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Charaf Eddine Watheq Malti, Imad Abdelhamid El Haci, Faiçal Hassani, Mathieu Paoli, Marc Gibernau, et al.. Composition, Chemical Variability and Biological Activity of Cymbopogon schoenanthus Essential Oil from Central Algeria. Chemistry and Biodiversity, 2020, 17 (6), 10.1002/cbdv.202000138. hal-02999044

HAL Id: hal-02999044

https://hal.science/hal-02999044

Submitted on 12 Nov 2020

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Chemistry and Biodiversity, 2020, 17 (6), 10.1002/cbdv.202000138

Composition, chemical variability and biological activity of *Cymbopogon*schoenanthus essential oil from Central Algeria

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Abstract. *Cymbopogon schoenanthus* (L.) Sprengel (Poaceae) is an aromatic plant whose aerial parts and rhizome produced an essential oil with pleasant odor. A chemical variability has been observed depending of the countries where the plant grows wild, including Algeria. The chemical compositions of 24 oil samples isolated from plants harvested in Central Algeria have been investigated. Twenty of these were dominated by *cis*- and *trans-p*-menth-2-en-1-ols (22.6% \pm 3.6 and 14.3% \pm 1.7, respectively) beside four atypical compositions. Otherwise, aerial parts and rhizomes produced similar essential oils. Lastly, a fair antimicrobial activity was measured against *Staphylococcus aureus* strain, while the antioxidant potential was low.

 $\textbf{Keywords}: \textit{Cymbopogon schoenanthus} \bullet \textbf{essential oil composition} \bullet \textbf{chemical variability} \bullet \textbf{antimicrobial activity} \bullet \textbf{Algeria}$

Introduction

The genus *Cymbopogon* is comprised of 144 species and it is widely distributed in the tropical and subtropical regions of Africa, Asia and America.^[1] Beside *C. flexuosus* and *C. citratus* (lemongrass), commercially cultivated for their essential oils, *Cymbopogon schoenanthus* (L.) Sprengel (Poaceae) grows wild in most tropical countries of Africa and Asia.^[2-4]. It is known since the antiquity. Indeed, when the tombs of certain Egyptian kings of the 20th and 21st dynasties (3,000 years ago) were opened in 1881, the scent of *Cymbopogon schoenanthus* grass was readily detected by smell.^[5]

The species *Cymbopogon schoenanthus* (L.) Sprengel is constituted of two sub-species, *C. schoenanthus* ssp. *schoenanthus* [synonyms = ssp. *laniger* (Desf.) Maire & Weiller; *Andropogon schoenanthus* Linnaeus] and ssp. *proximus* (Hochst. ex A. Rich.) Maire & Weiller. We will focus our study on *C. schoenanthus* ssp. *schoenanthus*.

Cymbopogon schoenanthus is a compact, evergreen, perennial grass forming dense tussocks at the base and numerous erect 60-80 cm stems. The leaves are linear and strongly curved. Each spike contains a single flower. Flowering takes place in the spring. The roots have a pleasant aromatic smell. The plant grows in arid regions and it is found on the gravelly-stony soils of djebels.

Cymbopogon schoenanthus is used as traditional medicine to treat digestive diseases: aerophagia, flatulence and urinary decrease, analeptic drink for new mother after childbirth, bad breath, gumboils and urinary incontinence. ^[6] In the Djanet area, the young leaves are eaten with a salad or cooked with meat. Straw was used in the Sahara to fill mattresses.

Various organs of the plant produce an essential oil: aerial parts, including leaves, stems, and flowers (inflorescences) as well as roots. Their composition has been investigated and demonstrated a strong variability. For instance:

- Commercial C. schoenanthus oil sample analyzed in Brazil contained mainly geraniol (62.5%) followed by geranial and neral;^[7]
- The major constituents of C. schoenanthus leaf oil from Indian Thar Desert were found to be 2-undecanone (14.68%) and limonene (19.54%);[8]

- Wild and cultivated aerial parts of *C. schoenanthus* from Uttarakhand region North Himalaya produced an essential oil containing *cis*-sabinene hydrate (30.1 and 31.6%), *trans*-sabinene hydrate (17.1 and 14.5%), *cis*-piperitol (9.6 and 18.5%), *trans*-piperitol (8.5 and 7.2%) beside piperitone, limonene and *p*-cymene;^[9]
- An oil sample from Saudi Arabia contained, as major components, piperitone (14.6%), β-elemene (11.6%), α-eudesmol (11.5%), elemol (10.8%), β-eudesmol (8.5%) beside the unusual cyclohexanemethanol (11.6%) and 2-naphthalenemethanol (7.1%),β-10]
- In contrast, the composition of an oil sample of Iranian origin (aerial parts) was dominated by piperitone (62.0%) beside α-terpinene (7.1%).[11]

Piperitone was the major component of *C. schoenanthus* oils from central Africa. For instance, it accounted for 58.9-68.5% in Beninese oils, followed by δ-2-carene (11.5-15.5%). High contents of piperitone were also found in Togolese oils (61.0-69.8%) followed by δ-2-carene (15.1-23.4%; 5.4% one sample) and sometimes by β-elemol and eremoligenol. An oil sample from Burkina Faso contained piperitone (42.0%), 2-carene (8.2%), elemol (6.2%). Another Burkinabe oil sample contained piperitone at lesser extent (18.48%) beside elemol (18.3%), eudesm-7(11)-en-4-ol (17.1%), α-eudesmol (10.7%). Piperitone-rich oils have been also reported in Sudan (47.7-71.5%) beside δ-2-carene (4.5-10.0%), β-elemol (2.7-9.0%) and/or intermedeol (6.1-17.3%).

In contrast with the compositions of African *C. schoenanthus* oils reported below, two oil samples isolated from plants harvested in Southern Tunisia displayed quite different compositions (leaves, 6 samples/roots, 2 samples, %): limonene, 22.1-27.3/26.0-10.5; β -phellandrene (13.4-16.3/15.9-8.2), δ -terpinene (7.4-21.2/9.7-4.3), α -terpineol (9.1-11.7/9.4-6.8), β -eudesmol (0.2-5.3/2.1-14.2), α -eudesmol (0.4-5.5/4.3-0.3), juniper camphor (2.7-4.1/3.3-8.2).[26]

Among African countries, Algeria is an immense territory stretched from the Mediterranean coast to the arid lands of Southern Sahara. Wild *C. schoenanthus* has been reported in various regions from North to South Algeria and recently the compositions of several oil samples have been reported, evidencing a chemical variability:

- The first study dates back to 2005 and it concerned a supercritical CO₂ extract. The main components were *cis-p*-menth-2-en-1-ol (17.78%), *trans-p*-menth-2-en-1-ol (10.83%) (Both not yet mentioned in other countries), beside β-elemol (13.71%), *cis*-piperitol (9.14%) and *trans*-piperitol (6.26%);^[27]
- An essential oil sample from Aïn Sefra region (about 250 Km South of Tlemcen city, dried leaves), confirmed *cis-p*-menth-2-en-1-o1 (18.6), *trans-p*-menth-2-en-1-ol (9.5%), as major components beside elemol (7.4%), piperitone (7.2%) and limonene (7.0%), [28]
- A similar composition was reported for an essential oil sample isolated from aerial parts harvested in Tébessa region, Eastern Algeria (*cis-p*-menth-2-en-1-ol 15%, *trans-p*-menth-2-en-1-ol 13%, *trans-p*-menth-2-e
- Menthenols were the main components of essential oils isolated from plants harvested in El Menia, Béchar, Ghardaïa and Ouargla regions (*cis-p*-menth-2-en-1-ol 12.1-15.1%, *trans-p*-menth-2-en-1-ol 6.7-9.2%, *trans*-piperitol 5.4-7.7%, *cis*-piperitol 3.3-6.9%, 2-carene 13.4-20.4%, limonene 0-13.0%, elemol 4.9-9.9%, β-eudesmol 2.8-7.7%);^[30]
- In contrast, essential oil isolated from plants harvested in Southern Algeria contained piperitone as main component. Indeed, piperitone reached 63.3% in oil from Illizi province, near Djanet, followed by β -eudesmol 9.3% and elemol 6.9% [31] Similarly, the contents of piperitone were 63.2 and 55.1% in oils from Djanet and Tamanrasset, respectively, accompanied by 2-carene (9.3 and 16.7%) and elemol (9.5 and 4.8%); [30]
- Lastly, totally different compositions have been recently reported for oil samples isolated from aerial parts and roots of plant harvested in Ghardaïa region: guaiol (20.4%), *cis*-β-terpineol (16.2%), hinesol (10.5%), *cis*-sabinene hydrate (10.0%) for aerial part oil; and agarospirol (14.2%), *cis*-β-terpineol (12.6%), 4-carene (6.9%), *cis*-sabinene hydrate (6.6%) and guaiol (5.9%), for root oil.^[32]Leaves collected from Oued Sbaa, El-Menia (Ghardaïa and at 267 km South-West of Ghardaïa, respectively) contained α-eudesmol (17.9%), *trans*-dauca-4(11),7-diene (10.7%), α-muurolol (= δ-cadinol, 6.6%), hinesol (6.1%).^[33]

Obviously, this review of the literature evidenced a chemical variability within *C. schoenanthus* essential oils, including Algerian oils. In short, the compositions of *C. schoenanthus* oils from Southern Algeria (Illizi and Tamanrasset provinces) were dominated by piperitone, like oils from Benin, Togo, Burkina Faso or Sudan. In contrast, oils from central to Northern Algeria (Béchar, Ghardaïa, Ouargla, Aïn Sefra and Tébessa provinces) contained mainly *cis*- and *trans-p*-menth-2-en-1-ols, not identified in *C. schoenanthus* oils from other countries (India, Saudi Arabia, Iran, Tunisia). It could be highlighted that menth-2-en-1-ol isomers bring some originality to Algerian *C. schoenanthus* oils.

However, the composition of only one or two oil samples was reported for every location of harvest and therefore, it was not possible to evidence homogeneity or a chemical variability within a given locality. The knowledge of homogeneity/chemical variability is crucial for an eventual commercialization of Algerian *C. schoenanthus* essential oil. Therefore, the aim of the present study was to isolate and to analyze a sufficiently large number of oil samples to evidence homogeneity or chemical variability within a location of harvest.

Results and Discussion

Two locations of harvest have been chosen in central Algeria, in provinces of Béchar and Ghardaïa. The whole plant (aerial parts and rhizomes) has been used to isolate essential oil. Indeed, we observed that it is easier to harvest the whole plant than pick up aerial parts without rhizomes. Therefore, our work has been divided as follow:

- 1. Analysis of three oil samples by combination of chromatographic and spectroscopic techniques (GC/RI, GC/MS, ¹³C-NMR) in order to identify as much as possible components;
 - 2. Comparison of oil samples isolated separately from aerial parts and rhizomes of the same individual plant (6 samples);
 - 3. Analysis of 24 oil samples isolated from plants (whole plant) harvested in Béchar (10 samples) and in Ghardaïa (14 samples);
 - 4. Antimicrobial activity, antioxidant activity.

Detailed analysis of three oil samples

Three *C. schoenanthus* oil samples isolated from the whole plant, one sample from Béchar, the two other from Ghardaïa, have been submitted to GC in combination with Retention Indices on two columns of different polarity, GC/MS and 13 C-NMR, following a computerized method developed at the University of Corsica. ^[34-36] In total, 48 compounds have been identified. The compositions of the three samples were dominated by various alcohols bearing the *p*-menthane skeleton: *cis-p*-menth-2-en-1-ol (22.6-28.5%), *trans-p*-menth-2-en-1-ol (15.4-16.3%), *cis*- and *trans*-piperitols (5.4-8.5% and 8.8-8.9%, respectively). Monoterpene hydrocarbons were represented by δ -2-carene (8.6-17.0%) and limonene (2.4-5.9%). β -Elemol (5.6-7.4%) was the main oxygenated sesquiterpene accompanied by β -eudesmol, α -eudesmol, intermedeol and juniper camphor (up to 3.5% each).

Table 1: Chemical composition of three oil samples isolated from the whole plant of *C. schoenanthus*.

Compounds ^[a]	RI _a [b]	RI _p [c]	B17 ^[d]	G16 ^[e]	G17	Identification mode	
Compounds	Kl _a '"	Kip ¹⁻⁷	E10	E1	E1	identification flode	
α-Pinene	932	1016	Tr	-	0.1	RI, MS	
Camphene	942	1063	Tr	Tr	0.2	RI, MS	
δ-2-carene	995	1130	8.6	13.1	17.0	RI, MS, ¹³ C NMR	
α-Terpinene	1008	1178	0.2	0.2	0.2	RI, MS	
<i>p</i> -Cymene	1010	1269	0.5	1.4	1.5	RI, MS, ¹³ C NMR	
Limonene*	1020	1199	5.9	2.4	2.5	RI, MS, ¹³ C NMR	
β-Phellandrene*	1020	1208	1.3	2.3	2.4	RI, MS, ¹³ C NMR	
(Z)-β-Ocimene	1026	1230	0.4	0.3	0.4	RI, MS	
(E)-β-Ocimene	1038	1247	0.2	0.1	0.2	RI, MS	
Terpinolene	1078	nd	0.1	-	-	RI, MS	
enchol	1098	1578	0.3	0.1	0.1	RI, MS	
cis-p-Menth-2-en-1-ol	1108	1559	26.6	28.5	22.6	RI, MS, ¹³ C NMR	
cis-p-Mentha-2,8-diene-1-ol	1116	1662	0.1	-	0.1	RI, MS	
trans-p-Menth-2-en-1-ol	1124	1624	16.1	16.3	15.4	RI, MS, ¹³ C NMR	
Terpineol-4	1160	1597	-	0.3	0.2	RI, MS	
a-Terpineol	1171	1692	3.8	1.2	0.9	RI, MS, ¹³ C NMR	
cis-Piperitol	1180	1674	7.1	8.5	5.4	RI, MS, ¹³ C NMR	
trans-Piperitol	1191	1741	8.9	8.8	8.8	RI, MS, ¹³ C NMR	
Nerol	1209	1795	0.1	0.1	-	RI, MS	
Piperitone	1229	1722	0.3	0.9	0.6	RI, MS, ¹³ C NMR	
Geraniol	1239	1842	0.1	0.3	0.3	RI, MS	
3-Elemene	1385	1584	0.4	0.3	0.5	RI, MS, ¹³ C NMR	
γ-Muurolene	1467	nd	Tr	-	0.1	RI, MS	
Selina-4,11-diene	1473	1667	Tr	Tr	0.1	RI, MS	
Germacrene D	1476	1701	Tr	Tr	Tr	RI, MS	
3-Selinene	1479	1710	0.1	0.1	0.1	RI, MS	
δ-Selinene	1482	1685	Tr	0.2	0.1	RI, MS	
4-epi-Cubebol	1485	1879	0.1	0.1	0.1	RI, MS	
a-Selinene	1488	1716	0.1	Tr	0.1	RI, MS	

γ -Cadinene	1502	1751	0.3	0.2	0.3	RI, MS
δ-Cadinene	1510	1749	Tr	0.1	0.1	RI, MS
β-Elemol	1534	2074	5.6	6.4	7.4	RI, MS, ¹³ C NMR
Germacrene B	1549	1819	0.1	Tr	Tr	RI, MS
Spathulenol	1560	2115	0.2	Tr	Tr	RI, MS
5-epi,7-epi-α-Eudesmol	1590	2132	0.2	0.1	0.2	RI, MS
Selina-6-en-4α-ol#	1601	2155	1.3	0.6	0.5	RI, MS, ¹³ C NMR
Alismol	1606	2245	0.1	0.1	0.1	RI, MS
Eremoligenol	1610	2185	0.1	Tr	Tr	RI, MS
γ -Eudesmol	1613	2159	0.1	0.1	0.2	RI, MS
τ-Muurolol	1619	2171	0.4	0.5	0.4	RI, MS, ¹³ C NMR
τ-Cadinol	1621	2161	0.6	0.1	0.5	RI, MS, ¹³ C NMR
α -Cadinol	1625	2220	0.3	0.1	0.1	RI, MS
β-Eudesmol*	1633	2220	1.7	1.8	3.7	RI, MS, ¹³ C NMR
Valerianol*	1633	2204	0.4	0.2	0.3	RI, MS
α-Eudesmol	1639	2211	1.2	1.1	2.3	RI, MS, ¹³ C NMR
Intermedeol	1641	2223	1.0	0.6	0.2	RI, MS, ¹³ C NMR
Juniper camphor	1677	2287	1.9	0.8	0.9	RI, MS, ¹³ C NMR
Geranyl linalool	1694	nd	Tr	Tr	Tr	RI, MS
Total (%)			96.9	98.1	97.0	

^[a]: Order of Elution and percentages are given on apolar column (BP-1), excepted those whose names are followed by an asterisk, percentages on polar column (BP-20). ^[b]RI_a: Retention indices measured on apolar column (BP-1). ^[c]RI_b: Retention indices measured on polar column (BP-20). ^[d]B17: Béchar 2017, ^[e]G16: Ghardaïa 2016. ^[a]C-NMR = compound identified by NMR at least in one oil sample. Tr: Traces. #Selina-6-en-4 α -ol = Eudesm-6-en-4 α -ol.

Oil samples isolated from aerial parts and rhizome of the same plant

Six *C. schoenanthus* plants have been harvested in Béchar, aerial parts and rhizomes have been separated manually, and they have been hydrodistilled separately. The twelve oil samples have been analyzed by GC/RI, some by GC/MS and/or by ¹³C-NMR. The contents of the major components have been reported in Table 2, as mean value (M), standard deviation (SD) and minimum-maximum (min-max).

Table 2: Chemical composition of 6 oil samples isolated from aerial parts and 6 oil samples isolated from rhizomes of the same individual plants.

Compounds ^[a]	RI _a ^[b]	RI _p [c]	AP ^[d]					R ^(e)			
compounds."	Ki _a	Kip	M ^[f]	SD[a]	Min ^[h]	Max ^[i]	M ^[f]	SD[a]	Min ^[h]	Max ^[i]	
δ-2-Carene	995	1130	9.0	3.7	2.1	12.6	7.6	1.4	4.8	8.5	
<i>p</i> -Cymene	1010	1269	2.1	0.4	0.3	0.7	2.6	1.1	0.4	0.8	
Limonene*	1020	1199	1.0	0.3	1.6	2.5	1.0	0.3	1.6	4.1	
β-Phellandrene*	1020	1208	0.3	0.1	0.6	1.4	0.4	0.1	0.7	1.4	
(Z)-β-Ocimene	1026	1230	0.1	0.1	0.2	0.5	0.1	0.1	0.3	0.5	
cis-p-Menth-2-en-1-ol	1108	1559	24.0	8.6	10.5	31.6	28.1	3.3	21.8	31.7	
trans-p-Menth-2-en-1-ol	1124	1624	15.5	4.8	7.2	19.9	18.5	1.8	15.8	20.2	
α-Terpineol	1171	1692	1.7	0.6	1.1	2.7	2.5	1.3	1.4	3.8	
cis-Piperitol	1180	1674	7.5	3.4	3.2	12.6	8.5	1.6	5.7	10.8	
trans-Piperitol	1191	1741	9.3	2.8	4.6	12.0	11.1	1.2	9.7	12.5	
Piperitone	1229	1722	0.6	0.2	0.2	0.9	0.7	0.2	0.4	0.8	
β-Elemol	1534	2074	4.5	3.6	0.5	9.8	2.6	1.8	1.4	6.1	
Selina-6-en-4α-ol#	1601	2155	2.0	1.6	0.8	5.1	1.5	0.1	1.4	1.5	
β-Eudesmol	1633	2220	2.1	1.6	0.3	4.0	1.2	1.1	0.4	3.3	
α-Eudesmol	1639	2211	1.4	1.1	0.1	3.0	0.7	0.4	0.4	1.5	
Intermedeol	1641	2223	1.5	1.0	0.7	3.3	1.2	0.2	1.0	1.6	

Juniper camphor 1677 2287 3.2 3.3 0.6 9.4 2.0 0.5 1.5 2.9

[a]: Order of elution and percentages are given on apolar column (BP-1), excepted those whose names are followed by an asterisk, percentages on polar column (BP-20). [b] RI_a: Retention indices measured on apolar column (BP-1). [c] RI_p: Retention indices measured on polar column (BP-20). [d] AP: Aerial parts. [e] R: Rhizomes. [f] M: Mean value. [g] SD: Standard deviation. [h] Min: Minimum. [i] Max: Maximum. Tr: Traces. #Selina-6-en-4α-ol.

All twelve compositions were dominated by *cis-p*-menth-2-en-1-ol (24.0% \pm 8.6 and 28.1% \pm 3.3, respectively) and *trans-p*-menth-2-en-1-ol (15.5% \pm 4.8 and 18.5% \pm 1.8, respectively). Other components present at appreciable content were *cis*- and *trans*-piperitols, β -elemol, selina-6-en-4 α -ol, and juniper camphor. Although slight differences have been observed, they were only quantitative and it could be concluded that aerial parts and rhizome produced similar essential oils.

Variability of 24 oil compositions

Twenty-four plants have been harvested, 10 in Béchar, 14 in Ghardaïa and hydrodistilled. Yields varied from 0.5 to 1.8%. The oil samples have been analyzed by GC/RI, 5 samples by GC/MS and 5 samples by ¹³C NMR, 3 samples by both techniques. The 24 compositions have been submitted to statistical analysis.

The dendrogram reported below displayed a large group of 20 oil samples with similar composition. For every component with appreciable content mean value, standard deviation as well as minimum and maximum contents have been reported on table 3, which contains also the major components of the last four compositions, classified as "atypical" since they do not constitute a minor group.

Concerning the main group, cis-p-menth-2-en-1-ol (M = 22.6% \pm 3.6) and trans-p-menth-2-en-1-ol (M = 14.3% \pm 1.7) were the major components followed by three monoterpenes namely δ -2-carene (M = 9.5% \pm 3.1), trans-piperitol (M = 8.3% \pm 0.9), cis-piperitol (M = 7.2% \pm 1.8) and a sesquiterpene, β -elemol (M = 7.3% \pm 3.1). Other oxygenated sesquiterpenes present at appreciable contents were β -eudesmol, α -eudesmol, juniper camphor, selina-6-en-4 α -ol and intermedeol.

Concerning the four, out of 24, atypical oil samples, two of these are from Béchar station, the two others from Ghardaïa station. Compared to the mean compositions of the main group, the two samples from Béchar (B19 E5 and B19 E4) contained more limonene (10.0 and 12.1% vs. 2.9% \pm 2.0), α -terpineol (7.4 and 8.3% vs. 2.2% \pm 1.7), and a little bit more intermedeol while δ -2-carene was present at lesser extent (3.8% vs. 9.5% \pm 3.1). The first atypical sample from Ghardaïa (G16 E2) appeared among the richest oil samples in menthenol isomers (25.2% and 18.5%, respectively) while *trans*-piperitol was abundant (12.8% vs. 8.3% \pm 0.9) and in contrast, the content of δ -2-carene was very low (1.0%). Lastly, the second oil sample from Ghardaïa (G17 E3) displayed the lowest content of menthenol isomers (11.6 and 8.2%, respectively) and it was characterized by its very high content of β -elemol (20.7% vs. 7.3% \pm 3.1) and β -eudesmol (7.2% vs. 3.0% \pm 1.4).

Table 3: Mean components of 20 oil samples isolated from the whole plant of C. schoenanthus and compositions of four atypical oil samples.

Compounds ^[a]	RI _a [b]	RI _p [c]	M ^[d] 20	SD ^[e] 20	Min ^[f]	Max ^[g]		Aty	pical	
Compounds	NI _a ,	Νip	samples	samples	WIII	Max	B19 E5	B19 E4	G16 E2	G17 E3
δ-2-Carene	995	1130	9.5	3.1	5.4	17.0	3.8	3.8	1.0	3.6
Limonene*	1020	1199	2.9	2.0	1.4	7.2	10.0	12.1	0.3	3.5
β-Phellandrene*	1020	1208	1.4	0.5	0.8	2.4	1.1	1.8	0.5	1.0
(Z)-β-Ocimene	1026	1230	0.4	0.4	0.1	1.6	0.7	0.6	0.1	0.1
(<i>E</i>)-β-Ocimene	1038	1247	0.2	0.2	0.1	0.7	0.3	0.3	Tr	Tr
cis-p-Menth-2-en-1-ol	1108	1559	22.6	3.6	15.0	28.5	17.1	17.8	25.2	11.6
trans-p-Menth-2-en-1-ol	1124	1624	14.3	1.7	10.8	17.0	9.6	11.4	18.5	8.2
α-Terpineol	1171	1692	2.2	1.7	0.9	5.8	7.4	8.3	1.3	2.7
cis-Piperitol	1180	1674	7.2	1.8	3.9	11.0	7.3	5.1	8.6	3.3
trans-Piperitol	1191	1741	8.3	0.9	6.5	10.1	5.6	6.4	12.8	5.1
Piperitone	1229	1722	0.9	0.5	0.3	2.2	0.3	0.1	1.1	0.5
β-Elemol	1534	2074	7.3	3.1	2.3	13.6	7.7	6.2	5.5	20.7
Selina-6-en-4α-ol#	1601	2155	1.2	0.5	0.4	2.4	0.5	0.4	1.3	1.5
β-Eudesmol	1633	2220	3.0	1.4	1.2	5.7	5.1	4.6	2.5	7.2
α-Eudesmol	1639	2211	1.7	0.8	0.6	3.8	2.2	2.2	1.3	4.0
Intermedeol	1641	2223	1.1	0.5	0.2	2.7	4.2	3.7	1.8	1.5
Juniper camphor	1677	2287	1.5	0.7	0.5	2.8	0.5	0.3	1.8	2.5

[a]: Order of elution and percentages are given on apolar column (BP-1), excepted those whose names are followed by an asterisk, percentages on polar column (BP-20). [b] Rl_s : Retention indices measured on apolar column (BP-1). [c] Rl_s : Retention indices measured on polar column (BP-20). [d] Rl_s : Retention i

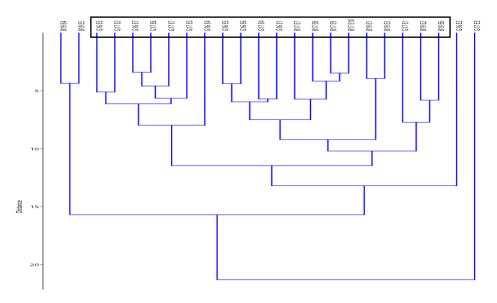


Figure 1. Dendrogram of hierarchical cluster analysis of the 24 C. schoenanthus essential oil samples.

Antimicrobial activity of C. schoenanthus EO

Various studies reported on the antimicrobial activity of Algerian C. schoenanthus essential oil. However, results differed from paper to paper:

- For instance, zone of inhibition of piperitone-rich (63.3%) *C. schoenanthus* essential oil (from Illizi province) against bacterial strains varied from 10.5 ± 0.7 mm to 21.75 ± 0.3 mm and was equal to 12.0 ± 1.4 mm for yeast. [31] Piperitone-rich (63.2% and 55.1%) oil samples from Djanet and Tamanrasset showed inhibition zones of 15 ± 1.2 mm to 38 ± 0.7 mm against various microbial strains; [30]
- Another oil sample, whose composition was dominated by guaiol (20.4%) and cis- β -terpineol (16.2%), displayed zones of inhibition of Gram + bacteria in the range of 6.0 ± 0.0 16.2 ± 0.3 mm and Gram bacteria in the range $7.3 \pm 0.3 13.7 \pm 0.6$ mm.^[32]
- A sesquiterpene-rich oil sample (α -eudesmol, 17.9%; trans-dauca-4(11),7-diene, 10.7%), from El-Menia was tested against bacterial strains (zone of inhibition 9.00 \pm 0.50 21.67 \pm 0.58 mm), filamentous fungi (zone of inhibition 6.50 \pm 0.50 26.33 \pm 0.58 mm) and a yeast (zone of inhibition 7.33 \pm 0.58 mm), filamentous fungi (zone of inhibition 6.50 \pm 0.50 26.33 \pm 0.58 mm)
- Lastly, essential oils from Béchar and Ghardaïa were checked. They contained various components at appreciable and similar contents, 2-carene, limonene, *cis-p*-menth-2-en-1-ol, *trans-p*-menth-2-en-1-ol and elemol. Zones of inhibition were measured (mm) against microbial strains: *S. aureus*, 27 \pm 0.6/22 \pm 0.3 mm; *B. subtilis*, 24 \pm 1.0/20 \pm 0.4 mm; *E. coli* 16 \pm 0.7/19 \pm 1.2 mm; *C. albicans* 29 \pm 0.2/32 \pm 1.3 mm; ^[30]

In order to obtain sufficient quantity of essential oil to carry out antimicrobial experiments, various oil samples with similar compositions have been mixed leading group I and group II, whose main components are reported below (Table 4). As observed in oil samples previously investigated, the compositions of both samples were dominated by *cis-p*-menth-2-en-1-ol (20.2 and 25.0%, respectively) and *trans-p*-menth-2-en-1-ol (14.0 and 15.4%, respectively) while other components (δ -2-carene, *cis*-piperitol, *trans*-piperitol, δ -elemol) accounted for 5-10% each.

Table 4: Main components of oil samples group I and group II

Compounds (%)	Group I	Group II	Compounds (%)	Group I	Group II
δ-2-carene	9.6	8.7	Piperitone	1.0	1.0
<i>p</i> -cymene	1.4	1.2	β-elemol	8.1	5.4
Limonene*	1.8	3.8	Selina-6-en-4α-ol	1.1	1.0
$\beta\text{-Phellandrene*}$	1,5	1.3	γ-Eudesmol	1.2	0.6
cis-p-Menth-2-en-1-ol	20.2	25.0	β-Eudesmol	4.0	2.3
trans-p-Menth-2-en-1-ol	14.0	15.4	α-Eudesmol	1.5	1.3
α-Terpineol	1.2	2.9	Intermedeol	1.5	1.1
cis-Piperitol	5.7	8.0	Juniper camphor	1.8	1.4

trans-Piperitol 8.4 8.9

Both oil samples have been checked against various microbial strains, bacteria, yeast and filamentous fungi (Table 5). Concerning bacterial strains, diameters of inhibition zone varied from 8.0 ± 0.0 mm to 10.3 ± 0.6 mm for *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *Staphylococcus aureus* was much more sensitive with diameters of inhibition equal to 19.3 ± 1.2 mm and 30.7 ± 0.6 mm, for oil samples GI and GII, respectively. The higher activity of essential oil GII could be highlighted.

Concerning the yeast *Candida albicans*, a fair activity of both oils was observed, oil GI being a little bit more active than GII (24.0 \pm 3.5 mm and 19.5 \pm 6.4 mm, respectively). Lastly, concerning filamentous fungi, a fair activity of both oil samples was observed against *Aspergillus flavus* (13.5 \pm 2.1 mm and 24.5 \pm 0.7 mm) and *Fusarium oxysporum* (19.0 \pm 1.4 mm and 32.5 \pm 0.7 mm). It could be pointed out that zones of inhibition were in the same range than those measured by *Aous et al.*^[30](essential oils from Béchar and Ghardaïa, respectively) against microbial strains: *S. aureus*: 27 \pm 0.6/22 \pm 0.3 mm; *E. coli*: 16 \pm 0.7/19 \pm 1.2 mm; *C. albicans*: 29 \pm 0.2/32 \pm 1.3 mm.

Table 5: Inhibition zones (mm) of oil collective samples group I and group II against various microbial strains.

Minubial studios	Essential oil	(15 μL/disc)		Positive controls			
Microbial strains	Group I	Group I Group II		NY (30 μg/disc)	FLU (25 μg/disc)		
Bacterial strains							
Escherichia coli	$10.0 \pm 0.0^{[a]}$	9.7 ± 0.6	34.7 ± 0.6	_	_		
Klebsiella pneumoniae	8.0 ± 0.0	10.3 ± 0.6	27.7 ± 0.6	_	_		
Staphylococcus aureus	19.3 ± 1.2	30.7 ± 0.6	30.0 ± 0.0	_	_		
Pseudomonas aeruginosa	8.0 ± 0.0	8.0 ± 0.0	30.0 ± 0.0	_	_		
Yeast							
Candida albicans	24.0 ± 3.5	19.5 ± 6.4	_	16.0 ± 0.0	6.0 ± 0.0		
Fungi							
Aspergillus flavus	13.5 ± 2.1	24.5 ± 0.7	_	22.3 ± 0.6	6.0 ± 0.0		
Fusarium oxysporum	19.0 ± 1.4	32.5 ± 0.7	_	16.0 ± 1.0	6.0 ± 0.0		

[[]a]: Mean values (three experiments) ± Standard deviation of the growth inhibition zones, in mm, including the disc diameter of 6 mm. CIP: Ciprofloxacin, FLU: Fluconazole. NY: Nystatin were used as positive controls.—: Not tested.

Taking into account these good results, we measured the Minimum Inhibitory Concentrations (MIC) of oil samples GI and GII against the most sensitive strains (Table 6). Indeed, MIC values allow a better comparison with antimicrobial activity of selected essential oil reported in literature than diameters of inhibition. Essential oils GI and GII displayed more or less similar antimicrobial activity, against one bacterial strain, one yeast and two filamentous fungi, slight differences being observed in some cases. A moderate activity against *Aspergillus flavus*, *Fusarium oxysporum* and overall *Candida albicans* was obtained with both oil samples. A fair activity was observed against *Staphylococcus aureus* with a MIC value of 0.5 μL/mL. The observed MIC value was comparable to that obtained with Algerian carvacrol-rich oils from *Thymus fontanesii* (MIC = 0.3 mg/mL)^[37] and *Thymus ciliatus* (MIC = 0.9 mg/mL). In the absence of phenol derivatives, it could be assumed that this fair activity could be attributed to the high amount of various alcohols bearing the *p*-menthane skeleton (*cis-p*-menth-2-en-1-ol, *trans-p*-menth-2-en-1-ol, *cis*- and *trans*-piperitols). However, oxygenated sesquiterpenes such as β-elemol and β-eudesmol could also contribute to this activity in synergism with major components.

 $\textbf{Table 6:} \ \textbf{Minimum} \ \textbf{Inhibitory Concentrations (MICs) of C.} \ \textit{schoenanthus} \ \textbf{of GI and GII collective samples against microbial strains.}$

Microbial strains	MIC (μL/mL)					
WICCODIAI Strains	Sample Group I	Sample Group II				
Staphylococcus aureus	0.5	0.5				
Candida albicans	4.0	3.0				
Aspergillus flavus	2.0	2.0				
Fusarium oxysporum	2.0	2.0				

Antioxidant activity of C. schoenanthus EO

The antioxidant ability of essential oils is generally associated with the presence of phenolic compounds such as thymol and carvacrol. Nevertheless, it has also been shown that essential oils containing mainly oxygenated monoterpenes displayed an appreciable antioxidant activity.

Moreover, the antioxidant activity of the monoterpene hydrocarbons fraction has been demonstrated. Concerning C schoenanthus essential oils, a low antioxidant activity has been observed [Aous et al, 2019]. However, IC_{50} value was not measured.

Therefore, the *in vitro* antioxidant activity of *C. schoenanthus* essential oils against DPPH free radical scavenging has been assessed and compared to that of ascorbic acid. The inhibitory potential of the essential oils against DPPH depended gradually increased, between 3.83 ± 0.16 to $93.78 \pm 0.11\%$, with an increasing concentration of essential oil (0.125 to 256 mg/mL) (Figure 2). Both oil samples exhibited similar antioxidant power, although IC₅₀ value varied from sample to sample (IC₅₀ = 10.66 ± 0.1 and 16.30 ± 0.3 mg/mL, Table 7). Whatever the sample, the antioxidant potential of *C. schoenanthus* essential oil was significantly lower than that of ascorbic acid employed as positive control (IC₅₀= 0.02 ± 0.001 mg/mL).

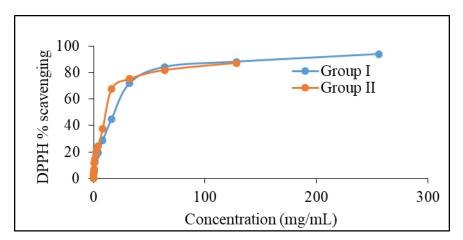


Figure 2. Scavenging effect on DPPH of C. schoenanthus oil samples.

Table 7: Antioxidant test measured by DPPH radical scavenging capacity: IC₅₀ values of C. schoenanthus essential oil.

IC₅₀ (mg/mL)	Group I	Group II	Ascorbic acid
50 (···· 5 ····-,	16.30 ± 0.3	10.66 ± 0.1	0.02 ± 0.001

Conclusions

Cymbopogon schoenanthus is an aromatic plant whose aerial parts and rhizome produced an essential oil with pleasant odor. Various compositions of the essential oil have been reported depending of the countries were the plant grows wild. Such a chemical variability has been observed in Algeria where a composition dominated by cis- and trans-p-menth-2-en-1-ols occurred, not found in other countries. We demonstrated that in two locations of harvest, Béchar and Ghardaïa (Central Algeria), the essential oil composition is homogenous for 20 oil samples out of 24, and therefore, this is a positive point for an eventual production and domestication of C. schoenanthus essential oil. Otherwise, a fair antimicrobial activity was measured against Staphylococcus aureus strain, while the antioxidant potential was low.

Experimental Section

Plant Material and Oil Distillation

Whole plant (WP), rhizome (R), aerial parts (AP: stems and leaves) from 30 individual plants *C. schoenanthus* were collected in two locations of Algeria (Figure 3), sixteen plants were sampled at Béchar province (July 2017: B17 AP E1 - 6; B17 R E1 - 6, B17 WP E7 - 10 and in April 2019: B19 WP E1 - 6) and fourteen plants were sampled at Ghardaïa province (seven in April 2016: G16 WP E1 - 7 and seven in January 2017: G17 WP E1 - 7). Identification of the plants was performed by Dr. F. Hassani (University of Tlemcen, Algeria). A voucher specimen has been deposited with the Laboratory of Natural Products (Department of Biology, University of Tlemcen) under the accession No. P. 111. Dry plants (60 - 120 g) were submitted to hydrodistillation for 2 h. Yields have been calculated from dry material (w/w).

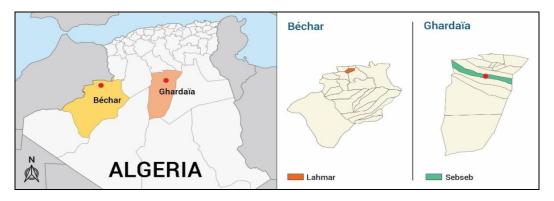


Figure 3. Sampling locations of Cymbopogon schoenanthus from Algeria.

Analytical GC

GC analyzes were performed on a Perkin-Elmer Clarus 500 gas chromatograph (FID) equipped two fused silica capillary columns (50 m x 0.22 mm, 0.25 μ m film thickness), BP-1 (polydimethyl siloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min, injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: hydrogen (0.8 ml/min); split: 1/60. The relative proportions of the oil constituents were expressed as percentages obtained by peak area normalization, without using correcting factors. Retention indices (RI) were determined relative to the retention times of a series of n-alkanes with linear interpolation ("Target Compounds" software from Perkin-Elmer).

GC/MS Analysis

The essential oils were analyzed with a Perkin-Elmer TurboMass detector (quadrupole), directly coupled to a Perkin-Elmer Autosystem XL, equipped with a fused-silica capillary column (50 m x 0.22 mm i.d., film thickness 0.25 μ m), BP-1(dimethylpolysiloxane). Carrier gas, helium at 0.8 mL/min; split, 1/60; injection volume, 0.5 μ L; injector temperature, 250 °C; oven temperature programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal (20 min); lon source temperature, 250 °C; energy ionisation, 70 eV; electron ionisation mass spectra were acquired over the mass range 40-400 Da.

13C-NMR Analysis

¹³C NMR analyzes were performed on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.623 MHz for ¹³C, equipped with a 5 mm probe, in deuterated chloroform (CDCl₃), with all shifts referred to internal tetramethylsilane (TMS). ¹³CNMR spectra were recorded with the following parameters: pulse width (PW), 4 μs (flip angle 45°); acquisition time, 2.73 s for 128 K data table with a spectral width (SW) of 220 000 Hz (220 ppm); CPD mode decoupling; digital resolution 0.183 Hz/pt. The number of accumulated scans ranged 2 000-3 000 for each sample (around 40 mg of oil in 0.5 mL of CDCl₃). Exponential line broadening multiplication (1.0 Hz) of the free induction decay was applied before Fourier transformation.

Identification of Components

Identification of the components was based: i) on comparison of their GC retention indices (RIs) on polar and apolar columns, determined relative to the retention times of a series of n-alkanes with linear interpolation ('Target Compounds' software of Perkin-Elmer), with those of authentic compounds and with reference data^[40] on computer matching against commercial mass spectral libraries^[40-43] and (iii) on comparison of the signals in the ¹³C-NMR spectra of essential oils with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software. [34-36] In the investigated samples, individual components were identified by NMR at contents as low as 0.3 - 0.4%.

¹³C-NMR (δ, ppm) of *cis*- and *trans-p*-menth-2-en-1-ol. *Cis* isomer: 133.56 (CH); 133.23 (CH); 67.48 (C); 42.12 (CH); 37.28 (CH₂); 31.74 (CH); 29.65 (CH₃); 21.64 (CH₂); 19.66 (CH₃); 19.31 (CH₃), *Trans* isomer: 134.68 (CH); 131.19 (CH); 69.60 (C); 41.71 (CH); 38.03 (CH₂); 31.77 (CH); 28.49 (CH₃); 23.59 (CH₂); 19.72 (CH₃); 19.39 (CH₃). ¹³C-NMR values fitted perfectly with those reported. ^[44]

Data Analysis

Principal Components Analysis (PCA) and Hierarchical clustering (Ward's method) were performed by XIstat (Adinsoft, France). [47]

Antimicrobial Activity of the Essential Oil

Microbial Strains

Antimicrobial activity of the essential oil (collective sample) were evaluated against one Gram positive bacteria (*Staphylococcus aureus* ATCC 6538) and three Gram negative bacteria (*Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853), one yeast (*Candida albicans* ATCC 10231) and two filamentous fungi (*Fusarium oxysporum* MNHN 963917 and *Aspergillus flavus* MNHN 994294).

Screening of Antimicrobial Activity

The agar diffusion method was used for the determination of antimicrobial activity of the EOS. [45] Briefly, a suspension of the tested microorganisms (1 mL of a suspension at 10^6 cells/mL for bacteria and yeast, 10^7 cells/mL for *S. aureus* and 10^4 spores/mL for filamentous fungi) was spread on the solid media plates, using Mueller-Hinton agar for bacteria, Sabouraud dextrose for yeast and PDA for filamentous fungi. Filter paper discs (6 mm in diameter) were impregnated with 15 μ L of the oil and 5 μ L of DMSO and placed on the surface of inoculated plates. The activity was determined by measuring the inhibitory zone diameter in mm after incubation for 24 h at 37 °C for bacteria, 24-48 h at 30 °C for yeast and 5 days at 25 °C for filamentous fungi. Fluconazole (FLU 25 μ g/disc), nystatin (NY 30 μ g/disc) were used as reference antifungal against yeast and filamentous fungi and ciprofloxacin (CIP 10 μ g/disc) was used as positive controls against bacteria. DMSO was used as negative control. Each test was performed in triplicate.

Determination of minimum inhibitory concentration (MIC)

The MIC was defined as the lowest concentration of tested sample that resulted in complete inhibition of visible growth. Dilutions of oil were made in culture medium over the concentration range 0.25- $4 \mu L/mL$. 1 mL of standardized suspension was added. Inoculated plates were incubated at 37° C for 24 h for bacteria, 24-48 h at 37° C for yeast and 5 days at 25° C for filamentous fungi. MICs were determined as the minimum concentration with no visible growth. Each test was performed in duplicate or in triplicate.

DPPH Radical Scavenging Activity

The antioxidant activity was measured on a sample of essential oil (Collective sample). The antioxidant activity of *C. schoenanthus* EO was measured on the basis to scavenge of the 2.2-diphenyl-1-picrylhydrazil (DPPH) free radical, according to the experimental protocol described by Kouame *et al.*^[46] A volume of 2.5 mL with various concentrations (256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 mg/mL) of the EO in absolute ethanol were added to 1 mL of an ethanolic solution of DPPH at 0.03 mg/mL. For each concentration, a blank was prepared. In parallel, a negative control is prepared by mixing 2.5 mL of absolute ethanol with 1 mL of ethanolic solution of DPPH. After incubation in the dark for 30 min at room temperature, the absorbance was measured against a blank at 517 nm. The activity of the EO was compared to ascorbic acid as a positive control. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

DPPH scavenging activity (%) = [(Acontrol-Asample)/Acontrol] X100

Acontrol is the absorbance of the negative control; Asample is the absorbance of the tested sample.

The concentration of the EO required for the 50% reduction in the initial concentration of DPPH (IC₅₀) was calculated from the graph plotted of percentage inhibition against essential oil concentrations. Each test was performed in triplicate.

Acknowledgements

This work was conducted within the cooperative program between the Universities of Abou Bekr Belkaïd (Tlemcen) and Pascal Paoli (Corsica). C. Bekhechi thanks University of Corsica for a research grant as associate professor (September 2018). C. Bekhechi also acknowledges Prof. N.A. Mokhtari-Soulimane (Dean, Faculty of Life Sciences, Tlemcen) for attribution of a travel grant (September 2019) and Dr F. Hassani, University of Tlemcen, for his help on plant identification.

Author Contribution Statement

C.B. conceived and designed the experiments; C.E.W.M., I.A.E.-H. and F.H. performed sampling, extractions and identification of the plant; M.P. performed GC, MS and NMR experiments; M.G. performed statistical analyses; J.C., C.B. and F.T. contributed to the preparation of the manuscript.

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Graphical abstract

