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Analysis of Aroma and Phenolic Components of Selected Achillea Species

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Abstract. Dried flowers and leaves of four different *Achillea* species grown wild in several provinces of Iran, including one species collected from three different locations of Isfahan province were analyzed for the headspace volatile components, total phenolics (TP) and tartaric esters (TE). Capillary gas chromatography/mass spectrometry (GC/MS) combined with a purge and trap method was used for quantification of aroma components. Over 70 compounds were determined in the samples. Flower samples from all species contained 2-methyl butanal, α-pinene, α-thujene, camphene, hexanal, β-pinene and 1,8-cineole; however, the major constituents of the aerial parts were determined as α-pinene, camphene, DL-limonene and 1,8-cineole. The largest number of aroma components were found in *Achillea tenuifolia* Lam. and *Achillea millefolium* L. In all species, except *A. millefolium*, the leaves contained more TP and TE than flowers. However, *A. wilhelmsii* from Semirom in Isfahan province showed the highest values for TP and TE.

Key words: *Achillea eriophora*, *Achillea millefolium*, *Achillea tenuifolia*, *Achillea wilhelmsii*, aroma compounds, bioactives, gas chromatography, mass spectrometry, medicinal herb, phenolics, tartaric esters

Introduction

The herb *Achillea*, which belongs to the family Compositae (Asteraceae), is a genus with more than 100 species all around the world. These plants are medicinal perennial rhizomous herbs that are native to Europe and Western Asia, although they are also found in Australia, New Zealand and North America [1]. *A. millefolium* is the best known species, commonly named yarrow, and was used as traditional medicine in Europe and by native Americans for treating swollen tissues and wounds [2, 3].

Many species of *Achillea* have been analyzed for their bioactives with more than 100 components found, including volatiles (mono- and sesquiterpenes or their lactones) in headspace vapor or essential oils and higher-molecular-weight phenolics or flavonoids from the extracts ([2, 4–22]).

Some investigators [7–10, 14, 23] have analyzed and reported the major volatiles of several Iranian *Achillea* species as being α-pinene and β-pinene, 1,8-cineole, camphor, camphene, α-terpineol, caryophyllene, ascaridole and bornyl acetate. Several species of *Achillea* have been analyzed for their total phenolics (TP) and flavanoids in alcoholic extracts [2, 24–26]. Total phenolic compounds in *A. millefolium* was reported at 5.3 mg of gallic acid per gram dry matter.

The importance of bioactives in *Achillea* species, such as mono- and sesquiterpenes or phenolics and flavonoids, and their effects on animal and human health have been reported by several investigators [2–4, 17, 27–31]. The metabolism of terpenoids has been reviewed and discussed by McGarvey and Croteau [32]. The literature on the biological activities of sesquiterpenes present in the Compositae plant family, being associated with anti-tumor, antimicrobial, and cytotoxic effects, was reviewed by Rodriguez et al. [27]. Several investigators [29, 30] reported significant effects from the extracts of *A. wilhelmsii* or *A. talagonica* grown in Iran as being antilipidemic, antihypertensive or immunosuppressive for humans or laboratory animals respectively.

The aim of this study was to collect four different species of *Achillea*, grown wild in different provinces of Iran, including one from three locations of Isfahan province to analyze and compare the volatile components in the headspace vapor above leaves or flowers. Also, since there has been little information in the literature on their extract bioactives, we decided to extract and analyze their TP and tartaric esters (TE).

Materials and Methods

Plant Material

The plant materials of different *Achillea* species were harvested and collected in June 2002 from different locations or provinces of Iran, as set out later. The plants were refrigerated at 4.4 °C overnight at the herbarium in Isfahan University of Technology (IUT), Isfahan, Iran. The plants were dry cleaned, sorted as to leaves, flowers, stems, and roots. Different parts were air-dried separately on stainless steel tables in the shade and at ambient conditions (25–30 °C and about 10–15% RH) for 5 days to their final moisture content. The dried tissues were packed separately in three layers of low-density polyethylene bags and sent to the Agriculture and Agri-Food Research Center in Summerland, BC, Canada, for analysis in October 2002. Leaves and flowers were analyzed in this research.

The locations of harvest were as follows:

A. tenuifolia Lam. from mountains and ranch lands of Daran, Isfahan.

A. millefolium L. from mountains, agricultural fields and orchards of Roode Hen, Damavand, Tehran
A. eriophora DC. from mountains and ranch lands of Amol, Mazandaran.
A. willhelmsii C. Koch from Lavark Farm, Agricultural Research Station of IUT in Najafabad, Isfahan.
A. willhelmsii C. Koch from Gardaneh Rokh mountains and ranch lands of Shahre Kord, Isfahan.
A. wilhelmsii C. Koch from mountains, ranch lands of Semirom, Isfahan.

Purge and Trap Conditions

Headspace volatile compounds of dried samples were trapped using the procedures described by Mazza and Cottrell [35] with some modifications. The sample chamber consisted of a 150 ml water-jacketed three-neck Wheaton jar, equipped with a Waring blender head to enable the sample to be stirred during the purge cycle. The chamber was left at room temperature (22 ± 1 °C). Nitrogen purge gas was used with connections from the chamber to a Tekmar 2000 sampler (Cincinnati, OH) via Teflon tubing. Volatiles from the chamber were trapped on a 100 mg Tenax TA trap (60/80 mesh) packed in a deactivated glass 6 mm o.d. tube. Purge gas flow rate was set at 43 ml/min.

Parameter settings for the Tekmar 2000 were as follows: pre-purge time, 0 min; purge time for all samples, 2.0 min desorb preheat, 195 °C; desorb 5.0 min at 200 °C; bake 15.0 min at 225 °C; valve temperature, 250 °C; transfer line 250 °C; and mount temperature, 110 °C. Before the start of the desorb cycle, the GC oven door was opened, and about 10 cm of the transfer line (immediately before the union to the GC column) was immersed in a Dewar flask of liquid nitrogen. At the end of desorb cycle, the Dewar was removed, the oven door closed, and the GC run was initiated.

Sample Preparation

Dried leaves or flowers (ca. 20 g) of each *Achillea* species were ground to a fine homogenous powder with a small coffee grinder, and 0.5 g of each powder was weighed and promptly transferred to the purge and trap jar. Each sample was blended vigorously with 40 ml distilled deionized water inside the Wheaton jar blender for 1 min before the purging cycle started and then with a slower blending during the 2 min purge time to enable the volatiles to be released from the plant tissue and trapped on the Tenax TA trap. Two replicates were used for each sample.

GC/MS Analysis

A Hewlett-Packard 5890A gas chromatograph (now Agilent Technologies, Santa Clara, CA) equipped with a Hewlett-Packard 5970 mass-selective detector was used to analyze all samples. The trapped volatiles from each sample were introduced from the Tekmar 2000 sampler via a heated 0.32 mm deactivated fused silica transfer line, connected by a ZDV union to a 60 m × 0.32 mm DB-Wax column (J & W Scientific, Folsom, CA) with a 0.25 μm film. Prepurified grade helium was used as carrier gas for the column and for the Tekmar transfer line at a head pressure of 30 psig. The transfer line from GC to MSD was set at 260 °C. The oven temperature program was as follows: initial temperature, 35 °C (hold for 5.0 min); temperature program rate, 5.0 °C/min; final temperature, 220 °C (hold for 1.0 min). Total run time was 43.0 min. MSD parameters, in scan mode were mass range 35–300 amu; threshold 1500; sample rate 2.2 scans/s; solvent delay, 3.0 min; and EM voltage, 1200 V.

The GC and MSD were controlled by, and spectra collected by an HP ChemStation. Mass spectral identification was done using the Wiley MS database and by comparison of the chromatograms with those of earlier researchers in this laboratory [16, 35]. Levels of aroma components were determined from the average of two replicate chromatograms, calculated and expressed as the area units of their abundance per gram dry matter.

Extraction and Determination of Phenolics and Tartaric Esters

Dried flowers or leaves (ca. 3.0 g) were weighed and transferred to a heavy duty Waring blender equipped with a stainless steel water-jacketed jar. Eighty milliliters of 80% (v/v) ethanol was added and the mixture was blended for 8.0 min then vacuum filtered through No. 541 Whatman filter paper. The solids were re-extracted with 60 ml of 80% ethanol as discussed earlier and the filtrates combined and made to a final volume of 250 ml. Ten milliliters of each extract was filtered through a 0.45 μm membrane filter and kept in a capped plastic vial at −30 °C for measurement of phenolics. Two replicate extractions of each sample were made. Total phenolics and TE were measured using a modified version of Glories' method [34] as described by [35]. In a test tube, 0.25 ml of 0.1% HCl in 95% ethanol, 4.55 ml of 2% HCl and 0.25 ml of standard solution or filtered extract was added, mixed by vortex and allowed to stand for approximately 15 min before reading the absorbance at 280 and 320 nm. The absorbance at 280 nm was used to estimate TP content and 320 nm used to estimate TE. Standards (Sigma Chemical Co., St. Louis, MO) used were chlorogenic acid for TP and caffeic acid for TE, both made up in 95% ethanol. The TP and TE values were calculated and expressed as milligram per gram dry matter using standard curves at five concentrations covering the range, 100–1600 μM for chlorogenic acid and 50–1200 μM for caffeic acid.

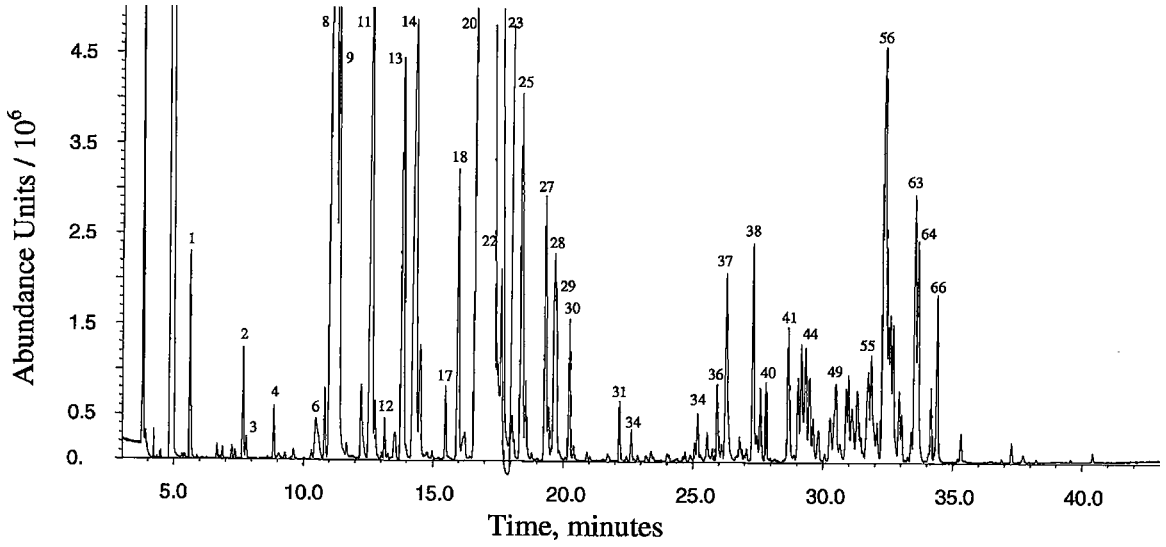

Figure 1. GC/MS of headspace aroma compounds in air-dried *A. tenuifolia* flowers.

Dry Matter Determination

For dry matter (DM), a laboratory vacuum oven was used. One to two grams of duplicate samples were weighed in aluminum drying dishes, dried for about 7 h at 58 °C and a vacuum of 27 in. of Hg. Dry matter content ranged from 97 to 98%.

Statistical Analysis. For statistical analysis of TP and TE in leaves and flowers of the plant, one-way ANOVA followed by Tukey's comparison test for means was performed using Minitab version 12.21 (Minitab Inc., State College, PA). Significance of difference was defined at $p < 0.05$.

Results and Discussion

GC/MS Analysis

Figures 1 and 2 show typical chromatograms for the headspace volatiles of *A. tenuifolia* flowers and leaves. Other samples showed a similar trend for all species with lower abundance values. Table 1 represents the quantified values for the volatile components of different *Achillea* species per gram dry matter. The numbers in the first column refer to the peaks that were analyzed and identified (Figures 1 and 2).

Each peak for a volatile component was identified from its mass spectrum using the mass-spectral database library and

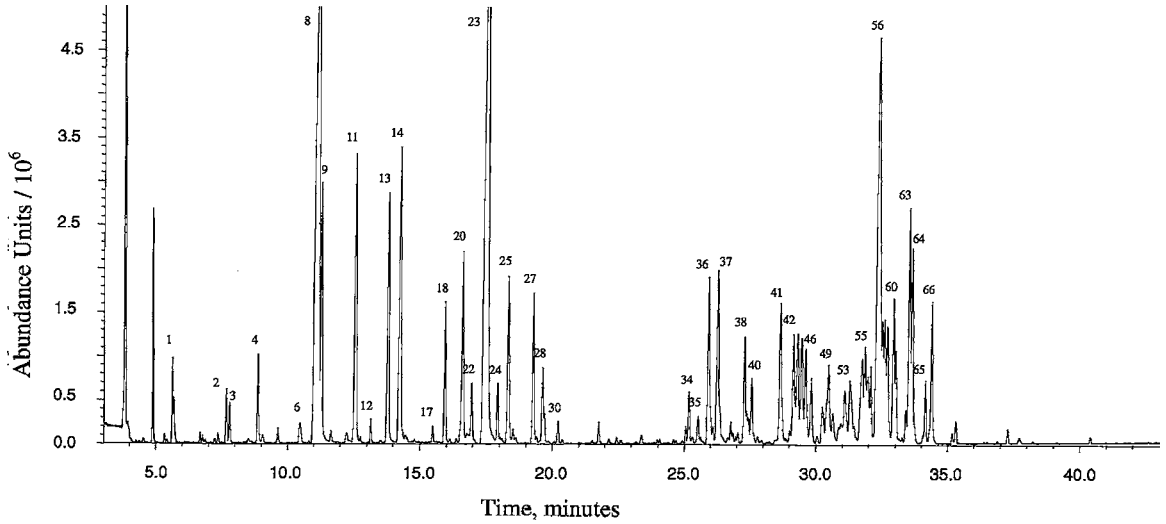

Figure 2. GC/MS of headspace aroma compounds in air-dried *A. tenuifolia* leaves.

Table 1. GC/MS analysis of aroma components in leaves (L) and flowers (F) of six species of *Achillea* grown in Iran[a]

Peak[b]	Compounds	Retention time (min)	ID[c]	*A. tenuifolia*		*A. millefolium*		*A. eriophora*		*A. wilhelmsii* Lavark		*A. wilhelmsii, Gardaneh* Rokh		*A. wilhelmsii* Semirom	
				L	F	L	F	L	F	L	F	L	F	L	F
1	Propanal, 2-methyl	5.63	t + m	27	74	6	9			8	26	24	20	14	108
2	Butanal, 2-methyl	7.67	t + m	17	41	9	9	6	8	3	3	7	13	10	16
3	Butanal, 3-methyl	7.80	t + m	28	8	9	14			6			7	9	7
4	Furan, 2-ethyl	8.87	m	17	18	25		6	6	16		9	6		
5	Pentanal	9.63	t + m			11	8			13					
6	Tricyclene	10.48	m	14	35					9	24				
7	2-Methylpropyl acetate	10.87	t + m										40		16
8	α-Pinene	11.17	t + m	653	933	96	1350	198	255	999	1100	213	499	381	665
9	α-Thujene	11.28	m	130	241	7	136	27	45	6	184	9	20	17	21
10	α-Fenchene	12.36	m				20								
11	Camphene	12.58	t + m	176	352		136	10	15	163	208	7	22	19	28
12	Hexanal	13.15	t + m	8	12	14	15		9	16	8	10	11	7	9
13	β-Pinene	13.82	t + m	147	288	30	674	17	40	23	41		15		21
14	β-Terpinene	14.27	m	193	366		381	20	34	16	17				6
15	2-Methyl-1-butyl acetate	14.43	t + m									23	109	8	66
16	Sabinene	14.48	t + m			6	400			7					
17	α-Phellandrene	15.49	t + m	7	25		56								
18	α-Terpinene	15.95	t + m	69	151		205	35	55	8	9				
19	2-Methylbutanoic acid, 2-methylpropyl ester	16.09	m												14
20	DL-Limonene	16.62	t + m	116	532		200	13	20	30		13	45	16	29
21	2-Methylpropanoic acid, 2-methylbutyl ester	16.80	m									19	102	15	47
22	β-Phellandrene	16.96	t + m	38	113		142	8	11						
23	1,8-Cineole	17.56	t + m	713	1161	64	950	408	253	116	84	37	33	107	49
24	2-Hexanal, [E]-	17.95	t + m	22	11										
25	γ-Terpinene	18.35	t + m	99	228		325	26	35						
26	3,7-Dimethyl-1,3,6-octatriene	18.60	m				60								
27	*p*-Cymene	19.29	t + m	77	151	16	170	130	191	63	90	23	77		
28	α-Terpinoline	19.64	m	43	114		124								
29	2-Methylbutanoic acid, 2-methylbutyl ester	19.70	m	5	46				37		44	21	90	26	77
30	3-Methylbutanoic acid, pentyl ester	20.22	m	9	57								68		26
31	3-Methylbutanoic acid, 3-methyl-3-butenyl ester	22.15	m	2	20			4	10						
32	Alloocimene	22.60	t + m				100								
33	Unknown	24.91	m									92	143	111	133
34	α-Cubenene	25.19	t + m	21	17										
35	Unknown terpene	25.55	m	10	10										
36	Unknown	25.94	m	83	35										
37	α-Copaene	26.29	m	99	111										
38	Camphor	27.31	t + m	52	88		23			112	101				
39	3,7-Guaiadiene	27.39	m	11	12										
40	Isoledene	27.59	m	31	33										
41	α-Ylangene	28.67	m	81	72										
42	β-Cubenene	29.16	m	62	55										
43	Lavandulyn acetate	29.29	m									9	25		
44	*trans*-Caryophyllene	29.37	t + m	63	76		328								

Table 1. Continued

45	Unknown terpene	29.48	m	65	47	12
46	α-Selinene	29.63	m	56	23	
47	β-Selinene	29.84	m	36	18	
48	Unknown terpene	30.27	m	24	26	
49	Unknown terpene	30.49	m	62	46	
50	Aromadendrene	30.66	m	18		
51	Cadinene isomer	31.11	m	44	33	
52	α-Humulene	31.27	t + m			20
53	epi-Bicyclosesquiphellandrene	31.31	m	58	54	
54	Cadinene isomer	31.76	m	75	80	
55	Cadinene isomer	31.87	m	36	37	
56	Cadinene isomer	32.38	m	370	333	21
57	α-Copaene	32.52	m	58	58	
58	Cadinene isomer	32.60	m	61	66	
59	α-Muurolene	32.71	m	62	56	
60	Germacrene B	32.95	m	85	34	
61	[E]-Farnesene	33.03	m	20	10	15
62	Cadinene isomer	33.42	m	16	24	
63	δ-Cadinene	33.55	t + m	126	137	14
64	γ-Cadinene	33.66	m	102	98	13
65	Cubebene isomer	34.17	30	31		
66	Muurolene isomer	34.41	m	75	79	

[a] Values are the average of area units (divided by 10,00,000) from two replicate samples per gram of dry matter.
[b] Numbers correspond to those shown on chromatograms.
[c] Identification of components. t + m: ID by retention time of standards plus mass spectrum, m: ID by mass spectrum only.

comparison of the results with retention times and spectra from similar chromatograms of earlier investigators in our laboratory [16, 33]. However, not all identifications could be confirmed by comparison with an authentic standard.

As shown in Table 1, *A. tenuifolia* was the richest in volatile components in its flowers and leaves among all species, followed by *A. millefolium*, which was rich particularly in its flowers. All species contained α-pinene, camphene, β-pinene, α-thujene, DL-limonene and 1,8-cineole. Among Iranian species of *Achillea* (Table 1), *A. eriophora*, *A. millefolium* and *A. tenuifolia* contained 1,8-cineole and α-pinene and β-pinene as their major aroma components. Weyerstahl et al. [9] analyzed *A. eriophora* collected from Pars Province in Iran and confirmed the same major components in its essential oil fraction. Another investigator [14] reported that camphor was the major volatile compound in the water distillate of flowers of *A. tenuifolia* which were collected north of Tehran. However, in this study camphor was not detected as a headspace constituent of *A. tenuifolia* collected from Isfahan province, but was found in other species. Afsharypuor et al. [7, 8] identified 21 constituents in the volatiles of *A. millefolium* from northeast of Tehran and reported a high percentage of sesquiterpenes, 55.4%, in the essential oil. However, in this research, 32 components were determined in the volatiles of *A. millefolium* with the majority being α-pinene and β-pinene, 1,8-cineole, sabinene, terpinene isomers, DL-limonene and *trans*-caryophyllene, particularly in the flower portions. Although the flowers of *A. millefolium* contained high concentrations of sabinene in their volatiles, in other species, this component was only detected in the leaves of *A. wilhelmsii* from Lavark. Figueiredo et al. [36] reported that sabinene was dominant in the essential oil of one of the two populations of *A. millefolium* collected in Spain.

Several other species of Iranian *Achillea* have been analyzed [10, 23] and their major constituents reported to be 1,8-cineole, α-pinene and β-pinene, camphor, α-thujone and camphene.

Nemeth et al. [37] classified the *Achillea crithmifolia* of different locations grown in Hungarian mountains as camphor, cineole or achillenol types and they reported that the difference of constituents in species or the same population was genotypic rather than phenotypic. In our study of headspace volatiles, camphor was detected in *A. tenuifolia* and in *A. wilhelmsii* from Lavark but not in that species from Gardeneh Rokh or Semirom.

The headspace volatile components of *A. filipendulina* Lam., grown in Canada and analyzed by GC/MS in our laboratory (unpublished results) resulted in about threefold more concentration of components in fresh leaves than flowers. Among 16 major volatiles determined, α-pinene, camphene, 1,8-cineole, *cis*-2,7-dimethyl-4-octene-2,7-diol, and *p*-cymene were the most predominant in fresh leaves.

Total Phenolics and Tartaric Esters

The values calculated from standard curves are expressed as mg/g DM for TP and TE. Duplicate readings from two replicate extracts of each leaf or flower were used for calculating TP or TE. Table 2 presents the contents of TP and TE of the dried flowers and leaves which were analyzed. TP values for flowers were statistically the same for five of the six *Achillea* species investigated; and the data indicates more TP than TE in all *Achillea* species. In most species, the levels of TP and TE were higher in the leaf than in the flower, except for *A. millefolium* and *A. wilhelmsii* from Semirom in which the levels were almost the same. It is also evident that there is as much variation in the levels of both TP and TE between samples of the same species collected from different sites as there is between species.

From the genus *Achillea*, yarrow was the first species to be analyzed for its polyphenolics and flavanoids [2]. Several other species have been analyzed for their flavonoid composition [25, 38]. In the aerial parts of *Achillea pannonica*, [25] isolated rutin, apigenin-7-*O*-glucopyranoside, luteolin-7-*O*-glucopyranoside, apigenin-7-*O*-rutinoside, acacetin-7-*O*-rutinoside, and luteolin-7,4′-*O*-beta-diglucoside.

Recently, we also analyzed flowers and leaves of *A. filipendulina* Lam. grown in Canada for TP and TE (unpublished results). The ethanolic extracts of the leaf samples

Table 2. Total phenolics (TP) and tartaric esters (TE) in flowers or leaves of different *Achillea* species grown in Iran

Species	TP (mg/g DM)		TE (mg/g DM)	
	Flower	Leaf	Flower	Leaf
A. tenuifolia	30.2 ± 4.6^{a}	56.6 ± 6.5^{b}	6.20 ± 1.09^{a}	13.7 ± 1.9^{c}
A. millefolium	$35.7 \pm 2.4^{a,b}$	32.7 ± 3.6^{a}	12.9 ± 0.9^{b}	$11.7 \pm 1.9^{b,c}$
A. eriophora	$38.9 \pm 6.8^{a,b}$	53.6 ± 4.7^{b}	6.72 ± 0.58^{a}	10.6 ± 1.1^{b}
A. wilhelmsii (Lavark)	27.4 ± 0.7^{a}	33.2 ± 7.6^{a}	5.60 ± 0.18^{a}	6.90 ± 1.68^{a}
A. wilhelmsii (Gardaneh Rokh)	$36.1 \pm 3.4^{a,b}$	49.5 ± 3.8^{b}	7.65 ± 0.41^{a}	$13.3 \pm 0.9^{b,c}$
A. wilhelmsii (Semirom)	58.3 ± 14.8^{c}	58.5 ± 6.1^{b}	11.8 ± 3.4^{b}	13.9 ± 1.5^{c}

*Values are means $\pm$ standard deviations ($n = 4$). Different letters in the same columns indicate a significant difference ($p < 0.05$).

contained more TP and TE than the flowers among fresh or dried samples. TP content of the fresh flower had the highest value, 47.0 mg/g DM, while dried samples contained 22.0–36.0 mg/g DM. Thus, the TP and TE levels in dry flowers and leaves of *Achillea* species grown in Iran are comparable to values reported for *A. filipendulina* grown in Canada.

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