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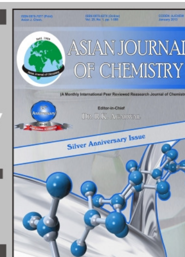

Chemical Composition and Antimicrobial Activity of *Artemisia tournefortiana* Rchb. Essential Oil

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The water distillation of the aerial parts of *Artemisia tournefortiana* has been analyzed by a combination of GC-FID and GC/MS in early flowering stage. The main components of *Artemisia tournefortiana* were (Z)-nerolidol (22.4 %), β-caryophyllene (15.6 %) and santolina triene (10.1 %). The antimicrobial activity of the oil was determined against eight bacteria and two fungi strains. The oil was active against all the tested strains. The oil has shown maximum zone of inhibition to *Staphylococcus aureus*. *Staphylococcus aureus* was the most sensitive microorganisms to the essential oil (having MIC value 125 and MBC value 250 µg/mL). The potent antimicrobial activity of oil could be associated with terpenes alcohols.

Key Words: *Artemisia tournefortiana*, Asteraceae, Essential oil, (Z)-nerolidol, β-Caryophyllene, Santolina triene, Antimicrobial activity.

INTRODUCTION

The genus *Artemisia* is one of the largest and widely distributed genera of the family Asteraceae. Thirty-four species of this genus are found in Iran, among which two are endemic: *A. melanolepis* Boiss. and *A. kermanensis* Podl.[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]. Some species are used in folk medicine; *A. annua* (Qinhaosu) is a traditional medicine herb of China. It is presently being cultivated on a commercial scale in China and Vietnam for its antimalarial sesquiterpene lactone artemisinin[4,5] and its essential oil. *A. austriaca* and *A. spicigera* are odorous herbs and used as antiseptics and stomachics in folk medicine[6]. The large genus *Artemisia* has been studied chemically by many researchers for example acetylenic compounds[7], flavonoides[8], coumarins[9] and sesquiterpene lactones[10]. The extract of the aerial parts of *A. tournefortiana* from Iran, was afforded three eudesmanolides[11]. Sanz *et al.*[12] found a new *cis*-eudesmanolide and three new eudesmane acids in extract of the aerial parts of *A. tournefortiana*. Also tourneforin, a novel eudesmanolide from *A. tournefortiana*, has been reported[13]. A numerous reports appear in the literature on *Artemisia* essential oils and antimicrobial activity[14-16]. Although the volatile constituents of *A. tournefortiana* from Iran previously has been reported in late flowering stage[17] but in this work, the essential oil of plant was analyzed in early flowering stage and antimicrobial activity of oil was determined for the first time.

EXPERIMENTAL

The aerial parts of *A. tournefortiana* was collected during the early flowering stage in Firuzkuh, Hesarbon, province of Tehran, Iran, in September 2009. A voucher specimen (No. 59077) has been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Isolation of the oil: The air-dried aerial parts *A. tournefortiana* of the plant (in the shade at room temperature, 172 g) was subjected to water distillation using a Clevenger-type apparatus for 3 h. After decanting and drying over anhydrous sodium sulfate, the corresponding yellowish coloured oil was recovered in yields of 0.2 % v/w. The sample were stored in dark glass bottles in a freezer (-5 ºC) until further use and analyze.

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 ºC) and a flame ionization detector (250 ºC). 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 ºC for 3 min and then heated to 220 ºC with a 5 ºC/min rate and kept constant at 220 ºC 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 ºC for 3 min and programmed to 220 ºC at a rate of 5 ºC/min and kept constant at 220 ºC 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/sec.

Identification of components: The compounds were identified by comparison of KI from DB-5 column with those reported in the literature and by comparison of their mass spectra with either the Wiley library or with published mass spectra[18-20]. The Kovats indices for all the components were determined according to the Van Den Dool method, using *n*-alkanes as standards[21].

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 tests: The antimicrobial activity of the essential oil was evaluated by a disc diffusion method using Mueller-Hinton and Sabouraud Dextrose agar respectively[22,23]. 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 ºC 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. tournefortiana* 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 ºC for 24 h and at 28 ºC for 72 h for the fungal strains. Ethanol (95 %) was used as a negative control in all the plates while ampicillin (10 mg/disc), gentamycin (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[24]. 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 ºC and 24 h for bacteria and at 30 ºC 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 ºC for 24 h and 30 ºC for 48-72 h respectively. After incubation the concentration at which no visible growth was seen was noted as minimum bactericidal concentration (for bacteria) and minimum fungicidal concentration (for fungi). All the experiments were carried out in triplicate and the mean calculated.

RESULTS AND DISCUSSION

The volatile components obtained from *A. tournefortiana* in early flowering stage are listed (Table-1). In this table, the percentage and retention indices of the components are given. Thirty compounds were obtained that representing 95.1 % of the total constituents in the essential oil. The main components were characterized by (Z)-nerolidol (22.4 %), β-caryophyllene (15.6 %) and santolina triene (10.1 %). Sesquiterpene hydrocarbons constitute the major fraction of the oil (26.3 %), while oxygenated monoterpenes accounted to 9.3 %. Oxygenated sesquiterpenes, monoterpene hydrocarbons and other compounds amounted to 22.4, 21.2 and 9.3 % of the oil, respectively. (Z)-nerolidol the main component of the oil of *A. tournefortiana*, is also characteristic of the oils of some species of *Artemisia*[25,26]. Likewise, caryophyllene derivatives are charactrized in the oils of many *Artemisia* genuses[14,17,27,28]. To best of our knowledge, santolina triene was not found in the oil of other species *Artemisia* as the major constituents. This compound is also obtained from other species as a minor componenets[29,28,30]. In previous studies on Iranian *A. tournefortiana* in late flowering stage, the oil were rich in trans-thujone (47.0 %), sabinene (16.5 %) and β-pinene (8.3 %)[17]. Other investigations about essential oils, also confirm our findings indicating the different oil components depending on the species, environment, specially flowering stage[31]. Subsequently, the influence of environmental factors as well as the quality of the seeds on the composition of essential oils can not be ruled out. The factors, such as day and night temperatures, photoperiod and light intensity are important in essential oil compositions[32]. Results of the antimicrobial activities of the oil are shown in Table-2. Antimicrobial activity was determined against eight bacteria and two fungi strains. The results showed that this oil was active against all of the tested strains. The oil has shown maximum zone of inhibition against *Staphylococcus aureus*. On the other hand, *Staphylococcus aureus* was the most sensitive microorganisms to the essential oil (having MIC value 125 and minimum bactericidal concentration value 250 μg/mL). Previous studies showed that terpene alcohols are well-known antimicrobial compounds isolated from different plant species[33]. The potent antimicrobial activity of oil could be associated with terpenes alcohols such as (Z)-nerolidol. The synergistic effects of these constituents 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[34]. In similar study, the chemical composition of the essential oil from *A. iwayomogi* Kitamura was analyzed by means of GC and GC/MS[15]. 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 β-

caryophyllene (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. In other report, the chemical composition and antimicrobial activity of the essential oil obtained from *A. lavandulaefolia* DC. were investigated[14]. Ninety-nine compounds accounting for 94.9 % of the essential oil were identified. The major compounds in the essential oil were β-caryophyllene (16.1 %), *cis*-chrysanthenol (7.0 %), 1,8-cineole (5.6 %), borneol (5.3 %), *trans*-β-farnesene (5.1 %), camphor (4.9 %), yomogi alcohol (4.5 %), α-terpineol (3.9 %) and α-humulene oxide (3.3 %). The essential oil and some of its major compounds were tested for antimicrobial activity against 15 different genera of oral bacteria. The oil exhibited considerable inhibitory effects against all obligate anaerobic bacteria (MIC values, 0.025 to 0.05 mg/mL; minimum bactericidal concentration values, 0.025 to 0.1 mg/mL) tested, while its major compounds demonstrated different degrees of growth inhibition.

TABLE-1
PERCENTAGE COMPOSITION OF AERIAL PARTS OF *A. tournefortiana* IN EARLY FLOWERING STAGE

Compound	RI[a]	(% W/W)[b]
Isopropyl acetate	648	t
Pentanal	697	t
Isopropyl butyrate	842	t
Santolin triene	923	10.1
α-Pinene	939	4.8
Isopropyl tiglate	973	4.3
Sabinene	976	0.5
α-Terpinene	1018	0.3
p-Cymene	1026	7.7
Limonene	1031	1.0
Hyacinthin	1043	t
γ-Terpinene	1062	4.5
n-Penthyl butyrate	1093	0.4
Ethyl heptanoate	1095	0.5
trans-Chrysanthemol	1143	3.6
Lavandulol	1166	0.9
Terpinene-4-ol	1177	1.4
α-Terpineol	1189	1.2
δ-Elemene	1339	2.6
α-Cubenene	1351	t
β-Caryophyllene	1418	15.6
Aromandendrene	1439	0.5
(E)-β-farnesene	1458	0.8
γ-Gurjunene	1473	0.5
γ-Muurolene	1477	1.2
En-in-dicycloether	1499	3.5
γ-Cadinene	1513	0.2
Geranyl isobutyrate	1514	2.2
δ-Cadinene	1524	4.8
(Z)-Nerolidol	1534	22.4
Total		95.5
Group components		
Monoterpene hydrocarbons		21.2
Oxygenated monoterpenes		9.3
Sesquiterpene hydrocarbons		26.2
Oxygenated sesquiterpenes		22.4
Other compounds		16.4

t = trace < than 0.05 %, [a]Retention indices, as determined on a HP-5 MS column, [b]Percentages obtained by FID peak-area normalization

Conclusion

In this research, 30 major and minor constituents of essential oils from *A. tournefortiana* were identified. The compound contents differed for the early or late flowering stages. The *A. tournefortiana* oil showed very good activity against *Staphylococcus aureus*. Therefore, the essential oil of *A. tournefortiana* can be used as medicinal plant in traditional applications because it has main components such as (Z)-nerolidol with potent antimicrobial activities.

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TABLE-2
ANTIMICROBIAL ACTIVITY OF THE AERIAL PARTS OIL OF *A. tournefortiana* AND ANTIBUTICS AGAINST STANDARD MICROORGANISMS

Microorganisms	Inhibition zone (mm)				MIC	MBC	MFC
	Oil	AMP	GEN	FLU			
Bacillus cereus (ATCC 6633)	12.0 ± 0.3	NI	20.0 ± 0.2	ND	500	1000	-
Bacillus subtilis (ATCC 6633)	18.0 ± 0.1	25.0 ± 0.2	20.0 ± 0.1	ND	250	500	-
Citrobacter spp	10.0 ± 0.6	23.0 ± 0.2	22.0 ± 0.7	ND	250	500	-
Entrobacter spp	10.0 ± 0.2	10.0 ± 0.1	20.0 ± 0.1	ND	500	1000	-
Escherichia coli (ATCC 25922)	15.0 ± 0.1	NI	25.0 ± 0.3	ND	125	500	-
Klebsiella pneumoniae (ATCC 27736)	10.0 ± 0.3	NI	20.0 ± 0.4	ND	250	500	-
Pseudomonas aeruginosa (ATCC 27852)	10.0 ± 0.2	NI	20.0 ± 0.2	ND	250	500	-
Staphylococcus aureus (ATCC 25923)	20.0 ± 0.3	NI	25.0 ± 0.2	ND	125	250	-
Aspergillus niger (ATCC 9142)	15.0 ± 0.2	ND	ND	20.0 ± 1.2	500	-	1000
Candida albicans (ATCC 6258)	15.0 ± 0.1	ND	ND	18.0 ± 0.3	250	-	500

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 µg/mL.

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