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Full Length Research Paper

Chemical composition, antibacterial and antioxidant activities of a new essential oil chemotype of Algerian *Artemisia arborescens* L.

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The aim of the present study is to investigate the chemical composition, antibacterial and antioxidant activities of three essential oils extracted by hydrodistillation from the aerial parts of *Artemisia arborescens* L., which was collected from three different regions near Tlemcen city in the West Northern of Algeria: Beni Snous, Bidar and Chetouane. The chemical composition was investigated using both capillary gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) techniques. Fifty-two compounds were detected, a total of fifty compounds, representing 81.8 to 90.2% of the total oils were identified in the three samples of essential oils. The essential oils of *A. arborescens* were rich in camphor (Beni Snous: 72.2%, Bidar: 50.3% and Chetouane: 32.8%). The present composition of the essential oils, with camphor as the only most abundant component, was considered as a new chemotype of *A. arborescens* growing in West Northern of Algeria. Antibacterial activity of the essential oils against gram-positive and gram-negative bacteria, was tested using the diffusion method and by determining the inhibition zone. The results showed that the oils had a great potential antibacterial activity against some bacteria. The maximum zone of inhibition was obtained against *Enterococcus faecalis* (22 mm). In contrast, the oils were ineffective on the inactivation of *Lysteria monocytogenes* and *Escherichia coli*. Antioxidant capacity was assessed by *in vitro* tests using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and significant activity was found for these *A. arborescens* oils.

Key words: *Artemisia arborescens* L., Asteraceae, essential oil, chemical composition, chemotype, camphor, antibacterial activity, antioxidant activity.

INTRODUCTION

The genus *Artemisia* (Family Asteraceae, tribe Anthemideae) belongs to a useful group of aromatic and medical plants comprising a variable number of species (from 200 to over 400, depending on the authors). The genus is wide spread on the Northern hemisphere, but only 10 taxons were identified on Southern hemisphere (Verdian-Rizi et al., 2008; Abou et al., 2010; Radoslaw et al., 2007).

Referring to phytochemistry, *Artemisia* species are distinguished by the presence of essential oils, polyacetylenes as well as lignans, sesquiterpene lactones and

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flavonoids (Radoslaw et al., 2007).

Various species of *Artemisia* have been characterized for their biological activities. They are considered to produce most medicinally important secondary metabolites. Several studies using *Artemisia* spp. showed a series of antimicrobial and antioxidant activities (Ahameethunisa and Hopper, 2010; Baykan et al., 2012).

Artemisia species are frequently utilized for treatment of diseases such as inflammation, hepatitis, cancer and infections by malaria, fungi, bacteria and viruses. They are also reported to possess antidiabetic effect (Jeong-Dan, 2007; Nezhadali et al., 2008).

Artemisia is one of the genera of Asteraceae family with many important medicinally valuable essential oils that have been widely used for a variety of medicinal purposes for many years (Ahameethunisa and Hopper, 2010).

Artemisia arborescens L. is a medicinal and aromatic plant from Asteraceae family. It is an evergreen shrub which grows wild in the maritime cliffs and the bushes of littoral hills (Lai et al., 2006; Quezel and Santa, 1962, 1963). This plant has anticatarrh, mucolytic, anti-inflammatory, antiallergenic, antihistaminic and choleretique (increases bile production) properties. It indicated for bronchial cattarh and for asthma, problems of the skin and insufficient bile production (Rosé and Earle, 1996).

The study of Saddi et al. (2007) demonstrated the antiviral activity of the essential oil *in toto* obtained from *A. arborescens* L. against herpes simplex virus 1 and 2 (HSV-1 and HSV-2). The mode of action of the essential oil as antiherpesvirus agent seems to be particularly interesting in consideration of its ability to inactivate the virus and to inhibit the cell-to-cell virus diffusion.

A. arborescens L. essential oil also demonstrated pesticidal activity against *Aphis gossipy* (a pest of citrus fruits), adult and young *Bemisia tabaci*, and *Lymantria dispar* L. (pest of *Quercuc suber*) and was efficiently encapsulated in cross-linked alginate beads for a controlled release into the soil (Lai et al., 2006).

The chemical composition of *A. arborescens* L. essential oil has been intensively investigated throughout the world. The chemical composition of the essential oil of *A. arborescens* L. growing in Morocco revealed the presence of β-thujone and camphor as the main constituents (Codignola et al., 1984; Pappas and Sheppard-Hanger, 2000).

The essential oil of *A. arborescens* L. from USA was identified and the major constituents were chamazulene and camphor (Tucker et al., 1993; Pappas and Sheppard-Hanger, 2000)**.** Analysis of essential oil of this plant collected from Italy (Sardinia) by gas chromatography-ion trap-mass (GC/ITMS) was reported and the characteristic components were camphor and β-thujone (Lai et al., 2006). Abderrahim et al. (2010) analyzed the aerial part essential oil of *A. arborescens* L. from Bejaia province of East of Algeria. The most abundant components were chamazulene and β-thujone.

Recently, Militello et al. (2011) examined the chemical composition of *A. arborescens* L. essential oil growing wild in Sicily (Italy) and the capability to inhibit some food-borne pathogen bacteria. Oxygenated monoterpenes (57.32%) constituted the main fraction, with β-thujone as the main compound, followed by the sesquiterpene hydrocarbon chamazulene. Undiluted essential oil showed a large inhibition spectrum against strains of *Lysteria monocytogenes*, whilst it was ineffective against *Enterobacteria* and *Salmonellas*. The minimum inhibition concentration (MIC) was evaluated for the two most sensitive strains (*L. monocytogenes* 186 and 7BO) at two cellular concentrations (10^6 and 10^7 CFU/ml). The lowest MIC (0.625 µl/ml, dilution of oil with acetone) was found for strain *L. monocytogenes* 186 at 10^6 CFU/ml.

In addition, the essential oil of *A. arborescens* L. from Turkey was identified and the major constituents were camphor and chamazulene. The essential oil was also tested for antimicrobial activity using the disc-diffusion method against bacteria and fungus. The oil was significantly active against *Candida albicans* (18 mm) (Baykan et al., 2012).

Reviewing the current literature, there are no previous studies of the antioxidant activity of *A. arborescens* L. essential oil using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The aim of this work was, in the first step, to investigate the chemical composition of essential oils from aerial parts of *A. arborescens* L. collected near Tlemcen (the West Northern of Algeria) during flowering stage and the results were compared with previous reports. In the second step, the antibacterial and antioxidant activities of these essential oils were evaluated.

MATERIALS AND METHODS

Plant material

The aerial parts of *A. arborescens* L. were collected from three regions near Tlemcen in the West Northern of Algeria: Beni Snous (853 m, 34°38'32"N, 1°33'45"W), Bidar (131 m, 35°4'0"N, 2°6'0"W) and Chetouane (573 m, 34°55'14"N, 1°17'29"W), during the flowering period (May, 2010).

Botanical identification of plant was conducted by Prof. Noury BENABADJI and a voucher specimen of the plant was deposited in the Herbarium of the Laboratory of Botany, Department of Biology, University of Aboubekr Belkaïd, Tlemcen, Algeria.

Before extraction, plants were extended by ground, in one layer, in an open room protected from the sun. During drying time, plants were turned over to allow homogeneous drying.

Essential oil isolation

The essential oils were isolated by hydrodistillation from the flowering aerial parts in Clevenger-type apparatus for 5 h.

Dichloromethane was used to recover the oils from the extractor apparatus. The organic phase was dried using anhydrous sodium sulphate and then the solvent was evaporated under reduced pressure in a rotary evaporator. The oil yield was expressed w/w versus dry matter. The essential oils were stored in sealed vials in the dark at +4°C before analysis and bioassays tests. The essential oils obtained were blue with a strong odor.

Essential oil analysis

***Gas chromatography* (GC)**

GC analysis was performed using a Perkin-Elmer Clarus 600 GC apparatus (Walhton, MA, USA) equipped with a single injector and two flame ionization detectors (FID). The analysis was carried out using two fused silica capillary columns (60 m × 0.22 mm i.d.; film thickness 0.25 µm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). The operating conditions were as follows: Injector and detector temperatures were maintained at 280°C. Helium was used as carrier gas (1 ml/min), the injection volume was 0,1 µl, split ratio was adjusted at 1:80, the oven temperature was programmed from 60 to 230°C at the rate of 2°C/min and then held isothermally at 230°C for 30 min.

***Gas chromatography-mass spectrometry* (GC/MS)**

The oils were investigated using a Perkin Elmer TurboMass quadrupole analyzer, directly coupled to a Perkin Elmer Autosystem XL equipped with two fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 µm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions were the same as ealier described. Ion source temperature, 150°C; energy ionization, 70 eV; electron ionization mass spectra were acquired with a mass range of 35 to 350 Da; scan mass, 1 s. Oil injected volume, 0.1 µl.

Component identification

Identification of the components was based:

1) On comparison of their GC retention indices (RI) on non-polar and polar columns. with those of authentic compounds or literature data (Arômes Library, 1987-2011; König, 2001; Adams, 2001; Velasco-Negueruela et al., 2002; Dabiri and Sefidkon, 2003; Bendimerad et al., 2005; Cha, 2007; Dib et al., 2010; Khamsan et al., 2011).
2) On comparison of their recorded mass spectra with those of a computer library provided by instrument software and MS literature data (Arômes Library, 1987-2011; König, 2001; Adams, 2001; NIST, 1999; Mc Lafferty et al., 1988, 1994; Hochmuth, 2006). RI were determined with C_7 to C_{25} alkane standards as reference,

Antibacterial activity

Bacterial strains

Antibacterial activities of *A. arborescens* essential oils were tested against 11 strains of bacteria: gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 10876), *Enterococcus faecalis* (ATCC 49452), *L. monocytogenes* (ATCC 15313) and gram-negative bacteria: *P*seudomonas *aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 13311), *Acinetobacter baumanii* (ATCC 19606), *Citrobacter freundii* (ATCC 8090), *Proteus mirabilis* (ATCC 35659), *Klebsiella pneumoniae (*ATCC 700603). The microorganisms were obtained from “Institut Pasteur de Paris (IPP)”.

Screening for antibacterial activity

Antibacterial activity was tested by the agar-well diffusion method (Bagamboula et al., 2004; Mighri et al., 2010). All bacterial cultures were first grown on Muller Hinton infusion (MHI) agar plates at 37 °C for 18 to 24 h prior to inoculation onto the nutrient agar. One or several colonies of similar morphology of the respective bacteria were transferred into API suspension medium (Biomérieux) and adjusted to 0.5 McFarland turbidity standard with a Densimat (Biomérieux).

The inoculums of the respective bacteria were streaked onto MHI agar plates using a sterile swab. A sterile filter disc (diameter 6 mm, Whatman paper No. 3) was placed. The disc was impregnated by the tested essential oils (10 µl/disc). The treated Petri dishes were placed at 4°C for 1 to 2 h and then incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the zone of growth inhibition around the discs after 24 h of incubation at 37°C.

The diameter of the zones of inhibition around each of the discs was taken as measure of the antibacterial activity. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

Antioxidant activity

DPPH radical scavenging assay

The hydrogen atoms or electron-donating ability of the corresponding essential oils was determined from the bleaching of purple-colored methanol solution of DPPH (Hatano et al., 1988). This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits and Bucar, 2000; Tepe et al., 2005). Radical scavenging activity of essential oils was measured by slightly modified method of Mighri et al. (2010) and Braca et al. (2002) as later described. Different concentrations of each essential oil were prepared in methanol (10, 20, 40, 60, 80 and 100 mg/ml). A solution of DPPH in methanol (24 µg/ml) was prepared and 2 ml of this solution was added to 50 µl of essential oil solution in methanol at different concentrations (10, 20, 40, 60, 80 and 100 mg/ml). The solution of DPPH was prepared daily before measurements. The sample solutions were kept in the dark at ambient temperature and the absorbance was measured at 517 nm at different times using a spectrophotometer (Thermo spectronic Heλiosγ) against methanol. The blank sample was used as 2 ml of DPPH solution (24 µg/ml in methanol) with 50 µl of methanol. Decreasing of the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity (% of inhibition). This activity is given as percent DPPH radical scavenging, which is calculated with the following equation:

$$\% \text{ DPPH radical scavenging} = [(A_0 - A_t)/A_0] \times 100$$

A_0, Absorbance of blank (t = 0 min); A_t, absorbance of tested sample solution at the time t.

The experiment was performed in triplicate and the average absorbance was noted for each measure. The same procedure was

followed for the positive control: ascorbic acid (AA) and tannic acid (TA). The methanol was used for baseline correction.

RESULTS AND DISCUSSION

Chemical composition

The essential oils were obtained by hydrodistillation using a Clevenger apparatus, from the aerial parts of *A. arborescens*, collected from different geographical origins near Tlemcen, a city in the West Northern of Algeria. The blue essential oils obtained (yields (w/w) in relation to the dry weight of the plant: Beni Snous (EOBS): 0.52%, Bidar (EOBD): 0.31% and Chetouane (EOCT): 0.64%) were analyzed by GC and GC/MS techniques. The relative percentages and the RI of the detected components were shown in Table 1. From the data obtained, fifty compounds were identified representing: EOBS: 90.2%, EOBD: 88.2% and EOCT: 81.8% of the total oils. Among these compounds, 13 monoterpene hydrocarbons, 14 oxygenated monoterpenes, 9 sesquiterpenes hydrocarbons, 9 oxygenated sesquiterpenes and 2 phenylpropanoïds were identified.

In addition to our results, the yields of the essential oils, obtained from different geographical origins varied considerably (Table 1). The highest oil yield was obtained from *A. arborescens* L. harvested in EOCT (0.64%, w/w). Baykan et al. (2012), Abderrahim et al. (2010) and Lai et al. (2007) studied the essential oil of *A. arborescens* and found that the yields were 1.20, 0.87 and 0.80%, respectively. Comparatively, it was seen that we have obtained low yields.

As shown in Table 1, all studied oils were dominated by the oxygenated monoterpenes (EOBS: 78.4%, EOBD: 61.8% and EOCT: 50.9%). The camphor was found to be the major constituent in all essential oils (EOBS: 72.2%, EOBD: 50.3% and EOCT: 32.8%). The other main compounds with concentrations higher than 4% were Terpinen-4-ol (EOBD: 6.1% and EOCT: 8.9%), Myrcene (EOBD: 7.4%) and Chamazulene (EOCT: 8.7%).

In one recent report on chemical composition of the essential oil of *A. arborescens* L. collected in Bejaia (East Northern of Algeria), β-Thujone (27.8%) and Chamazulene (30.2%) were determined as the predominant constituents which were present in small quantities in our research. By contrast, camphor, characterized as the main constituent of our studied oils, was not identified in the essential oil from Bejaia (Abderrahim et al., 2010). This compound was identified, for the first time, in the essential oil of *A. arborescens* L. of Algeria. For further comparison, the camphor (35.73%) and β-thujone (23.97%) chemotype was also found in the essential oil of *A. arborescens* L. from Sardinia, Italy (Lai et al., 2006), while the highest contents of β-Thujone (45.04%) and chamazulene (22.71%) were characteristic of essential oil isolated from Sicily, Italy (Militello et al., 2011). In addition, the major constituents of the essential oil of *A. arborescens* L. from Turkey were camphor (33.39%) and chamazulene (21.05%) (Baykan et al., 2012). Moreover, the Moroccan essential oil consisted, mainly of β-Thujone (30.06%) and camphor (21.67%) (Codignola et al., 1984; Pappas and Sheppard-Hanger, 2000). The chamazulene (39.60%) and camphor (16.71%) type oil was reported in USA (Tucker et al., 1993; Pappas and Sheppard-Hanger, 2000).

The present composition of the essential oils, with camphor as the only most abundant component (32.80 to 72.20%) was considered as a new chemotype of *A. arborescens* growing in West Northern of Algeria.

In conclusion, there are considerable qualitative and quantitative differences between the composition and the yield of essential oils from Algerian, Italian, Turkish, Moroccan and American (USA) origins. This variability can be attributed to the geographical region, climatic conditions, period of collection of the plant, state of plant (fresh or dry) and the method of extraction of the essential oil.

Antibacterial activity

The antibacterial activities of *A. arborescens* essential oils originating from the West Northern of Algeria were evaluated by paper disc diffusion method against 11 bacteria.

Table 2 showed that these oils have variable antibacterial activity against all tested strains. From our results, the variation in quantities of different components might be responsible for the different antibacterial activities. The inhibition zones were in the range of 7 to 22 mm. All bacterial strains showed less susceptibility to the EOBD. Among all gram-positive bacteria growths, the maximum zone of inhibition was recorded against *E. faecalis*: 22 mm, followed by *B. cereus*: 15 mm and *S. aureus*: 15 mm. Furthermore, the oils were ineffective against *L. monocytogenes*. On the other hand, seven different gram-negative bacterial strains were tested and among these microorganisms, *S. typhimurium* showed maximum zone of inhibition: 18 mm, followed by *C. freundii*: 15 mm and *K. pneumoniae*: 15 mm. In contrast, the oils did not show bacteria inhibitory effects on *E. coli*.

The essential oil of *A. arborescens* showed weak to good bacteria inhibitory effects on the test micro-organisms. The antibacterial activity of *A. arborescens* essential oils would be related to their oxygenated monoterpenes components which constitute more than 50.9% of the oils. Indeed, in essential oils, it was shown that monoterpenes hydrocarbons and oxygenated mono-terpenes are able to destroy cellular integrity resulting in respiration inhibition and permeability alteration (Cox et

Table 1. Chemical composition of *A. arborescens* L. essential oils from the West Northern of Algeria.

No.	Compound	IR^a_{Lit}	IR^b	IR^c	Essential oil[d]		
					EOBS	EOBD	EOCT
	Monoterpene hydrocarbon				(5.2)	(15.9)	(10.5)
1	α-Thujene	922	922	1020	0.1	0.1	0.1
2	α-Pinene	931	930	1018	0.1	0.5	0.4
3	Camphene	943	943	1061	tr	0.5	0.6
4	Sabinene	964	964	1116	0.9	0.7	0.1
5	β-Pinene	970	969	1106	0.1	0.1	tr
6	Myrcene	979	980	1154	2.0	7.4	2.7
7	α-Phellandren	997	997	1158	0.1	0.1	0.1
8	α-Terpinene	1008	1009	1173	0.2	1.0	0.9
9	p-Cymene	1011	1012	1261	0.7	1.4	1.3
10	Limonene	1020	1021	1193	0.3	0.5	0.4
11	γ-Terpinene	1047	1048	1238	0.6	2.6	3.1
12	Terpinolene	1078	1078	1275	0.1	0.5	0.7
13	1,4-Dihydro-p-menth-2-ene	1245	1242	2071	tr	0.5	0.1
	Oxygenated monoterpene				(78.4)	(61.8)	(50.9)
14	1,8-Cineole	1020	1021	1205	0.1	0.2	0.3
15	Trans-sabinene-hydrate	1051	1053	1454	0.1	0.1	2.3
16	Cis-sabinene-hydrate	1083	1085	1509	2.1	2.3	3.4
17	α-Thujone	1089	1085	1387	0.2	0.3	0.4
18	β-Thujone	1103	1097	1411	0.9	1.1	1.0
19	Trans-pinene-hydrate	1110	1109	1387	0.2	0.3	0.5
20	Camphor	1123	1124	1505	72.2	50.3	32.8
21	Cis-pinene-hydrate	1130	1132	1411	0.1	0.1	0.1
22	Borneol	1148	1149	1694	tr	0.3	0.4
23	Terpinen-4-ol	1161	1163	1590	2.1	6.1	8.9
24	α-Terpineol	1179	1180	1694	tr	0.4	0.2
25	Cis-piperitol	1181	1190	1674	0.1	0.1	0.2
26	Trans-piperitol	1193	1195	1676	0.1	0.1	0.1
27	Périllaldéhyde	1248	1245	1785	0.2	0.1	0.3
	Sesquiterpene hydrocarbon				(2.6)	(6.4)	(14.8)
28	α-Copaene	1379	1376	1483	tr	0.2	0.2
29	β-Bourbonene	1385	1383	1509	0.1	tr	0.1
30	Trans-caryophyllene	1424	1414	1584	0.4	0.3	0.7
31	γ-Muurolene	1471	1468	1677	0.1	0.1	0.2
32	Germacrene -D	1480	1474	1694	1.5	2.1	3.5
33	(E,E)-α-farnesene	1498	1495	1736	0.2	0.3	0.8
34	γ-Cadinene	1507	1504	1742	0.1	0.1	0.1
35	δ-Cadinene	1516	1512	1752	0.1	0.1	0.5
36	Chamazulene	1713	1707	2360	0.1	3.2	8.7

al., 2000; Zouari et al., 2010). However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to the antibacterial activity exhibited. In the oils, the possible compounds synergistic and antagonistic effects would play an important role in bacterial inhibition and should also be taken into consideration.

From these results, it is concluded that the essential oils of *A. arborescens* have a capacity to inhibit the growth of both gram-positive and gram-negative bacterial

Table 1. Contd.

	Oxygenated sesquiterpene				(3.0)	(2.9)	(4.1)
37	Bornyl butyrate	1453	1449	1709	0.1	0.1	0.1
38	Elemol	1535	1532	2061	1.0	1.1	1.6
39	Caryophyllene oxide	1576	1574	1978	0.1	0.1	0.3
40	Geranyl isovalerate	1587	1586	1893	0.5	0.2	0.3
41	Aromadendrene oxide 2	1617	1616	1993	0.2	0.2	0.3
42	α-Eudesmol	1619	1620	2185	0.4	0.2	0.4
43	T-Cadinol	1632	1630	2164	0.2	0.3	0.1
44	Himachalol	1644	1636	2208	0.3	0.4	0.7
45	α-Bisabolol	1672	1665	2208	0.2	0.3	0.3
	Phenylpropanoid				(0.3)	(0.2)	(0.4)
46	Methyl eugenol	1367	1373	2007	0.2	0.2	0.3
47	Isochavicol isobutyrate	1541	1541	2132	0.1	Tr	0.1
	Others				(0.7)	(1.0)	(1.1)
48	Toluene	749	751	943	0.6	0.3	0.8
49	6-Methyl hept-5-en-2-one	963	961	1327	0.1	0.5	0.1
50	Jasmone	1364	1366	1885	tr	0.2	0.2
	Unidentified compounds				(1.6)	(1.5)	(3.3)
51	Unknown 1	-	1589	2284	0.4	0.2	1.0
52	Unknown 2	-	1997	2321	1.2	1.3	2.3
	Total detected				91.8	89.7	85.1
	Total identified				90.2	88.2	81.8
	Yield (%)				0.52	0.31	0.64

[a]Retention indices of literature on the apolar column reported from Arômes Library (1987-2011), König (2001), Adams (2001), Velasco-Negueruela et al. (2002), Dabiri and Sefidkon (2003), Bendimerad et al. (2005), Cha (2007), Dib et al. (2010) and Khamsan et al. (2011). [b]Retention indices on the apolar Rtx-1 column. [c]Retention indices on the polar Rtx-Wax column. [d]Percentages are given on the apolar column except for components with identical RI (percentages are given on the polar column), tr, trace (< 0.05%); EO, essential oil of aerial part of *A. arborescens*, BS, Beni Snous; BD, Bidar; CT, Chetouane.

Table 2. Antibacterial activity of *A. arborescens* L. essential oils from the West Northern of Algeria.

Microorganism	**Inhibition zone diameter (mm)**		
	EOBD	**EOBS**	**EOCT**
Gram-positive bacteria			
S. aureus	8.0	14.0	15.0
B. cereus	n.a	15.0	13.5
E. faecalis	10.0	16.5	22.0
L. monocytogenes	n.a	n.a	n.a
Gram-negative bacteria			
P. aeruginosa	8.0	10.5	13.5
E. coli	n.a	n.a	n.a
S. typhimurium	n.a	18.0	18.0
A. baumanii	9.0	12.0	12.0
C. freundii	8.0	15.0	15.0
P. mirabilis	9.0	13.5	13.5
K. pneumoniae	7.0	15.0	14.0

EO, Essential oil (10 µl/disc) of aerial part of *A. arborescens*; BD, Bidar; BS, Beni Snous; CT, Chetouane; n.a, not active.

strains. Further, they showed an interesting activity for some tested strains.

Antioxidant activity

To the best of our knowledge, there are no available reports on antioxidant activity of essential oil from *A. arborescens* using DPPH method. Antioxidant properties of essential oils were evaluated to find a new natural source of antioxidant. The potential antioxidant activity of the *A. arborescens* essential oils was determined on the basis of the scavenging activity of the free stable radical DPPH and their activity was compared to the synthetic antioxidant: AA and TA which were used as antioxidant references. The results obtained at different concentrateions (10, 20, 40, 60, 80 and 100 mg/ml) are shown in Figure 1.

The results showed that the inhibitory activity of the most samples was higher than 50%, after 210 min of incubation time at room temperature. It is possible to conclude that the essential oils isolated from *A. arborescens* were able to give a proton to the stable radical DPPH that is, the principle of this method for the determination of antioxidant activity.

The best percentages of antioxidant activities were observed for the synthetic antioxidants AA and TA, generally used in food industry, more evident for low concentrations. At 40 and 60 mg/ml, the percentages of DPPH reduction reached 100% after 15 min of incubation time.

For essential oils, the antioxidant capacity was dependent on the concentrations tested. As shown in Figure 1, the antioxidant activity of EOBS increased with the increase of their concentrations from 10 to 100 mg/ml. For high concentration of EOBS (100 mg/ml), after 210 min of incubation time at room temperature the percentage of DPPH reduction reached 87.07%, while for low concentration (10 mg/ml) the percentage was 36.04%. The EOBD and EOCT showed at 20 mg/ml, the highest antioxidant capacity (EOBD: 84.16% after 15 min of incubation time and EOCT: 80.23% after 25 min of incubation time). Above this concentration (20 mg/ml), the antioxidant activity of EOBD and EOCT decreased. The same phenomenon was observed for the AA and TA, that they produced at 40 and 60 mg/ml their optimum antioxidant effect.

From these results, we can conclude that the antioxidant activity depends to the chemical composition of EOBS, EOBD and EOCT. The results showed that the inhibitory activities of essential oils EOBS (87.07% at 100 mg/ml), EOBD (84.16% at 20 mg/ml) and EOCT (80.23% at 20 mg/ml) were comparatively admirable for their inhibition capacity when compared to the positive controls AA (100% at 40 and 60 mg/ml) and TA (100% at 40 and 60 mg/ml).

Table 1 showed that essential oils of *A. arborescens* were markedly rich in oxygenated terpenes which may act as radical scavenging agents. It seems to be a general trend that the essential oils which contain oxygenated monoterpenes and/or sesquiterpenes have greater antioxidative properties (Tepe et al., 2004; Zouari et al., 2010). Furthermore, some researchers showed that some essential oils poor in phenolic compounds also, may have antioxidant potentials (El-Massry et al., 2002). Antioxidant activities of essential oils from aroma-tic plants are mainly attributed to the compounds present in them. This can be due to the high percentage of main but also to the presence of others constituents in small quantities or to synergy among them.

While the antioxidative activity of natural compounds is widely described, little information is reported on their kinetic behavior in the oxidation process. This represents however an important factor in the antioxidative activity process. In terms of reaction kinetics, Yen and Duh (1994) postulated that the more rapidly the absorbance decreased, the more potent were the antioxidant activity of the sample. Halliwell (1990) reported that the antioxidant power results first from the capacity to prevent the autoxidation of free radical mediated oxidation of the substrate in low concentration and second, that the resulting radical after scavenging must be stable. Es-Safi et al. (2007) also showed the evolution of remaining DPPH with time of each compound's family (flavonoids, iridoids,…). It showed that the compounds have a low kinetic behavior. Figure 1 showed the evolution of the percentage of reduction of DPPH with time of each sample at different concentrations (EOBS, EOBD, EOCT, AA and TA). It showed that AA, TA and EOBD have a rapid kinetic behavior. At 15 min, it reached the maximum percentage of reduction. For the AA, this confirms the result obtained by Cal et al. (2003). In contrast, others samples of essential oils at different concentrations have intermediate or slow kinetic behavior. According to Yen and Duh (1994), the samples AA, TA and EOBD are the more potents.

The free radical scavenging activity is usually expressed as percentage of DPPH inhibition but also by the antioxidant concentration required for 50% DPPH reduction (IC_{50}). Basically, a higher DPPH radical scavenging activity is associated with a lower IC_{50} value.

IC_{50} value was determined from plotted graph of scavenging activity against the different concentrations of *A. arborescens* essential oils, AA and TA. The scavenging activity was expressed by the percentage of DPPH reduction after 60 min of reaction. The measurements were triplicated and their scavenging effect was calculated based on the percentage of DPPH scavenged (Blois et al., 1958; Es-Safi et al., 2007; Singh et al., 2008). The obtained results are shown in Figure 2. The results show that EOBD (6.26 mg/ml) was the most potent of all the

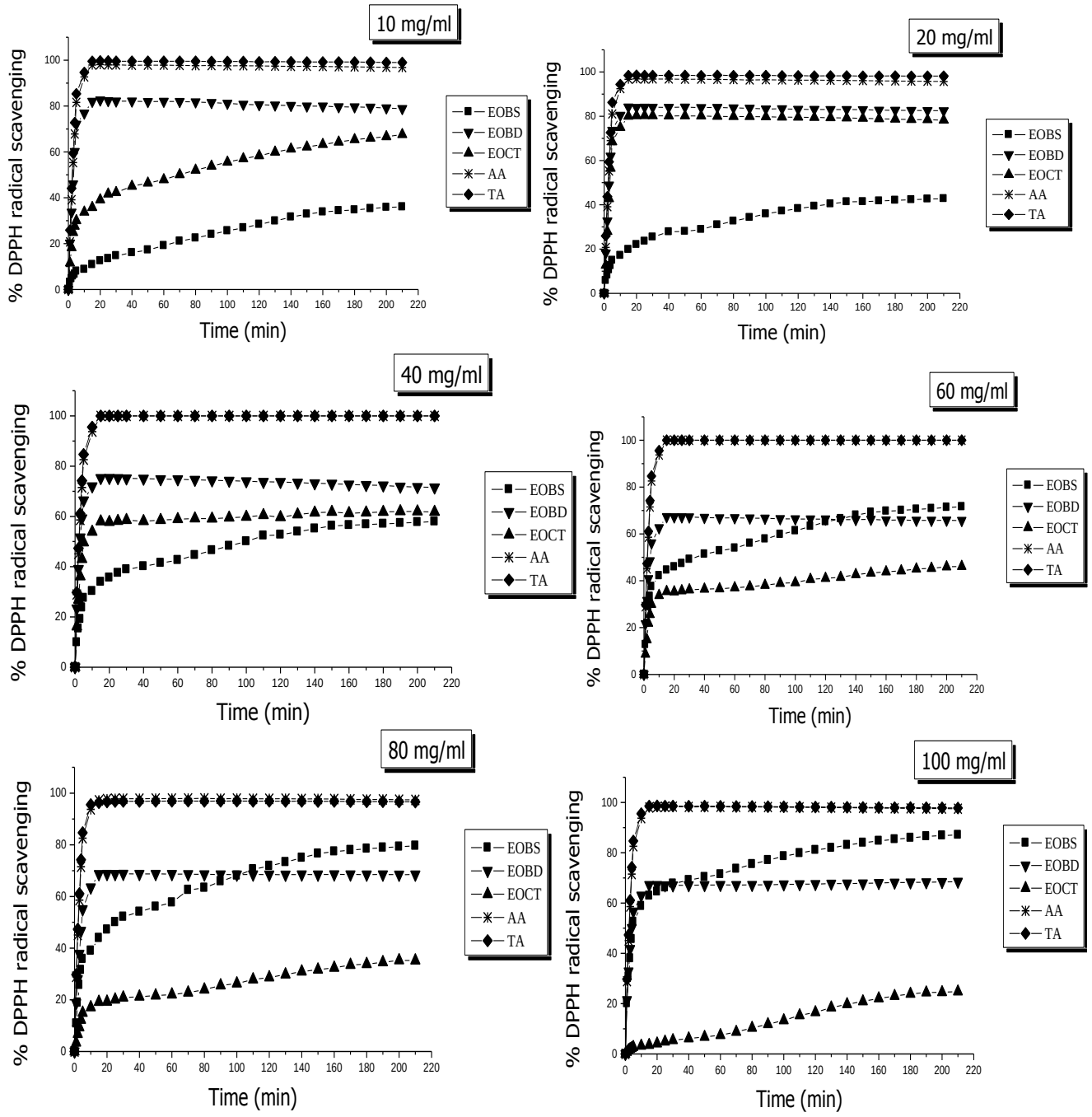

Figure 1. Percentage of DPPH radical scavenging as a function of reaction time for essential oils extracted from the aerial parts of *A. arborescens* L. and tested at different concentrations. EO, Essential oil of aerial part of *A. arborescens*; BS, Beni Snous; BD, Bidar; CT, Chetouane; AA, ascorbic acid; TA, tannic acid.

essential oils. It showed similar activity to AA (5.33 mg/ml) and TA (5.33 mg/ml). EOCT (10.67 mg/ml) also showed good activity compared to AA (5.33 mg/ml) and TA (5.33 mg/ml), while EOBS (53.43 mg/ml) exhibits weak activity.

Conclusion

Chemical characterization, antibacterial screening and antioxidant activity studies on plant based essential oils could lead to a discovery of new natural bioactive

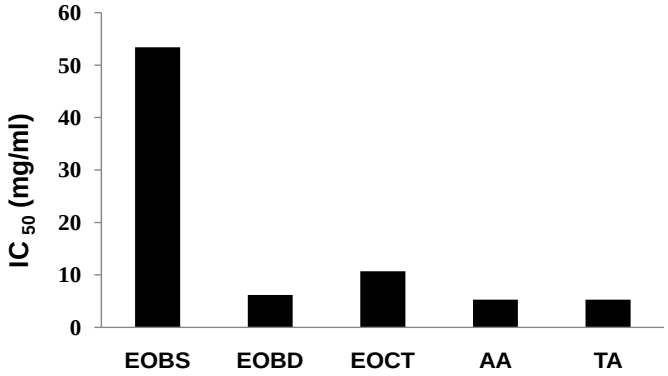

Figure 2. The concentration IC_{50} (mg/ml) of essential oils needed to decrease by 50% the initial DPPH absorption at 517 nm. Free radical scavenging activity was measured after 60 min of reaction. EO, Essential oil of aerial part of *A. arborescens*; BS, Beni Snous; BD, Bidar; CT, Chetouane; AA, ascorbic acid; TA, tannic acid.

substances. This work allowed the identification of new chemotype of *A. arborescens* growing in West Northern of Algeria and confirmed the tremendous chemical variability of the Algerian *A. arborescens*. The camphor found as the only most abundant component, is not described in the published literature. Camphor was described for the first time in the essential oil of Algerian *A. arborescens*. Our results indicated also that these essential oils have *in vitro* a strong antibacterial activity against *E. faecalis* and *S. typhimurium*. In contrast, the oils were ineffective on the inactivation of *L. monocytogenes* and *E. coli*. The study has also shown that the essential oils have *in vitro* a strong antioxidant activity when compared to the positive control. These results could support the use of plant by traditional healers to treat various infective diseases. These properties indicate the possibility of exploitation of essential oil of *A. arborescens* for food and pharmaceutical industries.

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