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Variability in Essential Oil Content and Composition of *Achillea tenuifolia* Lam. Populations in Field Conditions

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ABSTRACT

***Achillea tenuifolia* Lam. belongs to the Asteraceae family that is one of the most popular aromatic plants in Iran with great variation in both morphology and essential oil components. The aim of this study was determination of essential oil content and composition of *A. tenuifolia* populations in field condition. Seeds of 17 populations of *A. tenuifolia* collected from their natural habitats were cultivated under the same environmental conditions in the research farm of Alborz Research Station, Karaj, Iran. The aerial parts of the plants were collected at full flowering stage and dried in shade (room temperature) and their essential oils were obtained by hydro-distillation. The oils were analyzed by GC and GC/MS. Based on the results, the oil yield varied from 0.16 to 1.59% (w/w dried weight). Populations from Divandareh with 1.59% and Khoy2 with 0.16% had the highest and lowest oil yield, respectively. According to the cluster analysis, the populations were placed in two clusters. Germacrene D was the main compound in all oils, but the essential oils of populations in cluster 1 contained higher amount of germacrene D (up to 64.5% in Semnan population). α-Humulene (up to 15%) and 1,8-cineole (up to 11.7%) were the other major components in the oil of cluster 1 populations. The lower amounts of these three compounds (in addition to E-β-farnesene and piperitone) and higher amount of more volatile compounds like p-cymene, β-phellandrene, camphor, and α-thujone and presence or absence of other minor compounds placed the other populations in cluster 2. Each cluster divided in two groups because of different percentages of some component such as cubenol, viridiflorol, methyl hexadecanoate and phytol. Therefore, based on the demand for processing, the proper population can be chosen for vast cultivation.**

Keywords: Aromatic plants, Asteraceae family, Cluster analysis, Germacrene D, Medicinal plant.

INTRODUCTION

According to distribution of genus Achillea, two main centers of diversity occur in S.E. Europe and S.W. Asia. Diversified essential oil compositions from Balkan Peninsula have been numerously reported (Turkmenoglu *et al*., 2015). However, report on essential oils of Achillea species growing in Iran, which is one of the main centers of diversity, is very limited.

Achillea tenuifolia Lam. is one of the Asteraceae (Compositae) spices. In Iran, 19 species of this medicinal plant grow wild (Rechinger, 1963). *A. tenuifolia* is a native perennial herb with 20–90 centimeter height, with long and narrow leaves without petioles, distributed in western and northern

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regions of Iran (Rechinger, 1983; Ghahreman, 2003; Salehi Surmaghi, 2015).

In Iranian traditional medicine, some herbs like *A. tenuifolia*, commonly known as yarrow, are implicated as appetite enhancers (Nematy *et al*., 2016).

Achillea species have been used in folk medicine for thousands of years due to numerous medicinal properties such as anti-inflammatory, wound healing, spasmolytic and choleretic uses (Nemeth, 2010; Nemeth and Bernath, 2008; Hoffman, 2003; Chou *et al*., 2013; Vitalini *et al*., 2013). In addition, Achillea are used as spices and additives in food products, while essential oil and extracts of some species are used for preparation of digestive teas and cosmetic products (Nemeth and Bernath, 2008; Vitalini *et al*., 2013).

Some investigations showed that the major volatile compounds of several Iranian *Achillea* species were α- and β-pinene, 1,8-cineole, camphor, camphene, α-terpineol, caryophyllene, ascaridole, and bornyl acetate (Afsharypuor, 1996a,b; Weyerstahl *et al*., 1997; Rustaiyan *et al*., 1998; Rustaiyan *et al*., 1999; Aghjani *et al*., 2000; Shafaghat *et al*., 2009).

Literature review showed some researches about essential oil composition and antimicrobial effects of *A. tenuifolia*. The main components of flower oil of *A. tenuifolia*, collected from Khalkhal-Ardabil were limonene (23.2%) and α-cadinol (18.2%), while those of leaf and stem oils were limonene (23-25%) and α-pinene (13-14%) (Shafaghat *et al*., 2009). Isoascaridol, 1,8-cineol and camphor were reported as main components *of A. tenuifolia* aerial parts oil from Turkey (Toncer *et al*., 2010).

The leaves and flowers of *A. tenuifolia* Lam. have shown antioxidant properties (Aghajani *et al*., 2000). The essential oil is formed in secondary trichomes of leaves, stem, and specially flowers. The essential oil of *A. tenuifolia* flowers and leaves had antibacterial effects against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis* (Talebi Varnosfaderani *et al*., 2017). Previous investigation showed that the seed oil of *A. tenuifolia* Lam. contained 1.7% linolenic acid (Goli *et al*., 2008). Therefore, because of some components such as 1,8-cineole, limonene, camphor and sesquitepenes in the essential oil of this species and approved medicinal properties such as antibacterial and antioxidant effects, we decided to crop different population of it for comparing their essential oil content and composition to find the best population(s).

Other researches showed essential oil variation between different populations of one Achillea species. For example, in the oils of five accessions of *A. millefolium* collected from different ecological regions of Iran, camphor (16-40%), 1,8-cineole (10-20%) and borneol (4-21%) were the three major compounds (Ebrahimi *et al*., 2012). Analysis of the essential oils obtained by hydro-distillation from the aerial parts of eleven Achillea species in Turkey showed 1,8-cineole, p-cymene, viridiflorol and nonacosane as the main components. The chemical principal component analysis identified three species groups and a subgroup (Turkmenoglu *et al*., 2015).

According to the results of cluster analysis, based on percentages and essential oil constituents of *A. nobilis* L., studied populations were divided in 2 main clusters (Azimi *et al*., 2016). To reveal genetic variation of *A. millefolium* ssp. elbursensis, seeds of five populations from different agro-ecological zones of Iran were collected and grown in the same place. The results indicated high variation in their essential oil compositions (Ebrahimi *et al*., 2012).

Three groups were revealed according to the dendrogram of *A. tenuifolia* accessions based on morphological and molecular traits (Rahimmalek, 2012). Molecular study mentioned that *A. tenuifolia* has the highest gene diversity compared to *A. filipendulina*, *A. millefolium*, *A. santolina*, and *A. biebresteinii* (Rahimmalek, 2012; Rahimmalek *et al*., 2009b).

In this research, because of variation in essential oil composition of *A. tenuifolia* species in habitat (Aghajani *et al*., 2000;

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Jaimand and Rezaee, 2001; Dokhani *et al*., 2005; Rahimmalek *et al*., 2009a), for the first time, seeds of seventeen populations of *A. tenuifolia* were collected from their natural habitats and cultivated under the same environmental conditions to study their genetic diversity based on their essential oils.

MATERIALS AND METHODS

Plant Material

Seed materials of 17 wild populations of *Achillea tenuifolia* from different origins/sources of Iran were provided by National Natural Resources Gene Bank, Iran (Table 1). The seeds were sown in Jiffy pots in March 2015. After seed emergence, seedlings were transferred to field in Alborz Research Center, Karaj, Iran. This station is located between longitude 51° 31' east and latitude 35° 42' north with an altitude of 1,291 m asl The average annual rainfall is 248 mm and the average temperature is 16.21°C, with an absolute maximum of 44°C and absolute minimum of -8°C. The station has a loam-textured soil with a pH of 7.5-8.5. Around 24.22% of the annual rainfall occurs in winter and March is the rainiest month of the year. The average annual relative humidity of the research station is 40-50% with very cold semi-arid climate. The field experiment was arranged in a completely randomized block with three replications. Each plot included 36 spaced plants (0.50×0.50 m) in a single row. Fertilizer application rates were 100 kg ha^{-1} Phosphorus (P) at sowing. The field was irrigated once a week during summer.

In order to compare essential oil content and composition, foliage of all *A. tenuifolia* populations was harvested at the flowering stage in mid-June 2016. The plant materials were kept at room temperature, at 22-25°C, in shade for at least one week until dried and their moisture content was less than 5%. To determine the final moisture content of each sample at the time of essential oil distillation, after determination of seeds dry matter, 80 g of aerial parts including stem, leaf and flower were subjected to hydro-distillation using Clevenger-type glass apparatus for 2 hours. Essential oil yield was calculated based on the dry weight of the sample, taking into account the moisture content. The essential oils were kept in the refrigerated glass until analysis.

Hydro Distillation

Essential oils were obtained by hydro distillation method from 60-80 g dried plant samples by a Clevenger type apparatus. Distillation was continued for approximately 2.5 hours. The essential oils were stored in dark glass bottles at 4°C until analysis. Essential oil yields were determined based on dried weight of plant materials by calculating humidity percentage. So, at the same time of distillation, 5 grams of each sample was weighted and placed in the oven at 50°C for 24-48 hours (until completely dried) and weighted again after drying.

Essential Oil Analysis

The essential oils were analyzed by GC and GC/MS. 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 (Shibamoto, 1987; Davies, 1990; Adams, 1995). Mass spectra from the literature were also compared (Adams, 1995). The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

GC and GC-MS:

The analysis of GC was conducted by Thermo-UFM Ultra-Fast Gas Chromatograph equipped with a Ph-5 fused silica column (10 m×0.1 mm id, film thickness 0.40 μm). Oven temperature was

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held at 60°C for 3 minutes and then programmed to 280°C at a rate of 40°C min^{-1}. Detector (FID) temperature was 285°C and injector temperature was 285°C. Helium was used as carrier gas at 0.5 mL min^{-1}. The oils were manually injected to GC without dilution. The percentages of compounds were calculated by the area normalization method, without considering response factors. Quantitative data were obtained electronically from FID area percent data.

The analyses of GC-MS were performed in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m×0.25 mm id, film thickness 0.25 µm) to identify all oil components. Oven temperature was 50-240ºC at a 4ºC min^{-1} rate, transfer line temperature of 260ºC, helium as a carrier gas with a velocity of 31.5 cm s^{-1}, split ratio 1:60, ionization energy of 70eV, 1 second scan time, and 40-300 amu of mass range.

Statistical Analysis

The descriptive statistics characteristics including mean, maximum, minimum and standard deviation for each of the studied compounds of 17 populations of *A. tenuifolia* were estimated using the SAS software (SAS Institute Inc. 2003). Euclidean distances of populations were calculated based on analytical data of *A. tenuifolia* oils; and cluster analysis was conducted based on Unweighted Pair Group Method with Arithmetic Averaging (UPGMA) method using Minitab software version 14.

RESULTS AND DISCUSSION

Yield of Essential Oils

The essential oil yields of 17 studied populations from *A. tenuifolia* were 0.16 to 1.59% (Table 1). The highest and lowest oils yield were obtained from populations of Divandareh (1.59%) and Khoy2 (0.16%), respectively. Variation ranges of the essential oil yields are shown in Table 1.

Essential Oil Components

Totally, 27 compounds were identified in essential oils of all plants (Table 2). Germacrene D was the major compound with maximum and minimum amounts in the oils of Semnan2 (64.5%) and Khoy 1 (30%) populations, respectively (Table 3).

Cluster Analysis

Cluster analysis, based on the essential oil components, divided the populations to two clusters (Figure 1). Germacrene D, was the main compound in oils of all populations, and populations of cluster 1 with mean value of 50.3% had higher amount of this compound than cluster 2 with mean value of 44.2%.

One of the other fundamental differences between populations of cluster 1 and 2 was the percentages of α-humulene and 1,8-cineole, whose amounts were considerably higher in cluster 1 with means of 2.7% and 6.3% than cluster 2 with means of 0.8% and 2.1%, respectively (Table 3).

Populations of Sanandaj 3 in cluster 2 and Semnan 1 in cluster 1 were rich sources of 1,8-cineole (%11.7) and α-humulene (15%), respectively. Also, in the populations of cluster 1, E-β-farnesene and piperitone had the highest amount, while the highest content of α-thujone, β-phellandrene, p-cymene and α-pinene was found in populations of cluster 2 (Table 3).

Differences between Groups in Cluster 1

The populations of cluster 1 were divided into 2 groups based on the nine major compounds. In the groups 1 and 2, there were two and six populations, respectively (Figure 1). Percentage of germacrene D in group 2 (54.7%) was more than group 1 (37%). The combinations of α-humulene, cubenol and viridiflorol in group 1 is multiplied by group 2. Thus, Kahak population with means of 20.5 and 4.2% had

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Table 1. List of *Achillea tenuifolia* Lam populations, origin, yield of essential oil, 1,000 grain weight, and viability percentage.

No.	Origin		Viability%	1000-Grain weight	Essential oil yield of cultivated population (%)
	province	POP			
1	Ghom2	Kahak	86	0.17	0.41
2	Semnan1	Semnan1	90	0.18	0.25
3	Kordestan4	Divandareh	100	0.25	1.59
4	Kordestan3	Sanandaj3	100	0.1	0.62
5	Semnan3	Semnan3	96	0.04	0.40
6	Semnan2	Semnan2	94	0.14	0.66
7	Ghom1	Ghom	28	0.31	0.48
8	Azarbayjan2	Salmas	100	0.18	1.07
9	Azarbayjan4	Orumieh	100	0.16	0.50
10	Golestan2	Gorgan	60	0.92	0.27
11	Azarbayjan3	Khoy2	100	0.17	0.16
12	Azarbayjan1	Khoy1	100	0.07	0.29
13	Kordestan2	Sanandaj 2	88	0.32	0.40
14	Kordestan5	Dehgalan	100	0.3	0.49
15	Kordestan1	Sanandaj 1	100	0.64	0.29
16	Kordestan6	Sanandaj4	100	0.57	0.85
17	Golestan1	Kolaleh	76	0	0.47

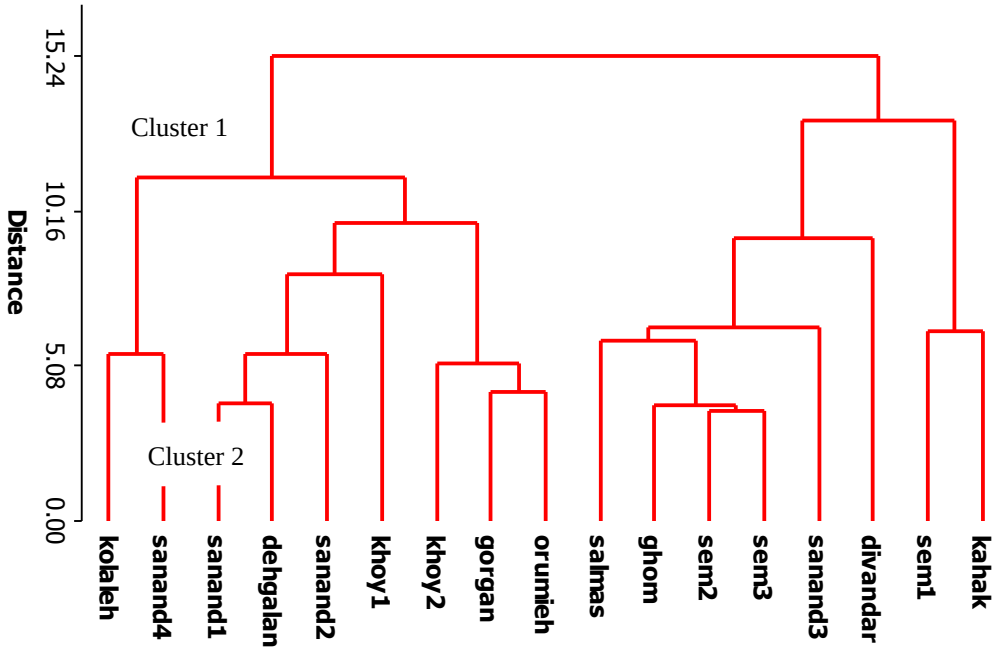

Figure 1. Dendrogram of clusters analysis by Ward method in 17 populations of *Achillea tenuifolia* Lam.

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Table 2. Descriptive statistics of mean, max, min, coeff of variation, and standard deviation of components for 17 *Achillea tenuifolia* Lam populations. [a]

No	Compounds	RI[a]	Total mean	MAX	MIN	CV	STD DEV
1.	α-Pinene	940	2.1	4.6	0.0	58.1	1.2
2.	Camphene	953	0.7	1.5	0.0	68.3	0.5
3.	Sabinene	976	1.5	5.8	0.0	110.0	1.6
4.	myrcene	992	0.2	0.9	0.0	158.6	0.3
5.	p-Cymene	1027	2.3	3.5	0.9	36.5	0.8
6.	β-phellandrene	1028	3.6	7.8	1.0	56.0	2.0
7.	1,8-cineole	1032	4.1	11.7	0.0	82.0	3.4
8.	Artemisia ketone	1062	1.5	2.9	0.0	57.2	0.8
9.	Terpinolene	1089	1.3	4.1	0.0	75.4	1.0
10.	α-Thujone	1103	2.5	8.8	0.0	107.0	2.6
11.	Trans-p-menth-2-en-1-ol	1142	1.7	7.0	0.0	89.9	1.6
12.	Camphor	1147	4.7	9.5	0.0	47.7	2.3
13.	Terpinen-4- ol	1178	1.3	3.7	0.2	74.5	1.0
14.	α-Terpineol	1190	0.4	2.4	0.0	135.9	0.6
15.	Piperitone	1254	0.9	3.7	0.0	132.0	1.2
16.	α-Copaene	1380	0.6	1.3	0.0	55.8	0.3
17.	E-caryophyllene	1418	0.5	1.0	0.0	70.1	0.3
18.	Z-β-farnesene	1444	1.5	3.5	0.4	52.4	0.8
19.	α-Humulene	1455	1.7	15.0	0.0	207.2	3.4
20.	E-β-farnesene	1458	2.1	4.1	1.1	32.5	0.7
21.	**Germacrene D**	1487	**47.1**	**64.5**	**30.1**	**22.5**	**10.6**
22.	Bicyclogermacrene	1502	2.9	3.8	1.5	23.2	0.7
23.	Yiridiflorol	1595	3.5	20.5	0.3	148.5	5.1
24.	Cubenol	1647	1.0	4.2	0.3	107.4	1.0
25.	14-Hydroxy-α-muurolene	1785	1.4	6.3	0.0	124.6	1.7
26.	Methyl hexadecanoate	1920	1.4	11.9	0.0	254.6	3.7
27.	Phytol	1943	1.0	16.8	0.0	408.2	4.1

[a] Bold items indicate predominant component of 17 Achillea tenuifolia populations.

the highest amounts of viridiflorol and cubenol, respectively. Also, the highest amount of α-humulene was found in Semnan 1 (%15). Group 1 had a higher percentage of Z-β-farnesene, while the amount of α-pinene and 1,8-cineole in group 2 were significantly higher than group 1. In group 1, population with average of 3.5% and Sanandaj 3 with average of %11 had the highest amount of α-pinene and 1,8-cineole, respectively. In addition, terpinolene and piperitone in group 2 and Z-β-farnesene in group 1 were the remarkable compounds.

Differences between Groups in Cluster 2

The mean level of germacrene D in group 1 was higher than group 2. The level of compounds such as sabinene, β-phellandrene, trans-p-menth-2-en-1-ol, terpinen-4-ol, α-thujone, and viridiflorol in group 1 was higher than group 2.

Thus, three compounds, namely, β-phellandrene (%7.8), trans-p-menth-2-en-1-ol (%2.8) and terpinen-4- ol (3.7%) were highest in the Khoy1 population. Sanandaj 1 population had the highest percentage of sabinene (5.8%).

The essential oil of populations in group 2 from cluster 2 had a significant amount of methyl hexadecanoate or phytol. The highest amount of methyl hexadecanoate (11.9%) was found in Kolaleh population and phytol (16.8%) in Sanandaj4 population. In addition, the amount of compounds such as 1,8-cineole and 14-hydroxy-α-muurolene

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Table 3. Essential oil compositions of 17 *Achillea tenufolia* Lam populations.[a]

Compounds	Cluster 1											Cluster 2											
	Kahak	semnan1	Mean group 1	Divandareh	Sanandaj3	Semnan3	Semnan2	Ghom	Salmas	Mean group 2	Mean cluster 1	Oromieh	Gorgan	Khoy2	Khoy1	Sanandaj 2	Dehgalan	Sanandaj 1	Mean group 1	Sanandaj4	Kolaleh	Mean group 2	Mean cluster2
α-Pinene	-	0.4	0.2	2.8	3.1	1.8	1.1	1.8	3.5	2.4	1.8	1.1	1.5	3.0	1.8	0.9	3.2	4.6	2.3	2.5	2.1	2.3	2.3
Camphene	-	-	-	1.1	-	0.7	0.5	0.4	0.9	0.6	0.5	0.8	0.8	1.5	0.9	-	0.7	1.1	0.8	0.7	1.2	1.0	0.9
Sabinene	-	0.7	0.4	1.4	1.2	1.8	0.6	0.3	2.8	1.4	1.1	-	1.6	0.8	0.9	1.4	5.1	5.8	2.1	0.9	1.0	1.0	1.8
Myrcene	-	-	-	0.8	-	-	-	-	-	0.1	0.1	-	-	0.9	0.8	-	-	-	0.3	0.4	0.7	0.6	0.3
p-Cymene	0.9	1.0	1.0	3.1	1.7	1.5	1.2	1.9	2.2	1.9	1.7	3.4	3.2	2.7	3.5	2.6	3.0	2.9	3.0	2.2	2.1	2.2	2.8
β-phellandrene	1.0	1.5	1.3	6.9	2.6	1.6	1.5	2.1	2.5	2.9	2.5	4.0	3.8	4.7	7.8	5.7	5.5	4.3	5.1	2.7	2.6	2.7	4.6
1,8-Cineole	-	4.3	2.2	5.6	11.7	8.3	4.6	7.5	8.3	7.7	6.3	1.4	4.0	1.5	-	2.6	-	1.6	1.6	3.7	4.4	4.1	2.1
Artemisia ketone	-	1.3	0.7	1.8	-	2.1	1.4	-	2.9	1.4	1.2	1.7	2.4	1.7	2.2	1.5	1.0	1.7	1.7	1.1	1.9	1.5	1.7
Terpinolene	-	0.6	0.3	4.1	1.2	0.8	1.0	0.8	2.3	1.7	1.4	1.0	1.3	0.5	2.2	1.2	0.3	1.8	1.2	0.9	1.5	1.2	1.2
α-Thujone	-	1.0	0.5	2.8	-	1.1	0.8	1.7	2.2	1.4	1.2	3.2	-	1.8	4.5	8.8	5.8	6.9	4.4	1.0	-	-	3.9
Trans-p-menth-2-en-1-ol	-	0.9	0.5	2.2	0.9	1.2	1.3	1.0	2.6	1.5	1.3	2.7	0.7	1.3	2.8	1.9	0.9	1.7	2.6	0.9	0.4	0.7	2.2
Camphor	2.7	4.2	3.5	7.3	-	5.1	3.2	2.3	3.0	3.5	3.5	7.5	5.2	6.5	9.5	5.4	4.8	4.3	6.2	3.6	5.7	4.7	5.8
Terpinen-4-ol	0.8	0.6	0.7	0.9	2.7	0.4	0.2	0.4	1.2	1.0	0.9	1.8	1.2	0.9	3.7	2.3	2.2	2.0	2.0	0.3	0.8	0.6	1.7
α-Terpineol	-	-	-	0.3	0.7	0.3	-	0.2	0.2	0.3	0.2	0.7	-	0.2	2.4	0.6	0.5	0.5	0.7	0.2	0.2	0.2	0.6
Piperitone	-	1.1	0.6	3.0	0.4	3.7	0.9	2.4	2.6	2.2	1.8	-	-	-	-	0.8	-	-	0.1	0.8	-	0.4	0.2
α-Copaene	1.3	1.2	1.3	0.6	0.4	0.6	0.7	0.9	0.6	0.6	0.8	0.3	0.3	0.5	0.6	0.5	-	0.3	0.4	0.6	0.3	0.5	0.4
E-caryophyllene	0.9	0.7	0.8	0.7	-	0.6	0.5	0.7	0.3	0.5	0.6	-	-	-	0.4	1.0	0.6	0.9	0.4	0.4	0.3	0.4	0.4
Z-β-farnesene	3.5	1.7	2.6	1.4	1.0	2.0	2.5	0.8	2.7	1.7	2.0	1.9	0.9	1.5	1.1	1.0	0.8	0.4	1.1	1.5	1.2	1.4	1.1
α-Humulene	0.9	15.0	8.0	-	1.0	1.2	1.1	1.1	1.1	0.9	2.7	0.6	0.8	1.2	0.7	0.7	0.6	0.8	0.8	0.6	1.0	0.8	0.8
E-β-farnesene	1.9	2.3	2.1	4.1	2.5	2.5	2.5	2.2	2.4	2.7	2.6	2.1	1.9	2.4	1.2	1.7	2.0	1.4	1.8	1.1	1.6	1.4	1.7
Germacrene D	30.1	44.1	37.1	42.7	60.3	53.8	64.5	60.2	46.4	54.7	50.3	48.4	59.5	49.9	30.5	48.8	37.4	43.0	45.3	32.8	48.6	40.7	44.3
Bicyclogermacrene	3.5	3.6	3.6	2.6	3.1	2.9	3.7	3.3	2.3	3.0	3.1	3.8	3.3	3.3	1.9	2.9	2.5	2.3	2.9	1.5	2.3	1.9	2.6
Viridiflorol	20.5	10.5	15.5	1.1	0.9	1.0	0.9	1.8	0.7	1.1	4.7	1.7	1.5	3.6	2.9	2.6	7.4	1.1	3.0	0.4	0.3	0.4	2.4
Cubenol	4.2	2.8	3.5	0.3	0.5	0.5	0.5	0.9	0.4	0.5	1.3	0.4	0.5	1.2	1.0	0.6	1.7	0.4	0.8	0.4	0.3	0.4	0.7
14-Hydroxy-α-muurolene	2.5	-	1.3	0.4	-	0.3	0.2	0.4	0.8	0.4	0.6	0.5	1.7	1.2	2.0	0.6	-	2.3	1.2	6.3	4.5	5.4	2.1
Methyl hexadecanoate	-	-	-	-	-	-	-	-	0.3	0.1	-	0.4	0.6	-	0.4	-	-	0.4	0.3	10.4	11.9	11.2	2.7
Phytol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.8	-	8.4	1.9
Oil yield	0.41	0.25	0.33	1.59	0.62	0.40	0.66	0.48	1.07	0.80	0.57	0.50	0.27	0.16	0.29	0.40	0.49	0.29	0.34	0.85	0.47	0.66	0.50
Total percentage	74.6	99.5	87.1	98.0	95.9	95.8	95.4	95.1	95.2	95.9	91.5	89.5	96.7	92.8	85.2	96.1	86.0	92.5	88.2	93.7	96.9	95.3	91.7

[a]shadow columns indicate mean groups while dark columns indicate mean clusters.

were higher in group 2. In summary, in this study, to reveal genetic variation of essential oil content and compositions, 17 *A. tenufolia* Lam populations were collected from different agro-ecological zones of Iran and were grown in the same place. Essential oil extraction and analysis showed the average oil yield was 0.54% and its variation was from 0.16 to 1.59%. According to previous research on different accessions of *A. nobilis* L., oil yields were 0.33–1.44% (Azimi *et al*., 2016). The oil yield for aerial parts of *A. millefolium*, *A. biebersteinii* and *A. wilhelmsii* from different locations of East-Azarbayjan Province was reported to be 0.35, 45 and 0.56%, respectively (Dehghan and Elmi, 2015). Essential oil yield of 19 accessions belonging to six different *Achillea* species obtained by hydro-distillation ranged from 0.1 to 2.7% in leaves (Rahimmalek *et al*., 2009a). The oil yields of *A. tenufolia* populations in the present study were similar to other Achillea species and showed a wide range (0.16 to 1.59%) like them.

The remarkable compounds in the oils were germacrene D (47.1%), camphor (4.7%) and 1,8-cineole (4.1%). In the oils of different accessions of *A. millefolium* ssp. Elbursensis, the major compounds were camphor (16-40%), 1,8-cineole (10-20%) and borneol (4-21%) (Ebrahimi *et al*., 2012). Major components in essential oil of *A. millefolium* were 1,8 -cineole, camphor, borneol and α-pinene (Hofmann *et al*., 1992; Smelcorevic *et al*., 2010). According to previous study on *Achillea aucheri* Boiss at different growing altitudes in Damavand, Iran, the main compounds included isopulegol (16.39%), yomogi alcohol (10.92%), (Z)-ß-ocimene (9.84%), camphor (5.65%), 1,8-cineole (4.98%), linalool (4.81%), γ-terpinolene (3.66%), α-fenchene (3.60%), and camphene (3.20%) (Sardrodi *et al*., 2017).

Based on the cluster analysis result, the populations were placed in two clusters. In previous study, accessions of *A. tenuifolia* collected from different geographical regions of Iran were placed in three groups based on morphological and molecular markers (Rahimmalek *et al*., 2012).

The level of genetic variability plays an important role in improvement of breeding programs. The genetic variations of a plant species may depend on different factors such as the pollination system, pollinators, number of individuals within species, self-incompatibility, and method of propagation (Fracaro and Echeverrigaray, 2006; Rahimmalek *et al*., 2009c).

Previous studies showed that the composition of *A. millefolium* essential oils was affected by climate and the environmental influences (Nadim *et al*., 2011).

Since *A. tenuifolia* does not have any rhizome (in contrast to *A. millefolium*) and seeds are the only way of its propagation, a wide variety of this plant is found in nature (Lofgren, 2002; Gharibi *et al*., 2011).

In this study, comparison of essential oil content and composition of *A. tenuifolia* Lam. accessions by cluster analyses showed important information about the diversity of Iranian *A. tenuifolia* accessions.

CONCLUSIONS

Different populations of *A. tenuifolia* cultivated in the same climatic condition showed big variation in their essential oil yields (0.16 to 1.59%). Therefore, to obtain the highest oil yield, selection of Divandareh population, which produced 10 times more oil compared to some other populations, can be suggested for large scale cultivation. After Divandareh, Azarbaijan2 population produced high oil yield (1.07%), while other populations in both clusters contained less than 1 percent oil. Analysis of the essential oils of different populations showed two clusters with different amounts of germacrene D, 1,8-cineole and other components. Therefore, due to the use of this oil in different products and industries, one or a group of populations can be

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selected for cultivation and oil extraction in this climatic situation.

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تنوع در بازده و ترکیبات اسانس جمعیت‌های مختلف *Achillea tenuifolia* Lam. در شرایط زراعی

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چکیده

Achillea tenuifolia Lam. از خانواده Asteraceae یکی از گیاهان معطر و محبوب معطر ایران است که دارای تنوع زیادی در مورفولوژی و اجزای اسانس است. هدف از این مطالعه، بررسی کمیت و کیفیت اسانس جمعیت‌های مختلف *A. tenuifolia* در شرایط زراعی بود. برای این منظور بذر جمعیت‌های مختلف این گیاه از رویشگاه‌های طبیعی جمع‌آوری و در شرایط اقلیمی یکسان در ایستگاه تحقیقات البرز، کرج کشت شدند. در مرحله گلدهی کامل، اندام هوایی گیاهان جمع‌آوری شده و پس از خشک شدن در سایه به روش تقطیر با آب اسانس‌گیری شدند. اسانس‌ها توسط دستگاه کروماتوگرافی گازی (GC) و کروماتوگرافی گازی متصل به طیف سنج جرمی (GC/MS) مورد تجزیه و شناسایی قرار گرفتند. بر اساس نتایج حاصله، بازده اسانس بین ۰/۱۶٪ تا ۱/۵۹٪ بر حسب وزن خشک گیاه متغیر بود. جمعیت با منشاء دیواندره با ۱/۵۹٪ و خوی-۲ با ۰/۱۶٪ اسانس بیشترین و کمترین بازده اسانس را داشتند. آنالیز کلاستر بر اساس نوع و ترکیبات متشکله اسانسها، همه جمعیت‌ها را در دو خوشه قرارداد. جرماکرن دی ترکیب اصلی اسانس همه جمعیت‌ها بود، ولی اسانس جمعیت‌های کلاستر ۱ مقدار بیشتری از این ترکیب (تا ۶۴/۵٪ در جمعیت سمنان) را دارا بودند. آلفا-هومولن (تا ۱۵٪)، ۸،۱-سینئول (تا ۱۱/۷٪) ترکیبات عمده بعدی در جمعیت‌های کلاستر ۱ بودند. مقادیر کمتر از این سه ترکیب (علاوه بر ترانس-بتا-فارنزن و پیپریتون) و مقادیر بالاتر ترکیبات فرارتر مثل پارا-سیمن، بتا-فلاندرن، کامفور و آلفا-توژون و حضور یا عدم حضور برخی ترکیبات جزئی، بقیه جمعیت‌ها را در کلاستر ۲ قرار داد. جمعیت‌های هر کلاستر، به دلیل تفاوت در درصد ترکیباتی مثل کوبنول، ویریدیفلورول، متیل هگزادکانوات و فیتول به دو گروه تقسیم شدند. بنابراین بر اساس نوع تقاضا برای ترکیبات مختلف اسانس، می‌توان جمعیت مناسب را برای کشت وسیع در شرایط اقلیمی مشابه توصیه نمود.

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