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Artemisia spicigera Essential Oil: Assessment of Phytochemical and Antioxidant Properties

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Abstract

Background: Essential oils (EO), also called volatile odoriferous oil, are aromatic oily liquids extracted from different parts of plants. In general, the constituents in EOs are terpenes, aromatic compounds (aldehyde, alcohol, phenol, methoxy derivatives, and so on), and terpenoids (isoprenoids). Essential Oils have been known to possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products.

Objectives: The aim of this study was to assess the quantity and quality of compounds, with active chemical and antioxidant properties, of *Artemisia spicigera* essential oil (EO) due to the effect of geographic location and season of harvest on the phenolic compounds of the plant. The plant was collected from east Azarbayjan province, Iran (both before and after the flowering stage).

Materials and Methods: *A. spicigera* EO was analyzed by gas chromatogram/mass spectrometry (GC-MS). The antioxidant activity and total phenolic content before and after flowering were evaluated by the Folin Ciocalteu method. Also, the yields of essential oil as a percentage based on the level of dry plant and the volume of extracted oil was determined.

Results: Analysis of *A. spicigera* EO by gas chromatogram-mass spectrometry showed that spachulenol 1 H cycloprop (18.39%) and bicyclo hexan-3-en, 4-met (26.16%), were the prominent EOs of *Artemisia* before and after the flowering stage; the total phenolic EO before and after the flowering stage was 23.61 ± 1.08 μg/mL and 17.71 ± 0.9 μg/mL, respectively. Also level of flavonoid content before and after the flowering stage was 37.27 ± 1.70 μg/mL and 29.04 ± 1.30 μg/mL, respectively. This EO was able to reduce the stable free radical 2, 2-diphenol,1-picryl hydrazyl (DPPH) with an IC_{50} of 86.14 ± 2.23 and 96.18 ± 2.61 μg/mL, before and after flowering, respectively. Yield of EO before and after flowering was 0.5% and 0.6%, respectively.

Conclusions: Results have shown that *A. spicigera* EO before and after flowering has antioxidant properties and therefore can be used in combination with other preservatives to protect food materials against a variety of oxidative systems.

Keywords: Essential Oils, Antioxidants, Gas Chromatography, *Artemisia spicigera*

1. Background

In the recent years, it has been proved that free radicals are the most important oxidative factors in food products (that are induce changes in nutritional value and chemical compositions) (1, 2). In addition to the adverse effects on the organoleptic properties of food with destruction of vitamins and essential fatty acids, in the body, these toxic compounds can lead to adverse effects such as inflammatory diseases, diabetes mellitus, ischemic heart and brain disease, cancer, immune deficiency and aging (3, 4). Therefore, the use of antioxidants for reduction of the rate of oxidation in foods is necessary, which if used properly can increase the shelf life of food products. Plants contain phenolic compounds and other compounds that have antioxidant potential. Since the activity of antioxidant compounds and natural extracts have been identified by a variety of methods, it is currently important to determine which of these natural antioxidants have greater efficiency (5). Flavonoids and other phenolic compounds are available in many plants and act as antioxidant, anti-microbial and anti-inflammatory agents and vasodilators, as indicated by a number of previous studies (6). These compounds can generally be seen in fruits, vegetables, leaves, nuts, seeds, roots and other plant parts. Due to the various properties of these phenolic compounds, they have become a point of interest in the food, chemical, pharmaceutical and medical fields (7). The *Artemisia* plant genus is one of the most important plants in the pastures of Iran, which has many species and forms large areas of the country's steppe and semi-steppe region's natural covering. This genus has 34

species of medical plants, which are spread throughout the country. These species include *A. melanolepis* and *A. kermanesis*. Other species of the genus *Artemisia*, in addition to Iran, grow in the Caucasus, Siberia, Turkmenistan, Afghanistan, Pakistan, Central Asia, Tibet and Europe (8). *Artemisia spicigera* as a member of the Asteraceae family is a perpetual genus, which is frequent under circumstances of 300 mm annual rainfall. *Artemisia* is abundant in the north and northwest regions of Iran. In Iranian traditional medicine, *Artemisia* is known to have astringent, antiseptic, anti-parasitic and anti-poisoning properties (9). Several studies on the essential oil yields from other species of the genus *Artemisia* have been performed previously. Chemical analysis of Essential Oil (EO) has shown that cineol crysanton, alphapinen and micetilen form the major parts EO (10, 11). Chemical EOs are formed from poly-propanoids, mono and sesquiterpene oils and aromatic compounds (11).

Considering the importance of medicinal plants, especially native medicinal plants of our country and also considering the impact of geographic location and harvest season of the plant on active chemical compounds, the aim of this study was to evaluate the quantity and quality of phenolic compounds and antioxidant properties of EO of *Artemisia* before and after flowering. Furthermore, these EO were studied due to their richness in our country as well as their accessibility and affordability.

2. Objectives

The aim of this study was to determine the quantity and quality of active chemical compounds and antioxidant properties of *A. spicigera* EO collected from East Azerbaijan (before and after flowering).

3. Materials and Methods

3.1. Plant Material

Samples of *A. spicigera* were collected during year 2015 from Bostan-Abad of East Azerbaijan province (Iran) before and after flowering. The species of the collected plant was confirmed and deposited at the herbarium of the department of pharmacy, university of Tabriz, Iran.

3.2. Preparation of Essential Oil

The dried plants (100 g) were hydro-distilled for three hours using a Clevenger type apparatus. The essential oil was then dehydrated over anhydrous sodium sulfate and after passing through a filter with a pore size of 0.45 μm, it was stored in sealed vials at 4°C (12).

3.3. Determination of the Yield of Plant Essential Oil

The amounts of dried plant used in the experiment and the yield of essential oil before and after flowering (as a percentage) were determined.

3.4. Gas Chromatogram-Mass Spectrometry (GC/MS)

The EO was analyzed by a gas chromatogram. The chromatograph (Agilent 6890 UK) was equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness) and the data were taken under the following conditions: initial temperature of 50°C, temperature ramp of 5°C/minute, 240°C/minute to 300°C (holding for three minutes), and injector temperature at 290°C. The carrier gas was helium and the split ratio was 0.8 mL^{-1}/minute. For confirmation of analysis results, the essential oil was also analyzed by GC/MS (Agilent 6890 gas chromatogram equipped with an Agilent 5973 mass-selective detector; Agilent UK) and the same capillary column and analytical conditions as above. The MS was run with the electron-ionization mode and ionization energy of 70 eV (13, 14).

3.5. Assay for Total Phenolic Content

Measurement of total phenolic materials was carried out using Folin-Ciocalteu reagent (Sigma-Aldrich) and gallic acid (Sigma-Aldrich) as the standard (15). Furthermore, 0.1 mL of the mentioned EO was transferred to an Erlenmeyer flask followed by the addition of 46 ml of distilled water and 1 mL of Folin-Ciocalteu reagent and the content was severely mixed. After three minutes, 3 mL of 2% sodium carbonate was added and the mixture was placed on an agitator screen with medium intensity for two hours and its absorption was read at 760 nm. This stages accomplished for standard soloution of gallic acid (0 - 1000 μg per 0.1 mL) and will be drowsed a standard curve equation according to the following equal (16).

$$\text{Absorption rate} = 0.0012 \times (\text{micro; g})\,(\text{gallic acid}) + 0.0033 \quad (1)$$

3.6. Flavonoid Contents

In order to measure the content of the flavonoids, different concentrations of EO were prepared and 0.5 mL of each concentration was poured into a test tube and to each tube 500 μL of 2% aluminum chloride was added and the tubes were left at room temperature for one hour and the absorbance was measured at 420 nm by a spectrophotometer apparatus. Using this method a standard curve of quercetin was drawn with a range of 5 to 60 mg and the total flavonoid content was calculated based on milligrams of quercetin per gram of essential oil (17).

3.7. Determination of Antioxidant Activity (DPPH Assay)

Power of dehydroghenation of extracted is measured by decolourization of solution of diphenyl Picryl hydrazyl. In this spectrometry evaluation, stable radical diphenyl Picryl hydrazyl (Sigma-Alderich) was used as the reactant. Fifty milliliters of different concentrations of essential oil was added to 5 mL of methanolic solution

(0.004%). After 30 minutes at room temperature, absorption at a wavelength of 517 nm was read and compared with the control. The inhibition free radical DPPH based on percentage (I%) was calculated as follows:

$$I\% = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100 \quad (2)$$

Where A was the blank (containing all reactants except EO) and A ample was the absorption solution containing different concentrations of EOs. The concentration of essential oil that showed 50% inhibition (IC_{50}) was given based on the percentage of inhibition against the concentration of essential oil, respectively. Synthetic antioxidant Boutill hydroxyl anysol (BHT) was used as the positive control. The tests were performed in triplicates (18, 19).

4. Results

4.1. The Yields of Essential Oil

The oil was isolated by hydro distillation from the dried plant before and after flowering. The yield of the EO before flowering was 0.5% (v/w) and after flowering this was 0.6% (v/w).

4.2. Constituents of Artemisia spicigera Essential Oil (Before and After Flowering) Using GC/MS

The evaluation of the results of this study showed that the yield of EO of *A. spicigera* before and after flowering was 0.5% and 0.6% based on the dry weight of the plant, respectively. The major compound of EO before and after flowering, along with retention time and the percentage of each compound are shown in Tables 1 and 2. Before flowering 33 compounds were detected that made up 94.32% of EOs, and the most common components were (-) -1H spachoulenol cycloprob (18.39%), epizunaren (9.64%), germakerin D (6.33%) and trans-caryophyllene (6.17%). Also after flowering, 13 compounds were identified that in to form 96.23% of components of EO. Most of the components of the EO after flowering formed bicycle [3, 1, 0] Hexan-3-en, 4 - met (26.16%), 1, 8 cineole 2-oxabicyclo [2, 2…] (26.15%), camphor bicyclo [2, 2, 1] heptane (17.46%) and beta tougun (12.86%).

4.3. Total Phenolic and Flavonoid Contents

Content of total phenol of EO in the stages of before and after was 23.61 ± 1.08 and 17.71 ± 0.9 micrograms gallic acid on milliliter EO.

Content of total flavonoeid of EO in the stages of before and after was 37.27 ± 1.7 and 29.04 ± 1.3 micrograms Quercetin on milliliter EO.

4.4. 2, 2-Diphenyl, 1-Picryl Hydrazyl (DPPH) Assay

Antioxidant activity was determined using the DPPH method. The level of IC_{50} for EO before flowering was 86.14 ± 2.23 (87.57 to 105.5) micrograms per milliliter and after flowering this was 96.18 ± 2.61, both of which were weaker than Butylated Hydroxytoluene (BHT) (Table 3). The results showed that with increasing concentrations of EO inhibition free radicals to be increased.

Table 1. Constituents of *Artemisia spicigera* Essential Oil (Before Flowering) Identified Using Gas Chromatogram/Mass Spectrometry[a]

Compound No.	Compound Name	RT, min	Percentage
1	Bicyclo [3.1.1] hept-3-en-2-ol, 4,..	8.6	6.76
2	p-Mentha-1, 5-dien-8-ol 2, 4-Cy...	9.02	3.76
3	Bicyclo [3.1.1] hept-2-ene-2-metha...	9.61	3.34
4	Bicyclo [3.1.1] hept-3-en-2-one, 4...	9.96	1.57
5	Trans-(+)-carveol	10.11	1.24
6	Acetic acid, 1, 7, 7-trimethyl-bic...	11.5	1.28
7	BetaETA. Bourbonene	13.4	1.42
8	Trans-Caryophyllene Bicyclo [7...	14.26	6.17
9	Trans-.beta.-Farnesene (E)-.b...	14.82	1.6
10	Alpha-Caryophyllene	14.9	1.41
11	1 Germacrene-D	15.65	6.33
12	Bicyclogermacrene [8.1...	15.94	2.91
13	1H-Cycloprop[e]azulene, 1a, 2, 3, 4...	16.32	1.46
14	Beta.-cadinene	16.39	1.05
15	Delta.-Cadinene Naphthalene,...	16.46	1.75
16	Butyl hydroxy toluene	16.68	1.06
17	Alpha-agarofuran	17	1.41
18	Caryophyllene oxide 5-Oxatric...	17.13	1.8
19	Germacrene B (CAS) 1, 5-Cyclod...	17.22	1.03
20	(-)-Spathulenol 1H-Cycloprop [...	18.08	18.39
21	Salvial-4(14)-en-1-one	18.18	1.14
22	Vieridiflorol Viridiflorol...	18.31	1
23	Caryophyllene oxide	18.44	2.05
24	2- Naphthalenemethanol, 1, 2, 3, 4, 4...	18.64	3.02
25	1H-Cycloprop [e] azulene, 1a, 2, 3, 4...	18.76	1.17
26	Junipiene 1,4-Methanoazulene, ...	18.95	1.7
27	Naphthalene, 1, 2, 3, 5, 6, 7, 8, 8a-oc...	19.02	1.16
28	Epizonaren	19.37	9.62
29	7-Epi-.alpha.-selinene	19.46	3.49
30	Caryophylleneol-II Bicyclo [7.2...	19.6	1.74
31	Aromadendrene, dehydro-	19.71	1.26
32	12-Norcyercene-B	19.83	1
33	2-Pentadecanone, 6,10,14-trimethyl-	22.27	1.23
Total			94.32

[a]This table includes basic compounds that had the highest percentage and minor compounds were avoided.

Table 2. Constituents of *Artemisia spicigera* Essential Oils After Flowering Using Gas Chromatogram/Mass Spectrometry[a]

Compound No.	Compound Name	RT, min	Percentage
1	Camphene Bicyclo [2.2.1] heptan...	4.85	2.3
2	1, 8-Cineole 2-Oxabicyclo [2.2....	13.09	26.15
3	Bicyclo [3.1.0] hexan-3-one, 4-met...	15.65	26.16
4	Beta-thujone	7.96	12.86
5	Camphor Bicyclo [2.2.1] heptan-...	8.47	17.46
6	Trans-decalin, 2-methyl-	8.54	1.48
7	Pinocarvone 6, 6-dimethyl-2-me...	8.63	1.6
8	Endo-Borneol Bicyclo [2.2.1] he...	8.71	2.39
9	Bicyclo [2.2.1] heptan-2-one, 1, 7,...	8.87	2.79
10	Benzenemethanol (CAS) Benzyl ...	9.24	1.54
11	Chrysanthenyl acetate	10.56	1.19
12	Bicyclo [2.2.1] heptan-2-ol, 1, 7, 7...	11.12	1.35
13	Phenol, 5-methyl-2-(1-methylethy...	11.17	0.96
Total			96.23

[a]This table includes basic compounds that had the highest percentage and minor compounds were avoided.

Table 3. IC_{50} of Essential Oil and Controls[a]

Essential Oil	IC50, μg/mL
***A. spicigera* (before flowering)**	86.14 ± 2.23
***A. spicigera* (after flowering)**	96.18 ± 2.61
Vitamin C	2.15 ± 0.23

[a]The results (means ± SD) are significantly different ($P < 0.05$).

5. Discussion

The results of the investigation of the chemical compounds of *A. spicigera* EO in the present study were somewhat in accordance with other investigations. In most studies similar to the present study, compounds such as 1H spachoulenol cycloprob (18.39%), epizunaren (9.64%), germakerin D (6.33%) and trans-caryophyllene (6.17%), respectively, were the major components of the *A. spicigera* EO before flowering. Also, bicyclo [3, 1, 0] hexan-3-en, 4-met (26.16%), 1, 8 cineole 2-oxabicyclo (26.15%), camphor bicyclo heptane (17.46%) and beta tougun (12.86%) after flowering were the major components of EO. In this study, 33 compounds were detected before flowering, including 94.3% EO while 13 compounds were detected after flowering including 96.23% EO. The yield of the EO before flowering was 0.5% (v/w) while after flowering this was 0.6% (v/w).

The level of phenolic compounds of *A. spicigera* EO before and after flowering was 23.61 ± 1.08and 17.71 ± 0.9 mg of gallic acid per gram of EO, respectively. Also the levels of flavonoeid compounds of EO before and after flowering, were 37.27 ± 1.70 and 29.04 ± 1.30 μg per mL, respectively. Level of IC_{50} of *A. spicigera* EO before flowering was 86.14 ± 2.23 μg per mL and after flowering this was 96.18 ± 2.61 μg per mL.

Currently, researchers are interested to study medicinal plants for extraction of natural antioxidants for usage instead of synthetic antioxidants. Natural antioxidants are healthier, have greater benefits and fewer side effects (20).

Among natural food additives that can be used in many foods, EOs are a good option with a plant origin that have antibacterial, antifungal, antioxidant, and anti carcinogenic properties (21).

In one study it was reported that free radical scavenging increases with increasing EO concentration and the obtained extract from the aerial parts of *Artemisia*, collected from regions of the Alborz Golestanak, provide 50% inhibition (IC_{50} of 30.6 ± 612 μg/mL) (22). In another study, the level of IC_{50} of the methanol extract from the aerial parts of *Artemisia*, collected from different areas of East Azarbaijan, was 29.74 to 64 .18 μg per mL (23). In another report, the level of inhibition of the free radical form of 2, 2 diphenyl 1-picrylhydrazyl of *Artemisia*, collected from Babak city of Kerman, was 71.6 ± 1.7 μg per mL (24).

According to the study of Mahmoudi, the level of total phenol of aerial parts of *Artemisia*, collected from different areas of Golestanak, was 194.7 ± 9.9 mg of gallic acid per gram of extract (22).

In the study of Khalaji et al. the level of phenol of the methanol extract of aerial parts of *Artemisia*, collected from different regions of East Azerbaijan, was 1.4 to 2.3 μg per 100 μg extract (23). In the mentioned study, the level of total flavonoid of the methanol extract of the aerial parts of *Artemisia*, collected from areas of Golestanak Alborz, was 0.6 ± 12.4 mg quercetin per gram of extract (22).

Also in the study of Khalaji and et al. the level of flavonoids of methanol extract of aerial parts of *Artemisia*, collected from different regions of East Azerbaijan, was 0.4 to 2.1 μM quercetin per 100 gram extract (23).

In year 2011 a study showed that aqueous extracts of the genus of *Artemisia* afra Jacq reduced Maloven-di-aldeid (MDA) and increased Super Oxide Dismotaz (SOD), glutathione reductase and glutathione peroxidase as enzymatic antioxidants in diabetic experimental animals (25).

The study conducted on aqueous extracts of *Artemisia* sieberi vulgaris in Egypt showed that IC_{50} was equal to 10 μg per mL. Level of Phenol aqueous extract of *Artemisia* sieberi vulgaris was 7.96 ± 0.76 mg gallic acid per gram of extract. The level of its flavonoid was 3.4 mg rutin per gram of extracts (26).

The conducted study on ethanol extract of *Artemisia* seeds in Nigeria showed that the level of IC_{50} was 150.33 ± 1.5 μg per mL (27).

The difference observed in the antioxidant properties of medicinal plants in various studies can be due to differences in the compounds of the mentioned plant (under the effect of genetics, water, air, harvest season, etc.), especially in the amount of phenolic and polyphenol compounds, so that there was a direct correlation between the level of phenol and antioxidant activity of medicinal plants (15).

Considering that *Artemisia* is native in Iran, and has easy and cheap access, consumption of this plant (a source of phenolic compounds) as an antioxidant in food and pharmaceutical industries, is favorable.

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Footnotes

Authors' Contribution: Peyman Ghajarbeygi, Azar Mohammadi, Razzagh Mahmoudi and Morteza Kosari-Nasab developed the original idea and the protocol, abstracted and analyzed the data, wrote the manuscript, and were the guarantors.

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