

COMPOSITION, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *ARTEMISIA HERBA-ALBA* ESSENTIAL OILS FROM ALGERIA

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ABSTRACT

The essential oils of three individual plants of *Artemisia herba-alba*, growing wild in tree different locations from Bordj Bou Arréridj in East Algeria has been extracted by hydrodistillation and a microwave distillation process. The main components were α -thujone (35.1 and 31.2% in HD and MD oils, respectively) for Draâ Ech, davanone (39.6-37.3%) and 1, 8-cineole (9.2-7.8%) for Bordj Ghédir, chrysanthenone (29.6-43.8%), camphor (18.6-16.0%), and α -thujone (15.9-8.6%) for Bordj Bou Arréridj. In comparison with HD, MD allows to obtain oil in a very short time, with the comparable qualities and substantial saving of energy. The minimum inhibitory concentration of various essential oils shows a power inhibitor not exceeding 10 $\mu\text{g/mL}$ against all microbial strains. The three chemotypes were slightly active, and the weak DPPH radical scavenging activity of these oils could be attributed to the absence of some phenolic components.

Keywords: *Artemisia herba-alba*, essential oils, microwave distillation, antibacterial activity, antioxidant activity.

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1. INTRODUCTION

Artemisia herba-alba Asso also known as "desert wormwood" is a prominent plant of the Irano-Turanien steppes of Spain, North Africa and the Middle East [1]. In Algeria, it abounds over large areas of the steppes and Sahara desert [2]. This plant is used as aromatisant for coffee and in folk medicine for treatment of colds, coughing and intestinal disturbances [3].

Investigations on the medicinal properties of *A. herba-alba* extracts reported antidiabetic, leishmanicidal, antibacterial, and antifungal properties [4, 5]. A survey carried out among the South Algerian population showed that *A. herba-alba* is the most recognized aromatic and medicinal plant [2]. In Algeria, this greenish-silver perennial shrub is characterized by its drought tolerance, essentially due to its leaves polymorphism and root architecture [2]. Over last decades, studies on *A. herba-alba* were focused on its essential oils. Their composition in different parts of the world revealed a high level of polymorphism and led to the definition of several chemotypes. The essential oil from *A. herba-alba* Asso populations has previously been analyzed [6-25].

In this paper, the essential oils of various *A. herba-alba* populations have been analyzed. The two aims of the present work are to extend the qualitative and semi-quantitative analysis of *A. herba-alba* oils from East Algeria isolated by hydrodistillation and microwave distillation, and to compare their chemical compositions. The microwave hydrodistillation has been compared with those obtained by conventional hydrodistillation. Appropriate comparisons were made in terms of isolation yields and rates, essential oil composition, antimicrobial activities, antioxidant activity and energy consumption.

The antibacterial effect of *Artemisia herba-alba* Asso essential oil on different bacterial species was done through the agar dilution method for determination of Minimum Inhibitory Concentration (MIC). In addition, the compositions of volatile compounds were determined to use these data to deduce which components are likely to contribute to the activities of the whole oils and to determine any structural relationships between the components and their antibacterial activity.

2. RESULTS AND DISCUSSION

3.1. Chemical Composition

The yields of essential oil extracted from *A. herba-alba* with the different isolation methods are respectively 0.31-0.50 % and 0.15-0.31% for the HD and MD (Table 1). The total extraction times and energy consumption of HD and MD essential oils were also reported. MD was clearly quicker than conventional HD. The isolation took 15 min, whilst 2.5h were required by hydrodistillation. These results mean a substantial saving of time and quantity of water and energy. A total of 142 compounds (Table 1) were identified in *A. herba-alba* essential oils using the two techniques. HD and MD enabled the detection of most volatile active compounds in *A. herba-alba* essential oil from Bordj Bou Arreridj region, in three locations such as α -thujone, chrysanthenone and β -thujone for DC (Draâ Ech Chih), davanone, 1,8-cineole and davana ether for BG (Bordj Ghedir), chrysanthenone, camphor and α -thujone for BB (Bordj Bou Arreridj), but their proportions depend on the isolation technique. Lightly higher amounts of compounds are present in the essential oils of the aromatic plant isolated by HD in comparison with MD. The MD oil is more concentrated in ketones compounds for BB and DC. The essential oil of *A. herba-alba* isolated either by HD and MD contains the same dominant components. Ketones, are the main abundant components in the essential oils with equivalent relative amounts for both extraction methods: α -thujone (35.1% HD vs 31.2% MD) and chrysanthenone (25.4% HD vs 31.6% MD) for DC; chrysanthenone, (29.6% HD vs 43.8% MD), camphor (18.6% HD vs 16.0% MD), and α -thujone (15.9% HD vs 8.6% MD) for BB and davanone (39.6% HD vs 37.3% MD) for BG. This investigation has shown that the six oils were rich in ketones (79.0% - 40.8%) and ether-oxides were highest in BG (18.2 HD v.s 16.3% MD). The Algerian *A. herba-alba* oil from this high table-lands region is characterized in this work by the chemotype α -thujone-chrysanthenone (Draâ Ech Chih), chrysanthenone-camphor- α -thujone (Bordj Bou Arreridj) and davanone (Bordj Ghedir).

3.2. Antioxidant activity

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. DPPH radical is a stable free radical and accepts an electron or

hydrogen radical to become a stable diamagnetic molecule. The scavenging ability of essential oils and positive control BHT is presented in Table 2.

The three chemotypes were slightly active and the weak DPPH radical scavenging activity of these oils could be attributed to the absence of some phenolic components, which may play an important role.

3.3. Antimicrobial activity

The minimum inhibitory concentrations (MIC) of essential oils from *A. herba-alba* against fourteen species of microorganisms by the agar dilution method are summarised in Table 3. They show a good inhibitory potency against all bacterial strains (MIC not exceeding 10 µg/ml). The essential oils showed a good activity against all microorganisms tested. The data obtained from the agar dilution method using *A. herba-alba* essential oil from BG indicated that *Penicillium expansum* and *Pseudomonas aeruginosa* were the most sensitive microorganisms tested (MIC < 0,5 µg/ml) and the essential oil from BB for *Penicillium expansum* as well. Overall, the essential oil from BB displayed a broad antimicrobial spectrum and exerted a much stronger antimicrobial effect against filamentous fungi and yeasts than against Gram-positive and Gram-negative bacteria. The Gram-negative bacteria, *Agrobacterium tumefaciens*, *Escherichia coli* and *Pseudomonas aeruginosa* were more resistant to the essential oil from DC, along with *Aspergillus ochraceus* (fungus) and *Candida albicans* (yeast). The antimicrobial activity of the essential oil could be due to the major components as thujones, 1,8- cineole and camphor [26]. It is also possible that components present at lower concentrations might be involved in some type of synergy with the other active compounds [27, 28]. The antimicrobial activities of the *A. herba-alba* essential oils obtained by HD or MD were similar.

Table 1. Chemical composition (%) of *Artemisia herba-alba* oils isolated by HD and MD

N°	Compounds	RI ¹	RI ²	BG (%)		BB (%)		DC (%)	
				HD	MD	HD	MD	HD	MD
1	santalinatriene ^{a,g,i,j}	899	1028	0,3	0,2	tr	tr	tr	tr
2	tetra hydrofuran (2,5-diethenyl-2-methyl) ^{a,d,g,i}	908		0,5	0,3	-	-	-	-
3	tricyclene ^{a,d,g,h,i,j}	917	1009	tr	tr	0,3	0,1	0,2	0,1
4	α -thujene ^{a,d,e,g,h,k}	920	1026	tr	tr	0,1	tr	0,2	0,3
5	α -pinene ^{a,b,c,d,e,f,g,h,i,j,k}	927	1022	0,2	0,1	1,2	0,5	0,3	0,2
6	ethyl tiglate ^d	936	1224	0,1	tr	-	-	-	-
7	camphene ^{a,b,c,d,e,f,g,h,i,j}	941	1061	0,9	0,5	4,1	1,4	1,2	1,0
8	thuja-2,4(10)-diene ^{g,j}	948	1120	-	-	-	-	0,1	0,1
9	verbenene ^h	949		-	-	0,2	0,1	-	-
10	isopropyl tiglate ^g			tr	tr	-	-	-	-
11	5,5-dimethyl-2(5H) furanone ^d	956	1559	0,5	0,3	-	-	-	-
12	sabinene ^{a,b,d,e,f,g,h,i,j,k}	966	1116	tr	tr	0,6	0,2	0,8	0,6
13	β -pinene ^{a,b,c,d,e,f,g,h,i,j}	969	1100	tr	tr	0,2	0,1	0,1	0,1
14	1-octen-3-ol ^{g,h,i}	971	1431	0,1	tr	0,1	0,1	tr	tr
15	1,6-dimethyl hepta-1,3,5-triene	974	1218	-	-	0,5	0,3	0,2	0,2
16	octan-3-one ^{g,h}	978	1241	tr	tr	-	-	-	-
17	myrcene ^{a,b,d,g,h,k}	982	1162	1,1	1,0	tr	tr	tr	-
18	trimethyl-1,3,5-benzene ^{a,h}	986	1257	-	-	tr	tr	tr	tr
19	yomogi alcool ^{a,b,e,f,g}	988	1331	-	-	0,2	0,2	0,5	0,6
20	α -phellandrene ^{a,d,f,g,h}	994	1165	-	-	tr	tr	tr	tr
21	o-isopropenyl toluene	1009	1271	-	-	0,1	0,1	tr	tr
22	α -terpinene ^{a,c,d,f,g,h,i,j,k}	1011	1181	0,1	0,1	tr	tr	tr	tr
23	allyl tiglate ti	1012		-	-	0,1	tr	0,2	0,1
24	propanoic acid-2-methyl-2-methyl butyl ester	1013	1196	tr	tr	0,1	0,1	0,2	0,1

N°	Compounds			BG (%)		BB (%)		DC (%)	
		RI ¹	RI ²	HD	MD	HD	MD	HD	MD
25	trimethyl-1,2,3-benzene ^{a,h}	1015	1287	-	-	tr	tr	tr	tr
26	p-cymene ^{a,b,c,d,e,f,g,h,i,j,k}	1019	1250	0,7	0,5	0,7	0,5	1,8	1,5
27	β-phellandrene ^{a,g,h}	1023	1207	tr	tr	-	tr	0,2	0,2
28	1.8-cineole ^{a,b,c,d,e,f,g,h,i,j,k}	1028	1206	9,2	7,8	4,9	2,9	1,4	1,2
29	santalina alcohol ^{a,e,f,g,i}	1036	-	0,4	0,3	tr	tr	-	-
30	lavender lactone ^g	1042	1126	0,1	0,1	-	-	-	-
31	(Z) arbusculone ^{a,d,i}	1049		0,4	0,3	-	-	-	-
32	bergamal ti	1051		-	-	tr	tr	tr	tr
33	(E) decahydro naphtalene ti	1053		-	-	tr	tr	tr	-
34	γ-terpinene ^{a,c,d,e,f,g,h,i,j,k}	1055	1235	0,3	0,1	0,3	0,1	0,5	0,3
35	lavender lactone iso	1062	1245	tr	tr				
36	cis-sabinene hydrate ^{e,i,j}	1066	1473	tr	0,1	tr	tr	tr	tr
37	(E) arbusculone ^{a,d,i}	1068		0,9	0,6	-	-	-	-
38	cis butanoic acid-3-hexenyl ester ^g	1070	-	-	-	0,2	0,2	0,2	0,1
39	artemisia alcohol ^{a,e,f,g,i}	1081	1480	0,2	0,2	0,2	0,1	0,2	0,2
40	terpinolene ^{a,c,d,f,g,h,i,k}	1087	1276	tr	0,1	-	-	0,1	tr
41	trans-sabinene hydrate ^{a,c,j}	1095	1452	0,4	0,5	tr	tr	-	-
42	linalool ^{a,d,e,g,j}	1099	1471	0,5	0,4	-	-	-	-
43	filifolone ^{a,h,j}	1103	1420	tr	tr	tr	tr	tr	tr
44	α-thujone ^{a,b,e,f,g,h,i,j,k}	1105	1405	0,7	0,4	15,9	8,6	35,1	31,2
45	β-thujone ^{a,b,e,f,g,h,i,j,k}	1116	1420	0,6	0,3	5,1	3,6	9,7	8,3
46	isophorone ^a	1120	1349	-	-	-	-	0,3	0,1
47	(Z)-p-menth-2-en-1-ol ^{d,g,i}	1124	-	0,3	0,2	-	-	-	-
48	(Z)-pinene hydrate ^{b,g}	1125	-	tr	tr	-	-	-	-
49	chrysanthenone ^{a,c,d,e,f,g,h,i,j,k}	1127	1501	tr	tr	29,6	43,8	25,4	31,6
50	terpinene 1-ol ^g	1132	1561	-	-	tr	tr	-	-
51	trans-pinocarveol ^{a,b,d,e,f,g,h,i,k}	1134	1622	-	-	-	-	0,1	0,1

N°	Compound	BG (%)		BB (%)		DC (%)			
		RI ¹	RI ²	HD	MD	HD	MD	HD	MD
52	(E)-p-menth-2-en-1ol ^d	1142	-	0,1	0,1	tr	0,1	0,1	0,1
53	(E)-pinene hydrate ^b	1143	-	0,1	tr	tr	tr	-	-
54	camphor ^{a,b,c,d,e,f,g,h,i,j,k}	1148	1512	0,5	0,3	18,6	16,0	4,7	4,5
55	(E)-verbenol	1149	1648	tr	tr	-	tr	0,1	0,1
56	ipsidienol ^g	1150	-	0,1	0,1	-	-	-	-
57	isothujanol ^{b,h}	1155	-	-	-	tr	0,2	0,1	0,1
58	isoborneol ^{f,g}	1159	1652	-	-	tr	tr	-	-
59	sabina ketone ^{g,i,j}	1160	-	-	-	tr	tr	tr	tr
60	(E)-pinocamphone ^g	1164	-	-	-	tr	tr	0,1	0,1
61	(Z)-chrysanthenol ^{a,d,e,f,h,j}	1166	-	-	-	tr	tr	-	-
62	pinocarvone ^{a,e,g,h,i,j,k}	1167	1569	0,1	0,2	2,4	2,7	1,0	0,9
63	borneol ^{a,b,c,d,e,f,g,h,i,k}	1169	1671	0,2	0,2	1,9	1,8	0,6	0,6
64	δ -terpineol ^e	1169	1631					0,2	0,2
65	lavandulol ^{d,f,g,i}	1170	-	1,2	0,9	-	-	-	-
66	α -pellandrene-8ol ^{g,i}	1173	-	0,1	tr	tr	tr	-	-
67	santalanyl acetate ^{a,e}	1176	-	0,1	0,1	0,1	0,1	0,1	0,1
68	(Z)-pinocamphone ^e	1178	-	tr	tr	0,1	0,1	0,1	-
69	terpinen 4-ol ^{a,c,d,e,f,g,h,i,j,k}	1181	1588	0,9	0,7	0,7	0,5	0,9	0,7
70	thuj-3-en-10-al ^g	1185	1565	-	-	0,1	tr	0,2	0,2
71	p-cymen-8-ol ^{a,c,d,e,g,i}	1187	1832	-	-	tr	0,1	0,1	tr
72	α -terpineol ^{a,b,c,d,e,g,h,i,k}	1192	1672	0,1	0,1	0,2	0,2	0,2	0,2
73	myrtenal ^{a,c,e,f,g,h,i,j}	1196	1614	0,3	0,2	0,4	0,3	0,4	0,3
74	cis-piperitol ^{a,b,c,d,e,g,h,i}	1197	1723	tr	tr	tr	tr	tr	tr
75	myrtenol ^{a,c,e,f,g,h,i,j}	1199	1774	0,4	0,3	tr	tr	tr	tr
76	verbenone ^{a,b,f,g,h,i,j,k}	1205	1685	-	-	tr	tr	-	-
77	trans-piperitol ^{a,b,c,d,e,f,g,h,i,j}	1208	1740	0,1	0,1	0,5	0,4	0,6	0,6
78	trans-carveol ^{a,f,g,h}	1221	1818	-	-	0,1	0,1	0,1	0,1

N°	Compound			BG (%)		BB (%)		DC (%)	
		RI ¹	RI ²	HD	MD	HD	MD	HD	MD
79	cis-sabinene hydrate acetate ^g	1222	-	0,2	0,1	-	-	-	-
80	nerol ^f	1226	1777	-	-	-	-	tr	tr
81	cis-carveol ^{a,g,h,j}	1232	1849	-	-	tr	tr	0,1	0,1
82	nordavanone ^{a,d,g,i}	1233	-	0,5	0,3	-	-	-	-
83	cumin aldehyde ^{a,c,d,g,h,i}	1243	1770	-	-	tr	tr	tr	tr
84	trans-ocimene	1244	1811	-	-	0,1	tr	0,1	0,1
85	carvone ^{a,f,g,h,i,j}	1245	1721	-	-	0,3	0,2	0,3	0,2
86	carvotanacetone ^{a,g}	1249	1713	-	-	tr	tr	tr	-
87	piperitone ^{a,b,c,f,g,h,i}	1255	1710	0,1	0,1	0,3	0,4	0,9	0,8
88	cis-chrysanthenyl acetate ^{a,d,e,g,h,i,j,k}	1266	1607	0,1	0,1	0,3	3,1	0,3	0,2
89	neo-thujyl acetate ti	1281	-	-	-	tr	tr	-	-
90	cis-verbanyl acetate ^h	1288	-	0,3	0,2	0,1	0,2	0,1	0,1
91	lyratyl acetate ^{a,d}	1290	1611	tr	tr	-	-	tr	tr
92	isobornyl acetate	1291	1571	tr	tr	-	-	-	-
93	bornyl acetate ^{a,b,c,d,e,g,h,i,j}	1293	1573	0,2	0,2	0,3	0,4	0,2	0,1
94	lavandulyl acetate ^{d,f,g}	1295	-	0,1	0,1	-	-	-	-
95	sabinyl acetate ^{a,g,i}	1296	1619	tr	tr	tr	0,5	0,1	tr
96	trans-pinocarvyl acetate ti	1301	-	tr	-	-	-	0,1	0,1
97	carvacrol ^{d,f,i}	1302	2150	-	-	-	-	tr	tr
98	hexyl tiglate	1325	1702	tr	tr	-	-	-	-
99	filifolide A ^{a,h}	1326	-	tr	tr	0,2	0,1	0,2	0,2
100	terpinene-4ol acetate ^a	1342	-	-	-	-	-	tr	tr
101	α -terpinyl acetate ^{a,b,c,d,e,f}	1343	-	tr	tr	tr	tr	tr	tr
102	eugenol ^{d,g,h,k}	1351	2155	tr	tr	0,1	0,2	-	-
103	lratyl propionate ^a	1352	-	0,4	0,5	tr	tr	0,1	0,1
104	α -copaene ^{a,d,e,f,g,h,i}	1366	1475	0,3	0,2	0,2	0,1	0,6	0,5
105	β -bourbonene ^g	1383	-	tr	-	-	-	-	-

N°	Compound	BG (%)		BB (%)		DC (%)		
		RI ¹	RI ²	HD	MD	HD	RI ¹	
106	β -elemene ^{a,f}	1387	1579	tr	tr	tr	tr	tr
107	β -cubebene ^{c,e,f,g}	1386	1535	tr	tr	tr	-	0,1 0,1
108	(Z)-jasmone ^{g,i}	1393	1932	tr	-	tr	-	tr -
109	methyl eugenol ^{a,g,h}	1399	1999	tr	-	tr	tr	tr tr
110	isocaryophyllene ^{c,g}	1416	1595	tr	tr	0,1	tr	0,1 0,1
111	davana furane ^{a,g,i}	1419	1758	0,5	0,4	-	-	- -
112	β -cedrene ^g	1423	-	0,2	0,1	-	-	- -
113	β -caryophyllene ^{a,c,d,f,h,k}	1425	2038	-	-	0,1	tr	0,1 0,1
114	β -gurjunene ^g	1433	-	-	-	tr	tr	0,1 0,1
115	α -humulene ^{a,c,f,g,h,i}	1460	-	0,2	0,2	0,1	tr	0,2 0,1
116	alloaromadendrene ^{a,f,g}	1466	1628	0,1	0,2	0,1	tr	0,1 0,1
117	(E)-ethyl cinnamate ^{f,g}	1471	-	-	-	0,1	tr	0,1 0,1
118	β -chamigrene ^g	1477	-	-	-	tr		0,1 tr
119	cis-davanone ti	1478	-	0,1	0,1	-	-	- -
120	γ -murolene ^{a,d,g,h,k}	1480	1691	1,1	0,8	0,8	1,0	0,2 0,5
121	D germacrene ^{f,h,i,j,k}	1484		-	-	tr	tr	tr tr
122	β -selinene ⁱ	1488	1651	-	-	0,1	-	tr -
123	bicyclogermacrene ^{g,i,j}	1500	1718	1,2	1,1	0,4	0,3	0,2 0,2
124	davana ether ^{a,d,g,i,k}	1501	-	5,1	4,9	-	-	- -
125	α -murolene ^{a,f,g,h}	1503	1708	-	-	tr	tr	0,1 0,1
126	Cubebol	1518	-	-	-	tr	tr	0,1 0,1
127	davana ether ^{a,g,i} (isomer)	1523	-	1,5	1,8	-	-	- -
128	davana ether ^{a,d,g,i} (isomer)	1525	-	0,6	0,6	-	-	- -
129	δ -cadinene ^{a,c,d,f,h,k}	1527	1742	-	-	0,1	tr	0,1 0,1
130	cadina-1,4-diene	1533	2057	-	-	tr	tr	tr tr
131	artedouglacia oxide A ^g	1537	-	0,1	0,1	-	-	- -
132	davanone ^{g,k}	1557	-	0,5	0,3	-	-	- -

N°	Compound	BG (%)		BB (%)		DC (%)			
		RI ¹	RI ²	HD	MD	HD	RI ¹		
133	artedouglacia oxide D ti	1569	-	0,5	0,2	-	-	-	-
134	davanone ^{g,i} (isomer)	1560	2015	1,1	1,0	-	-	-	-
135	ledol ^{a,j}	1563	2020	0,3	0,3	0,1	0,1	0,1	tr
136	trans-nerolidol ^{d,f,g,i}	1564	2031	0,1	0,1	tr	tr	0,1	0,1
137	spathulenol ^{b,d,f,g,h,i,k}	1569	2115	0,1	tr	0,4	0,2	1,5	1,1
138	caryophyllene oxide ^{d,f,g,h,i,k}	1583	1977	1,2	0,9	0,2	0,1	1,3	1,0
139	globulol ^{g,i,j}	1585	2065	-	-	tr	tr	0,1	0,1
140	β -copaen 4- α ol ^{g,i}	1588	2141	-	-	0,6	0,4	0,3	0,2
141	davanone ^{a,d,e,g} (isomer)	1590	2015	39,6	37,3	-	-	0,6	0,4
142	viridiflorol ^f	1594	2075	-	-	0,4	0,2	0,1	0,1
Isolation time (min)				150	15	150	15	150	15
Energy consumption (Kwh)				1.75	0.21	1.75	0.21	1.75	0.21
Identified compounds				94	90	100	98	96	92
Yield %*				0.50	0.31	0.46	0.20	0.31	0.15
Identified components (%)				79.5	70,1	95.8	94.0	98.0	95.2
Monoterpene hydrocarbons				3.6	2.6	7.7	3.0	5.5	4.4
Sesquiterpene hydrocarbons				3.6	3.0	2.2	1.5	2.1	2.2
Ketones				44.4	40.8	73.0	76.3	78.8	79.0
Alcohols				5.7	4.6	5.5	4.9	6.4	5.6
Ether oxides				18.2	16.3	5.1	3.0	2.7	2.2
Esters				1.7	1.3	1.1	4.6	1.5	1.0
Others				2.3	1.5	1.2	0.7	1.0	0.8

tr : trace < 0.05%; ti: tentatively identified; RI¹ and RI²: temperature programmed indices referred to n-alkanes C₇-C₂₈, determined respectively on HP5-MS and HP-Wax capillary columns according retention to Van Den Dool and Kratz; HD: hydrodistillation; MD: Microwave distillation; DC: Draâ Ech Chih ; BG: Bordj Ghedir and BB: Bordj Bou Arreridj

* Yield expressed as in grams of oil per 100g of plant material

a : (Benjilali, 1980 and 1981); b : (Boutekdjiret, 1992); c : (Feuerstein, 1988); d : (Salido, 2001 et 2004); e : (Segal, 1987); f : (Dob, 2006), g : (Dahmani-Hamzaoui, 2005 and 2010); h: (Vernin, 1994 and 1995); i: (Haouari, 2009); j: (Paolini, 2010); k: (Bezza, 2010).

Table 2. Radical scavenging activity of the essential oils of *Artemisia herba alba* against DPPH radical .

Sample ^b	Radical scavenging (%) ^a	
	HD ^c	MD ^c
BG	5.65	5.66
BB	13.23	13.06
DC	9.53	9.56
BHT	64.72	

^a DPPH: Scavenging percentage values are means of three replicates and the RSD is less than 1%.

^bBG: Bordj Ghedir ; BB: Bordj Bou Arreridj ; DC : Draâ Ech Chih ; BHT : 2,6-di-*tert*-Butyl-4-methylphenol (used as reference compound)..

^c HD: hydrodistillation; ^c MD: Microwave distillation.

Table 2. Antimicrobial activity of the essential oils of *Artemisia herba-alba*

Microorganism	Minimum inhibitory concentration (MIC) ($\mu\text{g/mL}$)		
	DC	BG	BB
<i>Bacillus subtilis</i> ATCC6663	5	5	5
<i>Bacillus coagulans</i> CIP6625	5	5	5
<i>Micrococcus luteus</i> ATCC9314	5	5	5
<i>Staphylococcus aureus</i> CIP7625	2	5	1
<i>Agrobacterium tumefaciens</i> N°2410	10	5	5-10
<i>Escherichia coli</i> CIP54.8	10	5	5
<i>Pseudomonas aeruginosa</i> CIPA22	10	<0,5	2
<i>Mucor ramannianus</i> NRRL1829	5	2	2
<i>Aspergillus ochraceus</i> CINRA	10	5	5
<i>Fusarium oxysporum</i> f. sp. <i>albedinis</i> CURZA	5	5	1
<i>Penicillium expansum</i> 8932	1	<0,5	<0,5
<i>Fusarium oxysporum</i> f. sp. <i>lini</i> CINRA	5	5	1
<i>Candida albicans</i> CLM	10	10	5
<i>Saccharomyces cerevisiae</i> ATCC4226	2	2	1

ATCC: American Type Culture Collection; CURZA: collection de l'Unité de Recherche sur les Zones Arides (Alger); CIP: collection de l'Institut Pasteur de Paris, France; CINRA: collection de l'Institut National de Recherche Agronomique de Dijon, France; NRRL: Northern Regional Center, Peoria (U.S.A); CLM: collection du Laboratoire de Microbiologie de l'ENS de Kouba, Alger.

DC: Draâ Ech Chih; BG: Bordj Ghedir; BB: Bordj Bou Arreridj

3. EXPERIMENTAL

2.1. Plant material

The aerial parts of *A. herba-alba* were collected in June 2007 at the flowering stage. The three samples were from Bordj Bou Arreridj region (BB), at 240 km east of Algiers, Draâ Ech Chih (DC) and Bordj Ghedir (BG) are respectively at 13km and 25km far from Bordj Bou Arreridj, in high table-lands of Algeria. The climate in Algeria is of Mediterranean type on all the northern fringe which includes the Littoral and the Tellian Atlas (hot and dry summers, wet and cool winters), semi-arid on the high-lands in the center of the country and desert as soon as the we cross the chain of the Saharan Atlas. The climatic situation of region studied was: climate (Summer very hot and winter very cold), and temperature January / July ($-3^{\circ}\text{C}/40^{\circ}\text{C}$), Rainfall (300-700 mm / yr) and Altitude (302 – 1885m).

The plant was authenticated by Dr. R. Amirouche, and voucher specimens of the different samples were stored in the Herbarium of the Vegetal Biology Departement, University of Sciences and Technology Houari Boumediene, Bab Ezzouar, Algiers. Plant samples were air-dried (3 – 6 days), minced, and subjected immediately to oil isolation.

2.2. Isolation of the essential oils

2.2.1. Microwave Hydrodistillation

Microwave hydrodistillation (MD) was performed at atmospheric pressure in a microwave laboratory oven, as described previously [29, 30]. Plant material (100g) was heated using a fixed power density of 1000 W for 15 min with 30 ml of distilled water. The direct interaction of microwaves with biological water releases the essential oils trapped inside the cells of plant tissues. The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C until used. Extractions were performed at least three times and the mean values are reported.

2.2.2. Hydrodistillation

Plant material (100g) was submitted to hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia and extracted with 3L of water for 2.5h.

The essential oil was collected, dried under anhydrous sodium sulphate, and stored at 4°C

until used. Extractions were performed at least three times and the mean values are reported.

2.3. Gas chromatography analysis

GC analysis was carried out using a Hewlett-Packard 6890N gas chromatograph equipped with a flame ionisation detector (FID), under the following operation conditions: vector gas, N₂; injector and detector temperatures, 250 °C and 320 °C, respectively; injected volume 0.2 µl; split-less mode; HP5MS (30 m x 0.25 mm LD., film thickness 0.25 µm; constant gas flow 0.3 mL/min) and HP wax (60 m x 0.32 mm LD., film thickness 0.25 µm; constant flow 0.9 ml/min); the oven temperature program was 60 °C for 8 min, rising to 250 °C at 2 °C/min, then held for 30 min at 250 °C; Retention indices were determined with C₅-C₂₈ alkanes standards as reference. Relative amounts of individual components are based on peak areas obtained without FID response factor correction.

2.4. Gas chromatography/mass spectrometry analysis

GC/MS analysis was carried out using an Agilent 6890N coupled to an Agilent 5973A mass spectrometer. Samples were analysed on a fused-silica capillary column HP5MS (30 m x 0.25 mm LD., film thickness 0.25 µm) and HP wax (60 m x 0.32 mm LD., film thickness 0.25 µm). Carrier gas He, injector and detector temperatures, 250 °C flow rate 0.5mL/min; split 1:20; injection volume 0.1µl; oven temperature progress from 60 to 250°C at 2°C/min; ionisation mode used was electronic impact at 70eV; electron ionisation mass spectra were acquired over the mass range 35-400 µ.

2.5. Identification components

Component identification was confirmed by comparison of mass spectral fragmentation patterns with those stored in the MS data bank (NIST 2002, Wiley 7), laboratory mass spectra libraries built up from pure substances, and with previously published spectra, and verified by comparison of linear retention indices of the identified compounds with published index data [31-33] on apolar and polar columns.

2.6. Antioxidant activity

The antioxidant activity of the different essentials oils was determined according to the ability of the tested samples to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH') [34], by off-line spectrophotometric measurements. Methanolic solutions (2.4 ml) of DPPH' (10⁻⁵M)

with an absorbance at 515 nm of 0.800 ± 0.030 AU were mixed with methanolic solutions (1.2 ml) of samples at a concentration of 1000 $\mu\text{g/ml}$. Triplicate samples were shaken and allowed to stand for 15 min in the dark at room temperature, and the decrease of absorbance at 515 nm was measured using a Perkin-Elmer Instruments, Norwalk, CT, USA). The radical scavenging activity of the tested samples, expressed as DPPH \cdot scavenging percentage, was calculated by the following formula:

$$\left[\frac{(A_A - A_B)}{A_B} \right] \times 100$$
, where A_B is the absorbance of the blank sample ($t = 0$), and A_A is the absorbance of the tested sample after 15 min.

2.7. Antimicrobial activity

Bacterial strains used in this study were obtained from American Type Culture Collection (ATCC), USA: *Bacillus subtilis* ATCC6663, *Micrococcus luteus* ATCC9314 and *Saccharomyces cerevisiae* ATCC4226; collection de l'Unité de Recherche sur les Zones Arides (CURZA), Alger: *Fusarium oxysporum* f. sp. *albedinis* ; collection de l'Institut Pasteur de Paris (CIP), France: *Bacillus coagulans* CIP6625, *Staphylococcus aureus* CIP7625, *Escherichia coli* CIP54.8 and *Pseudomonas aeruginosa* CIPA22; collection de l'Institut National de Recherche Agronomique de Dijon (CINRA), France: *Fusarium oxysporum* f. sp. *Lini*, *Aspergillus ochraceus* ; Northern Regional Center, Peoria (NRRL), USA: *Mucor ramannianus* NRRL1829; collection du Laboratoire de Microbiologie de l'ENS de Kouba (CLM), Algerie : *Candida albicans*.

The agar dilution method was employed to determine the antimicrobial activity of the essential oils. For these essays, cultures of the following microorganisms were used: four Gram-positive (*Bacillus subtilis*, *Bacillus coagulans*, *Micrococcus luteus* and *Staphylococcus aureus*) and three Gram-negative (*Agrobacterium tumefaciens*, *Escherichia coli* and *Pseudomonas aeruginosa*) and five fungi (*Mucor ramannianus*, *Aspergillus ochraceus*, *Fusarium oxysporum* f. sp. *albedinis*, *Penicillium expansum* and *Fusarium oxysporum* f. sp. *lini*) and two yeasts (*Candida albicans* and *Saccharomyces cerevisiae*). The agar dilution method was used: a final concentration of 1% (v/v) Tween-80 was incorporated into the agar after autoclaving to enhance oil solubility. Briefly, a series of two fold dilutions of each oil, ranging from 0.5 to 75 $\mu\text{g/ml}$, was prepared with 1% (v/v) Tween-80. Each Petri dish of 5 mm

in diameter contains 3 ml of nutrient agar medium and a known concentration of essential oil was dried prior to inoculation with 2 μ l of spots containing approximately 3×10^6 cells (for bacteria and yeasts) or spores (for fungi) of each organism. The experiment was repeated twice to confirm the inhibitory effect.. Minimum inhibitory concentrations (MIC) were determined after 24 h incubation at 37°C for bacteria and yeasts and 48 h for filamentous fungi. The MIC was determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar plate [35, 36],.

4. CONCLUSION

In conclusion, this work completes our chemical composition study on *A. herba-alba* essential oils from Algeria. Among the 142 constituents isolated by HD and MD and identified by GC and GC/MS, a great number has not been reported previously, to our knowledge. Overall, the essential oils from *A. herba-alba* displayed a broad antimicrobial spectrum and exerted a largest inhibition against all the tested microorganisms.

In comparison with HD, microwave extraction can produce essential oil in concentrated form, free from any residual solvents, contaminants or artefacts. The new systems developed to date indicate that microwave extraction offers net advantages in terms of selectivity, with shorter extraction times and better essential oil compositions, and is environmentally friendly. In this article we have discussed how microwave extraction highly accelerated the extraction process, without causing considerable changes in the volatile oil composition and properties, phenomena which were previously described. The MD offers important advantages over traditional alternatives: shorter isolation times, same yields, reduced cost; less energy consuming; cleaner features, and a better possibility of the natural aroma reproduction of the essential oil compared to HD.

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