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ORIGINAL ARTICLE

The Effects of Altitude on Productivity and Formative Components of Essential Oils of *Artemisia absinthium* L. (Iran)

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ABSTRACT
Productivity and growth of plants in terrestrial ecosystems and natural habitats influence by different factors such as the altitude from sea level. Common wormwood (Artemisia absinthium L.) species belongs to the Asteraceae (Compositae) family. Vegetative parts and floriferous twig of the species is used to treat the digestive and neural system disorders, and applying in food and chemical industries. The species has anti-bacterial nature. The aim of current research is surveying of the altitude effects on productivity and formative components of essential oils of Artemisia absinthium. In this research, aerial collected parts in flowering period from three altitude points was shrivelled in vitro thermal position, and extracting of essence then was done by Hydrodistilation. The compositions of the essential were identified and analyzed using GC and GC/MS and by measuring the Retention Index and Mass spectrums. Data set was analysed by one way ANOVA method in SPSS v.17 software. It was also used Duncan method to grouping of variables. Achievement results showed that the essential efficiency for altitudes 700-800 (1), 1100-1200 (2), and 1900-2000 (3) level is respectively 0.96±0.007%, 0.91±0.007%, and 0.94±0.007% so that the highest percentage is for first level and the least one is for second level. Generally, 54 components were found in three altitude levels that elements of Sabinene, beta-Pinene, α-Thujone, Germacrene D, and 1-2'-methyl-1'-propenyl) 7-[2'-propynyl) tricycle [3.1.0.0 (2,6)] hexane were the most compositions in the species. It was also found out that the best situation of growing the species and obtaining the desirable productivity in essence was first altitude level. Arising of quality and quantity in the essential oil at this altitude level showed that the environmental condition effects on the essence in this level apropos of the others.
Keywords: Artemisia absinthium L., Essential efficiency, Sea level, Chemical components, Iran

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INTRODUCTION

Antiquity of knowing of medicinal herbs attributes is out of historic mind. One of the important reasons of this archaism is inveterate beliefs of people in different areas about using of medicinal plants [23]. In recent years, it has been noted to herbal medicines and their origins as medicinal plants. It is because of lateral harms of chemical drugs and consequently trending of the man to using of natural products in order to keep their health [17]. The medicinal plants of rangelands on the analogy of silvical areas have less efficiency in area unit, but regarding to their diversity, these species are swept in vast areas in Iran's rangelands. The aromatic medicinal herbs have found special positions to plant at global increasing approach [13] that they can be applied in treatment and medicinal, food industries, and ornamental and therapeutic objects especially the economy without recumbence upon petroleum as using accurate programming [7]. Nowadays, estimating of effective matters of medicinal herbs is so important considering to emphasising of economic and remedial aspects of medicinal plants in global outlook of phytotherapy [5]. Although growth and development, and quality and quantity of the effective matters in medicinal herbs are conducted by genetic processes, it influenced by environmental factors including light, temperature, sea level, and irrigation [21]. As these factors, regarding plant dispersion and geography, are caused to change the growing of medicinal plants and their quality and quantity of effective matters [21]. For the matter of the environmental effects on growth and effective matters of medicinal plants, it is important to manage the medicinal herbs field to obtain the optimal primary and

secondary metabolites in plants. Hence, the maximum production can be attained by selecting of congruous plants and desirable environmental condition [12].
The *Asteraceae* family is of the important bacciferous families in the entire world with more than 20000 species which mostly disperse the Mediterranean and arctic areas.*Artemisia* genus has 34 species as annual and perennial herbs which two species are endemic of Iran and grow weedy form in the country [30]. The species of *Artemisia absinthium* L. is from *Asteraceae* family with 40-60 cm, innumerous stems, perpendicular and be terminated to length and foliaceous inflorescence. Geographical dispersion of *Artemisia absinthium* L. in Iran is limited to Azerbaijan, Golestan, Mazandaran, Khorassan, and Yazd provinces. The species spreads the other places such as India, Russia, and Europe. The most dispersion of the species is around 1300-2100 m a.s.l. with semi dry cool to semi humid climate and light soil texture [30]. This species, as traditional usages in medicine, performances in many fields such as increaser of choler latex, reinforcer of immunity system of body, antitussive, effect to asthma, stimulator of the food digestion, nutrition digestion, anti-rheumatism, reducer of edema and inflammation, prophylactic factor to influenza, and fever-cutter, and its essence has antibacterial role as well. Rabiei et al. (2003) have studied on essential oil of four species of *Artemisia* in north of Iran and have pointed out that the main component of them is monoterpenoid and its main monoterpene is called alpha-Phellandrene. Azad'bakht et al. (2004) in their research on essential components of *Artemisia aucheri* in Mazandaran found out that sesquiterpene hydrocarbons were the basic group of composition. Surveying and analysing of the essence of two species from *Artemisia* by Semnani et al. (2005) have shown that the fundamental components of the essence in *Artemisia absinthium* are beta-Thujone, p-Cymene, and beta-Pinene and consequently it is Camphor, 1,8-Cineole, and Borneol for *Artemisiascoparia*. It has been researched on some species of Thymus genera that their essential quality and quantity have been influenced by environmental factors such as altitude, soil diversity, weather, organic matters' rate, calcium and texture of soil [7]. The studies of Ghavam'Arabian [10] and Azarnivand et al [4] about ecological impaction on the essential quality and quantity of *Achillea millefolium* have shown that the effective matter percentage in the different altitude was various. While having the highest amounts of the effective matter from each species can represent basic role to providing of ornamental, medicinal, and industrial matters, determination of optimal circumstance to plant and product the species is memorable. Hence, this research endeavors to determine the altitude effects on rate and productivity of the essential oil in *Artemisia absinthium* L. species.

MATERIALS AND METHODS

A. Collection of herbs and extraction of essential

In order to determining of the best altitude in highly production of the essential oil in *Artemisia absinthium*, aerial parts of the species were collected in Savadkooh region (Iran) form from some altitudes such as 700-800 m (1), 1100-1200 m [2], and 1900-2000 m [3]. Sampling was done in June 2012 with two repetition and collected plants then were dried in vitro condition. 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 [10]. 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 Sulfate, and kept in the fridge at 4°C until it was injected into Gas Chromatography (GC) [2].

B. 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 [1].

C. Data analysis

In order to compare the percentage of the essential composition of *Artemisia absinthium* in three sites of Alborz Mt. (Iran), one-way ANOVA method was employed using SPSS v.17 software. Duncan test was administered to compare the means of these sites.

RESULTS

Percentage and chemical composition of the essential oil

Comparing of the essential components percentage in *Artemisia absinthium* from three altitudes showed that the highest percentages depend to first level (700-800 m) and the least one refers to 1900-2000 m (third class). In first level altitude, 31 components (52.33 % of essential content) in the essential oil were identified while it were 30 (89.83 %) and 35 (79.47 %) components for second (1100-1200 m) and third

(1900-2000 m) altitudes levels, respectively. Generically, 54 components were identified for three altitudes levels that the main components were Sabinene, beta-Pinene, α-Thujone, Germacrene D, 1-2'-methyl-1'-propenyl)7-[2'-propenyl) tricycle [3.1.0.0 (2,6)] hexane (Table 1).

Table 1. chemical composition of the essential oil in *Artemisia absinthium* L. from three altitudes

Row	Component name	Composition percentage			Retention Index
		700-800	1100-1200	1900-2000	
1	α -Pinene	0.804±0.003	-	0.54±0.003	912
2	Sabinene	9.45±0.002	9.37±0.02	8.33±0.003	960
3	β- pinene	2.03±0.03	0.84±0.02	1.01±0.002	970
4	β-Myrcene	0.27±0.004	-	0.19±0.002	979
5	α- terpinene	0.22±0.004	-	0.18±0.003	1008
6	β-phellandrene	0.38±0.04	0.42±0.002	0.35±0.003	1021
7	1,8- cineole	0.47±0.004	-	0.24±0.002	1023
8	γ- terpinene	0.46±0.002	0.29±0.001	0.28±0.002	1046
9	α-Thujone	0.26±0.002	30.7±0.04	0.14±0.04	1126
10	β-Thujone	1.67±0.003	-	22.28±0.04	1131
11	β-Terpinene	-	6.27±0.04	-	1131
12	Cis-Sabinol	0.23±0.003	0.61±0.02	-	1139
13	Isothujol	-	2.44±0.04	2.40±0.03	1148
14	4-Terpineol	-	-	0.49±0.002	1166
15	Anothole	-	1.58±0.004	-	1286
16	Naphthalene, 1,2- dihydro 1,1,6- trimethyl	-	0.88±0.002	-	1350
17	α-Copaene	0.35±0.002	0.23±0.02	0.38±0.004	1374
18	β-Bourbonene	0.502±0.001	0.55±0.003	-	1382
19	β -copanene	-	-	0.68±0.002	1382
20	α-cedrene	0.12±0.002	0.128±0.001	0.15±0.002	1409
21	β-Caryophyllene	-	0.27±0.02	-	1418
22	α-Amorphene	0.26±0.02	0.27±0.002	0.31±0.002	1421
23	δ-Selinene	-	0.367±0.002	0.25±0.05	1443
24	Germacrene D	3.05±0.003	2.42±0.002	0.35±0.001	1482
25	β-Selinene	0.58±0.002	1.54±0.002	0.43±0.002	1486
26	Bicyclogermacrene	0.14±0.002	0.303±0.002	-	1494
27	Naphthalene,1,2- dihydro- 4,5,7- trimtyhl	-	1.41±0.002	-	1514
28	Naphthalene	5.57±0.04	-	0.38±0.001	1518
29	1-Naphthalenol, 1,2,3,4-tetrahydro- 2,5,8-trimethyl	-	7.64±0.002	-	1518
30	Naphthol, 2,5,8- trimethyl	-	-	8.65±0.002	1518
31	δ-Cadinene	-	0.39±0.002	0.68±0.002	1522
32	Calacorene	-	-	0.34±0.002	1525
33	Cis-α-bisabolene	-	0.13±0.003	-	1540
34	Butanoic acid, 3,7- dimethyl- 2,6-octadienylester	-	0.67±0.001	-	1580
35	Geranylisovalerate	-	-	2.01±0.02	1580
36	γ-Cadinene	0.19±0.004	-	-	1605
37	2,2,3-trimethylnaphthalen-1(2H)-one	6.29±0.001	6.35±0.003	-	1624
38	Benzene, (2-ethyl-4-methyl-1,3-pentadienyl)	7.18±0.002	-	-	1637
39	1-2'- methyl- 1'- propenyl) 7- [2'- propynyl) tricycle [3.1.0.0 (2,6)]hexane	5.74±0.002	5.34±0.003	4.46±0.002	1637
40	α-Cadinol	0.837±0.002	0.75±0.002	-	1644
41	Cadinene	0.211±0.002	-	-	1644
42	1H-Indene, 3-ethyl-1-(1-methylethyl)	-	-	12.20±0.03	1667
43	Benzene, (4,5,5-trimethyl-1,3-cyclopentadien-1-yl)	1.18±0.03	-	0.48±0.004	1690
44	β -Costal	-	-	0.22±0.001	1695
45	Camazulene	1.16±0.002	-	0.98±0.001	1734
46	Valerenol	0.294±0.003	0.54±0.002	0.68±0.003	1767
47	Azulene	0.27±0.002	1.86±0.03	-	1801
48	Pyrethrone	0.28±0.002	-	-	1889
49	Nonadecane	-	-	0.32±0.02	1900
50	Zingiberene	-	-	0.28±0.002	1950
51	2- bromethanenitrile	1.907±0.002	-	-	-
52	α- curcumen	-	5.29±0.003	0.13±0.001	2012
53	Heneicosane	-	-	0.25±0.02	2100
54	Thymene	-	-	8.43±0.002	-
Total percentage of components		**52.33**	**89.83**	**79.47**	**-**

The essential efficiency

The average of the essential efficiency in *Artemisia absinthium* L. from three altitudes, including 700-800, 1100-1200, and 1900-2000 meters were respectively 0.96±0.007%, 0.91±0.007%, and 0.94±0.007% that the highest one was for first level (700-800 m) and the least one was for second one (1100-1200 m) (Table 2, Fig. 1). The altitude effects on the essential efficiency has been signified at P<0.05 (Table 2) and the Duncan test has also grouped them into the maximum (first level) and the least (second level) essential efficiency (Table 3).

Table 2. ANOVA result for the three altitude effects on common compositions percentages of essential oil in *Artemisia absinthium* L.

Component as variable reference	F-ratio
Essential output	25.33*
Sabinene	2741.70**
β- pinene	1190.02**
α-Thujone	516811.55**
Germacrene D	2662002.77**
1-2'- methyl- 1'- propenyl) 7- [2'- propynyl) tricycle [3.1.0.0 (2,6)]hexane	51889**

** $P_{value} < 0.01$, * $P_{value} < 0.05$

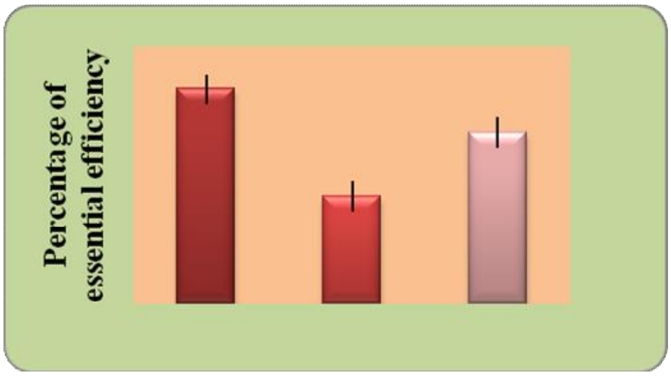

Fig.1. The average of the essential efficiency in *Artemisia absinthium* L. from three altitudes

Secondary medicinal components

Regarding of table 2, the altitude effects on common and main components of the essential oil in *Artemisia absinthium* L. was signified in P<0.01 level. In current research, the percentage of Sabinene component has swayed between 8.33±0.003 % and 9.45±0.002 % and comparing of the mean by Duncan test has also showed that Sabinene percentage is divided into three groups (Table 3) which the first level is in high rank. Beta-Pinene is one of the main components in this plant which grouping of it showed that the most level of beta-Pinene is in the first level of altitude. Another element was α-Thujone which is high rank in the second level (1100-1200 m). Germacrene D was another main composition that its mean was between 0.35±0.001 % and 3.05± 0.003 % and grouping of this matter showed that the first altitude level has it the most degree. And finally, 1-2'-methyl-1'-propenyl) 7-[2'-propenyl) tricycle [3.1.0.0 (2,6)] hexane was the last main component of the species which its mean was 4.46±0.002 % to 5.74±0.002 % and the most level of this component was found in the first level altitude. With relation to figure 2, the highest and least components were α-Thujone from the second (1100-1200 m) and third (1900-2000 m) altitudes levels, respectively.

Table 3.comparing of the means from efficiency and common components in *Artemisia absinthium* L. from three altitude levels

Altitudes (m)	number of components	total percentage of components	Essential output	Sabinene	beta-Pinene	α-Thujone	Germacrene D	A
700-800	31	52.33	0.96±0.007% a	9.45±0.002% a	2.03±0.03% a	0.26±0.002% b	3.05±0.003% a	5.74±0.002% a
1100-1200	30	89.83	b0.91±0.007%	9.37±0.02% b	0.84±0.02% c	30.7±0.04% a	2.42±0.002% b	5.34±0.003% b
1900-2000	35	79.47	a0.94±0.007%	8.33±0.003% c	1.01±0.002% b	0.14±0.04% c	0.35±0.001% c	4.46±0.002% c

A: 1-2'-methyl-1'-propenyl) 7-[2'-propenyl) tricycle [3.1.0.0 (2,6)] hexane; *a,b*, and *c* alphabets show the group division that *a* is the most and *c* is the least level.

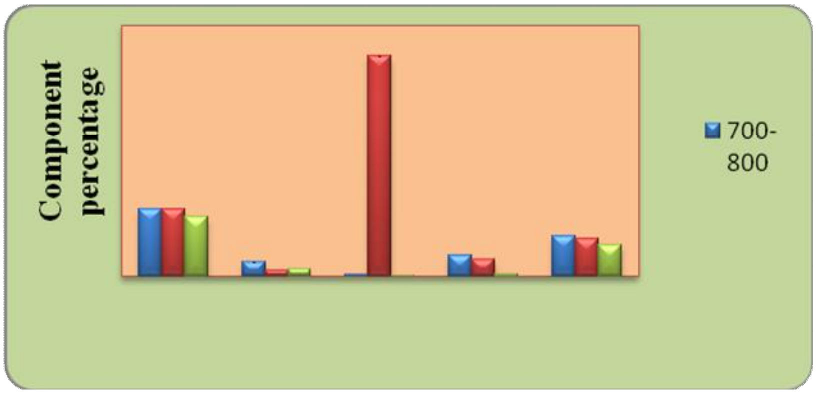

Fig. 2. The effects of three altitude levels on common composition percentages of essential oil from *Artemisia absinthium* L. species

DISCUSSION AND CONCLUSION

Altitude is of important factors to changing of terrestrial ecosystems so that regarding to increasing or decreasing of it, some environmental elements, including temperature, relative humid, wind speed, available water, and radiation rate will be changed. As a result, these changing of plant environments will alter many ecophysiological reactions in the plants bodies. The species of *Artemisia absinthium* L. is also a plant which influenced under edaphic factor and formation sort. The species grows on light soil textures with semi dry cool and semi mountainous humid climates. Hence, it is expected that with changing of the ecological niches, the essential rate and its components kind will also be altered. In connection with this matter, the results have shown that from 54 components in the essential oils from *Artemisia absinthium* L., some main elements percentages, regarding to the altitude changes, were fluctuated as the constituents e.g. Sabinene, beta-Pinene, α-Thujone, Germacrene D, 1-2'-methyl-1'-propenyl)7-[2'-propenyl) tricycle [3.1.0.0 [2,6]] hexane are numerable. These ingredients apply frequently in medicinal and food industries. The current result is connect with other researchers reports such as surveying of Rabiei *et al* [24] about the essences of four species of *Artemisia* genera from north of Iran, Azadbakht *et al* [3] concerning on the essences of *Artemisia aucheri* from Mazandaran, and Semnani et al (2005) about the essences of *Artemisia* genus. The essential quality of current result accordance with the others reports, but the percentages of essential components had differenced. It is because of microedaphic and microtopograhic features of the study area where is different with the others studies places.As it has been observed in the result section, with relation to increasing of the altitude in Savadkooh area, component numbers and percentage of total ingredients were different and statistical comparisons has also shown that the altitude effects on percentage of the essential elements were significant at $P<0.01$ level. Although the direct effects of altitude on the essential components of medicinal plants has been reported by many researchers, including Ghavam'Arabian [10] and Azarnivand et al [6] on *Achillea millefolium*, Najaf'Poor Navaei [19], Djamshidi et al [7], Habibi et al [12], Kazemizadeh et al [15], and Pluhar et al [22] on *Thymus* genus; the increasing of components numbers and its essential percentages in Savadkooh area is because of dominant condition of climate. This area has a sort of dry climate on the basis of Emberger's method. The area locates on central Alborz zone where is in connect with central Iran's climate because of cross section of this area toward to central Iran's climate which allows it to elongate to the area [14]. Hence, coldness and dryness climate in the area is caused to change the species ecophysiology and consequently the rate and percentages of the essential oils of *Artemisia absinthium* L.

Based upon results, the highest essential oil was found in 700-800 m and statistical comparison of this matter has also shown that the essential percentage from this altitude level was the maximum level. It is known that from down to up altitudes, the essential percentages will be decreased as the total rate of essential components will be increased. The reports of other researches, including Habibi et al [12] and Djamshidi et al [7], confirm this. The first level altitude of the study area apropos of upland altitudes, however, has enough soil and atmosphere humid [14]. Therefore, the down altitude has more essential efficiency than the others. Some essential components were just found in one altitudinal level. For instance, constituents of 2- bromethanenitrile, γ-Cadinene, and Benzene, (2-ethyl-4-methyl-1,3-pentadienyl) were only in 700-800 m, elements of β-Terpinene, Anothole, and 1-Naphthalenol, 1,2,3,4-tetrahydro- 2,5,8- trimethyl were only in 1100-1200 m, and ingredients of Naphthol, 2,5,8-trimethyl, 1H-Indene, 3-ethyl-1-(1-methylethyl), and Thymene were only found in 1900-2000 m. The element of α-

Thujone, as a main component, however was encountered in all three levels of altitudes. The component was the highest rates in the 1100-1200 m. In connection with this matter, Saez (1995) has pointed out that some organic elements and primary essences of *Thymushyemalis* have influenced by environmental effects factor. Kazemizadeh et al [15] have also reported that the quality and quantity of essential components in aerial parts of *Teucriumhyrcanicum* in different altitudes was because of ecological features of vegetative regions. And finally, it can be demonstrated that exploitation condition and consequently increasing of soil organic matters rate was caused to heighten the α-Thujone element in body of *Artemisia absinthium* L. in altitude 1100-1200 m.

Considering of results and field observations, the optimum essential efficiency refers to 700-800 m altitude where many rural people and ranchers settle in this area. Moreover, the species of *Artemisia absinthium* L. applies frequently in traditional medicine. Therefore, it is recommended that regarding to ecological management of this area, planting and establishing of the species in field scales can protect the species in the main habitat and increase the economic incomes of families who live in the area.

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