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Variability of the essential oil content and composition among the wild populations of *Achillea biebersteinii* Afan. from Iran: occurrence of new nepetalactones chemotypes

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In this study, the chemical composition of essential oils from twenty-three wild populations of *Achillea biebersteinii* Afan., growing wild in different parts of Iran, were determined by means of gas chromatography (GC) and GC–mass spectrometry (GC–MS) analyses. Based on the dry weight of samples, the oil content of studied populations varied from 0.44% (Nenor) to 1.62% (Golool) with an average value of 0.81%. In total, twenty-five compounds were identified in the essential oil of all samples corresponding about 92.5–99.1% of the chemical composition. All essential oils were characterized by a high amount of monoterpenoid compounds (99.1–99.2%), especially oxygenated ones (48.5–90%), which were almost dominant in all cases and with a trace amount of sesquiterpenes (0–0.3%). 1,8-Cineole (6.5–68.3%) was generally found as the principal component of most essential oils, followed by *p*-cymene (1.4–38.6%). The noticeable point of present study was the occurrence of nepetalactones chemotypes in plants of the *Nepeta* species. These compounds were detected in the essential oils of fourteen of twenty-three populations, which were mostly at least one of the three major components (4.3–43.3%) too.

Keywords: *Achillea biebersteinii* Afan.; essential oil; 1,8-cineole; *p*-cymene; nepetalactones; (*E*)-chrysanthenyl acetate; camphor

Introduction

The genus *Achillea* L. (commonly known as yarrow) belongs to the family Asteraceae and comprises more than 100 species worldwide. These often medicinal and rhizomatous perennial plants are native to Europe, Western Asia and North Africa, although they are also found in Australia, New Zealand and North America (1, 2). In traditional systems of medicine, *Achillea* species have a long history of use as medicinal plants mainly due to their anti-inflammatory, anti-spasmodic, diaphoretic, diuretic, carminative, tunic, vermifugal and emmenagogic properties, and are used as a cure for hemorrhage, pneumonia, rheumatic and abdominal pains, stomach ache and wounds (3–5). Nowadays, different medicinal properties of these plants such as spasmolytic, choleretic, anti-inflammatory and wound healing are documented (6). In recent years, the anticancer activity of essential oils isolated from some *Achillea* species has been reported and shown that can modulate macrophages activities (7). Due to the hair growth promotion properties, yarrow essential oils are used in cosmetic industries for the production of hair shampoos and creams (8).

In the flora of Iran, the genus *Achillea* is represented by nineteen species, of which seven are endemic. One of these species is *A. bieberstenii* Afan., which occurs naturally in many parts of the country in the central, north, northwest, west and northeast with the local name of 'Bumadarane Zard'. This plant is a perennial villose herb, 10–100 cm height, with radiate heads that are borne in large dense compound corymbs on the erect stems (3). To date, many investigations considered the volatile oil of *A. biebersteinii* from the chemical constituents to biological activities points of view (4, 5, 9–12). Based on the results of these studies, there is a considerable chemical polymorphism in the essential oil of this plant. These oils show different biological activities including antibacterial, antifungal, antioxidant, insecticidal, herbicidal and wound healing (5, 10, 13, 14). It is clear that the essential oil content and composition of medicinal and aromatic plants, and so their biological activities, are influenced by both intrinsic and external factors such as genetic background, climatic conditions, plant growth stages and type of plant part, as well postharvest processing of plant materials and method of extraction (15–18). A wide intraspecific genetic variation has been reported in the *A. biebersteinii* plants growing in Iran (19). This diversity may be originated from the adaptation process of these plants to the environmental conditions and

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their allogamy manner in pollination, which may has impact on the composition and biological activity of extracted essential oil (15, 20).

Research on the essential oil composition of different *Achillea* species leads to the identification of many chemotypes in these plants based on the major chemical components (16, 21). In Iran, most of the studies on the genus *Achillea* were done using one species, originating from a limited geographical area, and there is no comprehensive research considering variation in the essential oils of wild populations of *A. biebersteinii*. Therefore, the main aim of the present study was to expand the knowledge on the essential oil content and composition of twenty-three populations of *A. biebersteinii* growing wild in the different parts of the country, in order to find various chemotypes of this species, which may be used as initial materials for breeding programs and use in relevant industries.

Experimental

Plant materials and extraction of essential oils

The aerial parts of twenty-three wild populations of *A. biebersteinii* were collected at the full flowering stage from their natural habitats in different parts of Iran between April and May 2009 (Figure 1). Geographical and climatic conditions of each habitat were obtained from the nearest meteorology station (Table 1). Voucher specimens were deposited at the Ferdowsi University of Mashhad Herbarium, Mashhad, Iran.

For extraction of essential oil, an air-dried sample of each population (50 g) was separately hydrodistilled using a Clevenger-type apparatus for 3 hours, according to the method recommended in *British Pharmacopoeia* (22). To determine the oil content of plant materials, the experiment was repeated three times. After isolation, all essential oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis.

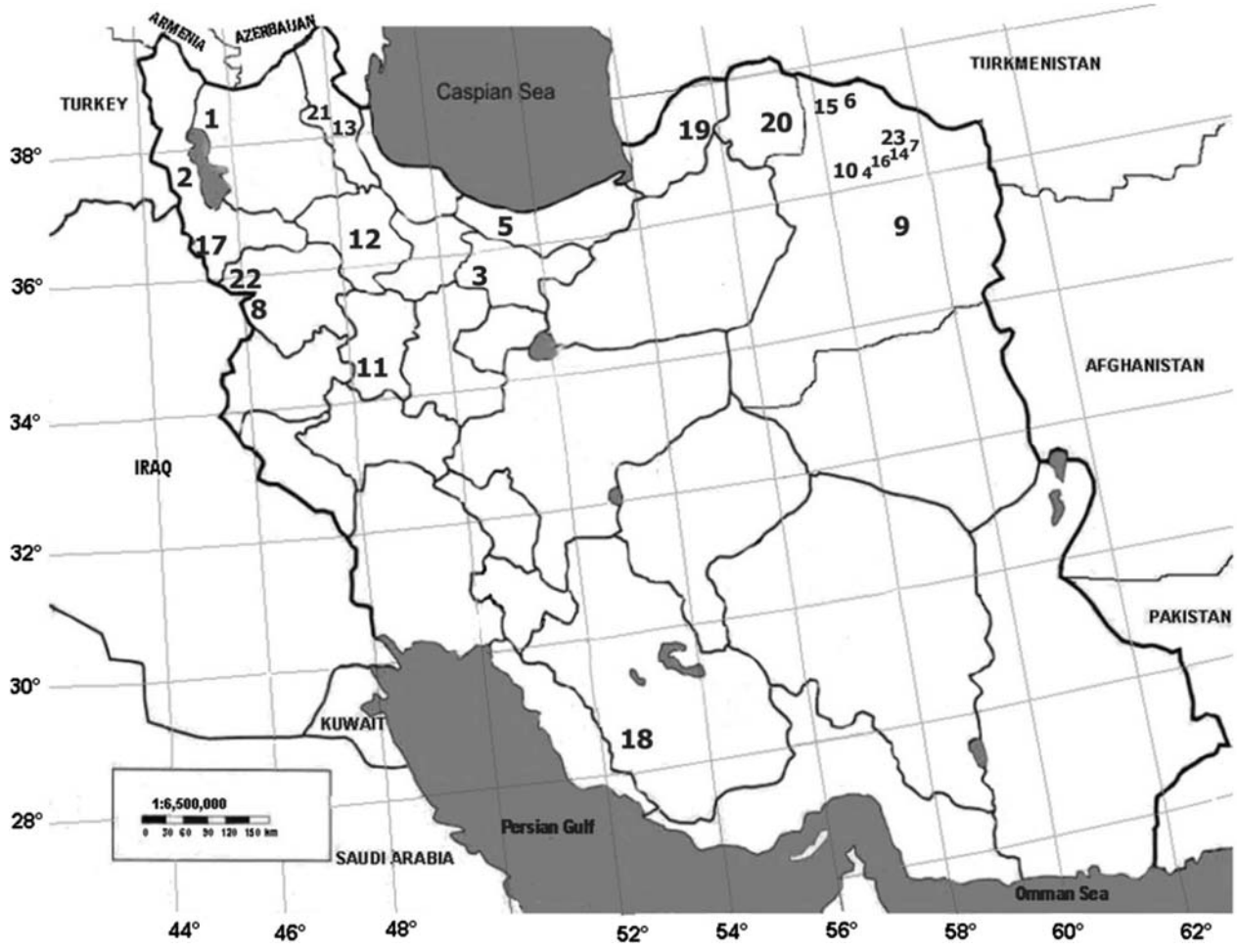

Figure 1. Collection sites of studied *Achillea biebersteinii* populations. For codes, see Table 1.

Table 1. Origin, geographical, climatic of natural habitats of twenty-three Iranian wild populations of *Achillea biebersteinii*.

Population no.	Site name (origion)	Longitude	Latitude	Altitude	T_{min}	T_{max}	H_{min}	H_{max}	H_{total}	P_{annal}	Sun
1	Mishoodagh, East Azarbaijan	45°38’	38°19’	2450	6.9	18	0.37	0.71	0.54	288.9	2794.3
2	Ghasemloo, West Azarbaijan	44°43’	37°29’	1340	5.4	17.6	0.42	0.78	0.6	341	2829.3
3	Mohammad Shahr, Tehran	50°32’	35°48’	1140	7.8	21.2	0.32	0.69	0.47	243.8	2959.7
4	Buzhan, Khorasan-e-Razavi	59°03’	36°13’	1600	6.7	21.8	0.3	0.7	0.49	239.8	3072.2
5	Siahbisheh, Mazandaran	51°33’	36°23’	1990	6.3	14.8	0.47	0.8	0.63	503.4	1959.4
6	Chelmir, Khorasan-e-Razavi	58°34’	37°31’	1584	6.8	19.7	0.4	0.8	0.58	272.4	2714
7	Goojgi, Khorasan-e-Razavi	59°56’	36°31’	2100	7.1	21.1	0.37	0.74	0.55	255.2	2892.4
8	Zaribar, Kordestan	46°08’	35°32’	1285	5	20.6	0.34	0.77	0.53	991.2	2967.9
9	Aman Abad, Khorasan-e-Razavi	59°32’	35°58’	1210	7.1	21.1	0.37	0.74	0.55	255.2	2894.4
10	Adag, Khorasan-e-Razavi	58°53’	36°11’	1260	6.7	21.8	0.3	0.7	0.49	239.8	3072.2
11	Eberoo, Hamedan	48°28’	34°41’	2250	3.3	19.1	0.36	0.77	0.54	316.6	2929.1
12	Zanjan, Zanjan	48°45’	36°30’	1640	4	18	0.37	0.75	0.54	313.1	2843.2
13	Sardabeh, Ardabil	48°15’	38°37’	1840	2.8	15.3	0.53	0.89	0.71	303.9	2454.3
14	Azghad, Khorasan-e-Razavi	59°24’	36°19’	1800	7.1	21.1	0.37	0.74	0.55	255.2	2892.4
15	Golool, North Khorasan	58°11’	37°37’	2100	6.8	17.5	0.4	0.7	0.53	252.7	2714
16	Golmakan, Khorasan-e-Razavi	59°13’	36°29’	1315	6.6	20.2	0.35	0.69	0.48	212.6	2898.2
17	Piranshahr, West Azarbaijan	45°04’	36°41’	1842	6.2	17.9	0.37	0.71	0.51	672.7	2766.4
18	Firooz Abad, Fars	52°37’	28°48’	1600	10.1	26.7	0.36	0.65	0.49	416.6	3358.6
19	Tangehgol, Golestan	55°49’	37°23’	220	11.7	23.9	0.55	0.82	0.68	564.1	2439.1
20	Havar, North Khorasan	57°11’	37°28’	2980	6.8	19.7	0.4	0.8	0.4	272.4	2714
21	Meshkin Shahr, Ardebil	47°38’	38°24’	1394	5.9	15.4	0.45	0.75	0.6	383.9	2503.2
22	Nenor, Kordestan	46°00’	35°52’	1830	8.7	18.6	0.34	0.58	0.44	689.3	2884.6
23	Ortokand, Khorasan-e-Razavi	59°51’	36°48’	1480	7.1	21.1	0.45	0.75	0.55	255.2	2892.4

Notes: $T_{min,}$ average of minimum temperature in year (°C); T_{max}, average of minimum temperature in year (°C); H_{min}, average of minimum relative humidity in year (%); H_{max}, average of maximum relative humidity in year (%); H_{total}, total relative humidity in year (%); P_{annals}, total of precipitation in year (mm); Sun, total of sunshine hours.

Oil analysis procedure

Gas chromatography (GC) analyses were performed using a Shimadzu GC-9 A gas chromatograph equipped with a DB-5 (dimethylsiloxane, 5% phenyl) fused silica column (J & W Scientific Corporation) (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 50°C for 5 minutes and then programmed to 240°C at a rate of 3°C/minute. Flame ionization

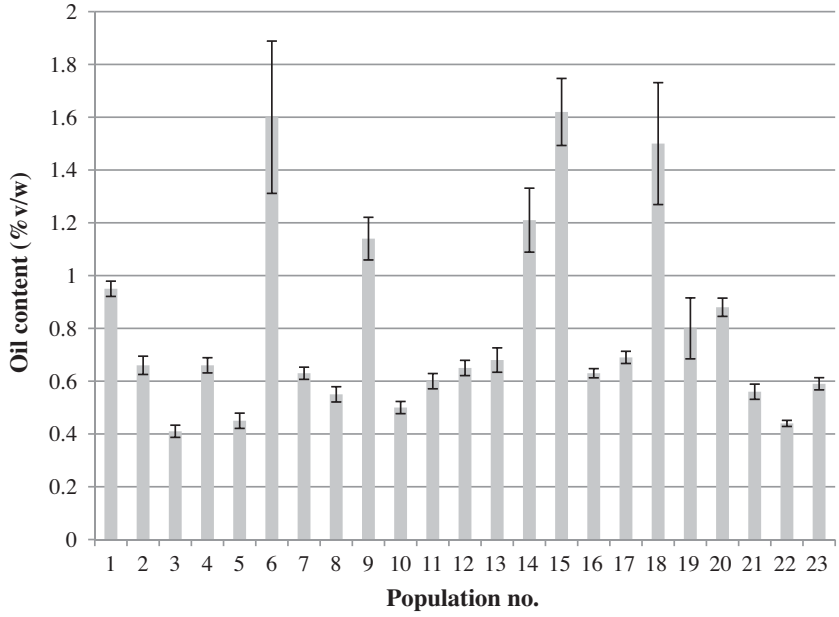

Figure 2. Variation of oil content (%v/w) among different Iranian wild populations of *Achillea biebersteinii*.

Table 2. Chemical composition of essential oils (%) of twenty-three Iranian wild populations of *Achillea biebersteinii*.

No.	Compounds	RIs	RI	Population number																						Method of identification
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
1	α-Thujene	930	931	tr	0.1	–	0.2	–	0.1	tr	0.1	tr	0.1	0.1	tr	0.3	0.1	0.2	–	0.1	0.1	–	–	0.1	0.1	0.1	RI, MS
2	α-Pinene	939	939	2.4	3.0	0.3	2.7	0.6	1.2	1.2	1.5	1.3	1.7	2.0	1.1	3.4	2.5	2.6	1.7	2.3	1.1	1.6	2.6	2.0	3.6	1.7	RI, MS, CoI
3	Camphene	954	952	6.7	3.0	0.1	1.2	0.4	0.4	0.3	0.5	0.2	0.9	2.2	0.6	1.8	0.7	0.4	0.5	1.9	0.3	1.0	2.1	2.9	4.9	0.1	RI, MS, CoI
4	Yomogi alcohol	999	998	0.3	0.4	2.3	0.6	0.3	0.6	0.4	0.1	0.4	0.6	0.2		2.1	0.5	0.9	0.5	–	0.4	1.1	0.7	0.7	0.4	0.6	RI, MS
5	α-Phellandrene	1003	1002	1.2	1.8	–	1.6	0.6	0.4	0.7	0.8	0.5	1.3	1.3	0.7	1.8	1.4	1.3	0.8	1.5	0.3	1.8	1.4	1.1	2.1	0.4	RI, MS
6	α-Terpinene	1017	1018	–	–	0.2	3.7	3.0	6.7	3.2	1.1	3.4	4.3	0.1	0.2	0.4	3.9	5.0	3.5	0.1	5.3	0.7	0.1	0.2	0.2	11.6	RI, MS
7	*p*-Cymene	1025	1025	1.9	2.5	20.8	17.7	24.1	20.3	17.4	14.2	19.5	19.8	1.4	3.0	2.2	16.9	19.7	17.5	2.6	38.6	12.6	2.6	2.6	2.7	34.8	RI, MS, CoI
8	1,8-Cineole	1031	1033	38.5	51.9	45.2	54.9	6.5	12.8	25.2	43.1	14.1	40.9	38.0	21.0	54.2	53.0	42.6	30.8	65.0	14.0	51.5	68.3	36.9	59.5	19.5	RI, MS, CoI
9	*Artemisia* ketone	1062	1063	–	–	–	–	–	0.2	–	–	0.1	–	0.1	0.2	0.6	–	–	–	–	–	0.1	–	0.2	–	–	RI, MS
10	*Artemisia* alcohol	1084	1083	0.3	–	1.2	–	–	0.1	0.1	–	–	0.2	–	–	0.3	–	–	0.1	–	–	0.4	0.2	0.2	–	–	RI, MS
11	*trans*-Sabinene hydrate	1098	1096	8.1	3.1	–	–	0.3	0.1	0.1	–	–	–	0.5	0.4	2.0	0.1	–	0.5	0.6	–	–	0.4	1.0	–	–	RI, MS
12	Linalool	1097	1098	–	–	–	0.1	–	0.2	0.2	–	0.1	0.1	–	–	0.2	–	0.2	–	–	–	0.4	–	–	–	0.2	RI, MS, CoI
13	β-Thujone	1112	1112	–	–	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	4.5	28.9	0.5	–	RI, MS
14	*cis-p*-Menth-2-en-1-ol	1122	1120	4.6	2.5	0.6	0.7	2.0	2.2	2.3	2.8	2.3	1.6	2.8	4.6	0.1	0.8	0.9	1.9	0.8	2.4	2.8	1.0	4.9	0.2	1.8	RI, MS
15	Chrysanthenone	1128	1125	–	0.8	–	0.2	0.9	0.5	0.7	1.0	0.6	0.5	1.9	2.8	0.2	0.2	0.2	0.4	0.7	0.3	0.6	0.7	–	0.2	0.3	RI, MS
16	Camphor	1146	1143	29.3	14.4	1.2	3.9	2.1	1.6	2.7	3.3	1.4	4.1	12.2	3.6	20.4	3.5	1.4	3.3	9.8	1.5	2.9	8.8	11.1	19.6	1.3	RI, MS, CoI
17	*cis*-Chrysanthenol	1164	1162	–	–	–	–	–	0.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	RI, MS
18	Terpinen-4-ol	1177	1177	2.5	0.8	–	0.7	0.3		0.9	0.8	0.7	0.9	1.9	1.0	4.0	0.9	0.9	0.7	1.3	0.3	0.6	1.4	0.9	1.0	0.6	RI, MS, CoI
19	α-Terpineol	1189	1189	1.1	0.9	–	1.4	0.3	0.6	1.9	0.2	0.5	1.4	1.9	0.5	3.6	1.9	2.2	0.7	1.1	–	1.4	2.1	0.8	1.5	0.6	RI, MS, CoI
20	*cis*-Chrysanthenyl acetate	1265	1262	–	13.8	20.4	1.7	45.1	7.3	8.9	14.5	6.8	3.7	28.7	55.8	0.4	2.3	3.6	4.3	10.6	6.7	2.7	0.2	3.7	0.2	3.5	RI, MS
21	4aα-7α-7aα-Nepetalactone	1360	1358	–	–	–	–	7.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	RI, MS
22	4aα-7α-7aβ-Nepetalactone	1387	1385	–	–	4.3	7.8	–	–	–	14.3	–	14.9	–	–	–	10.4	15.6	30.2	–	27.6	10.9	–	–	–	20.0	RI, MS
23	4aα-7β-7aα-Nepetalactone	1392	1390	–	–	–	–	–	40.9	25.9	–	43.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	RI, MS
24	Lavandulyl 2-methyle butyrate	1512	1513	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	RI, MS
25	Spathulenol	1578	1576	–	–	–	–	–	–	0.3	–	tr	0.1	–	–	–	0.1	0.1	–	–	–	0.1	–	–	–	0.1	RI, MS
Monoterpene hydrocarbons				12.3	10.4	21.4	27.0	28.8	29.0	22.9	18.2	24.9	28.1	7.1	5.7	9.8	25.5	29.1	24.0	8.5	45.8	17.7	8.8	8.7	13.5	48.7	
Oxygenated monoterpenes				84.7	88.7	75.2	72.1	65.6	67.8	69.3	80.1	70.3	69.0	88.1	90.0	88.0	73.5	68.4	73.4	89.8	53.2	75.5	88.4	89.4	83.0	48.5	
Oxygenated Sesquiterpene				–	–	–	–	–	–	0.3	–	–	0.1	–	–	–	0.1	0.1	–	–	–	0.1	–	–	–	0.1	
Total identified				96.9	99.1	96.6	99.0	94.3	96.8	92.5	98.3	95.3	97.2	95.2	95.8	97.8	99.1	97.6	97.4	98.3	98.9	93.2	97.3	98.1	96.6	97.3	
Essential oil content (%v/w)				1.0	0.7	0.6	0.7	0.5	1.6	0.6	0.6	1.1	0.5	0.6	0.7	0.7	1.2	1.6	0.6	0.7	1.5	0.8	0.9	0.6	0.4	0.6	

Notes: RI, retention indices in elution order from DB-5 column; RIs, standard RI from Adams (23); MS, mass spectrometry; CoI, co-injection; tr, less than 0.05%.

detector (FID) temperature was 265°C and injector temperature was 250°C. Helium was used as carrier gas with a linear velocity of 32 cm/second. The percentages of compounds were calculated by the area normalization method, without considering response factors.

GC–mass spectrometry (GC–MS) analyses were carried out in a Varian 3400 GC–MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm); oven temperature was 50–240°C at a rate of 4°C/minute, transfer line temperature 260°C, carrier gas helium, with a linear velocity of 31.5 cm/second, split ratio 1:60, ionization energy 70 eV, scan time 1 second, and mass range 40–300 amu.

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (23). Mass spectra from the literature were also compared (23). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes.

Statistical analysis

Differences in the oil content of the populations in question was tested with analysis of variance (ANOVA) and means further compared using Duncan's multiple range test at $p<0.05$. To determine the relationships of populations, principal component analysis (PCA) and hierarchical clustering were served on the basis of their essential oil composition. For this purpose, all constituents identified were subjected to the multivariate analysis. To avoid the dependency of percentage data, they

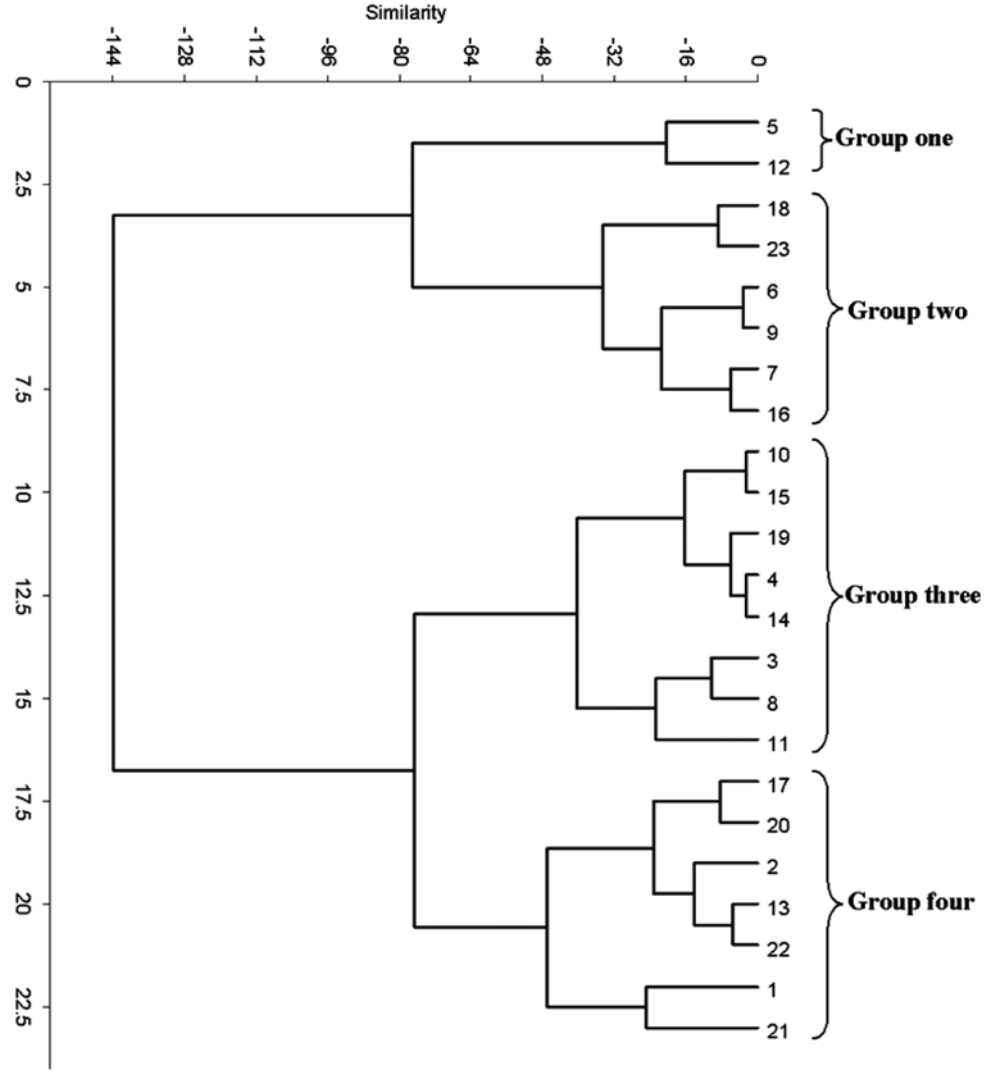

Figure 3. Dendrogram of the similarities among the twenty-three Iranian wild populations of *Achillea biebersteinii*, based on chemical data of essential oil.

were standardized prior to analysis. For hierarchical clustering, the Euclidean distance was employed to calculate the similarity between populations and the Ward's method. Analyses of data were performed using SAS 9.1, SPSS 11 and PAST 1.93 statistical computer software.

Results and discussion

There were significant differences among the essential oil yield of *A. biebersteinii* plants collected from different parts of country. Based on the dry weight of samples, the oil content of flowering aerial parts of studied populations ranged from 0.44% (Nenor) to 1.62% (Golool) with a mean of 0.81% (Figure 2). In previous studies, the essential oil content of *A. biebersteinii* from different parts of the world has been reports to be 0.20% (Jordan) and 0.63–0.70% from Turkey (4, 5, 9).

GC and GC–MS allowed the identification of twenty-five constituents in the essential oil of all samples representing about 92.5–99.1% of the total composition. The identified components and their relative percentages are presented in Table 2, where the components are listed in order of their elution from the DB-5 column. Oxygenated monoterpenes constituted the principal fraction of almost all the oils analyzed (48.5–90.0%), except Ortokand, in which monoterpene hydrocarbons (48.7%) were dominant. On the other hand, sesquiterpenes were not present or in trace amounts (0.1–0.3%) in the essential oil of studied populations. Although 1,8-cineole was generally found to be the main constituent, there was a considerable variation among the chemical composition of essential oils. While this compound amounted only 6.5% of the oil from Siahbishe, 68.3% of the essential oil of Havar, as the richest population, was made of 1,8-cineole. The second most abundant compound of our oil samples was the aromatic monoterpene hydrocarbon *p*-cymene with the highest and lowest concentration in the essential oils from Firooz Abad (38.6%) and Eberoo (1.4%), respectively. (*E*)-Chrysanthenyl acetate was also one of the major components of many essential oils analyzed in this study with the percentages ranging from 0.2 (Nenor) to 55.8% (Zanjan). In contrast, lavandulyl 2-methyle butyrate (0.1%) and (*E*)-chrysanthenol (0.8%) were the rarest compounds, which presented only in the essential oil of Ortekand and Chelmir populations, respectively.

Based on the data from percentage composition of essential oils, in this study PCA and hierarchical clustering were used to determine the relationships between the twenty-three studied populations (Figures 3 and 4). Cluster analysis grouped the twenty-three populations into four main groups corresponding to their principal constituents. Members of the first group (5 and 12) contained (*Z*)-chrysanthenyl acetate (45.1 and 55.8%, respectively) as the main constituent of essential oils, whereas plants of the second group (18, 23, 6, 9, 7 and 16) had the high amounts of nepetalactones (20.0–43.3%). The third group consisted of populations 10, 15, 19, 4, 14, 3, 8 and 11, in which 1,8-cineole was dominant and nepetalactones were present in considerable amounts in their essential oils. Finally, populations producing high levels of 1,8-cineole (36.9–68.3%) and camphor (8.8–29.3%), namely 17, 20, 2, 13, 22, 1 and 21, were placed together in group four. On the other hand, three groups of related populations were detected based on their spatial arrangement in three-plot analysis. As

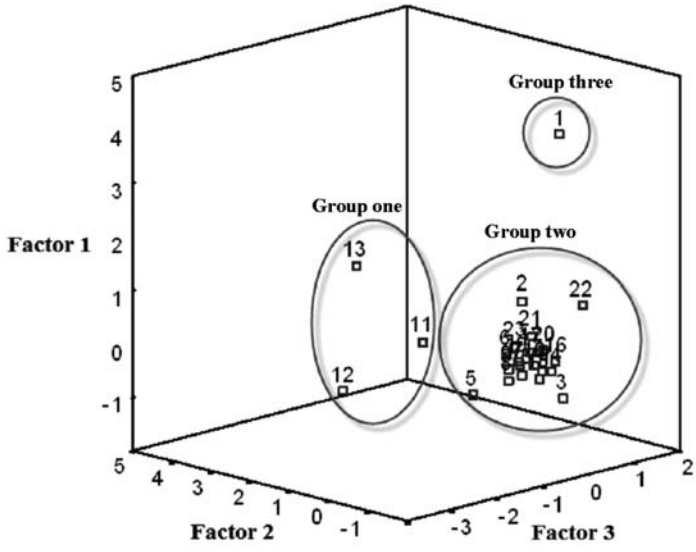

Figure 4. Three plot of twenty-three Iranian wild populations of *A.biebersteinii* along the first, second and third factor.

Table 3. Correlation coefficient between the essential oil constituents twenty-three Iranian wild populations of *Achillea biebersteinii*.

No.	Essential oil composition	Compound number																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	α-Pinene	1																								
2	Camphene	**0.50***	1																							
3	Yomogi alcohol	−0.04	**0.61****	1																						
4	α-Phellandrene	0.20	−0.01	−0.14	1																					
5	α-Terpinene	0.30	**0.86****	**0.55****	−0.05	1																				
6	*p*-Cymene	0.21	−0.25	**−0.52***	−0.09	**−0.45***	1																			
7	1,8- Cineole	0.08	**−0.56****	**−0.67****	0.06	**−0.65****	**0.82****	1																		
8	*Artemisia* ketone	0.19	**0.70****	0.41	0.23	**0.77****	**−0.53***	**−0.59****	1																	
9	*Artemisia* alcohol	**0.41***	0.19	−0.02	**0.46***	0.12	−0.14	−0.27	−0.04	1																
10	*trans*-Sabinene hydrate	−0.27	−0.33	−0.02	**0.78****	−0.25	−0.29	0.00	0.11	0.07	1															
11	Linalool	0.02	0.31	**0.77****	−0.04	0.20	−0.33	**−0.43***	0.18	0.08	0.08	1														
12	β-Thujone	0.24	0.00	−0.30	0.36	0.12	0.37	0.23	−0.06	0.38	0.08	−0.18	1													
13	*cis*-*p*-Menth-2-en-1-ol	−0.06	0.04	0.21	0.05	0.03	−0.20	−0.26	0.04	0.13	0.08	0.03	−0.17	1												
14	Chrysanthenone	−0.35	−0.33	0.23	**−0.42***	−0.24	−0.17	−0.18	**−0.44***	−0.02	−0.10	0.41	−0.15	**0.43***	1											
15	Camphor	0.13	**0.67****	**0.93****	0.02	**0.57****	**−0.54****	**−0.71****	**0.43***	0.25	0.00	**0.79****	−0.21	0.13	0.12	−0.14	1									
16	*cis*-Chrysanthenol	0.00	−0.19	−0.14	0.00	−0.27	0.32	0.13	−0.32	0.26	−0.05	−0.08	0.24	−0.05	0.02	−0.04	0.02	1								
17	Terpinen-4-ol	0.41	**0.61****	**0.51***	0.20	**0.50***	−0.40	**−0.59****	0.38	**0.59***	−0.07	**0.54****	0.08	−0.01	−0.04	−0.40	−0.10	0.41	1							
18	α-Terpineol	**0.48***	**0.69****	0.22	0.29	**0.67****	−0.20	**−0.45***	**0.55***	**0.44***	−0.12	0.10	0.38	−0.04	−0.40	−0.10	0.41	−0.15	**0.73****	1						
19	*cis*- Chrysanthenyl acetate	−0.35	**−0.51***	−0.24	−0.24	−0.34	−0.21	−0.06	**−0.42***	−0.02	−0.03	−0.13	−0.32	−0.13	0.34	**0.80****	−0.23	−0.05	−0.18	−0.37	1					
20	4aα,7-α,7aα-Nepetalactone	−0.25	−0.34	−0.14	−0.10	−0.17	0.04	0.21	−0.39	−0.10	−0.11	−0.05	−0.15	−0.05	0.00	0.10	−0.15	−0.05	−0.19	−0.23	**0.52***	1				
21	4aα-7α,7aβ-Nepetalactone	0.14	−0.17	**−0.42***	−0.03	−0.24	**0.55****	**0.66****	−0.18	−0.31	−0.10	−0.27	0.09	−0.18	−0.11	−0.23	**−0.45***	−0.16	−0.30	−0.24	−0.27	−0.16	1			
22	4aα,7-β,7a-α-Nepetalactone	−0.10	−0.31	−0.27	−0.09	−0.40	0.28	0.20	**−0.49***	0.23	−0.12	−0.15	0.28	−0.10	0.07	−0.02	−0.27	**0.61****	−0.24	−0.14	−0.08	−0.08	−0.28	1		
23	Lavandulyl 2-methyle butyrate	0.00	−0.06	−0.17	0.00	−0.26	**0.69****	**0.42***	−0.23	−0.10	−0.11	−0.09	0.27	−0.05	−0.04	−0.10	−0.17	−0.05	−0.10	−0.14	−0.11	−0.04	0.30	−0.08	1	
24	Spathulenol	0.01	−0.12	−0.28	−0.04	−0.05	0.28	0.24	−0.09	−0.18	−0.04	−0.20	**0.47***	−0.12	−0.07	−0.08	−0.28	−0.10	−0.09	0.30	−0.17	−0.10	0.06	0.25	0.10	1

Notes: *Significant at the 5% probability level, **significant at the 1% probability level.

can be seen in Figure 4, group one comprised populations 11, 12 and 13, in which their common traits were found to be the high amounts of (*Z*)-chrysanthenyl acetate (0.4–55.8%), 1,8-cineole (21.0–54.2%) and camphor (3.6–20.4%). Group two formed by all remained populations except population 1, inwhich 1,8-cineole (38.5%), camphor (29.3%) and camphene (6.7%) were the characteristics of its essential oil.

A simple correlation was computed among all of the chemical constituents present in the essential oils from twenty-three populations (Table 3). According to the results obtained, there were many negative and positive correlations among the chemical components. A significant positive correlation, for instance, was observed between the percentages of camphor and camphene, while the correlation between amounts of *p*-cymene and camphene was negative.

Comparing the results of presents study on the essential oil of *A. biebersteinii* with those reported previously, Kordali et al. (11) reported similarly high levels of oxygenated monoterpenes (84.0%) with 1, 8-cineol (38.1%) and camphor (23.6%) as the major components along with a small amount of sesquiterpenes (6.4%) in the sample from Erzurum, Turkey. Accordingly, 1,8-cineol (9.6–22.3%), camphor (4.7–38.1%), chrysantenone (8.2%) and *p*-cymene (31.6%) have been also reported as the principal constituents of *A. biebersteinii* essential oils by several other authors. In some cases, however, the analyzed essential oils were dominated by piperitone, ascaridole, borneol and carvenone oxide, which were not present in our samples at all (4, 5, 9–12). Also, in another study from Iran on the chemical composition of this plant, 1,8-cineole, carvacrol and piperitone were reported as the major constituents of *A. biebersteinii* (24).

The presence of nepetalactones in the essential oils of fourteen out of twenty-three studied populations was interesting. Apart from essential oil from the Mohhamad Shar population (4.3%), they were at least one of the three major components (7.8–43.3%). Nepetalactones are iridoid monoterpenoids that occur in the form of eight stereoisomers, four diasteroisomers and their corresponding enantiomers (25). Although there is little information on the physiological functions of these compounds in nature, it is shown that they are insect repellants and may have roles in the protection of plants from herbivorous insects (26). Antibacterial, antifungal and insect repellency or toxicity properties of different *Nepeta* species have widely been reported and nepetalactones proposed to be responsible for such activities (25, 27). In addition, the feline attractant properties observed in several *Nepeta* species are related to the presence of these compounds (28). Despite the negligible structural differences, nepetalactone isomers differ in biological activity; for example,

cis,*trans*-nepetalactone exhibits stronger antibacterial activity against *Helicobacter pylori* than the *trans*,*cis* stereoisomer (25). In addition to nepetalactones, there are many other compounds in the essential oils of studied populations with significant biological activities. Among them are linalool, 1,8-cineol, camphor, spathulenol, terpinen-4-ol and α-pinene, compounds with well known biological activity (29, 30).

Conclusion

As observed in Table 2, there is a considerable variation in the essential oil content and composition of twenty-three populations in question. The noticeable point of present study was the occurrence of nepetalactones chemotypes in plants of the *Nepeta* species (31). Variability was also observed between the results of the present study and those reported earlier on the same species. This variation can be related to factors such as genetic differences, different environmental conditions, time of sampling and postharvest processing of plant materials. The variation observed in the chemical profile of essential oils may a result, at least to some extent, of the genetic backgrounds of these populations, which allowed selection of populations with special biological activities as starting materials for use in the breeding programs of this plant. However, more studies are needed to evaluate the biological activities of the essential oils of the twenty-three studied populations and the genetic base for the observed chemical variation prior to use as materials for the genetic improvement of *A. biebersteinii*.

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References

1. A. Chevallier, *The Encyclopedia of Medicinal Plants*. Dorling Kindersley, London (1996).
2. A. Huber Morath, *Achillea*. In: *Flora Iranica compositae VI – Anthemideae*. Edit., K.H. Rechinger, 158, 49–71, Ackademiche Druck-U, Graz, Austria (1986).
3. A. Zargari, *Medicinal Plants*, Vol. 3. Tehran University Publication, Tehran (1996).
4. A. Esmaeili, F. Nematollahi, A. Rustaiyen, N. Moazami, S. Masoudi and S. Bamasian, *Volatile constituents of* Achillea pachycephala, A. oxyodonta *and* A. biebersteinii *from Iran*. Flavour Fragr. J, **21**, 2353–3256 (2006).
5. O. Baris, M. Gulluce, F. Sahin, H. Ozer, H. Kilic, H. Ozkan, M. Sokmen and T. Ozbek, *Biological activities of the essential oil and methanol extract of* Achillea biebersteinii *Afan. (Asteraceae)*. Turk. J. Biol, **30**, 65–73 (2006).
6. B. Benedek, B. Kopp and M.F. Melizg, *Achillea millefolium L. s.l. Is the anti-inflammatory activity mediated by proteaseinhibition?* J. Ethnopharmacol, **113**, 312–317 (2007).
7. S. Paulo, *Effect of the essential oil of* Achillea millefolium *L. in the production of hydrogen peroxide and tumor necrosis factor-α in murrain macrophases*. Br. J. Pharmacol, **41**, 401–405 (2005).
8. M. Lewis, *What is Yarrow* – Achillea millefolium *essential oil?* Alternative Medicine, Complementary Health Directory and Resources, UK (2006).
9. A. Bader, G. Flamini, P.L. Cioni and I. Morelli, *Essential oil composition of* Achille santolina *L. and* Achillea biebersteinii *Afan. collected in Jordan*. Flavour Fragr. J., **18**, 36–38 (2003).
10. A. Sokmen, M. Sokmen, D. Daferera, M. Polissiou, F. Candan, M. Unlu and H.A. Akpulat, *The in vitro antioxidant and antimicrobial activities of the essential oil and methanol extracts of* Achillea biebersteinii *Afan. (Asteraceae)*. Phytother. Res, **18**, 451–456 (2004).
11. S. Kordali, A. Cakir, T.A. Akcin, E. Mete, A. Akcin, T. Aydin and H. Kilic, *Antifungal and herbicidal properties of essential oils and n-hexane extracts of* Achillea gypsicola *Hub-Mor. and* Achillea biebersteinii *Afan. (Asteraceae)*. Ind. Crop. Prod, **29**, 562–570 (2009).
12. A. Rustaiyan, H. Komeilizadeh, M.S. Shariatpanahi, A.R. Jassbi and S. Masoudi, *Comparative study of essential oils of three* Achillea *species from Iran*. J. Essent. Oil Res, **10**, 207–209 (1998).
13. O. Calmasur, S. Kordali, O. Kaya and I. Aslan, *Toxicity of essential oil vapours obtained from* Achillea *spp. to* Sitophilus granarius *(L.) and* Tribolium confusum *(Jacquelin du Val)*. J Plant Dis Prot., **1**, 37–41 (2005).
14. E. Kupeli Akkol, U. Koca, I. Pesin and D. Yilmazer, *Evaluation of the wound healing potential of* Achillea biebersteinii *Afan. (Asteraceae) by* in vivo *excision and incision models*. eCAM Advance Access published. **39**, 1–7 (2009).
15. V.H. Heywood, *The conservation of genetic and chemical diversity in medicinal and aromatic plants*. In: *Biomolecular Aspects of Biodiversity and Innovative Utilization*. Edit., B. Sener, pp. 13–22, Biodiversity: Kluwer Academic/Plenum Publishers, New York (2002).
16. O. Gudaityt and P.R. Venskutonis, *Chemotypes of* Achillea millefolium *transferred from 14 different locations in Lithuania to the controlled environment*. Biochem. Syst. Ecol, **35**, 582–592 (2007).
17. K. Msaada, K. Hosni, M.B. Taarit, T. Chahed, M.E. Kchouk and B. Marzouk, *Changes on essential oil composition of coriander (*Coriandrum sativum *L.) fruits during three stages of maturity.* Food Chem, **102**, 1131–1134 (2007).
18. F. Sefidkon, K. Abbasi and G. Bakhshi-Khaniki, *Influence of drying and extraction methods on yield and chemical composition of the essential oil of* Satureja hortensis. Food Chem, **99**, 19–23 (2006).
19. M. Rahimmalek, B.E. Sayed Tabatabaei, A. Arzani and N. Etemadi, *Assessment of genetic diversity among and within Achillea species using amplified fragment length polymorphism (AFLP)*. Biochem. Syst. Ecol, **37**, 354–361 (2009).
20. G.W. Bourdot, *A Study of the Growth and Development of Yarrow* (Achillea millefolium *L.*). Lincoln College, New Zealand (1980).

21. D. Mockute and A. Judzentiene, *Variability of the essential oils composition of* Achillea millefolium *ssp*. millefolium *growing wild in Lithuania*. Biochem. Syst. Ecol, **31**, 1033–1045 (2003).
22. *British Pharmacopoeia*, 2, p. 137, HMSO, London (1988).
23. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th edn. Allured Publ. Corp., Carol Stream, IL (2007).
24. A. Ghani, M. Azizi, M. Hassanzadeh-Khayyat and A.A. Pahlavanpour, *Essential oil composition of* Achillea eriophora, A. nobilis, A. biebersteinii *and* A. wilhelmsii *from Iran*. J. Essent. Oil Bearing Plants, **11**, 460–467 (2008).
25. J. Nestorovic, D. Misic, B. Siler, M. Sokovic, J. Glamoclija, A. Ciric, V. Maksimovic and D. Grubisic, *Nepetalactone content in shoot cultures of three endemic* Nepeta *species and the evaluation of their antimicrobial activity.* Fitoterapia, **81**, 621–626 (2010).
26. F.E. Regnier, G.R. Waller, E.J. Eisenbraun and H. Auda, *The biosynthesis of methylcyclopentane monoterpenoids – II: Nepetalactone*. Phytochemistry, **7**, 221–230 (1968).
27. G. Gkinis, O. zakou, D. Iliopoulou and V. Roussis, Chemical *composition and biological activity of* Nepeta parnassica *oils and isolated nepetalactones*. Z. Naturforsch., **58c**, 681–686 (2003).
28. H.L. De Pooter, B. Nicolai, L.F. De Buyck, P. Goetghebeur and N.M. Schamp, *The essential oil of* Nepeta nuda. *Identification of a new nepetalactone diasteroisomer*. Phytochemistry, **26**, 2311–2314 (1987).
29. J.C. Matasyoh, J.J. Kiplimo, N.M. Karubiu and T.P. Hailstorks, *Chemical composition and antimicrobial activity of essential oil of* Tarchonanthus camphoratus. Food Chem, **101**, 1183–1187 (2007).
30. K. Vagionas, K. Graikou, O. Ngassapa, D. Runyoro and I. Chinou, *Composition and antimicrobial activity of the essential oils of three* Satureja *species growing in Tanzania*. Food Chem, **103**, 319–324 (2007).
31. F. Sefidkon and Z. Jamzad, *Essential oil composition of four Iranian* Nepeta *species* (N. cephalotes, N. bornmuelleri, N. mirzayanii *and* N. bracteata. J. Essent. Oil Res., **19**, 262–265 (2007).