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Full Length Research Paper

Composition, antimicrobial and antioxidant activities of the essential oil of *Artemisia kermanensis* Podl., an endemic species from Iran

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The essential oil of the aerial parts of *Artemisia kermanensis* was analyzed by GC-FID and GC-MS methods. Twenty-five compounds, accounting for 92.3% of the total oil, were identified. The main components of the oil were isoborneol (21.5%) and camphor (9.8%). The oil was found to be rich in regards to oxygenated monoterpenes (80.2%). The antimicrobial activity of oil was determined against eight bacteria and two fungal strains. The results of inhibition zone showed that this oil was active against some of the tested strains. As the results, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most sensitive microorganisms to the oil (having MIC value 1.25 mg/ml). The oil antioxidant activities were measured by DPPH assay and ß-carotene-linoleic acid tests. The essential oil significantly reduced the concentration of DPPH free radical (71.6%), with an efficacy higher than that of trolox (48.1%). Also, a 50% inhibition was noted in formation of peroxidation products in ß-carotene bleaching test. The activity of the oil may be due to the presence of phenolic and major compounds.

Key words: *Artemisia kermanensis,* Asteraceae, essential oil composition, isoborneol, camphor, antimicrobial activity, antioxidant activity.

INTRODUCTION

Artemisia (Asteraceae) is one of the largest and most widely distributed genera of the approximately 60 genera in the Anthemideae tribe. 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 (Mozaffarian, 1996; Rechinger, 1980).

Some compounds from the genus have shown antimalarial, antiviral, antitumoral, antipyretic, antihemorrhagic, anticoagulant, antianginal, antioxidant, anti-hepatitis, antiulcerogenic, antispasmodic, antihepatitis, antiulcerogenic, antispasmodic, anticomplementary and interferon inducing activity (Rustaiyan et al., 2009a; Tan et al., 1998). *A. annua* (Qinhaosu) is a traditional medicinal herb in China. It is now cultivated on a commercial scale in China and Vietnam for its anti-malarial sesquiterpene lactone, artemisinin (Klayman et al., 1984; Li et al., 1982) and essential oil. *A. austriaca* and *A. spicigera* are odorous herbs used as antiseptics and stomachics in folk medicine (Guoevenalp et al., 1998). *A. vestita* is a herb that has been widely used in traditional Tibetan and Chinese medicine for treating inflammatory diseases such as rheumatoid arthritis and contact dermatitis anepsis (Sun et al., 2006). *A. dracunculus* has been used orally as an antiepileptic and its anticonvulsant potential has been assessed (Sayyah et al., 2004). Studies on *Artemisia* have ascertained the presence of coumarins (Rybalko et al., 1977), acetylenic

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compounds (Bohlman et al., 1972) and sesquiterpene lactones (Rustaiyan et al., 1989). Numerous reports appear in the literature on the essential oils of different species of *Artemisia*, its antimicrobial and antioxidant activities (Hong, 2004; Khaled et al., 2002; Lopez-lutz et al., 2008; Verdian-rizi, 2009). The volatile constituents of *A. kermanensis* aerial parts from kerman region, Iran, previously reported (Rustaiyan et al., 2009b). In this study, for the first time, we investigated the chemical composition, also the antimicrobial and antioxidant activities of *A. kermanensis* cultivated in Shahrebabak region from Iran.

MATERIALS AND METHODS

Plant material

The aerial parts of *A. kermanensis* were collected during the flowering stage in Shahrebabak region, province of Kerman, Iran, in October 2009. Voucher specimens have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Isolation of the oil

The aerial parts (140.0 g) of the *A. kermanensis* plant were air-dried at room temperature in the shade and the oil was obtained by hydrodistillation using Clevenger-type apparatus for 3 h. After decanting and drying over anhydrous sodium sulfate, the corresponding yellowish colored oil was recovered in yields of 0.8% (v/w). The sample were then kept in bottles covered in aluminium foil at 4°C to prevent the negative effect of light, especially direct sunlight.

GC-FID

Gas chromatography-Flame ionization detector analysis was performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless (ratio 1:30), injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 ml/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

GC-MS

Analysis was performed using a Hewlett- Packard 5973 with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C for 5 min. The flow rate of helium as carrier gas was (1 ml/min). MS were taken at 70 eV, mass range, 30 to 350 amu, and scan time 2 scans/s. The compounds were identified by comparison of RRI, DB5 with those reported in the literature and by comparison of their mass spectra with either the Wiley library or with published mass spectra (Adams, 2000; Massada, 1976; Ramaswami, 1988). The retention indices for all the components were determined according to the Van Den Dool method, using *n*-alkanes as standards (Van Den Dool and Kratz, 1963).

Microorganisms

The bacteria included *Bacillus cereus* (ATCC 6633), *Bacillus subtilis* (ATCC 9372), *Enterobacter* spp*, Escherichia coli* (ATCC 25922), *Citrobacter* spp*, Klebsiella pneumoniae* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 27852) and *Staphylococcus aureus* (ATCC 25923), and fungi, *Aspergillus niger* (ATCC 9142) and *Candida albicans* (ATCC 6258). The microorganisms were obtained from the Research center of science and industry, Tehran, Iran.

Antimicrobial assay

The antibacterial and antifungal activity of the essential oil was evaluated by a disc diffusion method using Mueller-Hinton and Sabouraud Dextrose agar respectively (Collins and Lyne, 1987; Murray et al., 1995). A suspension of the tested microorganism (0.1 ml of a suspension of the tested microorganisms, containing 1.5×10^8 CFU/ml) was spread on the solid media plates. Mueller-Hinton and Sabouraud dextrose agar sterilized in a flask and cooled to 45–50°C were distributed to sterilized Petri dishes with a diameter of 9 cm (15 ml). A serial dilution of the oil was prepared in Mueller-Hinton and Sabouraud dextrose broth for bacteria and fungi respectively. The filter paper discs (6 mm in diameter) were individually impregnated with 15 µl of the *A. kermanensis* essential oil and then placed onto the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37°C for 24 h and at 28°C for 72 h for the fungal strains. Ethanol (95%) was used as a negative control in all the plates while ampicillin (10 mg/disc), gentamicin (10 mg/disc) for bacteria and fluconazol (20 mg/disc) were used as positive controls. The diameters of the inhibition zones were measured in millimetres.

For MIC assay, two controls were included with each batch of test. The first was a negative control, which contained the test material but not the organisms to check for contamination of the test material (NCCLS, 2000). The positive control contained microorganisms without the test material. The standardized suspension of bacteria and fungi were inoculated in to each tube and the final concentration in them was adjusted to 1.0×10^6 CFU/ml for strains. The tubes were incubated at 37°C and 24 h for bacteria and at 30°C and 48-72 h for fungi. The lowest oil concentration, which completely inhibited microbial growth, was the minimum inhibitory concentration (MIC) when compared to the control. To determine the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC), for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on Mueller-Hinton agar (for bacteria) and Sabouraud dextrose broth agar (for fungi) by streaking. Tubes inoculated with bacteria and fungi were then incubated at 37°C for 24 h and 30°C for 48-72 h respectively. After incubation the concentration at which no visible growth was seen was noted as MBC (for bacteria) and MFC (for fungi). All the experiments were carried out in triplicate and the mean calculated.

Radical-scavenging capacity (DPPH assay) of the oil

The hydrogen atom or electron donation abilities of the extracts and pure compounds were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenylpicrylhydrazyl (DPPH).

This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits and Bucar, 2000). In order to find out the effective dose of oil, different concentrations (0, 5, 10 and 20% v/v in methanol) were added to DPPH reaction mixture and the concentration-dependent inhibition in DPPH radical scavenging capacity was recorded (Data not shown). Based on this, further experiments were carried out with 20% (v/v) of the oil. Then, fifty µl of the essential oil in methanol were added to 5 ml of DPPH solution (0.004% DPPH in methanol). Trolox (1 mM, 500 µM and 250 µM), a stable antioxidant, was used as reference. After incubation for 30 min at room temperature, the absorbance was read against the blank at 517 nm. The following formula was used to estimate the inhibitory effects of the oil extract in percent (I%):

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100.$$

Where, A blank is the absorbance of the control reagent (containing all reagents except the test compound), and A sample is the absorbance of the test compound. All the assays were carried out in triplicate.

ß-Carotene-linoleic acid assay of the oil

Antioxidant activity of essential oils was determined using the ß-carotene bleaching test as described by Taga et al. (1984). Approximately 10 mg of ß-carotene (type I synthetic) was dissolved in 10 ml of chloroform. Two milliliters of the carotene-chloroform solution was pipetted into a boiling flask containing 20 mg linoleic acid and 200 mg Tween 40. Chloroform was removed using a rotary evaporator at 40°C for 5 min. Then, the residue was dissolved in 50 ml of distilled slowly with vigorous agitation, to form an emulsion. Five milliliters of the emulsion were added to a tube containing 0.2 ml of essential oil solution, prepared according to Choi et al. (2000). The absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without ß-carotene. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min. A sample containing 0.2 ml of ethanol instead of essential oils was used as control. Butylated hydroxyl toluene (BHT), a stable antioxidant, was used as a synthetic reference. The antioxidant activity was expressed as inhibition percentage with reference to the control after 60 min of incubation, using the following equation:

$$AA = 100(DRC - DRS)/DRC,$$

where AA = antioxidant activity, DRC = degradation rate of the control = [ln(a/b)/60], DRS = degradation rate in presence of the sample = [ln(a/b)/60], a = absorbance at time 0, b = absorbance at 60 min.

RESULTS AND DISCUSSION

The percentage and retention indices of the essential oil components obtained from aerial parts of *A. kermanensis* are listed in Table 1. As shown in Table 1, analysis of the aerial parts oil of *A. kermanensis* resulted in the identification of 25 constituents, representing 92.3% of the oil. The main components of the oil were isoborneol (21.5%) and camphor (9.8%). The aerial parts of *A. kermanensis* were found to be rich in regards to oxygenated monoterpenes (80.2%). This sample was poor in hydrocarbon monoterpenes: 1.2%, sesquiterpene hydrocarbons: 2.6%, oxygenated sesquiterpenes: 5.1% and other compounds (mainly phenol derivatives): 5.9%. In some researches on the essential oils of other *Artemisia* species, borneol is characteristic of the oils of *A. nilagrica* (Uniyal et al., 1985.), *A. frigida* (Atazhanova et al., 1999) and *A. iwayomogi* (Hong, 2004). In other studies, 1,8-cineole and bornane derivatives were reported as the main constituents. 1,8-cineole (45.5, 27.8, 25.7, 19.0 and 14.3%) and camphor (16.7, 37.9, 35.0, 44, and 45.5%) were obtained to be the major constituents of the oils of *A. sieberi* (Weyerstahl et al., 1993), *A. diffusa* (Khazraei-Alizadeh and Rustaiyan, 2001), *A. aucheri* (Mohammadpoor et al., 2002) and *A. scoparia* (Morteza-Semnani and Akbarzadeh, 2006). In our research, isoborneol and camphor predominated in the oils of *A. kermanensis* but 1,8-cineole was only found as a minor constituent. In previous report, the water distilled essential oil of aerial parts of *A. kermanensis* cultivated in Kerman region has been reported (Rustaiyan et al., 2009b). The oil was rich in davanone (21.4%), 1,8-cineole (16.0%), chrysanthenone (14.8%) and carvacrol acetate (9.3%).

Results of the inhibition zone of the oil are shown in Table 2. The oil has shown maximum zone of inhibition against *Klebsiella pneumoniae*. Also, in Table 2, results of MIC, MBC and MFC of the oil were collected. *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most sensitive microorganisms to the essential oil (having MIC value 1.25 mg/ml and MBC value 2.5 mg/ml respectively). Previous studies showed that camphor and borneol are well-known antimicrobial compounds isolated from different plant species (Pattnaik et al., 1997). The antimicrobial activity of *A. kermanensis* oil could, in part, be associated with camphor and borneol. The synergistic effects of these chemicals with each other and minor constituents of the essential oil should be taken into consideration for the activity. The mechanism of action of terpenes is not fully understood but it is thought to involve membrane disruption by the lipophilic compounds (Tzakou et al., 2001). In other research, the composition of the essential oil obtained from Iranian *A. annua* L. was analyzed by GC and GC/MS (Verdian-rizi, 2009). Thirty-two components were identified in the essential oil with camphor (48.00%), 1,8-cineole (9.39%), camphene (6.98%) and spathulenol (4.89%) as major components. The essential oil was evaluated for antibacterial and antifungal activities. The activity was more pronounced against fungal organisms than against Gram-positive and Gram-negative bacteria. In similar study, the chemical composition of the essential oil from *A. iwayomogi* Kitamura was analyzed by means of GC and GC-MS (Hong, 2004). Eighty-five constituents were identified representing 96.23% of the total oil. Camphor (19.31%), 1,8-cineole (19.25%), borneol (18.96%), camphene (4.64%), and beta-caryophyllene

Table 1. Percentage composition of aerial parts of *A. kermanensis* essential oil.

Compounds	RI[a]	(%)[b]
ethyl isovalerate	861	0.5
camphen	958	1.2
yomogi alcohol	1000	3.6
p-cymene	1031	2.7
1,8-cineole	1037	3.5
santolina alcohol	1041	5.8
artemisia alcohol	1089	4.4
cis-thujone	1107	7.6
trans-thujone	1121	4.2
camphor	1155	9.8
isoborneol	1165	21.5
terpinene-4-ol	1184	0.6
carvotanacetone	1253	6.0
chrysanthenyl acetate	1271	0.7
neo-3-thujyl acetate	1281	0.7
bornyl acetate	1294	4.2
trans-verbenyl acetate	1301	5.2
neoiso-dihydro carveol acetate	1365	0.8
Z-jasmone	1402	1.6
germacrene B	1576	2.6
longipinalol	1579	1.1
khusimone	1611	0.8
beta-oplopenone	1617	0.7
volgarone B	1662	1.5
himachalol	1665	1.0
Total		92.3
Group components		
Monoterpene hydrocarbons		1.2
Oxygenated monoterpenes		80.2
Sesquiterpene hydrocarbons		2.6
Oxygenated sesquiterpenes		5.1
Other compounds		3.2

[a]Retention indices, as determined on a DB-5 HAV column. [b]Percentages obtained by FID peak-area normalization.

(3.46%) were found to be the major components. Furthermore, the oil exhibited antibacterial activity against six Gram-(+) and six Gram-(-) bacteria in tests using the broth dilution method.

The antioxidant properties of the *A. kermanensis* essential oil measured by DPPH and ß-carotene bleaching assays are presented in Figure 1. When compared to a standard antioxidant agent, i.e. trolox, it was found that the essential oil significantly reduced the concentration of DPPH free radical (71.6%), with an efficacy higher than that of trolox (48.1%). Likewise, a 50% inhibition was noted in formation of peroxidation products in ß-carotene bleaching test (Figure 1). This result together with the oil composition may suggest that antioxidant activities of the oil are likely attributed to its principle compounds (borneol and camphor) and phenolic compounds (Taga et al., 1984). Phenolic compounds such as thymol and carvacrol show potent antioxidant and DPPH radical scavenging activities (Ruberto and Baratta, 2000). The weak antioxidant activities of the non-phenolic components were also supported by other studies on essential oils of *Artemisia* species such as *A. absinthium*, *A. biennis*, *A. cana*, *A. dracunculus*, *A. frigida*, *A. longifolia* and *A. ludoviciana* (Lopez-lutz et al., 2008). In similar report, the volatile components of the aerial parts of *A. judaica* were isolated via

Table 2. Antimicrobial activity of *A. kermanensis* oil and antibiotics against standard microorganisms.

Microorganisms	Inhibition zone (mm)				MIC	MBC	MFC
	Oil	AMP	GEN	FLU			
Bacillus cereus (ATCC 6633)	10.0±0.1	NI	20.0±0.2	ND	5.0	10	-
Bacillus subtilis (ATCC 6633)	NI	25.0±0.2	20.0±0.1	ND	1.25	2.5	-
Citrobacter spp	NI	23.0±0.2	22.0±0.7	ND	ND	ND	-
Entrobacter spp	NI	10.0±0.1	20.0±0.1	ND	2.5	5.0	-
Escherichia coli (ATCC 25922)	NI	NI	25.0±0.3	ND	2.5	5.0	-
Klebsiella pneumoniae (ATCC 27736)	15.0±0.3	NI	20.0±0.4	ND	2.5	5.0	-
Pseudomonas aeruginosa (ATCC 27852)	NI	NI	20.0±0.2	ND	1.25	2.5	-
Staphylococcus aureus (ATCC 25923)	12.0±0.5	NI	25.0±0.2	ND	1.25	2.5	-
Aspergillus niger (ATCC 9142)	10.0±0.9	ND	ND	20.0±1.2	2.5	-	5.0
Candida albicans (ATCC 6258)	10.0±1.1	ND	ND	18.0±0.3	ND	-	ND

NI: No inhibition, ND: not determined, AMP: ampicillin, GEN: gentamycin, FLU: fluconazol, MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration, MFC: minimum fungicidal concentration, MIC, MBC and MFC of compounds are indicated in mg/ml.

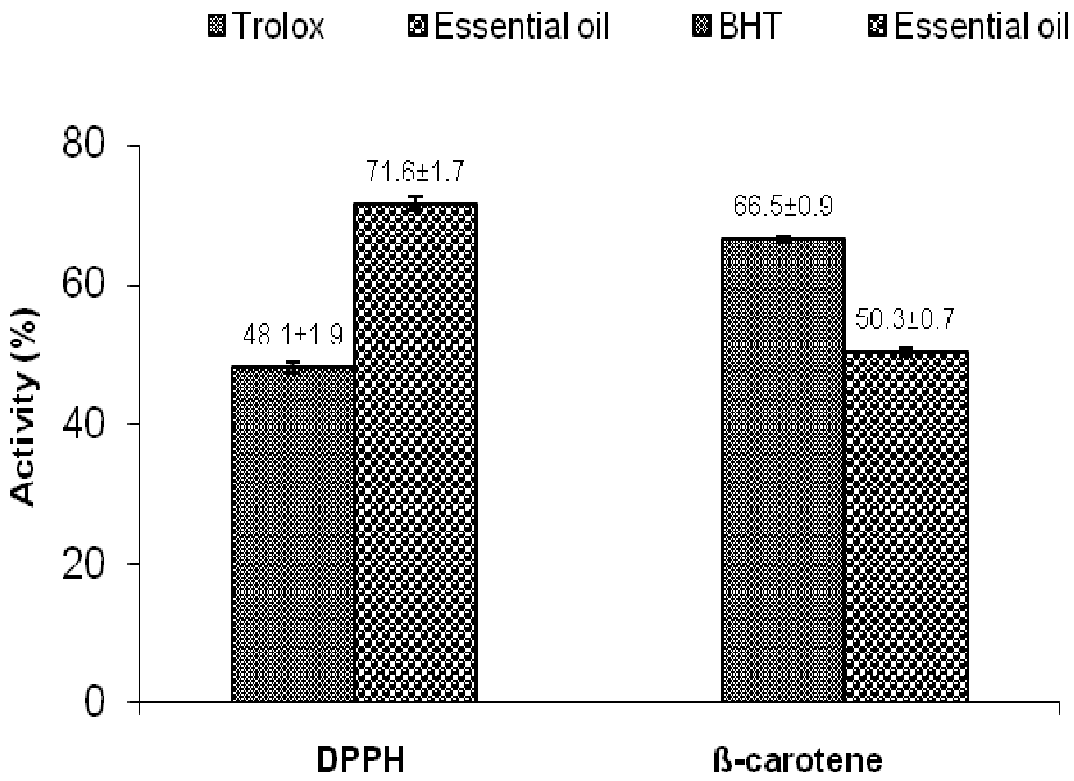

Figure 1. Free radical-scavenging and antioxidant activities of *A. kermanensis* essential oil in comparison with those of the references.

hydrodistillation and analysed by GC-MS (Khaled et al., 2002). Piperitone (45.0%), trans-ethyl cinnamate (20.8%) and ethyl-3-phenyl propionate (11.0%) were the predominant components, followed by spathulenol (6.27%), cis-ethyl cinnamate (5.64%), 2,6-dimethyl phenole (1.39%) and methyl cinnamate (1.06%). The oil showed antioxidative activity, determined by thiocyanate and scavenging effect on 1,2 diphenyl picrylhydrazyl (DPPH) methods. Its activity may be due to the presence of 2,6-dimethyl phenol (1.39%) and camphor (0.38%).

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