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Comparative Study of Antioxidant Activity of Different Extracts and Essential Oil Compositions Obtained from *Artemisia sieberi* Besser using Two Different Methods

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ABSTARACT

This study deals with essential oils of the aerial parts of *Artemisia sieberi* Besser which was obtained by hydrodistillation (HD) and simultaneous water steam distillation-organic solvent extraction (SDE) methods and compared regard to their components. The major compounds of the oil obtained by hydrodistillation were verbenol (16.09 %) and myristicin (13.76%). However, by using SDE, E-epoxy ocimen (14.02 %) and verbenol (13.93%) were detected as the major components. SDE obtained essential oil was very higher than that of hydrodistillation method based on the yield. Antioxidant activities of various extracts from *A.sieberi* were also evaluated via DPPH radical scavenging assay; total phenolic compounds were also measured using Folin-ciocalteu reagent. The results exhibited that, water extract of *A.sieberi* has considerable antioxidant activity and it seems that this activity drops off sharply with reducing polarity of extraction solvent.

Key words: *Artemisia sieberi*; essential oil; antioxidant; DPPH; total phenolic compounds.

INTRODUCTION

Artemisia sieberi Besser, belongs to the Asteraceae family, is a widely distributed plant in Iran[1-2]. In Iranian folk medicine, *Artemisia sieberi* is introduced to be useful for spasmolytic effects[3-4], vermicide and also for strengthening stamina and relieving cold symptoms. *A. sieberi* oil like some other species of *Artemisia* has potential to be used as an insecticide[5]. Animal dermatophytosis and clinical improvement with 3% solution of *A. sieberi* oil have been observed which is in comparison with clotrimazole[6]. Antidiabetic, antimalarial and antimicrobial effects of this plant have been reported previously[7-11].

As mentioned above, because of its medicinal properties, this plant is undoubtly a good candidate for evaluating phytochemicals or biological activities.

A couple of studies in the literature have reported composition of the essential oil of *Artemisia sieberi*, extracted by hydrodistillation method and

also extraction using supercritical carbon dioxide from different locations of Iran[12-17]. It is clear that production of secondary metabolites in plants is firmly depended on ecological conditions[18] and, in addition, yield of oil extraction and the chemical composition of the obtained essential oil may change as the method of extraction changes. According to the above descriptions and lack of enough reports about phytochemical and biological properties of this plant in the literature, this research focuses on comparative study on the chemical constituents of volatile oil obtained by SDE and general hydrodistillation extraction methods from *A. siberi* growing wild in Kashan, central Iran. Antioxidant activities of water, ethanol and chloroform extracts of this plant have also been assessed.

EXPERIMENTAL

Plant materials

The plants were collected from Karkas Mountains of Kashan, Iran, at an altitude about 1800 m in April 2010. The voucher specimens of the plant were confirmed in the Herbarium of Research Center of Barij Essence Pharmaceutical Company of Kashan (code: 1_108).

Essential oil isolation

The aerial parts of examined plants were dried in shadow at room temperature. Hydro-distillation and simultaneous water steam distillation-organic solvent extraction (SDE) methods[17] were used in this study. The extracted oils were then dehydrated by anhydrous sodium sulphate, to yield 0.3% and 1.13% of brown-red oil respectively.

GC-Mass analysis

The isolated Essential oils were analyzed using GC/FID for quantitative analysis and GC/MS to qualitative analysis on a HEWLETT-PACKARD 6890 gas chromatograph coupled with a mass detector (HEWLETT-PACKARD model 6973 HP). HP-Innowax capillary column (60 m × 0.32 mm, 0.50 mm film thickness) was used as the oil analyser. The mass spectra were obtained by electron ionization at 70 eV. The oven temperature was programmed as follows: 80°C (3 min), 80-230°C (3°C /min), 230°C (5 min), 230-250°C (3°C /min) and 250 °C (10 min).

The injection temperature was 250 °C. The carrier gas (helium) flow rate was 1 ml/min. The sample (1 µl) was injected with a split ratio of 1/90. Retention indices were calculated for all components using a homologous series of n-alkanes injected in conditions equal to those of the samples. Identification of components of Eos was based on retention indices (RI) relative to n-alkanes and computer matching with the Wiley 275.L and Wiley7n.L libraries. Also, comparisons of the fragmentation pattern of the mass spectra were made with data published in the literature[18].

Preparation of extracts

Thirty grams of the powdered aerial parts of the plant were soxhlet-extracted with 300 ml solvent. Solvent removal using rotary evaporation and drying the residue using a vacuum oven at 50 °C yielded 14.0% w/w of water, 14.0% w/w of ethanol and 9.5% w/w of chloroform extracts with the colours brown, green and light green respectively. Extracts were kept in dark at 4 °C until be tested.

Antioxidant activity

For evaluating antioxidant activities of samples, DPPH bleaching and total phenol measuring assays were used.

DPPH bleaching assay

In this assay the antioxidant activity was determined by measuring DPPH free radical scavenging ability.

To plot BHT standard curve, absorbance of DPPH methanol solution in the presence of various concentrations of BHT after 40 min in 517 nm was measured and inhibition of DPPH free radical in percent (I %) was calculated as follows:

$$I\ \% = (Ablank-Asample/Ablank) \times 100 \quad ...(a)$$

Where Ablank is the absorbance of the control reaction, containing all reagents except the test compound. Inhibition percentage of the samples (1 mg/ml of water, ethanol and chloroform extracts) and BHT standard were calculated similarly and all tests were carried out in triplicate to improve accuracy. Ability of DPPH radical scavenging was calculated as %I.

Total phenol measuring

Total phenolic constituents of the aforesaid extracts of *A. sieberi* were determined based on the absorbance values of the extract reacted with Folin-Ciocalteu reagent and compared the results with gallic acid as standard solution[19] with slight modifications. This assay was carried out in order to obtain the total phenolic contents of the extracts according to the µg gallic acid equivalent (GAE) per 1mg of extract. For this purpose, a solution containing 1 mg crude extract in ethanol was exposed to 1 ml of Folin-Ciocalteu reagent in the presence of 3 ml of a 2% Na_2CO_3 in a 50 ml volumetric balloon. The mixture was allowed to stand for 2 h with intermittent shaking (roughly every 30 minutes). Absorbance was measured at 760 nm. The same procedure was repeated for different concentrations of standard gallic acid solutions (0–1000 mg per 0.1 ml). The equation for the standard curve obtained was found to be like eq. (1).

$$\text{Absorbance} = 0.0012 \times \text{Gallic acid (µg)} + 0.0033 \quad ...(1)$$

Gallic acid equivalent of the samples was calculated as µg of gallic acid per mg of sample.

RESULTS AND DISCUSSION

The yield of hydrodistillated essential oil (HDEO) was 0.3% for *Artemisia sieberi* Besser from Kashan in comparison with 1.13% which obtained from SDE prepared essential oil (SDEO) which is almost 4 times more than that of HDEO.

Table 1: essential oils compositions of *Artemisia sieberi* Besser aerial parts from Kashan, Iran

No.	Compound Name	RI[c]	Comp[a].(%)	Comp[b].(%)
1.	Verbenene	1151	-	1.38
2.	1,8 Cineole	1235	-	0.14
3.	Yomogi alcohol	1397	5.21	6.68
4.	Artemiseole	1413	5391	10.41
5.	β-Thujone	1465	-	4.76
6.	α-Thujone	1477	4.02	7.071
7.	Artemisia alcohol	1514	2.01	3.05
8.	Camphor	1549	1.09	9.03
9.	trans-Chrysanthenyl acetate	1602	-	1.12
10.	Bornyl acetate	1603	2.24	1.26
11.	cis-Verbenol	1673	16.09	14.15
12.	α-Phellandren-8-ol	1679	7.44	4.98
13.	Lavandulol	1688	0.78	-
14.	Borneol	1721	6.61	2.11
15.	cis-Chrysanthenol	1765	1.83	-
16.	E-Epoxy ocimen	1847	9.77	14.24
17.	p- Cymen-8-ol	1865	0.92	-
18.	CisCarvone Oxide	1919	1.65	-
19.	2E-Hexenyl butanoate	1936	-	2.66
20.	Caryophyllene oxide	2016	1.17	-
21.	2-Pentadecanone, 6,10,14-trimethyl-	2144	1.35	-
22.	Spathulenol	2153	1.51	0.66
23.	Eugenol	2196	2.05	2.18
24.	Thymol	2207	1.86	0.63
25.	6-Methoxy-elemicin	2231	1.51	0.56
26.	Carvacrol	2238	1.72	0.46
27.	Elemicin	2252	1.56	0.71
28.	Myristicin	2292	13.76	3.78
29.	Apiole	2518	5.00	1.37

Table 2: Extraction yields and antioxidant activity of extracts of *Artemisia siberi* from Kashan, Iran

Sample	Yield of extaction	DPPH %I	Totalphenolics: Gallicacid eq(µg/mg sample)
Water extract	14.0%	69.00 ± 0.40	66.14 ± 0.91
Ethanol extract	14.0%	55.73 ± 1.00	59.61± 0.54
Choloroform extract	9.5%	13.47 ± 0.92	36.30 ± 0.39
BHT	-	87.10 ± 0.82	-

Compositions of the essential oils were evaluated using GC-MS analysis. The major components in the HDEO were found to be verbenol (16.09 %), myristicin (13.76%,), E-epoxy ocimen (9.77%) and α-phellandren-8-ol (7.44%). These are very different from the other reported components detected in *A. siberi* of other locations (10,11,13) these components are also , very different from those of SDEO with E-epoxy ocimen (14.02 %), verbenol (13.93%), artemiseole (10.25%) and camphor (8.89%) as the majors. As can be seen, although total number of components detected in both mentioned essential oils were almost equal, their types and percentages were very different (Table 1); for example some compounds such as lavandulol (0.78%), cis chrysanthenol (1.83%), p- cymen-8-ol (0.92%), cis carvone oxide (1.65%) caryophyllene oxide (1.17%) and 6,10,14-trimethyl 2-pentadecanone, (1.35%) detected in HDEO, were not seen in SDEO. On the other hand, verbenene (1.36%), 1, 8 cineole (0.14%), β-thujone (4.69%), trans-chrysanthenyl acetate (1.10%) and 2E-hexenyl butanoate (2.62%) which were found in SDEO, were not seen in HDEO.

Compounds such as myristicin and verbenol observed in the mentioned plant have been reported to be the most important contributors in insecticiding effects of the essential oil of *Artemisia* species.

Comparing these results, it can be revealed that method of essential oil isolation has a significant role on extraction yield, type and amount of extracted compounds.

Antioxidant activities, based on DPPH free-radical assay, for water, ethanol and chloroform extracts (Table 2) exhibit higher activity of water extract in the order of water> ethanol > chloroform. Total phenolic contents of these extracts also obey this order, but the differences are not as significant as antioxidant activities. Considering these findings and the yields of extraction, it seems that water as a safe and eco-friendly solvent, which is used locally in domestic consumptions, is the best solvent for extracting bioactive materials as far as antioxidant activity is concerned.

CONCLUSION

According to these results it needs to point out that the extraction solvent and method has generally a significant influence on properties and amount of active components of the plant extracts. In addition, as it is observed in this research, the yield of essential oil extraction for SDE method is much higher than general hydrodistillation technique; therefore, it can be concluded that the use of this especial techniques can be useful particularly for plants with low-content essential oil, such as *Artemisia* species.

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