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### **ORIGINAL ARTICLE**

# Essential oil from *Artemisia herba-alba* Asso grown wild in Algeria: Variability assessment and comparison with an updated literature survey

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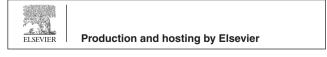
#### KEYWORDS

Asteraceae; Artemisia herba-alba Asso; Essential oil; Chemical variability; Algeria **Abstract** The chemical variability of the essential oils of *Artemisia herba-alba* Asso aerial parts, collected at Algeria was evaluated. *A. herba-alba* populations were collected in four regions, Benifouda; Bougaa; Boussaada and Boutaleb, at two different periods, July (flowering phase), and October and November (vegetative phase). The essential oils were isolated by hydrodistillation and analyzed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). The essential oils yield ranged between 0.2% and 0.9% (v/d.w.). Fifty components were identified in *A. herba-alba* oils, oxygen-containing monoterpenes being dominant in all cases (72–80%). Camphor (17–33%),  $\alpha$ -thujone (7–28%) and chrysanthenone (4–19%) were the major oil components. Despite the similarity in main components, three types of oils could be defined, (a)  $\alpha$ -thujone : camphor (23–28: 17–28%), (b) camphor : chrysanthenone (33:12%) and (c)  $\alpha$ -thujone : chrysanthenone (24:19:19%). The comparison between the present data and an updated survey of the existing literature reinforces the major variability of *A. herba-alba* essential oils and stresses the importance of obtaining a defined chemical type crop production avoiding the wild harvest.

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#### 1. Introduction

Asteraceae Martinov (= Compositae Giseke) is a family of herbs, shrubs or trees, commonly known as Aster or Compositae family, comprising about 1535 genera and 23,000 species. *Artemisia* (wormwood, tarragon), one of the most economically important and widespread of this family genus, includes 400 species (Judd et al., 2002).

Dobignard, (1977) has shown the taxonomic complexity of A. herba-alba lato sensu described in North Africa, and the

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Country / Plant part	CS	PMS	IP	Oil yield	Main components ( $\geq 5\%$ )	Reference
Algeria						
Aerial part	Wild			0.1–0.7	Chrysanthenone 5–55, $\alpha$ -thujone t-26, $\beta$ - thujone 6–16, camphor 6–16, bornyl acetate t- 8, 1,8-cineole t-6	Boutekedjiret et al. 1992 in (a)
n.r.	Wild			0.7	Camphor 2–48, $\alpha$ -thujone 2–27, chrysanthenone 5–23, $\beta$ -thujone 2–22, 1,8- cineole 8–18	Vernin et al. 1995 in (a)
Leaves and stems	Wild	Dry	Н	1.0 (w/w)	Camphor 19, <i>trans</i> -pinocarveol 17, chrysanthenone 16, $\beta$ -thujone 15	Dob and Benabdelkader (2006)
n.r.	Wild	n.r.	Н	n.r.	β-Thujone 32–41, camphor 16–25, cineol 0.1– 10	Benabdellah et al. (2006)
Aerial parts	Wild	n.r.	$\mathrm{SD}^*$	1.5–3.3 (w/w)	Chrysanthenone 31–54, camphor 11–27, filifolone 5–9, 1,8-cineole 2–9	Boutemak et al. (2009)
Aerial parts flowering phase	Wild	Dry	Н	0.6 (w/w)	Camphor 49, 1,8-cineole 13, borneol 7, pinocarvone 6, camphene 5	Dahmani-Hamzaoui and Baaliouamer (2010)
Flowering tops	Wild	Dry	Н	1.0 (v/w)	<i>cis</i> -Chrysanthenyl acetate 25, α-thujone 8, 2 <i>E</i> ,3 <i>Z</i> -2-ethyliden-6-methyl-3,5-heptadienal 8, verbenone 7, myrtenyl acetate 7, chrysanthenone 5	Bezza et al. (2010)
Egypt Leaves Israel and Sinai	Wild	Fresh	SD	1.6 (v/w)	Carvone, piperitone (no% given)	Saleh et al. (2006)
Leaves, stems and flowers	Wild	Dry	SD	0.1–1.7 (v/w)	1,8-cineole 5–50, thujone n.d. 27, camphor 0.1–25, <i>cis</i> -chrysanthenol n.d. 25, <i>cis</i> - chrysanthenyl acetate n.d. 25, iso-thujone n.d. 12, borneol n.d. 11, artemisia alcohol n.d. 10, santolina alcohol n.d. 6, yomogi alcohol n.d. 9, xanthoxylin n.d. 9, lyratol n.d. 6, terpinen- 4-ol 1–5	Feuerstein et al. (1986)
n.r.	Wild	Fresh	Н	0.1–1.9	<i>cis</i> -Chrysanthenyl acetate n.d. 69, $\beta$ -thujone t- 44, camphor n.d. 42, $\alpha$ -thujone n.d. 41, <i>cis</i> - chrysanthenol n.d. 30, 1,8-cineole 0.2–27, <i>cis</i> - chrysanthenyl propionate n.d. 7, camphene n.d. 5	Fleisher et al. (2002)
Jordan Leaves, stems and flowers	UG	Dry	Н	1.3 (v/w)	α-Thujone 16, santolina alcohol 13, artemisia ketone 12, β-thujone 9, trans-sabinyl acetate 5, germacrene-D 5, caryophyllene acetate 5	Hudaib and Aburjai (2006)
Morocco Aerial parts	Wild	Dry	Н	n.r.	α-Thujone 2–74, β-thujone 1–84, camphor 5–	Benjilali and Richard (1980)
Agrial parts	Wild	Dev	Н	2.0	70 Chrysenthenone 21, compher 24, comphere 5	Quashikh at al. (2000)
Aerial parts Aerial parts flowering phase	Wild	Dry Fresh	H H	2.0 1.3–3.3 (v/w)	Chrysanthenone 31, camphor 24, camphene 5 $\alpha$ -Thujone n.d. 74, chrysanthenone n.d. 53, camphor 9–46, $\beta$ -thujone n.d. 16, borneol t- 10, 1,8-cineole 0.3–8, camphene 0.2–8	Ouachikh et al. (2009) Paolini et al. (2010)

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Leaves	Wild	Dry	Н	022	Verbenol 22, bisabolene oxide 18, farnesene epoxide 17, $\beta$ -thujone 6, camphor 5	Tilaoui et al. (2011)
Spain	XX7'1 1	D	CD	0.7		$\Gamma_{}$ (1000)
Leaves	Wild	Dry	SD	0.6	Camphor 15, 1,8-cineole 13, α-terpineol 6, borneol 5, chrysanthenone 5, terpinen-4-ol 5	Feuerstein et al. (1988)
Aerial parts flowering phase**	Wild	Dry	Н	0.8(w/w)	Davanone 18, <i>p</i> -cymene 14, 1,8-cineole 10, chrysanthenone 7, <i>cis</i> -chrysanthenyl acetate 6, $\gamma$ -terpinene 6, myrcene 5	× /
Flowering tops	Wild	Dry	SD	0.4–2.3 (w/w)	Davanone n.d. 51, chrysanthenone n.d. 36, <i>cis</i> -chrysanthenol n.d. 28, 1,8- cineole 2–26, <i>p</i> -cymene 5–21, <i>cis</i> - chrysanthenyl acetate n.d. 18, $\alpha$ -pinene t-17, camphor n.d. 17, myrcene 1–11, bornyl acetate n.d. 9, $\gamma$ -terpinene 1–6, $\gamma$ - muurolene 1–5, spathulenol 1–5, davana ether n.d. n.d. 5	
Tunisia	*****		**	10(1)	D: 00 : 10	N
Leaves	Wild	n.r.	Н	1.9 (v/w)	Pinocarvone 38, isoamyl 2- methylbutyrate 20, α-copaene 12, limonene 11	Neffati et al. (2008)
Leaves and flowers	S	Dry	Н	0.7–1.9 (v/w)	α-Thujone n.d. 42, β-thujone n.d. 24, 1,8-cineole 1–28, sabinyl acetate n.d. 23, davanone n.d. 20, camphor n.d. 18, davana ether isomers n.d. 16, chrysanthenone n.d. 17, borneol n.d. 11, <i>cis</i> -chrysanthenyl acetate n.d. 10, yomogi alcohol n.d. 10, terpinen-4-ol 1– 9, germacrene-D n.d. 7, bicyclogermacrene 1–6, <i>cis</i> -sabinol n.d. 6, davana ether n.d. 6, 3- hydroxyisodavanone n.d. 5, <i>trans</i> - pinocarveol n.d. 5	
Leaves and flowers	EF	F/D	Н	1.7–2.5 (v/w)	β-Thujone 18–25, α-thujone 13–23, camphor 9–13, chrysanthenone 7–11, 1,8-cineole 7–9, <i>trans</i> -sabinyl acetate 4– 7, terpinen-4-ol 3–5	Mighri et al. (2009a)
Aerial parts (flowering phase and vegetative phase)	EF	Dry <sup>***</sup>	Н	1.6–2.2 (v/w)	β-Thujone 16–34, α-thujone 14–24, 1,8- cineole 6–12, camphor 5–10, <i>trans</i> - sabinyl acetate 3–6, terpinen-4-ol 2–5	Mighri et al. (2009c)
Leaves and flowers	EF	Dry	Н	0.9–2.4 (w/w)	β-Thujone 20–34, α-thujone 12–26, 1,8- cineole 6–23, camphor 5–12, chrysanthenone 1–9, <i>trans</i> -sabinyl acetate 1–7, terpinen-4-ol 2–5	Mighri et al. (2009b)
Leaves and flowers	Wild	Dry	Н	1.1–2.3	β-Thujone 17–58, α-thujone 7–44, 1,8- cineole 6–17, camphor 4–11, <i>trans</i> - sabinyl acetate 5–7, chrysanthenone 3–7	
						(continued on next page)

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 Table 1
 (continued)

Country / Plant part	CS	PMS	IP	Oil yield	Main components ( $\geq 5\%$ )	Reference
Leaves and flowers	EF	Dry	Н	n.r.	β-Thujone 14–58, α-thujone 6–49, 1,8- cineole 5–18, camphor 4–11, <i>trans</i> - sabinyl acetate 3–8	Mighri et al. (2010b)
Leaves and flowers	Wild	Dry	Н	1.2-4.9 (v/w)	<ul> <li>α-Thujone n.d. 80, chrysanthenone n.d.</li> <li>65, camphor n.d. 48, <i>trans</i>-sabinyl acetate n.d. 44, 1,8-cineole 1–24, davanone n.d. 21, β-thujone n.d. 18, trans-pinocarveol n.d. 15, borneol n.d.</li> <li>11, <i>cis</i>-chrysanthenyl acetate n.d. 11, camphene n.d. 10, <i>p</i>-cymene n.d. 9, germacrene-D 1–5, terpinen-4-ol n.d. 6, pinocarvone n.d. 5</li> </ul>	Boukrich et al. (2010)
Leaves and flowers	Wild	Dry	Н	1.5 (v/w)	<i>cis</i> -Chrysanthenyl acetate 11, α-thujone 9, sabinyl acetate 9, davana ether 6, chrysanthenone 5	Zouari et al. (2010)
Aerial parts	Wild	Dry	Н	n.r.	α-Thujone 25, germacrene-D 15, camphor 11, 1,8-cineole 9, β-thujone 8, lepidozene 6, chrysanthenone 5, sabinyl acetate 5	Kadri et al. (2011)

\* (a) data in Dob and Benabdelkader (2006). CS: Collection site. UG: University garden. S: Sub-cultured plants with different origins. EF: Experimental field. PMS: Plant material status. IP: Isolation Procedure. H: hydrodistillation. SD: steam-distillation. F/D: Fresh and different drying processes.

\* SD: under different experimental conditions.
 \*\* *Artemisia herba-alba* Asso ssp. *valentine* (Lam.) Marcl. was evaluated in this case.
 \*\*\* Dry: Different harvests. n.d.: not detected. n.r.: not reported. t = trace (<0.05%).</li>

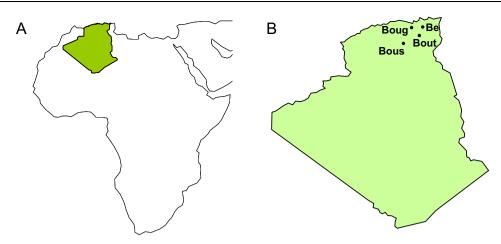


Figure 1 Algeria geographical location (a) and collection sites (b) of *Artemisia herba-alba*. Benifouda (Be); Bougaa (Boug); Boussaada (Bous) and Boutaleb (Bout).

need for a taxonomic study of the whole group. In the present study we followed the traditional criterion of this species delimitation. Eleven spontaneous *Artemisia* species are present in the Algerian flora (Quezel and Santa 1963). *Artemisia herbaalba* Asso = [*Artemisia aragonensis* Lam., *Seriphidium herbaalba* (Asso) Soják] (Greuter, 2006–2009), commonly known as white wormwood or desert wormwood (Arabic name *chih*), is a greyish-strongly aromatic dwarf shrub native to the South western Europe, Northern Africa, Arabian Peninsula and Western Asia.

The economic value, the local medicinal uses, the disappearance in some areas due to pasture and over-collection, as well as the potential use to restore degraded ecosystems support the large number of studies on *A. herba-alba*. A recent review detailed the distribution, taxonomy, morphology, phytochemistry and biological activities of *A. herba-alba* and its different extracts (Mohamed et al., 2010).

Among *A. herba-alba* phytochemical constituents, essential oils have been extensively studied, with several chemotypes being recognized. The variability from the essential oils iso-lated from *A. herba-alba* collected at Algeria, Israel, Morocco and Spain was revised by Dob and Benabdelkader in 2006, but, since then, many other studies reinforced its high chemical polymorphism (Table 1, and references therein). Plants were collected from wild in different countries, grown under controlled experimental field conditions, collected at different harvesting times, subject to different drying periods and processes, extracted fresh or dry, by hydrodistillation or steam-distillation under different experimental conditions, and different aerial plant parts have been used (Table 1).

The aim of the present study was both to evaluate the chemical composition of the essential oils isolated from

*A. herba-alba* collected at different locations in Algeria, and to compare these data with an updated survey on the chemical variability of this species essential oils.

#### 2. Experimental

#### 2.1. Plant material

The aerial parts of *A. herba-alba* were collected during the flowering (July, 2008) and vegetative phase of the plant (October and November, 2008) at different localities in Algeria (Benifouda; Bougaa; Boussaada and Boutaleb), characterized by diverse geographic and climate conditions (Fig. 1, Table 2). Plant material was dried in the dark, at room temperature. Certified voucher specimens have been deposited in the Herbarium of the Faculty of Nature and Life Sciences at F. A. University, Setif, Algeria.

#### 2.2. Essential oil isolation

The essential oils were isolated from the dried plant material by hydrodistillation for 3 h, at a distillation rate of 3 ml.min<sup>-1</sup>, using a Clevenger-type apparatus according to the European Pharmacopoeia (Council of Europe, 2007). The essential oils were stored at -20 °C in the dark until analysis.

#### 2.3. Essential oil analysis

#### 2.3.1. Gas chromatography (GC)

Gas chromatographic analyses were performed using a Perkin Elmer Autosystem XL gas chromatograph equipped with two

Table 2	Data on altitude.	precipitation and	temperature	range at the	collection sites of Alg	erian Artemisia herba-alba.

Average/Year	Artemisia herba-alba	Artemisia herba-alba						
	Boussaada (Bous)	Boutaleb (Bout)						
Altitude (m)	821	914	459	1321				
Precipitation (mm)	500	500	17	300				
Temperature (°C)	2–38	2-38	3–42	-2-40				

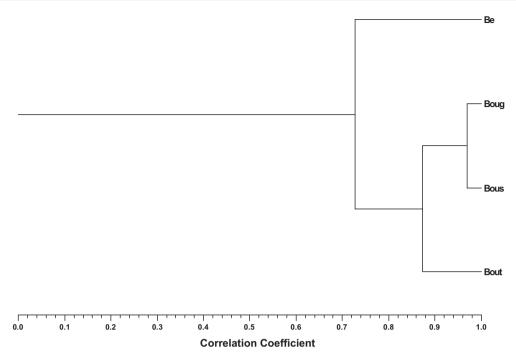
Data gathered from official Headquarter Maps [Carte d'État Major (CEM); CEM SoukNadjaa paper 169, CEM SaintArnaud, paper 98, CEM AinRoua, paper 69] and from the National Meteorology Office (Office National de la Météorologie).

 Table 3
 Percentage composition of the essential oils isolated by hydrodistillation from Artemisia herba-alba collected at different sites in Algeria.

Components	RI	Artemisia herba-alba					
		Benifouda Nov-08	Bougaa Jul-08	Boussaada Oct-08	Boutaleb Nov-08		
Santolina triene	911	0.7	0.2	1.3	t		
Tricyclene	921	0.2	0.3	0.2	t		
α-Thujene	924	t	t	t	t		
α-Pinene	930	0.4	0.8	0.8	t		
Camphene	938	7.1	4.2	4.1	0.7		
Sabinene	958	0.2	0.2	0.7	0.3		
1-Octen-3-ol	961	t	t	t	t		
β-Pinene	963	0.1	0.3	0.3	t		
1,2,4-Trimethyl benzene	978	0.4	0.4	0.2	0.2		
1-Decene	995	t	t	t	t		
α-Phellandrene	995	0.2	0.8	0.3	t		
1,2,3-Trimethyl benzene	1001	0.2	0.7	t	1.0		
α-Terpinene	1002	0.3	0.1	0.1	0.6		
<i>p</i> -Cymene	1002	0.8	0.9	0.4	0.6		
1.8-Cineole	1005	8.6	8.2	9.8	3.0		
Limonene	1005	t	t.2	5.8 t	5.0 t		
Santolina alcohol	1009			0.7			
		t	t		t		
γ-Terpinene	1035	0.3	0.8	0.6	0.3		
<i>trans</i> -Sabinene hydrate	1037	t	0.8	0.4	0.2		
Filifolene*	1074	3.9	1.0	1.1	2.8		
α-Thujone	1074	6.9	28.1	27.7	23.5		
β-Thujone	1081	1.9	7.8	3.4	3.0		
Chrysanthenone*	1081	12.2	3.9	7.6	19.0		
α-Campholenal	1088	t	0.1	0.4	0.3		
trans-p-2-Menthen-1-ol	1095	0.7	0.5	1.0	0.5		
Camphor	1095	33.1	22.8	17.3	18.7		
trans-Pinocarveol	1106	1.1	0.7	0.5	0.3		
cis-Verbenol	1110	0.9	0.3	t	0.4		
trans-Verbenol	1114	1.1	0.1	0.7	t		
Pinocarvone	1121	1.7	1.5	1.1	0.6		
Borneol	1134	2.5	2.0	1.9	1.5		
Terpinen-4-ol	1148	0.6	0.6	1.0	0.7		
Myrtenal	1153	0.2	0.2	t	0.2		
Myrtenol	1168	t	0.1	0.6	t		
trans-Carveol	1189	t	0.1	0.3	t		
<i>cis</i> -Carveol	1202	0.1	0.2	t	0.1		
Carvone	1202	t	0.1	0.1	t		
<i>cis</i> -Ocimenone	1200	t	0.1	0.2	t		
Piperitone	1200	0.4	0.3	t.	0.2		
<i>cis</i> -Chrysanthenyl acetate	1211	0.5	0.3	1.2	0.2 t		
	1241	0.3	0.2	0.2	0.2		
Bornyl acetate Carvacrol	1285	0.3	0.3				
				t 0.1	t 0.1		
β-Copaene	1426	t	t				
β-Ylangene	1435	t	t	0.2	t		
allo-Aromadendrene	1456	t	t .	t	t		
γ-Muurolene	1469	2.4	0.7	3.5	7.1		
Bicyclogermacrene	1487	0.7	0.4	1.1	2.5		
δ-Cadinene	1505	0.1	t	0.2	0.5		
Spathulenol	1551	0.1	0.4	0.2	1.0		
Ledol	1580	t	t	0.1	0.2		
% of identification		91.2	91.9	91.6	90.3		
Grouped components							
Monoterpene hydrocarbons		14.2	9.6	9.9	5.3		
Oxygen-containing monoterpenes		73.1	79.7	76.1	72.4		
Sesquiterpene hydrocarbons		3.2	1.1	5.1	10.2		
Oxygen-containing sesquiterpenes		0.1	0.4	0.3	1.2		
Others		0.6	1.1	0.2	1.2		
Oil Yield (%, v/dry weight)		0.79	0.94	0.72	0.16		

RI = Retention index relative to C<sub>9</sub>–C<sub>16</sub> *n*-alkanes on the DB-1 column, t = trace (<0.05%).

\* Identification based on mass spectra only.



**Figure 2** Dendrogram obtained by cluster analysis of the percentage composition of the essential oils isolated from *Artemisia herba-alba* samples based on correlation and using unweighted pair-group method with arithmetic average (UPGMA). Benifouda (Be); Bougaa (Boug); Boussaada (Bous) and Boutaleb (Bout).

polarities were installed: a DB-1 fused-silica column (polydimethylsiloxane.  $30 \text{ m} \times 0.25 \text{ mm}$  i.d., film thickness 0.25 um: J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column [(50% phenyl)-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.15 µm; J & W Scientific Inc.]. Oven temperature was programed, 45-175 °C, at  $3 \,^{\circ}\text{C}\,\text{min}^{-1}$ , subsequently at  $15 \,^{\circ}\text{C}.\text{min}^{-1}$  up to  $300 \,^{\circ}\text{C}$ , and then held isothermal for 10 min; injector and detector temperatures, 280 °C and 300 °C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of  $30 \text{ cm.s}^{-1}$ . The samples were injected using split sampling technique, ratio 1:50. The volume of injection was 0.1 µL of a pentane-volatiles solution (1:1). The percentage composition of the essential oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each sample, without using correction factors.

#### 2.3.2. Gas chromatography–Mass spectrometry (GC-MS)

The GC–MS unit consisted of a Perkin Elmer Autosystem XL gas chromatograph, equipped with DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; J & W Scientific, Inc.), and interfaced with a Perkin–Elmer Turbomass mass spectrometer (software version 4.1, Perkin Elmer, Shelton, CT, USA). Injector and oven temperatures were as above; transfer line temperature, 280 °C; ion source temperature, 220 °C; carrier gas, helium, adjusted to a linear velocity of 30 cm.s<sup>-1</sup>; split ratio, 1:40; ionization energy, 70 eV; scan range, 40-300 u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C<sub>9</sub>–C<sub>16</sub> *n*-alkane indices and GC-MS spectra from a homemade library, constructed based on the analyses of reference oils, laboratory-synthesized components and commercial available standards.

#### 2.4. Statistical analysis

The percentage composition of the isolated essential oils was used to determine the relationship between the different samples by cluster analysis using Numerical Taxonomy Multivariate Analysis System (NTSYS-pc software, version 2.2, Exeter Software, Setauket, New York) (Rohlf, 2000). For cluster analysis, correlation coefficient was selected as a measure of similarity among all accessions, and the Unweighted Pair Group Method with Arithmetical Averages (UPGMA) was used for cluster definition. The degree of correlation was evaluated according to Pestana and Gageiro (2000) and classified as very high (0.9-1), high (0.7-0.89), moderate (0.4-0.69), low (0.2-0.39) and very low (<0.2).

#### 3. Results and discussion

The aerial parts of *A. herba-alba* were collected at different localities in Algeria (Benifouda; Bougaa; Boussaada and Boutaleb), characterized by diverse geographic and climate conditions (Fig. 1, Table 2).

A. herba-alba populations studied afforded oils in a yield ranging from 0.2% (Boutaleb) to 0.9% (Bougaa) (v/d.w.), respectively, (Table 3). The oil yields recorded in the present study were within the ranges reported in the literature [0.1–4.9% (v/w), (Table 1)], and, as referred by Mighri et al. (2009b), where higher at the flowering phase.

*A. herba-alba* identified oil components are listed in Table 3 in order of their elution on the DB-1 column. Monoterpenes (78–89%) and particularly oxygen-containing monoterpenes (72–80%) dominated all oils. Sesquiterpenes ranged from 2–11% (Table 3).

A. herba-alba essential oils percentage composition was used to determine the relationship between the different samples, and allowed the definition of two clusters, Fig. 2. Cluster I was a one sample group as included only the Benifouda (Be) oil, which was dominated by camphor (33%) and chrysanthenone (12%), Table 3. Cluster II included the more correlated oil samples (S<sub>corr</sub> > 0.88) from Bougaa (Boug), Boussaada (Bous) and Boutaleb (Bout).  $\alpha$ -Thujone, which was < 7% in Be oil, was dominant in Cluster II sample oils (24–28%). Boug, Bous and Bout samples from Cluster II showed also large percentages of camphor (17–23%), and a wide range in chrysanthenone (4–19%) relative amount, Table 3.

Overall, the essential oil profiles of all samples were similar  $(S_{\text{corr}} > 0.7)$ , although with some variation in the relative amount of the three main components, camphor (17-33%),  $\alpha$ -thujone (7-28%) and chrysanthenone (4-19%). Despite the global resemblance, three types of oils could be defined, (a)  $\alpha$ -thujone : camphor (Bougaa and Boussaada plants oils), (b) camphor : chrysanthenone (Benifouda plant oil) and (c)  $\alpha$ -thujone : camphor : chrysanthenone (Boutaleb plant oil), Fig. 2.

No relationship could be drawn between the chemical composition of *A. herba-alba* essential oils and the four regions of Algeria (Benifouda; Bougaa; Boussaada and Boutaleb) where the samples were collected, nor with the altitude of the collection sites, temperature or humidity ranges (Tables 2 and 3, Fig. 2). This was confirmed by the fact that the most similar essential oil profiles were those isolated from plants collected during the flowering phase in Bougaa and during the vegetative phase in Boussaada (Tables 2 and 3, Fig. 2).

A review of the existing literature on *A. herba-alba* essential oils afforded a large number of studies, particularly in the last two years (Table 1). Although the isolation procedure was similar in most cases, the plant parts, the physiological stage, the plant status (fresh or dry), and the geographical origin were quite diverse, in addition to the use of collective samples and to the possibility of existing different subspecies. Only seldom studies have used individual plants, and even in those cases no clear correlation between plant oil types and environmental conditions was established (Fleisher et al., 2002).

With high percentage variability,  $\alpha$ -thujone (n.d. 80%) and  $\beta$ -thujone (n.d. 58%) were reported in studies from all countries (Table 1). Although not mentioned in Table 1, because this table includes only components with a relative amount  $\geq$  5%, the occurrence of  $\alpha$ -thujone and  $\beta$ -thujone was also reported in one study from Spain (Villar et al., 1983; in Salido et al., 2001). With exception of the work published on Jordanian A. herba-alba (Hudaib and Aburjai, 2006) (Table 1), chrysantenone (n.d. 65%), camphor (n.d. 49%) and 1,8-cineole (n.d. 28%) occurrence was always mentioned in, at least, some studies from all other countries (Table 1). In addition, with a more restricted occurrence, davanone (Spain and Tunisia) and *cis*-chrysanthenyl acetate (Israel, Sinai, Spain and Tunisia) attained also relatively high percentages (n.d. 51% and n.d. 69%, respectively) (Table 1). Usually one of these seven compounds or some of them, in different proportions, dominate A. herba-alba essential oils, which is in agreement with the results here reported for samples collected at different Algerian sites.

The chemical variability of essential oils from Algerian *A*. *herba-alba* emphasizes the importance of evaluating individual plant samples, as well as the worth of avoiding wild plant material collection, not only due to the innate variation, which has a negative market impact, but also to impair biodiversity deple-

tion. In view of this, it seems most adequate to recognize which chemovariety best fits the market demands and develop sustainable culture methodologies for local *A. herba-alba* crop production.

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#### References

- Benabdellah, M., Benkaddour, M., Hammouti, B., Bendahhou, M., Aouniti, A., 2006. Inhibition of steel corrosion in 2 M H<sub>3</sub>PO<sub>4</sub> by artemisia oil. Appl. Surf. Sci. 252, 6212–6217.
- Benjilali, B., Richard, H., 1980. Etude de quelque peuplements d'Armoise blanche du Maroc, *Artemisia herba-alba*. Riv. Ital. E.P.P.O.S. 62, 69–74.
- Bezza, L., Mannarino, A., Fattarsi, K., Mikail, C., Abou, L., Hadji-Minaglou, F., Kaloustian, J., 2010. Chemical composition of the essential oils of *Artemisia herba-alba* issued from the district of Biskra (Algeria). Phytothérapie 8, 277–281.
- Boukrich, F., Zouari, S., Neffati, M., Abdelly, C., Liu, K., Casanova, J., Tomi, F., 2010. Chemical variability of *Artemisia herba-alba* Asso growing wild in Semi-arid and Arid Land (Tunisia). J. Essent. Oil Res. 22, 331–335.
- Boutemak, K., Bezzina, M., Périno-Issartier, S., Chemat, F., 2009. Extraction by steam distillation of *Artemisia herba-alba* essential oil from Algeria: kinetic study and optimization of the operating conditions. J. Essent. Oil Bearing Plants 12, 640–650.
- Council of Europe (COE) European Directorate for the Quality of Medicines (2007). European Pharmacopoeia, 6th ed. COE, Strasbourg.
- Dahmani-Hamzaoui, N., Baaliouamer, A., 2010. Chemical composition of Algerian Artemisia herba-alba essential oils isolated by microwave and hydrodistillation. J. Essent. Oil Res. 22, 514–517.
- Dob, T., Benabdelkader, T., 2006. Chemical composition of the essential oil of *Artemisia herba-alba* Asso grown in Algeria. J. Essent. Oil Res. 18, 685–690.
- Dobignard, A., 1977. Nouvelles observations sur la flore du Maroc. 3. Candollea 52, 119–157.
- Feuerstein, I., Danin, A., Segal, R., 1988. Constitution of essential oils from *Artemisia herba-alba* population of Spain. Phytochemistry 27, 433–434.
- Feuerstein, I., Müller, D., Hobert, K., Danin, A., Segal, R., 1986. The constitution of essential oils from *Artemisia herba-alba* populations of Israel and Sinai. Phytochemistry 25, 2343–2347.
- Fleisher, Z., Fleisher, A., Nachbar, R.B., 2002. Chemovariation of Artemisia herba alba Asso aromatic plants of the Holy Land and Sinais. J. Essent. Oil Res. 14, 156–160.
- Greuter, W. (2006–2009): Compositae (pro parte majore). In: Greuter, W. & Raab-Straube, E. von (ed.): Compositae. Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity. http://ww2.bgbm.org/EuroPlusMed/ [accessed 07.11].
- Hudaib, M., Aburjai, T.A., 2006. Composition of the essential oil from *Artemisia herba-alba* grown in Jordan. J. Essent. Oil Res. 18, 301– 304.
- Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F., Donoghue, M.J., 2002. Plant systematics: a phylogenetic approach, 2nd ed. Sinauer Associates Inc., USA.

- Kadri, A., Zarai, Z., Békir, A., Gharsallah, N., Damak, M., Gdoura, R., 2011. Chemical constituents and antioxidant activity of the essential oil from aerial parts of *Artemisia herba-alba* grown in Tunisian semi-arid region. Afr. J. Biotechnol. 10, 2923–2929.
- Mighri, H., Akrout, A., Casanova, J., Tomi, F., Neffati, M., 2009a. Influence of drying time and process on *Artemisia herba-alba* Asso essential oil yield and composition. Journal of Essential Oil Bearing Plants 12, 358–364.
- Mighri, H., Akrout, A., Casanova, J., Tomi, F., Neffati, M., 2009b. Impact of season and harvest frequency on biomass and essential oil yields of *Artemisia herba-alba* cultivated in southern Tunisian. Exp. Agric. 4–5, 499–508.
- Mighri, H., Akrout, A., El-jeni, H., Zaidi, S., Tomi, F., Casanova, J., Neffati, M., 2010a. Composition and intraspecific chemical variability of the essential oil from Artemisia herba alba growing wild in a Tunisian arid zone. Chem. Biodivers. 7, 2709–2717.
- Mighri, H., Akrout, A., Neffati, M., Tomi, F., Casanova, J., 2009c. The essential oil from *Artemisia herba-alba* Asso cultivated in Arid Land (South Tunisia). J. Essent. Oil Res. 21, 453–456.
- Mighri, H., Hajlaoui, H., Akrout, A., Najjaa, H., Neffati, M., 2010b. Antimicrobial and antioxidant activities of *Artemisia herba-alba* essential oil cultivated in Tunisian arid zone. C. R. Chim. 13, 380– 386.
- Mohamed, A.E.-H.H., El-Sayed, M.A., Hegazy, M.E., Helaly, S.E., Esmail, A.M., Mohamed, N.S., 2010. Chemical constituents and biological activities of *Artemisia herba-alba*. Records of Natural Products 4, 1–25.
- Mohsen, H., Ali, F., 2009. Essential oil composition of *Artemisia herba-alba* from Southern Tunisia. Molecules 14, 1585–1594.
- Neffati, A., Skandrani, I., BenSghaier, M., Bouhlel, I., Kilani, S., Ghedira, K., Neffati, M., Chraief, I., Hammani, M., Chekir-Ghedira, L., 2008. Chemical composition, mutagenic and antimutagenic activities of essential oils from (Tunisian) Artemisia campestris and *Artemisia herba-alba*. J. Essent. Oil Res. 20, 471– 477.

- Ouachikh, O., Bouyanzer, A., Bouklah, M., Desjobert, J.-M., Costa, J., Hammouti, B., Majidi, L., 2009. Application of essential oil of Artemisia herba alba as green corrosion inhibitor for steel in 0.5M H<sub>2</sub>SO<sub>4</sub>. Surf. Rev. Lett. 16, 49–54.
- Paolini, J., Ouariachi, E.M.E., Bouyanzer, A., Hammouti, B., Desjobert, J.-M., Costa, J., Muselli, A., 2010. Chemical variability of *Artemisia herba-alba* Asso essential oils from East Morocco. Chem. Pap. 64, 550–556.
- Pestana, M. H., Gageiro, J.N. (2000). Análise de dados para ciências sociais. A complementaridade do SPSS. Edições Sílabo, Lisboa, Portugal.
- Quezel, P., Santa, S., 1963. Nouvelle flore de l'Algérie et des régions désertiques méridionales. CNRS, Paris.
- Rohlf, J.F., 2000. NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System. Applied Biostatistics, New York.
- Saleh, M.A., Belal, M.H., El-Baroty, G., 2006. Fungicidal activity of Artemisia herba alba Asso (Asteraceae). J. Environ. Sci. Health 41, 237–244.
- Salido, S., Altarejos, J., Nogueras, M., Sánchez, A., 2001. Chemical composition of the essential oil of *Artemisia herba-alba* Asso ssp. valentine (Lam.) Marcl. J. Essent. Oil Res. 13, 221–224.
- Salido, S., Valenzuela, L.R., Altarejos, J., Nogueras, M., Sánchez, A., Cano, E., 2004. Composition and infraspecific variability of *Artemisia herba-alba* from southern Spain. Biochem. Syst. Ecol. 32, 265–277.
- Tilaoui, M., Mouse, H.A., Jaafari, A., AboufatimaI, R., ChaitI, A., Zyad, 2011. A. Chemical composition and antiproliferative activity of essential oil from aerial parts of a medicinal herb *Artemisia herba-alba*. Rev. Bras. de Farmacognosia 21, 781–785.
- Zouari, S., Zouari, N., Fakhfakh, N., Bougatef, A., Aydi, M.A., Neffati, M., 2010. Chemical composition and biological activities of a new essential oil chemotype of Tunisian *Artemisia herba alba* Asso. J. Med. Plants Res. 4, 871–880.