Study on Chemical Composition of Essential oil and Anti-oxidant and Anti Microbial Properties of *Artemisia haussknechtii*

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Abstract

Background: ***Artemisia*** **species with common Persian name of Dermaneh are found all over Iran and are used for treatment in infectious diseases such as malaria, hepatitis and other diseases. Some** ***Artemisia*** **species are used in traditionally as tonic and anti-helmintic in north of Iran.**

Objective: The aim of this study was to investigate chemical composition of the essential oil of ***Artemisia haussknechtii.*** **Also potential antioxidant and anti microbial activities of the essential oil and ethanolic extract were studied.**

Methods: The essential oil was prepared by hydrodistillation and analyzed by GC and GC/MS instruments. Antioxidant activity was evaluated by methods; namely DPPH, free radical scavenging, FTC system and total phenolic compounds analyzing. The antimicrobial activities of the extract were individually tested against a panel of microorganisms using disc diffusion method and MIC (minimum inhibitory concentration) measurement.

Results: Forty-eight components were identified constituting 98.35 of total oil. Camphor (12.4%), α-Terpineol (9.93%), Davana ether (6/24%), and Bornyl acetate (3.77%) were the major components. Good antioxidant activity of extract; increasing with the increment of concentration of plant extract was revealed. Ethanolic extract of ***Artemisia haussknechtii*** **inhibited both gram-positive and gram- negative bacteria. MIC of the extract against yeast was the lowest (2.5 µg/ml).**

Conclusion: A known anti-bacterial compound (camphor) was one of major components in the essential oil, ethanolic extract showed good anti-oxidant activity and also extract inhibited growth of both gram positive and gram negative bacteria and fungi. These findings supported some traditional use of this plant.

Keywords: ***Artemisia haussknechtii,*** **Essential oil, Anti-oxidant, Anti microbial**

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Introduction

Artemisia species with common Persian name of Dermaneh are found all over Iran [1, 2]. All *Artemisia* Spp. are used for treatment of infectious diseases such as malaria, hepatitis and other diseases causing by helminthes, fungi, bacteria and viruses [3, 4, and 5]. The genus *Artemisia* is represented by 34 species growing in different parts of Iran, of which 2 species are endemic. Some *Artemisia* species are used traditionally as tonic, and as anti-helmintic in north of Iran [6].

Artemisia haussknechtii is used in dyspepsia and other gastrointestinal disorders by local people in the western part of Iran; province of Kermanshah. Antimicrobial effects of some endemic *Artemisia* in Iran such as *A. diffusa*, *A. oliveriana*, and *A. turanica* were reported [7].

Antibacterial activities *of A. scoparia*, *A. capillaries* and *A. Lavandulifolia* have proven [8]. Antimicrobial activities of these plants relate to high percentage of oxygenated monoterpenes (camphor and 1, 8-cineol) and monoterpene hydrocarbons (α-pinene).

Some reports on the antioxidant properties of these species are documented. Essential oils of two species (*A. absyssinca* and *A. afra*) are tested for antioxidant activity using TIC screening method [9]. Significantly higher antioxidant activity and flavonoid contents were observed in the *Artemisia judaica* [10]. Any report has not been published about the antimicrobial and antioxidant properties of this plant.

Material and Methods

Plant Material

Artemisia haussknechtii was collected from Oramanat area, Kermanshah (western part of Iran) in June 2007 and identified in Kermanshah research institute of Forests and Rangelands. The fresh plants were sliced and air dried with active ventilation at ambient temperature.

Isolation of the Essential Oil

Air-dried aerial parts of *A. haussknechtii* (100g) were subjected to hydrodistillation in a Clevenger – type apparatus for 4 hours period. After decanting and drying of the oil on anhydrous sodium sulfate, it was kept refrigerated until analysis.

GC and GC- MS Analyses

GC analysis was carried out using a Hewlett-Packard 6890 with HP-5 capillary column [phenyl methyl Siloxane; 25 m × 0.25 mm × 0.25 μm]. The oven temperature was programmed as following: 60 to 240 °C at 4 °C/min increment rate; injector temperature, 250 °C; detector temperature 260 °C; carrier gas He (1.5 ml/min); split ratio 1:25.

GC-MS analyses were carried out applying a Hewlett-Packard 6859 with a quadropol detector, on a HP-5 column (see GC), operating at 70 ev ionization energy, and using temperature program and carrier gas as mentioned above.

Retention indices were calculated by using retention times of n-alkanes that were injected after the oil at the same chromatographic conditions according to the Van den Dool's method [11]. The compounds were identified by comparison of relative retention indices (RRI, DB-5) with those reported in the literature [12] and by comparison of their mass spectra with the Wiley library [13]. The quantification of the oil was based on one analysis.

Extract Preparation

In order to extraction, according to conventional procedures [14] about 50g of

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powdered plant was treated with 100 ml ethanol at room temperature with stirring. This procedure was repeated five times until the extraction solvent became colorless. The obtained extract was filtered over Whatman No.1. Paper filter and the filtrates were collected, and then ethanol was removed in vacuum at temperature not exceeding 40°C.

Ferric Thiocyanate (FTC) Method:

The previously described method was used [15]. A mixture of 4.0 mg of plant extract dissolved in 4 ml of absolute ethanol; 4.1 ml of 2.52 % linolenic acid in absolute ethanol; 8 ml of 0.05 M potassium dihydrogen phosphate buffer (pH= 7.0) and 3.9 ml of water was placed in a vial with a screw cap and then placed in a dark oven at 40°C. A volume 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate were added to 0.1 ml of this solution. Precisely, 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color measured at 500nm every 24h until one day after the absorbance of control reached its maximum. A mixture without a plant extract was used as negative control.

DPPH Assay:

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 1, 1-Diphenly 2-picryl hydrazyl (DPPH). This spectrophotometeric assay uses the stable radical DPPH as a reagent [16].

One ml of various concentrations of the extracts in ethanol was added to 4 ml of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 512 nm. Inhibition of free radical by DPPH in percent (I %) was calculated with the following equation:

I (%) = [(A blank _ A sample)/ A blank] *100

where A blank is the absorbance of the control reaction (containing all reagent except the test compound).

A sample is the absorbance of the test compound.

Phenolic Compounds

Total Phenolic content of the plant extract was determined using Folin – Ciocalteu reagent. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The amount of total phenolic compounds in the plant extract was determined colorimetrically with the Folin–Ciocalteu (FC) reagent, and 1.5 ml of 20% sodium carbonate according to previous studies [14]. The reaction mixture contained 500 µl of 0.1% aqueous dilution of dry extract, 2.5 ml of freshly prepared 0.2 M FC reagent and 1.5 ml of sodium carbonate solution and was kept in the dark under ambient conditions for 2 hours to complete the reaction. Then absorbance of the resulting solution was measured at 765 nm in a UV–Vis spectrophotometer (model 8453 Hewlett Packard, Agilent Technologies, USA). The concentration of total phenolic compounds was expressed as mg of gallic acid equivalent (GAE) per g of dried extract (DE), using a standard curve of gallic acid. All measurements were carried out in five replicates.

Antimicrobial Assay

The antimicrobial activity of *A. haussknechtii* alcoholic extract was individually tested against a panel of microorganisms, including *Bacillus subtilis* (ATCC 465), *Enterococcus faecalis* (ATCC 29737), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis*

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(ATCC 12228), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763). Bacterial strains were cultured overnight at 37 ºC in Mueller Hinton broth (Merck co., Germany). Yeast was cultured overnight at 28 ºC in Sabouraud dextrose broth (Merck co., Germany). For determination of antimicrobial activities, disc diffusion method and MIC (minimum inhibitory concentration) measurement were employed. The MIC of ethanolic extract against the test microorganisms was determined by the Micro dilution method [17].

Result and Discussion

Essential oil composition

Forty-eight components, which accounted for 98.35% of the oil, were identified (Table 1).

Camphor (12.4%), α-Terpineol (9.93%), Davana ether (6.24%), and Bornyl acetate (3.77%) were the major components. As shown in Table 1., other components were in small amounts. First most prevalent compound in *A. haussknechtii* (camphor) was seen in *A. roxburghiaha* (15.2%) (18), *A. khorassanica* (13.9%) (19), *A. kopetdaghensis* (9.8%) [20].

The result of one research using hydrodistillation and head space liquid phase microextraction techniques showed 56 components in the essential oil of *A. haussknechtii* which were collected from Yazd province in Iran and camphor (40.83%), 1,8-Cineole (26.84%), cis-davanone (4.77%), and linalool (4.44%) were the main components [21]. Difference between our finding and previously reported research may be resulted from geographic origin and the environmental conditions on the plant.

Antioxidant activity of ethanolic extract of *A. haussknechtii*

The ethanolic extract was subjected to screening for its possible antioxidant activity. Namely DPPH free radical scavenging, FTC system, and total phenolic compounds were used for this purpose. Ethanolic extraction of *A. haussknechtii* yielded 22.1 w/w of dried extract and its DPPH scavenging activity inhibition of linoleic acid peroxidation are given in Table 2.

Table 1- Components of *A. hahssknechtii* essential oil identified by GC and GC/MS

NO	RT*	% of abundance	NAME	RRI**
1	16.02	0.06	δ- Terpinene	933
2	16.48	1.33	α- Pinene	941
3	17.33	0.71	Camphene	955
4	18.76	0.75	Anethole	979
5	18.92	1.44	β- Pinene	982
6	20.16	3.58	Yomogi alcohol	996
7	21.8	0.57	α- Terpinene	1020
8	21.57	3.29	p- Cymene	1030
9	21.98	3.73	1,8 – Cineole	1037
10	22.15	1.25	trans- β- Ocimene	1040
11	23.73	0.5	δ- Terpinene	1063
12	24.79	1.98	Artemisia alcohol	1088
13	24.64	2.33	Linalool	1106

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Continue Table 1- Components of *A. haussknechtii* essential oil identified by GC and GC/MS

NO	RT*	% of abundance	NAME	RRI**
14	27.01	1.04	Isoterpinolene	1137
15	27.46	1.41	trans Limonene oxide	1139
16	28.27	12.4	Camphor	1157
18	29.25	4.55	Ipsdienol	1165
19	29.4	4.99	Borneol	1179
20	29.81	1.47	Terpinen – 4-ol	1187
21	30.17	0.64	p- Cymen-8-ol	1195
22	30.44	9.93	α- Terpineol	1200
23	30.75	0.9	Myrtenol	1206
24	31.818	0.87	Nonanal	1222
25	32.85	1.64	Cuminal	1251
26	33.31	2.78	Geraniol	1260
27	33.69	1.56	Neral	1268
28	34.60	1.67	Isopulegol acetate	1286
29	34.98	3.77	Bornyl acetate	1295
30	35.39	2.22	p- Cymen -7-ol	1304
31	35.39	1.65	Carvacrol	1314
32	36.1	3.13	trans Ascoridole	1320
33	88.29	3.24	Neryl acetate	1369
34	40.97	0.62	Cominyl acetate	1429
35	43.87	6.24	Davana ether	1497
36	45.88	0.79	Artedouglasia oxide	1539
37	46.86	2.75	Nerolidol	1571
38	47.20	0.61	Geranyl propionate	1580
39	47.91	0.72	Spathulenol	1598
40	48.16	1.96	Caryophyllene oxide	1604
41	48.80	0.87	Globulol	1620
42	49.33	0.77	cis -Arteannuic Alcohol	1634
43	49.50	0.92	3-Nonyne	1639
44	50.21	1.87	Caryophylla-4(12), 8(18)-dien-5-beta-ol	1658
45	50.73	0.35	β- Eudesmol	1672
46	51.20	0.6	8-Hydroxy-iso-bornyl-iso butyrate	1683
47	51.41	2.03	n-Tetradecanol	1690
48	51.63	0.87	Valevanone	1695
49	52.95	2.89	Geraniol	1732
Sum	98.35			

* Retention Time (minutes), ** Relative Retention Indices

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Table 2- Yield, total amount of plant phenolic compounds, and total antioxidant capacity of ethanol extract of *A. haussknechtii*

Extraction yield (w/w)	Total phenolic count (TPC)	FTC	DPPH
22	103.8 mg/g	103.2	33.4

The ability of the ethanolic extract of *A. hausskenchti* to inhibit lipid peroxidation is determined and the results are shown in Fig. 1.

Ethanolic extract of *A. haussknechtii* showed absorbance values greater than the controls (without plant extract) indicating the presence of antioxidant activity. This study revealed that the antioxidant activity of the extract was in the increasing tread with the increasing concentration of plant extract.

DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging capacity of the extract. As antioxidant donates protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging capacity. Scavenging activity of ethanolic extract of *A. haussknechtii* against 1, 1-diphenyl-2-picrylhydrazil radical is shown in Fig. 2. The experimental data revealed free radical scavenging effect for all concentrations which applied.

Fig. 3 is the concentration- response curve for inhibition of the absorbance of DPPH radical at 3 different time points (5, 10, 30 min) for *A. haussknechtii* extract. All concentrations showed free radical scavenging activity. The inhibition value increased with increasing concentration, for example, the extract showed 87.8 and 78.5 inhibition with 0.25 mg/ml and 0.5 mg/ml of concentrations, respectively.

The IC50 value for plant extract, defined as the concentration of extract causing 50 percent inhibition of absorbance, was determined from the concentration – response curve plotted for inhibition of the absorbance of DPPH radical at 517 nm. Since IC50 is the measure of inhibitory concentrations, a lower IC50 value would reflect greater antioxidant activity of sample. Hence, ethanolic extract of *A. haussknechtii* displayed higher DPPH with lower IC50 values 0.15 mg/ml (Fig. 3). As for our literature survey could ascertain, here is no report dealing with antioxidant properties of *A. haussknechtii*.

Antimicrobial activity

The result of Antibacterial and antifungal activities of ethanolic extract of *A. haussknechtii* are presented in table 3. Ethanolic extract of *A. haussknechtii* exhibited moderate activities against Gram-positive bacteria and Gram-negative bacteria.

Ethanolic extract of *A. haussknechtii* show strong activity against yeasts (*Candida albicans*, *Saccharomyces cerevisiae*); that is ethanolic extract of *A. haussknechtii* specially inhibited *C. albicans*, *S. cerevisiae* (MIC=2.5) ($p<0.05$).

Among Gram-positive bacteria MIC of ethanolic extract of *A. haussknechtii* against bacteria is lowest for *Staphylococcus epidermidis* and *Bacillus subtilis* ($p<0.05$).

MIC observed in *S. epidermidis* and *B. subtilis* was 4 times greater than that seen in *E. coli*, this difference in inhibition may be due to differing composition of bacterial membranes and their permeability to antimicrobial component such as camphor, 1,8-cineole, and α-pinene

This plant inhibits growth of bacteria and fungi at low concentrations; therefore this plant like other species of genus Artemisia has some antimicrobial component (s).

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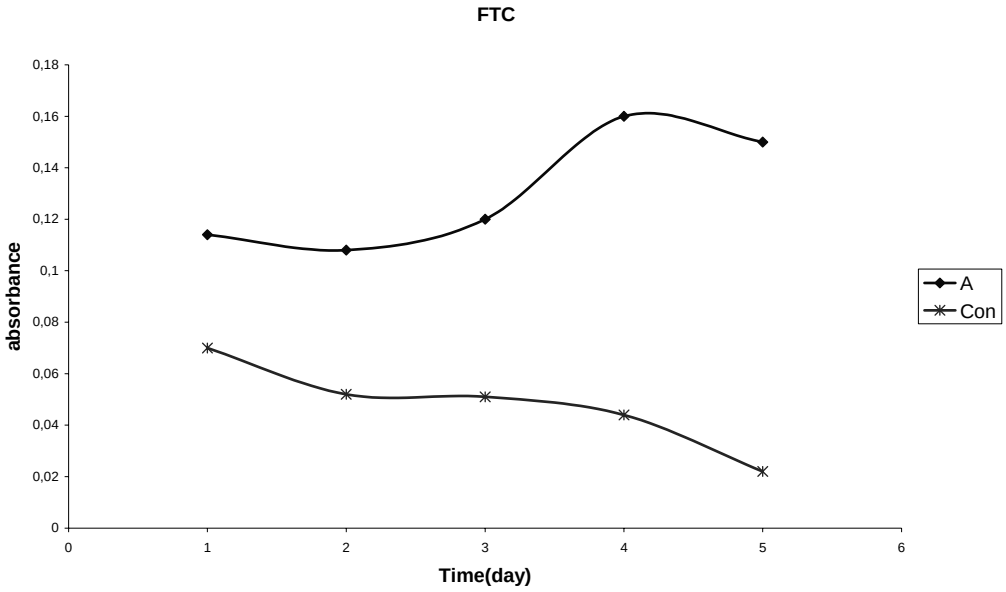

Fig. 1- Antioxidative activity of *A. haussknechtii* extract in linoleic acid system measured by ferric thiocyanate method. Values represent means ± SE (n=3)

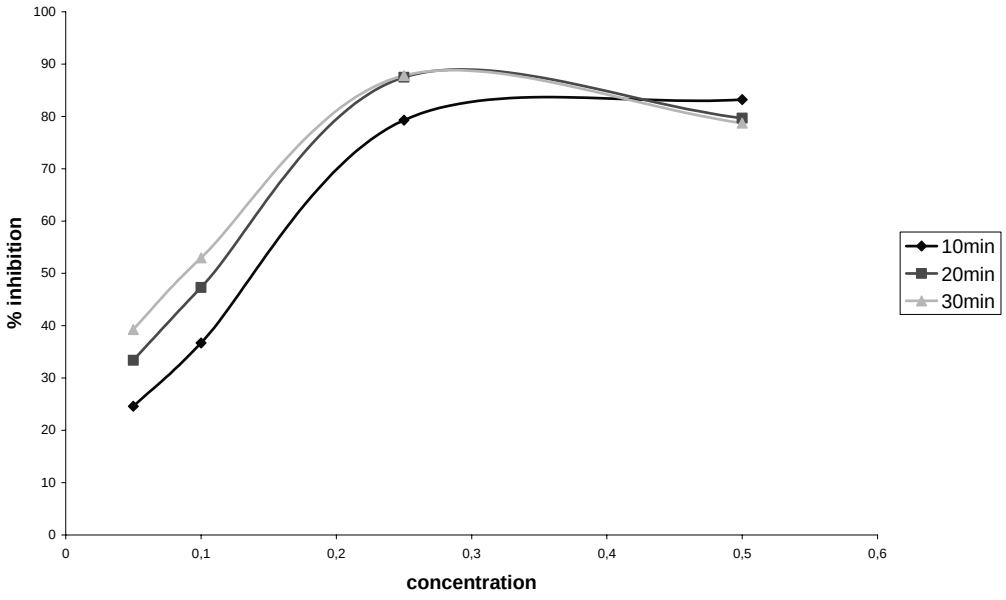

Fig. 2- Scavenging Capacity of *A. haussknechtii* extract on DPPH determined by measuring absorbance of the reaction mixture at 515 nm at tree different time points (10, 20 and 30 min)

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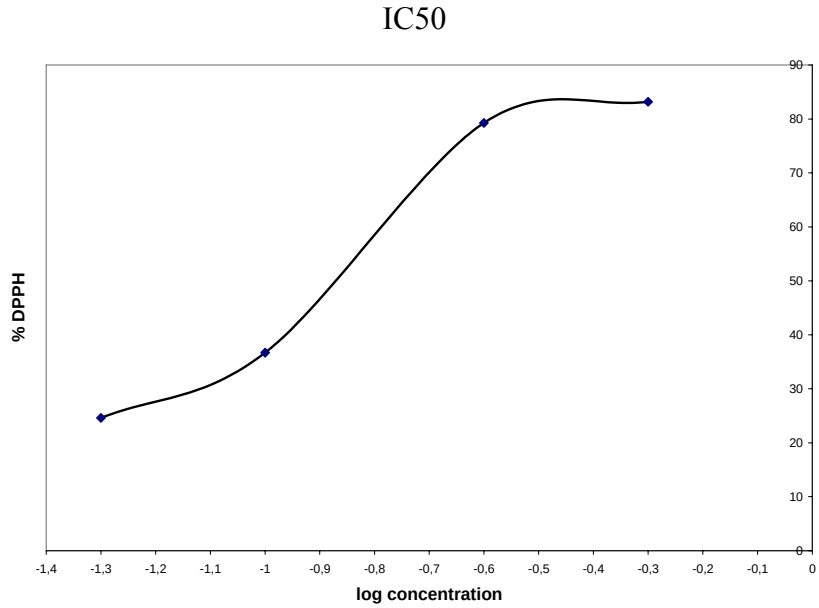

Fig. 3- IC50 value in DDPH assay

Table 3- Inhibition zone (mm) and MIC (µg/ml) values of ethanolic extract of *A. haussknechtii* in comparison with control

Microorganism	Ethanolic extract		Tetracycline (30 µg/disc)		Gentamicin (10 µg/disc)		Nystatin (30 µg/disc)	
	IZ[1]	MIC[2]	IZ	MIC	IZ	MIC	IZ	MIC
B. subtilis	23	3.75	21	3.2	-	nt[3]	nt	nt
E. faecalis	13	15	9	6.4	-	nt	nt	nt
S. aureus	17	7.5	20	3.2	-	nt	nt	nt
S. epidermidis	20	3.75	34	1.6	-	nt	nt	nt
E. coli	15	15	-	nt	23	3.2	nt	nt
K. pneumoniae	13	15	-	nt	20	3.2	nt	nt
P. aeroginosa	12	15	-	nt	12	6.4	nt	nt
C. albicans	14	5	nt	nt	nt	nt	18	3.2
S. cerevisiae	19	2.5	nt	nt	nt	nt	18	1.6

[1]Inhibition Zone
[2]Minimum Inhibitory Zone
[3]Not Tested

The antimicrobial properties of Artemisia components such as Borneol, 1,8-Cineole, α-pinene, and Camphor were demonstrated by some researchers [22, 23]. *A. haussknechtii* inhibits growth of fungi at low concentrations ratio to that of bacteria ($p<0.05$).

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