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COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF *ARTEMISIA JUDAICA*, *A. HERBA-ALBA* AND *A. ARBORESCENS* FROM LIBYA

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Abstract: The essential oils obtained by hydrodistillation from the aerial parts of *Artemisia judaica* L., *Artemisia herba-alba* Asso. and *Artemisia arborescens* L. (cultivated) from Libya, were analyzed by GC and GC-MS. The antimicrobial properties were determined using the broth microdilution method against eight bacterial species: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC10240), *Listeria monocytogenes* (NCTC7973), *Staphylococcus aureus* (ATCC6538), *Escherichia coli* (ATCC35210), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhimurium* (ATCC13311), *Enterobacter cloacae* (human isolates) and eight fungal species: *Aspergillus niger* (ATCC6275), *A. ochraceus* (ATCC12066), *A. versicolor* (ATCC11730), *A. fumigatus* (ATCC1022), *Penicillium ochrochloron* (ATCC9112), *P. funiculosum* (ATCC10509), *Trichoderma viride* (IAM5061) and *Candida albicans* (human isolate). The major constituents of *A. arborescens* oil were sesquiterpene hydrocarbons (47.4%). Oxygenated monoterpenes were the dominant constituents in the *A. judaica* and *A. herba-alba* oils (54.2% and 77.3%, respectively). Camphor (24.7%) and chamazulene (20.9%) were the major components in the essential oil of *A. arborescens*, chrysanthenone (20.8%), *cis*-chrysanthenyl acetate (17.6%) and *cis*-thujone (13.6%) dominated in the *A. herba-alba* oil, and the major constituents in the *A. judaica* oil were piperitone (30.21%) and *cis*-chrysanthenol (9.1%). The best antimicrobial activity was obtained for *A. judaica* oil and the lowest effect was noticed in *A. arborescens* oil. The effect of the tested oils was higher against Gram (+) than Gram (-) bacteria. All three oils showed the best antibacterial activity against *Listeria monocytogenes* and the lowest against *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae,* compared to streptomycin and ampicillin. All three oils showed better antifungal activities than ketoconazole, except *A. arborescens* oil against *Aspergillus niger*.

Keywords: *Artemisia judaica*; *Artemisia herba-alba*; *Artemisia arborescens*; essential oils; antimicrobial activity

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INTRODUCTION

Artemisia L. is a large, diverse and economically important genus of the family Asteraceae (Hayat et al., 2009). It has more than 500 species (the number varies depending on the authors (Bremer and Humphries, 1993; Ling, 1982; 1991a; 1991b; 1994; Oberprieler, 2001; Valles and Garnatge, 2005; Jafri and El-Gadi, 1983). The wind-polinated genus *Artemisia* has a cosmopolitan distribution, displaying highest diversity in temperate areas of the northern hemisphere and in several taxa in the southern hemisphere where it grows in arid and semiarid habitats (Hayat et al., 2009). In the flora of Libya, there are five species of this genus: *Artemisia herba-alba* Asso, *A. judaica* L., *A. arborescens* L. (cultivated), *A. monosperma* Delile and *A. campestris* L. (Jafri and El-Gadi, 1983).

Artemisia judaica is a perennial small fragrant shrub with pubescent leaves (Dob and Chelghoum, 2006). Reported medicinal effects of the plant include improved vision, cardiovascular health, capillary strength, connective tissue structure, appearance of skin and enhanced immune system functions, as well as decreased risk of atherosclerosis, cancer, arthritis and gastrointestinal disorders (Jafri and El-Gadi, 1983; Abdalla and Abu-Zagra, 1987; Khafagy et al., 1988; Khafagy and Tosson, 1968). *Artemisia herba-alba*, commonly known as *sheeh*, is a herb or shrub distributed in north Africa (Libya), and most of Europe (Jafri and El-Gadi, 1983). *Artemisia herba-alba* is used in the traditional medicine of the northern Badia region of Jordan, in the form of a decoction against fever and menstrual and nervous problems (Alzweiri et al., 2011). *Artemisia arborescens* is a morphologically variable species (or aggregate species) with grey-green to silver leaves. It is native to various habitats of the Mediterranean region, where it occurs as a shrub growing up to 1 m in height. According to popular folklore, it is used as an anti-inflammatory remedy (Ballero et al., 2001). A scientific study concerning the aspects of the therapeutic uses of the essential oil of *A. judaica*, *A. arborescens* and *A. herba-alba* from Libya, as well as their chemical composition, remains scarce and incomplete. Because of this, our investigation aimed to determine the chemical composition of the essential oil from the aerial parts of *A. judaica*, *A. arborescens* and *A. herba-alba* from Libya, and to assess their antimicrobial activity.

MATERIALS AND METHODS

Plant material

The aerial parts of *A. judaica*, *A. herba-alba* and *A. arborescens* were collected during the flowering stage in February 2012 in Libya. *Artemisia judaica* was collected in Western Hamada (N29°33'44.97"; E10°14'15.13"), *A. herba-alba* and *A. arborescens* were collected in Zintan (N31°57'21.19"; E12°13'6.35" and N31°56'13.95"; E12°16'0.18", respectively). Voucher specimen accession numbers BEOU AJU04022012; BEOU AHA28022012 and BEOU AAR20022012, respectively, were deposited in the Herbarium of the Institute of Botany, University of Belgrade, Faculty of Biology.

Essential oil extraction and analysis

Plant material of each species, dried at room temperature (150 g), was fragmented, and essential oils were isolated by 3-h hydrodistillation using a Clevenger-type apparatus, according to the procedure described in Ph. Eur. 6 (European Pharmacopoeia 6th Edition, 2007).

Table 1. Composition of essential oils of the aerial parts of *A. arborescens, A. herba-alba* and *A. judaica.*

No	RI	Components	***A. arborescens* (%)**	***A. herba-alba* (%)**	***A. judaica* (%)**
1	829	Ethyl-2-methylbutyrate	-	-	tr
2	864	1-Methylcycloheptene	-	0.3	-
3	882	4,5,6-Pyrimidinetriamine	-	-	tr
4	886	2,5-Diethenyl-2-methyl-tetrahydrofuran	-	0.5	-
5	886	4,6-Dimethyl-2-pyrimidinamine	-	-	tr
6	897	Tricyclene	tr	tr	-
7	901	*α*-Thujene	tr	tr	-
8	909	*α*-Pinene	1.0	0.3	tr
9	921	2-Cyclohexyldecane	-	0.8	2.9
10	927	Camphene	1.6	0.7	-
11	927	Phenylethanolamine	-	-	tr
12	936	Thuja-2,4(10)-diene	-	0.3	-
13	958	Sabinene	tr	-	-
14	962	4-Hydroxy-6-(methylamino)pyrimidin	-	-	tr
15	963	*β*-Pinene	tr	0.2	-
16	979	Myrcene	0.8	-	-
17	980	Dehydro-1,8-cineole	-	-	0.2
18	980	2,5,5-Trimethyl-1-hexen-3-yne	-	0.3	-
19	985	Mesitylene	-	0.8	tr
20	1000	*α*-Phellandrene	0.4	0.1	tr
21	1003	2-Acetyl-3,4,5,6-tetrahydropyridin	-	-	0.9
22	1003	Ni	-	0.2	-
23	1010	*α*-Terpinene	0.3	tr	tr
24	1017	*p*-Cymene*	0.5	0.5	1.7
25	1019	*o*-Cymene*	-	1.3	0.5
26	1020	Limonene	0.2	-	-
27	1021	*β*-Phellandrene	-	-	tr
28	1023	1,8-Cineole	-	2.8	tr
29	1031	*γ*-Vinyl-*γ*-valerolactone	-	0.4	0.3
30	1041	*cis*-Arbusculone	-	2.0	0.9
31	1048	*γ*-Terpinene	0.6	-	0.2
32	1050	2,3-Dimethylhexane	-	tr	-
33	1052	Ni	-	-	0.2
34	1055	*cis*-Sabinene hydrate (*cis* for IPP vs OH)	0.9	-	-
35	1058	*trans*-Arbusculone	-	1.2	0.4
36	1060	*cis*-Linalol oxide	-	-	0.2
37	1076	α-Terpinolene	tr	-	tr
38	1076	2-Acetylthiophene	-	0.2	-
39	1087	Ni	-	-	0.5
40	1089	Linalool	6.0	-	-
41	1091	2-Methylbutyl 2-methyl butyrate	tr	-	-
42	1091	Filifolone	-	4.8	-
43	1095	*cis*-Thujone	-	13.6	1.2
44	1103	2-Methyl-6-methylene-1,7-octadien-3-one	tr	-	-
45	1104	*trans*-Thujone	-	4.0	0.2
46	1109	Ni	-	-	0.3

Table 1 continued

No	RI	Components	***A. arborescens*** **(%)**	***A. herba-alba*** **(%)**	***A. judaica*** **(%)**
47	1110	*p*-Menth-2-en-1-ol	tr	-	-
48	1116	Chrysanthenone	-	20.5	-
49	1124	Isocyclocitral	-	-	0.2
50	1127	Ni	-	-	0.4
51	1128	*trans*-Pinocarveol	-	3.3	-
52	1137	Camphor	24.7	1.8	0.3
53	1138	Ni	-	0.3	-
54	1147	Sabina ketone	-	tr	-
55	1152	*cis*-Chrysanthenol	-	2.1	9.1
56	1155	Borneol	0.2	tr	-
57	1157	*p*-Mentha-1,5-dien-8-ol	-	tr	-
58	1157	(*Z*)-1,3,5-Hexatriene	-	-	0.2
59	1168	Terpinen-4-ol	1.2	0.4	0.3
60	1171	Dec-1-en-3-ol	-	tr	-
61	1177	Ni	-	-	0.5
62	1178	*p*-Cymen-8-ol	-	0.4	-
63	1178	2-Ethenyl-6-methyl-5-hepten-1-ol	0.3	-	-
64	1182	*α*-Terpineol	0.3	-	0.4
65	1187	(*E*)-2,3-Epoxycarane	-	-	0.2
66	1188	Myrtenal	-	0.3	-
67	1194	1,2-Dimethyl-3-(1-methylethenyl)-cyclopentanol	-	-	tr
68	1201	*γ*-Terpinene	-	-	tr
69	1202	Verbenone	-	0.7	-
70	1214	*trans*-Carveol	-	tr	-
71	1223	*nor*-Davanone	-	1.3	-
72	1230	Hexyl 2-methyl butanoate	-	tr	-
73	1237	Carvone	-	tr	-
74	1249	Piperitone	-	0.8	30.2
75	1257	*cis*-Chrysanthenyl acetate	-	17.7	3.6
76	1266	Ni	-	0.2	-
77	1268	Perilla aldehyde	tr	-	-
78	1269	Ethenylcyclohexane	-	-	0.2
79	1280	Isobornyl acetate	-	tr	-
80	1281	2,2,6-Trimethyl-1-(3-oxo-but-1-enyl)-7-oxa-bicyclo	-	-	0.3
81	1286	Bornyl acetate	4.9	-	-
82	1288	*trans*-Sabinyl acetate	-	3.1	-
83	1288	Thymol	-	-	0.6
84	1294	*trans*-Pinocarvyl acetate	-	tr	-
85	1294	2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methylethyl)-	-	-	0.3
86	1299	Carvacrol	-	0.4	1.6
87	1303	Ni	-	0.8	0.2
88	1308	Ni	-	0.3	-
89	1314	Flifolid A	-	1.2	-
90	1320	Myrtenyl acetate	-	tr	-
91	1344	Ni	-	0.2	-
92	1370	*α*-Copaene	0.4	-	-

Table 1 continued

No	RI	Components	***A. arborescens*** **(%)**	***A. herba-alba*** **(%)**	***A. judaica*** **(%)**
93	1371	(*Z*)-Ethyl cinnamate	-	-	tr
94	1379	(*E*)-Methyl cinnamate	-	tr	-
95	1380	*β*-Bourbonene	tr	-	-
96	1394	(*Z*)-Jasmone	-	0.8	-
97	1399	Cyanomethylbenzene, 2-fluoro-4,5-dimethoxy-	-	-	tr
98	1401	Methyleugenol	tr	tr	-
99	1411	(*Z*)-*threo*-Davanafuran	-	tr	0.4
100	1415	(*E*)-Caryophyllene	0.8	tr	-
101	1440	Ni	-	0.3	-
102	1441	Ni	-	-	0.9
103	1445	N-[(4-hydroxy-3-methoxyphenyl)methyl]acetamide	-	-	0.2
104	1447	2-Fluoro-4,5-dimethoxy-cyanomethylbenzene	-	-	tr
105	1149	*α*-Humulene	tr	-	-
106	1454	Ni	-	0.6	-
107	1455	Ethyl cinnamate	-	-	3.8
108	1461	Ni	-	0.4	-
109	1462	Benzene, 1-fluoro-3-isothiocyanato	-	-	3.8
110	1471	Thiophene, 2,5-bis(2-methylpropyl)	-	-	1.4
111	1475	Cyclohexanecarboxamide, N-furfuryl	-	-	0.3
112	1478	*γ*-Muurolene	-	0.5	-
113	1479	Germacrene D	4.4	-	-
114	1480	1-Propyl-2-hydroxymethylimidazole	-	-	tr
115	1490	Ni	-	0.3	-
116	1490	Davana ether 1	-	-	2.8
117	1494	Bicyclogermacrene	tr	0.5	tr
118	1506	(*E,E*)-*α*-Farnesene	0.3	-	-
119	1510	Davana ether 2	-	1.0	7.9
120	1511	*α*-Carocorene	1.8	-	-
121	1521	*δ*-Cadinene	tr	tr	-
122	1527	Davana ether	-	-	3.0
123	1532	Artedouglasia oxide A	-	-	0.6
124	1545	Laciniata furanone H	-	tr	tr
125	1556	Artedouglasia oxide D	-	-	tr
126	1560	(*E*)-Nerolidol	-	tr	-
127	1574	Spathulenol	tr	1.3	1.7
128	1578	Artedouglasia oxide B	-	-	0.7
129	1579	Ni	-	0.3	-
130	1579	Caryophyllene oxide	0.2	-	-
131	1583	Davanone	-	tr	tr
132	1588	Davanol D1	-	-	0.2
133	1599	Geranyl isovalerate	0.2	-	-
134	1599	Naphthalene, 1-difluoroboryloxy-2-acetyl	-	-	0.8
135	1604	Ni	0.7	-	0.3
136	1609	3-Cyclohexen-1-carboxaldehyde, 3,4dimethyl-	-	-	0.3
137	1614	Ni	-	0.3	0.4
138	1616	Isomeric aromatic hydrocarbon $C_{14}H_{18}$ #	4.7	-	-
139	1630	Isomer $C_{14}H_{18}$ #	6.3	-	-

Table 1 continued

No	RI	Components	*A. arborescens* (%)	*A. herba-alba* (%)	*A. judaica* (%)
140	1634	Ni	-	-	0.7
141	1639	*epi*-α-Cadinol	-	-	0.4
142	1640	Ni	0.2	-	-
143	1648	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis-	-		1.8
144	1659	Isomer $C_{14}H_{18}$#	3.9	-	-
145	1675	2-Propenal, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	-	-	1.1
146	1688	Ni	0.4	-	-
147	1693	4-Cuprenen-1-ol	-	tr	-
148	1738	Chamazulene	20.9	-	-
149	1982	Arborescin	0.3	-	-
150	2007	Ni	0.7	-	-
151	2017	$C_{21}H_{34}$#	5.8	-	-
		Total	95.6	97.5	92.5
		Monoterpene hydrocarbons	-	-	2.0
		Oxygenated monoterpenes	37.3	77.3	54.2
		Sesquiterpenes hydrocarbons	47.4	1.7	2.9
		Oxygenated sesquiterpenes	1.7	2.3	17.0

RI, Retention indices relative to n-alkanes on HP-5 MS; %, Relative percentage obtained from peak area; Tr, Compositional values < 0.1% (traces); Ni, Not identified; * Overlaid peaks; # Tentatively identified

GC and GC-MS analyses were performed on an Agilent 7890A GC system equipped with a 5975C MSD and flame ionization detector (FID), using an HP-5 MS column (30 m × 0.25 mm × 0.25 μm). Injection volume was 1 μL and injector temperature was 220°C with 200:1 split ratio. Carrier gas (He) flow rate was 1.0 mL/min at 210°C (constant pressure mode). Column temperature was linearly programmed in a range of 60-300°C at a rate of 3°C/min, with a final 10-min hold. The transfer line was heated at 280°C. The FID detector temperature was 300°C. EI mass spectra (70 eV) were acquired in an m/z range of 40-550. Library search and mass spectral deconvolution and extraction were performed using NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software version 2.64 using retention index (RI) calibration data analysis parameters with a "strong" level and 10% penalty for compounds without an RI. The retention indices were experimentally determined using the standard method involving retention times of n-alkanes, injected after the essential oil under the same chromatographic conditions. The search was performed against Adams, NIST05 and Wiley 7 mass libraries. Percentage (relative) of the identified compounds was computed from GC-FID peak area.

Antimicrobial activity

Gram-negative bacteria *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterobacter cloacae* (human isolate) and Gram-positive bacteria: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes* (NCTC 7973), and *Staphylococcus aureus* (ATCC 6538) were used. The microorganisms were obtained from the Mycological laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia.

The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by the microdilution method (Espinel-Ingroff, 2001). Briefly, fresh overnight bacteria culture was adjusted by spectrophotometer to a concentration of 1×10^5 CFU/mL. Dilutions of inocula were cultured on a solid medium to verify the absence of contamination and check the validity of the inoculum. Different concentrations of essential oils with 0.1% of Tween 80 were carried into the wells containing 100 μL of Tryptic Soy Broth (TSB), and afterwards, 10 μL of inoculum were added to all the wells. The microplates were incubated for 24 h at 37°C. The MIC of the samples was detected following the addition of 40 μL of iodonitrotetrazolium chloride (INT) (0.2 mg/mL) and incubation at 37°C for 30 min. The lowest concentration that produced significant inhibition in the growth of bacteria compared to the positive control was identified as the MIC. The minimum inhibitory concentrations (MICs) obtained from susceptibility testing of various bacteria to tested extracts were determined also by a colorimetric microbial viability assay based on reduction of a INT color and compared with the positive control for each bacterial strain (Tsukatani et al., 2012; Clinical and Laboratory Standards Institute Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2009). MBC was determined by serial subcultivation of 10 μL into microplates containing 100 μL of TSB. The lowest concentration showing no growth after the subculturing was read as the MBC. Standard drugs, namely streptomycin and ampicillin, were used as positive controls.

For the antifungal bioassays, eight fungi were used: *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus fumigatus* (ATCC 1022), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061), and *Candida albicans* (human isolate). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia. The micromycetes were maintained on malt agar and the cultures stored at 4°C and subcultured once a month (Booth, 1971). In order to investigate the antifungal activity of the compounds, a modified microdilution technique was used (Espinel-Ingroff, 2001). The fungal spores were washed from the surface of the agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in the final volume of 100 μL per well. The inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum. Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The essential oils investigated were dissolved in 0.1% Tween 80 (v/v) and added to broth Malt medium with inoculum. The microplates were incubated in a rotary shaker (160 rpm) for 72 h at 28°C. The lowest concentrations without visible growth (monitored using a binocular microscope) were defined as the MICs. The minimum fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μL of tested compounds dissolved in medium and inoculated for 72 h in microtiter plates containing 100 μL of broth per well and further incubation 72 h at 28°C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum. Commercial fungicides, bifonazole (Srbolek, Belgrade, Serbia) and ke-

toconazole (Zorkapharma, Šabac, Serbia), were used as positive controls (1-3500 μg/mL). All experiments were performed in duplicate and repeated three times.

RESULTS AND DISCUSSION

Essential oils composition

The essential oil of the aerial parts of *A. judaica,* yield 0.62% (w/w), was lemon yellow in color and had a strong smell. GC and GC-MS analyses resulted in the identification of 75 compounds, making up 92.5% of the oil (22 components were found in traces). The essential oil of the aerial parts of *A. herba-alba,* yield 0.90% (w/w), was golden yellow in color and had a strong smell. GC and GC-MS analyses resulted in the identification of 74 compounds, making up 97.5% of the oil (23 components were found in traces). The essential oil of the aerial parts of *A. arborescens,* yeld 0.75% (w/w), was ink blue and with a somewhat milder smell than the previous two species. GC and GC-MS analyses resulted in the identification of 48 compounds, making up 95.6% of the oil (15 components were found in traces). All the components are listed in Table 1, in order of elution. The results showed that sesquiterpene hydrocarbons dominate the essential oil of *A. arborescens* (47.4%), while oxygenated monoterpenes were dominant in the essential oils of *A. judaica* and *A. herba-alba* (54.2% and 77.3%,

Table 2. Antibacterial activity of essential oils of the aerial parts of *Artemisia arborescens*, *A. herba-alba*, *A. judaica*, Streptomycin and Ampicillin.

	BACTERIA	*A. arborescens*	*A. herba-alba*	*A. judaica*	Str	Amp
		MIC	MIC	MIC	MIC	MIC
		MBC	MBC	MBC	MBC	MBC
Gram (+)	***Staphylococcus aureus***	0.500	0.050	0.050	0.050	0.100
		1.250	0.125	0.125	0.100	0.150
	Bacillus cereus	0.250	0.050	0.050	0.125	0.100
		0.500	0.125	0.125	0.250	0.150
	Micrococcus flavus	0.500	0.500	0.050	0.250	0.100
		1.250	1.250	0.125	0.500	0.150
	Listeria monocytogenes	0.050	0.050	0.050	0.150	0.150
		0.125	0.125	0.125	0.300	0.300
Gram (-)	Pseudomonas aeruginosa	1.250	1.250	1.250	0.500	0.300
		2.500	2.500	2.500	0.100	0.500
	Salmonella typhimurium	1.250	0.050	0.050	0.050	0.100
		2.500	0.125	0.125	0.100	0.200
	Escherichia coli	2.500	2.500	1.250	0.050	0.150
		2.500	2.500	2.500	0.100	0.200
	Enterobacter cloacae	1.250	0.250	0.250	0.050	0.150
		2.500	0.500	0.500	0.100	0.200

MICs and MBCs (mg/mL), mean value of two measurements; Str, Streptomycin was used as stock solution 0.1 mg/mL; Amp, Ampicillin was used as stock solution 0.1 mg/mL

respectively). The investigated essential oil of *A. arborescens* from Libya was characterized by an exceptionally high percentage of camphor (24.7%) and chamazulene (20.9%), followed by aromatic hydrocarbon isomers $C_{14}H_{18}$ (14.9% – three compounds), linalool (6.0%), $C_{21}H_{34}$ (5,8%), bornyl acetate (4.9%) and germacrene D (4.4%). The essential oil of the aerial parts of *A. arborescens* from Italy was characterized by camphor (35.7%), *β*-thujone (23.9%) and chamazulene (7.6%) as the main components (Lai et al., 2007), while the essential oil of the aerial part of plants from Algeria was characterized by a high percentage of chamazulene (30.2%) and *β*-thujone (27.8%) (Abderrahim et al., 2010). The essential oil of *A. herba-alba* from Libya was characterized by an exceptionally high percentage of chrysanthenone (20.8%), *cis*-chrysanthenyl acetate (17.6%) and *cis*-thujone (13.6%), followed by filifolone (4.8%), *trans*-thujone (4.0%), *trans*-pinocarveol (3.3%) and *trans*-sabinyl acetate (3.1%). The main components of the essential oil from the aerial parts of *A. herba-alba* from Jordan were *trans*-sabinyl acetate (5.4%), germacrene D (4.6%), α-eudesmol (4.2%) and caryophyllene acetate (5.7%) (Hudaib and Aburjai, 2006). The essential oil of *A. herba-alba* from Libya lacks germacrene D and *α*-eudesmol, while chrysanthenone (20.8%), *cis*-chrysanthenyl acetate (17.6%) and *cis*-thujone (13.6%) emerged as the dominant components. The dominant component of the essential oil of *A. judaica* from Libya was piperitone (30.2%). In the essential oil of the aerial parts of *A. judaica* from Algeria, piperitone makes up almost half of the oil: 53.5% (Stoyka, 2002), 61.9% (Dob and Chelghoum, 2006). In addition to piperitone, the essential oil was also characterized by a high percentage of terpinen-4-ol (4.6%), bornyl acetate (3.0%) (Soković et al., 2002), chrysanthenone (9.8%) and *cis*-chrysanthenyl acetate (7.4%) (Stoyka, 2002).

Antimicrobial activities of essential oils

Essential oils of the aerial parts of *A. herba-alba*, *A. arborescens* and *A. judaica* were tested for antibacterial and antifungal activity. The antimicrobial potential was investigated against eight bacterial and eight fungal species. Antibacterial activity is presented in Table 2. The MIC of all the tested oils ranged between 0.05-2.5 mg/mL and the MBC 0.125-2.5 mg/mL. The effect of the tested oils was higher against Gram (+) than Gram (-) bacteria. The most resistant bacteria was *Escherichia coli* (MIC/MBC - 1.25-2.5 mg/mL), while *L. monocytogenes* (MIC/MBC - 0.05-0.125 mg/mL) was the most susceptible.

The essential oil of *A. arborescens* displayed lower activity than the other two oils tested, while the oil of *A. judaica* exhibited the best activity among all of them. The commercial antibiotic streptomycin, used as a control, displayed antibacterial activity at 0.05-0.5 mg/mL (MIC) and 0.1-0.3 mg/mL (MBC), while ampicillin exhibited MIC at 0.10-0.30 mg/mL and MBC at 0.15-0.50 mg/mL.

The essential oils of *A. herba-alba* and *A. judaica* showed the same antibacterial activity as streptomycin, but higher than ampicillin, against *S. aureus*. The same oils exhibited stronger antibacterial activity than both antibiotics against *B. cereus*, while in the case of *S. typhimurium* these oils showed better activity only against ampicillin. The essential oil of *A. judaica* showed higher antibacterial activity than both antibiotics against *M. flavus*. All three oils showed better antibacterial potential than streptomycin and ampicillin against *L. monocytogenes*. The oils possessed lower antibacterial capacity than both antibiotics against *P. aeruginosa*, *E. coli* and *A. cloacae*.

The results of the antifungal activity of the tested oils are presented in Table 3. The MIC ranged between 0.03-0.25 mg/mL, while the MFC was in the range of 0.06-0.5 mg/mL. The commercial antifungal agent bifonazole showed MIC at 0.10-0.2 mg/mL and MFC at 0.20-0.25 mg/mL. Ketoconazole displayed fungistatic activity at 0.20-2.50 mg/mL and fungicidal effect at 0.30-3.50 mg/mL. The essential oil of *A. judaica* exhibited higher antifungal potential than bifonazole against *A. versicolor*, *A. ochraceus*, *A. niger* and *Penicillium* species. The other two oils showed almost the same or slightly lower antifungal activity than bifonazole. All the oils tested showed better antifungal effect than ketoconazole, except in the case of *A. arborescens* oil against *A. niger*.

The obtained results showed that the essential oil of *A. arborescens* possessed lower antimicrobial activity than the other two oils. Considering that the essential oil *A. judaica* has the highest content of oxygenated compounds, it is expected to have the best antibacterial and antifungal effect. Oxygenated monoterpenes exhibit high antimicrobial activity on whole cells. In contrast, hydrocarbon derivatives have lower antimicrobial activity because of their lower solubility and diffusion through the medium (Knobloch et al., 1986). According to their structural type, hydrocarbons are relatively inactive in relation to their hydrogen-bonding capacity and solubility in water (Griffin et al., 2000). Ketones, aldehydes and alcohols are active, but with differing specificity and levels of activity, which is related to the present functional

Table 3. Antifungal activity of essential oils of the aerial parts of *Artemisia arborescens*, *A. herba-alba*, *A. judaica*, Bifonazole and Ketoconazole.

FUNGI	***A. arborescens***	***A. herba-alba***	***A. judaica***	**Bif**	**Ketoc**
	MIC	MIC	MIC	MIC	MIC
	MFC	MFC	MFC	MFC	MFC
	0.250	0.125	0.125	0.150	0.200
Aspergillus fumigatus	0.250	0.250	0.250	0.200	0.500
	0.125	0.060	0.030	0.100	0.200
Aspergillus versicolor	0.250	0.125	0.060	0.200	0.500
	0.125	0.060	0.060	0.150	1.500
Aspergillus ochraceus	0.250	0.125	0.125	0.200	2.000
	0.250	0.250	0.060	0.150	0.200
Aspergillus niger	0.500	0.250	0.125	0.200	0.500
	0.125	0.060	0.060	0.150	1.000
Trichoderma viride	0.250	0.125	0.250	0.200	1.000
	0.060	0.030	0.030	0.200	0.200
Penicillium funiculosum	0.060	0.060	0.060	0.250	0.500
	0.060	0.060	0.030	0.200	2.500
Penicillium ochrochloron	0.125	0.125	0.060	0.250	3.500
	0.125	0.060	0.060	0.100	0.200
Candida albicans	0.250	0.250	0.250	0.200	0.300

MICs and MFCs (mg/mL), mean value of two measurements; Bif, Bifonazole was used as a stock solution 1 mg/mL; Ketoc, Ketoconazole was used as a stock solution 1 mg/mL

group, but also associated with hydrogen-bonding parameters in all cases. Previous results have shown that greater antimicrobial potential could be ascribed to oxygenated terpenes, especially phenolic compounds (Soković et al., 2002; Couladis et al., 2004; Soković et al., 2005; Soković and van Griensven, 2006; Soković et al., 2010).

CONCLUSIONS

The presented results support future research into the antimicrobial properties and synergistic effects of the components (piperitone, camphor, chamazulene, chrysanthenone, *cis*-chrysanthenyl acetate, *cis*-thujone, etc.) of essential oils belonging to different groups of compounds (monoterpenes, sesquiterpenes) found in species of the genus *Artemisia*, for their potential application in medicine, agriculture and food industry.

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