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Essential oil Composition and Antioxidant Properties of Artemisia sieberi Besser in Two Enclosure and Grazed Sites at Three Phenological Stages

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ABSTRACT

Aims The use of plants for treatment and food returns to ancient times. This study was conducted to examine the essential oil composition and antioxidant activity of the essential oil of Artemisia sieberi Besser at 3 phenological stages and effect of grazing livestock on these properties.

Materials & Methods For this purpose, 5 shrubs from two sites (under grazing site and enclosure site) were selected randomly at 3 phenological stages. In laboratory operations, essential oil of the species was extracted in by SDE method.

Findings The results of GC/MS analysis indicated 45 compounds for grazed site with essential oil content 98.73%, and 42 compounds for enclosure site with essential oil content 98.54%. Also, α-tujune (21.63%), α-Pinene (19.53%), and Camphene (10.34%) were the main compounds in the enclosure site and α-tujune (23.70%), α-Pinene (20.33%), and Camphene (12.60%) were the main compounds in the grazed site. The results of screening antioxidant activity using two assays (DPPH and β-carotene-linoleic acid) showed that the free radical scavenging activity of essential oil (IC50 µg ml-1) in the first phenological stage (vegetative stage) was more than other phenological stages in two sites. Also, the inhibition of essential oil in the grazed sites had a significant difference with essential oil's inhibition in the enclosure site.

Conclusion Effect of grazing on the composition and properties of the essential oil of this species can be considered in the planning of livestock grazing management. We wish our future research on this plant leads to the finding of new natural antioxidant compounds.

Keywords Essential Oil; Phenological Stages; GC-MS; Antioxidant Activity; Artemisia

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Introduction

The use of natural plant compounds in recent years has attracted the attention of most people so that many researchers are also isolating these compounds from plants to eliminate microorganisms and pathogens [1].

Plant products are also known to possess potential for food preservation [2-5]. For example, the oxidation of lipids, which occurs during raw material storage, processing, heat treatment, and further storage of final products, is one of the basic processes causing rancidity of food products, leading to their deterioration. Changes in lifestyle and nutrition patterns have led oxidized lipids to have adverse effects on human organs [6, 7], and this has led to increased use of synthetic antioxidants, such as BHT and BHA considering the adverse effects of these substances on human health; this has raised concerns among people and government officials [8, 9]. With these interpretations, it is important to find and use natural compounds that have antioxidant effects.

Artemisia belongs to the family Asteraceae (tribe Anthemideae) and it is distributed in rangeland areas of Iran [10]. Artemisia species have also been studied all over the world and found to possess antibacterial, anti-tumor, anti-tuberculosis, antioxidant, anti-inflammatory, and anticholinesterase activities [11, 12].

Until the discovery of antibiotics, Artemisia was a frequent component of herbal tea mixtures, recommended to patients with tuberculosis to prevent sudation, and it was found to be an active ingredient in combined plant preparations for the treatment of chronic bronchitis. It has also been used as medication against perspiration, fever, rheumatism, and in treating mental and nervous conditions as well as an insecticidal [13, 14]. Several Artemisia species are also used against stomach ache, headache, wounds, and skin infections and the most common use of Artemisia species is against colds, also have been sold commercially, not only for use in therapy but also as a spice to flavor meats [15, 16]. Some of the phenolic compounds of plants belonging to this genus have also shown excellent antioxidant activity, as well as scavenging activity of active oxygen, as in superoxide anion radicals, hydroxyl radicals, and singlet oxygen [17], inhibiting lipid peroxidation [18].

Since some plant species are used by humans and livestock jointly, it is important to investigate the impact of the use of each group on this common food source. On the other hand, the impact of grazing on growth and plant compounds is important to decide on the starting livestock grazing and choosing the length of grazing period.

The present research reports (i) the chemical composition of the essential oil of *Artemisia sieberi* in 3 phenological stages at 2 sites (under grazing and enclosure sites), (ii) *in vitro* antioxidant of essential oil using 2 complementary assays methods namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, and β-carotene/linoleic acid bleaching assay.

Materials and Methods

This study was conducted in the step rangelands between the Kashan and Ghamsar cities of Isfahan province in 51° 10’ to 56° 28’ eastern longitudes and 33° 52’ to 33° 41’ northern latitudes with an area of 2500 hectares. The dominant species in this area is *Artemisia sieberi* and the average rainfall is 115 mm and has an irregular distribution. According to the Doumarten methodology, the climate of this region is semi-dry.

Plant material: The 5 shrubs from each phenological stage of each site (grazed and enclosure) were randomly selected at 3 phenological stages, the enclosure site is part of a military garrison that has not been grazed by livestock from 10 years ago. The aerial parts of *Artemisia sieberi* were collected at 3 phenological stages (vegetative, flowering, and seeding) from November 2016 to August 2017. An authenticated specimen of the plant was deposited in the herbarium of Natural Essential Oils Institute, University of Kashan, Iran.

Extraction and isolation of the essential oil: 100 g of aerial parts of plant samples that were dried in the shade at the room temperature was ground and their volatile fraction was prepared by a modified Likens-Nickerson’s simultaneous distillation and extraction (SDE) method [19], and microscale simultaneous distillation extraction apparatus (Ashke Shishe, Tehran, Iran) was used. Dried plant samples were homogenized with distilled water and the homogenates were subjected to SDE apparatus for 1 h, using pentane (chromatography grade reagent, Merck) as solvent. The yellowish oil was first dissolved in Diethyl ether (1 ml) in order to extract the

organic phase. Diethyl ether solution was, then, dehydrated by anhydrous sodium sulfate (Na_2SO_4). The solvent was evaporated at room temperature under vacuum and the remaining oil was maintained under atmospheric nitrogen at -5°C.

Gas Chromatography (GC) analysis: The essential oil obtained from aerial parts of A.siberi was analyzed, using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm film thickness; (Restek, Bellefonte, PA) equipped with an FID detector. Oven temperature was kept at 80°C for 5 min initially, and then raised at the rate of 3°C /min to 250°C. Injector and detector temperatures were set at 220°C and 290°C, respectively. Helium was used as carrier gas at a flow rate of 1 ml/min, and diluted samples (1/1000 in n-pentane, v/v) of 1.0 µl were injected manually in the splitless mode. Peaks area percents were used for obtaining quantitative data.

Gas Chromatography/Mass Spectrometry (GC/MS) analysis: GC/MS analysis of the essential oil was carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (Ionization energy: 70 eV) operating under the same conditions as described above. Retention indices were calculated for all components, using a homologous series of n-alkanes injected in conditions equal to samples ones [20].

Antioxidant activity

DPPH assay: The DPPH assay usually involves hydrogen atom transfer reaction, but, based on kinetic data, an electron transfer mechanism has also been suggested for this assay [21, 22]. Radical-scavenging activity (RSA) of the plant essential oil and extracts was determined, using a published DPPH radical scavenging activity assay method [23] with minor modifications. Briefly, stock solutions (10 mg ml^{-1} each) of the essential oil and the synthetic standard antioxidant BHT were prepared in methanol. Dilutions are made to obtain concentrations ranging from 1 to 5×10^{-10} mg ml^{-1}. Diluted solutions (1 ml each) were mixed with 1 ml of a freshly prepared 80 µg ml^{-1} DPPH methanol solution and allowed to stand for 30 min in the dark at room temperature for any reaction to take place. Ultraviolet (UV) absorbencies of these solutions were recorded on a spectrometer (Cintra 6, GBC, Australia) at 517 nm, using a blank containing the same concentration of oil or extracts or BHT without DPPH. Inhibition of free radical DPPH in I% was calculated as follows [8]:

Equation 1:

$$I\% = [(Ablank - Asample)/Ablank] \times 100$$

A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as Mean±SD of triplicates.

β-Carotene/linoleic acid bleaching assay: In this assay, antioxidant activity was determined by measuring the inhibition of volatile organic compounds and conjugated diene hydroperoxides arising from linoleic acid oxidation. The method described by Miraliakbari and Shahidi [24], was used with slight modifications. A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 ml chloroform, 25 µl of linoleic acid, and 200 mg Tween 40. The chloroform was evaporated under vacuum and 100 ml of oxygenated distilled water was, then, added to the residue. The samples (2 g l^{-1}) were dissolved in DMSO and 350 µl of each sample solution was added to 2.5 ml of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50°C for 2 h, together with 2 blanks, one containing the antioxidant BHT as a positive control and the other containing the same volume of DMSO instead of the extracts. The test tube with BHT maintained its yellow color during the incubation period. The absorbencies were measured at 470 nm on an ultraviolet spectrometer (Cintra 6, GBC, and Australia). Antioxidant activities (inhibition percentage, I %) of the samples were calculated, using the following equation [8]:

Equation 2:

$$I\% = (A - \beta carotene\ after\ 2\ h\ assay / Ainitial\beta - carotene) \times 100$$

$A_{\beta\text{-carotene after 2 h assay}}$ is the absorbance of β-carotene after 2 h assay remaining in the

samples and $A_{initial\ \beta\text{-carotene}}$ is the absorbance of β–carotene at the beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as Mean±SD of triplicates.

Findings

Since the *Artemisia sieberi* species is not usually fed to livestock in the first and second stages of phenology, and the aim of this study was to compare the enclosure and grazed sites, so the results of the third stage (seeding) are presented in Table 1; so, after the air-dried herbal parts of the plant were subjected to hydrodistillation, using a by modified Likens-Nickerson's simultaneous distillation and extraction (SDE) method and the yellow-colored oil was obtained in the yield of 0.59% (w/w) and 0.53% (w/w) for grazed and enclosure sites, respectively. The resulted showed that 45 compounds consisting up to 98.73 % of the essential oil were identified by GC and GC/MS analysis for grazed site, and 42 compounds consisting up to 98.54 % for enclosure site (Table 2).

The plant essential oil included α-tujune (21.63%), α-Pinene (19.53%), and Camphene (10.34%) in enclosure site and α-tujune (23.70%), α-Pinene (20.33%), and Camphene (12.60%) for in grazed site (Diagrams 1 and 2).

Table1) Chemical composition of *A. sieberi* essential oil in grazed site in seeding stage

Compound [a]	Composition (%)	RI [b]	RI [c]
Tricyclene	0.24	915	927
alpha-thujene	23.70	926	939
alpha-pinene	20.33	941	954
6-methyl-5-octene-2-one	0.85	971	979
Camphene	12.67	988	991
Sabinene	0.31	1014	1017
beta-pinene	0.87	1022	1025
Myrcene	2.66	1026	1029
alpha-phelandrene	3.02	1029	1031
alpha-terpinene	0.60	1047	1060
3-none-2-one	0.29	1073	1089
4-methy-4-vinylbutyrolactone	1.49	1091	1097
p-cymene	0.27	1128	1126
1,8cineol	0.55	1140	1139
lialic alchol	0.21	1164	1165
gama-terpinene	2.83	1168	1169
cis-sabinene hydrate	1.82	1180	1177
Verbenol	1.03	1194	1189
Linalool	18.68	1291	1289
trans-sabinene hydrate	1.03	1394	1381
beta-thujone	1.66	1407	1441
Cis-p-menth-2-en-1-ol	4.54	1424	1419
1-terpineol	1.46	1458	1455
Ipsdienol	0.33	1480	1500
Camphor	1.21	1555	1569
cis-limonene oxide	2.89	1577	1578
Nonanol	1.38	1583	1585
Borneol	0.96	1592	1593
Menthol	0.43	1629	1610
p-cymene-8-ol	2.98	1650	1615
terpin-4-ol	1.03	1670	1630
fenchyl alchol	1.66	1679	1645
p-menth-2-en-8-ol	4.54	1682	1658
Nordavanone	1.46	1696	1665
Citronellol	0.33	1710	1678
Neral	1.21	1723	1689
Carvone	2.89	1735	1578
4-tepinyl acetate	1.38	1742	1693
Pipertone	0.96	1755	1698
linalyl acetate	0.43	1763	1712
Geranial	0.96	1777	1725
Decanol	0.43	1785	1733
Thymol	2.98	1830	1823
Undecanal	1.03	1845	1835
Tricyclene	1.66	1868	1850
Total	98.73		

[a] Compounds listed in order of elution from HP-5MS column; [b] Relative retention indices to C_8-C_{24} *n*-alkanes on HP-5MS column; [c]Literature retention indices

Table 2) Chemical composition of *A. sieberi* essential oil in enclosure site in seeding stage

Compound [a]	Composition (%)	RI [b]	RI [c]
Tricyclene	0.24	915	927
alpha-thujene	21.63	926	939
alpha-pinene	19.53	941	954
6-methyl-5-octene-2-one	0.85	971	979
Camphene	10.34	988	991
Sabinene	0.31	1014	1017
beta-pinene	0.87	1022	1025
Myrcene	2.66	1026	1029
alpha-phelandrene	3.02	1029	1031
alpha-terpinene	0.60	1047	1060
3-none-2-one	0.29	1073	1089
4-methy-4-vinylbutyrolactone	1.49	1091	1097
p-cymene	0.27	1128	1126
1,8cineol	0.53	1140	1139
lialic alchol	0.21	1164	1165
gama-terpinene	2.83	1168	1169
cis-sabinene hydrate	1.82	1180	1177
Verbenol	1.03	1194	1189
Linalool	18.68	1291	1289
trans-sabinene hydrate	1.03	1394	1381
alpha-thujone	1.66	1407	1441
Cis-p-menth-2-en-1-ol	4.54	1424	1419
1-terpineol	1.46	1458	1455
Ipsdienol	0.33	1480	1500
Camphor	1.21	1555	1569
cis-limonene oxide	2.89	1577	1578
Nonanol	1.38	1583	1585
Borneol	0.96	1592	1593
Menthol	0.43	1629	1610
p-cymene-8-ol	2.98	2224	2218
terpin-4-ol	0.76	1630	1670
fenchyl alchol	0.79	1645	1679
p-menth-2-en-8-ol	0.87	1658	1682
Nordavanone	1.96	1665	1696
Citronellol	1.71	1678	1710
Neral	1.73	1689	1723
Carvone	1.35	1578	1735
4-tepinyl acetate	1.42	1693	1742
Pipertone	1.75	1698	1755
linalyl acetate	1.63	1712	1763
Geranial	0.77	1725	1777
Decanol	0.85	1733	1785
Total	98.54		

[a] Compounds listed in order of elution from HP-5MS column; [b] Relative retention indices to C_8-C_{24} *n*-alkanes on HP-5MS column; [c]Literature retention indices

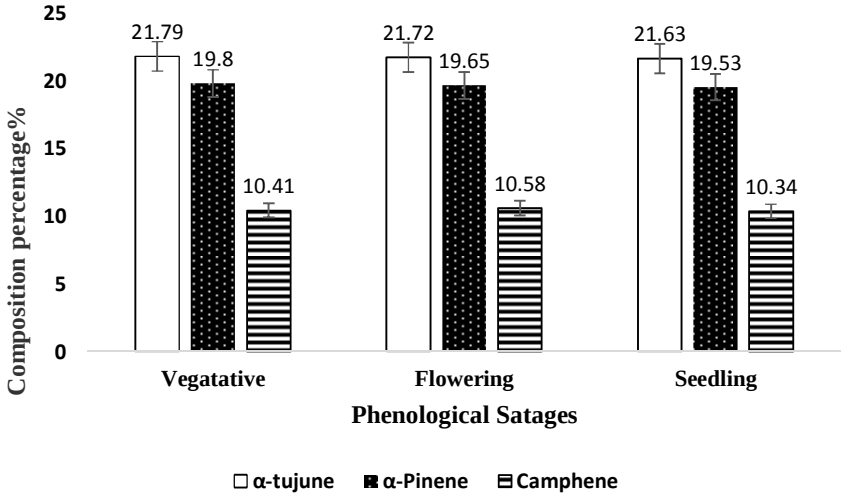

Diagram 1) Three major components of *Artemisia sieberi* essential oil in enclosure site at different phonological stages

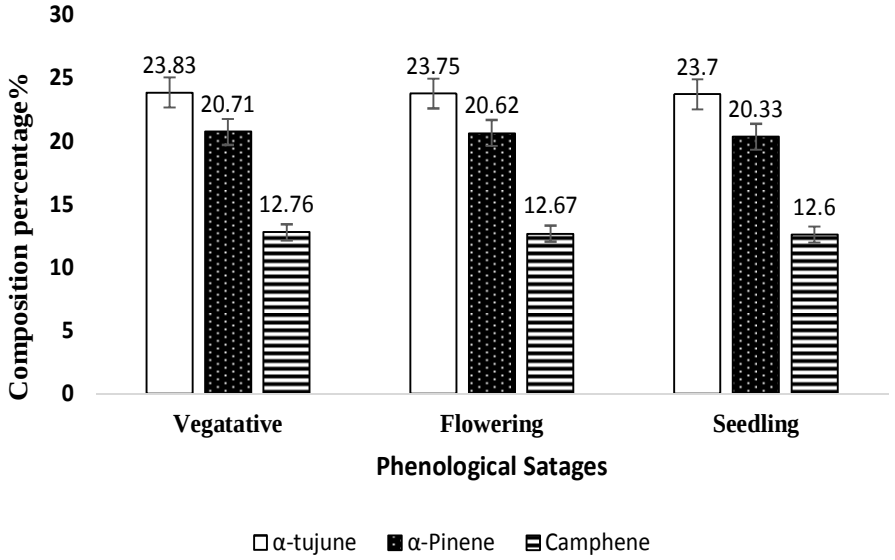

Diagram 2) Three major components of *Artemisia sieberi* essential oil in grazed site at different phonological stages

Antioxidant activity: The essential of A.sieberi was subjected to screening for their antioxidant activity, using 2 complementary test systems, namely DPPH free radical scavenging and β-carotene/linoleic acid systems. Compared to synthetic standard antioxidant BHT (IC_{50}=19.87 µg/ml), potential ability of the antioxidants to delay lipid peroxidation by reacting with chain propagating peroxyl radicals faster than the reaction of these radicals with proteins or fatty acid side chains is usually evaluated by β-carotene/linoleic acid test. Free radical scavenging capacities of the plant samples, measured by DPPH assay, since the reaction followed a concentration-dependent pattern, only concentrations of active samples providing 50% inhibition (IC_{50}). IC50 values of BHT are used as the positive control for Plant's essential oil. The percent inhibitions of linoleic acid oxidation of the essential oil and extracts of A.sieberi in 3 phenological stages at 2 sites are listed in Table 3.

Table 3) Average of (DPPH) scavenging and β-carotene/linoleic acid assays of A.sieberi essential oil in three phonological satages at 2 grazed and enclosure sites

Sample	Phonology	DPPH IC_{50} (µg ml^{-1})	β-carotene/linoleic acid Inhibition (%)
Grazed Site	3	248.04 ± 0.43	62.54 ± 0.032
	2	230.52 ± 0.4	56.86 ± 0.41
	1	225.86 ± 0.26	68.35 ± 0.35
Enclosure site	3	253.08 ± 0.21	54.76 ± 0.089
	2	246.12 ± 0.22	56.50 ± 0.45
	1	238.09 ± 0.76	59.32 ± 0.12
BHT		19.65 ± 0.82	87.35 ± 0.083

Discussion

To the best of our knowledge, there are many reports on the chemical composition of the essential oils isolated from the plants belonging to the genus Artemisia [25-27]. Most of these reports indicate that α-tujune and α-Pinene are the main and/or characteristic constituents of Artemisia essential oils, which are detected in our study. These results might have arisen from several differences in climatically, seasonal, geographical, and environmental factors as mentioned by Perry *et al*. [28].

According to a study carried out by Habibi *et al*., α-pinene, α-tujune, and camphene were the major constituents of Artemisia essential oil, which are natives of Iran, being consistent with the results of this research [29]. Also, Bagheri *et al*. studied the effect of different grazing intensities on the essential oils of *Artemisia sieberi*; their results showed that the different grazing intensities on essential oil composition in this species [30].

According to the results obtained by both tests (DPPH and β-carotene-linoleic acid), the antioxidant activity from A.sieberi essential oil in grazed site was more than in enclosure site (Table 3). Also, in both sites, antioxidant activity in the first phenological stage (vegetative) was more than other stages (flowering and seeding). This result was not reported before about this plant spices but many researchers conducted some research, which is close to the results of this research [31, 32].

Various results were obtained from antioxidant activity evaluation of other Artemisia species

essential oil such as of A. tridendata and A.potentillifolia in Turkey showed considerable DPPH radical scavenging activity with (IC50=69.44 µg ml^{-1}) that of BHT (IC50=80.50 µg ml^{-1}) in the DPPH system, and showed great lipid peroxidation inhibition (IC50=30.4 µg ml^{-1}) in the β-carotene-linoleic acid system [33].

Conclusion

The analysis of essential oils showed that the percentage of essential oil and number of essential oil compounds were different in 3 phenological stages. Also, the results indicated that the most of the essential oil composition in 3 phenological stages were common, but the changes in the essential oil ingredient content were obvious; these changes were clear in enclosure and grazed sites, too.

Today, a lot of different plant species are used as nutritional additives due to their antioxidant properties to improve the immunity against the diseases. In this regard, our study can be considered as the first report on the *in vitro* antioxidant activity of the essential oil of A.sieberi. The effect of grazing on the composition and properties of the essential oil of this species can be considered in the planning of livestock grazing management. Finally, according to the appreciable antioxidant activity of the essential oil in the β-carotene/linoleic acid test, we wish our future research on this plant lead to the finding of the new natural antioxidant compounds.

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