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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE VOLATILE OILS FROM *Artemisia turcomanica*

Z. Habibi,[1*] M. Yousefi,[1] M. Mohammadi,[1] F. Eftekhar,[2] T. Biniyaz,[1] and A. Rustaiyan[3]

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The genus *Artemisia* from the family Asteraceae is a genus of small herbs or shrubs and is represented by 34 species growing in different parts of Iran, of which two are endemic [1]. This perennial herb, distributed in different regions of Iran and China, has been of great interest to Iranian and Chinese folk medicine.

To the best of our knowledge, a detailed investigation of the aroma volatiles of *A. turcomanica* Gand. has not yet been undertaken. Previously two new germacranolides and several known sesquiterpene lactones have been isolated from the methanol extract of *A. turcomanica* [2]. The aim of the present work was to investigate the chemical profile of *A. turcomanica* essential oils by gas chromatography–mass spectrometry (GC–MS) and to determine their antibacterial activities.

Thirty-nine volatile components, which were identified on the basis of their mass spectra characteristics, retention indices, and ^{13}C NMR spectra, are presented in Table 1. The yields of the essential oils were 1.85% and 0.1% for stems and leaves, respectively. The volatile components identified accounted for 96.4% and 90.7% of the oil compositions.

After 3 h of distillation, the essential oil of leaves had a bright yellow color. It was extracted with diethyl ether and then dried with sodium disulfate.

The oil of the leaves contained 39 compounds with a yield of 1.85% (w/w), representing 96.4% of the total oil. The main constituents included linalool (16.5%), *cis*-chrysanthenyl acetate (15.3%), 1,8-cineole (13.4%), camphor (13%), and bornyl acetate (9.2%).

Eighteen compounds were identified in the volatiles from the stem of *A. turcomanica,* comprising 90.7% of the total oil in 0.1% (w/w) yield. The oil was rich in *cis*-chrysanthenyl acetate (29%), bornyl acetate (18%), camphor (9%), 1,8-cineole (6.9%), linalool (6.9%) and *trans*-nerolidol (6.2%).

Bornane derivatives (camphor, borneol, and bornyl acetate) and menthane derivatives such as 1,8-cineole have been shown to be the major characteristic components of many species of *Artemisia* such as *A. annua, A. vulgaris, A. diffusa, A. santonicum, A. spicigera, A. afra, A. asiatica, A. austriaca*, and *A. pedemontana* [3, 4]. Borneol and bornyl acetate were also identified in our *Artemisia* oils. As can be seen in Table 1, the amount of oxygenated monoterpenes was highest in leaves of the plant (80.8%). In comparison with the leaves the content of sesquiterpenes and oxygenated sesquiterpenes increase in the stems.

Recently, the chemical composition and antimicrobial and antioxidant activities of the essential oils isolated from aerial parts of seven wild sages from Western Canada (*A. absinthium* L., *A. biennis* Willd., *A. cana* Pursh, *A. dracunculus* L., *A. frigida* Willd., *A. longifolia* Nutt., and *A. ludoviciana* Nutt.) were investigated by GC–MS. High contents of 1,8-cineole (21.5–27.6%) and camphor (15.9–37.3%) were found in *A. cana*, *A. frigida*, *A. longifolia*, and *A. ludoviciana* oils. The oil of *A. ludoviciana* was also characterized by a high content of oxygenated sesquiterpenes with a 5-ethenyltetrahydro-5-methyl-2-furanyl moiety, of which davanone (11.5%) was the main component. *A. absinthium* oil was characterized by high amounts of myrcene (10.8%), *trans*-thujone (10.1%), and *trans*-sabinyl acetate (26.4%). *A. biennis* yielded an oil rich in (*Z*)-β-ocimene (34.7%), (*E*)-β-farnesene (40.0%), and the acetylenes (11.0%) (*Z*)- and (*E*)-en-yn-dicycloethers. *A. dracunculus* oil contained predominantly phenylpropanoids such as methyl chavicol (16.2%) and methyl eugenol (35.8%) [5].

1) Department of Chemistry, Faculty of Science, Shahid Beheshti University, GC, Tehran, Iran, fax: 89 21 22431663, e-mail: z_habibi@sbu.ac.ir; 2) Department of Microiology, Faculty of Biological Sciences, Shahid Beheshti University, GC, Tehran, Iran; 3) Department of Chemistry, Scinece & Research Campus, Islamic Azad University, Tehran, Iran. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 690–692, September–October, 2010. Original article submitted May 14, 2009.

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TABLE 1. Chemical Composition of *A. turcomanica* Oils, %

Compound	RI	Leaves	Stem	Compound	RI	Leaves	Stem
α-Thujene	930	0.43	–	Isobornyl propanoate	1385	0.07	–
α-Pinene	939	0.31	–	*cis*-Jasmone	1393	1.75	0.9
Camphene	954	4.43	0.5	*trans*-Caryophyllene	1419	0.39	–
Sabinene	975	0.13	–	(*Z*)-β-Farnesene	1443	0.06	–
β-Pinene	979	0.07	–	Geranyl propanoate	1478	0.18	
β-Myrcene	991	1.13	–	α-Selinene	1498	0.6	–
trans-Dehydroxylinalool oxide	993	0.08	–	(*Z*)-α-Bisabolene	1507	0.07	–
α-Terpinene[a]	1017	0.1	–	Geranyl isobutanoate	1515	0.24	–
p-Cymene	1025	3	1.1	Elemol	1550	0.26	0.4
1,8-Cineole[a]	1031	13.4	6.9	*trans*-Nerolidol	1563	0.74	6.2
γ-Terpinene	1060	0.56	0.2	Spathulenol	1578	1.15	2.8
cis-Linalool oxide[a]	1087	1.27	–	Caryophyllene oxide	1583	0.52	1
Terpinolene	1089	1.53	–	β-Eudesmol	1651	2.84	2.5
Linalool[a]	1097	16.5	6.9	Selin-11-en-4-α-ol	1660	1.61	3.3
Camphor[a]	1146	13	9.3	(2*E*),(6*E*)-Farnesyl acetate	1847	0.07	0.6
Pinocarvone	1165	0.13	–	Heneicosane	2100	0.17	Tr.
4-Terpineole	1177	2.58	0.7	Monoterpene hydrocarbons		6.69	1.8
α-Terpineol	1189	0.61	–	Oxygenated monoterpenes		80.8	72.1
Isobornyl formate	1239	0.13	–	Sesquiterpene hydrocarbons		1.12	–
Carvone	1243	1.13	–	Oxygenated sesquiterpenes		7.61	16.8
***cis*-Chrysanthenyl acetate**[a]	1265	15.3	29	Normal alkanes		0.17	Tr.
Bornyl acetate[a]	1289	9.15	18	Total		96.4	90.7
α-Terpinyl acetate	1349	0.63	0.4	Yield, w/w %		1.85	0.1

RI: kovat′s retention index, Tr.: trace (<0.05%).
[a]Identification method: RI, MS, ^{13}C NMR.

Analysis of *A. vulgaris* L. essential oil revealed the presence of 88 components, and the extracted oil was rich in camphor (16.8%), α-thujone (11.3%), germacrene D (7.2%), camphene (6.5%), 1,8-cineole (5.8%), and β-caryophyllene (5.4%) [6]. The main constituents of *A. haussknechtii* Boiss. from Iran included camphor (41.01%), 1,8-cineole (32.35%), *cis*-davanone (3.68%), 4-terpineol (2.99%), linalool (2.84%), β-fenchyl alcohol (2.72%), and borneol (2.58%) [7]. The chemical composition of *A. absinthium* oils extracted from plants grown in USA showed β-thujone (17.5–42.3%) and *cis*-sabinyl acetate (15.1–53.4%) as the main components [8]. Studies on *A. absinthium* and *A. dracunculus* oils from Turkey also showed chamazulene (17.8%), nuciferol butanoate (8.2%), nuciferol propionate (5.1%), and caryophyllene oxide (4.3%) as the main components, and the oils contained mainly (*Z*)-anethole (81.0%), (*Z*)-β-ocimene (6.5%), (*E*)-β-ocimene (3.1%), limonene (3.0%), and methyl eugenol (1.8%). Both oils had inhibitory effects on the growth of bacteria and fungi and also showed moderate to weak antioxidant activities, respectively [4, 9].

We investigated the antibacterial activity of our oil preparations against six bacterial laboratory standards. The results are presented in Table 2. The essential oils were active against *Bacillus subtilis* and *Staphylococus aureus* and were moderately active against *Klebsiella pneumoniae* and *Escherichia coli*. Two essential oils were inactive against *Pseudomonas aeruginosa* and *Enterococcus faecalis*. It can be seen that the greatest antibacterial activity belonged to the essential oil of leaves.

Oxygenated monoterpenes such as 1,8-cineole, camphor, terpinen-4-ol, linalool, α-terpineol, and borneol, which are representative components in some oils investigated, were reported to exhibit antimicrobial activity [10, 11]. Other *Artemisia* oils rich in camphor and 1,8-cineole were previously demonstrated to have potent antimicrobial activities *in vitro* [4, 9]. It is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might also influence the antimicrobial activity exhibited. Possible synergistic and antagonistic effects of compounds in the oil should also be taken into consideration [6].

Plant Material and Isolation Procedure. The aerial parts of wild-growing *A. turcomanica* were collected during the flowering stage in May 2008 from Dare gaz-Kalat road (North Khorasan Province, Iran). Voucher specimens have been deposited at the herbarium of the Research Institute of Forests and Rangelands by Dr. Mozaffarian, Tehran, Iran in Voucher No. AR-139-B. The air-dried stems and leaves of the plant (100 g) were powdered, and the volatile fraction was isolated by hydrodistillation for 3 h using a Clevenger-type apparatus.

TABLE 2. Antimicrobial Activity of the Oils

Microorganism	Inhibition zone diameter, mm[a]					
	Leaves	Stem	Gentamycin	Tetracycline	Ampicillin	Penicillin
Staphylococcus aureus	19.3	15	17	22	34	32
Bacillus subtilis	23.6	16	23	25	24	24
Enterococcus faecalis	–	–	8	11	30	11
Escherichia coli	15.3	9	20	19	16	–
Klebsiella pneumoniae	11.3	–	20	34	–	–
Pseudomonas aeruginosa	–	–	21	10	–	–

[a]Diameter of inhibition zones (mm) including diameter of sterile disk (6 mm).
(–), Inactive; (7–14), moderately active; (>14), highly active.

Oil Analysis Procedure. Gas chromatography and Gas chromatography–mass spectroscopy (GC–MS) analysis were performed according to our previous work [12].

Antibacterial Susceptibility. The antibacterial activity of the essential oils was studied using the disc diffusion method against six laboratory standards, including *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 9372, *Enterococcus faecalis* ATCC 15753*, Escherichia coli* ATCC 9763, *Klebsiella pneumoniae* ATCC 3583, and *Pseudomonas aeruginosa* ATCC 27852 [13]. Ten microliters of each oil was placed on the disc prior to being placed on bacterial lawns. The control antibiotic discs were gentamycin (10 μg), tetracycline (30 μg), ampicillin (10 μg), and penicillin (10 μg). Plates were incubated at 37°C for 24 h, at which the diameters of inhibition zones were measured in mm. Each assay was repeated three time, and the means were calculated.

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