



Phytochemical characteristics and antioxidant activity of two *Artemisia* species (*Artemisia aucheri* Boiss. and *Artemisia haussknechtii* Boiss.)

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ABSTRACT

Background & Aim: *Artemisia* (local name “Dermaneh”), is one of the most economically important and widespread of Asteraceae family and are used for treatment in infectious diseases such as malaria, hepatitis and other diseases. This study was conducted to determine phytochemical characteristics and antioxidant activities of essential oils from different parts of *Artemisia aucheri* Boiss. and *Artemisia haussknechtii* Boiss. collected from the Zagros mountains, Southwestern Iran.

Experimental: The essential oils of different parts of these plants were extracted by hydro-distillation and analyzed using GC/MS. The antioxidant activity was determined according to α, α -diphenyl- β -picrylhydrazyl (DPPH) method.

Results: In total, 60 volatile components were identified, representing 90% of total oils. Results indicated significant differences among the different parts of plants for the main constituents in the essential oils. The major compounds in the essential oil from the stem and leaves of *A. aucheri* were *Artemisia* ketone, Germacrene-D, Alpha-terpinene, Santolinatrien and major compounds in the essential oil from the flowers *A. aucheri* were *Artemisia* ketone and Nerolidol. While, Beta-eudesmol, Germacrene-D, Caryophyllene, Alpha-eudesmol, 1,8-cineole, Caryophyllene oxide and Borneol were the main components identified in the stem and leaves of *A. haussknechtii* and 1,8-cineole, Borneol, Alpha-eudesmol and Bornyl acetate were major compounds in the essential oil the flowers of *A. haussknechtii*.

Recommended applications/industries: It was interesting to note that the essential oil of arial parts of *A. aucheri*, *A. haussknechtii* exhibited potent antioxidant activity, and thus hold a good potential for use in the food and pharmaceutical industries.

1. Introduction

The genus *Artemisia* is one of the largest and most widely distributed genera of the family Astraceae (Compositae). It is a heterogenous genus, consisting over 800 diverse species distributed mainly in the temperate zones of Europe, Asia and North America (Chauhan et al., 2010). Thirty-four species have been reported in Iran (Mozaffarian, 1996). These species are

perennial, biennial and annual herbs or small shrubs (Mehrddad et al., 2007; Watson et al., 2002). Several *Artemisia* species have medicinal importance and are used in traditional medicine for the treatment of a variety of diseases such as hepatitis, cancer, inflammation and infections by fungi, bacteria, and viruses (Demirci et al., 2004; Kim et al., 2002).

Alsomany *Artemisia* species have a high economic value in several fields and have been used as food plants, tonics, antimalarials, antihelmintics, antidiabetics and in treating wounds, bronchitis, ulcers, and tuberculosis (Uzun 2004; Baytop, 1999; Akalin, 1993; Tumen, 1989).

There are several reports concerning the antimalarial, antioxidant, cytotoxic, antipyretic, analgesic, antidiabetic, antimicrobial, and antifungal activities of different *Artemisia* species (Korkmaz, 2002; Kalemba, 2002; Tan, 1998; ESCOP, 1997). Many species are economically important as food, forage, ornamentals or soil stabilizers in disturb habitats. Some taxa are toxic or allergenic and some others are invasive weeds which can adversely affect harvests (Tan et al., 1998; Pareto, 1985). An exhaustive literature survey on phytochemical reports of the genus *Artemisia* reveals that the *Artemisia* species comprise mainly terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes (Kundan and Anupam, 2011). The most of *Artemisia* species have a distinctive taste and smell that is due to the presence of monoterpene and sesquiterpene compounds, especially in leaves and flowers (Allahtavakoli et al., 2010), which in many cases are the reason for their application in folk medicine.

To the best of our knowledge, there are no reports documenting the antioxidant activity and chemical

composition of *A. aucheri* and *A.haussknechtii* essential oil collected of Zagros Mountains, Iran. In the literature, there are only a few papers dealing with the essential oil composition and properties of these species from different geographic origins. So, the aim of the present study was both to (a) evaluate the chemical composition of the essential oils isolated from different parts of *A. aucheri* and *A. haussknechtii* collected from alpine area of Zagros Mountains, in Chaharmahalva Bakhtiari province, Southwest of Iran and (b) to determine the antioxidant and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of the essential oils of these plants.

2. Materials and Methods

2.1. Plant material

The leaves, flowers and stems of *A. aucheri* and *A.haussknechtii* collected from natural habitats (in Chaharmahal va Bakhtiari province, southwestern Iran. The plants were identified by taxonomic references (Rechinger, 1969) (Voucher specimen No. 2616, 1722, respectively). Soil physical and chemical characteristics, including pH, electrical conductivity (EC), organic carbon (OC%) and soil texture were determined (Table 1). Climatic data of the locations were determined using data collected by the nearest meteorology station.

Table 1. Geographical and climate of natural habitats of *A. aucheri* and *A. haussknechtii*.

No.	Altitude	Latitude	Longitude	P	T	pH	E.C.	O.C	Sand	Silt
1	2349	495270	3573889	358.7	12.4	5.46	0.494	3.39	14	39
2	2943	524253	3476493	295.4	14.2	7.17	0.98	3.66	11	45

P: Annual precipitation (mm), T: Average temperature (°C); E.C.: electrical conductivity (dS.m⁻¹); O.C.: organic carbon (%), and Sand, Silt and Clay in %. Meteorological information was obtained from weather stations located within the study area and the surrounding zone; each value in the mean of 10 to 15 year data. Soil characteristics are based on average of samples taken from three farms in each region. 1: *A. aucheri*, 2: *A. haussknechtii*.

2.2. Sample preparation

The fresh plants were dried for six days at room temperature (30 ± 5 °C). Dried plant materials were grinded, and 100 g of tissue was distilled with 1 L water for 3 h using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia (British Pharmacopoeia, 1988). The separated oils were dried over anhydrous sodium sulfate, and stored in dark glass bottles at 4 ± 2 °C prior to use.

2.3. Identification of the oil constituents

The essential oil was analyzed using an Agilent 7890 A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30.00 m × 0.25 mm, 0.25 µm film thickness). Oven temperature was kept at 60°C for 4 min initially, and then raised at the rate of 4°C/min to 260°C. Injector and detector temperatures were set at 290°C and 300°C,

respectively. Helium was used as carrier gas at a flow rate of 2 mL/min, and 0.1 μ L samples were injected manually in the split mode. Peaks area percent were used for obtaining quantitative data. The gas chromatograph was coupled to an Agilent 5975 C (Agilent Technologies, Palo Alto, CA, USA) mass selective detector. The EI-MS operating parameters were as follow: ionization voltage, 70 eV; ion source temperature, 200°C. Retention indices were calculated for all components using a homologous series of *n*-alkanes (C₅-C₂₄) injected in conditions equal to samples ones. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system)(Adams, 2007).

2.4. Antioxidant test

The DPPH radical scavenging activity of essential oils was determined using the method proposed by Hung *et al.* (2005). The extracts at concentrations of 16 to 500 μ g/mL were mixed with an equal volume of 0.2 mM methanol solution of DPPH. The disappearance of the DPPH after 30 min of incubation at room temperature was determined spectrophotometrically at 515 nm. Methanol was used as blank sample. The absorbance of the DPPH radical without antioxidant served as the control and was measured. The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC₅₀) was calculated graphically and the percentage inhibition was calculated according to the equation:

$$\% \text{ inhibition} =$$

$$\left[\frac{AC(0) - AA(t)}{AC(0)} \right] * 100$$

where AC₍₀₎ is the absorbance of the control at t = 0 min and AA_(t) is the absorbance of the antioxidant at t = 30 min. The food preservative butylhydroxyanisole (BHA) was used as positive control.

2.5. Statistical analyses

The data was statistically analyzed using one-way ANOVA by the program SPSS (19.0), and comparison

of the means of the main constituents of essential oils evaluated by Duncan's multiple range test at *p*<0.01 level. Analytical data for cluster analysis were treated by means of the statistical package Minitab release 13.

3. Results and discussion

3.1. Chemical composition of essential oil

According to GC and GC-MS analysis of essential oils, 60 volatile components were identified in different parts of *A. aucheri* and *A.haussknechtii*, representing 90% of total oils (Table 2). All compounds are listed in order of their retention indices on the column. The analysis of essential oils detected the major compounds, santolinatrien, alpha-terpinene, p-cymene, limonene, 1,8-cineole, artemisia ketone, borneol, bornyl acetate, beta-Caryophyllene, germacrene-D, nerolidol, caryophyllene oxide, beta-eudesmol, alpha-eudesmol. The main components of essential oils in different parts of *A. aucheri* were as follows; for stems: Artemisia ketone (13.38%), Germacrene-D (13.23%), Borneol (3.35%), 1,8-cineole (8.3%), *trans*-verbenol, Caryophyllene oxide (2.66%) and Beta-eudesmol (2.48%), for leaves: Artemisia ketone (30.19%), Alpha-terpinene (11.92%), Santolinatrien(8.38%) and p-Cymene(5.57%) and for flowers: Artemisia ketone (52.94%) and Nerolidol (6.28%).

The main components of essential oils in different parts of *A.haussknechtii* were as follows; for stems: Beta-eudesmol (10.28%), Alpha-eudesmol (7.20%), Germacrene-D (6.63%) and Caryophyllene oxide (5.55%), for leaves: 1,8-cineole (12.04%), Caryophyllene oxide (9.61%), Borneol (5.67%) and for flowers: 1,8-cineole (11.73%), Borneol (5.91%) and Bornyl acetate (5.79%). Comparison of the oil compositions of different parts *A. aucheri* and *A. haussknechtii* showed that, although most of the compounds were present in all of the oils, their percentages were different. Moreover, there are variations in the volatile oil content and composition of these plants with different areas that were related to a variety of factors, such as season, plant age and different plant parts.

Table 2. The main constituents of the essential oils from different parts of *A. aucheri* and *A.haussknechtii*.

Components	<i>A. aucheri</i>			<i>A.haussknechtii</i>		
	stems	leaves	flowers	stems	leaves	flowers
Borneol	3.35	1.31	0.81	4.11	5.67	5.91
Caryophyllene oxide	2.66	0.00	0.59	5.55	9.61	1.47
Beta-eudesmol	2.48	0.13	1.44	10.28	5.31	2.62
1,8-cineole	2.1	0.71	0.6	3.18	12.04	11.73
Santolinatrien	2.33	8.38	1.45	1.69	0.69	1.08
Alpha-terpinene	2.26	11.92	1.62	0.20	0.50	0.81
p-Cymene	0.53	5.57	0.54	0.22	0.61	0.68
Bornyl acetate	0.43	0.88	0.40	2.01	3.19	5.79
Alpha-eudesmol	0.36	2.05	1.06	1.02	0.25	4.33
Artemisia ketone	13.38	30.19	52.94	0.00	0.38	1.02
Nerolidol	2.15	2.84	6.28	0.00	0.34	0.11
Germacrene-D	13.23	0.17	0.54	6.63	0.64	0.3
Caryophyllene	1.25	2.21	1.58	5.38	1.25	0.25
Alpha-eudesmol	0.87	1.35	2.13	7.20	1.36	1.08

[†]Retention indices (RI) relative to C5-C24 n-alkanes on apolar HP-5 MS column.

Numerous studies have been reported on the analysis of the essential oil compositions from various species of *Artemisia*. Asghari *et al.* (2012) reported the main components of essential oil from the seeds of *Artemisia aucheri* were: decane, p-cymene, 1,8-cineole, linalool, p-mentha-8-ol, triene, borneol, lavandulol, bornyl acetate, chrysanthenyl acetate, dehydroaromadenderene and caryophyllene oxide. In other report the main components of essential oil of *A. aucheri* were verbenone (21.5%), camphor (21.0%) 1,8-cineole (8.3%) and *trans*-verbenol (8.1%) (Sefidkon *et al.*, 2002). Mahboubi and ghazian Bidgoli (2009) reported the major components of *A. aucheri* were geranyl acetate (17.2%), E-citral (17.1%), linalool (12.7%), geraniol (10.7%), Z-citral (10.5%). Also, Khanahmadi *et al.* (2009) reported that Camphor (12.4%), α -Terpineol (9.93%), Davana ether (6.24%), and Bornyl acetate (3.77%) were the major components in *A.haussknechtii*. Moreover, Jalali *et al.* (2007) reported that the main constituents of essential oil *A. haussknechtii* included camphor (41%), 1,8-cineole (32.3%), *cis*-davanone (3.7%), 4-terpineol (3%), linalool (2.8%), β -fenchyl alcohol (2.7%) and borneol (2.6%).

The published reports on the chemical composition of the essential oil from other members of the *Artemisia* indicated the major constituents were, piperitone (32.4%), camphor (20.6%) and (E)-ethyl cinnamate (8.2%) in *A. judaica* L. (Hany, 2012), artemisia ketone (42.1%), germacrene B (8.6%), borneol (6.1%) and *cis*-chrysanthenyl acetate (4.8%) in *A. indica* and camphor (19.4%), *trans*-pinocarveol (16.9%), chrysanthenone (15.8%) in *A. herba-alba* (El-Hamd *et al.*, 2010).

A report by Verdian-rizi *et al.* (2008) indicated the main constituents of the essential oil of *A. annua* L. from Iran were 1,8-cineole (9.39%), camphene (6.98%) and spathulenol (4.89%). The major constituents of the essential oil of *A.chamaemelifolia* were vulgarone B (38.8%), santolinyl acetate (10.5%) and 14-liydroxy-9-epi-P-caryophyllene (8.4%)(Morteza-Semmani *et al.*, 2008).

Also, Imelouane *et al.* (2010) reported the major constituents of the essential oil of *A. herba-alba* in Morocco were camphor (43.07%), camphene (7.2%), 1,8-cineole (7.08%), filifolone (7.04%), borneol (4.88%), and bornyl acetate (3,79%). The major constituents of the oil of *A. nanschanica* were terpenoids (70.86%), thujone (21.3%), heptadiene (16.52%), and linalool (10.94%). The content of 1,8-cineole (9.43%), and camphor (6.66%). Previous research showed that alpha-pinene (10.2%), 1,8-cineole (10.1%), artemisia ketone (11.4%) and camphor (24.6%) were the main components of the essential oil of *A. biennis* grown in Iran (Nematollahi *et al.*, 2006). Also some researchs showed that bornane derivatives (camphor, borneol and bornyl acetate) and 1,8-cineole are major characteristic components of many species of *Artemisia* genus, such as: *A. annua*, *A. vulgares*, *A. diffusa*, *A. santonicum*, *A. spicigera*, *A. afra*, *A. asiatica*, *A. austriaca* and *A. pedemontana* (Kordali *et al.*, 2005; Perez-Alonso *et al.*, 2003; Kalemba *et al.*, 2002; Guvenalp *et al.*, 1998).

The Hierarchical cluster analysis of the main components grouped essential oil of the different parts into two distinctive clusters (Fig. 1). Cluster I was a one sample group as included the stem and leaves *A. haussknechtii* and also stem and flower *A. aucheri*.

Cluster II included the leaves of *A. aucheri* and flower of *A. haussknechtii*.

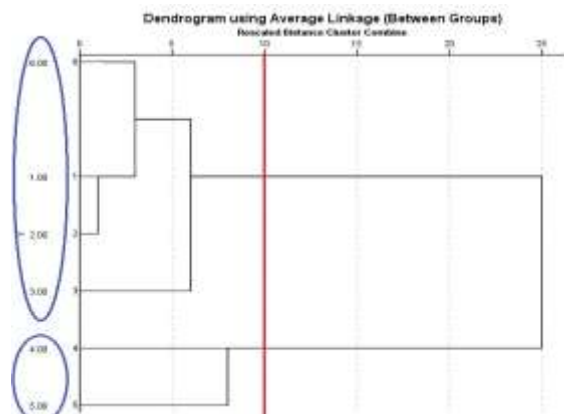


Fig. 1. Dendrogram obtained by hierarchical cluster analysis (HCA), based on the main compositions of the essential oils of different parts of *A. aucheri* and *A. haussknechtii*.

1. *Artemisia haussknechtii* × stem. 2. *Artemisia aucheri* × stem. 3. *Artemisia aucheri* × flower. 4. *Artemisia aucheri* × leaves. 5. *Artemisia haussknechtii* × flower. 6. *Artemisia haussknechtii* × leaves.

3.2. Antioxidant test

Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods and biological systems. Antioxidants retard oxidation and are sometimes added to meat and poultry products to prevent or slow oxidative degradation of fats. Antioxidant agents are effective due to different mechanisms such as free radical scavenging, chelating of pro-oxidant metal ions or quenching singlet-oxygen formation (Mariutti *et al.*, 2008).

The potential antioxidant activity of the essential oils was determined by the scavenging activity of the stable free radical DPPH. The DPPH is a stable free radical, which has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants and loses its purple colour when it accepts an electron from an antioxidant molecule (Hu *et al.*, 2004). This is a quick, reliable and reproducible method to assess the *in vitro* antioxidant activity of pure compounds as well as plant extracts (Mosquera *et al.*, 2007). The effect of antioxidants on DPPH is based on their ability to donate a hydrogen atom to DPPH, thus converting the radical into a stable molecule (Diouf *et al.*, 2009). The lower IC₅₀ value indicates a stronger ability of the extract to act as a DPPH scavenger while the higher IC₅₀ value indicates a lower scavenging activity of the

scavengers as more scavengers were required to achieve 50% scavenging reaction. In our study the antioxidant activities of essential oils from different parts of *A. aucheri* and *A. haussknechtii* were expressed as IC₅₀ with a high IC₅₀ value indicating the oil acts as moderate to weak to DPPH scavenger (Fig. 2).

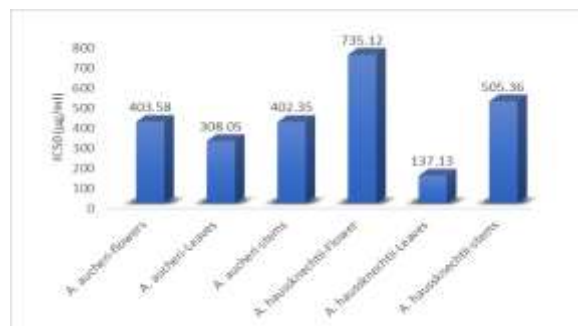


Fig. 2. The antioxidant activity of essential oil of different parts of *A. aucheri* and *A. haussknechtii* using DPPH assay (IC₅₀ = µg/ml).

IC₅₀ values were between 1.37 and 7.35 mg/mL for the essential oils, while 0.32 mg/mL for BHT (positive control). The radical scavenging activity of *Artemisia* volatile oil was found to be slightly lower than that of BHT which is known as a very efficient synthetic antioxidant agent and widely used in food technology (Potterat, 1997). The moderate to weak antioxidant and DPPH radical scavenging activities of *Artemisia* oil can be attributed to the absence of some components that have antioxidant activity. The oils isolated from plant species consist of the various constituents. Therefore, determination of the component(s) responsible for activity is very difficult. Studies have shown that antioxidant effects *Artemisia* is due to the presence of flavonoid, phenolic and terpene compounds. So that, researchers reported that this plant contains monoterpenes compounds such as camphor and 1, 8-cineol which are antioxidant compounds (Amjad *et al.*, 2013).

Moreover, the sesquiterpene lactone compounds especially parthenolide, have antioxidant properties (Amjad *et al.*, 2013). When we compared the results of different parts, it was apparent that the antioxidant activity is strongly affected by the essential oil composition. It is well known that the concentration of biologically active constituents varies in the plant different parts, which directly reflects this activity.

Free-radical scavenging activity of *Artemisia* species extract and essential oils has been reported in previous studies. Khanahmadi *et al.* (2009) reported that

ethanolic extract of *A. haussknechtii* exhibited high free radical scavenging activity ($IC_{50} = 0.15$ mg/ml). Also, Rachid *et al.* (2013) indicated the IC_{50} value of the essential oil of *A. indica* was 19.6 μ g/mL. Moreover, Akrou *et al.* (2012) reported that essential oil of *A. campestris* exhibited moderate free radical scavenging activity ($IC_{50} = 3.17$ μ L extract/mL). Variation among the antioxidant activity of different essential oils is due to differences in the antioxidant activity of their constituents which differentially extract by solvents with different polarity (Marinova *et al.*, 1997). According to previous studies, polar extracts have the higher free radical scavenging activity than non-polar ones which can be related to the presence of phenolic acids and flavonoids (Kamkar *et al.*, 2010).

4. Conclusion

In this investigation, the chemical composition and antioxidant activities of essential oils extracted from different parts of *A. aucheri* and *A. haussknechtii* were evaluated. To our knowledge, this is the first study to provide data that these essential oils possess antioxidant activities. Among the tested different parts, the essential oils from leaves of *A. haussknechtii* showed the highest antioxidant activities. Also, the chemical composition of these essential oils is described in detail. The oils obtained from *Artemisia* species are quite interesting from a pharmaceutical standpoint because of their antioxidant properties. Based on the present results, different parts from these plants can be targeted for specific medicinal and/industrial uses. Further investigations are in progress to compare the levels of activity in *Artemisia* oils and some of their constituents with the objective of identifying plant substances for future antioxidant formulations.

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