ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 11:2 (2017) 114-129

records of natural products

Essential Oils Extracted Using Microwave-Assisted Hydrodistillation from Aerial Parts of Eleven *Artemisia* Species: Chemical Compositions and Diversities in Different Geographical Regions of Iran

Majid Mohammadhosseini*

[1]*Department of Chemistry, College of Basic Sciences, Shahrood Branch, Islamic Azad University, Shahrood, Iran*

(Received February 18, 2016; Revised October 29, 2016; Accepted November 8, 2016)

Abstract: This study aimed to assess the chemical compositions of essential oils (EOs) extracted through microwave-assisted hydrodistillation from aerial parts of 11 *Artemisia* species growing wild in different regions in Northern, Eastern, Western, and Central parts of Iran. The EOs were subsequently analyzed via GC and GC-MS. The percentage yields of the EOs varied over the range of 0.21-0.50 (w/w%). On the basis of these characterizations and spectral assignments, natural compounds including camphor, 1,8-cineole, camphene, α-pinene, β-pinene, β-thujone, and sabinene were the most abundant and frequent constituents among all studied chemical profiles. Accordingly, oxygenated monoterpenes, monoterpene hydrocarbons, and non-terpene hydrocarbons were the dominant groups of natural compounds in the chemical profiles of 13, 4, and 2 samples, respectively. Moreover, five chemotypes were identified using statistical analyses: camphene, α-pinene and β-pinene; 1,8-cineole; camphore and 1,8-cineole; camphore and camphore and β-thujone.

Keywords: 1,8-cineole; *Artemisia*; camphor; chemotype; essential oil; (MAHD).

1. Introduction

Artemisia, commonly known as "wormwood", is a famous genus of the *Asteraceae* family belonging to the *Anthemideae* tribe. This genus comprises about more than 300 species [1], most of which are rich sources of flavonoids. To date, around 160 specific flavonoid compounds have been reported, the most abundant of which include flavone derivatives, such as apigenin and luteolin. Moreover, the high occurrence of these valuable compounds is a proper criterion to address some issues related to taxonomic classifications in plant sciences [2].

According to literature, more than 30 species of the *Artemisia* genus are native to Iran, the most endemic of which are *A. kermanensis*, *A. melanilepis*, and *A. sieberi*. The most important habitats of different *Artemisia* species are concentrated in many parts of the Middle East, Eastern Asia, and Australia. Specific climatic regions in India are conducive for growing *Artemisia* plants on a large scale [3].

* Corresponding author: E-Mail: majidmohammadhosseini@yahoo.com; Phone: +98-23-32394530; Fax: +98-23-32394537

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 12/24/2016 EISSN:1307-6167

In some ancient cultures of Europe, several *Artemisia* essential oils are frequently prescribed as proper antidotes against severe poisons and as effective agents to treat gastric disorders. Notably, different *Artemisia* species possess high amounts of valuable natural compounds, including monoterpenoids [4, 5] and sesquiterpenoids [5-9].

Prescription of medicinal plants has a very long history in the traditional Iranian and folk medicine [10]. In Persian botanical nomenclature, plants from the *Artemisia* genus are called Darmeneh [11]. Different *Artemisia* species reportedly possess promising phytochemical activities, including antidiabetic [12], hepatoprotetive [13], antioxidant [14], antifungal [15-17], nephroprotective [13], anti-inflammatory [18], anticoccidial [19, 20], antimalarial [21], antioxidant [14], cardioprotective [13], and anticancer [22]. In addition, *Artemisia* species exhibit potent pesticidal activities against Anopheles insect larvae, and EOs extracted from these plants may be used to control the egg hatching, spawning, and mortality of grain beetle larvae. However, additional investigations are warranted to advance the applicability of these medicinal plants to an industrial scale [23]. The local tribes and nomads in Iran often call these plants "Joshan." They often use different parts of these medicinal plants in local foods and cookery because of their spicy and fragrant flavors. These people believe in the great therapeutic capabilities of these herbal drugs as strong tonics, carminatives, wound disinfectants, appetizers, anti-gastritis, and anthelmintic agents [24, 25]. The *Artemisia* plants are also effective wound-dressing agents in local medicine. Moreover, decoctions of some *Artemisia* species have been recommended as potent alternatives in killing intestinal parasites, such as pinworm and *Ascaris* [26].

Over the recent decades, researchers have introduced and developed various effective techniques to isolate versatile natural products from raw medicinal plants [23]. The most promising advantages of these methodologies involve minimizing the operation time and the total usage of organic solvents while increasing the separation yield and the general qualities of the extracted oils. Among the proposed techniques, microwave-based ones serve as the best alternatives for traditional and classical approaches [27]. Microwave-assisted hydrodistillation (MAHD) involves the application of microwave radiations to plant samples in a separation chamber [28]. Extractions based on microwave beams were first suggested by Ganzler et al. [29] in the mid-1980s. MAHD can be conveniently combined with conventional hydrodistillation and is recommended as a versatile technique to isolate EOs from various aromatic and herbal plants. This technique is environmentally friendly, time saving, and economical [30-34].

To the best of my knowledge, this study is the first to compare the chemical compositions of EOs extracted by MAHD from aerial parts of 19 populations related to 11 *Artemisia* species growing wild in different regions of Iran.

2. Materials and Methods

2.1. Plant Collection and Identification

Plant samples in the full flowering stage were harvested within April-June 2015 in 19 localities across 13 provinces of Iran, including Khorasan Razavi, Golestan, Semnan, North Khorasan, Mazandaran, Ardabil, East Azarbayjan, West Azarbayjan, Hamadan, Markazi, Isfahan, Yazd, and Lorestan (Table 1). The precise location of each sampling area and the geographical coordinates were determined using a standard Global Positioning System (Vista Garmin) receiver. As anticipated, the collected plant samples had remarkable morphological variability in the common characteristics of their aerial parts. The plants were identified by a local botanist basing on the information given in *Flora Iranica* [35], and voucher specimens had been subsequently submitted to the Herbarium of the Research Institute of Forests and Rangelands (RIFR) in Tehran, Iran. In this regard, the voucher numbers of the studied species ranged from AS00451 to AS00469 as listed in Table 1.

2.2. Microwave assisted hydrodistillation (MAHD)

A comprehensive detail concerning the application of MAHD is available in our previous papers [36, 37]. In brief, a microwave oven from Samsung, South Korea, regulated at a frequency of 2450 MHz and having a sufficient internal capacity to house the glass Clevenger set-up, was employed and was connected to the condenser placed outside the system. It was found that 50 g portions of the dried aerial parts were adequate to finalize extraction.

Table 1. Origin, location and essential oil yield (w/w%) from 19 accessions belonging to eleven *Artemisia* species growing wild in Iran.

Sample number	Botanical name	Sampling area		Height (m.s.l) [a]	Latitude and longitude	Essential oil yield w/w (%)
		Province	Locality			
S1	*A. sieberi*	Khorasan Razavi	Torbat-e-Heidariyeh	1333	35° 16' 26" N, 59° 13' 10" E	0.44
S2	*A. sieberi*	Khorasan Razavi	Sabzevar	977.6	36°15'N, 57°40'E	0.39
S3	*A. absinthium*	Golestan	Ramian	320	37.0161° N, 55.1411° E	0.41
S4	*A. absinthium*	Golestan	Minudasht	901	37.2289° N, 55.3747° E	0.33
S5	*A. absinthium*	Semnan	Abr Forest	1453	36.4181° N, 54.9764° E	0.28
S6	*A. annua*	North Khorasan	Quchan	1350	37°10'N, 58°27'E	0.50
S7	*A. annua*	Mazandaran	Noor	1-10 [a]	36.5736° N, 52.0139° E	0.45
S8	*A. dracunculus*	Ardabil	Pars-Abad Moghan	32	39.6036° N, 47.8815° E	0.41
S9	*A. dracunculus*	East Azarbayjan	Osku	1600	37.9158° N, 46.1236° E	0.38
S10	*A. aucheri*	West Azarbayjan	Mahabad	1320	36°50'N, 45°45'E	0.23
S11	*A. aucheri*	West Azarbayjan	Miandowab	1314	35°37'N, 53°39'E	0.39
S12	*A. aucheri*	West Azarbayjan	Urmia	1332	37.5553° N, 45.0725° E	0.31
S13	*A. kulbadica*	Hamadan	Hamadan	1741	34°52'N, 48°32'E	0.21
S14	*A. diffusa*	Markazi	Arak	1700	34°00'N, 49°40'E	0.30
S15	*A. tschernieviana*	East Azarbayjan	Osku	1600	37.9158° N, 46.1236° E	0.47
S16	*A. lehmanniana*	Isfahan	Isfahan	1570	32.6333° N, 51.6500° E	0.24
S17	*A. deserti*	Yazd	Yazd	1230	32°0'N, 55°0'E	0.33
S18	*A. incana*	Lorestan	Khorram Abad	1347	33°30'N, 48°25'E	0.29
S19	*A. incana*	Mazandaran	Noor	1-10 [b]	36.5736° N, 52.0139° E	0.21

[a] Meters from sea level
[b] Above the Persian Sea (25 m below the free sea level)

2.3. GC and GC/MS analysis

GC was performed on a Shimadzu 15A gas chromatograph equipped with a spilt/splitless injector at a ratio of 1:30 and a flame ionization detector operated at 250 °C. High-purity nitrogen was utilized as the carrier gas (1 mL/min), and the employed capillary column was of DB-5 type. In the suggested temperature programming for the GC instrument, the column temperature was kept at 60 °C for 3 min, heated to 220 °C at a rate of 5 °C/min, and then kept constant at this temperature for 5 min. Relative percentage quantities considering the concerned peak area of each constituent were calculated with the aid of a CR5 Shimadzu CR pack discarding correction factors. Furthermore, GC/MS-based separations were carried out using a Hewlett-Packard 5973 instrument equipped with an HP-5MS column. The general dimensions of this column were 30 m × 0.25 mm with a film thickness of 0.25 μm. The defined temperature programming for the GC/MS apparatus was exactly the same as that described for the GC technique, with the mean flow rate of He (carrier gas) set at 1 mL/min. Moreover, the final temperatures of the column and detector (MS) were regulated at 230 °C and 250 °C, respectively. All mass spectra were obtained at an ionization voltage (E_i) of 70 eV over a mass range of 30-350 amu. The electron multiplier was run under a voltage of 1800 eV. The average number of scan times was two times per second. Library search was carried out using the NIST and Wiley 275 GC/MS libraries. Kovats retention indices relative to C_9-C_{24} *n*-alkanes with previously published data based on our findings in similar reports were also considered [27, 38-51]. Relative percentage amounts of the separated compounds were calculated from the total ion chromatography by the computerized integrator.

2.4. Statistical analysis

Hierarchical cluster analysis (HCA) and dimension reduction analyses were performed using SPSS software package version 14. To obtain proper clusters, Average Linkage (between groups) and Ward's minimum variance approaches were successfully utilized.

3. Results and Discussion

3.1. Essential oil yield (w/w%)

As shown in Table 1, the mean yields of the EOs in *A. sieberi*, *A. absinthium*, *A. annua*, *A. dracunculus*, *A. aucheri*, and *A. incana* were in the ranges of 0.39-0.44, 0.28-0.41, 0.45-0.50, 0.38-0.41, 0.23-0.39, and 0.21-0.29, respectively. Meanwhile, the yields of the other species were between 0.21 and 0.47. All of these values were in terms of the amount (g) of the isolated EO per gram of dried plant. The northern provinces of Iran, namely, Golestan, North Khorasan, Ardabil, and East and West Azarbayjan, exhibit greater annual rainfall and higher relative humidity compared with the central or southern provinces of the country. Such crucial environmental factors influence the percentage diversities of the isolated oils. Despite some dissimilarities, most of the mean yields of the volatile oils are due to the samples gathered from the northern parts of Iran. On the basis of the weight percentage data in Table 1, a dendrogram was constructed using the Average Linkage (between groups) method with a squared Euclidean distance (Figure 1). This dendrogram consists of four clusters. The first cluster contains four populations (S13, S19, S10, and S16), the second cluster has six (S4, S17, S5, S18, S12, and S14), the third cluster has five (S3, S8, S2, S11, and S9), and the last cluster has four (S1, S7, S15, and S6). Each cluster consists of oils having similar and/or identical w/w percentages.

3.2. Chemical profiles of EOs extracted by MAHD

The volatile constituents obtained from the 19 populations belonging to the 11 *Artemisia* species are listed in Table 2. With the proposed MAHD method, 108 organic compounds were recognized in the studied profiles representing 99.0-99.9% of their total compositions.

In terms of general categories, the main constituents of *Artemisia* volatile oils (Table 2) were camphor (trace-40.3%), 1,8-cineole (trace-19.3%), camphene (0.2%-24.2%), α-pinene (trace-13.9%), β-pinene (trace-23.6%), β-thujone (trace-11.3%), and sabinene (trace-18.6%). Camphor was the most abundant constituent among all the oil samples, followed by 1,8-cineole, camphene, α-pinene, β-pinene, β-thujone, and sabinene in sequential order. Oxygenated monoterpenes showed the highest frequency in the EO compositions of samples 1, 2, 4-7, 10, 12, 14, and 16-19. Meanwhile, monoterpene hydrocarbons dominated in S3, S11, S13, and S15. Moreover, non-terpene hydrocarbons methyl chavicol and (*Z*)-anethole were the major constituents in S8 and S9 from *A. dracunculus*. Similar to previous studies, oxygenated monoterpenes [52-61], monoterpene hydrocarbons [62], and non-terpene hydrocarbons [63] were prevalent in the EOs.

The broad ranges of relative percentages for the constituent components in Iranian *Artemisia* species account for the presence of considerable inter-species and intra-species chemical variations within the chemical profiles. However, some chemical components were identified only in certain species. As shown in Table 2, some natural compounds were less common in all EO profiles than the other constituents; these compounds include chrysanthenone, *trans*-*p*-menth-2-en-1-ol, (*E*,*E*)-allo ocimene, isomenthol, *cis*-pinocarveol, methyl chavicol, *cis*-carveol, cuminal, geranial, *cis*-verbenyl acetate, hexyl hexanoate, methyl cinnamate, β-panasinsene, β-longipinene, α-cedrene, spirolepechinene, artedouglasia oxide B, δ-undecalactone, gymnomitrone, *epi*-zizanone, and vetiselinenol. The largest fractions of unusual compounds occurred in the oils separated from S1 and S2, which were collected in Torbat-e-Heidariyeh and Sabzevar regions with unique desert conditions. The observed variations in the chemical compositions of the EOs can be attributed to the diverse geographic distributions of the plant samples, physicochemical variables, and related environmental parameters. In consideration of the aforementioned factors, substantial changes in the structure of the oils can be justifiable.

3.3. Chemotypes of Artemisia for the most abundant constituents

To check the relationship between the EOs of the 19 *Artemisia* species, a dendrogram was screened for the corresponding relative percentages of the six dominant constituents (Figure 2). In this regard, an HCA approach was performed using Ward's minimum variance strategy. As shown in Figure 2, the first cluster included five accessions (8, 9, 6, 7, and 13), of which two species belonged to *A. annua* and *A. dracunculus* and one species corresponded to *A. kulbadica* from the Hamadan region. The most frequently occurring compounds in the EOs of these samples had high contents of α-pinene, camphene, and β-pinene, as well as low quantities of linalool, bornyl acetate, eugenol, and (*E*)-caryophyllene. However, samples 7 and 13 were separated from the others because of some dissimilarities, such as the absence of myrcene, α-terpinolene, and spathulenol in the profile of S7. Sabinene was present in the chemical compositions of samples 7 and 13, whereas only traces of this frequently occurring compound may be found in the profiles of samples 6, 8, and 9.

The second cluster consisted of five accessions (4, 5, 19, 15, and 3). A simple comparison of the respective chemical compositions revealed that, similar to the first cluster, high amounts of α-pinene, camphene, and β-pinene were found in the corresponding EOs.

By contrast, 1,8-cineole and sabinene were highly prevalent in these five accessions. In addition, myrcene, *p*-cymene, linalool, β-thujone, borneol, terpinene-4-ol, bornyl acetate, and thymol were common in all samples. Localities 15 and 3 separated from the main cluster because, unlike samples 4, 5, and 19, only traces of β-elemene, (*E*)-caryophyllene, and phytol may be present in the profile of sample 15. Moreover, geranyl acetate (2.4%) and methyl jasmonate (1.2%), together with low amounts of myrtenol (0.2%), D-carvone (0.1%), and calarene (0.3%), were found in the profile of S3.

The third cluster consisted of accessions 2, 18, 1, and 16, which belonged to *A. sieberi* (Sabzevar region), *A. incana*, *A. sieberi* (Torbat-e-Heidariyeh region), and *A. lehmanniana*, respectively. From the frequency point of view, these oils were characterized by dominant natural compounds, such as camphene, 1,8-cineole, and camphor, with camphor ranking first among all

chemical profiles. The other common compounds were γ-terpinene, linalool, terpinene-4-ol, and bornyl acetate.

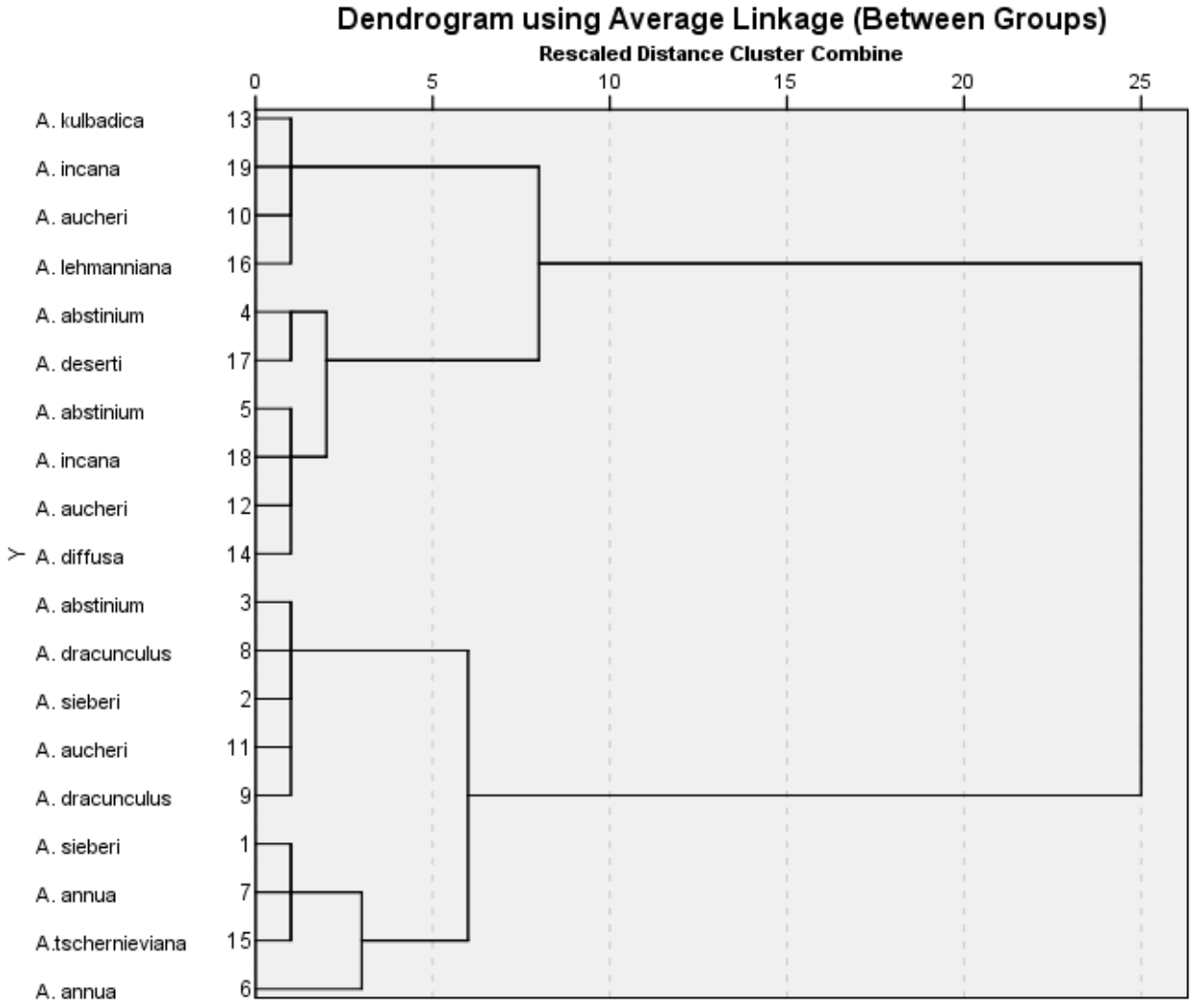

Figure 1. Dendrogram of eleven species of *Artemisia* (19 EOs) in Iran according to oil percentage using average linkage (between groups) approach.

Different from the profiles of S2 and S18, that of S1 lacked spathulenol, whereas that of S16 lacked α-terpinene, borneol, terpinene-4-ol, and geranyl acetate. Unlike the profiles of S2 and S18, that of S16 contained natural compounds such as α-thujene, artemesia alcohol, isomenthol, (*Z*)-anethole, carvacrol, hexyl hexanoate, eugenol, α and β-copaene, β-selinene, capilene, bicyclogermacrene, elemicin, (*E*)-nerolidol, (*E*)-isoelemicin, (*Z*)-isoelemicin, and (*E*)-asarone.

The fourth cluster was formed by accessions 10 (*A. aucheri*) and 11 (*A. aucheri*) from Mahabad and Miandowab regions (West Azarbayjan Province), respectively. All of the most abundant natural compounds in Table 2 (highlighted in bold) were found within the chemical compositions of the EOs relating to accessions 10 and 11. The EOs of these two samples were rich in monoterpene hydrocarbons (e.g., α-pinene, camphene, and β-pinene) and oxygenated monoterpenes (e.g., 1,8-cineole, β-thujone, and camphor). However, camphor was the most abundant compound in these two samples.

Table 2. Chemical composition of essential oils (%) of 19 accessions belonging to 11 *Artemisia* species [a].

No.	Compound	RI [b]	Sample number																	
			S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19
1	α-Thujene	924	0.4	-[i]	0.5	-	0.8	-	-	0.1	-	0.6	2.0	0.8	1.2	-	0.5	0.3	4.1	-	0.6
2	α-Pinene	932	**3.3**	**-**	**1.6**	**2.6**	**3.3**	**5.1**	**13.2**	**10.1**	**7.2**	**3.3**	**4.1**	**2.2**	**2.3**	**1.1**	**6.1**	**13.9**	**2.3**	**5.4**	**2.6**
3	Camphene	946	**4.6**	**3.1**	**24.2**	**1.1**	**2.5**	**3.8**	**2.4**	**0.2**	**1.1**	**19.4**	**16.7**	**2.1**	**0.3**	**4.1**	**1.4**	**1.2**	**0.2**	**1.5**	**2.1**
4	Sabinene	969	**0.6**	**-**	**12.1**	**18.6**	**9.3**	**-**	**0.9**	**-**	**-**	**1.0**	**0.3**	**-**	**18.4**	**0.4**	**1.0**	**6.7**	**0.8**	**0.2**	**0.5**
5	β-Pinene	974	**0.7**	**-**	**3.5**	**2.7**	**8.1**	**2.5**	**6.1**	**2.5**	**0.9**	**1.1**	**0.9**	**12.3**	**2.5**	**2.1**	**23.6**	**1.3**	**3.6**	**0.9**	**3.0**
6	Myrcene	988	0.4	-	3.7	1.2	0.6	1.2	-	1.7	0.2	-	0.5	-	2.8	0.7	2.3	0.7	0.4	0.5	0.1
7	Dehydro-1,8-Cineole	988	0.3	-	-	-	-	-	0.8	-	0.5	-	-	-	0.2	-	-	-	0.1	0.4	0.9
8	α-Phellandrene	1002	-	-	3.1	2.8	17.4	0.9	-	-	3.5	0.6	22.2	-	1.6	0.4	0.9	-	0.3	0.9	-
9	α-Terpinene	1014	1.8	0.6	0.7	0.8	0.2	0.4	-	-	1.1	-	-	-	3.4	0.2	3.3	-	-	1.0	-
10	*p*-Cymene	1020	2.3	1.2	0.5	0.5	0.9	-	-	-	-	0.3	0.8	1.1	0.5	0.1	9.5	-	0.4	-	2.4
11	Limonene	1024	-	-	1.1	-	0.1	-	-	0.3	1.1	-	-	-	1.0	-	12.5	1.6	0.9	1.3	0.6
12	1,8-Cineole	1026	**19.3**	**13.8**	**17.1**	**13.6**	**16.7**	**2.9**	**4.9**	**-**	**0.6**	**3.5**	**7.8**	**0.7**	**0.1**	**0.5**	**8.4**	**10.8**	**9.5**	**12.1**	**15.4**
13	(*Z*)-β-Ocimene	1032	-	-	0.6	0.2	0.1	-	0.7	0.5	2.8	-	-	-	0.9	19.6	0.3	4.6	-	0.1	-
14	(*E*)-β-Ocimene	1044	-	-	-	-	-	-	-	1.8	1.2	-	-	-	0.2	-	0.1	0.9	0.4	0.4	-
15	γ-Terpinene	1054	1.5	0.6	0.8	0.5	-	0.1	-	-	-	0.4	-	0.2	2.1	-	1.1	0.2	-	0.9	0.5
16	Artemisia ketone	1056	-	-	-	0.1	4.2	10.1	13.6	-	-	-	-	-	-	0.7	-	-	0.2	-	-
17	*cis*-Sabinene hydrate	1065	0.4	0.6	0.1	0.4	1.1	-	-	-	-	-	-	-	-	-	0.6	-	0.1	-	-
18	Artemesia alcohol	1080	0.3	-	-	0.1	0.1	8.7	5.9	-	-	-	-	-	-	-	1.3	0.1	-	-	-
19	α-Terpinolene	1086	0.4	-	-	-	0.2	1.1	-	0.5	5.4	-	-	-	3.3	1.1	3.2	2.9	0.3	0.1	0.5
20	Linalool	1095	0.8	0.9	0.5	0.5	1.7	2.5	0.2	0.7	0.4	1.3	-	-	0.2	-	1.1	1.3	0.6	0.9	0.3
21	*trans*-Sabinene hydrate	1098	0.5	0.6	0.1	0.1	-	-	0.3	-	0.9	-	-	-	-	-	0.7	-	-	-	0.4
22	α-Thujone	1101	-	-	5.1	0.8	-	-	-	-	0.2	10.1	0.1	2.2	4.1	6.6	0.1	1.1	4.1	2.8	5.1
23	Filifolone	1106	0.7	0.6	0.1	-	0.1	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-
24	β-Thujone (trans-Thujone)	1112	**-**	**-**	**0.7**	**1.4**	**1.9**	**-**	**-**	**-**	**-**	**8.7**	**4.4**	**9.1**	**11.3**	**10.1**	**0.4**	**8.7**	**10.6**	**3.1**	**0.6**
25	*cis*-p-Menth-2-en-1-ol	1118	0.3	-	-	-	-	-	-	-	-	0.1	0.3	-	-	-	0.2	-	-	0.4	0.1
26	Chrysanthenone	1124	0.5	0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	(*E*,*E*)- Allo	1128	-	-	-	-	-	-	-	-	2.5	-	-	-	-	-	-	-	0.2	-	-

28	ocimene *trans*-*p*-menth-2-en-1-ol	1136	0.2	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-
29	*trans*-Verbenol	1140	0.6	0.7	-	0.1	0.3	-	-	-	-	-	-	-	-	-	0.5	-	0.1	-	-
30	Camphor	1141	**24.0**	**30.8**	**0.6**	**-**	**4.1**	**0.6**	**-**	**-**	**0.2**	**35.1**	**26.4**	**40.3**	**-**	**38.5**	**0.7**	**19.6**	**33.4**	**28.1**	**10.3**
31	Isopulegol	1145	-	-	0.2	0.2	-	0.2	0.8	-	-	-	0.1	-	-	-	-	-	-	1.6	-
32	Pinocarvone	1160	-	-	-	2.1	-	3.6	0.6	-	-	-	-	-	-	-	-	-	-	-	-
33	*cis*-Chrysanthenol	1160	-	-	1.1	5.4	7.3	0.1	-	-	-	-	-	-	-	-	0.3	-	-	-	-
34	δ-Terpineol	1162	-	-	-	2.1	-	-	-	-	-	0.5	-	5.4	1.6	0.9	0.6	-	0.5	0.7	3.4
35	Borneol	1165	2.8	2.8	1.6	1.0	1.3	5.1	1.5	-	0.2	2.5	0.9	3.1	4.1	-	0.9	-	0.8	5.4	8.9
36	Artemisyl acetate	1169	-	-	0.1	-	0.2	-	0.9	-	-	-	-	-	-	-	0.1	-	-	-	-
37	Terpinene-4-ol	1174	3.2	3.0	0.1	0.8	0.5	0.3	-	-	-	-	-	-	0.6	0.4	2.0	-	1.1	2.1	0.5
38	Isomenthol	1179	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	0.4	0.1	-	-
39	*cis*-Pinocarveol	1182	-	-	-	-	-	2.2	2.6	-	-	-	-	-	-	-	-	-	-	-	-
40	α-Terpineol	1186	1.9	2.3	1.2	1.6	1.7	1.2	1.0	-	-	-	-	-	1.2	-	0.3	-	-	-	-
41	Myrtenol	1194	0.4	0.3	0.2	-	-	-	0.1	-	-	-	-	-	-	0.2	-	-	-	-	-
42	Methyl Chavicol	1195	-	-	-	-	-	-	-	41.1	17.8	-	-	-	-	-	-	-	-	-	-
43	*trans*-Carveol	1215	-	-	0.7	1.2	0.1	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-
44	*cis*-Carveol	1226	0.3	-	-	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	D-Carvone	1239	0.3	0.4	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	2.6	-	-
46	α-Citral	1240	-	-	0.2	-	1.3	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-
47	Cuminal	1242	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	Car-3-en-2-one	1244	0.2	-	0.1	0.2	-	-	0.2	-	-	-	-	-	-	-	-	-	0.4	1.8	0.3
49	*trans*-Geraniol	1249	0.7	0.9	-	-	0.9	1.1	0.9	-	-	-	-	-	-	-	-	-	0.7	-	-
50	Chrysanthenyl acetate	1261	0.3	-	0.2	0.8	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	Geranial	1264	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	*cis*-Verbenyl acetate	1280	-	0.4	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	(*E*)-Anethole	1282	-	-	-	-	-	-	-	10.2	8.6	0.4	-	-	-	-	-	0.1	-	-	-
54	Bornyl acetate	1284	1.5	2.0	0.3	5.5	0.7	2.6	1.3	1.2	2.0	2.3	-	-	1.4	1.2	0.9	0.7	3.6	1.6	16.5
55	Thymol	1289	-	-	0.1	0.3	0.2	0.1	-	-	0.4	-	-	-	0.2	0.5	0.7	-	0.2	5.1	0.7
56	Carvacrol	1298	-	-	-	-	-	-	-	2.4	4.5	1.2	-	2.1	0.3	1.3	-	0.8	0.7	-	0.6
57	α-Terpinyl acetate	1346	-	-	-	-	-	-	-	0.6	1.5	-	-	-	0.1	-	0.4	1.9	2.1	0.1	0.2
58	Eugenol	1356	-	-	-	0.7	0.3	5.5	0.3	0.1	1.1	-	-	-	1.6	-	1.1	0.4	-	-	-
59	α-Copaene	1374	-	-	-	-	-	-	-	1.2	2.3	-	-	-	0.3	-	0.6	0.3	0.6	-	-

60	Methyl cinnamate	1376	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
61	Geranyl acetate	1379	2.9	3.7	2.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-
62	β-Panasinsene	1381	1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
63	Hexyl hexanoate	1382	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	0.6	-	-	-
64	β-Cubebene	1387	-	-	0.2	0.5	-	0.3	0.9	-	-	0.5	-	-	0.4	-	-	-	-	0.4	-
65	β-Elemene	1389	-	-	1.1	5.3	0.9	2.1	0.2	-	0.1	2.4	-	-	1.3	-	-	-	1.1	1.2	0.6
66	*cis*-Jasmone	1390	1.3	1.8	0.6	0.4	0.2	0.8	0.5	-	-	-	-	-	-	-	-	-	0.5	-	-
67	β-Longipinene	1400	-	2.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	Methyl eugenol	1403	-	-	-	-	-	-	-	10.2	13.5	-	-	-	0.3	-	-	-	0.6	-	-
69	α-Gurjunene	1409	-	-	-	2.0	0.2	0.7	0.2	-	-	-	-	-	-	-	-	-	0.9	-	-
70	α-Cedrene	1410	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	-
71	(*E*)-Caryophyllene	1417	0.3	-	0.8	0.5	0.8	2.6	7.1	0.5	0.7	-	2.4	-	2.3	-	-	1.1	0.4	8.1	3.4
72	β-Copaene	1430	-	-	-	-	0.1	-	-	0.1	-	-	-	-	0.3	1.4	1.9	0.8	-	-	4.3
73	β-Gurjunene	1431	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-	0.2
74	Coumarin	1432	-	-	0.1	-	0.6	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-
75	Aromadendrene	1439	-	-	0.3	2.5	-	1.1	0.4	-	-	-	-	0.4	0.5	-	3.4	-	1.9	4.5	0.3
76	β-Calarene	-	-	-	-	0.3	-	-	-	0.6	-	0.8	-	-	-	0.2	-	-	-	-	-
77	Spirolepechinene	1449	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
78	Davana ether	1450	2.8	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79	β-Cadinene	1472	-	-	0.1	3.6	1.1	2.5	0.1	-	-	-	-	-	0.6	-	-	-	-	-	-
80	γ-Gurjunene	1475	-	-	-	-	0.4	0.4	0.3	-	-	-	-	-	-	-	0.1	-	-	-	-
81	Cadina-1,6-diene	1475	-	-	-	-	0.1	0.6	-	-	-	1.0	-	-	-	-	-	-	-	-	-
82	γ-Himachalene	1481	-	-	0.5	1.5	-	0.8	-	-	-	-	-	-	0.4	-	-	-	-	-	-
83	Germacrene-D	1484	-	-	-	4.3	-	12.1	16.7	-	0.8	0.2	-	5.8	3.1	0.6	0.3	-	3.0	2.2	4.8
84	β-Selinene	1489	-	-	0.5	1.2	-	2.1	1.3	-	-	-	-	-	2.2	1.1	-	0.1	0.2	-	-
85	Capillene	1493	-	-	-	-	-	-	-	3.1	2.5	-	-	-	-	-	-	0.6	-	-	-
86	α-Selinene	1498	-	-	-	-	-	1.2	-	-	-	-	-	-	0.2	-	0.7	-	-	-	-
87	Bicyclogermacrene	1500	-	-	-	-	-	-	-	0.9	0.1	-	-	-	0.9	-	-	1.2	0.8	-	0.8
88	γ-Cadinene	1513	-	-	-	-	-	-	-	0.5	0.3	0.3	2.5	10.4	17.2	-	-	2.2	0.9	0.7	4.1
89	Elemicin	1555	-	-	-	-	-	-	-	1.5	7.5	-	-	-	-	-	-	3.9	-	-	-
90	Germacrene-B	1559	-	-	-	-	-	-	-	0.9	0.4	-	-	-	0.8	-	0.2	2.6	-	0.8	2.1
91	(*E*)-Nerolidol	1561	0.3	-	-	0.4	0.5	0.3	-	1.1	-	-	-	-	-	2.9	-	0.5	-	-	-
92	Davanone B	1564	0.5	-	2.1	0.4	1.3	2.8	3.8	-	-	0.6	-	-	-	-	-	-	-	-	-
93	(*E*)-Isoelemicin	1568	-	-	-	-	-	-	-	2.5	2.3	-	-	-	-	-	-	3.1	-	-	-
94	(*Z*)-Isoelemicin	1568	-	-	-	-	-	-	-	-	0.2	0.5	3.4	1.1	-	-	-	1.2	-	-	-

95	Spathulenol	1577	-	1.7	1.1	-	0.6	0.6	-	0.1	1.5	-	-	-	0.5	2.7	0.3	0.2	0.6	1.7	-
96	Artedouglasia oxide B	1581	0.3	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
97	Caryophyllene oxide	1582	0.6	-	0.7	0.6	-	0.5	-	-	-	-	4.1	-	-	-	4.7	-	-	-	-
98	*cis*-Davanone	1587	10.0	19.8	3.2	0.3	0.1	0.5	1.4	-	-	-	-	-	-	-	-	-	-	-	-
99	δ-Undecalactone	1593	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	Gymnomitrone	1631	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
101	Methyl jasmonate	1648	0.2	-	1.2	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-
102	Valerianol	1656	-	-	1.1	-	0.3	0.2	0.5	-	-	-	-	-	-	-	-	-	-	-	-
103	*epi*-Zizanone	1668	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
104	(*E*)-Asarone	1675	-	-	-	-	-	-	-	3.1	0.4	-	-	-	-	-	-	0.6	-	-	-
105	α-Bisabolol	1685	-	-	-	0.9	-	1.2	0.4	-	-	1.5	-	-	-	-	-	-	-	-	-
106	Vetiselinenol	1730	1.9	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
107	(*Z*)-Nerolidyl isobutyrate	1783	-	0.5	-	-	0.7	1.0	2.3	-	-	-	-	-	-	-	-	-	1.5	-	-
108	Phytol	1942	-	-	0.1	2.9	1.9	2.4	0.8	-	-	0.4	-	-	-	-	-	-	0.7	0.4	1.9
MH[c]	Number		10	3	12	10	12	8	5	9	11	8	8	6	14	10	14	11	12	12	10
	Percentage (%)		16	5.5	52.4	31	43.5	15.1	23.3	17.7	27	26.7	47.5	18.7	40.5	29.8	65.8	34.3	13.9	13.2	12.9
OM[d]	Number		25	18	25	25	22	17	20	5	11	10	7	7	13	11	19	10	22	16	16
	Percentage (%)		63.9	66.4	34.7	40.3	45.9	42.5	37.0	5.1	11.4	65.3	40.0	62.9	25.4	60.9	20.2	45.4	72.3	66.7	64.2
SH [e]	Number		2	2	8	10	7	12	11	6	8	5	2	3	15	3	8	7	9	7	9
	Percentage (%)		1.7	2.6	3.8	21.6	3.6	26.5	28.3	4.1	5.5	4.4	4.9	16.6	30.7	3.1	7.8	8.3	9.8	17.9	20.6
OS[f]	Number		8	6	5	5	6	9	5	2	1	2	1	0	1	2	2	2	2	1	0
	Percentage (%)		17	25.3	8.2	2.6	3.5	7.2	8.4	1.2	1.5	2.1	4.1	0	0.5	5.6	5	0.7	2.1	1.7	0
OD [g]	Number		0	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0	1	1	1
	Percentage (%)		0	0	0.1	2.9	1.9	2.4	0.8	0	0	0.4	0	0	0	0	0	0	0.7	0.4	1.9
NH[h]	Number		2	0	1	1	2	1	2	8	9	3	1	1	2	0	1	8	1	0	0
	Percentage (%)		0.9	0	0.1	0.7	0.9	5.5	1.5	71.8	53.9	1	3.4	1.1	1.9	0	1.1	10.5	0.6	0	0
	Total percentage (%)		**99.5**	**99.8**	**99.3**	**99.1**	**99.3**	**99.2**	**99.3**	**99.9**	**99.3**	**99.9**	**99.9**	**99.3**	**99.0**	**99.4**	**99.9**	**99.2**	**99.4**	**99.9**	**99.6**

[a] The characterized compounds have been listed based upon their retention indices on an HP-5 MS capillary column, [b] Kovats indices reported in the reference, [c] MH: Monoterpene hydrocarbons,[d] OM: Oxygenated monoterpenes, [e] SH: Sesquiterpene hydrocarbons, [f] OS: Oxygenated sesquiterpenes, [g] OD: Oxygenated diterpenes, [h] NH: Non-terpene hydrocarbons, [i] Trace levels

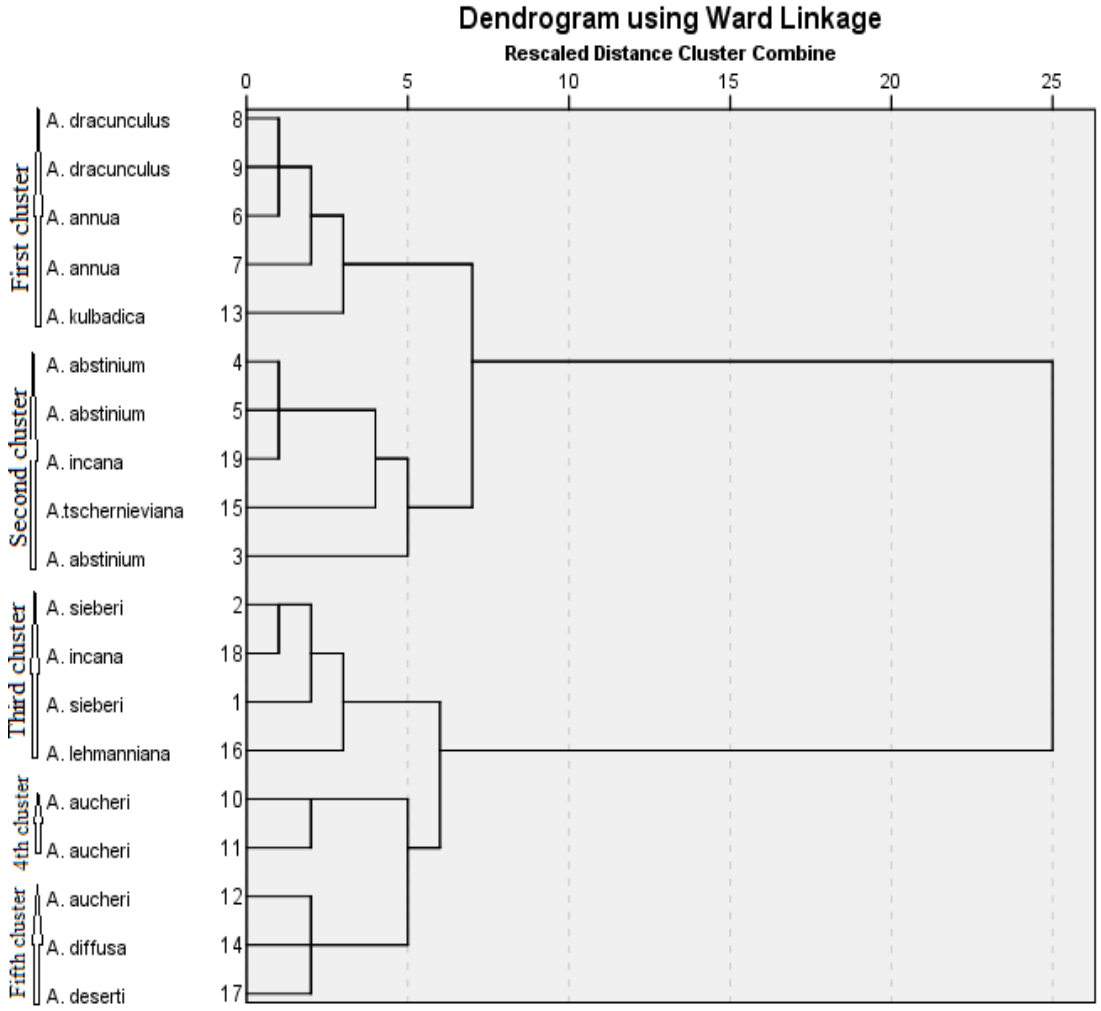

Figure 2. Dendrogram of *Artemisia* EOs related to nineteen accessions of Iran regarding the six dominant compounds in their profiles using Ward's minimum variance approach.

Finally, the last (fifth) cluster consisted of the EOs of S12, S14, and S17. The frequency of its major constituents was similar to that of the fourth cluster with one exception resulting from the absence of sabinene in S12. In each of these three populations, camphor and β-thujone dominated the chemical profiles.

3.4. Principal component analysis extraction method (PCAEM)

In recent years, PCAEM has gained much attention in the rational classification of compounds and in the determination of outliers in the main dataset [64, 65].

Application of PCAEM to seven major common components in the 19 profiles of the EOs extracted using MAHD revealed possible variations in chemical compositions of the EOs from *Artemisia* plants. As shown in Table 3, the first three principal components included a total variance of 73.05%. Furthermore, the first, second, and third extraction communalities were referred to as sabinene, camphor, and β-thujone, respectively. The table also displays that the first PC accounted for 27.191% of the total variance positively and negatively correlating with β-thujone (0.938) and 1,8-cineole (-0.844), respectively. Conversely, camphene positively correlated with the second PC,

whereas the minimum correlation coefficient of PC2 was related to α-pinene. Regarding the third PC encompasing 22.824% of the total variance, the highest and the lowest correlations were referred to camphor and sabinene, respectively.

Table 3. The results of principal component analysis extraction method considering seven dominant natural compounds in all profiles of nineteen species of *Artemisia* genus applying rotated component matrix [a, b].

Number	Major compounds in EOs	Extraction communalities	Component		
			1	2	3
1	Camphor	**0.932**	0.422	0.388	**0.777**
2	1,8-Cineole	0.750	**-0.844**	0.190	-0.046
3	Camphene	0.492	-0.214	**0.656**	0.127
4	α-Pinene	0.532	0.001	**-0.702**	0.200
5	β-Pinene	0.539	-0.282	-0.665	-0.131
6	β-Thujone	**0.919**	**0.938**	0.191	0.054
7	Sabinene	**0.949**	0.089	0.157	**-0.957**
		Total	2.266	1.475	1.372
		Variance%	32.369	21.071	19.607
	Initial Eigenvalues	Cumulative %	32.369	53.440	73.047
		Total	2.266	1.475	1.372
		Variance%	32.369	21.071	19.607
	Extraction Sums of Squared Loadings	Cumulative %	32.369	53.440	73.047
		Total	1.903	1.612	1.598
		Variance%	27.191	23.032	22.824
	Rotation Sums of Squared Loadings	Cumulative %	27.191	50.223	**73.047**

[a] Rotation method: Varimax with Kaiser Normalization
[b] Rotation converged in 5 iterations

4. Conclusion

The current study describes the major natural compounds in 19 MAHD-extracted EOs from *Artemisia* plants in the full flowering stage. Different accessions of Iran were selected for the collection of the plant samples over a wide variety of geographical coordinates. The climatic conditions of the sampling areas were specific and different from each other. In the identified chemical profiles of S1, S2, S4-S7, S10, S12, S14, and S16-S19, the dominant groups of constituents were oxygenated monoterpenes. Meanwhile, monoterpene hydrocarbons had the highest frequencies in S3, S11, S13, and S15, and non-terpene hydrocarbons such as methyl chavicol, methyl eugenol, and (*Z*)-anethole dominated in S8 and S9. Natural compounds such as camphor, 1,8-cineole, camphene, α-pinene, β-pinene, and β-thujone were prevalent in all profiles. Although plants growing near each other may logically display similar chemical profiles, the observed dissimilarities between the chemical profiles of the EOs could be attributed to the plant habitats, climatic conditions during plant growth, soil composition, and relative humidity of the sampling areas, among others. With the HCA approach, the following five chemotypes were recognized by qualitative and quantitative differences in the percentages of the six main constituents present in all of the EO samples which were showed in Figure 2 and Table 1.

In PCAEM, sabinene was ranked seventh among the EO samples. Accordingly, promising results were obtained using the first three principal components, which comprised about 73% of the total variance. The availability of diverse chemotypes allows us to develop and improve cultivars to produce and process EOs comprising valuable natural compounds, such as camphor, 1,8-cineole, and β-thujone. This outcome can be considerably generalized to a large industrial scale depending on the market and trade needs. However, such an idea necessitates further investigations among larger populations through selection of proper breeding methods, good chemotaxonomic characterization, and preparation of homogenous cultivars.

Acknowledgments

Technical and financial supports provided by the Office for Research Affairs of the Islamic Azad University, Shahrood Branch are acknowledged.

Supporting Information

Supporting Information accompanies this paper on www.acgpubs.org/RNP

References

[1] K. Morteza-Semnani, and M. Akbarzadeh (2005). Essential oils composition of Iranian *Artemisia absinthium* L. and *Artemisia scoparia* Waidst. et Kit, *J. Essent. Oil Res.* **17**, 321-322.
[2] L. Belenovskaja (1996). *Artemisia*: the flavonoids and their systematic value. In: D.J.N. Hind & H.J. Beentje (Eds.). Compositae: Systematics International Compositae Conference, Kew: Royal Botanic Gardens, pp. 253-259.
[3] A. Singh, R.A. Vishwakarma, and A. Husain (1988). Evaluation of *Artemisia annua* strain for higher artemisinin production, *Planta Med.* **34**, 475-477.
[4] A. Rustaiyan, A. Bamoniri, M. Raffatrad, J. Jakupovic, and F. Bohlinan (1987). Eudesmane derivatives and highly oxygenated monoterpenes from Iranian *Artemisia* species, *Phytochemistry* **26**, 2307-2310.
[5] M. Nekoei, M. Mohammadhosseini, and H. Akhlaghi (2012). Chemical composition of the volatile oils from the aerial parts of *Artemisia annua* L. (Asteraceae) by using head space solid phase microextraction and hydrodistillation methods prior to gas chromatographic-mass spectrometric determination: A comparative investigation, *J. Essent. Oil-Bear. Plants* **15**, 926-933.
[6] P. Weyerstahl, S. Schneider, H. Marschall, and A. Rustaiyan (1993). The essential oil of *Artemisia sieberi* Bess., *Flav. Fragr. J.* **8**, 139-145.
[7] P. Weyerstahl, S. Schneider, H. Marschall, and A. Rustaiyan (1993). New bisabolene derivatives and salsoleneketone from *Artemisia sieberi* Bess., *Liebigs Ann. Chem.* **193**, 111-116.
[8] A. Rustaiyan, H. Sigari, J. Jakupovic, and M. Grenz (1989). A sesquiterpene lactone from *Artemisia diffusa*, *Phytochemistry* **28**, 2723-2725.
[9] A. Rustaiyan, K. Zare, M.T. Ganji, and H.A. Sadri (1989). A melampolide and two dihydro artemorin derivatives from *Artemisia gypsacea*, *Phytochemistry* **28**, 1535-1536.
[10] M.B. Pasha Zanousi, M. Nekoei, and M. Mohammadhosseini (2016). Composition of the essential oils and volatile fractions of *Artemisia absinthium* by three different extraction methods: Hydrodistillation, solvent-free microwave extraction and headspace solid-phase microextraction combined with a novel QSRR evaluation, *J. Essent. Oil-Bear. Plants*, **19**, 1561-1581.
[11] M. Mohammadhosseini, A. Akbarzadeh, H. Hashemi-Moghaddam, A. Mohammadi Nafchi, H.A. Mashayekhi, and A. Aryanpour (2016). Chemical composition of the essential oils from the aerial parts of *Artemisia sieberi* by using conventional hydrodistillation and microwave assisted hydrodistillation: A comparative study, *J. Essent. Oil-Bear. Plants* **19**, 32-45.
[12] F. Irshaid, K. Mansi, and T. Aburjai (2010). Antidiabetic effect of essential oil from *Artemisia sieberi* growing in Jordan in normal and alloxan induced diabetic rats, *Pak. J. Biol. Sci.* **13**, 423-430.
[13] F.I. Irshaid, K. Mansi, A. Bani-Khaled, and T. Aburjia (2012). Hepatoprotetive, cardioprotective and nephroprotective actions of essential oil extract of *Artemisia sieberi* in alloxan induced diabetic rats, *Iran. J. Pharm. Res.* **11**, 1227-1234.
[14] F.I. Irshaid, K.A. Tarawneh, J.H. Jacob, and A.M. Alshdefat (2014). Phenol content, antioxidant capacity and antibacterial activity of methanolic extracts derived from four Jordanian medicinal plants, *Pak. J. Biol. Sci.* **17**, 372-379.
[15] A.R. Khosravi, H. Shokri, M.H. Darabi, A. Kashani, P. Mansouri, and A. Naser (2009). Comparative study on the effects of a new antifungal lotion (*Artemisia sieberi* essential oil) and a clotrimazole lotion in the treatment of pityriasis versicolor, *J. Mycol. Med.* **19**, 17-21.
[16] A.R. Khosravi, H. Shokri, S. Kermani, M. Dakhili, M. Madani, and S. Parsa (2011). Antifungal properties of *Artemisia sieberi* and *Origanum vulgare* essential oils against *Candida glabrata* isolates obtained from patients with vulvovaginal candidiasis, *J. Mycol. Med.* **21**, 93-99.

[17] S. Mashhady-Rafie, S. Baradaran-Alizadeh, and M. Bayat (2013). Comparison of the therapeutic effects of nano-essence of medical herb *Artemisia sieberi* with the ointment of Ketoconazole in guinea pig infected by *Microsporum canis*, *Int. Res. J. Biol. Sci.* **2**, 5-10.
[18] M.H. Dashti, A. Morshedi, M. Dehghan, and M.A. Bagherinasab (2011). The effect of *Artemisia sieberi* Besser on inflammatory and neurogenic pain in mice, *J. Med. Plants* **10**, 48-57.
[19] H.A. Arab, S. Rahbari, A. Rassouli, M.H. Moslemi, and F. Khosravirad (2006). Determination of artemisinin in *Artemisia sieberi* and anticoccidial effects of the plant extract in broiler chickens, *Trop. Anim. Health Pro.* **38**, 497-503.
[20] J.K. Katadj, K.P.K. Abadi, S. Bahadoran, and M. Cheraghchibashi (2011). Comparison the anticoccidial effects of artemisinin granule prepared from *Artemisia sieberi* extract with monensin in experimental broiler chicken coccidiosis, *Planta Med.* **77**, 1458-1458.
[21] H. Nahrevanian, B. Sheykhkanlooye-Milan, M. Kazemi, R. Hajhosseini, S. Soleymani-Mashhadi, and S. Nahrevanian (2012). Antimalarial effects of Iranian Flora *Artemisia sieberi* on *Plasmodium berghei in vivo* in mice and phytochemistry analysis of its herbal extracts, *Malar. Res. Treat.* **2012**, 1-8.
[22] S.A. Emami, N. Vahdati-Mashhadian, R. Vosough, and M.B. Oghazian (2009). The anticancer activity of five species of *Artemisia* on Hep2 and HepG2 cell lines, *Pharmacologyonline* **3**, 327-339.
[23] M. Mohammadhosseini (2016). A Comprehensive Review on New Methods for Processing, Separation and Identification of the Essential Oils, Islamic Azad University of Shahrood Press, Shahrood, Iran.
[24] A. Zargari (1996). Medicinal Plants, Tehran University Publication, Tehran.
[25] R. Omidbaigi (2012). Production and Processing of Medicinal Plants, 6th ed., Behnashr, Astan Ghods Razavi Press, Tehran.
[26] A. Ghasemi (2009). Medicinal and Fragrant Plants, Identification and Evaluation of Their Effects, Islamic Azad University, Shahrekord Press, Shahrekord, Iran.
[27] M. Mohammadhosseini, and M. Nekoei (2014). Chemical compositions of the essential oils and volatile compounds from the aerial parts of *Ferula ovina* using hydrodistillation, MAHD, SFME and HS-SPME methods, *J. Essent. Oil-Bear. Plants* **17**, 747-757.
[28] F. Chemat, and G. Cravotto (2013). Microwave-Assisted Extraction for Bioactive Compounds, Theory and Practice, Springer, New York.
[29] K. Ganzler, A. Salgo, and K. Valko (1986). Microwave extraction: A novel sample preparation method for chromatography, *J. Chromatogr.* **371**, 299-306.
[30] L.N. Thach, T.H. Nhung, V.T.N. My, and H.A. Tran (2013). The new rich source of rotundifolone: *Mentha aquatica* Linn. var. crispa oil from microwave-assisted hydrodistillation, *J. Essent. Oil Res.* **25**, 39-43.
[31] M. Mohammadhosseini, and M. Nekoei (2014). Chemical compositions of the essential oils and volatile compounds from the aerial parts of *Ferula ovina* using hydrodistillation, MAHD, SFME and HS-SPME methods, *J. Essent. Oil-Bear. Plants* **17**, 747-757.
[32] Y.Q. Liu, H.W. Wang, S.L. Wei, and Z.J. Yan (2012). Chemical composition and antimicrobial activity of the essential oils extracted by microwave-assisted hydrodistillation from the flowers of two *Plumeria* species, *Anal. Lett.* **45**, 2389-2397.
[33] N. Manika, C. Singh, C. M. Darokar, S. Singh, and G. Bagchi (2016). Compositional characters and antimicrobial potential of *Artemisia stricta* Edgew. f. *stricta* Pamp. essential oil, *Rec. Nat. Prod.* **10,** 40-46.
[34] H. Shamkhani, N. Nasiri, A. Aliahmadi, and A. Sonboli (2016). Essential oil composition and antibacterial activity of *Tanacetum hololeucum* from Iran, *Rec. Nat. Prod.* **10**, 818-823.
[35] K.H. Rechinger (1963). Flora Iranica, Akademische Druke-U. Verlagsanstalt, Wien, Austria.
[36] M. Mohammadhosseini, A. Akbarzadeh, A. Shafaghat, H. Hashemi-Moghaddam, A. Mohammadi Nafchi, and H. Ashouri (2016). Chemical composition of the essential oils from flowers and leaves of *Marsdenia erecta* using microwave assisted hydrodistillation technique, *J. Essent. Oil-Bear. Plants* **19**, 863-874.
[37] M. Nekoei, and M. Mohammadhosseini (2016). Chemical compositions of the essential oils from the aerial parts of *Achillea wilhelmsii* using traditional hydrodistillation, microwave assisted hydrodistillation and solvent-free microwave extraction methods: Comparison with the volatile compounds obtained by headspace solid-phase microextraction, *J. Essent. Oil-Bear. Plants* **19**, 59-75.
[38] H. Akhlaghi, M. Nekoei, M. Mohammadhosseini, and A. Motavalizadehkakhky (2012). Chemical composition of the volatile oils from the flowers, stems and leaves of *Prangos latiloba* Korov. using the head space solid phase microextraction method prior to analysis by gas chromatography-mass spectrometry, *J. Essent. Oil-Bear. Plants* **15**, 328-335.
[39] M. Mohammadhosseini, B. Mahdavi, and H. Akhlaghi (2013). Characterization and chemical composition of the volatile oils from aerial parts of *Eryngium bungei* Bioss. (Apiaceae) by using traditional hydrodistillation, microwave assisted hydrodistillation and head space solid phase microextraction methods prior to GC and GC/MS analyses: A comparative approach, *J. Essent. Oil-Bear. Plants* **16**, 613-623.

[40] M. Mohammadhosseini, M. Nekoei, H.A. Mashayekhi, and J. Aboli (2012). Chemical composition of the essential oil from flowers, leaves and stems of *Haplophyllum perforatum* by using head space solid phase microextraction, *J. Essent. Oil-Bear. Plants* **15**, 506-515.
[41] M. Mohammadhosseini, A. Pazoki, and H. Akhlaghi (2008). Chemical composition of the essential oils from flowers, stems, and roots of *Salvia multicaulis* growing wild in Iran, *Chem. Nat. Comp.* **44**, 127-128.
[42] M. Mohammadhosseini, H.A. Zamani, H. Akhlaghi, and M. Nekoei (2011). Hydrodistilled volatile oil constituents of the aerial parts of *Prangos serpentinica* (Rech.f., Aell. Esfand.) Hernnstadt and Heyn from Iran and quantitative structure-retention relationship simulation, *J. Essent. Oil-Bear. Plants* **14**, 559-573.
[43] A.R. Motavalizadehkakhky, A. Shafaghat, H.A. Zamani, H. Akhlaghi, M. Mohammadhosseini, J. Mehrzad, and Z. Ebrahimi (2013). Compositions and the *in vitro* antimicrobial activities of the essential oils and extracts of two *Achillea* species from Iran, *J. Med. Plants Res.* **7**, 1280-1292.
[44] H. Hashemi-Moghaddam, M. Mohammadhosseini, and M. Salar (2014). Chemical composition of the essential oils from the hulls of *Pistacia vera* L. by using magnetic nanoparticle-assisted microwave (MW) distillation: Comparison with routine MW and conventional hydrodistillation, *Anal. Methods* **6**, 2572-2579.
[45] M. Nekoei, and M. Mohammadhosseini (2014). Application of HS-SPME, SDME and cold-press coupled to GC/MS to analysis the essential oils of *Citrus sinensis* CV. Thomson Navel and QSRR study for prediction of retention indices by stepwise and genetic algorithm-multiple linear regression approaches, *Anal. Chem. Lett.* **4**, 93-103.
[46] M. Mohammadhosseini (2015). Chemical composition of the volatile fractions from flowers, leaves and stems of *Salvia mirzayanii* by HS-SPME-GC-MS, *J. Essent. Oil-Bear. Plants* **18**, 464-476.
[47] H. Hashemi-Moghaddam, M. Mohammadhosseini, and M. Basiri (2015). Optimization of microwave assisted hydrodistillation on chemical compositions of the essential oils from the aerial parts of *Thymus pubescens* and comparison with conventional hydrodistllation, *J. Essent. Oil-Bear. Plants* **18**, 884-893.
[48] M. Mohammadhosseini (2015). Chemical composition of the essential oils and volatile fractions from flowers, stems and roots of *Salvia multicaulis* Vahl. by using MAHD, SFME and HS-SPME methods, *J. Essent. Oil-Bear. Plants* **18**, 1360-1371.
[49] M. Mohammadhosseini, B. Mahdavi, and M. Shahnama (2015). Chemical composition of essential oils from aerial parts of *Ferula gummosa* (Apiaceae) in Jajarm Region, Iran using traditional hydrodistillation and solvent-free microwave extraction methods: A comparative approach, *J. Essent. Oil-Bear. Plants* **18**, 1321-1328.
[50] R.P. Adams (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, Allured Pubishing Co., Carol Stream, IL., USA.
[51] L. Ahamdi, and M. Mirza (2001). Chemical composition of essential oils from two Iranian species of *Artemisia*, *J. Essent. Oil Res.* **13**, 30-30.
[52] M. Bailen, L.F. Julio, C.E. Diaz, J. Sanz, R.A. Martinez-Diaz, R. Cabrera, J. Burillo, and A. Gonzalez-Coloma (2013). Chemical composition and biological effects of essential oils from *Artemisia absinthium* L. cultivated under different environmental conditions, *Ind. Crop Prod.* **49**, 102-107.
[53] P. Blagojevic, N. Radulovic, R. Palic, and G. Stojanovic (2006). Chemical composition of the essential oils of Serbian wild-growing *Artemisia absinthium* and *Artemisia vulgaris*, *J. Agric. Food. Chem.* **54**, 4780-4789.
[54] E. Ghasemi, Y. Yamini, N. Bahramifar, and F. Sefidkon (2007). Comparative analysis of the oil and supercritical CO_2 extract of *Artemisia sieberi*, *J. Food Eng.* **79**, 306-311.
[55] S. Kordali, I. Aslan, O. Calmasur, and A. Cakir (2006). Toxicity of essential oils isolated from three *Artemisia* species and some of their major components to granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), *Ind. Crop Prod.* **23**, 162-170.
[56] R.D. Bidgoli, M. Pessarakli, G.A. Heshmati, and A.H. Ebrahimabadi (2013). Effects of topographic factors of the site on the essential oil compounds of *Artemisia aucheri* aerial parts grown in a mountainous region, *Commun. Soil Sci. Plant Anal.* **44**, 2618-2624.
[57] S.K. Mohammadpoor, M. Yari, A. Rustaiyan, and S. Masoudi (2002). Chemical constituents of the essential oil of *Artemisia aucheri* Boiss. a species endemic to Iran, *J. Essent. Oil Res.* **14**, 122-123.
[58] M.R. Akhgar, P. Rajaei, and O. Alizadeh-Saljoughi (2013). Chemical composition of the essential oil of *Artemisia lehmanniana* Bunge growing wild in Iran, *J. Essent. Oil-Bear. Plants* **16**, 641-645.
[59] B. Cetin, H. Ozer, A. Cakir, E. Mete, M. Tosun, E. Ozturk, T. Polat, and A. Kandemir (2009). Chemical composition of hydrodistilled essential oil of *Artemisia incana* (L.) Druce and antimicrobial activity against foodborne microorganisms, *Chem. & Biodiv.* **6**, 2302-2310.
[60] A. Rustaiyan, S. Masoudi, and M. Kazemi (2007). Volatile oils constituents from different parts of *Artemisia ciniformis* Krasch Et M. Pop. ex Poljak and *Artemisia incana* (L.) Druce. from Iran, *J. Essent. Oil Res.* **19**, 548-551.

[61] K. Morteza-Semnani, M. Saeedi, and M. Akbarzadeh (2008). The essential oil composition of *Artemisia tschernieviana* Besser, *J. Essent. Oil Res.* **20**, 109-111.
[62] S. Kordali, R. Kotan, A. Mavi, A. Cakir, A. Ala, and A. Yildirim (2005). Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils, *J. Agric. Food. Chem.* **53**, 9452-9458.
[63] K. Heberger (1999). Evaluation of polarity indicators and stationary phases by principal component analysis in gas-liquid chromatography, *Chemometr. Intell. Lab. Syst.* **47**, 41-49.
[64] S. Wold, K. Esbensen, and P. Geladi (1987). Principal Component Analysis, *Chemometr. Intell. Lab. Syst.* **2**, 37-52.