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NPC Natural Product Communications

2013
Vol. 8
No. 3
407 - 410

Chemical Composition and Biological Activities of the Essential Oil from *Artemisia herba-alba* Growing Wild in Tunisia

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Received: November 9th, 2012; Accepted: January 17th, 2013

Aromatic plants can interfere in the Mediterranean ecosystem, mainly by the introduction in the environment of volatile compounds. For this reason, we studied the chemical composition and the possible phytotoxic and antimicrobial activities of the essential oil extracted from leaves of Tunisian Artemisia herba-alba Asso. The chemical composition of the essential oil, obtained by hydrodistillation, was analyzed by GC and GC-MS. In all, 24 compounds were identified. The main components were camphor (39.1.%), chrysanthenone (15.0%) and cis-thujone (7.8%). The essential oil was evaluated for its in vitro phytotoxic activity against germination and initial radical growth of Raphanus sativus L., Lepidium sativum L., Sinapis arvensis L., Triticum durum L. and Phalaris canariensis L. seeds. The radicle elongation of the five seeds was affected to different extents by the oil, while germination was not affected. The oil, when tested against eight selected bacterial strains, showed low antimicrobial activity. The chemical composition of the oil of A. herba-alba can help in the chemosystematics of this complex genus. However, the recorded biological activities seem to be neither ecologically nor medicinally significant.

Keywords: *Artemisia herba-alba* Asso, Essential oil, GC/MS, Phytotoxicity, Antimicrobial activity.

The Mediterranean basin is characterized by a large quantity of aromatic plants, producing essential oils and metabolites with low molecular weight. These plants belong to different botanical families and their presence is important in deciding the potential interference within the ecosystem [1].

The genus *Artemisia*, family Asteraceae, tribe Anthemideae, is comprised of between 200 to over 400 species, distributed throughout the world [2]. The genus may be divided into sections Artemisia and Dracunculus [3]. *Artemisia* has been an important genus in the search for new bioactive components, being rich in monoterpenes and sesquiterpenes [4]. *Artemisia* species have been used in folk medicine by many cultures since ancient times as anti-inflammatory, antibacterial, antifungal and antiviral drugs [2].

Artemisia herba-alba Asso, known also as "desert wormwood" or "shih" in Arabic, is an aromatic and medicinal shrub, 20 to 40 cm high, growing wild in arid areas of the Mediterranean basin. In Tunisia, this species is found from the mountains around Jebel Oust to the south of the country [5]. In traditional medicine, the plant is used to treat colds, intestinal diseases, diabetes and hypertension [6].

Many papers on the composition of the essential oil of *A. herba-alba*, from different parts of the world, are reported in the literature [2,4,7,8]. Its composition revealed a high level of polymorphism and led to the definition of several chemotypes [9], from Morocco to Algeria and southern Spain [2,7]. In Tunisia (semi-arid and arid land), a different composition in essential oil was observed, dominated either by a single component (α-thujone, β-thujone, 1,8-cineole, camphor, chrysanthenone or *trans*-sabinyl acetate) or characterized by the occurrence of two or more of these compounds [2,7-11]. The essential oil is known for its medicinal properties [2], mainly antibacterial [2,9-12]. The possible phytotoxicity of the essential oil was also showed [13]. Duke and coworkers [14] reported that *Artemisia* species produce many terpenoid compounds. In particular, sesquiterpenoid lactones are biologically active as fungicides, herbicides, antimicrobials, insecticides, and insect antifeedants. Moreover, the antimalarial sesquiterpenoid, artemisinin, from *A. annua*, is also a potent phytotoxin [14].

In continuation of our studies on the chemistry of essential oils from plants collected in the Mediterranean area, and their possible phytotoxic and antimicrobial activities [1,15], we studied the chemical composition of the essential oil from *A. herba-alba*, and its possible *in vitro* effects against germination and initial radical elongation of *Raphanus sativus* L. (radish), *Lepidium sativum* L. (garden cress), *Sinapis arvensis* L. (wild mustard), *Triticum durum* L. (wheat) and *Phalaris canariensis* L. (canary grass). The antibacterial activity of the essential oil was also evaluated against eight bacterial strains [*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATTC 25923), *S. epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATTC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 10031) and *Proteus vulgaris* (ATCC 13315)].

Hydrodistillation of the leaves of *A. herba-alba* furnished a pale yellow oil in 0.84% yield, on a dry weight basis. The chemical composition is summarized in Table 1, in which 24 identified compounds are listed according to their elution order on a HP-5MS column. Oxygenated monoterpenoids amounted to 78.6%, among

Table 1: Chemical composition of the essential oil from *Artemisia herba-alba*.

N.	Components[a]	RI[b]	RI[c]	%	Identification[d]
1	1,8-Cineole	1022	1213	0.7	RI,MS,Std
2	*cis*-Thujone	1094	1430	7.8	RI,MS
3	*trans*-Thujone	1106	1449	2.2	RI,MS
4	Chrysanthenone	1114		15.0	RI,MS
5	*trans*-Pinocarveol	1126	1664	1.3	RI,MS
6	Camphor	1130	1532	39.1	RI,MS,Std
7	*trans*-Sabinol	1135	1720	1.7	RI,MS
8	*trans*-Verbenol	1139	1683	0.3	RI,MS
9	Eucarvone	1150	1932	0.8	RI,MS
10	*Iso*-borneol	1154	1633	4.0	RI,MS
11	*cis*-Chrysanthenol	1157		2.6	RI,MS
12	Viridene	1160		0.5	RI,MS
13	Terpinen-4-ol	1167	1611	0.8	RI,MS
14	Dihydrocarveol	1183	1755	0.3	RI,MS
15	Myrtenol	1187	1804	0.4	RI,MS
16	Verbenone	1197	1723	0.4	RI,MS
17	*cis*-Chrysanthenyl acetate	1255		0.9	RI,MS
18	*trans*-Linalool oxide acetate	1277		0.3	RI,MS
19	α-Terpinen-7-al	1289		0.4	RI,MS
20	γ-Terpinen-7-al	1292		0.5	RI,MS
21	(Z)-Patchenol	1298		3.1	RI,MS
22	(E)-Patchenol	1304		9.5	RI,MS
23	Spathulenol	1562	2150	0.8	RI,MS,Std
24	Ar-Turmerone	1583		0.5	RI,MS
	Total identified			93.0	

[a]Order of elution is given from an apolar column (HP-5 MS); [b]Retention index on the apolar HP-5 MS column; [c]Retention index on the polar HP Innowax column; [d]Method of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those of authentic samples or from literature; Std, by co-injection of an authentic compound.

which camphor (39.1%), chrysanthenone (15%) and *cis*-thujone (7.8%) were the most abundant; total oxygenated sesquiterpenes was 13.9% {9.5% (E)-patchenol, 3.1% (Z)-patchenol, 0.8% spathulenol and 0.5% ar-turmerone}. The composition of our sample appears to be similar to that of the oil from arid Tunisian regions, in which oxygenated monoterpenes were the major components [2]. In another study, the main components were cineole, thujone, chrysanthenone, camphor, borneol, chrysanthenyl acetate, sabinyl acetate, davana ethers and davanone [2]. The essential oil obtained from the aerial parts of the plant growing in M'sila-Algeria contained camphor (19.4%), *trans*-pinocarveol (16.9%), and chrysanthenone (15.8%) as main compounds [2]. Imelouane and coworkers [12] reported that the most abundant compound of the essential oil of the species from eastern Morocco was camphor (43.1%). A thujone-camphor oil was also observed in another Moroccan sample [16]. Ghanmi and coworkers [17] reported the chemical composition of *A. herba-alba* from Guercif (eastern region of Morocco), whose main components were chrysanthenone and camphor. The essential oil from *A. herba alba*, collected in the North Sahara desert, contained mainly camphor (49.3 - 48.1%) [18]. Among oxygenated sesquiterpenes, spathulenol was also found to be a minor constituent in our oil, but Zouari and coworkers [11] reported this compound in relatively high amount.

The essential oil was evaluated for its phytotoxic activity against germination and radical elongation of radish and garden cress, two species frequently utilized in biological assays, and of wild mustard, wheat and canary grass, three weed species (Table 2).

The oil was inactive against germination, but active against radical elongation of the five tested seeds. At doses of 2.5 and 0.25 μg/mL, the essential oil of *A. herba-alba* significantly inhibited the radicle elongation of wheat. At the highest doses tested, the essential oil showed stimulatory activity of radicle elongation of radish. Only Escudero and coworkers [13] have reported the inhibitory effects of an aqueous extract of fresh *A. herba-alba* shoots and roots against germination of *Helianthemum squamatum* (L.) Dum Cours.

Table 2: Phytotoxic activity of the essential oil of *A. herba-alba* against germination and radical elongation of *S. arvensis*, *T. durum*, *P. canariensis*, *L. sativum* and *R. sativus* 120 h after sowing. Data are expressed in cm. Results are the mean ± standard deviation (SD) of three experiments.

Sinapis arvensis	Germinated seeds ± SD	Radical elongation ± SD
Doses		
Control	8.0±1.0	1.3±0.6
0.062 μg/mL	8.7±1.2	2.0±1.1*
0.125 μg/mL	8.7±1.5	1.3±0.6
0.25 μg/mL	8.0±2.0	1.6±1.0
0.625 μg/mL	8.7±0.6	1.3±0.6
1.25 μg/mL	8.0±1.7	1.3±0.6
2.5 μg/mL	9.7±0.6	1.1±0.6
Triticum durum	Germinated seeds ± SD	Radical elongation ± SD
Doses		
Control	9.0±0.0	5.9±2.7
0.062 μg/mL	8.7±0.6	4.1±2.4*
0.125 μg/mL	9.0±0.0	4.7±2.2
0.25 μg/mL	7.7±2.3	3.7±2.0**
0.625 μg/mL	7.7±1.2	4.4±2.6
1.25 μg/mL	8.7±1.2	4.5±2.2
2.5 μg/mL	8.0±1.7	3.9±2.2**
Phalaris canariensis	Germinated seeds ± SD	Radical elongation ± SD
Doses		
Control	8.3±1.2	3.2±1.4
0.062 μg/mL	8.7±1.2	3.4±1.2
0.125 μg/mL	9.7±0.6	3.1±1.2
0.25 μg/mL	8.7±0.6	3.2±1.3
0.625 μg/mL	8.7±0.6	2.3±1.5*
1.25 μg/mL	8.7±0.6	3.1±0.8
2.5 μg/mL	8.0±0.0	3.2±1.0
Lepidium sativum	Germinated seeds ± SD	Radical elongation ± SD
Doses		
Control	9.3±0.6	2.4±1.3
0.062 μg/mL	9.3±1.2	1.9±1.3
0.125 μg/mL	9.0±0.0	1.6±1.1**
0.25 μg/mL	9.0±1.0	2.2±1.8
0.625 μg/mL	8.0±1.0	1.8±1.0
1.25 μg/mL	8.3±1.5	1.9±1.4
2.5 μg/mL	9.7±0.6	2.4±1.4
Raphanus sativus	Germinated seeds ± SD	Radical elongation ± SD
Doses		
Control	9.7±0.6	4.0±2.1
0.062 μg/mL	9.7±0.6	4.6±2.5
0.125 μg/mL	9.7±1.2	5.0±2.2
0.25 μg/mL	10.0±0.0	5.7±2.5**
0.625 μg/mL	9.3±1.2	5.3±2.5*
1.25 μg/mL	9.3±0.6	6.0±2.2***
2.5 μg/mL	9.0±0.0	5.5±2.1**

Note: *) $p < 0.05$; **) $p < 0.01$; ***) $p < 0.001$ *vs.* control

Table 3: Antibacterial activity of *A. herba-alba* essential oil.

	Artemisia herba-alba		
Microorganisms	MIC[a] (μg/mL)	MBC[b] (μg/mL)	Ch[c]
Gram-positive bacteria			
Bacillus subtilis ATCC 6633	50	100	12.5
Staphylococcus aureus ATCC 25923	100	>100	25
Staphylococcus epidermidis ATCC 12228	25		3.12
Gram-negative bacteria			
Streptococcus faecalis ATCC 29212	50	100	25
Escherichia coli ATCC 25922	50		12.5
Klebsiella pneumonia ATCC 10031	100	>100	50
Proteus vulgaris ATCC 13315	50	100	25
Pseudomonas aeruginosa ATCC 27853	>100		100

[a]Minimum Inhibitory Concentration; [b]Minimum Bactericidal Concentration. [c]Antibiotic, Chloramphenicol for bacteria.

Li and coworkers [19] reported that the volatile compounds released from leaves of *Artemisia frigida* Willd. and aqueous extracts of leaves and roots, inhibited seed germination and seedling growth of three dominant species [*Leymus chinensis* (Trin.) Tzelev, *Stipa krylovii* Roshev, and *Cleistogenes squarrosa* (Trin.) Keng.] in the Inner Mongolia steppe. Jassbi and coworkers [20] demonstrated that the roots of *Artemisia tridentata* (Natt.) subspecies *tridentata*

release volatiles into the rhizosphere and showed that such compounds have phytotoxic effects on seed germination and seedling growth of the co-occuring plant, *Nicotiana attenuata* Torr. Camphor, 1,8-cineole, nerol, and neryl isovalerate were phytotoxic and released as the major constituents [20].

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values of the essential oil against 8 selected bacterial strains are reported in Table 3.

The essential oil showed inhibitory activity against the Gram-positive pathogens, *S. epidermidis* and *B. subtilis*. Among Gram-negative bacteria, the growth of *S. faecalis*, *E. coli* and *P. vulgaris* was inhibited by the oil. The antimicrobial properties of *A. herba alba* essential oil have been reported in several studies [2,9-12], Gram-positive bacteria being more sensitive. The growth of Gram-negative bacteria (for example *E. coli*) was partially inhibited [11]. The oil exhibited high inhibitory activity against *S. aureus* and *S. epidermidis*. This activity is probably related to the high content of oxygenated monoterpenoids (78.6%), as camphor, 1,8-cineole and thujone are three monoterpenes with well documented antibacterial and antifungal properties [12].

The essential oil from *A. herba-alba* leaves was mainly composed of oxygenated compounds and its composition can be useful in the chemotaxonomy of the species and the genus. However, the weak phytotoxic and antimicrobial activities seem to be neither ecologically nor medicinally significant.

Experimental

Plant material: Leaves of *Artemisia herba-alba* were collected from Makther Seliana (Tunisia) in April 2011. Five samples were collected from more than 5 different plants, mixed for homogenization, and used in 3 replicates for essential oil extractions. The plants were identified by Dr H. Lamia and a voucher specimen was deposited at the Herbarium of the Laboratory of Forestry Ecology at the National Institute of Research on Rural Engineering, Water and Forest (Tunisia).

Essential oil isolation: One hundred g of dried leaves were ground in a Waring blender and then subjected to hydrodistillation for 3 h, according to the standard procedure described in the European Pharmacopoeia [21]. The oil was solubilized in *n*-hexane, dried over anhydrous sodium sulfate and stored under N_2 at +4 °C in the dark until tested and analyzed.

***GC-FID analysis*:** The GC analysis was carried out on a Perkin-Elmer Sigma-115 gas chromatograph equipped with a flame ionization detector (FID) and a data handling processor. The separation was achieved using an apolar HP-5 MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Column temperature: 40°C, with 5 min initial hold, and then to 270°C at 2°C/min, 270°C (20 min); injection mode splitless (1 µL of a 1:1,000 *n*-pentane solution). Injector and detector temperatures were 250°C and 290°C, respectively. A fused silica HP Innowax polyethylenglycol capillary column (50 m × 0.20 mm i.d., 0.25 µm film thickness) was also used for analysis. In both cases, helium was the carrier gas (1.0 mL/min).

***GC/MS analysis*:** GC/MS analysis was performed on an Agilent 6,850 Ser. II apparatus, fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm i.d., 0.33 µm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization energy voltage 70 eV; electron multiplier voltage energy 2,000 V. Mass spectra were scanned in the range 40–500 amu, scan time 5 scans/s. Gas chromatographic conditions were as reported in the previous paragraph; transfer line temperature, 295°C.

***Identification of essential oil components*:** Most constituents were identified by gas chromatography by comparison of their Kovats retention indices (Ri) with either those of the literature [22,23] or with those of authentic compounds available in our laboratories. The Kovats retention indices were determined in relation to a homologous series of *n*-alkanes (C_{10}–C_{35}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with either those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature [22,24] and a homemade library. The relative concentrations of components were obtained by peak area normalization. No response factors were calculated.

Phytotoxicity assay: A bioassay based on germination and subsequent radicle growth was used to study the phytotoxic effects of the essential oil on seeds of *Raphanus sativus* L. cv. "Saxa", *Lepidium sativum* L. and the three weed species *Sinapis arvensis* L., *Triticum durum* L. and *Phalaris canariensis* L. The seeds of radish and garden cress were purchased from Blumen srl (Piacenza, Italy), while mustard, wheat and canary grass were collected from wild plants. The seeds were surface sterilized in 95% ethanol for 15 s and sown in Petri dishes (Ø = 90 mm), containing 5 layers of Whatman filter paper, impregnated with either distilled water (7 mL, control) or tested solution of the essential oil (7 mL), at the different assayed doses. The germination conditions were 20 ± 1°C, with natural photoperiod. The essential oil, in water–acetone mixture (99.5:0.5), was assayed at doses of 2.5, 1.25, 0.625, 0.25, 0.125 and 0.062 μg/mL. Controls performed with water-acetone mixture alone showed no appreciable differences in comparison with controls in water alone. After 120 h (on the fifth day), the effects on radicle elongation were measured in cm.

Antibacterial activity

***Microbial strains**:* The oil was tested against 3 Gram-positive bacteria, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATTC 25923), and *S. epidermidis* (ATCC 12228), and five Gram-negative bacteria, *Streptococcus faecalis* (ATTC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 10031) and *Proteus vulgaris* (ATCC 13315). The strains were maintained on tryptone soya agar (Oxoid, Milan, Italy); for the antimicrobial tests, tryptone soya broth (Oxoid, Milan, Italy) was used.

Dilution-agar method: The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), using the broth dilution method. In order to facilitate the dispersion of the oil in the aqueous nutrient medium, it was diluted with Tween 20 at a concentration of 10%. Each strain was tested with sample that was serially diluted in broth to obtain concentrations ranging from 100 μg/mL to 0.8 μg/mL. The sample was previously sterilized with a 0.20 μm Millipore filter. The sample was stirred, inoculated with 50 μL of physiological solution containing 5 x 10^6 microbial cells, and incubated for 24 h at 37°C. The MIC value was determined as the lowest concentration of the sample that did not permit any visible growth of the tested microorganism after incubation. Control, containing only Tween 20 instead of the essential oil, was not toxic to the microorganisms. Cultures, containing only sterile physiologic solution Tris buffer, were used as positive control. MBC was determined by subculture of the tubes with inhibition in 5 mL of

sterile nutrient broth. After incubation at 37°C, the tubes were examined. When no microbial growth was observed, the sample denoted a bactericidal action. Chloramphenicol was used as reference drug [15].

Statistical analysis: Means and standard deviations (SD) of the samples were calculated. Each determination was carried out with 3 replicates, using Petri dishes containing 10 seeds each. Data are expressed as the mean ± SD for both germination and radicle elongation. Data were ordered in homogeneous sets, and the Student's *t* test of independence was applied. For MIC and MBC determinations, the results are expressed as mean ± SD [25].

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