Pharmacognosy Research [Phcog Res.]
Vol 1, Issue 3, May-Jun, 2009 Page 120-124
[Rapid Publication]
A Supplement to Pharmacognosy Magazine – [Phcog Mag.]
Rapid publication process initiated: 20 February, 2009
Accepted: 9 April, 2009

PHCOG RES.: Research Article

Chemical Composition and Antimicrobial Activity of *Artemisia tschernieviana* Besser from Iran

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ABSTRACT:
The oil obtained from hydrodistillation of the aerial parts of *Artemisia tschernieviana* was analyzed by GC and GC/MS. The main constituents of the 30 identified components were p-cymene (21.3%), ß-pinene (17.8%), α-pinene (9.4%), γ-terpinene (9.1%), (Z)-cis-ocimene (8.8%), and α-cadinol (8.1%). This species is rich in monoterpenes. Antimicrobial activity was determined against six bacterial strains and one fungal strain. The results show that this oil is active against all the tested strains.
Key words: *Artemisia tschernieviana* Besser; Essential oil; Antimicrobial activity; p-cymene; ß-pinene; α-pinene; γ-terpinene; (Z)-beta-ocimene; α-cadinol

INTRODUCTION

Artemisia (Asteraceae) is one of the largest and most widely distributed genus 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 of them have been reported in Iran and some are endemic (1-2). Some substances from the genus have shown antimalarial, antiviral, antitumoral, antipyretic, antihemorrhagic, anticoagulant, antianginal, antioxidant, antihepatitis, antiulcerogenic, antispasmodic, anticomplementary and interferon-inducing activity (3-4). *A. annua* (Qinhaosu) is a traditional medicinal herb in China. It is now cultivated on a commercial scale in China and Vietnam for its antimalarial sesquiterpene(a lactone artemisinin) (5-6) and essential oil. The extract of the aerial parts of *A. diffusa* collected in the Province of Khorassan (Iran) afforded, in addition to several eudesmanolides, a new type of sesquiterpene lactone (Tehranolide) with an endoperoxide group that probably has the same effect as the antimalarial agent artemisinin. The antimalarial properties of the extract and the fraction that contains sesquiterpene lactones including Tehranolide have been reported (7-8). *A. austriaca* and *A. spicigera* are odorous herbs used as antiseptics and stomachics in folk medicine (9). *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 (10). *A. dracunculus* has been used orally as an antiepileptic in which its anticonvulsant potential has been assessed (11). Studies on *Artemisia* has ascertained the presence of coumarin (12), acetylenic compounds and sesquiterpene lactones (13-18). Although literature f the essential oils of different species of *Artemisia* (19-37) and its antimicrobial effects (22-23-30-34-36) is prevalent, no studies have been reported on the oil of *A. tschernieviana*. Therefore, we decided to investigate its chemical compositions and antimicrobial activity.

MATERIAL AND METHODS

Plant material - The aerial parts of *A. tschernieviana* were collected during the flowering stage in the

Behshahr province of Mazandaran, Iran, in October 2007. Voucher specimens (no. 88489) have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Preparation of oil

The air-dried aerial parts of *A. tschernieviana* (110.0 g) were subjected to separate hydrodistillation for 3 h using a Clevenger-type apparatus. After decanting and drying over anhydrous sodium sulfate, the corresponding yellowish oil was recovered in yields of 0.80 % w/w.

GC-MS analysis

The oil was analyzed using a Hewlett-Packard 5973 with a HP-5MS column (30 m x 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 readings were taken at 70 eV, mass range, 30 to 350 amu, and scan time, 2 scan/ sec. The constituents of each oil was identified by comparing their mass spectra and retention indices (RIs) with those in the literature and those authentic samples (37). GC analysis was performed on a Shimadzu 15A gas chromatograph equipped with a spilt/spiltless (ratio 1:30), injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 mL/min) and DB-5 (50 m x 0.2 mm, film thickness 0.32 pin) was used as the capillary column . The column temperature was kept at 60°C for 3 min and then heated to 220°C with a 5°C/min rate and maintained constant at 220°C for 5 min. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without using correction factors.

Microorganisms and Antimicrobial Studies

The antibacterial and antifungal activity of the essential oil was evaluated by the disc diffusion method using Mueller-Hinton and Sabouraud Dextrose agar, respectively (38). The bacteria included *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Entobacter aerogenes* (ATCC 49469), *Klebsiella pneumoniae* (ATCC 27736), *Bacillus creus* (ATCC 6633), *Bacillus subtilis* (ATCC 9372), and the fungi included *Candida albicans* (ATCC 6258). The microorganisms were obtained from the Research Center of Science and Industry, Tehran, Iran.

A serial dilution of the oil was prepared in Mueller-Hinton and Sabouraud dextrose broth for bacteria and fungi, respectively. The oil was diluted using water and ethanol solvents. The solvents, at an appropriate concentration, were also used as a negative control. The standardized suspension of bacteria and fungi was inoculated into each tube. The tubes were incubated at 37°C for 24 h for bacteria and at 30°C for 48-72 h for fungi. The lowest oil concentration, where there was no visible growth, was the minimum inhibitory concentration (MIC) when compared to 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 that did not show any growth and inoculated on Mueller-Hinton agar (for bacteria) and Sabouraud Dextrose Broth agar (for fungi) by streaking. Plates inoculated with bacteria and fungi were then incubated at 37 ^{0}C for 24 h and 30 ^{0}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 was calculated.

RESULTS AND DISCUSSION

The percentage and retention indices of the components of the volatile constituents obtained from *A. tschernieviana* are listed in Table 1. Thirty compounds, representing 98.3% of the total constituents in the oil of *A. tschernieviana,* were characterised by p-cymene (21.3%) and ß-pinene (17.8%) followed by α-pinene (9.4%), γ-terpinene (9.1%), (Z)-cis-ocimene (8.8%), and α-cadinol (8.1%). Monoterpene hydrocarbons constitute the major fraction of the oil (72.9%), while sesquiterpene hydrocarbons accounted for 0.9%. Oxygenated monoterpenes and oxygenated sesquiterpenes amounted to 10.4% and 14.0% of the oil, respectively. This *Artemisia* is rich in monoterpenes.

In some studies on the essential oils of other *Artemisia* species, p-cymene (17.4%, 16.8%, 16.5%, and 16.5%) is also characteristic of the oils of *A. scoparia* (19), *A. absinthium* (20-21), and *A. khorassanica* (22).

Pinane derivatives are found in the oils of some *Artemisia* species, for example, α-pinene is found in the oils of *A. annua* (23) and *A. biennis* (24), ß-pinene in *A. absinthium* (21, *A. campestris* (25-26), *A. scoparia* (27), and *A. moorcroftiana* (28), and γ-terpinene in *A. scoparia* (19).

(Z)-ß-ocimene, the main component of the oil of *A. tschernieviana*, is also characteristic of the oils of *A. glauca* (29) and *A. afra* (30).

In another study, ß-Pinene (24.0-49.8%) was found to be the main component of the oil of *A. campestris* from Tunisia (31). Monoterpene hydrocarbons were shown to be the major fraction (58.8-88.6%), and were

Table 1 :GC-MS analysis of the essential oil of A. tschernieviana

Compound	RI	*A. tschernieviana*
α-Pinene	939	9.4
Camphene	953	0.1
Sabinene	976	t
β-Pinene	980	17.8
Myrcene	991	1.2
α-Terpinene	1018	0.2
p-Cymene	1026	21.3
1,8-Cineole	1033	t
(Z)- β-Ocimene	1040	8.8
(E)- β-Ocimene	1050	4.9
γ-Terpinene	1062	9.1
Iso Terpinolene	1084	0.1
Linalool	1098	2.3
trans-Thujone	1114	1.4
Chrysanthenone	1123	0.6
Camphor	1143	1.4
Terpinen-4-ol	1177	1.1
α-Terpineol	1189	0.6
Citronellol	1228	t
Bornyl acetate	1285	1.0
Thymol	1290	0.2
trans-Pinocarvyl acetate	1297	0.3
Citronellyl acetate	1354	0.3
Geranyl acetate	1383	1.1
(E)-β-Farnesene	1458	t
Bicyclogermacrene	1494	0.9
(E)-Nerolidol	1564	1.6
Spathulenol	1576	3.5
α-Cadinol	1653	8.1
α-Bisabolol	1683	0.9
Monoterpene hydrocarbons		72.9
Oxygenated monoterpenes		10.4
Sesquiterpene hydrocarbons		0.9
Oxygenated sesquiterpenes		14.0
Total		99.2

[a] *Retention indices as determined on a DB-5 column using the homologous series of n-alkane*

Table 2 . Antimicrobial activity of the oil of A. tschernieviana against standard microorganisms

Microorganisms	Inhibition zone (mm)	MIC	MBC	MFC
Staphylococcus aureus (ATCC 5923)	10	312.5	625	-
Escherichia coli (ATCC 25922)	15	1250	2500	-
Klebsiella pneumoniae (ATCC 27736)	12	625	625	-
Entobacter aerogenes (ATCC 49469)	17	625	1250	-
Bacillus subtilis (ATCC 9372)	12	312.5	625	-
Bacillus creus (ATCC 6633)	10	1250	2500	-
Candida albicans (ATCC 6258)	10	312.5	-	650

MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration,
MFC: minimum fungicidal concentration.
MIC, MBC and MFC of compounds are indicated in µg/ml.

mainly composed of ß-pinene (24.0-49.8%), α-pinene (5.9-12.5%), ß-cymene (3.4-9.4%), limonene (4.9-9.3%), (Z)-ß-ocimene (0.2-5.5%), and γ-terpinene (2.0-6.5%).

In a similar research, from four areas of south-eastern Tunisia (32), ß-pinene (24.2-27.9%), p-cymene (17.4-22.3%), and α-pinene (4.1-11.0%) were observed to be the major constituents.

Camphor and 1,8-cineole were reported as the main constituents of many species of *Artemisia* (13); however, in our research these compounds were found in traces and as minor constituents, respectively.

Cadinane derivatives are characterized in the oils of *A. campestris* (31 and 33) whereas in our study α-cadinol was detected.

Results of the antimicrobial activities of the oil are shown in Table 2. Antimicrobial activity was determined against six bacterial strains and one fungal strain. The results show that this oil is active against all the tested strains. The oil has shown a maximum zone of inhibition against *Entobacter aerogenes*. *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans* were the most sensitive microorganisms to the essential oil (having an MIC value of 312.5 µg/ml).

Previous studies showed that terpene alkoholes are well-known antimicrobial compounds isolated from different plant species (34). The antimicrobial activity of the oil could, in part, be associated with terpene alkoholes such as linalool. These results may partly justify the traditional use of this *Artemisia*.

Composition and antimicrobial activity of the volatile oil of *A. kopetdaghensis* from Iran were investigated (35). The minimum inhibitory concentration was determined and the oil showed a moderate antimicrobial activity.

In another study, the essential oil of *A. lavandulaefolia* exhibited considerable inhibitory effects against all obligate anaerobic bacteria tested, while its major compounds demonstrated different degrees of growth inhibition (36).

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