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Comparative Survey on the Essential Oil Composition and Antioxidant Activity of Aqueous Extracts From Flower and Stem of Achillea Wilhelmsii From Taftan (Southeast of Iran)

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A B S T R A C T

Background: Achillea wilhelmsii (Asteraceae) is a Permanent herb, belongs to the genus Achillea (Compositae) Which grows wild in some regions of Iran including Taftan area of Sistan and Baluchestan, in southeast of Iran.
Objectives: The purpose of this study was to determine the comparative chemical composition and antioxidant activities of the essential oils extracted from the flowers, stem and leaves of Achillea wilhelmsii.
Material and Methods: In this study, the chemical composition of essential oils of the flower and stem of Achillea wilhelmsii from Taftan were obtained by the hydrodistillation method and analyzed by gas chromatography and gas chromatography mass spectroscopy.
Results: Sixty one compounds were identified in the essential oil of the flower. The major compounds were Camphor (27.99%), Sabinyl acetate (6.56%), Terpinene-4-ol (6.43%) Camphene (6.43%) and Alpha-Pinene (5.47%). Forty eight compounds were identified in the essential oil of the stem and leaves, which the major compounds were Camphor (34.49%), Alpha-Pinene (8.16%), Camphene (7.87%), Terpinene-4-ol (5.70%), 1,8-Cineole (3.32%). In addition, the antioxidant activity of ethanolic extract was evaluated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method.
Conclusions: According to antioxidant activity outcomes, the amount of IC50s of aqueous extracts of flower and stem and also butylated hydroxyl toluene (BHT) as standard were 232.34, 63.25 and 45.59 ppm respectively. Ultimately, it was highlighted that antioxidant activity of aqueous extract of flower was weaker than stem.

Keywords: Achillea wilhelmsii; Essential Oil; Camphor; Antioxidant Activity

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▸Implication for health policy/practice/research/medical education:
The extract of leaves and stem may be useful in the treatment of human diseases in which free radical production play a major role. In addition this extract could be used as a preventive factor on undesirable changes in the flavor and nutritional quality of food. The variety of essential oil can be used in healthy implications such as antioxidant, pharmaceutical and cosmetic purposes and perfume industries and it is a source of high percentage of camphor.

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1. Background

Achillea wilhelmsii (Asteraceae) is a permanent herb, belonging to the genus *Achillea* (Compositae) Which grows wild in some regions of Iran including Taftan area of Sistan and Baluchestan in southeast of Iran. The *Achillea* which belongs to the family Compositae (Asteraceae), comprises more than 120 species. These plants are native to Europe and Western Asia, although they are grown in Australia, New Zealand, and North America. Nineteen species of the genus *Achillea* (Compositae) are represented in the flora Iranica which *Achillea wilhelmsii* grows wild around farms and roadsides in many parts of Iran (1, 2). Berenjask is the local name for *Achillea wilhelmsii* around the Taftan semi active volcano in southeast of Iran. The plant has several medicinal usages in these areas. Local people used the plant for treatment of abdominal pain, stomachache, infant poisoning and wound healing. There are several reports regarding the pharmacological properties of this genus, such as antioxidant (3, 4, 5, 6), antiacid (7), antispasmodic (8), antihyperlipidemia (9), antihypertensive (10) and antitumoral activities (11). Cosmetic and hygiene industries have also used it to elicit tenderness and softness of the skin and to treatment skins inflammations with cream formulations (12). *Achillea* is rich in flavonoids and sesquiterpene lactones, and monoterpenoids which have antioxidant activities (13,14). The largest single component of volatile oil extracted from *Achillea millefoium* is chamazulene which has been shown to have anti-inflammatory and antiallergic effects (15, 6). Immunosuppressive effects on humoral immune responses have been reported with an aqueous extract of *Achillea talagonica* (16).

To date *Achillea wilhelmsii* has been analyzed from different parts of Iran such as Fars (17), Mazandran (18) and Kerman (19) for both the composition of essential oil and different pharmacological properties. But there are no reports of such an analysis on *Achillea wilhelmsii* from Taftan area in Sistan and Baluchestan, which is an important geographical zone for medicinal plants.

2. Objectives

The purpose of this study was to determine the comparative chemical compositions and antioxidant activities of the essential oils extracted from the flowers, stem and leaves of *Achillea wilhelmsii*.

3. Material and Methods

3.1. Plant Materials

A wilhelmsii was collected in June, 2010 from Taftan area of Sistan and Baluchestan province in Iran during the flowering stage. Taxonomic determination of the plant was confirmed by the Department of Botany of Shahid Beheshti University of Medical Sciences. Collected plant materials were dried in the shade and the flowers and aerial parts were separated from the roots. The voucher specimen has been deposited at the herbarium of the Department of Biology of the University of Sistan and Baluchestan.

3.2. Isolation of the Essential Oil

The flower and stem were dried and milled into a fine powder and 40 g was subjected for 2 h of hydrodistillation using a Clevenger-type apparatus. The obtained essential oil was collected, and dried over anhydrous sodium sulphate and kept at 4°C until analysis.

3.3. GC-MS Analysis

Analyses of the essential oil was performed using a varian gas chromatography 3600 with DB5 (methyl phenyl siloxane, 30mm X 0.25mm i.d.); the carrier gas was helium; split ratio 1:15 and flame ionization detector. Temperature program was from 60°C (2min) to 240°C at 5°C/min, injector temperature of 250°C and detector temperature of 260°C. GC-MS was used on a cross-linked 5% methyl phenylsiloxane (HP-5, 30m X 0.25mm id, 0.25μm film thickness. Carrier gas was helium, split ratio 1:15 with quadrupole mass spectrometer operating at 70ev ionization energy. The retention indices for every component were calculated by using retention time of n-alkenes (C8-C25) which were injected after the essential oil under the same condition. The components were identified by comparing retention indices (RRI, DB-5) with those of standards. The results were also confirmed by comparing their mass spectra with the published mass spectra or Wiley library.

3.4. Preparation of Aqueous Extract

Forty gram of flower and stem of *A. wilhelmsii* was finely ground using a homogenizer and was extracted with distilled water at room temperature for 24 hours. This mixture was then filtered using Whatman No.42 filter paper to remove debris and a volatile extract was then evaporated at 40°C using a rotary evaporator.

3.5 Determination of AntioxidantAactivity by DPPH Method

The DPPH radical scavenging activity of the extracts from A. wilhelmsii flowers, stem and leaves were measured according to the procedure described by Brand-Williams (20). Briefly, 1 ml samples of various concentrations of the extracts in ethanol were separately added to a 1 ml solution of DPPH radical in ethanol (final concentration of DPPH was 0.1 mM). The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 20 minutes. Then, the absorbance of the resulting solution was measured at 517 nm using a UV-Vis, Specords 100 spectrophotometer. Inhibition of free radical DPPH was calculated as percentage [IP (%)] as follows:

IP (%) = 100 × (A blank - A sample) / A blank

Where a blank is the absorbance of the control (containing all reagents except the test compound) and a sample is the absorbance of the test compound. The antioxidant activity of the extracts was expressed as the IC50. IC50 values (μg/ml) denote the concentration of sample, which is required

to scavenge 50% of DPPH free radicals. This was obtained by interpolation and using linear regression analysis. For the calculation of these values, Microsoft Excel software was used. Percent inhibition after 20 minutes was plotted against concentration, and the equation for the line was used to obtain the IC50 value. A lower IC50 value indicates greater antioxidant activity. BHT was used as positive control and the sample solution without DPPH was used as blank.

4. Results

4.1. Chemical Composition of the Essential Oil

The yield of volatile oils of *Achillea wilhelmsii* obtained by hydrodistillation of the finely powdered flower and stem were 0.5 and 0.2 % (v/w) respectively. The oils were light yellow and with a perfumery odor. The chemical composition of the oils is presented in *Table 1*. Determination of individual components was based on comparing their relative retention times with those of authentic samples on HP-5MS capillary column, and their mass spectra of peaks to be matched with those obtained from authentic samples and/ or the Wiley NIST 7 library spectra and existing data (21). The GC/MS chromatogram of the oil of flower revealed the presence of monoterpenes (22.84 %), oxygenated monoterpenes (55.31%), sesquiterpenes (0.68%), oxygenated sesquiterpenes (5.81%) and (8.24%) as other compounds and for stem revealed the presence of monoterpenes (28.82 %), oxygenated monoterpenes (58.17%), sesquiterpenes (0.40%), oxygenated sesquiterpenes (5.97%) and 3.62%) as other compounds.

4.2. Antioxidant Activity

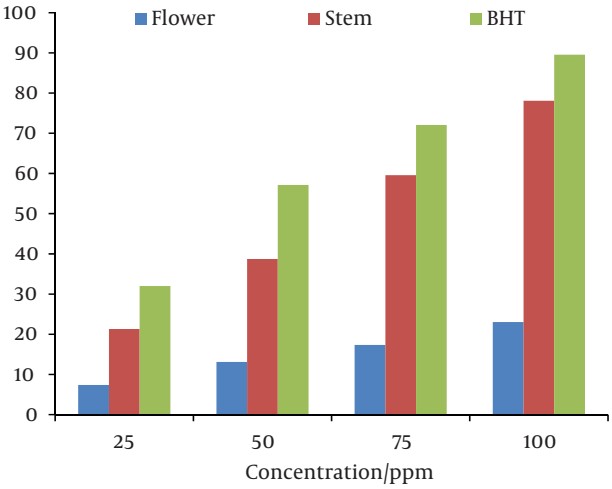
The scavenging effect of aqueous extract of *Achillea wilhelmsii* under investigation on DPPH radicals are shown in *Figure 1*. The reduction ability of DPPH radicals formation was determined by the decrease in its absorbance at 517 nm induced by antioxidants. Antioxidants effect on DPPH radical scavenging is seemed to be related to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (22).

Table 1. Comparison of Chemical Composition of Essential Oils of Flower and Stem in Achillea Wilhelmsii

No.	Name of Compounds	Stem	Flower	RT, Min	Oil of Stem, %	Oil of Flower, %
1	Tricyclene	+	+	10.29	0.38	0.38
2	Alpha- Thujene	+	+	10.58	1.26	1.35
3	Alpha- Pinene	+	+	10.95	8.16	5.47
4	Camphene	+	+	11.36	7.87	6.43
5	Sabinene	+	+	12.85	2.15	1.36
6	Beta- Pinene	+	+	12.96	1.62	1.40
7	6- methyl- 5- Hepten- 2- one	-	+	13.49	-	0.05
8	2- pentyl- Furan	-	+	13.77	-	0.06
9	Isoamylisobutyrate	+	+	14.87	0.17	0.47
10	Alpha- Terpinene	+	+	15.02	2.34	2.14
11	O- Cymene	+	-	15.43	3.27	-
12	P- Cymene	-	+	15.48	-	3.93
13	1,8- Cineole	+	+	15.72	3.32	1.69
14	Gamma- Terpinene	+	+	17.19	2.32	2.60
15	*Trans*- Sabinene hydrate	+	+	17.58	0.68	0.36
16	Alpha- Terpinolene	+	+	18.65	0.56	0.62
17	*cis*- Sabinene hydrate	+	-	19.31	1.09	-
18	Alpha- Thujone	+	-	19.51	4.21	-
19	Linalool	+	+	19.74	0.50	1.36
20	Beta- Thujone	+	+	20.03	0.77	4.92
21	*Trans*- Mentha- 2,8- Dien-1- ol	+	-	20.56	0.94	-
22	(E)-P-2-Menthol-1-ol	-	+	20.76	-	1.06
23	Camphor	+	+	21.59- 22.09	34.49	27.99
24	Pinocarvone	+	+	22.32	0.34	0.80
25	Borneol	+	+	22.59	2.16	0.99
26	Phellandral	-	+	22.87	-	0.96

27	Terpinene- 4- ol	+	+	23.21	5.70	6.43
28	Alpha- Thujenal	+	+	23.38	0.14	0.27
29	Alpha- Terpineol	+	+	23.76	0.59	0.43
30	Myrtenol	+	+	23.91- 23.99	0.37	0.60
31	Terpinene- 3- ol	+	-	24.55	0.21	-
32	*Cis*-Piperitol	-	+	24.59	-	0.27
33	*Trans*- (+)- carveol	+	+	25.07	0.23	0.40
34	Pulegone	+	-	25.96	0.17	-
35	2-Methyl-3-phenylpropanal	-	+	26.00	-	0.34
36	Geraniol	-	+	26.62	-	0.06
37	Linderol	+	+	28.17	0.18	0.08
38	1-Phenyl-1-butanol	-	+	28.35	-	0.05
39	Sabinyl acetate	+	+	28.54	2.26	6.56
40	Thymol	+	-	28.63	0.51	-
41	Cyclofenchene	-	+	28.91	-	0.66
42	Carvacrol	-	+	29.06	-	0.09
43	Eugenol	+	+	31.34	0.09	0.12
44	Gamma-Terpineol	-	+	31.60	-	0.13
45	Geranyl acetate	+	+	32.51	0.10	0.07
46	*cis*- Jasmone	+	+	33.09	0.10	0.12
47	2,4(10)- Thujadien	+	+	33.89	0.15	0.07
48	*Trans*- Caryophyllene	+	+	34.09	0.11	0.10
49	Hexylidencyclohexane	-	+	34.26	-	0.19
50	Bicyclogermacrene	+	-	37.28	0.14	-
51	Isobutyl-beta-phenyl propionate	-	+	37.41	-	0.05
52	Tetradecanal	-	+	37.80	-	0.05
53	Neryl acetate	+	-	39.81	0.11	-
54	Nerolidol	+	+	40.00	1.99	1.86
55	*Trans*- Geraniol	+	-	40.38	0.22	-
56	Spathulenol	+	+	40.54	0.31	0.12
57	Caryophyllene oxide	+	+	40.73	2.64	2.19
58	Neopentyl- beta-phenyl propionate	+	+	41.57	0.12	0.17
59	Widdrene	-	+	42.36	-	0.20
60	Alpha- Caryophylladienol	+	+	42.61- 42.75	0.19	0.38
61	Caryophylla-4(12),8(13)-diene-5β-ol	-	+	42.80	-	1.24
62	13-Tetradecanolide	+	+	43.26	0.52	0.49
63	Caryophylla- 3,8(15)- dien- 5-beta- ol	+	-	43.53	0.14	-
64	(1S,2R,5S)-Menthol	-	+	43.55	-	0.20
65	Caryophyllenol	-	+	44.06	-	0.40
66	E,E- alpha- Farnesene	+	+	48.99	0.15	0.38
67	Phytone	-	+	50.14	-	0.06
68	Palmitic acid	+	+	50.29	0.24	0.30
69	Octadecane	-	+	64.15	-	0.05
70	Pentacosane	-	+	69.34	-	0.04
71	Eicosane	-	+	71.96	-	0.03
72	Oleic acid	-	+	73.74	-	0.06

Figure1. Comparison of Scavenging Effect of BHT and Aqueous Extracts on DPPH Radicals



Abbreviation: IP; Inhibitory Percent

5. Discussion

The high content of oxygenated compounds might explain the characteristic and fragrant odor of the oil. Our results showed that the main constitute in the essential oil of the flower and stem of *Achillea wilhelmsii* from Taftan area were camphor with 34.49% in the leaves and stems, and 27.99% in the flowers. Another study on the plant which performed in Mazandaran province showed that the major components of the essential oil of the leaves and flowers of *A. wilhelmsii* were camphor with 24.1% and 21.2% respectively (18).Where as the amount of this component in the plant which collected from Kerman was 9.0% and in the plant from Kazeroon in Fars province was 2.2% (17, 19). The main constitute of the oil of *A. wilhelmsii* from Kazeroon was carvacrol (25.1%), while the main component in the oil of the plant from Kerman was caryophyllene oxide (12.5%). Caryophyllene oxide proportions in the essential oil of A. wilhelmsii from Taftan were 2.64% in stem and leaves and 2.19% in flower. Carvacrol was not seen in the essential oil of stem and leaves and presented in only-trace amount (0.09%) in the oil of flower. 1, 8 cineol which consists 3.32% of the essential oil of the stem has also been reported as the major constituent of the oil of *A. wilhelmsii* from Egypt and Turkey (5,17). General comparison of the essential oil from *A. Wilhelmsii* from different parts of Iran and other countries such as Egypt and Turkey shows similarity in chemical constituents, but with different percentages. These variations might be due to growth conditions, genetic variation, geographical variations and analytical procedures.

Studies have revealed that monoterpens have potent insecticides effects against stored – product insects (23, 24). Our study showed that *A. wilhelmsii* from Taftan has major monoterpens compounds. These compounds consist of camphor, carvacrol, 1, 8 cineole, α- pinene, piperitenone oxide and terpineol which have been shown to have insecticidal effects against some major insects that infect the stored crops. Therefore, the essential oil of A. wilhelmsii from Taftan could be a valuable alternative to chemical control strategies which have undesirable effects such as environmental pollution and direct toxicity to people. Regarding environmental problem and human health, this plant could be an alternative source of insecticides agents because many of its components have little or no harmful effects on humans and environment (24, 25). Furthermore, A. wilhelmsii is full of flavonoids and sesquiterpene lactones, which have been revealed to be effective in lowering blood lipids and hypertension (9).

Another major finding of this study was high antioxidant activity of the aqueous extract from the leaves and stem of *A. wilhelmsii* from Taftan. IC50 of aqueous extracts of flower and stem were compared to the IC50 of BHT. IC50 of flower, stem and BHT were 232.34, 63.25 and 45.59 respectively, which showed that antioxidant activity of the aqueous extract of flower is weaker than stem. Antioxidant activity is usually due to phenolic compounds which are presented in stem and leaves more than flowers due to their synthesis and metabolism process in these parts of the plant. Therefore, the extract of leaves and stem may be useful in the treatment of human diseases in which free radical production plays a major role. In addition this extract could be used as a preventive factor on undesirable changes in the flavor and nutritional quality of food.

In conclusion, *Achillea wilhelmsii* is emerging as one of the most important medicinal plants in Sistan and Baluchestan of Iran as well as many parts of the world. Preparations containing its essential oil could commercialize the plant as a valuable source for a variety of healthy implications such as antioxidant, insecticides effects, pharmaceutical and cosmetic purposes and perfume industries. If a high percentage source of camphor is needed, the *A. Wilhelmsii* from Taftan would be the best species available in Iran. Further studies are needed to be performed to determine which constituents are responsible for antioxidant activity. Also the antioxidant activity of the plant from Taftan needs to be examined by different in vitro tests.

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Authors' Contribution

Ali Shahraki has cooperated 90% and Mehdi Ravandeh has cooperated 10% in this study.

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