

Chemical composition and general toxicity of essential oils extracted from the aerial parts of *Artemisia armeniaca* Lam. and *A. incana* (L.) Druce growing in Iran

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Abstract

The essential oils of the aerial parts of *A. armeniaca* and *A. incana*, collected from Arasbaran area (East Azarbaijan province, Iran) were extracted by hydrodistillation and analyzed by GC-MS. In total, 16 and 40 constituents were identified and quantified in the oils of *A. armeniaca* and *A. incana* representing 80.5% and 84.6% of the oils, respectively. The essential oil of *A. armeniaca* was mainly composed of non-terpene hydrocarbons (24.8%). The major components of the oil were α -pinene (10.7%), nonadecane (10.0%), 6,10,14-trimethyl-2-pentadecanone (9.4%), spathulenol (7.8%) and Z-verbenol (5.8%). The essential oil of *A. incana* was dominated by oxygenated monoterpenes (41.6%), with camphor (20.4%), 1,8-cineol (10.3%), Z-verbenol (8.7%), β -thujone (8.3%) and α -thujone (5.6%), as major components. The essential oils were also subjected to general toxicity assay using brine shrimp lethality method. The toxicity profile of both oils indicated some degree of toxicity in comparison with podophyllotoxin.

Keywords: *Artemisia incana*; *Artemisia armeniaca*; General toxicity; Monoterpene; Brine shrimp

INTRODUCTION

The genus *Artemisia*, an important member of the Asteraceae family, is mostly distributed in Europe, North America, Asia and south Africa (1,2), and represented in Iranian flora by 34 species (3). According to the literature, this genus is rich in monoterpenoids, sesquiterpenoids, coumarins and flavonoids (4). *A. armeniaca*, locally known as "Dermane ye Armanestani" is an Iranian evergreen or semi-evergreen sub-shrub of the genus *Artemisia* (5,6). In our previous studies on this genus, we had reported the isolation and structure elucidation of two new coumarin-hemiterpene ether glycosides (5), four prenylated coumarins and some known flavonoids from the aerial parts of *A. armeniaca* (6). The other species in our work, *A. incana*, is a perennial herb usually growing on sloppy rocks (7).

Previous works have been carried out on chemical composition of essential oils of

A. incana from different geographical locations. In continuation of our studies on *Artemisia spp*, we have now conducted to evaluate the general toxicity activities of the essential oils of *A. armeniaca* and *A. incana* collected from Iran (East Azarbaijan province) by relative test as well as comparing the chemical composition of the oils with previous investigations.

MATERIALS AND METHODS

Plant material

The aerial parts of *A. armeniaca* and *A. incana* were collected from Arasbaran, East Azarbaijan province, Iran, in August 2008. The identity of the plants was confirmed by anatomical examination in comparison to the herbarium specimens. voucher numbers TBZ-fph 528 and TBZ-fph 527 are retained in the School of Pharmacy, Tabriz University of Medical Science, Tabriz, Iran.

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Isolation of the essential oils

Air dried and ground aerial parts of *A. armeniaca* and *A. incana* (40 and 70 g, respectively) were submitted to hydrodi-stillation for 4 h, using a Clevenger type apparatus. Because of low amounts of oils in the plants, xylene was used to take up the essential oils. The oils were dried over anhydrous sodium sulfate and stored at 4°C for further use (8).

GC-MS analyses

The essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS-QP5050A, DB-1 capillary column. Operating conditions were as follows: Carrier gas, helium with a flow rate of 1.3 (*A. armeniaca*), 1.0 (*A. incana*) ml/min; column temperature, 2 min in 50°C, 50-275°C at 5.0°C/min and finally 3 min in 275°C (*A. armeniaca*), 2 min in 100°C, 100-275°C at 1.0°C/min and finally 3 min in 275°C (*A. incana*); injector temperature, 250°C; detector temperature, 280°C; volume injected, 1 µl of oil in xylene (0.1 %); split ratio 1:49 (*A. armeniaca*), 1:62 (*A. incana*). The mass operating parameters were as follows: ionization potential, 70 eV; ion source temperature, 260°C; solvent delay 2.0 min, mass range 30-600 amu. Components of the essential oils were identified by comparison of their mass spectra with those of the spectrophotometer database using the NIST 107, NIST 21, NIST 69 and Wiley 229 mass spectral database or with authentic compounds. The identification of compounds was confirmed by comparison of the fragmentation pattern and their retention indices (RI).

Brine shrimp lethality assay

The general toxicity of the essential oils was monitored by Brine shrimp lethality bioassay described by Meyer and coworkers with some modifications (9,10). Essential oils were dissolved in DMSO and diluted with artificial seawater so that the final DMSO concentration did not exceed 1%. Seven different concentrations of essential oils were obtained by serial dilution. Then 10 nauplii (hatched brine shrimp, *Artemia salina*, Sera, Turkey) were transferred to each test and

control (containing DMSO, xylene and seawater) tubes. 24 h after introducing the shrimps, the number of survivals at each dosage was counted and recorded. LD₅₀ values were determined from the best-fit line plotted concentration versus percentage lethality. Podophyllotoxin was used as a positive control.

Statistical analysis

The percentage lethality was calculated from the mean survival of larvae of in oil treated tubes and control ones?. The data were reported as mean ± standard deviation. LD₅₀ values were calculated by best-fit line method.

RESULTS

Essential oils from aerial parts of *A. incana* and *A. armeniaca* were analyzed by GC-MS. Forty and sixteen components representing 84.6% and 80.5% of the total oils of *A. incana* and *A. armeniaca* were respectively identified (Table1).

In the case of *A. incana*, monoterpenes made up the higher contribution (78.3%) with oxygenated dominating (41.6%) while the content of sesquiterpenes amounted to 6.3%. Among these compounds, the main ones were camphor (20.4%), 1,8- cineol (10.3%), Z-verbenol (8.7%), β-thujone (8.3%) and α-thujone (5.6%).

In the case of *A. armeniaca*, non-terpene hydrocarbons, monoterpene hydrocarbons, oxygenated monoterpenes and oxygenated sesquiterpenes represented 24.8%, 13.6%, 8.7% and 11.7% of the essential oil respectively. Other common constituents (21.7%) were non-terpene derivatives such as aldehydes and ketones. α-pinene (10.7%), nonadecane (10.0%), 6,10,14-trimethyl-2-pentadecanone (9.4%), spathulenol (7.8%) and Z-verbenol (5.8%) were the most abundant components of the essential oil.

In brine shrimp lethality assay, compared with positive control (Podophyllotoxin, LD₅₀= 2.69 µg/ml) the essential oils of *A. incana* and *A. armeniaca* showed weak to moderate toxicity against brine shrimp with LD₅₀ values 49.98 ± 2.81 and 56.94 ± 2.37 µg/ml, respectively.

Table 1. Composition of the essential oils of the aerial parts of *A. armeniaca* and *A. incana*

Compound	RI ^a	<i>A. incana</i> (%)	<i>A. armeniaca</i> (%)	Identification method ^b
tricyclen	919	0.3	-	RI+MS
benzaldehyde	925	-	1.6	RI+MS
α -pinene	930	1.8	10.7	RI+MS
camphene	938	2.3	-	RI+MS
sabinene	970	0.1	-	RI+MS
myrcene	985	-	2.9	RI+MS
yomogi alcohol	991	1.6	-	RI+MS
p-cymene	1007	1.5	-	MS
benzene acetaldehyde	1008	-	3.1	RI+MS
1,8-cineol	1015	10.3	-	RI+MS
artemisia ketone	1040	4.9	-	RI+MS
artemisia alcohol	1071	0.8	-	RI+MS
Z-linalool oxide	1080	0.3	-	RI+MS
nonanal	1087	-	4.4	RI+MS
α -thujone	1088	5.6	-	RI+MS
β -thujone	1090	8.3	-	RI+MS
camphor	1095	20.4	-	MS
pinocarveol	1106	-	2.9	RI+MS
isothujol	1110	0.1	-	RI+MS
Z-verbenol	1111	8.7	5.8	RI+MS
borneol	1142	3.4	-	MS
4-terpineol	1142	1.5	-	MS
α -terpineol	1150	0.7	-	MS
myrtenol	1177	0.3	-	RI+MS
E-piperitol	1192	0.2	-	RI+MS
grandisol	1196	3.6	-	RI+MS
E-carveol	1198	0.1	-	RI+MS
cuminal	1200	0.1	-	RI+MS
carvotanacetone	1225	0.1	-	RI+MS
piperitone	1248	0.3	-	RI+MS
thymol	1270	0.1	-	RI+MS
bornyl acetate	1275	0.3	-	RI+MS
carvacrol	1277	0.2	-	RI+MS
α -terpinyl acetate	1350	0.2	-	RI+MS
Z-jasmone	1362	0.1	-	RI+MS
methyl eugenol	1370	0.1	-	RI+MS
β -selinene	1476	0.2	-	RI+MS
E-nerolidol B	1547	0.3	-	RI+MS
spathulenol	1551	1.5	7.8	RI+MS
caryophyllene oxide	1561	0.5	3.9	MS
globulol	1576	1.0	-	RI+MS
viridiflorol	1588	0.4	-	RI+MS
β -eudesmol	1619	2.0	-	RI+MS
α -bisabolol oxide B	1670	0.1	-	MS
α -bisabolol	1690	0.3	-	MS
6,10,14-trimethyl-2-pentadecanone	1822	-	9.4	MS
nonadecane	1900	-	10.0	RI+MS
phytol ^c	2000	-	3.1	MS
heneicosane	2100	-	3.7	MS
10-methyl eicosane	2100	-	3.9	MS
docosane	2200	-	4.4	RI+MS
tricosane	2300	-	2.9	RI+MS
Total compounds		40	16	
Oxygenated monoterpenes		41.6%	8.7%	
Monoterpene hydrocarbons		36.7%	13.6%	
Oxygenated sesquiterpenes		6.1%	11.7%	
Sesquiterpene hydrocarbons		0.2%	0%	
Non-terpene hydrocarbons		0%	24.8%	
Others		0%	21.7%	
Total identified		84.6%	80.5%	

^a Compounds listed in order of elution from a DB-1 column, ^b Identification Method (RI= Retention Indices, MS= Mass spectroscopy, ^c Correct isomeric form not identified.

DISCUSSION

Previous studies exhibited that 1,8-cineol and bornane derivatives are main characteristic constituents of many species of the *Artemisia* genus (11-14). In 2005, α -thujone (28.7%), 1,8-cineol (20.0%) and camphor (10.0%) were reported as main components in the essential oil of the aerial parts of *A. incana* collected in the vicinity of Ahar (East Azarbaijan province, Iran) (15). According to other report by Rustaiyan et al, 1,8-cineol (23.3%), chrysanthenone (21.3%) and danavone (19.3%) were the main components of the essential oil of this plant collected in Bojnord (North Khorasan province, Iran) (15). Moreover, the oil from Turkey represented camphor (19.0%), borneol (18.9%), 1,8-cineol (14.5%), bornyl acetate (7.8%) and α -thujone (4.8%) as principle components (7). The comparison of our results with literature shows remarkable differences in terms of chemical composition of the oils. It is notable that chrysanthenone and danavone were present just in the oil composition of *A. incana* collected in Bojnord. However, the percentage of borneol (3.4%) and bornyl acetate (0.3%) was quite lower in our examined oil compared with that of Turkish *A. incana* oil (18.9%, 7.8% respectively). Z-verbenol was found at a relatively high level (8.7%) in our study whereas it was not detected in the oil composition of *A. incana* in the previous works. Conversely, α -thujone was present at a low level (5.6%) in our study compared with that of oil composition of *A. incana* collected in Ahar (28.7%). Compared with the oil of *A. armeniaca* in the previous study (2), we found considerable differences in terms of chemical composition of the oil (Table1). For example, absence of 1,8-cineol (20.6%) was noted. Presence of non-terpene hydrocarbons and phytol was not reported in the previous research. Presence of α -pinene as the principle component, is the main similarity of both essential oils. Pinane derivatives have been detected in oils of many *Artemisia* species for example, α -pinene was found in the oils of *A. dracuncululus* (16) and *A. annua* (17). The variations in the essential oils composition of *A. incana* and *A. armeniaca* in comparison

with previous reports might be attributed to a variety of factors such as harvesting season, geographic location, climatic condition, altitude, chemotype or subspecies, reproductive stage, choice of plant part and extraction method (11,18,19). These findings showed that the genus *Artemisia* had a considerable variation in volatile oil composition. The brine shrimp lethality bioassay is considered as a rapid, simple, inexpensive and effective method for preliminary assessment of toxicity and also as a guide for the detection of cytotoxic, anti-tumor and pesticidal compounds (10). In the case of *A. incana*, the most likely components which can be responsible for the toxicity are believed to be α -thujone and β -thujone. Nausea, vomiting, insomnia, restless, vertigo and tremors were reported as side effects of thujone. High dose of thujone have been demonstrated to cause convulsions, paralysis, brain damage, renal failure and death (20). α -Thujone blocks the receptors for GABA in the CNS and believed that this is the main mechanism of neurotoxicity of α -thujone (21). Weak toxicity of the essential oil of *A. armeniaca* might be related to α -pinene (22) or caryophyllene oxide (23).

CONCLUSION

In summary, the current study, evaluated the general toxicity of the essential oils of *A. incana* and *A. armeniaca* for the first time, and also compared the chemical composition of the oils with previous reports.

ACKNOWLEDGMENT

The authors would like to acknowledge Amir Hossein Talebpour for identification of plant materials.

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