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Chemical composition, antioxidant and antimicrobial activities from extracts of *Cymbopogon schoenanthus* L. (Spreng) of Algeria

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Abstract

The main objective of this study is to investigate the chemical composition, antioxidant and antimicrobial activities of the essential oil and methanolic extract of *Cymbopogon schoenanthus* L. of south Algeria. Essential oil of *Cymbopogon schoenanthus*were extracted by hydrodistilation, and their chemical composition were identified by GC/MS Antioxidant activity of methanol extracts has been done by using DPPH essay. The antimicrobial activity of essential oil was realised by agar disc diffusion method. The resultants present in aerial partabout21 components accounting more than 76,94% of the total essential oil were identified, the major compounds are constituted by the Guaiol 20,44%,Cis-beta-terpineol 16,23%, Hinesol 10,55%, Cis-sabinene hydrate 9,98% and in root part about 25 components accounting more than 60,94% of the total essential oil were identified, the major compounds are constituted by the Agarospirol 14,21% cis-, beta,-terpineol 12,61%, (+)-4-Carene 6,92%, cis-sabinene hydrate 6,62%, Guaiol 5,88%. IC 50 values observed for DPPH essay were 168,28 ug/ml. In the other hand, this oil was found effective against all tested strains, this activity was ranging from 16,22±3,166 mm with *Staphylocoque aureus* ATCC 25923. These results provided evidence that the studied plant might indeed be potential sources of natural antioxidant and antimicrobial agents.

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Introduction

Plant-dérive antioxydants, especially, the phenolics have gained considerable importance due to their potential health benefits. Epidemiological studies have shown that consumption of plant based food containing antioxidants is beneficial to health because it down-regulates many degenerative processes and can effectively lower the incidence of cancer and cardio-vascular diseases. On other hand the oxidation in food processing industries causes rancidity or deterioration of food which results in discoloration, change in taste, shortened shelf life and loss of nutritional value of processed foods (Kim *et al*., 2011). In living organisms, free radicals such as ROS and RNS are produced as a result of oxidation process which causes damage to cell structures (Kumar *et al*., 2012). Antioxidants are the substances that neutralize free radicals, generated as a result of oxidation process and prevent damage to the human body and foods (Devasagayam *et al*., 2004).

Nature has provided an entire store-house of remedies to treat all ail mints of mankind. Plants and plant products use as medicines could be traced as far back as the foundation of human civilization. Antimicrobial activity of therapeutic plant has turn out to be a world wide concern. There is a continuous and urgent need to find out new antibacterial compounds for new communicable diseases. Consequently, researchers are increasingly turning their attentiveness to conventional medicine and probing for new leads to develop enhanced drugs agaçant broad range microbial infections incliner bacterial and fungal (Akbar *et al*., 2014; Majid *et al*.,2013).

These bioactive compounds are actually combinations of secondary products present in the plant. They have been used as food préservatives, pharmaceuticals, alternative medicines and natural therapies for centuries. These compounds are mostly alkaloids, steroids, tannins, phenolic compounds, flavonoids, resins and fatty acids. These compounds are odorous, complex, volatile compounds produced by special cells or groups of cells and concentrated in one particular region of plant such as the leaves, bark and stems (Ahmad *et al.* 2013).

In Sahara of Algeria, the florais very rich in medicinal plants which produce valuable natural substances such as essential oil. Actually, essential oil and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi purpose functional use (Boukhris *et al*., 2012). Many essential oils also have been confirmed to possess the antioxidant activity (Zhang *et al*., 2006). As part of the study evaluation of the biological effectiveness of the medicinal plants, *Cymbopogon schoenanthus*. This plant is a subspontaneous grass, tropical-afro-asiatic, which isused as traditional medicines to treat digestive diseases: aerophagia, flatulence and urinarydecrease, analeptic drink for new mother after child birth, bad breath, gumboils and urinary in continence (Hammiche *et al*., 2006). The leaves of this herb, when fresh and young are consumed in salads and also used for preparation of traditional meat recipes. The white centre of the leaves is used to impart a flavor to curries (Khadri *et al*., 2008). In addition, it is used as a source of essential oil (Cheel *et al*., 2005). As well as the role of oil in lamb sex perimentally infected with Haemonchus contortus (Katiti *et al*.,2012). Activities anti microbial (Koba *et al*., 2004). Actives antioxidant (Khadri *et al*., 2008).

The study presented a study of the antioxidant and antibacterial activities associated with the chemical composition of essential oili solated from *Cymbopogon schoenanthus* (Poaceae), and methanolic extract

Materials and methods

Plant material

Cymbopogon schoenanthus L. was collected during the flowering phase (July 2015) from Ghardaia is located within the Sahara Desert in northern-central of south Algeria (32°29'25.35"N ;3°40'25.87"E). The plant material were cleaned chopped into pieces and derided in air.

Extraction of the essential oil

Essential oil was extracted from air-dried parts of *Cymbopogon schoenanthus*by hydrodistillation for (3h) using a Clevenger apparatus type.

The yield of each essential oil was determined on average over the three replicates. These oils were kept at 4°c until analysis (Bruneton, 1999).

Preparation of methanol extract

Total methanol extract of *Cymbopogon schoenanthus* was prepared by maceration technique, the dried and powder of plant (aerial or roots parts) (5g) were macerated with (20ml) of methanol at room temperature 3 time (24 hours×3). After filtration, the extract was concentrated using a rotary evaporator at a maximum temperature of 45°C, the residuals obtained were divided, a half part was stored in a freezer at – 4°C until further study (Harar, 2012).

Antioxidant activity

The free radical scavenging capacity methanol extract of *Cymbopogons choenanthus* was evaluated with the methodology described by Blois (1958) as elaborated by El mastas (2007). The solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM) was prepared and 1 ml of DPPH solution was added to 1 ml of the solution of methanol extract at different concentrations (200, 400, 600, 800µg/ml), absorption was measured at 517 nm up to 30 min or until it remained constant. The scavenging capacity of DPPH radical was calculated using the following formula. Where, Acontrol (Ac) is the absorbance of the control reaction and A sample (AS), is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged Ascorbic acid was used as standards.

$$\%\ inhibition\ = \frac{Ac - As}{Ac} \times 100$$

The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect percentage against extracts concentrations (Shimada *et al,*. 1992).

Gas chromatography-mass spectrometry (GC-MS)

Analysis by GC/MS was carried out using a Varian GC 3800 equipped with a SPB1 capillary column (30 mm, 0.25 mm, 0.25 mm) and a "Mass Selective MS Saturn Series 2200, column SPB-1.

The temperature of the detector was 250°C, and the injector 210°C, the oven temperature was programmed as before and the transfer line temperature was 280°C and operating under the GC condition programmed heating at 55°C for 1 min to 150°C for 3min to 250°C for 8 min . The injector temperature was 250°C. Helium was the GC carrier gas at.

Phytochemical test

The methanol extracts were subjected to phytochemical tests for secondary metabolites, tannins, saponins, steroid, alkaloids and glycolsides in accordance with Trease and Evans (1987) and Harborne (1998) with little modification.

Evaluation of antibacterial activity

In this study, five strains Gram negative bacteria *Pseudomonas aeruginosa ATCC 25922, Vibrio cholerae, Salmonella enterica, Serratiamarcescen* and *Escherichia coli ATCC 27853* and four strains Gram positive bacteria *Micrococcus luteus, Staphylococcus epidermidis, Staphylococcus aureus (ATCC 25923), Enter ococcus faecalis* and *Bacillus cereus* provided from hospital El hakim saadan Biskra. This assay was carried out using the disc agar diffusion method with a little modification (Phaiphan, 2014). Tested strains grown on Müller-Hinton agar at 37 °C for 18 h for bacteria were suspended in a saline solution (0.9% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/mL). The suspension was used to inoculate 90 mm diameter Petri plates containing medium cited above. Sterile paper discs No. 1(6 mm diameter) was impregnated with 10µL of essential oil after sterilisation the disc laded on the surface of agar plates. Before incubation the incubation conditions were at 37°C for 24h for bacteria. Antimicrobial activities were evaluated by measuring the inhibition zone diameters.

The work was achieved in aseptically conditions. Spiramyc in 100µL/disc, (Cephotaxime) 30µL/disc, Ampicillin 10µL/disc were used as positive control to determine the sensitivity of Gram-negative and Gram-positive bacteria respectively (Schinor, 2007).

All tests were performed in triplicate for each micro-organisms strain and the final results of inhibition zone measured in millimetre were presented as the average.

Statistical analysis

Analysis of variance (ANOVA) was performed on the data obtained using Co Stat-Statistics Software version 6.4. The significance of the differences among treated samples was evaluated using the LSD test for comparisons the means ± standard deviation (SD) of the diameter of inhibition. Each experiment has three replicates and three determinations were conducted and the significance level for all measurements was considered at $p<0.05$.

Results and discussion

Air-dried parts of *Cymbopogon schoenanthus* were subjected to hydrodistillation using a Clevenger-type apparatus. Liquid, gilded yellow and penetrating strong odour essential oil was obtained with a yield of 4,45 % (w/w), based on dry weight of the plants, but in the other study was obtained with a yield of 2,6 % (Khadri *et al.*, 2008).

Chemical Composition of Essential Oils

Chemical analysis of essential oils of aerialpart revealed that both contain monoterpenes and sesquiterpenes.

However they differ in their major components *Cymbopogon schoenanthus* is 21composed mainly of Guaiol 20, 44%, Cis-beta-terpineol 16,23%, Hinesol 10, 55%, Cis-sabinene hydrate 9,98% (Table 1).

Table 1. Chemical composition of the essential oil of *Cymbopogon schoenanthus* in the aerial part.

Peaks	RT	Peak Name	Area	compound %
1	4,354	p-Xylene	18421	0,19%
2	4,858	o-Xylene	9921	0,10%
3	5,785	3-Carene	35371	0,36%
4	7,818	(+)-4-Carene	751962	7,70%
5	8,075	.alpha.-Phellandrene	34695	0,36%
6	8,442	1, 3-Cyclohexadiene	17642	0,18%
7	8,788	tert-Butylbenzene	23208	0,24%
8	8,894	Cyclobutane	148930	1,52%
9	9,051	Eucalyptol	181411	1,86%
10	9,144	Octatriene	87519	0,90%
11	9,538	alpha-pinene	42840	0,44%
12	12,85	cis-,beta,-terpineol	1,59E+06	16,23%
13	13,652	cis-sabinene hydrate	974718	9,98%
14	30,413	Guaiol	2,00E+06	20,44%
15	31,378	Cubenol	19785	0,20%
16	31,757	(-)-Globulol	21293	0,22%
17	33,578	.delta.-Selinene	236739	2,42%
18	33,733	Agarospirol	175049	1,79%
19	34,005	.tau.-Cadinol	77345	0,79%
20	34,598	Hinesol	1,03E+06	10,55%
21	42,396	Isoaromadendreneepoxide	46463	0,48%

But in the root part about 25 components accounting more than 60,94% of the total oil were identified , the major compounds are constituted by the Agarospirol 14,21% cis-, beta,-terpineol 12,61%, (+)-4-Carene 6,92%, cis-sabinene hydrate 6,62%, Guaiol 5,88% (Table 2). Onadja *et al.*, (2007) were obtained the major compounds are constituted by the Pipériton 42%, and Bassole *et al.*, (2001) is observed that the Piperiton with 70,2%, In the other the major compounds are constituted by the Geraniol 62,5% (Katiki *et al.*, 2012).

The difference Chemical analysis of essential oils of *Cymbopogon schoenanthus* might be attributed to age of the plant and plant part studied, as well as to the time the plant collection and soil climate factors (El-massry *et al.*, 2002).

Phytochemical screening

Investigations on the physiochemical screening of *Cymbopogon schoenanthus* plant extracts revealed the presence ofsteroid, tannins, Reducing sugars,

alkaloids and flavonoids. These compounds are known to be biologically active (Table 3). Our results are in close agreement with that reported by Amina *et al*., (2013), and El-kamali; El-amir (2010).

Table 2. Chemical composition of the essential oil of *Cymbopogon schoenanthus* the root part.

Peaks	RT	Peak Name	Area	Compound %
1	4,34	p-Xylene	22244	0,184
2	4,84	m-Xylene	13731	0,113
3	5,771	3-carene	14359	0,119
4	6,257	Camphene	10464	0,086
5	7,798	(+)-4-Carene	837834	6,917
6	8,055	.alpha.-Phellandrene	31725	0,262
7	8,875	Limonene	246445	2,035
8	8,945	.beta.-Phellandrene	99307	0,82
9	9,03	Eucalyptol	19695	0,163
10	9,126	1R-.alpha.-Pinene	29494	0,243
11	12,837	cis-,beta,-terpineol	1,53E+06	12,605
12	13,637	cis-sabinene hydrate	802080	6,622
13	16,545	2-Cyclohexen-1-ol	401283	3,313
14	18,524	2-Cyclohexen-1-one	57283	0,473
15	23,812	Caryophyllene	112805	0,931
16	24,976	Azulene	33933	0,28
17	25,445	Isoledene	178517	1,474
18	27,845	Cedran-diol	127795	1,055
19	29,428	Epiglobulol	52700	0,435
20	30,395	Guaiol	712302	5,88
21	31,466	(-)-Spathulenol	11515	0,095
22	31,557	Caryophylleneoxide	9020	0,074
23	33,225	Selina-6-en-4-ol	268285	2,215
24	33,744	Agarospirol	1,72E+06	14,206
25	36,45	Carotol	40782	0,337

Table 3. Phytochemicals found in methanolic extract of *Cymbopogon schoenanthus*.

Phytochemicals	Aerial part	Root part
Flavonoids	-	-
Saponin	-	-
Steroids	+	+
Reducingsugars	+	+
Tannins	+	+
Alkaloids-wagnersreagents	+	+
Alkaloids-Draghandroff-reagents	+	+
Volatile oils	+	+

Key: + = present, - = absent.

Antioxidantactivity

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Shirwaikar *et al*., 2006). Radical scavenging activities are very important to prevent the deleterious role of free radical in different diseases including cancer. DPPH free radical scavenging is an accepted mechanism by which antioxidants act to inhibit lipid peroxidation. This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. Our results revealed that the metabolic extract of *Cymbopogon schoenanthus* had the similar free radical scavenging activity when compared with standard ascorbic acid (Fig. 1); (Table 4). The results indicated the proton donating ability of the extractives which could serve as free radical inhibitors or scavengers and can also be served as primary antioxidants.

Table 4. Scavenging effects of methanol extracts on DPPH radical.

Concentration (µg /ml)	Percentage of inhibition
100	31.09±0.011
200	61.19±0.007
300	82.83
400	90±0.008

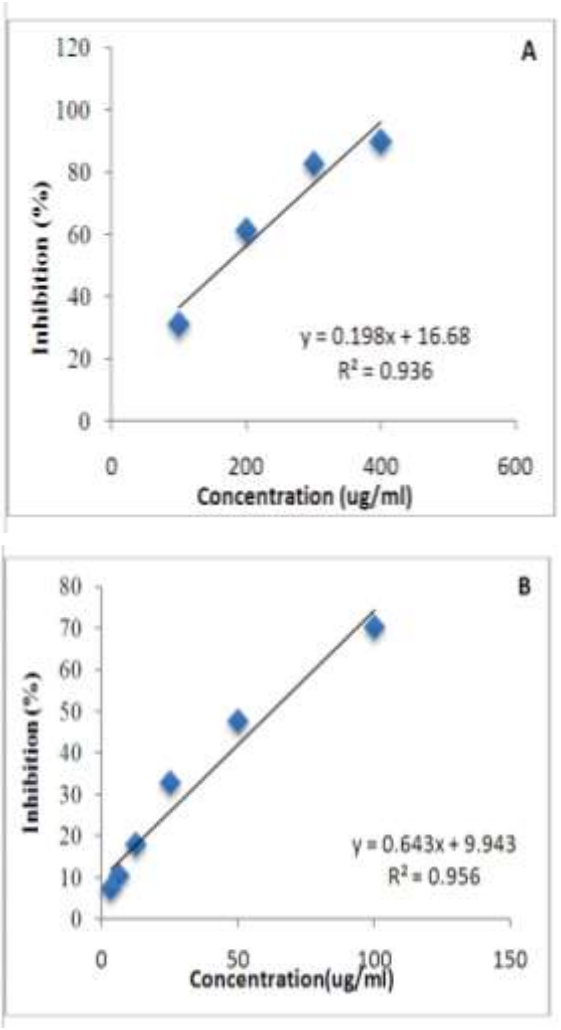

Fig. 1. (A, B). Reducing power of antioxidant activity methanolic extracts of *Cymbopogon schoenanthus* (A) and Ascorbic acid (B).

IC_{50} for DPPH radical-scavenging activity was 168.28µg/ml. The IC_{50} values for Ascorbic acid, 62µg/ml.While another study indicated that the antioxidant activity estimated that IC_{50} for DPPH radical-scavenging activity was 17,1µg/ml. (Khadri *et al*., 2010). The difference results might be the existing of poly phenol contents of the extractives and its anti-oxidant properties (Huang *et al*., 2005).

Antibacterialactivity

The lipophilic character of the hydrocarbon skeleton and the hydrophilic character of their functional groups are of main importance in the antimicrobial action of essential oil components (Griffin *et al*., 1999). Due to these data we were interested to study antimicrobial activity of the essential oil. The results were summarized in (Table 5), which showed that essential oil extracted from of *Cymbopogon schoenanthus* prevented the growth of all tested microorganisms with an inhibition zone medium diameter. The obtained inhibition on bacteria strains varied from 6,0 to 16,23±0,252 mm with a highest inhibition zone recorded for *Staphylococcus aureus*. It should be mentioned that there are no background antibacterial studies on *Cymbopogon schoenanthus*, while in genus *Cymbopogon* some studies have been reported as 16.23±0,252mm for *Staphylococcus aureus*, 13,73±0,643mm for *Vibrio cholerae* ,12.33±0,765mm for *Serratia marcescen* , and 12.10±0,173mm for *Salmonella enterica*, 11,10±0,854mm for *Escherichia coli* ATCC 27853, 10.67±0,681mm for *Staphylococcus epidermidis*, 9.77±0,404 mm for *Enterococcus faecalis*, 7.33± 0,289mm for *Pseudomonas aeruginosa* ATCC 25922 , and 6mm for *Bacillus cereus* .This low activity of the essential oils tested could be related to their chemical composition indeed, the study of the antibacterial activity of certain constituents of Essential oils has distinguished: phenolic compounds with high antimicrobial activity such as Thyme and caracole (Cosentino *et al*., 1999; Gergis *et al*., 1990). The constituents with low antibacterial activity which are: pulegone, Menthone, 1,8-cineole, p-cymene, isomenthone, myrcene, pinene, piperitone, Liminene, linalool, terpinene, sesquiterpenes and Terpenic (Lattaoui and Tantaoui, 1994; Carson *et al*., 1995; Chalchat *et al*., 1995).

Table 5. Zone of Inhibition of bacterial strains against of essential oil *Cymbopogon schoenanthus* and positive control.

Bacteriastrains		Zone of inhibition (mm)			
		Essential oil (10 µL/disc)	Spiramycin 100 µL/disc	(Cephotaxime) 30 µL/disc	Ampicillin 10 µL/disc
Gram negative bacteria	*P. aeruginosa*	7.33 ± 0,289^{c}	18 ±0,866^{b}	±29 1,32^{a}	ND
	V. cholerae	13.73± 0,643^{c}	±10 1,323^{d}	±37 0,50^{a}	25,10 ±1,852^{b}
	S. enterica	12.10± 0,173^{c}	±10 0,3^{c}	±321,323^{a}	±202,0^{b}

	S. marcescen	12.33 ± 0,764^{c}	±13,50,5^{c}	±350,5^{a}	20± 2,326^{b}
	E. coli	11.10 ± 0,854^{c}	10 ±0,2^{c}	35±1,20^{a}	22± 1,114^{b}
Gram positive bacteria	*M. luteus*	12.43± 0,929^{c}	±100,954^{d}	±360,30^{a}	±25 1,819^{b}
	S. epidermidis	10.67± 0,681^{d}	±170,866^{c}	±340,954^{a}	±240,889^{b}
	S. aureus	16.23± 0,252^{d}	23,97± 1,70^{c}	35,20±1,31^{b}	43,03 ± 1,19^{a}
	E. faecalis	9.77 ± 0,404^{c}	ND	±391,0^{a}	±181,323^{b}
	B. sereus	6 ± 00^{d}	±14,50,50^{c}	36,10± 0,854^{a}	±25 1,30^{b}

Means of three replicates ± SD (standard deviation) followed by at least one same letter are not significantly different according to LSD test at $p < 0.05$.ND = not detected.

Conclusion

The phytochemical screening of *Cymbopogon schoenanthus*. plante extracts revealed the presence of steroids, tannins, reducing sugars, alkaloids and flavonoids. Air-dried parts of *Cymbopogon schoenanthus* were subjected to hydro distillation using a Clevenger-type apparatus. Liquid, gilded yellow and penetrating strong odour essential oil was obtained with a yield of 4.45% (w/w), based on dry weight of the plants. The chemical analysis of essential oils of part aerial revealed 21 composed mainly of Guaiol 20, 44%. In part of root about 25 components accounting more than 60, 94% of the total oil was identified; the major compounds are constituted by the Agaro-spirol 14, 21%. For the antioxidant activity, IC_{50} values observed for DPPH essay were 168,28ug/ml. In the other hand, this oil was found effective against all tested strains, this activity was ranging from 16,23±0,252mm with *Staphylocoque aureus*.

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