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THE CHEMICAL COMPOSITION OF ESSENTIAL OIL OF ARTEMISIA SIEBERI BY GC/MC: FOCUSED ON BIODIVERSITY

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Abstract

TEIMOORY, H., G. AMIN, B. RIAZI, M. KARAMI, M. Z. H. ABADI and M. REZAEI, 2014. The chemical composition of essential oil of *Artemisia sieberi* by GC/MC: focused on biodiversity. *Bulg. J. Agric. Sci*., 20: 1334-1339

This research has comparatively studied the effects of changes in height, ecological and environmental factors on the type and amount of the chemical compounds of *Artemisia sieberi Besser* in three different ecological niches of Kashan: near Marjanab Salt Lake (high saltiness), near the sandy hills of Marjanab Desert (sandy), and mountain hills of Ghamsar road - Kashan – (gritty). Essence extraction was done by distillation of water. Decomposition and identification of the consisting elements of the essence was carried out through GC, GC/Mass tools. High amount of precipitations, height above the sea level, concentration of the plant, concentration of the accompanying species, low saltiness of the soil and also high amount of the main elements (microelements) in Ghamsar region is indicative of a better ecological condition which resulted in the high concentration of the total extracted essence, the percentage of the compounds of the essence and the strong scent of the extracted essence in this region. The region near the gritty hills which had the weakest soil form the point of view of having soil nutrients, lower average concentration of *Artemisia sieberi Besser* and average concentration of the accompanying species had also the lowest total amount of extracted essence, lowest percentage of the components of the essence and the lowest scent. The highest compound belonged to the compound of Borneol was witnessed in the mountain regions of Ghamsar.

Key words: *Artemisia siberi*, Ecology, Essence change, Biodiversity, Borneol

Introduction

Distribution and habitat of plants is influenced by environmental conditions, as it is the chemical combinations found in the plant. Artemisia sieberi is an herb perfectly known and used in Iran and the world traditional medicine. The plant is widely distributed and found in arid and semi-arid regions at height range 950-3000 m from sea level (Sadeghy, 1992). Artemisia sieberi is a perennial bush herb, which form considerable colonies in Iran and many other parts of the world. The perfect habitat of the plant is determined by factors such as land surface, climate, soil, grazers, and human activities (Mozafarian, 1996). The present study is an attempt to determine and introduce the topographic and earth science factors, which dictate distribution and location of the species. It also deals with the combination of the extract of the plant (quantitative and qualitative) collected from the region in vicinity of Maranjab Desert Salt Lake, Maranjab Desert sand dune, and mountainous hills of Ghamsar-Kashan RD.

Artemisia is considered as a good feed for many kind of animal; which, according to Hunter et al. (Hunter and Grant, 1971) and Letch et al. (Welch et al., 1981), it is due to monoterpenoid and other combinations found in the plant. A study on A.herba-alba species collected from Sinai and Israel des-

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ert showed that Siniol, Thujone, Borneol, and Pinene were the main groups of combinations found in the plant; so that their concentration was a factor of their habitat (Feuerstein et al., 1988). The Pasture Technical Office (1991) started to collect and code plants in the pasture and commented on the capacity of Artemisia and *A. sieberi* in particular. Rabie et al. (2007) confirmed the title *A. sieberi* after phytochemical studies on 34 species of Artemisia in Iran and Artemisia herba-alba from Spain and *A. siberi* from Palestine (Rabie et al., 2007). Mirza et al. (1998) extracted and studied essence of species of Artemisia genus and *A. sieberi* in particular from quantitative and qualitative viewpoint(Mirza et al., 1998). Mozafarian et al (Mozafarian, 1988). Determined are the habitats of the plant in Iran. Distinguishing feature of this study is its concentration on differences between the three habitats concerning climate factors and their effects on Artemisia bio-diversity and changes in the combinations of its essence. Some of the goals of the present study are to find and identify the topographic and earth science factors influencing distribution and place of the species. In addition, variations in the combination of the essence in the regions under study and the relation of between the changes in the essence and other factors are studied here.

Materials and Methods

Regions

A-Regions in vicinity (<20 km on average) of Maranjab Desert Salt Lake (high saltiness)

The habitat is located in the south of the salt lake and northeast of Aran, Bidgol, and Kashan cities and adjacent to the historical Karvan Sara of Marnajab. To northeast is the national park of the desert. The salt lake located in the north is called by the natives "Darya" (the sea), which is one of the salt mines in Iran. The best access to the lake passes from Kashan to Aran and then to Bidgol and then a GPS and a local guide are needed. The region under study is located at 34°, 29', 73" north latitude and 51, 49', 65" east longitude. Average height from sea level is 788m and the area is 1044ha in extent (Figure 1).

B-Regions in vicinity (50m on average) of sand dune of Maranjab Desert

The spot under study is located close to sand dune in lower part of the region and adjacent to the salt lake (region A). It is at 34°, 18', 13" north latitude and 51°, 49', 59" east longitude with average height of 800m from sea level and an area of 1608ha (Figure 1).

C-Mountainous hills regions along with Ghamsar-Kashan RD (sandy)

The habitat, a mountainous region, is located between Kashan and Ghamsar. It stretches along the Ghamsar-Kashan RD, and the number of bushes increases as the height from sea level increases. In addition to *A. sieberi*, other species of the genus are abundant. The region is located at 33°, 45', 50" north latitude, and 51°, 28', 53" east longitude. The height from sea level is about 1651m and the area is 6042 ha in extent (Figure 2).

Sampling

Sampling was carried out through random systematic sampling in each region and using quadrate of definite number; 20 samples of different sizes were collected randomly and the obtained herbarium specimens were studied in Faculty of Pharmacology, Tehran University of Medical Sciences. The results showed that the specimens were from *Artemisia seiberi* Basser subsp.

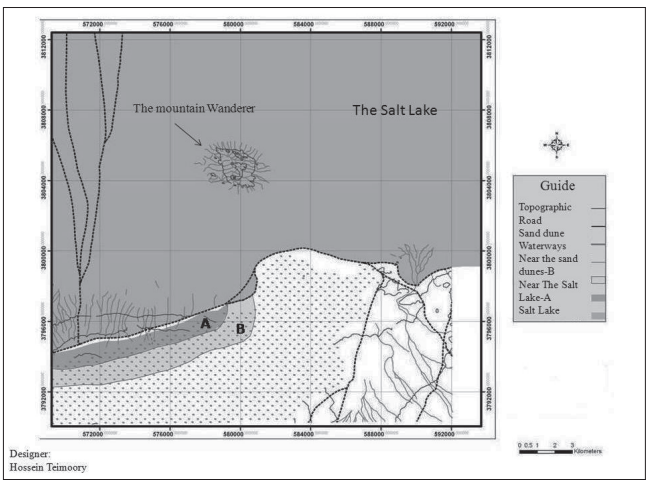

Fig. 1. Distribution of *A. sieberi* in Maranjab Desert habitat; regions A and B

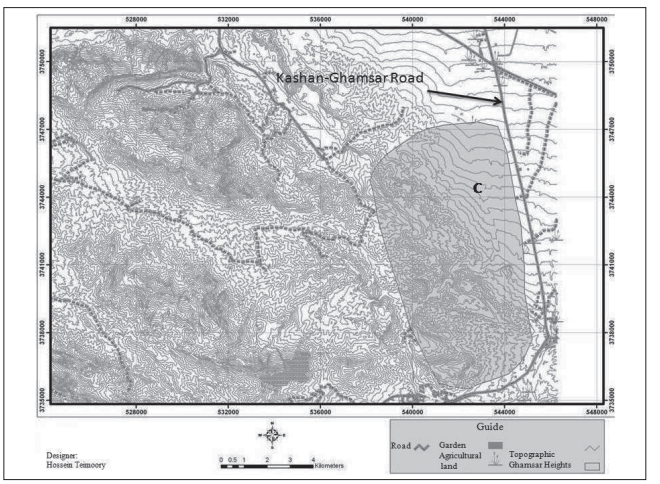

Fig. 2. Distribution of the *A. seiberi* species in the region C

Ecology

Earth study parameter measurement (i.e. height, crown coverage, density, abundance, and presence.

The soil samples were collected at the depth of 30cm (5 samples from each region) from the quadrates(Salardini, 1985). The highest stem of each bush was used as the measure of height of the plant. Crown coverage was obtained as the average of larger diameter (W) and small diameter (w) of the plant crown. Afterward, the area was calculated from area formula for circle. *A. sieberi* bushes usually had symmetrical and ordered crown form in shape of circle and oval.

Crown coverage area = $[(½ w + ½ W)]^2 *3.14$

Size and shape of the quadrates assumed in the regions were obtained using the two following methods:

- Minimum area, which is used to show features of the population, was not the same in different regions. Thus, minimum area (m^2) for different types of the plant in the three regions was obtained using an experimental table (see the references). The regions, by definition, are classified as barren region. The obtained figures for the area were between 2-10 m^2 (Asry, 2011).
- Using quadrate sampling program though Vigret in Ecological Methodology software, five square quadrates (2×2) with minimum relative variance in relative cost were used as the optimum frame in the three regions under study(Krebs, 1999) (Table 1).

Extraction of essence: based on flowering phenology stages, blossoming of the bushes was observed in 11 Oct. 2009 and 20 specimens were randomly collected at the same date from each region randomly within the quadrates. The essence was extracted from these specimens.

Combination of the essence: the essence combination was studied in GC/Mass and GC devices in herb research department of Jahad Daneshgahi. For all the spectrums given for GC/Mass Quats index was obtained for all the spectrums based on the normal patterns and spectrum inhibition index. Comparing the results with the data from Adams (Adams, 2001)and direct observation of the spectrums from the three regions using Wiley 275 databank, the combination of the essences were identified after confirming the objects. It is noticeable that the combinations common among the essences from the three regions were taken into consideration and other combinations were left aside. The GC devise was a Youngline Acm600 and GC/Mass was Agilent 6890 and Agilent 5973.

Results and Discussion

A-Regions in vicinity (<20km on average) of Maranjab Desert Salt Lake (high saltiness-Ecology: soil of the region A was of average loamy sand comprised of sand (58%), clay (17%), and Silt (25%). Saltiness was 3.67 ds/m, PH equal with 7.7, with 8.5% lime (in case of neutralized materials), and 420 meq/gr of plaster. Organic content (organic carbon) was 0.13% (Table 2). Density (number of plant in unit area) was 0.95 m^2 and average crown coverage of the samples was 0.44 m^2. In addition, abundance of A.sieberi was 3.7 plants (number of plants divided by number of plots), presence of the plant was 100%, and total essence yield was 2.409 ml/100g.

B-Regions in vicinity (50m on average) of sand dune of Maranjab Desert

Ecology: soil of the region B was of average loamy sand comprised of sand (57%), clay (17%), and Silt (26%). Saltiness (electric conductivity) was 2.77 ds/m, pH in saturated mud equal with 8, with 16.5% lime. Organic content (or-

Table 1
Size frame based on Vigret method

Frame size, m^2	Variance	Relative variance	Cost	Relative cost	Relative variance× Relative cost
0.5×0.5	53	13.94	6.7	1	13.94
1×1	21	5.52	12	1.79	9.89
1.5×1.5	15	3.94	13.2	1.97	7.77
2×2	3.8	1	20	2.98	2.98

Table 2
Nutrients in the sample from region A (micro/macro elements)

Total N, %	Absorbable phosphor, m.p.p(ava)P	Absorbable potassium, m.p.p(ava)K	Ferrite, gk/g	Zinc, gk/g	Copper, gk/g	Manganese, gk/g	Sodium, gk/g	Chlorine, gk/g
0.016	3.4	120	3.5	0.69	0.87	3.4	29.5	27

ganic carbon) was 0.13% (Table 3). Density was 0.95 m^2 and average crown coverage of the samples was 0.59 m^2. In addition, abundance of *A. sieberi* was 3.6 plants, presence of the plant was 100%, and total essence yield was 1.769 ml/100gr.

C-Mountainous hills regions along with Ghamsar-Kashan RD (sandy

Ecology: soil of the region A was of average loamy sand comprised of sand (60%), clay (16%), and Silt (24%). Saltiness was 1.19 ds/m, PH equal with 7.8, with 15.5% lime, and rate of plaster was zero. Organic content (organic carbon was 0.25%) (Table 4). Density area was 1.8 m^2 and average crown coverage of the samples was 0.41m2. In addition, abundance of *A. sieberi* was 7.2 plants, presence of the plant was 100%, and total essence yield was 2.768 ml/100g.

Surveys of *A. sieberi* showed that the plant is the ruling species at height 788 m to 1650 m. There is a negative relation between height from sea level and growth of the bush (Hunter and Grant, 1971), our results are consistent with the fact. So that, the bushes at heights of Ghamsar reduces to 25.6cm on average (Table 5). Given that abundance or frequency of A.sieberi was high at the three regions under study, one may conclude that tolerance of the species is high and it can be found under different microclimatic conditions. Thus, the plant can be placed at fifth level of plant population coverage (81-100%) at the sampling sites, which are considered as arid and semi-arid zones with homogenous population (Moghadam, 2008). Concerning crown coverage area, maximum coverage was found for regions B and C; while regarding density of Artemisia region C and region B had maximum and minimum density in square meter respectively. In addition, relative coverage of *A. sieberi* in region C was highest comparing with other regions. Taking into consideration the average number of other plant species, region B and C had the lowest and highest density; while average density of total number of neighbor plant species was highest in region B and lowest in region C. Classifying, bushes shorter than 50 cm as small specimen (Moghadam, 2008), *A. sieberi* is considered as a small species in the three regions under study.

Table 3
Nutrients in the sample from region B (micro/macro elements)

Total N, %	Absorbable phosphor, m.p.p(ava)P	Absorbable potassium, m.p.p(ava)K	Ferrite, gk/g	Zinc, gk/g	Copper, gk/g	Manganese, gk/g	Sodium, gk/g	Chlorine, gk/g
0.013	4.6	210	2.7	0.66	0.84	2	20.5	22

Table 4
Nutrients in the sample from region A (micro/macro) elements

Total N, %	Absorbable phosphor, m.p.p(ava)P	Absorbable potassium, m.p.p(ava)K	Ferrite, gk/g	Zinc, gk/gm	Copper, gk/g	Manganese, gk/g	Sodium, gk/g	Chlorine, gk/g
0.028	4.6	340	2.2	0.59	0.78	2.5	8	7

Table 5
Number of A. sieberi based on region

No	Region A, cm			Regions B			Regions C		
	Number of specimen	Small diameter	Large diameter	Number of specimen	Small diameter	Large diameter	Number of specimen	Small diameter	Large diameter
1	5	30	50	30	60	6	15	50	8
2	4	38	56	30	30	1	45	50	15
3	6	20	40	20	25	1	30	30	3
4	2	30	34	60	46	7	27	40	4
5	2	40	60	50	65	3	30	43	6
SD[1]	-	-	1078	-	-	2.79	-	-	4.76
RD[2]	-	-	46.84%	-	-	77.5%	-	-	66.11%

1-Std. deviation, 2-Relative distribution

Highest average height of the samples was observed in regions A and; the environment and season were suitable for the plant.

Comparative survey of *A. sieberi's* essence combinations based on bio-factors in the three regions

The results of photo-chemical analysis showed that *A. seiberi's* yield of essence and its combination, as a function of ecological factors, floristic factors, were different in the three regions. Sadeghi (Sadeghy, 1992) in a thesis titled "nutrition value survey based on few chemical combinations among identified species of Artemisia in pastures of Iran" concluded that essence yield is subject to environmental factors. Essence and its combinations are of the internal factors that dictate abundance of species of the genus. Mirhaji (Mirhaji, 2000), in a thesis, conducted an ecological comparison of Artemisia species in Semnan Province and pointed out high essence yield of *A. seiberi* in the regions under study and concluded that environmental factors such as height, temperature, moisture are effective on the yield and combination of the essence.

These results are consistent with the results in this work (Table 6). Results of this work showed that specific combination of essence from regions A and C were Alph Pinene, camphene, Verbenene, Beta Thujone, and Borneol; this is probably due to high rate of nutrient elements (micro-element) and organic carbon in the region. Arbabi Nazarlou (Arbabi Nazarloo, 2004) concluded in a study that there is a direct relation between increase in content of nitrogen in soil and Thujone, while this relation in case of Pinene is vice versa; increase in nitrogen content is commonly observed in industrial regions. This study also revealed that rate of Alph-Thujone in regions C and A was considerably higher than that of region B, while Alpha-Pinene was considerably lower in the C and A regions. Taking into account the descending rate of saltiness from the Salt Lake to sand dunes, and heights of Ghamsar while simultaneously the yield of essence and rate of its content ascend, one may conclude that rate of saltiness is factors in this change. However, despite laboratory condition, there are more than one factor in play in the nature, including a set of environmental and physiological factors and specific condition of the plant(Islami, 2010). Thus, factors such as saltiness, rate of plaster, phosphor, potassium, precipitation, height from sea level are effective on the changes of *A. seiberi's* total essence yield and its combination in the three regions under study (Teimoory, 2010).

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Table 6
Essence combination based on region

No	Elements	GC, % Regions A	KI(RRI)	GC, % Region B	KI(RRI)	GC, % Regions C	KI(RRI)
1	Alpha – Pinene	0.361	924	0.165	942	0.36	946
2	Camphene	2.411	941	1.511	947	2.47	976
3	Verbenene	6.381	970	1.25	969	6.27	988
4	Para – Cymene	0.916	1038	2.083	1044	0.56	1054
5	1,8-Cineole	0.734	1051	0.085	1055	0.15	1057
6	Gama – Terpinene	0.127	1128	7.114	1061	0.46	1070
7	Alpha – Thujone	13.459	1131	3.092	1132	13.3	1154
8	Beta – Thujone	7.327	1165	4.611	1149	6.95	1173
9	Camphore	12.333	1171	13.863	1167	12.2	1188
10	Borneol	40.813	1182	19.326	1172	41.9	1190
11	Chrysanthenyl acetate	2.834	1248	2.761	1225	2.86	1252
	Total	87.69%		55.86%		87.48%	

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