



Variability of the chemical composition and the antioxidant activity of the essential oils of two subspecies of *Artemisia campestris* L. growing in Algeria

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Abstract

The aim of the present work was to investigate the chemical composition variability of the essential oils (EOs) of two subspecies of *Artemisia campestris* L. The studied subspecies were ssp. *eu-campestris* (Briq. & Cav.) and ssp. *glutinosa* (J. Gay ex Besser) Batt. Several samples coming from different regions of collection were adopted. The plant material was harvested at full flowering phenological status. Essential oils obtained by hydrodistillation of the aerial parts of the studied plant were analyzed by GC and GC–MS. Subsequently, the antioxidant activity of these EOs were also determined in vitro using three different assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals scavenging activity, ferric reducing antioxidant power and β -carotene bleaching test. The results of the EOs analysis showed the presence of monoterpenes hydrocarbons in high amounts in the majority of studied subspecies samples. The EOs samples presented large variability of the chemical compositions for both studied subspecies; besides, a new chemotype (capillene/capillin) was identified for the ssp. *eu-campestris* of this plant which grows in the location of Aflou (Laghouat).

Keywords *Artemisia campestris* · Essential oil · Variability · Aerial parts · Chemotype · Capillene

Introduction

The genus *Artemisia*, widespread over the world, growing wild over the Northern Hemisphere belongs to the Asteraceae family. Eleven species of *Artemisia* can be found in Algerian flora [1, 2]. In Algeria, *A. campestris* L. is a plant known as “Dgouft” and it grows wild on the steppe and desert regions [3]. In Arab folk medicine, *A. campestris* has been used as febrifuge, vermifuge, anticancer, against digestive troubles, gastric ulcer, and menstrual pain [3, 4]. Infusion, maceration and decoction of leaves and flowers of *A. campestris* were the preparation modes for the oral administration [5]. Numerous studies in the literature have reported the chemical composition of the EOs extracted from different parts of *A. campestris* subspecies (Table 1).

These reports showed that this plant displays an intraspecific variation in the terpenic composition, which is dependent upon the phenological stage, plant part, geographic location, chemotype or subspecies. Actually, *A. campestris* EO exhibited various chemotypes, mainly correlated to the place of growing of the plant, the environmental variables and the subspecies studied (Table 1). Nevertheless, large part of these studies didn't specify clearly the plant parts used or the subspecies investigated.

Thus the aim of the present study was to determine the variability of the chemical composition and the antioxidant activity of the EOs from the aerial parts of different subspecies of *A. campestris* coming from various Algerian regions and locations. Actually, there were only two subspecies which were identified from the collected individuals of the selected regions: ssp. *eu-campestris* (Briq. & Cav.) and ssp. *glutinosa* (J. Gay ex Besser) Batt. All samples were collected at the full flowering period to compare oil compositions of plants at the same phenological stage.

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Table 1 Major constituent (%) of *Artemisia campestris* essential oils from various countries, according to the literature

<i>A. campestris</i> subspecies	Organ	Main components (%)	Place of origin	Yield (%)	References
<i>spp. glutinosa</i>	Aerial parts	(Z,E)-farnesol (10.3), cedrol (5.4) and verbenone (3.8)	Algeria	0.1	[3]
ns	Aerial parts	α -Pinene (15.66), myrcene (18.19), <i>trans</i> - β -ocimene (13.93), m-cymene (9.00), camphor (6.46), γ -terpinene (5.32)	Algeria	1.0	[6]
ns	Aerial parts	α -Terpenyl acetate (18.8%), α -pinene (18.4%), camphor (9.2%), camphene (7.7%), limonene (5.2%), borneol (5.2%)	Algeria	0.66	[7]
ns	Aerial parts	α -Pinene (9.9), sabinene (17.0), β -pinene (25.6), myrcene (3.3), p-cymene (4.1), limonene (6.6)	Algeria	ns	[8]
ns	Leaves	β -Pinene (24.0–49.8), α -pinene (5.9–12.5), p-cymene (3.4–9.4), limonene (4.9–9.3), spathulenol (1.2–8.9), γ -terpinene (2.0–6.5), eudesmol (1.0–6.4) and (Z)- β -ocimene (0.2–5.5)	Tunisia Southern	0.65–1.2	[9]
ns	Leaves	β -Pinene (24.2–27.9), p-cymene (17.4–22.3) and α -pinene (4.1–11.0)	Tunisia South eastern	0.65	[10]
<i>var. glutinosa</i> <i>Gay ex Bess</i>	Aerial parts	β -Pinene (41.0), p-cymene (9.9), α -terpinene (7.9), limonene (6.5) and myrcene (4.1)	Tunisia Southern	1.5	[11]
ns	Leaves and/or flowers	β -Pinene (34.2), β -myrcene (6.0), limonene (8.2), (Z)- β -ocimene (4.6), (E)- β -ocimene (4.3) γ -terpinene (6.1), germacrene D (7.3)	Tunisia Southeast	ns	[12]
ns	Leaves	α -Pinene (12.5), β -pinene (45.8), limonene (7.7), p-cymene (4.6)	Tunisia Southern	1.0	[13]
<i>spp. glutinosa</i>	Aerial parts (different stages)	γ -Terpinene (\leq 46.5), capillene (33.1), 1-phenyl-2,4-pentadiene (\leq 29.7) and spathulenol (\leq 11.3)	France	0.4–1.4	[14]
<i>spp. glutinosa</i>	Flowers and leaves	β -Pinene (6.9–57.2), germacrene D (0.4–28.6), bicyclogermacrene (1.0–14.5) and myrcene (1.7–11.2)	Italy Southern	0.2 0.6	[15]
<i>spp. campestris</i> and <i>borealis</i>	Aerial parts	α -Pinene (\leq 16.5), β -pinene (\leq 10.7), caryophyllene oxide (\leq 18.2), spathulenol (\leq 18.7), <i>epi</i> -cubanol (\leq 14.2) and 1,8-cineole (\leq 19.2)	Italy North west	ns	[16]
ns	Aerial parts	Spathulenol (9.2), β -pinene (9.1), α -pinene (3.4), limonene (2.5), germacrene D (3.3), 4-hydroxy-9- <i>epi</i> - β -caryophyllene (3.0)	Serbia	0.2	[17]
ns	Flowers, leaves and stems, separately	α -Pinene (23.9, 23.0, 29.2) and spathulenol (23.9, 15.8, 29.2) in the flower, leaf and stem oils, respectively; bicyclogermacrene (12.0) in the flower and β -pinene (12.6) in the leaf oil	Iran	0.49 0.18 0.05	[18]
ns	Aerial parts at flowering stage	α -Pinene (41.0), β -pinene (29.7), limonene (6.4) and sabinene (4.5)	Republic of Bashkortostan	ns	[19]
<i>spp. campestris</i>	Aerial parts (leaves and inflorescences)	α -Pinene (tr- 6.8), β -pinene (tr-5.5), <i>cis</i> -pinane (0.8–6.0), β -caryophyllene (1.6–6.2), germacrene D (3.5–15.0), caryophyllene oxide (8.5–38.8), humulene epoxide II (0.7–8.1)	Lithuania	0.03–0.08	[20]

Table 1 (continued)

<i>A. campestris</i> subspecies	Organ	Main components (%)	Place of origin	Yield (%)	References
<i>ssp. campestris</i>	Aerial parts	α -Pinene (0.8–11.4), β -pinene (0.1–13.8), β -caryophyllene (0.7–10), γ -curcumene (0.5–14.8), germacrene D (1.2–31.2), caryophyllene oxide (0.8–16.1), humulene epoxide II (0.3–11.1)	Lithuania	0.03–0.08	[21]
<i>subsp. campestris</i>	Inflorescences, flowers, leaves, stems and roots	α -Pinene (4.5, 2.2, 5.0, 1.0, 0.1), (E)- β -caryophyllene (6.5, 5.9, 4.2, 4.1, nd), germacrene D (24.2, 28.0, 27.8, 9.7, 0.1), γ -humulene (4.9, 6.2, 8.2, 4.0, 0.7), (E,E)- α -farnesene (3.9, 4.3, 3.5, 2.6, 0.5), (Z)-falcarnol (1.3, 1.6, 0.4, 19.0, 38.8)	Poland	0.16 0.19 0.11 tr 0.1	[22]
ns	Aerial parts at flowering stage	Spathulenol (10.19%), β -eudesmol (4.05%), p-cymene (3.83%), δ -cadinene (3.67%), β -pinene (2.82%), caryophyllene oxide (2.30%) and salvia-4(14)-en-1-one (2.51%)	Morocco Eastern	0.4 “w/w”	[23]

ns name of subspecies not indicated (not specified), nd not detected, tr traces

Materials and methods

Plant material

The aerial parts of individuals from various *A. campestris* L. populations were randomly collected at full flowering stage from two different localities in Algeria and from different altitudes characterized by diverse geographic and climate conditions reported in Table 2. Populations 1, 2, 3, 4, 5, 6, 7 and 8 were located at the Northeast Central of Algeria (Laghouat region) with a superior arid climate, while populations 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18 were localized at the North central of Algeria (Djelfa region) characterized by an inferior semi-arid climate. Shortly after that, the fresh samples were dried in the shade, at ambient temperature for 20 days. Separated from stems, aerials parts were subjected to essential oil extraction.

The plants were identified by Mr. Brahim Guit, doctor at the department of Agronomy from the University of Djelfa. The subspecies botanical identification revealed that the samples encompassed two different subspecies: *ssp. eu-campestris* (Briq. & Cav.) and *ssp. glutinosa* (J. Gay ex Besser) Batt. (Table 2). Voucher specimens (AC-Lgh-x/09/15, AC-Afl-x/09/15 and AC-Djf-x/09/15, respectively) were deposited in the herbarium of the Fundamental Sciences Research Laboratory at Laghouat University.

Essential oil extraction

The dried vegetable matter was submitted to hydrodistillation for 3 h, using a Clevenger-type apparatus. In this operation, water was used as a solvent for the extraction operation. After extraction completion, each essential oil sample was dried over anhydrous sodium sulfate and stored in sealed vials protected from light at -20 °C, until analysis.

Essential oil analyses

Gas chromatography (GC)

The analysis of the essential oil samples was carried out using a Chrompack CP 9002, gas chromatograph equipped with a flame ionization detector (FID) and a capillary DB-5 column (30 m x 0.32 mm; 0.25 μ m film thickness); injector and detector temperature were maintained at 250 and 280 °C, respectively.

A volume of 0.5 μ L solution, prepared by 10% EO dilution in dichloromethane (CH₂Cl₂), was injected in split mode (50:1), helium was used as carrier gas at a flow rate of 1 mL/min. The column temperature was programmed at: 50 °C for 3 min, then 2 °C/min to 250 °C, and then left at 250 °C for

Table 2 Description of the collection sites of two subspecies of *Artemisia campestris* L. in two different locations in Algeria

Sample No	Locality	Plant Subsp.	Latitude (S)	Longitude (W)	Altitude (m)
Laghouat region					
S1	Milok 1	<i>glutinosa</i>	33°50'35"	02°45'44"	800
S2	Milok 2	<i>glutinosa</i>	33°50'35"	02°45'34"	800
S3	El-Hadjeb 1	<i>glutinosa</i>	33°56'43"	02°37'10"	995
S4	El-Hadjeb 2	<i>glutinosa</i>	33°56'43"	02°37'10"	995
S5	M'Seka	<i>glutinosa</i>	34°04'45"	02°34'33"	1050
S6	Aflou 1	<i>eu-campestris</i>	34°06'27"	02°06'03"	1403
S7	Aflou 2	<i>eu-campestris</i>	34°06'27"	02°06'03"	1403
S8	Aflou 3	<i>eu-campestris</i>	34°06'27"	02°06'03"	1403
Djelfa region					
S9	Djelfa 1	<i>glutinosa</i>	34°13'710"	03°05'809"	961
S10	Djelfa 2	<i>glutinosa</i>	34°16'146"	03°06'787"	987
S11	Djelfa 3	<i>glutinosa</i>	34°16'146"	03°06'787"	987
S12	Djelfa 4	<i>glutinosa</i>	34°16'146"	03°07'299"	990
S13	Djelfa 5	<i>glutinosa</i>	34°16'146"	03°07'299"	990
S14	Djelfa 6	<i>glutinosa</i>	34°22'173"	03°10'598"	1046
S15	Djelfa 7	<i>glutinosa</i>	34°27'159"	03°13'640"	1156
S16	Djelfa 8	<i>glutinosa</i>	34°27'159"	03°13'640"	1156
S17	Djelfa 9	<i>glutinosa</i>	34°43'810"	03°11'790"	1038
S18	Djelfa 10 (Aïn-Maabed)	<i>glutinosa</i>	34° 44'713"	03°11'220"	1024

10 min. The percentages of the constituents were calculated by electronic integration of FID peak areas, without the use of correction factors. Mean percentage of compounds in *A. campestris* L. essential oils represented the average calculated on five individuals ($n = 5$). Linear retention indices (LRI) were calculated for separate compounds relative to (C_9 – C_{25}) *n*-alkanes.

Gas chromatography/mass spectrometry (GC/MS)

The isolated volatile compounds were analysed by GC/MS, using a shimadzu GC/MS-QP2010 ultra, and equipped with fused DB-5 capillary column (the same used in the GC/FID analysis). The oven temperature was programmed at: 50 °C for 3 min, then 2 °C/min to 250 °C, and then left at 250 °C for 10 min. The injection port temperature was 250 °C and that of the detector was 280 °C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 mL min⁻¹. The mass spectrometer conditions were as follow: ionization voltage, 70 eV; ion source temperature, 150 °C; electron ionization mass spectra were acquired over the mass range 50–550 m/z.

Antioxidant activity

The antioxidant activity was compared with the following positive controls: ascorbic acid, α -tocopherol and butylated hydroxyanisole (BHA), and assessed by three different tests, i.e. DPPH assay, The ferric reducing antioxidant power

(FRAP) and β -carotene bleaching method. All assays were performed in triplicate and in the dark using amber light flasks.

DPPH assay

The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant. DPPH solutions show a strong absorption band at 517 nm with a deep violet color. The absorption vanishes and the resulting decolouration is stoichiometric with respect to degree of reduction. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant [24–27]. 120 μ L of the extracted oil dilutions in ethanol was added to 1 mL of 120 μ M solution of DPPH [28]. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm (Shimadzu UV/Vis 1601 apparatus). Inhibition of DPPH free radicals in percent (I%) was calculated as follows:

$$I(\%) = \left[1 - \frac{A_{\text{sample}}}{A_{\text{blank}}} \right] \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. All tests were carried out

in triplicate. The BHA, α -tocopherol and ascorbic acid were used as positives controls.

Antioxidant capacity determined by ferric reducing antioxidant power (FRAP)

The FRAP assay was done according to Benzie and Strain with some modifications [29]. The stock solutions included 300 mmol/L acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$) and 16 mL $C_2H_4O_2$, pH 3.6, 10 mmol/L 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution in 40 mmol/L HCl, and 20 mmol/L $FeCl_3 \cdot 6H_2O$. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37 °C before using. Ethanolic solutions of samples of 100 μ L were added to 3 mL of FRAP reagent. The absorbance at 593 nm (Shimadzu UV/Vis 1601 apparatus) was read after 30 min. A standard curve was prepared using different concentrations of vitamin C; Data were expressed relative to the value obtained with ascorbic acid from calibration curve and then expressed as ascorbic acid equivalents AEAC. The antioxidant activity expressed as AEAC value and defined as the concentration of vitamin C in mg/mL which gives similar antioxidant activity power of a given extract sample of 1 mg/mL of concentration. Higher AEAC values demonstrated higher antioxidant activities. In order to make comparisons, vitamin E and BHA were also tested under the same conditions as standard antioxidant compounds, and their powers were naturally expressed as AEAC values. All experiments were carried out in triplicate unless otherwise stated, result are expressed as means \pm SD.

Antioxidant activity by β -carotene bleaching method

The antioxidant activity of each fraction was evaluated using the β -carotene-linoleate model system, as described by Sun and Ho [30], with some modifications. Briefly, 2 mg of β -carotene was dissolved in 10 mL chloroform and 1 mL β -carotene solution was mixed with 20 mg of Corn oil and 200 mg of Tween 80 emulsifiers. Chloroform was then evaporated, and the resulting mixture was immediately diluted with 50 mL of distilled water. To an aliquot of 5 mL of this emulsion, 0.2 mL of each extract or ascorbic acids and α -tocopherol were added and mixed well. The absorbance at 470 nm, which was regarded as t_0 , was measured, immediately, against a blank consisting of the emulsion without β -carotene. The capped tubes were placed in a water bath at 50 °C, and the absorbance was measured after every 15 min up to 120 min. For the positive control, sample was replaced with ascorbic acid and α -tocopherol. A negative control consisted of 0.2 mL of distilled water or solvent instead of extract or reference antioxidant was used. All samples were assayed in triplicate. The antioxidant activity (AA) was

measured in terms of successful bleaching of β -carotene by using the following equation;

$$AA = \left[1 - \left(\frac{A_0 - A_t}{A_0 - A_{0t}} \right) \right] \times 100$$

where A_0 and A_{0t} are the absorbance values measured at zero times during the incubation for each fraction and control, respectively. A_t and A_{0t} were the absorbance values measured for each fraction and control, respectively, after incubation for 120 min. The results were expressed as IC_{50} .

Statistical analyses

Cluster analysis

Cluster analysis was performed using Agglomerative Hierarchical Clustering AHC (Ward's technique) with Euclidean distance measure. The calculus was performed using a set composed of 18 different samples (individuals). The total number of adopted variables was 11 (representing simply the major identified essential oil components in all represented individuals, or the most influencing parameters that could make a difference i.e. some minor compounds).

Principal component analysis (PCA)

The PCA was performed using both the same individuals and variables adopted for Agglomerative Hierarchical Clustering method.

Results

Essential oil composition

The chemical compositions of the different EO samples of two subspecies of *A. campestris* L. are summarized in Tables 3 and 4. Eighteen components representing the most important chemicals were identified using GC and GC-MS. The total percentages for identified components were varying from 64.34 to 89.24%, and from 72.15 to 86.94% for both subspecies *glutinosa* and *eu-campestris*, respectively. The results showed also that the EO samples were rich in monoterpene hydrocarbons for both subspecies with intervals compositions which varied from 40.30 to 82.28% for ssp. *glutinosa* and from 29.47 to 51.74% for ssp. *eu-campestris*, respectively. The oxygenated monoterpenes contents were very low for both subspecies (ssp. *glutinosa*: 0.0–3.47%, ssp. *eu-campestris*: 0.52–0.61%). Similarly, the contents of total sesquiterpenes were varying from low to moderate values (ssp. *glutinosa*: 3.41–15.8%, ssp. *eu-campestris*: 6.0–9.45%). Furthermore, in the case of

Table 3 Percentage of the major components in the essential oil of two subspecies of *Artemisia campestris* collected in different regions from Algeria

Compounds	Region of Laghouat										Region of Djelfa									
	LRI ^L					LRI ^C					ssp. <i>et-campestris</i>					ssp. <i>gluthosa</i>				
	S1 (%)	S2 (%)	S3 (%)	S4 (%)	S5 (%)	S6 (%)	S7 (%)	S8 (%)	S9 (%)	S10 (%)	S11 (%)	S12 (%)	S13 (%)	S14 (%)	S15 (%)	S16 (%)	S17 (%)	S18 (%)		
α -Pinene	932	937	5.11	5.48	2.44	2.02	3.30	5.91	11.56	9.97	6.94	8.48	14.88	3.85	4.74	8.70	7.64	8.03	4.01	8.04
Sabinene	969	980	tr	tr	tr	tr	tr	0.42	0.79	0.59	1.90	tr	tr	1.00	tr	tr	tr	0.97	tr	3.21
β -Pinene	974	987	31.20	24.38	28.54	18.6	31.65	8.95	8.20	9.60	34.76	40.18	32.63	40.36	24.94	33.97	23.22	38.11	31.58	28.08
β -Myrcene	988	998	5.77	3.50	3.78	1.4	3.80	3.30	6.53	3.78	7.89	5.16	3.70	2.56	1.71	4.66	6.12	3.52	6.39	7.13
<i>p</i> -Cymene	1020	1030	3.91	4.58	6.44	8.47	6.16	3.32	10.25	10.64	6.96	7.96	6.97	6.93	11.56	7.85	7.90	11.63	8.34	6.84
Limonene	1024	1037	8.76	6.21	10.28	5.5	11.14	2.70	4.50	3.73	11.54	10.69	8.83	9.24	7.49	10.24	7.55	8.69	12.06	5.98
<i>cis</i> - β -Ocimene	1040	1043	1.96	0.98	tr	1.11	5.58	1.64	2.85	2.60	1.88	1.10	1.66	1.55	1.06	2.19	1.64	1.30	4.70	4.24
<i>trans</i> - β -Ocimene	1050	1054	2.40	1.6	1.29	1.19	2.79	1.31	2.84	1.02	4.35	2.56	1.51	2.89	1.13	2.55	2.95	2.01	3.54	5.09
γ -Terpinene	1057	1066	2.46	2.01	9.57	2.01	9.06	1.92	4.22	0.70	6.06	6.15	4.15	5.17	3.49	5.74	5.35	3.83	7.35	6.35
4-Terpineol	1186	1185	3.05	1.52	2.84	3.47	1.63	0.52	0.60	0.61	1.21	1.57	tr	0.64	1.53	2.05	2.10	1.17	1.65	0.85
Geranyl acetate	1379	1395	1.07	0.33	2.37	0.98	1.28	0.14	0.25	0.2	0.42	0.34	0.59	0.25	0.93	0.34	0.62	0.47	1.05	0.30
α -Curcumene	1479	1479	4.52	5.61	2.78	1.75	2.73	0.71	1.77	0.72	1.88	1.74	1.68	2.20	2.04	1.72	4.20	2.15	1.93	0.68
Capillene	1493 ^R	1520	-	-	-	-	-	46.93	12.03	17.76	-	-	-	-	1.26	-	-	-	-	0.68
Spathulenol	1577	1595	1.97	4.65	0.80	5.01	0.45	5.63	3.11	7.14	1.00	0.76	1.58	0.85	5.04	0.98	3.56	3.04	1.37	2.95
Viridiflorol	1592	1616	0.65	1.11	2.00	2.23	0.26	0.29	0.37	0.70	0.19	0.16	0.47	0.66	0.71	0.24	0.43	0.55	0.19	0.31
Geranyl isovalerate	1606	1630	1.97	3.79	1.24	3.91	1.45	0.76	0.66	0.97	1.32	0.80	1.40	1.92	2.83	1.52	2.63	1.41	1.00	0.56
Capillin	1637	1650	0.24	0.53	0.21	0.63	-	1.44	0.87	3.11	-	tr	0.15	-	0.36	0.11	0.33	0.13	0.16	0.31
β -Eudesmol	1649	1647	2.19	4.43	3.14	6.06	0.95	1.05	0.75	0.89	0.94	0.75	1.73	1.21	2.67	1.03	1.14	1.17	0.87	0.68
Total identified			77.23	70.71	77.72	64.34	82.23	86.94	72.15	74.73	89.24	88.4	81.93	81.28	73.49	83.89	77.38	88.18	86.19	82.28
Yield (%) d.w.			0.84	0.87	0.95	0.93	0.86	1.18	1.23	1.21	0.67	0.76	0.77	0.87	0.76	0.87	0.68	0.78	0.83	0.67
Monoterpene hydrocarbons			61.57	48.74	62.34	40.3	73.48	29.47	51.74	42.63	82.28	82.28	74.33	73.55	56.12	75.9	62.37	78.09	77.97	74.96
Oxygenated monoterpene			3.05	1.52	2.84	3.47	1.63	0.52	0.60	0.61	1.21	1.57	0.00	0.64	1.53	2.05	2.10	1.17	1.65	0.85
Total monoterpenes			64.62	50.26	65.18	43.77	75.11	29.99	52.34	43.24	83.49	83.85	74.33	74.19	57.65	77.95	64.47	79.26	79.62	75.81
Sesquiterpenes			4.52	5.61	2.78	1.75	2.73	0.71	1.77	0.72	1.88	1.74	1.68	2.2	2.04	1.72	4.2	2.15	1.93	0.68
Oxygenated sesquiterpenes			4.81	10.19	5.94	13.30	1.66	6.97	4.23	8.73	2.13	1.67	3.78	2.72	8.42	2.25	5.13	4.76	2.43	3.94

Table 3 (continued)

Compounds	Region of Djelfa																	
	Region of Laghouat							Region of Djelfa										
	LRI ^L			ssp. <i>glutinosa</i>				ssp. <i>eu-campestris</i>				ssp. <i>glutinosa</i>						
	S1 (%)	S2 (%)	S3 (%)	S4 (%)	S5 (%)	S6 (%)	S7 (%)	S8 (%)	S9 (%)	S10 (%)	S11 (%)	S12 (%)	S13 (%)	S14 (%)	S15 (%)	S16 (%)	S17 (%)	S18 (%)
Total sesquiterpenes	9.33	15.8	8.72	15.05	4.39	7.68	6.00	9.45	4.01	3.41	5.46	4.92	10.46	3.97	9.33	6.91	4.36	4.62
Terpeneesters	3.04	4.12	3.61	4.89	2.73	0.90	0.91	1.17	1.74	1.14	1.99	2.17	3.76	1.86	3.25	1.88	2.05	0.86
Aromatic homo-monocyclic	0.24	0.53	0.21	0.63	0.00	48.37	12.9	20.87	0.00	0.00	0.15	0.00	1.62	0.11	0.33	0.13	0.16	0.99

L LRI obtained from literature [31], R LRI obtained from literature [14], C LRI determined experimentally (calculated), *T* < 0.1%, – not detected

ssp. *eu-campestris*, the oxygenated sesquiterpenes percentage range was significantly higher than the sesquiterpene hydrocarbons (4.23–8.73 vs. 0.71–1.77%). The identification results showed that the percentages of the majority of main identified components (α -pinene, β -pinene, *p*-cymene, capillene and spathulenol) were not the same for all studied samples referring to ssp. *eu-campestris*. In the same manner, the ssp. *glutinosa* was also characterized by different main component percentages: α -pinene, β -pinene, β -myrcene, *p*-cymene, limonene, γ -terpinene. Besides, it was found that the EOs of ssp. *glutinosa*, contained weak percentages of terpenesters which accounted for only two chemicals: geranyl acetate (0.25–2.37%) and geranyl isovalerate (0.56–3.91%). Similarly, the EOs of ssp. *eu-campestris* presented also very weak percentages of terpenesters (geranyl acetate: 0.14–0.25%; geranyl isovalerate: 0.66–0.97%).

The samples of Aflou location (ssp. *eu-campestris*) presented high amounts (reported for the first time) of total homomonocyclic aromatics (12.9–48.37%, which accounted for only two components capillene and capillin). For this subspecies, the percentages of capillene (12.03–46.93%) were by far larger than of capillen (0.87–3.11%). Alternatively, for the samples of ssp. *glutinosa*, the total percentage variation of both capillene and capillin was very low and accounting for 0.0–1.62%.

The EO yields for ssp. *eu-campestris* ($1.21 \pm 0.03\%$) were higher than obtained for ssp. *glutinosa* ($0.81 \pm 0.09\%$). In other hand, there were no practical intraspecific variation of the EO yields for each subspecies depending on the region or the location from where they were collected (Table 3). It was found that for the samples of ssp. *glutinosa* (Laghouat), the EOs yielded $0.89 \pm 0.05\%$; these values were slightly higher than obtained for region of Djelfa, from where the yields were in the range of $0.77 \pm 0.08\%$.

The comparison of obtained yields with those of literature reveals some similarities and some differences (Table 1). In fact, comparison were performed with only papers that clearly specified the subspecies used (Table 1).

First, for the ssp. *glutinosa* the obtained yields (0.67–0.95%) were considerably higher than reported earlier from Algeria (0.1% at a different region of study), but were lower than the yields reported from Tunisia (1.5%). More interestingly, previous work from France reported large yields variation for the aerial parts of this subspecies (0.4–1.4%) which were in agreement with our results. Secondly, for the ssp. *eu-campestris*, the obtained EO yields (1.18–1.23%) were significantly higher than those reported for Lithuania (0.03–0.08%).

Antioxidant activity

The methods chosen are the most commonly used for the determination of antioxidant activities of plant extracts

Table 4 Range variation of the percentage of the essential oil of two subspecies of *Artemisia campestris* collected in different regions from Algeria

Compounds	<i>ssp. eu-campestris</i>				<i>ssp. glutinosa</i>			
	Min (%)	Max (%)	Mean (%)	SD (%)	Min (%)	Max (%)	Mean (%)	SD (%)
α -Pinene	5.91	11.56	9.15	2.91	2.02	14.88	6.24	3.28
Sabinene	0.42	0.79	0.60	0.19	0.97	3.21	1.77	1.05
β -Pinene	8.02	9.60	8.92	0.70	18.6	40.36	30.81	6.31
β -Myrcene	3.3	6.53	4.54	1.74	1.40	7.89	4.47	1.92
<i>p</i> -Cymene	3.32	10.64	8.07	4.12	3.91	11.63	7.50	2.09
Limonene	2.7	4.5	3.64	0.90	5.50	12.06	8.95	2.07
<i>cis</i> - β -Ocimene	1.64	2.85	2.36	0.64	0.98	5.58	2.21	1.49
<i>trans</i> - β -Ocimene	1.02	2.84	1.72	0.98	1.13	5.09	2.52	1.16
γ -Terpinene	0.7	4.22	2.28	1.79	2.01	9.57	5.25	2.32
4-Terpineol	0.52	0.61	0.58	0.05	0.64	3.47	1.81	0.82
Geranyl acetate	0.14-	0.25	0.20	0.06	0.25	2.37	0.76	0.56
α -Curcumene	0.71	1.77	1.07	0.61	0.68	5.61	2.51	1.30
Capillene	12.03	46.93	25.57	18.72	0.68	1.26	0.97	0.41
Spathulenol	3.11	7.14	5.29	2.04	0.45	5.04	2.27	1.65
Viridiflorol	0.29	0.70	0.45	0.22	0.16	2.23	0.68	0.64
Geranyl isovalerate	0.66	0.97	0.80	0.16	0.56	3.91	1.85	1.01
Capillin	0.87	3.11	1.81	1.16	0.11	0.63	0.29	0.17
β -Eudesmol	0.75	1.05	0.90	0.15	0.68	6.06	1.93	1.56
Yield (%) d.w.	1.18	1.23	1.21	0.03	0.67	0.95	0.81	0.09

Table 5 Antioxidant activities of the essential oil of aerial parts of two subspecies of *Artemisia campestris*

Region	Subspecies	Sample name	DPPH assay		FRAP assay		β -Carotene assay	
			IC ₅₀ (mg/mL)	SD	AEAC (mg/mL)	SD	IC ₅₀ (mg/mL)	SD
Laghouat	<i>ssp. glutinosa</i>	Milok 1	20.79	0.66	0.030	0.002	na	na
		Milok 2	26.07	0.40	0.026	0.001	0.13	0.01
		El-Hadjeb 1	21.76	0.67	0.027	0.001	0.10	0.01
		El-Hadjeb 2	19.84	1.02	0.023	0.001	0.46	0.01
		M'Seka	19.12	0.20	0.029	0.000	0.09	0.01
	<i>ssp. eu-campestris</i>	Aflou 1	5.65	0.49	0.036	0.003	0.32	0.03
Aflou 2		9.26	0.60	0.029	0.001	0.14	0.04	
Aflou 3		16.64	0.67	0.026	0.001	0.35	0.02	
Djelfa	<i>ssp. glutinosa</i>	Djelfa 1	17.43	0.59	0.030	0.001	0.18	0.01
		Djelfa 2	31.94	1.55	0.023	0.001	0.25	0.02
		Djelfa 3	13.07	0.17	0.026	0.001	0.13	0.01
		Djelfa 4	22.62	0.55	0.027	0.001	0.79	0.02
		Djelfa 5	20.52	0.45	0.023	0.001	0.27	0.00
		Djelfa 6	22.00	0.08	0.028	0.001	0.47	0.01
		Djelfa 7	17.57	0.76	0.032	0.002	0.18	0.01
		Djelfa 8	15.45	0.38	0.022	0.002	0.32	0.02
		Djelfa 9	17.58	0.33	0.033	0.001	0.12	0.02
		Djelfa 10	13.32	0.60	0.033	0.001	0.19	0.00
BHA			0.0056	0.0002	1.2	0.09		
Vitamin C			0.0047	0.0001	–	–	na	–
Vitamin E			0.0055	0.0001	0.10	0.04		

na the essential oil presented no activity in the studied concentrations range

and/or EOs. The results of antioxidant activity determined in vitro using three different assays are presented in Table 5.

Free radicals scavenging activity (DPPH assay)

The antioxidant activity of the EOs was determined in vitro using DPPH (1,1-diphenyl, 2-picrylhydrazyl) assay. The results are summarized in Table 5 and Fig. 1. The obtained values of IC₅₀ suggested low antioxidant activity when compared with synthetic antioxidants (BHA, vitamins C and E); but the overall activity range values were very interesting in comparison with recent report for the same plant from Tunisia (in which subspecies was not provided), where the reported IC₅₀ was equal to 94.5 mg/mL (lower IC₅₀ value, means higher activity) [12]. In addition, there was a large variability of the values of IC₅₀ for the different adopted samples, the IC₅₀ values range was (5.65–31.94 mg/mL). The minimum value of IC₅₀ recorded for the sample Aflou 1 represented the highest antioxidant activity. In the opposite side, the maximum IC₅₀ value was reflecting the lowest antioxidant power (practically inactive), in which this time was registered for Djelfa 2 sample. In general, the ssp. *eu-campestris* EOs exhibited higher antioxidant activity (IC₅₀ = 5.65–16.64 mg/mL) in comparison with ssp. *glutinosa*, (IC₅₀ = 13.07–31.94 mg/mL), especially for samples of Aflou 1&2 (rich in capillene).

FRAP assay

The results of FRAP assay are presented in Table 5 and schemed in Fig. 2. The reducing power values of tested individuals for both subspecies were very close to each other's with AEAC range variation of [0.022–0.036].

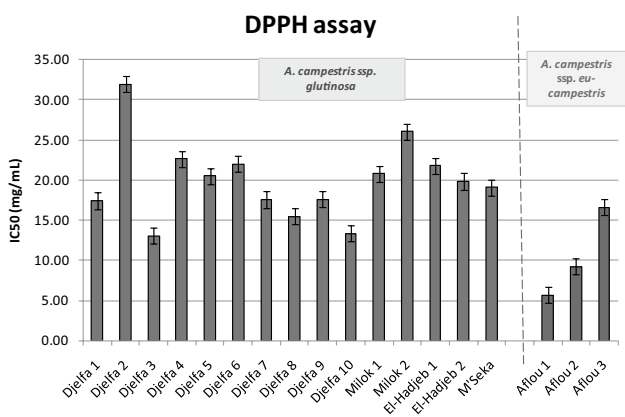


Fig. 1 Representation of the values of antioxidant activity of the essential oil of two subspecies of *Artemisia campestris* using DPPH assay

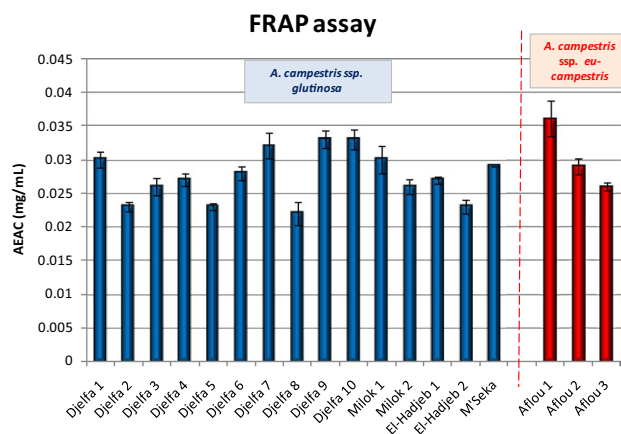


Fig. 2 Representation of the values of antioxidant activity of the essential oil of two subspecies of *Artemisia campestris* using FRAP assay

Bleaching ability of β-carotene in linoleic acid system

In β-carotene-linoleic acid system, β-carotene undergoes a rapid discoloration in the absence of an antioxidant. The presence of an antioxidant such as essential oils can hinder the extent of β-carotene destruction by “neutralizing” the linoleate free radicals and any other free radicals formed within the system. The results of this test are summarized in Table 5 and Fig. 3. Except for sample of Milok 1 which exhibited no measurable activity in the range of studied concentrations, the values of IC₅₀ were varying from 0.09 to 0.79 mg/mL. The determined activity values were independent on the region of collection and on the subspecies considered.

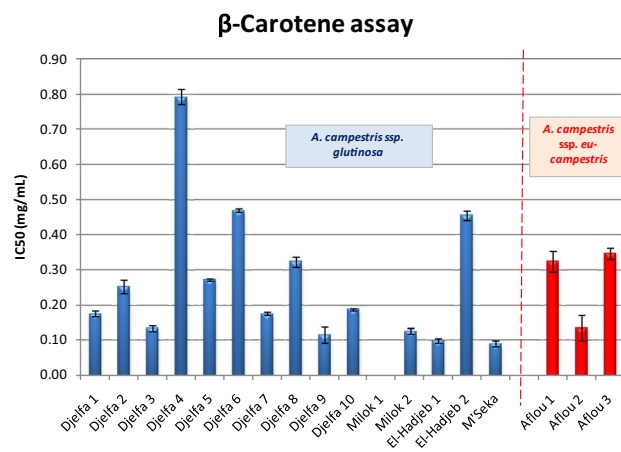


Fig. 3 Representation of the values of antioxidant activity of the essential oil of two subspecies of *Artemisia campestris* using β-carotene assay

Discussion

Essential oil composition

It was observed some large intraspecific variability in the percentages of the main components for both studied subspecies (Table 4). More explicitly, the ssp. *eu-campestris* was characterized by large variations in the following main components: α -pinene (5.91–11.56%), *p*-cymene (3.32–10.64%) and capillene (12.03–46.93%). Similarly, the ssp. *glutinosa* was also characterized by large variations in the following main components: α -pinene (2.02–14.88%), β -pinene (18.60–40.36%), β -myrcene (1.40–7.89%), *p*-cymene (3.91–11.63%), limonene (5.50–12.06%) and γ -terpinene (2.01–9.57%). Inversely, it was noticed that the contents of β -pinene was practically steady in all studied samples for ssp. *eu-campestris* (8.2–9.60%). According to these ranges variations, and taking in consideration each subspecies separately, it seemed clear that some major components in some populations were acting as minor components in some other populations, which confirms the variability theory of the chemical composition of the EOs for the two subspecies of *A. campestris* growing in different regions of Algeria.

Moreover, the occurrence of new EO component capillene (not reported earlier) with high percentages (up to 46.93%) suggested the presence of a new chemotype (capillene/capillin) for the EO of *A. campestris* ssp. *eu-campestris*. Finally, for the ssp. *glutinosa*, the variability of chemical composition of their EOs seemed to be independent to the region of collection.

For better understanding of similarities and/or differences between the studied samples (populations) in term of EO compositions variability for the two subspecies, cluster analysis was performed using Agglomerative Hierarchical Clustering AHC. The results of AHC schemed in the dendrogram of the Fig. 4, showed two clearly distinguished clusters “or groups” of samples (cluster I: Aflou samples: ssp. *eu-campestris*) and (cluster II: Djelfa + Laghouat samples “other than Aflou location”: ssp. *glutinosa*). Besides, these two clusters could be also separated in two different sub-clusters (sub-clusters); which indicated an existing dissimilarity of the studied samples (proves the existence of some variability of the chemical composition, for both subspecies, as well as the occurrence of new chemotype variability for ssp. *eu-campestris*). The comparison of the chemical composition of ssp. *eu-campestris* (Aflou samples “cluster I”) indicates roughly close similarities of the percentages of most major and minor components for samples Aflou-2&3. These two samples were distinguished from the sample Aflou-1 (characterized mainly by the highest content of capillene), which indicates a presence

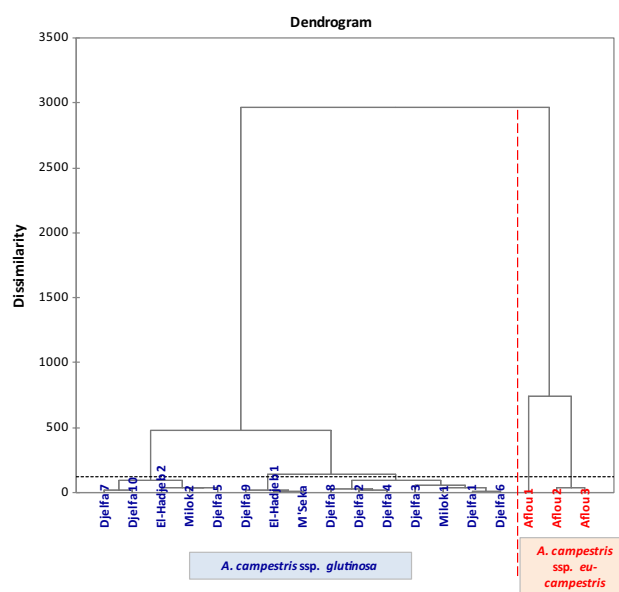


Fig. 4 Dendrogram obtained from a cluster analysis of 18 samples of essential oils of two subspecies of *Artemisia campestris* growing in Algeria. Samples are clustered using Ward’s technique with a Euclidean distance measure

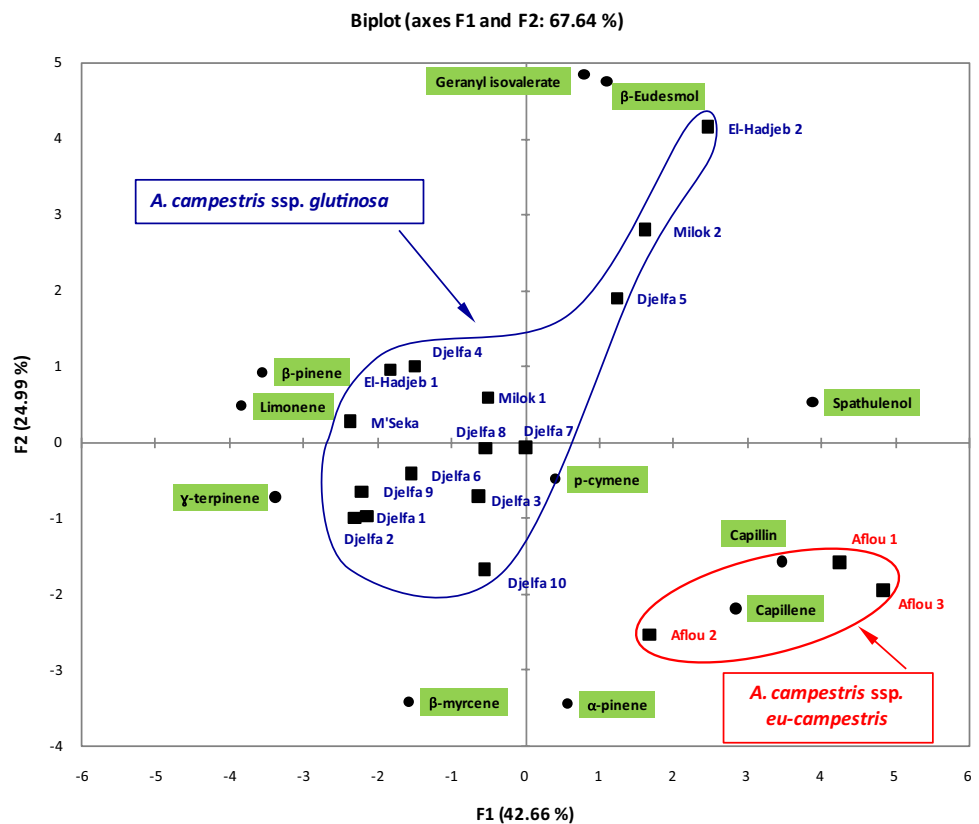
of at least two sub-clusters (based on the current number of samples). For the rest of samples which represented ssp. *glutinosa* (cluster II), the distance between the two sub-clusters (i and ii) was very low (Fig. 4), which means a very similar percentage distribution of the adopted chemical compounds in all population of this cluster (II), as consolidated by the data of Tables 3 and 4.

According to the results of AHC analysis, the most influencing difference between the two clusters (two subspecies) is that at the opposite of cluster (II), capillene was present at very high percentages in cluster (I). In the same context, the comparison of sub-clusters (i) “Djelfa 5,7,10, El-Hadjeb-2 and Milok-2” and (ii) “remaining populations of cluster II” reveals some similarities which could not be easily highlighted by this clustering method.

In order to determine a detailed similarities or differences between subspecies and/or between samples of the same subspecies, PCA method was performed to try to identify the most influencing components that can distinguish or regroup the studied samples taking in consideration the subspecies factor.

These above observations (occurrence of two main clusters representing the two subspecies) were also confirmed by the results of PCA (Fig. 5), and which allowed us to discuss the similarities and the differences upon the chemical compositions for each case. Unfortunately, the low differentiation between the two sub-clusters (i) and (ii) previously obtained by AHC analysis for ssp. *glutinosa* (Fig. 4)

Fig. 5 Two dimensional plot on axes F1 and F2 using PCA of 18 samples of essential oils of two subspecies of *Artemisia campestris* growing in Algeria



was not improved by the results of PCA method (Fig. 5), i.e. presence of interferences between sub-clusters.

Loading factors for principal axes F1 and F2 (representing 67.64% of the total information), are given in Fig. 5. The F1 axis, which represents 42.66% of the total information, is strongly and positively correlated with spathulenol (94.9%), capillin (84.6%) and capillene (69.5%). This same axis is strongly negatively correlated with limonene (− 93.6%), β -pinene (− 86.6%) and γ -terpinene (− 82.4%).

Axis F2, which represents 24.99% of the total information, is negatively correlated with both α -pinene (− 64.4%) and β -myrcene (− 63.7%). This same axis is strongly and positively correlated with only two compounds: geranyl isovalerate (90.3%) and β -eudesmol (88.6%).

As previously demonstrated with AHC method, there is appearance of the same formerly main clusters of individuals, in which this time are, also clearly distinguished (Fig. 5). The most influencing differences between clusters (I) and (II) “i.e. between ssp.” were mostly the same as stated earlier with AHC method. In addition the cluster (I) “ssp. *eu-campestris*” is also characterized by lower contents of β -pinene (8.2–9.6%) and limonene (2.7–4.5%). The sub-cluster (ii) is correlated with high percentages of major compounds β -pinene (28.54–40.36%) and limonene (8.7–12.06%); but characterized by relatively roughly lower percentages of minor compound spathulenol

(0.45–3.04%) in comparison with the rest of the samples of this subspecies.

The most important thing about investigated samples was the presence of large variability’s of the percentages for most identified chemical components (especially for ssp. *glutinosa*), i.e. major components were minor ones for some samples and vice versa (Tables 3 and 4).

According to literature, in *Artemisia*, acetylenic compounds (capillene and capillin) were previously identified in the essential oils of *A. capillaris* [32, 33] and *A. dracunculus* [34]. Most recently capillene was detected in *A. campestris* ssp. *glutinosa* with contents that depended on the studied part at different phenological status (Vegetative, before anthesis, full flowering and seed-bearing) ranging from 8.9 to 33.1% [14]. The maximum value was reported for the seed-bearing stage (33.1%). In the full flowering stage, this compound accounted for the value of 22.3%. Regarding capillin component, it was not reported detected at all in the investigated sample’s plant.

For the current investigation, high proportions of capillene (12.03–46.93%) was recorded for the EOs of ssp. *eu-campestris*, in addition, the ketone acetylenic compound (capillin) was also detected but with low proportions (0.87–3.11%). This is the first report that reveals the existence of both capillene and capillin in the EOs of *A. campestris* ssp. *eu-campestris* from Algeria. This is also

the first time that this plant reported to have a content of capillene that can reach 46.93% at the full flowering stage.

The comparison of the main components identified from *A. campestris* ssp. *glutinosa* EOs at flowering stage provided by literature [14] and the current investigation *A. campestris* ssp. *eu-campestris* from Aflou locations, shows major differences in main compounds and their percentages. The main components of *A. campestris* ssp. *glutinosa* reported from France were γ -terpinene (20.8%), 1-phenyl-2,4-pentadiyne (29.7%) and capillene (22.3%). Inversely, the main components of *A. campestris* ssp. *eu-campestris* for this study were α -pinene (5.91–11.56%), β -pinene (8.20–9.60%), *p*-cymene (3.32–10.64%), spathulenol (3.11–7.14%) and capillene (12.03–46.93%). In other words, and besides the presence of capillene as major compound in both compared different subspecies, γ -terpinene was identified in our study as minor component (0.7–4.22%). Furthermore, 1-phenyl-2,4-pentadiyne was not detected in all in EO's samples. This large difference was expected since the two compared subspecies were different, but the comparison was performed for the only reason that capillene was solely reported for this subspecies from France.

Now, if we compare previous reports of the same plant variety (*A. campestris* var. *glutinosa*) from Algeria “rich in (Z,E)-farnesol, cedrol” and from France “rich in γ -terpinene, capillene, 1-phenyl-2,4-pentadiyne and spathulenol”, we could notice that the major components are completely different (Table 1).

After a deep analysis of previous reports (Table 1), it was found that the EOs coming from Tunisia (leaves part and ssp. not specified) [9] were roughly similar to those of Djelfa and Laghouat (ssp. *glutinosa*), this is true only when considering the range variations of the similar components in both countries. Inversely, the EOs composition of Morocco (ssp. not specified) [23] and the region of Boussaada in Algeria (ssp. not specified) [7] were completely different to the current investigation.

Antioxidant activity

The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, and prevention of continued hydrogen abstraction, free radicals scavenging, reducing capacity, and binding of transition metal ion catalysts. It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods and different substrates are used. In addition, samples may exhibit different antioxidant powers (low to high) depending on the nature of the adopted test for the same reason explained earlier.

Free radicals scavenging activity (DPPH assay)

Previous reports that dealt with the antioxidant activity of EOs of *A. campestris* using DPPH assay were essentially coming from North West Africa countries (Mediterranean area): Tunisia [12, 13], Morocco [23] and Algeria [8]. Comparison of our results with values of literature (in most cases ssp. not provided) was somehow very tricky, since different reaction parameters were adopted, and were related to several modified protocols employed different initial DPPH solution concentrations, different solvents “methanol, ethanol, etc.”, and different reaction times “30 min, 60 min, etc.”; resulting in diverged IC_{50} values for the same plant. Besides, and according to previous reports, some inconsistencies were also spotted; as an example the IC_{50} values of the EOs of *A. campestris* when reported twice by the same author (using the same exact protocol) [12, 13], they were found to be largely different: 1.875 then 94.5 mg/mL. At first sight the result of IC_{50} values obtained from literature seemed to be difficult to compare (IC_{50} = 1.875 mg/mL [13], 94.5 mg/mL [12], mg/mL, 9.96 mg/mL [8], 0.69 mg/mL [23]). In this same context, and as above mentioned, and due to the difficulty to compare the antioxidant capacity of natural product with literature, a simple standard formula was proposed that overlaps this problem [35].

$$IC_{50}^{Standard} = IC_{50} \times \left[\frac{A_{100}}{A_0} \right]$$

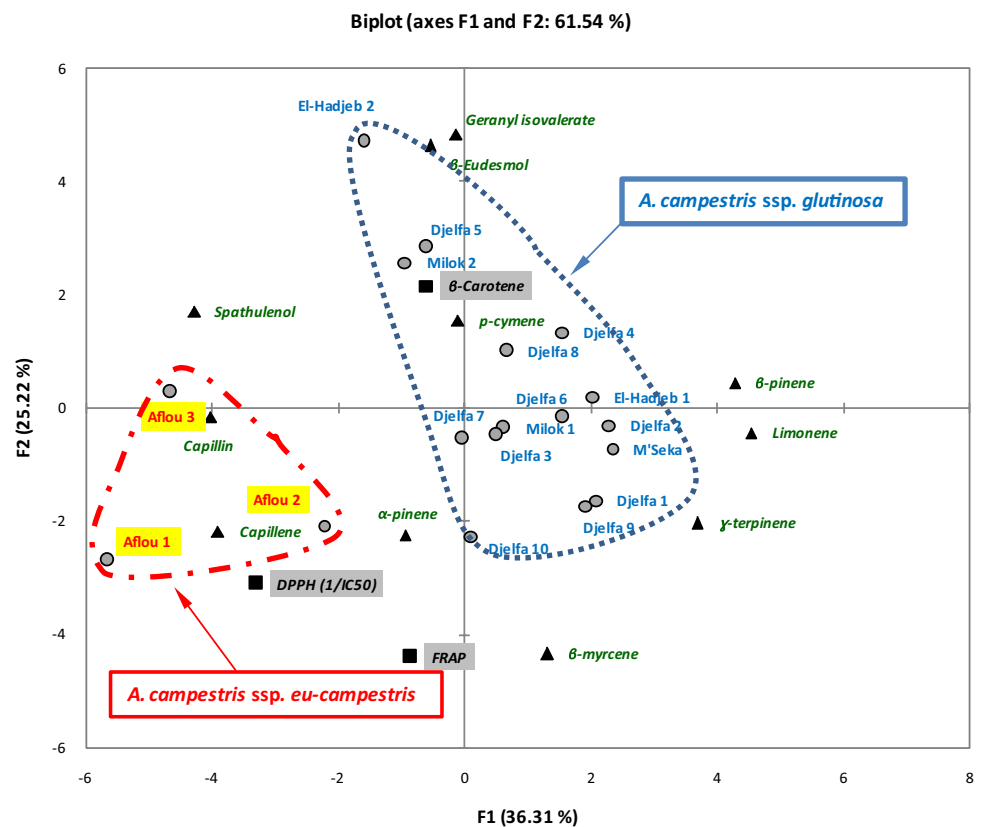
where A_{100} is the absorbance corresponding to arbitrary initial DPPH concentration (125 μ M was adopted for this study).

When this above formula was applied for the values obtained from literature we found after recalculation that the new standard values of $IC_{50}^{Standard}$ were equal to 2.34; 118.125; 20.75 and 1.5 mg/mL, corresponding to the previous reports of IC_{50} original values [8, 12, 13, 23], respectively. The antioxidant activity of Morocco EO “1.5 mg/mL” was very similar to Tunisia EO “2.34 mg/mL” (first report published). Although, the antioxidant activity power of EO reported earlier from Algeria “20.75 mg/mL” was lower (higher value) than those reported from Tunisia “2.34 mg/mL” and Morocco “1.5 mg/mL”, this activity was specifically similar to most of our investigated samples. Finally, and upon new standard IC_{50} calculated using literature values, our obtained antioxidant activity range values (5.65–31.94 mg/mL) was found to cover most of previous published values, which indicates the variability of the antioxidant activity of this investigated EO growing in Algeria.

FRAP assay

The AEAC range variation values from 0.022 to 0.036 represent approximately between 45 and 27 times less active

Fig. 6 Two dimensional plot on axes F1 and F2 using PCA of 18 samples of essential oils of two subspecies of *Artemisia campestris* growing in Algeria along with their antioxidant activity



power than vitamin C since it was adopted for comparison. The most active EO was Aflou 1 sample (ssp. *eu-campestris*) and the lowest activity was recorded for the samples of Djelfa 8, 2 and 5 (ssp. *glutinosa*). In another hand, the translation of determined AEAC values for antioxidant of reference BHA and vitamin E means first, that BHA exhibited slightly higher antioxidant activity than vitamin C (activity increased by almost 20%); inversely, vitamin E exhibited almost ten times lower activity power than vitamin C. This comparison means that the EOs were exhibiting interesting reducing power in comparison with vitamin E (practically just 3 to 5 times lower activity), but more lower power when compared with BHA (antioxidants of reference). As best of our knowledge, no FRAP assays were performed in earlier reports dealing with the EO of this plant. This time again, and as determined earlier for the DPPH assay, the samples of Aflou 1&2 (ssp. *eu-campestris*) were exhibiting again high antioxidant powers.

Bleaching ability of β-carotene in linoleic acid system

Only few previous reports were dedicated to the evaluation of the antioxidant activity of the EO using β-carotene assay, essentially from Tunisia [12] and Morocco [23], in which they obtained similar Antioxidative Activity AA equal to 20.9 and 82.2%, respectively (corresponding to an initial

concentration of EO equal to 1 mg/mL). Comparison of our results with those of literature is not possible since we adopted in this study a different expression value of this activity, in which we used IC₅₀ (mg/mL) instead of AA%. Nevertheless, these obtained IC₅₀ values presented very interesting antioxidant activity (low values of IC₅₀).

Correlations between the composition of the EOs and their antioxidant activity

Since the results of the investigated antioxidant activities (using three different tests) showed large variations in the values of their powers, it was mandatory to try to find some existing correlations between the chemical compositions of the EOs and their antioxidant activities powers; in addition correlations between different tests were also investigated. This was carried out by applying statistical methods involving PCA (Fig. 6). It was found that highest value of antioxidant activities for both DPPH and FRAP assays were correlated mainly with higher percentages of the following major components α-pinene, capillene and β-myrcene. At the opposite and for the β-carotene assay it seems for this test, that the presence of higher amounts of β-eudesmol, geranyl isovalate, spatulenol and p-cymene were probably (or at least partially) responsible for the higher activity. Finally, it could be noticed from the result of PCA that the antioxidant

activities measured by both DPPH and FRAP assays were inversely correlated with those measured using β -carotene assay. In other words, the high activity found for β -carotene assay for the sample were faced with low activities in DPPH and FRAP assays. In the mean time, DPPH and FRAP assays were positively correlated with each others: samples exhibiting high activities in DPPH assay were also exhibiting high activities in FRAP assays. These conclusions were expected, since the different employed antioxidant activity assays were characterized by different mechanisms of actions and different solvent environments (organic, aqueous or emulsion water-organic solvent).

Conclusion

The data obtained demonstrated clearly the chemical variability in the essential oil composition of *A. campestris* in Algeria. This study reveals the presence of new chemotype (capillene) in the EOs of this plant for the ssp. *eu-campestris* (Briq. & Cav.) collected from the locality of Aflou (Region of Laghouat). For the first time, capillene has been reported and with highest percentage ever found among the major constituents in *A. campestris* essential oils from the location of Aflou-Algeria. Moreover, capillin was also reported for the first time as minor compound of *A. campestris* ssp. *eu-campestris*. The study has expanded our chemotaxonomic knowledge of the *A. campestris* subspecies in some Algerian territories. In conclusion, the presence of aromatic polyacetylenes chemotaxonomically characterizes *A. campestris* ssp. *eu-campestris* from the Aflou locations (Laghouat) in Algeria. The antioxidant activity was investigated using three different assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals scavenging activity, FRAP and β -carotene bleaching test; the main result revealed the occurrence of some variability of the antioxidant powers which depended essentially on the determined variability of the EOs composition.

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