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RESEARCH ARTICLE

QUANTITATIVE AND QUALITATIVE COMPARISON OF ESSENTIAL OILS COMPOUNDS IN THREE *ARTEMISIA* SPECIES FOR CENTRAL RANGELAND OF IRAN

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ABSTRACT

One of the things that affect livestock behavior is herbal compounds, including essential oils, so the aim of this study is the comparison three Artemisia species in terms of essential oils composition coincident with the time of grazing the livestock from these species. For this purpose three Artemisia species including (*Artemisia sieberi* Bess*, Artemisia kermanensis Podl*, *Artemisia khorassanica* podl) From habitats with similar conditions were studied. In this study, 5 plant bases were selected randomly from each species and the essential oils were obtained by SDE of air-dried samples and were analyzed by GC-MS. The results showed that the about 88% of essential oil compounds were common in these three species and *Artemisia kermanensis Podl* had the more essential oil percentage (98.47%) and *Artemisia sieberi* had lowest essential oil percentage (73.21%) while the rate of grazing on the *Artemisia sieberi* Bess. was higher in the same vegetative stage. 6% of the compounds were observed only in the *Artemisia kermanensis* species, which is probably the presence of these compounds are the reason for the lack of feeding by the livestock of this species.

INTRODUCTION

One of the most important herbs in Iran is Artemisia which forms a large part of Iran's vegetation, as with the Astragalus forms more than 60% of Iran's natural vegetation. Artemisia L. is a genus of small herbs and shrubs found in northern temperate regions. It belongs to the important family Compositae (Asteraceae), one of the most numerous plant groupings, which comprises about 1,000 genera and over 20,000 species. Within this family, Artemisia is included in the tribe Anthemideae and comprises over 500 species, which are mainly found in Asia, Europe and North America (Zargari, 1998). A large number of members of the Anthemideae tribe are important as cut flowers and ornamental crops, as well as medical and aromatic plants, many of which produce essential oils used in folk and modern medicine, and in the cosmetics and pharmaceutical industry (Mozaffarian, 2008). The genus Artemisia L. (commonly known as wormwood), one of the largest and most widely distributed genus of the family Asteraceae, includes perennial, biennial, and annual herbs plus small shrubs (Weyerstahl *et al.,* 1993, Iranshahi *et al.,* 2007). The genus is of special interest because many Artemisia species have botanical and pharmaceutical properties, characterized scents and tastes due to the content of monoterpenes and sesquiterpenes. The plants have folk and conventional medicine applications (Kordali *et al.,* 2005a,b).

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The major classes of phytoconstituents of Artemisia species are terpenoids, flavonoids, coumarins, caffeoylquinic acids, and sterols. Bora and Sharma, (2011) making the genus an important source of biological compounds used in insecticides, antimalarials, cytotoxins, antihepatotoxic, fungicides, antibacterials, and allelochemicals (Rechinger, 1998, Bora and Sharma, 2013). A notably important drug found in this genus is artemisinin, the antimalarial drug isolated from A. annua (Allard, 1999). Other species of Artemisia have also been noted for their potential use at in-depth investigations on biological activities, especially those species that affect the central nervous and cardiovascular systems (Sun *et al.,* 2006). This genus comprises more than 400 species, and is predominantly distributed in the northern temperate region of the world in the 0–50 cm precipitation area. Thirty-four species have been reported in Iran and among which two are endemic: *A. melanolepis* Boiss. and *A. kermanensis* Podl (Omidbeigi, 2005; Sanadgol, 2002).

A. sieberi: In one experiment in the middle of Iran (Kashan city), the most frequent constituents were 1,8-cineole= 18.30% and camphor= 42.50%; in another evaluation done in the northwest of Iran (West Azerbaijan province) the most frequent constituents were camphor= 34.94% and betathujone= 35.66% (Sayyah, 2004; Teixeira, 2004; Adams, 2007).

A. kermanensis: In one evaluation on this species in the south of Iran (Kerman province), the most frequent constituents were: 1,8- cineole= 26.93% and camphor= 16.97% (Tan *et al*, 1998).

A. khorasanica: In some evaluations, all of which were done in the northeast of Iran, different constituents were found in this species; however among them were some shared components. Those components consist of: 1,8-cineole (17.75% in one experiment and 33.90% in another one), camphor (13.90% in one experiment and 12.60% in another one), alpha-thujone (43.40% in one and 11.90% in another one), beta-thujone (16.20% in one and 20.10% in another one) and davanone (12.20% in one and 36.40% in another one) (Uniyal *et al.,* 1985; Van *et al.,* 1963).

MATERIALS AND METHODS

Plant material: The plant materials obtained from three Artemisia species including (*Artemisia sieberi* Bess*, Artemisia kermanensis Podl*, *Artemisia khorassanica* podl) from natural habitats with similar climatologically conditions and altitude in Iran. These three Artemisia species taken from steppe rangelands of Kashan, kerman and southern Khorasan province respectively. In this study, 5 plant bases were selected randomly from each species in the end of the phenological stage (seedling). And dried at room temperature and shadow and prepared for essential oil operation.

Oil Isolation Procedure: The essential oil of air-dried samples (100g) of each species was isolated by hydrodistillation for 1 h, using a SDE(Simultaneous Distillation Extraction) The air-dried aerial parts of the plant were powdered and the volatile fraction was prepared by a modified Likens-Nickerson's simultaneous distillation and extraction (SDE) method (31, 32). SDE device conducted extraction of essential oils with an organic solvent such as pentane. A microscale simultaneous distillation extraction apparatus (Ashke Shishe, Tehran, Iran) was used. Dried powdered plant was homogenized with distilled water and the homogenate subjected to SDE apparatus for 1 h using pentane (chromatography grade reagent, Merck) as solvent and then extract was concentrated with nitrogen.

GC analysis: GC analysis was performed by using a Thermoquest gas chromatograph with a flame ionization detector (FID). The analysis was carried out using fused silica capillary Innowax column (60 m × 0.25 mm i.d.; film thickness 0.25 µm). The operating conditions were as follows: injector and detector temperatures were 230˚C and 250˚C, respectively. Nitrogen was used as carrier gas at a flow rate of 4 ml/min; oven temperature programme, 80˚C – 230˚C at the rate of 3˚C/min, and finally held isothermally for 10 min. GC-MS analysis GC-MS analysis was performed by using Thermoquest-Finnigan gas chromatograph equipped with an above mentioned column and coupled to a TRACE mass quadrupole detector. Helium was used as carrier gas with the ionization voltage of 70 ev. Ion source and interface temperatures were 200˚C and 250˚C, respectively. Mass range was from m/z 43-456. Gas chromatographic conditions were as given for GC.

Identification of Compounds: The constituents of the essential oil were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C5–C10) and the oil on an Innowax column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Vajs *et al.,* 2004). For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Antioxidant activity

DPPH assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay usually involves hydrogen atom transfer reaction but, based on kinetic data, an electron transfer mechanism has also been suggested for this assay. Radical-scavenging activity (RSA) of the plant essential oil and extracts was determined using a published DPPH radical scavenging activity assay method (Sarker *et al.,* 2006), 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 percent (I%) was calculated as follow equation 1.

$$\text{Equation 1:}\quad I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

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 means ± 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 (2008), 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 two blanks, one contained the antioxidant BHT as a positive control and the other contained 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, Australia). Antioxidant activities (inhibition percentage, I %) of the samples were calculated using the following equation:

$$\text{Equation 2:}\quad I\% = (A_{\beta\text{-carotene after 2 h assay}}/A_{\text{initial }\beta\text{-carotene}}) \times 100$$

$A_{\beta\text{-carotene after 2 h assay}}$ is the absorbance of *β*-carotene after 2 h assay remaining in the samples and $A_{\text{initial }\beta\text{-carotene}}$ 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 means ± SD of triplicates.

RESULTS

Essential oil percentage: The analysis of essential oils showed that the percentage of essential oil and number of essential oil compounds were different in these three Artemisia species (Table 1), also the results of this research indicated that the almost 88% of the essential oil composition of these three species was common, The 5 of compounds were observed only in the *Artemisia khorassanica* podl species essential oil, while 3 of composition was observed only in the *Artemisia kermanensis* Podl species essential oil (Figure1), (Table2), In the other hand, the tendency of livestock to feed on these three species showed that usually, the livestock feed *Artemisia sieberi* species more than two other species and does n't show much interest for grazing from the *Artemisia kermanensis* Podl species, of course, when the livestock have a normal physiological and metabolic conditions and have n't been hungry for a long time (Tzakou *et al.,* 2001, Verdian-rizi 2009).

It seems the selection of livestock from these three species to be highly relevant to their essential oil compounds, Although the amount of these compounds has decreased in the third phonological stage, but preventing livestock feeding on these species in the first and second stage of vegetation. The main components of the three A. species essential oil were alpha-terpinen, alpha-thujene, and camphen (Figure2). The aerial parts of (*Artemisia sieberi* Bess*, Artemisia kermanensis Podl*, *Artemisia khorassanica* podl) essential oil were found to be rich in regards to oxygenated monoterpenes (68.2%), (75.5%) and (80.3%) (Kordali *et al.,* $2005_{a,b}$). Of course, the percentage of compounds, -8.1 Cineol, Camphon, Chrysanthenone, Alpha-Painan, Sabine, Para-cymene, cis-chrysanthenone, 4-terpinene, hadn't significant differences In the three A. species *essential* oil (Sayyah *et al.,* 2004).

Antioxidant activity: The essential of Artemisia species were subjected to screening for their antioxidant activity, using two complementary test systems, namely DPPH free radical scavenging and *β*-carotene/linoleic acid systems. Compared to synthetic standard antioxidant BHT (IC_{50} = 19.55 µg/ml), potential ability of the antioxidants to delay lipid peroxidation by reacting with chain propagating peroxyl radicals faster than

Table 1. Percentage of essential oil compositions of *three Artemisia species*

N	Compound	RI	*A. khorassanica*	*A. kermansis*	*A. sieberi*
1	7-methyl-1-octene	860	2.8	1.9	3.5
2	2,5-divinyl-2-methyl-tetrahydrofuran	909	6.9	6.3	5.6
3	4,4-dimethyl but-2-enolide	919	1.9	1.2	2.3
4	Tricyclene	925	1.8	1.9	2.5
5	alpha-thujene	929	10.3	7.3	6.2
6	alpha-pinene	937	5.8	5.4	3.0
7	6-methyl-5-octene-2-one	940	3.1	3.0	0.2
8	Camphene	951	16.9	14.3	11.2
9	Sabinene	969	1.3	1.1	4.9
10	beta-pinene	976	5.3	4.9	2.4
11	Myrcene	980	2.8	2.4	1.9
12	alpha-phelandrene	999	6.9	5.7	11.6
13	alpha-terpinene	1011	11.6	8.6	6.2
14	3-none-2-one	1000	11.6	9.8	6.7
15	4-methy-4-vinylbutyrolactone	1004	2.8	2.5	1.4
16	p-cymene	1014	6.9	-	-
17	1,8cineol	1023	1.9	1.4	9.3
18	lialic alchol	1028	11.6	8.5	5.6
19	gama-terpinene	1049	-	9.3	-
20	cis-sabinene hydrate	1055	-	5.6	-
21	Verbenol	1076	3.1	2.7	2.5
22	Linalool	1081	2.5	-	-
23	trans-sabinene hydrate	1085	3.1	2.7	0.2
24	alpha-thujone	1100	5.5	5.5	17.7
25	Cis-p-menth-2-en-1-ol	1108	26.6	25.4	25.8
26	1-terpineol	1109	-	3.1	-
27	Ipsdienol	1125	2.1	5.5	5.5
28	Camphor	1126	5.3	28	28
31	Borneol	1152	2.8	-	-
32	Menthol	1156	6.9	2.1	2.1
33	p-cymene-8-ol	1158	1.9	3.5	3.5
34	terpin-4-ol	1161	11.6	9.8	9.8
35	fenchyl alchol	1169	3.1	4.6	4.6
36	p-menth-2-en-8-ol	1171	5.5	5.8	5.8
37	Nordavanone	1199	2.2	2.3	1.7
38	Citronellol	1201	4.3	-	-
39	Neral	1210	4.9	5.8	5.8
40	Carvone	1214	-	3.1	3.1
41	4-tepinyl acetate	1216	2.1	2.9	2.9
42	Pipertone	1228	5.3	4.7	4.7
43	linalyl acetate	1229	3.1	3.9	3.9
44	Geranial	1237	5.5	5.6	5.6
45	Decanol	1245	6.8	-	-
	Others	-	7.8	6.9	5.9
	Total	-	98.47	85.34	73.21

Table 2. The essential oil compositions that only observed in *A. khorassanica* and *A. kermanesis* species

Compound%	*A. sieberi*	*A. kermanesis*	*A. khorassanica*
6.9	*NA*	NA	p-cymene
2.5	*NA*	NA	Linalool
4.3	*NA*	NA	Citronellol
6.8	*NA*	NA	Decanol
2.8	*NA*	NA	Borneol
9.3	*NA*	gama-terpinene	NA
5.6	*NA*	cis-sabinene hydrate	NA
3.1	*NA*	1-terpineol	NA

Table 3. Antioxidant Activity of three spicies of Artemisa by (DPPH) scavenging and *β*-carotene/linoleic acid assays

Sample	Species	DPPH IC_{50} (µg/ml)	*β*-carotene/linoleic acid Inhibition (%)
Essential oil	A.si	244.04 ± 0.43	65.54 ± 0.032
	A.kh	232.52 ± 0.4	57.86 ± 0.41
	A.ke	228.86 ± 0.26	69.35 ± 0.35
BHT		19.55 ± 0.82	89.35 ± 0.083

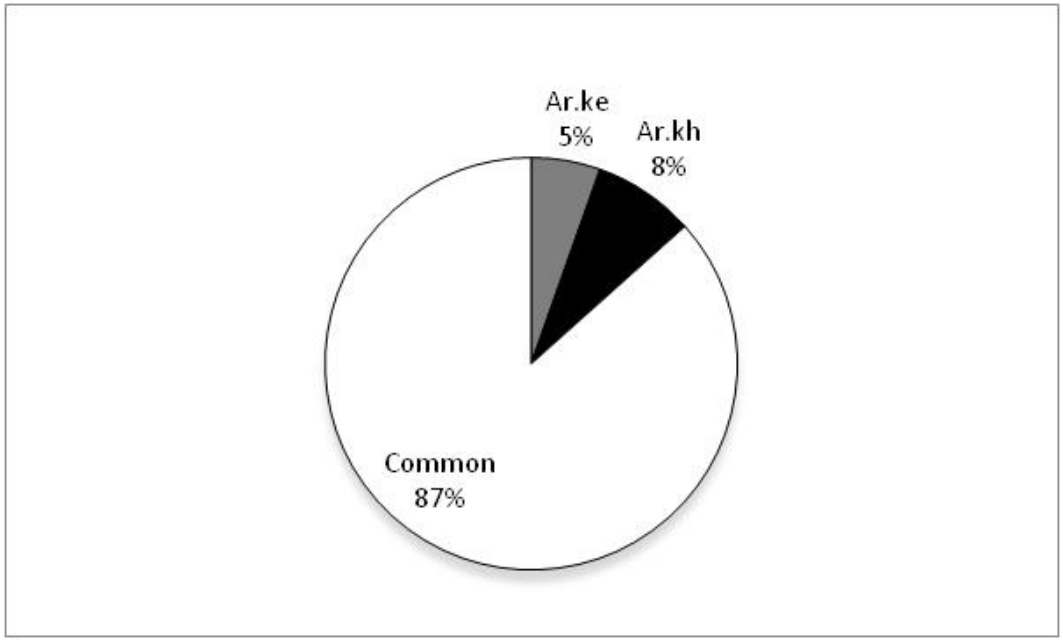

Figure 1. Distribution essential oil composition in three A. species

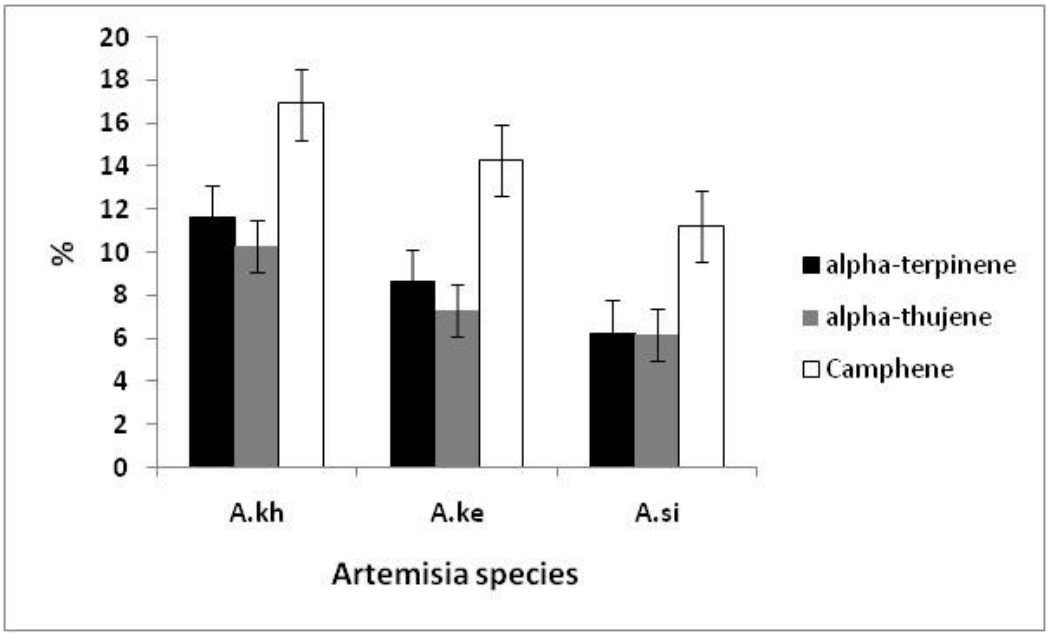

Figure 2.The percentage of main essential oil components in three A. species

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}). Plantl's essential oil, and positive control (BHT) IC_{50} values .The percent inhibitions of linoleic acid oxidation of the essential oil of three spicies of Artemisa listed in Table 3.

DISCUSSION

The percentage and retention indices of the essential oil components obtained from aerial parts of A. species are listed in (Table 1). As shown in Table 1, analysis of the aerial parts oil of A. species resulted in the identification of 45 constituents, representing 73.21%, 85.34% and 98.47% of the (*Artemisia sieberi* Bess*, Artemisia kermanensis Podl*, *Artemisia khorassanica* podl) essential oil (Taga *et al.,* 1984, Omidbeigi 2005, Sanadgol 2002). Livestock grazing planning based on essential oil combinations can be a new horizontal in rangeland management. Although the amount of essential oil compounds in the third phonological stage has in the three species of Artemisia decreased, but the judging about of grazing livestock from these plant species needs further research, in order to know exactly which compounds causes the livestock did n't eat these species. Perhaps biochemical defense in the plants with essential oil is a way to combat animal grazing. According of the results obtained by both tests ((DPPH and β-carotene-linoleic acid), the antioxidant activity from *A.sieberi* essential oil was more than two other Artemisia species. (Table 3) This result not repotted before about this plant spices but many researcher conducted some research which is close to the results of this research (Sachetti *et al.,* 2005), (Solgi *et al.,* 2017).

Conclusion

Various results were obtained from antioxidant activity evaluation of other *Artemisia* species essential oil such as of *A. tridendata* and *A.potentillifolia* in Turkey were 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 (Kivrak *et al.,* 2009). Given that 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 various Artemisia essential oil in same habitat and environmental conditions.

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