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ORIGINAL PAPER

Chemotypes and radical scavenging activity of the essential oils from *Artemisia arborescens* L. growing in three areas of Bejaia (Algeria)

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Abstract

Artemisia arborescens L. is a medicinal and aromatic plant used in traditional medicine for its therapeutic properties to treat lung diseases, gastrointestinal disorders, diabetes and inflammations. The leaves of *A. arborescens* L. were harvested in Amizour, Cap Bouak and Sahel (Bejaia, Algeria) and their essential oils were analyzed by Gas Chromatography/Flame Ionization Detector and Gas Chromatography/Mass Spectrometry. The main constituents were β-thujone in the essential oil of Cap Bouak (59.8%), camphor in the volatile oil of Sahel (58.7%) and chamazulene in that of Amizour (31.4%). The radical scavenging activity of these essential oils was evaluated against DPPH and ABTS radicals by calculation of IC_{50}. The best activity against DPPH and ABTS radicals were that of Sahel essential oil with IC_{50} DPPH = 133.0 µg/mL and IC_{50} ABTS = 211.6 µg/mL. The Spearman test revealed no significant correlations between IC_{50} DPPH and the chemical composition of the essential oil from *A. arborescens* L., while correlations with IC_{50} ABTS indicated that hydrocarbon monoterpenes, oxygenated monoterpenes and hydrocarbon sesquiterpenes promote activity against ABTS radical. These results can give prospects of using essential oils from *A. arborescens* L. as a natural adjunct in medicine formulations to treat diseases caused by oxidative stress.

Keywords *Artemisia arborescens* L. · Essential oils · Chemical composition · Radical scavenging activity · IC_{50}

Abbreviations

ABTS	2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	2,2-Diphenyl-1-picrylhydrazyl
BHA	Butylated hydroxy anisole
BHT	Butylated hydroxy toluene
HM	Hydrocarbon monoterpenes
HS	Hydrocarbon sesquiterpenes
NT	Non terpenic compounds
OM	Oxyginated monoterpenes
OS	Oxyginated sesquiterpenes

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Introduction

Artemisia arborescens L. is a shrub of the *Asteraceae* family that grows from 40 to 100 cm high. It is called '*chadjeret meriem*'' or '*echiba*'' in the vernacular language, and is very common on hillsides and ocean cliffs, in rockeries and bush woods of the coast [1, 2]. The plant (branches and leaves), given in internal use, possess aperitif, digestive, vermifuge, diuretic, antipyretic, sedative and abortive properties. It is used as stomachic, antispasmodic, cholagogue, anthelmintic, tonic, anti-diabetic, emmenagogue, antirheumatic and anti-inflammatory remedies. It is also used to treat gastrointestinal disorders, epilepsy, pulmonary diseases, cold and toothache. The plant in the form of poultice is used externally, as a remedy against snake and scorpion bites [3–7]. The essential oil of *A. arborescens* L. and the various extracts obtained by different solvents (methanol, ethanol, etc.) have antibacterial, antifungal, antioxidant, antiviral, cytotoxic and allelopathic activities [8–12].

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The essential oil from *A. arborescens* L. reported in the literature were classified into two chemotypes. The first chemotype was dominated by chamazulene with proportions between 30 and 50% and the second was β-thujone and camphor chemotype with proportions above 50% of these two oxygenated monoterpenes.

In Italy, the chamazulene chemotype was obtained with proportions between 26.6 and 51.8%, when the β-thujone and camphor chemotype was obtained with 50.0 to 64.0% [13–21]. In Algeria, Abderrahim et al. [22] shown that the essential oil from *A. arborescens* L. growing wild in the Bejaia area belongs to the chamazulene chemotype with proportions of 30.2%. Younes et al. [23] demonstrated the existence of the second chemotype (β-thujone and camphor) in the essential oils from another region (Tlemcen) with proportions of 51.4% and 73.1%. In Tunisia, Bouzenna and Krichen [24] and Dhibi et al. [25] obtained the chamazulene chemotype with proportions of 31.9% and 33.4% respectively. In Morocco, Codignola [26] and El-Montassir et al. [27] obtained the β-thujone and camphor chemotype with 69.0 to 78.0% and 56.5% respectively. In Libya, Janackovic et al. [28] have shown that the chemotype of their sample was dominated by chamazulene with 20.9%. In Lebanon, El-Bayrouthy et al. [29] obtained the β-thujone and camphor chemotype with 69.2%. Baykan Erel et al. [30] in Turkey and Pappas and Sheppard-Hanger [31] in the USA obtained the chamazulene chemotype respectively with 21.1% and 39.6%. In Canada, Van Tol et al. [32] obtained the β-thujone and camphor chemotype with 60.0%.

The aim of this study is to determine the major constituents of essential oils isolated from the leaves of *A. arborescens* L. harvested in three localities of Bejaia area (Algeria) and to classify them according to their chemotype. Thereafter, the radical scavenging activity of these essential oils against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals will be evaluated by measuring the inhibitory concentrations of 50% (IC_{50}).

Materials and methods

Plant material

The leaves of *A. arborescens* L. were gathered at flowering stage in three localities from Bejaia area (between May and June 2015): Amizour (36°37′19.47″N, 5°00′14.32″E, altitude of 581 ms), Cap Bouak (36°45′39.32″N, 5°06′08.78″E, altitude of 104 ms) and Sahel (36°47′33.41″N, 5°01′04.07″E, altitude of 10 ms). The collected samples were identified by the Museum of the National Park Gouraya (Bejaia, Algeria) and a specimen was deposited in Laboratory of Organic Materials, Faculty of Technology, University of Bejaia (Algeria). The voucher numbers were KBAA 064 for Amizour sample, KBAA 065 for Cap Bouak sample and KBAA 066 for that of Sahel. The leaves were dried in the shade at ambient temperature. Once the mass of the samples becomes constant, a fine and homogeneous powder was obtained using a laboratory grinder.

Isolation of essential oils

A sample of 100 g of powder from dried leaves of *A. arborescens* L., added to 750 mL of distilled water was loaded into the Clevenger apparatus to isolate the essential oil by hydrodistillation for 4 h. The essential oil was recovered by decantation and the organic phase was dried over anhydrous sodium sulfate (Cheminova Int. S.A., Madrid, Spain; 99% purity). The obtained essential oil was kept at 4 °C in an opaque and sealed bottle. Each distillation was repeated three times, and the yields with standard deviations were calculated. Distillation yield was calculated as the mass of the essential oil per mass unit of the dried plant.

Chemical composition

Gas chromatography/flame ionization detector analysis

The Gas Chromatography/Flame Ionization Detector (GC/FID) was performed on an Agilent 6890 apparatus (Agilent Technologies Manufacturing Gmbh & Co. KG. Waghäusel, Germany) provided with a capillary column HP5-MS (5%-phenyl)-methylpolysiloxane (30 m length, 0.25 mm internal diameter and 0.25 µm film thickness). The column was programmed at 50 °C for 5 min then increased to 320 °C with a rate of 5 °C/min and kept at this temperature for 5 min. The injector and flame ionization detector (FID) were programmed respectively to 280 °C and 300 °C. The sample was diluted in acetone (Sigma-Aldrich. St. Louis/Missouri, USA; 99.8% purity) to 1/25 (v/v) and 1 µL was injected in split mode with a fraction of 1/60. Hydrogen was used as carrier gas with a rate flow of 1 mL/min. A series of C_7–C_{30} *n*-alkanes (Sigma-Aldrich. St. Louis/Missouri, USA; 99.9% purity) was injected in the same conditions as our samples, in order to calculate the retention indices of each constituent. The proportions of the compounds were calculated by internal normalization.

Gas chromatography/mass spectrometry analysis

The Gas Chromatography/Mass Spectrometry (GC/MS) was performed on an Agilent 7890/5975 instrument (Agilent Technologies Manufacturing Gmbh & Co. KG. Waghäusel, Germany) under the same conditions as gas chromatography/Flame Ionization Detector (GC/FID). The acquisition of the mass spectra were obtained for m/z values between 33

and 550 in electron impact mode with an ionization energy of 70 eV and the ion multiplier at 1800 V. The temperature of the ion source and MS quadrupole were 230 °C and 150 °C respectively. Identification of essential oil components was performed on the MSD ChemStation G1701EA E.02.02.1431 by comparing the retention indices and mass spectra of each element with different databases (Adams and Wiley) [33, 34].

Radical scavenging activity

Evaluation of the radical scavenging activity of essential oils from *A. arborescens* L. was performed in vitro using two assays: scavenging DPPH and ABTS radicals. DPPH and ABTS were provided from Sigma-Aldrich (St. Louis/Missouri, USA) with 85% and 98% purities respectively. The essentials oils were diluted in methanol (Sigma-Aldrich. St. Louis/Missouri, USA; 99.7% purity) and the various solutions were tested at 100 µg/mL. Then, a series of concentrations, between 0 and 1 mg/mL, was used to calculate the 50% inhibitory concentrations (IC_{50}) using a polynomial regression of order two. For all tests, the measurements were performed three times and the average with standard deviations were calculated.

DPPH radical scavenging activity

An essential oil has the ability to give hydrogen in synthetic radical DPPH (2,2-diphenyl-1-picrylhydrazyl) violet color at oxidized form to reduce it to DPPH-H (2,2-diphenyl-1-hydrazine picryl) yellow color. The evaluation of the anti-radical activity of essential oils against the radical DPPH was performed according to Hemalatha et al. protocol [35]. Three mL from different concentrations of each essential oil were mixed with 1 mL of the solution of DPPH at 0.1 mmol/L. Three mL of methanol and 1 mL of DPPH were used as negative control. After incubation at 37 °C in the dark for 30 min, the absorbance was determined at a wavelength of 517 nm. The percentage inhibition of DPPH radical was determined by the formula below:

$$\%\ inhibition\ of\ DPPH = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

with $A_{control}$:absorbance of negative control; A_{sample}: absorbance of the essential oil.

Tocopherol, BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were used as standard (positive controls) and were purchased from Sigma-Aldrich (St. Louis/Missouri, USA) with 95.5%, 98.5% and 98% purities respectively. The absorbance measurements were performed on a spectrophotometer UNICO model 1200 (Dayton/New Jersey, USA).

ABTS radical scavenging activity

The decrease in absorbance caused by the essential oil reflects the capture capacity of the cationic radical $ABTS^{\cdot+}$ (green blue color) transforming the cation $ABTS\text{-}H^{\cdot+}$ (colorless) by a hydrogen donation. Capture of radical cation $ABTS^{\cdot+}$ was determined by the method of Re et al. [36]. The solution of the radical cation $ABTS^{\cdot+}$ was prepared by mixing 2.45 mmol/L of ABTS with 7 mmol/L of potassium persulfate (Biochem Chemopharma. Cosne-Cours-sur-Loire, France; 98% purity). After 16 h of incubation, the solution $ABTS^{\cdot+}$ was diluted with methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm (negative control). A volume of 10 µL of essential oil at the tested concentration is added to 1 mL of the $ABTS^{+\cdot}$ solution. Absorbance was measured at 734 nm after 6 min incubation in the dark. The percentage inhibition of the radical cation $ABTS^{+\cdot}$ was determined using the following formula:

$$\%\ inhibition\ of\ ABTS = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

with $A_{control}$: absorbance of negative control; A_{sample}: absorbance of the essential oil.

Trolox (Sigma-Aldrich. St. Louis/Missouri, USA; 97% purity) was used as a positive control (standard). The absorbance measurements were performed on a spectrophotometer UNICO Model 1200 (Dayton/New Jersey, USA).

Statistical analysis

The statistical analysis (Anova/Manova) was performed with Statistica software (StatSoft, Inc. 1999. Statistica, version 5.5). The analysis of variance (Anova) was performed by applying the Post Hoc comparisons with $p < 0.05$. The Principal Component Analysis (PCA) was determined using the Spearman correlation coefficients (r) at $p < 0.05$. The Spearman test was also applied to different error ratio ($p < 0.01$ and $p < 0.001$) to estimate the significance of correlations. All data represent the mean of three tests ± standard deviations (SD).

Results and discussion

Essential oils yields

Table 1 groups essential oils yields from leaves of *A. arborescens* L. for different regions. Statistical analysis (Anova) showed a significant difference between the yields of essential oils of *A. arborescens* L. leaves from Cap Bouak and Sahel, and a greater difference with that of Amizour. The lower yield was from Cap Bouak (0.16%), followed by Sahel

Table 1 Essential oils yields from leaves of *A. arborescens* L.

Locality	Yield (%)
Amizour	0.62 ± 0.06^{c}
Cap Bouak	0.16 ± 0.02^{a}
Sahel	0.45 ± 0.03^{b}

The letters a, b and c correspond to the significant difference among the essential oils yields

(0.45%) and the highest value was obtained with the leaves of Amizour (0.62%).

Comparing to the consulted literature, the yields of essential oils isolated from leaves of *A. arborescens* L. vary between 0.1 and 1.8%. Lower values was obtained in Italy with 0.1% [13] and 0.3% in the United States [31]. Several findings from various regions of Italy have slightly higher yields between 0.3 and 0.5% [18, 19, 21]. A study focused on three areas of Tlemcen (Algeria) also gave average yields with 0.3, 0.5 and 0.6% [23]. Higher values were obtained in Libya (0.8%) [28], Italy (0.8%) [15], Tunisia (0.9%) [25], Algeria (0.9%) [22], Turkey (1.2%) [30], Italy (1.4%) [16] and Lebanon (1.7%) [29]. The greatest value was obtained in Tunisia with 1.8% [24].

The yields we obtained with the essential leaves of *A. arborescens* L. were small compared to literature including Cap Bouak with 0.16% and Sahel slightly higher at 0.45%. The yield of Amizour is higher with 0.62% of the same order as the highest value obtained in Tlemcen (0.6%) [23], but remains low compared to the result already reported in 2010 that was 0.9% [22].

The yield of essential oils depend on the temperature, the humidity level, the duration of sunshine, the nature of the soil, the part of the used plant (organ), the vegetative cycle of the plant and the extraction method [37, 38].

Chemical composition of essential oils

After integration of the chromatograms and identification of components of different essential oils of *A. arborescens* L., the components were classified by terpene groups. The results were summarized in Table 2. Monoterpenes (hydrocarbon and oxygenated) dominate in the chemical composition of essential oils of *A. arborescens* L. from Cap Bouak and Sahel regions with proportions of 71.5% and 81.8% respectively. Sesquiterpenes (hydrocarbon and oxygenated) of the three regions (Amizour, Cap Bouak and Sahel) were minorities and vary between 13.3 and 24.4%. Non terpenic compounds predominate in the essential oil of Amizour with 40.3%, while they are in the minority in Cap Bouak and Sahel regions (1.8% and 2.4%). The essential oils of *A. arborescens* L. belong to two chemotypes. The first chemotype dominant monoterpene with a composition of β-thujone and camphor exceeding 50% (Fig. 1). We got this chemotype in Cap Bouak with 61.3% and 70.7% at Sahel. The second chemotype is non terpenic dominance with a chamazulene proportion exceeding 30% (Fig. 1). That chemotype was obtained with essential oil of Amizour with 31.4%.

In the consulted literature, the chemotype of monoterpenic dominance (β-thujone and camphor) was obtained in Italy with proportions between 50.0 and 64.0% [13–15, 18, 19], in Algeria with rates between 51.4 and 73.1% [23], in Morocco with percentages between 56.5 and 78.0% [26, 27], in Lebanon with 69.2% [29] and in Canada with 60.0% [32]. The chemotype dominated by chamazulene was obtained in Italy with rates between 26.6 and 51.8% (the highest value) [16–21], in Algeria with 30.2% [22], in Tunisia with proportions between 31.9 and 33.4% [24, 25], in Libya with 20.9% (the lowest value) [28], in Turkey with 21.1% [30] and in the United States with 39.6% [31].

DPPH radical scavenging activity

The radical scavenging activity of studied samples was expressed as IC_{50}, defined as the concentration of the essential oil necessary to reduce or inhibit 50% of DPPH radical solution. The best activity against DPPH radical was obtained with the lowest value of IC_{50}. For comparative purposes, standard antioxidants were used, they can be natural (tocopherol, flavonoids, carotenoids, ascorbic acid, etc.) or synthetic (3-tert-butyl-4-hydroxyanisole (BHA), 3,5-dit-ertiobutyl-4-hydroxytoluène (BHT), etc.). For anti-radical activity tests against DPPH: tocopherol, BHA and BHT were used as positive controls (standards). The results of radical scavenging activities against DPPH radical obtained with essential oils of *A. arborescens* L. were summarized in Table 3.

The Anova analysis showed a significant difference between the radical scavenging activities against DPPH radical of the essential oils from *A. arborescens* L. The best activity was that of Sahel essential oil ($IC_{50} = 133.0$ µg/mL), followed by that of Amizour with an IC_{50} of 137.9 µg/mL and the lowest activity was that of the essential oil from Cap Bouak ($IC_{50} = 148.1$ µg/mL). The antiradical activity of the used standards against DPPH remains high compared to that of the essential oils of *A. arborescens* L. with IC_{50} between 2.0 and 7.2 µg/mL. The anti-radical activity therefore falls into the following increasing order: Cap Bouak < Amizour < Sahel < Tocopherol < BHT < BHA.

Ornano et al. [19] obtained an IC50 against DPPH radical of essential oil *A. arborescens* L. more than 200 µg/mL, without specifying the exact value. Younes et al. [23] determined the IC50 of three samples from the region of Tlemcen (Algeria) that are 6.26 × 103, 10.67 × 103 and 53.43 × 103 µg/mL. The radical scavenging activity against DPPH of essential oils from *A. arborescens* L.

Table 2 Chemical compositions of essential oils isolated from *A. arborescens* L.

No	RI[a]	LRI[b]	Compound	Composition (%)		
				Amizour	Cap Bouak	Sahel
1	933	932	*α*-Pinene	–	–	0.9
2	947	946	Camphene	–	–	2.4
3	1003	1002	*α*-Phellandrene	–	0.4	0.2
4	1024	1020	*p*-Cymene	–	1.5	1.5
5	1028	1025	*β*-Phellandrene	–	1.0	0.6
6	1031	1026	Eucalyptol	–	0.4	–
7	1059	1054	*γ*-Terpinene	–	0.3	0.3
8	1100	1098	Linalool	–	0.3	0.9
9	1106	1101	*α*-Thujone	0.6	2.1	0.4
10	1117	1112	*β*-Thujone	28.9	59.8	12.0
11	1147	1141	Camphor	–	1.5	58.7
12	1168	1165	Borneol	–	–	0.9
13	1179	1174	Terpinen-4-ol	1.9	2.4	1.5
14	1188	1179	*p*-Cymen-8-ol	–	–	0.9
15	1193	1186	*α*-Terpineol	–	0.3	0.4
16	1278	1269	Perilla aldehyde	0.6	0.4	0.1
17	1295	1293	Undecan-2-one	2.1	–	–
18	1298	1294	Perilla alcohol	0.5	–	–
19	1302	1297	Carvacrol	–	1.1	0.1
20	1380	1374	*α*-Copaene	0.1	0.6	0.5
21	1389	1387	*β*-Bourbonene	–	0.7	0.3
22	1395	1389	*β*-Elemene	–	0.5	0.2
23	1406	1403	Methyl eugenol	t	0.3	0.3
24	1411[c]	-	3,4 dimethyl-Cinnoline	0.4	–	–
25	1414	1409	*α*-Gurjunene	–	1.2	0.5
26	1425	1417	*β*-Caryophyllene	0.6	1.6	0.9
27	1459	1452	*α*-Humulene	–	0.8	0.4
28	1466	1458	*allo*-Aromadandrene	–	0.6	0.2
29	1481	1478	*γ*-Muurolene	–	0.8	0.4
30	1486	1484	Germacrene-D	1.2	0.7	0.4
31	1492	1489	*β*-Selinene	–	1.1	0.6
32	1500	1498	*α*-Selinene	–	1.4	0.3
33	1505	1500	*α*-Muurolene	–	0.3	0.6
34	1511[c]	-	*nor β*-Calamenene	0.4	–	–
35	1519	1513	*γ*-Cadinene	–	1.1	0.5
36	1528	1522	*δ*-Cadinene	–	3.0	1.2
37	1554	1548	Elemol	–	0.4	0.8
38	1575	1565	Ledol	–	0.3	–
39	1584	1577	Spathulenol	–	1.2	0.7
40	1590	1582	Caryophyllene oxide	0.6	2.4	1.8
41	1600	1594	Salvial-4(14)-en-1-one		0.8	0.4
42	1610	1606	Geranyl isopentanoate		0.9	0.4
43	1618	1608	Humulene-1,2-epoxide	–	0.3	0.3
44	1620[c]	–	2,2,3-trimethylnaphthalen-1(2H)-one	2.1	–	–
45	1630[c]	–	Fonenol	0.9	–	–
46	1649	1640	*epi-α*-Muurolol (*τ*-Muurolol)	–	0.2	–
47	1652	1646	*iso*-Spathulenol	–	0.3	–
48	1658	1649	*β*-Eudesmol	8.4	0.7	0.5
49	1661	1652	*α*-Cadinol	–	2.1	1.1

Table 2 (continued)

No	RI[a]	LRI[b]	Compound	Composition (%)		
				Amizour	Cap Bouak	Sahel
50	1693	1687	Eudesma-4(15),7-dien-1-β-ol	–	0.4	0.3
51	1695[c]	–	Phenyl hydroquinone	0.9	–	–
52	1737	1730	Chamazulene	31.4	1.5	2.1
53	1740[c]	–	3,3′-dimethyldiphenyl	2.9	–	–
54	2008	2023	Catalponol	5.7	–	–
Total (%)				90.5	97.7	97.5
Hydrocarbon monoterpenes (HM)				0.0	3.2	5.9
Oxygenated monoterpenes (OM)				32.6	68.3	75.9
Hydrocarbon sesquiterpenes (HS)				2.0	14.4	7.0
Oxygenated sesquiterpenes (OS)				15.7	10.0	6.3
Non terpenic compounds (NT)				40.3	1.8	2.4

t traces (< 0.05)

[a]Calculated retention indices on HP5-MS column

[b]Literature retention indices on DB-5 column Adams library (33)

[c]Identification only with mass spectra Wiley library (34)

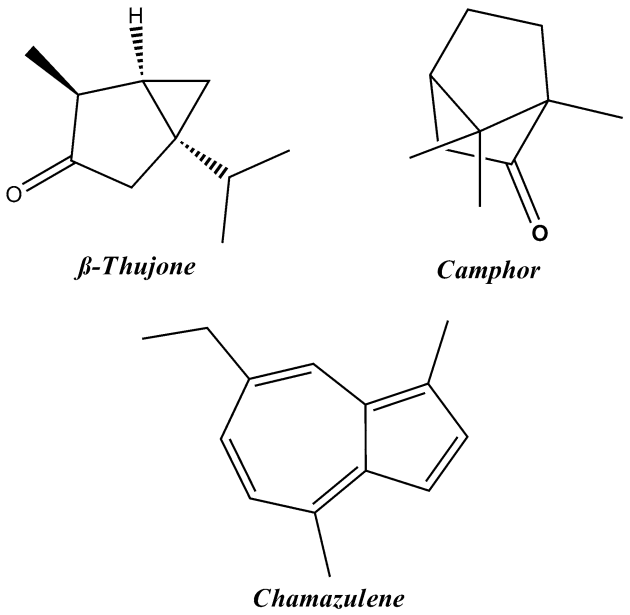

Fig. 1 Chemical structures of the mains compounds of the essential oils from *A. arborescens* L.

(Amizour, Cap Bouak and Sahel) were better than that obtained in Italy and Tlemcen (Algeria).

The Spearman test revealed no significant correlations between the IC_{50} DPPH and the chemical groups in the essential oil of *A. arborescens* L. However, hydrocarbon monoterpenes and non terpenic compounds have contributed, even so weakly, to activity against the radical DPPH (Table 4).

Table 3 Radical scavenging activity of essential oils isolated from *A. arborescens* L. against DPPH radical

	IC_{50} DPPH (µg/mL)	R^2
Amizour EO	137.9 ± 3.1^b	0.918
Cap Bouak EO	148.1 ± 0.7^c	0.926
Sahel EO	133.0 ± 1.0^a	0.876
Tocopherol	7.2 ± 0.1	0.948
BHA	2.0 ± 0.1	0.848
BHT	3.8 ± 0.4	0.887

The letters a, b and c correspond to the significant difference among the IC_{50} DPPH of the essential oils

EO essential oil

The application of the PCA shows the low dependence between the different chemical families of *A. arborescens* L. and radical scavenging activity against DPPH (Fig. 2).

ABTS radical scavenging activity

In addition to inhibitory concentrations of 50% of the radical cation ABTS in solution (IC_{50}), the effectiveness of essential oils to trap this radical was evaluated in TEAC (Trolox Equivalent Antioxidant Capacity) which corresponds to the concentration of Trolox producing the same effect of reduction of ABTS per gram of essential oil. More the value of TEAC is high more the essential oil is active. The results of the radical scavenging activities against the radical cation ABTS obtained with essential oils of *A. arborescens* L. were summarized in Table 5.

The Anova analysis showed a significant difference between the regions for the radical scavenging activities

Table 4 Matrix of correlations between IC_{50} DPPH and chemical composition of *A. arborescens* L.

	HM	OM	HS	OS	NT	DPPH
HM	1.00					
OM	0.95***	1.00				
HS	0.45	0.70*	1.00			
OS	− 1.00***	− 0.96***	− 0.49	1.00		
NT	− 0.88**	− 0.99***	− 0.82**	0.90***	1.00	
DPPH	− 0.27	0.03	0.71*	0.23	− 0.20	1.00

HM hydrocarbon monoterpenes, *OM* oxyginated monoterpenes, *HS* hydrocarbon sesquiterpenes, *OS* oxyginated sesquiterpenes, *NT* non terpenic compounds, *DPPH* IC_{50} DPPH

*$p < 0.05$; **$p < 0.01$, ***$p < 0.001$

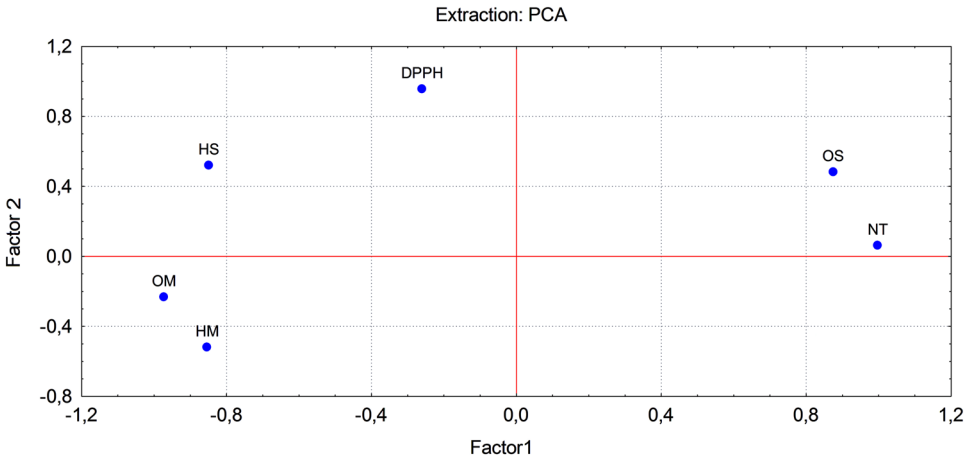

Fig. 2 PCA between IC_{50} DPPH and chemical composition of *A. arborescens* L.

Table 5 Radical scavenging activity of essential oils isolated from *A. arborescens* L. against the ABTS radical cation

	IC_{50} ABTS (µg/mL)	R^2	TEAC
Amizour EO	269.9 ± 3.2^c	0.991	0.19 ± 0.00
Cap Bouak EO	221.5 ± 3.1^b	0.924	0.23 ± 0.00
Sahel EO	211.6 ± 0.5^a	0.894	0.24 ± 0.00
Trolox	50.5 ± 2.9	0.990	1.00 ± 0.06

The letters a, b and c correspond to the significant difference among the IC_{50} ABTS essential oils. TEAC: Antioxidant Concentration Trolox Equivalent expressed in g Trolox equivalent per gram of essential oil; EO: Essential Oil

of the essential oils from *A. arborescens* L. against the ABTS radical cation. The highest activity was that of Sahel essential oil ($IC_{50} = 211.6$ µg/mL), followed by that of Cap Bouak with an $IC_{50} = 221.5$ µg/mL and the lowest activity was that from Amizour ($IC_{50} = 269.9$ µg/mL).

Ornano et al. [19] obtained an IC_{50} ABTS of the essential oil from *A. arborescens* L. of 21.9 µg/mL. TEAC values of different essential oils from *A. arborescens* L. vary between 0.19 and 0.24 g of Trolox/g of essential oils show that the scavenging activity against ABTS is relatively medium.

The Spearman test gives a significant correlation ($p < 0.05$) between the IC_{50} ABTS and hydrocarbon sesquiterpenes (HS). Very highly significant correlations ($p < 0.001$) were obtained with hydrocarbon monoterpenes (HM) and oxygenated monoterpenes (OM) (Table 6).

The PCA summarizes the findings earlier. Note on the chart that the three chemical families that are opposed to the IC_{50} ABTS (HM, OM and HS) promote radical scavenging activity against ABTS. This was confirmed by the high negative correlation coefficients found for these chemical families and IC_{50} ABTS. By against, the two other chemical groups (OS and NT) have an inverse effect on the scavenging activity against ABTS and were those which occupy the same position as the IC_{50} ABTS in the correlation circle (Fig. 3).

Conclusion

The essential oils of *A. arborescens* L. belong to two chemotypes. The first dominant monoterpene with a composition of β-thujone and camphor exceeding 50%. This chemotype was obtained in Cap Bouak with 61.3% and Sahel with 70.7%. The second chemotype is non terpenic dominance with a

Table 6 Matrix of correlations between IC_{50} ABTS and chemical composition of *A. arborescens* L

	HM	OM	HS	OS	NT	ABTS
HM	1.00					
OM	0.95***	1.00				
HS	0.45	0.70*	1.00			
OS	− 1.00***	− 0.96***	− 0.49	1.00		
NT	− 0.88**	− 0.99***	− 0.82**	0.90***	1.00	
ABTS	− 0.95***	− 1.00***	− 0.70*	0.96***	0.98***	1.00

HM hydrocarbon monoterpenes, *OM* oxyginated monoterpenes, *HS* hydrocarbon sesquiterpenes, *OS* oxyginated sesquiterpenes, *NT* non terpenic compounds, *ABTS* IC_{50} ABTS

*$p < 0.05$; **$p < 0.01$, ***$p < 0.001$

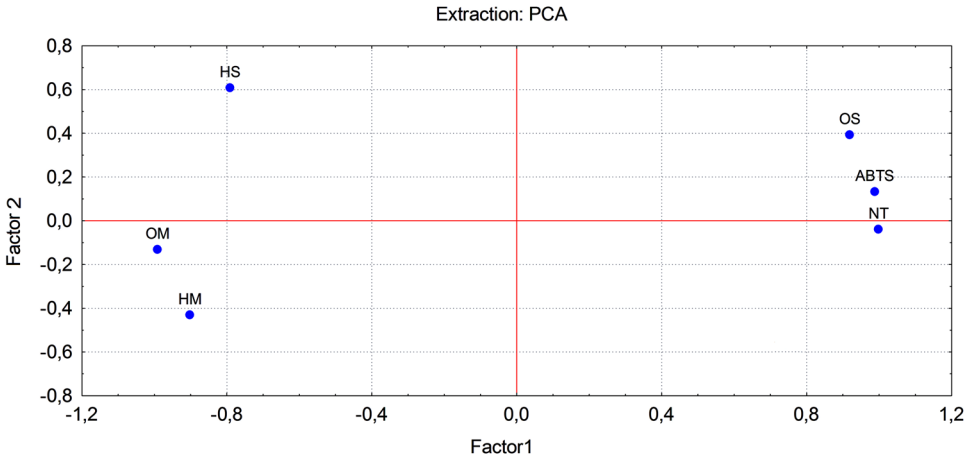

Fig. 3 PCA between IC_{50} ABTS and chemical composition of *A. arborescens* L.

chamazulene proportion exceeding 30%. This chemotype was obtained with essential oil from Amizour with 31.4%.

The principal component analysis indicates the chemical families contributing to elevation or reduction of radical scavenging activities against DPPH and ABTS of the essential oils from *A. arborescens* L. no chemical group was contributed significantly to activity against the radical DPPH, while HM, OM and HS promoted that against ABTS.

As prospects, we wish to extend this study to other locations in Bejaia region to complete the classification of chemotypes of the essential oils isolated from *A. arborescens* L. In addition, other complementary methods: in vitro (Ferric Reducing Antioxidant Power (FRAP), Total Antioxidant Capacity (TAC), etc.) and in vivo would be needed to explain the molecular and cellular mechanisms involved in the radical scavenging activity.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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