surh et al., 2005) so thatfor a very long time, plants have played an important role in the treatment of many diseases (Fallah-Hoseini et al., 2006). The herbs that have received the most scientific attention inregard to influencing psychological processes have been drawnfrom the traditional medicines (Leite et al., 1986). As aresult, there needs to be a significant investment in human clinicaltrials to substantiate many of the hypothesised health benefits.Nevertheless, this review provides encouragement for furtherscientific inquiry (Dragland et al., 2003).In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Farnsworth, 1966).

Phlomis genus, as one of the medicinal plants, has much medicine application such as induction of insulin release (Hasani-Ranjbar et al., 2008). The genus belongs to Labiatae (Lamiaceae) family with 70 annual and perennial species which disperse in Asia regions including Iran, Afghanistan, Turkmenistan, and Iraq (Rechinger, 1982; Morteza-Semnani et al., 2006). Seventeen species from Phlomis genus is endemic of Iran territory that grows naturally (Rechinger, 1982; Morteza-Semnani et al., 2006). Phlomis cancellata is a perennial and annual species which is widely scattered in rangelands of Khorassan, Golestan, and Mazandaran provinces (Rechinger, 1982; Morteza-Semnani et al., 2006). Furthermore, it is used as medicinal herb (Akhlaghi and Motevalizadeh Kakh'ky, 2010), and has antibacterial property (Deylamsalehi et al., 2013). The most researches have focused on chemical compositions of the herb such as Morteza-Semnani et al. (2006) have reported 53 components of the herb essence and have pointed out that Germacrene-D (25.6%) and □-Pinene (6.4%) were the best part of chemical compound. The phytochemical analysis of the Phlomis cancellata has done by extracting of essence using Hydrodistilation method that four major elements are identified in the herb essence such as Germacrene-D, β-Caryophyllene, Bicyclogermacrene, and β-selinene as Akhlaghi and MotevalizadehKakh'ky (2010) have reported. Hexadecanoic acid and Germacrene-D compounds are recognised as the main elements in the herb essence (Deylamsalehi et al., 2013). Evaluation of researchers' studies on ecological effects on the quality and quantity of essence showed that this matter of aromatic plants has recently noticed by researchers. For instance, the study of Haider et al., (2009) on the effects of altitude on the essence of Artemisia roxburghiana Besser var. purpurascens has showed that the most efficiency rate and the chemical compounds in the essential oilof the species related to low altitude. Other researches showed that there was reversely correlation between soil nitrogen and the essence function of Achillea millefolium L. subsp. millefolium (Azarnivand et al., 2010). Another research has been also done on the essential oils of five associations of Thymus migricus in Azerbaijan province (Iran) and showed that between the effects of soil and altitude on the essence have significantly differed and altitude had the most affection (Yavari et al., 2010). The quality and quantity of the essence in Artemisia sieberi in four altitude level have been studied and results showed that the altitude level has effective consequence on the essential oil, especially the altitude 1280 m (Behtari et al., 2012). Singh et al. (2013) have pointed out that reason of variety in chemical compositions of the essential oils in Ocimumamericanum L. in different habitats (Himalaya Mountains) is differences of soil properties and microclimate circumstances. At least, it has showed that PH factor of soil has not had any affection on the quality and quantity of the essential oil in Agathosmabetulina (Ntwana et al., 2013).

Northern aspect of Alborz Mountains is a place where many aromatic and medicinal plants are growing. It has been known that different environmental factors e.g. altitude and physicochemical of soil (Verport et al., 2000) have abysmally role to change the growth pattern and consequently phytochemical variation in plants (Omidbaygi, 2005). Hence, current research intends to analysis the quality and quantity of essential oil in Phlomis cancellata regarding to ecological parameters effects of location on the herb essence in northern of Alborz Mountains (Iran).

MATERIAL AND METHODS

Site traits

In order to investigation of the effects of ecological factors on the chemical compounds of essential oil in Phlomis cancellata, Kandi'Chall in 55 km from south of Chalous in northern Alborz Mt. was selected. The geographical position of the study area is limited in $36^{o}23^{'}03^{''}$E and $51^{o}10^{'}41^{''}$ with 600 mm average annual rain and upland cool climate regarding Emberger's method. General slope percentage of the area is 30 % with sandy-clay-silty soil texture of all areas. The most vegetation cover refers to Phlomis cancellata Bunge. along with other species such as Berberis vulgaris L., Stachys lavandulifolia Vahl., Stachys byzanthina C. Koch., and Festuca ovina L.

Herbal sampling

Vegetative organs of Phlomis cancellata were sampled at flowering period from five altitudinal levels such as 2000, 2200, 2400, 2600, and 2800 m a.s.l. The samples were dried in vitro condition after cleaning and separating of the herbal organs from thatches (Omidbaygi, 2005). Afterward, the dried samples were milled and as much as 100 gr of the essential oil in the herb's aerial parts extracted using Clevenger instrument with Hydrodistilation method for 3 hours and regarding three repeats, the essence efficiency (volume percentage to dried weight) were calculated (Demirci et al, 2008;Goncalves et al, 2010). In order for the essential not to be mixed with water, 1 mili-litter of pentane solvent was poured into the store inlet of the essential.Considering the moisture percentage, the essential output was measured in dry weight (w/w).The essential, when extracted, is collected and distilled using Sodium Sulphate, and kept in the fridge at $4^{\circ C}$ until it was injected into Gas Chromatography (GC) (Vagionas et al, 2007; Ahmadi et al, 2010).

Edaphic sampling

At the outset, some soil profiles with 0- 30 cm depth were drilled and soil sampling were done on each altitudinal level. The samples were transferred to laboratory and after sieving with 2 mm sieve (Allison, 1965), they then analysed; physical analysis of soil in order to determine the soil texture was done by Bouyoucos hydrometer method (Mclean, 1988). Chemical analysis, however, were done using pH meter to determine the acidity rate (Jones, 1980) and Electrical Conductivity Meter was employed to obtain the Electrical Conductivity

(EC) (Rhoads, 1982). Moreover, the nitrogen rate was determined by kjeldahl method (Lull, 1959), absorbable potassium and phosphor were obtained by saturated distillate (Olsen, 1954), and finally, the organic carbon and lime rates were achieved by titration method (Allison, 1965).

GC-FID and GC/MS traits

The experiment is employed the N6890 Gas Chromatography (GC) (Agilent Ltd., US), with FID detector (ionisation detector by Hydrogen flame), with HP-5 column in 30 meters length and 0.25 millimetre internal diameter, plus with constant phase layer in 0.25 micron. The conveyer gas was Helium and temperature of injection position was 250^{o}C which in the direction of thermal programing, it is used 50-250^{o}C with increasing 5^{o}C per every minute. In this study, it also used B5975 GC/MS (Agilent Ltd., US), with 70 volt-electron as detector. The employed column in HP-5 was same thermal programing and traits in GC.

Essential analysis

The extracted essential oil was first injected into the GC. The most suitable programing of thermal column then was obtained for complete separation of the essential oil. In addition, the relative percentage and Deterrence Index of each component was measured. Then, the essential oil was analyzed using GC/MS in order to identify its composition. The components were identified using under area of mass spectrometry curve, and were compared with the standard compositions and the data in the mass database Wiley275.L (Adams, 2007).

Statistical analysis

Collected data was normalised by Kolmogorov-Smirnov test and then in order to analyse the physicochemical properties of soil on the herb essence in fivealtitudinal levels of Alborz Mt. (Iran), Pearson correlation coefficient method (Sharma, 1996) was employed using SPSS v.16 software.

RESULTS

A) Analysis of altitude effects on quality and quantity of the herb essence

Average function of the extracted essential oil from the altitudinal levels such as 2000, 2200, 2400, 2600, and 2800 meters were 31, 21, 35, 22, and 24 %, respectively (Fig. 1) which 2400 and 2200 m have the most and the least one. Comparing of chemical compounds of these altitudes arrangement were also 48, 43, 30, 22, and 20 elements, respectively (Fig. 2). As it is seen, the first and last altitude levels have the most and the least elements, correspondingly.

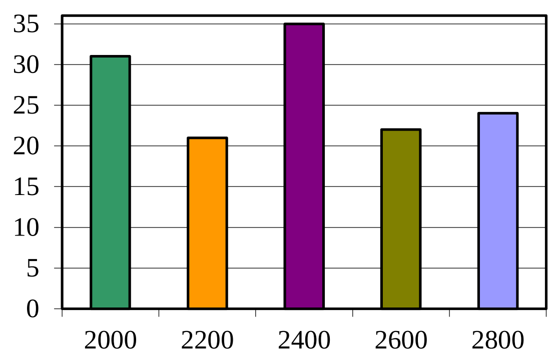

Figure1. comparing of the essence efficiency (%) of the herb essential oil in different altitudes

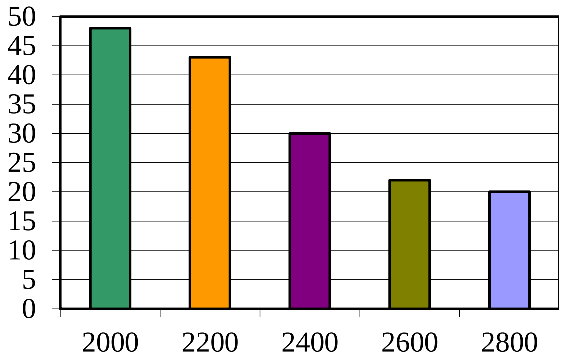

Figure2. Number of the chemical elements in the essential oil of the herb in different altitudes

The qualitative evaluation showed (Table 1) that the main elements in all altitudinal classes were six chemical compounds such as α-Pinene, β-Bourbonene, β-Elemene, Germacrene-D, Germacrene B, and t-Muurolol. The Germacrene-D element was the most in all altitude and only this composition was positively signified to the altitude. Furthermore, in the 2400 meter altitude, three components were the most elements including α-Pinene, β-Bourbonene, and β-Elemene as Germacrene B, and t-Muurolol compounds were high in the 2800 and 2600 meters, as well (Fig. 3). Except the mentioned compositions, some valuable elements with more than 2% of whole compounds were seen including Trans Caryophyllene, Bicyclogermacrene, β-elemene, Farnesene <E-B->, Phytol, Linoleicacid(Table 1).

Table 1. introducing of chemical compounds of the herb essence in different altitudinal classes

row	Compound name	altitudinal classes (m)					Retention Index	Identifiable way
		2000	2200	2800	2600	2400		
1	a-Thujene	1	1.4	-	-	0.4	930	RI , MS
2	a-Pinene	1	3.6	1	2	4	930	RI , MS
3	Sabinene	1.8	1.2	-	-	-	975	RI , MS
4	b-Pinene	2.5	1.4	-	-	-	980	RI , MS
5	Myrcene	2	1.2	-	-	-	992	RI , MS , CoI
6	Limonene	0.8	1.9	-	-	-	1029	RI , MS
7	Ocimene <E-b->	0.5	0.4	-	-	-	1049	RI , MS
8	Camphenilone	1.3	0.9	-	-	-	1083	RI , MS , CoI
9	Terpinolene	1.5	0.8	-	-	-	1091	RI , MS
10	Linalool	1.7	0.6	-	-	-	1097	RI , MS
11	Nonanal	0.4	0.6	-	0.9	-	1116	RI , MS
12	a-Cubebene	1	1.8	-	-	-	1349	RI , MS
13	a-copaene	0.9	-	-	-	4	1376	RI , MS , CoI
14	a-Ylangene	0	0.6	-	-	-	1377	RI , MS
15	b-Bourbonene	1.5	2.1	1.5	3.5	5.1	1388	RI , MS
16	b-Elemene	3	3.5	2.5	2.8	7.5	1390	RI , MS , CoI
17	b-Cubebene	0	1.3	-	-	-	1391	RI , MS , CoI
18	Trans caryophyllene	3.3	5.8	11	10.5	-	1395	RI , MS , CoI
19	a-Cedrene	1.4	1.2	-	0.8	-	1414	RI , MS , CoI
20	(E)-caryophyllene	1.5	-	-	-	1.4	1419	RI , MS
21	Ionone<(E)-a->	1.8	1.5	-	-	-	1430	RI , MS
22	g-elemene	3.2	-	2.7	3	-	1436	RI , MS , CoI
23	Aromadendrene	1.5	-	-	-	1.3	1441	RI , MS
24	Farnesene <E-B->	2.4	-	3.1	2	-	1452	RI , MS
25	a-Humulene	1.2	1	2	2.5	-	1454	RI , MS , CoI
26	Germacrene-D	30.1	35	43.3	39.5	37.2	1485	RI , MS
27	d-Cadinene	-	2.9	1.8	-	0.6	1494	RI , MS
28	Bicyclogermacrene	2	-	6	4.7	2.3	1494	RI , MS
29	g- selinene	-	1.5	-	0.9	-	1497	RI , MS , CoI
30	a-zingiberene	0.7	1	-	-	-	1498	RI , MS
31	b-bisabolene	1.4	0.8	-	-	-	1507	RI , MS
32	Calamene<trans->	1.4	1.3	-	-	-	1526	RI , MS , CoI
33	Nerolidol<(Z)->	0.6	0.9	-	-	-	1534	RI , MS
34	Germacrene B	5.2	5.5	10	6	1	1567	RI , MS
35	Spathulenol	1.9	1.2	1.1	-	1.1	1578	RI , MS , CoI
36	Caryophyllene oxid	-	0.2	1.5	1.4	0.4	1583	RI , MS , CoI
37	n-Hexadecane	-	-	-	-	1.1	1600	RI , MS , CoI
38	Tetradecanal	-	0.9	-	-	-	1615	RI , MS
39	a-cadinol	-	-	-	-	1.2	1656	RI , MS
40	t-Muurolol	3.1	3.7	2.7	4	2.3	1668	RI , MS , CoI
41	n-Heptadecane	-	-	-	-	2.1	1700	RI , MS , CoI
42	Eremophilone	0.4	0.6	-	-	-	1736	RI , MS , CoI
43	Octadecane	1.5	-	-	1.7	1.7	1800	RI , MS , CoI
44	Benzyl salicylate	1.2	0.9	-	-	-	1869	RI , MS , CoI
45	n-Nonadecane	0.7	-	-	-	0.4	1900	RI , MS , CoI
46	Farnesyl acetone <5E , 9E>	0.9	0.5	-	-	-	1917	RI , MS
47	Phytol	3.8	2.7	1	2.5	-	1942	RI , MS
48	Hexadecanoic acid	1.5	0.9	-	-	1.8	1959	RI , MS , CoI
49	Eicosane	-	-	-	1.4	1.5	2004	RI , MS
50	Dibutyhl phthalate	0.5	0.7	1.5	3	1.3	2035	RI , MS
51	Octadecanol<n->	-	0.5	-	-	-	2081	RI , MS
52	Heneicosane <n->	1.5	1.2	-	-	0.3	2100	RI , MS , CoI
53	Methyl octadecanoate	-	-	-	-	8.6	2127	RI , MS
54	Linoleic acid	2.4	-	-	3.5	2.8	2132	RI , MS
55	Docosane	-	1.2	-	-	3.7	2200	RI , MS
56	Tricosane	-	-	2	-	1.3	2300	RI , MS , CoI
57	Tetracosane	0.5	-	1	-	0.8	2400	RI , MS , CoI
58	Pentacosane	0.3	0.3	1.6	0.5	1.2	2500	RI , MS
59	Heptacosane	-	0.9	1.1	1	-	2700	RI , MS
60	Nonacosane	0.2	-	-	-	0.5	2900	RI , MS , CoI
	Number of compounds	48	43	20	22	30	-	-
	Total (%)	99	98.1	98.4	98.1	98.9	-	-
	Monoterpenes (%)	11.1	11.9	1	2	4.4	-	-
	Sesquiterpenes (%)	61.7	65.3	83.9	76.2	60.4	-	-
	Oxygenated sesquiterpenes (%)	9.8	9.3	6.3	7.9	5	-	-
	Oxygenated monoterpenes (%)	1.7	0.6	-	-	-	-	-
	Other compounds	14.7	11	7.2	12	29.1	-	-

RI: Retention Index, MS: Mass spectrum, CoI: Simultaneous Injection of standard samples

B) Analysis of chemical compounds of the herb essence

The chemical analysis of the herb showed that sesquiterpene element was the most compound on the analogy of the others and it was the highest and the least one in 2800 m and 2400 m, respectively. Interaction between the soil properties and the essence efficiency as correlation coefficient between them, were given in tables 2 and 3. As it is observed, the α-Pinene element has also reversely correlation to clay in the first altitude class and has directly correlation to phosphor in the last altitude level (table 2). In this case, the Electrical Conductivity (EC) and nitrogen have also reversely correlation to second, and third associations and has directly ration to fifth association (P<0.001). The β-Bourbonene, however, has directly correlated to the EC and nitrogen in first altitude class (P<0.05). The β-Elemene in the first, second, and third altitudinal classes has correlated to the nitrogen (positively, P<0.05) and silt (negatively, P<0.01), the phosphor (positively, P<0.05), and the potassium (negatively, P<0.05) elements (table 2).

Table 2. Correlation coefficient of three elements with soil properties in five altitudinal classes

Elements name	α-Pinene					β-Bourbonene					β-Elemene				
altitude / soil elements	2000	2200	2400	2600	2800	2000	2200	2400	2600	2800	2000	2200	2400	2600	2800
EC	-0.27^{ns}	-0.82*	0.55^{ns}	0.24^{ns}	0.72**	0.72*	0.62^{ns}	-0.41^{ns}	-0.47^{ns}	-0.41^{ns}	0.50^{ns}	-0.49^{ns}	0.39^{ns}	0.06^{ns}	0.18^{ns}
pH	-0.44^{ns}	-0.16^{ns}	0.67^{ns}	-0.33^{ns}	0.49^{ns}	-0.41^{ns}	0.27^{ns}	-0.67^{ns}	0.65^{ns}	-0.09^{ns}	0.06^{ns}	-0.27^{ns}	0.13^{ns}	-0.15^{ns}	0.59^{ns}
N	-0.33^{ns}	0.39^{ns}	-0.74*	0.04^{ns}	0.84**	0.06^{ns}	-0.24^{ns}	0.23^{ns}	0.38^{ns}	-0.18^{ns}	0.71*	-0.07^{ns}	-0.68^{ns}	0.24^{ns}	0.08^{ns}
P	-0.02^{ns}	0.41^{ns}	0.30^{ns}	0.24^{ns}	0.86**	0.34^{ns}	-0.26^{ns}	0.07^{ns}	0.14^{ns}	-0.15^{ns}	0.20^{ns}	0.79*	-0.02^{ns}	0.26^{ns}	-0.28^{ns}
K	-0.68^{ns}	0.50^{ns}	-0.69^{ns}	-0.37^{ns}	-0.6^{ns}	-0.26^{ns}	-0.40^{ns}	-0.22^{ns}	0.15^{ns}	0.10^{ns}	0.047^{ns}	0.36^{ns}	-0.79*	-0.14^{ns}	-0.29^{ns}
Lime	0.18^{ns}	-0.22^{ns}	0.24^{ns}	0.17^{ns}	0.10^{ns}	-0.05^{ns}	0.52^{ns}	-0.04^{ns}	0.17^{ns}	0.03^{ns}	-0.53^{ns}	-0.48^{ns}	0.13^{ns}	0.16^{ns}	0.51^{ns}
sand	-0.48^{ns}	-0.41^{ns}	0.08^{ns}	0.13^{ns}	0.21^{ns}	-0.28^{ns}	-0.03^{ns}	0.27^{ns}	0.10^{ns}	0.25^{ns}	0.27^{ns}	0.29^{ns}	0.56^{ns}	-0.05^{ns}	0.32^{ns}
silt	0.37^{ns}	0.09^{ns}	-0.28^{ns}	-0.46^{ns}	-0.43^{ns}	0.28^{ns}	-0.15^{ns}	-0.48^{ns}	0.62^{ns}	0.04^{ns}	-0.94**	0.14^{ns}	-0.04^{ns}	-0.22^{ns}	-0.05^{ns}
clay	-0.80*	0.16^{ns}	0.12^{ns}	-0.25^{ns}	-0.15^{ns}	-0.36^{ns}	-0.34^{ns}	0.26^{ns}	0.23^{ns}	-0.28^{ns}	0.01^{ns}	0.17^{ns}	0.54^{ns}	0.00^{ns}	-0.01^{ns}

ns: non-significant, *: P<0.05, and **: P<0.01

The element of Germacrene D in first association (2000 m) has reversely meaningful correlation to clay factor while its situation in second association (2200 m) is directly significant correlation. The element has directly meaningful relation to lime, silt, potassium, and EC in the second, third, and fourth associations, respectively (table 3). The Germacrene B element has also correlated to the EC in altitude 2600 m (P<0.05). The t-Muurolol element, however, has reversely correlated to silt (P<0.05) in first altitudinal class and positively correlated to the clay (P<0.01) in altitude 2200 m (table 3).

Table 3. Correlation coefficient of another three main elements with soil properties in five altitudinal classes

Elements name	Germacrene D					Germacrene B					t-Muurolol				
altitude / soil elements	2000	2200	2400	2600	2800	2000	2200	2400	2600	2800	2000	2200	2400	2600	2800
EC	0.15^{ns}	0.23^{ns}	0.02^{ns}	-0.22^{ns}	0.24^{ns}	-0.05^{ns}	-0.64^{ns}	-0.27^{ns}	0.75*	-0.11^{ns}	0.31^{ns}	-0.23^{ns}	-0.35^{ns}	-0.39^{ns}	0.15^{ns}
pH	-0.65^{ns}	-0.14^{ns}	0.45^{ns}	-0.40^{ns}	0.47^{ns}	0.05^{ns}	-0.2^{ns}	-0.57^{ns}	-0.04^{ns}	-0.24^{ns}	0.39^{ns}	-0.22^{ns}	-0.65^{ns}	0.38^{ns}	0.59^{ns}
N	-0.00^{ns}	-0.03^{ns}	-0.2^{ns}	0.06^{ns}	-0.03^{ns}	0.66^{ns}	0.59^{ns}	-0.13^{ns}	0.09^{ns}	0.22^{ns}	0.60^{ns}	0.07^{ns}	-0.04^{ns}	0.11^{ns}	-0.38^{ns}
P	-0.19^{ns}	-0.08^{ns}	-0.37^{ns}	-0.34^{ns}	-0.04^{ns}	-0.09^{ns}	0.20^{ns}	-0.15^{ns}	0.24^{ns}	-0.30^{ns}	-0.03^{ns}	-0.22^{ns}	-0.14^{ns}	-0.35^{ns}	-0.35^{ns}
K	-0.53^{ns}	0.01^{ns}	0.06^{ns}	0.87**	-0.17^{ns}	0.07^{ns}	0.39^{ns}	-0.62^{ns}	-0.38^{ns}	0.34^{ns}	0.18^{ns}	-0.05^{ns}	-0.25^{ns}	0. 68^{ns}	-0.26^{ns}
Lime	0.13^{ns}	0.86**	-0.17^{ns}	-0.21^{ns}	0.06^{ns}	-0.40^{ns}	0.02^{ns}	-0.06^{ns}	-0.11^{ns}	0.23^{ns}	-0.51^{ns}	0.32^{ns}	0.27^{ns}	-0.35^{ns}	0.31^{ns}
sand	-0.49^{ns}	-0.18^{ns}	-0.15^{ns}	-0.69^{ns}	-0.45^{ns}	-0.14^{ns}	-0.16^{ns}	0.60^{ns}	0.35^{ns}	0.33^{ns}	0.17^{ns}	-0.18^{ns}	0.66^{ns}	-0.46^{ns}	-0.26^{ns}
silt	0.12^{ns}	0.13^{ns}	0.79*	0.00^{ns}	-0.40^{ns}	-0.51^{ns}	-0.06^{ns}	-0.24^{ns}	-0.13^{ns}	0.09^{ns}	-0.70*	-0.09^{ns}	0.24^{ns}	0.52^{ns}	-0.18^{ns}
clay	-0.74*	0.51^{ns}	-0.08^{ns}	0.38^{ns}	0.36^{ns}	0.27^{ns}	0.44^{ns}	0.60^{ns}	-0.05^{ns}	0.34^{ns}	-0.17^{ns}	0.92**	0.70^{ns}	0.31^{ns}	0.07^{ns}

ns: non-significant, *: P<0.05, and **: P<0.01

As it is seen in figure 3, the Germacrene-D element was the highest rates in all altitudinal classes, especially in 2800 meter. However, the α-Pinene and t-Muurolol compounds were the least rates in 2000 and 2800 meters, especially α-Pinene was the least one in 2000 and 2800 meters, and t-Muurolol was also the least one in the 2400 m.

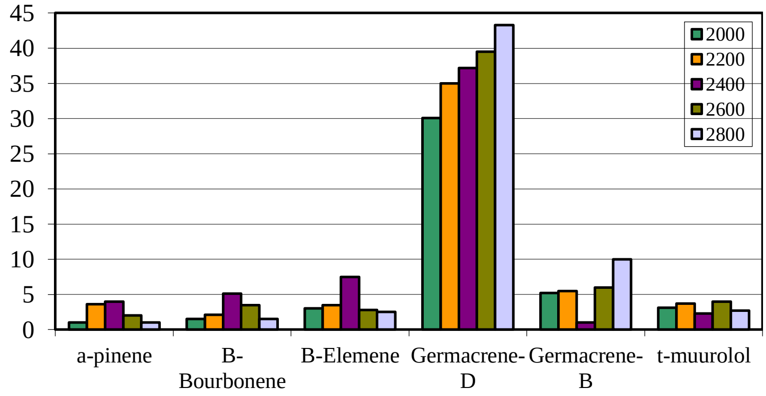

Figure3. Quantitative comparison of the main chemical elements of the herb essence in five altitudinal classes

From whole chemical compounds, monoterpene, sesquiterpene, oxygenated sesquiterpene, and oxygenated monoterpene were the most valuable compositions as well as the a-pinene, B-elemene, Germacrene-B, Germacrene-D and t-muurolol in different industries. The sesquiterpene element was the most abundant compounds in all altitudinal classes, especially in 2800 m, while the oxygenated monoterpene was the least one and it was stayed away from 2400, 2600, and 2800 meters (Fig. 4).

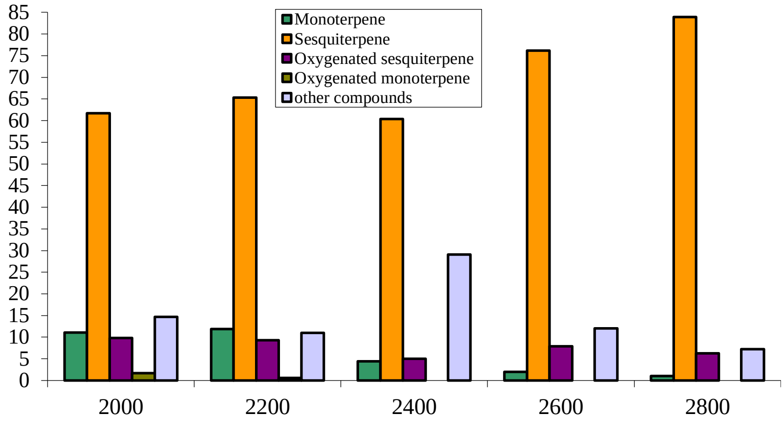

Figure4. Comparing of structure of the chemical compounds in the herb essence from five altitudinal classes

DISCUSSION AND CONCLUSION

The ecological parameters of habitat, such as altitude and physicochemical properties of soil, not only can impact on vegetative growth and form of plants (Omidbaygi, 2005), but they can also change the quality and quantity of the essential oil's compounds in aromatic and medicinal plants (Weiss and Edwards, 1980).Consequently it can diverse the phytochemical compositions and form new chemo-type in plants (Verport et al., 2000) as it has been proved by different researches' report (e.g. Behtrai et al., 2012; Singh et al., 2013) and current results. As physical and chemical analysis sections have showed, the average rates of essence efficiency have changed along with increasing of altitudinal levels. Some microclimate condition will be created in different altitudinal levels and the position of sampling is effective to the rate of essence efficiency. For instance, south aspect of upland receives the most sunshine and hence it can make dense the essence of a plant. In this case, some altitudinal levels conducted this way. The amount of chemical compounds has also decreased along with increasing of the altitudinal levels. As it is known, in upland, we have less humidity and more sunshine rays, especially ultra-violate ray which is so powerful to make dense the physiologic fluids of plants as the essential chemical composition of Phlomiscancellata has followed this version. A number of researchers have reported that increasing of altitudinal levels can decrease the amount of the chemical compounds of essence that they include Morteza-Semnani et al. (2006), Haider et al. (2009), Yavari et al. (2010), Akhlaghi and MotevalizadehKakh'ky (2010), Behtari et al. (2012), and Deylamsalehi et al. (2013). And they have also emphasised on the Germacrene-D element as the most compounds in the essential oils as current study proves their reports. This element has reversely correlated to the clay in 2000 m. The soil texture of this altitudinal level was mostly formed by the clay. The Germacrene-D element in the second, fourth, and third classes was also correlated to the absorbable potassium and the silt, respectively, as the soil texture in these altitudes were changed to loam and silty-loam.

Five main compounds, including α-pinene, β-bourbonene, β-elemne, Germacrene-B, and t-muurolo were found in the essential oil of Phlomis cancellata. The α-pinene element has correlated to the clay (reversely) and the phosphor (directly) in the last altitudinal level. Moreover, the EC and nitrogen cases had also correlated to the α-pinene in second and third (reversely) and fifth (directly) altitudes. Results of Azarnivand et al. (2010) have also showed same consequences as they have reported that the essential oil of Achillea millefolium L. subsp. millefolium is affected by the soil nitrogen. The β-elemne element has reversely correlated to the silt and potassium in three altitudinal classes and has positively related to the EC, nitrogen and phosphor in other classes. Yavari et al. (2010) have pointed out that from all edaphic parameters; the potassium is the case which has impacted on the chemical compounds of Thymus migricus species. The t-muurolol element with silt in the first class (reversely) and the lime and clay in the second class has highly correlated. The β-bourbonene, however, will be changed by the EC and nitrogen rate in the first altitude, as well. In the end, Germacrene-B has directly affected by the potassium and EC in the fourth altitudinal class.

Regarding the results, if goal of extracting of the essential oil from Phlomis cancellata is the highest essence efficiency, the altitude 2400 m is the best point to collect the herb, but the most desirable quality of the essential oil of the herb refers to first altitude class. Moreover, the sesquiterpenes elements have the highest rate in all altitudinal levels and other compounds were the least ones. The current results also showed that the ecological parameters of habitat location, e.g. altitude and physiochemical properties of soil factors, had the most roles to change the quality and quantity of the essential oil in Phlomis cancellata in the area. Another great obtained result is that regarding to mentioned outcomes, precise collecting place of medicinal plants can reduce the pressure of natural environments of the species and can also give the highest percentages of essential oils from the medicinal plants.

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