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Original Article

Evaluating the Diversity of the Essential Oil Constituents of *Artemisia* Accessions from Iran

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ABSTRACT

Artemisia L., the largest genus from the Anthemideae tribe and Asteraceae family, comprises almost 500 species, which is one so important in traditional Persian medicine. Of these species, *A. absinthium* and *A. aucheri* have numerous uses in various fields such as pharmaceutical, agricultural, cosmetics, sanitary, perfumes, and food industries. Due to their characteristic features in terms of chemical composition and usefulness, this paper aims to present the results of the chemical composition of essential oils extracted by the hydrodistillation from *A. absinthium* and *A. aucheri*. The plant material was collected from different geographical areas of Iran. The qualitative and quantitative essential oil analysis was performed by the GC/MS. The percentages show the presence of chemical compounds represented in the majority by camphor, sabinen, linalool, hydroxy dihydro- lavandulyl acetate, and Geraniol. Hydrocarbon and oxygen monoterpenes, especially ketones, were the essential chemical groups in the *Artemisia* essential oil from different parts of Iran.

INTRODUCTION

Artemisia is a plant of the Asteraceae family, and between 200- 400 species have been identified worldwide. However, its diversification center is in Central Asia, representing about 150 species in China, 50 in Japan, and 35 in Iran. At the same time, the speciation areas are northwest America, Irano-Turanian, Pakistan, and the Mediterranean region [1]. Few species have been reported from Africa and Europe [2]. It is one of the most important plant species in Iran. Species of this genus, due to adaptation to arid and semi-arid regions, resistance to cold and drought in the environment, a particular form of the semi-arid plant, and supply of fodder for livestock and wildlife are essential. Some of the species of this genus are economically important, used in medicine, industry, and as soil stabilizers, while others are intrusive, which are inauspicious to crop yield [3, 4].

Many species of *Artemisia* are fragrant and rich in essential oils. *Artemisia* unique fragrance is due to monoterpenes and sesquiterpenes [5]. Some *Artemisia* species treat high blood pressure, diabetes, and gastrointestinal disorders [6]. Artemisinin, extracted from *Artemisia*, is used to treat malaria, cancer, fever, and coronavirus disease 2019 (COVID-19) [7]. According to the study by Yao and Chen [8], the *Artemisia* species possess anti-bacterial, anti-fungal, anti-inflammation, anti-tumor, and anti-pathogenic activities and are used for the treatment of hepatitis, ulcer, and hyperlipidemia. It is also effective in the treatment of leishmaniasis [9]. Besides the uses mentioned above, the plant extracts are used in asthma, skin diseases, constipation, and enhancing digestion in the stomach [10].

A. aucheri is one of the most famous species of *Artemisia* in Iran. This perennial plant is grayish-green with a height of 25-50 cm. It has leaves lamina with ovate or nearly round petioles, yellow flowers, and capitol inflorescences [11]. It is of great interest in the traditional medicine of Iran and China. Antibacterial, antifungal, antioxidant, anti-parasitic, and anti-inflammatory properties have been reported for this species [12].

A. absinthium is a shrubby plant having a height of 1 meter. It is a perennial herb. Basal and lower stem leaves are long and have leaf stalks, petiole length is about 10 cm, the lamina is extensively ovate, its length is 8-15cm, and width is 4-8 cm 2-3-pinnatisect [13, 14].

Plants' products have been screened for health purposes because many people have indulged openly or ramblingly in the traditional usage of different products of plant origin. Essential oils are among the important secondary metabolites of medicinal and aromatic plants. They have many usages in various industries and fields, from the pharmaceutical and cosmetic to the food and aromatherapy industries. Many studies have shown that *Artemisia* species display significant intraspecific variations in the essential oil constituents. In the current study, we described the distribution of the genus *Artemisia* in Iran and its composition in detail. The chemical composition of these oils varies from species to species depending on the environmental conditions [14]. We try to identify *Artemisia* species with particular attention to their pharmacological potential and phytochemistry.

MATERIALS AND METHODS

Plant Materials

The *Artemisia* species from Iranian provinces were collected and identified by the laboratory staff of Iranian Biological Resource Center (IBRC) as described in Table (1). All accessions were obtained under national and international guidelines and the plants were collected under their supervision.

Aerial parts of each *Artemisia* accession were collected before the flowering stage, dried at room temperature, fine powder before hydro distillation.

Essential Oil Extraction

Fifty grams of air-dried powdered plants (aerial parts) were subjected to hydro-distillation using a Clevenger apparatus (flask capacity 1000 mL, model TF-1000 ml) for 3-4 hrs (until the essential oil volume remained constant) with 400 mL of distilled water. The extracted oil was weighed and stored at 4 °C until used.

Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GC–MS) An Agilent 6890N gas chromatograph with a 5973 mass-selective detector was used for gas chromatography-mass spectrometry analysis (Agilent Technologies, CA, USA). The essential oil components were separated on a HP-5 MS capillary column (30 m × 0.25 mm i.d. and 0.25 µm film thickness) (5% −diphenyl- 95% -dimethylsiloxane). Helium gas at a flow rate of 1.0 mL /min was used as a carrier. The oven temperature was initially adjusted to 40 °C (held for 3 min), raised to 250 °C by a 4 °C /min rate, and held at this temperature for 10 min.

Identification of Components

The essential oils' constituents were identified by calculating their retention indices under temperature-programmed conditions for n-alkanes (C_5-C_{20}, C_{20}-C_{40}) and oil on a HP-5 MS capillary column under the same chromatographic conditions. Identification of individual compounds was made by comparing their mass spectra with those of the internal reference mass spectra library or with authentic compounds, confirmed by comparing their retention indices with the authentic compounds or those reported in the literature [15, 16]. For quantification purposes, relative area percentages obtained by FID were used without correction factors.

RESULTS AND DISCUSSION

The analysis of the essential oils showed that the total percentage of essential oil and the number and diversity of oil compounds varied in those two *Artemisia* species with three ecotypes (Table 2).

Table 1 Geographical origins of *A. absinthium* L. and *A. aucheri* Boiss.

Code	IBRCno	Species	Province	Latitude	Longitude	Altitude (m) asl
n.1	P1000394 IBRC	*A. absinthium* L.	Gilan	36º54'22.2"	49º54'46.6"	1606
n.2	IBRC P1000023	*A. absinthium* L.	Golestan	37º17'50.4"	55º18'44.8"	68
n.3	IBRC P1000563	*A. absinthium* L.	Semnan	35º59'00.2"	53º01'51.7"	902
n.4	IBRC P1007313	*A. aucheri* Boiss.	Semnan	36º28'48.1"	54º33'4.6"	2377
n.5	IBRC P1000557	*A. aucheri* Boiss.	Mazandaran	35º56'57.6"	53º00'23.8"	1178
n.6	IBRC P1006442	*A. aucheri* Boiss.	Isfahan	31º12'4.8"	51º42'41.7"	2313

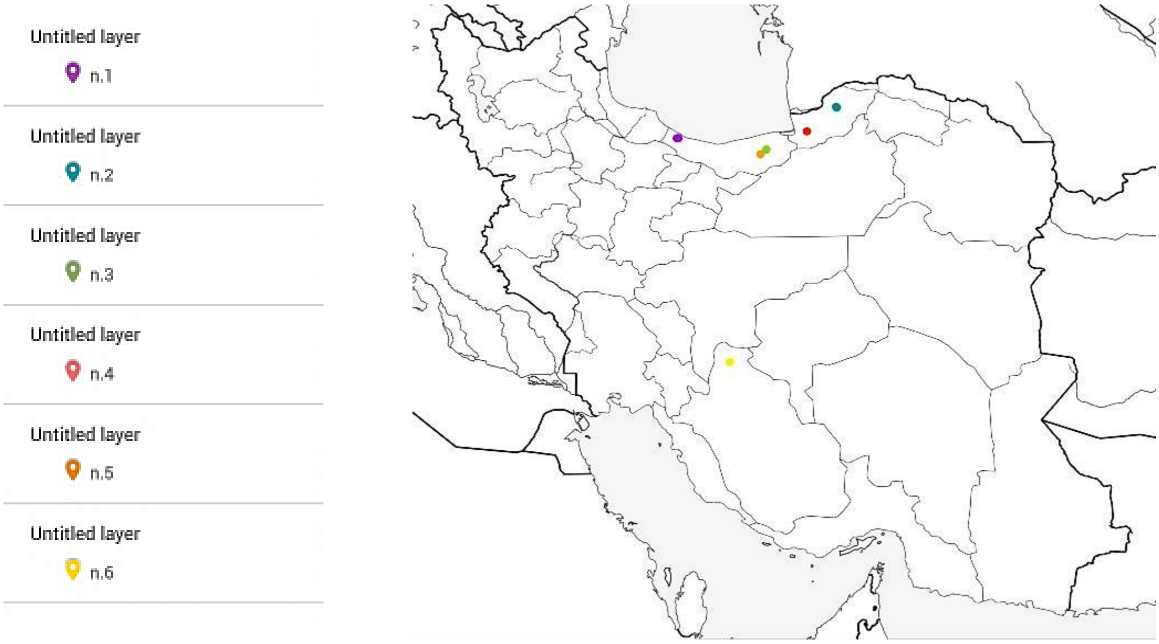

Fig. 1 Collection sites of *Artemisia spp*. from Google Earth.

Chemical Diversity of Essential Oils

Results showed that the percentage of essential oil and the number of oil compounds varied in two *Artemisia* species with three ecotypes (Table 2). Also, the results of this research indicated that almost 56.48% of the essential oil composition of ecotypes was common. Some components were just in one population. For example, 6 compounds were observed only in the *A. absinthium* from Gilan, while 5 compounds only in the *A. absinthium* from Golestan, 14 compounds only in the *A. absinthium* from Semnan, 8 compounds only in the *A. aucheri* from Semnan, 5 compounds only in the *A. aucheri* from Mazandaran, and 9 compounds only in the *A. aucheri* from Esfahan (Fig. 2, Table 2).

Essential oils compounds were analyzed using GC-MS. According to the analysis of chromatograms, the essential oil compositions of *Artemisia* ecotypes are listed in Table 2.

91.22% of the total oil for *A.absinthium* that was collected from Gilan was identified. δ -Cadinene 13.31%, n- Decane 7.97%, Camphor 6.94%, and Germacrene D 6.54% were the major components. 96.78% of *A. absinthium* essential oil compounds collected from Golestan was identified. In this population, the most compounds were Sabinen 15.75%, n-Decane 11.46%, o-Cymene 11.43%, and Cis -thujone 10.3%. For the other population of *A. absinthium* was collected from Semnan 93.24% of total essential oil was identified. Cis -thujone 29.43%, Sabinen 9.49%, and Camphor 6.98% were the major components.

Almost all the compounds (92.92%) in *A. aucheri* essential oil collected from Semnan were identified. The main compounds of oil were Linalool 15.12%, Nerol 11.79%, and Geraniol 6.37%. For the other population collected from Mazandaran, 94.39% of the total essential oil components were identified. Hydroxy dihydro- lavandulyl acetate 15.25%, Linalool 13.66%, and Nerol 8.72% were the major components. 98.02% of the total oil for *A. aucheri* collected from Isfahan was identified. The most compounds were Hydroxy dihydro- lavandulyl acetate 44.19%, Linalool 15.51%, Geraniol acetate 7.11%, and Camphor 4.15%.

According to a study by Benchaar *et al*. [17], α-pinene, Cis- thujone, and camphene were the significant constituents of *Artemisia* essential oils native to Iran, which is consistent with the results of this research. The other reports showed, a total of 54 compounds were identified in *A. absinthium* essential oil, with the most abundant constituents being eucalyptol (25.59%), linalool (11.99%), and β-myrcene (10.05%) [18].

Table 2 Percentage of essential oil compositions of two *Artemisia* L. species with three ecotypes

N	Compound	RI	*A. absinthium* (Guilan)	*A. absinthium* (Golestan)	*A. absinthium* (Semnan)	*A. aucheri* (Semnan)	*A. aucheri* (Mazandaran)	*A. aucheri* (Isfahan)
1	Santolina triene	906	-	-	0.11	-	-	0.57
2	α -Pinene	932	-	2.88	0.87	0.16	-	-
3	Camphene	946	2.93	-	3.31	0.44	-	0.66
4	Sabinen	969	3.97	15.75	9.49	0.66	-	-
5	β- pinene	974	-	-	0.84	-	-	-
6	β-myrcene	988	0.74	-	-	0.42	-	-
7	Mesitylene	994	-	-	0.22	-	-	-
8	n-Decane	1000	7.97	11.46	1.17	-	-	-
9	α- Phellandrene	1003	-	4.01	1.27	-	-	-
10	1-p-menthene	1021	-	-	-	0.16	-	-
11	o-Cymene	1022	-	11.43	-	1.44	-	-
12	1,8-cineole	1026	1.97	-	4.91	0.73	-	1.22
13	β-Cymene	1033	-	-	-	-	6.24	0.43
14	γ-Terpinene	1054	-	-	1.13	-	-	-
15	Terpinolene	1086	-	0.49	1.15	-	-	-
16	Linalool	1088	1.48	0.70	0.25	15.12	13.66	15.51
17	p- Cymenene	1089	0.86	5.37	0.97	-	-	-
18	Cis- thujone	1101	2.90	10.30	29.43	0.55	-	-
19	Trans- thojone	1112	-	1.37	2.57	5.18	3.64	-
20	Camphor	1141	6.94	4.38	6.98	4.46	4.90	4.15
21	Neo-isopulegol	1144	0.93	-	-	-	-	-
22	Sabina ketone	1154	-	-	-	1.29	-	0.17
23	Borneol	1165	1.19	-	2.73	-	-	-
24	Rosefuran epoxide	1173	-	-	0.54	-	-	-
25	Terpinen-4-ol	1174	2.46	0.75	1.92	-	-	-
26	α -Terpineol	1186	-	-	0.45	-	-	0.25
27	Methyl chavicol	1195	-	-	-	0.86	-	-
28	n-Dodecane	1200	4.38	5.75	-	0.94	-	1.93
29	α-fenchyl acetate- Endo	1218	0.90	-	-	-	-	-
30	Nerol	1227	-	-	-	11.79	8.72	2.04
31	Neral	1235	-	-	0.27	4.71	2.91	1.04
32	Cumin aldehyde	1238	-	-	0.23	-	-	-
33	Carvotanacetone	1244	-	-	0.44	-	-	-
34	Geraniol	1249	-	-	0.92	6.37	4.43	-
35	Geranial	1264	-	-	-	5.05	0.55	-
36	n-Decanol	1266	-	-	-	-	-	0.20
37	Perilla aldehyde	1269	-	-	0.27	-	-	-
38	Thymol	1289	0.65	-	-	-	-	-
39	Carvacrol, ethyl ether	1298	-	-	0.37	-	-	-
40	Trans-dihydro- α -Terpinyl acetate	1300	-	-	1.11	-	-	-
41	Hexyl tiglate	1330	-	-	-	0.28	-	-
42	α.-Terpinyl acetate	1346	2.20	-	-	-	-	-
43	α -Cubebene	1348	-	-	-	0.61	-	-
44	Neryl acetate	1359	-	-	-	-	4.38	-
45	Longicyclene	1371	-	-	-	-	0.90	-
46	α -Copaene	1374	-	0.71	-	-	-	-
47	β-Patchoulene	1379	-	-	0.6	-	-	-
48	Geranyl acetate	1379	-	-	-	-	0.67	7.11
49	β -Cubebene	1387	-	-	2.24	-	-	-
50	β -Bourbonene	1387	-	-	0.19	-	-	-
51	(Z)- Jasmone	1392	2.12	-	0.55	0.83	-	-
52	Phenyl ethyl isobutanoate	1393	-	-	-	1.04	-	0.99
53	n -Tetradecane	1400	1.52	2.28	0.34	-	-	-
54	α- Funebrene	1402	-	-	-	-	1.10	-
55	Methyl eugenol	1403	-	-	-	-	-	0.46

56	Italicene	1405	-	-	-	-	-	1.69
57	α -Gurjunene	1409	-	-	-	-	2.78	0.34
58	α - Cedrene	1410	-	0.34	0.21	-	-	-
59	(E) -Caryophyllene	1417	0.68	0.96	1.43	-	-	-
60	Copaene - β	1419	1.16	-	0.47	-	-	-
61	β -Cedrene	1419	-	0.59	-	-	-	-
62	hydroxy dihydro- lavandulyl acetate	1436	-	-	-	-	15.25	44.19
63	Aromadendrene	1439	0.94	-	2.33	1.16	2.27	0.17
64	(Z)- β-Farnesene	1440	0.72	-	0.60	-	-	-
65	α -Humulene	1452	-	-	-	0.16	-	-
66	(E)-β-Famesene	1458	-	-	-	-	-	0.64
67	Ar-Curcumene	1479	-	-	0.11	0.36	-	0.08
68	Germacrene D	1480	6.54	2.28	-	-	-	-
69	γ-Himachalene	1481	-	-	-	0.53	-	0.09
70	β -Selinene	1489	-	2.04	-	0.98	-	1.53
71	cis-β-Guaiene	1492	-	-	-	-	0.63	0.44
72	Viridiflorene	1496	-	-	0.62	0.26	1.81	-
73	Benzyl tiglate	1497	-	-	-	-	-	1.37
74	α -selinene	1498	-	-	-	0.89	-	0.61
75	γ- Patchoulene	1502	-	-	-	2.17	-	-
76	α -Farnesene	1505	0.38	0.47	0.30	0.32	-	0.33
77	β -Bisabolene	1505	-	-	-	5.1	-	1.65
78	Cis - α -bisabolene	1506	-	-	0.39	-	0.24	-
79	β-Sesquiphellandrene	1521	-	-	-	1.19	2.36	0.23
80	δ -Cadinene	1522	13.31	2.34	1.99	-	-	-
81	(Z)- Nerolidol	1531	3.18	-	0.36	-	-	-
82	α -Calacorene	1544	-	4.18	-	-	-	-
83	Elemol	1548	-	-	-	1.09	-	-
84	Geranyl butanoate	1562	-	-	-	0.58	-	0.40
85	Spathulenol	1577	3.02	0.89	1.32	2.69	3.08	0.83
86	sesquisabinene hydrate	1578	-	-	-	5.46	4.21	1.37
87	Caryophyllene oxide	1582	1.76	2.08	1.19	1.83	-	2.87
88	Thujopsan-2-β-ol	1586	-	1.41	-	-	-	-
89	Thujopsan-2-α-ol	1586	-	-	-	0.95	0.28	0.44
90	n-Hexadecane	1600	-	0.72	-	-	-	-
91	α- Corocalene	1622	-	0.85	-	2.56	-	-
92	Eremoligenol	1629	-	-	1.26	-	-	-
93	Epi-α -Cadinol	1638	-	-	-	-	1.37	-
94	Allo-aromadendrene epoxide	1639	6.93	-	0.56	-	-	-
95	Epi-α- -Muurolol	1640	3.58	-	1.83	-	-	-
96	Vulgarone B	1649	-	-	-	-	-	0.90
97	2,3-dihydro-Farnesol	1688	-	-	-	0.65	-	-
98	Chamazulene	1730	-	-	0.43	-	-	-
99	β -costol	1765	-	-	-	0.29	2.20	0.25
100	13-hydroxy- Valencene	1767	-	-	-	-	-	0.41
101	(2Z,6E)- Farnesyl acetate	1821	0.34	-	-	-	-	-
102	(2E,6E)- Farnesyl acetate	1845	-	-	-	0.39	0.85	-
103	Cembrene	1937	-	-	-	-	-	0.10
104	Phytol	1942	-	-	-	-	-	0.11
105	Hexadecanoic acid	1959	-	-	-	-	2.22	0.05
106	Kaurene	2042	-	-	-	-	0.82	-
107	Methyl linoleate	2095	-	-	-	0.22	1.92	0.20
108	Oleic acid	2141	2.57	-	-	-	-	-
	Total	-	91.22	96.78	93.24	92.92	94.39	98.02

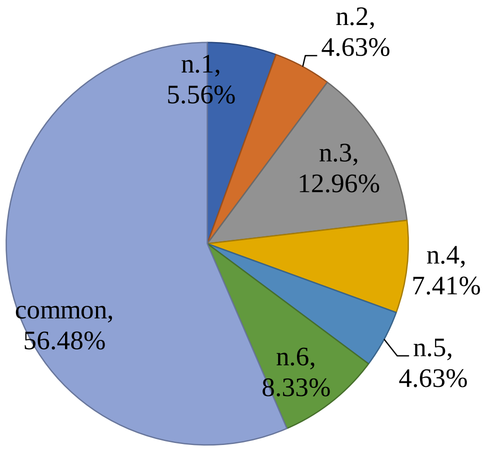

Fig. 2 Distribution of essential oil composition in two *Artemisia* L. species with three ecotypes

Morteza-Semnani and Akbarzadeh [19] reported that trans-thujone (35.1%), p-cymene (16.5%), β-pinene (7.3%), and 7-ethyl-5, 6-dihiydro-1, 4-dimethyl azulene (5.5%) were abundant in the essential oil obtained from *A. absinthium* plants growing in Iran. In another study, trans-thujone (35.6%) was dominant in the essential oil produced by *A. absinthium* growing in Morocco; although α-pinene, sabinene, linalool, camphor, and n-decanal were also found in this essential oil [20], shown in the present study but with different percentages. Another study shows that, the major components of the essential oil of *A. aucheri* were camphor (51.0%) and 1, 8-cineol (25.0%). The results suggest that *A. aucheri* essential oil possesses biologically active constituent (s) that have significant activity against acute inflammation and have central and peripheral antinociceptive effects which support the ethnomedicinal claims of the plant application in the management of pain and inflammation [21]. However, the presence, absence, and number of compounds differ in all samples due to the differences in plant habitats. Reports on the chemical composition of the essential oils isolated from the plants belonging to the genus *Artemisia* indicate that Cis-thujone is the main constituent of *Artemisia* essential oils [22], which strongly supports the findings of our research.

Linalool was observed in all samples; its amount in *A. aucheri* was higher than in *A. absinthium*. Linalool is a monoterpene compound commonly found as a significant component of essential oils of several aromatic species, many of which are used traditionally as sedatives. About 70% of the terpenoids of floral scents are represented by linalool. Over 200 species of plants produce linalool, mainly from the families Lamiaceae (mint and other herbs), Lauraceae (laurels, cinnamon, rosewood), and Rutaceae (citrus fruits). Also, birch trees and other plants from tropical to boreal climate zones and fungi produce this chemical compound [23].

Camphor was the other main compound in all samples. Camphor is a waxy, flammable, transparent solid with a strong aroma. It is a cyclic monoterpene, which sublimates at room temperature and melts at 180 °C. It is used for its scent, embalming fluid, topical medication, manufacturing chemicals, and religious ceremonies. It is practically insoluble in water but soluble in alcohol, ether, chloroform, and other organic solvents. This compound is one of the essential parts of *Artemisia* oil [24].

Sabinen, a monoterpene accumulated in natural organisms, is the other compound in all samples except *A. aucheri* in Mazandaran and *A. aucheri* in Esfahan.

Geraniol is a commercially important terpene alcohol found in the essential oils of several aromatic plants. Geraniol appears as a clear to pale-yellow oil which is insoluble in water but soluble in most organic solvents. It is emitted from flowers of many species and is present in the vegetative tissues of many herbs [25]. Geraniol is known to be derived from geranyl diphosphate (GPP) by related synthases based on a common ionization-dependent reaction mechanism [26].

One study showed that the geraniol and geranyl acetate levels in lemongrass (*Cymbopogon flexuosus*) fluctuated during leaf development. The geranyl acetate level decreased from ∼ 59 to ∼ 3%, whereas the level of geraniol increased from ∼ 33 to ∼ 91% during the leaf growth period. These fluctuations indicated the role of an esterase in converting geranyl acetate to geraniol during leaf development [27]. These results are congruent with a previous study by Dubey and Luthra 2001[28]. Accordingly, the presence of this substance in *A. aucheri* is due to genetics, and its fluctuations are due to climate, harvest time, and vegetative stage.

Terpenes are a large and diverse class of organic compounds mainly produced by various plants. They are generated from common precursors, IPP (Isopentenyl pyrophosphate) and DMAPP

(Dimethylallyl pyrophosphate). They are produced from the methyl erythritol 4-phosphate (MEP) or mevalonate (MVA) pathway [29].

In general, there are different reports in different parts of the world regarding the essential oil components of *Artemisia* species [30-33]. Their study also shows a noticeable difference in the oil composition. This difference can result from the factors as follows: the diversity of the species studied, ecotype, chemotype, plant genotype, ecological conditions, harvest time, type of harvestable organ, essential oil extraction method, and identification of effective compounds.

The difference in the quantity and quality of the active ingredients of plant essential oils is related to the region's climatic conditions, soil type, altitude, and even the time of plant collection [34]. It appears that seasonal studies produce similar results. Many studies have shown that *Artemisia* species display significant intraspecific variations in the essential oil constituents. Various factors are involved in the diversity of essential oil compounds; such as pH, climatic factors and etc. In some cases, the variation in the volatile components of these plants may occur during plant ontogeny or growth at different altitudes [35].

CONCLUSION

The variations among natural populations of *Artemisia* showed that add to the impact of plant inheritance, it conjointly encompasses a high adaptation potential so that a variety of climatic conditions like altitude are among different populations. Several pieces of evidence can justify periodic fluctuations in the composition and yield of plant essential oils. The plant develops, and the structure of its cells, and tissues changes, leading to the change in various chemical compounds existing in its organs. These changes can affect the chemical interactions that control the production of essential oils. Differences in the composition of essential oils of different plant parts may be due to distinct secretory structures that are non-uniformly distributed throughout the plant. Observation of these differences can be due to factors such as Altitude ASL conditions on the composition of essential oils of different populations of a species.

There were variations in the main components of essential oil among species and ecotypes. These variations are probably related to different environmental conditions of the plants and might have arisen from several differences in climate, seasonal, and geographical. So, due to the various compounds in essential oils, different ecotypes can be used in different industries. Thus, these two species are the superior species with the best medicinal value, which can be introduced to cultivation in different regions. It can be clearly seen that the composition of essential oils is primarily intrinsically and dependent on genotype, and secondly, it is the effect of environmental factors and stressors.

According to the present study results, the essential chemical groups present in the essential oil of two species of *Artemisia* from different parts of Iran were hydrocarbon monoterpenes and oxygen monoterpenes, especially ketones.

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