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Extraction of *Artemisia aucheri* Essential Oils and Evaluation of their Chemical Composition and Antioxidant Activity

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Abstract

Introduction: Plants are an excellent source of biologically active phytochemical compounds that act as antioxidants to combat free radicals. The aim of this study is to chemically analyze the essential oil compounds (EOs) and evaluate the antioxidant capacity of *Artemisia aucheri* flowers *in vitro*.

Materials and Methods: *Artemisia aucheri* was collected from the Kashan region of Isfahan Province, Iran. The flower essential oil was extracted using a Clevenger apparatus, and its components were separated and identified using gas chromatography-mass spectrometry (GC-MS).

Results: The antioxidant properties of the flower essential oil were evaluated using the DPPH free radical scavenging method and the β-carotene-linoleic acid bleaching test. Data analysis was performed using SPSS 20 software. The major compounds identified in the flower essential oil were camphene (31.05%), β-myrcene (26.18%), and camphor (15.41%). The DPPH assay showed that the essential oil exhibited significant free radical inhibition, while the β-carotene assay used ascorbic acid as a positive control.

Conclusions: Research indicates that many factors influence the primary composition of plants, including climatic conditions, soil type, and genetic factors. Due to the hydrophilic nature of the DPPH assay, the IC_{50} value of the essential oil was higher than that of ascorbic acid, indicating lower activity in this assay. Conversely, due to the lipophilic nature of the β-carotene assay, the IC_{50} value of the essential oil was lower than that of ascorbic acid, suggesting stronger antioxidant activity in lipophilic environments.

Keywords: *Artemisia aucheri*, DPPH Method, β-Carotene Method, Antioxidant Activity, Essential Oil

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Introduction

Essential oils (EOs) are fragrant, volatile liquids obtained from various plant parts, including leaves and flowers. These oils consist of multiple compounds with distinct chemical structures and possess characteristic aromas. Due to their volatility at room temperature, they are also known as volatile oils, aromatic oils, or ester oils. Essential oils exhibit diverse biological activities, including antibacterial, antifungal, anti-inflammatory, anticancer, antispasmodic, and analgesic effects.[1]

Artemisia is an aromatic shrub belonging to the family Asteraceae (Compositae), widely distributed in arid and semi-arid regions worldwide, including the desert areas of Iran. The aerial parts of the plant—stems, leaves, flowers, fruits, and seeds—emit a strong and distinctive wormwood fragrance. Many species within this genus possess notable medicinal properties, attributed to the high concentrations of essential oils and aromatic compounds in their tissues.[2] Typically, *Artemisia* species grow to heights between 25 and 50 cm and are commonly found in Isfahan province.[3]

Many *Artemisia* species flower in late summer or autumn, producing aromatic essential oils. Key bioactive compounds in these plants include 1,8-cineole and thujone, which have demonstrated therapeutic effects such as antiparasitic, expectorant, and anti-inflammatory activities. Additionally, these compounds have been investigated for their potential in dissolving cholesterol gallstones and repelling insects.[4] In the essential oil of *Artemisia aucheri*, these compounds constitute a significant portion (approximately 11.3%), indicating the presence of 1,8-cineole as a major constituent.[5] Other important monoterpenes such as α-pinene, camphene, and β-pinene exhibit anti-inflammatory, antibacterial, and antifungal properties, effectively inhibiting microbial growth.[6]

Moreover, *Artemisia* species are rich sources of biologically active phytochemicals that function as antioxidants to combat free radicals. These secondary metabolites, often polyphenolic in nature, are synthesized by plants to mitigate reactive oxygen species (ROS) and are distributed throughout various plant tissues. Acting as antioxidants, they help detoxify and reduce the damaging effects of free radicals within biological systems. Antioxidants play a critical role in counteracting the adverse effects of oxidative stress in tissues.[7]

Polyphenolic antioxidants are present in all plant parts, including skin, stems, leaves, roots, flowers, pods, and seeds.[8] They protect target molecules via several mechanisms including: a) Neutralizing reactive oxygen species through enzymatic catalysis or direct chemical reactions; b) Reducing the formation of reactive species; c) Chelating metal ions that catalyze the conversion of less reactive substances into more reactive radicals; d) Repairing damaged biomolecules; and e) Removing severely damaged molecules and facilitating their replacement with healthy ones.

Disruption of antioxidant defenses can impair physiological functions and accelerate aging and disease progression. Therefore, antioxidant supplementation may help mitigate oxidative damage.[9] Several analytical methods exist to assess the antioxidant and radical scavenging capacities of natural substances. This study employs two widely used assays—the DPPH radical scavenging test and the β-carotene-linoleic acid bleaching test—both suitable for evaluating antioxidant potential in food industry applications.[10] While numerous studies have examined the antioxidant properties of various plant species globally and within Iran, research on *Artemisia aucheri* Boiss., a species endemic to Iran, remains limited. This study aims to evaluate the antioxidant properties of *Artemisia aucheri* floral essential oil through *in vitro* assays and to chemically characterize its constituents.

In a related study, Mojarrab et al. (2021) investigated the antioxidant activities of five extracts (petroleum ether, dichloromethane, ethyl acetate, ethanol, and ethanol-water) from the aerial parts of *Artemisia princeps* using ferric ion chelating, DPPH radical scavenging, and β-carotene bleaching assays. They identified ethyl caffeate and spinacetin 3-rutinoside as bioactive compounds, with ethyl caffeate demonstrating significant antioxidant activity, particularly in DPPH radical scavenging, as well as in scavenging superoxide ions and nitric oxide.[11]

Given the limited data on *Artemisia aucheri*, this study collected plant material from the Kashan region of Isfahan and focused on analyzing the floral essential oil's chemical composition and antioxidant capacity using DPPH and β-carotene-linoleic acid assays.

Materials and Methods

Collection and Preparation of the Plant

Artemisia aucheri flowers from late summer until early October. In mid-September 2021, flowering branches of *Artemisia aucheri* were collected from the areas surrounding Kashan, Isfahan Province, Iran (Figure 1). The plant was scientifically identified and authenticated at the Herbarium of Forests and Meadows, Isfahan Province.

Figure 1. The *Artemisia oucheri* Collected from Kashan Region.

The flowering branches were carefully separated from the stems and spread evenly on newspapers to dry in a dark, well-ventilated environment at room temperature. After complete drying, the plant material was ground using an electric grinder. The powdered samples were then stored in airtight containers, wrapped in aluminum foil to protect against light exposure, and kept refrigerated at 4 °C until further analysis.

Identification and Analysis

The chemical composition of the essential oil was analyzed using a gas chromatography-mass spectrometry (GC-MS) system equipped with an Agilent 5975 mass selective detector with an electron ionization (EI) source, coupled to an Agilent 5975 gas chromatograph. Separation was performed on a 30-meter HP-5MS capillary column with an inner diameter of 0.25 mm and a film thickness of 0.25 µm. The inlet temperature was set at 280 °C, the ionization source temperature at 150 °C, the quadrupole analyzer temperature at 230 °C, and the interface temperature between the GC and MS at 280 °C. Helium was used as the carrier gas at a constant flow rate of 1.2 ml/min. The oven temperature program started at 50 °C for 3 minutes, increased to 200 °C at a rate of 8 °C/min, then ramped to 290 °C at 12 °C/min, where it was held for 3 minutes. To analyze the volatile essential oil components, a headspace system was applied by exposing samples to 85 °C for 30 minutes.

Extraction Method

Essential oil extraction was performed from the aerial parts and flowers of the plant. After harvesting, the plant materials were air-dried in the shade and ground using an electric grinder. A total of 100 grams of the powdered plant material was subjected to hydrodistillation using a Clevenger apparatus for 3 hours. The essential oil separated as a distinct layer on top of the water. After separation, the essential oil was collected, stored in small amber containers wrapped with aluminum foil to protect from light, and refrigerated at 4 °C until further use. The essential oil yield was calculated as volume per weight (v/w). All chemicals and solvents used were of analytical grade and purchased from Merck (Germany), while DPPH, beta-carotene, linoleic acid, and ascorbic acid were obtained from Sigma-Aldrich (USA).

DPPH Radical Scavenging Assay

The antioxidant capacity of the essential oil was evaluated by measuring its ability to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical. Various volumes of essential oil (10, 20, 40, 60, 80, and 100 µl) were mixed with pure ethanol. Then, 125 µl of DPPH solution (prepared by dissolving 0.02 g DPPH in 100 ml ethanol) or a blank sample was added. The mixtures were covered with aluminum foil and shaken at 200 rpm for 30 minutes in the dark. Absorbance was measured at 517 nm using ethanol as a blank control. The IC_{50} value, representing the concentration of essential oil required to scavenge 50% of the DPPH radicals, was calculated. Ascorbic acid was used as a positive control for comparison.

β-Carotene-Linoleic Acid Bleaching Assay

The antioxidant activity was also assessed by the β-carotene-linoleic acid bleaching method, which measures the inhibition of conjugated diene hydroperoxide formation during linoleic acid oxidation. Beta-carotene acts as an indicator; its color fades upon oxidation, which can be measured spectrophotometrically.

Initially, 0.5 mg of β-carotene was dissolved in chloroform, followed by the addition of 20 mg linoleic acid and 200 mg Tween 40. The mixture was shaken at 200 rpm for 30 minutes, forming a yellowish milky emulsion. Different volumes of essential oil (10, 20, 40, 60, 80, and 100 µl) and ethanol were added. Then, 2.5 ml of the emulsion was mixed with 350 µl of each sample. The tubes were sealed with aluminum foil and shaken at 200 rpm for 30 minutes. Absorbance was measured at 490 nm immediately (initial absorbance). Samples were then stored in the dark for 48 hours, after which absorbance at 490 nm was measured again (final absorbance). Pure ethanol served as a negative control, and ascorbic acid as a positive control.

Statistical Analysis

All experiments were performed in triplicate. Data were analyzed using one-way ANOVA and two-way variance analysis with SPSS software version 20. Results are presented as mean ± standard deviation, and p-values less than 0.05 were considered statistically significant.

Results

Chemical Analysis of Artemisia aucheri Flower Essential Oil

Following the acquisition of chromatograms and mass spectra, the compounds present in the essential oil (EO) were identified by analyzing their mass fragmentation patterns and retention indices. The quantitative composition of the EO constituents was determined based on peak areas (Figure 2). The detailed results of the chemical analysis of *Artemisia aucheri* flower essential oil are presented in Table 1.

DPPH Assay

The antioxidant activity of *Artemisia aucheri* essential oil (EO) was evaluated using the DPPH radical scavenging method. The relationship between EO concentration and absorbance at 517 nm, as well as between concentration and percentage of free radical inhibition, is presented in Figure 3. The results demonstrate that as the concentration of the essential oil increased, the absorbance decreased correspondingly, indicating greater scavenging of DPPH radicals. Concurrently, the percentage of free radical inhibition increased with increasing EO concentration, confirming a dose-dependent antioxidant effect.

Comparison of Antioxidant Capacity of Essential Oil and Control Using DPPH Assay

The antioxidant capacity of *Artemisia aucheri* flower essential oil (EO) was compared to that of the control (ascorbic acid) using the DPPH radical scavenging assay. As shown in Figure 4, both the antioxidant activity and concentration of EO and control increased concurrently, resulting in a decrease in absorbance at 517 nm for both groups. Correspondingly, the percentage of free radical inhibition

Table 1. Essential Oil Compounds Identified in *Artemisia oucheri* Flowers

Row	Name Composition	Percent	Inhibition Index (Rt)
1	Cyclopentane, 1,1-dimethyl-	0.294	5.021
2	Isoterpinolene	1.181	6.123
3	3-Carene	1.015	6.511
4	α-Pinene	4.46	6.674
5	Camphene	31.047	7.005
6	β-Pinene	6.766	7.598
7	β-Myrcene	26.774	7.929
8	1,8-Cineole	11.348	8.758
9	Linalool	0.442	10.18
10	Camphor	15.41	11.077
11	Borneol	0.452	11.491
12	Iso-Bornyl acetate	0.274	13.659
13	β-Caryophyllene	0.537	15.693

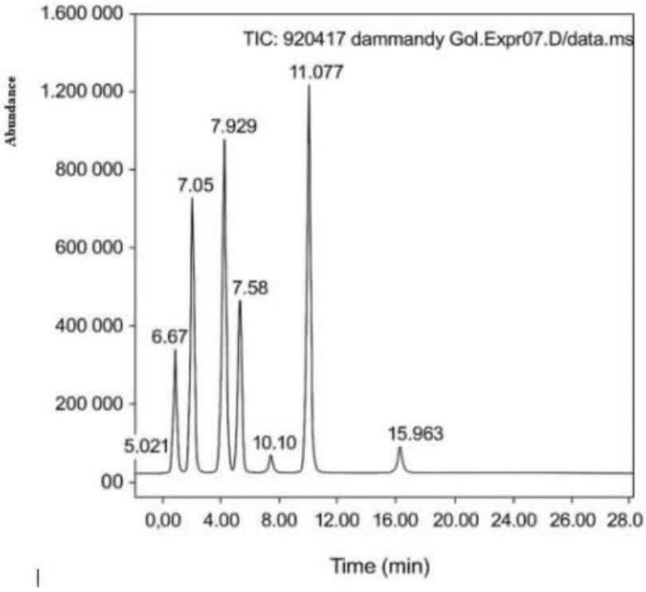
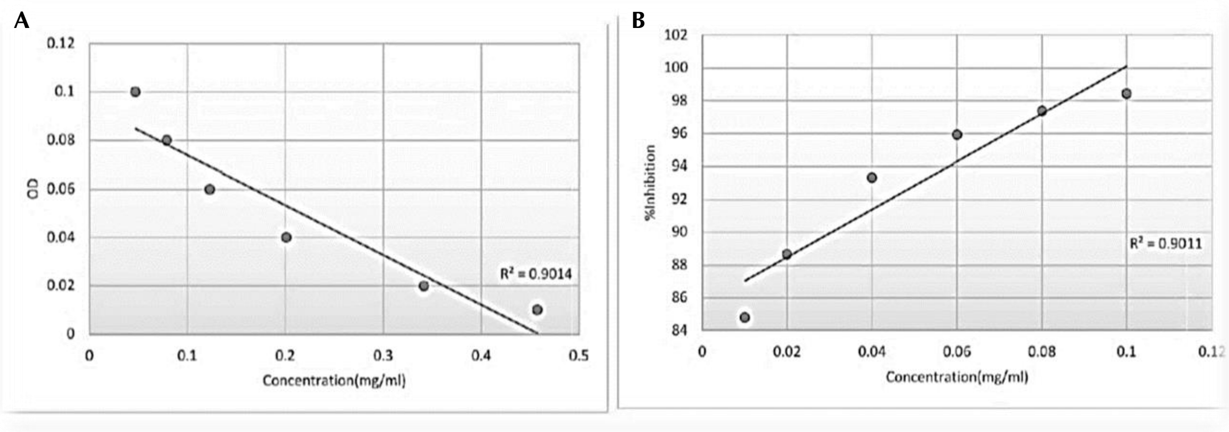


Figure 2. Gas Chromatogram Spectrum Analysis of Essential Oil in *Artemisia oucher.*



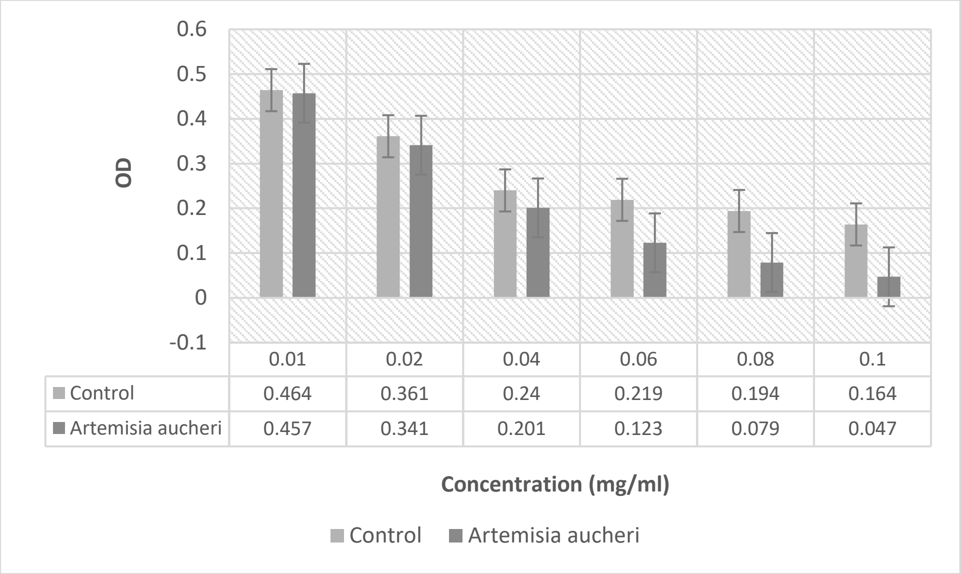
Figures 3. The effect of different concentrations of flower-based essence on antioxidant activity, measured by changes in light absorption **(A)** and as inhibition percentage **(B)**.

increased with rising concentrations of EO and control, as illustrated in Figure 5.

The IC_{50} values, which represent the concentration required to inhibit 50% of the initial DPPH radicals, were determined as 31.27 µg/ml for the essential oil and 37.54 µg/ml for the control. Since a lower IC_{50} value indicates stronger antioxidant activity, the essential oil demonstrated significantly higher free radical scavenging ability compared to the control ($p<0.05$).

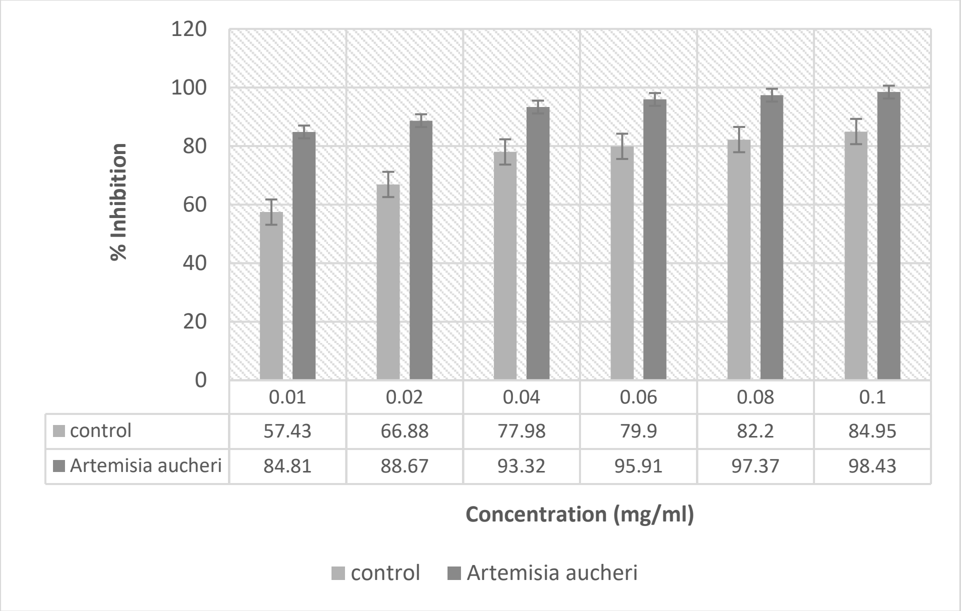
Assessment of Antioxidant Activity Using the β-Carotene Bleaching Method

The antioxidant capacity of *Artemisia aucheri* essential oil (EO) was evaluated using the β-carotene-linoleic acid bleaching assay. This method is based on the oxidation of linoleic acid, which generates free radicals that attack β-carotene molecules, leading to a loss of color. The extent of β-carotene discoloration thus reflects the antioxidant activity of the sample, as antioxidants inhibit the oxidation process and preserve the color. In this assay, ascorbic acid was used as a positive control, while ethanol served as the negative control. As shown in Figure 6, increasing concentrations of *Artemisia aucheri* essential oil corresponded to enhanced antioxidant activity, evidenced by a slower rate of β-carotene bleaching. The results clearly demonstrate a dose-dependent increase in antioxidant capacity with rising EO concentrations. This method is widely accepted for evaluating antioxidant activity, particularly in lipid-rich environments, as β-carotene is a lipid-soluble antioxidant. The inhibition of β-carotene oxidation by the essential oil suggests its potential to prevent lipid peroxidation, which is relevant for food preservation and health applications.



	0.01	0.02	0.04	0.06	0.08	0.1
■ Control	0.464	0.361	0.24	0.219	0.194	0.164
■ Artemisia aucheri	0.457	0.341	0.201	0.123	0.079	0.047

Figure 4. Comparison of Antioxidant Capacity of ESO with Ascorbic Acid as Control Using the DPPH Radical Scavenging Assay.



	0.01	0.02	0.04	0.06	0.08	0.1
■ control	57.43	66.88	77.98	79.9	82.2	84.95
■ Artemisia aucheri	84.81	88.67	93.32	95.91	97.37	98.43

Figure 5. Comparison of Antioxidant Capacity with Ascorbic Acid as Control based on Inhibition Percentage.

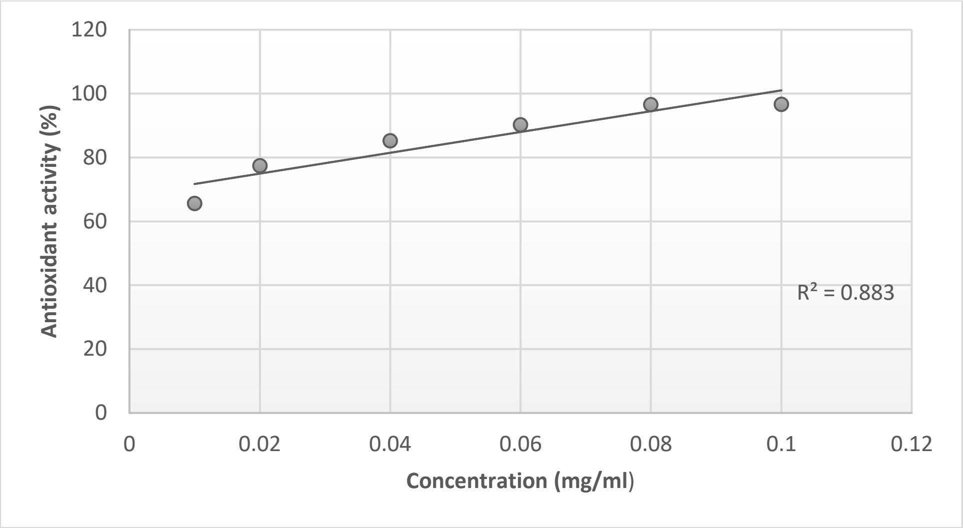
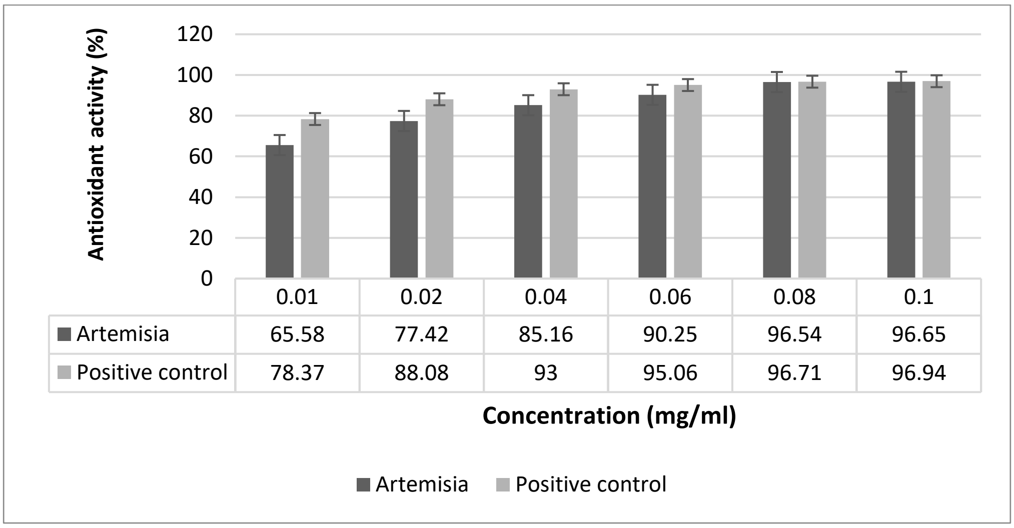


Figure 6. Evaluation of Antioxidant Capacity of *Artemisia aucheri* Essential Oil Using the β-carotene-linoleic Acid Bleaching Assay.

Comparison of Antioxidant Activity Between Essential Oil and Control Using the β-Carotene Assay

The antioxidant activities of *Artemisia aucheri* essential oil (EO) and the control (ascorbic acid) were compared using the β-carotene-linoleic acid bleaching method. Significant differences in antioxidant activity were observed at concentrations below 80 µg/ml for both samples. Specifically, at 80 µg/ml EO concentration, the differences became more pronounced.

As shown in Figure 7, increasing the concentrations of both EO and control resulted in enhanced antioxidant activity. The IC_{50} values were calculated as 51.73 µg/ml for the essential oil and 51.57 µg/ml for ascorbic acid. Statistical analysis indicated no significant difference between the antioxidant capacities of the EO and the control (p>0.05). These results suggest that the antioxidant potency of *Artemisia aucheri* essential oil is comparable to that of ascorbic acid under the conditions of the β-carotene assay.



	0.01	0.02	0.04	0.06	0.08	0.1
■ Artemisia	65.58	77.42	85.16	90.25	96.54	96.65
■ Positive control	78.37	88.08	93	95.06	96.71	96.94

Figure 7. Comparsion of Antioxidant Activity of *Artemisia aucheri* Essential Oil (EO) and the Ascorbic Acid as Control based on Concentration Using the β-carotene-linoleic Acid Bleaching Method.

Discussion

This study focused on *Artemisia aucheri* collected from the Kashan region in Isfahan Province. The chemical composition of the flower essential oil was analyzed, and its antioxidant activity was evaluated using DPPH and β-carotene-linoleic acid assays. Previous research on *Artemisia turcomanica* from Razavi Khorasan reported major components such as 1,8-cineole (19.23%), camphor (15.55%), α-pinene (1.17%), and camphene (1.2%). In contrast, the essential oil of *Artemisia aucheri* in this study was dominated by camphene (31%), 1,8-cineole (11.3%), β-pinene (6.7%), and α-pinene (4.4%). These variations in chemical profiles can be attributed to factors such as geographical location, seasonal changes, altitude, and climatic conditions.[21] For example, Amir Karimi et al. found that *Artemisia vulgaris* extracts collected in spring contained higher levels of octane, decane, and kaempferol compared to autumn samples.[13] Quercitrin, a flavonoid, was detected only in spring extracts of *Artemisia anguicida*. Similarly, Mehdi Yunsi et al. identified 85 compounds in *Artemisia anguicida*, with camphor (9.91–34.44%), α-thujone (19.22–42.63%), and 1,8-cineole (12.57 –31.87%) as major constituents. Novel compounds such as dabanone, α-cadinol, verbene, and ortho-ocimide were also reported for the first time.[14] Comparative studies on *Artemisia princeps* essential oils from China and Japan revealed different dominant compounds, including beta-pinene, germacrene D, gamma-terpinene, limonene, and β-caryophyllene, highlighting the influence of geographical origin. The main compounds identified in *Artemisia aucheri* essential oil in this study—1,8-cineole, camphor, α-pinene, β-pinene, borneol, and linalool—are consistent with previous reports, though their relative abundances vary.[15]

In a 2011 study by Kadri et al. conducted a study on the antioxidant activity of Artemisia ESO using the DPPH method and concluded that the IC_{50} of the plant ESO was 50 µg/ml and the IC_{50} of the positive control BHT was 37/8 µg/ml. This indicates that the antioxidant activity of the plant ESO is lower than the positive control. Meanwhile, the IC_{50} of Artemisia ESO was 31.27 µg/kg and the IC_{50} of the positive control (ascorbic acid) was 37.54 µg/ml. The results show that ESO from Artemisia plant has higher antioxidant activity compared to the positive control. Also, in the β-carotene-linoleic acid method in plant *Artemisia anguicida*, the antioxidant activity of ESO was lower than that of the positive control, which is consistent with the plant *Artemisia anguicida*. In another study, the antioxidant activity of ESO from the plant *Artemisia anguicida* was examined using the DPPH method. This revealed that the IC_{50} of ESO from this plant was 150.33 µg/ml, and the IC_{50} of the positive control was 6.1 µg/ml. Thus, the antioxidant activity of the plant ESO is lower than that of the positive control. However, the IC_{50} of *Artemisia anguicida* flower essence is 31.27 µg/ml, and the IC_{50} of the positive control is 37.54 µg/ml. The results show that *Artemisia anguicida* flower essence has higher antioxidant activity than the positive control.[16]

DPPH radical was used to measure the antioxidant activity of *Artemisia anguicida*. It was observed that ESO inhibited DPPH radical with $IC_{50} = 1.310$ µg/ml and the positive control with $IC_{50} = 2.76$ µg/ml. This indicates a higher antioxidant activity than the positive control, but ESO from the plant *Artemisia aucheri* has a stronger free radical inhibition ability than the positive control.[17] The antioxidant activity of ESO from *Artemisia aucheri* was investigated using the DPPH method. This indicates that the free radical inhibition ability of ascorbic acid as a positive control was higher than that of the essential oil. However, ESO from *Artemisia aucheri* has a higher free radical inhibition ability than the positive control.[18] A study on the antioxidant activity of ESO from *Artemisia anguicida*, carried out using the DPPH method, concluded that ESO from this plant inhibits a lower percentage of free radicals than ascorbic acid. The percentage of free radical inhibition of ESO from the plant Artemisia princeps is higher than the positive control.[19] A study by Parvin Jahanbani et al. further measured the antioxidant activity of five different extracts (petroleum ether, dichloromethane, ethyl acetate, ethanol, and ethanol water) of the aerial parts of Artemisia princeps using three methods to measure ferric ion chelation (FIC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and beta-carotene (BCB) decolorization test. In this study, the presence of ethyl caffeate and 3-rotinoside spinastin was detected for the first time in Artemisia princeps. As one of the antioxidants, spinastin contributes to the antioxidant capacity of spinach. Spinach leaves showed significant antioxidant capacity in the DPPH test. In addition, spinastin gentiobioside showed moderate ABTS radical scavenging activity comparable to BHT activity. Ethyl caffeate has valuable effects in scavenging superoxide anion radicals, nitric oxide, and DPPH, and also significantly inhibits PC12 neuronal cell death caused by hydrogen peroxide at concentrations of 5 and 25 µM. The results of this study demonstrated the strong cleaning power of Artemisia ethanol extract in the DPPH method. It has been reported that the TPC of Artemisia ethanol extract is higher than that of *Artemisia anguicida* and *Artemisia japonica* extracts. These results may be partly related to the presence of different types of phytochemicals in the extract.[11]

French In the study of Asma Mahdizadeh et al., the extract of *Artemisia Oucheri* was also shown to have antioxidant activity in Campylobacter jejuni infection. which exerts this antioxidant activity by influencing the expression of genes involved in oxidative stress such as COX2, iNOS, SOD, GPx. Thus, the administration of 10 mg/body weight/day of *Artemisia aucheri* extract in free form or encapsulated in a nanoliposome significantly increased the expression of SOD and GPx and decreased the expression of COX2 and iNOS genes. It can be said that the regulation of inflammation and anti-oxidant genes is related to the antioxidant and anti-inflammatory activity of the phenolic compounds in the extract of *Artemisia aucheri,* including gallic acid, caffeic acid, 1 'syringic acid, cinnamic acid, catechin, ellagic acid, and crystal.[20] On the other hand, this was shown in the study of Mahbobeh Irani et al. that nanoemulsions from the plant *Artemisia Oucheri* can show antioxidant properties. The results of this study showed that the nanoemulsion synthesized from *Artemisia aucheri* ESO has a strong potential to inhibit DPPH and ABTS radicals.[21]

Conclusion

This phytochemical study evaluated the antioxidant properties of *Artemisia aucheri* floral essential oil using two established assays including the DPPH free radical scavenging and the β-carotene-linoleic acid bleaching methods, respectivly. GC-MS analysis identified camphene (31.05%), β-myrcene (26.18%), and camphor (15.41%) as the major constituents of the essential oil. The DPPH assay revealed that the essential oil exhibited superior free radical scavenging activity compared to other tested samples. In contrast, ascorbic acid showed the strongest antioxidant activity in the β-carotene-linoleic acid assay. The differences in antioxidant performance between assays are attributed to their distinct physicochemical properties. The hydrophilic nature of the DPPH assay favored higher radical scavenging by the essential oil (IC_{50} = 31.27 µg/ml) compared to ascorbic acid (IC_{50} = 37.54 µg/ml). Conversely, in the lipophilic β-carotene-linoleic acid assay, the essential oil showed slightly lower activity (IC_{50} = 51.73 µg/ml) than the positive control (IC_{50} = 51.57 µg/ml), consistent with polarity-dependent antioxidant behavior. Environmental factors such as climate, soil composition, and genetic variability influence the chemical profile and bioactivity of *Artemisia aucheri* essential oil. The identified bioactive compounds, particularly camphene, β-myrcene, and camphor, contribute significantly to its antioxidant potential. Further pharmacological and therapeutic investigations are recommended to explore the full potential of *Artemisia aucheri* essential oil and its constituents.

Authors' Contributions

All authors contributed equally to data collection and analysis. All authors read and approved the final version of the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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