



Evaluation of the essential oil composition and antioxidant activity of *Achillea eriophora* as a medicinal plant

Malek taheer Maghsoodlou^{1*}, Jafar Valizadeh², Javad Mottaghipisheh¹, Nahid Rahnesan¹

¹Department of Chemistry, University of Sistan and Baluchestan, Zahedan, Iran;

²Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran;

*Email: mt_maghsoodlou@chem.usb.ac.ir

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ABSTRACT

Background & Aim: *Achillea* (Fam. Asteraceae) is a medicinal plant and different species of it have several uses in the folk medicine all around the world. *Achillea* species are used for feverish conditions, common cold, digestive complaints, slow healing wounds and skin inflammations. This study aims to investigate the essential oil composition and antioxidant activities from the aerial parts of *Achillea eriophora* collected from Khash city in Sistan and Baluchestan province.

Experimental: Chemical composition of the essential oils was obtained by hydro-distillation method using a Clevenger type apparatus and analyzed by GC/MS.

Results and Discussion: In the essential oil of *Achillea eriophora* 33 compounds representing 100%, were identified in which Camphor (21.55%), Artemesia ketone (13.84%), Alpha-Thujone (11.85%), Borneole (8.94%), Yomogi alcohol (7.74%), 1,8-cineole (5.19%), Terpinene-4-ol (4.23%) and Myrtenol (3.10%) were the major constituents. In addition, the antioxidant activity of ethyl acetate and ethanolic extracts of *Achillea eriophora* was analyzed using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging. According to antioxidant activity outcomes, the amount of IC₅₀ of ethyl acetate and ethanolic extracts of the aerial parts of *Achillea eriophora* and also butylated hydroxyl toluene (BHT) as a standard was 245.20 ppm, 89.25 ppm and 45.58 ppm, respectively. Ultimately, it was highlighted that the antioxidant activity of ethyl acetate extract was weaker than ethanolic extract. The antioxidant activity of both of them were also weaker than BHT as a synthetic antioxidant. Some compounds like 1, 8-cineole and terpinen-4-ol can be responsible for antioxidant and antimicrobial activity, while Camphor and borneol have not been proven to be strong antioxidant agents as emphasized elsewhere.

Recommended applications/ industries: The results of the present study showed that *Achillea eriophora* from Sistan and Baluchestan may be considered as source of natural antioxidant to be used in medicinal and food products to promote human health and prevent diseases.

1. 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 (Bua-In and Paisooksantivatana, 2009; Jaimand and Rezaee, 2001; McKay and Blumberg, 2006). *Achillea* (Fam. Asteraceae) comprises more than 100 species, which are mainly distributed in Europe, Asia, Australia and North America (Rechinger, 1963). Nineteen species of this genus are found in different geographical and ecological regions of Iran (Asgarirad *et al.*, 2010; Gharibi *et al.*, 2011; Rustaiyan *et al.*, 1998). *Achillea* species have been used as anti-inflammatory, antispasmodic, diaphoretic, diuretic, emmenagogue agents and for treatment of hemorrhage, pneumonia, rheumatic pain, and wounds since ancient times (Ghasemi *et al.*, 2008). *Achillea* is rich in flavonoids, sesquiterpene lactones, and monoterpenoids which have antioxidant activities (Jaimand and Rezaee, 2001; Saeidinia *et al.*, 2005). The largest single component of volatile oil extracted from *Achillea millefolium* is chamazulene which has been shown to have anti-inflammatory and antiallergic effects (Nemeth and Bernath, 2008). Immunosuppressive effects on humoral immune responses have been reported with an aqueous extract of *Achillea talagonica* (Rezaeipoor *et al.*, 1999). To date *Achillea eriophora* has been analyzed from different parts of Iran such as Fars (Jaimand and Rezaee, 2007) and Shoushtar (Farhoudi and Mehrnia, 2013) for both the composition of essential oil and different pharmacological properties. But there are no reports of such an analysis on *Achillea eriophora* from Khash area in Sistan and Baluchestan, which is a geographical zone for medicinal plants. For this reason, the importance of this plant as an herbal medicine, the purpose of this study was to determine the comparative chemical compositions and antioxidant activities of the essential oils extracted the aerial parts of *Achillea eriophora*.

2. Materials and Methods

2.1. Plant Materials

Achillea eriophora was collected from Khash in Sistan and Baluchestan province during the flowering

stage. Identification of the plant was done by the Medicinal and Ornamental plant Research Institute, University of Sistan and Baluchestan, Zahedan, Iran. Collected plant materials were dried in the shade and the aerial parts were separated from the roots.

2.2. Isolation of the Essential Oil

The aerial parts of *Achillea eriophora* were dried and milled into a fine powder. The extraction procedure used was hydrodistillation using a Clevenger type apparatus. For the extraction, 500 g of the cleaned, air-dried and powdered of the aerial parts of *Achillea eriophora* were hydrodistilled in a Clevenger type apparatus for 4h. The essential oil was dried over anhydrous Na₂SO₄ (Merk), stored in a dark glass bottle and kept at 4 °C until analysis.

2.3. GC/MS Analysis

The essential oils were analyzed on an Agilent 6890 gas chromatograph interfaced to an Agilent 5973 N mass selective detector (Agilent Technologies, Palo Alto, USA). The GC column was HP-5MS fused silica capillary with a (5% phenyl) – polymethylsiloxane stationary phase, film thickness of 0.25µm, a length of 30 m and an internal diameter of 0.25 mm. The data were acquired under the following conditions: initial temperature was 60°C; velocity programming 3°C/min; injector temperature 280 °C and detector temperature 280 °C. The carrier gas was helium at a flow rate of 1 ml/min and the split ratio was 1:15. For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. The retention indices were calculated for all volatile constituents by using retention time of n-alkanes which were injected after the essential oil under the same condition. The components were identified by comparing retention indices with those of standards. The results were also confirmed by comparing their mass spectra with the published mass spectra or Wiley library.

2.4. Preparation of ethyl acetate and ethanolic extracts

A portion of dried plant material (10 g) was extracted with ethanol and ethyl acetate at room temperature for 24 hours and, then filtered using Whatman No.42 filter paper to remove debris. After filtration and solvent evaporation, extracts were stored in sealed vials at 4 °C until biological testing.

Table 1. Chemical composition of the *Achillea eriophora* essential oil from Khash.

compound	% ¹	RI ²	compound	%	RI
Artemesia triene	0.328	923	Terpinen-4-ol	4.236	1174
Comphene	0.354	946	Alpha-Terpineol	0.649	1186
Dehydrocineole	0.527	993	Myrtenol	3.105	1194
Yomogi alcohol	7.745	999	Cis-p-Mentha-1(7), 8-dien-2-ol	0.351	1227
Alpha- Terpinene	1.314	1014	2 (5H)-Furanone,5-(2-methyl-3-methylen-4-buthyl)-	1.034	1268
P-Cymene	0.585	1020	Bornyl acetate	0.645	1287
1,8-cineole	5.190	1028	Lavandulyl aetate	0.435	1288
Gamma-Terpinene	1.562	1054	Terpinen-4-ol-acetate	0.362	1229
Artemesia ketone	13.849	1080	Eugenol	0.328	1356
Artemesia alcohol	3.646	1080	Methyl eugenol	2.111	1403
Terpinolen	0.451	1086	Lungi pinocarvone	0.415	1569
Alpha-Thujone	11.854	1103	Caryophyllene oxide	0.366	1582
Beta-Thujone	3.126	1116	3-phenyl propenoic acid, 3-methyl buthyl ester	0.601	1593
Cis-p-Menth-2-en-1-ol	0.431	1118	Benzen propanoic acid, 2-penthyl ester	0.692	1594
Comphor	21.559	1141	Caryophylla 4(12), 8(13)-dien-5-beta-ol	0.319	1639
Pinocarvone	1.863	1160	Exacyclotetradecan-2-1,14-methyl	1.018	1650
Borneol	8.948	1165	-	-	-

1. compound percentage

2. Retention indices

2.5. Antioxidant evaluation

2.5.1. DPPH radical-scavenging activity

The DPPH radical scavenging activity of the extracts from the aerial parts of *Achillea eriophora* were measured according to the procedure described by Brand-Williams (Brand-Williams et al., 1995). Briefly, 1 ml samples of various concentrations of the extracts in ethanol and ethyl acetate were separately added to a 1 ml solution of DPPH radical in ethanol (final concentration of DPPH was 0.1 mM). The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 minutes. Then, the absorbance of the resulting solution was measured at 517 nm using a UV-Vis, Specords 100 spectrophotometer. Inhibition of free radical DPPH was calculated as percentage [IP (%)] as follows: $IP (\%) = 100 \times (A \text{ blank} - A \text{ sample}) / A \text{ blank}$

Where: Ablank: absorption of blank sample; A sample: absorption of testing extract solution.

The antioxidant activity of the extracts was expressed as the IC50. IC50 values (ppm) denote the concentration of sample, which is required to scavenge

50% of DPPH free radicals. This was obtained by interpolation and using linear regression analysis. For the calculation of these values, Microsoft Excel software was used. Percent inhibition after 30 minutes was plotted against concentration, and the equation for the line was used to obtain the IC50 value. A lower IC50 value indicates greater antioxidant activity. The synthetic antioxidant Butylated Hydroxy Toluene (BHT) was also used as positive control.

3. Results and discussion

3.1. Chemical composition of the essential oil

The oils were isolated by hydro-distillation and analyzed by capillary gas chromatography, using flame ionization and mass spectrometric detection. Determination of individual components was based on comparing their relative retention times with those of authentic samples on HP-5MS capillary column, and their mass spectra of peaks to be matched with those obtained from authentic samples and/ or the Wiley NIST 7 library spectra and existing data (Adams, 2007). The obtained results of the identified compounds in the

essential oil from the aerial parts of *Achillea eriophora* with their percentage and coefficient of quarts (RI) were shown in Table 1. The yield of volatile oil of *Achillea eriophora* was 2.1 % (v/w). The chromatographic analysis of the extracted volatile oil of *Achillea eriophora* revealed the presence oxygenated monoterpenes (86.88%), hydrocarbon monoterpenes (5.121 %), other oxygenated compounds (3.553 %), oxygenated sesquiterpenes (0.781 %) and as other compounds (3.664 %). The high content of oxygenated compounds might explain the characteristic and fragrant odor of the oil. The results showed in the essential oil of *Achillea eriophora* 33 compounds representing 100 %, were identified in which Camphor (21.55 %), Artemisia ketone (13.84%), Alpha-Thujone (11.85 %), Borneole (8.94 %), Yomogi alcohol (7.74%), 1,8-cineole (5.19%), Terpinene-4-ol (4.23 %) and Myrtenol (3.10 %) were the major constituents.

Chemical composition of the essential oil of *Achillea* species growing in different geographical locations has been extensively studied. Farhoudi and Mehrnia (2013) reported sabinene (21.1 %), 1, 8-Cineole (18.3 %), α -bisabolol (10.6%), terpinene-4-ol (8.6 %), α -pinene (6.7 %), β -pinene (4.0 %), p-Cymene (3.21 %) and chamazulene (2.1 %) as the major compounds in *Achillea eriophora* essential oil from Shoushtar (Farhoudi and Mehrnia, 2013). The comparison of chemical compositions of *Achillea eriophora* from Shoushtar and Khash was presented in Table 2. Saberi-ameli *et al.* (2007) identified 32 components in the oil of *Achillea eriophora* from Kerman. The major components were Camphor (46.43 %), 1, 8-Cineole (9.85 %), Alpha-Thujone (8.16 %), Comphen (4.88 %) and Beta-Thujone (4.66%) (Saberi-ameli *et al.*, 2007). In another studies Nadim *et al.* (2001) and Rustaiyan *et al.* (1998) found sabinene, α -pinene, 1, 8-cineole, chamazulene, borneol, β -pinene and terpinene-4-ol as the major chemical compounds in *Achillea* species essential oil (Nadim *et al.*, 2001; Rustaiyan *et al.*, 1998). The comparison of results showed 1, 8-cineole was a main compound in species of *Achillea* but with different percentages. In addition, Camphor (46.43 %) and 1, 8-Cineole (9.85 %) which appeared as major constituents in the oil of *Achillea eriophora* from Kerman were present in low concentration (Camphor 21.59 % and 1, 8-cineole 5.19 %) in *Achillea eriophora* from Sistan and Baluchestan. Whereas the amount of Camphor and 1, 8-Cineole components in the plant which collected from

Shoushtar was 0.86 % and 18.3 %, respectively. Although α -pinene, β -pinene and sabinene have been reported as main compounds in many species of *Achillea*, but there were not identified in *Achillea eriophora* from Sistan and Baluchestan. In total, great quantitative and qualitative variations in volatile composition of *Achillea eriophora* were seen between this and other studies. These variations may be due to the influence of geographical differences, environmental conditions, physiological differences, different extraction, analytical procedures and genetic factors (Kokkini *et al.*, 2004; Hassanpouraghdam *et al.*, 2010).

3.2. Antioxidant Activity

Medicinal plants that traditionally used in folk medicine are particularly interesting for investigation of their antioxidant effects. A number of methods and modifications have been proposed for antioxidant activity assessment. In this study, the antioxidant activity of ethyl acetate and ethanolic extracts of *Achillea eriophora* was analyzed using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging. The reduction ability of DPPH radical formations was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Antioxidant effects on DPPH radical scavenging are seemed to be related to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The results indicated, the amount of IC₅₀ of ethyl acetate and ethanolic extracts of the aerial parts of *Achillea eriophora* and also butylated hydroxyl toluene (BHT) as a standard was 245.20 ppm, 89.25 ppm and 45.58 ppm respectively, which showed the antioxidant activity of the of ethanolic extract was higher than ethyl acetate extract. The antioxidant activity of both of them was also weaker than butylated hydroxyl toluene as a synthetic antioxidant. Ho (2010) reported some chemical compounds like 1, 8-cineole and terpinene-4-ol can be responsible for antioxidant and antimicrobial activity (Ho, 2010). Although, Camphor and borneol have been reported as main compounds in *Achillea eriophora* from Sistan and Baluchestan but none of them have been proven to be strong antioxidant agents as emphasized elsewhere.

Table 2. The comparison of chemical composition of the *Achillea eriophora* essential oil from Khash and Shoush

Compound	% Khash	% Shoushtar	Compound	% Khash	% Shoushtar
Artemesia triene	0.328	-	Terpinen-4-ol	4.236	8.6
Comphene	0.354	-	Alpha-Terpineol	0.649	0.64
Dehydrocineole	0.527	-	Myrtenol	3.105	-
Yomogi alcohol	7.745	-	Cis-p-Mentha-1(7), 8-dien-2-ol	0.351	-
Alpha- Terpinene	1.314	-	2 (5H)-Furanone,5-(2-methyl-3-methylen-4-buthyl)-	1.034	-
P-Cymene	0.585	3.21	Bornyl acetate	0.645	-
1,8-cineole	5.190	18.3	Lavandulyl aetate	0.435	-
Gamma-Terpinene	1.562	-	Terpinen-4-ol-acetate	0.362	-
Artemesia ketone	13.849	-	Eugenol	0.328	0.44
Artemesia alcohol	3.646	-	Methyl eugenol	2.111	-
Terpinolen	0.451	-	Lungi pinocarvone	0.415	-
Alpha-Thujone	11.854	0.71	Caryophyllene oxide	0.366	-
Beta-Thujone	3.126	-	3-phenyl propenoic acid, 3-methyl buthyl ester	0.601	-
Cis-p-Menth-2-en-1-ol	0.431	0.34	Benzen propanoic acid, 2-pentyl ester	0.692	-
Camphor	21.559	0.86	Caryophylla 4(12), 8(13)-dien-5-beta-ol	0.319	-
Pinocarvone	1.863	-	Exacyclotetradecan-2-1,14-methyl	1.018	-
Borneol	8.948	1.73	-	-	-

Moreover, Antioxidant activity is usually due to phenolic compounds which are presented in stem and leaves more than flowers due to their synthesis and metabolism process in these parts of the plant. However, the phenolic compounds which are responsible for antioxidant activity, need to be investigated in other studies.

4. Conclusion

This study indicated that *Achillea eriophora* from Sistan and Baluchestan may be used as a valuable source for pharmaceutical and cosmetic purposes and perfume industries. It also can be considered as a source of natural antioxidant to be used in medicinal and food products to promote human health and prevent diseases. However, further studies are needed to be performed to investigate the antioxidant activity of *Achillea eriophora* from Khash in Sistan and Baluchestan province by different in vitro tests.

5. Acknowledgments

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6. Conflict of interest

There is not any conflict of interest in this study.

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