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Journal of Chemical and Pharmaceutical Research, 2015, 7(2):16-22

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Chemical composition and antioxidant properties of Lorestan province *Artemisa persica*

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ABSTRACT

The present work was identified the chemical composition compounds and compared the various antioxidative activities artemisa persica area parts hydroalcholic extract (AAHE) and artemisia persia arial parts essential oil (AAEO). This experimental study, which carried out in 1391 in Lorestan Medical respectively. Arial parts of artemisa persica were prepared and then radical scavenging activity, total antioxidant capacity, total phenol and flavonoid samples was assessed. Also the components of AAEO were analyzed with gas chromatography/mass spectrometry (GC/MS). It was demonstrated that total antioxidant capacity AAHE and AAEO is (25.123±5.18; 1.05±0.55) nmol of ascorbic acid equivalents/g extract or essential oil, phenol content (326.68±10.07; 131.14±3) mg of gallic acid equivalents (GAE)/g extract or essential oil, and flavonoid content (6.3±0.94; 1.4±0.65) mg of of quercetin equivalents/g extract or essential oil. Also GC/MS data and retention indices for reference essential oil airial parts samples were used to identify 54 constituents. These compounds make up a total of 70.33 percent essential oil. Alpha- terpinene; thymol; 3-bromomethyl- 3- oxetanemethanol; 4- ethoxy- tricyclo[5.2.1.0(2, 6)] deca- 3,8 diene- 3-c; nerolidol and phenol are the most of compounds of AAEO. This study showed that artemisa persica has good antioxidant properties and artemisa persica is a source easily accessible of natural antioxidants such as alpha-terpinene and thymol and it may be suitable for use in food and pharmaceutical applications.

Keywords: Antioxidant activity, Essential oil, Chemical composition, *Artemisa persica*

INTRODUCTION

Oxidative stress caused by an imbalance between productions of free radicals within the body's antioxidant defense mechanisms is achieved. Lipid peroxidation in organisms present in the walls of living cells from free radicals is the most important goals. Therefore, the presence of peroxides, especially free radicals play a key role in the number of virulent diseases such as diabetes, heart disease - cardiovascular, cancer, aging and other diseases [1].

Free radicals are chemically unstable atoms or molecules that can cause extensive damage to cells as a result of imbalance between the generation of reactive oxygen species (ROS) and the antioxidant [1]. ROS or reactive nitrogen species (RNS) and their excess have a harmful effect, such as the peroxidation of the membrane lipids, aggression to tissue proteins and membranes, on damage to DNA and enzymes [1]. Therefore, they can be related to some pathologies, such as cancer, coronary diseases, cataract, arthritis and AIDS as well as age-related degenerative brain disorders [2].

The beneficial effects of antioxidants on promoting health is believed to be achieved through several possible mechanisms, such as direct reaction with and quenching free radicals, reduction of peroxides, chelation of transition metals, and stimulation of the antioxidative enzyme defense system [2].

Currently, there is a great interest in the study of antioxidant substances mainly due to the findings concerning the effects of free radicals' in the organism. Phenolic plant compounds have attracted considerable attention for being the main sources of antioxidant activity. The antioxidant activity of phenolic is mainly due to their redox properties, which allow them to act as reducing agents, chelating metal, hydrogen donors, and singlet oxygen quenchers. The antioxidant activities of phenolic play an important role in the balance oxidant and antioxidant and adsorption or neutralization of free radicals [3]. Several synthetic antioxidants are commercially accessible but have been reported to be toxic [4]. Plants have been reported to exhibit antioxidant activity due to the presence of antioxidant compounds such as phenolic, proanthocyanidins and flavonoids [5]. Hence the agents that can scavenge these reactive oxygen species can be beneficial in the treatment of various diseases such as diabetes.

The genus *artemisia* belongs to the asteraceae family and comprises more than 400 species [6]. Several *artemisia* species have been used in various traditional and folk medicines as a treatment for fever and malaria [7]. *Artemisia* persica is the only species in this genus and the species name persica means from Iran. *Artemisia* persica have been used in traditional medicine to treat various infections and inflammations [8].

It is an effective remedy for common cold, bronchitis, rheumatism problems, being specifically helpful in treating cough [8-10]. The leaves are antibacterial, anti malaria, antiviral, diarrhea, abscesses, and fever anti colic, nose bleeds, otitis and headache. *Artemisia* species with the local name of ''Dermaneh'' are distributed all over the country [8-10]. *Artemisia* persica Boiss. is an indigenous plant that grows mainly in the Zagros Mountains, south-western, southern and central parts of Iran [10].

Recent pharmacological and biological studies have also shown several activities, such as antioxidant, antiviral, antifungal, cancer chemopreventive, anti-diabetic, antispasmodic and hypotensive from *artemisia persica [8, 11]*. Different study showed that *artemisia persica* has good effects as herbal drug. This effects related to chemical composition of *artemisia persica*.
Since the the chemical composition compounds and evaluated the various antioxidative activities of Lorestan *artemisia persica* area parts essential oil have not previously been reported; the objectives of the present study were to investigate the chemical composition compounds and various antioxidative activities of AAHE and AAEO. Since the various antioxidative activities of Lorestan AAHE and AAEO and chemical composition compounds of Lorestan *artemisia persica* leaves essential oil have not previously been reported; the objectives of the present study were to investigate Various antioxidative activities of Lorestan AAHE and AAEO and chemical composition compounds of Lorestan AAEO.

EXPERIMENTAL SECTION

Isolation of the essential oil from *artemisa persica* aerial parts:
Artemisa persica were prepared July 2013 from farms between Aleshtar and Nahavand city in Garin in the Lorestan province (western Iran). Aerial parts of the plants were collected during flowering stage and were air-dried at ambient temperature in the shade separately. Hydroalcoholic extract of leaves at the Research Center of Lorestan University of Medical Sciences was prepared. Also, aerial parts were hydro -distilled using a clevenger apparatus for 4 hours, giving yellow oil in 1% yield. The oil was dried over anhydrous sodium sulfate and stored at 4ºC. The voucher specimen was deposited at Herbarium of the Agriculture and Natural Resources Research Center of Lorestan Province, Khoramabad, Iran (no. 12106).

DPPH free radical-scavenging activity: DPPH free radical-scavenging activity of the test samples was determinated according to the method of Blois [12]. In brief, 4 ml of DPPH radical solution in ethanol (1mM) was mixed with 1 ml of *artemisa persica* extract or essential oil solution in ethanol containing 0.01–1000 µg/ml of AAHE or AAEO; and after 30 min, the absorbance was measured at 517 nm. This activity was given as percentage DPPH scavenging that is calculated as %DPPH scavenging= [(control absorbance- AAHE or AAEO absorbance) / (control absorbance)] ×100. The 50% inhibition concentration (IC_{50}), i.e. the concentration of AAHE or AAEO that was required to scavenge 50% of radicals, was calculated.

Total antioxidant activity: Total antioxidant activity of the test samples was determinated according to the method of prrieto et al. In brief, 0.3ml of sample was mixed with 3.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95º C for 90 min under water bath. Absorbance of all the sample mixtures was measured at 695 nm.

The total antioxidant activity was calculated according to the following equation that was obtained from the standard ascorbic acid graph: Absorbance − 0.026 ascorbic acid. The total antioxidant activity was expressed as the number of equivalents of ascorbic acid (μmol g^{-1}) [13].

Total phenolic content measurement: Total phenols content in the obtained extracts was determined by using the Folin–Ciocalteu's phenol reagent, according to a previously described procedure [14] with some modifications. Briefly, 200 μL of the extract solution was mixed with 1.5 mL of Folin-Ciocalteau reagent (previously diluted 10-fold with distilled water). After 3 min, 1.5 mL sodium bicarbonate solution (60 g L^{-1}) was added to the mixture. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Analytik Jena 200-2004 spectrophotometer). Gallic acid was used for constructing the standard curve (1–3500 μg/ml). and a standard curve obtained with the following equation:

Absorbance = 0.0012 × gallic acid+ 0.0033.

The results are expressed as mg of gallic acid equivalents (GAE)/g of extract.

Total phenols of the extract, as gallic acid equivalents (GAE)/g of extract, was determined by using the absorbance of the extract measured at 760nm as input to the standard curve and the equation. All tests were carried out in triplicate and phenolic contents as gallic acid equivalents were reported as means SD of triplicate determinations.

Total flavonoids content measurement: Total flavonoid contents were determined using the method of Ordon ez et a**l [15]**. A volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Quercetin was used for constructing the standard curve (1–50 μg/ml).

The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph: Absorbance − 0.026 quercetin. The results are expressed as mg of quercetin equivalents /g of extract.

Gas chromatography/ mass spectrometry

Fid- GC was carried out using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane. 25 m, 0.25 mm i.e., ratio, 1:25, and flame ionization detector. Temperature programmer: 60 ºC (2 min) rising to 240 ºC at 4 ºC/min: injector temperature 250 ºC, detector temperature, 260 ºC. GC-MS was performed using a Hewlett-Packard 6859 with a quadruple detector, on a HP-5 column, operating at 70 eV ionization energy, using the same temperature programmer and carrier gas as above. Retention indices were calculated by using retention times of n-alkanes that were injected after the oil at the same chromatographic to Van Den Dool method [16].

Identification of components

The linear retention indices for all the compounds were determined by conjection of the sample with a solution containing the homologous series of C8-C22 n-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database.

Statistical analysis: The data were presented as mean ±SD of three experiments performed in duplicate. These parameters were obtained using the student's *t*-test (using SPSS 13.0 statistical software) for independent data and the differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The polyphenol compounds exhibit a wide variety of beneficial activities in mammals including antidiabetic [17], antibacterial [18], immune stimulating [19], antiallergic [20], antihypertensive [21], antiischemic, antiarrhythmic [22], antithrombotic [23], hypocholesteromic, hepatoprotective [24], anti-inflammatory [25], anticarcinogenic [26, 27]. Flavonoids are an important group of polyphenols with antioxidant, antiviral, antibacterial [28] and anti-inflammatory activities [25, 29]. The present investigations have demonstrated a strong correlation between antioxidant activities and chemical composition compounds content of *artemisa persica area parts*.

Antioxidant activity

DPPH scavenging assay:

A stable free radical 2, 2′-diphenyl-1-pycrylhydrazyl (DPPH) has widely been used in the assessment of radical scavenging activity of plant extracts, natural compounds and foods [30]. The antioxidant activity of AAHE and

AAEO were evaluated by the DPPH radical scavenging capacity. Table 1 shows the percentage of DPPH radicals scavenging capacity AAHE, AAEO and BHT as reference. In the DPPH scavenging assay, the IC_{50} (the concentration required to scavenge 50% of radical) values of AAHE, AAEO and BHT as reference were 165.73±4.52; 16470.67±34 and 3.88±1µg/ml µg/ml, respectively. The data obtained show that AAHE and AAEO are free radical scavenger and may act as primary antioxidant, which can react with free radicals by donating hydrogen. Free radical scavenger activity of AAHE more than AAEO. The data obtained show that *artemisa persica* is free radical scavenger and may act as primary antioxidant, which can react with free radicals by donating hydrogen. Iqbal et al reported that DPPH antioxidant activity of hexane extract and water extract of Artemisia *annua* leaves better than methanol extract and ethanol extract of Artemisia *annua* leaves [31].

Table 1. The IC_{50} (the concentration required to scavenge 50% of radical) values of hydroalcholic extract (AAHE) and *artemisa persica area parts* essential oil (AAEO) and BHT

Each point represents the mean of five experiments

Parameter	Mean	S.D
AAHE (µg/ml)	165.73*	4.52
AAEO (µg/ml)	16470.67	34
BHT (µg/ml)	3.88*#	1.00

**P < .05 as compared with AAEO. #P < .05 as compared with AAHE.*

Researches showed the presence of different flavonoids, phenolic compound, such as alpha- terpinene; thymol; nerolidol and phenol in *artemisa persica area parts [32]*. These compounds may be the main cause of its considerable radical-scavenging activity. Researchers are recently interested in investigation and research into extraction of natural antioxidants such as coenzyme Q10, rosmarinic acid, tannins and flavonoids, from medical herbs to replace synthetic antioxidants [33, 34].

Natural antioxidants are safer and more beneficial and have fewer side effects than synthetic antioxidants [35]. Phyotochemical with antioxidant effects include some cinnamic acids, oleuropein, coumarins, flavonoids, eremophilene and tannins [36, 37].

Therefore, the herbs which have high amount of these compounds are taken into consideration in order to inhibit diseases related to oxidative stress such as coronary heart disease and diabetes [38]. Conducting research on herbal antioxidants and evaluating and comparing their antioxidant effects, as well as newer and more valuable sources of natural antioxidants can be found and used in food and pharmaceutical applications.

Total antioxidant activity: The phosphomolybdenum method has been widely used in the assessment of total antioxidant activity of plant extracts, natural compounds and foods. The total antioxidant activity of AAHE and AAEO were 25.123±5.18 and 1.05±0.55 nmol of ascorbic acid equivalents/g AAHE or AAEO (Table 2). The difference in the amount of antioxidant of extracts may be attributed to the differences in the amount and kind of existing antioxidant compounds in them.

Baykan Erel et al reported the total antioxidant capacity of various *artemisa* species [39]. Total antioxidant capacity of *artemisa* arsirrthinus better than others artemisa species such as *artemisa* arborascens, *artemisa* vulgaris, *artemisa* scoparia and *artemisa* sartonicum [39].

Our recent results indicated that *artemisa persica airal parts* are found to possess a good antioxidant activity.

Total phenols: The total phenol of AAHE and AAEO were 326.68±10.07 and 131.14±30 f Gallic acid equivalents/g AAHE or AAEO (Table 2). Total phenol shown by the AAHE or AAEO may be due to the presence of different phenolic compounds. Iqbal et al reported that total flavonoid content of methanol extract and water extract of Artemisia *annua* leaves well than hexane extract and ethanol extract of artemisia *annua* leaves [31]. Baykan Erel et al reported the total phenol content of various *artemisa* species. Total phenol content of *artemisa* arsirrthinus better than others artemisa scoparia species such as *artemisa* arborascens, *artemisa* vulgaris, *artemisa* and *artemisa* sartonicum [39]. Also Rashid et al reported the total phenol of *artemisa persica airal parts* extract prepared was 407 of Gallic acid equivalents/g extract [40].

Our recent results indicated that *artemisa persica airal parts* are found to possess a good antioxidant activity.

Total flavonoids: The total flavonoid of AAHE and AAEO were 6.3±0.94 and 1.4±0.65 mg of quercetin equivalents/g AAHE or AAEO (Table 2). Total flavonoid shown by the AAHE or AAEO may be due to the presence of different flavonoids such as catechin, epicatechin and myricetin.

Table 2. The total antioxidant activity, total phenols content and total flavonoids content of AAHE and AAEO
Abbreviations as in table 1
Each point represents the mean of five experiments.

Parameter	AAHE	AAEO
Total antioxidant activity (nmol ascorbic acid equivalents/g AAHE or AAEO)	25.123±5.18	1.05±0.55
Total phenols (mg of gallic acid equivalents (GAE)/g AAHE or AAEO)	326.68±10.07	131.14±30
Total flavonoids (mg of of quercetin equivalents/100g AAHE or AAEO)	6.3±0.94	1.4±0.65

Baykan Erel et al reported the total flavonoid content of various *artemisa* species. Total flavonoid content of *artemisa* arsirrthinus better than others artemisa scoparia species such as *artemisa* arborascens, *artemisa* vulgaris, *artemisa* and *artemisa* sartonicum [39]. Also Rashid et al reported the total flavonoid of *artemisa Persia airal parts* extract prepared was 308 of Gallic acid equivalents/g extract [40].

Our recent results indicated that *persica airal parts* are found to possess a good antioxidant activity.

Chemical composition of *artemisa persica airal parts* essential oil

The yield of the essential oils obtained from *artemisa persica airal parts* was 1% (W/W) respectively. Results of the GC-MS analysis of the oils are shown in table 3.

Table 3. Chemical composition of the *artemisa persica area parts* essential oil (AAEO)

Number	Compound	Retention Time (min)	Yield%
1	Butanoic acid, 2- methyl-, ethyl este	493	0.48
2	Butanoic acid, 3-methyl, ethyl ester	547	0.71
3	Thymol	739	6.41
4	Alpha- pinene, (-)-	757	0.165
5	Ethyl tiglate	779	0.09
6	Butanoic acid, 2- methyl-, propyl ester	795	0.122
7	Comphene	807	4.4
8	Sabinene	847	0.09
9	2-beta-pinene	892	0.10
10	1,8- Epoxy-p-meth-2-ene	927	0.11
11	1- Phellandrene	973	0.08
12	Benzene, methyl (1- methlethyl) - (CAS)	1043	0.981
13	Eucalyptol	1086	0.99
14	(3r, 4 as, 8 ar)- 3-4, 4a, 7,8, 8a- hexahydro- Gamma- terpinene	1156	0.54
15	Benzene,1- methyl- 4-(1-methylethenyl)	1257	0.90
16	Terpineol, z-beta-	1284	0.23
17	Bicyclo [2.2.1] heptan-3-one, 6, 6- dimethyl –Hotrienol	1312	0.24
18	3- Fluoro-o-xylene	1328	0.12
19	1,3,8-p-Menthatriene	1340	0.47
20	1- Terpineol	1379	1.44
21	Menthofuran	1399	0.15
22	Benzofuran, 2, 3- dihydro- 2- methyl-	1433	0.49
23	Camphor	1479	0.12
24	2- methylene	1554	0.33
25	1-borneol	1572	1.2
26	3- cyclohexen- 1-ol, 4-methyl- 1- (1-methylethyl)-	1629	1.71
27	Piperitol isomerll	1780	1.13
28	Alpha- terpinene	1856	24.79
29	Cis piperitone oxide	1962	0.97
30	Thiophene, 2,5- dipropyl-	1972	1.36
31	3- Hydroxy- 4- methoxybenzoic acid	1987	0.39
32	4- Fluoromandelic acid	1997	0.33
33	2, 6 trimeth-	2025	0.88
34	(3s, 4as, 8ar)- 3,4,4a, 7,8, 8a- hexahydro-methyl- 6- (1- metyl)	2058	0.92
35	Phenol, 2- methyl- 5- (1- methylethyl)	2123	3.36
36	3-(But-3-enyl) - cycloheanone	2134	2.06
37	Phenol, 2- methoxy- 4- (2- propenyl)	2202	0.72
38	Alpha, alpha. 4-trimethylbenzyl ester of acetic	2322	0.71
39	1, 3- cyclohexadiene- 1 carboxaldehyde,1,1,3,6-tetra	2397	0.73
40	1,1,3,6-tetra	2428	0.54
41	Bornyl ester of n-pentanoic acid	2472	0.49
42	3-bromomethyl- 3- oxetanemethanol	2554	3.18
43	Nerolidol	2572	3.09
44	4- Ethoxy- tricyclo [5.2.1.0(2, 6)] deca- 3, 8 diene- 3-c	2603	0.76
45	Phenol, 4-(3-hydroxy- 1- propenyl)	2687	0.40
46	d-Nerolidol	2708	1.66
47	2, 6, 10- Dodecatrienal, 3, 7, 11- trimethyl-	2788	1.78

48	2- pentadecanone, 6, 10, 14- trimethyl-	2940	0.23
49	2- cyclohexen- 1- one, 2- hydroxy- 3- n- Hexadecasanoic acid	3075	0.67
50	Heneicosane	3231	0.12
51	9, 12-Octadecadienoic acid (z, z)- (CAS)	3315	0.06
52	Tricosane	3470	0.09
53	Pentacosane	3840	0.09
54	Heptacosane	4446	0.07

Fifty four compounds of AAEO were identified (70.33% of the total oils respectively).

The main constituents found in the *artemisa persica airal parts* were alpha-terpinene (24.79%); thymol (6.41%); 3-bromomethyl- 3- oxetanemethanol (3.18%); 4- ethoxy- tricyclo[5.2.1.0(2,6)] deca- 3,8 diene- 3-c (0.76%); nerolidol (3.09%); phenol, 2- methyl- 5- (1- methylethyl) (3.36%); 3-(but-3-enyl)- cycloheanone (2.06%); 2,6, 10- dodeca-trienal, 3, 7, 11- trimethyl- (1.78%); 3- cyclohexen- 1-ol, 4-methyl- 1- (1-methylethyl)- (1.71%); d-nerolidol (1.66%); 1-terpineol (1.44%); thiophene, 2,5- dipropyl- (1.36%).

Ghorbani-Ghouzhadi et al reported, the main constituents found in the *artemisa sieberi* aerial parts prepared from south of Khorasan province of Iran were β-thujone (19.79%); α- thujone (19.55%); camphor (19.55%) and verbenol (9.69%). The main constituents found in the *artemisa* Khorasanica were davanone (36.4%); ρ-cymene (16.55%); z-citral (8%) [41]. Others researchers reported the main constituents found in the aerial parts of *artemisia persica* prepared from the karkas mountain, Isfahan province, Iran were davanone (60.56%), *cis* chrysanthenyl acetate (8.65%), limonene (5.68%), α pinene (3.74%), davanone ether isomer + (3.6%) and α-thujene (3.6%) [42]. The results of this study indicate that the composition of volatile oil of *artemisia persica* is not similar to those which are reported from another study.

The observed differences may be probably due to using of different of parts of plant for analysis, different environmental and genetic factors, different chemotypes and the nutrional status of the plants as well as other factors that can influence the oil composition.

CONCLUSION

This study showed that essential oil of *artemisa persica airal parts* is a source easily accessible of natural antioxidants such as alpha-terpinene, Thymol, Nerolidol and Phenol and it may be suitable for use in food and pharmaceutical applications and a good alternative to reduce the risk of atherosclerosis and coronary heart disease and other free radical associated health problems.

Acknowledgement
The authors wish to thank Deputy of Research and Razi Herbal Research Center of Lorestan Medical University, Lorestan. Iran.

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