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Chemical Composition and Antimicrobial Activity of the Volatile Oil of *Artemisia khorassanica* from Iran

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Abstract

Artemisia khorassanica Podl. (Asteraceae) is a common perennial herb growing wild in northeastern parts of Iran. The essential oil of *A. khorasanica* was isolated by hydrodistillation in 1.25 (v/w) yield. The chemical composition of the essential oil was examined by gas chromatography and gas chromatography-mass spectrometry. Thirty-one compounds were identified, representing 79.6% of the total oil. The major constituents were 1,8-cineol (17.7%), camphor (13.9%), davanone (12.2%), and isogeraniol (5.7%). Minimum inhibitory concentration was determined using agar dilution method against eight bacteria and two fungal strains. The essential oil indicated a moderate antimicrobial activity.

Keywords: Antimicrobial, *Artemisia khorassanica*, Asteraceae, camphor, 1,8-cineol, davanone, essential oil, isogeraniol.

Introduction

The genus *Artemisia* is represented by 34 species growing in different parts of Iran, of which 2 species are endemic (Mozaffarian, 1996). Some *Artemisia* species known as Dermaneh in Iran are used in traditional medicine in gastritis, as tonic, and as antihelmentic. The decoction is used as an antiseptic in wounds (Zargari, 1996). *Artemisia* species, including *A. khorasanica* Podl. (Asteraceae) are used in dypepsia and other gastrointestinal disorders by local people in the northern part of Khorasan province. There are many reports on the essential oil compositions of different species of *Artemisia* (Matsuo et al., 1973; Abegaz & Yohannes, 1982; Nasr et al., 1983; Hifnawy et al., 1990; Khanina et al., 1991a,b, 1993; Carnat & Lamaison, 1992; Dung et al., 1992; Shanta et al., 1992; Arnold et al., 1993; Souleles, 1993; Chalchat et al., 1994; Figueiredo et al., 1994; Perfumi et al., 1995; Swiader & Krzyzanowska, 1997). Similarly, the essential oil compositions of six *Artemisia* species from Iran were reported in recent years (Weverstah et al., 1993; Rustaiyan et al., 2000; Ahmadi & Mirza, 2001; Khazraei-Alizadeh & Rustaiyan, 2001; Ahmadi et al., 2002; Sefidkon et al., 2002; Mohammadpoor et al., 2002) but the essential oil composition of *A. khorasanica* has not yet been reported. In the current study, the essential oil composition and antimicrobial activity of the oil of *A. khorasanica* were investigated for the first time.

Materials and Methods

Plant material and essential oil isolation

The plant material was collected from the Ghouchan mountains in Kkorasan province (Iran). The plant was identified by Mr. M.R. Joharchi at the Department of Botany, Ferdowsi University (Mashhad, Iran). A voucher specimen (no. 490) was deposited in the Herbarium of the Faculty of Pharmacy, Mashhad University of Medical Sciences. The aerial parts were air-dried at room temperature in the shade, and the oil was obtained by hydrodistillation using a Clevenger-type apparatus for 3 h, yielding 1.25% (v/w) of yellow oil. It was dissolved in *n*-pentane and dried over anhydrous sodium sulfate and stored at 4–8°C in the dark.

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Identification of the oil components

The gas chromatography (GC) analysis of the oil was carried out using Varian GC 3600 chromatograph with a DB-1 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm); carrier gas, helium, and a detector (FID). Oven temperature was programmed from 50 to 265°C at 2.5°C/min; injector and detector temperatures were 250°C. Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on a Varian 3400 GC-MS system equipped with a DB-1 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was as indicated for GC analysis. Injector and transfer line temperatures were 260°C; the carrier gas was helium with a linear velocity of 31.5 cm/s; split ratio, 1:10; ionization energy, 70 eV; scan time, 1 s; mass range, 35–400 amu. The retention indices for all the components were determined according to the Van Den Dool & Kratz (1963) method using *n*-alkanes as standard. Compounds were indentified by comparison of their retention indices (RI, DB-1) with those reported in the literature (Shibamoto, 1987; Davies, 1990) and by comparison of their mass spectra with those of computer library.

Antimicrobial assay

The antimicrobial activity of the essential oil was determined by a modification of the method reported by Zogda and Porter (2001). Minimum inhibitory concentrations (MICs) were evaluated against a panel of bacterial and fungal strains. The microorganisms tested included Gram-positive (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 29737), Gram-negative (*Enterobacter cloacae* PTCC 1003, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhi* PTCC 1185, *Serratia marcescens* Persian Type Culture Collection (PTCC) 1111, and *Shigella dysenteriae* PTCC 1188), and fungal strains (*Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231). Essential oil was mixed with DMSO (0.3 g/0.1 ml) and added to 30 ml of sterile molten

Table 1. Composition of the essential oil of *Artemisia khorassanica*.

Compounds	RRI (DB-1)	Percentage in oil	Methods of identification
α-Pinene	924	0.4	RI/MS
1,8-Cineol	1013	17.7	RI/MS
τ-Terpinene	1025	0.4	RI/MS
Filifolone	1075	0.4	RI/MS
Linalool	1088	1.5	RI/MS
Chrysanthnone	1092	0.8	RI/MS
Camphor	1117	13.9	RI/MS
Isogeraniol	1145	5.7	RI/MS
Terpin-4-ol	1157	3.9	RI/MS
β-Fenchyl alcohol	1168	3.3	RI/MS
Myrtenol	1173	0.7	RI/MS
cis-Ascaridol	1206	3.3	RI/MS
Geraniol	1233	0.5	RI/MS
Chrysanthenyl acetate	1238	1.1	RI/MS
endo-Bornyl acetate	1261	2.0	RI/MS
trans-Ascaridol	1269	2.3	RI/MS
trans-Sabinene hydrate acetate	1275	0.2	RI/MS
α-Terpinyl acetate	1324	0.7	RI/MS
cis-Jasmone	1359	4.7	RI/MS
Davanafurane	1386	0.1	RI/MS
trans-Caryophyllene	1398	0.6	RI/MS
α-Muurolene	1448	0.3	RI/MS
Ledene	1462	0.3	RI/MS
Eremophilene	1464	0.3	RI/MS
Sclareolide	1523	0.3	RI/MS
Caryophyllene oxide	1547	0.6	RI/MS
Davanone	1561	12.2	RI/MS
Ledol	1600	0.2	RI/MS
Globulol	1607	0.3	RI/MS
Plaustrol	1640	0.2	RI/MS
Davana ether	1710	0.7	RI/MS

RRI, Relative Retention Index; RI, Retention Index; MS, Mass Spectrometry.

Table 2. Antimicrobial activity of essential oil of *Artemisia khorassanica*.

Strains	MIC (ppm)
Bacillus subtilis ATCC 16404	1000
Enterobacter cloacae PTCC 1003	3000
Escherichia coli ATCC 8739	10,000
Pseudomonas aeruginosa ATCC 9027	1000
Salmonella typhi PTCC 1185	3000
Serratia marcescens PTCC 1111	3000
Shigella dysenteriae PTCC 1188	1000
Staphylococcus aureus ATCC 29737	3000
Aspergillus niger ATCC 16404	1000
Candida albicans ATCC 10231	3000

ATCC, American Type Culture Collection; PTCC, Persian Type Culture Collection; MIC, minimum inhibitory concentration.

Mueller-Hinton agar (MHA) at 45°C to give a 10,000 ppm concentration of oil. This medium was further diluted with sterile medium to produce the desired concentrations of oil ranging from 30 to 10,000 ppm. The highest concentration was 10,000 ppm because above this concentration, the DMSO concentration would increase to above 0.3% (v/v), which exhibited antimicrobial activity interfering with antimicrobial results of the oil. Six threefold serial dilutions were prepared in triplicate and poured into sterile 24-well plates. Each well was inoculated with about 1×10^4 microorganisms, applied as spots of about 3 mm in diameter. Bacterial strains were incubated at 37°C for 24 h. *Candida albicans* and *Aspergillus niger* were incubated at 25°C for 2 and 7 days, respectively. Two controls were included with each test batch. The first was a negative control that contained the test material but not the organisms to check for contamination of the test material. Positive control contained microorganisms without the test material. MICs were determined as the lowest concentrations that completely inhibited microbial growth.

Results and discussion

This is the first report on the composition of essential oil of *A. khorassanica*. The hydrodistillation of the aerial parts of *A. khorassanica* gave a pale green volatile oil in 1.25% (v/w) yield. The oil was analyzed by capillary GC and GC-MS. Thirty-one constituents, which accounted for 79.6% of the total oil, were identified (Table 1). All components in a relative amount $>0.1\%$ have been indentified. Major components were 1,8-cineol (17.7%), camphor (13.9%), davanone (12.2%), and isogeraniol (5.7%). The other notable minor components were *cis*-jasmone (4.7%), terpin-4-ol (3.9%), β-fenchyl alcohol (3.3%), and *cis*-ascaridol (3.3%). 1,8-Cineol and camphor were also found as the major components in other species of *Artemisia* from Iran (Khazraei-Alizadeh & Rustaiyan, 2001).

Antimicrobial activity of the oil was determined (Table 2). The oil was active against both Gram-positive and Gram-negative bacteria, being most active against *B. subtilis* and *P. aeruginosa* with MICs of 1000 ppm. It also indicated a moderate antifungal activity against *A. niger* and *C. albicans* with MICs of 3000 ppm. It has been reported that 1,8-cineol was active (Carson & Riley, 1995; Griffin et al., 1999; Kalemba et al., 2002) against bacteria and fungi. Camphor has also exhibited a moderate activity against bacteria and fungi (Griffin et al., 1999). Considering moderate antimicrobial activity of the essential oil observed in this study and moderate antimicrobial activity reported for both 1,8-cineol and camphor, the antimicrobial activity of the oil from *A. khorassanica* could be attributed to these two major components.

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