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(RESEARCH ARTICLE)

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Phytochemical analysis and biological activity of the essential oil of lemongrass (*Cymbopogon schoenanthus* (L.) Spreng) from the Algerian Sahara

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Abstract

The work focuses on the phytochemical and biological study (antimicrobial activity) of the essential oil (EO) from the underground parts of *Cymbopogon schoenanthus*, collected from the cultural park of Ahaggar in southern Algeria.

The extraction of the essential oil gives a yield of more than 4 ml per 100 g of dry matter (gdm). Chromatographic analysis of the essential oil of this species shows a richness in monoterpenes (59.7%) with a predominance of piperitone (50.25%) and secondarily α-elemol (11.06%).

The antimicrobial activity of the essential oil, tested on six microorganisms, shows a strong antifungal and antibacterial action. This bioactivity is mainly due to the richness of this essence in piperitone known for its effectiveness against microbial agents.

The limit test of toxicity shows that a dose of 2 g of essential oil/kg of body weight is not toxic.

Keywords: *Cymbopogon schoenanthus*; Essential oil; Piperitone; Antimicrobial activity; Toxicity

1. Introduction

Cymbopogon schoenanthus is a tufted plant, native to India and is very common throughout the Sahara. In the Ahaggar region, the plant grows in isolated clumps in the rocky beds of wadis and in mountain ravines [1-3]. *Cymbopogon schoenanthus* is used in folk medicine. Its medicinal properties have been known since antiquity and were already described by "Pliny the Elder" in his book Naturalis Historia (Natural History) [4]. It is also used in a poultice to treat camel wounds [4].

2. Material and methods

2.1. Plant Material

The plant material consists of roots and rhizomes of *Cymbopogon schoenanthus*. Several samples were collected over several years, during the months of September and October in the Ahaggar region at Djebel Oumhamt (50 km north of Tamanrasset, at an altitude of 1679 m).

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Figure 1 *Cymbopogon schoenanthus*, general overview (D. Boukhalfa)

2.2. Microbial material

Table 1 Tested reference bacterial strains

Name of strain	N° ATCC	Forms	Familly (Taxon)
Staphylococcus aureus	6538	CG+ Aerobi-anaerobic facultative	*Micrococcaceae*
Bacillus subtilis	9372	BG+Aerobic strict	*Bacillaceae*
Pseudomonas aerugenosa	9027	BG-Aerobic strict	*Pseudomonadaceae*
Escherichia coli	4157	CG+ Aerobi-anaerobic facultative	*Enterobacteriaceae*
Enterococcus faecium	6569	CG+ Anaerobic facultative	*Enterococcaceae*

Table 2 The fungal strains chosen for this study

Nom de souches	N° ATCC	Formes	Famille
Candida albicans	24433	Levure	*Cryptococcaceae*
Saccharomyces cerevisiae	2601	Levure	*Saccharomycetaceae*
Aspergillus brasiliensis	16404	Champignon	*Trichocomaceae*

2.3. Physicochemical equipment

2.3.1. Refractometer

The refractometer used for determining the refractive index of the essential oil is a CARL ZEISS refractometer, reference number 89717, manufactured in the former West Germany.

2.3.2. Hydrodistillation apparatus

Standardized apparatus for essential oil dosage in accordance with the European Pharmacopoeia 8th edition.

2.3.3. Physicochemical analysis equipment

The gas chromatograph used in this work is a Hewlett-Packard (HP) Palo-Alto CA, USA (Agilent technologies) 6800 plus.

- Injector: The sample is introduced with a micro-syringe. Column:
- Type: Hewlett Packard-5MS, nonpolar.
- Dimensions: 30 m long, 0.25 mm internal diameter, 0.25 µm film thickness Stationary phase: 5% phenyl 95% dimethylpolysiloxane.

- Detector: The detector used is a mass detector or Mass Spectrometer (MS): It is an HP (Agilent technologies) MSD 5973 triple quadrupole mass filter. It is of the brand HP (Agilent technologies), type MSD 5973, triple quadrupole mass filter (QQQ).

2.4. Animal material

Mice: Male or female mice with an average weight of 20 ± 2 g.

2.5. Extraction and dosage

Extraction of the essential oil by hydrodistillation. The dosage is performed directly on the device where the volume reading is carried out on the graduated tube which has a total volume of 1 ml, divided into 50 graduations, each of which corresponds to 20 µl.

Table 3 Operating conditions

Dry weight	Solvent (ml)	Extraction time
10	water + glycerin : (200+100)	4 Hours

2.6. Physical indices

- **Refractive index**: The refractive index at 20°C and density are determined by methods conforming to AFNOR standards, 2011 [5].
- **Relative density:** This is the ratio of the mass of a certain volume of an essential oil at 20°C to the mass of an equal volume of distilled water at 20°C [5].

2.6.1. Gas chromatography/mass spectrometry (GC/MS) separation

Operational conditions of the GC/MS analysis

- Injector: split-splitless set at 250 °C, injection mode: split 50:1, injected volume: 0.2 µl. The injector has a dual function: it vaporizes the sample, then it carries it into the gas flow at the head of the column.
- Analysis mode: Scan (from 34 to 450), Solvent used: Hexane, solvent delay: 4 min Interface temperature: 280 °C
- Type of ionization: Electron impact Filament intensity: 70 eV
- Type of mass analyzer: Quadrupole Quadrupole temperature: 150 °C, source temperature: 230 °C
- Column: Type: Hewlett Packard-5MS, non-polar Dimensions: length 30 m, internal diameter (I.D): 0.25 mm, film thickness 0.25 µm
- Stationary phase: 5% Phenyl, 95% dimethylpolysiloxane. Oven temperature: programmed at 60°C (8 min), at a rate of 2°C/min up to 250°C, for 10 minutes. Carrier gas: Helium purity: N 6, carrier gas flow rate: 0.5 ml/min.

2.6.2. Biological methods: Method for evaluating antimicrobial activity:

We used the agar diffusion method using sterile cellulose discs impregnated with essential oil called aromatograms, [6]

- **Determination of the minimum inhibitory concentration (MIC):** The minimum inhibitory concentrations (MIC) of essential oils were determined according to the method reported by Remmal et al. [7] and Satrani et al. [8]. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that can visibly inhibit the growth of a microorganism after 24 hours for bacteria and 48 hours for yeasts.
- **Determination of the minimum bactericidal concentration (MBC) and fungicidal concentration (MFC):** The minimum bactericidal concentration (MBC) is the lowest concentration of the essential oil capable of killing microorganisms after 24 h for bacteria and 48 h for yeasts (called MFC in this case) of incubation to obtain the destruction of 99.99% of the initial inoculum and leaving a percentage of surviving bacteria < 0.01% of the starting inoculum.
- **Limit toxicity test of *Cymbopogon schoenanthus* essential oil:** Limit test: dose equal to 2g/kg which does not cause mortality, indicating that the product is not toxic [9].

A limit test is performed by administering a dose of at least 2 g/kg of body weight to a group of 10 mice. No abnormalities or mortality (mortality rate must be equal to 0) should occur within 14 days following administration. The mice have free access to food (ONAB pellets and tap water), temperature: 20 to 24°C, humidity: 50%, lighting: 10 hours a day. The

essential oil is solubilized in a 1% Tween solution (in tap water), at a concentration of 0.86 ml of essential oil in 10 ml of 1% Tween, and 0.5 ml of this solution is administered to the mice using a gavage needle. The animals are observed carefully during the 14 days of the test.

3. Results

3.1. Physical indices

Table 4 Yield, refractive index, and relative density

Plant	Average Yield (ml /100 gdm)	IR_n^{x20}	Density
Cymbopogon schoenanthus	4±0.2	1.4849 ±0.0005	0.9266

3.2. Results of Gas Chromatography-Mass Spectrometry (GC-MS)

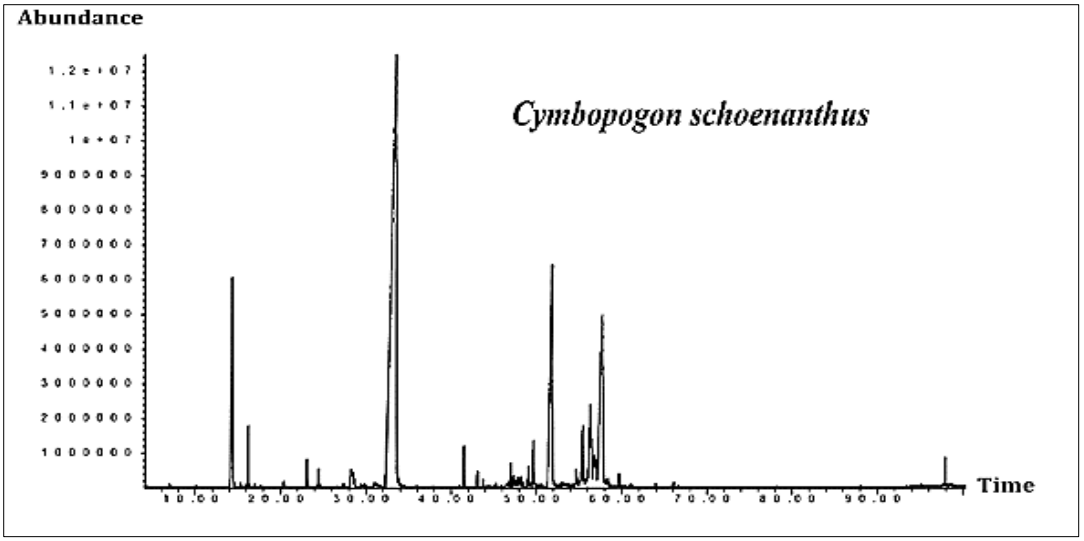

Figure 2 Chromatographic profile of *Cymbopogon schoenanthus* essential oil extract

Table 5 Chemical composition of *Cymbopogon schoenanthus* essential oil

N°	Identified compouds	TR	IR	IR ref.	%
01	p-xylen	06.98	869	865	0.04
02	l- δ-4-Carene	14.34	1003	1004	5.46
03	α-phellandren	14.54	1005	1007	0.08
04	α-terpinen	15.33	1016	1018	0.07
05	p-cymen	15.97	1025	1025	0.06
06	dl-limonen	16.22	1029	1030	1.18
07	cis-β-ocimen	16.93	1039	1046	0.06
08	p-menth-2-en-1-ol (quercivorol)	23.12	1125	1123	0.63
09	cis-β-terpinenol	24.47	1144	1141	0.45
10	l-α-terpineol	28.27	1197	1195	1.18

11	cis-piperitol	29.46	1214	1212	0.24
12	acide octanoique	31.09	1237	-	0.26
13	piperitone	33.59	1273	1268	50.25
14	β-elemene	41.51	1391	1391	0.82
15	trans-β-caryophyllen	43.08	1416	1418	0.37
16	(+)-calaren	43.79	1428.	1423	0.14
17	α-humulen	45.23	1451	1451	0.08
18	γ-sélinen	46.60	-	1473	0.12
19	α-Amorphen	46.75	1476	1475	0.12
20	germacren	47.00	1480	1480	0.58
21	β-selinen	47.31	1485	-	0.29
22	α-selinen	47.56	-	1494	0.53
23	α-muurolen	48.21	1499	1499	0.24
24	Leden	48.46	1504	1505	0.14
25	γ-cadinen	49.06	1514	1513	0.40
26	δ-cadinen	49.64	1524	1525	1.05
27	α-elemol	51.83	1561	1563	11.06
28	caryophyllene oxide	53.14	1583	1581	0.13
29	agarospirol	55.10	1617	1620	0.16
30	selina-6-en-4-ol	55.46	1624	1617	2.14
31	γ-eudesmol		1639	1630	3.34
32	iso spathulenol		1642	-	1.03
33	α-cadinol	56.83	1648	1652	1.85
34	β-eudesmol	57.59	1662	1656	6.36
35	α-eudesmol	56.29	1665	1658	6.02
36	juniper camphre	56.45	1700	1700	0.29
37	farnesol 1	61.06	1726	1725	0.12
Total (%)					97.34

3.2.1. Antimicrobial activity test

The essential oil of *Cymbopogon schoenanthus* has a very strong antimicrobial activity against fungal species *Candida albicans* and *Saccharomyces cerevisiae*, but this activity is weak against *Aspergillus brasiliensis*. This essential oil also has a strong activity against *Bacillus subtilis* and a moderate activity against *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity against *Pseudomonas aeruginosa* is weak.

Table 6 Antimicrobial activity of the essential oil of *Cymbopogon schoenanthus*

Inhibition diameter Microorganisms	**E.O. (diamètre(mm))**	**Contrôl (-)**	**Gentamycin**	**CMI (%)**	**CMB (%)**	**CMF (%)**
P. aeruginosa ATCC 9027	12	-	37	18.6	>18.6	-
E. coli ATCC 4157	14	-	25	1.16	4.65	-

B. subtilis ATCC 9372	25	-	34	0.29	1.16	-
S. aureus ATCC 6538	15	-	36	0.58	2.32	-
C. albicans ATCC 24433	44	-	25	0.29	-	0.29
S. cerevisiae ATCC 2601	40	-	-	0.29	-	0.29
A. brasiliensis ATCC 16404	11	-	-	1.16	-	1.16

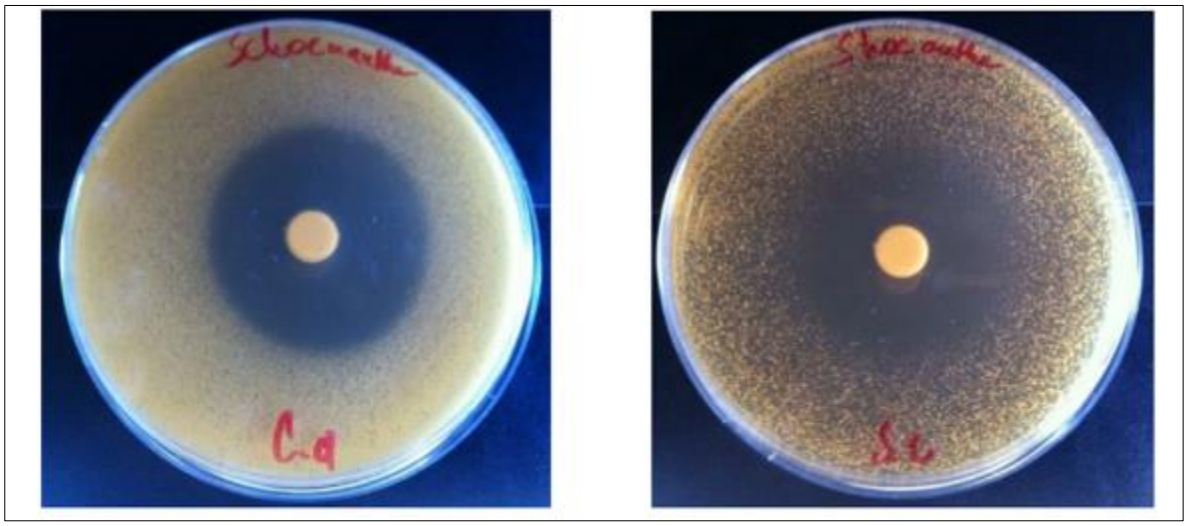

Figure 3 Antifungal activity of *Cymbopogon schoenanthus* essential oil on *Candida albicans*

3.3. Limit toxicity test of the essential oil of *Cymbopogon schoenanthus*:

Throughout the test period, no mice died due to extrinsic factors: Mortality rate: 0%. A dose of 2 g of EO/kg of body weight is not toxic, and no abnormalities were observed during the trial period. The LD50 of this essential oil is greater than 2 g/kg.

4. Discussion

4.1. Yield and Physical Indices

The extraction of essential oil from the rhizome of this species has yielded a considerable amount, sometimes exceeding 4%. This essential oil yield classifies *Cymbopogon schoenanthus* as one of the rare species in arid zones that provides such a yield. The low density of this oil (0.9266) makes it lighter than water. The higher refractive index (1.4849 ±0.000) complies with AFNOR standards [5].

4.2. Gas Chromatography-Mass Spectrometry

Thirty-seven compounds were counted in the *Cymbopogon schoenanthus* essential oil from Ahaggar, representing a total of 97.34%.

This analysis identified the different constituents, which are classified into two main groups: monoterpenes and sesquiterpenes.

Monoterpenes are the most significant fraction (59.66%), of which 52.75% are oxygenated monoterpenes. Pipéritone is the major compound (50.25%), followed by l-α-terpinéol (1.18%), p-menth-2-en-1-ol (quercivorol, (0.63%)), and cis-β-terpinéol (0.45%).

Hydrogenated monoterpenes represent only 6.91%, with δ-4-carene being the major compound (5.46%), followed by dl-limonene (1.18%), while other hydrogenated monoterpene derivatives are present in trace amounts. Citronella is free of pinenes.

Limonene and δ-4-carene are the monoterpenes that are partly responsible for the plant's scent and are also used in alternative medicine for their observed ability to reduce heartburn and acid reflux.

As for sesquiterpenes, they represent 37.38%, with the majority being oxygenated (32.50%). α-elemol is the dominant compound (11.06%), followed by β-eudesmol (6.36%), α-eudesmol (6.03%), γ-eudesmol (3.34%), selina-6-en-4-ol (2.14%), α-cadinol (1.85%), and iso-spathulenol (1.03%). Citronella is low in hydrated sesquiterpenes and contains only 4.88%, including δ-cadinene (1.05%), β-elemene (0.82%), germacrene D (0.58%), α-selinene (0.52%), and so on.

Other chemical compounds were detected in very small proportions, including monoterpene carboxylic acid (octanoic acid, 0.26%). Therefore, pipéritone represents half of the percentage of this essential oil (50.25%), but there is no citronellol or citral.

The composition of the essential oil in our sample is almost identical to that of the essential oil of *Cymbopogon schoenanthus* cultivated in Lomé (Togo). The latter is rich in pipéritone (68%) and carène-2 (16.48%), limonene (2.29%), β-caryophyllene (1.10%), β-élémène (0.82%), and β-eudesmol (0.79%). All of these compounds exist in our sample but in varying concentrations. However, if *Cymbopogon citratus* L. represents the chemo-type with citral and *Cymbopogon nardus* L. represents the chemo-type with citronellal/geraniol, then *Cymbopogon schoenanthus* from Ahaggar may represent the chemo-type with pipéritone/elémol [10], which suggests that the chemotropism of a species also varies with the geographical zone.

Literature data shows that aside from citronellal, which could be almost the only constant compound in various varieties, the rest of the chemical composition is highly variable and characteristic of one region to another. Citronella generally contains terpenic aliphatic alcohols and aldehydes (geraniol, citral) [11].

The essential oil of *Cymbopogon schoenanthus* collected in southern Tunisia and analyzed by GC-MS and ^{13}C NMR contains limonene (10.5-27.3%), β-phellandrene (8.2-16.3%), δ-terpinene (4.3-21.2%), and α-terpineol (6.8-11.00%)[12]. The analysis by GC-MS of the essential oil of *Cymbopogon nardus* (L), cultivated in Sri Lanka, showed that the major compounds are citronellal (42.0%), citronellol (20.8%), and geraniol (14.5%)[13]. The same study conducted on the essential oil of *Cymbopogon nardus* from Iran, to use this species as a preventive measure aimed at limiting the growth and development of microorganisms as an alternative to commercial antibiotics used in aquaculture, counted a total of 22 chemical compounds by GC-MS, with citronellal representing the main compound (29.6%) and 2,6-octadienal, 3,7-dimethyl-, (E)- (neral, 11.0%) [14]. These results indeed show that essential oils of the *Cymbopogon genus* are variable from one region to another except for citronellal, which is absent in our sample, making it unique and very different from other regions.

4.3. Microbiological activities

The antibacterial activity of this essential oil is very significant for certain strains; it has bactericidal power against all reference strains tested, except for *Pseudomonas aeruginosa*, which shows slight sensitivity. *Bacillus subtilis* has the lowest MIC (0.29%).

The antifungal activity is also significant; the fungistatic power of the essential oil against *Candida albicans* (MIC=MFC=0.29%) and *Saccharomyces cerevisiae* (MIC=MFC=0.29%) has been demonstrated.

These results are very interesting compared to the literature [10], where a study of the antimicrobial power of the essential oil of three *Cymbopogon sp*. cultivated at the Agropedagogical Experimentation Station of the Higher School of Agronomy at the University of Lomé (Togo) In vitro testing on 7 fungal strains and 7 bacterial strains responsible for mixed infections in dogs and cats revealed that all bacterial strains tested were insensitive to the studied essential oils. However, *Cymbopogon citratus* L. (citral chemotype) and *Cymbopogon nardus* L. (citronellal/geraniol chemotype) showed a very valuable fungistatic activity with minimum inhibitory concentrations (MICs) ranging from 75 to 200 $\mu g.ml^{-1}$ [15].

If we can assume that the inhibitory activity of the essential oils of *C. citratus* and *C. nardus* is due, for the former, to the monoterpene aldehydes (neral, geranial, citronellal) it contains, and for the latter to the chemically related alcohol (citronellol and geraniol) [10], the antibacterial and antifungal activities of the essential oil in our sample are mainly due to the sesquiterpene alcohols it contains (α-elémol, β-eudesmol, α-eudesmol and γ-eudesmol on the one hand, and the terpene hydrocarbons (δ-carene and dl-limonene) on the other hand.

Moreover, all of these compounds are well-known for their anti-infective properties [16,17].

4.4. Limit toxicity test

The limit toxicity test of the studied essential oil of *Cymbopogon schoenanthus* showed that it is not toxic when the dose is ≤ 2g/kg, this assay should be supplemented by the study of acute toxicity in order to determine the exact LD50 by increasing the dose to higher than 2g/kg.

5. Conclusion

The physico-chemical study of the underground parts of lemongrass showed a very high yield of essential oil (greater than 4ml/100gms) and a chemical composition rich in piperitone, which could consider it as a species with the piperitone chymotype.

The biological study revealed a fairly significant antibacterial and antifungal activity which could be exploited in therapy.

The non-toxicity of this essential oil has been confirmed.

In the end, all of these results obtained in vitro constitute only a first step in the search for biologically active substances from natural sources. Additional tests will be necessary and should be able to confirm the properties highlighted.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors and all co-authors declare that they have no conflicts of interest in connection with this document, and the material described is not in the process of being published nor is it intended for publication elsewhere.

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