

Antioxidant activity of methanol extracts of different species of *Artemisia* from Iran

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Summary

Synthetic food additives and commercial antioxidants have been criticized, mainly due to possible toxic effects. Therefore there is an interest for effective antioxidants from natural sources to prevent deterioration of foods, drugs and cosmetics.

Fourteen different *Artemisia* species (seventeen samples); *A. absinthium* L., *A. annua* L., *A. biennis* Willd., *A. diffusa* Krasch ex poljack, *A. fragrans* Willd., *A. kubadica* Boiss. & Buhse, *A. khorassanica* Podl., *A. kopedaghensis* Krasch., M. Pop. & Lincz. ex Poljack, *A. santolina* Schrenk, *A. siberi* Besser, *A. turanica* Krasch., *A. vulgaris* L., *Artemisia* sp1 and *Artemisia* sp2, from Asteraceae were collected from different parts of the country.

The antioxidant activity of the methanol extracts of these fourteen different species was evaluated. Antioxidant activity of each extract was measured using two different methods: the ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods.

The antioxidant activity of these extracts compared with the antioxidant activity of α -tocopherol (a natural antioxidant) and butylhydroxytoluene, (BHT, a synthetic antioxidant).

Results indicated that the methanol extracts of tested species possessed antioxidant activity when tested with both FTC and TBA methods. However, the extracts showed a stronger antioxidant activity when FTC method were used.

Keywords: medicinal plant, ferric thiocyanate test, thiobarbituric acid test, antioxidant activity, radical scavenging, *Artemisia* spp., Asteraceae

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Introduction

Oxidation is well known to be major cause of foods and materials degradation. The generation of reactive oxygen species (ROS) beyond the antioxidant capacity of a biological system gives rise to oxidative stress (1-2). Free radical oxidative stress has been implicated in the pathogenesis of a variety of human diseases such as aging, coronary heart disease, stroke, diabetes mellitus, rheumatic disease, liver disorders, multiple sclerosis, Parkinson's disease, autoimmune disease, Alzheimer's, AIDS and carcinogenesis (2-5). An increasing number of investigations have been carried out to find antioxidative drugs, which not only prolong the shelf life of food products but also participate as radical scavengers in living organisms.

Commercial antioxidants like other synthetic food additives have been criticized, mainly due to possible toxic effects. Therefore, in recent years there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical induced tissue injuries. Also there is considerable interest in the food industry and in preventive medicine in the development of natural antioxidants from botanical sources (6-10).

They can be an alternative to the use of synthetic compounds in food and pharmaceutical technology or serve as lead compounds for the development of new drugs with the prospect of improving the treatment of various disorders.

The genus *Artemisia* L. is one of the largest and most widely distributed of the Astraceae (Compositae). This genus is a large and heterogenous genus, numbering over 400 species distributed mainly in the temperate zone of Europe, Asia and North America (11-15). The genus in Iran has 34 species which 2 of them are endemic to the country (15-17).

Different species of *Artemisia* have a vast range of biological effects including antimalarial (18-20), cytotoxic (21), antibacterial, antifungal (18-20, 22-25) and antioxidant (19-20, 22, 24-29) activity. There are many reports about the antioxidant activity of various species of *Artemisia* (23, 24, 28-33). The antioxidant properties of the obtained extracts from these plants were also investigated in different areas (26, 32, 34-42).

The aim of the present study was to evaluate the antioxidant properties of the methanol extracts of fourteen different *Artemisia* species (seventeen samples), growing in north and north east of Iran. There are no previous reports about antioxidant activities of the extracts of these plants growing in these areas.

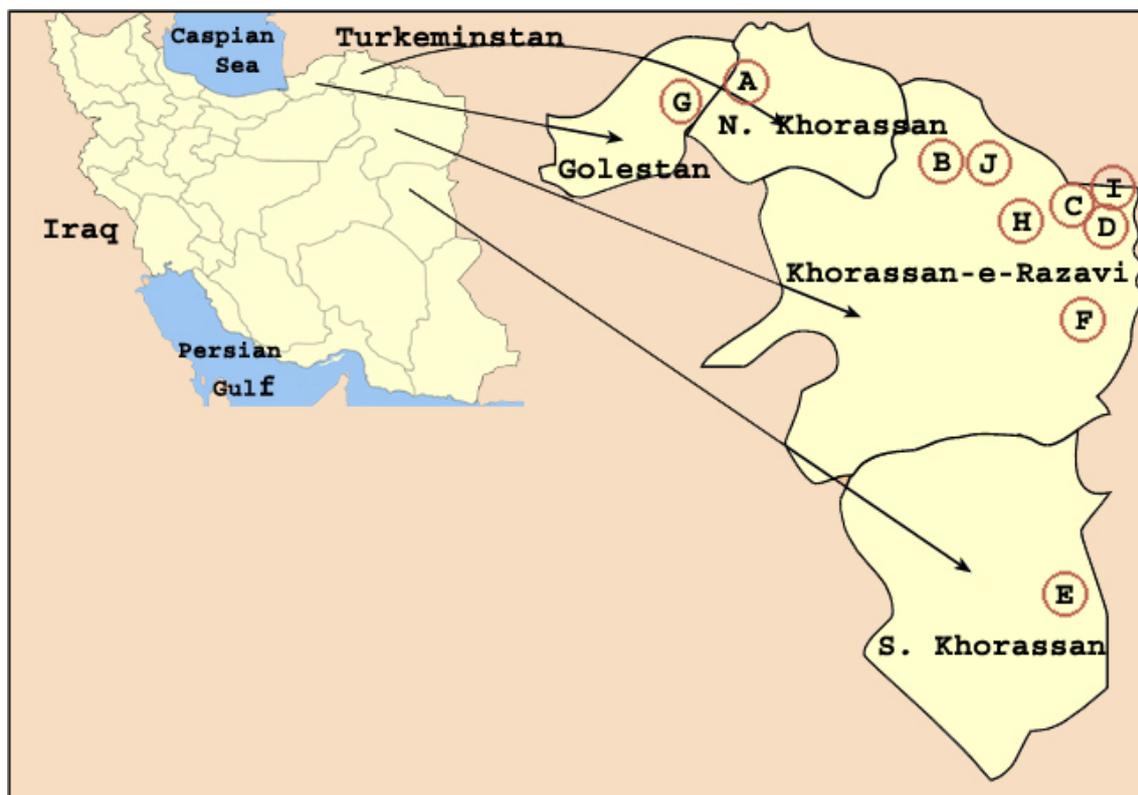
Materials and Methods

Plant material

Fourteen species of *Artemisia* collected from four different provinces in north and north east of Iran, (Fig. 1), (Khorasan Shomali, Khorasan Jonobi, Khorasan Razavi and Golestan Provinces) and their Persian names, (43), are as follow:

1- *A. absinthium* L. from Islamabad near Maraveh tapeh-Shahrabad road, Khorasan Shomali province, height 675 m (21 Oct. 2003). Its Persian name is "Afsentin".

- 2- *A. annua* L. from Islamabad near Maraveh tapeh-Shahrabad road, Khorasan Shomali province, height, 945 m (22 Oct. 2003). Its Persian name is "Gandwash".
 - 3- *A. biennis* Willd. from near Chovailly-Bajgiran road, Ghochan, Khorasan Razavi province, height 1774 m (15 Oct. 2003). Its Persian name is "Dermaneh Dosaleh".
 - 4- *A. diffusa* Krasch ex Poljack, from Mazdavand, between Sarakhs and Mashhad, Khorasan, Razavi province, height 940 m (13 Oct. 2004). Its Persian name is "Dermaneh Afshan".
 - 5- *A. fragrans* Willd. from Islamabad near Maraveh tapeh-Shahrabad road, Khorasan Shomali province, height 940 m (22 Oct. 2003). Its Persian name is "Dermaneh Moattar".
 - 6- *A. kubadica* Boiss. & Buhse from Islamabad, near Maravehtapeh-Shahrabad road, Khorasan Shomali province, height 940 m (23 Oct. 2003). Its Persian name is "Dermaneh Kubadi".
 - 7- *A. khorassanica* Podl. from near Chovailly-Bajgiran road, Ghochan, Khorasan Razavi province, height 1565 m (15 Oct. 2003) sample (1), from mountain near Cheshmeh Gilas village, Mashhad, Khorasan Razavi province, height 1155 m (19 Oct. 2003) sample (2) and from Sarakhs, near Iran and Turkmenistan border, Khorasan Razavi province, height 240 m (17 Oct. 2004) sample (3). Its Persian name is "Dermaneh Khorasani".
 - 8- *A. kopedaghensis* Krasch., M. Pop. & Lincz. ex Poljack from near Bazangan lake, Sarakhs, Khorasan, Razavi province, height 914 m (10 Oct. 2003), sample (1) and from near Chovailly-Bajgiran road, Ghochan, Khorasan Razavi province, height 1619 m (15 Oct. 2003) sample (2). Its Persian name is "Dermaneh Kopetdaghi".
 - 9- *A. santolina* Schrenk from between Khosph-Birjand, Birjand, Khorasan Jonobi, height 1460 m (25 Oct. 2003). Its Persian name is "Dermaneh Sefid".
 - 10- *A. siberi* Besser from Ghorogh Samie abad, Torbatjam, Khorasan Razavi province, height 909 m (20 July 2003). Its Persian name is "Dermaneh".
 - 11- *A. turanica* Krasch. from Ghorogh Samie abad, Torbatjam, Khorasan Razavi province, height 909 m (20 July 2003). Its Persian name is "Dermaneh Ghermez".
 - 12- *A. vulgaris* L. from Bojnord-Gorgan road, near Tangrah, Golestan province, height 645 m (12 Oct. 2003). Its Persian name is "Dermaneh Bernejst".
 - 13- *Artemisia* sp1, from near Bazangan lake, Sarakhs, Khorasan, Razavi province, height 914 m (10 Oct. 2003).
 - 14- *Artemisia* sp2, from near Nowrozi village, Ghochan, Khorasan, Razavi province, height 1300 m (15 Oct. 2003).
- Dr. V. Mozaffarian, Research Institute of Forest and Rangelands, Ministry of Jahad Keshavarzi, Iran, was identified these plants. Voucher specimens of the species have been deposited in the Herbarium of School of Pharmacy, Mashhad University of Medical Sciences (MUMS), Mashhad, Khorasan Razavi province, Iran. The collected materials were dried under shade and stored in a cool place until analysis.



Figur 1: Locations of collected *Artemisia* from Iran. A- *A. absinthium*, *A. annua*, *A. fragrans*, *A. kubadica* ; B- *A. biennis*, *A. khorassanica* (sample 1), *A. kopetdaghensis* (sample 2); C- *A. diffusa*; D- *kopetdaghensis* (sample 1); E- *A. santolina*, *A. sp1* ; F- *A. siberi*, *A. turanica*; G- *A. vulgaris*; H- *A. khorassanica* (sample 2); I- *A. khorassanica* (sample 3); *A. sp2*

Extraction of the samples

The shade dried aerial parts of each species (100 g) were chopped in small pieces and then crushed into powder by a blinder. Each sample was macerated in pure methanol for 24 hours. The samples then extracted using a percolator. The extracted solutions (seventeen samples) were concentrated at 50 °C under reduced pressure to dryness. The methanol extracts of each species were evaluated for their antioxidant activity. The presence of flavonoids (44), saponins (45), tannins and anthocyanine (46) were determined.

Antioxidant assays

Several reports have been on evaluation of antioxidant activity of various essential oils and extracts of different plants (6-9, 24-29, 34-42).

In this study the methanol extracts of aerial parts of each species were evaluated for their antioxidant activity (final concentration 0.02% w/v). Ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods were used to evaluate antioxidant activity (11). Vitamin E and butylated hydroxytoluene (BHT) (0.02%) were used as standards in both methods. Also one sample without antioxidant activity was used as control.

In these experiments for inhibition of linoleic acid peroxidation, the reaction mixture was composed of linoleic acid in ethanol, the sample solution, 0.05 mol/L phosphate buffer (pH 7.0) and water. Following the incubation, the degree of oxidation was measured according to the FTC and TBA methods. To calculate the percentage of the antioxidant activity, after reading the absorbance of the samples at 500 nm for FTC method and at 532 nm for TBA method, the percentage of activity was calculated according to the following equation:

$$AI (\%) = 100 \times (A_0 - A) / A_0$$

Where, A_0 is the absorbance of the control reaction (reaction containing no test compound) and A is the absorbance of the test compound. The values obtained for the control samples were taken for 100% lipid peroxidation.

All experiments were repeated three times and average was used for calculating the antioxidants activity of each sample. The antioxidant activity of the extracts and positive controls were compared by ANOVA-one way test, ($P \leq 0.05$), using SPSS program.

Results

Antioxidant assays:

The aerial parts of all fourteen different species (seventeen samples) of Iranian *Artemisia* were evaluated for their antioxidant activity. They showed strong antioxidant activity by both FTC and TBA methods (Figs. 2 and 3). The activity of BHT was about 100 % (the average absorbance of the BHT in both FTC and TBA method was 0.003 in comparison to the control sample absorbance which was 0.918 and 0.735 respectively). Using the FTC method the antioxidants activity of the extracts was mainly within the range of 92-99%. Antioxidant activity of the extracts was variable when they were tested by the TBA method and was within the range of 18-75%.

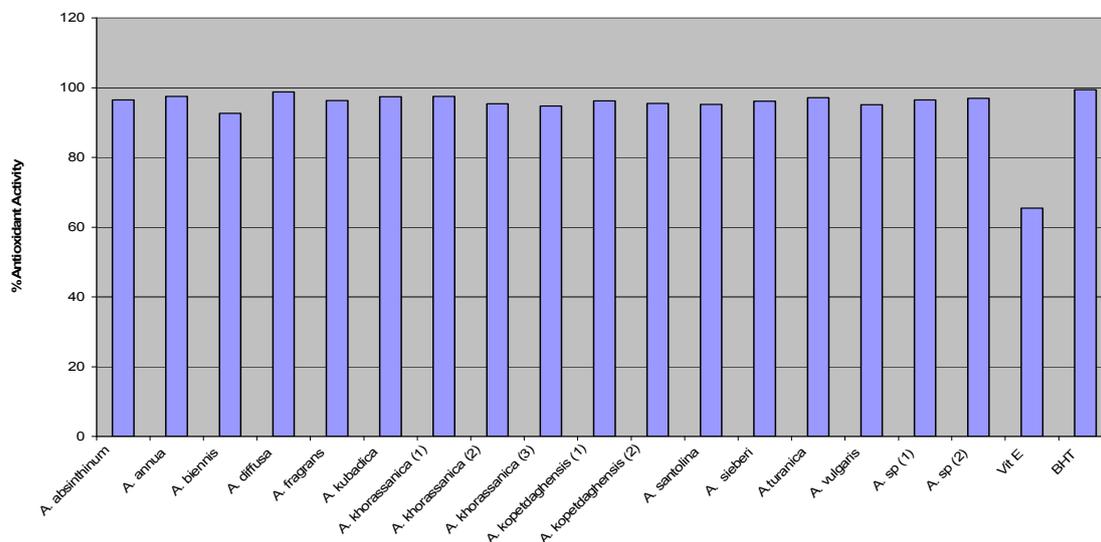


Figure 2: Antioxidant activity of methanol extracts of fourteen different *Artemisia* species (seventeen samples) growing in north and north east of Iran (final concentration 0.02% w/v) measured using FTC method (n=3).

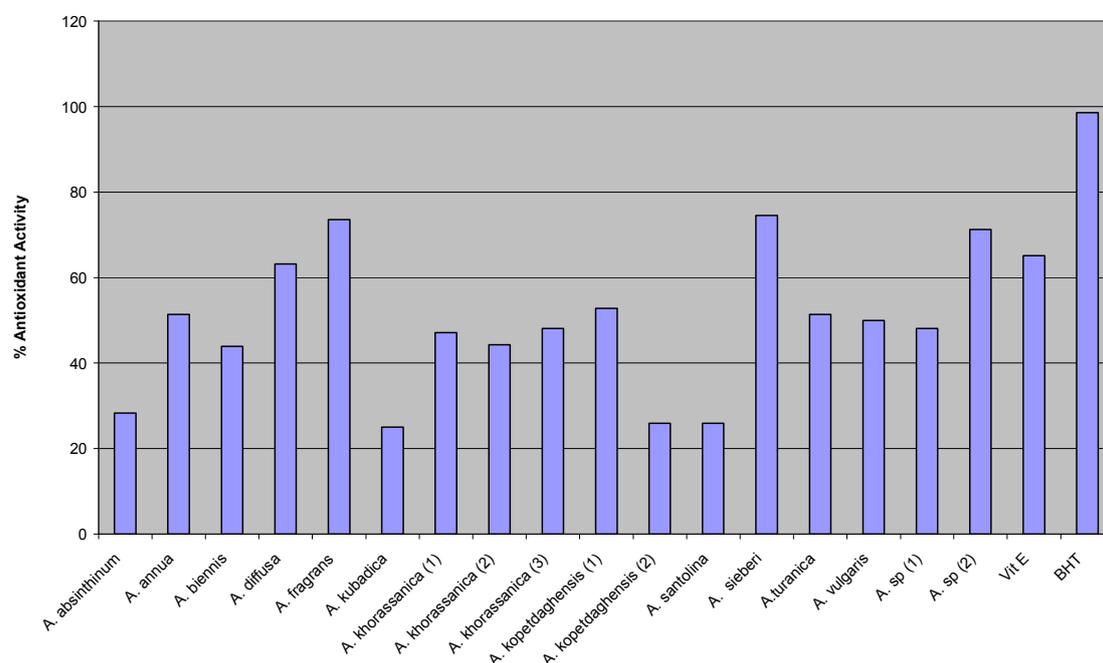


Figure 3: Antioxidant activity of methanol extracts of fourteen different *Artemisia* species (seventeen samples) growing in north and north east of Iran (final concentration 0.02% w/v) measured using TBA method (n=3).

Quantification of different compounds in the extracts:

The amounts of non-volatile components (from methanol extracts) of the aerial parts of all tested plants are shown in Table 1.

Discussion

In recent years there have been an increasing number of investigations to find antioxidative compounds. As it was stated before commercial antioxidants like other synthetic food additives have been criticized, mainly due to possible toxic effects. Therefore there is a strong need for effective antioxidants from natural sources as alternatives to synthetic food additives in order to prevent deterioration of foods, drugs and cosmetics. The extracts and essential oils of many plants have been investigated for their antioxidant activity. The methanol extracts obtained from the aerial parts of fourteen different species (seventeen samples) of Iranian *Artemisia*; *A. absinthium* L., *A. annua* L., *A. biennis* Willd., *A. diffusa* Krasch ex Poljack, *A. fragrans* Willd., *A. kubadica* Boiss. & Buhse, *A. khorassanica* Podl., *A. kopetdaghenensis* Krasch., M. Pop. & Lincz. ex Poljack, *A. santolina* Schrenk, *A. sieberi* Besser, *A. turanica* Krasch., *A. vulgaris* L., *Artemisia* sp1 and *Artemisia* sp2, were evaluated for their antioxidant activity in this study.

Table 1. Major components in methanol extracts of the aerial parts of different species of *Artemisia* from Iran

Chemical components (Average content)* Plant Name	Anthocyanins	Flavonoids	Saponins	Tannins
<i>A. absinthinum</i>	-	1+	-	2+
<i>A. annua</i>	-	2+	-	2+
<i>A. biennis</i>	-	1+	1+	2+
<i>A. diffusa</i>	2+	3+	-	3+
<i>A. fragrans</i>	1+	2+	-	2+
<i>A. kabadica</i>	1+	1+	-	3+
<i>A. khorassanica</i> (1)	1+	1+	-	1+
<i>A. khorassanica</i> (2)	-	1+	-	1+
<i>A. khorassanica</i> (3)	-	1+	-	2+
<i>A. kopetdaghensis</i> (1)	1+	3+	-	3+
<i>A. kopetdaghensis</i> (2)	1+	1+	-	2+
<i>A. santolina</i>	1+	2+	-	3+
<i>A. sieberi</i>	2+	2+	-	2+
<i>A. turanica</i>	1+	2+	1+	3+
<i>A. vulgaris</i>	-	2+	-	2+
<i>A. sp</i> (1)	1+	1+	1+	3+
<i>A. sp</i> (2)	2+	3+	1+	3+

* average content was rated from - to 4+; +, slightly positive; ++, moderately positive; +++, strongly positive; +++++, very strongly positive; -, not detected.

-Number (1), (2) and (3) indicates different collection area

The inhibition of lipid peroxidation in linoleic acid was evaluated by measuring the concentration of the TBA-reactive substances (TBARS) and ferric thiocyanate.

The FTC method measures the amount of peroxide produced during the initial stage of lipid oxidation. Subsequently at a later stage of lipid oxidation, peroxide decomposes to form carbonyl compounds that are measured by the TBA method. All the methanol extracts possessed quite strong antioxidant activity (low absorbance values) by both the FTC and TBA methods. The antioxidant activity was then compared with those of α -tocopherol (a natural antioxidant) and butylated hydroxytoluene, (BHT, a synthetic antioxidant).

Different extracts obtained from various *Artemisia* species exhibited strong antioxidant activity within the range of 92-99% by the FTC method (Fig. 2). Using the TBA method, all the extracts were also showed antioxidant activity within the range of 18-75% (Fig. 3). Methanol extracts of all the studied plants exhibited a very high antioxidant activity (close to BHT) when tested by FTC method (Fig. 2). When antioxidant activity of the samples was examined using the TBA method the results were quit variable and generally lower

than the amount detected with the FTC method. These differences may be due to their different antioxidant mechanisms. The higher results in FTC method might suggest that the amount of peroxide in the initial stage of lipid peroxidation was more than the amount of peroxide in the secondary stage. Since in FTC method the amount of peroxide at the beginning of lipid peroxidation, in which peroxide will react with ferrous chloride and form ferric ions, is measure. Ferric ions will then unite with ammonium thiocyanate and produce ferric thiocyanate. The substance is red, and denser color is indicative of higher absorbance. The TBA method measures free radicals present after peroxide oxidation. The antioxidant activity of methanol extracts of most of the samples tested when using the TBA method was nearly as high as the activity of α -tocopherol. The methanol extract of *A. sieberi* was shown the highest antioxidant activity (74.5%) while the methanol extract of *A. khorassanica* (sample 3) possessed the lowest activity (18.4%), (Fig 3). Antioxidant activity and the strength of this activity in each plant depend on the existence of various compounds in that plant. Antioxidative and radical scavenging activities of flavonoids are well studied (47). Some of phenolic compounds (anthocyanidines, catechines, flavones, flavonols and isoflavones), tannins (ellagic acid and gallic acid), phenylpropanoids (caffeic acid, coumaric acids and ferulic acid), lignans, catchol and many others are antioxidants (48). Several different essential oils obtained from various plants and their components have also been studied for their antioxidant activities (49). Figures 1 and 2 indicate different extracts obtained from aerial parts of different species of *Artemisia* from Iran showed different strength of antioxidant activity by FTC and TBA methods. The phytochemical analysis of the methanol extract of the tested plants indicated the presence of major phytochemicals, including anthocyanin, flavonoids, saponins and tannins (Table 1), which may have been responsible for part of the observed antioxidant activity (50). As it can be seen from Table 1, the amounts of non-volatile compounds (anthocyanines, flavonoids, saponins and tannins) in the aerial parts of these plants vary significantly. Variation in the amounts of various non-volatiles in different tested plant can be one of the reasons causing differences in antioxidant activity of the extracts obtained from the aerial parts of these plants. Finally while further investigations will be aimed at isolating and identifying the substances responsible for the antioxidant activity of plant extracts, using several different methods, but at this stage, methanol extracts of these plants can be considered as strong antioxidant agent.

Conclusions

Different extracts obtained from aerial parts of various *Artemisia* species from Iran showed different strength of antioxidant activity by FTC and TBA methods. Methanol extracts of all the studied plants exhibited a very high antioxidant activity (close to BHT) when tested by FTC method. The differences in the amounts of various non-volatiles can cause this variation in antioxidant activity of the extracts obtained from the aerial parts of these plants. However while further investigations in isolating and identifying the substances responsible for the antioxidant activity of the plant extracts, using several different methods is needed, but at this stage, methanol extracts of these plants can be considered as strong antioxidant agent.

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