



## The Essential Oil Composition and Antioxidant Activity of *Achillea* spp. Growing in the Southwest of Iran

Roozbeh Farhoudi<sup>1\*</sup> and Mohammad Amin Mehrnia<sup>2</sup>

<sup>1</sup>Department of Agronomy and Plant Breeding, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran

<sup>2</sup>Department of Food Industry, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran

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### Abstract

The composition of essential oil isolated from *Achillea eriophora*, *Achillea millefolium*, *Achillea biebersteinii* and *Achillea tenuifolia* growing wild in the south west of Iran, was analyzed. *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oils were characterized by sabinene, 1, 8-cineole, terpinene-4-ol,  $\alpha$ -bisabolol, p-Cymene,  $\beta$ -pinene and  $\alpha$ -pinene. The *A. biebersteinii* essential oil was characterized by sabinene, borneol, camphor, piperitone and  $\alpha$ -pinene. Antioxidant activity was analyzed using the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging and  $\text{Fe}^{3+}$  reducing power methods. Results indicated essential oil obtained from *A. eriophora*, *A. millefolium*, *A. tenuifolia* and *A. biebersteinii* exhibited a dose-dependent increase with a radical scavenging effect of 85.0%, 82.0%, 82.0% and 64.0% at 350  $\mu\text{g/ml}$ , which are close to the 1,1-diphenyl-2-picrylhydrazyl inhibition of the positive control Butylated Hydroxytoluene (88.0%) at the same concentration. It was shown that the *A. biebersteinii* essential oil exhibited the weakest antioxidant effect than Butylated Hydroxytoluene or other *Achillea* spp. essential oils. In this study chamazulene,  $\alpha$ -bisabolol and  $\alpha$ -bisabolol oxide percentage were higher in *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oil compared to *A. biebersteinii* essential oil and these compounds improved antioxidant capacity of *Achillea* spp.

**Key words:** *Achillea* spp., Essential oil, Radical scavenging,  $\text{Fe}^{3+}$  reducing power

### Introduction

In recent decades, the phytochemical constituents of plants have received much attention due to their potential use in nutraceuticals and drug industries. Spices and herbs are a part of daily food intake in many regions of the world. They have been used as natural sources of flavorings and preservatives [1-3]. Yarrow (*Achillea* spp.) belongs to Asteraceae family and more than 100 species have been recognized in this genus. The genus *Achillea* is well-known medicinal plants, widely used in folk medicine against gastrointestinal disorders such as lack of appetite. These plants are native to Europe and western Asia but are also found in Australia, New Zealand and North America [4]. Nineteen species of *Achillea* have been recognized in Iran distributed in different geographical and ecological regions [5-7]. *Achillea* spp. are diaphoretic, astringent, tonic, stimulant and mild aromatic and

produce a group of active compounds including isovaleric acid, salicylic acid, asparagine, sterols, flavonoids, tannins and coumarins. Major components in *Achillea* spp. oil are sabinene, 1, 8-cineole, camphor,  $\alpha$ -pinene,  $\beta$ -pinene, borneol and bornyl acetate [8].

The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging and atherosclerosis [9]. In biological systems, oxygen-derived free radicals have repeatedly been demonstrated to play a role in cellular injury through a chain reaction which leads to lipid peroxidation. Almost all organisms are well protected against free radical damage by oxidative enzymes, such as superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT), or by chemicals such as tocopherols, ascorbic acid, carotenoids, polyphenols and glutathione [10]. Thus, increasing antioxidant intake in human diet is

\*Corresponding author: Department of Agronomy and Plant Breeding, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran

E-mail Address: rfarhoudi@gmail.com

an important way to minimize such oxidative damages. Therefore, researches concerning essential oils as potential antioxidants for treatment of human diseases, prevention and treatment of free radical-related disorders, and preserving foods are important. Concomitantly, public attention to natural antioxidants has increased during the last years, and there is need to find natural sources of antioxidants that could replace synthetic antioxidants or at least reduce their use as food additives [11]. Plants showing potent antioxidant activity may be used as a safer source for inhibition of oxidative reactions. Asgarirad *et al.*, [5] reported direct relationship between phenol and flavonoid content of *Achillea tenuifolia* extracts and the antioxidant activity of this plant. They found the greater amount of phenolic compounds leads to more potent radical scavenging and lipid peroxidation inhibition activities as it was observed in *A. tenuifolia* polar extract. *Achillea millefolium* extract showed good free radical scavenging activity and ability to decrease the levels of intracellular reactive oxygen species (ROS) [12, 13]. Hernández-Ceruelos *et al.*, [14] found that chamomile essential oil is an efficient chemo protective agent against damage induced by daunorubicin in precursor cells of the germinal line of mice, and that its antioxidant capacity may induce this effect. The aim of this work is to investigate chemical composition of essential oils of *A. eriophora*, *A. millefolium*, *A. biebersteinii* and *A. tenuifolia* to evaluate their antioxidant activity by using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay and Fe<sup>3+</sup> reducing power assay.

## Material and Methods

### Plant material

Plant materials (leaf and head branches) of *Achillea eriophora* DC., *A. millefolium* L., *A. biebersteinii* Afanasiev and *A. tenuifolia* Saliseb. were collected from medical plant garden of Islamic Azad University, Shoushtar branch (32°3'0"N 48°51'0"E) in June 2011.

Clevenger apparatus was used to extract oils by hydro distillation of leaf and head branches for 3 hour according to the method described in British Pharmacopeia [15]. The oils were dried over anhydrous sodium sulphate and were kept in refrigerator until they were analyzed.

### GC/MS analysis conditions

For identification of components, Agilent gas chromatography model 6890 N, equipped with MSD model 5973 N and fused silica capillary column (HP-5MS, 30m- 0.25mm) were used for qualitative and quantitative analysis of oils. The GC oven temperature was held at 50 °C for 5 min, then programmed from 50 °C to 240 °C at a rate of 3 °C min<sup>-1</sup> and from 240 °C to 290 °C at a rate of 5 °C min<sup>-1</sup>, held for 2 min at 290 °C, using He gas as the carrier (1.0 ml min<sup>-1</sup>). The temperature of injector and detector were 240 °C and 280 °C. The percentage composition of the essential oils was computed from GC peak areas without using any correction factors. Qualitative analysis was based on comparison of retention times and indices on both columns and mass spectra using computer mass spectra libraries model Agilent Technologies 5973 Network and corresponding data available in the literature [16].

### DPPH radical scavenging assay

The ability of oil to scavenge free radicals of *Achillea* spp. essential oil was assayed by using a synthetic free radical compound 1, 1-diphenyl-2-picrylhydrazyl (DPPH), according to the method employed by Bersuder *et al.*, [17]. Briefly, 500 µl of each sample was mixed with 500 µl ethanol and 125 µl DPPH (0.02%) in 99.5% ethanol. After 60 min, the absorbance was measured at 517 nm using a spectrophotometer. The DPPH radical-scavenging activity is calculated as follows:

$$\text{Radical-scavenging activity} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100.$$

Where  $A_{\text{blank}}$  and  $A_{\text{sample}}$  are the absorbance of the control (blank) and the sample. The IC<sub>50</sub> value is defined as the amount of the antioxidant necessary to inhibit DPPH radical formation by 50%. As such, the synthetic antioxidant reagent Butylated Hydroxytoluene (BHT) was used as a positive control.

### Reducing power

The ability of oil to reduce iron (III) was determined according to Yildirim *et al.*, [18] with some modifications. An aliquot of 500 µl of each sample at different final concentrations was dissolved in ethanol and mixed with 1.25 ml of 0.2 M phosphate buffer reagent (pH 6.6) and 1.25 ml of 1% potassium ferricyanide. The mixture was incubated for 30 min at 50 °C followed by the addition of 1.25 ml of 10% (w/v) trichloroacetic acid. The mixture was then centrifuged at 1500 g for 10 min. Finally, 1.25 ml of the supernatant

solution was mixed with 1.25 ml of distilled water and 250  $\mu$ l of 0.1% (w/v) ferric chloride. After 10 min, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

## Results and Discussion

### Chemical composition

Gas-chromatographic analysis of the composition of *Achillea* spp. essential oil revealed very interesting profile of chemical constituents. The components of the oil, the percentage of each constituent and their retention indices are summarized in Table 1. In *A. eriophora* major compounds that were identified by gas chromatography–mass spectrometry (GC–MS) were sabinene (21.1%), 1, 8-Cineole (18.3%),  $\alpha$ -bisabolol (10.6%), terpinene-4-ol (8.6%),  $\alpha$ -pinene (6.7%),  $\beta$ -pinene (4.0%), p-Cymene (3.21%) and chamazulene (2.1%). In *A. millefolium* major compounds were sabinene (22.1%), 1, 8-Cineole (16.2%),  $\beta$ -pinene (11.1%),  $\alpha$ -bisabolol (8.3%),  $\alpha$ -pinene (4.8%), p-Cymene (2.9%) and Chamazulene (2.6%). In *A. biebersteinii* major compounds included sabinene (18.8%), borneol (8.2%), camphor (6.4%), piperitone (5.2%), 1,8-Cineole (4.8%),  $\beta$ -Myrcene (4.1%) and  $\beta$ -pinene (3.6%). In *A. tenuifolia* essential oil characterized by sabinene (20.5%), 1,8-Cineole (15.2%),  $\alpha$ -bisabolol (7.5%),  $\alpha$ -pinene (6.1%), terpinene-4-ol (4.64%),  $\beta$ -pinene (3.81%) and p-Cymene (2.94%). Results indicated *Achillea* spp. major oil components include sabinene,  $\alpha$ -pinene, camphor, borneol, 1,8-cineole,  $\beta$ -pinene, p-Cymene,  $\alpha$ -bisabolol, terpinene-4-ol and chamazulene which directly reduce inflammation and are both anti-inflammatory and antispasmodic actions [19,20]. Nadim *et al.*, [21] and Rustaiyan *et al.*, [7] found sabinene,  $\alpha$ -pinene, 1,8-cineole, chamazulene, borneol,  $\beta$ -pinene and terpinene-4-ol are major chemical compounds in *Achillea* spp. essential oil.

Smelcorevic *et al.*, [8] reported sabinene,  $\alpha$ -pinene, 1, 8-cineole and borneol are major chemical compounds in *A. millefolium* and *A. crithmifolia* which have antioxidant capacity. The essential oil also contains  $\beta$ -pinene,  $\alpha$ -pinene, 1, 8-cineole, terpinene-4-ol, abisabolonoxide A and chamazulene which have anti-inflammatory and antioxidant actions [1,19,22].

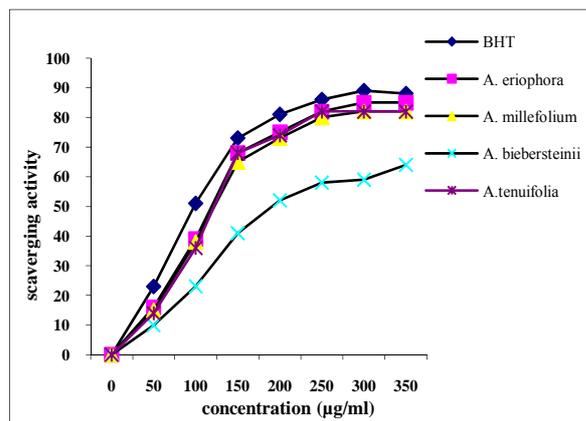
### DPPH radical-scavenging activity

The antioxidant activity of the tested essential oils of *Achillea* spp. were determined by different *in vitro* methods, such as the DPPH free radical scavenging assay and reducing power assay. The results were compared with the synthetic antioxidant BHT, which is an efficient synthetic antioxidant in food. All the assays were carried out in triplicate and the average values were considered [23].

Free radicals are often generated as by-products of biological reactions or from exogenous factors and cause damage on biological molecules like membrane lipids. Involvements of free radicals in pathogenesis of a large number of diseases are well documented. DPPH is a free-radical compound which has been widely used to test the free-radical scavenging ability of various samples. The model of scavenging the stable DPPH radical is a widely used method to evaluate free radical scavenging ability of various samples [23]. Results show the effective concentrations of the essential oil required to scavenge DPPH radical and the scavenging values as an inhibition percentage at various concentrations (Fig. 1). Results indicated essential oil obtained from *A. eriophora*, *A. millefolium* and *A. tenuifolia* exhibited a dose-dependent increase with a radical scavenging effect of 85.0%, 82.0% and 82.0% at 350  $\mu$ g/ml, which is close to the DPPH inhibition of the positive control BHT (88.0%) at the same concentration. essential oil obtained of *A. biebersteinii* exhibited a dose-dependent increase with a radical scavenging effect of 64.0% at 350  $\mu$ g/ml and it was shown that the *A. biebersteinii* essential oil exhibited the weakest antioxidant effects than BHT or other *Achillea* spp. essential oil. DPPH scavenging activity is usually presented by  $IC_{50}$  value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Comparing the DPPH scavenging activity of *A. eriophora* (134.2  $\mu$ g/ml), *A. millefolium* (132.0  $\mu$ g/ml) and *A. tenuifolia* (135.4  $\mu$ g/ml) and those expressed by BHT (100.0  $\mu$ g/ml), it was shown that the essential oil of these *Achillea* spp. exhibited the good antioxidant effects than *A. biebersteinii* (Fig. 1). Therefore, the antioxidant effect of the *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oils were about near that of the synthetic antioxidant BHT. The DPPH scavenging ability of *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oil can be attributed to the presence of some components that have antioxidant activity, for example:  $\alpha$ -pinene,  $\beta$ -pinene, 1,8-

Cineole,  $\alpha$ -bisabolol,  $\alpha$ -bisabolol oxide A and chamazulene [5,24].

As a consequences of exposure to exogenous chemicals, the reactive derivatives of oxygen, ascribed as ROS are continuously generated inside the human body. Normally the ROS generated are detoxified by antioxidants present in the body and there is equilibrium between the ROS generated and the antioxidants present. The strong antioxidant and DPPH radical scavenging activities of *D. buettneri* essential oil can be attributed to the presence of some components that have antioxidant activity like 1, 8-cineole,  $\alpha$ -pinene and  $\beta$ -pinene [25]. Results indicated some of *A. eriophora*, *A. Millefolium* and *A. tenuifolia* essential oil compounds like 1,8-Cineole,  $\alpha$ -bisabolol,  $\alpha$ -pinene and  $\beta$ -pinene were higher compared *A. biebersteinii* essential oil (Table 1) and it can be improve antioxidant activity of these plants [2,3]. For the *A. biebersteinii* essential oil, the weak activity could inevitably be expected, as the main constituents were camphor and it is not strong antioxidant compound [26].



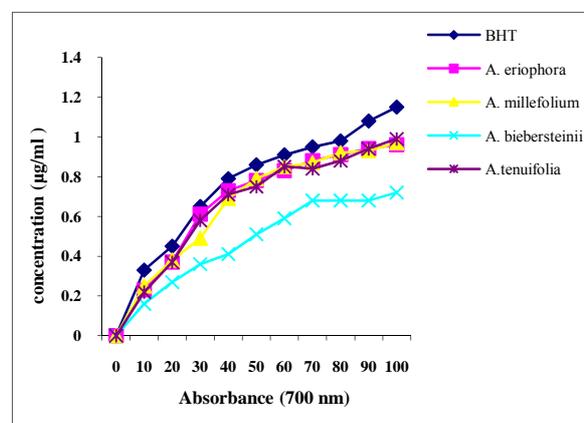
**Fig. 1** Free radical scavenging activity of *Achillea* spp. essential oil and positive control (BHT).

#### Reducing power

Antioxidant activity was also determined by ferric reducing power using a spectrophotometer at 700 nm. In this assay, the presence of reducers causes the transformation of  $\text{Fe}^{3+}$  into  $\text{Fe}^{2+}$  by donating an electron. Then, the amount of complex can be monitored by measuring the formation of Perl's Prussian blue ( $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$ ) at 700 nm. Reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. Increasing absorbance at 700 nm indicates an increase in reductive ability.

Fig. 2 illustrates dose-response curves for the reducing powers of the essential oils and synthetic

antioxidant BHT, an increase in the values can be seen increasing the concentration, which indicated an increase in the ferric reducing power. The *Achillea* spp. essential oil exhibited a lower reducing power compared of synthetic antioxidant BHT but Results showed *A. eriophora* (0.96%), *A. millefolium* (0.97%) and *A. tenuifolia* (0.99%) essential oil absorbance values were close to synthetic antioxidant BHT (1.15%) obtained at 100  $\mu\text{g/ml}$ . The EC50 (a concentration of which the absorbance is 0.5 value of BHT was 28.2  $\mu\text{g/ml}$  and EC50 of *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oils were 34.3  $\mu\text{g/ml}$ , 37.5  $\mu\text{g/ml}$  and 40.0  $\mu\text{g/ml}$  but EC50 of *A. biebersteinii* essential oil was 56.2  $\mu\text{g/ml}$ . These result showed absorbance value of *Achillea* spp. except *A. biebersteinii* essential oil was close to BHT (Fig. 2).



**Fig. 2** Reducing power of *Achillea* spp. as compared to BHT

Reports concerning the local applications and effects of the essential oils and extracts of several *Achillea* spp. have been cited in the literature [13,27,28]. In this study chamazulene,  $\alpha$ -bisabolol,  $\alpha$ -bisabolol oxide percentage were higher in *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oil compared to *A. biebersteinii* essential oil (Table 1).

Some chemicals compounds like chamazulene,  $\alpha$ -bisabolol and  $\alpha$ -bisabolol oxide were important compounds in chamomile essential oil [3,4].

Rekka *et al.*, [29] found chamazulene decreased lipid peroxidation and increased free radical scavenging capacity in target cell. Lis-Balchin *et al.*, [30] indicated some compounds in chamomile essential oil like chamazulene,  $\alpha$ -bisabolol,  $\alpha$ -bisabololoxide increase antioxidants activity of this plant.

**Table 1** Chemical composition and percentage composition of the *Achillea* spp. essential oil.

<i>A. eriophora</i>			<i>A. millefolium</i>			<i>A. biebersteinii</i>			<i>A. tenuifolia</i>		
Compound	RI <sup>2</sup>	% <sup>1</sup>	Compound	RI	%	Compound	RI	%	Compound	RI	%
Tricyclene	914.8	0.23	<i>Trans</i> -2-hexenal	898.3	0.95	$\alpha$ -pinene	931.7	2.6	$\alpha$ -thujene	926.1	0.75
$\alpha$ -thujene	920.9	0.71	$\alpha$ -Tricyclene	925.1	0.93	$\beta$ -pinene	974.8	3.6	$\alpha$ -pinene	937.1	6.1
$\alpha$ -pinene	928.1	6.7	Thuja-2,4(10)-diene	961.3	0.87	Sabinene	983.7	18.8	Sabinene	978.4	20.51
Thuja-2,4(10)-diene	955.9	0.26	1-Octen-3-ol	971.2	0.11	Myrcene	993.5	1.1	$\beta$ -myrcene	994.7	1.11
$\beta$ -pinene	971.6	4.0	Sabinene	977.1	22.1	B-myrcene	1001.4	0.76	Myrcene	996.4	0.77
Sabinene	977.1	21.1	$\beta$ -myrcene	1001.4	1.62	P-Cymene	1023.1	0.97	$\alpha$ -phellandrene	1009.5	0.54
$\alpha$ -phellandrene	1003.6	0.52	$\alpha$ -phellandrene	1004.2	0.71	1,8-Cineole	1035.1	4.8	$\acute{\alpha}$ -terpinene	1011.8	0.71
$\Delta$ -terpinene	1012.5	0.96	P-cymene	1018.7	2.9	Cis- $\beta$ -ocimene	1041.4	0.81	1,8-cineole	1036.3	15.2
P-cymene	1020.2	3.21	Cis- $\beta$ -ocimene	1026.9	1.27	<i>Trans</i> - $\beta$ -ocimene	1051.2	0.43	Limonene	1037.9	0.18
$\beta$ -phellandrene	1027.4	0.78	Limonene	1035.0	0.69	<i>Trans</i> -linalool oxide	1058.6	1.11	Cis- $\beta$ -ocimene	1051.8	0.52
1,8-Cineole	1031.2	18.3	1,8-Cineole	1037.8	16.2	<i>Trans</i> -sabinene Hydrate	1067.6	0.87	<i>Trans</i> - $\beta$ -ocimene	1056.7	0.78
Cis- $\beta$ -ocimene	1045.7	0.29	Cis-sabinene hydrate	1065.7	0.81	Fenchone	1081.4	1.54	<i>Trans</i> -sabinene hydrate	1061.5	0.91
<i>Trans</i> - $\beta$ -ocimene	1058.0	0.83	1065.4	0.89	<i>Trans</i> -linalool Oxide	1083.0	1.61	1079.8	1.82	Linalool	
Cis-sabinene hydrate	1067.5	0.76	1093.3	1.58	Linalool	1139.1	14.5	1128.6	1.17	Cis-menth-2-en-1-ol	
Linalool	1087.4	2.13	1164.4	1.86	Camphor	1141.8	0.92	1143.5	1.67	Camphor	
Cis-p-menth-2-en-1-ol	1117.3	0.34	1180.5	1.61	Borneol	1161.8	2.1	1151.8	1.62	Phenol,2-(1Z)-propenyl	
Camphor	1137.5	0.86	1183.5	4.64	Terpinen-4-ol	1171.1	12.0	1175.7	1.93	Borneol	
Borneol	1161.1	1.73	1187.5	1.81	$\alpha$ -terpineol	1193.4	0.14	1181.7	1.81	Terpinen-4-ol	
Terpinen-4-ol	1179.0	8.6	1198.8	0.73	Myrtenol	1201.3	0.46	1189.8	0.79	$\alpha$ -terpineol	
$\alpha$ -terpineol	1184.9	0.64	1229.8	1.52	Piperitone	1223.7	0.37	1197.9	1.85	Cis-dihydrocarvone	
Cis-dihydrocarvone	1195.6	0.11	1319.6	0.86	Carvacrol	1239.5	5.2	1206.5	0.71	<i>Trans</i> -dihydrocarvone	
<i>Trans</i> -dihydrocarvone	1202.7	0.23	1336.7	0.25	Eugenol	1268.3	0.71	1233.8	0.28	Eugenol	
Piperitone	1241.7	0.98	1368.9	0.63	(E)- $\beta$ -damascenone	1278.4	0.14	1258.3	0.54	Geraniol	
Nerylformate	1275.3	0.39	1376.4	0.81	$\beta$ -bourbonene	1317.2	0.87	1317.1	1.24	Carvacrol	
Carvacrol	1314.6	1.74	1427.3	1.96	Geranylacetone	1341.4	0.93	1342.8	0.81	$\acute{\alpha}$ -copaene	
Eugenol	1325.1	0.44	1431.9	0.17	Calarene	1371.8	1.13	1373.8	0.73	$\beta$ -copaene	
$\acute{\alpha}$ -copaene	1367.0	0.91	1446.8	0.28	$\acute{\alpha}$ -humulene	1365.4	0.63	1391.8	0.99	$\beta$ -cubebene	
$\beta$ -bourbonene	1388.4	0.31	1473.9	0.73	$\acute{\alpha}$ -muurolene	1406.4	0.18	<i>Trans</i> - $\beta$ -caryophyllene	1398.5	0.27	$\beta$ -bourbonene
<i>Trans</i> - $\beta$ -caryophyllene	1510.6	0.64	Cubebol	1437.8	0.53	Isogermacrene D	1408.1	0.11	<i>Trans</i> - $\beta$ -caryophyllene	1402.7	0.15

Table 1(Continue)

$\alpha$ -humulene	1530.5	0.93	Germacrene B	1478.4	0.25	$\beta$ -ionone	1421.9	0.78	E-caryophyllene	1443.1	0.74
A-murolene	1551.7	0.71	Z- $\beta$ -farnesene	1511.6	0.61	<i>Trans</i> -calamenene	1439.1	0.71	$\alpha$ -humulene	1471.6	0.22
Germacrene D	1565.0	0.96	Elemol	1558.5	0.18	Z- $\beta$ -farnesene	1474.3	0.33	$\alpha$ -murolene	1482.4	0.68
$\beta$ -bisabolene	1579.9	0.12	Spathulenol	1561.0	0.71	Elemol	1517.0	0.62	$\delta$ -cadinene	1505.1	0.29
Cubebol	1589.2	0.76	Caryophyllene oxide	1577.8	0.85	Spathulenol	1528.8	0.75	Germacrene B	1548.4	0.36
Elemol	1607.8	1.32	Torilenol	1589.4	0.61	Mintoxide	1544.1	0.69	Z- $\beta$ -farnesene	1559.6	0.17
Spathulenol	1626.9	0.87	12-epi-cedrol	1601.3	0.48	Germacrene-D	1571.4	1.31	Spathulenol	1581.6	0.22
Germacrene-D	1653.1	0.61	$\alpha$ -eudesmol	1624.3	0.32	Humulene epoxide III	1611.6	0.19	Humulene epoxide II	1591.5	0.46
Salvial-4(14)-en-1-one	1671.8	7.5	$\alpha$ -bisabolol	1678.8	3.1	$\alpha$ -bisabolol	1644.9	0.66	Vulgarone B	1600.7	0.16
Humulene epoxide II	1691.9	1.31	(E,E)-farnesol	1682.4	1.62	$\alpha$ -eudesmol	1653.0	0.11	Murolol T	1607.4	0.12
$\alpha$ -cadinol	1695.3	0.26	(Z,Z)-farnesol	1699.1	0.16	(Z,Z)-farnesol	1671.3	8.3	$\alpha$ -bisabolol	1653.4	0.23
$\alpha$ -bisabolol	1721.7	0.93	$\gamma$ -eudesmol acetate	1716.3	0.27	$\gamma$ -eudesmol acetate	1688.8	0.88	(E,E)-farnesol	1669.1	10.6
$\gamma$ -eudesmol acetate	1761.8	0.81	Cedryl acetate	1777.1	1.84	Chamazulene	1691.8	0.64	(Z,Z)-farnesol	1703.0	0.34
Cedryl acetate	1781.9	2.67	Chamazulene	1791.5	0.68	(E,E)-farnesyl acetate	1711.8	0.52	$\gamma$ -eudesmol acetate	1757.8	0.15
Chamazulene	2004.9	0.21	Manoyl oxide	2012.5	0.67	Manoyl oxide	1754.3	0.64	(E,E)-farnesyl acetate	1773.9	2.1
(E,E)-farnesyl acetate	1025.9	0.52	13-epi-manoyl oxide	2065.5	0.28	Manool	1781.2	2.6	Chamazulene	1798.2	0.21
Manoyl oxide	2071.1	0.17	13-epi-manool	2075.3	0.56	13-epi-manool	2057.6	0.78	Manool	2006.1	0.61
Total	-----	90.2	Total	-----	93.1	Total	-----	86.9	Total	-----	94.8
	1761.8	0.81	Cedryl acetate	1777.1	1.84	Chamazulene	1691.8	0.64	(Z,Z)-farnesol	1703.0	0.34

1: compound percentage 2: Retention indices

Ho [22] reported some chemical compounds like sabinene, 1,8-cineole, terpinen-4-ol and  $\alpha$ -pinene improved *Alpinia speciosa* antioxidant and antimicrobial activity. This study indicated that *Achillea* spp. may be considered as a good source of natural antioxidant to be used in medicinal and food products to promote human health and prevent diseases. Camphor, piperitone and borneol have been found as major compounds in some *Achillea* spp. and none of them have been proven to be strong antioxidant agents as emphasized elsewhere. In this study camphor, piperitone and borneol are large compounds in *A. biebersteinii* and results indicated *A. biebersteinii* essential oil did not have strong antioxidant capacity compared to other *Achillea* spp.

## Conclusion

This study indicated that *Achillea* spp. collected from south-west of Iran may be considered as a good source of natural antioxidants to be used in medicinal and food products to promote human health and prevent diseases. Totally 27 compounds and more than 90% of the oils were identified. Sabinene, 1,8-cineole  $\alpha$ -pinene,  $\beta$ -pinene,  $\alpha$ -bisabolol, and chamazulene were the main constituents of essential oil in *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oil displayed stronger antioxidant activity compared to *A. biebersteinii* essential oil.

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