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Full Length Research Paper

Chemical composition and antimicrobial activity of essential oils of different organs of three *Artemisia* species from Iran

Mohammad Bagher Pasha Zanousi[1]*, Parviz Aberoomand Azar[2] and Mahdi Raeesi[3]

[1]Department of Chemistry, Chalous Branch, Islamic Azad University, Chalous, Iran.
[2]Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[3]Department of Pharmacology, Chalous Branch, Islamic Azad University, Chalous, Iran

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In this study, the chemical composition of essential oils isolated from dried stems, leaves, and fruits of three wild sages from Northern Iran (*Artemisia absinthium* L.*, Artemisia annua* L.*, and Artemisia tschernieviana* B) were investigated by a combination of gas chromatography (GC) and gas chromatography (GC)/mass spectrometry (MS). A total of 83 components were identified accounting for 93.51 to 100.00% of the oil composition. *A. absinthium* oils were characterized by high amounts of β-thujone (24.27 to 40.91%), 1,8-cineole (8.10 to 14.09%), and sabinene(8.35 to 10.05%). *A. annua* yielded an oils rich in camphor (10.54 to 24.12%), borneol (1.48 to 27.78%), and 1,8-cineole (8.03 to 11.71%). β - pinene (13.65 to 22.37%), limonene (7.69 to 13.65%), and cubenol (3.50 to 15.43%) were the main components in the essential oils of *A. tschernieviana.* The total numbers of volatile compounds identified from *A. absinthium, A. annua, and A. tschernieviana* were 58, 84, and 86, respectively. In *A. absinthium* and *A. annua*, monoterpens were higher than the sesquiterpens, and oxygenated monoterpene compounds were the main constituents, but in oils of *A. tschernieviana*, monoterpene hydrocarbons were the main constituents. The results of the antimicrobial activity of all essential oils showed that the oils had varying degrees of growth inhibition against the microorganisms tested.

Key words: *Artemisia absinthium, Artemisia annua, Artemisia tschernieviana,* Compositae, essential oil composition, antimicrobial activity.

INTRODUCTION

Essential oils are volatile aromatic oils liquids isolated from plants by mainly hydrodistillation. Aromatic oils can be found in all the various parts of a plant, including the stem, leaves, flowers, seeds, and fruits. The principal use of the essential oil is in pharmaceutical, perfumes, cosmetics, and foods. The genus *Artemisia* belongs to a useful group of aromatic and medicinal plants comprising about 300 species found in the northern hemisphere (Morteza-Semnani et al., 2005). In Iran, the genus *Artemisia* of the family Compositae is represented by 40 species, including *Artemisia absinthium* L., *Artemisia annua* L., and *Artemisia tschernieviana* Bess (Bayramoglu et al., 2008; Kazemi et al., 2010; Mozaffarian, 1996). This large genus has been the subject of numerous chemical studies (Marco et al., 1998). The essential oil of aromatic herbs is traditionally obtained by hydrodistillation (Golmakani and Rezaei, 2008). Chemical analysis of *A. absinthium* has shown that its volatile oil is rich in thujone (α and β), which has been earlier reported as an anthelmintic (Mescher and Howlett, 1999; Morteza-Semnani and Akbarzadeh, 2005). In other research, analysis of the chemical composition of *A. annua* oils extracted from plants grown in USA showed β-thujone (17.5 to 42.3%) and cis-sabinyl acetate (15.1 to 53.4%) as the main components (Klayman et al., 1984). The chemical composition, antimicrobial and antioxidant

*Corresponding author. E-mail: mpashazanousi@yahoo.com.

activities of *A. absinthium* oil from turkey were also investigated. The results showed chamazulene (17.8%), nuciferol butanoate (8.2%), nuciferol propionate (5.1%), and caryophyllene oxide (4.3%) as the main components in *A. absinthium* oil. The oils had inhibitory effects on the growth of bacteria and fungi tested and also showed moderate to weak antioxidant activities (Kordali et al., 2005a,b., 2006). *Artemisia* species have been reported to contain number of coumarins, flavones, and terpenes (Ma et al., 2007). Many secondary metabolites of terpene peroxides are isolated from *A. annua* L., such as artemisia ketone, artemisinic alcohol, arteannuin B, and myrcene hydroperoxide (Bertea et al., 2005; Brown et al., 2003). The chemical composition, antimicrobial activity of the aerial part of *A. tschernieviana* oil from Iran, was investigated. The results showed, p-cymene (21.3%), β-pinene (17.8%), α-pinene (9.4%), γ-terpinene (9.1%), and (z)-cis-ocimene (8/8%) as the main components, and the oil was active against six bacterial stains and one fungal strain (Kazemi et al., 2009). Sabinyl acetate, is one of the major compounds characterized in the oils of *A. absinthium* in different geographical origin (Orav et al., 2006). In the oil of *A. annua,* camphor was the major compound (48.0%) (Verdian-Rizi et al., 2008).

The medicinal properties attributed to the oils of the genus *Artemisia* prompted us to investigate the chemical constituents and antimicrobial activity of the oils of different organs of *A. absinthium, A. annua,* and *A. tschernieviana* by hydrodistillation method.

EXPERIMENTAL

Plant materials

Stem, leaves and fruit of *A. absinthium, A. annua,* and *A. tschernieviana* were collected in September 2010 from the Kojur of Nowshahr, Province of Mazandaran, Northern Iran. Voucher specimens have been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Science, Tehran, Iran.

Oil isolation

The dried stems (100 g), leaves (100 g) and fruits (80 g) were separately subjected to hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia (1996), for 4 h. The essential oils were collected, dried under sodium sulphate and stored at 4°C until used. Essential oil yield was expressed in terms of the weight of the oil collected per gram of dry plant material.

Gas chromatography (GC) and GC/mass spectrometry (MS) analysis

GC analysis were 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 the 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^{-1} rate and was kept constant at 220°C for 5 min. Relative percentage amounts were calculated from peak area using a CR5 SHIMADSU CR PACK without the use of correction factors. GC/MS analysis were 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^{-1} and was kept constant at 220°C for 5 min. The flow rate of helium as carrier gas was 1 ml/min, final temperature at 230°C and detector temperature at 250°C; mass spectrometry (MS) were taken at 70 eV (E1), electron multiplier voltage at 1800 eV; mass range from 30 to 350 amu; and scan time at 2 scan/s.

Identification of components

The components of the oil were identified by the comparison of their mass spectra with those of the MS literature data or with authentic compounds and were confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature (Adams, 1995). The retention indices were calculated for all volatile constituents using a homologous series of C_9 to C_{18} n-alkanes (Adams, 1995; McLafferty and Stauffer, 1989).

Antimicrobial activity

The antimicrobial activity of the essential oils of stems, leaves, and fruits of *A. absinthium, A. annua*, and *A. tschernieviana* were individually tested against ten microorganisms, including, five Gram-positive bacteria: *Bacillus subtilis* ATCC 10702, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 15753, *Listeria monocytogenes* ATCC 25923, and *Staphylococcus epidermidis* ATCC1228*,* and five Gram-negative bacteria: *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 27852, *Salmonella typhimurium* CCM 5445, and *Enterococcus cloacae* ATCC 13047. The test bacteria were screened for susceptibility to the differently extracted essential oils using the standard agar cup well diffusion method as described by Deans and Ritchie (1987), using a cut off screening concentration of 10 mg/ml of the oils in hexan (Glauciemar et al., 2009; Neda et al., 2004). For this purpose, 10 µl of each oils was placed on the disc prior to being placed on bacterial lawns. The control antibiotic discs were, 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 times, and the means were calculated.

RESULTS AND DISCUSSION

Hydrodistillation of the dried stems, leaves, and fruits of *A. absinthium, A. annua,* and *A. tschernieviana* oils with yields of 0.35, 0.75, and 0.70%, w/w for *A. absinthium*, 0.28, 0.56, and 0.51% w/w for *A. annua*, and 0.25, 0.45, and 0.40% w/w for *A. tschernieviana*, respectively. The highest and the lowest yields were in the leaves and stems oils in *A. absinthium* and *A. tschernieviana* in the amounts 0.75 and 0.25%, respectively. 83 volatile

Table 1. Yield, extraction time, and chemical composition of *A.absinthium, A. annua,* and *A. tschernieviana* essential obtained by HD.

Compound[a]	RI[b]	Percentage composition (%)								
		A. absinthium			*A. annua*			*A. tschernieviana*		
		Stem	Leaf	Fruit	Stem	Leaf	Fruit	Stem	Leaf	Fruit
α-Thujene	930	-	-	0.26	-	-	-	-	0.66	0.46
α Pinene	939	1.59	1.85	4.41	5.16	14.27	4.30	8.52	9.19	6.96
Camphene	960	-	-	-	3.52	4.49	1.13	1.15	1.86	0.81
Sabinene	975	10.05	9.78	8.35	0.52	0.82	-	2.14	3.05	2.24
β-Pinene	979	6.29	6.28	8.46	-	2.45	-	22.37	21.68	13.65
1-Decene	990	-	-	-	-	-	-	-	-	0.34
Myrcene	991	-	0.56	0.33	-	-	-	-	-	-
dehydro-1,8-Cineole	991	-	-	-	-	-	1.59	-	0.23	0.42
α-Phellandrene	1003	2.79	2.84	2.20	0.32	-	-	-	-	-
α-Terpinene	1017	-	-	-	-	0.32	-	0.69	1.14	0.37
p-Cymene	1025	0.73	1.11	2.62	0.49	0.43	-	-	-	-
o-Cymene	1026	-	-	-	-	-	-	3.91	5.81	5.76
Limonene	1029	-	-	0.17	-	-	-	13.27	13.65	7.69
1,8-Cineole	1031	14.09	13.58	8.10	11.71	8.03	9.67	1.08	3.02	0.71
(Z)- β-Ocymene	1037	-	-	-	-	-	-	1.66	2.78	0.42
γ-Terpinene	1060	-	-	0.37	-	-	-	10.76	13.23	4.00
Artemisia ketone	1062	-	-	-	2.45	3.08	9.83	-	-	-
cis-Sabinene hydrate	1070	-	0.54	0.37	-	0.55	0.73	-	-	-
Artemisia alcohol	1084	-	-	-	-	0.52	1.39	-	-	-
Terpinolene	1089	-	-	-	-	-	-	-	0.62	-
Linalool	1097	-	0.64	0.73	-	-	-	1.63	2.82	0.42
trans-Sabinene hydrate	1098	-	-	0.24	-	0.35	-	-	-	-
α-Thujone	1102	1.93	2.21	1.77	-	-	-	-	-	-
β Thujone	1114	40.91	36.44	24.27	-	-	-	-	-	-
cis-para-Menth-2-en-1-ol	1122	-	-	-	0.80	-	-	-	-	-
α Campholenal	1126	-	-	-	-	1.32	-	-	-	-
iso-3-Thujanol	1138	1.01	1.26	2.30	-	-	-	-	-	-
trans-Pinocarveol	1139	-	-	-	-	-	9.66	-	-	0.92
trans-p-Menth-2-en-1-ol	1141	-	-	-	0.51	-	-	-	-	-
cis-Sabinol	1143	-	-	-	1.61	-	-	-	-	-
Camphor	1146	-	-	-	19.61	24.12	10.54	-	-	-
Isopulegol	1150	-	-	0.19	-	-	-	-	-	-
neoiso-3-Thujanol	1152	-	-	0.21	-	-	-	-	-	-
cis-Chrysanthenol	1164	8.92	8.87	18.92	0.34	-	-	-	-	-
Pinocarvone	1165	-	-	-	1.66	5.93	4.85	-	-	0.45
Borneol	1169	-	-	-	27.78	1.48	-	-	-	1.23
4-Terpineol	1177	0.91	1.12	2.41	1.42	1.49	1.67	1.43	2.00	1.13
α-Terpineol	1189	1.70	1.84	1.11	0.42	0.63	0.64	0.59	0.99	0.24
Myrtenol	1196	-	-	-	-	0.94	1.45	-	-	0.26
trans-Piperitol	1208	-	-	-	0.42	-	-	-	-	-
trans-Carveol	1217	0.77	1.05	3.67	-	0.55	-	-	-	-
Citronellol	1226	-	-	-	-	-	-	-	-	0.80
Carvone	1243	-	-	-	-	0.15	-	-	-	-
Geraniol	1253	-	-	0.31	-	-	-	-	-	-
cis-Chrysanthenyl acetate	1265	-	-	0.46	-	-	-	-	-	-
Bornyl acetate	1289	-	-	-	10.84	-	-	3.90	3.96	5.62
Citronellyl acetate	1353	-	-	-	-	-	-	0.40	0.33	0.28
Eugenol	1359	-	-	-	-	-	0.91	-	-	-
Neryl acetate	1362	-	-	-	-	-	-	-	0.25	-

Table 1. Contd.

α Copaen	1377	-	-	-	-	-	0.94	-	-	-
Geranyl acetate	1381	-	-	-	-	-	-	1.45	1.33	0.62
cis-Jasmone	1393	-	-	-	-	0.21	-	-	-	-
α -Gurjunene	1410	-	-	-	-	-	-	-	-	1.15
e β Caryophyllen	1419	-	-	-	3.23	1.32	4.81	-	0.49	-
(z)- β-Farnesene	1443	-	-	-	-	-	2.45	-	-	-
Dehydro-Sesquicineol	1471	-	-	-	-	-	-	0.57	-	-
γ-Gurjunene	1477	-	-	-	-	-	-	-	0.42	-
β-Chamigrene	1478	-	-	-	-	1.30	-	-	-	-
Geranyl propanoate	1478	1.28	1.63	-	-	-	-	-	-	-
Germacrene D	1485	-	-	0.49	-	3.46	-	-	-	-
β-Selinene	1490	-	0.44	0.64	0.75	6.43	0.45	0.50	-	-
cis-β-Guaine	1493	-	-	-	-	-	-	-	-	0.40
Cubebol	1494	-	-	-	1.20	-	4.30	-	-	-
Valencene	1496	-	-	-	-	-	4.41	0.45	-	-
α-Selinene	1498	-	-	-	1.08	-	-	-	-	-
Bicyclogermacrenel	1500	-	-	-	-	-	-	1.21	1.09	-
(z)-Nerolidol	1533	-	-	-	-	-	-	2.86	1.98	10.40
α-Calacorene	1546	-	-	0.83	-	-	-	-	-	-
Citronellyl propionate	1549	-	-	-	-	-	-	-	-	0.81
Geranyl butanoate	1564	-	-	0.21	-	-	-	-	-	-
Spathulenol	1578	-	-	0.24	-	-	1.39	1.26	0.65	10.00
Caryophellene oxide	1583	-	-	-	0.32	1.48	7.21	-	-	-
Anozol	1591	-	0.17	-	-	1.29	-	-	-	-
Viridiflorol	1593	-	-	-	-	-	-	0.83	0.31	1.42
Salvia-4(14)-en 1- one	1595	-	-	-	-	-	0.59	-	-	-
epi-α -Cadinol	1640	3.00	3.20	2.59	-	1.15	0.82	-	-	-
epi- α-Muurolol	1642	-	-	-	-	3.06	-	-	-	-
α-Muurolol	1646	-	-	-	-	-	-	0.67	-	-
Cubenol	1647	-	-	-	-	0.65	2.42	11.65	3.50	15.43
α-Bisabolol	1686	-	-	-	-	0.29	1.52	4.75	2.35	2.83
Chamazulene	1732	4.03	3.33	0.74	-	-	-	-	-	-
14-hydroxy -α-Muurolene	1780	-	-	-	-	1.33	3.88	-	-	-
α-Eudesmol-acetate	1795	-	-	-	-	1.78	-	-	-	-
Total	-	100.00	98.74	97.97	96.16	95.96	93.51	100.0	99.39	98.04
Monoterpene hydrocarbons	-	21.45	22.42	27.17	10.01	22.78	5.43	64.77	73.67	42.70
Oxygen-containing monoterpens	-	71.52	69.35	65.27	79.57	50.93	52.93	10.48	13.60	13.10
Sesquiterpene hydrocarbons	-	4.03	3.77	2.70	5.06	12.51	13.02	2.73	2.24	1.55
Oxygen-containing sesquiterpen	-	3.00	3.20	2.83	1.52	9.74	22.13	22.02	9.88	40.69
Yield (%,w/w-dry basis)	-	0.35	0.75	0.70	0.28	0.56	0.51	0.25	0.45	0.40

[a]Compounts presented in order of alution from the HP-5MS capillary column; [b]Kovat's retention index to n-alkans on the HP-5MS capillary column.

compounds components were identified on the basis of their mass spectra characteristics, retention indices and co- chromatography with available standards using HP-5MS capillary column. α-pinene, 1,8-cineole, 4-terpineol, and α-terpineol were in the essential oils of organs of all species. The percentages of each component were listed in Table 1. In *A. absinthium*, 16, 21, and 31 components were identified in the stem, leaf and fruit oils, which represented 100, 98.74, and 97.97% of the total compositions of the oil, respectively. In the stems, leaves and fruits, β-thujone (40.91, 36.44, and 24.27%), 1.8-cineole (14.09, 13.58, and 8.10%), sabinene (10.05, 9.78, and 8.35%) and cis-chrysanthenol (8.92, 8.87, and 18.92%) were the major components, respectively. In *A. annua,* 23, 34 and 27 components were identified in the stems, leaves and fruits oils, which represented 96.16, 95.96 and 93.51% of the total compositions of the oil, respectively. In the stems, leaves and fruits, camphor (19.61, 24.12, and 10.54%), 1.8-cineole (11.71, 8.03, and 9.67%), and α-pinene (5.16, 14.27, and 4.30%) were the major

Table 2. Antimicrobial activity of essential oils organs of *A. absinthium, A. annua* and *A. tschernieviana.*

Microorganism	Inhibition zone diameter (mm)[a]									Standard antibiotics	
	A. absinthium			*A. annua*			*A. tschernieviana*				
	Stem	Leaf	Fruit	Stem	Leaf	Fruit	Stem	Leaf	Fruit	Ampicillin	Penicillin
B. subtilis	16.2	24.2	25	14	19.2	21.8	-	11	12	26	26
L. monocytogenes	14	19.2	18.7	7	12	13	-	10	10	18	15
S. aureus	15	23	25	15	21	23	-	12	13	32	30
E. faecalis	7.8	9.5	9	7	10	12	-	-	-	26	16
E. epidermidis	17	21	20	14	19	18.5	-	11.9	11.5	20	20
E. cloacae	7.2	14	13	-	9	10	-	9	7	16	14
E. coli	7	10	10	-	9	8	-	12.3	12	12	6
K. pneumoniae	7.2	11	10	9	12	15	-	11	11	-	-
P. aeruginosa	-	-	-	-	-	-	-	-	-	-	-
S. typhimurium	-	-	-	-	8	10	-	-	-	22	19

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

components, respectively. In *A. tschernieviana*, 26, 28 and 32 components were identified in the stem, leaf and fruit oils, which represented 100, 99.39, and 98.04% of the total compositions of the oil, respectively. In the stems, leaves and fruits, β-pinene (22.37, 21.68, and 13.65%), limonene (13.27, 13.65, and 7.69%), cubenol (11.65, 3.50, and 15.43%) and α-pinene (8.52, 9.19, and 6.96%) were the major components, respectively. The volatile components identified accounted for 93.51 to 100% of the oils composition. The analyzed oils can be classified in four groups according to their chemical make-up. In *A. absinthium* and *A. annua,* oxygenated monoterpens were the main constituents for oils of stems, leaves, and fruits in the amounts (71.52, 69.35, and 65.27%) and (79.57, 50.93, and 52.93%), respectively. But in *A. tschernieviana,* monoterpene hydrocarbons were the main constituents for oils of stems, leaves, and fruits in the amounts (64.77, 73.67, and 42.70%). The highest percentage of oxygenated sesquiterpens was for oil of fruit of *A. tschernieviana* in the ratio of 40.69%. Previous research showed that bornane derivatives (camphor, borneol, and bornyl acetate) and 1.8-cineole were the major characteristic components of many *Artemisia* genus, such as: *A. annua, Artemisia vulgaris, Artemisia diffusa, Artemisia specigera, Artemisia afra, Artemisia asiatica*, and *Artemisia pedemontana* (Kazemi et al., 2009; Klayman et al., 1984; Kordali et al., 2005a). *A. absinthium* oil was characterized by high amount of myrcene (10.80%), trans- thujone (10.10%), and trans- sabinyl acetate (26.40%). Approximately, 71% of *A. absinthium* oil composition was identified. The remaining unidentified components were monoterpene esters and sesquiterpens (Lutz, 2008). Pinane derivatives were found in the oils of some *Artemisia* spp., for example, α-pinene were found in the oils of *A. annua* (Rassli et al., 2003) and *Artemisia biennis* (Nematollahi et al., 2006). On the other hand, the antimicrobial activities of the essential oils of different organs of *Artemisia* spp. were presented in Table 2. The results showed that the oils had varying degrees of growth inhibition against the microorganisms tested. The essential oils of *A. absinthium* were active against *B. subtilis, L. monocytogenes, S. aureus,* and *E. epidermidis* and moderately active against *E. faecalis, E. cloacae, E. coli,* and *K. pneumonia,* but had no effect on the growth of *P. aeruginosa,* and *S. typhimurium*. The essential oils of *A. annua*, were active against *B. subtilis, S. aureus*, and *E. epidermidis* and moderately active against *L. monocytogenes, E. faecalis, K. pneumonia*, and *E. cloacae, E. coli*, and *S. typhimurium*, except in stem that was inactive, but had no effect on the growth of *P. aeruginosa.* The essential oils of *A. tschernieviana*, were moderately against *B. subtilis, S. aureus, E. epidermidis, L. monocytogenes, K. pneumonia, E. cloacae*, and *E. coli,* but had no effect on the growth of *P. aeruginosa, E. faecalis*, and *S. typhimurium.* The essential oil of stem was also inactive on the growth all of the microorganisms.

It can be seen in Table 2, that the greatest antibacterial activity belonged to the essential oil of leaf of *A. absinthium,* and all of the essential oils were inactive against *P. aeruginosa.* On the other hand, the comparison of these findings with the control antibiotics (Ampicillin and Penicillin) showed that these essential oils have strong antibacterial activity against all micro-organisms, except *P. aeruginosa.*

Conclusion

Hydrodistillation was used to obtain the essential oils of stem, leaves, and fruit of three *Artemisia* species; *A.*

absinthium, A. annua, and *A. tschernieviana.* GC/MS analysis indicated the apparent difference in the volatile compound composition of essential oils between species. The total numbers of compounds identified from *A. absinthium, A. annua*, and *A. tschernieviana* were 58, 84, and 86, respectively. α-pinene, 1,8-cineole, 4-terpineol, and α-terpineol were in the essential oils of organs of all species. All oils consisted of monoterpens and sesquiterpens, but monoterpens were the main constituents. Antimicrobial properties of essential oils are of great interest in food, cosmetic, and pharmaceutical industries since their possible use as natural additives emerged from the tendency to replace synthetic preservatives with natural ones. This study has revealed that the oils obtained had varying degrees of growth inhibition against the microorganisms tested.

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