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Morphological properties and chemical composition of three *Calligonum* species Growing-wild in Tunisian Desert: A chemotaxonomic study

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Abstract

The essential oils of three wild species of *Calligonum* (*C. azel*, *C. arich* and *C. comosum*) growing in Tunisian desert were obtained by hydrodistillation and were analyzed in order to discern the differences and similarities between the volatiles chemical compositions of these species. A total of 110 compounds, which accounted for 93.95-99.68 % of the total composition of the oils, have been identified. The main constituents were viridiflorol (9.59 %) in *C. azel*, palmitic acid (20.06%) in *C. arich* and 9-Octadecenoic acid (19.76 %) in *C. comosum***.** Morphological similarity study shows two groups: the first one contains C. *azel* and C. *comosum* and the second group is formed by *C. arich*. Based on their chemical composition, two chemotaxonomic groups may be established: C. *azel* on one hand, *C. arich* and *C. comosum* on the other hand. As a consequence, the morphological diversity of these species doesn't reflect their chemical polymorphism.

Keywords: *Calligonum*; Essential oil; Polygonaceae; Tunisian desert; Chemotaxonomic significance; Morphological character

1. Introduction

Desertification is a land degradation problem of major importance in the world's arid regions. Approximately, three quarters of Tunisia are arid and desert regions [1]. Virtually all of the rangeland has suffered severe land degradation. Erosion continues on the extensive dry farm lands and desertification effects are evident in the form of soil fertility, soil compaction, and soil crusting [2]. Characterised by low rainfall, high evapotranspiration, high temperature and desiccating winds, Tunisian desert was colonised by several endemic plants that are likely new crop candidates for these arid lands [3]. These plants, producing significant yields of relatively high valued products such as pharmaceuticals and biologically active materials like essential oils, have lower water requirement [4]. Polygonaceae family is a source of bioactive compounds; some species have medicinal properties and uses: *Emex spinosa* is purgative and diuretic, its boiled leaf was used by African tribes for the cure of dyspepsia and biliousness, and to stimulate appetite [5]. *Rumex vesicarius* is stomachic, diuretic and astringent. Its roasted seeds are eaten for the cure of dysentery and the plant juice is used to check toothache and nausea and to promote appetite [6]. In the south of Tunisia, *Polygonum aviculare* was used as astringent, antidiarrheic, vulneraire, hemostatic while *Polygonum equisetiforme* was used for the disinfection of Plaies, cicatrisant and fortifiant [7].

Calligonum genus belongs to the Polygonaceae family, with some 80 species distributed throughout Western Asia, Southern Europe and North Africa [8]. Only *C. comosum* L'Hérit, *C. azel* Maire and *C. arich* Le Houérou occur in the Tunisian arid zone and the last one is endemic [1]. The three *Calligonum* species are dominant perennials in active sand dunes and stabilized sand field in the southern desert of Tunisia and grow naturally in the eastern Great Erg [1]. In tunisian Desert, Cauvet show that *C. comosum* was served to the treatment of the scabies of the camel [9]. The decoction

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of roots of this species was vermifuge [7]. In United Arab Emirates, *Calligonum comosum* is used by some healers to treat stomach ailments, the stems and leaves are chewed for curing toothache [10]. The fresh aerial parts of this species, collected from Abu Dhabi Emirates, show an anti-inflammatory and antiulcer activity [11]. Some compounds, extracted from *Calligonum comosum* L. growing in Egypt, present a cytotoxic and antioxidant activity [12]. Till date there has been scarce information about the morphological studies of *Calligonum comosum* [13].

In the Tunisian desert, the taxonomy of the *Calligonum* genus was described several times [1, 3, 7, 14-15] The Results of the recent study [15] show highlight differences in phenological and morphological patterns of the three *Calligonum* shrubs (*C. arich*, *C. azel* and *C. comosum*) that typically establish at different positions on dune crests, slopes and valley, respectively. To the best of our knowledge, there are no reports in the literature concerning the extraction of essential oils from the three *Calligonum* species growing in Tunisia. Therefore, the aim of the present work is to characterize the volatile constituents, extracted by hydrodistillation, from the aerial parts of *C. comosum*, *C. azel* and *C. arich* collected at the flowering stage and to search an eventual correlation between the chemical and the morphological polymorphism of the three studied species.

2. Material and methods

2.1. Plant materials

The aerial parts (green branches and flowers) of *C. comosum*, *C. azel* and *C. arich* were collected from the eastern Great Erg of Tunisia near the petrol station of El Borma (31° 36'N, 09° 27'E, at 231 and 277 m asl) at the flowering stage (March-April 2009) and identified according to the ''Flore de la Tunisie'' [16]. The studied site (El Borma) was dominated by *C. azel*, *C. arich and C. comosum* beside others species such as *Cleome arabica*, *Cornulaca monocantha*, *Euphorbia gugoniana*, *Helianthemum confertum*, *Retama raetam*, *Spartium saharae*, *Ephedra alata*, *Astragalus gombiformis* and *Stipagrostis pungens* [15]. Three samples of each *Calligonum* species were deposited at the herbarium of the Institute of Arid Areas in Medenine for chemical analysis (Tunisia).

2.2. Morphological study

Measurements of the different morphological traits were recorded on 10 plants for each species. Plant length and width, fruits length and diameter of each species were determined using a meter and a graduated ruler respectively. Also, nodes and internodes were determined using a digital calliper and a graduated ruler, respectively. Colour, aspect identification was also dealt for flowers, stems, anthers and stigmas. Number of anthers and stigmas has been examined by optic microscope. Other morphological traits were also recorded such as flowering and fruiting time (Table1).

Table 1 Morphological traits studied in the three *Calligonum* species

Morphological traits	Measurements (cm)		
	C. comosum	***C. azel***	***C. arich***
Plant length	75-220 (147.5)	350-550 (450)	430-810 (620)
Plant width	70-120 (95)	260-340 (300)	450-650 (550)
Old stem color	Grayish white	Grayish white	Brown
Green stem aspect	Rough	Smooth	Smooth
Inter-node length	2.2-6.8 (4.5)	3.4-9.6 (6.5)	5.2-12.8 (9)
Nodes width	0.07-0.20 (0.135)	0.10-0.35 (0.225)	0.10-0.45 (0.275)
Flower color	White	White	Red
Anthers color	Red	White	White
Stigmas color	Rose	White	White
Anthers number	12	16	14
Stigmas number	4	4	4
Flowering time	Middle march	End march	End april

Fruit length	0.7-0.9 (0.8)	1.0-1.2 (1.1)	1.2-1.4 (1.3)
Fruit diameter	0.25-0.45 (0.35)	0.26-0.50 (0.38)	0.21-0.35 (0.28)
Fruit hairs length	0.15-0.35 (0.25)	0.11-0.27 (0.19)	0.20-0.40 (0.30)
Fruit color	Brown	Brown	mallow
Fruiting time	First april	Midd april	Mid may

2.3. Essential oil extraction

The aerial parts of each sample were air dried for 15 days and submitted to hydro distillation for 4 h, in a Clevenger-type apparatus [17]. n-Hexane (2 ml) was used as the collector solvent [18]. After evaporation of the solvent under N_2 flow, the essential oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from the light at -20 °C before analyses. Three oil samples for each species were analyzed by GC-FID and GC-MS.

2.4. Essential oil analysis

2.4.1. Gas chromatography

A Hewlett-Packard 5890 series II gas chromatograph equipped with HP-5MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm; Hewlett-Packard) and connected to a flame ionization detector (FID) was used. The column temperature was programmed at 50 °C for 1 min, then 7 °C/min to 250 °C for 5 min, split: 1/60.. The injection port temperature was 240 °C and that of the detector 250 °C. The carrier gas was helium (99.995% purity) at a constant flow of 1.2 ml/min. The analysis was performed on 2 µl volume of each sample. Percentages of the constituents were calculated by electronic integration of FID peak areas, without the use of response factor correction. Retention indices (RI) were calculated for separate compounds relative to C_8-C_{26} n-alkanes mixture (Aldrich Library of chemicals standards) [19].

2.4.2. Gas chromatography - Mass spectrometry (GC/MS)

The isolated volatile compounds were analysed by GC/MS, using a Hewlett-Packard 5890 series II gas chromatograph. The fused HP-5MS capillary column (the same as that used in the GC analysis) was coupled to a HP 5972A masse-selective detector (Hewlett-Packard, Palo Alto, CA, USA). The oven temperature was programmed from 50 °C (1 min) to 250 °C (5 min) at 7 °C/min. The temperature of the injector port was held at 250 °C, split: 1/100, the temperature of the detector was set at 280 °C. The carrier gas was helium (99.995% purity), with a flow rate of 1.2 ml/min and the analysed sample volume was 2 µl. The mass spectrometer (MS) 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.

2.4.3. Volatile compounds identification

The essential oil compounds of were identified by comparing the mass spectra data with spectra available from the Wiley 275 mass spectra libraries (software, D.03.00). Further identification confirmations were made referring to retention indices (RI) data generated from a series of known standards of n-alkanes mixture (C_8-C_{26}) (Aldrich Library of chemicals standards) [19] and to those previously reported in the literature [20-28].

2.5. Cluster analysis

The cluster analysis (morphological traits and the identified chemical compounds) was based on similarity matrix and implemented according to the NTSYS software's WPGMA cluster (Weighed Pair Group Method using Arithmetic Average).

3. Results and discussion

3.1. Morphological study

The morphological traits of the three *Calligonum* species were shown in Table 1. This table and fig.1, shows that the size, both length and width, differ among species: *C. arich* is the taller and the larger while *C. comosum* is the shorter and the smaller (fig. 1). *C. arich* old stems are brown but *C. azel* and *C. comosum* have the same old stems colour: grayish white (fig.1). *C. azel* and *C. arich* green stems are smooth with an inter-node length reaching 9.6 and 12.8 cm, respectively

whereas *C. comosum* green stems is rough with an inter-node length reaching 6.8 cm (fig. 2). *C. arich* and *C. azel* nodes are generally swollen (0.1 to 0.45 cm width) while *C. comosum* nodes are slightly larger.

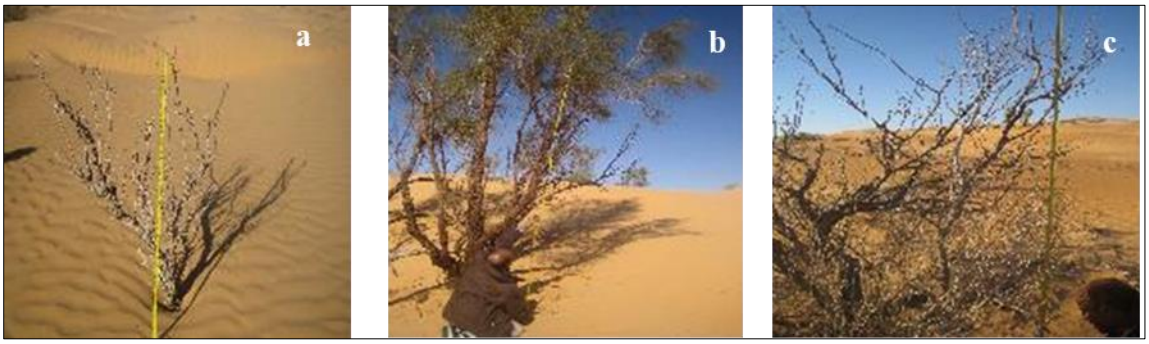

Figure 1 Length and width of the *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c)

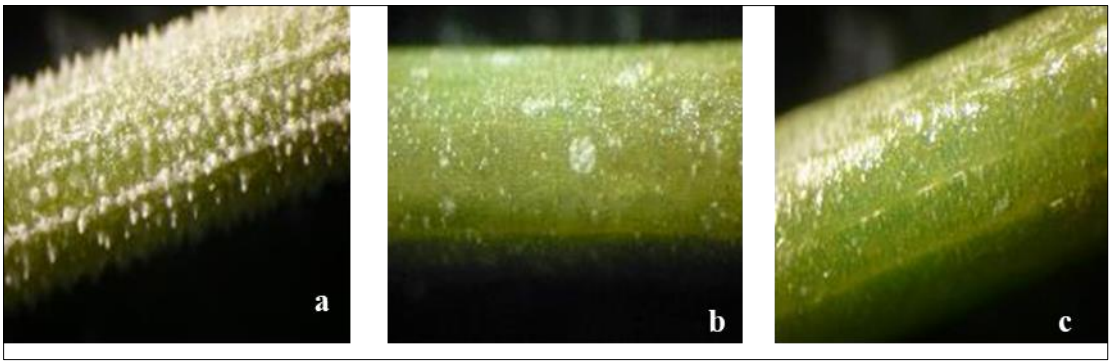

Figure 2 Green stems aspects of the *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c)

Generally, flowering time for *C. azel* and *C. comosum* is recorded in March and delayed about one month for *C. arich*. The flower colour is white for *C. azel* and *C. comosum* and red for *C. arich* (fig. 3). The three flower species possess the same number of stigmas (4) but differ in anthers number (12 for *C. comosum*, 16 for *C. azel* and 14 for *C. arich*) (fig. 3). Stigmas and anthers of *C. arich* and *C. azel* are white while *C. comosum* have red anthers and rosy stigmas (fig. 4).

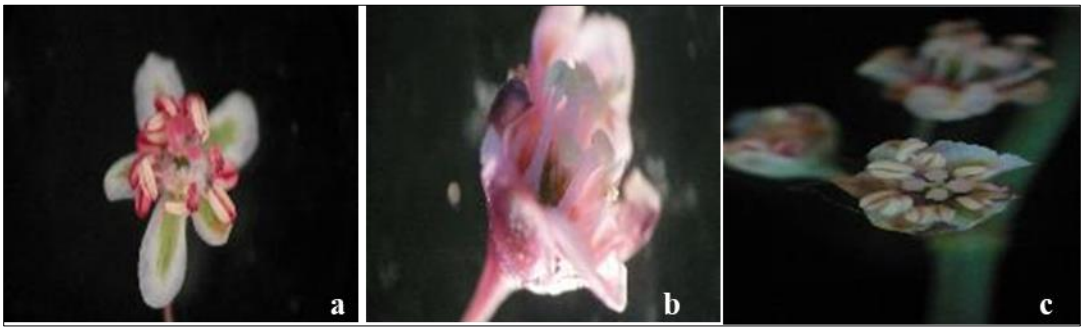

Figure 3 The flower color of the *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c)

For the three *Calligonum* species, fruiting time follows the flowering time two weeks later. The fruits length of all species is about one cm with a brown color for *C. azel* and *C. comosum* and a mallow colour for *C. arich* (fig. 5). All fruits are rectangular with longitudinal rows of hairs which have a length about 3 mm (fig. 6).

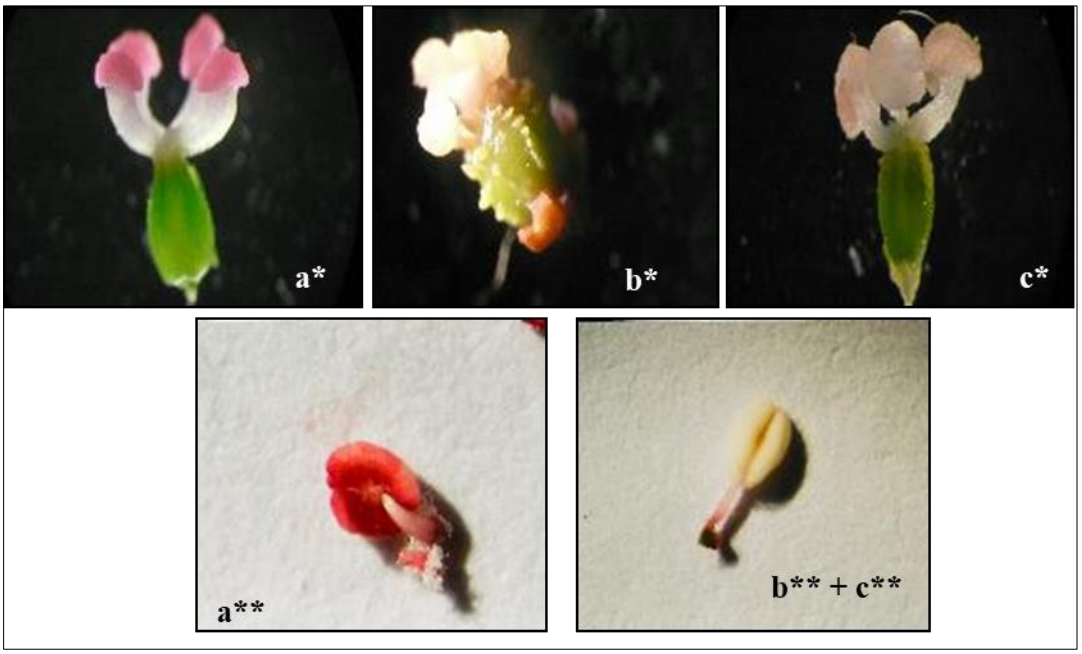

Figure 4 Stigmas* and anthers**of the *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c)

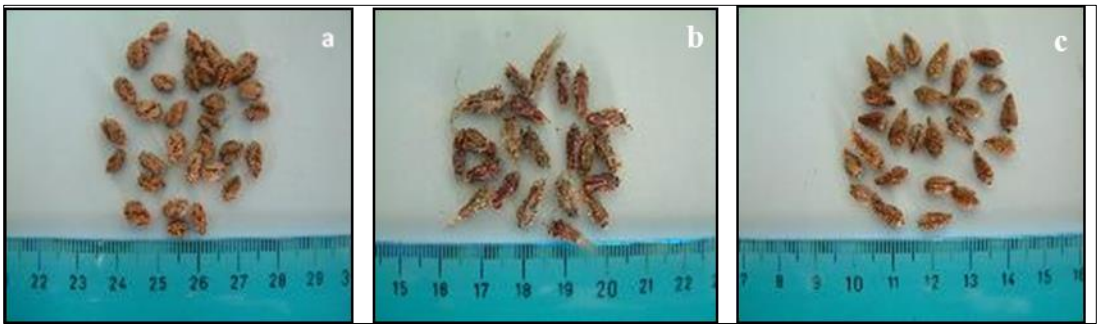

Figure 5 The fruits length and color of the *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c)

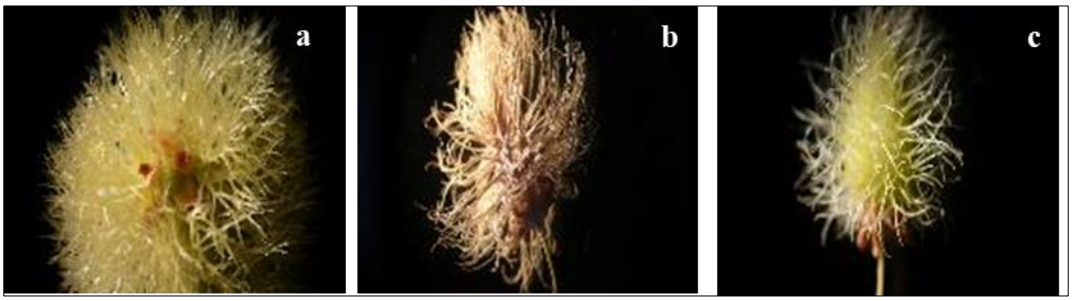

Figure 6 Fruit hairs length of the *Calligonum* species: *C. comosum* (a), *C. arich* (b), *C. azel* (c)

Morphological traits were also used to study the similarity between the three species. The dendrogram obtained in the cluster analysis of these morphological traits showed two groups (Fig. 7). The first is composed by *C*. *Comosum* and *C*. *azel* with a similarity of 29 % as they share some morphological traits (Stem, flower and fruit colour). The second consists of *C*. *arich* with a similarity of 15 %.

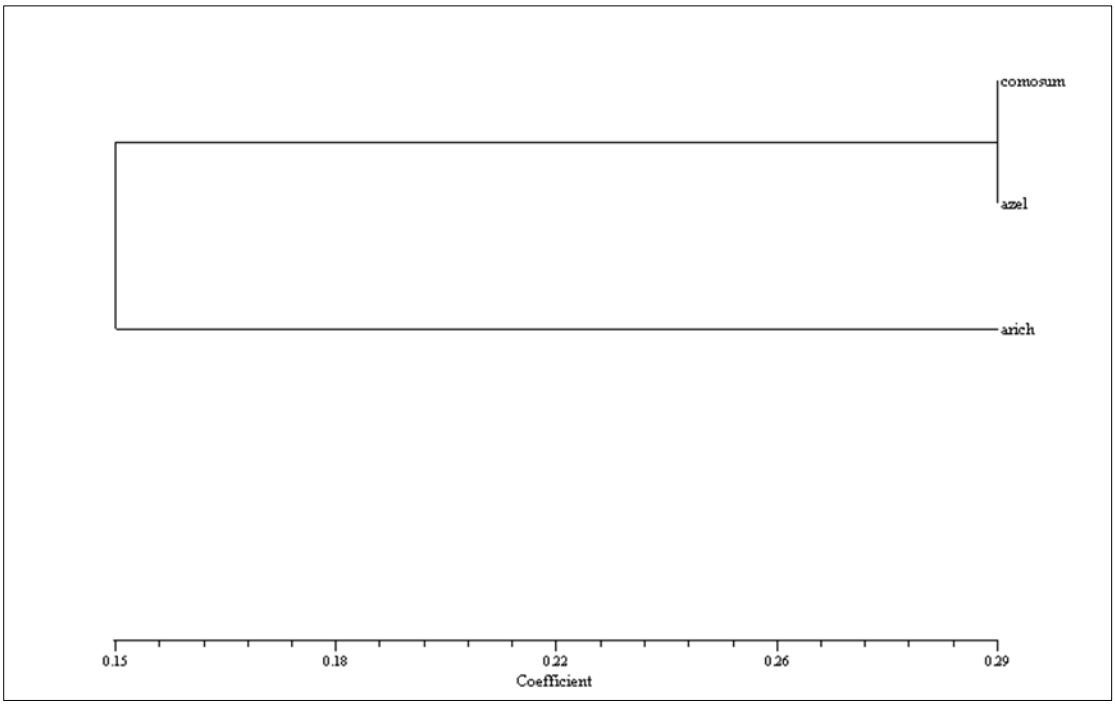

Figure 7 Dendrogram obtained in the cluster analysis of some morphological traits based on "WPGMA" method

3.2. Essential oils composition

The essential oils of the three species of *Calligonum* were obtained by hydrodistillation during the flowering phase (March-April 2009). The identified oil components from these three species (*C. arich, C. azel* and *C. comosum*,), representing 96.71% (19 compounds), 93.95% (64 compounds) and 99.68% (36 compounds) of the total oils, are listed in table 2 in order of their elution on the HP-5MS column. This table includes also their retention indices and the percentage composition.

Table 2 Essential oil composition of the aerial parts of three *Calligonum* species growing in Tunisian desert

	Compounds[a]	**RI**[b]	*%*[c]		
			C.	***C.***	***C.***
1	Hexanal	804	0.68	2.82	0.44
2	*trans*-2-hexenal	858	0.36	1.30	-
3	Heptanal	905	-	0.13	0.42
4	α-pinene	938	0.66	-	-
5	Camphene	952	0.18	-	-
6	Sabinene	977	0.15	-	-
7	β-pinene	980	0.16	-	-
8	6-Methyl-5- hepten-2-one	992	-	-	0.10
9	α-terpinene	102	0.29	-	-
10	*o*-cymene	102	-	0.14	-
11	*p*-cymene	102	0.17	-	-
12	Limonene	103	0.15	-	-
13	1.8-Cineole	103	0.15	-	-
14	*trans*-β-ocimene	105	0.35	-	-
15	γ-terpinene	106	1.14	1.32	0.11
16	α-terpinolene	109	0.26	-	-
17	Linalool	110	0.14	-	-

18	Nonanal	110	0.23	0.42	0.79
19	β-thujone	111	-	-	0.32
20	Camphor	114	0.36	-	0.14
21	Cyclooctanone	115	-	-	1.02
22	Borneol	117	0.32	-	-
23	Unidentified	117	-	-	0.80
24	4-Terpineol	118	0.63	-	-
25	β-Cyclocitral	122	-	0.13	
26	Unidentified	123	-	-	0.38
27	Methyl thymyl ether	123	0.42	0.86	-
28	Linalyl acetate	125	0.17	-	-
29	Chrysantenyl acetate	126	-	-	5.27
30	Vitispirane	128	0.29	5.94	-
31	Bornyl acetate	128	1.06	-	-
32	Sabinyl acetate	129	-	-	3.77
33	Carvacrol	130	0.49	10.46	-
34	1-Butyl-1H-1.2.4-triazole	131	-	-	0.36
35	Unidentified	133	-	-	1 .93
36	Bicycloelemene	134	0.57	-	0.27
37	Unidentified	136	-	-	0.23
38	α-Copaene	138	1.15	-	-
39	β-Bourbonene	139	0.4	-	-
40	β-Elemene	139	1.45	-	-
41	Unidentified	140	1.07	-	-
42	α-Gurjunene	141	3.41	-	-
43	*trans*-Caryophyllene	142	4.01	-	0.25
44	(*E*)-Geranyl acetone	145	-	0.17	-
45	α-Humulene	146	0.42	-	-
46	Unidentified	146	-	-	0.5
47	*allo*-Aromadendrene	146	1.66	-	-
48	α-amorphene	148	0.37	-	0.14
49	β-Ionone	149	-	0.63	-
50	Germacrene D	149	4.46	-	3.54
51	Aromadendrene	149	0.74	-	-
52	Bicyclogermacrene	150	2.47	-	3.35
53	Eremophilene	151	0.64	-	-
54	Davana-ether	152	-	-	10.76
55	γ-Cadinene	152	5.71	-	-
56	δ-Cadinene	153	2.29	-	0.45
57	β-Bisabolene	154	1.78	-	-
58	α-Calacorene	154	0.24	-	-
59	Elemol	156	6.94	-	-
60	Nerolidol	156	-	-	0.74
61	Lauric acid	157	-	1.78	0.27
62	Palustrol	158	0.58	-	-
63	*Cis*-Davanone	159	-	-	2.31
64	Caryophyllene oxide	159	4.38	-	-
65	Diethyl phthalate	159	-	15.36	3.5

66	Viridiflorol	161	9.59	-	0.31
67	*epi*-Globulol	161	1.55	-	-
68	Naphthalene. 1. 2. 3. 4. 4a. 7-hexhydro-1.6-dimethyl-4-(1-	162	1.08	-	-
69	Dillapiole	163	-	17.47	1.32
70	Unidentified	163	1.36	-	-
71	Unidentified	164	-	-	0.38
72	β-eudesmol	164	4.66	-	-
73	Unidentified	165	-	-	1.72
74	2-Isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	165	7.61	-	-
75	τ-Muurolol	166	-	-	0.77
76	α-Eudesmol	167	8.33	-	-
77	Caryophyllenol II (Caryophylla-2 (12), 6-dien-5β-ol)	167	0.3	-	-
78	Unidentified	167	-	-	0.26
79	Unidentified	168	-	-	0.59
80	Cadalene	168	0.48	-	-
81	Vulgarol B	170	-	-	0.53
82	14-Norcadin-5-en-4-one	170	0.54	-	-
83	Valerenol	172	0.68	-	-
84	Unidentified	172	-	-	0.28
85	Cadina-4.10 (15)-dien-3-one	175	0.79	-	-
86	Myristic acid	176	-	1.15	0.50
87	Hexadecanal	181	-	-	0.26
88	6.10.14-Trimethylpentadeca-2-one	184	-	-	0.18
89	Di-isobutylphthalate	186	0.36	2.27	0.66
90	Hexadecanol	187	-	-	0.21
91	Nonadecane	188	0.12	-	-
92	Unidentified	191	-	-	0.41
93	15-Hexadecanolide	193	-	-	0.75
94	Palmitic acid	195	-	20.06	15.04
95	(*Z*)-9.17-Octadecadienal	197	-	-	0.26
96	13-Octadecenal	198	-	-	0.41
97	Eicosane	199	-	-	0.20
98	1-Heneicosene	208	0.25	-	0.45
99	Heneicosane	210	1.43	-	0.44
100	γ-Palmitolactone	211	-	-	0.13
101	Phytol	212	0.35	-	0.85
102	(*Z*)-9-Octadecenoic acid	215	-	-	19.76
103	Ethyl linoleate	216	0.17	14.29	-
104	Octadecanoic acid	217	-	-	1.26
105	9-Tricosene	228	0.28	-	0.36
106	Tricosane	230	-	-	2.74
107	9-Octadecenamide	237	-	-	0.44
108	Unidentified	248	-	-	2.51
109	Pentacosane	250	0.47	-	1.41
110	Glycerol. 2-hexadecanoate	252	-	-	2.41
Tota			93.95	96.71	99.68
	Grouped components (%)				
	Hydrocarbons and functionalised hydrocarbons		4.35	59.75	54.13

	Monoterpene hydrocarbons		3.51	1.46	0.11
	Oxygenated monoterpenes		2.66	0 .13	9.50
	Sesquiterpenes hydrocarbons				
	Naphthalene sesquiterpenes		19.33	-	0.59
	Azulene sesquiterpenes		2.4	-	-
	Others sesquiterpenes hydrocarbons		19.21	-	7.41
	Oxygenated sesquiterpenes				
	Oxygenated Naphthalene sesquiterpenes		14.32	-	0.77
	Oxygenated Azulene sesquiterpenes		11.72	-	0.31
	Others oxygenated sesquiterpenes		12.76	5.94	14.34
	Diterpenes		0.35	-	0.85
	Phenolic derivatives		0.91	11.32	-
	Arylpropanoids		-	17.47	1.32
	Miscellaneous compounds		-	0.63	-
	Nitrogeneous compounds		-	-	0.36
	Unidentified		2.43	-	9.99

[a]Compounds are listed in order of their elution from a HP 5MS column; [b]RI, Retention indices calculated against C_8-C_{26} *n*-alkanes on the HP 5MS column; [c]Percentages obtained by FID peak-area normalization ; values represent an average of three determination

The isolated essential oils of each specie were complex mixture of mainly functionalised hydrocarbons (acids, esters, aldehydes …), monoterpenes, sesquiterpenes, arylpropanoids and Phenolic derivatives: a total of 110 component were identified from which only four compounds (hexanal, gamma terpinene, nonanal and diisobutylphthalate) are common for the three oils with a relatively low rates.

The chemotypes of the investigated essentials oils of *Calligonum* species are the following: in *C. azel*: alpha eudesmol (8.33 %) and viridiflorol (9.59 %); in *C. arich*: carvacrol (10.46 %), ethyl linoleate (14.29 %), diethyl phthalate (15.36 %), dillapiole (17.47 %) and palmitic acid (20.06 %) and in *C. comosum*: davana ether (10.76 %), palmitic acid (15.04 %) and (*Z*)-9-Octadecenoic acid (19.76 %)**.**

Comparing the chemical composition of the three oils within the same section many similarities are obvious. Hence, the oil of *C. azel* was characterized by a very large presence of terpenoids mainly sesquiterpenes hydrocarbons (40.94 %) and oxygenated sesquiterpenes (38.8 %) while the oil of *C. comosum* contain less amount of terpenoids mainly monoterpenes (9.61 %) and sesquiterpenes (33.42 %). In contrast, the main similarities among *C. arich* and *C. comosum* in their composition are related to the high percentages of hydrocarbons and functionalised hydrocarbons (59.75 % and 54.13 %, respectively), palmitic acid was found to be a major compound in both oil and could be used as a chemotaxonomic tool for the characterization of others Calligonum species. Among this class, (*Z*)-9-Octadecenoic acid (19.76 %) and ethyl linoleate (14.29 %) were assigned as the main component in *C. arich* and *C. comosum*, respectively.

In addition, the essential oil of *C. arich* presents a relatively high percentage of phenolic derivatives (11.32 %) and arylpropanoid (17.47 %). The last one was represented only by dillapiole which was considered as a larvicidal phytochemical compound [29]. Dillapiole could be also used for therapheutical purposes, particularly in the treatment of dermatophytosis [30]. Considered as a main component in the essential oil of *C. arich*, dillapiole was found only with a low rate in the essential oil of *C. comosum* (1.32%). Although Carvacrol was found in a small percentage in the essential oil from aerial parts of *C. azel* (0.49 %), this phenolic derivative compound was reported as a major constituent in the oil of *C. arich* (10.46 %) and was not been detected in *C. comosum* oil. This compound possessed high levels of antimicrobial activity [31].

The monoterpenes and sesquiterpenes distribution in these oils showed some differences. In fact, the monoterpenes hydrocarbon class was nearly represented in similar way in all cases but *C. comosum* oil contains much more oxygenated monoterpenes such as camphor. Detected only in the *C. comosum* oil, the two main compounds of oxygenated monoterpenes, were chrysantenyl acetate (5.94 %) and sabinyl acetate (3.77 %). Sesquiterpenes hydrocarbons were absent in *C. arich* and present in low amount in *C. comosum* (8 %) whereas *C. azel* is rich in sesquiterpenes hydrocarbons (40.94 %). Among this class, the naphthalene and others hydrocarbons sesquiterpenes were present in almost equal percentage (19.33 % and 19.21 %, respectively) in *C. azel* in which γ-Cadinène (5.71 %) and germacrene D (4.46 %) were assigned as the main hydrocarbons sesquiterpenes.

The percentage of oxygenated sesquiterpene was approximately twice more important in the essential oil of C. *azel* (38.8 %) than that of *C. comosum* (15.42 %). In particular, davana ether (10.76 %) was found as the main oxygenated sesquiterpenes in *C. comosum* while viridiflorol (9.59 %) and α-eudesmol (8.33 %) were the major oxygenated sesquiterpenes in *C. azel* which could be considered as the chemotype of this oil. On the contrary, the oxygenated sesquiterpene compounds were present in a relatively smaller proportion (5.94 %) in *C. arich*. Absent in the *arich* oil, the diterpene class was identified only with a very low rate in *C. azel* (0.35 %) and *C. comosum* (0.85 %).

As described in the literature [13], the fresh flowers of *C. comosum* can be eaten as it is high in sugar and nitrogeneous components. This class was identified in our *C. comosum* oil and was presented by 1-butyl-1H-1,2,4-triazole.

3.3. Cluster analysis of chemical compounds

The cluster analysis of the chemical compound descriptors was shown as a dendrogram indicating the estimated relations between the three species of *Calligonum* (Fig. 8). The dendrogram obtained showed two groups. The first one is formed by *C. arich* and *C. comosum* with a similarity of 38 % when using all chemical compound identified descriptors. However, using only major compound the similarity was only 30 %. These similarity percentages are low to consider *C. arich* and *C. comosum* as similar. This low similarity could be explained by the fact that Carvacrol was reported as a major constituent in the oil of *C. arich* (10.46 %) and absent in *C. comosum* oil. Besides, sesquiterpenes hydrocarbons compounds were absent in *C. arich* and present in low amount in *C. comosum* (8%). However, having high percentages (59.75 % and 54.13 %) of hydrocarbons and functionalised hydrocarbons respectively, *C. comosum* and *C. arich* could have a common ancestor. The second group is formed by *C. azel*. It is identified as different genetically from both species. In fact, the similarity was 18 % and 11 % for all and major chemical compound identified descriptors respectively. In fact, *C. azel* is the richest in sesquiterpenes hydrocarbons and oxygenated sesquiterpenes with 40.94% and 38.8 % respectively. Also, *C. azel* presented viridiflorol (9.59%) and α-eudesmol (8.33%) as the major oxygenated sesquiterpenes. The last was approximately twice more important in the essential oil of C. *azel* (38.8 %) than that of *C. comosum* (15.42 %). Thus, the former could be considered as the chemotype of this oil. Furthermore, *C. azel* showed a small percentage of Carvacrol in its essential oil.

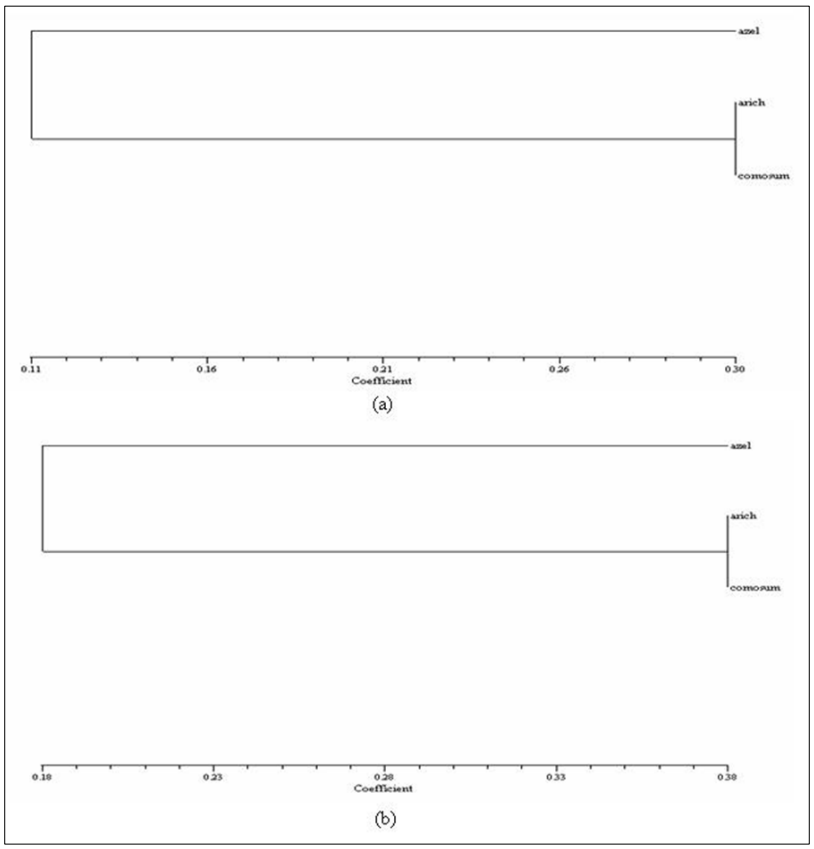

Figure 8 Cluster analysis of the three *Calligonum* species; (a): Dendrogram generated by the cluster analysis of the major chemical compound descriptors and (b): Dendrogram generated by the cluster analysis of all chemical compounds identified descriptors

The GC and GC-MS analysis of these three oils (*C. azel*, *C. comosum* and *C. arich*) resulted in the identification of 64, 36 and 19 constituents, respectively. So, the great difference in the number of constituents between species can be explained by the different positions of the studied species along the slope and its root distribution profile (fig. 9 and fig. 10). In fact, *C. arich* growing mostly on the upper part of the slope with a deep roots that can reach the ground water table and others very extended horizontal roots which exploit only surface soil water [3], while *C. comosum* exist in the foot of the slope (inter- dune) where there is always moisture on the soil surface [32]. So, these two species were less stressed and therefore they produce less secondary metabolites (19 and 36 constituents in *arich* and *comosum* oil respectively). In contrast, *C. azel* occurring mostly on the lower slope with only extended horizontal roots and more or less deep roots which face wind erosion [3]. For this reason, this species is often stressed and produce more secondary metabolites (64 constituents).

Figure 9 Photographs of El Borma site depicting the three *Calligonum* species in different slope positions: (b) *C. arich* in the upper slope, (a) *C. comosum* in foot slope and (c) *C. azel* in the lower slope

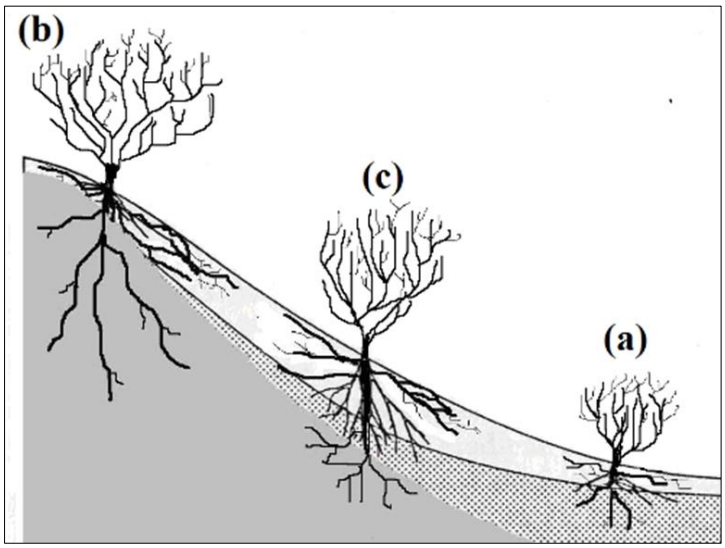

Figure 10 A Schema of *Calligonum* root depth in different slope positions in relation to soil moisture profile ((a): *C. comosum*, (b) : *C. arich*, (c) : *C. azel*). White, speckled and gray painted areas among solid lines represent the dry soil layer, moist soil layer and bedrock, respectively, on the slope under drought

Plant diversity or existence of different morphological forms is an important phenomenon on which relies further progress in crop improvement. The characterization, classification and analysis of these morphological forms have become inevitable activities for assessing their genetic diversity [33]. In our study, it is worth mentioning that morphological similarities show two groups: the first one contains C. *azel* and C. *comosum* and the second group is

formed by *C*. *arich*. Based on the chemical composition of the studied oils, two chemotaxonomic groups may be established: C. *azel* on one hand, *C. arich* and *C. comosum* on the other hand. As a consequence, the morphological diversity of the three studied species doesn't reflect their chemical polymorphism.

4. Conclusion

The essential oil composition of the three wild growing *Calligonum* species (*C. comosum*, *C. azel* and *C. arich*) from Tunisian deserts has not been reported before and therefore this study can be considered as the first information on the composition of essentials oils of the studied plant, indicating the existence of a chemical polymorphism in the genus *Calligonum*. The differences in essential oil composition detected between these species made the volatile fraction a reliable marker to distinguish between them and confirmed the phonological data at the base of their discrimination [15].

Further investigations are needed to reflect taxonomic relationships and chemical compositions of volatile compound in *Calligonum*, since its more than 80 species remain to be investigated.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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